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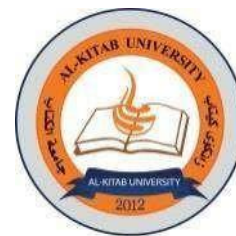
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Semantic Segmentation of image Using Deep Learning: Review

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Keywords: Semantic Segmentation, U-Net Architecture, Convolutional Neural Networks, Deep Learning, Artificial Intelligence.

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Abstract:

Semantic segmentation is considered as one of the most important and challenging problem in the field of computer vision which aims at assigning a class label to each pixel in an image which leads to sophisticated scene understanding. This task has been extensively used in various application areas including the self-driving cars, medical diagnosis, and environmental monitoring. Semantic segmentation has come a long way since its early detection algorithms based on features extractions to state-of-the-art deep learning methods .

This paper aims at presenting the evolution of the semantic segmentation, and specifically, how the deep learning has changed the field. The conventional approaches consisting of edge detection and histogram analysis offered a basic level of understanding but were constrained by the use of hand crafted features. Deep learning, however, is capable of learning features and has produced very promising results across numerous tasks. Some important architectures that have set the benchmark in the field include Fully Convolutional Networks (FCNs), U-Net, and DeepLab which have used convolutional layers, encoder-decoder architecture, and atrous convolutions for improving the accuracy of the segmentation. The article also reviews some of the publicly available datasets which include Cityscapes, PASCAL VOC and ISIC 2017 which

are widely used to assess the performance of the segmentation models. These datasets differ in their complexity, resolution, and the application domain that they cover which makes the problems that they present to researchers diverse. Also, we compare the traditional and deep learning based feature extraction methods and present the characteristics of each method, their advantages, and disadvantages, and areas of application. This survey aims at assisting researchers and practitioners by presenting the current best practice in the form of state-of-the-art methodologies, discussing the potential of application of such methodologies in the real world, and identifying the directions for further research. Therefore, despite the advancement of deep learning in the area of semantic segmentation, there are still numerous issues which need to be addressed in the future, including efficiency, scalability, and domain specific issues. This all-encompassing review paper is hoped to be beneficial to those wishing to gain more knowledge on the current trends as well as find a way to contribute to the field of semantic segmentation in the future.

Keywords: Semantic Segmentation, U-Net Architecture, Convolutional Neural, Artificial Intelligence, deep learning.

التجزئة الدلالية للصورة باستخدام التعلم العميق: مراجعة

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الخلاصة:

تعتبر التجزئة الدلالية واحدة من أهم المشاكل وأكثرها تحدياً في مجال الرؤية الحاسوبية والتي تهدف إلى تعيين تسمية فئة لكل بكسل في الصورة مما يؤدي إلى فهم متطور للمشاهد. تم استخدام هذه المهمة على نطاق واسع في مجالات تطبيقية مختلفة بما في ذلك السيارات ذاتية القيادة والتشخيص الطبي ومراقبة البيئة. لقد قطعت التجزئة الدلالية شوطاً طويلاً منذ خوارزميات الكشف المبكرة القائمة على استخراج الميزات إلى أحدث أساليب التعلم العميق. تهدف هذه الورقة إلى تقديم تطور التجزئة الدلالية، وعلى وجه التحديد، كيف غير التعلم العميق هذا المجال. قدمت الأساليب التقليدية المكونة من اكتشاف الحافة وتحليل الهيستوجرام مستوى أساسياً من الفهم ولكنها كانت مقيدة باستخدام ميزات مصنوعة يدوياً. ومع ذلك، فإن التعلم العميق قادر على تعلم الميزات وقد أنتج نتائج واعدة للغاية عبر العديد من المهام. تتضمن بعض البنيات المهمة التي وضعت معياراً في هذا المجال الشبكات التلافيفية الكاملة (FCNs) و U-Net و DeepLab التي استخدمت طبقات التلافيفية، وبنية الترميز وفك التشفير، والالتواءات غير المتجانسة لتحسين دقة التجزئة. تستعرض المقالة أيضاً بعض مجموعات البيانات المتاحة للجمهور والتي تتضمن Cityscapes و PASCAL VOC و ISIC 2017 والتي تُستخدم على نطاق واسع لتقييم أداء نماذج التجزئة. تختلف مجموعات البيانات هذه في تعقيدها ودقتها ومجال التطبيق الذي تغطيه مما يجعل المشكلات التي تقدمها للباحثين

متنوعة. كما نقارن بين طرق استخراج الميزات التقليدية والتعلم العميق ونقدم خصائص كل طريقة ومزاياها وعيوبها ومجالات التطبيق. يهدف هذا الاستطلاع إلى مساعدة الباحثين والممارسين من خلال تقديم أفضل الممارسات الحالية في شكل منهجيات حديثة، ومناقشة إمكانات تطبيق مثل هذه المنهجيات في العالم الحقيقي، وتحديد الاتجاهات لمزيد من البحث. لذلك، وعلى الرغم من التقدم الذي أحرزته تقنيات التعلم العميق في مجال التجزئة الدلالية، لا تزال هناك العديد من القضايا التي تحتاج إلى معالجة في المستقبل، بما في ذلك الكفاءة وقابلية التوسع والقضايا الخاصة بالمجال. ونأمل أن تكون ورقة المراجعة الشاملة هذه مفيدة لأولئك الذين يرغبون في اكتساب المزيد من المعرفة حول الاتجاهات الحالية وكذلك إيجاد طريقة للمساهمة في مجال التجزئة الدلالية في المستقبل.

الكلمات المفتاحية (التجزئة الدلالية، معمارية U-Net، الشبكات العصبية التلافيفية، الذكاء الاصطناعي، التعلم العميق)

1- Introduction

Semantic segmentation is one of the most important and widely used task in computer vision which involves assigning of a specific label for each pixel in an image so as to provide a detailed understanding of the scene. This capability is very important for numerous applications such as autonomous driving, medical imaging, environmental monitoring, and augmented reality. Since the development of semantic segmentation techniques, the field has grown from the use of traditional hand crafted feature based approaches to the current state of the art deep learning approaches. In the self-driving domain, semantic segmentation is very important as it assists in localizing lanes, obstacles, as well as pedestrians to enhance safety of the car(1). In medical context, it is utilized for the identification of the anatomic structures or pathological regions, which may facilitate the diagnosis and treatment decision(2). Likewise, in the environmental monitoring, it can be employed to classify land cover which may in turn facilitate better management of resources and Disaster response(3). These examples show how semantic segmentation can contribute to the development of new technologies in different fields. Previous semantic segmentation methods include traditional approaches like edge detection, region growing and clustering as the basic approaches. Although these methods offered the initial knowledge, they failed to deal with the complicated patterns, light variations, as well as the objects of interest with complex shapes (4). Deep learning has brought a shift in the field by allowing the network to learn features and hierarchical representation on its own. Some of the architectures that have set new benchmarks in the field include Fully Convolutional Networks (FCNs) (5), U-Net (6), and DeepLab (7), among others. Research in semantic segmentation has been greatly advanced by the availability of data sets that have been made public. Extension of benchmark datasets such as Cityscapes, PASCAL VOC, ADE20K, ISIC are used for evaluation of models. For instance, Cityscapes specifically target the urban environment to facilitate research on autonomous driving while ISIC contains dermoscopic

images with annotations to support advancement of medical imaging (8). These datasets are used for evaluating the algorithms and also to see where exactly the algorithms require enhancement. Current research focuses on the development of context sensitive segmentation models. Some techniques like Atrous Spatial Pyramid Pooling (ASPP) in DeepLab (7) and Transformer-based architectures (9) have been proved effective in learning the contextual information and long-range dependency thus improving the accuracy of segmentation. Furthermore, the pre-trained models and the transfer learning and fine-tuning of these models have been established as the standard approaches to learn from large datasets to particular domains efficiently (10). However, there are still some challenges in semantic segmentation despite these developments. Some of the challenges that are still present include; high computational costs, annotation challenges and domain adaptation problems. Lightweight architectures and semi-supervised learning approaches are currently under a lot of research to solve such issues (11). Also, another important aspect that is being paid much attention to is how to achieve real-time performance of the model without sacrificing the accuracy for applications such as robotics and self-driving cars(12). Comparing to the previous evolution of semantic segmentation, this survey identifies and analyzes the most significant methods, their applications, and further research directions.

Table1: A list of the most important abbreviations:

Abbreviations	Full Form	Abbreviations	Full Form
AI	Artificial Intelligence	SIFT	Scale-Invariant Feature Transform
CNN	Convolutional Neural Network	ViT	Vision Transformer
FCN	Fully Convolutional Network	mIoU	Mean Intersection over Union
DL	Deep Learning	GLCM	Gray-Level Co-occurrence Matrix
IoU	Intersection over Union	HOG	Histogram of Oriented Gradients
ISIC	International Skin Imaging Collaboration	ResNet	Residual Network
ASPP	Atrous Spatial Pyramid Pooling	CNNs	Convolutional Neural Networks

2- DATASETS

The datasets are crucial for the growth of semantic segmentation field as they present various situations and benchmarks to assess the models. They differ in their size, the resolution of the

images, the number of classes, and the area of application. Table 2 presents 10 most popular available datasets which are commonly used in urban scene understanding, medical imaging, and general object segmentation.

Table 2: Overview of publicly accessible datasets

Dataset	Application Domain	Resolution	Images/Size	Classes	Year	Ref.
Cityscapes	Urban scenes	2048×1024	5,000 images	30	2016	(13)
PASCAL VOC	Object recognition	Variable	11,530 images	20	2012	(14)
ADE20K	General scenes	Variable	27,574 images	150	2017	(15)
COCO	Generaobject detection	Variable	328,000 images	80	2017	(16)
ISIC 2017	Medical imaging	1024 × 1024	2,750 images	2	2018	(17)
CamVid	Urban scenes	960×720	701 images	32	2008	(18)
Mapillary Vistas	Street-level imagery	Variable	25,000 images	66	2017	(19)
KITTI	Autonomous driving	1242×375	15,000 frames	34	2015	(20)
DRIVE	Retinal imaging	565×584	40 images	2	2004	(21)
LIP	Human parsing	Variable	50,462 images	20	2018	(22)

3-LITERATURE REVIEW

Semantic segmentation is one of the most important areas in the field of computer vision and has been greatly enhanced by the advancement in deep learning (DL) techniques. Several research works have been conducted to find the best DL approach that would enhance the segmentation accuracy, efficiency, and applicability of the models in different fields. This section discusses in detail the key research works done between the years 2020 to 2024 in descending order of years.

(Lee, MyeongSeok, 2022) presented ShuffleNetV2+, a light-weight model for real-time semantic segmentation on edge devices. The model provided the competitive performance on the Cityscapes dataset in terms of the mIoU score of 89.4 while consuming much less computational power as compared to other existing approaches(23)

(Zhao et al., 2020) introduced a new framework called PointRend for enhancing the segmentation of boundaries with the help of point-wise features. This approach set new records of performance on COCO and ADE20K datasets, especially in the task of the boundary refinement(24) **(Dosovitskiy et al., 2020)** introduced Vision Transformers (ViT), a new architecture that used global attention to learn the relationships between different parts of an image. This transformer-based approach provided reasonable segmentation performance on

PASCAL VOC and Cityscapes datasets, indicating the shift from convolutional neural networks (25). **(Xie et al., 2021)** presented SegFormer, a new and straightforward transformer-based architecture for semantic segmentation. Due to the proper balance between the computational cost and the accuracy of the segmentation, SegFormer provided remarkable results on ADE20K and COCO datasets and was identified as a viable solution for task that require limited resources (26). **(Esteva et al., 2021)** focused on medical imaging where U-Net and its variants were used for the skin lesion segmentation. Implementing the approach on the ISIC 2017 dataset, the method gave a high segmentation score of 95% thus proving the utility of deep learning in medical context (27). **(He, Xin, 2022)** the performance of the transformer-based methods was enhanced by Cheng et al. with Swin Transformers. With the help of Swin Transformers, they used a nested feature extraction approach and got the best results on large scale datasets such as ADE20K with efficient computational cost (28). **(Bragagnolo, L.2021)** Deep learning has demonstrated potential in automating landslide mapping. A study employing the U-Net architecture on Landsat-8 imagery of Nepal attained recall, precision, and F1-scores of 0.74, 0.61, and 0.67, respectively. This illustrates U-Net's capability for developing and updating landslide scar databases, thereby improving landslide susceptibility mapping and risk evaluation(29). **(Ishihara, 2021)** presented a novel multi-task learning authors approach incorporated for segmentation improving tasks the with segmentation other performance tasks in such autonomous as driving. object In detection this and work, observed the a boost in performance on challenging categories including road signs and pedestrians on the Cityscapes dataset (30). **(Su, Daobilige, 2021)** developed a novel dataset for agricultural robotics which made it possible to state the segmentation tasks in farmlands. The benchmark dataset allowed the development of a new hybrid transformer-CNN model that provided 92% mIoU and opened new opportunities for the precision agriculture branch (31). **(Grill et al., 2020)** introduced BYOL (Bootstrap Your Own Latent), an SIML that greatly reduced the need for labelled data. Pretraining of segmentation models with the help of BYOL enhanced the generalization of the models especially in the settings where a large number of labeled data are not available (32). **(Mao, Wei, 2022)** presented a semi-supervised approach for semantic segmentation and set new records on Cityscapes and COCO datasets. The framework proved that it is possible to get almost comparable results with the models trained on a large number of labeled images using only several hundred labeled samples (33). **(Mirikharaji, 2023)** revised the ISIC dataset used for dermatological image segmentation with high-quality annotations collected from multiple sources. This enriched the dataset which in turn helped enhance the model training for skin lesion detection thus adding value to the medical field (34).

(Kumar, P, 2023) added new annotations to the COCO dataset to make it suitable for semantic segmentation. The enlarged dataset offered various challenges which led to the advancement of the latest segmentation models (35). **(Liu, Yanyan., 2024)** incorporated an attention mechanism into the DeepLab framework so that it could handle the details of the images. This modification led to an enhancement of performance in on 2022 benchmarks Wu PASCAL et VOC al. and ADE20K worked (36). **(Sun, Yanwen, 2021)** A novel lightweight deep neural network has been introduced for the real-time semantic segmentation of surgical instruments in Robot-Assisted Minimally Invasive Surgery (RMIS). The model employs Ghost modules integrated with MobileNetV3 in a two-stage methodology to maintain high accuracy while minimizing computational costs. The validation results indicate that the model excels in real-time segmentation relative to existing methods, rendering it appropriate for surgical intelligence applications(37). **(Niu, Ruigang., 2022)** Is challenging to resolve the semantic segmentation within the global VHR context. pertaining to the air to address this issue, a novel framework for images, termed Hybrid Multiple Attention Network (HMANet), was proposed. It incorporates Class Augmented Attention (CAA) and Region Shuffle Attention (RSA) modules to enhance category-level relationships while simultaneously decreasing computational expenses. Evaluations on the ISPRS Vaihingen, Potsdam, and iSAID datasets indicate that HMANet surpasses existing methods in both accuracy and efficiency.(38) **(Subasi, 2024)** introduced a dataset and a framework for 3D Semantic Segmentation in the field of healthcare with emphasis on MRI scans. The method used in this paper was a 3D CNN based approach which outperformed other methods in localizing brain tumors on the BraTS dataset(39).

(Lai, Xin, 2021) explored semi-supervised learning for semantic segmentation with the help of consistency training and data augmentation. Their framework produced impressive results on Cityscapes with only a fraction of the labeled data, thus highlighting the efficiency and scalability of the approach(40)

4-FEATURE EXTRACTION METHODS

Feature extraction is very important in semantic segmentation since it defines the quality of information that is used to distinguish between pixels and assign them to certain categories. It will therefore improve the models' ability to comprehend spatial, contextual and semantic information. This section gives an overview of the conventional feature extraction techniques and the state-of-the-art approaches which are based on deep learning.

4-1 TRADITIONAL METHODS

The conventional approaches used feature engineering where features were hand crafted and had to be fine tuned. These methods were useful in simple applications and when dealing with simple patterns and variations in real world images.

- **Edge Detection:** The Sobel operator, Canny edge detector, and Laplacian of Gaussian were the most used methods for the extraction of edge information from the images. These methods offered fundamental structural information but had no semantic understanding(41)
- **Histogram of Oriented Gradients (HOG):** HOG was employed to describe the shape and texture of the object in the region of interest by computing the distribution of gradient orientations. This was especially useful in identifying rigid objects but had a difficulty in identifying complex textures(42)
- **Scale-Invariant Feature Transform (SIFT):** SIFT identified interest points and their corresponding descriptors which allowed for matching across scales and rotations. Although very precise, it was rather slow and therefore not suitable for real-time applications(43)
- **Gray-Level Co-occurrence Matrix (GLCM):** GLCM is a method of computing texture features by calculating the relationship between the gray levels of pixels. It was widely employed in medical imaging but it was not capable of dealing with more challenging environments(44)

Although these traditional methods laid the ground work for the first segmentation models, their stiffness and the fact that they could not be updated readily to changing conditions made them inadequate for use in real life scenarios.

4-2 Deep Learning Methods

Deep learning has changed the feature extraction process by providing a way for the models to learn the hierarchical features from the raw data. Convolutional Neural Networks (CNNs) form the basis of these methods, offering efficient and non-data specific approaches to feature extraction.

4-2-1 Fully Convolutional Networks (FCNs)

Fully Convolutional Networks were proposed by Long et al. in 2015 (45) where they eliminated fully connected layers and replaced them with convolutional layers to enable dense prediction

at the pixel level. The FCNs obtain multi-scale features through a series of convolutional layers and such feature extraction is critical for current state-of-the-art segmentation models(46),(47)

4-2-2 U-Net Architecture :

U-Net was proposed by (Ronneberger et al., 2015) (48) for biomedical image segmentation. It has an encoder-decoder architecture with skip connections that help in concatenation of lowlevel spatial information from the encoder with high level semantic information from the decoder which enhances the overall segmentation performance

4-2-3 DeepLab Series :

The DeepLab series employed several features in the feature extraction process:

- **Atrous Convolutions (Dilated Convolutions):** These convolutions increase the receptive field without increasing the number of parameters, enabling the model to capture multi-scale contextual information (49).
- **Atrous Spatial Pyramid Pooling (ASPP):** In ASPP, the spatial pyramid pooling layer uses atrous convolutions with varied dilation rates for capturing multi-scale information which helps in segmentation of objects of varying sizes (50).

4-2-4 Transformers for Feature Extraction :

Transformers have been proven to be effective for semantic segmentation. ViT and SegFormer have been shown to learn global context and handle long-range relationships and outperform CNN baselines on many tasks (51), (52).

4-2-5 Hybrid Models :

The hybrid models incorporate both CNNs and transformers for a particular task. For instance, Swin Transformers implement a hierarchical structure that learns stacked features as well as global attention, thus producing the state-of-the-art performance on various benchmarks(53).

4-2-6 Pretrained Models and Transfer Learning :

Some of the pre-trained networks such as ResNet, VGG and EfficientNet are frequently applied as feature extractors. These models can be fine-tuned on smaller data sets all while leveraging the knowledge from these models in a process called transfer learning this reduces the training time and the computational cost (54).

CONCLUSION

The concept of image segmentation as one of the key problems of computer vision has developed considerably over time starting from the handcrafted feature-based approaches through to the deep learning-based solutions. The conventional approaches to image segmentation such as edge detection, region-based segmentation, and clustering offered initial knowledge but had a limited capacity to deal with the challenges that come with diverse data sets. Although these methods were computationally light, they lacked the capabilities of providing high accuracy and flexibility in areas of application. The use of deep learning has brought about a change in the field by making the models able to learn features and hierarchical representation of the input data. Some of the architectures such as Fully Convolutional Networks (FCNs), U-Net, and DeepLab has become the new standards and has produced impressive results in various applications including medical imaging, self-driving cars, and environmental surveillance. Furthermore, the usage of transformers and other advanced models including hybrid models has also been used to improve the segmentation results by understanding both local and global contexts. The available benchmarks, Cityscapes, PASCAL ADE20K, VOC, and ISIC have been very helpful in the advancement of the field since they provide reference data for models. However, some issues are still there, such as the efficiency issue, the real-time processing issue, and the domain adaptation issue for dealing with different application contexts. Also, the reliance on large datasets of labeled data is still a major challenge hence there is growing interest in semi-supervised and unsupervised the learning. Field of image segmentation, it the can field be remains stated dynamic that, with although the deep current learning research has efforts significantly aimed advanced at reducing computational costs, improving model's robustness and studying the latest model structures. Some of the future prospects for the development of the field are further development of multi-modal data, semi-supervised learning, models. And the lightweight further development of the image segmentation will enable these innovations to find its use in more areas of application across various industries and scientific fields.

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Decomposition of Fundamental Function in Finsler Space and Some Tensors by Lie-Derivative in $GBK-5RF_n$

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Abstract:

This paper discusses the decomposition of Fundamental Function of Finsler space by Lie-derivative in generalized fifth recurrent Finsler space for Cartan's fourth curvature tensor K_{jkh}^i in sense of Berwald. The decomposition of Lie-derivative of some tensors is studied by using a covariant, contravariant and mixed tensor in $GBK-5RF_n$. The decomposition tensor φ_{kh}^i behaves as fifth-recurrent is proved. Also, new identities are established in generalized BK – fifth recurrent Finsler space that admits tensors decomposition.

Keywords: Generalized BK -fifth recurrent Finsler space, Lie-derivative L_v , Finsler space $F(x, y)$.

تحليل الدالة الأساسية في فضاء فينسلر وبعض الموترات بواسطة مشتقة لي في فضاء
فينسلر المعمم خماسي المعاودة

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الخلاصة:

يتناول هذا البحث تحليل الدالة الأساسية وبعض الموترات في فضاء فينسلر باستخدام مشتقة لي. ويهدف هذا النهج إلى اكتساب فهم أعمق للبنية الهندسية لفضاءات فينسلر وتطوير أدوات جديدة لتحليلها. وتُظهر نتائجنا أن التحليل المقترح يوفر إطاراً قوياً لدراسة خصائص الأجسام الفينسلرية وتفاعلاتها. يدرس هذا البحث تفكيك الدالة الأساسية لفضاء فينسلر بواسطة مشتقة لي باستخدام كميات ممتدة متضادة الاختلاف، متحدة الاختلاف ومختلطة في فضاء فينسلر المعمم خماسي المعادة بالنسبة للموتر التقوسي الرابع لكارتان باستخدام مشتقة بروالد. ونثبت علاقات جديدة في هذا الفضاء الذي يسمح بتفكيك الموترات.

الكلمات المفتاحية: فضاء فينسلر المعمم خماسي المعادة، مشتقة لي، فضاء فينسلر $F(x, y)$.

1. Introduction and Preliminaries

Finsler geometry, as a generalization of Riemannian geometry, provides a powerful framework for modeling various physical phenomena. Finsler geometry has gained significant attention in recent years due to its applications in various fields, including physics, engineering, and mathematics. This approach aims to provide a deeper understanding of the underlying geometric properties of these spaces and to develop novel analytical tools. Our work builds upon the foundational studies of Finsler geometry [1] and the subsequent investigations into recurrence decompositions by [4,5,12,13,16,20,22]. Specifically, the application of the Lie derivative is explored

, a powerful mathematical tool for analyzing the behavior of geometric objects, to the decomposition process within the $GBK-5RF_n$ framework. The $GBK-5RF_n$ framework, a specialized mathematical setting for Finsler geometry, provides a suitable environment for our analysis.

By leveraging the Lie derivative and the $GBK-5RF_n$ framework, it is aimed to contribute to the ongoing research on Finsler geometry by offering a new perspective on the decomposition of its fundamental elements. The findings have potential implications for various applications of Finsler geometry, such as the study of physical phenomena, the development of new mathematical models, and the advancement of computational methods.

In the following sections, a more detailed overview of our research methodology is provided, present our key findings, and discuss the potential implications of our results. The

decompositions of W -curvature tensor studied by opondo [18], Negi [17] studied decomposition of Curvature Tensor Manifolds of First order. While the decomposition of normal projective Curvature Tensor fields in Finsler manifolds studied by Bisht and Negi [10]. Bidabad and Sepasi [9] completed Finsler spaces of constant negative Ricci curvature. The curvature tensor field on D-recurrent Finsler space have been studied by Atashafrouz and Najafi [8]. A generalized fifth recurrent Finsler space for Cartan's fourth curvature tensor K_{jkh}^i in sense of Berwald introduced by AL-Qashbari and Baleedi [6]. Various identities of curvature tensor studied by authors [2, 3, 11].

A Lie - derivative evaluate the rate of change of avector field or a tensor field along the flow of another vector or tensor field. AL-Qashbari and Baleedi [7] studied the Lie-derivative in GBK - $5RF_n$ and established various identities of tensors in Finsler space by Lie-derivative. Gouin [14] introduced some remarks on the Lie-derivative. The Lie- derivative of forms and its application was investigated by authors [15, 19, 21]. An n -dimensional Finsler space F_n endowed with the metric function $F(x, y)$ satisfying the request conditions [1]. The metric function F , the metric tensor g_{ij} and the Kronecker delta δ_h^i are given by

$$(1.1) \quad \begin{aligned} & \text{a) } g_{ij} y^i y^j = F^2, \quad \text{b) } g_{ij} y^j = y_i, \quad \text{c) } g_{hj} = \partial_h y_j, \quad \text{d) } y_i y^i = F^2, \\ & \text{e) } g_{ij} g^{ik} = \delta_j^k = \begin{cases} 1 & \text{if } j = k \\ 0 & \text{if } j \neq k \end{cases}, \quad \text{f) } \delta_h^i g_{ik} = g_{hk}, \\ & \text{g) } \delta_k^i y^k = y^i, \quad \text{h) } \delta_h^j = \partial_h y^j, \quad \text{i) } \delta_i^i = n \quad \text{and} \quad \text{j) } \delta_k^i y_i = y_k \end{aligned}$$

The torsion tensor H_{kh}^i and the deviation tensor H_h^i are defined as follows

$$(1.2) \quad \begin{aligned} & \text{a) } H_{kh}^i = K_{jkh}^i y^j = R_{jkh}^i y^j, \quad \text{b) } H_{kh}^i y^k = H_h^i, \quad \text{c) } H_k^i y^k = 0, \\ & \text{d) } H_{kh}^i = \partial_k H_h^i, \quad \text{e) } H_{kh}^i = \frac{1}{3} (\partial_k H_h^i - \partial_h H_k^i), \\ & \text{f) } \mathcal{B}_m H_{kh}^i + \mathcal{B}_h H_{mk}^i + \mathcal{B}_k H_{hm}^i = 0, \quad \text{g) } y_i H_{kh}^i = 0 \quad \text{and} \quad \text{h) } y^r \partial_j \partial_r H_{kh}^i = 0. \end{aligned}$$

Bianchi identities for the curvature tensor K_{jkh}^i is given by

$$(1.3) \quad K_{jkh}^i + K_{hjk}^i + K_{kjh}^i = 0.$$

The deviation tensor R_h^i are given by

$$(1.4) \quad R_{jkh}^i g^{jk} = R_h^i.$$

Berwald's covariant derivative of the vectors y^i and y_i are defined to be identically zero

$$(1.5) \quad \text{a) } \mathcal{B}_k y^i = 0 \quad \text{and} \quad \text{b) } \mathcal{B}_k y_i = 0.$$

The (h) hv-torsion tensor C_{ijk} possesses the following properties

$$(1.6) \quad C_{ijk} y^k = C_{kij} y^k = C_{jki} y^k = 0.$$

The unit vector l^i and associative vector l_i in the directions of y^i and y_i are defined by

$$(1.7) \quad l^i = \frac{y^i}{F}.$$

$$(1.8) \quad l_i = \frac{y_i}{F}.$$

$$(1.9) \quad l^i l_i = 1.$$

The vector y^i is Lie-invariant i.e.,

$$(1.10) \quad L_v y^i = 0.$$

Let us investigate a generalized fifth-order recurrent Finsler geometry satisfying condition [3]

$$(1.11) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i = a_{sqlnm} K_{jkh}^i + b_{sqlnm} (\delta_h^i g_{jk} - \delta_k^i g_{jh}) \\ - c_{sqlnm} (\delta_h^i C_{jkn} - \delta_k^i C_{jhn}) - d_{sqlnm} (\delta_h^i C_{jkl} - \delta_k^i C_{jhl}) \\ - e_{sqlnm} (\delta_h^i C_{jkq} - \delta_k^i C_{jhq}) - 2b_{qlnm} y^r \mathcal{B}_r (\delta_h^i C_{jks} - \delta_k^i C_{jhs}).$$

$$(1.12) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = a_{sqlnm} H_{kh}^i + b_{sqlnm} (\delta_h^i y_k - \delta_k^i y_h).$$

$$(1.13) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i = a_{sqlnm} H_h^i + b_{sqlnm} (\delta_h^i F^2 - y^i y_h).$$

$$(1.14) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i = a_{sqlnm} R_{jkh}^i + b_{sqlnm} (\delta_h^i g_{jk} - \delta_k^i g_{jh}) \\ - c_{sqlnm} (\delta_h^i C_{jkn} - \delta_k^i C_{jhn}) - d_{sqlnm} (\delta_h^i C_{jkl} - \delta_k^i C_{jhl}) - e_{sqlnm} (\delta_h^i C_{jkq} - \delta_k^i C_{jhq}) \\ - 2b_{qlnm} y^r \mathcal{B}_r (\delta_h^i C_{jks} - \delta_k^i C_{jhs}) + \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (C_{jt}^i H_{kh}^t) - a_{sqlnm} (C_{jt}^i H_{kh}^t).$$

The tensor product of two tensors, one of type (r_1, s_1) and the other of type (r_2, s_2) is a tensor of type $(r_1 + r_2, s_1 + s_2)$. Tensor decomposition is the process of decomposing a higher-rank tensor into a sum of simpler tensors, often of lower rank. These simpler tensors can be expressed in terms of products of contravariant and covariant basis vectors. The Cartan's fourth curvature tensor K_{jkh}^i is a mixed tensor of type (1,3) it may be written as a tensor product of a tensor of type (0,1) represented by y_j and a tensor of type (1,2) represented by φ_{kh}^i , or it may be written as a tensor product of a tensor of type (1,0) represented by y^i and a tensor of type (0,3) represented by φ_{jkh} , with the possibilities as

$$(1.15) \quad \text{a) } K_{jkh}^i = y_j \varphi_{kh}^i, \quad \text{b) } K_{jkh}^i = y^i \varphi_{jkh}, \quad \text{c) } K_{jkh}^i = y_k \varphi_{jh}^i \quad \text{and} \quad \text{d) } K_{jkh}^i = y_h \varphi_{jk}^i.$$

It can be represented as the tensor product involving a mixed tensor of rank (1,1), represented by X_j^i , and a covariant tensor of type (0,2), represented by φ_{kh} .

$$(1.16) \quad \text{a) } K_{jkh}^i = X_j^i \varphi_{kh}, \quad \text{b) } K_{jkh}^i = X_k^i \varphi_{jh} \quad \text{and} \quad \text{c) } K_{jkh}^i = X_h^i \varphi_{jk}.$$

The Lie-derivative of a general mixed tensor field $T_j^i(x, \dot{x})$ expressed in the form:

$$(1.17) \quad L_v T_{jkh}^i = v^m \mathcal{B}_m T_{jkh}^i - T_{jkh}^m \mathcal{B}_m v^i + T_{mjh}^i \mathcal{B}_j v^m + T_{jmh}^i \mathcal{B}_k v^m \\ + T_{jkm}^i \mathcal{B}_h v^m + \dot{\partial}_m T_{jkh}^i \mathcal{B}_r v^m y^r.$$

Taking the Lie-derivative of both sides of [(1.5)] and using [(1.5)e] when $i \neq j$, we get

$$(1.18) \quad L_v y_k = 0.$$

By applying (1.17) on the Cartan's fourth curvature tensor K_{jkh}^i and on the h(v)-torsion tensor H_{kh}^i and using [(1.5)a] and [(1.5)h,e], when $r \neq m$, we get

$$(1.19) \quad L_v K_{jkh}^i = v^m \mathcal{B}_m K_{jkh}^i - K_{jkh}^m \mathcal{B}_m v^i + K_{mkh}^i \mathcal{B}_j v^m + K_{jmh}^i \mathcal{B}_k v^m + K_{jkm}^i \mathcal{B}_h v^m, \quad \text{and}$$

$$(1.20) \quad L_v H_{kh}^i = v^m \mathcal{B}_m H_{kh}^i - H_{kh}^m \mathcal{B}_m v^i + H_{mh}^i \mathcal{B}_k v^m + H_{km}^i \mathcal{B}_h v^m, \quad \text{respectively.}$$

Taking the Lie-derivative of both sides of [(1.2) a], we get

$$(1.21) \quad L_v H_{kh}^i = L_v K_{jkh}^i y^j.$$

Using (1.10) in above equation, we get

$$(1.22) \quad L_v H_{kh}^i = y^j L_v K_{jkh}^i.$$

Using (1.19), [(1.2)a] and (1.10) in above equation, we get

$$(1.23) \quad L_v H_{kh}^i = v^m \mathcal{B}_m H_{kh}^i - H_{kh}^m \mathcal{B}_m v^i + K_{mkh}^i \mathcal{B}_j v^m y^j + H_{mh}^i \mathcal{B}_k v^m + H_{km}^i \mathcal{B}_h v^m.$$

In view of (1.23) and (1.20), we get

$$(1.24) \quad K_{mkh}^i \mathcal{B}_j v^m y^j = 0.$$

Using [(1.5)a] in above equation and since $K_{mkh}^i \neq 0$ and $y^j \neq 0$, we get

$$(1.25) \quad \mathcal{B}_j v^m = 0.$$

Taking the Lie-derivative of (1.7) and using [(1.1)d], (1.10) and (1.18) in (1.7), we get

$$(1.26) \quad L_v l^i = 0.$$

The main aim of this paper is to study the decomposition of Fundamental Function of Finsler space and some tensors by Lie-derivative in generalized fifth recurrent Finsler space. By employing the Lie derivative within the newly introduced $GBK-5RF_n$ framework, a novel approach for analyzing the geometric structure of Finsler spaces is developed.

2. A Decomposition of Lie-Derivative of Some Tensors by using a Covariant

Tensor y_j and Mixed Tensor φ_{kh}^i in $GBK-5RF_n$

The Lie-derivative is a fundamental tool in differential geometry and its applications. In this study, a novel decomposition of the Lie-derivative for a specific class of tensors is introduced. The decomposition of Cartan's fourth curvature tensor K_{jkh}^i in the form [(1.15a)] is studied, where y_j it is a non-vanishing covariant tensor and φ_{kh}^i is a decomposition tensor.

Taking $\mathcal{B}$ -covariant derivative of fifth order for [(1.15a)], with respect to x^m, x^n, x^l, x^q and x^s , then using [(1.5)b], we get

$$(2.1) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i = y_j (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{kh}^i).$$

Using [(1.15)a], (1.11) and [(1.1)e] in (2.1), we get

$$(2.2) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{kh}^i = a_{sqlnm} \varphi_{kh}^i.$$

In conclusion, it can be stated that

Theorem 2.1: In $GBK - 5RF_n$, the decomposition tensor φ_{kh}^i behaves as fifth-recurrent.

Using [(1.15)a] in the right side of (1.11), we get

$$(2.3) \quad \begin{aligned} \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i &= a_{sqlnm} y_j \varphi_{kh}^i + b_{sqlnm} (\delta_h^i g_{jk} - \delta_k^i g_{jh}) \\ &- c_{sqlnm} (\delta_h^i C_{jkn} - \delta_k^i C_{jhn}) - d_{sqlnm} (\delta_h^i C_{jkl} - \delta_k^i C_{jhl}) \\ &- e_{sqlnm} (\delta_h^i C_{jkq} - \delta_k^i C_{jhq}) - 2b_{qlnm} y^r \mathcal{B}_r (\delta_h^i C_{jks} - \delta_k^i C_{jhs}). \end{aligned}$$

Transvecting (2.3) by y^j , using [(1.1)b,d], [(1.2)a], [(1.5)a] and (1.6), we get

$$(2.4) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = a_{sqlnm} F^2 \varphi_{kh}^i + b_{sqlnm} (\delta_h^i y_k - \delta_k^i y_h).$$

Using [(1.1)e] in (2.4), we get

$$(2.5) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = a_{sqlnm} F^2 \varphi_{kh}^i.$$

Therefore, it can be concluded that

Theorem 2.2: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the torsion tensor H_{kh}^i decomposes according to (2.5).

By taking the Lie-derivative of both sides of the equation (2.5), yields

$$(2.6) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i) = F^2 L_v (a_{sqlnm} \varphi_{kh}^i) + a_{sqlnm} \varphi_{kh}^i L_v F^2.$$

Using (1.12) and [(1.1)e] in (2.6), we get

$$\begin{aligned} L_v F^2 &= \frac{1}{a_{sqlnm} \varphi_{kh}^i} [H_{kh}^i L_v (a_{sqlnm}) + a_{sqlnm} L_v H_{kh}^i \\ &- F^2 (\varphi_{kh}^i L_v a_{sqlnm} + a_{sqlnm} L_v \varphi_{kh}^i)]. \end{aligned}$$

Using (1.17) and (1.25) in above equation, we get

$$(2.7) \quad \begin{aligned} L_v F^2 &= \frac{v^m}{a_{sqlnm} \varphi_{kh}^i} [H_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m H_{kh}^i \\ &- F^2 (\varphi_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m \varphi_{kh}^i)]. \end{aligned}$$

Consequently, it can be concluded that

Theorem 2.3: In $GBK - 5RF_n$, the Lie-derivative for the quadrature fundamental function of Finsler space decomposes according to (2.7).

Transvecting (2.5) by y^k , using [(1.5)a] and [(1.2)b], we get

$$(2.8) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i = a_{sqlnm} F^2 y^k \varphi_{kh}^i.$$

Subtracting (2.8) from (2.5), we get

$$(2.9) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (H_{kh}^i - H_h^i) = a_{sqlnm} F^2 (1 - y^k) \varphi_{kh}^i.$$

Using [(1.2)d] in (2.9), we get

$$(2.10) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (\partial_k H_h^i - H_h^i) = a_{sqlnm} F^2 (1 - y^k) \varphi_{kh}^i.$$

Which can be written as

$$(2.11) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \partial_k H_h^i = a_{sqlnm} F^2 (1 - y^k) \varphi_{kh}^i + \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i.$$

Using (2.8) in (2.11), we get

$$(2.12) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \partial_k H_h^i = a_{sqlnm} F^2 \varphi_{kh}^i.$$

In view of (2.5) and (2.12), we get

$$(2.13) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \partial_k H_h^i = \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = a_{sqlnm} F^2 \varphi_{kh}^i.$$

Thus, it can be concluded that

Corollary 2.1: In $GBK - 5RF_n$, Berwald's covariant derivatives of the fifth order for the torsion tensor H_{kh}^i and for the partial derivative with respect to y^k for the deviation tensor H_h^i have the same decomposition.

In view of (2.8) and (2.12), we get

$$(2.14) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i = y^k \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \partial_k H_h^i = y^k a_{sqlnm} F^2 \varphi_{kh}^i.$$

Thus, it can be concluded that

Corollary 2.2: In $GBK - 5RF_n$, Berwald's covariant derivatives of the fifth order for deviation tensor H_h^i and for the partial derivative with respect to y^k for the deviation tensor H_h^i are decomposing in the same direction.

Upon taking the Lie-derivative of both sides of the equation (2.8), we get

$$(2.15) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i) = F^2 y^k L_v (a_{sqlnm} \varphi_{kh}^i) + a_{sqlnm} \varphi_{kh}^i L_v (F^2 y^k).$$

Using [(1.1)e], (1.13) and (1.10) in (2.15), we get

$$L_v F^2 = \frac{1}{a_{sqlnm} \varphi_{kh}^i y^k} [L_v (a_{sqlnm} H_h^i) - L_v [b_{sqlnm} (y^i y_h)] - F^2 y^k L_v (a_{sqlnm} \varphi_{kh}^i)].$$

Using (1.10), (1.17), (1.18) and (1.25) in above equation, we get

$$(2.16) \quad L_v F^2 = \frac{v^m}{a_{sqlnm} \varphi_{kh}^i y^k} [H_h^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m H_h^i - y^i y_h \mathcal{B}_m b_{sqlnm} - F^2 y^k (\varphi_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m \varphi_{kh}^i)].$$

In conclusion, it can be stated that

Theorem 2.4: In $GBK - 5RF_n$, the Lie-derivative for the quadrature fundamental function of Finsler space decomposes according to (2.16).

Using (1.12) and [(1.1)e] in (2.5), we get

$$(2.17) \quad H_{kh}^i = F^2 \varphi_{kh}^i.$$

it can be summarized that

Theorem 2.5: In $GBK - 5RF_n$, the $h(v)$ -torsion tensor H_{kh}^i decomposes according to (2.17).

By taking the Lie-derivative of both sides of the equation (2.17), we obtain

$$(2.18) \quad L_v H_{kh}^i = (L_v F^2) \varphi_{kh}^i + F^2 (L_v \varphi_{kh}^i).$$

Using (2.7), (1.17) and (1.25) in (2.18), we get

$$(2.19) \quad L_v H_{kh}^i = \frac{v^m}{a_{sqlnm}} [H_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m H_{kh}^i - F^2 (\varphi_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m \varphi_{kh}^i)] + F^2 v^m \mathcal{B}_m \varphi_{kh}^i.$$

Consequently, it can be concluded that

Theorem 2.6: In $GBK - 5RF_n$, the Lie-derivative for the $h(v)$ -torsion tensor H_{kh}^i decomposes according to (2.19).

In view of (2.17), we have

$$(2.20) \quad F = \frac{1}{\sqrt{\varphi_{kh}^i}} \left[\sqrt{H_{kh}^i} \right].$$

Taking the Lie-derivative of both sides of (2.20), we get

$$L_v F = \left[L_v \left(\frac{1}{\sqrt{\varphi_{kh}^i}} \right) \right] \left[\sqrt{H_{kh}^i} \right] + \frac{1}{\sqrt{\varphi_{kh}^i}} \left[L_v \sqrt{H_{kh}^i} \right].$$

Using (1.17) and (1.25) in above equation, we get

$$L_v F = \frac{1}{2\sqrt{\varphi_{kh}^i H_{kh}^i}} \left[v^m \mathcal{B}_m H_{kh}^i - \frac{H_{kh}^i v^m \mathcal{B}_m \varphi_{kh}^i}{\varphi_{kh}^i} \right].$$

Since the torsion tensor H_{kh}^i is skew-symmetric in its two lower indices h and k , then above equation can be written as

$$L_v F = \frac{v^m}{2\sqrt{\varphi_{kh}^i H_{kh}^i}} \left[\frac{H_{hk}^i \mathcal{B}_m \varphi_{kh}^i}{\varphi_{kh}^i} + \mathcal{B}_m H_{kh}^i \right].$$

If the torsion tensor H_{kh}^i and decomposition tensor φ_{kh}^i behaves as recurrent, then above equation can be written as

$$(2.21) \quad L_v F = \frac{v^m \lambda_m}{2\sqrt{\varphi_{kh}^i H_{kh}^i}} [H_{hk}^i + H_{kh}^i], \text{ where } \lambda_m \text{ is non-zero covariant vector field.}$$

it can be concluded that

Theorem 2.7: In $GBK - 5RF_n$, the Lie-derivative for the fundamental function of Finsler space decomposes according to (2.21) if the torsion tensor H_{kh}^i and decomposition tensor φ_{kh}^i behaves as recurrent.

Using [(1.1)e] in (2.3), we get

$$(2.22) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i = (a_{sqlnm} y_j) \varphi_{kh}^i .$$

In conclusion, it can be stated that

Theorem 2.8: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (2.22).

Using [(1.15) a] in (1.3), we get

$$(2.23) \quad y_j \varphi_{kh}^i + y_h \varphi_{jk}^i + y_k \varphi_{hj}^i = 0 .$$

Transvecting (2.23) by $(a_{sqlnm} y^j)$, using [(1.1)d], we get

$$(2.24) \quad a_{sqlnm} F^2 \varphi_{kh}^i + a_{sqlnm} y_h y^j \varphi_{jk}^i + a_{sqlnm} y_k y^j \varphi_{hj}^i = 0 .$$

Using (2.22) and (2.5) in (2.24), we get

$$(2.25) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i + y^j \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{hjk}^i + y^j \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{khj}^i = 0 .$$

Which can be written as

$$(2.26) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = -y^j \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (K_{hjk}^i + K_{khj}^i) .$$

In conclusion, it can be stated that

Theorem 2.9: In $GBK - 5RF_n$, that admits tensors decomposition, the Berwald's covariant derivatives of the fifth order for the torsion tensor H_{kh}^i and for the tensor $(K_{hjk}^i + K_{khj}^i)$ are in the opposite directions.

Using [(1.2)e] in (2.5), we get

$$(2.27) \quad \varphi_{kh}^i = \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (\partial_k H_h^i - \partial_h H_k^i) .$$

If and only if

$$(2.28) \quad F^2 = \frac{1}{3a_{sqlnm}} .$$

Hence, it can be concluded that

Theorem 2.10: In $GBK - 5RF_n$, the decomposition tensor φ_{kh}^i is giving by (2.27) if and only if the quadrature fundamental function of Finsler space is giving by (2.28).

Using (2.17) in [(1.2)f], we get

$$(2.29) \quad F^2 (\mathcal{B}_m \varphi_{kh}^i + \mathcal{B}_h \varphi_{mk}^i + \mathcal{B}_k \varphi_{hm}^i) = 0 .$$

Since the fundamental function of Finsler space is bigger than zero, so (2.29) can be written as

$$(2.30) \quad (\mathcal{B}_m \varphi_{kh}^i + \mathcal{B}_h \varphi_{mk}^i + \mathcal{B}_k \varphi_{hm}^i) = 0 .$$

Hence, it can be concluded that

Theorem 2.11: In $GBK - 5RF_n$, that admits tensors decomposition, we have identity (2.30).

By taking the Lie-derivative of both sides of the equation (2.22), we get

$$(2.31) \quad L_v(\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i) = \varphi_{kh}^i L_v(a_{sqlnm} y_j) + a_{sqlnm} y_j (L_v \varphi_{kh}^i) .$$

Using [(1.1)e] and (1.11) in (2.31), we get

$$L_v K_{jkh}^i = \frac{1}{a_{sqlnm}} \left[\varphi_{kh}^i L_v(a_{sqlnm} y_j) + a_{sqlnm} y_j (L_v \varphi_{kh}^i) - (L_v a_{sqlnm}) K_{jkh}^i \right] .$$

Using (1.17), (1.18) and (1.25) in above equation, we get

$$(2.32) \quad L_v K_{jkh}^i = \frac{v^m}{a_{sqlnm}} \left[\mathcal{B}_m a_{sqlnm} (\varphi_{kh}^i y_j - K_{jkh}^i) + a_{sqlnm} y_j \mathcal{B}_m \varphi_{kh}^i \right] .$$

In conclusion, it can be concluded that

Theorem 2.12: In $GBK - 5RF_n$, the Lie-derivative for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (2.32).

3. A Decomposition of Lie-derivative of Some Tensors by Using A Contravariant

Tensor y^i and a Covariant Tensor φ_{jkh} in $GBK-5RF_n$

This study explores a novel method for decomposing the Lie-derivative of specific tensors within the context of $GBK-5RF_n$ geometry. Our approach leverages the properties of a contravariant tensor y^i and a covariant tensor φ_{jkh} to provide a more refined analysis of these derivatives. The decomposition of Cartan's fourth curvature tensor K_{jkh}^i in the form [(1.15)b] is studied, where y^i is a non-zero contravariant tensor and φ_{jkh} is a decomposition tensor.

Using [(1.15)b] in the right side of (1.11), we get

$$(3.1) \quad \begin{aligned} \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i &= a_{sqlnm} y^i \varphi_{jkh} + b_{sqlnm} (\delta_h^i g_{jk} - \delta_k^i g_{jh}) \\ &- c_{sqlnm} (\delta_h^i C_{jkn} - \delta_k^i C_{jhn}) - d_{sqlnm} (\delta_h^i C_{jkl} - \delta_k^i C_{jhl}) \\ &- e_{sqlnm} (\delta_h^i C_{jkq} - \delta_k^i C_{jhq}) - 2b_{qlnm} y^r \mathcal{B}_r (\delta_h^i C_{jks} - \delta_k^i C_{jhs}) . \end{aligned}$$

Transvecting (3.1) by y^j , using [(1.1)b], [(1.2)a], [(1.5)a] and (1.6), we get

$$(3.2) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = (a_{sqlnm} y^i y^j) \varphi_{jkh} + b_{sqlnm} (\delta_h^i y_k - \delta_k^i y_h) .$$

Using [(1.1)e] in (3.2), we get

$$(3.3) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i = a_{sqlnm} y^i y^j \varphi_{jkh} .$$

From the previous steps, the following theorem can be concluded that

Theorem 3.1: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the h(v)-torsion tensor H_{kh}^i decomposes according to (3.3).

Taking the Lie-derivative of both sides of (3.3), we get

$$(3.4) \quad L_v(\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_{kh}^i) = y^i y^j L_v(a_{sqlnm} \varphi_{jkh}) + a_{sqlnm} \varphi_{jkh} L_v(y^i y^j) .$$

Using (1.12), [(1.1)e] and (1.10) in (3.4), we get

$$L_v(a_{sqlnm}H_{kh}^i) - y^i y^j L_v(a_{sqlnm} \varphi_{jkh}) = 0 .$$

Using (1.17) and (1.25) in above equation, we get

$$(3.5) \quad \mathcal{B}_m a_{sqlnm} (H_{kh}^i - y^i y^j \varphi_{jkh}) + a_{sqlnm} (\mathcal{B}_m H_{kh}^i - y^i y^j \mathcal{B}_m \varphi_{jkh}) = 0 .$$

Therefore, the proof of theorem is completed, it can be said that

Theorem 3.2: In $GBK - 5RF_n$, that admits tensors decomposition, we have identity (3.5).

Transvecting (3.2) by y_i , using [(1.1)d], [(1.2)g] and [(1.5)b], we get

$$(3.6) \quad (a_{sqlnm} F^2 y^j) \varphi_{jkh} + b_{sqlnm} y_i (\delta_h^i y_k - \delta_k^i y_h) = 0 .$$

Using [(1.1)b] in (3.6), we get

$$(3.7) \quad (a_{sqlnm} F^2) \varphi_{jkh} + b_{sqlnm} g_{ij} (\delta_h^i y_k - \delta_k^i y_h) = 0 .$$

Which can be written as

$$(3.8) \quad \varphi_{jkh} = (\delta_k^i y_h - \delta_h^i y_k) .$$

If and only if

$$(3.9) \quad F^2 = \alpha_{sqlnm} g_{ij} , \text{ where } \alpha_{sqlnm} = \frac{b_{sqlnm}}{a_{sqlnm}} , \text{ is non-zero covariant tensor of fifth order.}$$

So, the proof of theorem is completed, it can be said that

Theorem 3.3: In $GBK - 5RF_n$, the decomposition tensor φ_{jkh} is giving by (3.8) if and only if the quadrature fundamental function of Finsler space is giving by (3.9).

Using (2.5) and [(1.1)d] in (3.3), we get

$$(3.10) \quad y_i \varphi_{kh}^i = y^j \varphi_{jkh} .$$

In conclusion the proof of theorem is completed, it can be concluded that

Theorem 3.4: In $GBK - 5RF_n$, the admits tensors decomposition, we have the identity (3.10).

Transvecting (3.2) by y^k , using [(1.1)d], [(1.1)g], [(1.2)b] and [(1.5)a], we get

$$(3.11) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i = (a_{sqlnm} y^i y^j y^k) \varphi_{jkh} + b_{sqlnm} (\delta_h^i F^2 - y^i y_h) .$$

Taking the Lie- derivative of both sides of (3.11), we get

$$(3.12) \quad L_v(\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m H_h^i) = a_{sqlnm} \varphi_{jkh} L_v(y^i y^j y^k) + y^i y^j y^k (L_v a_{sqlnm} \varphi_{jkh}) \\ + L_v[b_{sqlnm} (\delta_h^i F^2 - y^i y_h)] .$$

Using (1.13) and (1.10) in (3.12), we get

$$L_v(a_{sqlnm} H_h^i) - y^i y^j y^k (L_v a_{sqlnm} \varphi_{jkh}) = 0 .$$

Using (1.17) and (1.25) in above equation, we get

$$(3.13) \quad \mathcal{B}_m a_{sqlnm} (H_h^i - y^i y^j y^k \varphi_{jkh}) + a_{sqlnm} (\mathcal{B}_m H_h^i - y^i y^j y^k \mathcal{B}_m \varphi_{jkh}) = 0 .$$

Thus, it can be concluded that

Theorem 3.5: In $GBK - 5RF_n$, the admits tensors decomposition, we have the identity (3.13).

In view of (1.12) and (3.2), we get

$$(3.14) \quad H_{kh}^i = y^i y^j \varphi_{jkh}.$$

Also, in view of (3.11) and (1.13), we get

$$(3.15) \quad H_h^i = y^i y^j y^k \varphi_{jkh}.$$

Thus, the proof of theorem is completed, it can be concluded that

Corollary 3.1: In $GBK - 5RF_n$, the torsion tensor H_{kh}^i and the deviation tensor H_h^i decompose according to (3.14) and (3.15), respectively.

Using [(1.2)d] in (3.14), we get

$$(3.16) \quad \partial_k H_h^i = y^i y^j \varphi_{jkh}.$$

Using (3.15) in (3.16), we get

$$(3.17) \quad \partial_k (y^i y^j y^k \varphi_{jkh}) = (y^i y^j \varphi_{jkh}).$$

In conclusion the proof of theorem is completed, it can be determined that

Theorem 3.6: In $GBK - 5RF_n$, the partial derivative with respect to y^k for the tensor $(y^i y^j y^k \varphi_{jkh})$ is $(y^i y^j \varphi_{jkh})$.

Using (3.14) in [(1.2)a], we get

$$(3.18) \quad R_{jkh}^i = y^i \varphi_{jkh}.$$

Transvecting (3.18) by y_i , using [(1.1)d], we get

$$(3.19) \quad y_i R_{jkh}^i = F^2 \varphi_{jkh}.$$

Which can be written as

$$(3.20) \quad F = \frac{1}{\sqrt{\varphi_{jkh}}} \left[\sqrt{y_i R_{jkh}^i} \right].$$

By taking the Lie-derivative of both sides of the equation (3.20), we get

$$L_v F = \left[L_v \left(\frac{1}{\sqrt{\varphi_{jkh}}} \right) \right] \left[\sqrt{y_i R_{jkh}^i} \right] + \frac{1}{\sqrt{\varphi_{jkh}}} \left[L_v \sqrt{y_i R_{jkh}^i} \right].$$

Using (1.17), (1.18) and (1.25) in above equation, we get

$$L_v F = \frac{v^m y_i}{2\sqrt{\varphi_{jkh} y_i R_{jkh}^i}} \left[\mathcal{B}_m R_{jkh}^i - \frac{R_{jkh}^i \mathcal{B}_m \varphi_{jkh}}{\varphi_{jkh}} \right].$$

Since the Cartan's third curvature tensor R_{jkh}^i , is skew-symmetric it their last two lower indices, then above equation can be written as

$$L_v F = \frac{v^m y_i}{2\sqrt{\varphi_{jkh} y_i R_{jkh}^i}} \left[\mathcal{B}_m R_{jkh}^i + \frac{R_{jkh}^i \mathcal{B}_m \varphi_{jkh}}{\varphi_{jkh}} \right].$$

If the Cartan's third curvature tensor R_{jkh}^i and decomposition tensor φ_{jkh} behaves as recurrent, then above equation can be written as

$$(3.21) \quad L_v F = \frac{v^m \lambda_m y_i}{2 \sqrt{\varphi_{jkh} y_i R_{jkh}^i}} [R_{jkh}^i + R_{jkh}^i] .$$

Thus, it can be concluded that

Theorem 3.7: In $GBK - 5RF_n$, the Lie-derivative for the fundamental function of Finsler space decomposes according to (3.21) if the Cartan's third curvature tensor R_{jkh}^i and decomposition tensor φ_{jkh} behaves as recurrent.

Using [(1.2)a] in (2.5), we get

$$(3.22) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (R_{jkh}^i y^j) = (a_{sqlnm} F^2) \varphi_{kh}^i .$$

Using [(1.1)d] and [(1.5)a] in (3.22), we get

$$(3.23) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i = (a_{sqlnm} y_j) \varphi_{kh}^i .$$

Taking the Lie-derivative of both sides of (3.23), we get

$$L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i) = \varphi_{kh}^i L_v (a_{sqlnm} y_j) + (a_{sqlnm} y_j) L_v \varphi_{kh}^i .$$

Using (1.17), (1.18) and (1.25) in above equation, we get

$$(3.24) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i) = y_j v^m (\varphi_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m \varphi_{kh}^i) .$$

In conclusion the proof of theorem is completed, it can be concluded that

Theorem 3.8: In $GBK - 5RF_n$, the Lie-derivative of Berwald's covariant derivative of the fifth order for the Cartan's third curvature tensor R_{jkh}^i decomposes according to (3.24).

Using [(1.1)e] in (1.14), we get

$$(3.25) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i = a_{sqlnm} R_{jkh}^i + \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (C_{jt}^i H_{kh}^t) - a_{sqlnm} (C_{jt}^i H_{kh}^t) .$$

Taking the Lie-derivative of both sides of (3.25), we get

$$(3.26) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m R_{jkh}^i) = R_{jkh}^i (L_v a_{sqlnm}) + a_{sqlnm} (L_v R_{jkh}^i) \\ + L_v [\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (C_{jt}^i H_{kh}^t)] - L_v [a_{sqlnm} (C_{jt}^i H_{kh}^t)] .$$

In view of (3.26) and (3.24), we get

$$L_v R_{jkh}^i = \frac{1}{a_{sqlnm}} [y_j v^m (\varphi_{kh}^i \mathcal{B}_m a_{sqlnm} + a_{sqlnm} \mathcal{B}_m \varphi_{kh}^i) - R_{jkh}^i (L_v a_{sqlnm}) \\ - L_v [\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m (C_{jt}^i H_{kh}^t)] + L_v [a_{sqlnm} (C_{jt}^i H_{kh}^t)]] .$$

Using (1.17), (1.18) and (1.25) in above equation, we get

$$(3.27) \quad L_v R_{jkh}^i = \frac{v^m}{a_{sqlnm}} [\mathcal{B}_m a_{sqlnm} (\varphi_{kh}^i y_j - R_{jkh}^i) + a_{sqlnm} y_j \mathcal{B}_m \varphi_{kh}^i] ,$$

if and only if the tensor $(C_{jt}^i H_{kh}^t)$ behaves as fifth recurrent.

The proof of theorem is completed, it can be concluded that

Theorem 3.9: In $GBK - 5RF_n$, the Lie-derivative for the Cartan's third curvature tensor R_{jkh}^i decomposes according to (3.27) if and only if the tensor $(C_{jt}^i H_{kh}^t)$ behaves as fifth recurrent.

Using [(1.15)b] and [(1.°)a] in (2.22), we get

$$(3.28) \quad y^i \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{jkh} = (a_{sqlnm} y_j) \varphi_{kh}^i.$$

Using (1.7) in (3.28), we get

$$(3.29) \quad (l^i F) \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{jkh} = (a_{sqlnm} y_j) \varphi_{kh}^i.$$

Transvecting (3.29) by l_i , we get

$$(3.30) \quad (l_i l^i F) \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{jkh} = l_i (a_{sqlnm} y_j) \varphi_{kh}^i.$$

Using (1.9) in (3.30), we get

$$(3.31) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m \varphi_{jkh} = \varphi_{kh}^i.$$

If and only if

$$(3.32) \quad F = l_i (a_{sqlnm} y_j).$$

In conclusion, it can be determined that

Theorem 3.10: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the decomposition tensor φ_{jkh} is equal the decomposition tensor φ_{kh}^i if and only if the fundamental function of Finsler space is giving by (3.32).

Using [(1.1)e] in (3.1), we get

$$(3.33) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i = (a_{sqlnm} y^i) \varphi_{jkh}.$$

The proof of theorem is completed, it can be concluded that

Theorem 3.11: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (3.33).

Taking the Lie-derivative of both sides of (3.33), we get

$$(3.34) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i) = \varphi_{jkh} L_v (a_{sqlnm} y^i) + a_{sqlnm} y^i (L_v \varphi_{jkh}).$$

Using [(1.1)e] and (1.11) in (3.34), we get

$$L_v K_{jkh}^i = \frac{1}{a_{sqlnm}} [\varphi_{jkh} L_v (a_{sqlnm} y^i) + a_{sqlnm} y^i (L_v \varphi_{jkh}) - (L_v a_{sqlnm}) K_{jkh}^i].$$

Using (1.17), (1.10) and (1.25) in above equation, we get

$$(3.35) \quad L_v K_{jkh}^i = \frac{v^m}{a_{sqlnm}} [\mathcal{B}_m a_{sqlnm} (\varphi_{jkh} y^i - K_{jkh}^i) + a_{sqlnm} y^i (\mathcal{B}_m \varphi_{jkh})].$$

The proof of theorem is completed, it can be determined that

Theorem 3.12: In $GBK - 5RF_n$, the Lie-derivative for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (3.35).

4. A Decomposition of Lie-derivative for Some Tensors by Using a Mixed Tensor X_j^i and A Covariant Tensor φ_{kh} in $GBK- 5RF_n$

This paper presents a novel decomposition of the Lie derivative for specific tensors, utilizing a mixed tensor. We provide a detailed analysis of this decomposition and explore its implications for some tensors. We study the decomposition of Cartan's fourth curvature tensor K_{jkh}^i in the form [(1.16)a], where X_j^i it is a non-vanishing mixed tensor and φ_{kh} is a decomposition tensor.

Using [(1.16) a] and [(1.1)e] in right side of (1.11), we get

$$(4.1) \quad \mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i = (a_{sqlnm} X_j^i) \varphi_{kh}.$$

In conclusion, it can be stated that

Theorem 4. 1: In $GBK - 5RF_n$, Berwald's covariant derivative of the fifth order for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (4.1).

In view of (4.1) and (2.22), we get

$$(4.2) \quad X_j^i \varphi_{kh} = y_j \varphi_{kh}^i.$$

In conclusion, it can be stated that

Corollary 4.1: In $GBK - 5RF_n$, that admits tensors decomposition, we have the identity (4.2).

Taking the Lie-derivative of both sides of (4.1), we get

$$(4.3) \quad L_v (\mathcal{B}_s \mathcal{B}_q \mathcal{B}_l \mathcal{B}_n \mathcal{B}_m K_{jkh}^i) = \varphi_{kh} L_v (a_{sqlnm} X_j^i) + a_{sqlnm} X_j^i (L_v \varphi_{kh}).$$

Using (1.11) and [(1.1)e] in (4.3), we get

$$L_v K_{jkh}^i = \frac{1}{a_{sqlnm}} \left[\varphi_{kh} L_v (a_{sqlnm} X_j^i) + a_{sqlnm} X_j^i (L_v \varphi_{kh}) - (L_v a_{sqlnm}) K_{jkh}^i \right].$$

Using (1.17) and (1.25) in above equation, we get

$$(4.4) \quad L_v K_{jkh}^i = \frac{v^m}{a_{sqlnm}} \left[\mathcal{B}_m a_{sqlnm} (\varphi_{kh} X_j^i - K_{jkh}^i) + a_{sqlnm} (\varphi_{kh} \mathcal{B}_m X_j^i + X_j^i \mathcal{B}_m \varphi_{kh}) \right].$$

In conclusion, it can be stated that

Theorem 4.2: In $GBK - 5RF_n$, the Lie-derivative for the Cartan's fourth curvature tensor K_{jkh}^i decomposes according to (4.4).

Transvecting (4.2) by y^j , using [(1.1)d], we get

$$(4.5) \quad F^2 \varphi_{kh}^i = y^j X_j^i \varphi_{kh}.$$

Which can be written as

$$(4.6) \quad \varphi_{kh}^i = y^j \varphi_{kh}.$$

If and only if

$$(4.7) \quad F^2 = X_j^i.$$

In conclusion, it can be stated that

Theorem 4.3: In $GBK - 5RF_n$, the decomposition tensor φ_{kh}^i is giving by (4.6) if and only if the quadrature fundamental function of Finsler space is equal the mixed tensor X_j^i .

Transvecting (3.10) by y^i , using [(1.1)d], we get

$$(4.8) \quad F^2 \varphi_{kh}^i = y^i y^j \varphi_{jkh}.$$

Using [Theorem 4.3] in (4.8), we get

$$(4.9) \quad X_j^i \varphi_{kh} = y^i \varphi_{jkh}.$$

Transvecting (4.9) by y_i , using [(1.1)d], we get

$$(4.10) \quad y_i X_j^i \varphi_{kh} = F^2 \varphi_{jkh}.$$

Which can be written as

$$(4.11) \quad \varphi_{jkh} = y_i \varphi_{kh}.$$

If and only if $[F^2 = X_j^i]$.

In conclusion, it can be stated that

Theorem 4.4: In $GBK - 5RF_n$, the decomposition tensor φ_{jkh} is giving by (4.11) if and only if the quadrature fundamental function of Finsler space is equal the mixed tensor X_j^i .

