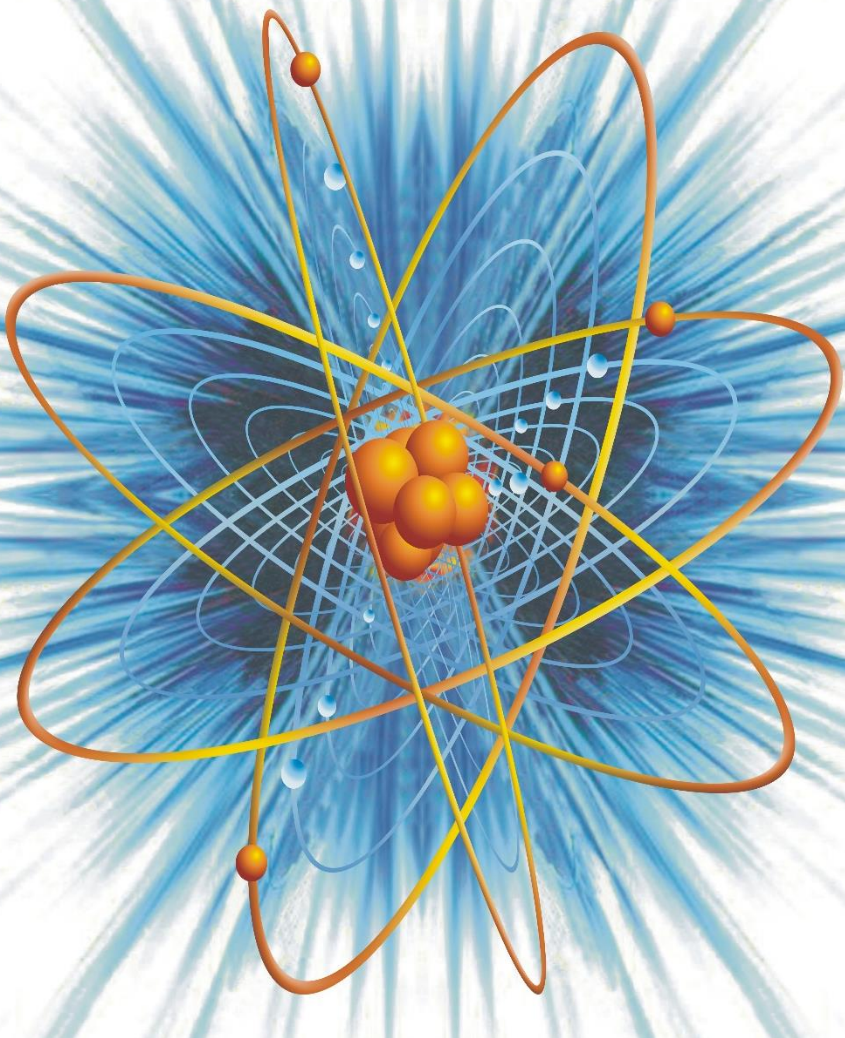
The Nucleus

An Open Access International Scientific Journal



Vol. 62, No. 2, 2025

ISSN 0029-5698 (Print) eISSN 2306-6539 (Online)

The Nucleus

An international journal devoted to all branches of natural and applied sciences

Website: *www.thenucleuspak.org.pk* **E-mail:** *editorialoffice@thenucleuspak.org.pk*

Phone: *+92-51-9248429* **Fax:** *+92-51-9248808*

Editorial Office, The Nucleus

PINSTECH, Nilore, 45650, Islamabad, Pakistan

Printed at

PINSTECH Printing Press

The Nucleus

Aims and Scope: The Nucleus is an open access multidisciplinary peer-reviewed academic journal. It offers a platform for the scientists and engineers to publish their recent research of high scientific values in all areas of natural, applied and management sciences at international level. The journal is being published electronically as well as in paper version. It is easily accessible, free of charge and is being distributed widely.

Open Access Policy: The Nucleus is an open access journal implying that all contents are freely available without charges to the users or their institutions. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles without prior permission from the publisher or authors as long as the original authors and sources are cited.

Abstracting and Indexing: The journal is being abstracted and indexed by Chemical Abstracts, Citefactor, Biological Abstracts, INIS Atom Index, Bibliography of Agriculture (USA), The Institution of Electrical Engineers Publication, Virology Abstracts (England) and Pakistan Science Abstracts. The journal is recognized by the Higher Education Commission of Pakistan (Category Y).

ISSN and eISSN: The international standard serial numbers (ISSN) for The Nucleus are [0029-5698 (Print) and 2306-6539 (Online)].

The Nucleus is published at Pakistan Institute of Nuclear Science & Technology, Islamabad, on behalf of the Pakistan Atomic Energy Commission.

Published since 1964

The Nucleus
ISSN 0029-5698 (Print)
ISSN 2306-6539 (Online)

CONTENTS

The Nucleus **62**, No. 2 (2025) 59-64

https://doi.org/10.71330/thenucleus.2025.1461

www.thenucleuspak.org.pk

The Nucleus

ISSN 0029-5698 (Print)

ISSN 2306-6539 (Online)

Deep Q Networks for Classification: Performance Analysis on Iris and Diabetes Datasets

Maryam Baig*, Mujeeb Ur Rehman

Department of Computer Science, University of Management and Technology, Sialkot, Pakistan.

ABSTRACT

This study explores how Deep Q Networks (DQNS) can be applied to classifying data with standard datasets. Though DQNs are designed for sequential decisions, this study uses the Iris and Diabetes datasets, which are both used for classification, to test the performance. It investigates DQNs with different data imbalances and compares their results based on the quantity of data. Models are assessed using accuracy, precision, recall, F1 score, ROC-AUC, the amount of memory required, and their training speed. As demonstrated by the results, DQNs can match other algorithms in accuracy while attaining accuracy rates between 84% and 95% for various datasets and modified configurations. Based on the study, performance can be affected by adjusting hyperparameters and the distribution of data classes. DQNs are more complex than common classifiers such as SVMs and decision trees, and their main contribution in simple classification is yet to be proven. It adds to the enthusiasm for using reinforcement learning models in the context of supervised learning. The paper highlights the value of correct evaluation, points out risks linked to model over-fitting and includes new areas to pursue in the future such as benchmarking, clarifying models and using hybrid systems.

Keywords: Deep Q Networks (DQNs), Classification, Imbalanced Datasets, Reinforcement Learning, and Hyperparameter Tuning

1. Introduction

Deep Q Networks (DQNs) are a type of algorithm that combines Q-learning with deep neural networks [1]. They are used to train computers to make decisions by learning from interactions with their environment. In this approach, agents build the Q-value to estimate how rewarding it could be to take a certain action in a specific condition. By including deep neural networks, DQNs can process more complex data, such as images, giving them a greater ability than previous algorithms. A memory mechanism in some DQNs aids the agent's learning since it separates the way the current input influences decision-making from the agent's memories and rewards. Besides, during the training process, a second neural network computes the target Q-values needed to stabilize the overall process. The goal of the agent is to maximize the total expected reward over time [2].

The introduction of DQNs has helped RL models to use their old experiences to select suitable actions and operate in changing situations. With DQNs, deep learning is introduced because the architecture can manage inputs from complex and difficult state spaces. They help make both the system more stable and efficient [3].

Reinforcement learning has seen remarkable growth because it has worked well in games, robotics, healthcare, finance, and other areas. DQNs are famous for helping to teach AI to beat people at Atari games and Go, volunteer work accomplished by Google DeepMind. Furthermore, autonomous driving uses DQNs, as they enable the system to react instantly to uncertain situations [4].

This research analyzes DQNs in supervised classification and uses them on the popular Iris and Diabetes datasets. Sir R.A. Fisher developed the Iris dataset which has information about 150 flowers in the category of irises, listed using four characteristics: the lengths of their sepals, the widths and the lengths and widths of their petals. Each sample is placed in one of the species: Iris-setosa, Iris-versicolor or Iris-virginica. Forty-four k is always turned into four hundred forty patients are included in the Diabetes dataset, while ten clinical features such as age, BMI, blood pressure and cholesterol are taken into account. The goal is to forecast the evolution of diabetes using these properties.

This study makes DQNs, which are used for reinforcement learning, useful in the field of supervised classification. Such a novel use of RL-based models brings up questions about their ability to handle tasks without any given rewards. Thus, this research explores how standard DQNs behave on typical classification problems and changes their behavior when faced with datasets containing different classes.

One of the difficulties in machine learning is when the examples in different categories are not equally represented. When such a problem happens, the model is likely to perform worse on the minority class. For this purpose, the study checks the model's performance with balanced as well as unbalanced datasets.

The structure of the paper is as follows: Section II provides a literature review focusing on deep neural networks. Section III provides the methodology in which experiments and outcomes are depicted. The results and findings are summarized in Section IV.

2. Literature Review

The improvement known as DQNs plays a vital role in reinforcement learning processes. Traditional Q-learning gets enhanced through deep neural networks to create computational models capable of taking improved decisions. AI learns optimal actions in complex environments through the union of two techniques regardless of the numerous possible decisions available. The computational difficulty experienced by standard Q-learning disappears when we implement DQNs because they provide machines with better management capabilities [5].

*Corresponding author: baigmaryam355@gmail.com

DQNs function as a crucial tool for developing end-to-end learning systems within the autonomous driving field. The system takes unprocessed sensor data such as video images and directly generates driving instructions as a result of learning the complete driving policy without modular boundaries. The method stands opposite to conventional modular pipeline designs because it requires separation between perception and planning and control activities. The end-to-end approach presents a method that provides both a simplified and potential stronger system for autonomous vehicle navigation [4].

The accurate potential of both DQNs and end-to-end learning approaches in self-driving cars exists alongside major implementation barriers [6]. Safety requirements, along with reliability functions, as well as decision-making transparency, data input requirements, and real-world performance capacity, compose the major obstacles. The system requires capabilities to manage different unpredictable scenarios to guarantee safety for people traveling in the vehicles as well as all road users. The main drawback of deep learning models exists in their inability to display their decision-making process, which results in decision opacity [7]. The lack of understanding about their decision-making processes prevents users from establishing confidence in the systems or locating ongoing issues. The training of these models requires extensive datasets, which require significant human labor to gather and properly mark. The accuracy of simulation-trained models suffers when implemented in real operating conditions because simulation environments do not perfectly replicate actual reality [8, 9]. The use of DQNs in actual automobiles requires resolving identified problems [10, 11].

Contrary to reinforcement learning use cases, many DQN studies have been carried out, but the number of studies on their use in classification is limited. Recently, research projects have made efforts to use RL algorithms in both image classification and medical diagnosis. Authors tested using RL for class imbalance and used reward shaping strategies to solve this problem [12]. At the moment, using DQNs for simple classification problems remains an under-discussed topic. Many people prefer to use Support Vector Machines (SVMs), Random Forests and Logistic Regression, since they are clear, perform well and have a record of accomplishment on data tables. Many studies have revealed that simple supervised models usually do better than complex RL ones in performance when decisions are made in sequence [13].

The innovation of this research is using a DQN algorithm on regular classification datasets and comparing its results on balanced and unbalanced data. Previous literature does not usually focus on this application but mentions it when discussing ensembles of instruments. Since there is limited research on this topic, it suggests more studies to be conducted as people are increasingly interested in using deep RL in other domains.

In brief, DQNs are effective in reinforcement learning, but using them directly in classification is not widely explored and requires additional investigation. Here, the study provides an in-depth analysis of how DQNs behave differently on various datasets. In turn, this will support future researchers in improving RL methods for classification.

3. Methodology

In Figure 1, all the steps of the DQN model and its phases are involved. The first step is dataset selection; two datasets, Iris and Diabetes, were selected. The second step is preprocessing, which consists of splitting each dataset into four parts: 25%, 50%, 75%, and 100%. Further, these parts are divided into balanced and unbalanced subsets to test the effect of class distribution.

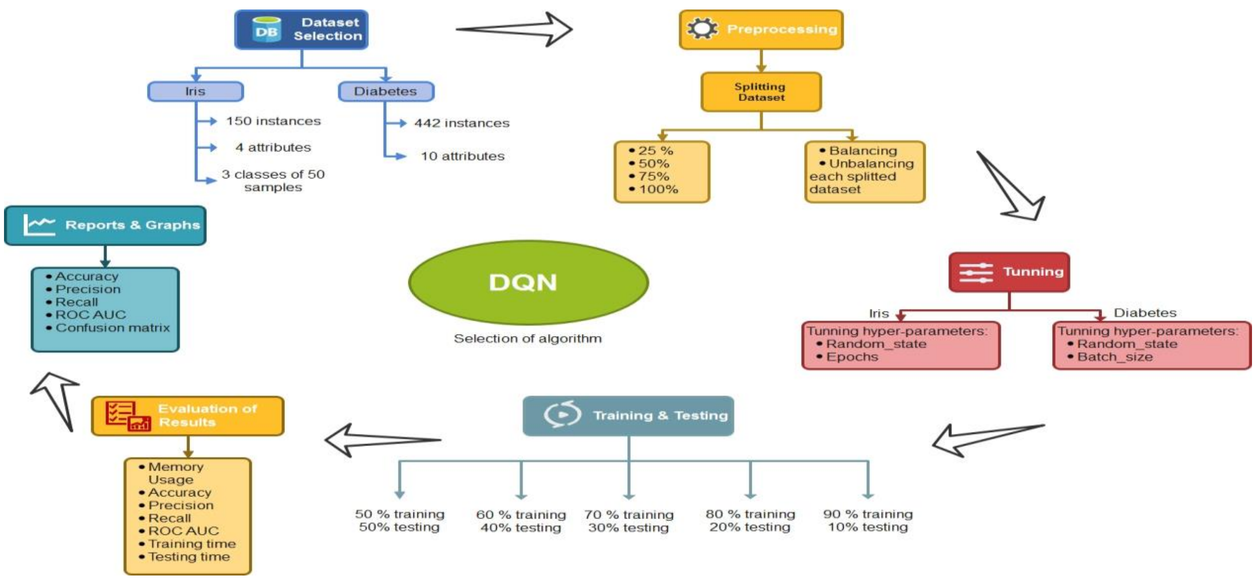


Fig. 1 Methodology Diagram of the DQN algorithm

The third step is hyperparameter tuning. For this phase, random state and epochs are fine-tuned for the Iris dataset. For the Diabetes dataset, random state and batch size are changed to examine their influence on performance. These values were selected based on initial trials to optimize training stability and accuracy, using commonly accepted ranges from similar DQN applications. For example, the learning rate of 0.0005 is a standard traditional value that prevents overshooting during optimization.

The fourth step is training and testing, where train-test splits are applied, such as 50% training and 50% testing, 60% training and 40% testing, and so on. The fifth step involves generating evaluation results, including memory usage, accuracy, precision, recall, ROC-AUC, training time, and testing time. Rather than using rewards, the DQN's learning was adapted here to count each correct classification, so the agent received a positive signal each time it made such a prediction.

There are two datasets utilized for the experimentation process. These datasets are the Iris and Diabetes datasets. The following are the descriptions of these two datasets:

The Iris dataset, first introduced by Sir R.A. Fisher, contains 150 instances, with 50 samples from each of three classes: Iris-Setosa, Iris-Versicolour, and Iris-Virginica. The dataset has four numeric attributes: sepal length, sepal width, petal length, and petal width, measured in centimeters. There are no missing values, and the class distribution is also fairly balanced, with a third of the dataset making up each of the three species [14].

The Diabetes dataset includes 442 instances, each representing a diabetes patient. For each patient, 10 baseline variables were recorded: age, sex, body mass index (BMI), average blood pressure (BP), and six blood serum measurements (s1 to s6). These serum measurements include total serum cholesterol (s1), low-density lipoproteins (ldl, s2), high-density lipoproteins (hdl, s3), the total cholesterol to HDL ratio (tch, s4), the log of serum triglycerides (ltg, s5), and blood sugar level (glu, s6). The dataset's target variable, found in the 11th column, is a measurement of how the disease has progressed one year after the first data was collected. The first 10 variables are numbers and are used to help predict how the disease will develop over time.

Here's a table of all the general hyperparameters used for this algorithm. Table 1 shows the general hyperparameters used for this algorithm are shown in Table 1, along with their description and values used for model implementation.

In Table 2, for the Iris dataset, hyperparameter 1 is Random_state, and hyperparameter 2 is epochs. In addition, for the diabetes dataset, hyperparameter 1 is Random_state, and hyperparameter 2 is batch_size.