Using (1.7) in (3.14), we get

$$(4.12) \quad H_{kh}^i = l^i l^j F^2 \varphi_{jkh}.$$

Using [Theorem 4.4] in (4.12), we get

$$(4.13) \quad H_{kh}^i = l^i l^j y_i X_j^i \varphi_{kh}.$$

Using (1.7) in (3.15), we get

$$(4.14) \quad H_h^i = l^i l^j F^2 y^k \varphi_{jkh}.$$

Using [Theorem 4.4] in (4.14), we get

$$(4.15) \quad H_h^i = l^i l^j y_i X_j^i y^k \varphi_{kh}.$$

In conclusion, it can be stated that

Theorem 4.5: In $GBK - 5RF_n$, the torsion tensor H_{kh}^i and the deviation tensor H_h^i decompose according to (4.13) and (4.15), respectively.

By taking the Lie-derivative of both sides of the equation (4.13), we get

$$L_v H_{kh}^i = y_i X_j^i \varphi_{kh} L_v(l^i l^j) + l^i l^j \varphi_{kh} L_v(y_i X_j^i) + l^i l^j y_i X_j^i L_v(\varphi_{kh}).$$

Using (1.17), (1.18), (1.25) and (1.26) in above equation, we get

$$L_v H_{kh}^i = l^i l^j \varphi_{kh} y_i v^m \mathcal{B}_m X_j^i + l^i l^j y_i X_j^i v^m \mathcal{B}_m \varphi_{kh}.$$

Using [(1.1)d] and (1.7) in above equation, we get

$$(4.16) \quad L_v H_{kh}^i = y^j v^m (\varphi_{kh} \mathcal{B}_m X_j^i + X_j^i \mathcal{B}_m \varphi_{kh}).$$

In conclusion, it can be stated that

Theorem 4.6: In $GBK - 5RF_n$, the Lie-derivative for the torsion tensor H_{kh}^i decomposes according to (4.16).

By taking the Lie-derivative of both sides of the equation (4.15), we get

$$L_v H_h^i = y_i X_j^i y^k \varphi_{kh} L_v(l^i l^j) + l^i l^j y^k \varphi_{kh} L_v(y_i X_j^i) + l^i l^j y_i X_j^i L_v(y^k \varphi_{kh}).$$

Using (1.10), (1.17), (1.18), (1.25) and (1.26) in above equation, we get

$$L_v H_h^i = l^i l^j y^k \varphi_{kh} y_i v^m \mathcal{B}_m X_j^i + l^i l^j y_i X_j^i y^k v^m \mathcal{B}_m \varphi_{kh}.$$

Using [(1.1)d] and (1.7) in above equation, we get

$$(4.17) \quad L_v H_h^i = y^j y^k v^m (\varphi_{kh} \mathcal{B}_m X_j^i + X_j^i \mathcal{B}_m \varphi_{kh}).$$

In conclusion, it can be stated that

Theorem 4.7: In $GBK - 5RF_n$, Lie-derivative for the deviation tensor H_h^i decomposes according to (4.17).

Transvecting (3.18) by y_i , using [(1.1)d], we get

$$(4.18) \quad y_i R_{jkh}^i = F^2 \varphi_{jkh}.$$

Using [Theorem 4.4] in (4.18), we get

$$(4.19) \quad y_i R_{jkh}^i = y_i X_j^i \varphi_{kh}.$$

Since y_i is a non-zero covariant tensor, then (4.20), can be written as

$$(4.20) \quad R_{jkh}^i = X_j^i \varphi_{kh}.$$

In conclusion, it can be stated that

Theorem 4.8: In $GBK - 5RF_n$, the Cartan's third curvature tensor R_{jkh}^i decomposes according to (4.20).

Using (4.20) in the right side of (3.27), we get

$$(4.21) \quad L_v R_{jkh}^i = \frac{v^m}{a_{sqlnm}} [\mathcal{B}_m a_{sqlnm} (\varphi_{kh} y_j - X_j^i \varphi_{kh}) + a_{sqlnm} y_j \mathcal{B}_m \varphi_{kh}].$$

In conclusion, it can be stated that

Theorem 4.9: In $GBK - 5RF_n$, the Lie-derivative for the Cartan's third curvature tensor R_{jkh}^i decomposes according to (4.21).

Taking the partial derivative with respect to y^r of both sides of (3.16), we get

$$(4.22) \quad \partial_r \partial_k H_h^i = \partial_r y^i y^j \varphi_{jkh}.$$

Using [(1.2)d] in (4.22), we get

$$(4.23) \quad \partial_r H_{kh}^i = \partial_r y^i y^j \varphi_{jkh}.$$

Taking the partial derivative with respect to y^j of both sides of (4.23), we get

$$(4.24) \quad \partial_j \partial_r H_{kh}^i = \partial_j \partial_r y^i y^j \varphi_{jkh}.$$

Transvecting (4.24) by y^r , using [(1.2)h], we get

$$(4.25) \quad y^r \partial_j \partial_r y^i y^j \varphi_{jkh} = 0. \text{ Since } y^r \neq 0, \text{ so above equation can be written as}$$

$$(4.26) \quad \partial_j \partial_r y^i y^j \varphi_{jkh} = 0.$$

Transvecting above equation by y_i , using [(1.1)d,h,i], we get

$$(4.27) \quad \partial_r F^2 \varphi_{jkh} = 0.$$

Using [Theorem 4.4] in above equation, we get

$$(4.28) \quad \partial_r (X_j^i y_i \varphi_{kh}) = 0.$$

In conclusion, it can be stated that

Theorem 4.10: In $GBK - 5RF_n$, the partial derivative with respect to y^r for the tensor $(X_j^i y_i \varphi_{kh})$ is vanishing.

Using [(1.16)a] in (1.3), we get

$$(4.29) \quad X_j^i \varphi_{kh} + X_h^i \varphi_{jk} + X_k^i \varphi_{hj} = 0.$$

Transvecting (4.29) by $(y^j y^h y^k)$, using [Theorem 4.3], we get

$$(4.30) \quad F^2 (y^h y^k \varphi_{kh}^i + y^j y^k \varphi_{jk}^i + y^j y^h \varphi_{hj}^i) = 0.$$

Since the fundamental function of Finsler space is bigger than zero, so (4.30) can be written as

$$(4.31) \quad y^h y^k \varphi_{kh}^i + y^j y^k \varphi_{jk}^i + y^j y^h \varphi_{hj}^i = 0.$$

Thus, it can be stated that

Theorem 4.11: In $GBK - 5RF_n$, that admits tensors decomposition, we have identity (4.31).

In view of (4.31) and (2.23), we get

$$(4.31) \quad y^h y^k = y_j.$$

In conclusion, it can be stated that

Theorem 4.12: In $GBK - 5RF_n$, that admits tensors decomposition, the product of different two contravariant vectors is a covariant vector.

Transvecting (4.31) by y^j and using [(1.1)d], we get

$$(4.32) \quad F = \sqrt{y^h y^k y^j}.$$

In conclusion, it can be stated that

Theorem 4.13: In $GBK - 5RF_n$, that admits tensors decomposition, the fundamental function of Finsler space is giving by (4.32).

Taking the partial derivative with respect to y^h of (4.31) and using [(1.1)c,h,i], we get

$$(4.33) \quad g_j = n y^k.$$

In conclusion, it can be stated that

Theorem 4.14: In $GBK - 5RF_n$, that admits tensors decomposition, the metric tensor g_j is giving by (4.33).

Transvecting (4.33) by y_k and using [(1.1)d], we get

$$(4.34) \quad F = \sqrt{\frac{g_j y_k}{n}}.$$

In conclusion, it can be stated that

Theorem 4.15: In $GBK - 5RF_n$, that admits tensors decomposition, the fundamental function of Finsler space is giving by (4.34).

5. A Illustrative Example

Let's assume we have a vector field representing the velocity of a rivers flow moving in a specific direction. If we have another vector field representing a force acting on the river (for example, a ship), then the Lie-derivative of the first field along the second field represents the rate of change of the rivers flow velocity due to this force.

If the force acts in the opposite direction of the flow, then the Lie-derivative will be negative, meaning the flow velocity will decrease. If the force acts in the same direction as the flow, then the Lie- derivative will be positive, meaning the flow velocity will increase.

For example, Let's assume a large tree trunk falls in to the river during the flow. Then the tensors will undergo decomposition into tensors of a lower order. For instance, the tensor of the fifth recurrent during decomposition maybe will return to the first, second, third or fourth recurrent.

6. Conclusions

In this paper the decomposition of Fundamental Function of Finsler space and some tensors by Lie-derivative in generalized fifth recurrent Finsler space for Cartan's fourth curvature tensor K_{jkh}^i in sense of Berwald has been introduced. Also, different identities and several theorems have been discussed under the decomposition. Based on the results of this study, we propose the following recommendations for future research:

1. Investigate the relationship between the proposed decomposition and other decomposition techniques in Finsler geometry, such as the decomposition of the curvature tensor.
2. Develop a computational algorithm to implement the decomposition procedure and apply it to specific examples of Finsler spaces.
3. Explore the potential applications of this decomposition in the study of Finslerian gravity theories, particularly in the context of modified gravity models.
4. Examine the role of the GBK framework in other areas of Finsler geometry, such as Finsler information geometry.

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Artificial seeds for In Vitro production of *Arabidopsis thaliana* Nossen (NO-o) plants

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Abstract

Somatic embryos are regarded the primary plant material for producing artificial seeds, and these seeds provide for agricultural institutions genetically uniform seeds, unlike the traditional seeds that often produce plants with different traits from the parent plant; in addition to the possibility of storing it for a long time *Arabidopsis thaliana*, locally known as mouse ears, is a promising model because it is self-fertile, which makes it conserved, and can produce thousands of seeds, which makes it ideal for mutation experiments. It is diploid ($10=2n$), and the identification of selectable traits is more straightforward because it has a relatively small genome (125 Mpb). The genome organization in *Arabidopsis thaliana* is simple and particularly suitable for genetic and molecular biology experiments.. This study produced *Arabidopsis thaliana* Nossen (NO-0) plants from the differentiation of callus tissues from its growing seedlings on MS medium (roots, stems, leaves). The B5 medium free of growth regulators successfully induced root callus, while the highest response for callus induction from stem segments was observed on B5 medium containing 0.05 mgL⁻¹ kinetin (Kin) and 0.5 mgL⁻¹ 2,4-Dichlorophenoxy acetic acid (2,4-D). The B5 medium containing 0.05 mgL⁻¹ (Kin) kinetin and 1.0 mg/L-1 (2,4-D) 2,4-Dichlorophenxy acetic acid (2,4-D) also affected leaf callus induction. The results of the statistical program SPSS version 16 also showed a significant difference at a probability level of 1% in the percentage of calyx creation

for the plant parts used .The MS medium containing 0.5 mgL⁻¹ Thidiazuron (TDZ) and 1.0 mgL⁻¹ 6-Benzyl adenine (BA) was effective in producing embryonic phases from transferred root callus. Meanwhile, the embryonic phases from stem and leaf callus were produced on MS medium supplemented with 0.5 mgL⁻¹ Thidiazuron (TDZ), resulting in a group of 290 shoots. A 2% sodium alginate concentration was used in experiments to encapsulate somatic embryos, yielding the best form of artificial seeds in spherical structures (Beads) and achieving a high conversion rate of 80% when stored for 20 days at 4°C, and 60% when stored for 20 days at 25°C. There are significant differences at the 5% level in the germination of seeds stored for 20 days at a temperature of 25°C. The number of plants resulting from the artificial seeds was 36 green shoots. The experiments were carried out over a period of six months in the Tissue Culture and Genetic Applications Laboratories at the College of Education for Pure Sciences/University of Mosul.

Keywords: Arabidopsis thaliana Nossen (NO-0), Artificial seeds, Somatic, Sodium alginate.

البذور الصناعية لإنتاج نباتات Arabidopsis thaliana Nossen (NO-0) خارج الجسم الحي

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الخلاصة:

تُعدُّ الأجنة الجسمية المادة النباتية الأساسية في إنتاج البذور الصناعية وأن هذه البذور توفر للمؤسسات الزراعية بذوراً متجانسة وراثياً على عكس البذور التقليدية التي تعرف بإنتاجها نباتات غالباً ما تكون مغايرة لصفات النبات الأم فضلاً عن إمكانية تخزينها لمدة طويلة و يعد Arabidopsis thaliana، المعروف محلياً بأذان الفأر نموذجاً واعداً كونه ذاتي الإخصاب مما يجعله محافظاً على صفاته، ويمكنه إنتاج آلاف البذور مما يجعله مثالياً لإجراء تجارب الطفرات وهو ثنائي المجموعة الكروموسومية $2n=10$ إن تحديد الصفات المنتخبة يكون أكثر وضوحاً لامتلاكه جينوم صغير نسبياً (125 Mpb) كما أن تنظيم الجينوم في Arabidopsis thaliana بسيط ومناسب بشكل خاص في تجارب الوراثة والبيولوجي الجزيئي، انتجت هذه الدراسة نباتات Arabidopsis thaliana Nossen(NO-0) من تميز كالس اجزاء بادراتها النامية على وسط ميوراشيك وسكوك (الجذور، السيقان، الاوراق)، وقد نجح وسط B5 الخالي من منظمات النمو في استحداث كالس الجذور في حين كانت اعلى استجابة لاستحداث الكالس من قطع السيقان على الوسط B5 المحتوي على 0.05 ملغم لتر⁻¹ (Kin) و 0.5 ملغم لتر⁻¹ 2,4-Dichlorophenxy acetic acid (2,4-D) وكان للوسط الزراعي B5 المحتوي على 0.05 ملغم لتر⁻¹ (Kin) و 1.0 ملغم لتر⁻¹ 2,4-Dichlorophenxy acetic acid (2,4-D) اثر في استحداث كالس الاوراق كما بينت نتائج البرنامج الاحصائي spss version 16 عن وجود فرق معنوي عند مستوى احتمال 1%. في نسبة استحداث الكالس للاجزاء النباتية المستخدمة وتألق الوسط MS المحتوي على (0.05) ملغم لتر⁻¹ Thidiazuron (TDZ) و 1.0 ملغم لتر⁻¹ 6-Benzyl adenine (BA) في تكوين الاطوار الجنينية من كالس الجذور المنقول اليه في حين تكونت الاطوار الجنينية لكالس السيقان والاوراق على وسط MS المدعم بـ (0.05) ملغم لتر⁻¹ Thidiazuron (TDZ)

ليكون مجموعة الافرع الخضرية المتكونة 290 فرعاً وقد اعتمد التركيز ٢٪ الجينات الصوديوم في التجارب لتغليف الأجنة الجسمية اذ اعطى افضل شكل للبذور الصناعية بهيئة تراكيب كروية (Beads) وملائماً جداً في اعطاء أعلى نسبة تحول conversion بلغت ٨٠٪ عند خزنها مدة ٢٠ يوم في ٤٠ ° مئوية و ٦٠٪ عند خزنها مدة ٢٠ يوم في ٢٥ ° مئوية وبلغت عدد النباتات الناتجة من البذور الصناعية ٣٦ فرعاً خضرياً. نفذت التجارب خلال ستة اشهر في مختبرات زراعة الانسجة والتطبيقات الوراثية في كلية التربية للعلوم الصرفة /جامعة الموصل

الكلمات المفتاحية: Arabidopsis thaliana Nossen (NO-0)، البذور الصناعية، الاجنة الجسمية، الجينات الصوديوم، الكالس.

Introduction:

The genus *Arabidopsis*, from the Brassicaceae strains family, includes nine species and eight subspecies, *Arabidopsis thaliana* is one of its most important species. It shows a wide range of features and genetic variations among of the wild type strains and provides unmatched information regarding genetic material, that makes it suitable for studying natural variations genetic experiments and molecular [1].

Wild *Arabidopsis* plants represent nearly homologous genotypes known as strains, which have been studied to describe genetic diversity and their morphological differences [2]. *Arabidopsis thaliana* plants are considered a promising model for studying plant genomics and expression, attracting botanists due to its high-quality genome sequence, short life cycle, and various phenotypes across different ecology patterns [3]. Controlled conditions and artificial growth mediums have been used to study its morphological traits, which cannot be replicated in natural environments [4].

Arabidopsis thaliana is a good model for elucidating the genome, that aims to correlate genotype and phenotype relationships, and through it, 36 new genes were identified, which likely associated with flowering traits [5].

Using basic methods to plant tissue culture for *Arabidopsis thaliana* is beneficial for producing large numbers of genetically similar plants in a relatively short growth period; and the production of callus and its cellular suspensions transplants, along with plant regeneration, serves as essential inputs for studying biochemical processes and cellular molecular processes [6]. The application of tissue culture in *Arabidopsis thaliana* (Col-0, No-0, Ler-0, Ws-0) has taken a significant place in plant regeneration, obtaining sterile plants, overcoming seed dormancy, and forming somatic embryogenesis and comparing the morphological traits of the resulting plants [7].

Artificial seeds represent a distinct technique in tissue culture, it relies on obtaining them from somatic embryos or other plant parts such as growing tips and buds. The storage of artificial seeds is a crucial factor that may influence conversion, preservation of genetic resources, and maintaining viability and germination potential even after storage periods [8]. Mature seeds of *Arabidopsis thaliana* are not viable, and their small size and light weight induce dormancy that prevents germination. The application of tissue culture in this plant type has taken a significant role in renewing plants and overcoming seed dormancy and forming somatic embryogenesis [9]. Production of good artificial seeds for the storage and propagation of rare plants with desirable genes is also vital to reduce costs associated with certain seeds or overcome seed dormancy or low viability [10].

2. Material and methods:

2.1 Seed Development

Arabidopsis thaliana (NO-0) seeds were sterilized after obtaining them from the Nottingham Arabidopsis Stock Center in the UK, by placing them in 97% alcohol for 3 minutes in a sterilized Eppendorf centrifuge tube (1.5 ml) by three consecutive washes with sterilized water for 2 minutes each to remove the sanitizer [11]. Seeds were dried on sterile filter paper to remove residual water and seeds were planted on solid MSO medium [12] in 100 ml glass bottles, with 2-5 seeds per bottle. The bottles containing the seeds were placed in a culture room under alternating light conditions (16 hours light/8 hours dark) at a temperature of $25 \pm 2^{\circ}\text{C}$.

2.2 Plant Parts and Their Culture

Thirty-day-old seedlings were used as a source for inducing callus of root, stem, and leaf using the following media:

S1: MSO

S2:B5

S3:B5 + 0.5 mgL^{-1} 2,4-D + 0.1 mgL^{-1} Kin

S4:B5 + 1.0 mgL^{-1} 2,4-D + 0.05 mgL^{-1} Kin

S5:B5 + 1.5 mgL^{-1} 2,4-D + 0.05 mgL^{-1} Kin

Callus induction from the plant parts was observed, after 30 days, measuring the fresh weight from samples kept in the culture room.

2.3 Production of Artificial Seeds

The production process of somatic embryogenesis involved culture of segments of callus of root, stem, and leaf with weighing 1g/piece on the following embryo production media:

M1: MSO

M2: MS + 0.5 mgL⁻¹ TDZ

M3: MS + 0.5 mgL⁻¹ TDZ + 1.0 mgL⁻¹ BA

M4: MS + 0.5 mgL⁻¹ TDZ + 1.5 mgL⁻¹ BA

Samples were kept in the culture room until embryonic phases (spherical, heart, torpedo, and cotyledon) were formed. The torpedo phase was used to produce artificial seeds using a 2% sodium alginate solution mixed with 1L of MS medium free of CaCl₂·2H₂O, with stirred continuously, and the pH was adjusted to 5-8. The solution was sterilized in a pressure apparatus for 20 minutes. The embryos were excised at the torpedo phase and then immersed in the 2% sodium alginate solution in a 150 ml beaker for one minute, and the plant parts were removed using a micropipette. Drops of sodium alginate with the plant parts were placed in a calcium chloride (CaCl₂·2H₂O) solution, left for 15 minutes to form beads, then washed 3 times with distilled sterilized water to remove the hardening agent. They were dried on sterile filter paper, transferred to Petri dishes, sealed tightly [13], and stored at 4°C and 25°C for 20 days. After the storage period, the beads were planted on solid MS medium for 3 weeks and then transferred to the culture room at 25 ± 2°C under 16 hours light/8 hours dark conditions.

2.4 Acclimatization of Resulting Plants

The resulting plants from the callus were transferred to MS medium free of growth regulators and MS containing 1.0 mg/L⁻¹ IAA. The plants with root clusters were washed with distilled sterilized water to remove any residual sodium alginate beads, then cultured in the same medium and kept under culture room conditions.

3. Results and Discussion

The surface sterilization method used showed high efficiency in germinating the seeds of *Arabidopsis thaliana* (NO-0), achieving a 100% germination rate and producing healthy seedlings within 7 days of planting. Thirty-day-old seedlings were used as a source for obtaining plant parts, as the ecology patterns of *A. thaliana* differ in their positive response to seed dormancy and variations in leaf shape, stem length, and flower morphology [14]. Root, stem, and leaf segments showed a clear response to callus induction when cultured on B5 medium. The roots planted on B5 medium Table 1. It was observed that the roots cultured in a medium free of growth regulators showed a distinctive response for callus induction within

5 days, callus was characterized by a yellowish-white color and friable texture **Figure 1-A**. Meanwhile, the B5 medium containing 2,4-D and Kin had a good effect in inducing callus from stems and leaves, with a friable texture and greenish-yellow color **Figure 1-B,C**. Additionally, it was observed there was an increase in the fresh weight of callus from the three segments successfully after 30 days of culture on the same medium **Figure 1- D,E,F** at a rate of 20 replicates per plant segment.

Table 1: Callus Induction from Plant Segments of Arabidopsis thaliana (NO-0)

Induction Media	Callus Induction Percentage (%)			Duration for Callus Induction (day)			Average Fresh Weight		
	Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves
S1	•	0	•	0	•	0	•	0	•
S2	100	0	•	5	0	•	٣,٨	0	•
S3	75	٩٥	90	٧	١٤	١٥	٣,٦	٣,٨	٤,٣
S4	٨٥	١٠٠	٩٥	٧	١٣	٩	٣,٤	٤,٠	٤,٥
S5	٦٥	٧٥	٩٠	١٠	١٧	١٢	٣,١	٣,٧	٤,١
Chi ²	٨٩,٢٧٦	٩٢,٨٥٢	٨٧,٣١٨	٧,٣٧٩	١١,٢١٧	١٠,١٢٨	٢,١١٨	٠,٨٥٧	٠,٨٥٧
p-value	***,٠٠	***,٠٠	***,٠٠	n.s, ١١٧	*, ٠١١	*, ٠١٨	n.s, ٧١٤	٠,٨٣٦ n.s	٠,٨٣٦ n.s

- Number of pieces used: 20 /plant segment

The data in **Table 1** above, derived from visual observations and weight measurements, reflect the variation in the color and structure of the callus, influenced by the type of added growth regulators and their concentrations, as well as the accumulated hormone content in the plant, particularly the high levels of auxins associated with callus production [15,16]. It has been reported that the relative level between 2,4-D and Kin is essential for inducing root callus of Arabidopsis thaliana, and the response of the leaves, due to their possession of active meristematic cells through their divisions, resulted in the production of parenchymal tissue characterized by its continuous vitality in the presence of abundant nutritional requirements and the repeated transfer to new media [17].

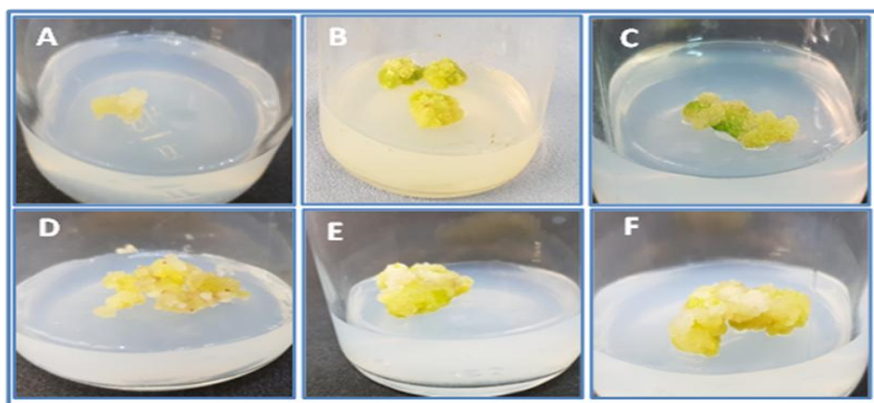


Figure 1: Callus of plant parts of *Arabidopsis thaliana* (NO-0) in culture media

A: Beginning of root callus induction D: Rate of root callus growth after 30 days

B: Beginning of stem callus induction E: Rate of stem callus growth after 30 days

C: Beginning of leaf callus induction F: Rate of leaf callus growth after 30 days

The callus induced from different segments was transferred to selected media for the production of somatic embryos, which are essential for generating artificial seeds. These embryos are produced from somatic cells of a single plant, unlike natural seeds that result from fertilized embryos from sexual reproduction between two different plants [18]. It was observed that the MS medium supplemented with growth regulators TDZ and BA influences in the production of green shoots **Table 2** from root callus **Figure 2-A**. Whereas, using TDZ at 0.5 mg L^{-1} has an effect in the production of embryonic phases from stem and leaf callus, starting with the spherical phase **Figure 2-B**, then the heart phase **Figure 2-C**, the torpedo phase **Figure 2-D**, and finally the cotyledon phase **Figure 2-E**, and leading to leaf production **Figure 2-F**.

Table 2: Production of green shoots and development of embryonic phases from callus of plant parts of *Arabidopsis thaliana* (NO-0)

Nutrient Medium (mg L^{-1})	Plant Segment	Rate of Somatic Embryos/ phase				Number of Shoots
		Spherical	Heart	Torpedo	Cotyledon	
M1	Leaves	33	30	10	10	70
M2		20	14	10	9	40
M3		20	10	10	10	30

Chi ²		3,308	8,169	1,429	1,824	21,00
p-value		n.s., 191	*, 0.17	n.s., 490	n.s., 402	**, 0.00
M1	Stems	20	20	10	11	60
M2		20	10	10	9	30
M3		10	12	10	9	30
Chi ²		2,00	2,080	1,429	0,276	10,00
p-value		n.s., 287	n.s., 303	n.s., 490	n.s., 871	**, 0.01
M1	Roots	10	10	9	9	10
M2		10	8	0	0	10
M3		0	0	0	0	0
Chi ²		10,692	7,003	6,40	6,40	11,610
p-value		**, 0.00	*, 0.29	*, 0.41	*, 0.41	**, 0.03

- Number of pieces used: 3 callus segments/treatment

The response of the ecology patterns of *A.thaliana* (Col-0, Ws-0, No-0, HR-10) varied in their germination rates and abilities to form new plants, with different rates, the highest rate recorded in Ws-0 and No-0 at 30.67% for callus induction and new plant production [19]. The cells of *A. thaliana* are capable of renewing the organs and tissues and even cells; in addition, they are able to produce walls and divisions for forming new plants [20]. Moreover, adding cytokinins is essential for the production of green shoots, and the cells of the wild types of *A. thaliana* are capable of forming buds on nutrient media containing varying ratios of cytokinins to increased auxins [20,21]. The formation of roots from the new plants posed a major obstacle in the process of developing complete plants, which was overcome by adding low concentrations of auxins [19], it was observed that rooting of resulting plants at 100%.

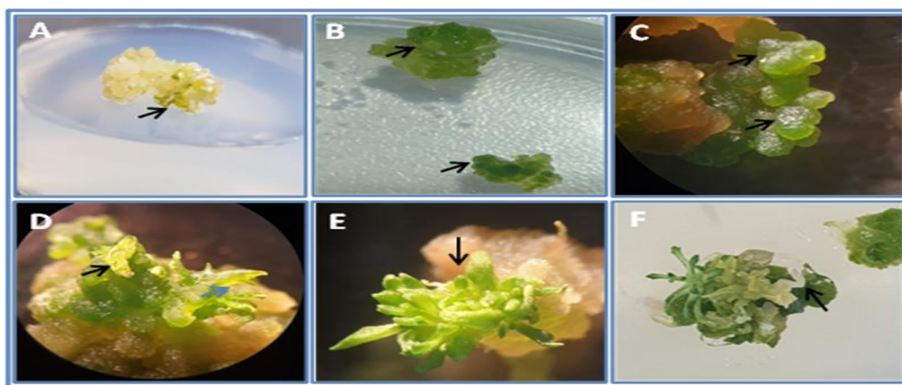


Figure 2: Emergence and Development of Somatic Embryos from *Arabidopsis thaliana* (NO-0) Callus

A: Formation of shoots from root callus.

B: Development of somatic embryos to the spherical .

C: Development of somatic embryos in (B) to the heart phase using a dissecting microscope.

D: Development of somatic embryos to the torpedo phase (see blue marker) and the cotyledon stage of the somatic embryos in (D) (see black markers) using a dissecting microscope..

E: Emergence of real leaves from the embryonic mass (indicated part) using a dissecting microscope..

F: Elongation of the formed shoots and rooting in medium MS + 0.1 mg L⁻¹ IAA

The physiological state of the plant parts used as a source for somatic embryos is a significant factor in their emergence and development. Other factors may also contribute to the successful formation of somatic embryos in this study, such as the polar accumulation of endogenous hormones in the plant part [23].

In this study, the torpedo phase was obtained from the embryonic phases formed from stem and leaf callus using sodium alginate and CaCl₂.2H₂O at 2%; and the interaction between them played a role in forming spherical structures (Beads) for artificial seeds (Figure 3,A). After storing the seeds at 4°C and 25°C (Figure 3-B), the highest conversion rate for the artificial seeds containing somatic embryos was 80% for samples stored at 4°C (Table 3). The presence of sodium alginate at specific concentrations contributed to forming cohesive gel-like coatings around the embryonic stages, protecting them from moisture loss and facilitating ion exchange between Na⁺ and Ca⁺⁺; and the hardness and softness of seeds are determined by the ionic exchange between sodium ions and calcium [24]. Although the commercial source of sodium genes is a determining factor that may lead to variations in the concentration ability of this substance for producing artificial seeds, several studies have indicated that a concentration of 2% of sodium genes is the optimal concentration, as observed in *Piper nigrum* L. [25].

Table 3: Effect of storage duration and temperature on the percentage of conversion of artificial seeds from somatic embryos of *A. thaliana* (No-o) to Complete-grown Plants.

Storage Duration (Day)	Conversion of Artificial Seeds (%)		Chi ²	p-value	Number of Resulting Plants		Chi ²	p-value
	4°C	25°C			4°C	25°C		
7	80	60	2.857	0.091 ^{n.s}	20	10	0.714	0.398 ^{n.s}
20	72	40	9.143	0.002**	10	10	0.0	1.0 ^{n.s}
Chi ²	0.421	4.0			3.33	1.0		
p-value	0.516 ^{n.s}	0.046*			0.068 ^{n.s}	0.317 ^{n.s}		

- Number of pieces used: 25 seeds/treatment

The highest conversion rate for artificial seeds stored at 25°C was 60% during 20 days into plantlets after 4 weeks after germinating them on MSO medium **Figure 3-C**. They started planting after 6 days **Figure 3-D**. The successful germination of artificial seeds of somatic embryos, which resulted in green shoots **Figure 3-F** and roots formation after 4 weeks **Figure 3-E**; and plants continued to grow normally. The high conversion rates for seeds stored at 4°C for the same period can be explained by reduced metabolic activity, especially respiration, and water and nutrient absorption at 25°C, which assists seeds in maintaining their viability during storage [26]. Several studies have indicated the effectiveness of storage at 4°C for various periods in preserving the viability of stored artificial seeds and their successful germination rates [27]. Increasing the storage duration of seeds at 4°C reduces the conversion rate of artificial seeds to complete plants, likely due to the process of impaired gas exchange in the plant tissues within the beads, and this leads to a decrease in oxygen levels in cells, which leads to an increase in carbon dioxide, affecting the potential energy of the plant tissue. Consequently, there is a decrease in vital processes such as respiration, protein synthesis, and other functions with increased storage periods due to the occurrence of respiration processes [28].

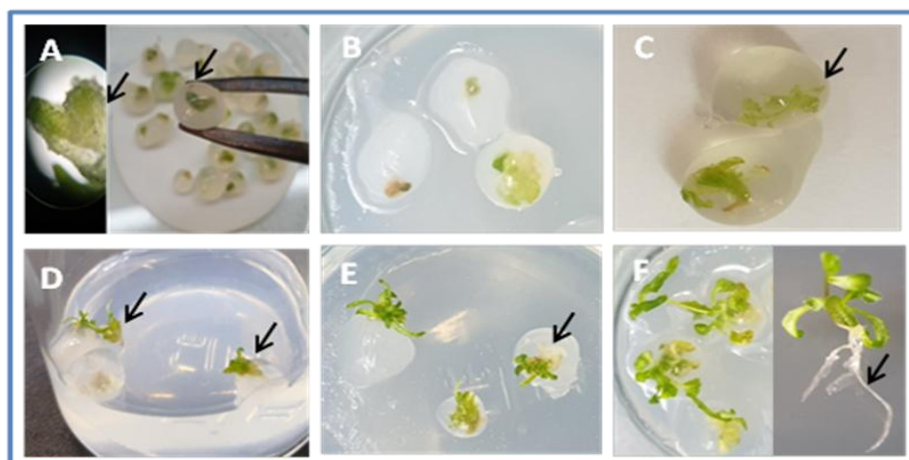


Figure 3: Encapsulation of somatic embryos of *A.thaliana* (No-0) and formation of artificial seeds, germination, and development of new plants

A: One of the embryonic phases in the artificial seed beads .

B: Artificial seed beads after hardening with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

C: Conversion of artificial seeds stored at 4° C after 6 days of planting .

D: Conversion of artificial seeds stored at 25°C after 6 days of planting .

E: Emergence of rooted shoots from the artificial seeds .

F: Plants produced from artificial seeds after four weeks of planting .

The change in color of the somatic embryos coated with sodium alginate to yellow or brown negatively affects the germination of seeds and the formation of plants; as the color change in nodal buds of *Aguilaria malacensis* trees coated with sodium alginate after prolonged storage and increasing their size, leading to decomposition due to inhibited respiration [29].

In the present study, the decrease in the conversion rate of seeds stored at room temperature 25° for 15.7 days is attributed to the drying of the seed coat and the retention of the plant part, which resulted in reduced oxygen levels and decreased moisture inside the storage containers [30]. A recent study [27] compared four genetic varieties of *Cicer arietinum* L. when the growing tips were wrapped and stored at 4° for 5 months. The results showed that the Giza4 and Giza88 varieties excelled, with germination rates of 70-75% for both classes, compared to the other two classes.

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A retrospective study on the detection and prevalence of intestinal protozoan parasites at Shekhan General Hospital, Iraq

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Keywords: Gastrointestinal Parasites, Shekhan General Hospital, Parasites, Duhok, Parasite detection.

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Abstract

Globally, intestinal parasites cause of a considerable amount of illness and death. Parasites in the gut are a serious public health concern in poor countries. Infections with intestinal parasites are among the most prevalent diseases worldwide. Low-quality drinking water and unclean personal and environmental conditions are often associated with them. This study aimed to determine the prevalence of gastrointestinal parasites from patients who attended Shekhan General Hospital, Shekhan District, Duhok, Iraq. The study conducted a retrospective cross-sectional analysis from January to November 2023. Overall, 228 recorded stool samples for eleven months were included in this study, and direct wet stool examinations were done for all stool samples to record intestinal protozoan stages. 228 stool samples were reported for eleven months between January and November 2023. Of them, 90 (39.47%) were recorded positive for intestinal protozoan parasites. The highest infection rate was found in September (8.3%), while the lowest rate of intestinal parasites was in November (0.0%). Regarding gender, the infection rate with parasitic infection was higher in males (22.3%) than in females (17.1%). Regarding a total of 228 samples the most prevalent age group aged <10 years (6.7%), the moderate one was in ages between 10-30 years (8.8%), and the lowest was in ages >30 years (3.9%). Finally, this study recorded that the most common

gastrointestinal protozoan parasites were *Giardia lamblia* (21.1%) and *Entamoeba histolytica* (17.1%). Infections can be prevented and controlled by practicing epidemiological surveillance, keeping oneself clean, and improving environmental cleanliness.

Keywords: Gastrointestinal Parasites, Shekhan General Hospital, Parasites, Duhok, Parasite detection.

الكشف بأثر رجعي عن طفيليات الأوليات المعدية المعوية وانتشارها في مستشفى شيخان العام، منطقة شيخان، دهوك، العراق

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الخلاصة:

هدفت هذه الدراسة إلى معرفة مدى انتشار الطفيليات المعدية المعوية بين عينات البراز بين المرضى الذين حضروا مستشفى شيخان العام، منطقة شيخان، دهوك، العراق. أجرت الدراسة تحليلاً مقطوعياً بأثر رجعي من يناير إلى نوفمبر ٢٠٢٣. تم الكشف عن تفاصيل جميع الطفيليات المعدية المعوية المبلغ عنها داخل عينة البراز عن طريق الفحص المباشر للبراز، وتم الحصول على الانتشار من وحدة التسجيل في مستشفى شيخان العام. بشكل عام، تم تضمين ٢٢٨ عينة براز مسجلة لمدة أحد عشر شهراً في هذه الدراسة، وتم إجراء فحوصات البراز الرطب المباشرة لجميع عينات البراز لتسجيل الأوليات الأولية والخراجات المعدية لمدة أحد عشر شهراً. تم حساب الطفيليات الأولية المعدية المعوية المكتشفة ومعدل انتشارها باستخدام التكرار والنسب المئوية. أخيراً، تم تقديم جميع البيانات كجداول. تم الإبلاغ عن ٢٢٨ عينة براز لمدة أحد عشر شهراً بين يناير ونوفمبر ٢٠٢٣. من بينها، تم تسجيل ٩٠ (٣٩,٤٧٪) إيجابية للطفيليات الأولية المعدية. تم العثور على أعلى معدل إصابة في سبتمبر (٨,٣٪)، في حين كان أدنى معدل للطفيليات المعدية المعوية في نوفمبر (٠,٠٪). وفيما يتعلق بنوع الجنس، كان معدل الإصابة بالعدوى الطفيلية أعلى لدى الذكور (٢٢,٣٪ في المائة) منه لدى الإناث (١٧,١٪ في المائة). وفيما يتعلق بمجموعه ٢٢٨ عينة والفئة العمرية الأكثر انتشاراً بين > ١٠ سنوات (٦,٧٪)، كانت العينة المعتدلة في الأعمار بين ١٠-٣٠ سنة (٨,٨٪)، وأقلها في الأعمار < ٣٠ سنة (٣,٩٪). أخيراً، سجلت هذه الدراسة أن طفيليات الأوليات المعدية المعوية الأكثر شيوعاً هي *Giardia lamblia* (٢١,١٪) و *Entamoeba histolytica* (١٧,١٪). يمكن الوقاية من العدوى والسيطرة عليها من خلال ممارسة المراقبة الوبائية، والحفاظ على نظافة النفس، وتحسين النظافة البيئية.

الكلمات المفتاحية: داء الجيارديا، داء الأميبا، طفيليات الجهاز الهضمي، فحص البراز، الانتشار

Introduction

Intestinal helminths and protozoan parasites, as well as other parasitic infections, are major global public health concerns that are primarily responsible for illness and disease, especially in affluent nations. These parasites affect at least thirty percent of people on the planet. In the

world, for example, 3.5 billion people are impacted, and these infections are responsible for over 450 million illnesses. Gastrointestinal infections in affluent nations are more frequently caused by protozoan infections than helminth infections [1,2]

Globally, parasitic diseases are common; their frequency varies according to sanitation standards and is linked to several factors such as poverty, malnourishment, overcrowding, and tropical weather [1]. A major contributing factor to the spread of parasitic diseases is inadequate food hygiene, contaminated water, and poor personal hygiene [2,3]. The main parasitological diagnosis for screening of morphological stages of the intestinal parasite is an examination of stool for detection of the first screening of both trophozoites and cysts morphological stages of intestinal protozoan, including *Entamoeba histolytica*, non-pathogenic *Entamoeba*, and *Giardia lamblia*, and the oocyst of *Crptosporidium parvum*, [4, 5] the second for screening of eggs of some intestinal nematodes such as *Enterobius vermicularis*, *Ascaris lumbricoides*, and *Ancylostoma duodenale*, and the larval one of nematode like *Strongyloides stercoralis*, [6] and finally for searching of segments and eggs of cestodes [7].

Gastrointestinal parasitic infections are globally significant public health problems in poor nations, [8] as a result of several risk factors such as inadequate sanitation, personal hygiene, clean drinking water, crowding, and malnutrition [9]. Gastrointestinal parasites affect people of all ages, mainly children, who are most frequently impacted due to their unhygienic habits and weakened immune systems [10]. Absorption problems, loss of weight, anemia, learning challenges, mental retardation, and intellectual issues are all linked to intestinal parasites in children [11]. Millions of toddlers and school-age children are generally at high risk of contracting helminthic and protozoan parasites, requiring an effective medication and a preventive measurement [12]. The World Health Organization reported that more than three billion people are infected with gastrointestinal parasites throughout the world, and most of them are children [13].

Till now there is no such study that recorded the most frequent gastrointestinal parasites in Shekhan District, Duhok City, Iraq. Most of the patients who attended the General Shekhan Hospital are primarily from low-socioeconomic communities and are at risk of parasite diseases. Shekhan District is widely renowned for its hot and humid weather, presence of immigrant camps, crowding, contamination of drinking water, and environmental and personal hygiene; all these provide an optimal environment for the spread of parasitic

diseases. Till now, no document has determined the most gastrointestinal protozoan parasites in Shekhan. Therefore, this study aimed to know the prevalence of common intestinal parasites among stool samples at Shekhan General Hospital, Shekhan District, Duhok, Iraq.

1. Materials and Methods

1.1. Study Design and Data Collection

To determine the detection of gastrointestinal protozoan parasites and their prevalence during eleven months (January to November 2023), a retrospective cross-sectional study was conducted. Details for all reported intestinal parasites were detected within the stool sample by direct stool examination, and the prevalence rate of infection was obtained from the register unit at Shekhan General Hospital. 228 stool samples were included in this study from January to November 2023, and direct wet stool examinations were done for all stool samples to record intestinal protozoan trophozoites and cysts for eleven months.

2.2. Inclusion and Exclusion Criteria

All patients who underwent an 11-month examination for gastrointestinal parasites during the study period were included in the analysis; however, data that did not include sociodemographic information, the year the stool examination was conducted, or information about the species and stage of gastrointestinal parasites had been excluded.

1.2. Data Analysis

All recorded data were entered and analyzed in the Microsoft Office Excel worksheet 2010. The detected gastrointestinal protozoan and its prevalence rate were calculated using frequency and percentages. Finally, all data were presented as tables.

Results

During the period of study (January to November 2023). 228 stool samples were requested for gastrointestinal parasite examination at Shekhan General Hospital. Ninety (39.47%) of them were recorded positive for intestinal protozoan parasites. The highest infection rate was found in September (8.3%), while the lowest rate of intestinal parasites was in November (0.0%). Regarding gender, the infection rate with parasitic infection was higher in males (22.3%) than in females (17.1%), as shown in Table 1.

Table 1: The gender distribution of samples per month and prevalence of intestinal parasites in this study:

Months	Total examined stool samples	Positive cases and their percentage		
		Total Positiv e No.	Males	Females
January	9	3(1.3%)	2(0.9%)	1(0.4%)
February	10	4(1.8%)	2(0.9%)	2(0.9%)
March	18	13(5.7%)	8(3.5%)	5(2.2%)
April	11	3(1.3%)	0(0.0%)	3(1.3%)
May	18	8(3.5%)	4(1.7%)	4(1.7%)
Jun	29	7(3.0%)	6(2.6%)	1(0.4%)
July	33	12(5.2%)	7(3.0%)	5(2.2%)
August	26	9(3.9%)	6(2.6%)	3(1.3%)
September	35	19(8.3%)	10(4.4%)	9(3.9%)
October	36	12(5.2%)	6(2.6%)	6(2.6%)
November	3	0(0.0%)	0(0.0%)	0(0.0%)
Total	228	90(39.4%)	51(22.3%)	39(17.1%)

Regarding

a total of 228 stool samples, the most prevalent age group aged <10 years (6.7%), the moderate one was in ages between 0-30 years (8.8%) and the lowest was in ages >30 (3.9%) as shown in Table 2.

Table 2: The distribution of intestinal parasites according to gender and age groups:

Age Groups	Total No. examined Stool sample	Positive Stool Samples		
		Total Positive No.	Males	Females
<10	115	61(26.7%)	37(16.2%)	24(10.5%)
10 - 30	75	20(8.8%)	13(5.7%)	7(3.1%)
>30	38	9(3.9%)	1(0.4%)	8(3.5%)
Total	228	90(39.4%)	51 (22.3%)	39 (17.1)

The most common intestinal parasites detected in this study were *G. lamblia*, *E. histolytica*, and *E. coli* (21.1%, 17.1%, and 1.3%) respectively, as shown in Table3.

Total No.	Positive Cases	%	<i>E. coli</i>	<i>E. histolytica</i>	<i>G. lamblia</i>
228	90	39.5%	3(1.3%)	39(17.1%)	48(21.1%)

Table 3: The detected intestinal protozoan parasites in this study and their rate:

3. Discussion

Infections with gastrointestinal parasites are a serious public health concern and are thought to be a primary cause of many infections. Gastrointestinal parasitic infections are linked to inadequate sanitation and unhygienic practices [14]. There is no such study that determines the reported gastrointestinal parasites and their prevalence rate in Shekhan District, Duhok, Iraq.

This retrospective study highlights that the infection rate with gastrointestinal parasitic disease was (39.4%). The prevalence rate with intestinal parasites according to the month was higher in September (8.3%), and a lower infection rate was recorded in November (0.0%). This finding is within the line of Mohammed et al., [15] and Konadu et al. [16], who reported as this study reported. The autumn season is wet and rainy, which makes sanitation extremely

unsafe and increases the risk of gastrointestinal parasite contamination in food and drink due to improper stool waste disposal [17].

Regarding gender, the infection rate with parasitic infection was higher in males (22.3%) than in females (17.1%). This result is in line with a study done in Duhok City, Iraq, by which a higher rate was recorded in males than in females [18]. The same rate was reported in males by Taher and Ali [19]. This finding disagrees with a study done in Saudi Arabia between the period of 2019 to 2023, which found that the higher infection rate in females can be explained by the fact that females are more likely than males to contract parasite infections during the infectious stages because of household responsibilities and lifestyle choices [20]. While, this result disagrees with the study result recorded by Getnet *et al.* [21], which found the highest rate in males than in females among schoolchildren at Amber Primary School, Ethiopia.

This study found that the most common was in the age group <10 years (6.7%), and the lowest was in ages >30 years (3.9%). This high rate in these ages was reported in Kirkuk City, Iraq, by Mohammed *et al.* [8]; also, a similar rate was reported by other researchers such as Ismael *et al.* [18], Taher and Ali [19], Kumar *et al.* [22]. This age group is more susceptible to infection because they spend most of their time outside the home and in contact with the contaminated environment.

According to the present study, the most frequently detected gastrointestinal parasites were *G. lamblia* and *E. histolytica*. This result agrees with Taher and Ali, who found that the most intestinal parasites were *G. lamblia* and *E. histolytica* [19]; also, a similar report was obtained by Duguma and Tekalign [23] and a retrospective study was done in Ghana for five years, which agrees with the result within this study [16, 24]. It is suggested that these two parasites were more frequent in this study because these parasites are transmitted by ingestion of contaminated food and grasses and drinking contaminated water [25, 26, 27].

In contrast to the current study's findings, a retrospective survey of intestinal parasites by Kurup & Gurdir [28] found a total incidence of 26.1% within four years. The most common protozoan infection was *Giardia intestinalis*, which was found to have a prevalence rate of 21.1% in this study. This is lower than findings from various types of literature that reported giardia prevalence of 2 to 3 percent in wealthy nations but up to 30 percent in the majority of underdeveloped countries [29].

Our findings for the prevalence of amebiasis were 17.1%, whereas a study conducted in Iraq found a prevalence of 3.78 percent [30]. The current study's findings are significantly lower than those of another similar study conducted at the University Hospital of Parma, which found an intestinal parasite prevalence of 16.6% [31] between 2006 and 2010. Intestinal parasite prevalence rates of 17.6%, 13.9%, 9.03, and 8.9% were reported in four Indian tertiary care facilities; our results were greater than those of other studies [32, 33]. Additionally, a study in north-central Iran between 2012 and 2016 found an intestinal parasite prevalence of 18.0%, which was less than what our study found [34].

Conclusion

Due to the lack of research on the prevalence of gastrointestinal parasites in Shekhan General Hospital, Duhok, Iraq, this investigation provided several interesting findings. This study revealed a low number of stool samples examined daily during the eleven months, and the rate of parasitic infection was 39.4%. The most frequent gastrointestinal parasites in this study were *G. lamblia* and *E. histolytica*. Also, our study highlights that the male sex, young people, and the humid season all impact positively on the prevalence rate of parasitic infection.

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Contribution Study Concept and Design

Shameeran Salman Ismael, Iman Salim Sulaiman, and Hayam Nori Awad

Analysis and interpretation of data

Shameeran Salman Ismael

Ethics

The study proposal was approved by the ethics and scientific committee of the Shekhan Technical College of Health/Duhok Polytechnic University and Shekhan General Hospital.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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Preparation and Study the Properties of Ni-Co Ferrite Substituted with Ag- ions Calcined at 1000°C

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Abstract:

Analysis of the structural and dielectric properties of Ag-substituted Ni-Co spinel ferrite, represented by the formula $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$, were examined for values of ($0.00 \geq x \leq 0.45$). An auto-combustion sol-gel process was used to synthesis this ferrite, which was then calcined at temperatures up to (1000°C). The ferrite samples underwent X-ray diffraction (XRD) research to determine their particle size, lattice characteristics, bulk density, and hopping length. The size of the crystallites varied between (32.952 nm and 43.926 nm), while the lattice parameter rose between 8.291 and 8.326 Å as the amount of silver ions increased. The X-ray density increased from 5.466 to 5.904 g/cm³, which was attributed to the larger molecular mass of silver (Ag) at 107.87 g/mol compared to cobalt (Co) at 58.93 g/mol. Dielectric properties were also studied, including both the real (ϵ') and imaginary (ϵ'') components of the dielectric constant, the dielectric loss represented by the loss tangent ($\tan \delta$), and electrical resistivity (ρ_{ac}). The results showed a decrease in both the real and imaginary dielectric constants, as well as a reduction in dielectric loss with increasing applied frequency. Additionally, a decrease in electrical resistivity was observed with increasing frequency.

Keywords: Sol-gel method, Dielectric properties, Silver-cobalt ferrite nanoparticles, Spinel ferrites, X-ray diffraction.

تحضير ودراسة خصائص فرايت النيكول-كوبالت المعوض بأيونات الفضة والمكسنة عند ١٠٠٠°C

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الخلاصة:

يدرس هذا البحث الخصائص التركيبية والعزلية لفرايت الكوبلت - الفضة، ذات الصيغة: $Ni_{0.4}Co_{(0.6-x)}Ag_2Fe_2O_4$ حيث ان $(0.00 \geq X \geq 0.45)$ باستخدام طريقة السول-جل، حيث تم تلدين النماذج عند درجات حرارة تصل إلى $(1000^\circ C)$. ويدرس البحث ايضا الخواص التركيبية للعينات المحضرة باستعمال فحص حيود الأشعة السينية (XRD) لتحديد حجم الجسيمات، وخصائص الشبكة البلورية، والكثافة الحجمية، وطول القفزات. حيث تراوح حجم البلورات بين ٣٢,٩٥٢ نانومتر و٤٣,٩٢٦ نانومتر، بينما زادت معامل الشبكية من ٨,٢٩١ إلى ٨,٣٢٦ مع زيادة تركيز أيونات الفضة. كما أظهرت نتائج تحليل حيود الأشعة السينية زيادة في كثافة الأشعة السينية من ٥,٤٦٦ إلى ٥,٩٠٤ جرام/سم^٣، ويرجع ذلك إلى الكتلة الجزيئية الأكبر للفضة (Ag) مقارنة بالكوبلت (Co)، وتمت دراسة الخواص الكهربائية أيضاً، المتمثلة ثابت العزل الكهربائي بنوعيه الحقيقي (ϵ') والخيالي (ϵ'')، بالإضافة إلى زاوية فقدان العزل ($\tan \delta$) والمقاومية الكهربائية (ρ_{AC}). أظهرت النتائج انخفاضاً في كل من الثابت العازل الحقيقي والتخيلي، فضلاً عن انخفاض في فقدان العزل مع زيادة التردد المطبق. كما لوحظ انخفاض في المقاومة الكهربائية مع زيادة التردد.

الكلمات المفتاحية: طريقة السول-جل، الخصائص العزلية، الفرايت الكوبلت - الفضة النانوي، الفرايت الاسبينيل، حيود الأشعة السينية (XRD).

1. Introduction:

A notable group of electrical materials, spinel ferrites are used in radio frequency circuits, factor gadgets of superior quality, cores of transformers, medium for magnetic recording, fluids that are magnetic, and other state-of-the-art technology because of their strong electrical resistance and minimal eddy current losses [1,2,3]. Cobalt ferrite nanoparticles demonstrate improved characteristics when integrated with noble metals such as silver (Ag) or gold (Au). The incorporation of silver into $Co-NiFe_2O_4$ is anticipated to augment its antimicrobial efficacy owing to the intrinsic antibacterial characteristics of silver [4,5]. The characteristics of these ferrites are affected by various factors, such as the preparation technique, sintering parameters, chemical composition, crystallite dimensions, and the distribution of cations in the tetrahedral

(A) and octahedral (B) lattice sites, represented chemically by the formula AB_2O_4 [6,7,8,9]. Among ferrite materials, nanoparticles of cobalt and silver stand out for their high electrical resistance, low tangent losses, and excellent dielectric properties. Therefore, that is critical to investigate their dielectric characteristics throughout a range of frequencies [10,11]. This research intends to examine the impact of integrating silver ions into the cobalt ferrite structure on its electrical properties. This is accomplished by examining the real (ϵ') and imaginary (ϵ'') components of the dielectric constant, the dielectric loss tangent ($\tan \delta$), and the electrical resistivity (ρ_{ac}) across an extensive frequency spectrum. This work enhances the comprehension of the correlation between structural composition and electrical qualities, aiding in the creation of materials with improved electrical performance for advanced technological applications. These applications encompass supercapacitors, insulating materials in integrated circuits, electrical sensors, high-frequency transformers, electromagnetic wave-absorbing materials, and electronic components utilized in wireless communication and microwave technologies [12].

2. Material and methods:

Sol-gel technique was used to prepare six distinct samples of $Ni_{0.4}Co_{(0.6-x)}Ag_{2x}Fe_2O_4$, with the value of x ranging from 0.00 to 0.45. Initially, the solution was prepared using a set of pure chemicals, such as iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$), cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), silver nitrate ($Ag(NO_3)$), nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$), and citric acid ($C_6H_8O_7 \cdot H_2O$). These materials were carefully chosen, as the metal nitrates act as oxidizing agents, while citric acid serves as a reducing agent, helping to form the final ferrite structure. Double distilled water was used throughout all experiments to ensure the solvent was free of impurities that could affect the reaction [13]. Subsequent to the solution's preparation, the mixture was incrementally heated on a hot plate to $90^\circ C$ for two hours, culminating in the formation of a dense gel. Continuous agitation during this phase was essential to guarantee the uniform distribution of the components within the mixture. As the solvent evaporated, the mixture became increasingly concentrated, evolving into a viscous brown gel, which serves as the precursor to the self-propagating combustion process [14].

The next step, the temperature was increased to $250^\circ C$ to initiate the self-combustion process, which removed the remaining water from the mixture and produced the burned ferrite powder. To ensure a fine and uniform powder, it was ground using a mortar and stirring mechanism, which helped improve the uniformity of the particles. The resultant powder was further heated to $400^\circ C$ for three hours to eliminate any residual organic compounds and to enhance the

sample's homogeneity. Subsequently, the powder was combined with polyvinyl alcohol (PVA) as a binder to fabricate circular discs of 13 mm in diameter and roughly 2 mm in thickness. The dry pressing process involved applying a pressure of 3 tons using a hydraulic press for one minute to form the discs. The resultant discs were sintered at 1000°C for three hours to enhance the characteristics of the samples. Subsequent to the sintering procedure, the discs were allowed to cool naturally to ambient temperature, so preparing them for the examination of their insulating qualities. An electrical impedance analyzer was employed to assess these properties subsequent to the sintering of the samples [15]. **Figure1** shows the steps involved in using the sol-gel method for preparation [16].

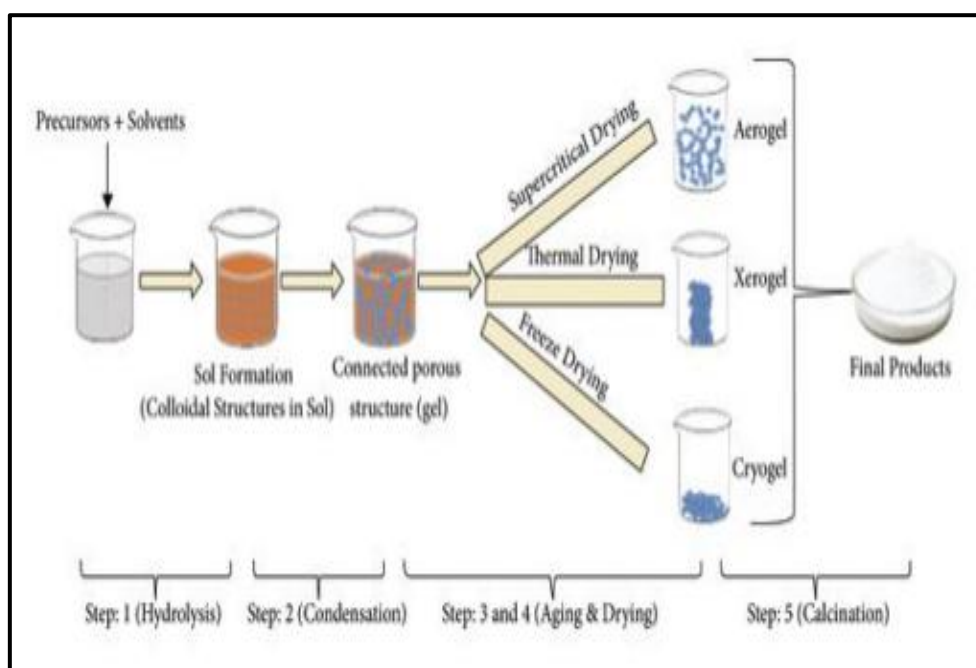


Figure1:Flowchart depicting the different phases of the sol-gel process: from precursor to aerogel (16)

3. X-ray diffraction analysis:

Figure 2 illustrates the XRD pattern for a sample with minimal Ag doping ($x = 0.00, 0.05, 0.15, 0.25, 0.35$ and 0.45). The lattice planes (111), (220), (311), (222), (400), (422), (511), (440), (620), (533), and (622) exhibit various configurations. The material exhibits a single-phase spinel structure, with the most prominent diffraction peak found at the (311) plane. The cubic spinel structure (ICDD 22-1086) matches the indexed peaks corresponding to the lattice planes According to [17]. The crystallite size (D) of the samples was calculated based on the Debye-Scherrer equation [18].

$$D = \frac{k\lambda}{(\beta \cos\theta)} \dots \dots \dots (1)$$

In this context, β signifies the full width at half maximum (FWHM) in radians, k indicates the form factor, which is set at 0.89, λ is the wavelength of the incident X-ray beam, and its value is 0.15406 nm, and θ represents the Bragg diffraction angle[19].

The lattice constant (a) of the cubic spinel structure was ascertained using the approach.

$$a = d\sqrt{h^2 + k^2 + l^2} \dots \dots \dots (2)$$

In this context, h, k, l denote Miller indices, while d represents interplanar distance, which may be calculated using Bragg's law: $2d \sin \theta = \lambda$

Variations in the lattice parameter as a result of silver ion accumulation. The following equation is used to determine the X-ray density of the ferrite nanoparticles that are produced [20]:

$$\rho_x = \frac{8M}{N_A a^3} \dots \dots \dots (3)$$

M denotes the molecular weight, a marks the lattice parameter, and N_A represents Avogadro's number.

The hopping length at the tetrahedral (A) site and octahedral (B) site is determined using the subsequent equations [21].

$$L_A = a \frac{\sqrt{3}}{4} \dots \dots \dots (4)$$

$$L_B = a \frac{\sqrt{2}}{4} \dots \dots \dots (5)$$

The structural properties were computed utilizing the aforementioned equation, as indicated with in **Table 1**

Table 1: X-ray diffraction (XRD) results for $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ ferrite nanoparticles calcined at 1000 °C.

x	D(nm)	a (Å)	ρ (g/cm ³)	L_A (Å)	L_B (Å)
0.00	32.952	8.291	5.466	3.590	2.932
0.05	39.165	8.303	5.499	3.596	2.936
0.15	39.771	8.302	5.614	3.595	2.937
0.25	43.929	8.308	5.715	3.598	2.938
0.35	43.928	8.323	5.797	3.604	2.943
0.45	43.926	8.326	5.904	3.605	2.944

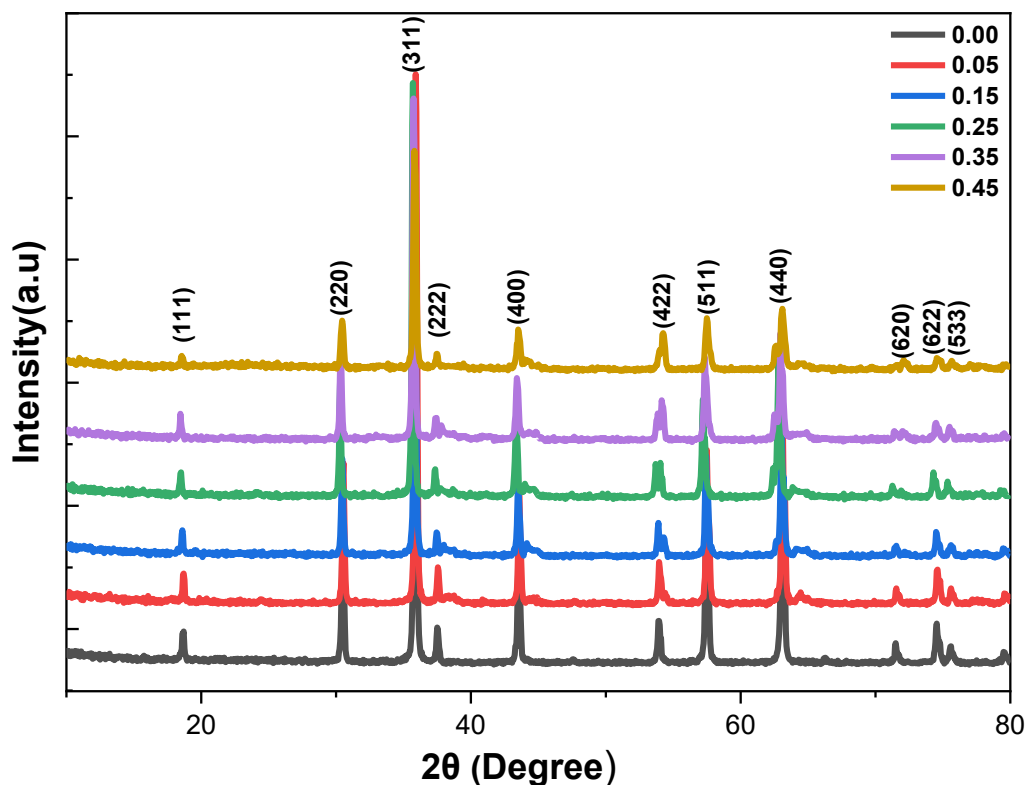


Figure 2: XRD pattern of nanoparticles $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{FeO}_{42}$.

3.1 Crystallite Size (D):

Equation 1 and X-ray diffraction (XRD) analysis were used to determine the crystallite size (D). The 311) peak and its full width at half maximum (FWHM) as well as its peak position (2θ) were used for the measurements. For the $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ ferrites at 1000°C , the particle sizes, depending on the varying x concentrations, ranged from 32.952 nm to 43.926 nm, as presented in **Table 1**. The results indicated that the crystallite size increases with the enhancement of silver doping in Ni-Co Fe_2O_4 , as the higher concentration of Ag^+ ions substituting Co^{2+} ions leads to a larger crystallite size. This increase is attributed to the larger ionic radius of Ag^+ (1.26 Å) compared to Co^{2+} (0.83 Å) ,and Fe^{2+} (0.92 Å) ions, This is consistent with the researchers' results [22,23]. As the silver concentration (x) increases in the crystal lattice, the available sites in the crystal structure are filled with silver ions. However, after a certain concentration (typically at $X = 0.25$ or higher), the network reaches a state of “saturation,” where most of the sites in the lattice are fully occupied. Therefore, silver can no longer significantly influence the expansion of the crystallite size beyond this point, leading to the stabilization of the crystallite size[24].

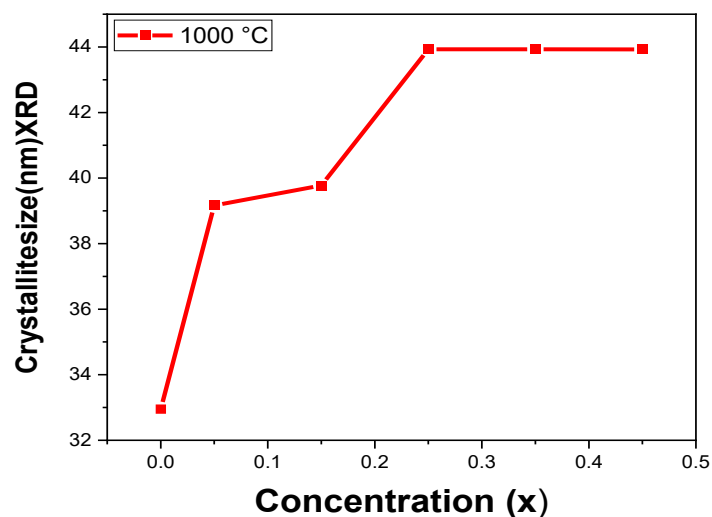


Figure 3: Variation in Crystallite Size (D) with Ag Concentration (x) for Ni-Co Ferrites Sintered at 1000°C.

3.2 Lattice Parameter (a):

The lattice parameter (a) for the sample at the (311) peak at 1000°C was calculated using Equation 2, and the results are shown in Table 1. As silver content increases, the lattice parameter of silver-cobalt ferrite go from 8.291 Å to 8.326 Å. According to the researchers, this is because the ionic radius of Ag^+ (1.26 Å) is larger than that of Co^{2+} (0.83 Å). Consequently, the lattice constant of cobalt ferrite that is substituted with silver is expected to increase as the concentration of silver increases [25, 26].

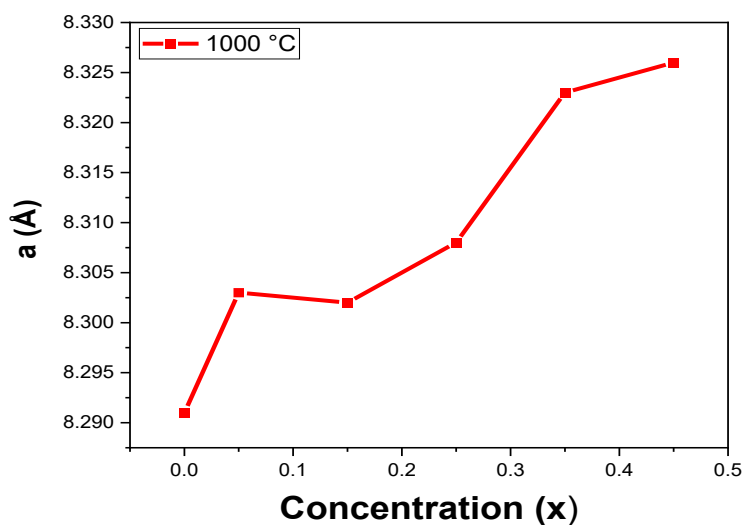


Figure 4: Lattice Parameter Variation as a Function of Ag Content (x) for Ni-Co Ferrites.

3. 3 Theoretical Density ($\rho_{x\text{-ray}}$):

Equation 3 was applied to determine the theoretical density values ($\rho_{x\text{-ray}}$) of the $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ ferrite phase at 1000°C across various silver concentrations ($x = 0.0$ to 0.45). **Figure 5** depicts the relationship between silver concentration and density. The disparity in molar masses between cobalt (Co^{2+}) and silver (Ag^+) is responsible for the observed shift in behavior. Comparing silver to cobalt (Co), the former has a molar mass of 107.87 g/mol and the latter stands at 58.93 g/mol . The density is increased when silver is used in place of cobalt because silver has a larger atomic weight, This is consistent with the researcher results[20].

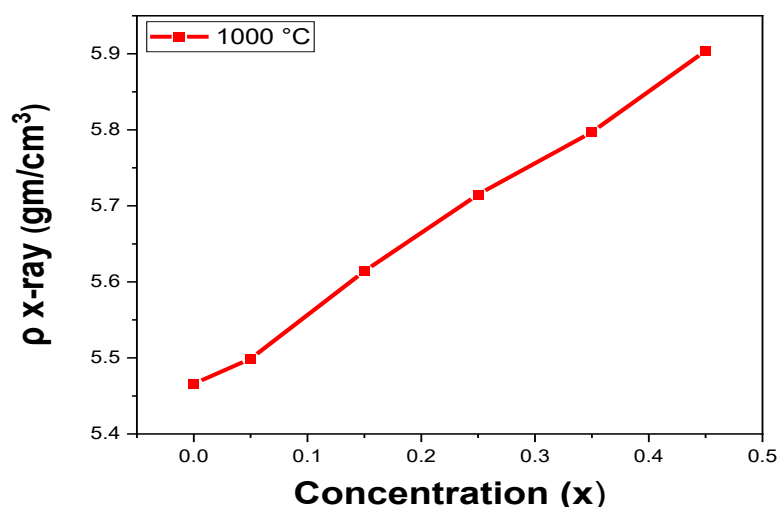


Figure 5: shows how the theoretical density values ($\rho_{x\text{-ray}}$) for Ni-Co ferrites vary with increasing Ag Content (x).

3. 4 Hopping Length (L):

The lengths of ion hop at the tetrahedral (A) site L_A and octahedral (B) site L_B were determined by calculating the interionic distances using the crystal lattice constant, as outlined in **Equations 4 and 5**. The calculated values for the synthesized Ag-Co ferrite sample at 1000°C are illustrated in **Figures 6** and **Table 1**. It is evident that the ionic jump lengths increase with augmented Ag content. Given that silver ions are larger than cobalt ions, their substitution results in an expansion of the lattice constant, thereby yielding a denser crystal structure[14]. The ionic jump lengths (L_A and L_B) exhibit a direct proportionality to the lattice constant (a), This results in a rise in jump lengths as silver concentrations increase. Furthermore, the ionic jump length (L_B) is somewhat shorter than (L_A) due to the more compact nature of the (L_B) sites, resulting in shorter jump distances; this is consistent with the researcher's results [27,28].

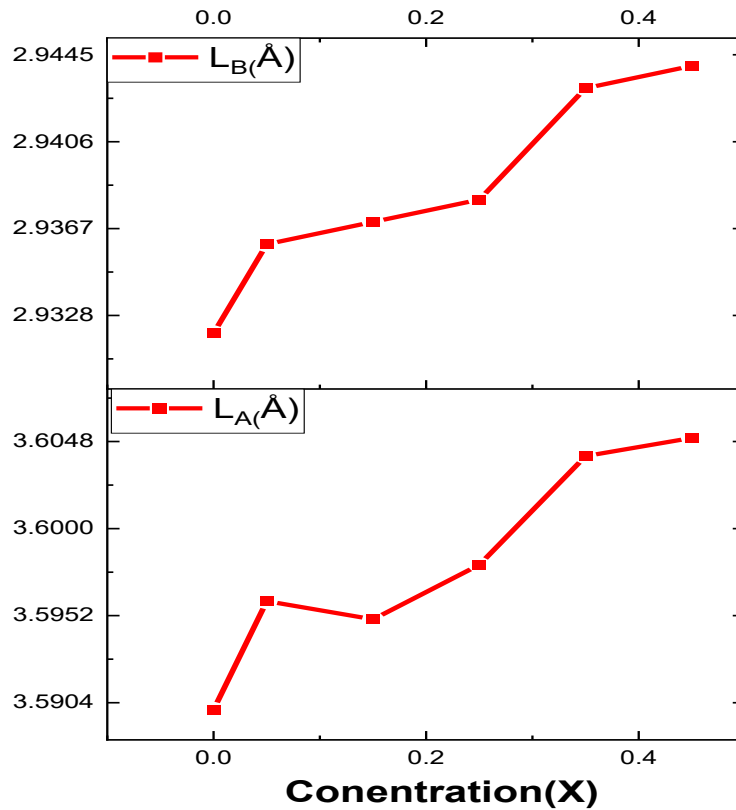


Figure 3: Shows the variation in jump lengths L_A and L_B for Ni-Co ferrites with increasing Ag content (x).

4. Electrical Characteristics:

The behavior of nanoparticles made from ferrite demonstrates the mechanism associated with the dielectric response to alternating electric fields and electrical conduction processes. These properties are influenced by several factors, including chemical composition, manufacturing process, the ratio of components, particle size, ionic charge, porosity, and the cation distribution between the tetrahedral (A-site) and octahedral (B-site) [29].

Calculations of the dielectric properties of the $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ sample at room temperature were carried out, including the dielectric constant (ϵ'), dielectric loss (ϵ''), dielectric loss angle ($\tan \delta$), and electrical resistivity (ρ_{ac}) as a function of frequency (500kHz – 5MHz) using the following equations [30].

$$\epsilon' = \frac{Cd}{\epsilon_0 A} \dots \dots \dots (6)$$

$$\tan \delta = \frac{1}{2\pi f R_P C_P} \dots \dots \dots (7)$$

$$\epsilon'' = \epsilon' \tan \delta \dots \dots \dots (8)$$

$$\rho = R_p \frac{A}{d} \dots \dots \dots (9)$$

In this context, C denotes the capacitance of the pellet in farads, d represents the thickness of the pellet in meters, A signifies the cross-sectional area of the pellet's flat surface, ϵ_0 refers to the permittivity constant of free space, R_p indicates the equivalent parallel resistance, and C_p stands for the equivalent parallel capacitance, A Surface area of the insulator, and d Thickness of the insulator

Figure (7-10) Illustrates the variation of the dielectric constant (ϵ'), the dielectric loss angle ($\tan \delta$), dielectric loss factor (ϵ''), and the electrical resistivity (ρ_{ac}) as a function of frequency.

4.1 Dielectric Constant (ϵ'):

Figure 7 shows how the real dielectric constant (ϵ') changes with frequency at ambient temperature, ranging from (50 Hz to 5 MHz) for $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ ferrite samples. As the frequency increases, a decrease in the dielectric constant (ϵ') is observed, which can be explained by **Equation 6**. It can be inferred that the ferrite samples contain conductive grains with non-conductive grain boundaries. Electron polarization occurs when electrons hop across the non-conductive grain boundaries, where they accumulate due to the increased resistance. At higher frequencies, the electrons fail to interact with the oscillating field, minimizing the probability of electrons encountering the grain boundaries, hence diminishing polarization, This aligns with the researcher's findings [31,32].

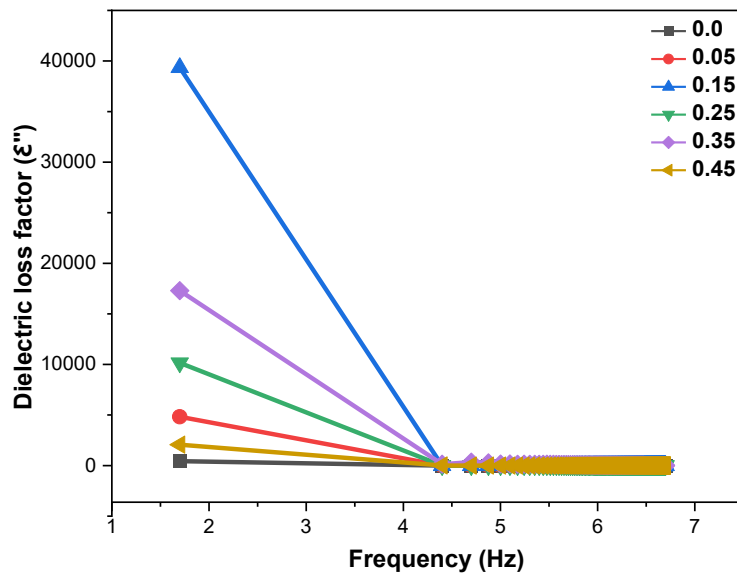


Figure 7:The dependence of dielectric constant (ϵ') on frequency for $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$.

4.2 Dielectric Loss Angle ($\tan\delta$):

The values of tangent loss ($\tan\delta$) are influenced by various elements, including chemical composition, Fe^{2+} concentration, and structural uniformity, all of which are contingent upon the composition of the ferrite samples and the calcination temperature.

Figure 8 illustrates the fluctuation of tangent loss relative to frequency for Ni-Co ferrite samples with varying silver ion (x) concentrations. The factor of dielectric loss is highest at low frequencies and declines with the rise in the applied electric field frequency, as explained by **Equation 7**. The inverse relationship between the dielectric loss factor and frequency arises from the substantial energy gained by dipoles from the applied electric field, which heightens the probability of collisions among these dipoles as they rotate at lower frequencies, resulting in diminished dielectric properties. With the increase in the applied field frequency, the capacity of the dipoles to track the fluctuations in the field diminishes, leading to a reduction in the energy absorbed by the dipoles from the applied field, and consequently, a decline in the dielectric loss factor, This is consistent with the researcher results [33].

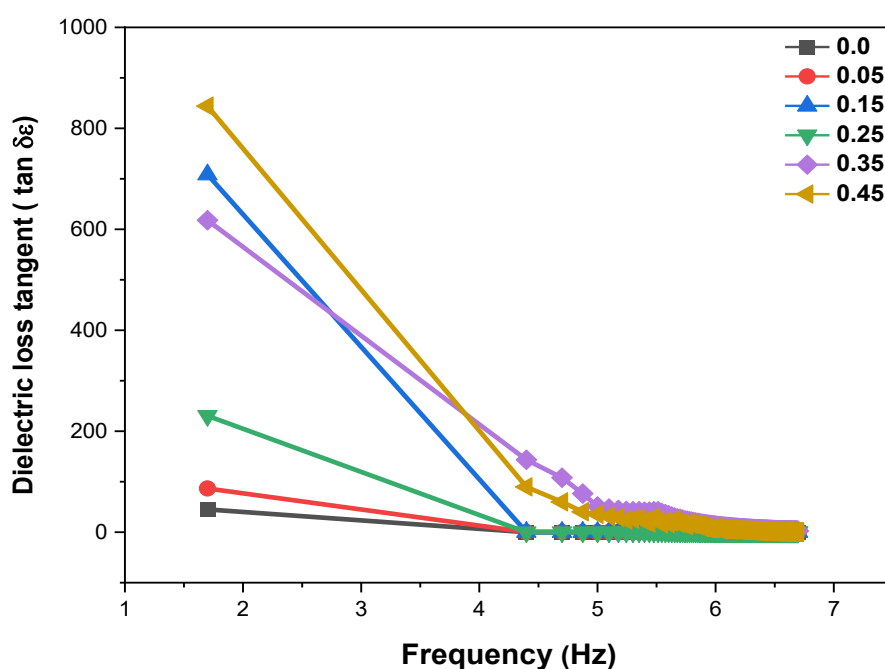


Figure 8: Displays the correlation between frequency and dielectric loss angle ($\tan\delta$) for spinel nanoparticles of $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_2\text{xFe}_2\text{O}_4$.

4.3 Dielectric Loss Factor (ϵ''):

The imaginary portion of the dielectric constant (ϵ'') representing the loss of dielectric energy, is calculated through **Equation 8** and is a critical parameter in evaluating the total core loss of ferrites. Consequently, lower dielectric losses are preferred for reducing core loss. The relationship between dielectric loss (ϵ'') and frequency for Ni-Co nano ferrite samples with different levels of Ag-ion content (x) is shown in **Figure 9**. The dielectric loss curve resembles the tangent loss shown in the previous figure. The increase in electron hopping results in a localized shift in the direction of the applied electric field, resulting in higher electric polarization and consequently enhancing the dielectric loss factor, This is consistent with the researcher's results [31,34].

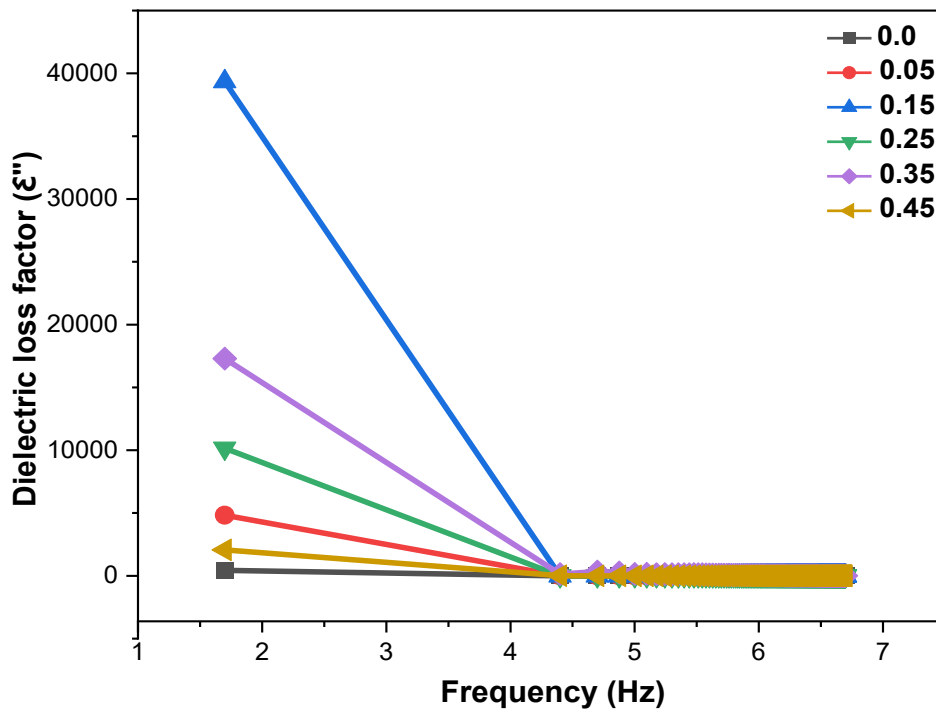


Figure 9: Relationship between the dielectric loss factor (ϵ'') and frequency for $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ spinel nanoparticles.

4.4 Resistivity (ρ_{ac}):

Using **Equation 9**, the electrical resistance was computed, where **Figure 10** shows the relationship between electrical resistance and frequency. As the frequency increases, a drop in electrical resistance is detected, following a pattern similar to the real value of the dielectric constant (ϵ') and the imaginary component of dielectric loss (ϵ''). This tendency suggests that electrical conductivity rises with increasing frequency. This alteration can be described based

on the conduction mechanism in ferrite materials, where electron transfer happens between (Fe^{2+}) and (Fe^{3+}) ions at the octahedral locations (site B). As the ions get closer together, this transfer increases, leading to electron hopping in internal displacements, resulting in polarization. This is congruent with the researchers' results [35,34].

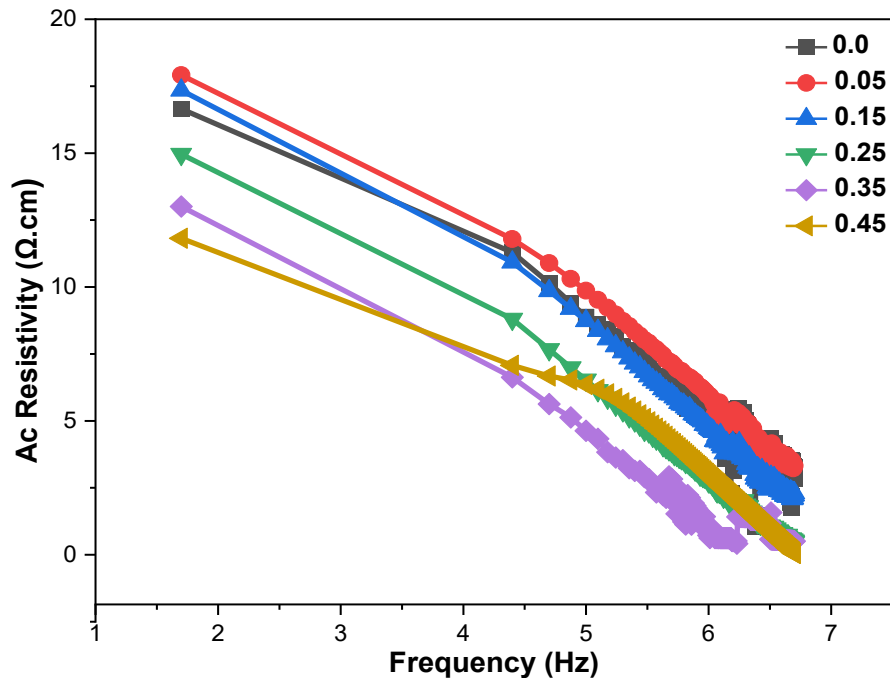


Figure 10: Resistivity (ρ_{ac}) variation with frequency for $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$ spinel nanoparticles.

5. Conclusions

This work studied the effect of substituting Co ion with Ag ion in the spinel ferrite structure $\text{Ni}_{0.4}\text{Co}_{(0.6-x)}\text{Ag}_{2x}\text{Fe}_2\text{O}_4$, synthesized using the auto-combustion sol-gel process and sintered at 1000°C . X-ray diffraction (XRD) studies demonstrated an increase in lattice constant and bulk density with increasing Ag content, which is due to the larger molecular mass of Ag. Dielectric investigations demonstrated a drop in both the real (ϵ') and imaginary (ϵ'') components of the dielectric constant, as well as a reduction in dielectric loss ($\tan \delta$) and AC electrical resistivity (ρ_{ac}) with increasing frequency. These discoveries contribute to the understanding of the relationship between the structural composition and electrical properties of the material, supporting applications in high-frequency circuits, sensors, integrated circuit insulation, and electromagnetic wave absorption. Additionally, introducing Ag may boost the antibacterial capabilities, expanding its potential for medical and electrical applications. Overall, this work helps to the creation of high-performance materials appropriate for sophisticated technological applications.

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The role of vitamin C as a potent Antioxidant and Pro-oxidation in Acute Myeloid Leukemia Treatment Progress

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Abstract:

Vitamin C has effects on cancer as both an antioxidant and a pro-oxidant. It serves as an antioxidant by reducing oxidative stressors and minimizing genetic damage. Conversely, high pharmacological doses of vitamin C can act as pro-oxidants impacts that are detrimental to cancerous cells. Moreover, vitamin C can modify the metabolomics and epigenetic profiles of cancer cells, leading to the elimination of cancer stem cells. Additionally, it can enhance immune system activity and suppress cancer transformation through various pathways.

The review included several studies that dealt with the effect of vitamin C on leukemia patients or associated it with treatment in vitro or in vivo to enhance patients' response to treatment and minimise side effects. It has been shown through previous and recent studies that the role of vitamin C is effective in treating leukemia patients. Additional researches are required to better understand the mechanistic and clinical dimensions of these effects.

Keywords: acute myeloid leukemia, antioxidant, pro-oxidant, vitamin c.

دور فيتامين سي كمضاد ومحفز للأكسدة في تقدم علاج سرطان الدم النقوي الحاد

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الخلاصة:

لفيتامين سي تأثيرات مضادة للأكسدة ومؤيدة للأكسدة على السرطان. فهو يعمل كمضاد للأكسدة عن طريق تقليل الجذور المؤكسدة ومنع التلف الجيني. في المقابل، يمكن للجرعات الدوائية العالية من فيتامين سي أن تمارس تأثيرات مؤيدة للأكسدة تضر بالخلايا السرطانية. علاوة على ذلك، يمكن لفيتامين سي تعديل السمات الأيضية والجينية للخلايا السرطانية، مما يؤدي إلى القضاء على الخلايا الجذعية السرطانية. بالإضافة إلى ذلك، يمكن أن يعزز نشاط الجهاز المناعي ويثبط تحول السرطان عبر مسارات مختلفة.

تضمنت الدراسة مراجعة العديد من الدراسات التي تناولت تأثير فيتامين سي على مرضى سرطان الدم أو المرتبطة بالعلاج في المختبر أو في الجسم الحي لتعزيز استجابة المريض للعلاج وتقليل الآثار الجانبية. وقد أظهرت دراسات سابقة وحديثة أن لفيتامين سي دوراً فعالاً في علاج مرضى سرطان الدم. هناك حاجة إلى أبحاث إضافية لفهم الأبعاد الميكانيكية والسريرية لهذه التأثيرات بشكل أفضل.

الكلمات المفتاحية: سرطان الدم النقوي الحاد، مضاد للأكسدة، مؤكسد، فيتامين سي.

1. Introduction:

L-ascorbic acid, often known as vitamin C (VC), is a micronutrient vital for overall human health. Ascorbic acid (AA) and dehydroascorbic acid (DHA) are its two main forms. As a potent antioxidant and enzymatic cofactor, VC is essential for many biological processes, such as metabolism of carnitine and catecholamines, dietary iron absorption, immune system support and collagen synthesis [1]. VC possesses antioxidant and pro-oxidant effects on many diseases, especially cancer. It works as an antioxidant by neutralizing reactive oxygen species and averting DNA damage. However, high pharmacological dosages of VC can have harmful pro-oxidant effects on cancer cells [2].

VC has the potential to change the metabolomics and epigenetic profiles of cancer cells, which results in the eradication of cancer stem cells. Moreover, it can boost the immune system and hinder cancer transformation through various pathways [3]. Oxidative stress, arises from an imbalance between pro-oxidant molecules and antioxidant defense systems, leading to DNA

damage and cellular alterations that drive cancer progression[4]. To reduce oxidative stress and cancer risk, antioxidant supplementation is recommended, especially with vitamin C [5].

Acute myeloid leukemia is characterized by the clonal proliferation of immature hematopoietic stem cells, or blasts, in the bone marrow. It a rare cancer that progresses rapidly. Uncontrolled expansion disrupts normal megakaryopoiesis and erythropoiesis, clinically appearing as relatively rapid bone marrow failure. The outcome is inadequate production erythrocyte and platelet [6,7]. As a result patients with AML many symptoms, such as, frequent infections, fatigue and easy bleeding or bruising, due to the insufficient production of normal blood cells. The aggressive nature of AML leads to a rapid decline in bone marrow function, making it a situation that threatens, life that, needs medical care, treatment and attention [8,9].

The review included many studies that confirm that vitamin C's pro-oxidative potential as well as its antioxidant qualities. The function of VC in cancer is discussed in the first section. The previous and recent studies highlight that VC has a therapeutic effect on Acute Myeloid Leukemia (AML) and is also advantageous for patients receiving radiation and chemotherapy when taken as a supplement. In addition, VC supplementation could be a potential combination therapy for the treatment of AML. However, the effectiveness of high-dose VC as an anticancer therapy is still debatable and requires further rigorous clinical trials for conclusive results.

2. Characteristics of Vitamin C

Organic compounds called vitamins are necessary for human metabolism. Notably, antioxidants included in vitamins A, E, and C protect against damage caused by free radicals.[10]. Ascorbic acid, is a water-soluble vitamin that is found naturally, in some foods, added to others, and sold as a nutritional supplement. Humans are dependent on food consumption because they are unable to manufacture\

VC, like most other vertebrates and are entirely reliant on food consumption. Consequently, it is a crucial nutritional component [11]. The recommended amount of VC per day ranges from 40 to 120 mg, based on factors such as physiological state, stress, and diseases. While Smokers, and those who are overweight may need up to 165 to 155 mg per day, according to recent research. [12,13].

3. Free radical

Numerous biological processes, including the intracellular bacteria elimination by phagocytic cells, especially macrophages and granulocytes, as well as some cellular signaling pathways, the mutagenic response, and redox regulation, depend on free radicals; many of these processes are necessary for life. However, excessive creation of free radicals leads to structural changes in cellular proteins that alter their activities, causing cellular dysfunction and interference with vital cellular functions. [14,15].

Increased reactive oxygen species (ROS) concentrations, a kind of free radical, can damage the integrity of various biomolecules, including proteins, lipids, and Deoxyribonucleic Acid. Specifically, ROS can damage proteins through altering amino acids at certain sites, peptide chain fragmentation, accumulation of cross-linked reaction products, alteration of electric charge, enzymatic inactivation, and proteolysis susceptibility[16]. The lipid membrane can be broken by ROS, which also increases the permeability and fluidity of the membrane. Finally, DNA damage includes deoxyribose oxidation, strand breaks, DNA-protein crosslinking, altering base, and nucleotide deletion [17].

4. Vitamin C as an antioxidant

The role of VC as an antioxidant and its effect in preventing chronic diseases associated with oxidative stress. VC works to reduce free radicals and reactive oxygen species (ROS) via two pathways. The first is by inhibiting nitrogen peroxides, blocking the formation of free radicals such as hydroxyl and proxy radicals. The second is by converting hydrogen peroxide to water through a reaction with ascorbate[18]. Additionally, vitamin C helps lower lipid peroxidation in cell membranes and increases the action of vitamin E (α -tocopherol) [19].

VC exhibits a defense against cardiovascular diseases such as hypertension and atherosclerosis, but its effects vary depending on its source and blood concentration levels[20]. Additionally, research has indicated that VC may be beneficial in preventing disorders of the nervous system such as Alzheimer's disease and depression, through its effects on nerve and brain function[21]. According to certain research, VC can affect cancerous cells, selectively killing them without affecting healthy cells, through the formation of hydrogen peroxide and free radicals. It has helped enhance the effectiveness of anti-tumor drugs like gemcitabine and adiotherapy, and reduced their side effects [22,23].

5. Vitamin C as an oxidant

VC influences several biological processes including hormone, collagen and carnitine synthesis, as well as, regulation of translation and gene transcription through a variety of mechanisms which including demethylation of DNA and histones, hydroxylation of transcription factors, tRNA, and ribosomal proteins, and tyrosine removal, Additionally, VC provides protection against free radicals, and reducing the frequency of undesired spontaneous differentiation of induced pluripotent stem cells [24] VC is particularly important for the activation of the ten-eleven translocation family (TETs) of dioxygenases, whose activity in DNA active demethylation appears to be downregulated in majority human malignancies [25]..

VC has a significant role in regulating human physiological processes, particularly cellular immune responses. It acts as an antioxidant molecule at physiological concentrations, preventing DNA damage and ultimately cancer development. Additionally, VC is involved in epigenetic regulation [12, 26]

6. Vitamin C as a Pro-oxidant

Linus Pauling and Ewan Cameron initially proposed the idea of using VC as a medicinal agent for cancer treatment in 1970 [27] High-dose VC is administered intravenously at a concentration greater than 10 g/infusion. Ascorbic acid serves a critical role in the antioxidant defense system as a nonenzymatic component. Its function is maintained by balanced dietary plasma concentrations.[28]. Even with oral supplementation, plasma ascorbate concentrations remain at micromolar levels. Ascorbate serves as an antioxidant against oxidative stress and modulates enzymes. At pharmacological doses, ascorbic acid acts as a pro-oxidant. However, at millimolar dosages and in certain clinical conditions, ascorbic acid's pro-oxidative activity can lead to DNA damage and ATP depletion [29].

7- The studies that showed the role of VC as a potential antioxidant and pro-oxidation of AMC

According to recent studies, high-dose Vitamin C may be useful in treating various tumors by targeting cancer cell growth mechanisms [30]. VC has shown specific cytotoxicity in human malignancies in preclinical studies. Its potential as an anticancer drug has been suggested. [22,31]. Intravenous high-dose VC may be used as a potential anti-cancer agent, according to pharmacological studies [32].

A study discovered that ascorbic acid can lead to apoptosis in AML cells. This is due to the build-up of H₂O₂ because of VC oxidizing reduced glutathione intracellularly [33]. A study found that VC is cytotoxic against leukemia in vitro. It can kill most types of acute and chronic myeloid leukemia cells without harming normal cells.[34].

One study showed that VC treatment significantly stops development of leukemia in primary human leukemia patient-derived xenografts by imitates the restoration ten-eleven translocation 2 (TET₂) in Tet2-deficient mouse HSPCS. Various enzymes that are involved in DNA demethylation, including TET₂, require vitamin C to remain active [35]. The concept crucial for AML patients due to demethylase enzymes play an important role in the growth and progression of AML [36].

Research aimed to find a biomarker for VC treatment in acute myelogenous leukemia by analyzing gene expression patterns and their relation to patient survival. The study discovered that low expression of SLC2A3, a gene involved in VC uptake, was linked to lower overall survival in AML patients and reduced effectiveness of VC in AML and lymphoma cells [37].

8. VC in combination treatments

VC at high doses has been the focus of numerous research projects recently as a pro-oxidative agent in combination with chemotherapy and radiotherapy. The pre-clinical and clinical literature on the use of high-dose VC in combination therapy is reviewed in this review. Other combination treatments have been studied. According to one previous study, the survival rate of mice with P388 leukemia increased upon receiving treatment with VC and vitamin B₁₂ [38].

Another investigation found that combining decitabine and low-level VC had a positive effect on leukemic cells and clinical results in elderly AML patients. In vitro experiments showed synergy between the two agents in inhibiting DNA methyltransferase and affecting gene expression. Patients who received the combination treatment had higher remission rates and longer survival than those who received decitabine alone. Low-dose VC improved treatment response and survival in elderly AML patients [39].

Additionally, administering Ascorbic Acid orally to patients with myeloid cancers undergoing DNMTi treatment can enhance the 5hmC/5mC ratio and normalize plasma vitamin C levels. This indicates that supplementing with VC may improve the biological impacts of DNMTis [40].

Also, the study demonstrated that in AML patients with reduced TET2 activity, VC administration could improve clinical remission and restore methylation patterns [41]. Another study examined the effect of VC on leukemia expressing the IDH1R132H mutation. The study observed that VC treatment decreased cell proliferation and increased the gene expression related to leukocyte differentiation. VC also caused changes in Histone alterations and DNA methylation, leading to the remodeling of transcription factor-binding sites and driving differentiation in the leukemia model [42].

Darwiche *et al.*'s study proposes that VC supplementation has potential using a combination of therapies to treat chronic lymphocytic leukemia. This is due to its ability to induce apoptotic cell death, enhance targeted therapies, and synergistically potentiate drug cytotoxicity in CLL B-cells targeting mitochondrial metabolism [43].

VC concentration inversely correlates with peripheral blast counts in AML patients, indicating vitamin C's potential for suppressing leukemia progression. [44].

A recent study found that ascorbic acid significantly enhances the response of leukemic cells to the histone methyl transferase inhibitor DZNep in AML by promoting apoptosis and differentiation. The enzyme D-3-phosphoglycerate dehydrogenase (PHGDH) and the anti-apoptotic gene BCL2 are downregulated by vitamin C, and the vitamin C-enhanced anti-leukemic action of DZNep is reversed by overexpression of PHGDH.[45].

9. Conclusions

Through a review of prior research, it was determined that VC possesses both pro-oxidant and antioxidant effects in relation to AML. It has a variety of effects, acting as a pro-oxidative cytotoxic agent, immunological modulator, anti-cancer epigenetic regulator, and cancer-specific agent, among other effects. Furthermore, it has a larger physiological role beyond its antioxidant properties, including involvement in hormone and carnitine synthesis, facilitation of iron absorption, and important roles in epigenetic processes. High dosages of VC function as a pro-oxidant, instead of an antioxidant. Additionally, it can act synergistically with standard therapies and reduce harmful adverse effects of chemotherapy. Strong clinical data studies are absent despite the rationale and evidence to support it., highlighting the need for more extensive awareness and research on high-dose VC as a non-toxic cancer treatment.

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The Role of Oxidative Damage in the Progression of Coronary Heart Disease: A Study of Biochemical Markers in Patients from Kirkuk City

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Abstract:

Cardiovascular diseases, particularly coronary heart disease (CHD), are among the most prevalent conditions associated with atherosclerosis, primarily affecting adults aged 40 years and older. Oxidative damage plays a crucial role in various pathological conditions, with reactive oxygen species (ROS) contributing significantly to CHD progression. This study included 60 CHD patients (30 males and 30 females) aged 40–69 years, all of whom were non-smokers, non-alcoholics, and free from comorbidities such as hypertension or diabetes. A control group of 30 healthy individuals (15 males and 15 females) aged 40–67 years was also included. Blood samples were collected from hospitals in Kirkuk for biochemical analysis.

The findings revealed a significant decrease ($P \leq 0.05$) in the levels of high-density lipoprotein (HDL), superoxide dismutase (SOD), vitamin E (VE), and total antioxidant capacity (TAC) in CHD patients compared to the control group. The measured values were 29.128 ± 2.001 mg/dL (HDL), 40.038 ± 7.245 U/mL (SOD), 26.136 ± 6.376 μ mol/L (VE), 216.19 ± 34.85 μ mol/L (TAC), whereas the control group exhibited 56.267 ± 8.890 mg/dL, 72.191 ± 2.729 U/mL, 39.251 ± 7.839 μ mol/L, and 422.66 ± 69.11 mg/mL, respectively. Additionally, gender showed a statistically significant effect ($P \leq 0.05$) on CHD risk, with

differences observed between male and female patients compared to the healthy group. Age also played a critical role, with significant variations ($P \leq 0.05$) across age groups, indicating an increased CHD risk with advancing age. Furthermore, body mass index (BMI) showed significant differences ($P \leq 0.05$) when comparing all biochemical variables between patients and the control group. The study highlights the significant role of oxidative stress and antioxidant depletion in CHD patients, with age, gender, and BMI contributing to disease progression. These findings emphasize the importance of early intervention strategies to manage oxidative stress and reduce CHD risk. The results revealed a significant decrease in antioxidants (SOD, vitamin E, TAC) and HDL levels in CAD patients compared to controls, confirming oxidative stress's role in disease progression. Age, gender, and BMI were linked to higher risk. These findings highlight the importance of early detection and risk factor management in heart disease prevention.

Keywords: Coronary heart disease, Oxidative stress, Antioxidants, Age-related risk, Biomarkers.

دور الضرر التأكسدي في تطور مرض الشريان التاجي: دراسة للعلامات البيوكيميائية لدى المرضى في مدينة كركوك

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الخلاصة:

تُعد أمراض القلب والأوعية الدموية، ولا سيما مرض الشريان التاجي (CHD)، من بين أكثر الحالات شيوعاً المرتبطة بتصلب الشرايين، حيث تصيب بشكل أساسي البالغين الذين تبلغ أعمارهم ٤٠ عاماً فأكثر. يلعب الضرر التأكسدي دوراً حاسماً في العديد من الحالات المرضية، حيث تساهم أنواع الأكسجين التفاعلية (ROS) بشكل كبير في تطور مرض الشريان التاجي. شملت هذه الدراسة ٦٠ مريضاً مصاباً بمرض الشريان التاجي (٣٠ ذكراً و٣٠ أنثى) تتراوح أعمارهم بين ٤٠ و٦٩ عاماً، وكان جميعهم من غير المدخنين، وغير المدمنين على الكحول، وخاليين من الأمراض المصاحبة مثل ارتفاع ضغط الدم أو السكري. كما تم تضمين مجموعة ضابطة مكونة من ٣٠ فرداً سليماً (١٥ ذكراً و١٥ أنثى) تتراوح أعمارهم بين ٤٠ و٦٧ عاماً. تم جمع عينات الدم من المستشفيات في كركوك لإجراء التحليل البيوكيميائي. كشفت النتائج عن انخفاض معنوي ($P \leq 0.05$) في مستويات البروتين الدهني عالي الكثافة (HDL)، وإنزيم ديسموتاز الفائق (SOD)، وفيتامين E (VE)، والسعة الكلية لمضادات الأكسدة (TAC) لدى مرضى الشريان التاجي مقارنة بالمجموعة الضابطة. حيث بلغت القيم المقاسة $29,128 \pm 2,001$ ملغم/ديسيلتر (HDL)، و $40,038 \pm 7,245$ وحدة/مل (SOD)، و $26,136 \pm 6,376$ ميكرومول/لتر (VE)، و $216,19 \pm 34,85$ ميكرومول/لتر (TAC)، بينما أظهرت المجموعة الضابطة قيماً بلغت $8,890 \pm 56,267$ ملغم/ديسيلتر، و $2,729 \pm 72,191$ وحدة/مل، و $7,839 \pm 39,251$

ميكرومول/لتر، و $422,66 \pm 69,11$ ملغم/مل، على التوالي. علاوة على ذلك، أظهر الجنس تأثيرًا إحصائيًا معنويًا ($P \leq 0.05$) على خطر الإصابة بمرض الشريان التاجي، مع وجود اختلافات بين المرضى الذكور والإناث مقارنة بالمجموعة السليمة. كما لعب العمر دورًا حاسمًا، حيث لوحظت فروق معنوية ($P \leq 0.05$) بين الفئات العمرية المختلفة، مما يشير إلى زيادة خطر الإصابة بمرض الشريان التاجي مع التقدم في العمر. بالإضافة إلى ذلك، أظهر مؤشر كتلة الجسم (BMI) فروقًا معنوية ($P \leq 0.05$) عند مقارنة جميع المتغيرات البيوكيميائية بين المرضى والمجموعة الضابطة. تسلط هذه الدراسة الضوء على الدور المهم للإجهاد التأكسدي واستنزاف مضادات الأكسدة لدى مرضى الشريان التاجي، مع مساهمة العمر والجنس ومؤشر كتلة الجسم في تطور المرض. تؤكد هذه النتائج على أهمية استراتيجيات التدخل المبكر لإدارة الإجهاد التأكسدي وتقليل خطر الإصابة بمرض الشريان التاجي. كشفت النتائج عن انخفاض كبير في مستويات مضادات الأكسدة (SOD ، وفيتامين E ، TAC) ومستويات HDL لدى مرضى CAD مقارنة بالضوابط، مما يؤكد دور الإجهاد التأكسدي في تطور المرض. تم ربط العمر والجنس ومؤشر كتلة الجسم بزيادة المخاطر. تسلط هذه النتائج الضوء على أهمية الكشف المبكر وإدارة عوامل الخطر في الوقاية من أمراض القلب.

الكلمات المفتاحية: مرض الشريان التاجي، الإجهاد التأكسدي، مضادات الأكسدة، الخطر المرتبط بالعمر، المؤشرات البيوكيميائية.

1. Introduction:

Cardiovascular diseases are among the most common diseases affecting the general population, especially adults aged 40 years and above. According to 2012 statistics, approximately 17.1 million deaths worldwide were attributed to cardiovascular diseases, particularly coronary artery disease [1]. Atherosclerosis is the pathological process underlying these diseases, severely affecting all blood vessels, including cerebral, coronary, and peripheral arteries [2]. Atherosclerosis is considered an inflammatory and chronic condition driven by innate immunity [3, 4], resulting from the deposition of lipids in the inner walls of blood vessels. These deposits can grow to the point of obstructing blood flow, leading to the formation of plaques or clots, which can cause strokes or aneurysms in the abdominal blood vessels, gangrene in one of the extremities, cardiac arrhythmias, myocardial infarction, pulmonary embolism, and finally, sudden death [5]. In this context, oxidative stress plays a significant role. Oxidative stress refers to a state of imbalance resulting from the excessive production of reactive oxygen species (ROS) and other related oxidizing agents. This imbalance leads to tissue injury and cellular dysfunction, particularly under pathological conditions. The overproduction of ROS contributes to the development of cardiovascular diseases and atherosclerosis [6]. ROS, which are continuously formed as byproducts of cellular metabolism, are the most abundant free radicals in biological systems. At low concentrations, ROS are essential for various physiological processes, such as enhancing the immune system, eliminating inflammation, and regulating cell differentiation. However, when their production exceeds reasonable limits, ROS can attack biological molecules such as

proteins, fatty acids, and nucleic acids, leading to oxidative damage, impairment of cellular functions, and interference with normal metabolic processes [7, 8]. Lipids, including animal fats and plant oils, are important contributors to coronary heart disease. The accumulation of lipids in adipose tissues leads to dyslipidemia, which is a risk factor for atherosclerosis in coronary arteries [9, 10]. This condition contributes to the progressive development of cardiovascular diseases, as lipids accumulate in the walls of blood vessels and form plaques, further exacerbating oxidative stress. To combat oxidative damage, antioxidants are crucial. These substances help protect cells from oxidative damage that could lead to conditions such as Alzheimer's disease, cancer, and cardiovascular diseases. The antioxidant system includes both enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants, such as catalase (CAT), superoxide dismutase (SOD), and peroxidase, work to neutralize harmful free radicals. Non-enzymatic antioxidants include vitamins, minerals, and natural compounds like polyphenols, flavonoids, and carotenoids, which are found in fruits and vegetables and help reduce the risk of cardiovascular diseases [11-14]. Among the key antioxidants, Superoxide Dismutase (SOD) plays a vital role in the body by converting superoxide radicals into less harmful molecules, like hydrogen peroxide and oxygen. Vitamin E (VE) also acts as a potent antioxidant, maintaining the integrity of cell membranes and protecting against ROS-induced damage, particularly in blood vessels. Its concentration is closely linked to blood lipid levels, and it is naturally present in eight forms, with alpha-tocopherol being the most effective [1, 12, 15]. Several risk factors contribute to the development of coronary heart disease (CHD), including increased oxidation of molecules, inhibition of nitric oxide (a vasodilator), and direct cytotoxic effects on endothelial cells. These factors include obesity, age, gender, high blood pressure, dyslipidemia, diabetes, and smoking [1, 16, 17]. CHD is more common in men under the age of 55, with a higher prevalence than in women. Most heart attacks occur after the age of 65, and the incidence increases with age. Women before menopause rarely suffer from heart attacks, likely due to the protective effects of hormones that diminish after menopause [18,19]. Age groups under 45 for men and over 55 for women are considered risk factors for cardiovascular diseases [20]. Smoking is another significant risk factor for CHD, as nicotine increases the stickiness of blood platelets, making thrombus formation more likely. Reducing smoking rates can lead to improvements in blood pressure, HDL levels, and lipid profiles [19, 21].

2. Materials and methods:

2.1. Sample Collection

The study included 60 patient samples (30 males, 30 females) aged between 40 and 69 years, who were randomly selected. They were non-smokers, non-alcoholics, and free from other diseases such as hypertension or diabetes. Control samples included 30 individuals (15 males, 15 females) aged between 40 and 67 years who were free from coronary artery disease but may have had other non-cardiac health conditions. Blood samples were collected from both patients and healthy individuals in hospitals in Kirkuk. In the assessment of coronary artery disease (CAD), clinicians employ a multifaceted approach that includes the evaluation of medical history, physical examinations, and diagnostic tests. Initially, they investigate symptoms such as angina or dyspnea and analyze risk factors including hypertension or diabetes mellitus. Standard diagnostic procedures encompass an electrocardiogram (ECG) to identify arrhythmias, a stress test to observe cardiac function under exertion, and laboratory analyses to evaluate lipid levels and myocardial enzymes. Furthermore, imaging modalities like coronary angiography yield comprehensive visual representations of arterial obstructions. Prompt identification of CAD is crucial for effective management and the mitigation of potential complications.

2.2. Initial Processing of Blood Samples

Blood was drawn from the antecubital vein using a sterile syringe (5 ml) and transferred into EDTA-containing gel tubes to prevent clotting. The samples were left at 37°C for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. The serum was separated and placed in clean, tightly sealed plastic tubes, which were stored at -86°C in a deep freezer until biochemical analysis was performed.

2.3. Estimation of Total Antioxidant Capacity (TAC) in Blood Serum

The FRAP (Ferric Reducing Antioxidant Power) method [22] was used to estimate TAC. This method is simple and effective for assessing antioxidant power. FRAP values were obtained by comparing the absorbance changes in the reaction mixture with those containing known concentrations of Fe (II) ions.

2.4. Estimation of Superoxide Dismutase (SOD) Level in Blood Serum

The level of SOD (Superoxide Dismutase) activity was estimated using the diagnostic kit (SOD Assay Kit-WST) prepared by the Japanese company Dojindo. The Nitro Blue Tetrazolium (NBT) method was used to determine SOD activity in the presence of the highly soluble salt Water-Soluble Tetrazolium (WST-1), which forms a water-soluble formazan dye upon reaction with the superoxide anion. SOD activity (U/mL) was measured colorimetrically at a wavelength of $\lambda = 450$ nm.

2.5. Estimation of Vitamin E Level in Blood Serum

The concentration of VE was estimated using the diagnostic kit (Human Vitamin E ELISA Kit) prepared by the company MyBioSource. The ELISA technique was used at ($\lambda=450$ nm).

2.6. Estimating high-density lipoprotein (HDL) concentration in serum

The enzymatic method was used to estimate high-density lipoprotein (HDL) concentration in serum using the Biolabo diagnostic kit [23] supplied by the French company Biolabo (HDL Kit). The principle of operation of this method is based on the quantitative sedimentation of chylomicrons and lipoproteins after adding phosphotungstic acid / MgCl_2), so that HDL remains in the filtrate alone after the centrifugation process.

2.7. Statistical analysis

The statistical analysis was performed using the Minitab 15 software, applying a significance level of ($p \leq 0.05$). I utilized my expertise in statistical planning to ensure the robustness of the analysis. Initially, I verified the assumptions of normality and homogeneity in the data. Once these conditions were met, the one-way analysis of variance (ANOVA) method was employed to compare the differences between the groups. This approach allowed for a thorough evaluation of whether any statistically significant differences existed among the groups. In addition to the ANOVA, I calculated key descriptive statistics, including the mean, standard deviation (SD), standard error, and significance level, to provide a comprehensive overview of the data distribution and the precision of the results.

3. Results and Discussion:

The concentrations of various parameters, including TAC, SOD, VE, and HDL, were measured in samples from patients with coronary artery disease and a control group. The study was organized into four main sections: The first section examined the total number of participants, consisting of 60 patients and 30 controls. The second section focused on gender distribution, with an equal percentage (50%) for each gender. The third section categorized participants into age groups: 40-49, 50-59, and 60-69 years and above. It was observed that 66.66% of both genders were aged 50 years or older. The mean age and standard deviation (SD) for the patients were calculated, with males having an average age of 56.0 ± 7.280 years, females having an average age of 58.430 ± 8.380 years, and controls of 52.233 ± 8.877 . The fourth classification was based on body mass Index (BMI, kg/m^2): BMI₁ (ages 18.5–24.9) had normal weight, BMI₂ (ages 25–29.9) were overweight, and BMI₃ (ages 30–39.9) were classified as obese. The (mean $\pm$ SD) BMI values for these groups were (23.707 ± 1.555) for BMI₁, (26.962 ± 1.296) for BMI₂, and (33.110 ± 2.37) for BMI₃. Additionally, the (mean $\pm$

SD) BMI for the healthy control group was (22.437 ± 1.874). This study compared oxidative stress markers (TAC, SOD, VE) and HDL levels between CAD patients and controls, while analyzing demographic patterns. Key findings revealed: 1) Significant antioxidant depletion in patients, 2) Majority (66.7%) of participants were ≥ 50 years old, 3) Patients showed higher average age and BMI than controls, and 4) Obesity prevalence (BMI3 group) was notable among patients. These results highlight age and metabolic factors as critical contributors to CAD progression.

3.1. The measurement of the average concentrations of TAC, SOD, VE, and HDL in the serum of patients with coronary atherosclerosis and the healthy control group.

The results, as shown in Table 1 and Figure 1, showed a significant decrease at the probability level ($P \leq 0.05$) in the levels of TAC, SOD, VE, HDL and concentrations in patients with coronary atherosclerosis. The results were (216.19 ± 34.85), (40.038 ± 7.245), (26.136 ± 6.376), and (29.128 ± 2.001), respectively, compared to the healthy control group, which had results (422.66 ± 69.11), (72.191 ± 2.729), (39.251 ± 7.839), and (56.267 ± 8.890), respectively.

Table 1: The concentrations level of TAC, SOD, VE, and HDL in the serum of patients with coronary atherosclerosis and the healthy control group.

Parameters (Units)	Patients (n=60) mean $\pm$ SD	Controls (n=30) mean $\pm$ SD
TAC ($\mu\text{mol/L}$)	216.19 ± 34.85	422.66 ± 69.11
SOD (U/mL)	40.038 ± 7.245	72.191 ± 2.729
VE ($\mu\text{mol/L}$)	26.136 ± 6.376	39.251 ± 7.839
HDL (mg/dL)	29.128 ± 2.001	56.267 ± 8.890

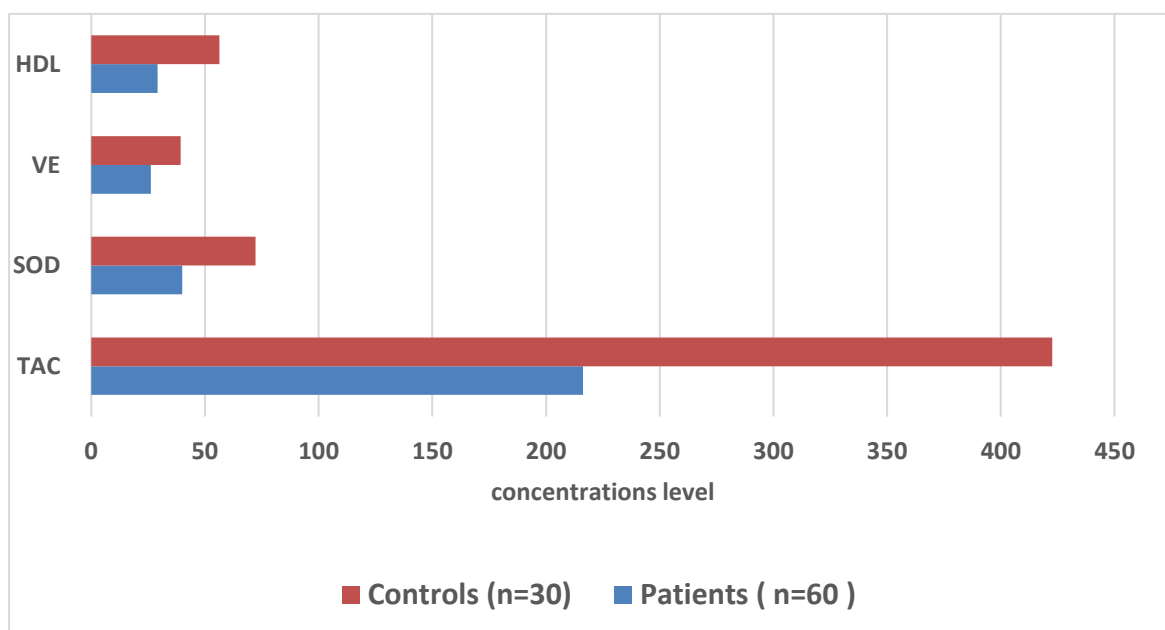


Figure 1: The concentrations level of TAC, SOD, VE, and HDL in the serum of patients with coronary atherosclerosis and the healthy control group.

The current results were consistent with the study of Karajibani, Mansour, et al [12] and Sözmen, Bülent, et al. [24] and Chikkanna Da, et al. [25] and Buczyński, A., et al. [26] where they observed a decrease in the levels of antioxidant concentrations (SOD TAC) and HDL. This is an indication that oxidative stress leads to the weakening and suppression of the antioxidant system in the body due to the increase in lipid peroxides (Malonaldehyde) which leads to a decrease in the activity of antioxidants SOD. This decrease can be explained by the effect of the increase in reactive oxygen species (ROS) derived from free radicals which inhibits the activity of SOD, because the lower the concentration of ROS, the greater the activity of SOD [24]. In light of these results, the most rational and scientifically supported recommendation for the general public is to use a balanced diet with an emphasis on fruits and vegetables rich in antioxidants and whole grains. The results of clinical trials in the secondary prevention of vitamin E have been encouraging, and studies have confirmed that vitamin E supplementation has an objective role in individuals with cardiovascular disease [27]. Contrary to the current study, there were no significant differences in plasma SOD in CHD patients compared to healthy controls. However, there was a consistent and significant difference in TAC and vitamin E levels in CHD patients compared to healthy controls [28]. Although (a) VE Tocopherol is an effective antioxidant and plays a protective role against CAD, its levels were observed to be higher in patients with CHD compared to healthy controls, which contradicts the antioxidant hypothesis. These results were inconsistent with the current study. One explanation for these conflicting results is that patients with CHD have been interested in managing their health through lifestyle modifications and taking therapeutic medications, in addition to their tendency to intentionally eat a healthy diet and take vitamins, especially VE. CHD patients may benefit from taking VE vitamin supplements on a regular basis after being diagnosed with coronary artery disease [29]. High levels of TC in the blood are considered among the most important risk factors for coronary artery disease (CHD), while high HDL cholesterol provides protection against these disorders [24]. Regarding beneficial treatments, statins remain the first-line treatment for dyslipidemia and have proven effective in lowering blood cholesterol levels and reducing the incidence of cardiovascular disease [30]. A significant decrease in antioxidant levels and an increase in blood lipids may be the cause of the suppression of the antioxidant system, lack of adherence to a healthy diet,

eating foods containing fats and red meat, not eating fruits and foods rich in vitamins, the patient's neglect of treatments, and a significant decrease in HDL levels.

3.2. Measurement of the Average Concentrations of TAC, SOD, VE HDL, and by Sex in the Serum of Male and Female Patients with Coronary Atherosclerosis and a Healthy Control Group.

The results showed a significant decrease ($P \leq 0.05$) in the levels of concentrations of (TAC, SOD, VE, and HDL) as shown in Table 2 and Figure 2, in male patients with coronary atherosclerosis. The results were $219.62 \pm 20.66 \mu\text{mol/L}$, $39.828 \pm 8.297 \text{ U/mL}$, $26.000 \pm 5.218 \mu\text{mol/L}$, $30.1421.387 \text{ mg/dL}$, respectively compared with the healthy group ($408.63 \pm 72.48 \mu\text{mol/L}$, $72.449 \pm 2.812 \text{ U/mL}$, $38.845 \pm 6.758 \mu\text{mol/L}$, $57.406 \pm 9.642 \text{ mg/dL}$), respectively. In female patients with coronary atherosclerosis, the concentrations of TAC, SOD, VE, and HDL significantly decreased, with values of $212.76 \pm 44.95 \text{ U/mL}$, $40.248 \pm 6.152 \text{ mg/dL}$, $26.271 \pm 4.527 \mu\text{mol/L}$, and $28.115 \pm 2.025 \mu\text{mol/L}$, , and, respectively, compared to the healthy control group. The results were statistically significant with $P > 0.05$, and the concentrations in the healthy group were $436.68 \pm 79.55 \mu\text{mol/L}$, $71.933 \pm 2.715 \text{ U/mL}$, $39.658 \pm 6.995 \mu\text{mol/L}$, and $55.127 \pm 8.244 \text{ mg/dL}$.

Table 2: The concentrations of TAC, SOD, VE HDL, and by Sex in the serum of male and female patients with coronary atherosclerosis and a healthy control group

Sex	Parameters(Units)	Patients (n=60) mean $\pm$ SD	Controls (n=30) mean $\pm$ SD
Male	TAC ($\mu\text{mol/L}$)	219.62 ± 20.66	408.63 ± 72.48
	SOD (U/mL)	39.828 ± 8.297	72.449 ± 2.812
	VE ($\mu\text{mol/L}$)	26.000 ± 5.218	38.845 ± 6.758
	HDL (mg/dL)	30.142 ± 1.387	57.406 ± 9.642
Female	TAC ($\mu\text{mol/L}$)	212.76 ± 44.95	436.68 ± 79.55
	SOD (U/mL)	40.248 ± 6.152	71.933 ± 2.715
	VE ($\mu\text{mol/L}$)	26.271 ± 4.527	39.658 ± 6.995
	HDL (mg/dL)	28.115 ± 2.025	55.127 ± 8.244

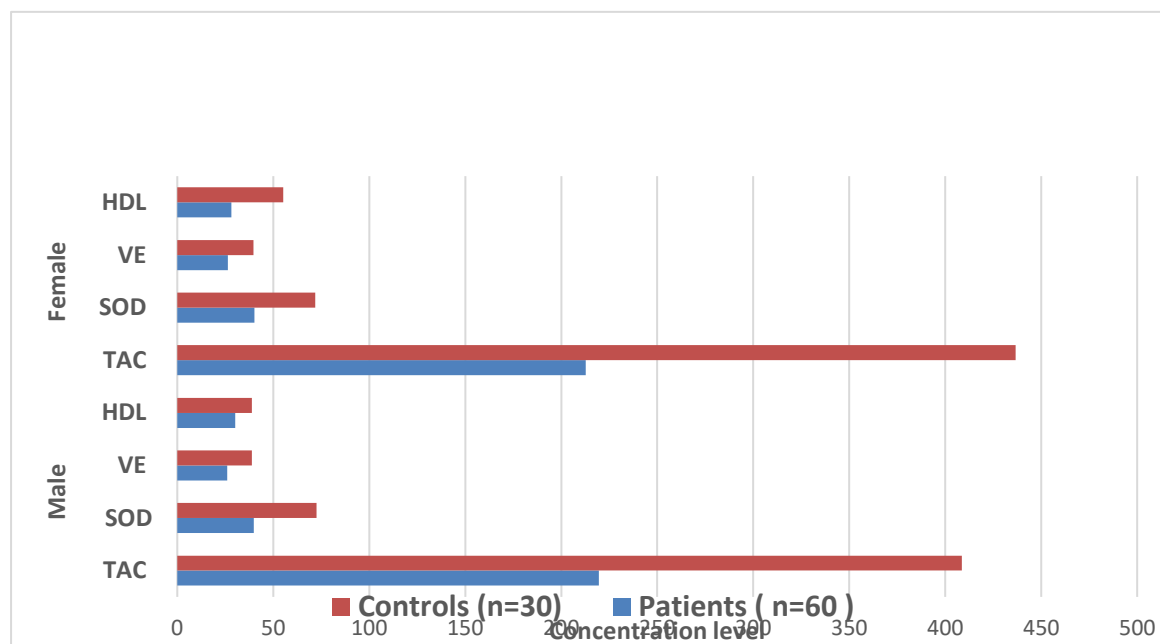


Figure 2: The concentrations level of TAC, SOD, VE HDL, and by Sex in the serum of male and female patients with coronary atherosclerosis and a healthy control group

When comparing only two groups of patients (males and females) with coronary atherosclerosis, no statistically significant differences were found at the probability level ($P > 0.05$). It was noted that TAC and HDL concentrations were higher in males than in females. SOD concentrations were higher in females than in males, and no significant changes in VE concentrations were observed between males and females. The researchers suggested that low levels of total antioxidants (TAC and HDL) may be involved in the development of atherosclerosis. There is some evidence that antioxidant therapy may be beneficial for the prevention of coronary heart disease (Souiden, Yosra, et al. [31]. Furthermore, a significant decrease in both SOD activity levels and total antioxidant TAC was found in patients with coronary atherosclerosis. This finding suggests that decreased levels of both SOD and TAC likely contributed to the development of coronary artery disease (CHD) stenosis in Tunisian men [32]. Free radicals play an important role in the body through various mechanisms, including androgenesis systems and essential metabolic processes such as oxidative phosphorylation and mitochondrial respiration, as well as environmental factors such as light, heat, and metals. The mechanisms underlying various coronary microvascular

dysfunctions are due to intima-reactive vasculature, low levels of endogenous estrogen, coagulation disorders, and abnormal inflammatory reactions [12]. A significant decrease in VE levels negatively impacts CHD patients due to a weakened system. They have antioxidant properties, as VE has an important role as an antioxidant due to its ability to stop the spread of oxidants produced during cellular metabolism and toxic chemicals. It also prevents the spread of lipid peroxidation in phospholipid membranes and protects membranes and arteries from ROS [33]. Studies have confirmed the increased consumption of fruits, green vegetables, vegetable oils (such as soybeans, corn, and sunflowers), whole grains, wheat germ, nuts, seeds, and leafy vegetables as antioxidants, as they affect the clinical outcomes of CHD patients. It has a broad ability to protect biological membranes and nucleic acids when exposed to attack by free radicals in cells [8, 34]. Researchers confirmed that CHD symptoms are delayed in women (Sutton-Tyrrell Kim et al.) [35] by 10 to 15 years compared to men due to the protective effect of ovarian hormones. The prevalence of carotid atherosclerosis is evident among premenopausal women and women 5-8 years after menopause, as they found a prevalence of plaques (deposits) of 25% among premenopausal women and 54% among postmenopausal women. Estrogen also has a regulating effect on a number of factors such as metabolism, lipids, inflammatory markers, and the coagulation system. It also enhances the direct vasodilatory effect through A and B receptors in the vascular wall. Furthermore, there are changes in lipid profiles around the time of menopause. The risk of CHD increases in women during the transition to menopause, and changes occur in the distribution of body fat, causing obesity as a result of the frequent accumulation of visceral fat after menopause, due to the imbalance. Hormonal [36], and the decrease in HDL levels after menopause is also affected by the lack of estrogen, which plays an important role in reducing harmful cholesterol levels and raising levels of HDL cholesterol [37]. Many studies have confirmed that raising HDL levels is a logical therapeutic goal and that any decrease in its concentration leads to an increase in cardiovascular diseases [5]. The hormone estrogen reduces HDL levels by reducing the activity of the triglyceride lipase enzyme in the liver, which breaks down HDL. Global studies have shown that the risk of atherosclerosis for both sexes is inversely related to HDL levels in the blood; the higher the HDL levels, the lower the cardiovascular risk factors. [38]. A low HDL concentration of less than 40 mg/dL in males and 50 mg/dL in females. It has been suggested that the difference in phenotypes between males and females may be determined by the action of sex hormones. In women, estrogen is the dominant sex hormone, and any decrease in its production before or after menopause alters lipid metabolism more favorably toward atherosclerosis by lowering HDL levels. It also has a

direct effect on endothelial cell function and vascular. We believe that men under the age of 45 are at greater risk of developing CHD than women of the same age. Since 66.66% of the study samples of both sexes were aged (50) years and above, where the average age of females (58.430 ± 8.380) was greater than the age of males (56.000 ± 7.280), this can be explained by the fact that women at this age lack the role of the estrogen hormone, which has an important role in the pre-menopausal stage in many physiological and metabolic processes of the body, and as a result. It was suggested to take nutritional supplements of vitamin E, as it is one of A powerful, essential fat-soluble antioxidant. It acts as an antioxidant, exclusively capturing free oxygen radicals, protecting lipids in cell membranes from oxidation, and preventing bad cholesterol from adhering to blood vessel walls

۳.۳. Measurement of TAC, SOD, VE, and HDL Concentrations by Age Group in the Serum of Patients with Coronary Atherosclerosis and Healthy Controls

Table 3 and Figure 3 showed a significant decrease at the probability level in TAC, SOD, VE, and HDL concentrations ($P \leq 0.05$) for patients with coronary atherosclerosis with age according to the three age groups compared to the age groups of the healthy group. We note that the levels of total antioxidants TAC decreased with age in patients with CHD, especially in the age group (50-59) years, which is greater than other age groups compared to healthy people, and its level increased in the age group (60-69), and this may be explained by the patient's prevention of the risk of contracting the disease, taking medications, and following a healthy diet rich in vitamins, while the levels of (SOD, HDL, VE) decreased gradually with age according to the age groups (40-49), (50-59), (60-69) years, respectively. Antioxidant concentrations (VE, SOD, TAC) gradually decreased with age. This indicates that oxidative stress in the cardiovascular system occurs when antioxidant capacity is insufficient to reduce ROS and other free radicals. Early observational studies focused on dietary antioxidants, such as beta-carotene-tocopherol and ascorbic acid, and demonstrated an inverse association between the intake of these antioxidants and cardiovascular disease [39]. SOD is an antioxidant enzyme that plays a vital role in the body, converting superoxide radicals to H_2O_2 and oxygen, eliminating free oxygen radicals that contribute to endothelial dysfunction in patients with coronary artery disease (CHD). SOD is also active in other tissues. After the conversion of superoxide radicals to H_2O_2 , the final product, catalase then converts H_2O_2 to water and oxygen [12,7]. Vitamin E acts as an antioxidant. It has anti-inflammatory activity and regulates the expression of proteins involved in the absorption, transport, and degradation of tocopherols, as well as the absorption, storage, and export of lipids such as cholesterol. It

plays an important role in combating atherosclerosis due to the oxidation of lipids accumulated within the blood vessels. Macrophages resulting from foam cells contribute to the formation of atheroma in arterial walls. Oxidative stress plays a crucial role in this process, leading to endothelial injury and plaque formation [40]. Studies have shown that with aging, lipid peroxidation is directly linked to systemic oxidative stress. Two important components of oxidative stress with aging are the decline in the abundance of dietary antioxidants and the accumulation of oxidation products of biological structures [41]. The present study focuses on key oxidative stress parameters, including TAC, SOD, VE, and HDL, across different age groups. Previous research suggests that oxidative stress markers tend to increase with age due to metabolic changes and reduced antioxidant defenses [42]. The findings of Towfighi et al. support this, as their study analyzed oxidative stress parameters in middle-aged CHD patients (35–54 years) by gender [20]. Since 66.66% of the current study samples for both sexes were 50 years old and above, the average age of females (58.43 ± 8.38) was higher than that of males (56.00 ± 0.28). This aligns with studies indicating that oxidative stress-related changes become more pronounced with aging [43], particularly in postmenopausal women, where decreased estrogen levels may contribute to alterations in antioxidant status.[44]. We believe that the main risk factors for cardiovascular disease are the increase in males under 45 years and females over 55 years. Engaging in physical exercise, maintaining a healthy diet, managing body weight, and taking appropriate medications can help mitigate oxidative stress and reduce potential risk factors associated with aging.