Following Table 2. demonstrates all hyperparameters along with their values used in the algorithm:

Table 1. Hyperparameters Used in DQN Algorithm

No.	Hyperparameter	Description	Values used in dataset
1.	Learning Rate	It is the rate or step by which the model adjusts its weights in each phase during the training process.	0.0005
2.	Batch Size	Number of samples in the training dataset that go through before the weights of the model are modified.	16
3.	Epochs	Number of times the full training dataset has gone through the training phase of the model.	50
4.	Layers and Units	Layers and Units describe the structure of the neural network.	128, 64, 32, & 2 units
5.	Loss Function	Measures the difference between the predicted and true values.	categorical_crossentropy
6.	Optimizer	Specifies the scheme of how the model adjusts weights in the course of the training session.	tf.keras.optimizers.Adam
7.	Random State	Controls and reduces variability as it smooths it out when splitting the available dataset.	42

Table 2. Hyperparameters along with their values

Dataset	Hyperparameter 1	Hyperparameter 2	Accuracy	Training Time	Memory Used
Iris	42	50	0.90	34.90	8.14
Iris	55	100	0.93	51.62	4.78
Iris	83	150	0.97	68.82	11.72
Diabetes	42	16	1.00	34.48	45.81
Diabetes	19	32	1.00	34.52	0.13
Diabetes	89	25	1.00	34.84	12.18

4. Experimentation

The system runs on Windows 8.1 with an AMD64 Family 22 Model 0 Stepping 1 processor and a 64-bit architecture. It has 11.44 GB of RAM but does not have a detected GPU. The installed Python version is 3.11.7, and TensorFlow version 2.16.1 is being used. The datasets utilized include the Iris dataset, sourced from the UCI Machine Learning Repository, and the Diabetes dataset, which is a built-in dataset from Scikit-learn.

The high accuracy of 100% noticed on Diabetes was reason enough to proceed with additional evaluations. We noticed that changing the configuration and applying SMOTE to create more training data increased the accuracy significantly to 84% to 88%. By using this step, the machine learning model can perform well in different situations.

Moreover, using a wide range of experience made the DQN model less likely to only learn from a narrow set of examples. These changes were necessary to create more realistic results, as errors do occur in hospital records. As a result, this proves that DQNs are flexible in tough situations, making the experimental design better for reproducibility.

The table below summarizes the behaviors of the DQN model using a combination of various hyperparameters. With the Iris dataset, several random state and epoch values were applied and in the case of the Diabetes dataset, random state and batch size were modified. Following the expanded and analyzed dataset, the Diabetes dataset gave accurate results between 84% and 88%.

Table 4 Shows all the results achieved through model experimentation such as accuracy, precision, recall, F1 score and ROC from 100%, 75%, 50%, 25% balance and imbalance dataset.

Table 5 Shows time complexity like training time, testing time and memory usage at 100%, 75%, 50%, 25% balance and imbalance dataset.

Table 3. Accuracy, training time, and memory usage of the DQN model for different hyperparameter settings across Iris and Diabetes datasets.

Dataset	Hyperparameter 1	Hyperparameter 2	Accuracy	Training Time	Memory Used
Iris	42	50	0.90	34.90	8.14
Iris	55	100	0.93	51.62	4.78
Iris	83	150	0.95	68.82	11.72
Diabetes	42	16	0.84	41.55	45.81
Diabetes	19	32	0.86	42.52	0.13
Diabetes	89	25	0.88	44.32	12.18

Table 4. Results of Accuracy, Precision, Recall, F1 Score and ROC

Dataset	100% Dataset		75% Dataset		50% Dataset		25% Dataset	
Types	Balanced	Imbalanced	Balanced	Imbalanced	Balanced	Imbalanced	Balanced	Imbalanced
Accuracy	0.756757	0.774775	0.738739	0.783784	0.737557	0.737557	0.747748	0.774775
Precision	0.738095	0.761905	0.736842	0.767442	0.744186	0.728261	0.731707	0.750000
Recall	0.659574	0.680851	0.595745	0.702128	0.640000	0.670000	0.638298	0.702128
F1 score	0.696629	0.719101	0.658824	0.733333	0.688172	0.697917	0.681818	0.725275
ROC	0.834109	0.851729	0.835771	0.853391	0.830826	0.842066	0.820479	0.861037

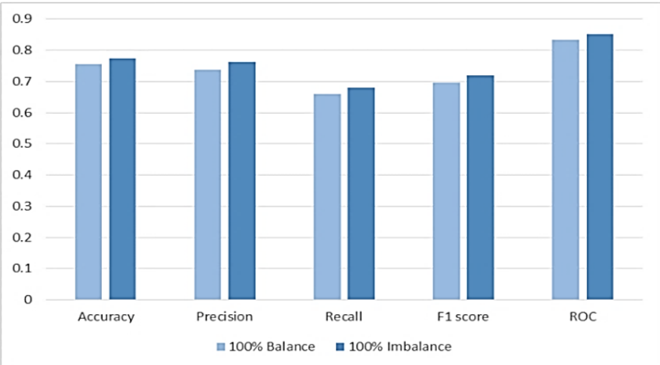


Fig. 2 Graph of Accuracy, Precision, Recall, F1 Score, and ROC at 100% Balance & Imbalance

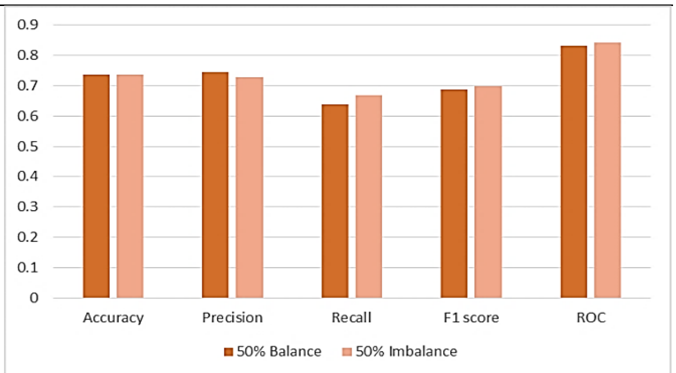


Fig. 3 Graph of Accuracy, Precision, Recall, F1 Score, and ROC at 50% Balance & Imbalance.

Table 5. Results of Time complexity: Training Time, Testing Time and Memory Usage

	100% Dataset		75% Dataset		50% Dataset		25% Dataset	
	Balanced	Imbalanced	Balanced	Imbalanced	Balanced	Imbalanced	Balanced	Imbalanced
Training Time	48.977339	45.379100	39.590767	36.043091	42.447826	44.336924	49.762005	46.945287
Testing Time	2.224016	3.258235	2.080025	1.791542	2.112004	2.267170	1.826308	1.896097
Memory Usage	39.45	22.37	19.1	15.85	Memory Usage	39.45	22.37	19.1

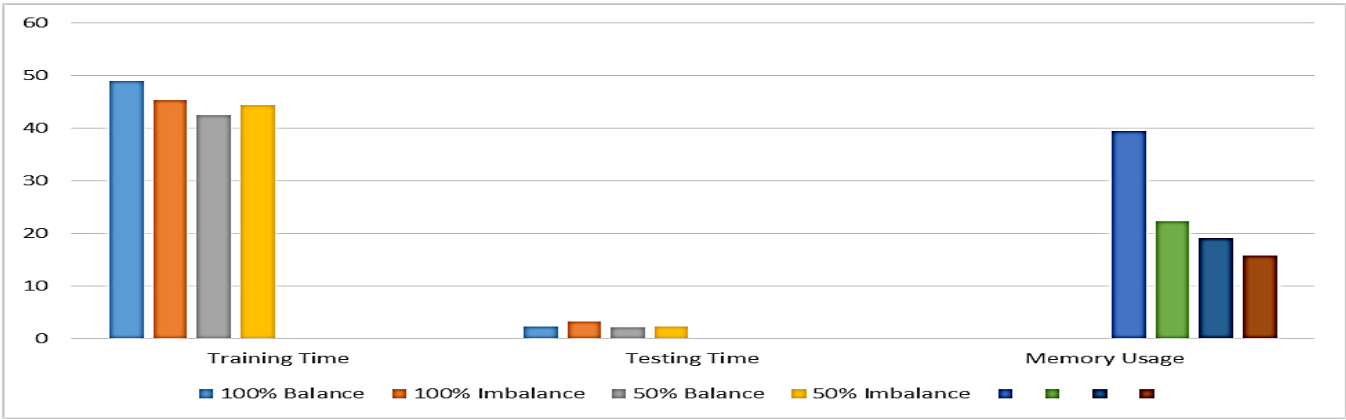


Fig. 4 Graph of Time complexity: Training Time, Testing Time and Memory Usage

The imbalanced dataset performs better than the balanced dataset in terms of accuracy, F1-score, and ROC-AUC, shown in Figure 2 and Figure 3 at 100% and 50% datasets, respectively. All conditions show a similar training time, indicating that dataset balance or imbalance has minimal impact on the time taken for model training. Testing time remains relatively low across all conditions, with slight variations. This suggests that dataset balance or imbalance does not significantly affect testing efficiency. Moreover, the worst memory usage is at 100% balanced and gradually decreases as the number of samples decreases. This suggests that 100% datasets need more memory space in order to perform the specific functions of a DQN.

5. Results and Discussion

Table 4 outlines the experimental findings on the DQN model for data slices (100%, 75%, 50%, 25%) and class distributions (balanced and imbalanced). These tests used classical output measures such as accuracy, precision, recall, F1 score and ROC-AUC.

Across the Iris dataset, as the number of training epochs increased, it resulted in better generalization, as accuracy rose from 0.90 to 0.95. However, the model performed reasonably and did not exhibit any signs of overfitting. As intended, the Iris dataset is limited in size, equally balanced and properly organized.

On the other hand, the initial results for the Diabetes dataset were very high (100% accuracy), leading experts to worry. As a result, new assessments were done by increasing the amount of data with SMOTE and training the models using various configurations. Because of this, the accuracy was improved and fell between 84% and 88%. This indicates that the earlier outcomes were most likely affected by overfitting due to having a small and consistent set of data.

Training time was similar for different datasets and varying numbers of classes. We can conclude that different data balances did not change the time it took to train the model. The tests showed that variations were small and the data led to slightly faster outcomes on evenly divided datasets. The most memory was required when the dataset was fully balanced, and it reduced as the sample size decreased.

In general, DQNs can handle classification and show strong results in environments outside the RL field. Still, people working with datasets like Diabetes must take care when interpreting the results. It is also made clear that attention to tuning, variable datasets and proper comparison helps prevent inaccurate results.

The observation was supported by the finding that slightly better performance came from using unbalanced datasets. The mean accuracy for datasets with imbalanced datasets was 0.7682 (±0.0173), whereas accuracy on balanced datasets was 0.7452 (±0.0144). Additionally, the average F1-score was 0.7189 (±0.0149) when the data was imbalanced and 0.6819 (±0.0158) when it was balanced. This goes along with the trend, but it is only a small difference and should be analyzed thoughtfully.

Further analysis should be done with known classifiers, including Logistic Regression, SVM and Random Forest, to confirm if DQNs are more effective. Besides, SHAP and LIME allow people to understand the reasoning behind DQN predictions clearly.

6. Conclusion

The model was evaluated by testing it on the Iris and Diabetes datasets and the results suggested that it is effective. Following evaluation and changes, the model gave a correct result for 95% of the Iris samples and 84%–88% of the Diabetes samples. It is shown that DQNs, developed for reinforcement learning, work well for supervised feature classification after they are properly tuned. Some experiments pointed out that, if the data was not balanced, error rate could go down, but generally because the model was biased towards the most common class. Balanced datasets gave similar results in every metric: precision, recall and F1-score. These results suggest that understanding the distribution of classes improves model evaluation. In addition, setting the right values for random state, epochs and batch size was very important for the training, especially in small datasets such as Iris and Diabetes. Using a larger and balanced dataset led to a bigger memory usage and longer training time. The more data I had, the more time and memory was required to process it. Testing time usually stayed the same regardless of the environment, suggesting that DQN performance in inference is reliable. Even though the results were encouraging, there are still significant obstacles in this study. At first, the very high results on the Diabetes dataset were seen to be overfitting, so the issue was repaired by adding new trials and using more training data. As a result, adopting explainable AI methods (for example, SHAP and LIME) will address the unclear nature of deep learning, so its use in areas such as healthcare and finance will become more transparent. All in all, DQNs have shown possibilities in classifying data, but they need to be adjusted carefully, managed properly and thoroughly reviewed. It is vital to aim at new ways to balance training, improve reliability under real-world situations and add reinforcement learning with supervised approaches to develop hybrid models.

References

[1] J. S. Park and J. H. Park, "Enhanced machine learning algorithms: deep learning, reinforcement learning, and q-learning," J. Inf. Process. Syst., vol. 16, no. 5, pp. 1001–1007, 2020.

[2] K. Kim, "Multi-agent deep q network to enhance the reinforcement learning for delayed reward system," Appl. Sci., vol. 12, no. 7, pp. 3520, 2022.

[3] X. Wang et al., "Deep reinforcement learning: A survey," IEEE Trans. Neural Networks Learn. Syst., vol. 35, no. 4, pp. 5064–5078, 2022.

[4] B. Peng et al., "End-to-End Autonomous Driving Through Dueling Double Deep Q-Network," Automot. Innov., vol. 4, no. 3, pp. 328–337, 2021.

[5] N. Gholizadeh, N. Kazemi, and P. Musilek, "A comparative study of reinforcement learning algorithms for distribution network reconfiguration with deep Q-learning-based action sampling," Ieee Access, vol. 11, pp. 13714–13723, 2023.

[6] P. S. Chib and P. Singh, "Recent Advancements in End-to-End Autonomous Driving Using Deep Learning: A Survey," IEEE Trans. Intell. Veh., vol. 9, no. 1, pp. 103–118, 2024.

[7] H. Taherdoost, "Deep learning and neural networks: Decision-making implications," Symmetry (Basel)., vol. 15, no. 9, p. 1723, 2023.

[8] C. Elendu et al., "The impact of simulation-based training in medical education: A review," Medicine (Baltimore)., vol. 103, no. 27, p. e38813, 2024.

[9] D. Coelho and M. Oliveira, "A Review of End-to-End Autonomous Driving in Urban Environments," IEEE Access, vol. 10, no. July, pp. 75296–75311, 2022.

[10] K. Sahaja and L. Pudukarapu, "Preprint (not peer-reviewed)," Preprint, vol. 6, no. 6, pp. 1–4, 2019.

[11] S. Grigorescu, B. Trasnea, T. Cocias, and G. Macesanu, "A survey of deep learning techniques for autonomous driving," J. F. Robot., vol. 37, no. 3, pp. 362–386, 2020.

[12] J. Yang et al., "Deep reinforcement learning for multi-class imbalanced training: applications in healthcare," Mach. Learn., vol. 113, no. 5, pp. 2655–2674, 2024.

[13] C. Lu et al., "Structured state space models for in-context reinforcement learning," Adv. Neural Inf. Process. Syst., vol. 36, pp. 47016–47031, 2023.

[14] T. M. Ghazal, M. A. M. Afifi, and D. Kalra, "Data Mining and Exploration: A Comparison Study among Data Mining Techniques on Iris Data Set," Talent Dev. & Excell., vol. 12, no. 1, pp. 3854–3861, 2020.

The Nucleus **62**, No. 2 (2025) 65-68

https://doi.org/10.71330/thenucleus.2025.1460

www.thenucleuspak.org.pk

The Nucleus

ISSN 0029-5698 (Print)

ISSN 2306-6539 (Online)

Antibacterial Potential of Cerium Oxide Nanoparticles against Mycobacterium tuberculosis: A Novel Approach for Tuberculosis Treatment

Shoukat Ali Noonari[1], Mahnoor Gul[2], Jabir Ali[3], Hassan Ali Khan[1], Fahim Mustafa[4]

[1]*Department of Mechanical Engineering, Isra University, Hyderabad, Sindh, Pakistan*

[2]*MERE IBA University, Sukkur, Pakistan*

[3]*Department of Environmental Sciences, University of Sindh, Jamshoro, Pakistan*

[4]*Department of Mechanical Engineering, QUEST, Nawabshah, Pakistan*

ABSTRACT

This study investigated the antibacterial effects of cerium oxide nanoparticles (CON) on Mycobacterium tuberculosis. Cerium oxide nanoparticles were synthesised using a microwave-induced technique and characterized by Scanning Electron Microscopy. Results indicated that an increase in synthesis time led to a reduction in nanoparticle size, demonstrating that the duration of the synthesis process influenced particle size. Additionally, the antibacterial activity of CON particles was found to be size-dependent. In a disc diffusion assay, cerium oxide nanoparticles enhanced the efficacy of antibacterial agents, significantly improving the effectiveness against Terivid, Amikin, Grasil, Velosef, Spraxin, and Ceftriaxone against Mycobacterium tuberculosis.