Table 3: The concentration level of TAC, SOD, VE, and HDL Concentrations by Age Group in the Serum of Patients with Coronary Atherosclerosis and Healthy Controls

Parameters (Units)	Ages (years)	Patients (n=60) mean $\pm$ SD	Controls (n=30) mean $\pm$ SD
TAC ($\mu\text{mol/L}$)	(40 - 49) years	219.10 $\pm$ 31.98	448.35 $\pm$ 71.99
	(50 - 59) years	181.10 $\pm$ 28.31	381.57 $\pm$ 21.99
	(60 - 69) years	203.48 $\pm$ 38.64	369.28 $\pm$ 16.94
SOD (U/mL)	(40 - 49) years	44.763 $\pm$ 7.822	71.510 $\pm$ 3.201
	(50 - 59) years	34.765 $\pm$ 9.186	70.782 $\pm$ 3.246
	(60 - 69) years	31.033 $\pm$ 8.261	69.616 $\pm$ 3.243

VE (umol/L)	(40 - 49) years	28.067 ± 5.644	40.946 ± 4.773
	(50 - 59) years	27.979 ± 6.920	39.067 ± 5.059
	(60 - 69) years	25.312 ± 4.872	37.820 ± 4.028
HDL (mg/dL)	(40 - 49) years	29.052 ± 1.265	56.673 ± 5.950
	(50 - 59) years	28.330 ± 2.888	54.613 ± 4.453
	(60 - 69) years	27.735 ± 1.593	53.750 ± 5.609

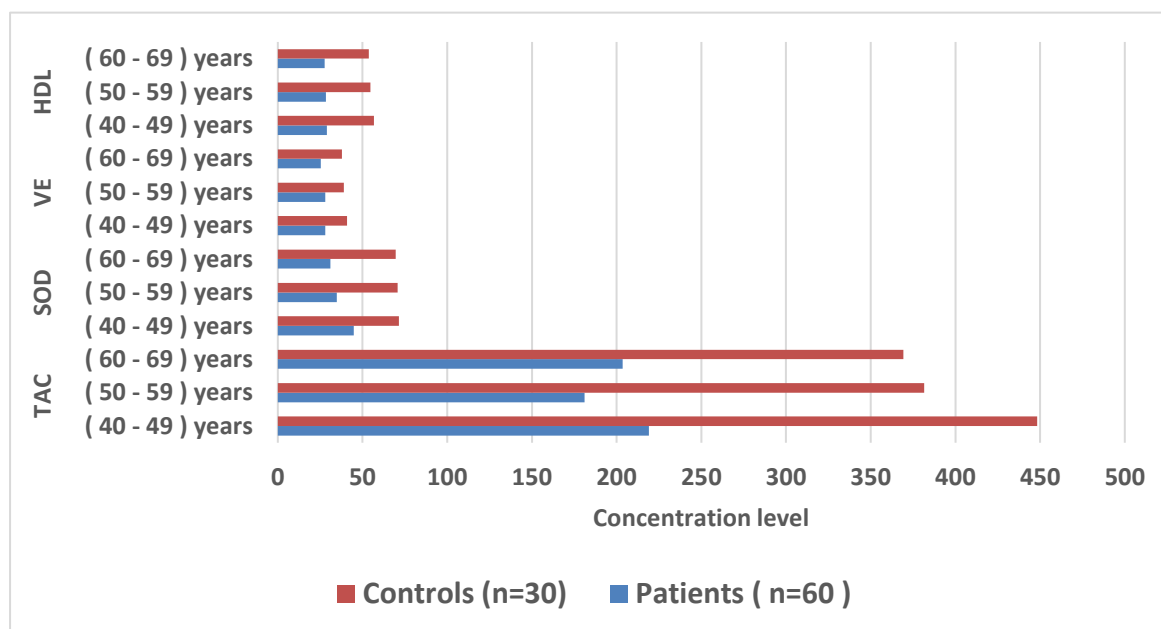


Figure 3: The concentration level of TAC, SOD, VE, and HDL Concentrations by Age Group in the Serum of Patients with Coronary Atherosclerosis and Healthy Controls

3.4. Measurement of TAC, SOD, VE , and HDL Concentrations in Blood Serum of Patients with Coronary Atherosclerosis and a Healthy Group Based on Body Mass Index (BMI).

The results, as shown in Table 4 below, showed that the body mass index (BMI) plays an important and influential role on antioxidants in coronary heart disease, as the concentrations of (TAC SOD, VE, HDL,) decreased significantly at the probability level ($P>0.05$) with an increase in BMI (BMI_1 , BMI_2) in patients with coronary atherosclerosis when compared with the BMI control group of healthy people. The three BMI groups did not show any significant differences between the groups of patients with coronary atherosclerosis.

Increased BMI is considered a marker of obesity and is one of the risk factors for the development of oxidative stress associated with several diseases, including atherosclerosis, in terms of the increase in oxidation products and the decrease in antioxidant levels [45]. The results of the current study showed an inverse relationship between antioxidants (V.E, SODAC) and blood lipids in coronary heart disease, with increased BMI (obesity) in patients compared to healthy people. This study was consistent with HirakawaYoichiro et al. [9], and Kushi, Lawrence H. et al. with a study [46] explained that obesity is a risk factor for CHD and

confirmed that patients who have one or more risk factors, such as high blood pressure, diabetes, and smoking, are more likely to die than other patients who do not have risk factors. They also noted that physical activity, the use of medications, or attention to health awareness reduce risk factors, and that body mass indexes and high waist-to-hip ratios were associated with the risk of cardiovascular disease [47]. The World Health Organization confirmed that 23% of the global burden of coronary artery disease (CHD) is attributable to overweight/obesity, with increased CHD mortality rates [9]. Postmenopausal women in otherwise healthy obese women have been associated with decreased antioxidant levels due to progressive loss of estrogen, increased body weight, and central obesity. 35.9% of postmenopausal women were overweight, 25% were obese, and 60.9% were morbidly obese. TAC levels were significantly lower in obese women than in overweight women, and serum log TAC levels were inversely associated with BMI [48]. Body weight increases in women during the first years of menopause due to altered body fat distribution, with central obesity occurring more frequently and visceral fat becoming more prevalent after menopause, with escalating comorbid risk factors and components of metabolic syndrome in women compared to men [49]. This study aligns with the large-scale international study by Hirakawa, Yoichiro et al. [9], which investigated the impact of body mass index (BMI) on the relationship between lipid variables and the risk of coronary artery disease. Their findings demonstrated that the risk of disease... Regardless of BMI, the association between lipid variables and coronary artery disease remained significant, highlighting the complex interplay between metabolic factors and cardiovascular health. The World Health Organization confirmed in 2002 that more than half of global obesity cases are attributed to dyslipidemia [9]. In recent years, numerous studies have demonstrated a strong association between obesity and oxidative stress and inflammation due to high levels of reactive oxygen species (ROS) and impaired antioxidant defenses, with increased inflammation resulting from excess fat accumulation leading to overweight and obesity [50].

Table 4: Measurement of TAC, SOD, VE , HDL, and Concentrations in Blood Serum of Patients with Coronary Atherosclerosis and a Healthy Group Based on Body Mass Index (BMI).

Parameters (Units)	BMI (Kg / m ²) (n=90) mean ± SD			
	Controls (n=30) mean ± SD	Patients (n=60) mean ± SD		
	BMI Controls (n=30) 22.437±1.874	BMI ₁ (n=20) 23.707±1.555	BMI ₂ (n=20) 26.962±1.296	BMI ₃ (n=20) 33.110±2.726
TAC (μmol/L)	422.66 ± 69.11	223.127 ± 32.15	217.95 ± 37.00	212.21 ± 29.17

SOD (U/mL)	72.191 ± 2.729	44.566 ± 8.166	37.025 ± 10.617	32.503 ± 9.171
VE (umol/L)	39.251 ± 7.839	27.351 ± 5.854	25.068 ± 6.067	24.967 ± 7.121
HDL (mg/dL)	52.267 ± 8.890	29.831 ± 2.046	29.740 ± 1.684	28.762 ± 3.189

4. CONCLUSION

This study highlights the significant impact of oxidative stress and antioxidant depletion in the development and progression of coronary heart disease (CHD). The findings revealed a notable decrease in the levels of HDL, vitamin E (VE), superoxide dismutase (SOD), and total antioxidant capacity (TAC) in CHD patients compared to the control group. These reductions suggest that oxidative damage plays a crucial role in CHD pathophysiology, contributing to disease severity and progression. Additionally, the study demonstrated that gender significantly affects CHD risk, with both male and female patients exhibiting distinct biochemical differences compared to healthy individuals. Age was also found to be a key determinant, as older individuals showed more pronounced biochemical changes, further supporting the notion that advancing age is a major risk factor for CHD. Moreover, body mass index (BMI) was strongly associated with oxidative stress markers, indicating that individuals with higher BMI are more susceptible to CHD due to increased oxidative burden and metabolic alterations. The observed reductions in antioxidant levels and enzymatic defense mechanisms suggest an imbalance between oxidative stress and the body's ability to neutralize reactive oxygen species (ROS). This imbalance may accelerate the progression of atherosclerosis and other CHD-related complications. Therefore, these findings emphasize the necessity of adopting early intervention strategies, such as lifestyle modifications, dietary supplementation with antioxidants, and weight management, to mitigate oxidative damage and reduce CHD risk. In conclusion, this study provides valuable insights into the biochemical alterations associated with CHD, reinforcing the importance of oxidative stress as a central factor in disease progression. Future research should focus on exploring potential therapeutic strategies aimed at enhancing antioxidant defenses and minimizing oxidative damage to improve cardiovascular health and patient outcomes. In summary, this research offers significant insights into the biochemical changes linked to coronary heart disease (CHD), emphasizing the critical role of oxidative stress in the advancement of the condition. Nonetheless, the conclusion currently lacks clarity regarding the practical implications of these findings for the diagnosis or prevention of coronary artery disease. A more definitive statement encapsulating the clinical implications and importance of the results is necessary to

effectively communicate the relevance of this study. Subsequent investigations should focus on specific explorations of diagnostic indicators and preventive strategies informed by oxidative stress assessments. Additionally, it is crucial to recognize the limitations inherent in this study, including sample size, demographic diversity, or external variables that may influence the applicability of the results. Addressing these limitations in future research is essential for enhancing the credibility and relevance of the outcomes.

5. References

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Study of the Synthesis of Materials with Specific Properties Water and Water Systems

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Abstract:

The features of water and aqueous systems used in the directed synthesis of materials with specified properties are shown. Water simultaneously plays the role of the initial medium, "building material" and "tool" in this synthesis. The conditions of directed crystallization and melting of ice are discussed as the basis for controlling the physicochemical transformations of components in aqueous systems. New methods of studying and using aqueous systems are considered - the "floating reactor" method and its based on the use of the Pettier and Seebeck effects.

Keywords: aqueous systems, crystallization, ice, melting, water.

دراسة تركيب المواد ذات الخواص المحددة للمياه والأنظمة المائية

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الخلاصة:

يستعرض هذا البحث خصائص الماء والأنظمة المائية المستخدمة في التركيب الموجه للمواد ذات الخصائص المحددة. حيث يلعب الماء دور الوسيط الأولي "مادة البناء" و"الأداة" في هذا التركيب. تتم مناقشة ظروف التبلور الموجه وذوبان

الجليد كأساس للتحكم في التحويلات الفيزيائية والكيميائية للمكونات في الأنظمة المائية. يقدم البحث طرقاً جديدة لدراسة الأنظمة المائية واستخدامها – طريقة «المفاعل العائم» وطريقة تعتمد على استخدام تأثيرات بيلتييه وزبيبيك

الكلمات المفتاحية: الماء، الأنظمة المائية، الجليد، التبلور، الذوبان.

1. Introduction:

Water scarcity is a global problem [1, 2] and industrial water is a major contributor to water consumption [3,4]. Recycling industrial water is an important means of saving water, but the repeated use of circulating cooling water causes the scaling ions in the water to form scale on the heat exchanger and pipe walls in the form of precipitation, resulting in a decrease in the heat exchange efficiency of the equipment [5, 6]. Water treatment agents are currently added to alleviate the scaling of circulating water treatment systems, as they offer certain advantages including simple operation, excellent effectiveness, and low cost [7]. The most commonly used agents include inorganic salts, organic phosphine compounds, natural macromolecules, and synthetic macromolecules. Among these scale inhibitors, inorganic salts and organic phosphine compounds have limited applications because they can easily promote the growth of bacteria and algae in the system, which can cause water eutrophication and environmental damage. Natural polymers are cheap and easy to obtain, but they have disadvantages; for example, they are needed in large amounts and are unstable at high temperatures in the process of application [8]. Thus, phosphorus-free synthetic macromolecule scale inhibitors have received extensive attention. These copolymers mostly contain carboxyl, hydroxyl, sulfonic acid, amino, and other anti-scaling functional groups, and a combination of different groups that can effectively prevent the weak hydrophilic groups from forming insoluble calcium gels. This process can also help to dissolve calcium gels, to effectively prevent scaling. IA is an unsaturated dicarboxylic acid, which contains the carbon double bond functional groups required for free-radical polymerization, as well as carboxylic acid groups on both sides of the double bond. The latter endows IA copolymers with excellent negative dispersion properties and the ability to complex with other ions [9]. Moreover, IA molecules do not contain phosphorus or nitrogen. As a result, they do not cause eutrophication of water bodies through enrichment, and are environmentally friendly. While polymers can be obtained from IA monomers through homopolymerization, and this particular polymer offers certain scale inhibition effects for calcium carbonate, its dispersion performance still needs to be improved. Sulfonic acid groups are strong acid groups with strong hydrophilicity, and they can enhance the solubility of a copolymer, effectively preventing the weak acid group from generating insoluble calcium sol gels. Electrostatic water treatment technology has additional advantageous characteristics

including low energy consumption, near-zero pollution, and high efficiency, with excellent application prospects that urgently need to be researched and developed (. In this study, SMAS, which contains sulfonic acid groups, was copolymerized with IA to obtain a scale inhibitor (IA/SMAS) with good scale inhibition and dispersion properties. The resulting product was free of phosphorus and nitrogen and environmentally friendly. The IA/SMAS polymers were characterized by various means. Afterward, the synergistic scale inhibition performance of the physical water treatment technology (electrostatic field water treatment technology) and the agent was also studied. Finally, this study also evaluated the IA/SMAS copolymer alone and its synergistic scale inhibition mechanism with electrostatic fields(10).

Aim of study

Consider new methods for studying aquatic electronics - the "floating reactor" method and a method based on the use of the Peltier and Zeppeck effects.

2. Material and methods:

An aqueous solution of the substance under study of a given volume and concentration is cooled to a temperature that guarantees the transformation of all the water in the solution into ice. The resulting ingot is then maintained at an ambient temperature of 300K and, as the melt forms, it is taken in certain portions (fractions), recording the volume of the fraction and the time of its formation. After room temperature has been established in all fractions of the melt, the concentration of the substance dissolved in water is determined in each of them. Various analytical methods are used for this - refractometry, potentiometry, and others. Based on the data obtained, graphs are plotted of the dependence of the volume of the formed melt on the time the ingot is kept at room temperature, and the concentration of the dissolved substance in the fraction on the volume of the formed melt for initial aqueous solutions of different concentrations. The nature of the curves on these graphs allows one to determine the type of phase diagram of the corresponding binary aqueous system and the conditions for separating and isolating the components from the solution. The obtained data can also be used to establish the conditions for growing crystals of these components with specified properties [11].

Moreover, the fractional melting technique can be used to grow crystals of a substance dissolved in water directly in the process of melting an ice ingot. For this, the initial solution, for example, a salt whose phase diagram of the binary system ("salt-water") is eutectic, must

correspond to a certain composition from the hypereutectic region of this system. That is, its concentration in the initial solution must exceed the eutectic. Such a solution is cooled to a temperature below the eutectic, and then the formed ingot is subjected to fractional melting using the technique described above. In the process of melt fractions selection, the remaining unmelted part of the ingot is enriched with salt, since the melt fractions being selected at the beginning of melting have a salt concentration close to its eutectic value, i.e. lower than the initial, hypereutectic concentration. Under a certain regime of melt fractions formation and selection, salt crystals begin to grow inside the ingot that has not yet melted. In this case, the ingot itself simultaneously acts as a “container”, “thermostat” and the starting material for crystal growth. At the same time, the internal structure of the ice ingot determines the conditions for the growth of salt crystals, their shape, composition and structure. Moreover, the melting process itself is inextricably linked with the crystallization process inside it. In order to consciously.

To control the above-described crystallization and melting processes and use them to synthesize materials with specified properties, it is necessary, first of all, to control and purposefully set the temperature-time parameters of these processes: the rate of cooling, heating, as well as the corresponding changes in the nature of the distribution of the concentration of components in the system. For this purpose, we have developed a set of methods based on the use of the Peltier and Seebeck effects, as well as the “floating reactor” method - a method of physicochemical transformations under hydrostatic conditions.

Traditional cooling methods are most often based on the use of freon technology. In this case, a certain volume is cooled, into which the initial solution is placed. Heat is removed from it from the surfaces that limit this solution. Ice crystallization begins from these surfaces, and the substances dissolved in water are displaced by it into the center of the solution volume. This nature of ice crystallization in the solution reduces the efficiency of separating the components and complicates the control of the processes occurring in the system. We have developed a method for controlled cooling of solutions using cooling pipes introduced into a given area of the solution volume. The heat flow through the cooling pipe is set and controlled by semiconductor thermoelectric modules operating using the Pelte and See beck effects [12]

The fundamental difference of this method is that ice crystallization in the solution begins not from its surface, but from a certain given, volume, which is determined by the shape dimensions, and material of the cold line. The intensity of crystallization and the structure of

the ice depend on the magnitude of the working current flowing through the thermoelectric modules. By changing the direction of the current, it is possible to change the direction of the heat flow between the modules and the solution, thereby combining the directed processes of crystallization and melting in a single technological cycle. Moreover, since the thermoelectric modules operate on the Peltier and Seebeck effects, the cold line (heat pipe) connecting them to the solution (reaction volume) simultaneously performs the functions of a working element transferring heat and a temperature sensor. During phase transformations that are controlled by heat flows through the cold line, for example, during crystallization.

When ice (or other components of the solution) is liquefied and melted, heat is released or absorbed, and the corresponding temperature changes, recorded by the cold pipe as a change in the heat flow through it, affect the electrical parameters of the semiconductor thermoelectric modules and their power source. In our particular device, this influence is clearly recorded by a voltage jump of the corresponding sign on the power supply unit, which maintains a specified value of the operating current through the module. Moreover, the sensitivity of the device to phase transformations is so high that it allows them to be monitored in a solution volume of less than 1 ml, for example, by applying just one drop of the solution to the surface of the cold pipe. This allows us to study phase transformations very effectively by combining precise recording of the transformation temperature with visual control of the process.

The use of thermoelectric devices (modules) for the implementation and control of various physicochemical transformations allows us to significantly increase the efficiency of the processes of separation and isolation of components in solutions, targeted synthesis of materials, for example, growing crystals with specified properties [13].

For example, we significantly modified the above-described method of fractional melting of ice. Moreover, its modification was carried out in two directions. The first direction is the development of the "floating reactor" method or the method of physicochemical transformations under hydrostatic conditions. Often, to control the processes occurring in solutions, it is necessary to operationally control the nature of the distribution of components in the volume of the system. Moreover, sometimes it is more important to control the dynamics (kinetics) of change, for example, the concentration of components in time and space, than its absolute values.

The method of such control was developed by us primarily to study the nature of the distribution of components in the process of fractional melting of ice ingots formed in aqueous solutions with different initial concentrations of components. Its essence is that the vessel with the ice ingot, after its removal from the refrigeration chamber, is lowered into another vessel with liquid, distilled water in our case, so that the vessel with ice - the "floating reactor" - floats freely in this liquid, and the design of both vessels allows, purposefully change the mass of substances in the vessels. In the case of fractional melting of ice, the sampling of each melt fraction from the inner vessel (see above) is accompanied by fixing the liquid level in the outer vessel.

The ratio of the volumes of the fractions sampled and the corresponding changes in the liquid level in the outer vessel makes it possible to determine the density of each fraction and, consequently, the concentration of the dissolved substance in it, and to plot the dependence of the concentration of components on time during the melting process. Naturally, these studies require compliance with a number of conditions. It is necessary to control the temperature, pressure, and also pre-calibrate the system using solutions with a known concentration and density.

The scope of application of this method can be significantly expanded, for example, to study processes associated with the evaporation of components, adsorption, ion exchange, etc. It can also be effectively used in combination with the previously described method of physicochemical transformations controlled by thermoelectric modules. If a coolant is introduced into the volume of the solution in a "floating reactor", it is possible to directly control the concentration of components in the phases undergoing the corresponding transformations during the crystallization or melting process, and a change in the position of the reactor relative to the coolant, determined by a change in the liquid level in the external vessel, for directional crystallization or melting of the solution components.

3. Results:

Figure 1 shows a typical picture of the distribution of the concentration of a substance dissolved in water (in this case, NaCl) by melt fractions during fractional melting of ice ingots formed from NaCl solutions with different initial concentrations

For initial compositions from the pre-eutectic region (more diluted) of the "H₂O–NaCl" system, the first fractions of the melt are enriched in salt compared to the initial concentration. As the initial concentration increases (approaches the eutectic), the degree of enrichment decreases, and with an initial concentration equal to the eutectic, the distribution among the melt fractions becomes almost uniform. Exceeding the eutectic concentration in the initial solution leads to a fundamental change in the Chirac.

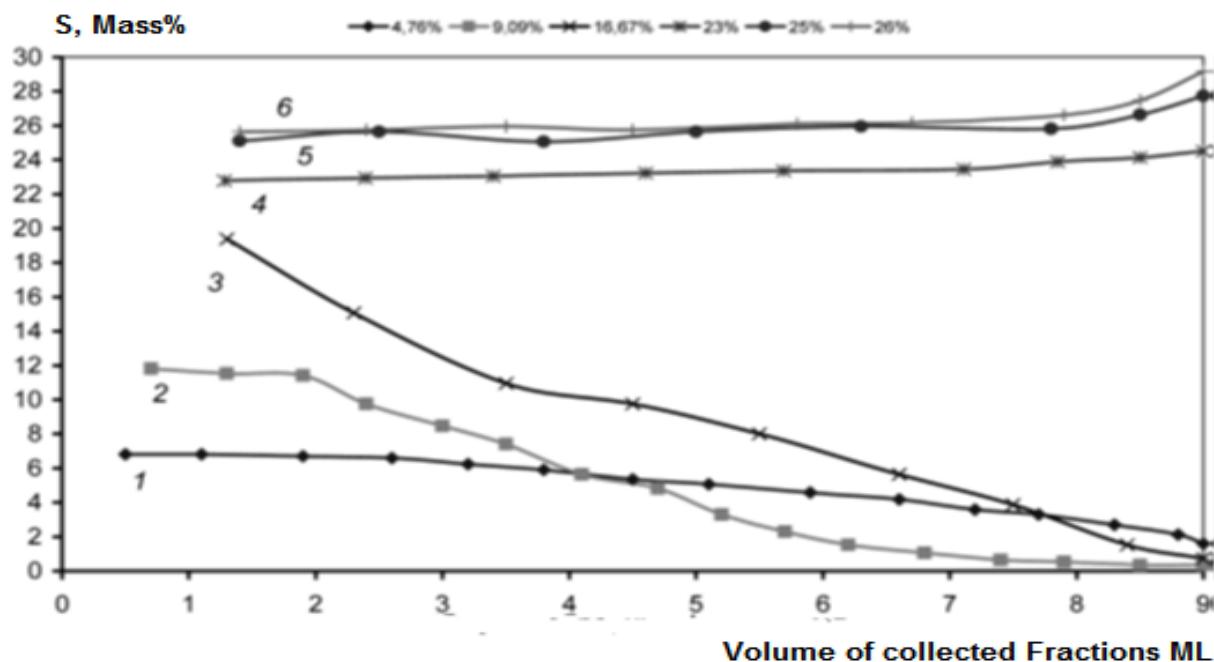


Fig. 1. Distribution of NaCl concentration by melt fractions for initial solutions of different concentrations

distribution of salt among melt fractions. The latter fractions are enriched with salt In [11, 13] it is shown that such a pattern of distribution of the concentration of a substance dissolved in water among melt fractions during fractional melting is characteristic of all substances we have studied, whose phase diagrams are eutectic. We have established that these include not only water-salt systems, but also a number of systems water–amino acid”, “water–glucose”, etc.

The study of aqueous solutions in the H₂O–NaCl system by the method of fractional melting in a floating reactor did not reveal any significant differences in the distribution of salt concentration among melt fractions compared to its classical version (i.e., in a stationary

vessel). At the same time, the determination of the density of melt fractions during fractional melting in a floating reactor by the method described above (see Fig. 2) showed a rather complex pattern of changes in the density of the solution in successive fractions. It is interesting that the “direct” determination of the density of fractions by weighing them on an analytical balance repeated this pattern with the only difference that the absolute values of density in this case turned out to be somewhat different from those for the floating reactor method.

Fig. 3 shows similar results for the "H₂O-fructose" system

The following can be noted regarding the complex nature of the melt density distribution by fractions (Fig. 2, 3):

1-The periodic change in density is not exclusively the result of experimental errors and inaccuracies, although they are naturally not excluded. These changes reflect, first of all, the objective picture.

.2-The objectivity of the results is confirmed by a good correlation between the dependencies obtained in two different ways - the "floating reactor" method and "direct" weighing on a scale

3-The periodicity of the change in the density of successive melt fractions is determined by the periodic pattern of distribution of components in the ice ingot formed during its crystallization.

4- The density of the melt depends on the temperature. The results obtained in the floating reactor method reflect the maximum correspondence to the melting ingot temperature. The process of weighing the selected fractions introduces inevitable changes into this correspondence. Therefore, the results, repeating qualitatively, differ somewhat in the absolute values obtained

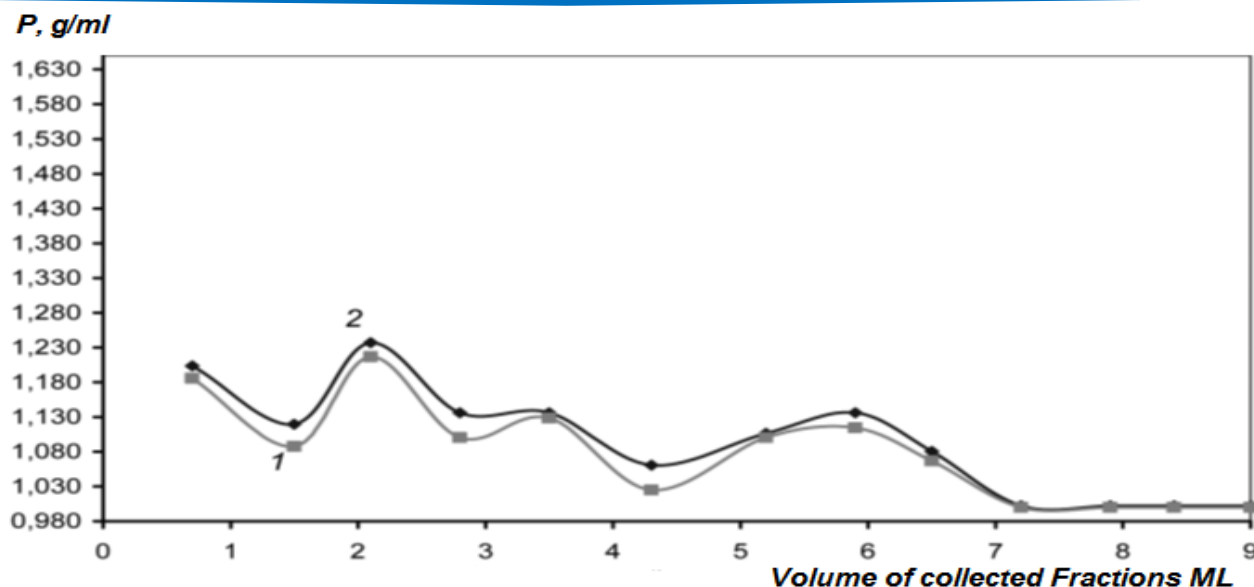


Fig. 2. Density of different melt fractions for the initial solution of 10% NaCl: 1 - pycnometric determination of density; 2 - determination of density by the “floating reactor” method.

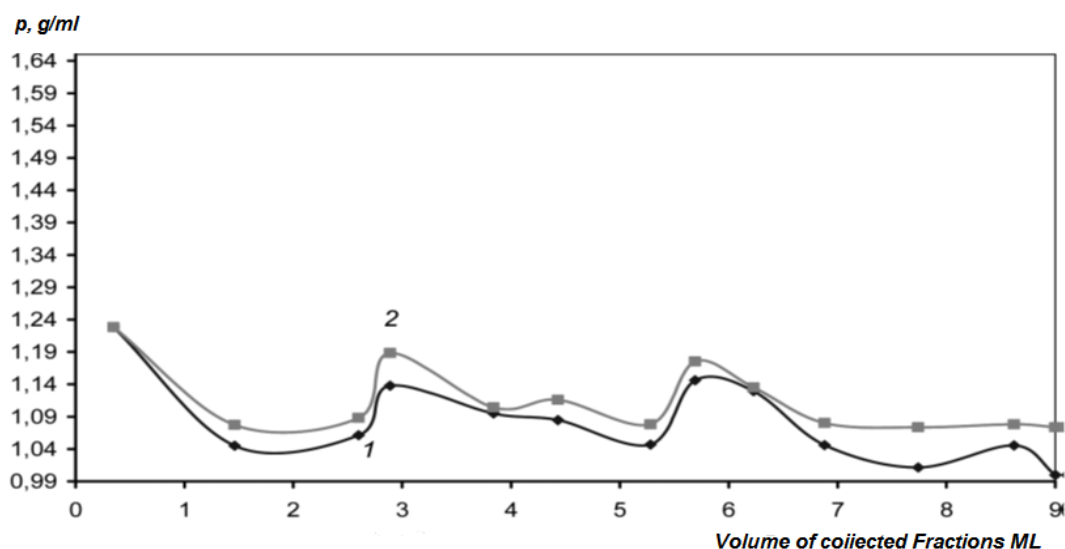


Fig. 3. Density of different melt fractions for the initial solution of 20% fructose: 1 — pycnometric determination of density; 2 — determination of density by the “floating reactor” method.

quantities. It can be said that the melt, separated from the ingot, loses the “connection” with it that exists in the melting process itself.

4. Discussion

Directed synthesis, i.e. synthesis of materials with specified properties, requires control of the processes of component distribution in a given volume - distribution of their mass, density, concentration. In aqueous media, the main "instrument" of control is the state of water which is primarily determined by temperature. A change in temperature changes the structure of water, the nature of the interaction of its molecules with each other and with particles of other substances. Very indicative from this point of view are transformations in systems described by eutectic phase diagrams, for example, "salt-water". In [14], it is shown quite convincingly, on the basis of many experimental results and theoretical reasoning, that the well-known "NaCl-H₂O" solutions turn into colloidal ones when the temperature drops to 0 °C and below — to the eutectic point. We believe that this phenomenon is not unique, it characterizes many eutectic systems, which we are convinced of, for example, by the results of our own experiments on the study of the composition, structure and properties of glycine and triglycine sulfate crystals - TGS, grown from aqueous solutions at different temperatures [15].

Comparing the complex of physicochemical properties of these crystals grown in the temperature range near room temperature and near 0 °C (and below), we came to the conclusion that significant and regular changes in these properties with a decrease in growth temperature are due, first of all, to a change in the state of water in those aqueous solutions in which this growth was carried out, as well as the state of water in the crystals themselves during and after their growth. According to modern concepts, even pure water is a micro heterogeneous system built from aggregates of different composition and structure. The sizes of these aggregates fluctuate from molecular to colloidal-dispersed . As the temperature decreases, the proportion and role of colloidal-dispersed states in water increases. The same tendency is characteristic of aqueous solutions of various substances. At room and higher temperatures, their structure fully meets the conditions for the existence of true solutions, that is, solutions in which the degree of dispersion is of an ionic-molecular nature, and individual ions or molecules of dissolved substances replace individual water molecules in the three-dimensional network of its hydrogen bonds. The solution is maximally homogeneous. When the temperature decreases, the water itself is the first to heterogenize, losing its hydrophilic properties - the tendency to form a single branched network of hydrogen bonds. Its hydrophobization occurs - the tendency for self-closure of hydrogen bonds, the formation of disunited aggregates, which, even being closely arranged with each other, do not create hydrogen bonds with each other. In such a structure, the expulsion of particles of dissolved substances from the nodes of the three-dimensional network into its voids prevails, which contributes to their unification with each other into their own

aggregates. This is the mechanism of formation of colloidal-dispersed phases, isolated from each other - water and substances previously dissolved in it. Such a process develops in the temperature range from room temperature to 0 °C and below, to the eutectic point. If we compare the crystallization conditions of the water itself and the substance dissolved in it, that is, in the pre- and post-eutectic regions of the system, we can distinguish two fundamentally different mechanisms of nucleation and growth of crystals , which are determined precisely by the difference in the structure of water and aqueous solutions in these regions. In the crystallization processes of interest to us, this difference must be associated first of all with the concepts of "saturation" and "super saturation" of the solution, which determine the conditions of nucleation, growth of crystals, their composition, structure, properties.

Graphically, in the language of phase diagrams these states are associated with the location of the "liquids" lines. On the eutectic phase diagram, they connect the eutectic point with the points corresponding to the melting temperatures of the pure components that make up the system. The liquids line itself determines the state of saturation of the solution with one of the components at its corresponding concentrations and temperatures. The state of the second component in this case — namely for eutectic type systems — changes regularly along these lines, in the regions adjacent to them, from far from saturation, at higher temperatures, to saturated near the eutectic. Here, the regions of saturation and super saturation of the system for both of its components overlap. If far from the eutectic, the states of saturation and super saturation of the system for each of its components are not connected with each other and are independent of each other, the "liquidus" lines go far from each other, then in the eutectic region they come closer together, intersecting (and ending) at the eutectic point, and the saturation regions for both components overlap, superimpose on each other, "interfere". In these regions, where the solution is simultaneously saturated with both components, the conditions for the nucleation and growth of crystals, their composition, structure, and properties change sharply and fundamentally. Let us consider, first of all, the behavior of the components in limited, local regions of the solution, both in the eutectic region of temperatures and compositions, and far from it. As was indicated above, at higher temperatures (far from the eutectic) the solution is "true", i.e. it consists of individual ions and molecules, and the formation of aggregates from them is hindered by thermal motion. When the system is saturated and supersaturated with one of the components, which is necessary for the nucleation and growth of its crystals, the solution remains far from being saturated with the second component, in relation to which it is still built as "true". In this case, the most important mechanism for the construction of the crystal is the

well-known mechanism of layer-by-layer growth , in which the building material is attached to the crystal "piece by piece", mainly in the form of individual molecules or ions, the role of aggregates pre-formed in the solution is, as a rule, insignificant. As a result, conditions are created for the growth of large, perfect, flat-faced single crystals. The capture of solution components by the growing crystal is reduced to a minimum (which, by the way, corresponds to the minimum mutual solubility of components in the solid state, characteristic of such systems).

In the region of states adjacent to the eutectic, i.e. when the temperature decreases, the solution begins to become saturated with both components at once the "liquids" (lines converge). At the same time, as already indicated above, the solution begins to disintegrate (micro heterogenize) into separate micro regions, built mainly from water molecules or molecules (ions) of the dissolved with each other. Each of them is "supersaturated" with one of the components. This component, striving to form its own structure, "phase" first in the form of a prototype" of the future crystal, pushes out the molecules of the second component from this region, since in the solid state they are practically insoluble in each other. In this case, the regions adjacent to the given one are enriched and supersaturated with this very second component, pushed out of it. Such a process, under appropriate conditions, begins to develop in an avalanche-like manner, like a chain reaction, encompassing new regions of the system. This is where the mutual connection of states (regions) supersaturated with both components at once manifests itself. The decomposition of the "true", homogeneous solution occurs, which can be called eutectic decomposition. Naturally, the conditions for the nucleation and preferential growth of crystals of one of the components in this region will be fundamentally different than at higher temperatures, discussed above. With some deviation of the solution composition from the eutectic, for example, into the hypereutectic region, it is possible to grow crystals of a substance dissolved in water, but they will not be built from individual molecules or ions, but from already formed aggregates (during eutectic decomposition of the solution) according to the so-called "block mechanism [16].

Their composition, structure and properties will differ significantly from those formed during layer-by-layer growth. As an example, we can cite the difference in the ferroelectric properties of TGS crystals grown by us purposefully according to both described mechanisms [15]. It should also be noted that the mechanism of "eutectic" decomposition of aqueous solutions is, in our opinion, essentially very close to spinodal decomposition [17], which is characteristic

mainly of solid solutions, but is also observed in liquid phases [18]. We pointed out the possibility of decomposition of eutectic solutions by the spinodal type earlier in [19]. In accordance with our ideas about the spinodal decomposition of eutectic solutions, it is possible to theoretically estimate the concentration and temperature at the eutectic point, that is, to predict it in the case where it is unknown in a given system from other sources, and, accordingly, to model in advance the conditions for crystal growth and synthesis of materials with specified properties. For such an assessment, it is necessary to compare the concentrations, densities of solutions and the densities of their individual components at different temperatures. Based on the results obtained, it is possible to estimate the volumes of the solution regions in which can simultaneously, that is, according to the described mechanism of eutectic, spinodal, decomposition from supersaturated states of the system both components for The experimental basis for such assessments for us is primarily the results of directed crystallization and subsequent fractional melting of ice in the studied aqueous systems using the methods described above that we have developed.

We consider it necessary to make some comments regarding the existing ideas about the states of "saturation" and "super saturation" of the system. As a rule, they are associated with the deviation of the temperature and concentration of components in the system from certain equilibrium values that are connected to each other, for example, by the "liquids".

or "solidus" lines on the phase diagrams of these systems. Understanding these concepts as purely thermodynamic, associated with the excess of free energy of the system over its equilibrium, i.e. minimum values, usually overlook the chemical nature of these concepts, their crystal chemical aspect, interpreted in a broad sense, as an aspect of structure. The approach of the state of the system to "saturated" and "supersaturated" fundamentally changes the nature of the interaction of the particles that make it up. The most important factor in this change is the tendency of particles to "aggregate", "condense". Such a change depends both on the internal structure of the system and on the parameters of the external impact on it. In this case, the key factors in this impact are the gradients (i.e. driving forces) of those parameters that affect the system, and the nature of their distribution and change in space and time.

5. Conclusions

Two new methods for studying phase transformations in water and aqueous systems have been developed the “floating reactor” method and a method based on the use of the Peltier and Seebeck effects. They allow one to control the distribution of components during directed crystallization and melting of ice and use these processes to synthesize materials with specified properties.

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Nanoparticles and Heterocyclic Compounds: Synthesis, Functionalization, and Applications: A Review

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Abstract:

Integrating nanoparticles (NPs) with heterocyclic compounds has emerged as a multidisciplinary innovation that spans materials science, medicinal chemistry, and nanotechnology. Nanoparticles, with their distinct physicochemical properties such as high surface area-to-volume ratio, surface reactivity, and functionalizability, provide an ideal platform for enhancing the performance and delivery of heterocyclic compounds. Heterocycles, which contain at least one atom other than carbon in their ring structure, are foundational to many pharmaceuticals, agrochemicals, and optoelectronic materials. When conjugated with or loaded onto nanoparticles, these compounds often exhibit improved solubility, stability, and biological availability. The nature of the interaction, whether through physical adsorption, covalent bonding, or coordination chemistry, significantly influences the functionality of the resulting hybrid nanomaterials. Applications range from targeted drug delivery and controlled release systems to heterogeneous catalysis and environmental sensing. Additionally, green synthesis approaches have enabled more sustainable production of these conjugates, using plant extracts and biological materials as reducing and stabilizing agents. However, challenges such as toxicity, environmental accumulation, and regulatory oversight remain barriers to large-scale implementation. This review explores the chemistry behind NP with heterocycle interactions,

the synthesis and characterization techniques employed, and the current and emerging applications in various scientific and industrial fields. By critically evaluating recent progress, this study provides insight into the potential of these hybrid systems and highlights future directions for their optimization and safe application.

Keywords: Catalysis, Drug Delivery, Nanoparticles, Heterocyclic Compounds, Green Synthesis.

الجسيمات النانوية والمركبات الحلقية غير المتجانسة: التحضير والوظيفة والتطبيقات: مراجعة

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الخلاصة:

ان أهمية الجسيمات النانوية (NPs) مع المركبات الحلقية غير المتجانسة يعتبر أحد الابتكارات المتعددة التخصصات، التي تشمل علوم المواد، والكيمياء الطبية، وتكنولوجيا النانو. حيث توفر الجسيمات النانوية بخصائصها الفيزيائية والكيميائية المتميزة مثل ارتفاع نسبة مساحة السطح إلى الحجم وتفاعلية السطح وقابلية تحديد الوظائف، منصة مثالية لتحسين أداء المركبات الحلقية غير المتجانسة وتوصيلها. تُعد المركبات الحلقية غير المتجانسة، التي تحتوي على ذرة واحدة على الأقل غير الكربون في بنيتها الحلقية، أساساً للعديد من المستحضرات الصيدلانية، والمواد الكيميائية الزراعية، والمواد البصرية الإلكترونية. عند اقترانها بالجسيمات النانوية أو تحميلها عليها، غالباً ما تُظهر هذه المركبات تحسناً في قابلية الذوبان، والاستقرار، والوفرة البايولوجية. تؤثر طبيعة التفاعل، سواءً من خلال الامتزاج الفيزيائي، أو الرابطة التساهمية، أو كيمياء التناسقية، بشكل كبير على وظائف المواد النانوية الهجينة الناتجة. تتراوح التطبيقات من توصيل الأدوية المُستهدَف وأنظمة الإطلاق المُتحكم بها، إلى التحفيز غير المتجانس والاستشعار البيئي. بالإضافة إلى ذلك، أتاحت مناهج التخليق الأخضر إنتاجاً أكثر استدامة لهذه المركبات، باستخدام مستخلصات نباتية ومواد بيولوجية كعوامل اختزال وتثبيت. ومع ذلك، لا تزال تحديات مثل السمية، والتراكم البيئي، والرقابة التنظيمية، تُعيق التنفيذ على نطاق واسع. يستكشف المقال الاستعراض التركيبي الكيميائي لجزيئات النيوكليوفوسفات مع تفاعلات الحلقات غير المتجانسة، وتقنيات التخليق والتوصيف المستخدمة، والتطبيقات الحالية والناشئة في مختلف المجالات العلمية والصناعية. من خلال التقييم النقدي للتقدم الأخير. ان هذه الدراسة تُقدم رؤية ثاقبة حول إمكانات هذه الأنظمة الهجينة، وتُسلط الضوء على التوجهات المستقبلية لتحسينها وتطبيقها الآمن.

الكلمات المفتاحية: التحفيز، توصيل الدواء، الجسيمات النانوية، المركبات الحلقية غير المتجانسة، التركيب الأخضر.

1. Introduction:

Nanotechnology, broadly defined as the science and engineering of materials at the nanometer scale (approximately 1–100 nm), represents one of the most revolutionary scientific advancements of the 21st century. These properties arise due to quantum effects, high surface-to-volume ratios, and enhanced surface reactivity. Consequently, nanotechnology is a discipline of fundamental scientific interest and a technological enabler across various industries. If one considers its rapid development, it becomes clear that it is poised to influence nearly every aspect of human life, from medicine and agriculture to electronics and environmental sustainability (1).

The foundational concept of nanotechnology lies in its ability to manipulate matter at the atomic or molecular level. Because of this precision, researchers and engineers can design materials with specific attributes tailored to distinct applications. For instance, nanoparticles, an essential nanotechnology component, can be synthesized with controllable size, shape, surface charge, and composition. If these parameters are finely tuned, nanoparticles can serve as drug carriers, catalysts, or environmental sensors, depending on the application's requirements (2).

Diagnostics, targeted medicine delivery, and regenerative therapies have advanced thanks to nanotechnology. Nanomedicine uses nanoparticles to deliver therapeutic chemicals to sick tissues, decreasing systemic toxicity and boosting treatment effectiveness. For example, liposomal nanoparticles and polymer-based nanocarriers are already used to treat cancers and inflammatory diseases in clinical settings and for biological activity, such as antibacterial (3, 4). Moreover, if nanoparticles are engineered with imaging agents, they can serve dual purposes as diagnostic tools and treatment monitors, a concept known as theragnostic. The capacity to combine therapeutic and diagnostic functionalities in a single nanosystem illustrates the profound impact of nanotechnology on healthcare. It is considered vital to agricultural and food systems as well as medical. Nano-fertilizers and insecticides reduce environmental pollution and increase nutrition delivery. Agrochemical waste might be reduced, and crop yields could be increased by replacing them with nanocarriers that release their payloads in a controlled manner. Developing nanosensors to monitor soil conditions, pathogens, and nutrient levels in real time may enable more accurate and sustainable farming. These improvements are crucial given the rising global food demand and climate change (5).

Nanotechnology develops high-strength, lightweight materials, energy storage devices, and innovative production methods in industry and energy. Carbon nanotubes and graphene-based nanomaterials improve the mechanical and electrical qualities of aeronautical, automotive, and civil engineering composites. Energy storage batteries and supercapacitors benefit from nanostructured electrodes. Scaling and integrating these materials might provide more durable, cost-effective, and environmentally friendly technology (6). It is also gaining popularity in environmental applications. Photocatalytic methods use nanomaterials like TiO₂ and ZnO to decompose pollutants and clean air and water. Water treatment uses magnetic nanoparticles to adsorb and remove heavy metals and dyes owing to their retrievability. If used appropriately, It may also reduce environmental damage and promote sustainability (7).

It enables creativity across fields and applications. Its ability to atomically design materials gives it unmatched control over functionality and performance. Nanotechnology's potential drives worldwide research and investment despite toxicity, environmental effects, and regulatory issues. If these challenges are overcome via multidisciplinary collaboration, nanotechnology will be a cornerstone of future scientific and technological growth (8, 9).

Recent nanoparticle synthesis research stresses precision and sustainability. Traditional chemical and physical nanoparticle production uses toxic chemicals and energy-intensive techniques. Alternative green synthesis employing plant extracts, microbes, or fungi is popular (10). These biological agents reduce and stabilize nanoparticles, enabling eco-friendly nanoparticle shape and size management. Such nanoparticles are biomedical and environmental-friendly owing to their low toxicity. Nanoparticles have transformed health, agriculture, and industry. Many antimicrobial coatings, medication transporters, and biosensors employ metallic nanoparticles like Ag, Cu, and Fe. High surface energy and flexible surface chemistry make them suitable for heterocyclic chemical functionalization, improving selectivity and reactivity. Catalysis, solar energy conversion, and environmental remediation use semiconductor and metal oxide nanoparticles. Whether top-down or bottom-up, modern nanomaterials help sustainable development, material efficiency, and precision-targeted medicine (11-14).

Hybrid systems in drug delivery, catalysis, biosensing, and optoelectronics are becoming more relevant. This work reviews synthetic methods, including conventional and green chemistry, to explain how nanoparticles might improve heterocycle stability, targeting efficiency, and reactivity. Nanoparticle-based conjugation with heterocyclic ligands can create

multifunctional systems with specific physicochemical features. This review will also discuss current advances. It also covers important issues, including toxicity, environmental sustainability, and repeatability, to help create next-generation nanomaterial for targeted and intelligent applications. This review aims to study the nanoparticle-of heterocyclic compound with functional groups, and evaluating the applications in different fields.

2. Literature Review

Vaishali D., Eco-friendly green synthesis methods for TiO₂-NPs that catalyze the formation of various heterocyclic compounds, including benzimidazole, indole, and pyrimidine derivatives, highlighting their potential for sustainable development in organic transformations (15).

Yaghoub P.; An eco-friendly, recyclable catalyst, molybdenum oxide nanoparticles (MoO₃ NPs), is used to synthesize physiologically active heterocycles under ecologically benign circumstances with high to outstanding yields (16).

Akanksha C.; An eco-friendly synthesis of diverse heterocycles using zirconia nanoparticles as catalysts in one-pot multicomponent reactions, emphasizing sustainable methodologies and the advantages of reduced reaction steps and enhanced efficiency in organic synthesis (17).

Digafie Z.; A green synthesis of pharmacologically important heterocycles using multicomponent reactions and magnetic nanocatalysts (MNCs), emphasizing eco-friendly methodologies, high atomic economy, and the use of biodegradable biopolymers in the preparation and recovery of these nanocatalysts (18).

Ritu M.; Figure 1 shows how green-supported nanomaterials were used to synthesize 1,4-DHP and its derivatives, polyhydroquinoline, benzopyranopyridines, and dihydropyridine (19).

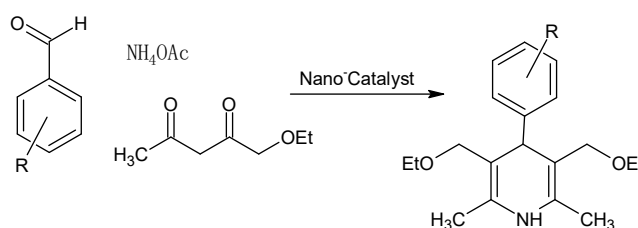


Figure 1: Green-supported nanomaterials in the synthesis.

Mahmoud N.; The green production of CuONPs utilizing *Tamarix gallica* leaf extract and their catalytic activity for N-arylation of nitrogen-containing heterocycles with aryl halides without ligands, as show in Figure 2 (20).

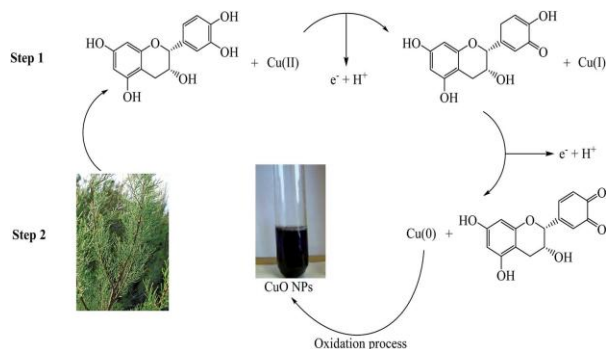


Figure 2: Role of CuO in the oxidation process.

Akber R.; 1H-1,2,3,4-tetrazoles synthesis by using CuO-NPs were immobilized on the surface of natural bentonite using *Thymus vulgaris* extract as a reducing and stabilizing agent (21).

Ayashkanta N.; The Fe₂O₃-cellulose-Mn nanocomposite was synthesized and used as an efficient catalyst to produce tetrazole derivatives rapidly with high yields and purity. Some compounds showed superior antifungal activity compared to standard drugs like Cefixime and Fluconazole, as shown in Figure 3 (22).



Figure 3: Role of Fe₂O₃ cellulose Mn nanocomposite to produce tetrazole derivatives.

Andreii S.; Novel tetrazole-chitosan derivatives were synthesized via metal-catalyzed cycloaddition in water. Their nanoparticles showed strong antibacterial activity, while the polymers exhibited superior catalytic performance in aldol reactions, making them effective catalysts and antimicrobial agents (23).

Dileep K.; A green method using nano-CuFe₂O₃ in water synthesized quinazolinone–tetrazole conjugates with confirmed C–S bond formation. Several compounds showed strong anticancer activity, especially 7o, outperforming etoposide against MD-AMB-231 cells by inducing G1 phase arrest (24).

Zahra G.; A green, efficient method for synthesizing indeno-pyrido-pyrimidines using a novel, reusable nanocatalyst in water offers mild conditions, high yields, and easy work-up. Various spectroscopic and analytical techniques confirmed product and catalyst structures (25).

Valiey E.; A novel mesoporous organosilica (APS-TDU-PMO) was developed and used to support Cu(II) nanoparticles, forming a recyclable catalyst (Cu@APS-TDU-PMO) for efficient, solvent-free tetrazole synthesis. The catalyst showed high activity, stability, and reusability over six cycles (26).

Hassan E.; A novel magnetic nanocatalyst (Cu(II)@Fe₃O₄@SiO₂-glucosamine) was synthesized and used in a green one-pot synthesis of 5-substituted tetrazoles, offering high yields, mild conditions, reusability, and eco-friendly, cost-effective advantages (27).

3. Classification of Nanoparticles:

In recent decades, Nanotechnology is revolutionizing health, electronics, environmental research, and energy. The research and use of nanoparticle materials with diameters of 1 to 100 nanometers drives this technological revolution. Nanoparticles vary from bulk materials in physical, chemical, and biological characteristics due to their high surface area-to-volume ratio and quantum effects. Consequently, the systematic classification of nanoparticles is essential for understanding their behaviors, tailoring their functionalities, and ensuring their safe integration into practical applications (28).

Given the vast array of nanoparticle types, various classification schemes have been developed, each based on distinct criteria. Nanoparticles may be categorized according to their

origin, including natural, incidental, and engineered nanoparticles. While natural nanoparticles are formed through geological or biological processes, incidental ones arise unintentionally as byproducts of industrial or combustion processes. Engineered nanoparticles, by contrast, are intentionally synthesized for specific uses. If one considers the increasing complexity of industrial applications, it becomes evident that a robust and comprehensive classification based on origin is critical to distinguish between intentional and unintended exposures, particularly in environmental and toxicological studies (29).

Nanoparticles may be carbon-based, metal-based, organic, inorganic, ceramic, or composite based on their chemical makeup. Each category is associated with particular attributes and functional capabilities. On the other hand, metal-based nanoparticles, such as gold or silver nanoparticles, are highly valued for their optical and antimicrobial properties. Because each type has distinct physicochemical characteristics, understanding this classification mode facilitates nanoparticle design for targeted applications. If materials scientists intend to harness specific properties, selecting nanoparticles based on composition becomes a foundational consideration (30).

Another widely accepted classification method pertains to morphology or structural configuration, encompassing spheres, rods, tubes, and dendritic structures. Nanoparticles' morphology is critical in determining their interaction with biological systems, catalytic performance, and surface functionalization potential. As a result, the morphological classification is especially relevant in biomedical and catalysis-related research. Moreover, suppose the size and shape of a nanoparticle influence its cellular uptake or biodistribution. In that case, controlling morphology during synthesis becomes desirable and necessary for biomedical efficacy and safety (31).

In addition, classification based on dimensionality namely, zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) nanoparticles offers further insights into their functional mechanisms. For instance, 0D nanoparticles such as quantum dots have unique optical properties that are size-dependent, whereas 2D materials like nanosheets possess high surface areas suitable for drug loading or sensing platforms. If research is to progress toward more sophisticated nanodevices, this dimensional framework provides a valuable perspective for designing multifunctional systems (32).

Finally, classification systems may also consider crystallinity, surface charge, solubility, and reactivity, depending on the use context. In pharmaceutical applications. Thus, such

secondary classifications often intersect with primary schemes to provide a multidimensional view of nanoparticle behavior (33).

In light of these diverse frameworks, it becomes clear that a universal classification system is challenging to establish, yet it remains indispensable for standardizing research and regulatory practices. If interdisciplinary communication is to be improved and innovation is fostered, then clear, consistent classification criteria must be agreed upon. Therefore, this review aims to critically analyze the existing classification schemes of nanoparticles, evaluate their relevance in different scientific domains, and propose integrative approaches that may better support future research and applications (33).

Chemistry and Significance of Heterocyclic Compounds

Heterocyclic compounds are a vast and structurally diverse class of organic molecules characterized by ring systems containing at least one heteroatom, commonly nitrogen, oxygen, or sulfur, alongside carbon atoms. Based on ring size and saturation, they are broadly classified into aliphatic and aromatic heterocycles, and further subdivided into five- and six-membered rings (Such as pyrrole, furan, thiophene, pyridine, and quinoline). Their structural versatility enables a wide range of physicochemical properties, including tunable polarity, hydrogen bonding potential, and electron-donating/withdrawing behavior. These features make heterocycles essential scaffolds in pharmaceutical chemistry, where they serve as core units in more than 75% of FDA-approved small-molecule drugs, including antibiotics, antivirals, anticancer agents, and CNS drugs. Their biological activity is often enhanced by the presence of heteroatoms, which facilitate receptor binding and metabolic stability. In nanotechnology, heterocyclic compounds are increasingly employed to functionalize nanoparticle surfaces, providing improved solubility, targeted delivery, and enhanced catalytic or sensing capabilities. For instance, nitrogen-containing heterocycles can coordinate with metal nanoparticles, forming stable metal–ligand complexes used in biomedical imaging or drug delivery. Their conjugation with nanomaterials enables the design of intelligent systems responsive to pH, temperature, or light. Thus, heterocycles represent a critical interface between molecular chemistry and nanoscale engineering, offering multifunctionality in both therapeutic and industrial applications (٣٤).

The Relationship Between Nanoparticles and Heterocyclic Compounds

Integrating nanoparticles (NPs) with heterocyclic compounds is a frontier in materials science, medicinal chemistry, and nanotechnology. Pharmaceuticals, agrochemicals, dyes, and electrical and photonic materials use heterocyclic compounds, which include rings with at least one element other than carbon (usually nitrogen, oxygen, or sulfur). Such chemicals may be conjugated or loaded onto nanoparticles to improve functionality, stability, and application breadth. The study of nanoparticle-heterocyclic system interactions, physical loading, or chemical reactions has grown fast as an interdisciplinary area (35).

Nanoparticles' drug delivery, catalysis, and sensing benefits explain why they are often coupled with heterocyclic compounds. Due to their high surface area-to-volume ratio and distinctive surface energy, nanoparticles are ideal for heterocycle immobilization or functionalization as carriers, supports, or reaction media. Thus, heterocyclic molecules may be stable, bioavailable, or reactive. A hydrophilic nanoparticle may increase the water solubility and control the release profile of a heterocyclic medication with low solubility. The nanoparticle's capacity to modify solvent and biological interface interactions is responsible for this increase (36). The behavior of the NP-heterocycle hybrid depends on the manner of integration—physical adsorption, covalent bonding, or coordination chemistry. Hydrophobic, π - π , or electrostatic interactions may benefit physical loading in reversible systems or controlled release applications. In contrast, covalent conjugation, employed in sensor and catalyst design, produces stronger hybrid materials. Covalent or coordination techniques are better for strong and stable binding in high-temperature or solvent-intensive conditions (37).

Chemically, heterocyclic compounds have several functional groups that interact with nanoparticles. Nitrogen-containing heterocycles may form metal–ligand complexes with metal nanoparticles, affecting catalytic activity and material durability. The nanoparticle surface offers a high density of active sites, while the heterocycle affects reactivity and selectivity, making such systems common in heterogeneous catalysis. Specific heterocycle-nanoparticle interactions are key to improving catalytic activity (38). Loading heterocyclic medicines onto nanoparticles for targeted and sustained drug delivery is popular in biomedicine. Biologically active heterocycles often have fast metabolism, membrane permeability, or systemic toxicity. However, encapsulating these compounds in biocompatible nanoparticles like liposomes, dendrimers, or polymeric nanoparticles may significantly enhance their pharmacokinetic characteristics. Surface modification of nanoparticles with heterocyclic ligands allows cell-specific targeting, particularly when the ligands imitate natural substrates or interact with overexpressed diseased tissue receptors (39).

In addition to loading, heterocycle-nanoparticle chemical reactions have been widely explored for innovative or responsive materials. Functionalized heterocycles may undergo nanoparticle-mediated oxidative coupling or click chemistry. These surface alterations attach heterocycles and provide novel functional domains that may be used in biosensing, where environmental cues are crucial. Reactive integration using heterocyclic chemicals is a flexible and customizable method for multifunctional nanomaterials (40). The heterocycle interaction also depends on the nanoparticle type: metallic, ceramic, carbon-based, or polymeric. Gold nanoparticles are biocompatible and easy to surface functionalize, making them appropriate for conjugation with thiol- or amine-containing heterocycles. Magnetic nanoparticles like Fe₃O₄ enable external manipulation via magnetic fields, enabling tailored medication administration or MRI. Nanoparticle selection is crucial for field responsiveness and biocompatibility (38).

Nanoparticles' loading or chemical interaction with heterocyclic compounds has opened new avenues for functional materials in catalysis, biology, sensing, and electronics. This interaction allows constructing more efficient, tailored, and environmentally or biologically sensitive systems. Therefore, this study seeks to cover the mechanics, techniques, and practical consequences of combining heterocyclic compounds with nanoparticles in diverse scientific fields (40).

Many heterocycle classes have been merged with nanoparticles (NPs) for various uses. Nitrogen-containing heterocycles including imidazoles, triazoles, tetrazoles, and pyridines are employed for catalysis, drug administration, and biosensing because they coordinate well with metal surfaces. Thiazoles and oxazoles coupled with metal oxide NPs are antibacterial and anticancer. Targeted cancer treatment often uses polymeric or gold NPs with quinoline and indole compounds. Furans and thiophenes are also studied in optoelectronics and sensing. Each heterocycle enhances nanoparticle performance in distinct sectors with unique binding and electrical features (38-40).

Several heterocyclic–nanoparticle systems have been developed for targeted applications. For example, triazole-modified gold nanoparticles have been used to detect Hg²⁺ ions via colorimetric changes, acting as selective biosensors for heavy metals (Ali, I., et al., 2022) (41). Tetrazole-functionalized Fe₃O₄ nanoparticles have been applied in anticancer delivery, showing enhanced uptake and apoptosis in breast cancer cells (Xue et al., 2021) (42). Imidazole-coated silver NPs exhibit potent antimicrobial activity by disrupting bacterial membranes. In biosensing, pyridine-functionalized quantum dots can detect glucose and dopamine by

fluorescence quenching or enhancement mechanisms. These systems quantify biomolecules, metal ions, or pH changes in biological and environmental samples, offering high sensitivity and selectivity due to the heterocycle–metal interface (42).

Functionalization of Nanoparticles Using Heterocyclic Ligands

Functionalising nanoparticles (NPs) using heterocyclic ligands may improve their surface chemistry, stability, and specificity for advanced applications. Due to their electron-rich nitrogen, oxygen, or sulphur atoms, heterocycles may form covalent bonds, coordination complexes, or non-covalent forces on nanoparticle surfaces. Covalent functionalisation improves biocompatibility and solubility by chemically grafting reactive heterocycle ligands like imidazole, triazole, or pyrrole onto NPs. Thiolated thiophene or pyridine derivatives may stabilise gold nanoparticles in biological environments by forming Au–S or Au–N linkages (Chakroborty, S., et al., 2024) (43). In metal oxide or magnetic nanoparticles, heterocyclic ligands such as tetrazoles and pyridines coordinate to surface metal ions to provide drug attachment or catalytic activity (Jawad et al., 2023; Geçer, M., et al., 2023) (44, 45). Surface changes such as π – π stacking, hydrogen bonding, or electrostatic interactions provide reversible and responsive functionalisation for drug release and biosensing. The heterocycle-modified NPs improve cancer treatment, antibiotic administration, and selective analyte detection. The conjugations of organic reactivity with nanomaterial precision create hybrid systems with customised physicochemical behaviour for biomedical and environmental applications (45).

We appreciate the reviewer’s observation regarding the lack of detailed strategies on the functionalization of nanoparticles using heterocyclic compounds, which indeed aligns with the central theme of our manuscript. To address this, we have now expanded the relevant sections to incorporate comprehensive insights into chemical methodologies for nanoparticle surface modification via heterocyclic ligands. Heterocycles offer diverse functional groups (e.g., amines, thiols, carboxyls) that can interact with nanoparticle surfaces through covalent bonding, coordination chemistry, or non-covalent interactions. Covalent strategies often involve carbodiimide-mediated coupling or click chemistry, such as azide-alkyne cycloaddition, allowing stable linkage of heterocycles to metal or polymeric nanoparticles. Coordination strategies utilize lone pairs on nitrogen, oxygen, or sulfur atoms within heterocyclic rings (e.g., imidazole, pyridine, triazole) to form metal–ligand complexes, particularly with gold, silver, or iron oxide surfaces. Additionally, non-covalent approaches include π – π stacking and hydrogen bonding, which are useful for reversible drug delivery or responsive systems. Specific

examples, such as the use of tetrazole-functionalized Fe₃O₄ NPs for anticancer delivery or pyridyl-modified gold NPs in catalysis, have been incorporated and cited. These functionalization techniques significantly enhance nanoparticle performance in biomedical, environmental, and catalytic applications, and thus are crucial to the synthesis–application continuum emphasized in our review (45).

Structural–Activity Relationship (SAR) of Heterocyclic–Nanoparticle Hybrids

The structural–activity relationship (SAR) helps explain how heterocyclic–nanoparticle hybrids' chemical characteristics affect their biological performance, especially in medication delivery and therapy. Solubility, membrane permeability, and metabolic stability are affected by structural characteristics such ring size, heteroatom type, substitution pattern, and degree of conjugation. Nanoparticles functionalised with nitrogen-rich heterocycles (e.g., tetrazoles, imidazoles) bind better to metal ions or cellular receptors, enhancing site-specific delivery. Aromatic heterocycles' electron-donating or withdrawing substituents affect bioavailability and cytotoxicity via affecting biological targets. Surface charge, cellular uptake, and intracellular trafficking are also affected by heterocycle orientation on nanoparticle surfaces. Hydrophilic substituents on heterocyclic moiety promote aqueous dispersion and systemic circulation, according to studies. SAR investigations show that hybrids with electron-rich moieties on five-membered rings increase apoptosis owing to improved ROS production and DNA intercalation in anticancer applications. SAR insights help chemists create hybrid materials for optimum therapeutic effectiveness, decreased off-target effects, and increased biocompatibility across biomedical platforms.

Methods for Preparing Nanoparticles on both Classical and Modern Approaches

Nanoscale material synthesis technologies have grown since the discovery of nanoparticles and the founding of nanoscience. Nanoparticles (NPs), particles between 1 and 100 nanometers, have distinct physicochemical features. Due to their improved surface reactivity, size-dependent optical properties, and quantum effects, nanoparticles are significant in medicine, electronics, catalysis, and energy storage. However, controlling nanoparticle size, shape, crystallinity, and surface functionality is crucial to their use in such applications. The invention and improvement of preparation procedures are key to nanomaterials research (46).

The first nanoparticle synthesis methods came from traditional chemistry and metallurgy. Colloidal gold and silver nanoparticles were made in the Middle Ages, but their creation methods remained unknown. Many empirical techniques were used in these early approaches, resulting in poor repeatability and particle characteristic control. However, these old methods helped scientists comprehend nanoscale material nucleation, growth, and stability (47, 48).

The need for high-performance nanomaterials has spurred the development of more advanced and controlled synthesis processes. Top-down and bottom-up nanoparticle preparation methods exist. Top-down methods use ball milling, laser ablation, or lithography to split bulk materials into nanoparticles. These technologies can create vast amounts of nanoparticles but have drawbacks such as wide particle size dispersion, high energy consumption, and milling medium contamination. If particle shape and purity must be controlled precisely, top-down approaches may be less suitable. Bottom-up methods use chemical reactions or self-assembly to make nanoparticles from atomic or molecular precursors. Examples include sol-gel synthesis, CVD, microemulsion, and hydrothermal/solvothermal processes. Bottom-up approaches are recommended for high uniformity and tunability applications because they better regulate size, shape, and surface chemistry. Bottom-up synthesis meets biocompatibility and particle size requirements for cellular absorption and biodistribution in biomedical applications (47).

Due to nanotechnology breakthroughs, green synthesis techniques have emerged, which lessen environmental and health risks. These eco-friendly approaches reduce and stabilize using plant extracts, microorganisms, or enzymes. Green synthesis approaches appeal to nanoparticle manufacturing that prioritizes sustainability and ecological effects. Because biological approaches are benign, they may retain delicate biomolecules' functional integrity, making them ideal for biomedical and pharmaceutical applications (48).

In addition to green chemistry, contemporary hybrid methodologies combine top-down and bottom-up approaches to improve efficiency and scalability. Plasma-assisted synthesis or laser pyrolysis can produce functional nanoparticles for commercial use with great accuracy and speed. The emergence of microfluidic synthesis platforms has enabled regulated and continuous nanoparticle production. These "lab-on-a-chip" devices accurately manage reaction parameters, reducing batch-to-batch variability. Microfluidic technology may improve nanoparticle production repeatability and scalability (46).

Despite these advances, the choice of nanoparticle production process depends on the application. Thus, material type, intended use, economic feasibility, and environmental concerns must be considered while choosing a process (47).

Nanoparticle preparation techniques have changed substantially since their discovery. Modern nanoscience was founded on conventional methods, but developing better, greener, and more exact synthesis methods has allowed nanoparticles to be widely used in science and industry. Nanoparticle synthesis will become even more integrated into our everyday technology if future developments focus on sustainability, scalability, and multifunctionality. Therefore, this study critically examines the many processes used to produce nanoparticles, analyzes their pros and cons, and examines future trends that may define the next generation of nanomaterial production (48).

4. Applications of Nanoparticles in Medical, Agricultural, and Industrial Fields

Nanoparticles are useful in numerous sectors due to nanotechnology's fast growth. The high surface-area-to-volume ratio, quantum effects, and increased surface reactivity of nanoparticles (NPs) make them useful in many sectors. Nanomaterials have improved efficiency, sustainability, and health in agriculture and industry performance. Nanoparticle integration might change 21st-century technology if these discoveries proceed responsibly and controllably (49).

Nanoparticles have revolutionized medical diagnostics, treatments, and medication delivery. One of their biggest contributions is targeted medication delivery, when pharmaceuticals are encapsulated or conjugated to nanoparticles to increase bioavailability and target particular tissues or cells. Lipid-based nanoparticles are employed in clinically approved medicines including mRNA vaccination platforms. Ligand-functionalized nanoparticles may transport therapeutic compounds to sick cells like malignant tissues, reducing systemic toxicity and improving treatment effectiveness. Metal nanoparticles like gold and silver NPs have also been researched for antibacterial and anti-inflammatory effects (50). These properties make them appropriate for wound dressings, medical device coatings, and photothermal and photodynamic therapy. Superparamagnetic iron oxide nanoparticles (SPIONs) also improve contrast and real-time viewing of interior structures in magnetic resonance imaging (MRI) .

Nanoparticles useful for the separation and purification of biological cells and substances. MRI can be improved by utilizing nanoparticle biosensors to diagnose various diseases. In phagokinetic investigations. Cellular imaging (51).

Nanoparticles in agriculture have also improved crop protection, soil quality, and resource management. Various types of nanoparticles have been used in agriculture. Nano-barcodes and nano-processors can help detect the quality of agricultural products. Li et al. (52) developed microscopic probes or bars to flag various diseases in the field. This method is cost-effective, quick, and efficient for disease detection. Nano science is crucial for studying plant hormonal regulation. Nano sensors on farms detect plant viruses and soil nutrient levels. Nano-encapsulated, mildly secreted nutrients are being used to reduce fertilizer usage and contamination in surrounding areas. Under the existing situation of pollution Nano pesticides and fertilizers are more effective and ecologically benign. Due to their tiny size and controlled release, these nano formulations may be used at lower dosages while maintaining or boosting effectiveness. Thus, chemical discharge into water sources is reduced, and ecological harm is reduced. Nano-based insecticides administered in exact quantities for particular pests may avoid resistance and sustain agricultural output. In addition, nano sensors are being developed to monitor soil health, nutrient levels, and pathogens in real time. These technologies can revolutionize precision farming by allowing data-driven decision-making, improving production and sustainability, as shown in Figure 4 (53). Nanoparticles (NPs) can purify irrigation water, lowering the danger of crop. Coagriculture can boost crop yields, minimize agricultural environmental impact, and improve food product safety and quality (54).

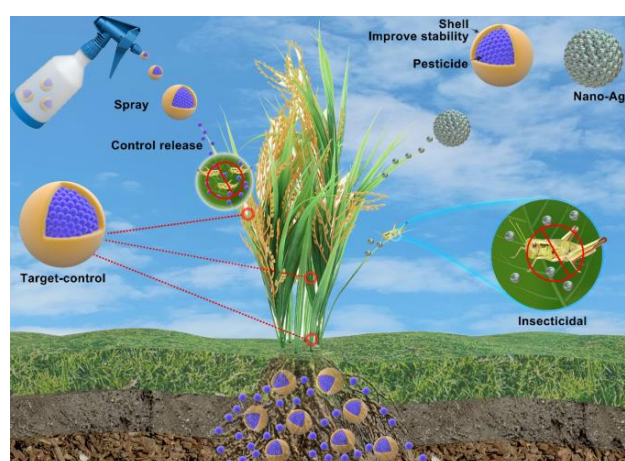


Figure 4: Nanomaterials loaded with pesticides (51).

Nanoparticles revolutionize industry, especially materials engineering, electronics, and environmental cleanup. Carbon nanotubes and graphene nanoparticles are utilized to make lightweight, strong materials. High electrical and thermal conductivity makes them excellent for next-generation flexible electronics and energy storage devices like supercapacitors and lithium-ion batteries. Additionally, ceramic and metal oxide nanoparticles are widely employed in catalysis to accelerate chemical processes without being eaten. This is used in the petrochemical and pharmaceutical sectors. Nanoparticles such as TiO₂ and ZnO are used in self-cleaning surfaces, UV-blocking coatings, and antimicrobial films. If nanoparticle-based coatings are widely adopted, industries might profit from longer material lifespans, lower maintenance costs, and improved product functioning (55).

Despite these potential uses, nanoparticle toxicity, environmental persistence, and regulatory supervision remain issues. Thorough risk evaluations and consistent synthesis, application, and disposal methods are essential. However, nanoparticles have significant medicinal, agricultural, and industrial advantages. Nanoparticles might solve numerous health, food security, and sustainable development issues if appropriately used. Powerful nanoparticles connect scientific innovation with practical application. Their benefits to human health, agricultural production, and industrial manufacture are growing. As research on nanoparticle interactions and safety advances, their integration into daily technology will likely produce more sophisticated and pervasive systems, resulting in more sustainable and efficient systems across numerous industries (55).

Applications of Nanoparticles in Cancer Therapy

Despite available treatments, cancer still kills millions of people annually. The usage of existing antineoplastic medications can lead to adverse side effects, compromising a patient's lifetime. Novel nanoparticle-based medications are gaining popularity due to their effectiveness, minimal side effects, and ability to target cancer cells. These activities could be attributed to the huge surface area of NPs, which enables the mixing of high pharmacological dosages (56). According to Khan and Fulekar, nanoparticles (NPs) are highly effective anticancer medicines due to their biocompatibility, selectivity, ease of manufacture, and low pH dissolution. Nanomaterials activate angiogenesis in human malignant melanomas skin cells via the caspase 3 pathway (57).

5. Applications of Nanoparticles in Vaccines

Conventional live-attenuated pathogen vaccines may return to pathogenic virulence, but inactivated vaccines generally restrict immune response. Nanoparticle-based vaccines may overcome vaccine limitations. Recent chemical and biological engineering advances have allowed for exact nanoparticle size, shape, functionality, and surface characteristics, improving antigen presentation and immunogenicity (58).

Applications of Nanoparticles in Electronics

Due to their lower cost than silicon printing, printed electronics have become popular. Electronics printed with different inks should flow quickly. These inks may include CNTs, organic, and ceramic nanoparticles (59). Electronic, photonic, and sensor materials may be made from one-dimensional semiconductors and metals due to their structural, electrical, and optical capabilities. Electronics companies find new semiconductor materials. Microchips like diodes and transistors replace vacuum tubes (60).

Other Applications of Nanoparticles

Nanoparticles (NPs) are employed in various consumer products such as water filters, deodorants, soaps, socks, food preservation, and room sprays, expanding the business sector of nanotechnology. NPs and their composites exhibit better catalytic activity for dye reduction and removal (61).

6. Conclusions

Nanoparticle-of heterocyclic compound integration offers great scientific and technological promise. Due to their physicochemical features, these hybrid systems improve medicine delivery, catalysis, sensing, and material creation. Nanostructure functionality and efficiency depend on the interaction mode—loading, coordination, or covalent bonding. Additionally, green synthesis technologies provide a biocompatible and sustainable means to make such materials. Toxicology, long-term stability, and consistent regulatory criteria remain issues. Multidisciplinary research and innovation must address these limits when using lab discoveries. Nanoparticle–heterocycle conjugates might solve medical, agricultural, and industrial problems while boosting sustainability and enhancing functional material design if explored ethically and strategically. Before widespread use, nanoparticle–heterocycle hybrid systems must overcome many difficulties despite their potential multifunctionality. Concerns include cytotoxicity, long-term environmental buildup, and poor biocompatibility, especially with metal-based nanoparticles. Scalable, repeatable synthesis techniques for green and eco-friendly processes are few. Future research should improve surface functionalisation, generate

stimuli-responsive hybrids, and use computer modelling to anticipate biological behaviour. Safety and sustainability need more comprehensive toxicological and life cycle evaluations. Addressing these shortcomings will improve these materials' medical, catalytic, and environmental remediation applications.

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Allelopathic Activity of *Ficus carica* and *Punica granatum* Leaves on Germination and Seedling Growth of *Vicia faba* L.

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Abstract:

The pot experiments were conducted in winter 2024-2025 at the Department of Biology green house, College of Science in the University of Mosul. Treatments were done alongside the pure leaf of (*Ficus carica* and *Punica granatum*) by adding soil to the pots with the rate of 10 g/kg soil. All the parameters applied in the experiments were randomly allocated in accordance with absolutely randomization of the design (CRD) to know their influence on the *Vicia. faba* L. seed germination and growth. The results indicated that leaves at 10% for *P. granatum* had a significant stimulatory in shoot length 37.5cm, root length at an average of 20.7 cm and its fresh weight 14.8 g. while *F.carica* had a significant increased growth parameters (germination 96% , shoot length and its fresh weight ,39.4 cm and 25.9 g) respectively, root length and its fresh weight(18.2 cm,13.6 g), as well as the number of nodes and flowers, compared to the control. The findings of this research suggest the potential of applying fresh leaf of *F. carica* and *P. granatum* as a bio stimulant for *V. faba* plants' germination and development.

Keywords: Allelopathy, Growth, Germination, Tree, *Vicia faba*.

(Immediately after the abstract, provide 5-7 keywords and arrange them alphabetically, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes).

التأثير الأليلوباثي لأوراق *Punica granatum* و *Ficus carica* على إنبات ونمو نبات *Vicia faba* L

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الخلاصة:

أُجريت تجارب أُلصص خلال موسم شتاء 2024-2025 في البيت الزجاجي التابع لقسم علوم الحياة، كلية العلوم، جامعة الموصل. طُبقت المعاملات بإضافة الأوراق الطرية لـ (*Punica granatum* و *Ficus carica*) إلى تربة الأُلصص بمعدل 10 غ/كغم تربة. وُزعت جميع المعاملات المستخدمة في التجارب عشوائيًا وفقًا لتصميم عشوائي كامل (CRD) بثلاث مكررات، وذلك لتحديد تأثيرها على إنبات بذور *Vicia faba* ونموها. أشارت النتائج إلى أن أوراق الأشجار بنسبة 10% من *P. granatum* سببت زيادة معنوية في طول المجموع الخضري بمعدل 37.5 سم وطول الجذر بمتوسط 20.7 سم و وزنه الطري 14.8 غم. بينما أظهرت *F. carica* زيادة معنوية في معايير النمو (نسبة الإنبات 96%، طول المجموع الخضري ووزنه الطري 39.4 سم و 25.9 غم على التوالي و طول الجذر ووزنه الطري 18.2 سم و 13.6 غم)، وكذلك عدد العقد والأزهار، مقارنة بمعاملة السيطرة. تشير نتائج البحث الحالي إلى إمكانية تطبيق الأوراق الطرية لـ *F. carica* و *P. granatum* كمحفز حيوي لإنبات وتطور نباتات *V. faba*.

الكلمات المفتاحية: التضاد الاحيائي، النمو، الانبات، الاشجار، الباقلاء

1.Introduction:

Allelopathy is an ecological phenomenon associated with the effect of chemicals substances released from a particular plant on the growth and development of other plants by promoting or inhibiting its growth[1] Allelopathic plants achieve the said allelopathic effect on the host plants by volatilization of stems and stem leaves, leaching of allelopathic substances, root exudations or release of allelopathic substances into the environment in the degradation of plant remains [2]. Allelochemicals are affected by a variety of biotic and abiotic variables, among others. pH, organic matter, nutrients, and moisture content in the soil are examples of

abiotic variables, age of the donor plant also affects the amount of released allelochemicals in the rhizosphere [3]. *V. faba* (*faba bean*) is the most important legume in most parts of the world especially in developing countries. It produces protein as a source of food. It contains a lot of some of the vitamins, minerals and dietary fiber as well. *Ficus* genus comprises approximately 850 species of woody trees, shrubs, vines, epiphytes, and hemi epiphytes belonging to the Moraceae family, results of phytochemical studies of *Ficus*, dry leaf extracts shown the presence of active substances, phenols, flavonoids, glycosides and tannins in aqueous extracts [4]. Pomegranate, scientifically known as *Punica granatum* L., belongs to the intriguing Punicaceae family, renowned for its diverse applications, ranging from medicinal uses to various industrial endeavors [5]. In a series of intriguing germination tests involving common commercial seeds like lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and pepper (*Capsicum baccatum*), it was observed that extracts from pomegranate (*Punica granatum*) exhibited fascinating allelopathic effects, influencing not only their germination but also impacting their growth in remarkable ways [6]. The effects of allelopathic compounds might be directly on the germination and growth of plants but they might also indirectly interfere with the root- soil interaction of plants i.e., the interaction of plants with mycorrhizal fungi. They include a number of diverse chemical compounds although majority of them are phenolic compounds which were identified to help seed germination of most of the plants [7]. Additionally, the options of phenolic allelopathic compounds manipulate several biochemical and physiological activities in plants like photosynthesis, cell division, enzyme reactions, plant water affinity, and nutrients transportation [8]. Due to the significance of the allelopathic occurrence in the agricultural domain and since researchers want to profit from this phenomenon present in nature as herbicides, insecticides or natural growth promoters and to avoid employing chemical ones because of their adverse impacts on the environment and to maintain clean agriculture devoid of chemicals. Therefore, the study aimed to use the tender leaves of *F. carica*, and *P. granatum* to study their effect on the germination and development of *V. faba*.

2. Material and methods:

2.1. Sample Collection

Samples of *F. carica* and *P. granatum* were collected from Mosul city and washed with distilled water and preserved for use in experiment.

2.2. Pot Experiment:

The study included an experiment in the Department of Biology, College of Science, University of Mosul, in 2024-2025 to study of allelopathic effects of fresh leaves of, *F. carica* and *P. granatum* in seed germination and growth of *V. faba* L., Thoroughly mix the fresh leaves of *F. carica* and *P. granatum* with the prepared sterilized soil in a pot containing 10 g of leaves / 3kg of loamy soil per pot. Three replicates were performed for each treatment, in addition to distilled water as a control treatment. 10 *V. faba* L seeds per pot were sown in the soil for two months and irrigated as needed [9] (The seeds of the plant used in this study were collected from the department examination and certification of seeds in Tikrit, Salah Aldeen, Iraq, Viable and healthy seeds, free from visible infections, and of uniform size were selected). Evaluate the germination percentage after 14 days by used equation [10].

$$\text{Germination \%} = \frac{\text{seeds germinated}}{\text{total seeds}} \times 100$$

2.3. Characteristics of the Study

Following 60 days after planting, certain growth indicators were assessed, including shoot length (cm), root length (cm), fresh weight of shoot (g) and fresh weight of root, number of flowers per plant, number of branches per plant, and number of nodes per plant [11].

Statistical Analysis: The research study was conducted with the plan of Random Complete Design and conducted a statistical analysis of data according to the SAS program. Duncan Multiple Range test was used at a 5% probability of differentiation between the average of transactions.

3. Results and Discussion

3.1. Characterization of *V. faba*

3.1.1 Germination and Growth

Data present in **Table (1)** indicate a significant stimulation in the germination, lengths of shoot and roots and their fresh weights of *Vicia faba* L., with the highest stimulating effect observed in the *F. carica* leaf treatment, where the readings were germination 96%, shoot length and its fresh weight, 39.4cm and 25.9g, respectively,

while the highest value of root length 20.7cm and fresh weight 14.8 gm was in *P. granatum* treatment, compared to the control treatment without addition.

Table 1: Effect of *F. carica* and *P. granatum* fresh leaves on germination & growth of *V. faba*

Treatments		Seed Germination (%)	Shoot length(cm)	Fresh Shoot weight (g)	Root length (cm)	Fresh Root weight(g)
Control		86.6b	35.0c	16.2b	17.8cb	10.5c
<i>P. granatum</i>		76.6c	37.5b	17.4b	20.7a	14.8a
<i>F. carica</i>		96.0a	39.4a	25.9a	18.2b	13.6b
Average effect of trees	<i>P. granatum</i>	81.6b	36.2b	16.7b	19.3a	14.7a
	<i>F. carica</i>	91.3a	37.2a	21.4a	17.0b	11.5b

* Similar letters indicate that there are no differences that are significant at the 5% level

Considering Duncan's Multiple Range Selection

3.1.2. Flower number

As shown in Fig. (1 and 2), the results of the effect of adding fresh leaves of *F. carica* and *P. granatum* to the soil at a concentration of (10%) on the number of flowers of *V. faba*. Plants, where the results showed an increase in the number of flowers with the effect of *F. carica* leaves, while plants grown in soils incubated with leaves of *P. granatum* were not affected.

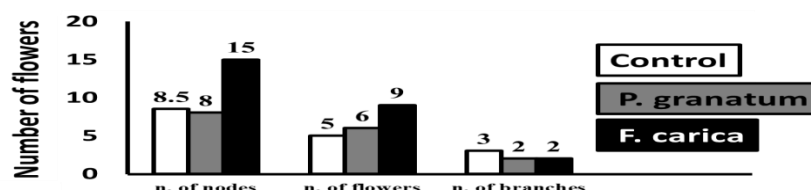


Figure 1: Effect of *F. carica* and *P. granatum* fresh leaves on flower number of *V. faba*.



Figure 2: Growth of *V.faba* L. of treatment with fresh leaves of *F. carica*, and *P. granatum*.

A: Plants treated with *F. carica* B: Plants treated with *P. granatum* C: Control

3.1.3. Nodes/ plant

The number of bacterial nodes in roots was increased after treating with leaves residues, shows the root nodes as a single multi-node, most of them have a blue or pink colour appearance, which in turn considered them active nodes, on the other hand, their size showed an enlarged size due to the effect of additional leaf of tree under study, and the highest increase was noticed after adding *p, granatum* leaves in **Fig.3**.



Figure 3: Nodes of *V. faba* root treated with fresh leaf of *F. carica*, and *P. granatum*.

A: Plants treated with *F.carica* B:Plants treated with *P.granatum* C:Control

The results showed a significant increase in the growth characteristics of the of *V. faba* plant due to the addition of *F.carica* and *P. granatum* tree leaves to the soil, *F. carica* leaves had the highest germination percentage of 96%, while *P. granatum* at the same concentration reached 76.6% compared to the control 86.6% This may be due to increased osmotic pressure in plant cells, which increases the cells' ability to absorb water It may also be that trace inorganic ions the activity of respiratory enzymes of *V. faba* cells stimulates the activity of respiratory enzymes and enhances the capacity of the plants to produce nutrients and consequently enhance germination of *V. faba* seeds [12]. Plant secondary products Allelochemicals are commonly termed as the secondary metabolic product. In the intricate world of plant metabolism, a significant portion of compounds is synthesized through the fascinating shikimic acid and acetate pathways. These pathways weave together in a complex

movement, shaping the very essence of how plants thrive and interact with their environment., are usually water-soluble and widely present in most plants, and in many tissues Allelochemicals in a dose that retard growth of one species can literally stimulate the same or another species at different concentration [13]. It was proposed that since allelochemicals exist in the leaves of *F.caria* and *P. granatum*, there is a possibility of their release into the soil that can promote growth of beans directly or indirectly[14]. [15] Other plant allelochemicals enhance the cultivation of the recipient plant by altering the proportion and the level of the hormones present in them. To some extent, other allelopathic substances in the leaf of the *F.caria* and *P. granatum* trees may also promote the growth of the *V. faba* in other ways. Allelopathic chemicals can alter the composition of microbial community within the soil and favor the growth of other plants and other allelopathic chemicals that are present in the tree leaves may stimulate colonization by mycorrhizal fungi and this facilitates the growth of the *V. faba* [16]. According to research results, reached similar results in a study conducted [17] the effect of aqueous extract of *F. caria* e leaves was most effective in growth promotion on mint (*Mentha haplocalyx Briq.*) in medium and low concentration. In other research [18] Phytochemical exploration into the extracts of *F. caria*, mulberry, and eucalyptus leaves revealed a treasure trove of active compounds. Within these aqueous extracts, one can find a vivid array of phenols, flavonoids, glycosides, and tannins. Each of these chemicals dances with potential, promising fascinating insights into their roles and benefits. The effect of these extracts on maize seed germination was very remarkable at different concentration rates. Aqueous extracts of leaves of the plants under the investigation produced better morphological growth and germination rates at low levels, whereas higher concentrations revealed marked toxicity to maize plants. Our research findings are consistent with [19] *Vitis vinifera* L. leaves contain essential elements for plant growth like calcium, magnesium, phosphorus, and iron, which favorably affected the morphological traits of the *V.faba* plant.

4. Conclusions

The results showed that adding fresh *F. carica*, and *P.granatum* leaves to the soil at a concentration of 10% has allelopathic effects that stimulate the germination and growth of the *V. faba* plant growing in it. However, *F. carica* leaves showed a significant increase over *P.granatum* leaves for most growth traits of *V. faba*. However, there are some limitations, the experiment took place in a controlled pot experiment hence it might not appear accurately in the field environments where type of soil, moisture content, and pests contribute to the allelopathic aspect of the plant. Moreover, the concentrations applied cannot be perfectly

reflective of the ones occurring in natural settings, which propagates towards the insufficient generalizability of the findings. Additional studies dealing with the field experiments to confirm these findings under the varying environmental conditions and the influences on other varieties of *V. faba* should be undertaken in the future.

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An Experimental Comparison of Different Machine Learning Algorithms for Detecting Type 2 Diabetes

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Abstract:

Artificial intelligence (AI) is transforming healthcare, with (ML) offering significant potential for early disease diagnosis and personalized treatment, particularly in diabetes management. This capability is crucial for enhancing diagnostic accuracy. However, accurately predicting type 2 diabetes remains challenging due to diverse patient populations and complex data, as traditional methods can be slow and miss subtle early indicators, leading to delayed interventions. There is a clear need for robust predictive models. This paper proposes and evaluates various ML algorithms and data mining techniques for effective type 2 diabetes prediction. We utilized a unique Iraqi dataset with comprehensive features including HbA1c, lipid profiles, age, and BMI. Our methodology involved rigorous data preprocessing, including cleaning and handling class imbalance with SMOTE. Eight ML algorithms were compared: Decision Tree (DT), Naïve Bayes (NB), Random Forest (RF), Gradient Boosting (GB), K-Nearest Neighbor (KNN), Logistic Regression (LR), and Artificial Neural Networks (ANN). Model performance was assessed using accuracy, precision, recall, F1-score, MAE, RMSE, and AVE, with cross-validation ensuring robustness. The DT algorithm achieved the highest performance, with an accuracy of 99.44%, outperforming all other models. This highlights DT's

effectiveness and its potential for accurate early diagnosis, confirming HbA1c, age, and BMI as key predictors. Future work should establish a national health database in Iraq and explore advanced deep learning techniques, such as Convolutional Neural Network and Recurrent Neural Network.

Keywords: ML, Decision Tree, Naïve Bayes, Random Forest, Gradient Boosting, K Nearest Neighbor, Logistic Regression and ANN.

استخدام خوارزميات الذكاء الاصطناعي للتنبؤ بمرض السكري من النوع الثاني

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الخلاصة:

يُحدث الذكاء الاصطناعي (AI) تحولاً جذرياً في قطاع الرعاية الصحية، حيث يقدم التعلم الآلي (ML) إمكانيات هائلة في التشخيص المبكر للأمراض والعلاج المخصص، لا سيما في إدارة مرض السكري. تُعد هذه القدرة حاسمة لتعزيز دقة التشخيص السريري. ومع ذلك، يظل التنبؤ الدقيق بمرض السكري من النوع الثاني تحدياً كبيراً نظراً لتنوع المجموعات السكانية للمرضى وتعقيد البيانات المتاحة، إذ قد تكون الأساليب التشخيصية التقليدية بطيئة وتفتقر المؤشرات المبكرة الدقيقة، مما يؤدي إلى تأخر التدخلات العلاجية. لذا، هناك حاجة ماسة لتطوير نماذج تنبؤية قوية وموثوقة.

يُقدم هذا البحث وتقييم مجموعة متنوعة من خوارزميات التعلم الآلي وتقنيات تنقيب البيانات بهدف التنبؤ الفعال بمرض السكري من النوع الثاني. لقد تم الاعتماد على مجموعة بيانات عراقية فريدة تتميز بخصائص شاملة، بما في ذلك الهيموغلوبين السكري (HbA1c)، وملفات الدهون (الكوليسترول، الدهون الثلاثية، HDL، LDL، VLDL)، والعمر، ومؤشر كتلة الجسم (BMI). تضمنت منهجيتنا معالجة مسبقة صارمة للبيانات، شملت التنظيف ومعالجة عدم توازن الفئات باستخدام تقنية SMOTE. تمت مقارنة أداء ثمان خوارزميات تعلم آلي: شجرة القرار (DT)، نايف بايز (NB)، الغابة العشوائية (RF)، تعزيز التدرج (GB)، أقرب جار (KNN)، الانحدار اللوجستي (LR)، والشبكات العصبية الاصطناعية (ANN). تم تقييم أداء النماذج باستخدام مقاييس الدقة، والضبط، والاستدعاء، ونتيجة F1، ومتوسط الخطأ المطلق (MAE)، ومتوسط الجذر التربيعي للخطأ (RMSE)، وتباين الأخطاء المطلقة (AVE)، مع ضمان متانة النتائج من خلال التحقق المتقاطع.

أظهرت خوارزمية DT أعلى أداء، حيث بلغت دقتها ٩٩,٤٤٪، متفوقة على جميع النماذج الأخرى التي تم اختبارها. يسلط هذا الإنجاز الضوء على فعالية خوارزمية DT وإمكاناتها في التشخيص المبكر الدقيق، مؤكداً أن HbA1c والعمر ومؤشر كتلة الجسم هي المؤشرات الرئيسية للتنبؤ بالمرض. يجب أن تركز الأبحاث المستقبلية على إنشاء قاعدة بيانات صحية وطنية شاملة في العراق، مع إمكانية دمجها مع قواعد البيانات العالمية، واستكشاف تقنيات التعلم العميق المتقدمة، مثل الشبكة العصبية التلافيفية (CNN) والشبكة العصبية المتكررة (RNN)، لتعزيز القدرات التنبؤية.

الكلمات المفتاحية: التعلم الآلي، شجرة القرار، نايف بايز، الغابة العشوائية، تعزيز التدرج، أقرب جار K، الانحدار اللوجستي، الشبكات العصبية الاصطناعية.

1. Introduction

Artificial intelligence (AI) has become a rising star and is rapidly progressing, with the potential for incredible impact on healthcare (1). Artificial intelligence (AI) has created opportunities and transformed medical perspectives and diagnosis, and treatment paradigms. These advancements offer healthcare providers new ways of facing and solving formidable hurdles to expenditure reduction, disease management, service accessibility, and therapeutic optimization. Notable AI-based technologies, such as (ML) and deep learning (DL), have contributed significantly to diagnostics, patient monitoring, drug discovery, drug development, and telemedicine (2).

Diabetes is divided into three main types: type 1, type 2, and gestational diabetes. Type 1 diabetes is considered to be an autoimmune disease, in which the immune system attacks and destroys the cells in the pancreas that make insulin. Type 2 diabetes arises due to the inability of the body to make enough insulin and the failure of the body's cells to utilize insulin properly. Gestational diabetes, which occurs in pregnant women during the 6th and 9th months of pregnancy, is caused by the hormones the placenta makes, which in turn make it hard for the body's insulin to work. The proportion of subjects with type 2 diabetes is increasing versus those with type 1 and gestational diabetes.

Diabetes Mellitus (DM) is also known as diabetes, and is a long-term condition affecting the body's ability to convert food into energy (3). Algorithms are created using ML concepts to generate predictive models of the risk of DM onset and its complications. Applicable to resources-finite needs, the evidence of the efficacy of digital therapeutics has been established, as well as a validated intervention for lifestyle in the management of diabetes. Then, patients are increasingly empowered to self-manage diabetes, such that patients and clinicians are benefiting from clinical decision support (4). In this paper, methods for augmenting synthetic samples to under-represented classes were applied across various ML and DL techniques. These algorithms include Decision Tree (DT), Naïve Bayes (NB), Random Forest (RF), Gradient Boosting (GB), K-Nearest Neighbor (KNN), Logistic Regression (LR), and Artificial Neural Networks (ANN).

In this work, some pre-processing approaches have been employed for the performance enhancement of ML models. The dataset was first cleaned by dropping missing values, duplicated entries and outliers. Categorical attributes were converted to numbers using Label Encoding. To handle the class imbalance problem, the Synthetic Minority Over-sampling Technique (SMOTE) was applied to create synthetic instances for underrepresented classes. To

normalize the features and make the contribution of all features in learning equal, the Standard Scaler was used to scale the values of the features. Feature importance analysis, particularly with the DT model, also demonstrated that HbA1c, AGE, and BMI were the most significant predictors for diagnosing type 2 diabetes. Eight types of ML algorithms were developed: DT, NB, RF, GB, KNN, LR, and ANN. The performance of each model was evaluated using various metrics, including accuracy, recall, precision, AVE, MAE, F1-score, RMSE, and AUC.

Additionally, the cross-validation technique was applied to generalize and confirm the robustness of the results. This paper aims to develop accurate predictive models for identifying individuals at risk for type 2 diabetes at an early stage through ML based on a dataset with rich medical data. Using the most important predictors and by comparing models, the study is aimed at building a practical decision support for caregivers, especially in other countries with population demographics similar to Iraq.

2. Material and methods

2.1 Materials

The method is applied extensively in this study to the data at hand. In addition, the study relied upon the data of July 2020 obtained from Iraq, as provided by Information Technology University, which can be accessed through the link:

<https://data.mendeley.com/datasets/wj9rwkp9c2/1/files/2eb60cac-96b8-46ea-b971-6415e972afc9>

The diabetes data reported for the following 1000 records is categorized into the following sugar registers: (Yes) Y (diabetic), N (Non-Diabetic), P (Pre-Diabetic). It contains 12 features, which are: (Age, Gender, Cr, BMI, Urea, Chol (LDL, VLDL) Fasting Lipid Profile), TG, (HDL) High-density lipoprotein Cholesterol, and HbA1c. In this study, there were three categories: diabetic (Y), pre-diabetic (P), and non-diabetic (N). In order to reduce the classification problem to a binary one, we combined the person with diabetes (Y) and pre-diabetic (P) classes into a (positive) “diabetic” class, and the non-diabetic (N) class remained unaltered. This approach is in line with previous literature that stressed the clinical importance of identifying diabetic and pre-diabetic patients, as they have a higher chance of developing full diabetes and its complications (Edgar et al., 2024)(5). Thus, the binary classification separates the subjects at risk from those with a normal metabolism.

2.2 Methods

We have designed an effective classifier using ML for diabetes prediction. The code was written in Python and heavily uses the Pandas package for pre-processing the data, while the Label Encoder class is from the sklearn package. The preprocessing step involves converting the next part of the text features, which are currently categorical, to numeric as well.

2.2.1 Data Preprocessing

Preprocessing is the initial step to organize the input data for processing. It includes several processing operations, such as merge, reorder, and manipulation of data to clean, prune, reduce, and discretize (6). Choosing the right methods is key, as good data pre-processing can vastly improve classification results. To prepare the data for analysis, preprocessing involves several vital operations: eliminating noise (Noisy Data), Data Cleaning (Data Cleansing), Data Transformation, Data Reduction, and Data Integration. High-quality data must be guaranteed before analysis is conducted. These preparatory steps have previously been assumed by the author for high-quality data generation (7).

2.2.2 Data Cleaning

Data cleansing is the act of identifying and correcting (or removing) dirty data from a database. It usually involves discovering missing values, outliers, and inconsistencies, and then dealing with them by either excluding or substituting them with valid values (8).

2.2.3 Data Transformation

Data transformation is reformatting data for the analysis at hand. Data preprocessing is necessary to convert raw measurements into a format that can be analyzed by the analyzer, enabling the verification of the diabetes dataset.

2.2.4 Synthetic Minority Oversampling Technique (SMOTE)

SMOTE has been one of the most popular and efficient methods for addressing class imbalance problems in various fields. The basic intuition in SMOTE is to generate more minority samples that are close to some existing minority examples in feature space (9). To mitigate the class imbalance between the diabetic and the non-diabetic classes, we used SMOTE. The dataset was initially divided into the training and test sets to avoid data leakage. It was further used exclusively on the training part of the data to create synthetic samples for

the minority class. This process allowed for a consistent set of evaluation metrics that were valid and unbiased.

2.2.5 Data Splitting

The `train_test_split` method from the Scikit-learn library was utilized to separate the data into a training set (80%) and a test set (20%). This ensures that the models are trained on a varied training set and effectively evaluated on the test set. Moreover, cross-validation was applied to mitigate bias in evaluation as well as guarantee the performance of the model on other datasets. Specifically, the data is split into five groups (five-folds). The model is trained on four of these groups and tested on the remaining group, and this process is repeated for each group.

2.2.6 Utilized ML algorithms

a. Decision Tree (DT)

The DT algorithm is a supervised ML technique that divides the data in terms of some parameter. DT is a technique to separate a dataset into smaller sets by applying rules. That is, it may be described as a method that classifies (large) amounts of data into small groups of data (10).

b. Naïve Bayes (NB)

The Bayesian classifier is a statistical classifier that uses conditional probability to categorize data into predefined categories. Both a descriptive and a predictive algorithm are considered to be NB. The categories of the untrained data are then predicted using the descriptive probability. The following are some advantages of this approach. It is simple to use to start. Furthermore, NB doesn't always require a lot of training data for categorization (11).

c. Random Forest (RF)

The RF is an ensemble algorithm, which is capable of tackling both classification and regression tasks. RF is a classifier that includes multiple DT classifiers, with the results determined by majority voting from the metaclassifier. Each tree has been trained with a random sampling of the entire training set. Unlike some other algorithms, in the RF procedure, there is no problem with fitting the data too much (overfitting). The rate at which it fails can be used to compute the generalization error directly without the use of

a cross-validation procedure. The RF model can handle both categorical and numeric data, including missing values and non-scaled data (9).

d. Gradient Boosting (GB)

GB is an ML algorithm used for regression and classification. It is a type of ensemble learning method that combines weak learners to form a strong one. Successively fitting decision trees reduces the loss function to the residuals of the current model. Each iteration will focus more on the mistakes made in the last iteration. The output of all trees included in the model is combined to yield the final prediction (12).

e. K Nearest Neighbor Algorithm (KNN)

KNN is a minimal and popular modeling technique. Classification problems, pattern recognition and regression through ML algorithms. KNN gets neighbors from data by using the Euclidean distance between points of data (13).

f. Logistic Regression (LR)

LR is a supervised learning model commonly used to solve the binary classification problem. It applies the logistic function to estimate the probability of the data point being assigned to one of the categories. There are many complicated generalizations of LR. It is a model of regression; it predicts the probability of an item being a certain type (13).

g. Artificial neural networks (ANN)

ANN is an intelligent method to diagnose and predict diseases. ANN is an ML methodology that imitates humans to learn and predict intricate issues with neural architecture (14). A neural network is trained by providing it with patterns and allowing it to modify its weights in accordance with a given learning rule. The operation of the artificial neural network is divided into two stages: learning and inference. Before a decision is taken, the ANN is educated on previous examples of evaluation. Learning takes place after continually adjusting the weights until the time-average mean square error reaches the given minimum allowed threshold. After training, the decision-making remains in the weights and connections, as the trained model is now ready to solve a new case (15). Two types of ANN are presented as shown below.

A. Hidden Layer Perceptron Neural Networks (HLNN)

A neural network model containing a hidden layer is proposed and implemented to enhance the predictive ability of the model. This neural network has a very simple network structure consisting of an input layer and an output layer. It is a Perceptron that uses Back propagation to learn weights from the data in an efficient manner. Forward propagation generates predictions and calculates the error between the actual and predicted value so that the weights can be adjusted accordingly. The method ensures continuous improvement and is thus a useful tool for data-driven operations.

B. Multilayer Perceptron Neural Networks (MPNN)

A neural network model with multiple hidden layers is proposed and implemented to capture the complex relationships between input features. It is implemented to enhance the model's performance. The Multilayer network consists of a dynamic system. The input layer has nodes equal to the number of characteristics, and the hidden layer contains 200 nodes. Every node multiplies its inputs with its weights and passes them through a sigmoid activation function. The output layer of the present model contains a single node, which takes data from the hidden layer as input and applies the sigmoid function to map the output between 0 and 1.

3. Model Evaluation

Several evaluation measurements evaluate the predicted results. The performance is measured by classification accuracy, confusion matrix, and F1-score. The classification accuracy is defined as the number of correctly matched ones over the total number of input patterns. The applied metrics are as given below:

1. Root mean square error (RMSE): It is a very popular way to check the accuracy of the model for predicting statistical data. The Root Mean Square Error (RMSE) scores are between 0.0 and 0.5, indicating that the model is able to predict the data correctly. It is formulated as follows (16):

$$\sqrt{RMSE} = \frac{1}{m} \sum_{i=0}^m (x_i - y_i)^2 \quad (1)$$

2. Mean absolute error (MAE): The mean absolute difference between the actual output and the predicted output for the entire dataset (16). MAE can be expressed as follows (17):

$$MAE = \frac{1}{m} \sum_{i=0}^m |xi - yi| \quad (2)$$

3. Kappa statistic: The kappa statistic quantifies the degree of concordance of the instances being classified by the ML classifier and conditions on the true labelled data as a ground truth to account for the expected accuracy of a random classifier. To see how well a classifier works for a given dataset, the following equation presents the kappa computation (16):

$$Kappa = \frac{OA - i}{1 - IE} \quad (3)$$

4. Accuracy is the proportion of correctly predicted positives, both true and false, out of all positives. It is composed as follows (18):

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \quad (4)$$

5. Precision: is the ratio of the number of true positive predictions to the total number of positive predictions made by the model. It is calculated as follows (18):

$$Precision = \frac{TP}{TP + FP} \quad (5)$$

6. Recall: It can be computed as the ratio of correctly estimated data points out of the total included true data points. Sensitivity is defined as follows (19):

$$Recall = \frac{TP}{TP + FN} \quad (6)$$

7. F1-score: The F1-score is the harmonic mean of sensitivity and precision, and is determined as follows (18).

$$F1_Score = 2 \times \frac{Precision \times Recall}{Precision + Recall} \quad (7)$$

8. Confusion Matrix: A confusion matrix describes the predicted and actual classifications. The performance of any system is usually tested on matrix-shaped data (20).

9. ANN Evaluation: Here, we address research questions regarding the evaluation of ANN models.

RQ1: How to tune the Batch size and number of epochs?

RQ2: How to tune the training optimization algorithm?

RQ3: How to tune learning rate and momentum?

RQ4: How to tune network weight initialization?

4. Results

According to several metrics, including Accuracy, Recall, F1-score, Precision, Cohen's Kappa, MAE, RMSE, and running time. The DT model had the best predictive performance compared to the rest of the models. It reached the highest values of accuracy (99.4%), recall (98.6%), precision (98.64%), and F1-score (98.60%), as well as the best Cohen's Kappa value (0.988), which denotes a very strong agreement with the reference labels. It was also the least error-prone (MAE = 0.005, RMSE = 0.07) and fastest in execution (13 ms), making it accurate and computationally efficient. The RF model had the same accuracy (99.4%) but lower values on the other measures. GB and KNN also achieved higher accuracies of 99.1% and 98.9%, respectively, but with higher residual errors. LR and NB achieved not bad results, obtaining, respectively, 97.8 and 97.9% accuracy. Among the presented ANN models, HLNN has the highest performance (97% accuracy), lowest error rate, and fastest processing time (0.4 ms). In comparison, MPNN has the lowest performance (95% accuracy) and has the longest processing time (64 seconds) compared to HLNN. Results from feature importance analysis also showed that HbA1c, AGE and BMI were the most important features for predicting type 2 diabetes. These features, in conjunction with the DT classifier, produced the highest accuracy (99.44%).

4.1 Tuning the Batch Size and Number of Epochs

The value of Batch Size is calculated, which is the number of training records passed to the model, i.e., the number of samples the model will treat. Even if the batch size is 20, the model will consider this value and calculate the error, then update the weights for each epoch. The best performance of 67% has been achieved precisely for payment size 20 and number of application training 100. The final result of this method is shown in Figure 1:

```
Best: 0.674040 using {'batch_size': 20, 'epochs': 100}
0.632317 (0.050247) with: {'batch_size': 10, 'epochs': 10}
0.650541 (0.029356) with: {'batch_size': 10, 'epochs': 50}
0.668903 (0.042884) with: {'batch_size': 10, 'epochs': 10}}
0.619373 (0.043666) with: {'batch_size': 20, 'epochs': 10}
0.646620 (0.039530) with: {'batch_size': 20, 'epochs': 50}
0.674040 (0.029795) with: {'batch_size': 20, 'epochs': 100}
0.611392 (0.057363) with: {'batch_size': 40, 'epochs': 10} .....
Accuracy: 61%
```

Figure 1: Network results for batch size and iteration count searches.

4.2 Tuning the Training Optimization Algorithms

It is trained with the aid of Kera's Library using a neural network. The search for this test is then employed to test various modifications or algorithms, adopting the one that achieves the best results. The current network is prepared using (EPOCH = 100) and the number of samples in each training step (Batch Size = 10), and the name of algorithms for optimization, which (SGD, RMSPROP, Adagrad, Adadelata, Adam, Adamax, Nadam) are used. The data is partitioned into three groups (Cross Validation). The resulting NADAM optimization algorithm was extracted. The best performance for the remaining optimizations was 67% (see Figure 2).

```
Best: 0.689711 using ('optimizer': 'Nadam')
0.653196 (0.001206) with: ('optimizer': 'SGD')
0.649290 (0.011847) with: ('optimizer': 'RMSprop')
0.585565 (0.100332) with: ('optimizer': 'Adagrad')
0.620639 (0.043876) with: ('optimizer': 'Adadelata')
0.688399 (0.009652) with: ('optimizer': 'Adam')
0.654539 (0.025063) with: ('optimizer': 'Adamax')
0.689711 (0.015514) with: ('optimizer': 'Nadam')
...
Accuracy: 0.7561517429938484
```

Figure 2: Network results for search optimization algorithms

4.3 Tuning Learning Rate and Momentum

This approach implements a neural network using TensorFlow/Keras, and additionally, the network search is used to obtain the best momentum and learning rate. The first step is to create a Model() and call the sequential function within it. This function is part of the Keras model from the serial layers, allowing you to obtain the model. In the last layer, a person's binarized classification is determined, whether they have sugar or not. The Sigmoid activation function is used in the final layer to fit the value between (0,1). The form is warped using Kera's Classifier to determine the optimal parameters. Then, the learning and momentum rates are adjusted, and several values are tested to identify the best one. The values of learning rate used are (Learn rate = [0.001, 0.01, 0.1, 0.2, 0.3]), where the best value of the learning rate is 0.1. For momentum, several values were tested, and the following were experimented with: Momentum [0.0, 0.2, 0.4, 0.6, 0.8, 0.9]. The model performed best when congestion was

moderate (0.8). The results obtained using (SGD) with learning rate (0.8) and momentum (0.1) for this approach are: The best results are obtained with 75% accuracy (see Figure 3).

```
Best: 0.769982 using ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.8)
0.725024 (0.014011) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.0)
0.723762 (0.016650) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.2)
0.746299 (0.035143) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.4)
0.746271 (0.021892) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.6)
0.747520 (0.017654) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.8)
0.751298 (0.029039) with: ('optimizer__learning_rate': 0.001, 'optimizer__momentum': 0.9)
0.746243 (0.007302) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.0)
0.746271 (0.019400) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.2)
0.750035 (0.028135) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.4)
0.752513 (0.018427) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.6)
0.733759 (0.005724) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.8)
0.755005 (0.006860) with: ('optimizer__learning_rate': 0.01, 'optimizer__momentum': 0.9)
0.756235 (0.011355) with: ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.0)
0.758746 (0.002197) with: ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.2)
0.732454 (0.028245) with: ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.4)
0.745023 (0.013619) with: ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.6)
0.769982 (0.014745) with: ('optimizer__learning_rate': 0.1, 'optimizer__momentum': 0.8)
```

Figure 3: Network results for momentum and learning rate searches

4.4 Tuning Learning Weight Initialization

In this work, a neural network is developed using Keras 46 and features a hidden layer with 12 neurons. In this layer, ReLU is also used. The output layer is one cell using Sigmoid, and the model is optimized by GridSearch function Cv. It's a method for finding the best values for the hyperparameters of the model by iterating through all the possible sets of values. Then there were several methods which are: ('uniform', 'lecun_uniform', 'normal', 'zero', 'glorot_normal', 'glorot_uniform', 'he_normal', 'he_uniform') and any of them that do better.

The data is divided into 10 parts, and cross-validation is used, with one part reserved for testing and the remaining nine parts for training the model. Between these types, the 'normal' type

gives the best performance compared with the rest, and then predicts (71% accuracy) and the generator output of this method, as mentioned in Figure 4.

```
Best: 0.718413 using ('model__init_mode': 'normal')
0.707945 (0.013441) with: ('model__init_mode': 'uniform')
0.689726 (0.021351) with: ('model__init_mode': 'lecun_uniform')
0.718413 (0.020539) with: ('model__init_mode': 'normal')
0.651889 (0.000643) with: ('model__init_mode': 'zero')
0.717075 (0.012976) with: ('model__init_mode': 'glorot_normal')
0.704013 (0.019934) with: ('model__init_mode': 'glorot_uniform')
0.685784 (0.006825) with: ('model__init_mode': 'he_normal')
0.683221 (0.025060) with: ('model__init_mode': 'he_uniform')
```

Figure 4: Network results for network weight configuration searches

5. Discussion

The current data set is obtained from Mendeley and has been properly synchronized to remove missing and outlier values. Categorical values are converted to numerical values, and duplicates are eliminated. Additionally, the technology of budget categories is used to increase the artificial samples of the less common category. Cross-validation is adopted, a statistical technique that splits data into a training dataset and a test dataset, assessing the model's compatibility and stability to be more accurate. From the evaluation standpoint, the accuracy, Precision, recall, F1 score, average absolute error, root mean square error, and average variance extracted (AVE) are used, as seen in Tables 1 and 2. An extensive exploration and simulation of machine learning algorithms are conducted to precisely diagnose diabetes at an early stage in the healthcare industry. A few ML techniques are selected, including RF, DT, GB, NB, KNN, LR, and ANN, Figure 9 illustrates the accuracy comparison among classification algorithms, showing that Decision Tree and Random Forest achieved the highest accuracy. With the current discovery, there is potential to change the computation of predicting and treating Diabetes, and ultimately enhance patient wellness . TP, or true positive, is the number of cases correctly classified as sick, indicating how well the current classifier is performing. (TN), A true negative is a case identified as having the disease, but it actually doesn't have the disease. False positives (FP) are cases where healthy conditions are incorrectly identified as unwell, and false negatives (FN) are cases where sick conditions are incorrectly considered healthy.

DT achieved the best overall performance across all studied algorithms and evaluation metrics. It obtained an accuracy of 99.4%, a recall and F1-score of 98.6%, and the best Cohen's Kappa value (0.988), surpassing the ensemble models, such as RF and GB. Confirming that DT was well adapted to this dataset, as it was able to handle mixed data types, model non-linear relationships, and provide rules that can be exhibited. RF and GB provided strong results as well, but with more complex models, they did not offer much improvement over DT, which performed just as well and faster. Performance results of neural network models varied the most over the compared methods. MPNN showed competitive F1 and precision, but is not suitable for real-time scenarios due to its very high runtime (64 s). In comparison, HLNN achieved a better trade-off between speed and accuracy, making it more suitable for fast diagnosis.

Future research should focus on: Establishment of a national database encompassing all health centers and clinics in Iraq, with the capability for integration with worldwide or neighboring databases, Creating a consolidated center between the Ministry of Health and the Ministry of Higher Education, It is recommended to utilize a CNN and RNN model that employs cutting-edge deep learning techniques.

Feature importance measures the significance of each feature in the decision-making process of the data tree. It is a value between 0 and 1 for each feature, where zero indicates “never used” and 1 indicates “accurate prediction of the target” (see Figure 5).

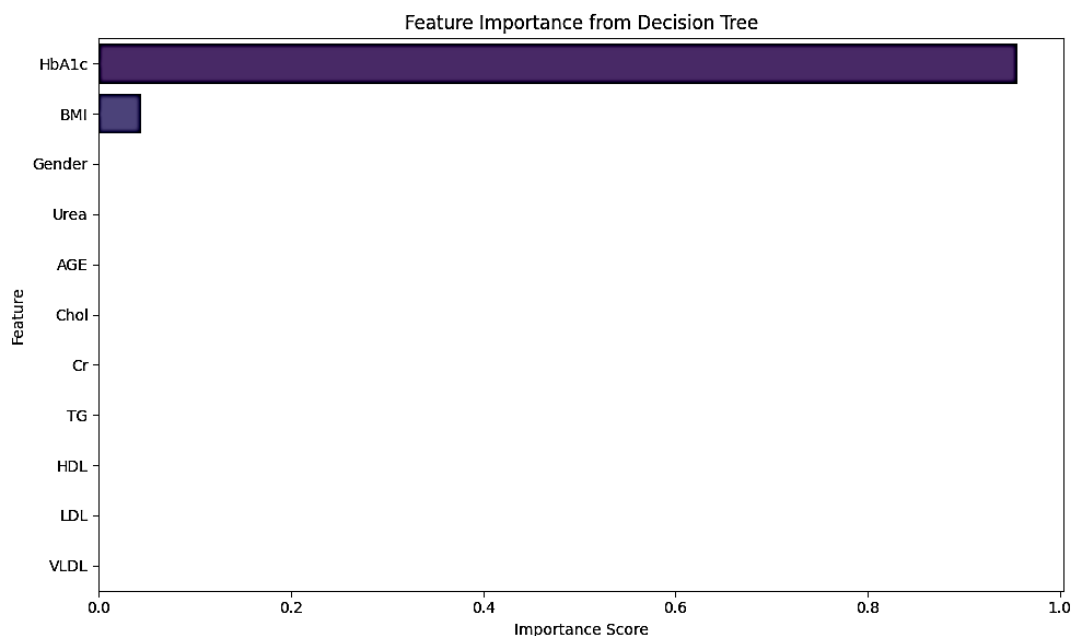


Figure 5: Feature importance in the DT model

The two most important features in the data tree are glycated hemoglobin (HbA1c) and body mass index (BMI). The major predictor for diabetes was HbA1c, which was a full and nearly perfect predictor, followed by BMI, which had a small effect. The other characteristics had virtually no additional effect, and HbA1c alone was sufficient to separate the samples correctly.

According to Figure 6, all the features in the LR model are listed from highest to lowest importance. The data are HbA1c, BMI, Cholesterol, TG, etc.

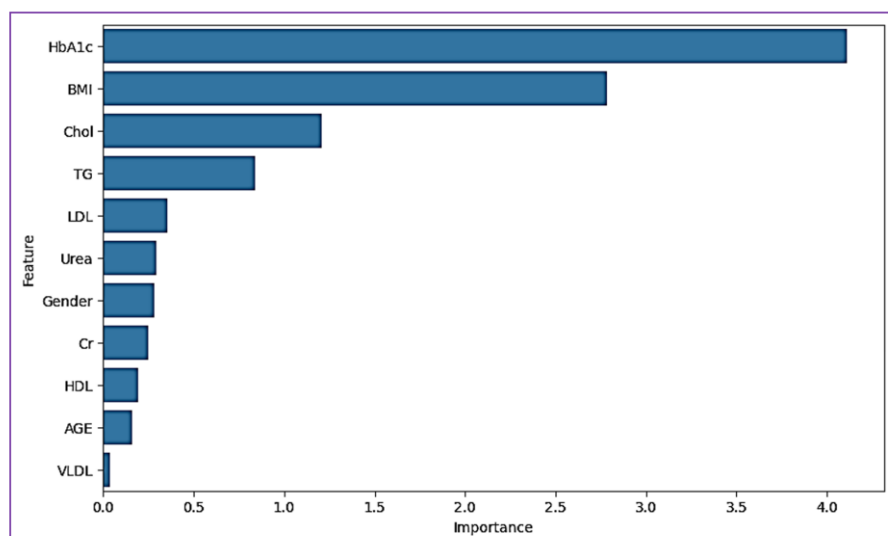


Figure 6: Feature importance in the LR model

The efficiency of the trained classifier model (RF classifier) was assessed using the error matrix (Confusion Matrix). This matrix provides an overview of the model's classification of each sample, as well as the correct and incorrect classifications for each class. The error matrix gathered here will be described and discussed. Figure 7 illustrates the confusion matrix, where the classification results of the RF algorithm on the binary diabetes data, where there are two classes: Class 0 (non-diabetic) and Class 1 (Diabetic).

The confusion matrix demonstrates the following measurements. Firstly, $TN = 179$, which represents the number of samples that actually belong to the uninfected class and were correctly classified. Secondly, $FP = 0$, which means no mistake is committed in classifying uninfected samples as infected. This is particularly good since we didn't have any false alarms. Thirdly, $FN = 2$, where two cases were incorrectly not identified as infected, despite being infected. Sensitivity to this kind of error is important in a medical use case. Lastly, $TP = 178$, representing the number of infected samples classified correctly. This figure illustrates the model's close performance in diagnosing infected individuals.

The confusion matrix, the overall accuracy index of the model, as well as more precise performance indicators, including precision, recall, and F1 score, were evaluated using the test set. These evaluations were further enhanced by using a 5-fold cross-validation to obtain a more comprehensive and stable evaluation of model performance.

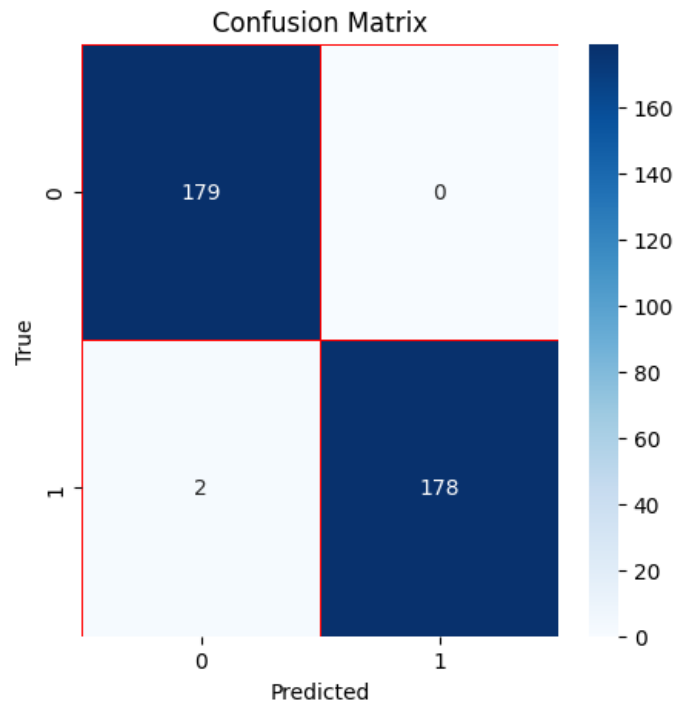


Figure 7: Confusion matrix of the RF model

This was supported by the high scores obtained in ROC AUC, with most models achieving 100% using this metric, such as RF, GB, and LR models. The DT, KNN and NB models also achieved a score of 99%. These findings indicate the discriminative value of the models in distinguishing patients with T2DM from those without T2DM. They also demonstrate that the models are utilizing more effective features in the data, beyond just superficial accuracy, which thus helps to boost their reliability in medical diagnostic scenarios. Figure 8 illustrates a cross-comparison of ROC curves in various models.

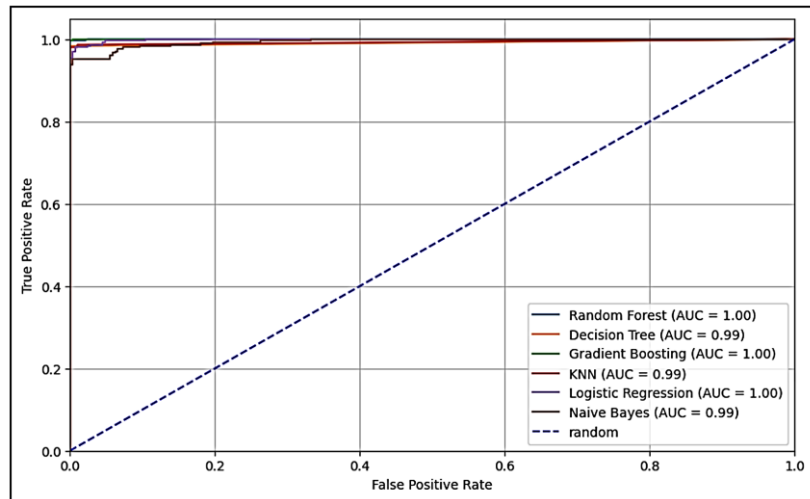


Figure 8: ROC curve of all models developed using the IRD dataset

Table 1: Performance evaluation metrics for the diabetes dataset

NO.	Algorithm	Accuracies	Recall	F_Score	Precision	Cohen kappa
1.	R F	0.994	0.983	0.983	0.984	0.988
2.	DT	0.994	0.9860	0.9860	0.9864	0.988
3.	GB	0.991	0.985	0.985	0.9863	0.983
4.	NB	0.979	0.9667	0.9666	0.968	0.959
5.	KNN	0.989	0.970	0.969	0.971	0.979
6.	LR	0.9781	0.970	0.969	0.971	0.956
7.	MPNN	0.9500	0.9609	0.9718	0.9829	0.7546
8.	HLNN	0.9700	0.9721	0.9831	0.9943	0.8528

Table 2: The error rate analysis

NO.	Algorithm	MAE	RMSE	AVE	Time
1.	R F	0.005	0.07	0.005	34ms
2.	DT	0.005	0.07	0.005	13ms
3.	GB	0.008	0.09	0.008	5.82ms
4.	NB	0.02	0.14	0.01	1.59ms
5.	KNN	0.010	0.10	0.010	11.9ms
6.	LR	0.02	0.14	0.01	0.54ms
7.	MPNN	0.05	0.22	0.04	64150ms
8.	HLNN	0.03	0.17	0.02	0.4ms

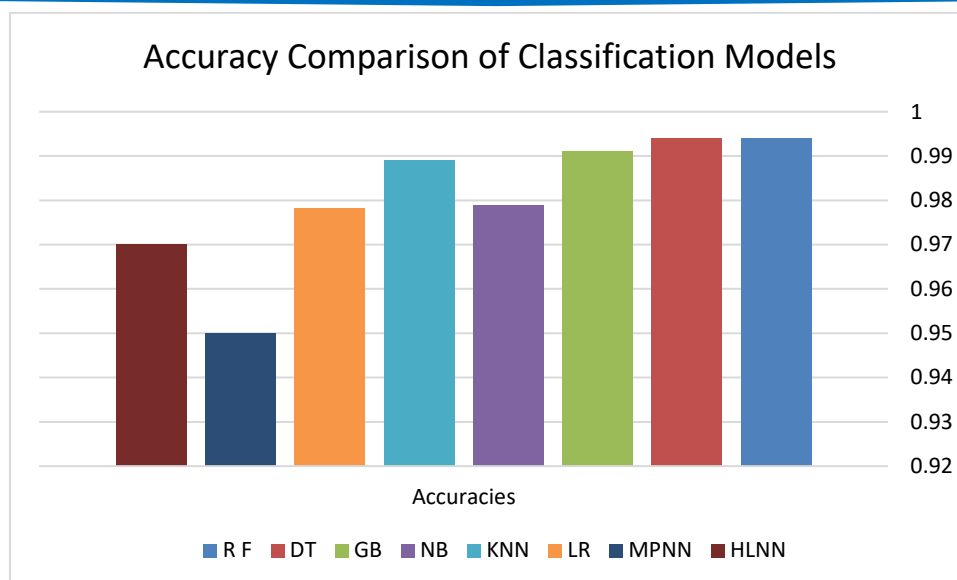


Figure 9: Comparison of the accuracy of algorithms

6. Conclusions

Diabetes is one of the most prevalent and highly prevalent chronic diseases worldwide. DM is a significant global health concern, with its prevalence rapidly increasing. Undiagnosed diabetes can lead to numerous complications, including retinopathy, nephropathy, neuropathy, and various vascular disorders. Both type 1 and type 2 diabetes are among the leading causes of mortality worldwide and are associated with renal disease, visual impairment, and cardiovascular conditions. Data mining techniques can enhance healthcare decision-making by facilitating accurate disease diagnosis and treatment, thereby reducing the burden on healthcare specialists. Using clinical data from Iraq, this study sought to develop and assess artificial intelligence models for the prediction of type 2 diabetes mellitus (T2DM).