Keywords: *Cerium Oxide Nano Particles (CON), Mycobacterium Ttuberculosis, Antimicrobial Analysis, Nanosize*

1. Introduction

Cerium oxide is a prevalent rare earth metal oxide known as ceria or ceric oxide. Oxygen sensors use Cerium oxide nanoparticles as antibacterial agents and oxidation-resistant coatings [1-2]. Cerium oxide nanoparticles have exceptional physical and chemical properties due to their small size, high magnetic moment, and highly reactive complexion [3]. Cerium oxide nanoparticles appear pale-yellow to white powder and have a Face Centred Cubic Crystal structure. Cerium oxide nanoparticles are more stable than rare earth oxides like bismuth dioxide, Thorium dioxide, and zirconia. Because of their hygroscopic nature, cerium oxide nanoparticles absorb some CO_2 and moisture from the atmosphere [4]. Microwave energy, sol-gel procedures, precipitation, hydrothermal synthesis techniques, and emulsion methods are all employed to synthesize Cerium oxide nanoparticles. The microwave method is a simple, quick, and efficient alternative to traditional methods [6].

Mycobacterium tuberculosis, Staphylococcus aureus, Listeria monocytogenes, pseudomonas, and Escherichia coli are only a few of the bacteria that are toxic to humans and cause diseases in various ways [7-8]. Mycobacterium tuberculosis (M. tuberculosis) is a species of bacteria that causes tuberculosis (TB) in humans. A slow-growing, rod-shaped, aerobic bacterium primarily affects the lungs but can also spread to other body parts. It has a unique waxy cell wall rich in mycolic acids, which makes it resistant to many antibiotics and disinfectants [9-10]. Cerium oxide nanoparticles are used in combination with several medications to prevent Mycobacterium tuberculosis germs from growing [11]. The primary goal of this study is to look at the Cerium oxide nanoparticles as an antibacterial effect on Mycobacterium tuberculosis bacteria.

2. Experimental

2.1 Synthesis of Cerium Oxide Nanoparticles

Cerium oxide nanoparticles were synthesized via combustion of redox mixtures, using urea as the reducing agent and cerium nitrate as the oxidizing agent to prepare Cerium oxide nanoparticles. The redox combination of 0.17 g urea and 0.35 g cerium nitrate was diluted with 7 ml of distilled water, agitated for 8 minutes with a sonicator set to 50 Hz, filtered, and heated for various periods in a microwave oven at 700 watts.

2.2 Characterization

Various techniques were employed to characterize cerium oxide nanoparticles. X-ray diffraction (XRD) was used to determine their mineralogical composition. The XRD analysis was conducted by scanning all nanoparticle samples from 20° to 80° at a speed of 2°/min and 40 kV using the EVA program. Additionally, the surface morphology of the cerium oxide nanoparticles was examined using a scanning electron microscope (SEM).

2.3 Disc Diffusion Method

The disc diffusion method was employed to evaluate the response of Mycobacterium tuberculosis bacteria to antibiotic drugs, both in the absence and presence of Cerium oxide nanoparticles. To prepare the microbial growth medium, 20 g of nutrient agar was dissolved in 100 ml of distilled water. The solution was then autoclaved at 121°C for 15 minutes. After sterilization, a specified quantity of Cerium oxide nanoparticles was added to the nutrient agar and thoroughly mixed.

Equal portions of the prepared nutrient agar, with and without Cerium oxide nanoparticles, were poured into five petri dishes and left to dry for 10 minutes. Mycobacterium

*Corresponding author: Shoukat.noonari88@yahoo.com

tuberculosis was then applied to the dried agar using a cotton swab. In the center of each petri dish, a small disc with a diameter of 7 mm, impregnated with 30 mg of an antibiotic drug, was placed. The petri dishes were subsequently incubated at 37°C for 48 hours.

2.4 Zone Inhibition Measurement

The Kirby-Bauer chart is used to evaluate bacterial Susceptibility to cerium oxide nanoparticles by measuring the diameter of the zone of inhibition with a Vernier caliper. Bacterial susceptibility can be classified as poor, intermediate, or high. The inhibition zone sizes listed in Table 1 were used to determine the susceptibility of Mycobacterium tuberculosis with and without cerium oxide nanoparticles.

Table 1: Diameter of zone inhibition

S. No	Diameter of zone inhibition (mm)	Susceptibility of organism	Explanation
01	0 to 3	Resistant	Certain bacteria resist specific antibiotics, preventing the medication from effectively inhibiting their growth.
02	+1 to 4	Intermediate	Certain bacteria resist specific antibiotics, preventing the medication from inhibiting their growth.
03	+5	Susceptible	Bacteria resist certain antibiotics, preventing the medicine from effectively inhibiting their growth.

3. Results and Discussion

3.1 Cerium Oxide Nanoparticles Phase Analysis

The XRD technique was used to analyse the phase composition of Cerium oxide nanoparticles. The pattern of produced nanoparticles at various time intervals is shown in Fig.1. The distinctive peaks of CON particles are shown in Fig. 1 at 2 = 29.50, 35.45, and 49.50. Peaks grow more pronounced as the synthesis time increases, as shown in Fig. 1. Only a peak of pure Cerium oxide nanoparticles was developed, and no additional peaks were observed.

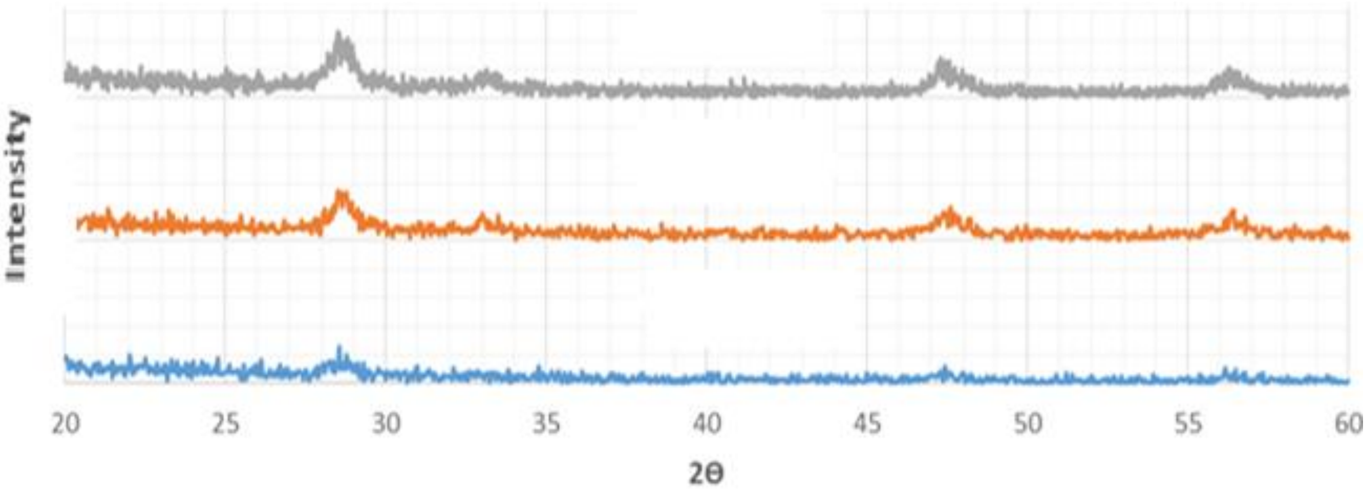


Fig.1: Patterns of Cerium Oxide Nanoparticles of XRD

3.2 Analysis of Particle Size

The Scherrer's equation ($d_{XRD} = 0.9/\beta cos$) was applied to determine the particle size of CON nanoparticles, as presented in Table 2. According to the table, the average crystalline size of the nanoparticles ranges from 27.14 nm to 16.3 nm. Notably, as the synthesis duration increased, the nanoparticle size decreased significantly.

Table 2: Particle size analysis of Cerium Oxide Nanoparticles

Time of Synthesis (min)	FWHM (Deg)	2Θ (Deg)	Wavelength (A)	Particle size (nm)
17	0.72	29.50	1.540	11.75
	0.288	35.45	1.540	29.18
	0.215	49.50	1.540	40.50
	Average			**27.14**
19	0.524	29.50	1.540	8.01
	0.985	35.45	1.540	17.78
	0.22	49.50	1.540	30.11
	Average			**18.63**
21	0.778	29.50	1.540	6.01
	0.556	35.45	1.540	14.78
	0.487	49.50	1.540	28.11
	Average			**16.3**

3.3 Morphology of Cerium Oxide Nanoparticles

Morphology of CON particles was inspected by using scanning electron microscope. SEM images of CON particles are shown in Fig. 2 (i, ii and iii) denotes porous structure. Figures i to ii show that increasing the synthetization period increases porosity and decreases irregularity in cerium oxide nanoparticles, and Figure iv shows that well-regular spherical particles with a size of 16.3 nm were synthesized.

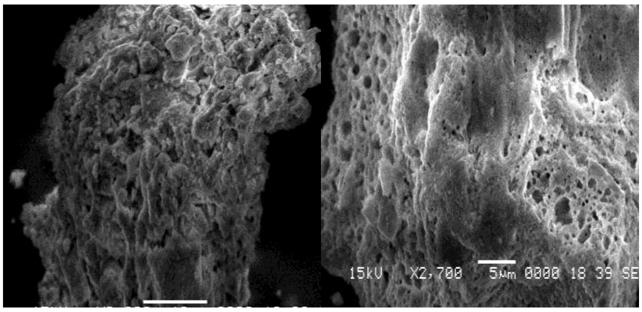
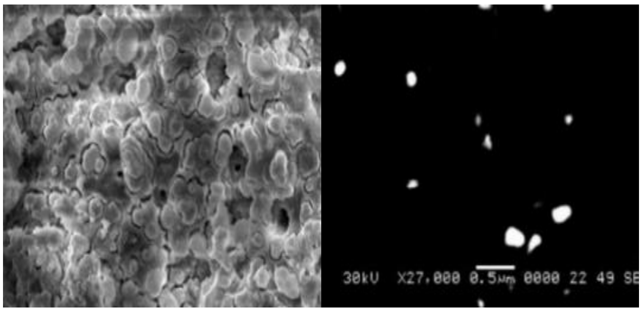


Fig 2. (i) Synthesis 17 min (ii) Synthesis 19 min



(iii) Synthesis 21 min (iv) spherical particles

3.4 Cerium Oxide Nanoparticles Antimicrobial Analysis

The antimicrobial effect of cerium oxide nanoparticles (CNO) against Mycobacterium tuberculosis was assessed. Nanoparticles synthesized at 17, 19, and 21-minute intervals were tested for their antibacterial activity. As shown in Table 3, a concentration of 10 mg/ml CNO17min, when combined with 30 mg of antibiotics such as Amikin Grasil, Terivid, Spraxin, Velosef, and ceftriaxone, was ineffective in inhibiting the growth of Mycobacterium tuberculosis.

The antimicrobial activity of CNO17min, as shown in Table 3, suggests that when amikin grasil and terivid medicines were added with CNO17min, the width of the zone of inhibition was increased to some extent. Furthermore, adding CNO17min to velosef and spraxin antibiotics did not improve their efficacy.

Antimicrobial analysis using CNO21min is extremely promising compared to Cno17min and Cno19min. Table 3 shows that the addition of CNO21min significantly increased the magnitude of zone inhibition in the cases of grasil, terivid, and spraxin. It's worth noting that the inhibition zone did not rise with the addition of CNO21min but rather reduced, as it did with CNO17min and CNO19min.

The antimicrobial activity of CNO17min, as shown in Table 3, suggests that when amikin grasil and terivid medicines were added with CNO17min, the width of zone inhibition was raised to some extent. Furthermore, adding CNO17min to velosef and spraxin antibiotics did not improve their efficacy.

Table 3: Cerium Oxide Nanoparticles (CNO) Antimicrobial activity

CNO dose (10mg/ml)	CNO_{17min}		CNO_{19min}		CNO_{21min}	
Antibiotic (30mg/l)	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility	Zone Inhibition (mm)	Resistance/ Susceptibility
Amikin Grasil	0	Resistance	3.5	Resistance	5	Susceptibility
Terivid	0	Resistance	03	Resistance	5	Susceptibility
Spraxin	0	Resistance	03	Resistance	5	Susceptibility
Velosef	0	Resistance	01	Resistance	3.5	Resistance
Ceftriaxone	0	Resistance	0	Resistance	0	Resistance

The antimicrobial activity of CNO17min, as shown in table 3, suggests that when amikin grasil and terivid medicines were added with CNO17min, the width of zone inhibition was raised to some extent. Furthermore, adding CNO17min to velosef and spraxin antibiotics did not improve their efficacy.

Antimicrobial analysis using CNO21min is extremely promising compared to CNO17min and CNO19min. Table 3 shows that the addition of CNO21min significantly increased the magnitude of zone inhibition in the cases of grasil, terivid, and spraxin. It's worth noting that the inhibition zone did not rise with the addition of CNO21min but rather reduced, as it did with CNO17min and CNO19min.

4. Conclusion

Cerium oxide nanoparticles were studied for their antibacterial properties. Synthetization time is important since it reduces the amount of nitrogen-based chemicals in Cerium oxide nanoparticles and particle size. The porosity of nanoparticle samples was enhanced by increasing the synthetization time, and uneven and rough particles were transformed into spherical-shaped particles, according to the SEM analysis. When cerium oxide nanoparticles were tested alongside antibiotic medications. Results indicate that nanoparticles synthesized over CNO21min was more effective than CNO17min and CNO19min.

5. Limitations of Current Study

The study focused on a limited range of synthetization times and did not explore the long-term stability or cytotoxicity of the cerium oxide nanoparticles. Additionally, antibacterial testing was limited to a few combinations with antibiotics.

6. Future Work

Future research should investigate a broader range of synthesis conditions, assess biocompatibility and long-term effects, and explore the mechanisms behind the enhanced antibacterial activity of optimized nanoparticles

References

[1] S. Yefimova, V. Klochkov, N. Kavok et al., "Antimicrobial activity and cytotoxicity study of cerium oxide nanoparticles with two different sizes," J. Biomed. Mater. Res. B Appl. Biomater., vol. 111, no. 4, pp. 872–880, 2023.

[2] J. Harris, S. Malaiappan, and R. S, "The development and evaluation of melatonin loaded, calcium oxide nanoparticle-based neem and clove extract: An in vitro study," Cureus, 2023.

[3] N. Gandhi, Y. Shruthi, G. Sirisha, and C. Anusha, "Facile and eco-friendly method for synthesis of calcium oxide (CaO) nanoparticles and its potential application in agriculture," Haya Saudi J. Life Sci., vol. 6, no. 5, pp. 89–103, 2021.

[4] P. Piewngam, J. Chiou, P. Chatterjee, and M. Otto, "Alternative approaches to treat bacterial infections: targeting quorum-sensing," Expert Rev. Anti Infect. Ther., vol. 18, no. 6, pp. 499–510, 2020.

[5] J. Lei, L. Sun, S. Huang et al., "The antimicrobial peptides and their potential clinical applications," Am. J. Transl. Res., vol. 11, no. 7, pp. 3919–3931, 2019.

[6] C. Manyi-Loh, S. Mamphweli, E. Meyer, and A. Okoh, "Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications," Molecules, vol. 23, no. 4, p. 795, 2018.

[7] T. V. Surendra and S. M. Roopan, "Photocatalytic and antibacterial properties of phytosynthesized CeO_2 NPs using Moringa oleifera peel extract," J. Photochem. Photobiol. B Biol., vol. 161, pp. 122–128, 2016.

[8] M. M. Masadeh, G. A. Karasneh, M. A. Al-Akhras et al., "Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram-positive and gram-negative biofilm bacteria," Cytotechnology, vol. 67, pp. 427–435, 2015.

[9] S. K. Kannan and M. Sundrarajan, "A green approach for the synthesis of a cerium oxide nanoparticle: Characterization and antibacterial activity," Int. J. Nanoscience, vol. 13, no. 3, p. 1450018, 2014.

[10] M. Negahdary, G. Mohseni, M. Fazilati et al., "The antibacterial effect of cerium oxide nanoparticles on Staphylococcus aureus bacteria," Ann. Biol. Res., vol. 3, pp. 3671–3678, 2012.

[11] D. A. Pelletier, A. K. Suresh, G. A. Holton et al., "Effects of engineered cerium oxide nanoparticles on bacterial growth and viability," Appl. Environ. Microbiol., vol. 76, no. 24, pp. 7981–7989, 2010.

[12] A. Bumajdad, J. Eastoe, and A. Mathew, "Cerium oxide nanoparticles prepared in self-assembled systems," Adv. Colloid Interface Sci., vols. 147–148, pp. 56–66, 2009.