The primary contribution is that the DT algorithm was shown to be the most effective model, outperforming all other tested models with a classification accuracy of 99.44%. The results emphasize the importance of features and data quality in improving model performance, particularly for biomarkers such as HbA1c and triglycerides. The results indicate that valuable clinical data is more important for diagnostic success than algorithm complexity. It also indicates the potential for investigating the construction of prototype models for intelligent medical electronic apparatus for diagnosing early diseases. The DT model is a strong candidate for integration into clinical decision support systems (CDSS), which are intelligent platforms that help medical professionals diagnose illnesses, provide treatments, and make well-informed decisions. This is due to the model's high accuracy and interpretability. Early diabetes risk

detection is made possible by integrating the suggested model into these systems, particularly in primary care and resource-constrained environments.

To improve generalizability and facilitate widespread clinical deployment, future research should concentrate on integrating real-time data streams from wearable devices, implementing longitudinal monitoring, and validating the models across various clinical populations.

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Comprehensive Review of Software-Defined Networking and Application of Machine Learning in Load Balancing

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Abstract:

This paper presents a comprehensive review and experimental analysis of machine learning algorithms applied to load balancing in Software-Defined Networking (SDN). From a review standpoint, it considers the rapid development of data centers and the increasing complexity of traffic, underscoring the shortcomings of traditional algorithms. The studies examined are systematically compared in terms of their pros and cons in real-life situations. In addition to the review, the paper presents original findings by assessing specific machine learning techniques, notably Artificial Neural Networks (ANN) and Deep Learning (DL) models, via tenfold cross-validation. The experimental results indicate that the ANN achieves the lowest average response time (1.955 ms), closely followed by the DL (1.962 ms), which demonstrates the highest stability across runs. SVM with $C=100$ gets the best classification accuracy in ten-fold cross-validation. DL comes in third, and ANN comes in last. When considering both latency and accuracy, DL offers the best overall trade-off between speed, stability, and accuracy for SDN load balancing. It beats traditional baselines (LR: 4.461 ms; SVM: 17.9–18.0 ms). These results, which are directly related to the method used, show that advanced ML methods are better for SDN load balancing. The paper also addresses

significant challenges, such as scalability and adaptability, and proposes future research directions for hybrid AI-driven models to enhance the efficiency of SDN-based network management.

Keywords: Software-Defined Networking, Load Balancing, Machine Learning, Deep Learning.

مراجعة شاملة للشبكات المعرفة بالبرمجيات وتطبيق التعلم الآلي في موازنة الأحمال

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الخلاصة:

يقدم هذا البحث مراجعة شاملة وتحليلاً تجريبياً لخوارزميات التعلم الآلي المطبقة على موازنة الأحمال في الشبكات المعرفة بالبرمجيات (SDN) ومن منظور المراجعة، يتناول البحث التطور السريع لمراكز البيانات والتعقيد المتزايد لحركة المرور، مبرزاً قصور الخوارزميات التقليدية. وقد جرى إجراء مقارنة منهجية بين الدراسات السابقة من حيث المزايا والعيوب في المواقف الواقعية. إضافةً إلى ذلك، يعرض البحث نتائج أصلية من خلال تقييم تقنيات محددة في التعلم الآلي، ولا سيما الشبكات العصبية الاصطناعية (ANN) ونماذج التعلم العميق (DL)، وذلك باستخدام التحقق المتقاطع ذي عشرة أقسام. وتشير النتائج التجريبية إلى أن ANN حققت أقل متوسط زمن استجابة (١,٩٥٥ ملي ثانية)، تليها مباشرةً DL (١,٩٦٢ ملي ثانية) التي أظهرت أعلى درجات الاستقرار عبر التكرارات. أما آلة متجه الدعم (SVM) عند $C=100$ فقد حققت أفضل دقة تصنيف في التحقق المتقاطع، فيما جاء أداء DL في المرتبة الثالثة و ANN في المرتبة الأخيرة. وعند النظر إلى كلٍّ من الكمون والدقة، قدّمت DL أفضل توازن إجمالي بين السرعة والاستقرار والدقة في موازنة الأحمال ضمن بيئة SDN، متفوّقةً على الخطوط الأساسية التقليدية (LR: 4.461 ملي ثانية؛ SVM: 17.9–18.0 ملي ثانية). وتُظهر هذه النتائج، المرتبطة مباشرةً بالمنهجية المستخدمة، أن أساليب التعلم الآلي المتقدمة أكثر كفاءة لموازنة الأحمال في SDN. كما يعالج البحث تحديات جوهرية مثل قابلية التوسع وقابلية التكيف، ويقترح اتجاهات بحثية مستقبلية نحو تطوير نماذج هجينة مدعومة بالذكاء الاصطناعي لتعزيز كفاءة إدارة الشبكات القائمة على SDN.

الكلمات المفتاحية: الشبكات المعرفة بالبرمجيات، موازنة الأحمال، التعلم الآلي، التعلم العميق.

1. Introduction:

The new revolution in networks is the SDN. In traditional networking devices, the control and data plane is integrated. With an increase in the network, the systems become complex and difficult to handle. This can be overcome with the help of SDN.

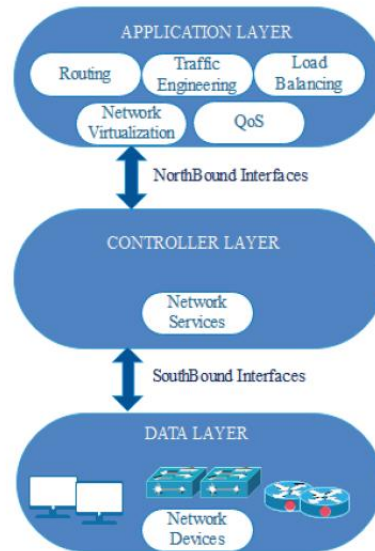


Figure 1: The generic SDN architecture [1].

SDN consists of three layers: data, control, and application. It is highly contrary to traditional network architecture where SDN separates the control plane from the data plane. The separation mainly allows for the management and programming of the network with much ease. The data layer forms the infrastructure comprising switches and routers, while the control layer is seen to be a "brain" since it centrally manages network operations through its interaction with both the data and application layers regarding networking terminology, an "openflow controller" can be thought of as a kind of supervisory application that works inside an SDN environment. The application layer hosts applications that may change the network structure by giving remote access to the controller through northbound APIs, for example, REST. Several southbound protocols such as BGP, NETCONF, XMPP, OVSDB, MPLS-TP, and especially OpenFlow enable communication with the data layer. Among them, the most used southbound protocol is OpenFlow. It allows for more flexible and efficient network management and operation. The major machines will connect to the edge level, while servers are placed at the aggregation and core levels.

- K value in fat tree structure determines how many servers each level will support and the total number of servers that can be connected.
- Decisions are based on the k parameter that helps to make more structured connections. It, therefore makes it rather straightforward to identify the network switch through which the traffic will flow.

Load balancing has two major concepts. First, it is an approach designed to prevent resource overload by shifting a load from a source to multiple sources for sharing in order to help achieve maximum scalability within the network. It is also applied to share one high-load operation among many node devices for parallel processing, which largely boosts up the whole performance. This technique reduces response time, consumption of resources, and load from any single source as well. Most frequently, examples of load balancing include server load balancing and link load balancing. Traditional techniques are based on both hardware and software. In conventional networks, however, there is a difficulty in having an overall view of network status. Thus, it may be difficult to apply the strategy of global load balancing easily. Furthermore, traditional approaches are not able to keep pace with the dynamics taking place concerning the conditions of networks. Another weakness of traditional methods lies in the reliance on specific hardware configurations, which may be expensive, unchangeable, and bound to certain vendors. By contrast, in SDN the load balancer is easily implemented in programmatic code at the controller, so more effective management of network load is achievable [2]. Therefore, two kinds of primary techniques for load balancing are the ones applied in IP networks and those deployed online in the context of an SDN environment. In traditional IP-based load balancing, this process is performed with the support of an LBR. A newly arrived packet first passes through an LBR at the entrance of the network that, after being processed, forwards the packet to one of the available servers based on recent traffic flow conditions by appending the IP address of the server to the packet. The packet is then routed along a computed path to the destination using predefined routing protocols. On the other hand, independently in each and every new packet, SDN performs real-time low-latency server selection and makes immediate decisions on routing with options to send packets across any of the available paths instantaneously [2]. SDN can then classify load balancing techniques as static, dynamic, or a hybrid. In static load balancing, the rules of routing are hardcoded into the load balancer. This might not be effective since it may hurt network performance due to a lack of real-time information about the network. Dynamic schemes, on the other hand, do better in terms of efficiency by distributing loads interactively in accordance with a programmed model; thus, they can adapt to changes in network conditions much more easily. This flexibility in the way traffic is handled makes SDN a fitter platform to implement advanced strategies for load balancing. Among many other fields, machine learning has now emerged as a transformative force on networking. The ability to analyze a huge volume of network data in real time opens the possibility of proactive and effective decision-making capability that becomes quite crucial

in SDN-based systems. These algorithms can predict traffic patterns based on learning from historical data and make decisions that considerably improve the strategy for load balancing. It has also been demonstrated by researchers that ML can improve the efficiency and effectiveness of SDN load balancing in order to handle issues such as traffic congestion and generally enhance network performance [2]. Load balancing is an important aspect of SDN because it ensures resources within the network are used optimally without causing any network bottlenecks or imbalances in traffic. The conventional algorithms in load balancing are based on static configurations that cannot adapt dynamically to the changing conditions of a network. With the integration of ML, the process of load balancing becomes flexible because such algorithms can be dynamically adjusted in accordance with real-time network performance metrics. Indeed, current research depicts that most of the deep learning models achieve substantial enhancements compared to the traditional techniques by reducing response times and improving system accuracy [3]. Despite all the advantages of machine learning integration in SDN load balancing, several challenges are still persisting. However, at this point, the delicacy of models, scalability-related problems, and need for immense computational power to train deep learning models stand in the way of their wide diffusion. Besides, ensuring those models support real-time decision-making without introducing latency into them is still a very active area of research. Several optimization techniques have been studied for reducing the computational overhead of these models for better efficiency in large-scale networks. ML becomes an instrumental factor in enhancing network performance with the evolution of SDN. The future research will be focused on the large-scale and efficiency enhancement of such models to deal with the tough situations occurring in modern DCNs. By integrating the centralized control principle of SDN with predictive capability from machine learning, more robust and adaptive load-balancing solutions can be built that can meet increasing demands from infrastructural underlying networks.

2. Related Work

A. Traditional load balancing techniques in SDN

- *Conventional Load Balancing Techniques*

Network performance management requires traditional load balancing methods, which utilize algorithms such as round-robin, equal-cost multipath routing, least connections, and random techniques. Every method has its own traits and situations where it works best. We use

different performance metrics to evaluate their effectiveness, as explained in. For instance, a round-robin approach fairly divides requests among servers, which helps prevent starvation but adds some overhead due to queuing [6][8]. Several researchers have thus suggested enhancements to these approaches. One approach is the implementation of load sharing through the duplication of controllers within Virtual SDN controllers [6]. The Virtual SDN architecture allows the master controller periodically to implement virtual IP addresses to other controllers in order to share loads. With failover [8][7] the distributed controllers can further split TCP and UDP traffic to make services even more reliable. The issue of controller placement is also examined in various studies of SDN environments, where the optimal locations are those that minimize latency and ensure seamless operation. The controller placement challenge is also addressed in various studies within SDN environments, where optimal locations minimize latency, ensuring reliability [9] [11]. Hierarchical architectures optimize controller and switch placements to improve load balancing [13]. These methodologies indicate significant improvements in both migration costs and overall performances, especially with increasing traffic [15][12]. Switch migration's role in load balancing has gained attention [6]. BalCon and BalConPlus are two methods that reduce migration costs while ensuring an even load distribution [14]. The Flow Stealer method is a lightweight approach that allows idle controllers to share workloads for a short time [16]. These kinds of new ideas make it easier to respond to changes in traffic in dynamic settings [18]. Adding energy-efficient technologies to load-balancing strategies is a significant step forward [19][20]. Using green energy helps make the best use of resources and reduces the need for traditional power sources. Advanced resource allocation processes are made possible by algorithms like QALB and hybrid flow-based methods [21][22]. In general, traditional load balancing methods are continually evolving to address new challenges and improve network performance [23][24].

- *Artificial Intelligence Based Load Balancing Techniques*

In these regards, artificial intelligence techniques utilize metaheuristics approaches in efficiently solving real-world problems of load balancing issues in SDN. AI includes several sub-classes, namely deep learning, neural networks, natural language processing, and decision-making strategies. Characterizing different AI-based techniques for Load Balancing is discussed along with a discussion on performance metrics utilized for evaluating such techniques. These AI techniques and methods will improve learning and decision-making in SDN and will thus facilitate effective load distribution across network resources. Researchers

have also developed heuristic methods to move switches from overloaded controllers to under loaded controllers. For instance, the heuristic in [24] incorporates movements and shifts within a search framework that assesses the potential of every step in migration. This method does not stop working when it detects that a simple migration is impracticable; instead, it investigates complex movements to achieve an optimal performance. Moreover, authors in [25] proposed a publish/subscribe system based on SDN that disseminates efficiently events using a global topology view and Huffman trees to minimize end-to-end latency. Other key techniques of optimization are essential to balance the loads and reduce congestion during different phases. In the work presented by [26], the first two phases create a sub-topology for performance enhancement, which is followed by load balancing. The authors in [27] developed the OCLB method, which would reduce the average response time of controllers using pre-calculated switch migrations based on real-time application data. Moreover, genetic optimization techniques, as shown in [28], can be used to minimize load imbalance in elastic optical networks using a phased approach that guarantees optimal solutions. A variety of techniques improves load-balancing strategies using AI methodologies like PSO and fuzzy logic. For example, the modified PSO technique in [29] efficiently selects the vehicular nodes for reducing power consumption whereas KKT conditions used in [30] help to select the appropriate controller during load balancing. The fuzzy logic in [31] analyzes server load parameters due to which dynamic adjustment of server performance is done to optimize the energy consumption and achieve load balance effectively. Lastly, game theory has been used in traffic balancing among the SDN controllers as obtained in [32]. These methods therefore train the networks to learn about the right flow rates to attain an equilibrium state that enhances load distribution. A greedy approach is employed in [33] for efficient switch migration with the aim of effectively balancing the load, considering migration costs and load variations. Moreover, the framework in [34] investigates multi-criteria decision-making for choosing the target controllers, thereby optimizing the process of load balancing in the environment of SDN.

Table 1: Comparative Summary of Traditional and AI-Based Load Balancing Techniques in SDN

Approach Type	Algorithms / Techniques	Characteristics	Strengths	Limitations	Common Evaluation Metrics
Traditional (Conventional)	Round Robin, ECMP, Least	Static rules based on topology or connection count	Simple to implement, low	Lack of adaptability to dynamic conditions, limited	Response time, end-to-end latency, resource

Approach Type	Algorithms / Techniques	Characteristics	Strengths	Limitations	Common Evaluation Metrics
Enhanced Traditional	Connections, Random, LBR		computational overhead	global view, dependency on specific hardware	utilization, switch migration cost
	BalCon, BalConPlus, Flow Stealer, Controller Placement Optimization	Dynamic migration or multi-controller deployment	Reduced migration cost, improved reliability	Still limited in traffic prediction and adaptability	Controller delay, scalability, service reliability
	ANN, Deep Learning, SVM, PSO, Fuzzy Logic, Game Theory, Genetic Algorithms	Learning-based and metaheuristic models	Dynamic adaptation to network changes, traffic prediction, multi-criteria optimization	High computational demand, model complexity, possible added inference latency	Classification accuracy, response time, throughput, energy efficiency
	Hybrid ML + Heuristics (e.g., OCLB, QoS-aware ML)	Integration of ML with heuristic/decision-making methods	Balance between high accuracy and low response time	Complex design and training, large data requirements	Scalability, flexibility, congestion avoidance success rate

B. MACHINE LEARNING IN SDN

SDN has a centralized controller that can see an overall picture of the network and hence allows for easier management and control. Machine learning techniques will further enhance these capabilities through advanced data analysis, optimization of the network, and automatic provisioning of services. This will, in turn, enable the controller to self-learn and optimally make decisions in response to changes in the network on its own. This section highlights various challenges that require the use of machine learning in overcoming many challenges related to SDN: classification of traffic, routing optimization, prediction of QoS and QoE, managing resources, and security. Further, we describe how machine-learning algorithms are put into place in the context of the SDN.

1) Elephant Flow-Aware Traffic Classification

Elephant flow-aware classification is used to classify two kinds of flows: elephant flows, which are long-lived and bandwidth-intensive, and mice flows, which are short-lived and delay-

sensitive. In data centers, roughly 80% of flows are mice flows, while most of the volume of data is carried by elephant flows. Classifying elephant flows is thus of vital importance for efficient traffic management. According to various researches, it is possible to allow the central SDN controller to apply other more effective traffic optimization techniques according to the classification result using machine learning techniques for classifying traffic at the network edge. One argued that within SDN, detection of elephant flows could be done by cost-sensitive learning. The approach comes with a two-phase strategy wherein it first measures the head packet in order to distinguish suspicious elephant flow from mice flow. Afterwards, in its second phase, it uses a decision tree to verify the flows if they are truly elephant or not. The proposed elephant flow detection system consists of three major components: Flow Collector, Real-time Detection Strategy, and Classifier. The Flow Collector is responsible for collecting the input to the system by capturing the IP packets from the SDN network, and the flows will be recognized depending on IP packet header inspection. Each flow is identified by using a five-tuple that represents source and destination information and statistical features of duration and packet count. In the processing stage, the Real-time Detection Strategy module will extract key features from the collected flows to classify them effectively. This module shall adopt a hybrid strategy that encompasses TCP flow characteristics and DPI attributes to select the relevant n-tuple features, essential in the correct identification of elephant flow. The objective of this module shall be to have an appropriate feature selection to develop an efficient and robust classification. The Classifier module classifies each n-tuple precisely into an elephant flow or not following the feature extraction. It may employ any statistical method or machine learning algorithm to do the classification; in [36], the C4.5 decision tree is used as the detection method. Another contribution proposed here is the cost-sensitive approach in order to improve the efficiency of the elephant flow detection, since accurate elephant flow detection can help with optimal resource allocation within the network and avoid congestion. This system enables the correct identification of elephant flows by users and the implementation of necessary optimizations in traffic management. Fig. 2.

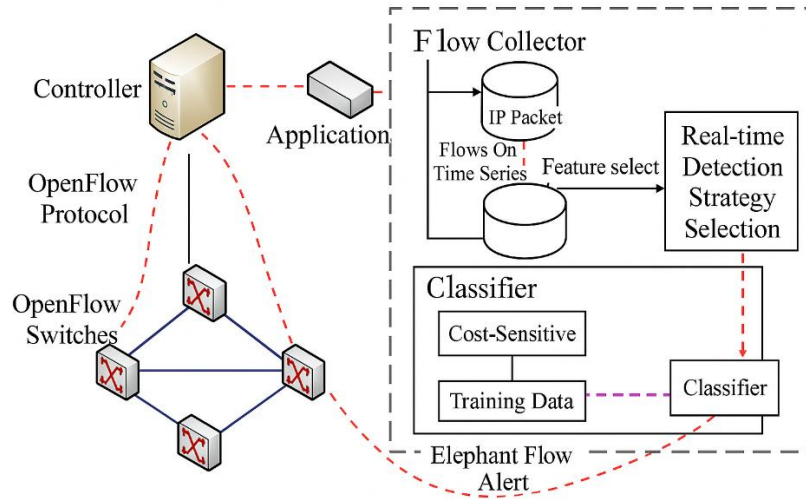


Figure 2: The System model detecting elephant flows[36].

In this section, we present a two-stage strategy for elephant flow detection, as illustrated in Fig. 2. This method is designed to quickly and efficiently identify elephant flows using statistical thresholds and flow features over time series.

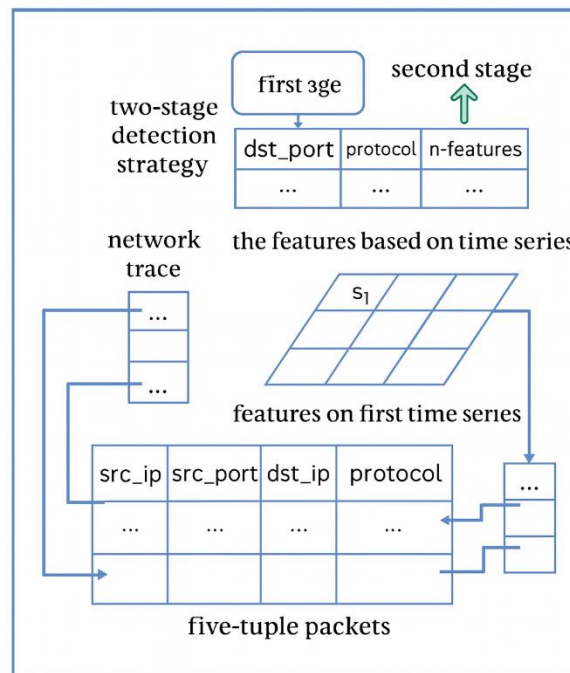


Figure 3: Two-Stage Elephant Flow Detection Strategy [36].

In the first stage, we distinguish large flows from small flows using head packet measurement. This differentiation is based on thresholds such as destination port and protocol.

The suspicious flows identified at this stage are forwarded to the second stage to enhance accuracy. In the second stage, n -features are extracted from the statistical data collected in the initial time series, including parameters like `c2s_pkts` and `s2c_psmx`. To optimize the feature set for the dataset, we employ a correlation-based filter (CFS), which plays a crucial role in the feature selection process [36]. This two-stage approach enables more precise and efficient detection of elephant flows, which is particularly significant for improving traffic management in SDN networks.

2) Application-Aware Traffic Classification

Machine learning methods for identifying internet traffic types typically work with data collected from specific protocols known as IPFIX. These protocols assist Internet Service Providers (ISPs) in gathering and processing traffic data from routers. IPFIX aggregates packets to provide information about flows, such as duration and bit rate, which are then used in a separate system for traffic classification [37]. Software-Defined Networks (SDN) offer new approaches for collecting and managing this data. OpenFlow, a protocol for SDN, directly gathers flow statistics and connects packets to flows based on specific rules. This allows information to be collected from various points in the network, ranging from low-traffic access switches to high-traffic core switches. One of the advantages of SDN is that statistics and information are sent directly to the controller, enabling it to respond quickly and automatically. For example, if the controller learns information about a traffic flow, it can swiftly apply new rules to manage that flow. Additionally, SDN allows for customization of the types and numbers of packets that need to be analyzed. In general, most of the procedures of traffic classification in SDN consist of several major steps. An SDN application connects to switches using the OpenFlow protocol and instructs them to forward all incoming traffic to the controller. These include recorded TCP packets through port mirroring or direct data collection, respectively, capturing important information such as transport protocol and flags of the TCP. It inspects the packets, identifies, counts, and logs the first five packets exchanged between the client and the server. After that, all the remaining packets are handled locally inside the switch, in order to minimize the load of Packet In messages at the controller (Fig 3). After data collection and analysis, it creates a labeled dataset to train machine learning models. Therefore, the data is divided into a training-testing set, and models trained on algorithms such as Random Forest and Gradient Boosting are used. For the measurement of the accuracy of these models, the results predicted by them are compared against the actual labels. Further, the average of these is

determined. The obtained results can finally be used for flow optimization and managing traffic in SDN networks, which can be further extended for legacy networks [37].

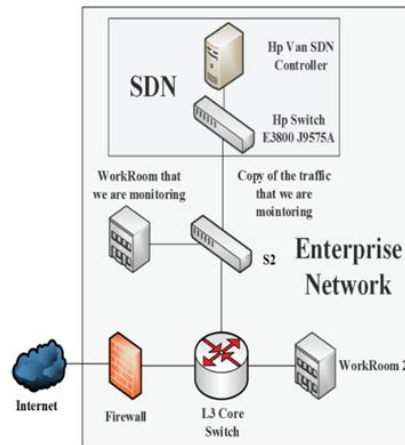


Figure 4: Enterprise Network Setup for Application-Aware Traffic Classification in SDN [37].

Application-aware traffic classification focuses on determining the specific applications associated with various traffic flows. Amaral et al. conducted research in enterprise networks to explore this concept. In their approach, a straightforward OpenFlow-based SDN system was established within an enterprise environment to collect traffic data. Various classification algorithms were employed to categorize the traffic flows based on the identified applications. A system known as MultiClassifier was introduced, which integrates both machine learning (ML) and Deep Packet Inspection (DPI) techniques to identify applications. When a new flow is detected, the ML classifier is initially utilized for classification. If the reliability of the ML classifier's output exceeds a predetermined threshold, this result is accepted as the final output of the MultiClassifier. Instead, the DPI method is employed. If the DPI classifier does not return a "UNKNOWN" result, its output is chosen as the final classification. In a related area, another study evaluated the process to classify applications that implement the UDP protocol. Specifically, it proposed a behavioral classification engine to improve the accuracy of application-aware traffic classification. Building upon this, we deployed the Support Vector Machine (SVM) algorithm to categorize UDP traffic based on NetFlow records, which include the number of packets and the total number of bytes. Simulation results indicated that this SVM-based classification engine achieved an accuracy rate of over 90%. Furthermore, research on mobile application classification introduced a framework called Atlas, which aims to identify mobile applications effectively. This framework uses a crowdsourcing approach to gather accurate data from end devices, which is then utilized to train a decision tree. The resulting

model is capable of identifying mobile applications associated with the analyzed traffic flows. Simulation outcomes demonstrated that Atlas achieved an average classification accuracy greater than 94% for the top 40 applications available on Google Play. In another research effort, deep neural networks (DNNs) were utilized to identify mobile applications. Data was collected from an experimental network regarding mobile network traffic, and five flow features destination address, destination port, protocol type, TTL, and packet size were selected to train an 8-layer DNN model. The simulation results revealed that this model attained a remarkable accuracy of 93.5% in recognizing 200 distinct mobile applications. Mobile applications often produce various types of traffic flows. For example, the Facebook application can generate video, voice, instant messaging (IM), and file sharing traffic flows. To facilitate a detailed and fine-grained classification of mobile application-aware traffic, it is crucial to identify both the specific mobile applications and the corresponding types of flows. A sophisticated system called TrafficVision was proposed for this purpose in an SDN-enabled wireless edge network. The TrafficVision Engine (TV Engine) is the most important part of TrafficVision. It is a crucial component of the overall design and operation of the SDN-enabled system. Figure 2 illustrates the architecture of the TrafficVision Engine (TV Engine), comprising three main components. The first thing it does is collect, store, and analyze flow statistics and ground-truth training data from end-user devices and access points. This basic step is crucial for ensuring that applications are correctly identified. Second, the TV Engine utilizes a decision tree classifier to identify various application names, such as Facebook, YouTube, and Amazon. It also utilizes a k-Nearest Neighbors (k-NN) classifier to distinguish between different types of flows, such as video content, audio files, and video chats. Together, these components facilitate a detailed and efficient mobile application-aware traffic classification system within SDN-enabled wireless edge networks.

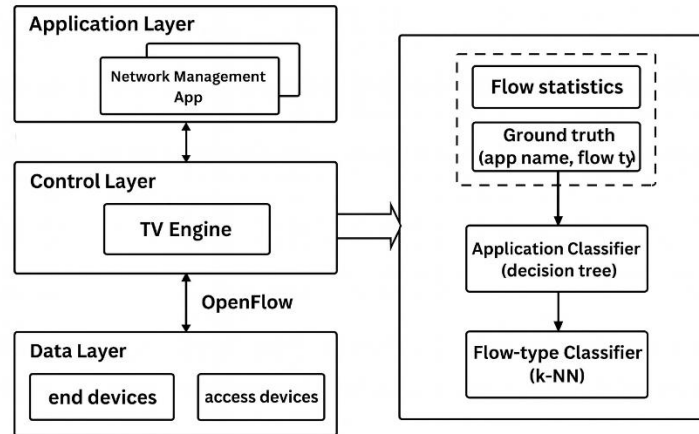


Figure 5: The architecture of Traffic Vision, which is designed for Software-Defined Networking (SDN), operates at a high level and includes the workflow of the TV Engine

The TV Engine performs three primary functions: (1) it gathers flow statistics and authentic training data from both end devices and access points, (2) it utilizes a decision tree classifier to determine the names of applications, and (3) it employs a k-nearest neighbors (k-NN) classifier to categorize different types of data flows.

3) Impact of QoS-Aware Traffic Classification on SDN Performance

QoS-aware traffic classification has a high impact on the performance of Software-Defined Networks. In this method, an SDN can manage flows according to their QoS class requirement, specified in terms of maximum delay, jitter, and packet loss rate. This way, network resources are utilized much more intelligently. Delay-sensitive traffic-that is, video calls or other similar real-time applications-will be processed quicker. Therefore, the network will be able to produce higher performance and yield better user experience in respect to various applications. It is hard to identify all the applications in the internet environment; QoS classification classifies the traffic flows according to QoS requirements rather than identifying each application. This enables SDNs to manage traffic in a more effective way without requiring precise knowledge of specific applications. Using semi-supervised learning and some techniques like Deep Packet Inspection, the network can have an exact classification and management even in unknown traffic flows, improving the accuracy of the classification and enhancing traffic management. Besides, the QoS-based classification helps SDNs in optimizing the utilization of network resources and forwarding the traffic to meet its actual needs, thereby avoiding congestion and improving overall network efficiency. By applying machine learning

and deep learning algorithms, SDNs manage to process large and complex datasets of flow traffic more efficiently to let the network become more intelligent towards dynamic and changing conditions. In summary, this approach makes for a very important role in augmenting the quality of the services and overall efficiency of the SDN networks. In [35], a QoS-aware traffic classification system is proposed which combines semi-supervised learning algorithms and DPI. Here, the DPI only labels some of the traffic flows from known applications, and then semisupervised learning algorithms, such as Laplacian SVM, use the labeled training data to classify the traffic flows from unknown applications. This provides the capability for the system to efficiently classify both known and unknown traffic into different QoS classes. Simulation results demonstrate that the achieved high accuracy of the developed traffic classification system attains more than 90% in the exactness of the classification for different kinds of traffic flows. This becomes very important for SDN systems because this allows the network to make more realistic decisions about each flow QoS requirements by optimizing resource allocation and traffic management. It not only enhances the precision and efficiency in traffic management but also tackles the challenges brought about by exponential growth in diversified internet applications. Due to the fact that it categorizes the traffic based on QoS needs rather than identifying each application, it proves highly effective in dynamic and variable environments such as SDNs, which may improve performance and reduce congestion in networks.

Table 2: Machine Learning-Based Traffic Classification Solutions in Software-Defined Networks (SDN)

ref	Objective	Learning Model	Dataset Input	Dataset Output
[36]	Elephant flow-aware classification	Decision tree	Basic five-tuple, statistical flow features	Elephant flow, mice flow
[37]	Application-aware classification	Random forest	12 flow features (packet size, inter-arrival time, flow duration, etc.)	8 applications (e.g., YouTube, Skype, Dropbox)
[38]	Application-aware classification	ML classifier	Not mentioned	Not mentioned
[39]	Application-aware classification	SVM	Basic five-tuple, Netflow records (packet, byte counts)	8 applications (e.g., PPstream, Skype, DNS)
[40]	Application-aware classification	Decision tree	Flow features (packet sizes, source/destination ports)	Top 40 apps in Google Play Store
[41]	Application-aware classification	Deep NN	Destination address, protocol type, TTL, packet size	200 mobile applications
[42]	Application-aware classification	Decision tree, k-NN	Flow features (per reference [7])	Dataset 1: 37 apps, Dataset 2: 45 apps
[35]	QoS-aware classification	Semi-supervised learning	9 flow features (packet inter-arrival time, Hurst parameter, etc.)	Four QoS classes (voice/video, streaming, etc.)

3. Machine Learning Techniques for Load Balancing

Machine learning has been realized as one of the innovative ways of load balancing in SDN. It involves the use of mathematical and statistical algorithms to analyze information and predict network behavior. Machine learning will enable the system to recognize the patterns in the traffic and select the best paths which data shall take based on pattern recognition. Such algorithms predict traffic behaviors by analyzing historical and current network data, thus helping network managers make better decisions regarding network traffic. Certain examples of machine learning applications in SDN involve identifying the pattern of traffic load and predicting conditions in the future. For instance, using traffic data gathered, it can be predicted using machine learning algorithms which links and nodes will be highly loaded in the future. Therefore, the SDN controller may make better decisions on the distribution of the traffic over the network and choose routes in order to avoid highly congested paths for routing data. Hence, the efficiency of the network improves and the possibility of bottlenecks and unexpected outages becomes low [43], In addition, machine learning further enhances the response to failures by enabling quick detection of link or node failures through highlighting appropriate alternative paths with which data transmission can continue. In that way, the network remains functional without degradation in performance. Overall, the application of machine learning in the traffic management of SDN networks contributes to load balancing optimization, greatly improving the reliability and efficiency of the network.

C. Supervised Learning Methods

The controller can identify all paths between two nodes and determine the optimal load conditions for each path by updating the network's topology information. This broad view enables the controller to make more informed decisions about managing traffic and balancing loads. Initially, network simulation is conducted to gather data, and then it is repeated to validate the results. The first step in this process is to start the Floodlight controller, which serves as the foundation for the network simulation. After that, Mininet is used to set up the network topology.

At this stage, the user inputs the source host, and then the least loaded host is selected as the destination. Traffic is generated between these hosts, and the Wireshark tool is utilized for monitoring this traffic [43], Next, Dijkstra's algorithm is employed to determine the shortest path(s) between the source and destination. The machine-learning module is enabled to select

the optimal path when multiple shortest paths are available. If not, the shortest path found is the best one. This process helps improve traffic flow and ensures that packets reach their destination as quickly as possible. To manage failures, the SDN controller first identifies link failures, particularly when a switch is unable to send a packet-in message to the controller within a predefined time period. In such cases, the SDN controller notifies the load balancer about the link failure, temporarily removing all paths that include the faulty link and identifying the least loaded path. Additionally, in the event of a node failure, the controller identifies the backup path and uses it to maintain connections between the source and destination hosts. These mechanisms enhance the efficiency and stability of the network while enabling effective load management. The flow diagram of the system is shown in Figure 6.

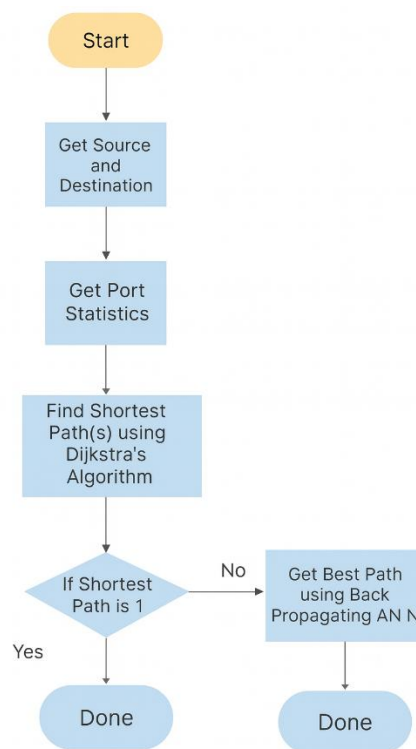


Figure 6: The Machine Learning Techniques for Load Balancing [43]

D. Deep learning models for load prediction and optimization

Deep learning is a way of coming up with machine learning in cascading layers for feature extraction from the input data towards making decisions. What leads the most among principles behind deep learning is that there is learning of data representations at gradually higher levels of abstraction. For almost all levels, higher-level abstract representations get derived from lower-level representations. This is what generally makes this hierarchical process of learning

effective. It can perceive, understand and learn complex representations directly from raw data, which finds an application in many fields [1].

Three or four elements or parts commonly show up in an application:

- 1) Representation, i.e. how the state of the environment can be represented in handy numerical formats serving as the input layer for the DL network.
- 2) How the results of the validation shall be represented or interpreted in such a way as to reflect the physical meaning of the output layer of the DL network.
- 3) How the reward value shall be calculated and updated, together with the determination of an appropriate reward function that will guide the iterative updates of weights in each neural layer.
- 4) DL architecture, which includes the number of hidden layers, material design for each layer, and layer inter-relations, is done. Deep learning has solved various open issues in many application areas including computer vision, mobile and wireless networks, natural language processing.

Model architecture: The flow chart of the experiment model architecture is depicted in Figure 7. First of all, the Mininet emulator is selected and then a k-ary fat-tree topology is created.

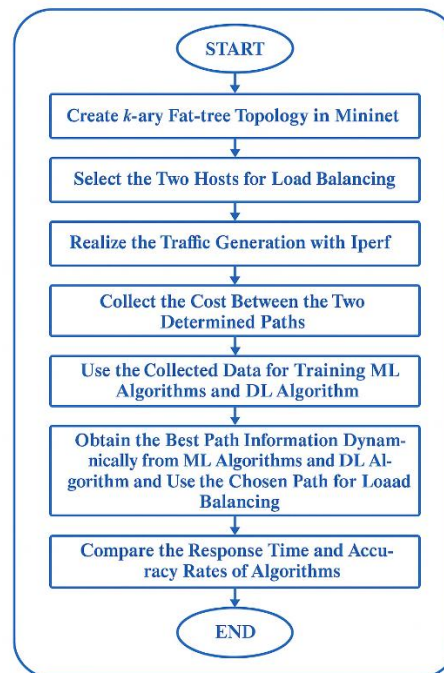


Figure 7: The Deep Learning Techniques for Load Balancing [2].

Then, two hosts are randomly chosen to initiate the load balancing process. For this, tx and rx transmission and receiving rates are enabled in Floodlight. Traffic between them is generated using Iperf. Finally, respective costs between the selected paths are gathered. Route information like IP addresses, switches, MAC addresses, and port mappings are accessible by using Rest-API. This data provides the cost between these two paths in milliseconds on a text file (.txt). This additional file can be retrieved in Ulu. On the Floodlight side, the costs are gathered, and configurations are modified for the enablement of Rest-API operations. The paths cost computation:

(1)

$$Cost = tx(transmitted) + rx(received)$$

Equation (1), adapted from [2], defines the cost as the sum of transmitted (tx) and received (rx) packets. This formulation has been widely used in SDN-related studies as a straightforward performance indicator, and in this work, we adopt it to ensure consistency with prior cost-based approaches. Total cost is calculated as the summation of all tx and rx values. The data so collected forms the training input for ML and DL. In-Port, Out-Port, Source IP, Destination IP, Source MAC, Destination MAC, etc. The program updates this information per minute, thus making it dynamic; once the best path through which data is to be sent is estimated using algorithms through ML and DL, then this information is used for load balancing. The block diagram of the load balancing mechanism used in the simulations is illustrated in Figure8.

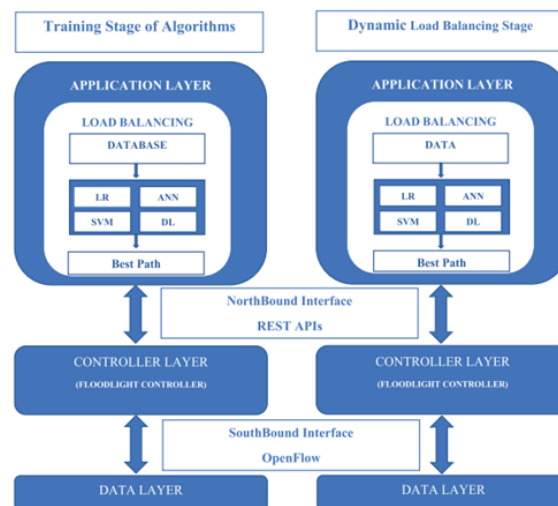


Figure 8: The load balancing mechanism in the simulations [2].

Two situations are looked at. In the first case, connections are made in MiniEdit, and two hosts (Host 1 [h1] and Host 4 [h4]) are chosen to share the load. The REST API gathers information about the costs of routes between these two hosts, which helps determine a route that incurs the least cost. The selected paths are h1, switch7 (s7)-switch4 (s4)-switch8 (s8) and h4, h1-s7-switch3 (s3)-s8-h4. Cost values for the paths are obtained using Rest-API by providing the MAC addresses of the switches. An example of a data entry might look like "02 :: 15 :: 01' : 288, ' 02 :: 0a :: 01' : 1,530." Based on the obtained route information and cost values, classification values are added to the database. A sample data row in the database is "288, 1,530, 0." In the second scenario, load balancing occurs over different routes than in the first scenario. Connections to MiniEdit are reestablished, and two new routes (h1–h3) and (h6–h7) are selected simultaneously. The REST API collects road cost information for these hosts, which helps them choose the most cost-effective routes. As in the first scenario, classification values are added to the database, with a sample data line looking like "1,254, 694, 1, and 81,659, 80,769, 1." A total of 800 training data points and 192 test data. points are generated. This database is then utilized to train ML and DL algorithms. For ML, algorithms like Logistic Regression (LR), Support Vector Machine (SVM), Artificial Neural Network (ANN), and DL are used to compare results during training and testing. Specifically, the SVM algorithm has a C parameter that can be set to 1 or 100. Regarding the ANN model, as shown in Figure 6, it has two layers: the first layer has five nodes, and the second layer has one node. The learning rate is set to 0.00025. The first layer employs the ReLU activation function, while the second layer utilizes the sigmoid activation function. The paths' input variables are shown as x0 and x1. In the case of DL, Figure 9 shows the four-layer model used.

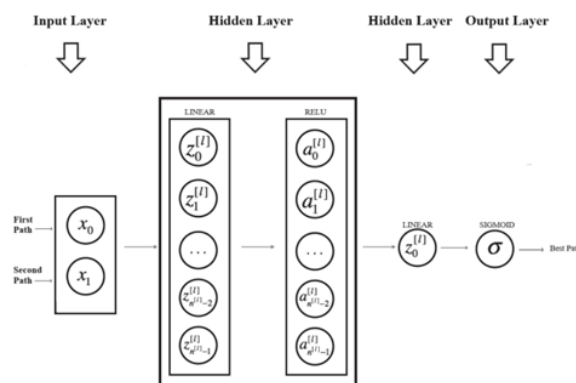


Figure 9: The DL model for load balancing [2].

This model has 20 nodes in the first layer, 7 nodes in the second layer, 5 nodes in the third layer, and 1 node in the fourth layer. The activation function for all layers except the fourth is the ReLU function. The activation function for the fourth layer is the sigmoid function. The input variables for the paths are still x_0 and x_1 , and the notation [1] shows which layer they belong to. The letter Z stands for the pre-activation parameter.

4. Experimental Evaluation

In their study, B. Babayiğit and B. Ulu used deep learning techniques to optimize load balancing in SDN within data centers. They tried several machine learning models, including deep learning techniques that improve the network performance-traffic management. The research study in the paper gives insight into how AI-based methodologies can enhance both scalability and responsiveness of SDN-based networks and, finally, provide an efficient way of solving load balancing strategies [2]. These measurements are response times in milliseconds and are shown in Figure 10 and listed in Table 3. The researchers tested the LR, SVM, with $C = 1$ and $C = 100$, ANN, and DL algorithms in their experiments. Therefore, these measurements give a view of each algorithm's performance under several different network conditions. The DL model for load balancing [2].

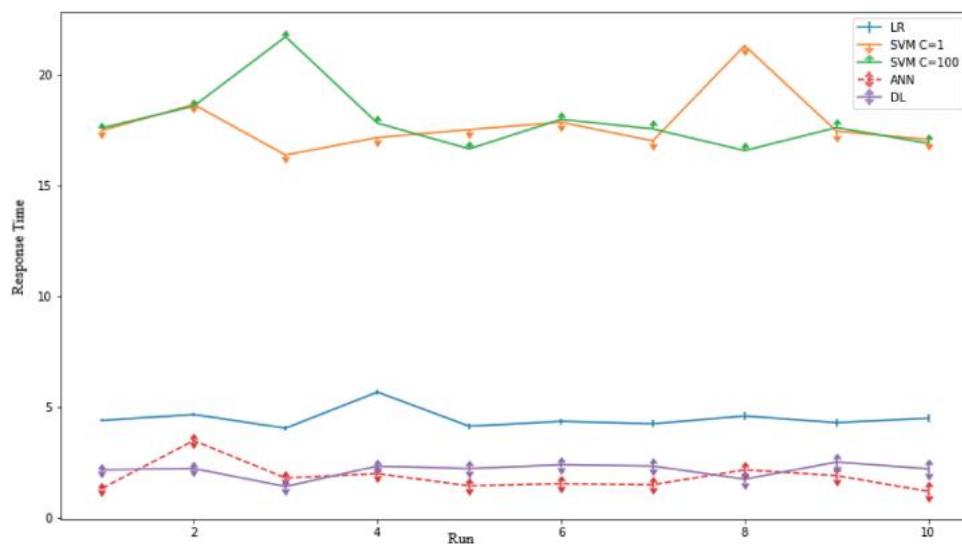


Figure 10: The response times for the LR, SVM (with $C = 1$ and $C = 100$), ANN, and DL algorithms in the second scenario [2].

Based on Figure 10, a clear performance gap is observed among the evaluated algorithms in terms of response time across ten runs. The SVM classifiers (C=1 and C=100) exhibit the highest response times, ranging from approximately 16 to 22 milliseconds, and show noticeable fluctuations, indicating lower stability. Logistic Regression (LR) gets average results, with response

Running	LR	SVM C = 1	SVM C = 100	ANN	DL	times of
1	4.20	17.32	17.79	2.10	1.97	
2	4.97	18.17	18.02	1.32	1.57	
3	4.09	16.53	20.98	1.82	1.63	
4	4.94	17.92	18.65	1.29	1.27	
5	5.11	17.46	17.08	1.94	2.37	
6	4.77	18.10	18.01	2.68	2.04	
7	4.03	19.40	16.56	1.68	2.70	
8	4.10	20.12	16.72	2.64	1.57	
9	4.25	18.96	18.12	2.44	1.75	
10	4.15	16.09	17.03	1.64	2.75	

about 4–5 milliseconds, and its performance is fairly stable. The Artificial Neural Network (ANN), on the other hand, typically has the fastest response times, which are usually between 1.5 and 2.5 milliseconds, although they vary slightly from run to run. Deep Learning (DL) models work almost as well as ANN, but they are more stable and don't change as much. Their response times are a little longer, ranging from 1.5 to 2.7 milliseconds. In general, ANN is the fastest algorithm, and DL is the most reliable and consistent. This shows that advanced ML techniques are better than older ones like SVM and LR for SDN load balancing.

Table 3: The response times (in milliseconds) for the LR, SVM (with C = 1 and C = 100), ANN, and DL algorithms in determining the optimal path for the second scenario [2].

This table presents a comparative analysis of Logistic Regression (LR), Support Vector Machine (SVM) with C=1 and C=100, Artificial Neural Networks (ANN), and Deep Learning (DL) in ten consecutive runs in terms of response time (milliseconds). The results reveal a significant performance gap among the evaluated methods. The SVM algorithms, under both C values, demonstrated the weakest performance; their response times ranged between 16 and 22 milliseconds, and in addition to having a high average, they showed considerable fluctuations across runs, indicating low stability. In contrast, LR showed moderate performance with response times in the range of 4–5 milliseconds. While slower than ANN and DL, LR maintained more consistent results compared to SVM.

In contrast, ANN and DL outperformed other methods. ANN consistently had the fastest response times, ranging from 1.3 to 2.6 milliseconds, resulting in the lowest average delay. DL performed nearly as well, but was more stable with less variation, posting response times from

1.2 to 2.7 milliseconds. These small differences suggest ANN is ideal for applications needing low latency, while DL is preferable for environments that value stability and adaptability. Overall, the table demonstrates that although traditional approaches such as LR and especially SVM are computationally simpler, they cannot compete with ANN and DL in terms of response time for SDN load balancing.

5. Challenges and Future Directions

Along with continuous growth in the area of machine learning and load balancing in SDN, a number of works remain to be pursued towards surmounting challenges. Among those are the responsiveness and adaptability enhanced by algorithms under dynamic environments. Network conditions fluctuate due to rapid changes in traffic loads, failures of nodes, and modification within network topologies. An algorithm showing quick adaptability with optimal performance against such changes has their importance in such scenarios. Another challenge is scalability: the network sizes are increasing each day, which increases the computational complexity of the load balancing algorithms, hence worst response time and less efficiency. These need scalable solutions that can ensure better performance on larger networks. Also, there is a need for more scope in integrating AI techniques within the existing architecture of SDNs. This integration can make different decisions for various processes in real time to help enhance the overall efficiency in load balancing mechanisms. Future directions may relate to exploring hybrid models leveraging the strengths of different machine learning techniques to exhibit superior performance. There is also the security perspective, which always remains a very critical concern. Since load balancing algorithms are achieved by the sharing of data and communications between nodes, the confidentiality and integrity of their data are crucial. It is highly recommended that studies on secure techniques for load balancing against eventual vulnerabilities be carried out in order to increase reliability within SDN environments in general.

6. Conclusion

Machine learning algorithms for load balancing in SDN show promise as a way to make networks more efficient and better manage resources. Although ANN and DL models have demonstrated better response times and accuracy, there are still some issues that need to be addressed before they can be applied in real-life scenarios. One significant issue is that deep

learning models are computationally expensive to run, requiring substantial processing power, memory, and time to train. In large-scale SDN deployments, these requirements may lead to scalability bottlenecks and increased operational costs unless optimized through techniques such as model pruning, quantization, or GPU/TPU acceleration.

Another challenge is the need for models to adapt to evolving traffic patterns. Recently, hybrid strategies that integrate traditional optimization methods (such as Dijkstra's algorithm and heuristics) with AI-driven models have demonstrated effectiveness. Combining static, rule-based techniques with deep reinforcement learning can achieve a balance between real-time adaptability and reduced computational load. Ensemble approaches that integrate ANN, SVM, and decision trees can further bolster system resilience to diverse traffic types.

Future research should focus on developing resource-aware AI models that can dynamically scale according to available computing resources while maintaining accuracy. Also, energy-efficient versions of DL models are needed to make deployment possible in real-world data center networks. These models could use edge computing or lightweight federated learning methods. To ensure that SDN load-balancing systems are more accurate, responsive, cost-effective, sustainable, and scalable, these problems must be addressed.

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Correlation between Vaginal Yeast Infection and Interleukin -10 gene Polymorphism in Women within Mosul City

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Abstract:

Vaginal candidiasis is a common condition among women due to genetic and environmental factors, which are associated with the causative agents of *Candida* species sp, leading to physical symptoms in affected women. Sixty-five samples (45 samples from women with vaginal candidiasis and 20 samples from healthy women) were collected from vaginal swabs and blood samples. Microscopic diagnostic tests were performed to investigate and identify *Candida* species. Tetra-ARMS PCR was also conducted to determine the association of the interleukin-10 gene polymorphism with vaginal candidiasis in women from the city of Mosul, northern Iraq. Furthermore, genetic variation results were analyzed using five molecular tests. The microscopic examination results of vaginal swabs revealed four types of yeast: *Candida albicans* represented 67% of sample frequency, *C. glabrata* 21%, *C. tropicalis* 7%, while *C. krusei* represented 5% of sample frequency. Moreover, the results of Tetra-ARMS-PCR of blood samples from infected women showed a higher percentage of the wild CC genotype (70%) compared to the mutant TT genotype (12%), while the heterozygous CT genotype was (18%) compared to the healthy group (55%, 5%, 40% respectively). The frequency of the (C) allele in infected samples was high (80%) compared to the mutant (T) allele (20%), whereas

in the healthy group, the frequencies of the C and T alleles were (75% and 25% respectively). The Hardy–Weinberg equilibrium test showed an environmental factor effect and the disappearance of the genetic factor.

Keywords: *Candidas spp*, vaginal yeast infection, Interleukin-10.

الارتباط بين عدوى الخميرة المهبلية وتعدد أشكال جين Interleukin -10 لدى النساء في مدينة الموصل

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المستخلص:

يعدّ داء المبيضات المهبلي حالة شائعة بين النساء بسبب العوامل الوراثية والبيئية، والتي ترتبط بالعوامل المسببة لأنواع المبيضات، مما يؤدي إلى أعراض جسدية لدى النساء المصابات. أُخِذَت خمسة وستين عينة (٤٥ عينة من النساء المصابات داء المبيضات المهبلية و ٢٠ عينة من النساء السليمات) من مسحات مهبلية وعينات دم. أُجريت الاختبارات التشخيصية المجهرية للتحقيق وتحديد أنواع المبيضات. كما تم إجراء تفاعل البوليميراز المتسلسل Tetra-ARMS لتحديد علاقة تعدد أشكال جين Interleukin-١٠ مع داء المبيضات المهبلية للنساء في مدينة الموصل، شمال العراق. علاوة على ذلك، تم تحليل نتائج التباين الجيني باستخدام خمسة اختبارات جزيئية. أظهرت نتائج الفحص المجهرى للمسحات المهبلية وجود أربعة أنواع من الخميرة؛ مثلت المبيضة البيضاء نسبة تكرار العينة ٦٧٪، و *C.glabrata* نسبة تكرار العينة ٢١٪، و *C.tropicalis* نسبة تكرار العينة ٧٪، بينما مثلت *C.Krusei* نسبة تكرار العينة ٥٪. فضلاً عن ذلك، أظهرت نتائج تفاعل البوليميراز المتسلسل رباعي التكافؤ (Tetra-ARMS-PCR) لعينات الدم المصابة نسبة أعلى من النمط الجيني CC البري (٧٠٪) مقارنةً بالنمط الجيني المتحور TT (12٪)، بينما كانت نسبة النمط الجيني المتغاير CT (١٨٪) مقارنةً بالمجموعة السليمة (٥٥، ٥، ٤٠٪) على التوالي. كان تكرار الأليلات (C) في العينات المصابة مرتفعاً (٨٠٪) مقارنةً بالأليل المتحور (20٪) (T)، بينما في المجموعة السليمة كان تكرار الأليلين C و T (٧٥٪، 25٪) على التوالي. أظهر اختبار توازن هاردي-وينبرغ تأثير العامل البيئي واختفاء العامل الوراثي. تعدّ فطريات المبيضات السبب الرئيسي لعدوى الخميرة المهبلية لدى النساء. إضافةً إلى ذلك، لوحظ تأثير العامل البيئي، كما لوحظ اختفاء التأثير الجيني لجين الإنترلوكين-١٠.

الكلمات المفتاحية: أنواع المبيضات، داء المبيضات المهبلية، الإنترلوكين-١٠

1. Introduction:

The abnormal growth of the female genital system's mucous membrane is a hallmark of vaginal yeast infection which results in the infection of the vagina with *Candida* sp. [1]. As it is able to inflict the disease, it possesses the relevant enzymes and metabolic systems that enable it to survive in the changing temperature of the host according to the epidemic and to overcome its defense mechanisms [2]. As *Candida* sp. has virulence factors that help it to stabilize and inflict the disease and confronting the host's defense mechanisms, including the morphological change and the formation of the biofilm, secreting the external enzymes and fungal toxins, the damage of the epithelial cells, resisting the phagocytosis and evading the immune cells and all this contribute to the development of the disease, the persistence of the epidemical factors and defeating the defense mechanisms of the host [3]. Moreover, depending on the species, the infection kind, and the host's response [4]. The most typical symptoms include intense itching in the vagina or around the vulva, thick, white discharge that resembles cottage cheese and is frequently odorless, burning or irritation when urinating or having sex, redness and swelling in the vaginal or vulvar area, vaginal pain or a pressure-like sensation, and in some extreme cases, skin cracking around the vagina [2]. This is due to factors including long-term use of antibiotics, hormonal changes, a weak immune system, uncontrolled diabetes, tight or non-cotton underwear, and psychological stress [4]. Recent research has shown that the host's genetic variety is crucial in determining the severity of the invasive fungal infection and its susceptibility to infection, indicating that genetic variances play a crucial role in fungal infections. The gene *IL-10* is located on the 2p36.3 chromosome and this gene encodes the cytokine protein, which adjusts the fungal infection through anti-inflammatory practices against the infection and it is produced mainly by the monocytes and to a lesser degree, it is produced by the lymphocytes. The cytokine has multiple effects in terms of immunological regulation, because it controls the expression of Th1 cytokines, MHC class II Antigen and macrophage costimulatory molecules. Additionally, it increases the persistence and frequency of B-cells. This cytokine also inhibits NF-Kappa B activity and helps to coordinate the JAK-STAT signal pathway [5,6]. As the occurrence of the mutation in the gene *IL-10* makes it vulnerable to the infection with the *Candida* sp. due to the defect or the absence of immunity to the fungi [7]. In addition to that, the increase of the infection of with the opportunistic fungal diseases could be due to the multiple genetic morphologies that control the diseases and the

environmental and genetic factors as well [8]. Research objectives are: to investigate the prevalence of vaginal yeast infections among women in Mosul, Iraq, isolate and identify the *Candida* species causing the infection using microscopic examination, study the relationship between interleukin-10 gene polymorphisms and vaginal yeast infections using Tetra-ARMS PCR, analyze the genetic distribution of the different types (CC, CT, TT) and compare them between infected and healthy women, assessing the frequency of alleles (C and T) and determining their relationship to the risk of infection, determining the influence of environmental and genetic factors on the spread of infection using the Hardy-Weinberg equilibrium test.

2. Materials and methods:

2.1. Specimen collection

Sixty-five specimens were collected from women at Al-Batoul Teaching Hospital in Mosul, Iraq, during the period from 22/1/2024 to 8/4/2024 after obtaining ethical approval from the College of Environmental Sciences, University of Mosul, under Protocol No. 62 dated 17/1/2024. (45) specimens of the specimens were taken from women with vaginal yeast infection (experimental group) and (20) who were infection from healthy women (control group) from vaginal swabs and blood, where (5) ml of each specimen was taken from venous blood and preserved in EDTA anticoagulant tubes. The ages of the women ranged between 20-50 years. [9].

2.2. Isolation and identification of fungi:

Vaginal swabs were cultured on Sabouraud Dextrose Agar and incubated at 37°C for 48 hours. Fungal colonies were examined microscopically using Lactophenol blue stain. Identification of *Candida species* was based on colony morphology and microscopic features [10,11].

2.3. Tetra ARMS-PCR:

After extracting the DNA (blood) of 65 specimens utilizing the [12]. Modified technique (Allele-specific amplification is a straightforward and effective technique for detecting single nucleotide polymorphisms SNPs), four microliters of DNA and one microliter of each IL-10 gene primer, supplied by MacroGen Company were added, as displayed in Table 1 [13].

Table 1: Primers that were utilized to determine genetic variation in the *interleukin-10* gene using PCR technology

Primer	Sequence	Band size	Annealing
F-outer	5- CCTCTTACCTATCCCTACTTCCACC -3	639 bp	61
R-outer	5- GACAACACTACTAAGGCTTCTTTGGTAA -3		
F-inner	5- GTAGTCTGCACTTGCTGAAAGCTT -3	393 bp	
R-inner	5- TCAGTGTTCCTCCCAGTTACAGTC -3	299 bp	

Table 2, the primers were added to the Master mix's contents before reaction tubes were put within the thermocycler to start reaction chain. utilizing an electrophoresis device, specimens were put onto 2% agarose gels, Red Safe dye was added, and DNA bands were then viewed under a UV lamp [14].

Table 2: the approved program in PCR

Number	The stage	The temperature	Duration	Number of cycles
1	First denaturation	95	5 min.	1
2	Denaturation	95	45 sec.	35
3	Annealing	61	1 min.	
4	Extension	72	1 min.	
5	Final extension	72	7 min.	1
6	Stop reaction	4	3 min.	1

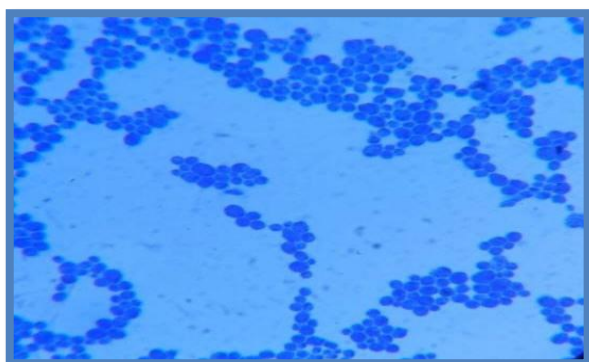
2.4. Analysis of statistics

The statistical analysis was conducted using the T-test (SPSS), and the acceptable level of statistical distinction was ($P > 0.05$). Additionally, the CI, P value, and Odds Ratio were computed.

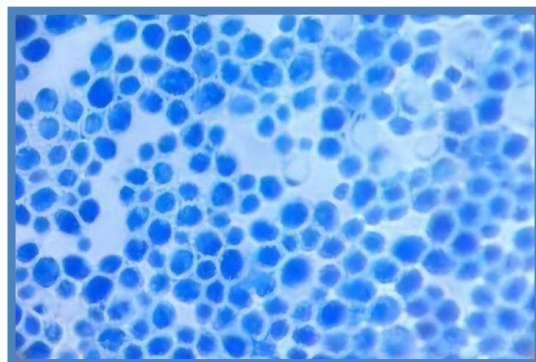
3. Results:

3.1. Isolation and Identification of Candida

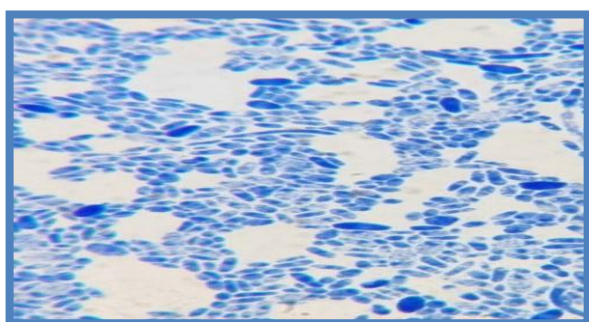
The results of the clinical specimens collected from the female genital system indicate that four types of *Candida* sp. were identified depending on the diagnostic characteristics in cultures and by the microscope as shown in the Figures 1-2. The percentage of *C.albicans* was the frequency (67%) then *C.glabrata* the frequency (21%), *C. tropical* the frequency (7%) and finally *C.krusei* the frequency (5%) .



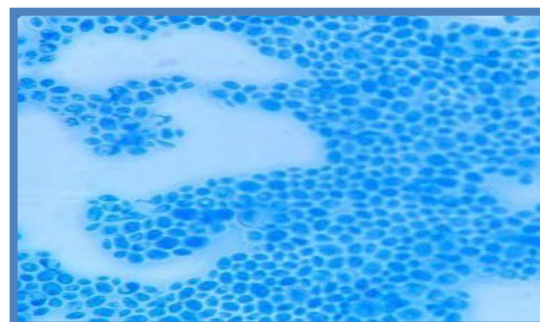
C. albicans



C.tropicalis



C.glabrata



C.krusei

Figure 1: *Candida* spp under a microscope using lactophenol blue under 100X.

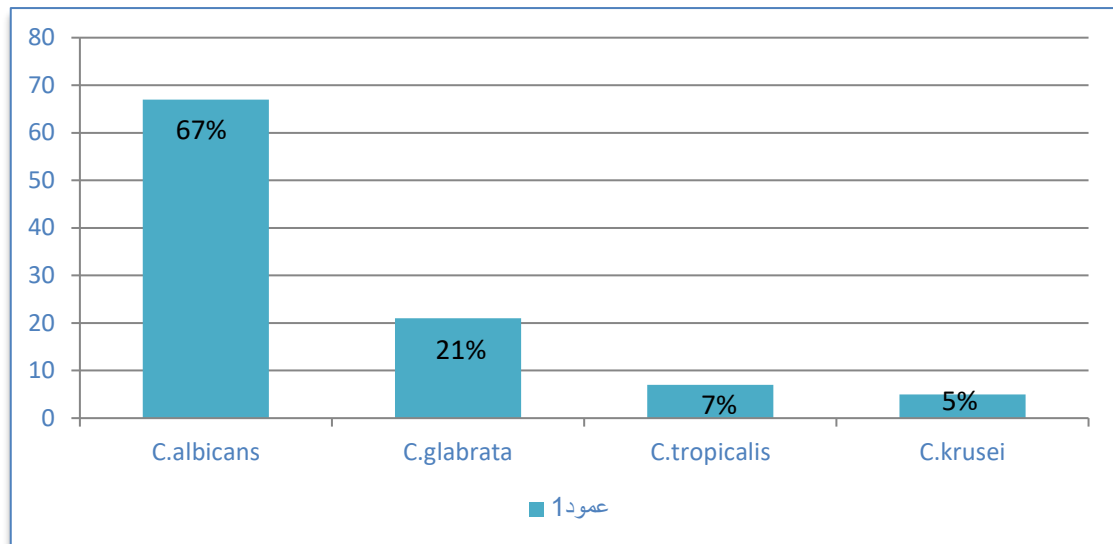


Figure 2: the percentages % of each type of *Candida spp.*

3.2. Results of Analysis of the genetic Contrast of the *Interleukin -10* and determining its relationship with the events of vaginal yeast infection

Five molecular tests were used to analyze the genetic variation

3.2.1. Identifying the proportion of observations of the different genotypes

The results of the TARMA PCR, demonstrated in Figure 3, showed that there is not a relationship between the patients who suffer from the frequent vaginal infections and the IL-10 gene's genetic variation.

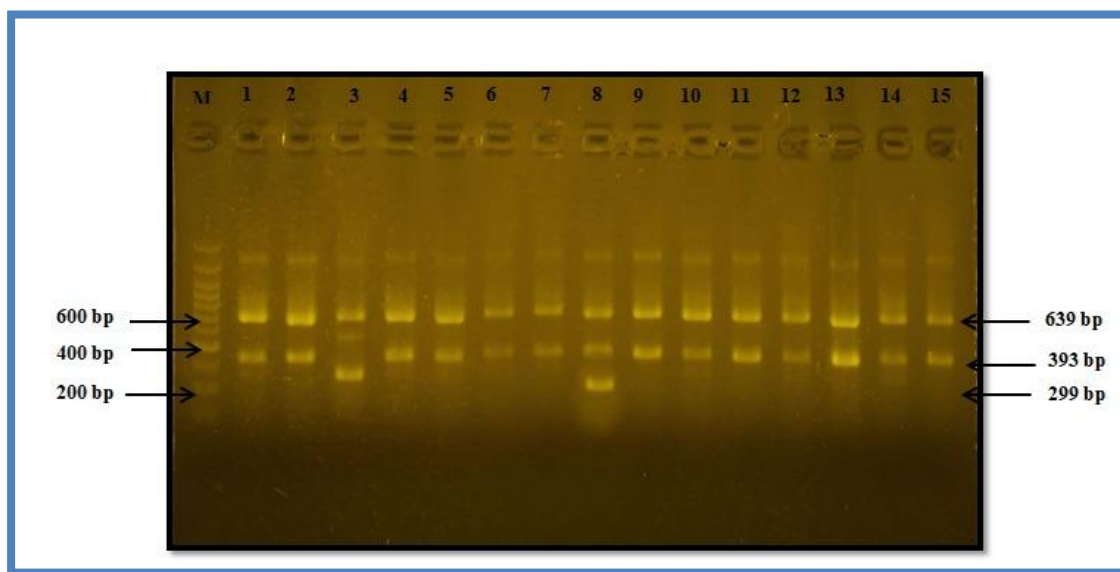


Figure 3: The product of the TARMS-PCR reaction for the genetic variation of the *IL-10* gene

From the PCR results, it was clear that there were three different genetic patterns: TT, CC and CT and the normal pattern is CC (393) bp and the hetero genotype (393) bp CT, while the mutant genotype (299) bp and (100) bp Marker the volume index supplied by Biolabs Company and it was isolated by the agarose gel with a concentration of 2. Also, Table 3 shows that the allele observation and the frequency of the various genetic patterns of IL-10 were evident in the women infected with the vaginal yeast infection and the frequency of the pattern (wild genotype) CC was (70%) compared to the (mutant genotype) TT (12%). As for the (hetero genotype) CT the percentage was (18%), which is low in contrast to the control group, which had the value of (55%) for the typical genotype, (40%) for the hetero genotype and (5%) for the genotype of the mutation. Also, the OR value of the genotype was (1.71) and so, it is higher than (1.0) at a likelihood of p (0.05)

Table 3: Distribution Interleukin -10 gene alleles and genotypes as a percentage

SNP1	Sick women		Healthy women		P-value	Odds ratio	Confidence interval 95%
	NO.	%	NO.	%			
CC	32	70	11	80	P = 0.6376	1.7188	0.1805 to 16.3676
CT	8	18	8	40			
TT	5	12	1	5			

3.2.2. Determining the allele frequency and the observation level

The research current results, for the women infected with the vaginal yeast infection, showed a frequency of the normal allele C (80%), which is considered high compared to the mutant allele T (20%). Regarding the control group, the normal allele's frequency was (75%) and for the mutant allele was (25%) as displayed in Table 4.

Table 4: the allelic frequency ratio

Allele	Sick women		Healthy women		P-value	Odds ratio	Confidence interval 95%
	No	%	No	%			
C	36	80	15	75	P = 0.5229	0.7500	0.3103 to 1.8129
T	9	20	5	25			

3.2.3 Analyzing the genetic variation results by Hardy Weinberg equilibrium test

The distribution of the genetic patterns of the patients were dealt with using Hardy Weinberg equilibrium test, which is the third analysis of variation in question in order to identify the severity of cases according to the recommended equation. When the results were compared, as shown in Table 5, between the observation percentage obtained and the observation percentage of HWE equilibrium, the value obtained was: p value = 0.002869, which is lower than $p < 0.05$, and this denotes that there is a variation in the within the study groups and that it is not covered by the equilibrium law.

Table 5: shows the results of the HWE balance test

Genotype	CC	CT	TT
Genotype observed	32	^	o
Anticipated genotype	28.8	14.4	1.8
P value = 0.002869		Chi squared value $X^2 = 8.88$	

Also, there was a variation in the recorded and the predicted genetic percentages and this indicates the effect of the environmental factor and the disappearance of the genetic factor in the cases studied.

3.2.4. Verify that the inheritance of this variation recessive in the study groups.

This indicates that the genetic variant apparent an apparent pattern as in the case of the normal genotype, as shown in Table 6.

Table 6: The study group's distribution of IL-10 polymorphisms using the recessive model

Genotype	Sick women	Healthy women	Odds ratio	Confidence interval	P-value
CT-CC	40 (88%)	19 (95%)	0.4211	0.0459 to 3.8591	P = 0.4441
TT	5 (12%)	1 (5%)			

3.2.5. Verify that the inheritance of this variation dominant in the study groups.

According to Table 7, this indicates that the variant genotype produces a protein similarly to the mutant genotype, and that this is dependent on the P value.

Table 7: IL-10 polymorphism distribution in the research group using the dominant model

Genotype	Sick women	Healthy women	Odds ratio	Confidence interval	P-value
CC	32 (75%)	9 (80%)	0.4965	0.1667 to 1.4792	0.2087
TT+CT	13 (25%)	11 (20%)			

When examining how the experimental group's and the control group's genotype percentages, when comparing the findings in the dominance distribution, it was evident that P value = 0.2087, which is lower than $P > 0.05$, differs from P value = 0.4441, which is greater than $P > 0.05$ in the recessive distribution test, i.e. the mutant genotype has a recessive effect on the disease case and its effect is not dominant .

4. Discussion:

A fungal infection of the vagina and vulva is called vaginal yeast infection, or vaginal candidiasis. It is brought on by an overabundance of a fungus known as *Candida spp.*, which is found in the body naturally but in balanced amounts. The infection typically arises when the vagina's normal bacterial and fungal balance is upset, allowing the *Candida spp.* fungus to proliferate [15,16]. Its virulence factors, which include adhesion, the secretion of decomposition enzymes, and the ability to transform from yeast to hyphal forms the epidemic filamentous shape—allow it to invade mucus tissues, which is essential for the disease to develop and for the formation of biofilms on the host's surfaces. This is why the percentage of

Candida albicans was found to have increased significantly [17]. The study's findings concurred with those of multiple other investigations, which indicated that the infection with *C.albicans* was the highest in terms of spread when vaginal swaps were taken from women, who are infected with vaginal yeast infection [18,19]. It was also noted that *Candida krusei* decreased to 5%. These results were similar to the results of Ahmed and Khan's study [20], who attributed the reason for its decrease compared to other species to the fact that the vaginal environment is acidic and unsuitable for its growth.

So far, there are no studies that link SNPs IL-10 gene to the hazard of vaginal yeast infection. This was the first research between polymorphisms in the Interleukin-10 gene and vaginal yeast infection [21]. When a genetic mutation (SNP) occurs in the IL-10 gene, the level or function of this cytokine may change, potentially affecting the body's ability to control *Candida spp.* infections. Some mutations may result in increased production IL-10, which may suppress the natural immune response against fungi, increasing the risk of infection. The anti-inflammatory cytokine interleukin-10 (IL-10) is crucial for controlling the immune response, inhibiting excessive inflammatory responses [22, 23, 24]. This study found the absence of factors genetic as well as the influence of environmental factors, some of the most important environmental factors include consuming foods high in sugar, using antibiotics, disrupting the circadian rhythm, stress, and depression, which weaken the immune system and make it harder for the body to fight off fungi. Hormonal changes during pregnancy, or the use of hormonal contraceptives affect vaginal acidity and facilitate yeast growth [25,26 ,27]. despite the high genotype CC, TT in women with vaginal yeast infection (70%-12%) compared to healthy women (55%-5%). This demands conducting many studies, such as examining known SNPs (-1082, -819, -592) of the Interleukin-10 gene or measuring the level of Interleukin-10 in vaginal secretions and linking it to genes, analyzing genetic interaction with other genes such as (IL-4 and TLR-2).

5. Conclusions:

Women in Mosul frequently get vaginal yeast infections, which are probably brought on by a mix of environmental and hereditary causes. The results of the microscopic analysis indicated that the most prevalent species was *Candida albicans*, which was followed by *Candida glabrata* and then, to a lesser degree, *Candida tropicalis* and *Candida krusei*. Genetic variations in the Interleukin-10 gene are linked to heightened vulnerability to vaginal yeast infections, particularly in women with the CC genotype, according to tetra-ARMS PCR

data. Hardy-Weinberg equilibrium analysis demonstrated that environmental factors had an impact on infection prevalence, with a discernible decrease in the genetic role. This study highlights the importance of molecular analysis and genetic polymorphisms in understanding the mechanism of susceptibility to infection, clearing the path for the future creation of individualized preventative or treatment plans. To further understand the immune gene interaction, future research should examine other immune genes linked to vaginal yeast infection, examine the relationship between genotypes and treatment response, which could aid in the development of tailored treatment plans based on the genetic profile of the patient, examine the impact of behavioral and environmental factors (such as personal hygiene, nutrition, and antibiotic use) on the frequency and severity of Candida infections, and confirm the overlap between fungal infections and the vaginal microbiome using contemporary genetic sequencing technologies (e.g., NGS).

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A Review of the Role of Shuttling Proteins in Herpesvirus Replication and Pathogenesis

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Abstract

Herpesviruses are complex DNA viruses that rely on host-cell machinery for replication and pathogenesis. One critical aspect of their lifecycle involves the transport of viral and host proteins between the nucleus and cytoplasm. This review aims to explore the role of shuttling proteins in herpesvirus infection, focusing on their transport mechanisms, interaction with nuclear transport receptors, and contribution to viral replication and immune evasion. the viral lifecycle is significantly impacted by the transportation of different proteins between the cytoplasm and nucleus throughout viral infection. Shuttling proteins usually consist of nuclear localization signals as well as nuclear export signals for the purpose of mediating proper positioning for themselves and other proteins. They are crucial components in nucleocytoplasmic information transmission inside the cells. The nuclear pore complex on nuclear envelope facilitates the nucleocytoplasmic transport mechanism, which is mediated by certain protein carriers. Ongoing research has progressively clarified which herpesvirus proteins function via nucleocytoplasmic shuttling. An outline of how shuttling proteins use nuclear transport receptors as well as nucleocytoplasmic shuttling signals for nucleocytoplasmic transport is given in the presented work. This research offers a resource for

comprehending herpesvirus infection pathogenesis and formulating novel anti-viral approaches. It also explains how herpesvirus shuttling proteins contribute to efficient infection of viruses through altering their life-cycle and engaging in innate immunity.

Keywords: Apoptosis, Herpesvirus, Innate immune escape, Nuclear export signal, Shuttling proteins.

مراجعة لدور البروتينات الناقلة في تضاعف فيروس الهربس و مسبباته المرضية

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المستخلص

تُعدّ عملية النقل النووي-السيتوبلازمي للبروتينات من الآليات الحيوية التي تؤثر بشكل مباشر على دورة حياة الفيروس. وتعتبر فيروسات الهربس من الفيروسات التي تمتلك تلك الآليات. حيث ان تعتمد العديد من البروتينات الفيروسية على إشارات الدخول والخروج النووي لضمان تموضعها الصحيح داخل خلية المضيف. هذه الإشارات تتيح للفايروسات أداء وظائفها في التعديل المناعي والتكاثر. علاوة على ذلك، تلعب بروتينات النقل دوراً محورياً في تنظيم هذه الحركة عبر المسام النووية وذلك باستخدام مستقبلات نقل متخصصة. في هذا السياق، يهدف العمل الحالي إلى تسليط الضوء على دور بروتينات النقل في آلية العدوى بفيروسات الهربس. بالإضافة الى ذلك التركيز على كيفية استخدامها لإشارات النقل النووي والمستقبلات المرتبطة بها، وذلك لفهم تأثيرها في تعديل دورة حياة الفيروس والتفاعل مع المناعة الفطرية.

الكلمات المفتاحية: موت الخلايا المبرمج، فيروس الهربس، الهروب من المناعة الفطرية، إشارة التصدير النووي، البروتينات الناقلة.

1. Introduction

Herpesviruses are enveloped viruses that have got large double-stranded DNA genomes found in nucleus. Herpesviruses are big, spherical, and have icosahedral symmetry, measuring between 150 and 200 nanometers. With an average range diameter of 100 nm, the icosahedral protein capsid is made up of 162 hollow hexagonal and pentagonal capsomeres with an electron-dense core that houses the double-stranded DNA genome. The nucleocapsid is made up of 125–240 kbp nucleotides. [1], as shown in **Figure 1**. They belong to the Herpesviridae family. These viruses have a biphasic lifecycle with lytic and latent phases, this enables them to cause chronic infections in their hosts. They could infect humans as well as other vertebrates. There are three subfamilies of herpesviruses based on characteristics including Alpha, Beta,

and Gamma. These characteristics play a crucial role in disease processes, cell tropism and latency sites. Alpha-herpesviruses are responsible for infecting animal. For example, Marek's disease virus, pseudorabies virus, and bovine herpesvirus-1. Moreover, they cause human infections, such as varicella-zoster virus and herpes simplex virus type 1 and 2. In sensory neurons, such viruses typically remain latent [2].

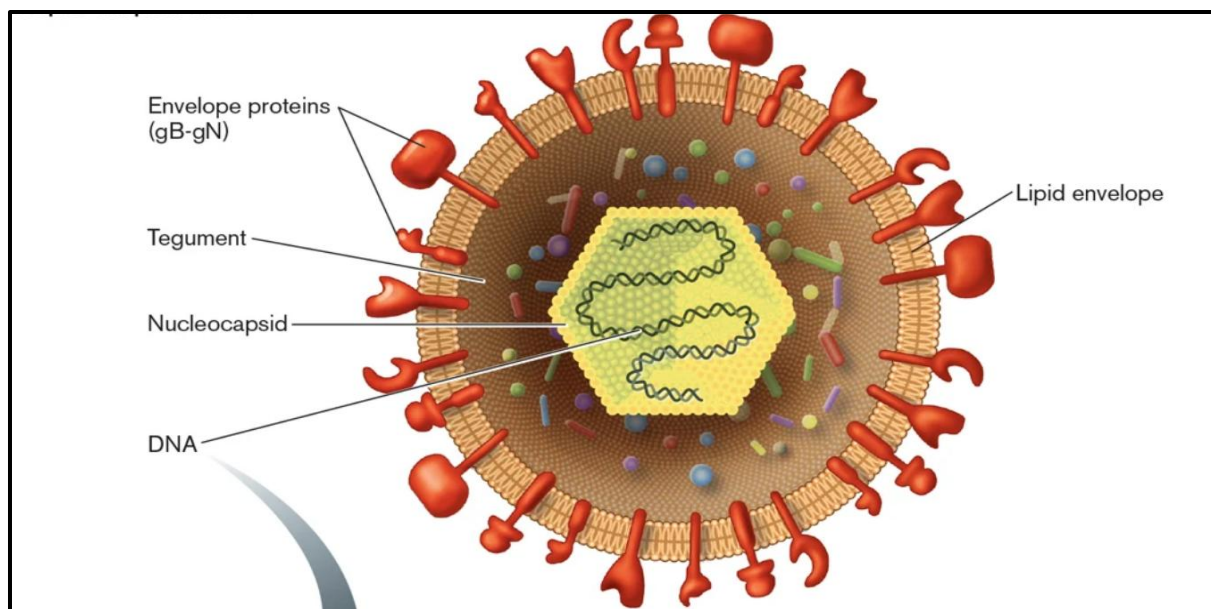


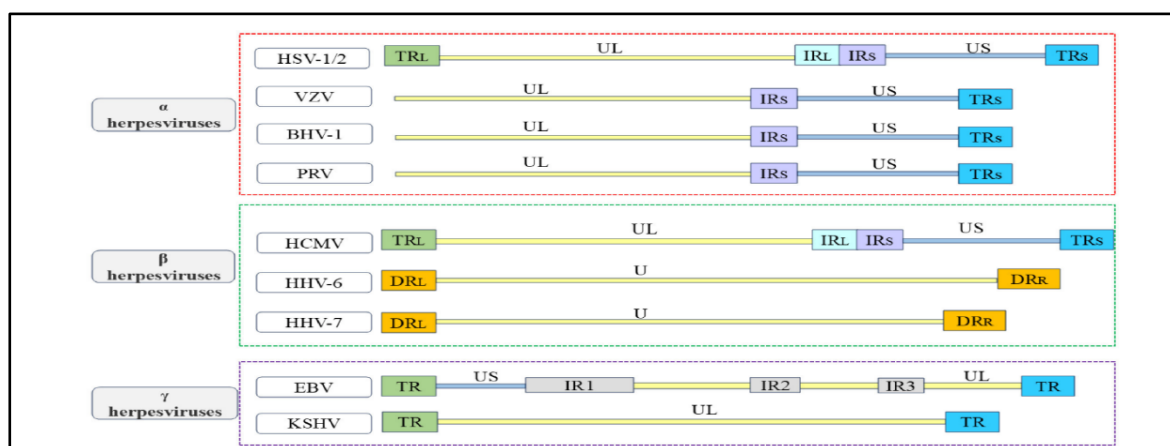
Figure 1: Structural Organization of Herpes Simplex Virus Type 1 (HSV-1) [1].

Human cytomegalovirus, human herpesvirus-6, and -7 are the primary members of the beta-herpesvirus group. They have a tendency to develop latency in mononuclear cells. Gamma-herpesviruses, on the other hand, usually remain dormant in lymphocytes. For example, Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus in humans, as well as alcelaphine herpesvirus 1 and herpesvirus saimiri in animals [3]. The capsid, a DNA core, a layer of tegument around it, and an outer lipid envelope make up herpesvirus particles structurally. The unique short and long regions of viral genome are surrounded by different repeat sequences. These consist of internal, direct, and terminal repetitions that differ amongst herpesvirus species and affect their genomic structure. The coordinated action of numerous viral proteins supports viral replication and propagation. These proteins must localize to specific cellular compartments by crossing barriers such as the plasma and nuclear membranes. Some strategies for viral life cycle and replication. In certain viral proteins there is the capacity of shuttling between cytoplasmic and nuclear compartments, such as important processes including regulation of host transcription, translation, immune evasion, mRNA transport, or virion assembly [4].

For instance, proteins such as UL47 of HSV-1 and BHV-1 may act in the nucleus or during the replication process and then migrate to the cytoplasm for virion assembly. These proteins, called the nucleocytoplasmic shuttling proteins, via their NLS (nuclear localization signals) and NES (nuclear export signals), alter their subcellular location. They are found throughout the cell and their spatial and temporal distribution directly affects the progression of the viral lifecycle; they are trafficked through interaction with host receptors and adaptor proteins [5].

Notably, many herpesvirus lytic genes are intronless. Since transcription takes place in the nucleus, the resulting mRNA must be efficiently exported to the cytoplasm. It is a process that is mediated by proteins like ICP27 which re-invigilate host-based RNA processing pathways in order to sustain viral transcripts to stay constant as well as be effectively translated. There are also other viral proteins, one of which is the HSV-1 VP19c, the HCMV UL94 and KSHV ORF45 that help in the virion assembly [6].

Herpesviruses maintain infection by the use of a defined set of specific proteins. The most notable of them (PRV UL46, HSV 1 g134.5, and HCMV UL94) align notably due to their ability



to disrupt the most immune signaling pathways. The Kaposi herpesvirus that is known as latency-associated nuclear antigen-2 (LANA2) serves as another immune-evasion determinant in latency [3]. Like nuclear importation through nuclear localization signals (NLS), and nuclear export, nucleocytoplasm-based transport protein fermented by nuclear export signals (NES), are essential to the coordination of nuclear cellular infection by herpesvirus. These proteins modulate temporally regulated viral gene expression as well as delayed development of the lytic phase. Thorough knowledge of these processes of transport has become central to understanding the pathogenesis of herpesvirus [5].

Figure 2: A summary of the Genome Structure of Herpesvirus [7].

2. The Mechanism of Nucleocytoplasmic Shuttling in Shuttling Proteins

The remaining shuttling proteins have been shown to have nucleocytoplasmic taxishm which is similar to that of the herpesvirus shuttling proteins. As further outlined below, individual steps are characterized by many protein protein interactions and influenced by complex strict regulatory systems [8].

2.1. Pathways of Nucleocytoplasmic Shuttling

The nuclear envelope that separates the cytoplasm and the nucleoplasm is a physical barrier that limits movement of macromolecules in between the nucleoplasm and the cytoplasm. The inner and outer nuclear membranes are penetrated and bridged by a number of supramolecular structures known as nuclear pore complexes, which are made up of nucleoporins and mediate nucleocytoplasmic transport [9].

2.2. Receptors Mediating Nucleocytoplasmic Transport

Nuclear transport receptors are specialized transport proteins that regulate the nucleocytoplasmic shuttling of proteins across nuclear pore complexes (NPCs). Karyopherins are essential for transporting materials between cytoplasm and nucleus. Karyopherins are divided into two primary categories depending on their functions: exportins and importins. Usually acting as heterodimers, importins are classified into two families: importin- β and importin- α . Finding the NLS on cargo proteins is the task of importin- α . Three functional domains are present in this molecule: Importin- β 1 interacts with an N-terminal importin- β -binding (IBB) domain. NLS-bearing cargo is recognized and bound by Armadillo (ARM) repetitions in a central region. C-terminal domain that promotes nucleocytoplasmic translocation by binding to nucleoporin 50 and the export factor CAS. Conversely, importin- β proteins have a role in the nuclear import of RNA and proteins [10]. They are distinguished by two conserved regions: an N-terminal domain unique to importin- β and a central HEAT domain (called after Huntingtin, Elongation factor 3, and protein phosphatase (2A)). As shown in **Figure 3**, members of the importin- β family include exportins and bidirectional transport receptors in addition to importins.

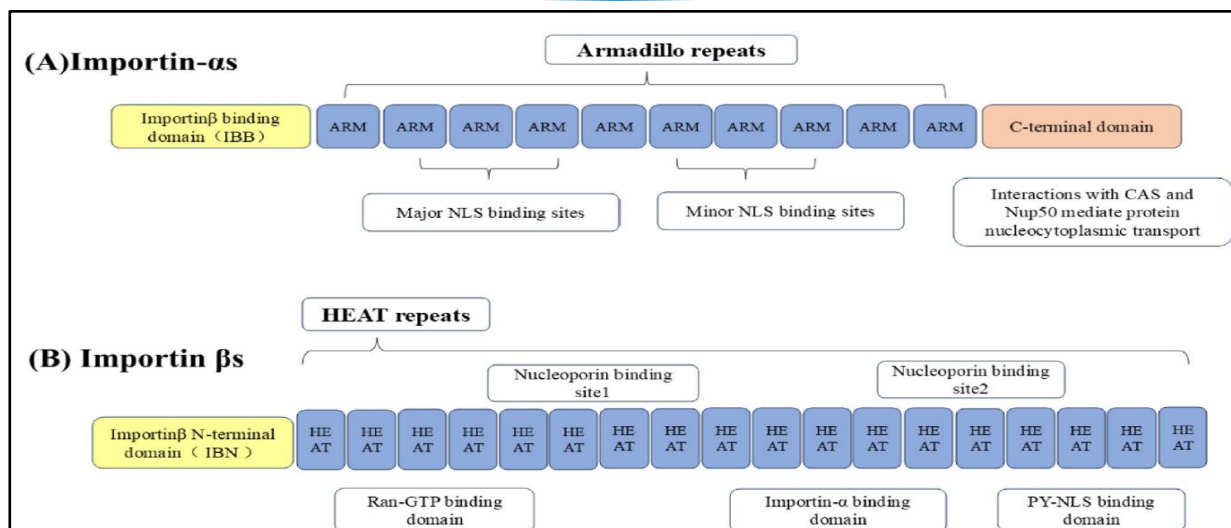


Figure 3: The two groups of importins are (B) importin-β and (A) importin-α [11].

2.3. Sources of Energy Driving Nucleocytoplasmic Transport

A dynamic flow of energy headlined by Ras-GTPases and Ran GTPases has been outlined within nuclear envelope, which is necessary to the transfer of shuttling proteins to the specific nuclear transport receptors. Ran majorly serves intercapillary and cytoplasmic protein and RNA transport, is the biggest Ras-GTPase superfamily and also serves as a operation regulator of the importin-β protein. To conclude, nuclear transport receptors, nuclear pore complex, Ran GTPases, and cargo proteins spatial, and temporal interaction facilitate the nuclear pore complex in accomplishing numerous biological functions [12].

2.4. Nucleo-cytoplasmic-shuttling proteins into nucleus

A particular device of the translocation of cytoplasm proteins to the nucleus is organized by a set of signaled sequences, one of which is the transport nuclear localization signal (TNLS). TNLS is a small peptide, which has a unique amino acid chain and which acts as a nuclear localization signal whose disposition coordinates in an orientation-specific translocation of proteins via the nuclear pore complex (NPC) to specified subcellular destinations. A wide range of proteins contain nuclear localization signals that have irregular structural motifs, herein referred to as noncanonical nuclear localization signals (ncNLSs). Varied range of ncNLS variants has been discovered.

These could be broadly classified as spatial epitope NLSs, proline and tyrosine-containing NLSs, and NLSs rich in arginine (IKNLSs). Importin5 recognizes the IK-NLS, whereas

transportin1 recognizes the PY-NLS, which is more complicated when compared to cNLS. A cytoplasmic cNLS–cargo is first accessible to binding to importin -a using the canonical nuclear import pathway and later it links to importin -b1 by its IBB domain to form a trimeric complex [13]. Importin -2 is then the one that assists in getting this trimer to the nucleus. GTP hydrolysis catalysed by the Ran GTPase provides the energetics needed by the trimer to traverse the nuclear pore complex. Integrations The activation of importin-2 and the cNLS cargo depends on both binding of Ran GTP to the trimer, with the aid of importin-2, i.e. release of importin-2 and subsequent conformational rearrangements of the trimer.

It is during the solid state of the internal skeleton that cargo is carried, and it is kept in the nucleus. Second to importin- 1 in mediating non-classical nuclear entry is transportin-1, which is often mediated through importin- 2 binding to non-canonical NLS signals. It seems that the selective importation of proteins that contain the PY-NLS motif is determined by Transportin-1. Conclusively, protein importation into the nucleus by NLS is an essential constituent that assures correct protein functioning [14].

2.5. Nucleocytoplasmic shuttling of proteins out of the nucleus

Nucleocytoplasmic shuttling of proteins must interact with exportins to facilitate the gradual transport regarding the shuttling protein from nucleus to the cytoplasm when it has completed its function. NES is a brief motif of hydrophobic or leucine-rich amino acids. CRM1, sometimes referred to as export 1 or Xpo-1, is the most well-identified of seven exportin types. CRM1 has a key role in nuclear export mechanism of shuttling proteins. CRM1-NES cargo RanGTP ternary export complex is first formed when NES on shuttling-protein links to the CRM-1. After attaching itself to a variety of nuclear pore proteins, the ternary complex passes through NPC and enters the cytoplasm [15]. Lastly, for the subsequent export cycle, CRM1 makes its way back to the nucleus via NPC. In conclusion, NES is crucial for shuttling protein transport, and the dynamic mechanisms that NES and NLS mediate serve as the foundation for shuttling proteins to carry out various tasks, as illustrated in **Figure 3**.

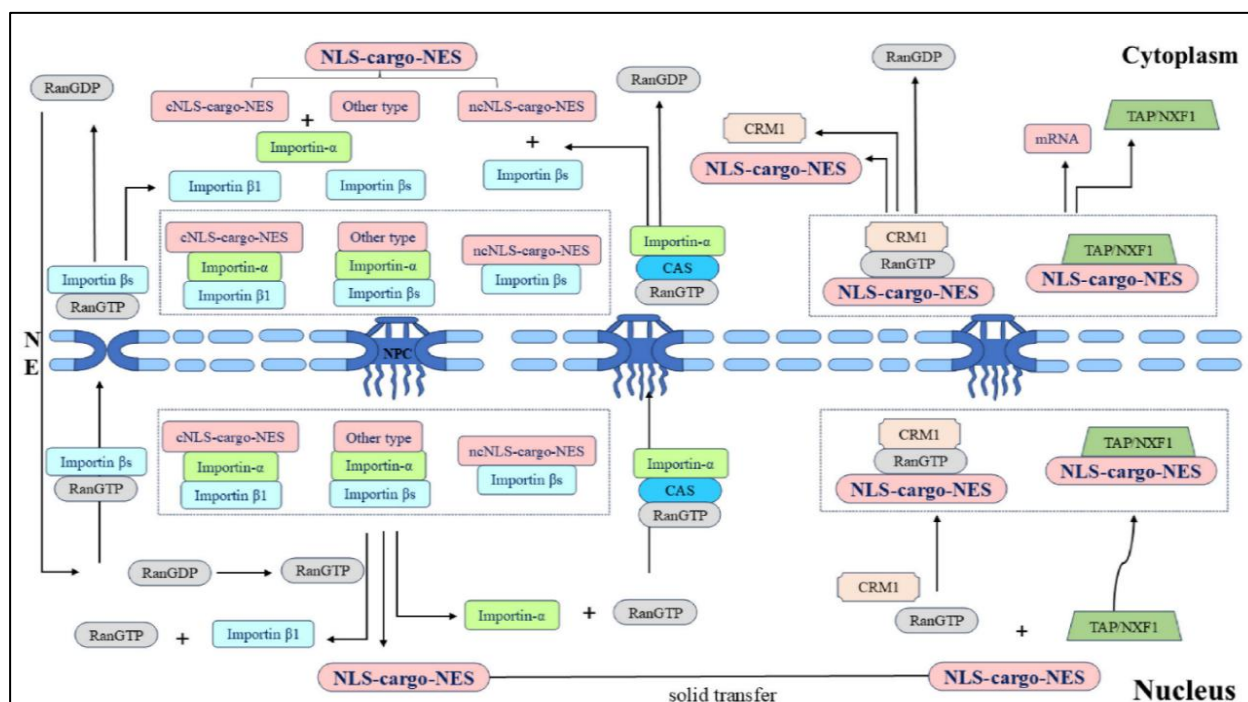


Figure 4: Diagrammatic representation of shuttling-protein NLS-cargo-NES protein structure nucleocytoplasmic transport and related protein molecular circulation [16].

3. Latency and reactivation mechanisms in herpesviruses

Herpesvirus DNA often persists as stable, circular episomal forms in the host cell nucleus throughout the latent phase. HHV-6 is an exception, as it integrates into the host chromosomes' telomeric regions. The expression regarding a single transcript known as latency-associated transcript (LAT) is what defines latency in HSV-1, whereas immediate-early protein ICP-0 is highly essential for starting the lytic phase and reactivation. Only VLT-ORF63 (VLT63) fusion transcript as well as the VZV latency-associated transcript exhibit latent gene expression in varicella-zoster virus (VZV) [11]. The tegument protein IE62, which is expressed by VZV, enters the nucleus after infection and activates immediate early, early, and late viral genes, causing lytic replication [17]. Different viral proteins are involved in the control regarding latency as well as reactivation in gamma and beta herpesviruses. The IE2 and IE1 proteins, which are encoded by UL122 and UL123, respectively, are crucial in HCMV. IE2 regulates the expression regarding downstream viral genes, IE1 is necessary to start lytic infection as well as reactivation. EBNA1, the first latency protein identified in EBV, serves to bind the viral episomes to host chromatin. Classified as latency I, 0, IIb, IIa, and III, EBV latency exhibits a variety of gene expression patterns. Rta (BRLF1) and Zta (BZLF1) are immediate-early transcription factors that regulate reactivation [13].

Through limited expression of viral genes, mainly LANA1, which stabilizes episomal maintenance, KSHV sustains latency. Transcriptional activator RTA, encoded by UL50, regulates the transition to lytic phase. Herpesviruses utilize several latency techniques. Alpha herpesviruses typically do not require viral anchoring proteins to establish latency in non-dividing neurons. By using viral proteins to secure genome to host chromosomes and guarantee their distribution throughout cell division, gamma and beta herpesviruses, at the same time, maintain latency in growing cells. The lifetime of herpesviruses in all three subfamilies is essentially the same, despite these variations. The shuttling proteins' function in influencing the course of reactivation and infection can be better understood by looking at the lytic phase [18].

3.1. Replicative Cycle of Herpesviruses

3.1.1 Mechanisms of Herpesvirus Entry into Host Cells

The entry process of alpha, beta, and gamma herpesviruses into host cells involves a complex interplay among several envelope glycoproteins, each designated by a letter preceded by the prefix “g” to indicate glycosylation. Initially, herpesviruses attach to the host cell surface using multiple glycoproteins and a range of cellular receptors; however, this attachment alone does not initiate viral entry [19]. Depending on the type of cell, the viral envelope either enters the cell by endocytosis or directly fuses with host cell membrane after attachment. Herpesviruses share a consistent core entry mechanism including the glyco protein gB and the gH–gL heterodimer, despite the fact that they infect a variety of host cells. However, in order to contact various host receptors, each herpesvirus subfamily uses unique combinations regarding glyco proteins, enabling them to target particular cell types as well as tissues [20].

3.1.2. Dissociation of Tegument Proteins and Capsid Transport

As soon as herpesviruses penetrate a host cell, tegument associated capsid virions and their proteins are transported into cytoplasm. One component of the tegument proteins dissociates rapidly from the capsid, where the rest of the components stay attached and interact with micro-tubules network to facilitate efficient capsid transportation to the nucleus. Upon reaching the nuclear envelope, an opening forms at one capsid vertex. Certain capsid proteins then engage with NPC components, particularly Nup214 and Nup358, ensuring proper docking and triggering genome release [21].

The densely packed, negatively charged viral DNA within the capsid generates substantial internal pressure, which has been hypothesized to be several magnitudes greater than regarding atmospheric pressure. This force pushes the DNA through the NPC, overpowering the selective blocking of the pore and allowing the genome to enter the nucleus. For alpha herpesvirus, e.g., HSV-1, proper function requires a stepwise dissociation of the tegument proteins. Initially, outer tegument proteins are released, followed by inner ones. VP16 is the first outer tegument protein to dissociate. It is followed by VP13/14, encoded by UL-47 gene, which serves as a shuttling protein as well. The next protein in this sequence is VP22. These proteins play roles in the viral replication process, with UL47 and UL49 representing genes uniquely associated with alpha herpesviruses. Conversely, the dynamics of dissociation in tegument proteins in varicella -zoster virus (VZV), 2 -herpesviruses, and 3 00 -herpes viruses are relatively ill-defined [22].

3.1.3. Transcription, replication, and capsid assembly of viral genomes

After viral DNA reaches the nucleus, linear genome replicates by a rolling circle process and goes through circularization, producing concatemeric DNA. The basic assembly unit, which eventually assembles into a mature nucleocapsid, is formed by the capsid proteins of transport into the nucleus after translation, transcription, and DNA replication are finished. Within the nucleus, transcription of viral genome takes place in a cascade-like pattern. Depending on their transcriptional timing, herpesvirus genes are divided into 3 classes, which are: immediate-early (IE), early (E), and late (L) genes. Prior to DNA replication, transcriptional activators initiate the transcription of IE genes. After being exported to cytoplasm, the resultant IE mRNA is translated into IE proteins. After returning to nucleus, such proteins trigger the transcription of E genes. The expression and transcription of L genes are then started by the E gene products. IE proteins use synchronized nuclear export and import mechanisms to promote the expression of E and L genes during this cycle [23].

As will be shown in more detail in the following sections. The members regarding to the ICP27 protein family are essential for the export of viral mRNA. It is thought that most of the capsid assembly pathway is shared by all herpesviruses. Similarly, there is a great degree of similarity in the function and structure of the main capsid proteins in Herpesviridae family. For instance, VP5 and VP23 are important capsid proteins in HSV-1. Following cytoplasmic synthesis, VP5 and UL26.5 combine to create a complex, and VP23 associates with VP19C to form a second

complex. These parts are brought into the nucleus by NLS located in VP5 and VP19C, where they come together to form the capsid structure.

The terminase complex recognizes specific cis-acting "pac" motifs on the concatemeric viral DNA, performing two cleavage events to generate a unit-length genome. This genome is then loaded into the preformed capsid through an interaction with the UL6 portal protein. Amongst those components, HSV1 VP19-C functions as a shuttling protein and will be discussed in greater detail in the following section [24].

3.1.4. Nuclear egress, secondary envelopment and release.

Following assembly, the nucleocapsid engages in interactions with the nuclear envelope and acquires a temporary envelope through budding through the inner nuclear membrane. This process is referred to as primary envelopment. Thus, perinuclear virions are created and placed in the perinuclear area. Following de-envelopment at outer nuclear membrane, such virions release naked, or non-enveloped, capsids into cytoplasm [25]. All of the herpesvirus subfamilies' nucleocapsids successively attach to tegument proteins once they are in the cytoplasm. After budding into trans-Golgi network, they acquire a secondary envelope and develop into mature virions. Depending on the type of herpesvirus, secondary envelopment usually occurs in Golgi apparatus, although it can occur in early endosomes or autophagosomes. Most people agree that the Golgi is the primary location for envelope acquisition. The intercellular transmission cycle is then completed when mature virions are encapsulated in cytoplasmic vesicles and transferred to the plasma membrane by exocytosis. Two conserved regulatory proteins are necessary for herpesviruses to mediate nuclear egress. UL31's Epstein-Barr virus homolog, BFLF2, is these among one; it functions as a shuttling protein and will be covered in more detail in a later section [26].

4. Herpesvirus Shuttling Proteins and Their Functional Roles Throughout the Viral Lifecycle

Herpesviruses from different subfamilies use slightly distinct mechanisms to infect host cells. Over time, they have developed specific strategies to manipulate nucleocytoplasmic transport pathways, optimizing conditions for viral replication. Nuclear export sequence elements are designed specifically to move the proteins to the cytoplasm. Host cell trafficking systems were essential for viral parasitism, and viral proteins took advantage of the NLS, NES, and other functional motifs to exploit nuclear transport receptors to enhance viral propagation. The herpesvirus lifecycle is nuclear-centered, with key processes such as transcription, genome

replication, and capsid assembly occurring in the nucleus. Regulating the movement of viral factors such as DNA polymerase, capsid protein monomers, transpeptidation factors, and other proteins into the nucleus becomes crucial. After the newly formed capsids have entirely assembled, the capsids get rid of the nucleus, and the tegument and glycoprotein coating complete their assembly. The NES elements regulate the translocation of viral proteins from the nucleus to other parts of the host cell to maintain its lifecycle.

In conclusion, herpesvirus-encoded shuttling proteins are indispensable in guaranteeing the precise subcellular localization of viral factors through the interplay between NLS, NES, and adequate transport machinery provided by the host. Any mutation in the pattern of NLS and NES variants disrupts the dynamic transport process and, as a result, completely impairs viral reproduction. A comprehensive summary of the role of shuttling proteins in the herpesvirus life cycle (Figure 4).

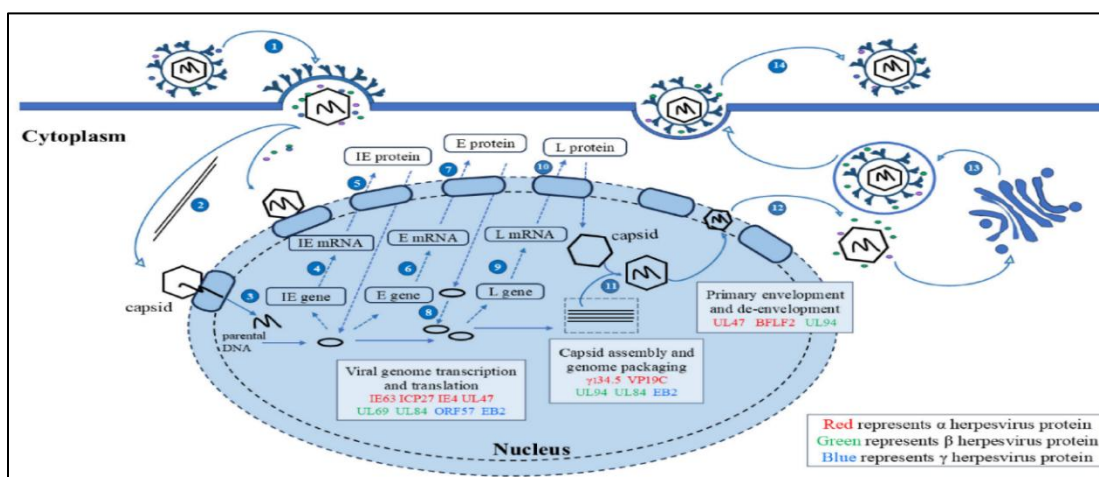


Figure 5: Herpesvirus shuttling proteins contribute to several stages of the viral life cycle [28].

4.1. Regulation of mRNA Transport by Herpesvirus Shuttling Proteins

mRNA transport in herpesviruses is a regulated process intimately tied to transcription, splicing, post-transcriptional modification and translation. These events are independent and interrelated steps in the host cell. The lack of introns in most herpesvirus genes requires them to utilize other means for nuclear export because export factor recruitment here cannot depend on splicing [29].

For that reason, the helter-skelter regulation of mRNA export plays significant roles in viral replication and pathogenicity. Various herpesviruses have evolved to foil multiple host export pathways, including Aly/REF, CRM-1, and TAP/NXF1, and encode regulatory proteins

that assist the export of viral transcripts from the nucleus. These are exigency viral export elements, which ensure the transfer of the transcripts from the nucleus to the cytoplasm. HSV-1 is a representative example of these proteins. ICP27, and its shuttling behavior, obliges functionality to the NLS, NES, and other useful zones. This viral protein recruits cellular terms, providing conveyance through TAP/NXF1. ICP27 straightforwardly ingenuity the trade of viral transcripts, including UL15, UL17, UL48, UL30, UL29, UL42, it can be. UL5. ICP27 mutation analysis supports that it is functional. Mutant analysis involving 16 ICP27 mutants uncovered its critical functioning. M11, M15, and M16 mutations are decisive, and they have substitutions of amino acid differences at locations 340/341, 465/466, and 488 [30].

Disruption of the M15 mutation in ICP27's worldly binding to Nup62 results in defective re-export and continuous shuttling. These viruses cannot induce late gene expression and replicate adequately. Similarly, a nuclear export signal NES-defective ICP27 mutant results in reduced shuttling and viral replication and reduced affinity for RNA binding, indicating the involvement of protein in export and gene expression. Finally, the VZV IE4 protein, another cyclical molecule with the world receptor TAP/NXF1, contains binding sites for cellular SR proteins. The three internal regions, Ra, Rb, and Rc, control its binding to RNA, and the host transcription factors p50 and TFIIB and the nuclear localization signal transmembrane, which is directed to the nucleus, are in the Rb domain [25].

As with RNA binding, when IE4 binds to RNA, the main fraction of is confined within the cytoplasm, precluding a question of spatiotemporal control. With the alpha herpesvirus UL47 protein family, HSV-1 and BHV-1 UL47 proteins use an importin β -dependent shuttling mechanism. UL47 proteins exhibit a conserved motif responsible for RNA interaction and nuclear delivery. At early times post-infection, HSV-1 UL47 forms nuclear domains adjacent to the transcription site. Thus, the protein is intimately involved in post-transcriptional processes. With the beta relative HCMV UL69, this means it functions as a posttranscriptional activator or RNA sign export factor [31].

It binds helicases UAP56 and URH49 through overlapping motifs, controlling nuclear targeting and RNA interactions. Mutations disrupting binding by either UAP56 or NES abolish virus shuttling, reducing replication and viral gene expression. Diverse homologs of UL69 across species, such as C69 in chimpanzees or Rh69 in rhesus cytomegalovirus, retain shuttling and homodimerization, but only cytomegaloviruses heterodimerize with host factors. Another related protein is HCMV UL84, which promotes cytoplasmic accumulation of early transcripts.

It enters the nucleus via interaction with importin- α and associates with UL44 in viral replication compartments. UL84 also functions as an RNA-binding protein, and NES-defective mutants show reduced export of IRS1 mRNA, a transcript critical for suppressing host protein synthesis via eIF2 dephosphorylation [32].

Predicted to belong to the DExD/H RNA helicase family, UL84 supports its involvement in nuclear export. Gamma herpesvirus proteins such as EBV EB2 utilize the DN domain to recruit REF, facilitating mRNA export. Additionally, the HVS ORF57 protein targets mRNA for export by localizing to the nucleolus. Mutations in its NLS prevent this localization and abrogate its function in mRNA export and gene activation, reinforcing the importance of nucleolar transit in viral transcript, processing as show in Figure 5.

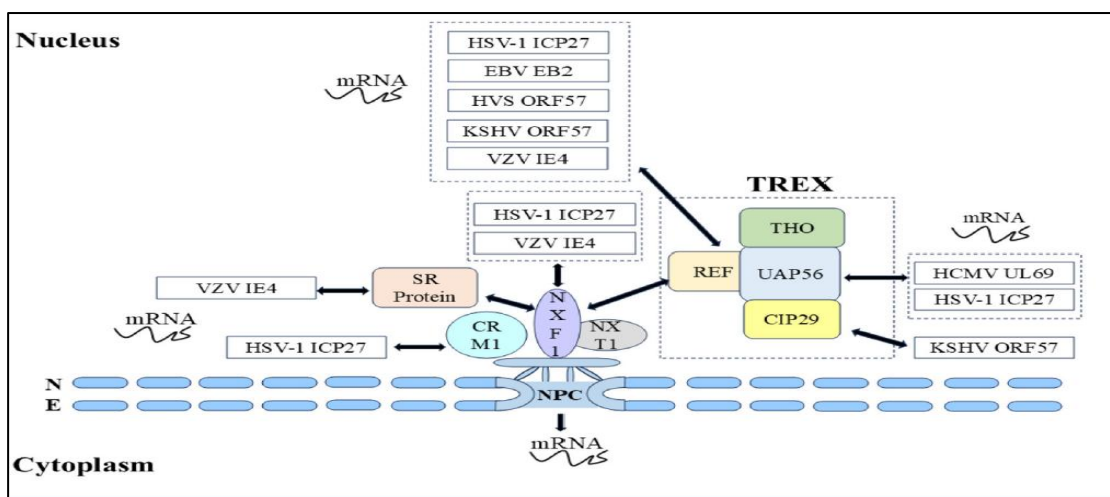


Figure 6: Mechanism of viral mRNA export by herpesvirus shuttling proteins [33].

5. Regulation of Apoptosis by Herpesvirus Shuttling Proteins

Apoptosis can be defined as a programmed cell death form that is characterized by specific bio-chemical and morphological changes. During the early phase of viral infection, apoptosis can limit viral propagation by eliminating infected cells, while in later stages, certain viruses harness apoptotic processes to facilitate replication and release. In bovine herpesvirus 1 (BHV-1), the UL47 protein selectively suppresses, the phosphorylation of structural maintenance of chromosomes 1 (SMC1) in the nuclei compartment, but has no effect on the processes that take place in the cytoplasm. This excessively activates the ATM/NBS1/SMC1 signaling cascade, a critical DNA damage response pathway and this drives the impairment of DNA repair. Research has shown that reduced phosphorylation of SMC1 compromises cellular survival following genotoxic stress [34].

In cells expressing the UL47 protein, apoptosis induced by DNA damage is significantly increased, suggesting that UL47 contributes to apoptosis by hindering SMC1 function and DNA repair. Another protein, VP22—encoded by the UL49 gene of BHV-1—is a tegument component with a NES at its C-terminal region that overlaps with a mitochondrial targeting sequence. Overexpression of VP22 in HeLa cells has shown to induce apoptosis, which was possibly due to the transfer of VP22 location from the cytoplasm towards the mitochondria; the mitochondrial pathways may thus play a role in the apoptosis induced by VP22. In gamma herpesviruses, the latency-associated nuclear antigen 2, actively transcribed only in viral-infected B cells, has been identified from KSHV and is responsible for initiating the apoptotic resistance; LANA2 inhibits the p53-dependent transcriptional activation of the protein kinase R pathway. A form of NLS, the nuclear localization signal, is critical for the apoptotic resistance by LANA2, whose mutants relocate this complex from the nucleus to the cytoplasm, thereby inhibiting the capability to suppress p53-mediated transcription. [31].

Subsequent research shows that LANA2 phosphorylation disrupts NES function and reduces its anti-apoptotic potential [35]. These findings underscore the importance of LANA2 nuclear retention in suppressing apoptosis and highlight the significance of its shuttling behavior in sustaining viral replication. Overall, herpesvirus shuttling proteins modulate apoptosis-related signaling nodes by relocating between the nucleus and cytoplasm. Through this dynamic movement, they can either promote or suppress apoptotic processes, depending on the stage of infection and the needs of the virus.

5.1. Herpesvirus Shuttling Proteins and Their Role in Immune Evasion

The significant capacity of Herpesviruses to induce lifelong infection and a strong level of host selectivity have distinguished Herpesviruses. Recognition of viral nucleic acids by pattern recognition receptors, in particular MDA5 and RIG-I which sense pathogen associated molecular patterns leads to activation of host cellular innate immune response, and communication of such signals by adaptor proteins, including MAVS and STING. Events of subsequent signaling lead to nuclear translocation of major transcription factors, such as NF- κ B, IRF-3, IRF-7 transcription factors, and results in the transcription of type I interferon (IFN-I) and pro-inflammatory cytokines. Subsequently after this, the JAK-STAT cascade is activated that augments the expression of interferon stimulated genes (ISGs) and therefore establishes an appropriate antiviral state. Conversely, the viruses in herpes have a synthesis amount of anti-host defense mechanisms [36].

The genomes of herpesviruses encode diverse sets of proteins that activate active immune evasion through inhibition of type I interferon synthesis, interference with downstream interferon -signaling cascades, and regulation of interferon -stimulated gene expression as well as blockage of essential factors of the innate immune responses, etc.. As an example, nuclear localization of the bovine herpesvirus 1 (BHV-1) transcriptional regulator ICP27 is essential in silencing the IFN β promoter in cells transfected; mutations in its N-terminal nuclear localization domains disrupt this silencing capability. Also, ICP27 can disrupt the maturation of host cellular mRNAs, by disrupting the 3'-end processing of transcripts required to express IFN- β , although this potential mechanism remains under study. The HSV-1 homolog ICP27 also activates innate immune signaling; it causes the relocation of the cellular protein Daxx out of the nucleus into the cytoplasm, thus leading to its association with the subunit of NF- κ B p65 and a suppression of NF- κ B transcriptional activity [25].

This modulation of Daxx localization contributes to viral evasion of intrinsic immune responses. STAT1, a key mediator in IFN-I signaling, is regulated by BHV-1 UL47. Lack of a functional NLS in UL47 prevents STAT1 from accumulating in the nucleus following interferon stimulation, thereby attenuating antiviral signaling. HSV-1 ICP27 also inhibits IFN-I responses by repressing the phosphorylation and nuclear translocation of STAT1. ICP27 mutants that lack either NES or NLS, disrupting the specific localization to cytoplasm after nuclear export, have been tested and found unable to effectively block expression of IFN. Some viral proteins also interfere with upstream immune signaling. UL46 protein of pseudorabies virus (PRV) modulates STING activity in the cytoplasm, undermining innate immune sensing [11]-[13].

Similarly, HSV-1 g134.5 inhibits the trafficking of RIG-I to mitochondria and STING to migrate from the endoplasmic reticulum to the Golgi. It also prevents IRF3 from being activated and entering the nucleus, resulting in the inhibition of IFN induction. In beta herpesviruses, the HCMV UL94 inhibits antiviral signaling by restraining STING. It blocks the drawing of TBK1 to STING composites, which is required to hasten IRF3 filial and IFN-I creation subsequently. It selectively interferes with later signal transmission, thwarting immune activators. In gamma herpesviruses, KSHV LANA2 regulates the NF- κ B by prompting its nuclear accretion, improving transcriptional productions. ORF45, another KSHV-made protein, prevents IFN-I assembly by confining IRF7 and averting its filial and nuclear localization. Altogether, these examples show how herpesvirus shuttling proteins orchestrate host immune processes by

altering the cytoplasmic subcellular localizations of signaling molecules and transcriptional agents. In doing so, they weaken the interferon reaction and help with viral endurance, displaying a suave viral strategy of immune repeal. algorithm: [37]

6. Conclusion and Future Trends

The review aims to explore the role of nucleocytoplasmic shuttling proteins in herpesvirus replication and pathogenesis. These proteins regulate mRNA transport, capsid formation, and early envelopment by moving between the nucleus and cytoplasm. Viruses use host nuclear export pathways in order to enable viral protein production and in order to avoid host defense mechanisms. Without interactions with nuclear localization and export signals (NLS/NES), genome replication and immune evasion are not achievable. In-depth knowledge of these pathways can be used to focus antiviral interventions on the shuttling pathways.

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Abbreviations list

Abbreviation	Description
HSV-1	Herpes Simplex Virus Type 1
HSV-2	Herpes Simplex Virus Type 2
PRV	Pseudorabies Virus
VZV	Varicella-Zoster Virus
BHV-1	Bovine Herpesvirus Type 1
HHV-6	Human Herpesvirus Type 6
HHV-7	Human Herpesvirus Type 7
HCMV	Human Cytomegalovirus
KSHV	Kaposi Sarcoma-Associated Herpesvirus
EBV	Epstein-Barr Virus
UL	Unique Long Segment
TRL	Terminal Repeat Long
IRL	Internal Repeat Long
US	Unique Short Segment
U segments	Heterogeneous Genomic Regions in HHV-6 and HHV-7
Importin-α	Alpha Importin (Group A)

Abbreviation	Description
Importin-β	Beta Importin (Group B)
IBB	Importin-β Binding Domain
CAS	Cellular Apoptosis Susceptibility Protein (Export Factor)
Nup50	Nucleoporin 50
ARM	Armadillo Repeat Motif
NLS	Nuclear Localization Signal
IBN	Importin-β-Specific N-terminal Domain
HEAT	HEAT Repeat Domain (Conserved Structural Domain)
LAT	Latency-Associated Transcript (defines latency in HSV-1)
ICP-0	Immediate-Early Protein 0 (triggers HSV-1 lytic phase and reactivation)
VLT63	VLT-ORF63 Fusion Transcript (latent expression in VZV)
IE62	Immediate-Early Tegument Protein (activates VZV gene expression for lytic replication)
IE1	Immediate-Early Protein 1 (encoded by UL123 in HCMV; initiates lytic infection)
IE2	Immediate-Early Protein 2 (encoded by UL122 in HCMV; regulates downstream viral genes)
EBNA1	Epstein-Barr Nuclear Antigen 1 (anchors EBV episomes to host chromatin)
Rta (BRLF1)	Replication Transactivator (EBV transcription factor for reactivation)
Zta (BZLF1)	Z Transactivator (EBV transcription factor for reactivation)
LANA1	Latency-Associated Nuclear Antigen 1 (maintains episomal stability in KSHV)
RTA (UL50)	Replication Transactivator (KSHV protein regulating lytic switch)

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Synthesis of Triazole-Imine Derivatives with Antibacterial and Anticancer Potential: An Experimental and in Silico Study

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Abstract:

This work condensed 4-hydroxybenzaldehyde and triazole-based primary amines to form triazole-imine compounds (A–D). Generated compounds revealed azomethine links in FTIR and NMR. *Streptococcus pneumoniae* and *Bacillus subtilis* antibacterial activity was measured by 0.1, 0.001, and 0.00001 mg/mL agar diffusion derivative C had the largest concentration-dependent inhibition zones of 23 mm against *S. pneumoniae* and 25 mm against *B. subtilis*. MTT also assessed PC-3 and MCF-7 cytotoxicity. The derivative A dose-dependently reduced PC-3 and MCF-7 cell viability by 4.99% and 8%, respectively, at 320 µg/mL. Docking DNA-associated protein (8RZX) explains these biological results. Docking data show stable binding conformations, with RMSD values around 2 Å and binding energies –2.5 kcal/mol, especially for derivative C. The majority of interactions were hydrogen bonding with phosphate groups and π – π stacking with nucleobases. Experimental and computational evidence suggest triazole-imine compounds are potential antibacterial and anticancer scaffolds.

Keywords: Molecular docking, Schiff bases, Triazole derivatives, Heterocyclic compounds.

تحضير مشتقات التريازول- إيمين كعوامل واعدة مضادة للبكتيريا والسرطان: دراسة

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الخلاصة:

في هذه الدراسة، تم تكثيف ٤-هيدروكسي بنزالدهيد مع الأمينات الأولية القائمة على التريازول لتكوين مركبات تريازول-إيمين (A-D). أظهر طيف الأشعة تحت الحمراء وطيف الرنين النووي المغناطيسي عن وجود روابط أزوميثين في المركبات المنتجة. تم قياس النشاط المضاد للبكتيريا ضد الميكروبات الرئوية (*Streptococcus pneumoniae*) والعصوية الرقيقة (*Bacillus subtilis*) باستخدام تراكيز ٠,١ و ٠,٠٠١ و ٠,٠٠٠١ ملغم/مل من مشتق الأجار C، حيث أظهر أكبر مناطق تثبيط معتمدة على التركيز، بقطر ٢٣ مم ضد الميكروبات الرئوية و ٢٥ مم ضد العصوية الرقيقة. كما تم تقييم سمية المركبين PC-3 و MCF-7 باستخدام اختبار MTT. أدى المشتق A إلى خفض حيوية خلايا PC-3 و MCF-7 بنسبة ٤,٩٩٪ و ٨٪ على التوالي، عند تركيز ٣٢٠ ميكروغرام/مل، وذلك بشكل متناسب مع الجرعة. ويُفسر ارتباط البروتين المرتبط بالحمض النووي (RZX^A) هذه النتائج البيولوجية. تُظهر بيانات الارتباط تكوينات ارتباط مستقرة، بقيم $RMSD$ تقارب ٢ أنغستروم وطاقات ارتباط تبلغ -٢,٥ كيلو كالوري/مول، وخاصةً بالنسبة للمشتق C. وكانت غالبية التفاعلات عبارة عن روابط هيدروجينية مع مجموعات الفوسفات وتفاعلات تكديس $\pi-\pi$ مع القواعد النيتروجينية. تشير الأدلة التجريبية والحسابية إلى أن مركبات التريازول-إيمين تُعدّ هياكل محتملة مضادة للبكتيريا ومضادة للسرطان.

الكلمات المفتاحية: الالتحام الجزيئي، قواعد شيف، مشتقات التريازول، المركبات الحلقية غير المتجانسة.

1. Introduction:

Heterocyclic compounds play a crucial role in medicinal chemistry due to their diverse biological activities [1]. Synthesis of heterocyclic chemical compounds is an important priority in organic chemical synthesis due to its many uses in health, pharmacology, electronic devices, agricultural production, and other associated domains [2]. Two essential requirements must be addressed by contemporary heterocyclic chemistry methods (reagents, techniques, strategies, reaction processes, chemical and instrumental auxiliaries, catalysts, etc.): "specificity" and "selectivity" (containing common prefixes like chemo, regio, or stereo) [3]. There are two isomers of the triazole ring, a notable five-membered heterocycle containing three nitrogen atoms: The aromatic system 1,2,3-triazole, also known as 1,2,4-triazole, is rich in electrons. Through weak interactions like coordination bonds, hydrogen bonds, ion-dipole interactions, cation stacking, hydrophobic effect, van der Waals force, and so forth. This kind of special

structure enables triazole derivatives to readily bind with a variety of enzymes and receptors in biological systems, leading to a wide range of biological activities[4-6].

Schiff bases, or imines, are essential organic structural motifs. Reversible condensation reactions between a carbonyl molecule and a primary amine generate imines, which have a carbon–nitrogen double bond. Drying agents, molecular sieves, or Dean–Stark traps remove water from the process [7]. Imines are valuable as catalytic ligands, intermediates in the synthesis of nitrogen-containing heterocycles and alkaloids, and players in coordination chemistry. Dantrolene, a muscle relaxant, and thiacetazone, an antituberculosis medication, contain imines [8]. Several organic laboratories have imine synthesis reactions, despite their abundance in biochemical processes in nature. In light of this, a lab study involving imine-based chemical synthesis is crucial[9].

Recent research has focused on triazole-associated Schiff bases with different substituents for antibacterial and anticancer properties. Few studies have linked spectroscopic confirmation, in vitro biological evaluation, and in silico docking against DNA-linked target proteins like PDB ID: 8RZX. Thus, there is little experimental and molecular study on how triazole-imine framework structural variation impacts binding affinity, antibacterial activity, and cytotoxicity.

New triazole-imine molecules are synthesized and tested for biological activity using complementary approaches. The primary research questions: How do triazole amine precursors affect Schiff bases' physicochemical and biological properties? Working with the 8RZX binding pocket, molecular docking may explain antibacterial and anticancer action differences. Answering these questions requires several steps. FTIR and NMR the target derivative structure following synthesis. Their antibacterial and cytotoxic properties against Gram-positive bacteria and PC-3 and MCF-7 cancer cells are tested. Finally, molecular docking examines binding modes, interaction energies, and structure–activity relationships. Triazole–imine molecules are intriguing bioactive candidates, and this work applies this integrated approach.

2. Material and methods:

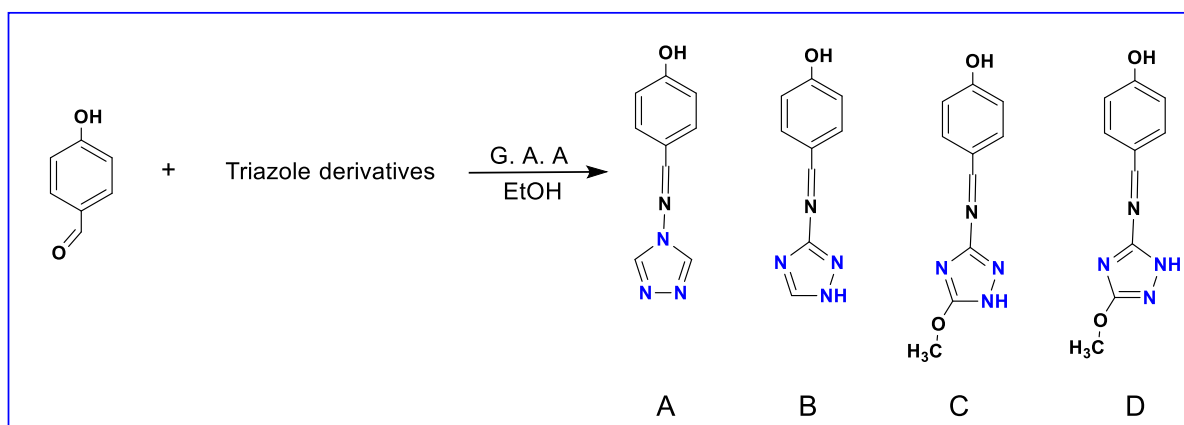
2.1 Material.

Sigma Aldrich provided a variety of amines, including 4-Amino-1,2,4-triazole, 3-Amino-1,2,4-triazole, 3-Amino-5-methylthio-1H-1,2,4-triazole, and 5-Amino-1H-[1,2,4]-triazole-3-carboxylic acid methyl ester. While 100% ethanol, glacial acetic acid, and 4-hydroxybenzaldehyde were acquired from BDH.

2.2 Synthesis of schiff base derivatives (A-D).

According to Jaafar, M. R., et al (2024) [10], a novel group of imine derivatives (A-D) was synthesized by reacting the same moles of 4-hydroxybenzaldehyde with varying derivatives of triazole that were classified as primary amines. 0.1 mole of 4-hydroxy benzaldehyde dissolved in 20 mL of absolute ethanol, and add 2-3 drops of glacial acetic acid; add the amine compound to this solution, and reflux for 3 hours. The mixture is then cooled to room temperature and filtered to collect the precipitate. To synthesize derivative A obtained from 4-amino-1,2,4-triazole; derivative B from 3-Amino-1,2,4-triazole; derivative C from 3-Amino-5-methylthio-1H-1,2,4-triazole; derivative D from 5-Amino-1H-[1,2,4]-triazole-3-carboxylic acid methyl ester, as shown in Scheme 1.

The physical properties of synthesized derivative A were obtained as a dark yellow precipitate and have melting point ($1^{\wedge}\text{v}^{\circ}\text{C}$). In contrast, derivative B was obtained as a yellow color and (161°C) with a melting point, and derivative C was yellowish-brown had a melting of (143°C). Finally, derivative D was formed in a dark yellow and 181°C .



Scheme 1: Synthesized triazole-imine derivatives (A-D).