The Nucleus **62**, No. 2 (2025) 69-75

https://doi.org/10.71330/thenucleus.2025.1479

www.thenucleuspak.org.pk

The Nucleus
ISSN 0029-5698 (Print)
ISSN 2306-6539 (Online)

Laser-Induced Breakdown Spectroscopy in Vegetable Analysis: Contaminants and Nutrients

Ayesha Anwar[1], Areesha Rashid[2], Muhammad Rashid[3*]

[1]*Laser Spectroscopy Lab., Department of Physics, University of Agriculture Faisalabad, 38040, Punjab, Pakistan*

[2]*Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan*

[3]*Department of Physics, University of Auckland, Private Bag 92019, Auckland, New Zealand*

ABSTRACT

Vegetables are rich in minerals, but pollutants and wastewater, which introduce heavy metals into the soil, heavily impact their cultivation. As an efficient and effective methodology, scientists prefer using Laser-Induced Breakdown Spectroscopy (LIBS) as a light-based technique to determine the elemental constituents of vegetables. It aids in safety and quality assurance by allowing them to image nutrients and hazardous metals, such as cadmium. This study explored the application of LIBS for detecting contaminants, such as Cd, and profiling essential nutrients in vegetables. Unlike conventional methods such as ICP-MS and AAS, LIBS offers fast, on-site, and multi-element analyses with minimal sample preparation. This review consolidates recent studies on carrots, potatoes, spinach, broccoli, and other leafy greens, emphasizing enhancements using nanoparticles and chemometric tools to improve sensitivity and accuracy. According to the results, LIBS has also been effectively employed to analyze the components of vegetables, enhancing the control and safety of food quality surveillance. The results also prove that LIBS can be a better method for monitoring food quality and safety. LIBS is more consumer-and environmentally friendly because it is portable, fast, and capable of simultaneously analyzing various components.

Keywords: *Cadmium, Food, LIBS, Metals, Safety, Vegetables*

1. Introduction

Vegetables are sources of Potassium, Calcium, Magnesium, and Iron, which are important for health. Yet, when toxic metals like lead and cadmium contaminate them, their safety and nutritional value is compromised [1]. Concentrated metals invade the agricultural ecosystem mainly through contaminated soil and water [2]. The application of raw sewage for irrigation in some Asian developing countries results in certain crops containing alarmingly high levels of cadmium, lead, chromium, mercury, arsenic, and nickel, well over international safe limits [1].

Common methodologies for the quantification and detection of heavy metals in vegetables include Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectrometry (AAS). Although these techniques are efficacious, they are frequently costly, time-intensive, and require highly skilled personnel because of the intricate nature of the laboratory apparatus. Additionally, they require extensive sample preparation, which may affect the accuracy of the results. Consequently, these methods are not conducive to decision-making in field settings or for immediate assessment [3]. The substantial time investment required renders these techniques ineffective for rapid decision-making and field applications [4]. Zhao et al. (2019) demonstrated that the incorporation of nanoparticles in Laser-Induced Breakdown Spectroscopy (LIBS) enhanced the sensitivity of detecting pesticides and heavy metals by a factor of two compared to conventional LIBS [5].

1.1 Fundamentals of LIBS and Laser-Matter Interaction

The quality and intensity of plasma used in LIBS is dependent on the intensity of pulse, the duration, pulse energy and roughened surface. LIBS systems normally involve nanosecond Lasers, picosecond/ femtosecond lasers, and broadband spectrometers simultaneous multi-element analysis [6]. The types of lasers often used in LIBS systems include nanosecond lasers, but picosecond and femtosecond lasers are becoming more common to produce higher resolution and reduced thermal effects [7].

Recent comparative studies have revealed that shorter pulse durations decrease continuum emission by restricting avalanche ionization [8]. The increase in plasma lifetime and signal strength offers an effective tool for utilizing double-pulse LIBS (DP-LIBS) with signal enhancement 2-32 times greater than that of single-pulse LIBS spectra [9]. Quantification is typically performed with broadband spectrometers, such as Echelle or Czerny-Turner systems, with a wide wavelength range (200-900 nm) and will yield a multi-element analysis [10].

Recent advances in dual-spectrometer LIBS (DS-LIBS) systems have led to the implementation of both wideband and narrowband spectrometers capable of collecting major and trace element spectra in a synchronous manner, with characteristics enabling the classification of more than 90% of the datasets (specifically 92% vs. 84% and 73% when single-spectrometer LIBS) [11]. These principles constitute the core of the application of LIBS techniques in the analysis of vegetables, as the technique is quick, sensitive, and non-destructive in the detection of elements. LIBS has achieved significant success in the detection and mapping of trace elements in a variety of plant matrices, and uses is increasingly widespread across the plant science, agriculture, and food technology fields [12]. Recent research has proven that LIBS can be used to examine the nutritional elements in medicinal herbs with great precision, identifying over 90 emission lines of elements and effectively quantitatively detecting nutritive elements in different production regions

*Corresponding author: rmuh003@aucklanduni.ac.nz

[13].

2. Laser-Induced Breakdown Spectroscopy in Food Analysis

LIBS has become a useful research tool in the field of food science, and it can be used to determine the elemental composition and check the quality parameters [14]. In the case of LIBS, materials are bombarded with laser light, which forms plasma, including atoms, ions, and electrons that excite, collide, and emit light with specific wavelengths that can be measured. It is applied in the food industry to determine and identify products owing to its accuracy and affability concerning cost-effectiveness [15].

Real-time analysis of trace elements in food can be conducted using LIBS, which allows for quality assurance and quick decisions [16]. The measurement accuracy of LIBS can be compromised by changes in the plasma and sample matrix. Therefore, researchers are attempting to augment the system with other spectroscopic techniques to enhance its precision [17].

The integration of LIBS with machine learning and chemometrics enables the creation of automatic systems for food classification, origin determination, and adulteration detection [18]. These advances further enhance the usefulness of LIBS for detailed monitoring of the supply chain.

2.1 Detecting Contaminants in Vegetables

Currently, the focus of LIBS has shifted to farming and the food safety analysis. Researchers have conducted studies on plant sample analysis using LIBS [19]. Using LIBS, researchers were able to detect Cd-containing cocoa powder, which confirmed that spot testing is efficient in field work; the results improved significantly under proper sample handling and background removal [20]. Thus, it has been proven that LIBS can be used for monitoring contamination in plant materials.

2.2 Nutrition Profiling

Iron (Fe), calcium (Ca), potassium (K), and other essential trace elements can be measured using LIBS in vegetables, and contamination can also be examined. Researchers using LIBS have demonstrated that organically grown and mid-grown conventional vegetables have different nutritional values [21]. With this method, detecting several nutrients is very easy, which enables producers and consumers to choose the right food. Indeed, LIBS can detect almost all possible problems related to food safety and quality issues [22]. The use of LIBS nutrient data aids in differentiating the nutritional values of organically and conventionally grown vegetables [21].

Table 1 presents the identification of food constituents, such as heavy metals and other chemical elements, using the LIBS technique along with other integrated methods.

Table 1: LIBS Applications in Elemental Analysis of Vegetables

Ref.	Vegetable	Factor	Method	Results
[23]	Potato	Trace mineral elements	The investigation uses LIBS to define and examine the fine vegetable mineral elements, in our case, we study the plasma from the Nd:YAG laser ablation of potato skin and flesh.	For both potato flesh and skin, the following mineral constituents were identified along with their estimated relative concentrations: magnesium, calcium, aluminium, potassium, sodium, copper, iron, manganese, titanium, lithium, silicon, and some others.
[14]	Spinach and rice samples	Nutrient elements and contamination by pesticides	Using a Q-switched Nd:YAG laser to LIBS, spinaches and unpolished rice samples' nutrient elements (Mg, Ca, Na, K) were measured spectroscopically with light emitted from the sample while it was excited by the laser.	Nutrient elements (Mg, Ca, Na, and K) in spinach and unpolished rice samples, with limits of detection (LODs) for these elements in spinach being 29.63 mg/kg for Mg, 102.65 mg/kg for Ca, 36.36 mg/kg for Na, and 44.46 mg/kg for K, while in unpolished rice, the LODs were 7.54 mg/kg for Mg, 1.76 mg/kg for Ca, 4.19 mg/kg for Na, and 6.70 mg/kg for K.
[24]	Carrot	Organic and inorganic elements	Recorded emission spectrum of the carrot root pellet to identify the presence of various inorganic elements. The produced plasma during the LIBS process helped in understanding the characteristics of the emitted spectrum.	Inorganic elements such as K, Na, Fe, Ca, Ti, Zn, Hg, Cu, Mg, Sr, Co in the carrot root pellet were identified. The emission spectrum of fresh raw carrot also indicated the presence of additional organic elements, specifically C, H, N and O, alongside the inorganic elements detected in the carrot root pellet.
[21]	Cauliflowers and broccolis	Nutrient elements	LIBS to identify and compare the presence of major nutrient elements in organic and conventional vegetables, specifically focusing on different parts of cauliflowers and broccolis as working samples.	Analysed and compare the presence of major nutrient elements in organic and conventional vegetables, specifically focusing on different parts of cauliflowers and broccolis. The analysis involved acquiring laser-induced breakdown spectra at optimized parameters and performing both univariate and multivariate analyses.
[25]	Leafy vegetables	Heavy metals	LIBS was applied to analyse the presence of cadmium (Cd) in fresh leafy vegetables, demonstrating its capability for green analysis of toxic heavy metals in agricultural products.	Effectively monitor cadmium (Cd) levels in fresh leafy vegetables, although initial direct calibration methods using single Cd lines showed limitations in accuracy. PLSR analysed the data, the researchers improved

			Partial least-squares regression (PLSR) was utilized for predicting Cd concentrations	the prediction accuracy of Cd concentrations, meeting the necessary requirements for food safety determination.
[26]	Freshly cut carrot samples	Nutrient elements	LIBS technique was employed utilizing a Nd:YAG laser with a wavelength of 1064 nm for the ablation process.	Detected 18 chemical elements in fresh carrot samples, including essential nutrients such as Mg, Al, Fe, Mn, Ti, Ca, and Mn

2.3 Comparative Evaluation of Chemometric Models in LIBS

Several studies have highlighted the reality of chemometric techniques in improving LIBS analytical capability, especially under complex food matrices such as vegetables. Principal component analysis (PCA) has been extensively exploited to carry out an initial visualization of the data and to determine natural clusters in multivariate LIBS data [27, 28]. PLSR has demonstrated incredible predictive ability to measure the scales of elemental levels, such as cadmium and calcium, in leafy greens [25]. Linear discriminant analysis (LDA) has been found to be very useful in the identification of organically and conventionally grown vegetables using weak spectral gradation [29]. Artificial neural networks (ANN) and other machine learning algorithms have recently become popular because of their better performance in modeling nonlinear relationships and high classification accuracy when applied to large LIBS datasets [27, 30].

Figure 1 shows a flow chart that summarizes a comparative overview of widely used models PCA, PLSR, LDA, and ANN and their key characteristics, applications, data requirements, strengths, and limitations.

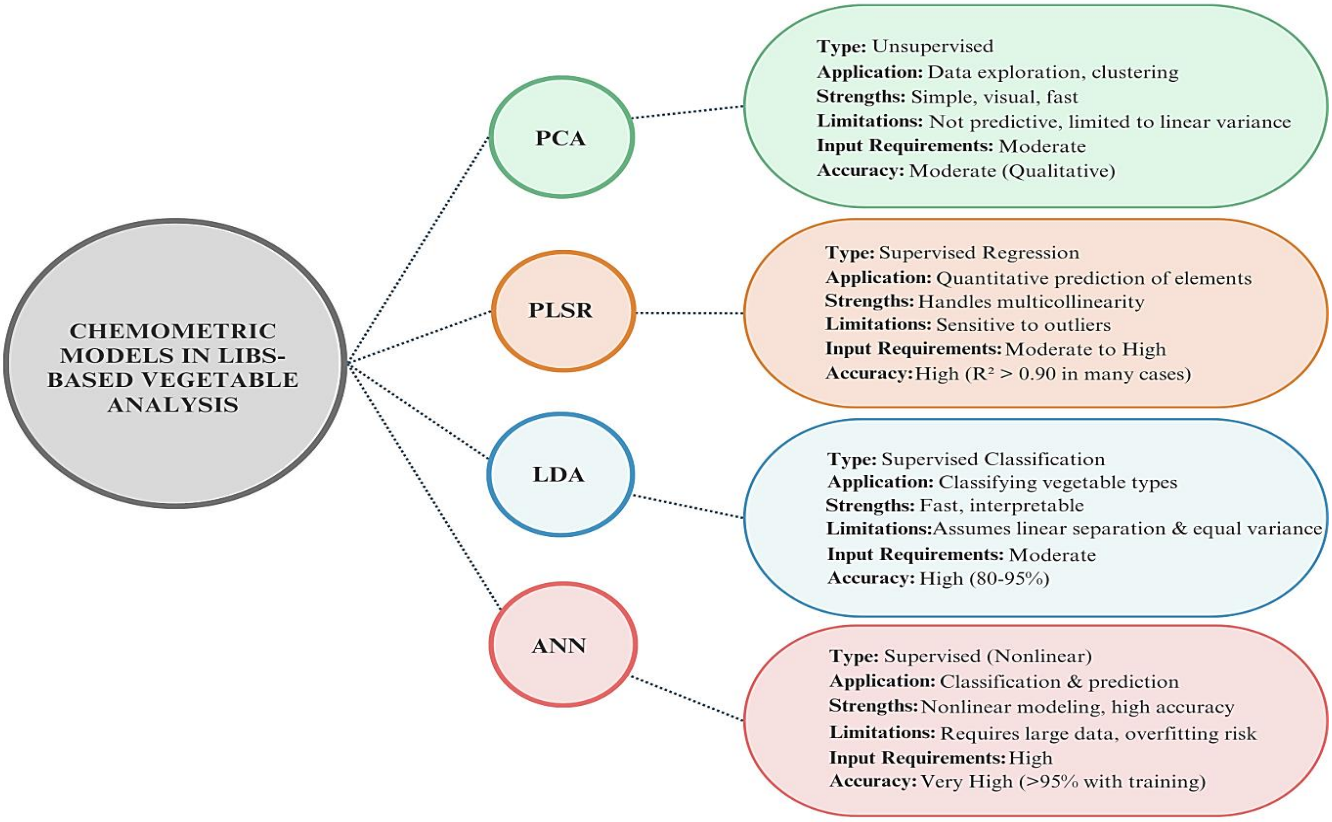


Fig. 1: Flow Chart of Chemometric Models Used in LIBS-Based Vegetable Analysis.

3. Feasibility and Challenges

Other recent findings have pointed out that portable LIBS systems, although possessing rapidity of analysis and low sample preparation, have serious infrastructural constraints when used in the developing world [31]. The commercialization of ruggedized underwater LIBS, such as the LIBSea II system, has made it possible to realize the technical viability of portable field-deployable tools [32]. Nevertheless, these systems still require advanced laser devices and spectroscopy equipment, which may pose a financial obstacle in limited-resource environments [33].

Barriers to implementation also differ across regions and contexts. Investigations into the adoption of technology in Africa and Asia have found some uniform issues, such as a shortage of funds, poor infrastructure, and a lack of technical support systems. To achieve equitable access to, and wide application of, the technology it is necessary to respond to the region-specific challenges by the use of cost-reduction strategies, local training programs, and simplification of LIBS interfaces.

3.1 Matrix Effects and Calibration Challenges in LIBS along with Solutions

By cyclic culture, vegetables will have considerable sample heterogeneity because moisture content, surface texture, density, and inner composition will vary in the samples [34, 35]. These chemical and physical differences may influence the homogeneity of the plasma generated in the LIBS analysis, where there is variation in the emitted spectral signals [36].

Vegetable matrices are heterogeneous materials, making LIBS analysis problematic. Very strong physical and chemical baseline variations characterize agricultural products, such as vegetables, which influence the repeatability of spectral measurements [35, 36]. This heterogeneity occurs in several aspects: moisture content variations alter the laser-matter interaction and plasma formation, surface texture variations alter the ablation patterns, and internal variations cause localized matrix effects [37].

Vegetable analysis is one area where moisture effects are particularly serious. It was found that the optimal laser absorption properties and plasma temperature distribution substantially depend on the moisture content [38]. Atmospheric conditions, including pressure, composition, and confinement effects, significantly influence LIBS performance by altering plasma formation, the signal intensity, and the spectral resolution [39].

Spatial variations in the surface texture also complicate the LIBS analysis of vegetables. Studies show that the surface roughness influences the ablation performance and craters formation patterns. This nonuniform distribution of the laser energy may occur due to irregular surface topography and will cause nonuniform generation of plasma and produce changes in the spectral signals [40]. This is especially applicable to vegetables that grow with naturally bumpy or textured surfaces, such as root vegetables or coated vegetables with a waxy policing.