2.3 Assessment of Antimicrobial Properties of Schiff bases Derivatives.

The cup-plate agar diffusion technique evaluates the antibacterial efficacy of derivatives A-C. *Streptococcus pneumoniae* and *Bacillus subtilis* isolated from clinical infections are designated as test organisms. Compounds are formulated in dimethyl sulfoxide at three concentrations: 0.1, 0.001, and 0.00001 mg/mL. Muller-Hinton agar plates are inoculated with bacterial suspensions, and 12 mm wells are filled with the test solutions. Ceftriaxone reconstituted in sterile water serves as the reference standard. Following one hour of pre-diffusion, the plates were incubated..." (add what happens next). The antibacterial efficacy of each chemical is evaluated by measuring the inhibition zone diameter in millimeters and comparing it to the reference medicine [11].

2.4 The Biotech MTT kit was used to assess several synthetic compounds for cytotoxicity.

Synthesized molecules were evaluated for cytotoxicity against prostate cancer PC3 cells with the MTT assay according to the manufacturer's instructions. MCF-7 cells were seeded at 4.5×10^5 per well in sterile 96-well plates containing 200 μL of growth medium. To improve cell adherence, plates were sealed with parafilm, lightly centrifuged, and incubated at 37 °C in 5% CO_2 for 24 hrs. Cells were subjected to a 2-fold serial dilution and treated with synthetic derivatives at concentrations of 0, 20, 40, 80, 160, and 320 $\mu\text{g/mL}$ after incubation. The controlled plate was incubated for 24 hours. Following drug exposure, each well received 10 μL of MTT solution, which was then incubated for 4 hours to allow metabolically active cells to transform MTT into insoluble formazan crystals. After incubation, remove the supernatant and dissolve the formazan in 100 μL of DMSO in each well to preserve the crystals. After five minutes of moderate swirling, the plates melted. 575 nm absorbance was measured using ELISA microplate readers (Bio-Rad, Germany). Cell viability was shown by optical density measurements, and the half-maximal inhibitory concentration (IC_{50}) of the derivatives was ascertained using statistical analysis. This technique assessed cytotoxic effects accurately, consistently, and dependably [12].

2.5 Molecular Docking Process

To investigate the binding mechanism and affinity, the chemically generated triazole–imine compounds (A–C) were docked with the biological target protein (PDB ID: 8RZX) using the Molecular Operating Environment (MOE) tool [13, 14]. **Figure 1**. Its three-dimensional crystal structure was taken from the Protein Data Bank and generated in MOE by removing crystallographic water molecules and co-crystallized ligands to reduce ligand binding interference. We inserted hydrogen atoms and energy-minimized the protein structure to decrease steric conflicts and maximize form [15]. MOE imported ligand chemical structures drawn in Chem Draw. Ligands were translated into three-dimensional conformations and geometry was adjusted using a force field to create stable structures. For docking, partial atomic charges and rotatable bonds were assigned for conformational flexibility [16]. The MOE docking simulation active binding area was defined by 8RZX binding pocket nucleotide residues. The placement strategy produced various active site ligand postures. A scoring function calculated the binding free energy. After energy reduction improved top-ranked postures, docking scores, interaction types, and RMSD values were gathered. The research

studied ligand-protein interactions, including hydrogen bonding, π - π stacking, and binding distances.

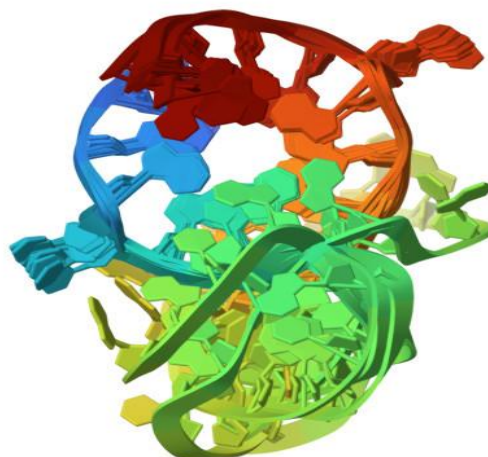


Figure 1: Chemical structure of 8RZX protein.

3.Results and Discussion

The reaction between 4-hydroxybenzaldehyde and different primary amines to produce Schiff base derivatives results in different yellow colors as precipitates. In these reactions, the glacial acetic acid plays a catalytic role to protonate the carbonyl group, and the amine derivative plays a nucleophilic role to attack the carbon of the carbonyl group. In FTIR spectroscopy, the primary amine and the carbonyl group of the aldehyde disappeared and showed a new peak for the imine group [17, 18].

4-(((4H-1,2,4-triazol-4-yl)imino)methyl)phenol (A).

The FTIR (cm^{-1}) spectrum shows a peak at 1625 for azo methane group [19], 3336 for the hydroxyl group as broad band, 3029 for the C-H of the aromatic ring, and 1580 for C=C of aromatic ring. ^1H -NMR (DMSO- d_6 as solvent) δ 9.15 (s, 1H, -OH), 8.49 (s, 1H, imine), 7.71 – 7.18 (m, 4H, Ar), as shown in Figure 2. ^{13}C -NMR (DMSO- d_6) the key peaks include 155.33 ppm (C-OH), 149.65 ppm (triazole carbon), and 138.87 ppm (ipso-carbon bonded to the linkage). Aromatic methine carbons (CH) appeared at 133.16 and 125.71 ppm, while the ortho-carbons shifted upfield to 114.56 ppm due to the electron-donating effect of the hydroxyl group [20], as shown in Figure 3.

4-(((1H-1,2,4-triazol-3-yl)imino)methyl)phenol (B).

The FTIR (cm^{-1}) spectrum shows a peak at 1641 for imine group, 3335 for the hydroxyl group, 3055 for the C-H of the aromatic ring and 1582 for C=C of aromatic ring [21]. ^1H NMR

(DMSO- d_6) δ 9.91 (s, 1H, -OH), 8.98 (s, 1H, imine), 8.15-7.24 (m, 5H, Ar), as shown in [Figure 4](#). ^{13}C NMR (DMSO- d_6) δ 161.01 for aromatic carbon that linked to hydroxyl group, 153.76 for carbon imine group, 149.50-113.81 for aromatic carbon [\[22\]](#), as shown in [Figure 5](#).

4-(((5-methoxy-1H-1,2,4-triazol-3-yl)imino)methyl)phenol (C).

The FTIR (cm^{-1}) spectrum shows a peak at 1631 for imine group, 3384 for the secondary amine, 3025 for the C-H of the aromatic ring and 1579 for C=C of aromatic ring [21]. $^1\text{H-NMR}$ ($\text{DMSO-}d_6$) δ 9.09 (s, 1H, -OH), 8.67 (s, 1H, imine), 7.75-7.24 (m, 4H, Ar), 5.29 (s, 1H, NH), 3.89 (s, 3H, methoxy)[11], as shown in Figure 6. $^{13}\text{C-NMR}$ (50 MHz, $\text{DMSO-}d_6$) δ 162.81 for aromatic carbon that linked to hydroxyl group, 157.70 for carbon triazine ring that linked to oxygen of methoxy group, 152.97 for carbon imine group, 148.02-108.61 for aromatic carbon, 61.26 for methoxy group [23], as shown in Figure 7.

4-(((3-methoxy-1H-1,2,4-triazol-5-yl)imino)methyl)phenol (D).

The FTIR (cm^{-1}) spectrum shows a peak at 1621 for imine group, 3363 for the secondary amine, 3068 for the C-H of the aromatic ring and 1598 for C=C of aromatic ring. ^1H NMR ($\text{DMSO-}d_6$) δ 9.01 (s, 1H, -OH), 8.11, (s, 1H, imine), 7.64-7.01 (m, 4H, Ar), 5.26 (s, 1H, NH) [24], 2.98 (s, 3H, methoxy) [10], as shown in **Figure 8**. ^{13}C NMR ($\text{DMSO-}d_6$) δ 162.52 carbon of imine group, 155.49 for carbon triazine ring that linked to oxygen of methoxy group, 155.27 aromatic carbon that linked to hydroxyl group, 154.07-122.95 carbons of aromatic group, 59.92 carbon of methoxy group [25], as shown in **Figure 9**.

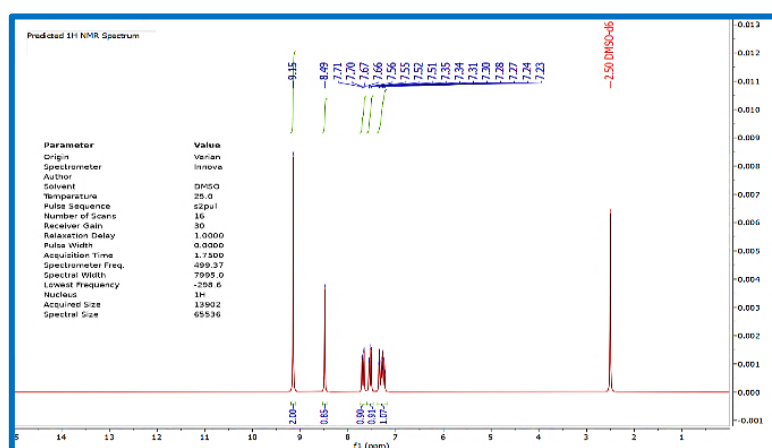


Figure 2: ^1H -NMR spectrum of derivative A.

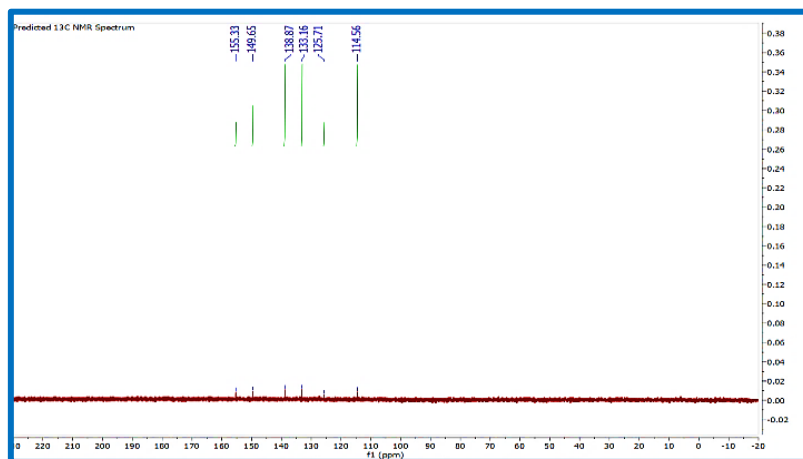


Figure 3: ^{13}C -NMR spectrum of derivative A.

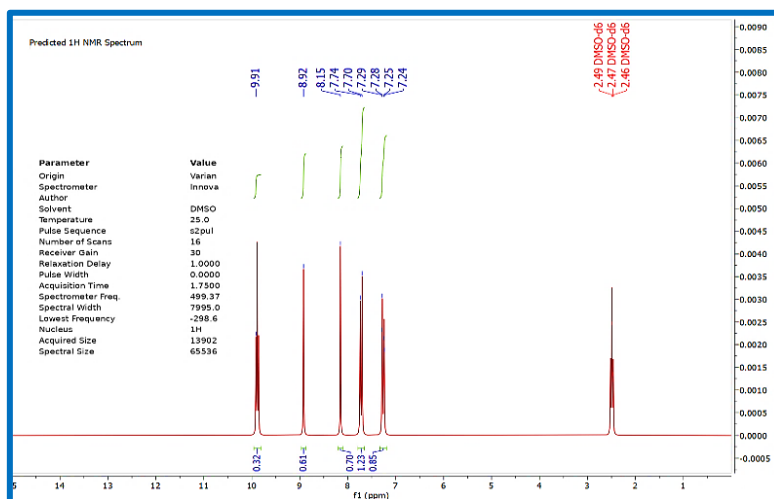


Figure 4: ^1H -NMR spectrum of derivative B.

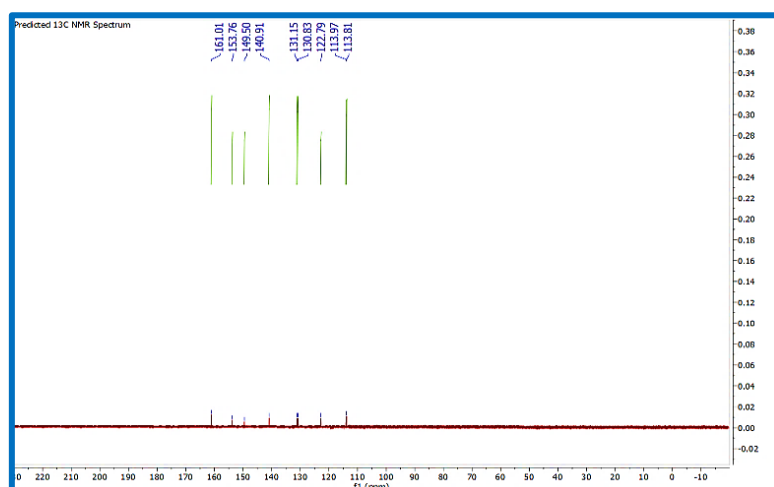


Figure 5: ^{13}C -NMR spectrum of derivative B.

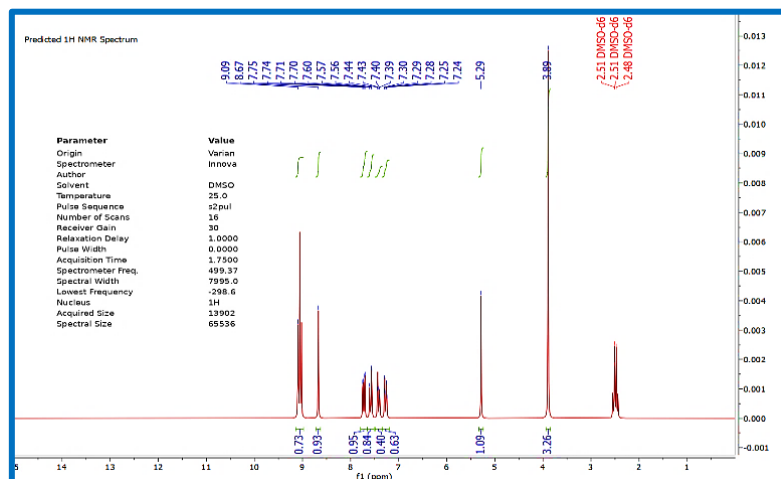


Figure 6: ^1H -NMR spectrum of derivative C.

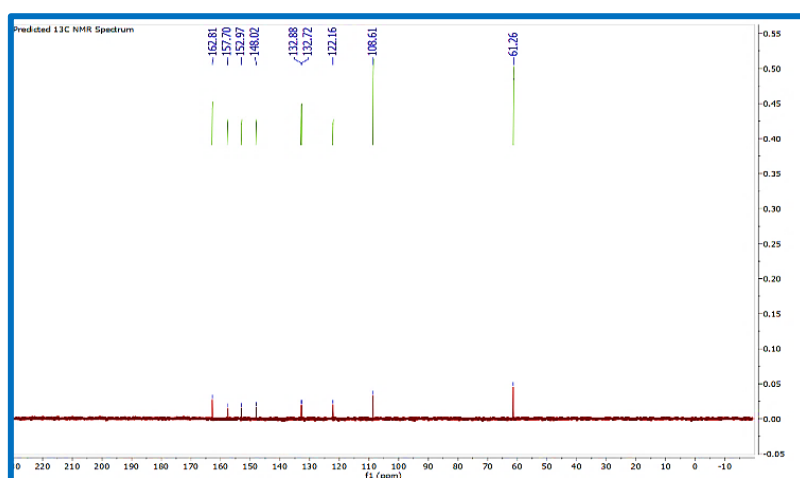


Figure 7: ^{13}C -NMR spectrum of derivative C.

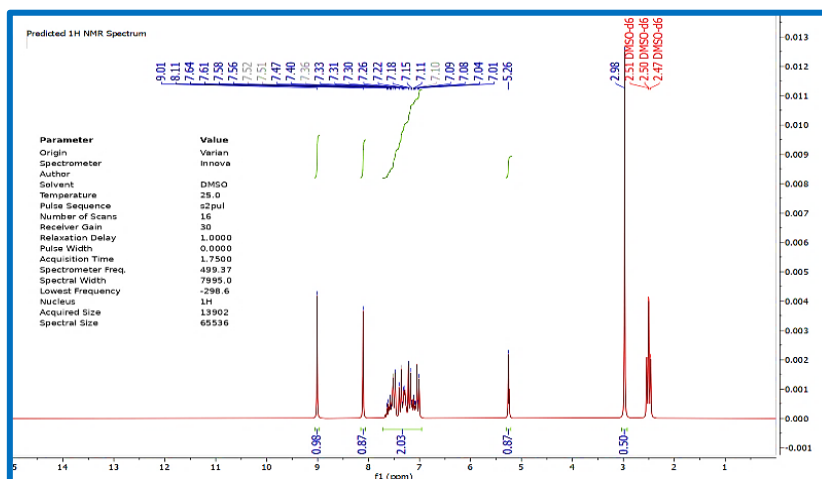


Figure 8: ^1H -NMR spectrum of derivative D.

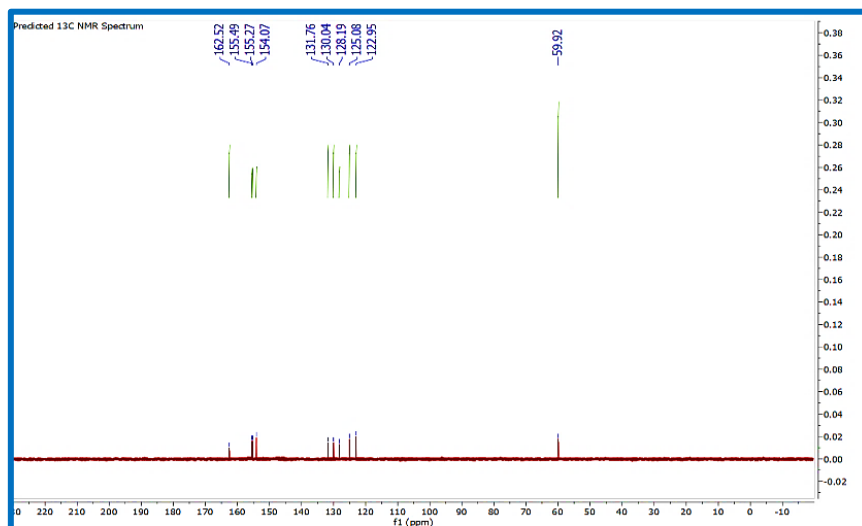


Figure 9: ^{13}C -NMR spectrum of derivative D.

The 8RZX protein has a well-defined binding pocket composed of DNA nucleotides such as DT, DC, DA, and DG. The pocket's mild polarity and aromaticity facilitate the stability of organic ligands by hydrogen bonding with phosphate groups and π - π stacking with nucleobases. The structural characteristics render 8RZX suitable for bioactive ligand evaluation by molecular docking [26].

Table 1 describes the physicochemical parameters of the active site of the 8RZX protein. The moderate size of the binding pocket accommodates small aromatic ligands without steric hindrance. Numerous nucleotide residues, particularly DG and DT units, provide a hybrid polar and aromatic milieu. Heteroatom as an aromatic ring ligands bind strongly because of these characteristics, selective and stable ligand binding and docking interactions of synthetic molecules are made possible by the pocket structure.

Table 1: Binding Pocket Characteristics of 8RZX Protein.

Site	Size	PLB	Hyd	Side	Residues
1	50	2.4	7	51	1:(DT1 DC12 DA13 DG14 DG15 DT16 DG17 DG18 DG19 DC20 DG21)

The synthetic 8RZX protein with synthesized derivatives (A-C) together with their docking contacts and binding energies are listed in **Table 2**. The hydrogen bonds that Compound A forms with DG21's OP1 show polar anchoring of the binding site. Compound B's π - π stacking and hydrogen bonding with DG19 and DG21 show enhanced stability via dual contact

mechanisms. The most intricate interactions are shown by compound C, which forms π - π stacking with DG21 and hydrogen bonds with DG19. Ligand-protein stability is indicated by moderate binding energies and contact distances. Heteroatoms and aromatic systems boost binding affinity, but compound C exhibits superior active site spatial complementarity compared to the others.

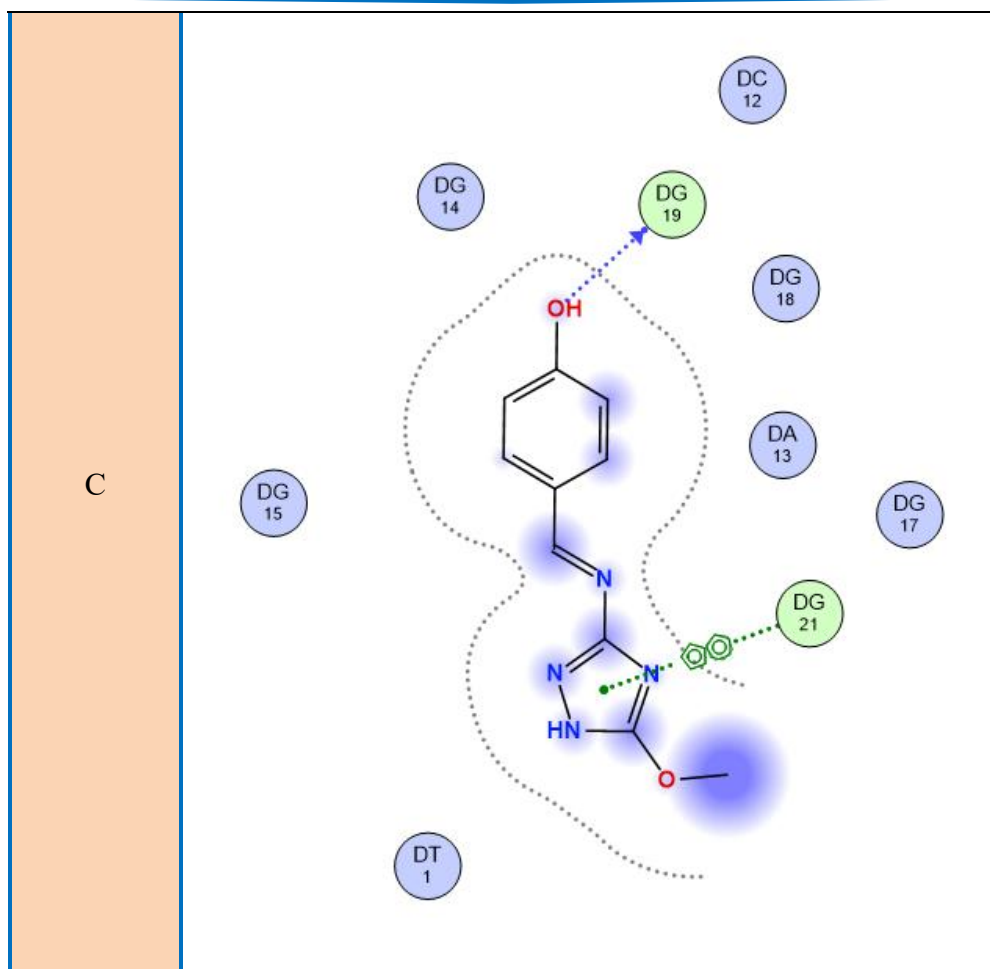
Table 2: Docking results for synthesized derivatives interaction with target protein.

Compound No.	Ligand	Receptor	Interaction	Distance (Å)	E (kcal/mol)
A	O 11	OP1 DG 21 (A)	H-donor	3.16	-2.5
B	O 11	OP1 DG 19 (A)	H-donor	2.97	-2.5
	5-ring	5-ring DG 21 (A)	π - π	3.96	
C	O 15	OP1 DG 19 (A)	H-donor	2.92	-2.1
	5-ring	5-ring DG 21 (A)	π - π	3.71	
	5-ring	6-ring DG 21 (A)	π - π	3.60	

Table 3 displays the medicines' energy components, RMSD values, and 8RZX receptor docking scores. With RMSD values close to 2 Å, all compounds show stable binding orientations. Compound C has the best docking, indicating stability throughout placement and refinement. Compounds A and B score well after docking and refining. Favorable intermolecular interactions that encourage spontaneous ligand-protein attachment are indicated by negative binding scores. Hydrogen bonding and π - π stacking in compounds are impacted by changes in functional groups and aromatic substitution. Research indicates that modifications to the ligand scaffold significantly influence binding at the 8RZX active site.

Table 3: Interaction details of the synthesized derivatives and the protein acceptor.

Compound No.	Image
A	
B	



Each of the derivatives inhibits *Streptococcus pneumoniae* in a dose-dependent manner. At the highest concentration, compound C (23 mm) has the greatest activity among the synthesized derivatives, followed by derivative D (20 mm), derivative B (17 mm), and A (15 mm). The optimal standard is 25 mm azithromycin. The inhibitory zones of derivative A significantly decrease at middle and low dosages, indicating less activity persistence. The derivative C is the most promising derivative against *S. pneumonia*, demonstrating sustained suppression comparable to the reference antibiotic at elevated doses, as shown in **Figure 10**.

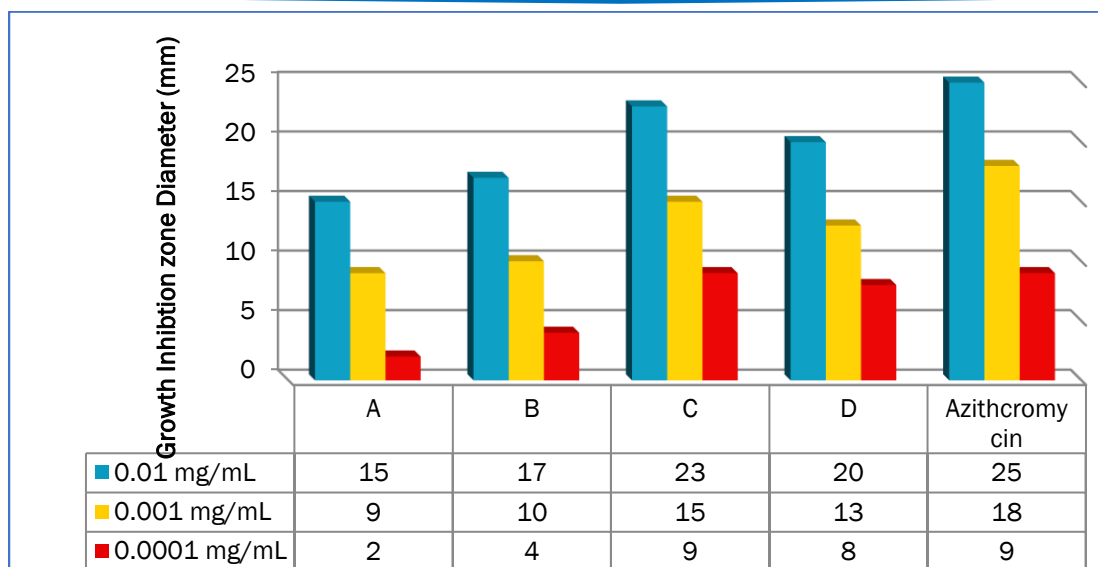


Figure 10: Synthesized derivatives activity of against *Streptococcus pneumoniae*.

Each synthesized derivative has concentration-dependent *Bacillus subtilis* antibacterial activity. At 0.1 dosage, derivative C (25 mm) and derivative D (23 mm) had the largest inhibitory zones, reaching azithromycin (24 mm). Both derivative A (18 mm) and derivative B (16 mm) demonstrated modest action at equal doses. At concentrations of 0.001 and 0.00001, inhibition zones markedly decrease for all derivatives, indicating reduced efficacy at lower doses. Derivatives C and D exhibit superior antibacterial efficacy compared to derivative A and derivative B, suggesting enhanced interaction with bacterial targets, as shown in **Figure 11**.

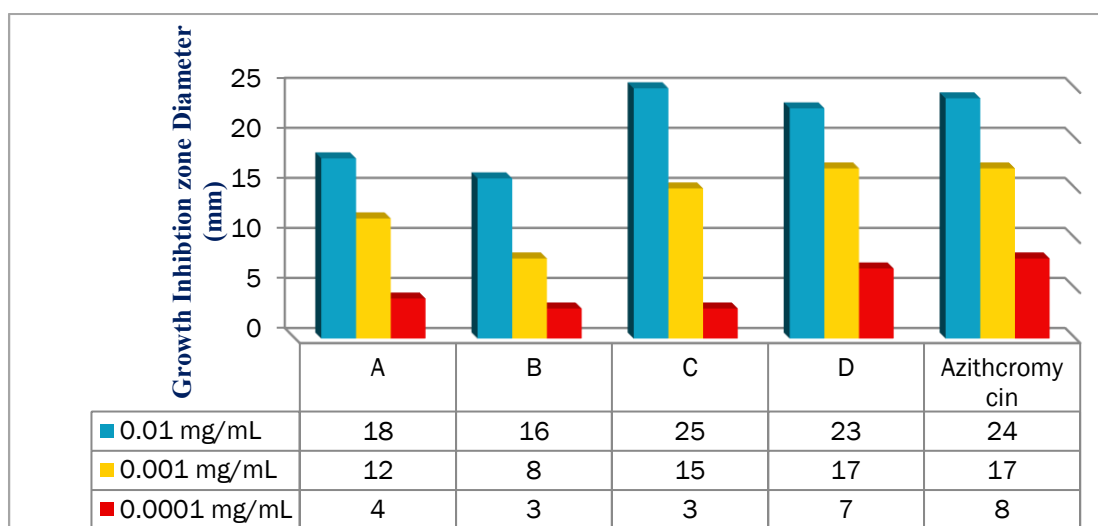


Figure 11: Antibacterial activity of synthesized derivatives against *Bacillus subtilis*.

This figure demonstrates A's concentration-dependent cytotoxicity against PC-3 cells. Cell viability decreases from 99.69% in the control group to 59.26% and 44.51% at 20 and 40 ppm. At the maximum dosage, viability is reduced to 4.99% by high dosages (80–320 ppm). This notable decline seems to have the potential to impede development and cause cell death.

Because of its consistent trend throughout concentrations, chemical A may combat prostate cancer at high doses, as shown in **Figures 12 and 13**.

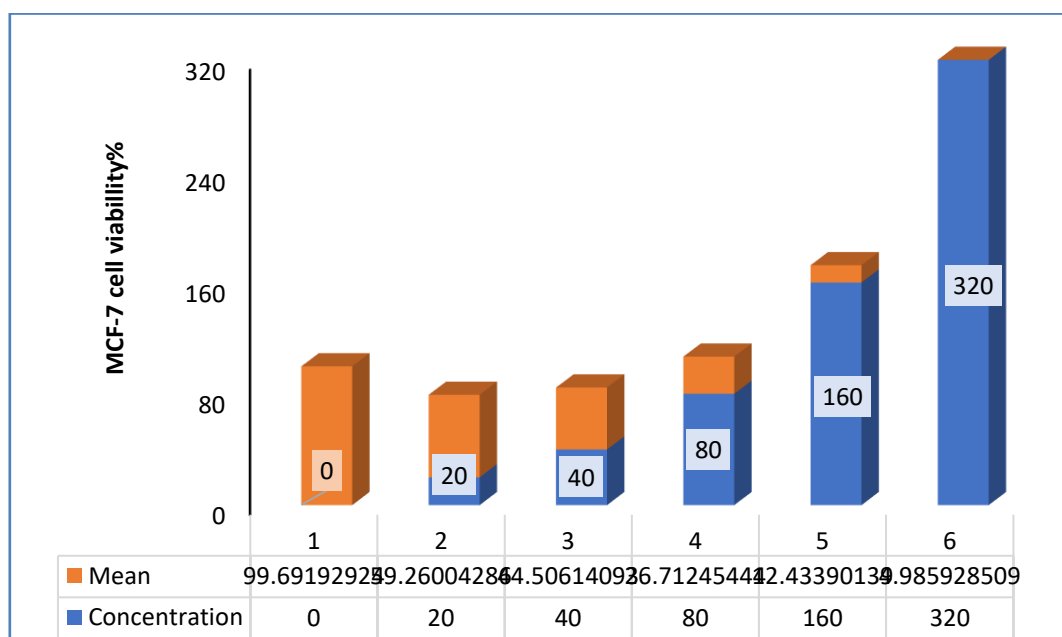


Figure 12: Cytotoxicity of MCF-7 Cells to increasing concentrations of derivative A.

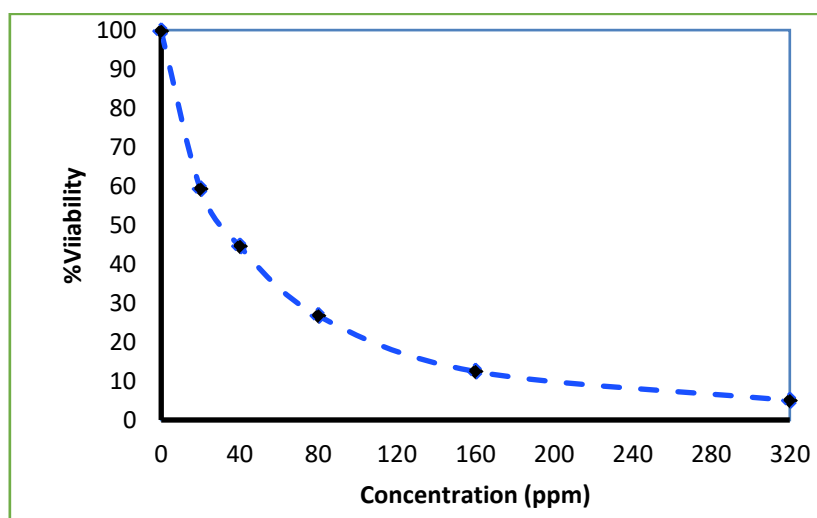


Figure 13: Dose response curve illustrating the cytotoxic effect of derivative A on MCF-7 Cells.

Concentration-dependent Compound C cytotoxicity against MCF-7 cells. Control group cell viability remains almost unaltered, indicating normal cell growth. Growth inhibition is seen between 20 and 40 ppm, when viability reduces to 61% and 50%. Higher dosages reduce viability to 33.5% at 80 ppm and 8% at 320 ppm. This slow fall reveals that derivative C restricts cell proliferation and may kill cells at high doses. The derivative C has exceptional antitumor potential against MCF-7 cells, as shown in **Figures 14 and 15**.

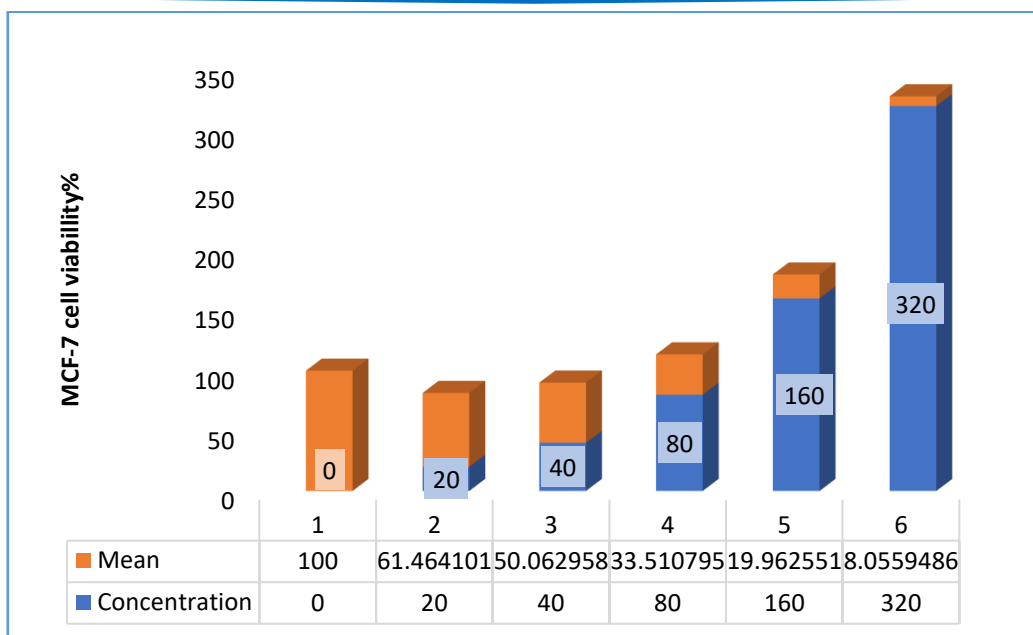


Figure 14: Cytotoxicity of MCF-7 Cells to increasing concentrations of derivative C.

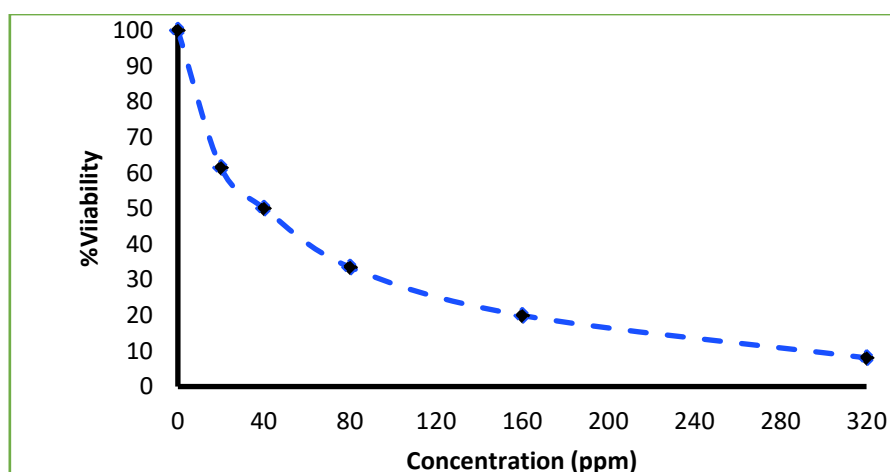


Figure 15: Dose response curve illustrating the cytotoxic effect of derivative C on MCF-7 Cells.

The predicted ADMET profiles for derivatives A-D (Figures 16-19) show drug-likeness and pharmacokinetics. All derivatives have molecular weights below 250 g/mol and pass Lipinski's rule of five without exception, indicating oral drug-like characteristics. The consensus LogP values of 1.1–1.8 imply balanced lipophilicity, which improves membrane permeability and water solubility. All compounds are predicted to be water-soluble, which aids formulation and absorption. Pharmacokinetic estimates imply oral uptake due to high gastrointestinal absorption for all derivatives. Anticancer and antibacterial compounds are unlikely to cross the blood-brain barrier, reducing central nervous system side effects. Without P-glycoprotein substrate behavior, efflux-mediated drug resistance is unlikely. Importantly, the compounds do not

inhibit critical cytochrome P450 enzymes, indicating low metabolic drug-drug interactions. The absence of PAINS alerts in medicinal chemistry improves confidence in experimental data's biological relevance, whereas the imine functionality Brenk alarm is expected and chemically justified. ADMET analysis confirms experimental findings and reveals that produced triazole-imine compounds have appropriate pharmacokinetic properties for optimization and biological evaluation.

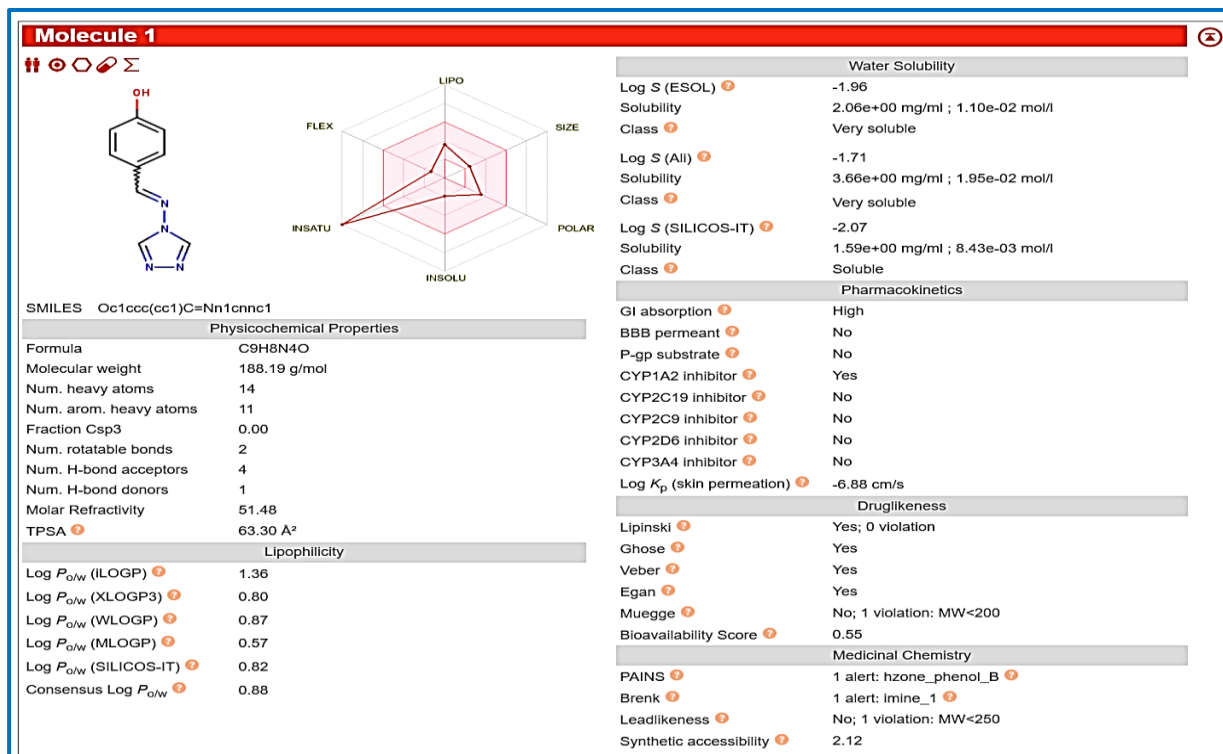


Figure 16: SwissADME Prediction for derivative A.

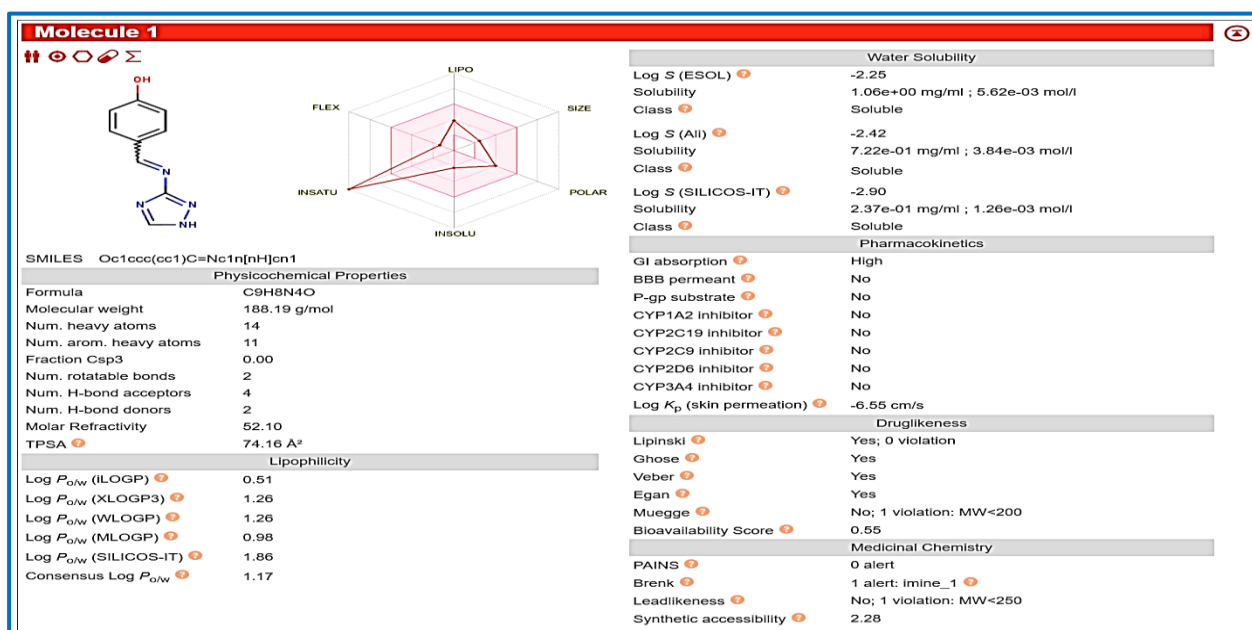


Figure 17: SwissADME Prediction for derivative B.

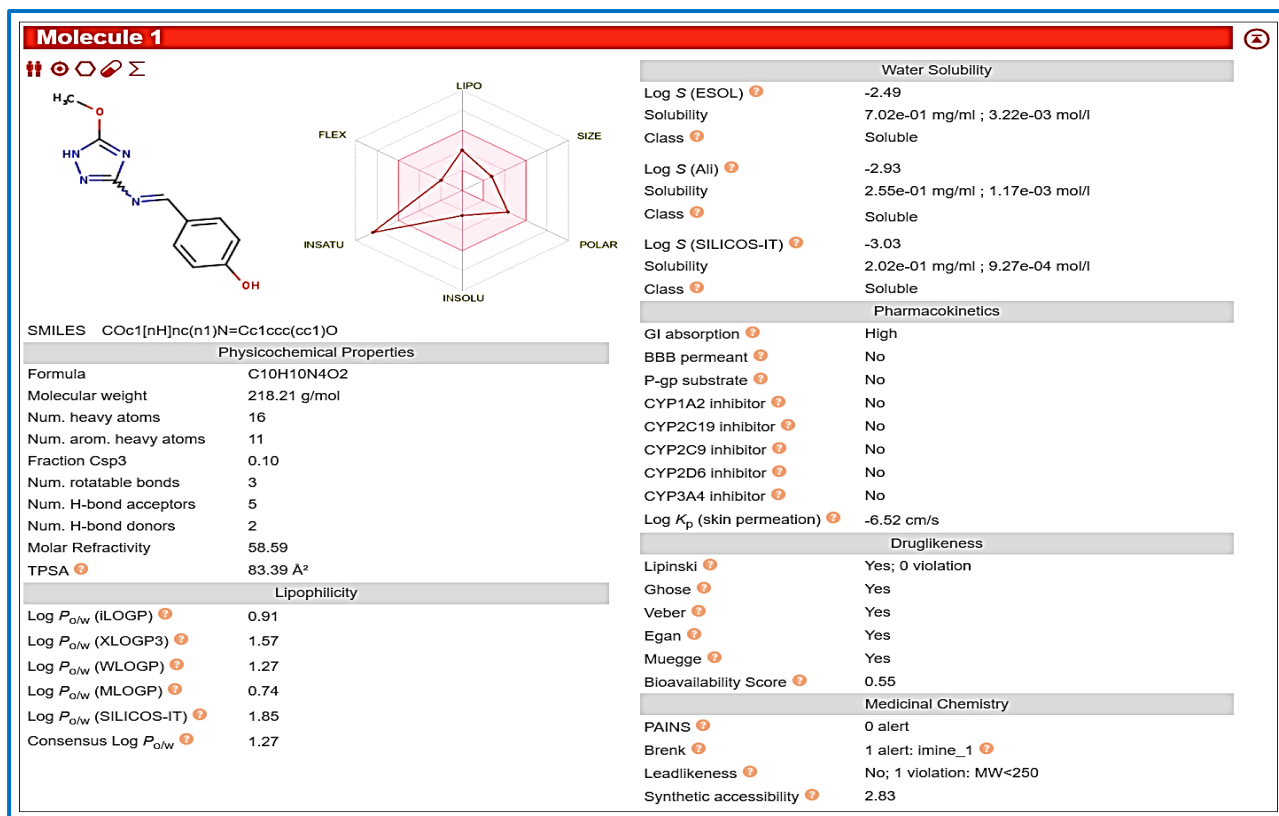


Figure 18: SwissADME Prediction for derivative C.

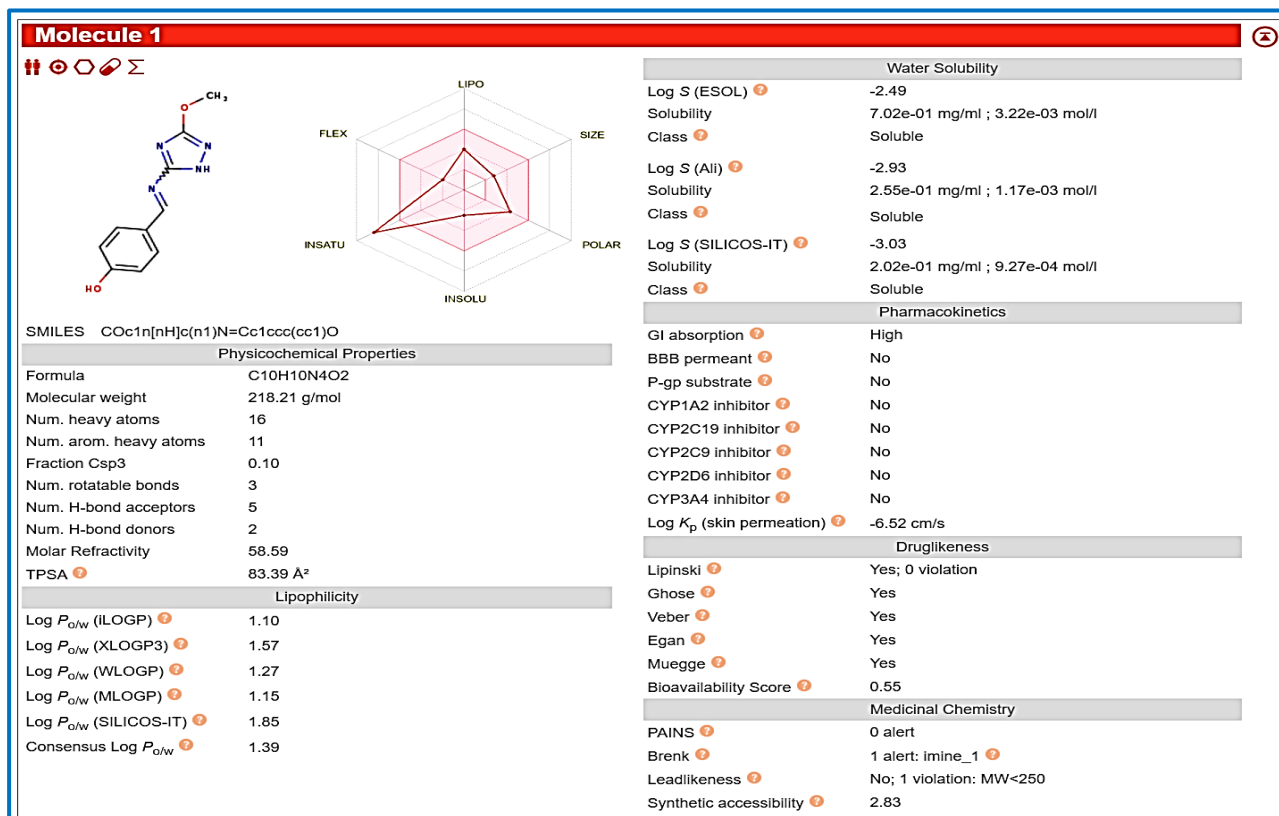


Figure 19: SwissADME Prediction for derivative D.

4. Conclusion

This study shows that triazole–imine derivatives are versatile and physiologically active heterocyclic compounds. Successful synthesis and spectroscopic analysis demonstrate moderate structural modification of triazole-based Schiff bases. Thus, substituent type and concentration considerably alter antibacterial and cytotoxic properties, as compound C suppresses bacteria better while compound A kills PC-3 and MCF-7 cells. Molecular docking studies show that ligand-protein interactions in the 8RZX binding pocket survive via hydrogen bonding and π – π stacking. These data suggest electrical dispersion and aromaticity affect biological performance. Although promising, the study requires in vivo confirmation and selectivity testing against normal cells. The next research will focus on biological applications such antifungal screening, mechanistic apoptosis, and in vivo anticancer testing. Molecular dynamics simulations are essential to study ligand stability over time. Expanding the compound library and relating physicochemical aspects to biology may enhance structure–activity connections. This research provides a solid experimental and theoretical foundation for rationally constructing triazole–imine-based therapies.

5. References

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Article Review: Anomalous Meissner Effect in Multiband Superconductors

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Abstract:

As a material transitions below its critical temperature, a defining characteristic of superconductivity is the Meissner effect, which is the removal of magnetic fields from the material. Within the Ginzburg-Landau and BCS models, this effect is thoroughly comprehended in traditional (single-band) superconductors. . In multiband superconductors like MgB₂ and iron-based superconductors, exceptions to this usual pattern of action have been found. Clustering of anomalous vortices, mixed-phase vortex states, paramagnetic. Meissner responses are all examples of such phenomena. Type-1.5 superconductivity, giant paramagnetic Meissner responses, and implications for advanced superconducting applications are the main topics of this paper's review of recent experimental and theoretical results on the anomalous Meissner effect in multiband superconductors.

Keywords: Meissner effect, superconductors, Multiband Superconductivity.

مقالة علمية: تأثير مايسنر الشاذ في الموصلات الفائقة متعددة النطاقات

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الخلاصة:

عندما تنخفض درجة حرارة المادة عن درجة حرارتها الحرجة تتحول الى مادة موصل فائقة ، تُعدّ ظاهرة مايسنر، التي تتمثل في إزالة المجالات المغناطيسية من المادة، سمةً مميزةً للموصلية الفائقة. ضمن نموذجي جينزبورغ-لانداو وBCS، تُفهم هذه الظاهرة فهماً دقيقاً في الموصلات الفائقة التقليدية (أحادية النطاق). وقد رُصدت اضطرابات في هذا النمط السلوكي القياسي في الموصلات الفائقة متعددة النطاقات مثل MgB_2 والموصلات الفائقة القائمة على الحديد. وتُعدّ تجمعات الدوامات الشاذة، وحالات الدوامات ذات الطور المختلط، واستجابات مايسنر البارامغناطيسية أمثلةً على هذه الظواهر. وتُشكل الموصلية الفائقة من النوع ١,٥، واستجابات مايسنر البارامغناطيسية العملاقة، وتداعياتها على تطبيقات الموصلات الفائقة المتقدمة، المواضيع الرئيسية التي تتناولها هذه الورقة البحثية في استعراضها للنتائج التجريبية والنظرية الحديثة حول ظاهرة مايسنر الشاذة في الموصلات الفائقة متعددة النطاقات.

الكلمات المفتاحية: تأثير مايسنر، الموصلات الفائقة، الموصلية الفائقة متعددة النطاقات.

1. Introduction:

The discovery of a material with zero electrical resistance was made possible by Heike Kamerlingh Onnes's groundbreaking 1911 explanation of superconductivity. The finding of superconductivity was an unforeseen byproduct of the experiment's primary objective, which was to liquefy helium—the final noble gas. Investigations exploring the relationship between temperature and resistivity [1]. At that time, various assumptions and speculations concerning metals' behavior at cold temperatures, ranging from constant declines to anomalous increases, that necessitate testing. At temperatures lower than 4.2 K, or -268°C, liquid mercury showed almost no resistance. The 1913 Nobel Prize in Physics went to Kamerlingh Onnes for his studies of materials at cryogenic temperatures rather than his work on superconductivity. A significant challenge in the generation, transmission, and distribution of energy is material resistance. Energy loss can be mitigated by employing appropriate conductors. The current target is to develop superconductors to facilitate the generation of alternative energy sources. Researchers are contemplating various strategies to enhance the synthesis of superconductors, which currently exist solely under low-temperature conditions [2]. The principal obstacle to widespread adoption of superconductors now lies in the fabrication of a superconductive material with the necessary properties for use in electrical and electronic applications [3].

2. Material and methods:

2.1 Conventional Meissner Effect

Superconducting materials have the Meissner Effect as one of their basic properties. Superconductivity, defined as the absence of internal magnetic fields and zero electrical resistance, occurs when a material's temperature drops below its critical temperature T_c . Superconductivity is proven to be a separate thermodynamic state by this behavior, which distinguishes superconductors from regular conductors. Under the standard Meissner state, when the temperature drops below T_c , the magnetic field is ejected from the superconductor's interior [4]. Therefore, there is practically no magnetic flux density within the material's bulk:

$$B = 0$$

Inducing electric currents close to the superconductor's surface is what causes the magnetic field to be expelled. By creating their own magnetic field, these surface currents block the external magnetic field from permeating the material. The London Equations give a theoretical account of this occurrence. An electric field's effect on a superconductor's current density, J_s , is described by the first London equation. E:

$$\frac{dJ_s}{dt} = \frac{n_s e^2}{m} E$$

In this context, n_s stands for the density of superconducting charge carriers, e for the electron charge, and m for the electron mass. For superconducting currents, the relationship to magnetic fields is defined by the second London equation:

$$\nabla \times J_s = -\frac{n_s e^2}{m} B$$

The relationship between these relations and Maxwell's equations demonstrates that, as one moves away from the surface of a superconductor, the internal magnetic field weakens rapidly, as follows:

$$B(x) = B_0 e^{-x/\lambda_L}$$

in where B_0 stands for the surface magnetic field and λ_L is the so-called London penetration depth. Here we see the range of values for the parameter that describes the depth to which a magnetic field can marginally permeate a superconductor before quickly fading away.

The BCS Theory, which explains how electrons create bound states called Cooper pairs, provides the microscopic explanation of superconductivity. Together, these pairs diffuselessly traverse the crystal lattice, resulting in the Meissner effect's magnetic field exclusion and zero electrical resistance. [5,6]

2.2 Multiband Superconductivity

A phenomenon known as multiband superconductivity occurs when multiple electronic bands, each with its own superconducting gap, surpass the Fermi level. The critical temperature is frequently increased above forecasts made using single bands when these gaps interact and produce a cooperative effect. One example of this occurrence is the presence of two separate band gaps in magnesium diboride (MgB_2), while superconductors based on iron display a more unusual pairing, known as $\pm$ pairing, in which the gap switches sign between the electron and hole pockets [7,8]. Unusual physical phenomena, such as non-standard heat capacity and superfluid density as well as complex vortex patterns, emerge as a result of this multiband nature, unveiling a more varied and tuneable domain for superconducting physics. Seven and eight. Figure 1 shows the transport of several bands and superconductors [9].

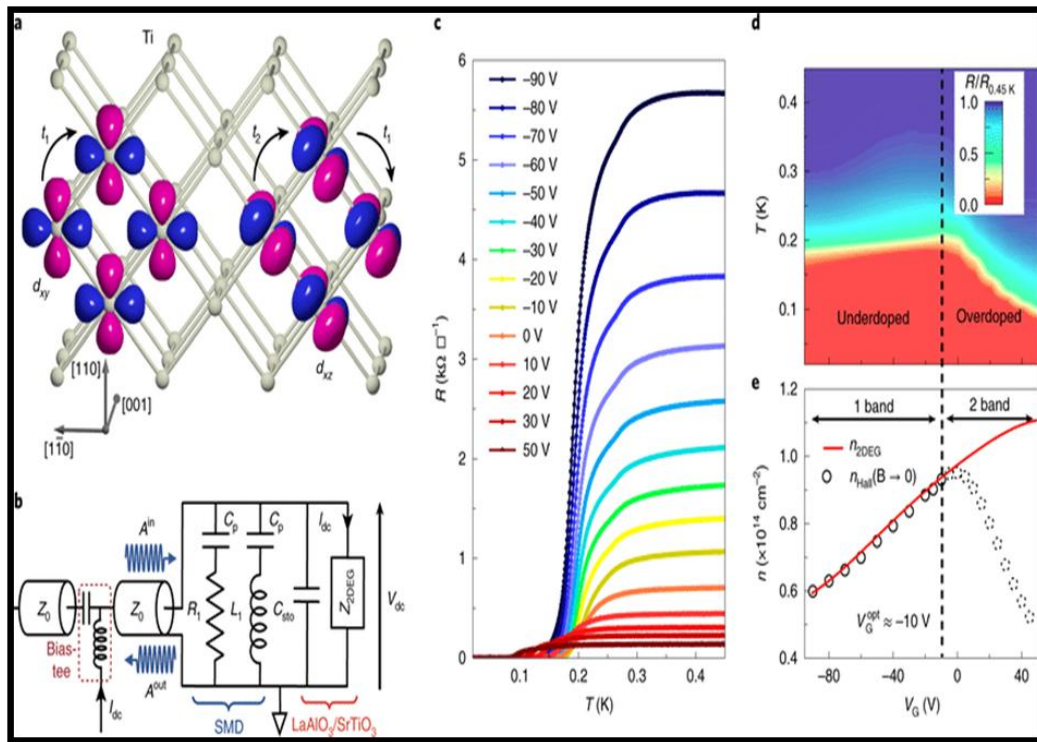


Figure 1: Superconductivity and multiband transport

3. The Paramagnetic Meissner Effect

3.1 Basic Mechanism

When field-cooling techniques are used to chill a superconductor, the paramagnetic Meissner effect occurs. Instead of releasing magnetic flux as expected, the material traps and compresses it in specific arrangements, leading to field amplification inside the sample. This appears to run counter to the basic principles of superconductivity since it causes a paramagnetic reaction.

3.2 Flux Compression and Trapping

An explanation that could be considered is that the magnetic flux is compressed and trapped during cooling. This trapped flux can take the shape of large vortices when the superconducting order parameter initially nucleates at the sample surface, and as the temperature decreases, its volume decreases, leading to regions where the magnetic field is amplified rather than emitted.

3.3 Giant Paramagnetic Meissner Effect

In 2015 it was shown that multiband superconductors can have a large paramagnetic response which is much larger than previous theoretical predictions. This important phenomenon occurs in the large crossover region between Type-I and Type-II superconductors, where Abrikosov vortices interact non-monotonically and multibody effects become significant. At this transition stage, different flux configurations can be blocked by surfaces. The resultant paramagnetic moment may be much larger than the diamagnetic Meissner response in the same applied field—approximately 30 times larger than previous theoretical predictions [10,11]. The effect changes with the applied magnetic field because of flux quantization and is superseded.

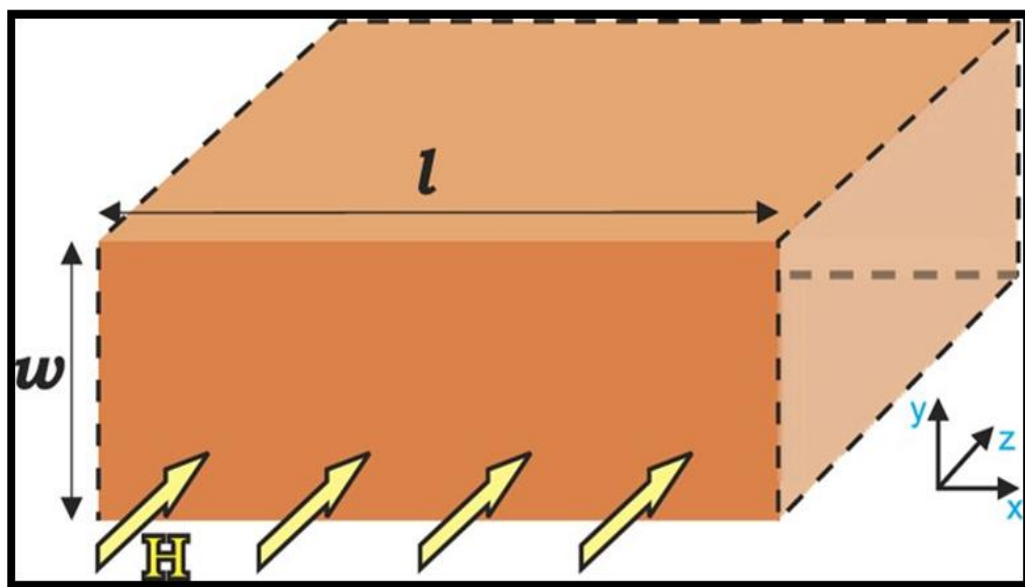


Figure 2 : Giant Paramagnetic Meissner Effect

4. Experimental Evidence

Various experimental methodologies can identify odd-frequency pairing and anomalous magnetic responses in multiband systems [12,13,14]:

- 1- Hybridization gaps in the electrical density of states indicate distinct signs of odd-frequency pairing [15].
- 2- Paramagnetic Meissner measurements directly assess the magnetic response.
- 3- Kerr effect assessments in materials exhibiting time-reversal symmetry violation
- 4-Angle-resolved photoemission spectroscopy (ARPES) can delineate superconducting gaps and ascertain nodal configurations [16,17].
- 5- Scanning tunneling microscopy elucidates regional discrepancies in gap magnitude [18]

5. Applications and Implication

5.1 Super current Diode Effects

In 2025, recently conducted studies on Josephson interferometers using multiband superconductors proved that magnetic fields can control the size and direction of supercurrent rectification independently. Important for cryogenic applications and superconducting quantum circuits, the nonreciprocal transport phenomena rely on the unusual magnetic response of multiband materials. electronics [19].

5.2 Disorder Robustness

Multiband superconductivity with flat bands has extraordinary disorder robustness, according to recent theoretical studies [20]. Even while the superconducting state could be disturbed by more typical sources, it does not. The stability of the Meissner effect in real materials and its practical uses are profoundly affected by this.

5.3 Quantum Computation

A significant step forward in topological quantum processing has been achieved with the discovery of materials like PtBi₂ that intrinsically support Majorana-bound states [21]. Particles known as Majorana fermions can be both their own antiparticles and fault-tolerant

quantum bits. New possibilities for quantum information processing arise from the ability to generate and manipulate these states in intrinsic topological superconductors.

6. Conclusion

The intricate interplay between different superconducting gaps, interband coupling, and vortex dynamics is illustrated by the anomalous Meissner effect in multiband superconductors. The finding of Type-1.5 behavior and large paramagnetic reactions provides deep physics with important practical consequences while also challenging long-held assumptions. To make full use of these phenomena in next-generation superconducting systems, further theoretical and practical study is essential.

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