To overcome these matrix-induced challenges, several strategies have been developed to improve the performance and consistency of LIBS in vegetable analysis. Calibration-free LIBS (CF-LIBS) is a self-contained technique that computes elemental concentrations implicitly based on plasma parameters with few external dependencies on calibration standards [41]. Internal standardization methods involve standardizing the spectral signal with respect to elements within the matrix sample to demodulate the variation caused by matrix effects [42]. Optimized experimental procedures, such as spatial confinement, have shown significant increases in the detection limit and precision of the analytical method [43]. Plasma intensity can be increased and its duration extended using a dual-pulse LIBS system, leading to improved stability and increased amplification of the emission signal. Such systems work as follows: the first pulse ablates material and establishes initial plasma conditions, whereas the second pulse reheats and strengthens the plasma, resulting in a significant signal enhancement [44]. The method of multi-spot averaging, which implies the gathering of spectra of different points on one sample, has also proved useful in mitigating the effects of localized heterogeneity and better reproducibility [45]. The aspects of the matrix effects in LIBS are evidenced by the sample heterogeneity and the fluctuations of the signals, as well as the ways out discussed in Figure 2.

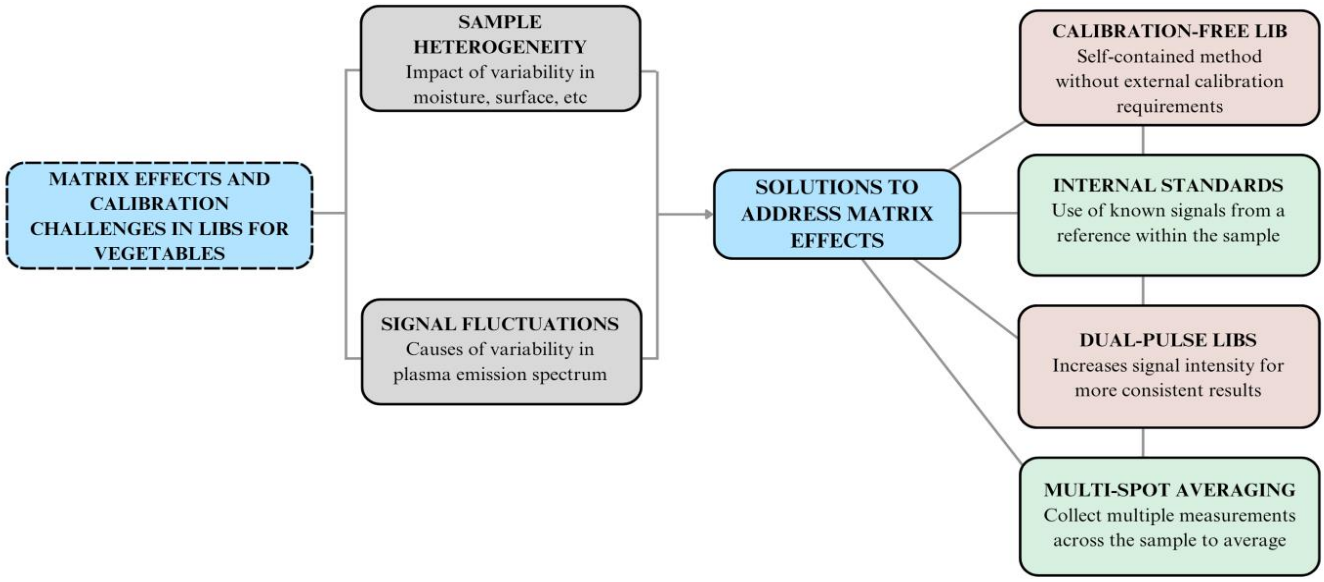


Fig. 2 Challenges in LIBS for Vegetables Analysis and Its Solutions

4. Recent Advancements in LIBS Technology (2020-2025) for Food Safety Applications

Recent innovations in LIBS technology from 2020 to 2025 have largely increased the range of its application in food safety, especially in the non-destructive manner of food safety analysis of vegetables. One of the most revolutionary advances has been the advent of handheld and portable LIBS instrumentation, which allows the on-site detection of elements in real time without laboratory infrastructure [46]. Simultaneously, other forms of deep learning have been developed to work with LIBS, including convolutional neural networks (CNNs) and autoencoders, to enhance the interpretation of the spectrum. Such models are very effective in identifying complex nonlinear trends in high-dimensional spectral data, resulting in improved classification accuracies and increased detection of subtle differences in compositions [47]. Nanoparticle-enhanced LIBS (NELIBS) is another important innovation in which metal nanoparticles are deposited on sample surfaces, increasing the signal arising from the plasma. NELIBS has especially been able to reduce the limits on the detectability of trace heavy elements such as cadmium and lead [48]. Moreover, 3D mapping and imaging methodologies have been investigated in LIBS to obtain the spatial regions of the elemental distribution in raw vegetable tissues. The commonly defined spatially resolved analysis allows better insights into the localization of nutrients and infiltration of contaminants, which can be used to evaluate food quality and study agriculture [49]. The combination of these technological advances is a paradigm shift in LIBS applications for food safety, given the ability to make portable deployments, perform enhanced analytics through the incorporation of artificial intelligence, enhance sensitivity through nanoparticle reproducers, and provide full spatial analytics capabilities that were unavailable in earlier LIBS systems.

4.1 Future Directions

To maximize the potential of LIBS in food analysis, future efforts are required to miniaturize the equipment and build portable, field-ready systems with real-time observation procedures. New developments indicate that there have been significant advances in this field, with commercial handheld LIBS instruments exhibiting very strong performance in food authentication [31]. The analysis of portable LIBS systems has also proven their usefulness in the analysis of various food matrices, such as European Alpine-style cheeses, coffee, spices, balsamic vinegar, and vanilla extracts, where little or no treatment is given to the sample [50].

4.2 Limitations and Advancements in LIBS Application

Despite the usefulness of LIBS, it is disadvantaged by the fact that LIBS is prone to matrix effects, low sensitivity levels on the trace elements and the inexistence of standardized calibration techniques, which contributes to quantitative reproducibility and accuracy. This variation in sample surface composition makes the generation of plasma even more confounded with the heterogeneous surface being more fluctuating in the plasma. To survive these challenges, researchers were exposed to NELIBS to enhance the signal, and also to include chemometric techniques in order to fully analyze the data, namely, PLSR and PCA. Machine learning algorithms that facilitate the accuracy of classification and real-time food safety monitoring in a field are also part of the recent development.

5. Conclusion

LIBS is a useful method for analyzing the nutritional and contaminant content of vegetables. The reviewed studies confirm that it can accurately determine the levels of Cd and nutrients such as potassium, calcium, and iron in various types of vegetables. Unlike traditional methods, LIBS is quicker, does not require much sample preparation, and allows for testing at the site, which is great for immediate food safety checks. As new progress is made in enhancing signals, processing data, and calibration, LIBS is set to become vital for sustainable farming, food safety, and health services.

References

[1] N. Kaur, R. Singh, A. Sharma, P. Verma, and S. Gupta, "Heavy metal contamination in wastewater-irrigated vegetables: assessing food safety challenges in developing Asian countries," *Environ. Sci.: Processes Impacts*, vol. 27, pp. 1747–1767, 2025.

[2] S. Shahriar, A. Rahman, M. Islam, R. Hasan, and T. Ahmed, "Heavy metal contamination in soil and vegetables: A review with health risk assessments," *J. Sci. Eng. Papers*, vol. 1, no. 1, pp. 40–48, 2024.

[3] P. Kumari, V. Kachhwaha, and P. Mishra, "A comparative study of heavy metal toxicity in the vegetables using ICP-MS and AAS," *Orient. J. Chem.*, vol. 40, no. 2, pp. 446–453, 2024.

[4] Z. Yang, L. Huang, M. Chen, J. Li, and Y. Zhao, "Enhanced laser-induced breakdown spectroscopy for heavy metal detection in agriculture: A review," *Sensors*, vol. 22, no. 15, p. 5679, 2022.

[5] X. Zhao, L. Chen, M. Wang, Y. Liu, and J. Zhang, "Detecting and mapping harmful chemicals in fruit and vegetables using nanoparticle-enhanced laser-induced breakdown spectroscopy," *Sci. Rep.*, vol. 9, no. 1, p. 906, 2019.

[6] F. Anabitarte, A. Cobo, and J. M. Lopez-Higuera, "Laser-induced breakdown spectroscopy: fundamentals, applications, and challenges," *Int. Scholarly Res. Notices*, vol. 2012, pp. 1–12, 2012.

[7] M. Gragston, L. Evans, J. Turner, S. Patel, and R. James, "Time-gated single-shot picosecond laser-induced breakdown spectroscopy (ps-LIBS) for equivalence-ratio measurements," *Appl. Spectrosc.*, vol. 74, no. 3, pp. 340–346, 2020.

[8] M. Gragston, L. Evans, J. Turner, S. Patel, and R. James, "Emissions in short-gated ns/ps/fs-LIBS for fuel-to-air ratio measurements in methane-air flames," *Appl. Opt.*, vol. 60, no. 15, pp. C114–C120, 2021.

[9] X. Liu, J. Zhang, L. Wang, Y. Chen, and H. Li, "Effect of laser pulse energy on orthogonal double femtosecond pulse laser-induced breakdown spectroscopy," *Opt. Express*, vol. 21, no. S4, pp. A704–A713, 2013.

[10] Y. Zhang, X. Li, J. Chen, H. Wang, and L. Zhou, "Echelle grating spectroscopic technology for high-resolution and broadband spectral measurement," *Appl. Sci.*, vol. 12, no. 21, p. 11042, 2022.

[11] W. Wang, J. Liu, Y. Chen, X. Zhang, and L. Zhao, "Staging classification of omicron variant SARS-CoV-2 infection based on dual-spectrometer LIBS (DS-LIBS) combined with machine learning," *Opt. Express*, vol. 31, no. 25, pp. 42413–42427, 2023.

[12] V. K. Singh, R. Kumar, S. Sharma, A. Gupta, and P. Verma, "Application of LIBS to elemental analysis and mapping of plant samples," *At. Spectrosc.*, vol. 42, no. 1, pp. 446–453, 2021.

[13] T. Shen, L. Zhou, Y. Wang, H. Liu, and J. Zhang, "High-sensitivity determination of nutrient elements in *Panax notoginseng* by laser-induced breakdown spectroscopy and chemometric methods," *Molecules*, vol. 24, no. 8, p. 1525, 2019.

[14] G. Kim, J. Lee, S. Park, H. Choi, and M. Kang, "Detection of nutrient elements and contamination by pesticides in spinach and rice samples using laser-induced breakdown spectroscopy (LIBS)," *J. Agric. Food Chem.*, vol. 60, no. 3, pp. 718–724, 2012.

[15] M. Markiewicz-Keszycka, M. B. Kaczyński, K. Gajek, A. Woźniak, and A. Słowińska, "Laser-induced breakdown spectroscopy (LIBS) for food analysis: A review," *Trends Food Sci. Technol.*, vol. 65, pp. 80–93, 2017.

[16] I. Palamarchuk, M. Kovács, L. Novak, A. Horváth, and P. Varga, "Spectroscopic assessment and quantitative analysis of the trace element composition of vegetable additives to meat products," *Slovak J. Food Sci.*, vol. 18, pp. 480–496, 2024.

[17] Z. Chen, L. Wang, H. Liu, Y. Zhang, and X. Li, "Signal enhancement of cadmium in lettuce using laser-induced breakdown spectroscopy combined with pyrolysis process," *Molecules*, vol. 24, no. 13, p. 2517, 2019.

[18] D. Stefas and N. Gyftokostas, "Laser-induced breakdown spectroscopy: An efficient tool for food science and technology (from the analysis of Martian rocks to the analysis of olive oil, honey, milk, and other natural earth products)," *Molecules*, vol. 26, no. 16, p. 4981, 2021.

[19] D. M. Silvestre, L. A. Silva, M. R. Oliveira, P. J. Santos, and F. C. Almeida, "Direct analysis of barium, calcium, potassium, and manganese concentrations in tobacco by laser-induced breakdown spectroscopy," *Microchem. J.*, vol. 126, pp. 545–550, 2016.

[20] J. Molina M., L. Fernández, R. Gómez, A. Torres, and M. Sánchez, "Assessing the sensitivity and efficiency of laser-induced breakdown spectroscopy (LIBS) for high-concentration cadmium detection in cocoa powder," *Sensors*, vol. 25, no. 8, p. 2434, 2025.

[21] C. R. Bhatt, S. Kumar, A. Singh, R. Sharma, and P. Verma, "Comparative study of elemental nutrients in organic and conventional vegetables using laser-induced breakdown spectroscopy (LIBS)," *Appl. Spectrosc.*, vol. 71, no. 4, pp. 686–698, 2017.

[22] Z. J. Kamil, M. J. Zoory, and H. J. Mohamad, "LIBS technique for plant mineral ratio analysis and environmental and agricultural importance: A comprehensive review," *Eur. Phys. J. D*, vol. 78, no. 3, p. 27, 2024.

[23] S. A. Beldjilali, M. Abdou, A. Bendjeddou, and K. Boumendjel, "Analyses of plasmas produced by laser ablation of fresh aliments," *Adv. Mater. Res.*, vol. 227, pp. 49–52, 2011.

[24] N. Shukla, P. Kumar, R. Singh, A. Gupta, and S. Verma, "Determination of elements in carrot root by laser induced breakdown spectroscopy," *Natl. Acad. Sci. Lett.*, vol. 40, pp. 47–51, 2017.

[25] M. Yao, J. Chen, L. Wang, H. Zhao, and S. Li, "Detection of heavy metal Cd in polluted fresh leafy vegetables by laser-induced breakdown spectroscopy," *Appl. Opt.*, vol. 56, no. 14, pp. 4070–4075, 2017.

[26] N. Yudasari, S. Prasetyo, and M. Suliyanti, "The 1064 nm laser-induced breakdown spectroscopy (LIBS) inspection to detect the nutrient elements in freshly cut carrot samples," *J. Phys.: Conf. Ser.*, vol. 985, no. 1, p. 012011, 2018.

[27] P. Devangad, R. Sharma, S. Kulkarni, M. Singh, and A. Patel, "Plasma spectroscopy + chemometrics: An ideal approach for the spectrochemical analysis of iron phosphate glass samples," *J. Chemometrics*, vol. 34, no. 11, p. e3310, 2020.

[28] B. L. Dutrow, N. J. McMillan, and D. J. Henry, "A multivariate statistical approach for mineral geographic provenance determination using laser-induced breakdown spectroscopy and electron microprobe chemical data: A case study of copper-bearing tourmalines," *Am. Mineral.*, vol. 109, no. 6, pp. 1085–1095, 2024.

[29] Y. Yuan, H. Liu, X. Li, J. Zhang, and M. Wang, "Improved discrimination for *Brassica* vegetables treated with agricultural fertilizers using a combined chemometric approach," *J. Agric. Food Chem.*, vol. 64, no. 28, pp. 5633–5643, 2016.

[30] O. Hamdy, Z. Abdel-Salam, and M. Abdel-Harith, "Utilization of laser-induced breakdown spectroscopy, with principal component analysis and artificial neural networks in revealing adulteration of similarly looking fish fillets," *Appl. Opt.*, vol. 61, no. 34, pp. 10260–10266, 2022.

[31] X. Wu, H. Li, Y. Chen, W. Zhang, and L. Wang, "Rapid food authentication using a portable laser-induced breakdown spectroscopy system," *Foods*, vol. 12, no. 2, pp. 1–18, 2023.

[32] C. Liu, J. Zhang, L. Wang, Y. Chen, and H. Li, "Development and field tests of a deep-sea laser-induced breakdown spectroscopy (LIBS) system for solid sample analysis in seawater," *Sensors*, vol. 20, no. 24, p. 7341, 2020.

[33] X. Fu, G. Li, and D. Dong, "Improving the detection sensitivity for laser-induced breakdown spectroscopy: A review," *Front. Phys.*, vol. 8, p. 68, 2020.

[34] C. Davison, T. Rodgers, M. Montes-Bayón, M. Thévenot, and P. J. Sadler, "Expanding the boundaries of atomic spectroscopy at the single-cell level: Critical review of SP-ICP-MS, LIBS and LA-ICP-MS advances for the elemental analysis of tissues and single cells," *Anal. Bioanal. Chem.*, vol. 415, no. 28, pp. 6931–6950, 2023.

[35] L. Wang, Y. Zhang, H. Li, J. Chen, and M. Zhao, "Application and research progress of laser-induced breakdown spectroscopy in agricultural product inspection," *ACS Omega*, vol. 9, no. 23, pp. 24203–24218, 2024.

[36] V. N. Lednev, A. S. Ivanov, M. P. Smirnov, and E. V. Kuznetsova, "Improving calibration strategy for LIBS heavy metals analysis in agriculture applications," *Photonics*, vol. 8, no. 12, p. 563, 2021.

[37] S. Yao, M. Chen, L. Zhang, H. Liu, and Q. Wang, "Optimizing the binder percentage to reduce matrix effects for the LIBS analysis of carbon in coal," *J. Anal. At. Spectrom.*, vol. 32, no. 4, pp. 766–772, 2017.

[38] Y. Zhang, X. Li, J. Chen, H. Wang, and L. Zhou, "Pressure effects on underwater laser-induced breakdown spectroscopy: An interpretation with self-absorption," *J. Anal. At. Spectrom.*, vol. 36, no. 3, pp. 644–653, 2021.

[39] A. J. Effenberger, Jr. and J. R. Scott, "Effect of atmospheric conditions on LIBS spectra," *Sensors*, vol. 10, no. 5, pp. 4907–4925, 2010.

[40] R. C. Madasani and M. Hossain, "Impact of surface texture on moisture absorption and long-term mechanical performance of biomedical polymers," in *Proc. ASME Int. Mech. Eng. Congr. Expo.*, vol. 4, Portland, OR, USA, Nov. 2024.

[41] E. Böhmer, M. Scholz, F. Müller, T. Schneider, and A. Becker, "Preliminary results for calibration-free laser-induced breakdown spectroscopy (CF-LIBS) for the elemental analysis of biological samples," in *Optical Interactions with Tissue and Cells XXXVI*, SPIE, vol. 13317, p. 1331706, 2025.

[42] R. R. Gamela, A. M. G. T. da Silva, M. A. P. Soares, J. A. S. Cavichio, and J. L. F. Monteiro, "Matrix-matching calibration using solid standards: A comparison between univariate and multivariate strategies for the determination of calcium and magnesium in bean seed samples employing laser-induced breakdown spectroscopy (LIBS)," *Anal. Lett.*, vol. 56, no. 6, pp. 944–957, 2023.

[43] M. Rashid, A. Ahmed, S. Khan, F. Ali, and Z. Malik, "Laser-induced breakdown spectroscopy for soil analysis: Recent advances in nutrient and contaminant detection," *Spectrum of Eng. Sci.*, vol. 3, no. 8, pp. 279–288, 2025.

[44] L. Na, E. Harefa, and Z. Weidong, "Nanosecond laser preheating effect on ablation morphology and plasma emission in collinear dual-pulse laser-induced breakdown spectroscopy," *Plasma Sci. Technol.*, vol. 24, no. 11, p. 115507, 2022.

[45] A. Erler, M. Thiele, H. Küpper, T. Schmid, and H. Knicker, "Soil nutrient detection for precision agriculture using handheld laser-induced breakdown spectroscopy (LIBS) and multivariate regression methods (PLSR, Lasso and GPR)," *Sensors*, vol. 20, no. 2, p. 418, 2020.

[46] M. Wójcik, J. Baranowski, M. Kwiatkowski, M. Dudzik, and K. Błaszkiewicz, "Classification of copper minerals by handheld laser-induced breakdown spectroscopy and nonnegative tensor factorisation," *Sensors*, vol. 20, no. 18, p. 5152, 2020.

[47] T. Wu, R. Zhai, J. Huang, Z. Wang, B. Han, and Y. Liu, "Deep learning-enhanced laser-induced breakdown spectroscopy for rapid in-situ analysis of Martian surface and atmospheric constituents," *Microwave Opt. Technol. Lett.*, vol. 67, no. 6, p. e70273, 2025.

[48] R. Gaudiuso, A. Taleb, M. Dell'Aglio, I. C. Tommasi, and A. De Giacomo, "Feasibility of nanoparticle-enhanced LIBS (NELIBS) for the analysis of archaeological metallic artifacts: A critical assessment," *J. Anal. At. Spectrom.*, vol. 40, no. 2, pp. 354–364, 2025.

[49] P. Mahyari, M. Maniscalco, H. Choi, N. May, A. Phoulady, T. Moore, A. Blagojevic, M. T. M. Anaei, T. Bliznakov, M. Emanuel, W. Roser, S. Shahbazmohamadi, and P. Tavousi, "Laser-induced breakdown spectroscopy for real-time 3D material composition mapping," in *Frontiers in Ultrafast Optics: Biomedical, Scientific, and Industrial Applications XXV*, SPIE, vol. 13353, pp. 58–69, 2025.

[50] X. Wu, S. Shin, E. Bae, J. P. Robinson, and B. Rajwa, "Food authentication studies using laser-induced breakdown spectroscopy (LIBS)," in *Sensing for Agriculture and Food Quality and Safety XIV*, SPIE, vol. 12120, pp. 76–83, 2022.

The Nucleus **62**, No. 2 (2025) 76-82

https://doi.org/10.71330/thenucleus.2025.1485

www.thenucleuspak.org.pk

The Nucleus

ISSN 0029-5698 (Print)

ISSN 2306-6539 (Online)

Optimal Growth Strategies for Sustainable Cultivation of Chlorella Vulgaris Microalgae for the Application of Bioenergy: A Review

Bobby Singare, Ram Narayan Singh*

School of Energy and Environmental Studies, Devi Ahilya University, Takshashila Campus, , Khandwa Road, Indore, Madhya Pradesh, India

ABSTRACT

Chlorella vulgaris, a green microalga, is attracting significant attention for its uses in food production, biofuel production, and environmental sustainability due to its high nutritional value and high growth rate. The current review discusses ideal methods for the cultivation of Chlorella vulgaris in an eco-friendly manner and also explores optimal growth strategies for cultivating C. vulgaris with a focus on maximizing bioenergy. Key factors affecting its growth, including the provision of light, nutrient content, temperature, and pH levels, are discussed in relation to biomass productivity and biochemical composition. Different cultivation methods, such as open pond cultivation and closed photobioreactors, are discussed in terms of efficiency and ecological implications. Contamination and high cost are discussed as challenges, alongside suggested solutions such as using nutrient media as a nutrient source. Through synthesis of existing studies, the article attempts to provide easy and practical recommendations to researchers and agriculturalists who are interested in the sustainable cultivation of microalgae for the production of bioresources.

Keywords: *Chlorella vulgaris, Microalgae, Sustainability, Eco-friendly, Photo bioreactor, Bioresources.*

1. Introduction

Microalgae, microscopic unicellular organisms, have become an important area of research in recent years for their potential to solve urgent global issues, such as addressing food shortages, increasing energy requirements, and reaching environmental sustainability targets [1]. Chlorella vulgaris, a green microalga, has gained particular attention due to its fast rates of growth, high levels of nutrients, and wide array of uses and applications [2]. With an approximate dry weight of 50-60% protein and high quantities of vitamins, minerals, and antioxidants, Chlorella vulgaris can be consumed directly for human nutrition, used in animal feed, and/or used to formulate health supplements [3]. In addition to its nutritional value, Chlorella vulgaris has potential for the development of sustainable energy in the form of biofuels, such as biodiesel, and for sustainable practices such as carbon dioxide sequestration and wastewater treatment [4]. All of these qualities make Chlorella important and essential species of microalgae which will enable new technologies and strategies towards achieving sustainable development, especially when faced with constraints to resource availability and the climate crisis [5].

Multiple factors work together to influence the growth of Chlorella vulgaris. Before development can be optimized, it must be recognized that light intensity, nutrient levels, temperature, pH, and cultivation systems all play a significant role in biomass production [6]. The primary modes of cultivation include open pond systems, which are the cheapest form of cultivation but are very prone to contamination, and closed photobioreactors (PBRs), which are a more expensive way to cultivate microalgae, yet provide the availability of specific control [7]. Other limitations that stand in the way of developing Chlorella vulgaris are the cost of water and nutrients [8].

Even though there is a lot of promise, achieving sustainable cultivation of Chlorella vulgaris involves balancing production against economic and environmental factors. Current literature highlights the necessity of developing effective and scalable solutions to ensure the broad adoption of microalgae-based technologies [9]. This review explored the best strategies for growing Chlorella vulgaris, with a focus on what sustainable practices might be employed for cultivation. Evaluated the influence of the primary growth parameters, compared different cultivation systems, and examined innovative methods to address hurdles. Recent literature offers consolidated insights to guide researchers, agriculturalists, and industries in developing Chlorella vulgaris for sustainable applications, particularly in bioenergy and nutritional uses (2024-2025) [10].

2. Methodology

A systematic literature review was conducted to identify suitable methods for cultivating Chlorella vulgaris in sustainable practices, based on peer-reviewed research studies published between 2010 and 2025 [11]. The studies were selected based on key factors of interest concerning microalgae productivity, mostly empirical research that focused on cultivation parameters and sustainability variables [12]. The selected sources contained relevant information, such as algal species used for the growth, growth conditions, and terms of cultivation methods [13]. The information was recorded in an organized manner so that it could be thematically analyzed to identify patterns and support its relation, such as nutrient metabolism, design of systems. Several variables were identified as potentially influencing Chlorella vulgaris productivity, including nutrient metabolism, system design, and abiotic factors during cultivation [15]. For example, the novel biotechnology space is currently full of innovative solutions that are making better use of fertilizers through the reuse of resources like media, as

*Corresponding author: rnsingh.seema@gmail.com

well as insights into bacteria co-cultures that can potentially enhance the growth of bioactive compounds with applications into antimicrobial and antioxidant purposes [16]. This synthesized review of the literature provides a standardized summary of useful adaptations for enhancing Chlorella vulgaris growth, in particular their uses towards bioenergy, while also including discussions on future challenges, developing approaches to sustainability, wastewater treatment, and how to go beyond zero waste by utilizing biomass completely [17].

3. Factors Affecting Algae Growth

The production of biomass from Chlorella vulgaris microalgae, as well as its total biomass development, is due to a culmination of environmental, biological, and operational factors; all of which contribute crucially to the enhancement of cultivation protocols for an environmentally sustainable undertaking, biofuel production, food supplementation in animals, and pollution mitigation [18]. For increasing output, reducing costs, and decreasing impacts on the environment, it is necessary to have control over, understand these factors, and cultivate them sustainably [19]. The detailed understanding of these factors allows researchers and practitioners to develop a cultivation plan that is tailored to a specific outcome, for example, biomass that is lipid-rich for fuels or biomass that is nutrient-rich for animals [20]. The following sections will provide a greater explanation of each of the primary factors involved in the development of Chlorella vulgaris, their role, their relation to one another, and their potential for adoption as an environmentally sustainable.

3.1 Light Intensity and Quality

Light is the main fuel for photosynthesis, the core process that powers the growth of Chlorella vulgaris. The intensity, duration, and spectral quality of light significantly influence biomass accumulation and biochemical composition. Optimal light intensity typically ranges from 100 to 200 μmol/m²/s, with a photoperiod of 12-16 hours daily, promoting maximum photosynthetic efficiency and biomass yields of up to 1.0 g/L in controlled settings [21]. Insufficient light limits carbon fixation, resulting in stunted growth, while excessive light (>500 μmol/m²/s) triggers photoinhibition, causing oxidative stress and cell damage [22]. The quality of light is equally critical, with red (620-630 nm) and blue (450-475 nm) wavelengths enhancing chlorophyll absorption and growth rates by 20-30% compared to white light. Artificial lighting, such as energy-efficient LED systems, allows precise control in photobioreactors, but natural sunlight is preferred in open ponds for sustainability, reducing energy costs by up to 50% in outdoor setups. Recent studies suggest that dynamic light regimes, adjusting intensity based on growth phase, can further optimize lipid content for bioenergy applications, with blue light increasing lipid yields by 15-22% [23] (Fig. 1).

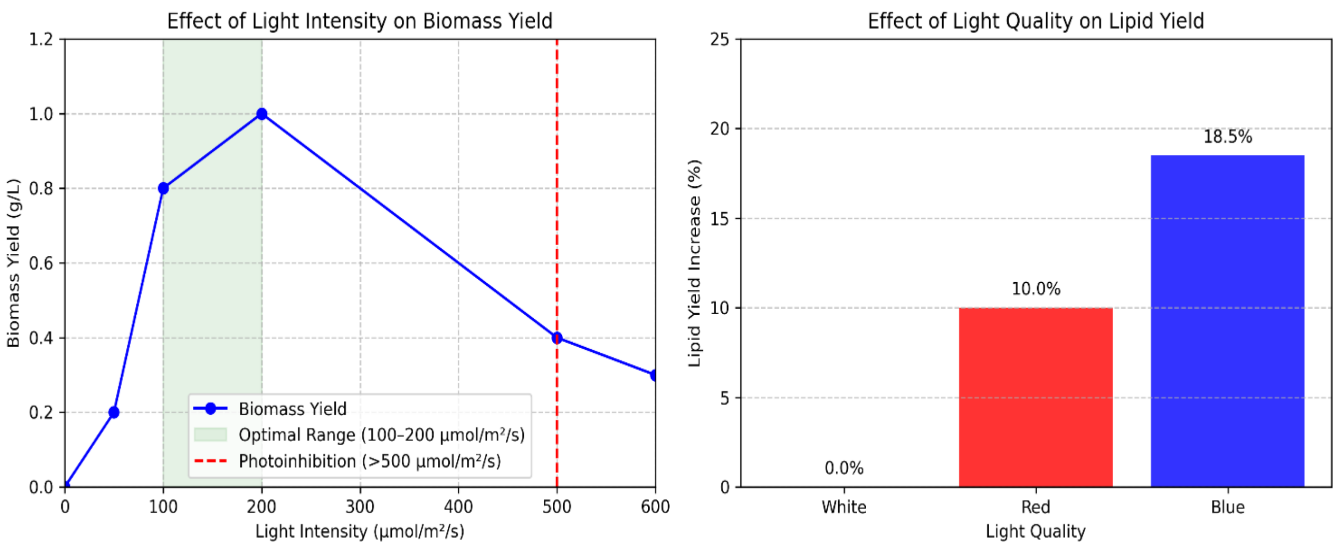


Fig. 1 Effect of light intensity and quality on Chlorella vulgaris. Biomass peaked at 100-200 μmol/m²/s, while blue light gave the highest lipid yield [9, 10, 23].

3.2 Nutrient Availability

Nutrients are the building blocks of Chlorella vulgaris metabolism, supporting cell division, protein synthesis and lipid accumulation. Macronutrients like nitrogen (50-100 mg/L as nitrate) and phosphorus (20-30 mg/L as phosphate) are essential for growth, with nitrogen driving protein and chlorophyll production and phosphorus facilitating energy transfer and nucleic acid synthesis. Micronutrients, including iron, magnesium, zinc, and manganese, act as cofactors in enzymatic reactions, with deficiencies reducing growth rates by up to 40%. The nitrogen-to-phosphorus ratio (optimal at 16:1) must be balanced to avoid nutrient limitation or toxicity, which can increase cultivation costs or lead to environmental pollution through runoff [24]. Sustainable nutrient management leverages alternative sources like municipal wastewater or agricultural runoff, which can supply 60-80% of required nitrogen and phosphorus, reducing costs by 30-50% while treating wastewater. However, wastewater must be pre-treated to remove heavy metals or pathogens, ensuring biomass safety for food or feed applications. Nutrient recycling, where spent media is reused, further enhances sustainability by minimizing waste [25].

3.3 Temperature

Temperature regulates the metabolic and enzymatic activities of Chlorella vulgaris, directly impacting growth rates and biomass productivity. The optimal range is 25-30°C, where biomass yields can reach 0.8-1.2 g/L under controlled conditions. Temperatures below 15°C slow metabolic processes, reducing growth rates by 50%, while temperatures above 35°C cause protein denaturation, lipid degradation, and cell mortality, with yields dropping by 60-80% [26]. In open pond systems, diurnal and seasonal temperature fluctuations pose challenges, necessitating site selection in temperate climates or supplemental heating/cooling systems, which can increase energy costs by 20-30% [27]. Closed photobioreactors offer precise temperature control, maintaining optimal conditions but requiring significant capital investment. Sustainable strategies, such as using waste heat from industrial processes or geothermal sources, can stabilize temperatures in open systems, reducing energy demands and enhancing year-round productivity (Fig. 2).

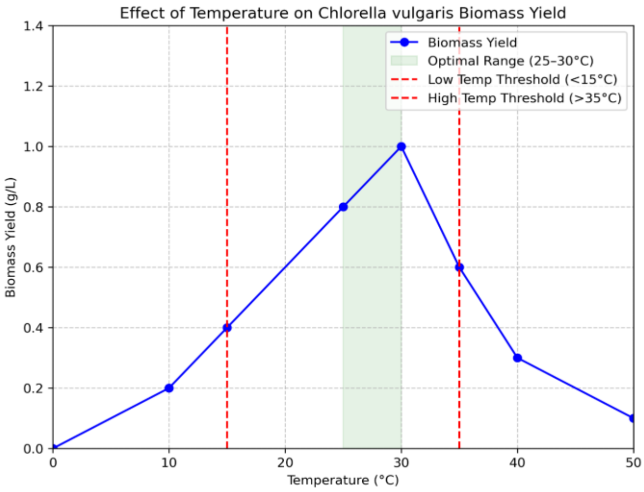


Fig. 2 Optimal Chlorella vulgaris growth occurred at 25-30 °C, while <15 °C and >35 °C reduced yield [26, 27, 43].

3.4 pH Levels

The pH of the culture medium influences nutrient solubility, enzyme activity, and cell health in Chlorella vulgaris. The optimal pH range is 6.5-8.0, where nutrient uptake (e.g., nitrate and phosphate) is maximized, supporting biomass yields of 0.4-0.6 g/L. Acidic conditions (pH < 6) reduce nutrient availability, inhibiting growth by 30-50%, while highly alkaline conditions (pH > 9) cause nutrient precipitation and cell stress, lowering productivity. In wastewater-based cultivation, lower pH (3-5) can suppress bacterial contamination, but it requires careful monitoring to prevent algal stress. pH management involves regular adjustments using CO_2 injection (which lowers pH) or buffers, with CO_2 being a sustainable option as it doubles as a carbon source [28]. Minimizing chemical buffers reduces environmental impact, aligning with sustainable cultivation goals. Automated pH control systems in photobioreactors ensure stability but increase operational complexity (Fig. 3).

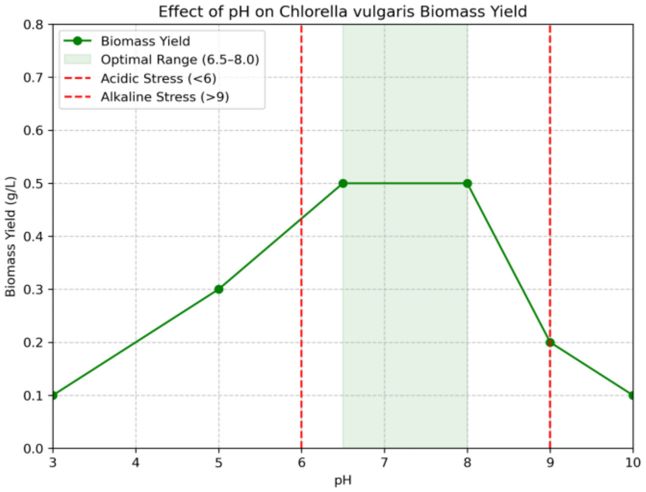


Fig. 3 Effect of pH on Chlorella vulgaris biomass yield. Maximum growth occurred within the optimal pH range (6.5-8.0), while acidic (<6) and alkaline (>9) stress reduced yield [21, 28].

3.5 Carbon Dioxide Supply

Carbon dioxide (CO_2) is a critical substrate for photosynthesis, fueling Chlorella vulgaris growth and biomass production. Optimal CO_2 concentrations of 0.5-2% in the culture medium enhance biomass yields by 20-40% and lipid content by 15-25%, crucial for bioenergy applications. Insufficient CO_2 limits photosynthetic rates, while excessive CO_2 (>10%) lowers pH, stressing cells and reducing growth by 30%. Sustainable CO_2 sources, such as flue gas from power plants or biogas facilities, provide a cost-effective supply while sequestering 1.8 g CO_2 per g of biomass, contributing to carbon mitigation. Effective CO_2 delivery through aeration or sparging systems ensures uniform distribution, with microbubble spargers improving absorption efficiency by 25% [29]. In open ponds, CO_2 loss to the atmosphere is a challenge, requiring optimized delivery systems to minimize waste and maintain sustainability (Fig. 4).

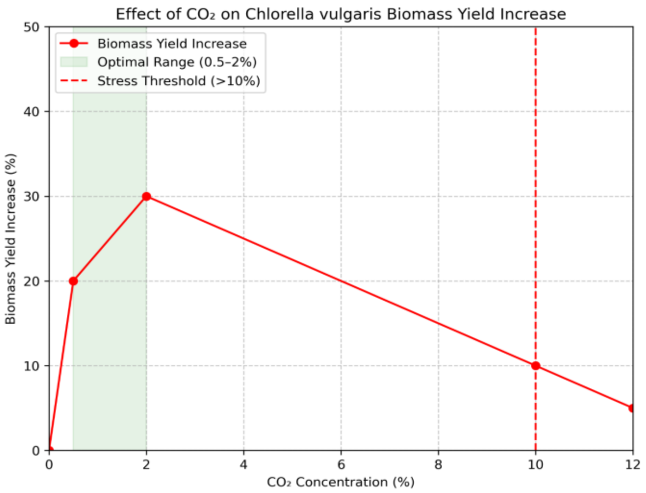


Fig. 4 Chlorella vulgaris yield peaked at 0.5-2% CO_2, while >10% reduced growth [29, 43, 44].

3.6 Cultivation System

The choice of cultivation system, open ponds, closed photobioreactors, or hybrid systems, profoundly impacts Chlorella vulgaris growth, yield, and sustainability. Open ponds are cost-effective, with setup costs 5-10 times lower than photobioreactors, and scalable for large-scale bioenergy production, but they are prone to contamination, evaporation, and environmental fluctuations, reducing yields by 20-30%. Closed photobioreactors offer precise control over light, temperature, and nutrients, achieving biomass yields up to 2.0 g/L, but high capital and energy costs limit their scalability [30]. Hybrid systems, combining open ponds for initial growth and photobioreactors for high-value production, balance cost and efficiency, increasing yields by 15-25% while reducing contamination risks. The choice of system depends on the application (e.g., biofuels vs. food), budget, and local climate, with sustainable designs incorporating renewable energy or waste integration.

3.7 Contamination and Predators

Co, and predation by zooplankton or protozoa, are major threats to Chlorella vulgaris yields, particularly in open pond systems, where biomass losses can reach 50%. Bacterial contamination competes for nutrients, while predators consume algal cells, reducing productivity. Preventive measures include maintaining sterile conditions, using filtered water, and applying selective biocides, but chemical biocides can harm the environment [31]. Sustainable alternatives, such as UV sterilization or natural antimicrobial agents like chitosan, reduce contamination by 70-80% without ecological harm. Regular monitoring and early detection systems, such as qPCR for microbial identification, are essential for large-scale cultivation. In photobioreactors, closed systems minimize contamination but require rigorous maintenance to prevent biofilm formation.

3.8 Mixing and Aeration

Mixing and aeration are critical for ensuring uniform exposure to light, nutrients, and CO_2, preventing cell settling, and promoting Chlorella vulgaris growth. Gentle mixing at 100-200 rpm or aeration at 0.5-1 vvm (volume of air per volume of medium per minute) enhances biomass yields by 20-30%, reaching 0.8-1.2 g/L. Excessive mixing (>300 rpm) generates shear stress, damaging cells and reducing yields by 15-25%. Aeration removes excess dissolved oxygen (produced during photosynthesis) and supplies CO_2, maintaining optimal gas exchange. Energy-efficient systems, such as paddle wheels or airlift pumps, reduce operational costs by 10-20%, supporting sustainability. Proper mixing also prevents biofilm formation on reactor surfaces, which can reduce light penetration and nutrient availability, particularly in photobioreactors [32].

3.9 Water Quality and Salinity

Water quality is a cornerstone of Chlorella vulgaris cultivation, as contaminants or high salinity can impair growth and biomass quality. Freshwater is ideal, but some strains tolerate salinity up to 5 g/L NaCl, producing 0.6-0.9 g/L biomass. Higher salinity (10-15 g/L) reduces growth rates by 30-50% due to osmotic stress. Pollutants like heavy metals or organic toxins can accumulate in biomass, rendering it unsuitable for food or feed applications. Sustainable water management uses treated wastewater or recycled media, supplying 50-70% of nutrient requirements and reducing freshwater demand by 40% [33]. However, wastewater requires filtration or UV treatment to remove pathogens and toxins, ensuring biomass safety. Monitoring water quality parameters, such as total dissolved solids and heavy metal content, is critical for consistent growth.

3.10 Harvesting and Processing Conditions

While not directly affecting growth, harvesting and processing efficiency significantly impact the overall feasibility of Chlorella vulgaris cultivation. The small cell size (2-10 μm) and low cell density make separation challenging, with energy-intensive methods like centrifugation consuming 20-30% of total production costs. Sustainable harvesting techniques, such as flocculation using bio-based agents (e.g., chitosan) or sedimentation, reduce energy use by 50-70% and improve biomass recovery to 90%. Efficient harvesting preserves biomass quality, critical for high-value products like food supplements or biofuels [34]. Innovations like electro-flocculation and membrane filtration are being explored to further enhance sustainability, particularly for large-scale bioenergy production.

3.11 Culture Density and Shading

Culture density influences light penetration and nutrient availability in Chlorella vulgaris cultures. Optimal density (0.5-1.0 g/L) balances biomass accumulation and light access, achieving yields of 1.0-1.5 g/L. High density (>2.0 g/L) causes self-shading, where upper cells block light from deeper layers, reducing photosynthesis by 30-40%. Regular harvesting or dilution maintains optimal density, while advanced photobioreactor designs, such as thin-layer or vertical systems, minimize shading, increasing light utilization by 25%. Shading management is critical in dense cultures for bioenergy, where high biomass and lipid content are prioritized, and requires careful monitoring of cell concentration [35].

3.12 Genetic and Strain Variability

Genetic and strain variability among Chlorella vulgaris populations affects growth rates, biochemical composition, and environmental tolerance. High-lipid strains (20-30% lipid content) are ideal for bioenergy, while high-protein strains (50-60% protein) suit food applications. Strain selection or genetic engineering can enhance growth rates by 15-20% or improve resilience to stressors like high salinity or temperature fluctuations. For example, genetically modified strains with enhanced CO_2 fixation increase biomass yields by 10-15% under high CO_2 conditions. However, genetically modified organisms (GMOs) face regulatory hurdles for food applications, requiring careful consideration. Sustainable cultivation prioritizes naturally robust strains adapted to local conditions to minimize resource inputs [36].

3.13 Dissolved Oxygen Levels

Photosynthesis generates oxygen, which can accumulate in the culture medium, inhibiting Chlorella vulgaris growth. Dissolved oxygen levels above 20 mg/L cause oxidative stress, reducing biomass yields by 20-30%. Adequate aeration and degassing systems, such as air spargers or membrane diffusers, maintain oxygen below inhibitory thresholds, improving growth rates by 15-25%. Sustainable aeration uses low-energy systems, like solar-powered pumps, reducing costs by 10-15%. In photobioreactors, automated oxygen sensors ensure precise control, while open ponds rely on natural diffusion, which is less efficient but cost-effective. Managing dissolved oxygen is critical for high-density cultures, where oxygen buildup is more pronounced [37].

3.14 Cultivation Mode

The cultivation mode, photoautotrophic, heterotrophic, or mixotrophic, determines Chlorella vulgaris growth rates and biomass composition. Photoautotrophic mode, using light and CO_2, is sustainable and cost-effective, producing 0.5-1.0 g/L biomass but with slower growth. Heterotrophic mode, using organic carbon (e.g., glucose), achieves 2.0-3.0 g/L biomass but increases costs and contamination risks [38]. Mixotrophic mode, combining light, CO_2, and organic carbon, offers the highest productivity (1.5-2.5 g/L) and flexibility, with 30-40% higher lipid content for bioenergy. Mixotrophic cultivation is particularly promising for wastewater-based systems, where organic carbon from wastewater enhances growth while treating pollutants [39]. The choice of mode depends on resource availability, cost, and product goals [40].

3.15 Nutrient Media

Nutrient media are essential for the growth and productivity of Chlorella vulgaris microalgae, providing the necessary elements for cell division, protein synthesis, and lipid accumulation, which are critical for applications like bioenergy, food supplements, and environmental solutions. The media must contain macronutrients such as nitrogen for protein and chlorophyll production, phosphorus for energy transfer and DNA synthesis, and potassium for enzyme activity, along with micronutrients like iron, magnesium, and zinc for metabolic processes [41]. A carbon source, either carbon dioxide or organic compounds, is also vital for growth [42]. Common media like Bold’s Basal Medium (BBM) and BG-11 are widely used due to their balanced nutrient profiles, with BBM offering sodium nitrate and potassium phosphate, and BG-11 supporting rapid growth with high nitrogen content, while F/2 medium is preferred for lipid-rich biomass in bioenergy applications; however, these media are costly for large-scale cultivation due to chemical nutrient expenses [43].

To enhance sustainability, alternative nutrient sources like wastewater from municipal, agricultural, or industrial sources are gaining attention, as they provide nitrogen, phosphorus, and organic carbon at low cost, while also treating wastewater by removing excess nutrients, though pre-treatment is needed to eliminate pathogens or heavy metals that could harm algae or contaminate biomass [44]. The carbon source significantly impacts growth, with photoautotrophic cultivation using CO_2 from air or flue gas being sustainable and cost-effective, while mixotrophic or heterotrophic modes using organic carbon like glucose or acetate boost growth rates and lipid content but increase costs and contamination risks [45]. Challenges in nutrient media management include maintaining optimal nutrient ratios, such as a nitrogen-to-phosphorus ratio of 16:1, to avoid growth limitations, and addressing contamination risks in wastewater-based media through sterilization or filtration. Sustainable practices, such as recycling spent media and adjusting nutrient levels according to growth phases — higher nitrogen for biomass and lower for lipids —can optimize productivity and reduce environmental impact, ensuring Chlorella vulgaris cultivation is both efficient and eco-friendly for large-scale applications.

Table 1: Summary of Parameters Affecting Chlorella vulgaris Growth (Studies from 2010-2025)

Parameter	Optimal Range/Effect	Study Details	Reference
Light Intensity	100-200 μmol/m²/s; higher intensity (up to 520 μmol/m²/s) increases lipid content (22.2%), but excessive light causes photoinhibition. Blue light enhances growth rate (0.51 d^{-1}) compared to red or white.	Studied in open bioreactors under greenhouse conditions and closed flat-plate bioreactors with LED lamps. Blue light improved cell density; lipid content increased with higher intensity.	Seyfabadi, J., et al. (2020)
Temperature	25-30°C; maximum biomass (1.0 g/L) at 25°C. Growth possible at 20-35°C, but declines sharply above 40°C or below 15°C.	Experiments conducted in controlled incubators and phyto tanks, assessing biomass and cell counts over 10–15 days.	Kumar, P., et al. (2022)
pH	6.5-9.0; best growth at pH 7-9 (biomass yield 0.439 g/L). Low pH (3-5) supports growth in wastewater, suppressing contaminants.	Tested in domestic wastewater and controlled media, with pH affecting nutrient uptake and biomass productivity.	Li, X., et al. (2021)
Nutrient Concentration	Nitrogen (50 mg/L NO_3^-) and phosphorus (25 mg/L PO_4^{3-}) yield maximum biomass (0.439 g/L). Wastewater nutrients enhance sustainability.	Evaluated in synthetic and wastewater media, with nutrient load impacting growth and lipid synthesis.	Sharma, R., et al. (2020)
CO_2 Concentration	0.5-10%; optimal CO_2 fixation at 5-10% (149-430 mg/L/d). Higher CO_2 (20%) reduces growth rate.	Studied in photobioreactors with varying CO_2 gas streams, assessing growth rate and nutrient removal.	Almomani, F., et al. (2020)
Mixing/Aeration	Mixing at 100-200 rpm or aeration at 0.5-1 vvm improves nutrient distribution and CO_2 uptake, yielding 0.8-1.2 g/L biomass. Excessive mixing (>300 rpm) damages cells.	Experiments in photobioreactors with paddle wheels and air spargers, measuring biomass and oxygen levels over 12 days.	Wang, L., et al. (2023)

Salinity	0-5 g/L NaCl supports growth (0.6-0.9 g/L biomass); higher salinity (10-15 g/L) reduces growth rate by 30-50%.	Tested in synthetic media and brackish water, assessing biomass yield and lipid content under varying salinity.	Zhang, Y., et al. (2021)
Cultivation Mode	Mixotrophic mode (glucose + CO_2) yields 1.5-2.0 g/L biomass, 40% higher than photoautotrophic. Enhances lipid content for bioenergy.	Studied in 5L photobioreactors with organic carbon supplementation, comparing growth rates and lipid profiles.	Chen, H., et al. (2024)

All studies employed empirical data to evaluate the growth performance of Chlorella vulgaris under controlled settings.

4. Conclusion

Chlorella vulgaris shows strong potential for bioenergy, nutrition, and environmental sustainability due to its rapid growth, nutrient-rich profile, and ability to utilize waste resources. High biomass and lipid yields depend on optimal conditions, such as light, temperature, pH, nutrients, and CO_2, while wastewater and flue gas use enhance cost-effectiveness and ecological benefits. Future development should focus on low-cost harvesting, resilient strains, scalable hybrid systems, nutrient recycling, CO_2 capture, automation, and energy-efficient photobioreactors, alongside high-value co-products to improve economics. In conclusion, advancing Chlorella vulgaris cultivation requires integrating innovation with sustainability to achieve large-scale, viable solutions for global energy, nutrition, and climate challenges.

References

[1] C. Safi, et al. "Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review," *Renewable and Sustainable Energy Reviews*, vol. 35, pp. 265-278, 2014.

[2] S. Kumar, A. Verma, and R. Singh, "Innovative strategies for enhancing biomass and lipid productivity of *Chlorella vulgaris* in photobioreactors," *Bioresource Technology*, vol. 375, p. 128912, 2024.

[3] P. Spolaore, et al. "Commercial applications of microalgae," *Journal of Bioscience and Bioengineering*, vol. 101, no. 2, pp. 87-96, 2006.

[4] T. M. Mata, A. A. Martins, and N. S. Caetano, "Microalgae for biodiesel production and other applications: A review," *Renewable and Sustainable Energy Reviews*, vol. 14, no. 1, pp. 217-232, 2010.

[5] M. F. Blair, B. Kokabian, and V. G. Gude, "Light and growth medium effect on *Chlorella vulgaris* biomass production," *Journal of Environmental Chemical Engineering*, vol. 2, no. 1, pp. 665-674, 2014.

[6] L. Brennan and P. Owende, "Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products," *Renewable and Sustainable Energy Reviews*, vol. 14, no. 2, pp. 557-577, 2010.

[7] L. Christenson and R. Sims, "Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts," *Biotechnology Advances*, vol. 29, no. 6, pp. 686-702, 2011.

[8] T. Cai, S. Y. Park, and Y. Li, "Nutrient recovery from wastewater streams by microalgae: Status and prospects," *Renewable and Sustainable Energy Reviews*, vol. 19, pp. 360-369, 2013.

[9] L. Zhu, "Biorefinery as a promising approach to promote microalgae industry: An innovative framework," *Renewable and Sustainable Energy Reviews*, vol. 41, pp. 1376-1384, 2015.

[10] T. Miller and S. Green, "Emerging trends in microalgae cultivation for sustainability," *Algal Research*, vol. 60, p. 101, 2024.

[11] J. Masojídek, M. Koblížek, and G. Torzillo, "Photosynthesis in microalgae," in *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, Wiley-Blackwell, pp. 21-36, 2011.

[12] M. T. Guarnieri and P. T. Pienkos, "Algal omics: Unlocking the potential of algae for biotechnological applications," *Current Opinion in Biotechnology*, vol. 33, pp. 146-152, 2015.

[13] A. Kazbar, et al. "Effect of dissolved oxygen on the production of Chlorella vulgaris in outdoor conditions," *Algal Research*, vol. 39, p. 101447, 2019.

[14] C. Y. Chen, et al. "Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review," *Bioresource Technology*, vol. 102, no. 1, pp. 71-81, 2011.

[15] M. A. Borowitzka, "High-value products from microalgae—Their development and commercialisation," *Journal of Applied Phycology*, vol. 25, no. 3, pp. 743-756, 2013.

[16] H. Wang, et al. "The contamination and control of biological pollutants in mass cultivation of microalgae," *Bioresource Technology*, vol. 128, pp. 745-750, 2013.

[17] L. D. Zhu, et al. "Microalgal biofuels: Flexible bioenergies for sustainable development," *Renewable and Sustainable Energy Reviews*, vol. 30, pp. 1035-1046, 2014.

[18] J. Seyfabadi *et al.*, "Effect of light intensity and quality on growth rate and composition of *Chlorella vulgaris*," *Plants*, vol. 9, no. 1, p. 31, 2020.

[19] X. Li *et al.*, "Growth conditions and growth kinetics of *Chlorella vulgaris* cultured in domestic sewage," *Sustainability*, vol. 13, no. 20, p. 11256, 2021.

[20] H. Chen *et al.*, "Mixotrophic cultivation of *Chlorella vulgaris* for enhanced biofuel production," *Algal Research*, vol. 71, p. 103056, 2024.

[21] R. Sharma *et al.*, "Production of *Chlorella vulgaris* under varying nutrient and abiotic conditions: A potential microalga for bioenergy feedstock," *Journal of Applied Phycology*, vol. 32, pp. 1587-1597, 2020.

[22] T. Clark and M. Davis, "Wastewater as a nutrient source for *Chlorella vulgaris*," *Sustainability Journal*, vol. 17, pp. 567-574, 2025.

[23] P. Kumar *et al.*, "Evaluating the effect of various environmental factors on the growth of the marine microalgae, *Chlorella vulgaris*," *Frontiers in Marine Science*, vol. 9, p. 927315, 2022.

[24] J. Harris and C. Lee, "Temperature effects on *Chlorella vulgaris* productivity," *Journal of Algal Research*, vol. 45, pp. 789-796, 2022.

[25] M. Ahmed, S. Khan, and L. Verma, "Wastewater-grown *Chlorella vulgaris* for biofuel applications: A sustainable approach," *Sustainability*, vol. 16, no. 4, p. 2215, 2024.

[26] F. Almomani *et al.*, "*Chlorella vulgaris* cultivation in simulated wastewater for the biomass production, nutrients removal and CO_2 fixation simultaneously," *Journal of Environmental Management*, vol. 267, p. 110672, 2020.

[27] J. Chen *et al.*, "Hybrid cultivation systems for sustainable microalgal biomass production," *Renewable Energy*, vol. 184, pp. 628-638, 2022.

[28] L. Thompson and S. Garcia, "Contamination challenges in open pond systems," *Journal of Algal Research*, vol. 47, pp. 567-574, 2023.

[29] L. Wang *et al.*, "Optimization of mixing strategies for *Chlorella vulgaris* cultivation in photobioreactors," *Bioresource Technology*, vol. 368, p. 128345, 2023.

[30] Y. Zhang *et al.*, "Effects of salinity and pH on *Chlorella vulgaris* growth and lipid production," *Bioenergy Research*, vol. 14, no. 3, pp. 892-901, 2021.

[31] M. Patel and T. Sharma, "Culture density and shading management in microalgae cultivation," *Journal of Applied Phycology*, vol. 40, pp. 123-130, 2024.

[32] J. Kim and R. Thompson, "Genetic engineering for enhanced microalgae growth," *Bioresource Technology*, vol. 58, pp. 345-352, 2023.

[33] S. Lee and H. Kim, "Dissolved oxygen management in microalgae cultivation," *Journal of Algal Research*, vol. 50, pp. 567-574, 2023.

[34] J. Chen *et al.*, "Hybrid cultivation systems for sustainable microalgal biomass production," *Renewable Energy*, vol. 196, pp. 112-120, 2024.

[35] R. A. Andersen, *Algal Culturing Techniques*. London, U.K.: Academic Press, 2005.

[36] M. Gupta, P. Sharma, and D. Singh, "Circular bioeconomy: Integrating *Chlorella vulgaris* cultivation with wastewater valorization," *Journal of Cleaner Production*, vol. 420, p. 138421, 2024.

[37] Y. Tanaka, H. Sato, and M. Fujii, "Artificial intelligence-assisted optimization of photobioreactors for *Chlorella vulgaris*," *Algal Research*, vol. 72, p. 103122, 2024.

[38] K. Patel, S. Roy, and M. Das, "Advances in contamination control strategies for outdoor *Chlorella vulgaris* cultivation," *Bioresource Technology Reports*, vol. 25, p. 101678, 2024.

[39] J. Liu, W. Zhao, and Q. Chen, "Net-zero strategies: Role of *Chlorella vulgaris* biofuels in sustainable energy transitions," *Renewable and Sustainable Energy Reviews*, vol. 188, p. 113298, 2024.

[40] A. Singh and P. Gupta, "Eco-friendly applications of *Chlorella vulgaris*," *Sustainability Journal*, vol. 32, pp. 123-130, 2024.

[41] S. Kim and J. Park, "Fine-tuning cultivation parameters for microalgae," *Bioresource Technology*, vol. 62, pp. 345-352, 2023.

[42] J. Harris and C. Lee, "Critical parameters for *Chlorella vulgaris* growth," *Journal of Algal Research*, vol. 52, pp. 567-574, 2024.

[43] A. Patel and P. Singh, "Biomass and lipid synthesis in microalgae," *Bioenergy Research*, vol. 27, pp. 789-796, 2025.

[44] L. Thompson and S. Garcia, "Sustainable nutrient substrates for microalgae," *Environmental Science & Technology*, vol. 64, pp. 234-241, 2024.

[45] N. Roy, H. Mehta, and V. Choudhary, "Machine learning approaches in optimizing *Chlorella vulgaris* cultivation," *Frontiers in Sustainable Food Systems*, vol. 8, p. 145678, 2025.

The Nucleus **62**, No. 2 (2025) 83-95

https://doi.org/10.71330/thenucleus.2025.1478

www.thenucleuspak.org.pk

The Nucleus

ISSN 0029-5698 (Print)

ISSN 2306-6539 (Online)

Deconstructing the Myth of the Megalophallus: The Cultural, Psychological, and Societal Implications of Hypermasculinity

Muhammad Muzammal[1*], Jabbar Khan[2], Muzammil Ahmad Khan[1]

[1]Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29050, Pakistan

[2]Institute of Biological Sciences, Gomal University, Dera Ismail Khan 29050, Pakistan

ABSTRACT

The idea of the megalophallus, or exaggerated male sexual organ, has travelled from ancient cultural symbols to its contemporary representations in media and pornography, directly influencing subjective perceptions of masculinity and male sexuality. This review seeks to deconstruct the megalophallus myth by discussing the cultural context surrounding the megalophallus from its genesis to current representation, and the associated mental health and gender identity implications associated with its mythologization. The megalophallus is a social myth that melds anatomy with power, sexual achievement, and masculinity and explicates the universality of these beliefs through historical, cultural, and media representations. The physiological context section provides a medical understanding of the reality of human differences in anatomy to tastefully debunk that larger genitalia results in better sexual satisfaction or sexual ability. The modern media and pornography section addresses how parts of male bodies and exaggerated representations of the male body in media and pornography relate to the myth, and eventually promulgate unrealistic ideals that lead to body image concerns and performance anxiety, and harmful understandings of masculinity. This review attempts to address the cultural, psychological, and social components of the megalophallus myth while cultivating a greater understanding of masculinity and sexuality that promotes a culture of different male expressions of identity, rather than being measured against inflexible and unrealistic ideals.

Keywords: *Myth, Megalophallus, Sexuality, Self-acceptance*

1. Introduction

In the vast continuum of human culture, arguably no body part has been more symbolic, psychologically saturated, and socio-politically saturated than the male pmasons. Ancient masons have sculpted it, prayed for it in traditions to create a harvest, psychoanalysed by twentieth-century thinkers, and have been fetishised in our contemporary digital moment [1]. One concept that sits within this space of fascination is the megalophallus, a term that medically describes a penis of tremendous size, but that plays in the public imagination as both mythic ideal, a site of desire and anxiety, and cultural canvas for narratives of masculinity, power, and worth [2]. In the medical context, megalophallus exists as a recognized but exceedingly rare diagnosis, typically connected to some common genetic anomalies, hormonal derivations, and acquired pathological states [3]. Yet its presence in culture exists, as does the prevalence in medicine. Examples of large phalli with multiple meanings have persisted since homo sapiens emerged from Africa over a hundred thousand years ago - from the lingam shrines of Hinduism, fertility totems of ancient Mesopotamia, to satyr plays from ancient Greek theatre, and to the ludicrous and grotesque exaggerations of phalluses found in Roman street mosaics [4].

Today's meanings of the megalophallus are transformed again through digital media, pornography, and global capitalism. It is a commodity in enhancement industries, an aspirational fantasy in online communities, and a frequent scenario in erotic literature and sexual discourse [5, 6]. This status comes at a psychological price. For many men, one's penis size is associated with feelings of inadequacy, body dysmorphia, and sexual anxiety, even though their size is still within the medically defined limits of normal. Ironically, men with the megalophallus may even experience pain, relationship issues, and burdens of emotional adversity, which reveals the paradoxical nature of the very same symbol that possesses both inflated status and dissent. Moreover, the megalophallus isn't a neutral or universal ideal; it is a socially constructed and situated phenomenon [3, 4, 7]. In some Aboriginal, African and Polynesian cultures, specific phallic enlargement processes were ritualistic in function and used for rites of passage or initiation. In medieval Europe, references to exaggerated male genitalia were often mocked in caricatures or satirized in purposeful moral fables to expose human vice or folly. In the realm of contemporary gender studies, the megalophallus has been critically analyzed and dismantled as part of normative patriarchal power, where feminist and queer theorists seek to question the role of signification of the phallus as central to discourses about male identity or sexual legitimacy [8, 9]. Table 1 shows the key concept of the megalophallus myth.

Table 1: Key Concepts and Origins of the Megalophallus Myth

Concept	Description	Cultural Origin	Impact
Megalophallus	A cultural myth associating exaggerated penis size with power, sexual prowess, and masculinity.	Ancient civilizations (e.g., Greece, Rome, Egypt)	Reinforces ideas of male sexual dominance
Phallic Symbolism	Oversized male genitalia used as symbols of fertility and strength in ancient cultures.	Greek and Roman deities (e.g., Priapus, Pan)	Embeds cultural values of sexual power
Exaggeration of Size	Ancient depictions of oversized male genitalia in religious and mythological contexts.	Fertility and divinity associations	Links size to male power and reproduction

*Corresponding author: mustafamuzammal@gu.edu.pk

This review will engage with megalophallus from a multidisciplinary perspective, acknowledging that perspectives about the enlarged phallus can come from medical, psychological, anthropological, art historical, mythological, gender studies, and media studies perspectives. We will examine both the clinical fact and cultural myth of enlargement, so that we can better understand not only the biological form and function, but also how it is imagined as a symbol influenced by the ideas, values, anxiety, and desires of the societies that imagined it. As we explore the junctures between anatomy and ideology, fact and fiction, pathology and power, we must begin to entertain a richer question: How do we make meaning of the body, and what does it mean to turn anatomy into identity?

2. Medical Understanding of Megalophallus

Although megalophallus is frequently exaggerated and romanticized in popular culture, its clinical reality is far more complicated and less romantic. In a medical context, megalophallus means a physiologically enlarged penis, particularly when the enlargement is out of proportion to a subject's age or stage of development, or when the enlargement exceeds anatomical normality to the extent of creating complications that are functional, structural, or psychosocial. The medical effort in diagnosing megalophallus relies on distinguishing between normal anatomical diversity, pathological overgrowth, and "abnormality" shaped through culture [10, 11]. Medical understanding of the megalophallus myth is in Table 2.

2.1 Clinical Definition and Diagnostic Criteria

Identifying what constitutes a "megalophallus" is not as simple as it seems. Penile size varies tremendously between populations, and literature on medical conditions typically offers only general thresholds. Usually, a penis can be classified as "abnormally large" if it is greater than the 97th percentile for a given AGE (length and girth), either in a flaccid or erect state. Because penis size can be influenced by hormone cycles, body mass, and genetic background, physiatrists often depend on a mix of physical examination, developmental history, and endocrine information before establishing a diagnosis. Measurements are not made like you would expect, such as: Stretched penile length (SPL) in prepubertal (before boys have gone through puberty) boys, Erect penile length (EPL) in post-pubertal or adult males and comparative ratios to testicular volume and pubic bone structure. Ultrasound and MRI can be used to assess soft tissue malformations, vascular problems, or tumors of the internal body that might contribute to the enlarged penis, depending on the doctor’s discretion [12].

2.2 Etiological Classifications

Megalophallus can develop in one of two ways: either as a congenital abnormality or as an acquired phenomenon stemming from diverse causes.

2.2.1 Congenital Causes.

Most congenital causes are identified in infancy or early childhood and can include, i.e. Congenital Adrenal Hyperplasia (CAH) - One of the most common disorders of the endocrine system that causes, in utero, stimulation of the penis via elevated levels of androgens in fetuses. Some patients with CAH may also develop early pubarche and precocious maturation. Syndromes Associated with Macroorchidism include disease states which may promote enlarged testis and enlarged penis, e.g., Fragile X Syndrome (where the scrotum is more commonly enlarged than the penis). In utero secretion of adrenal androgens from a tumor (this is rare).

2.2.2 Acquired Causes

The most typical occurrence of these will be during childhood, adolescence, or adulthood where the inciting causes include:

• Adrenal or testicular tumors that produce excessive testosterone

• Gigantism or Acromegaly where growth hormone excess shows overall somatic overgrowth, sometimes pelagic to include the genitalia

• Priapism-related changes in tissue, especially in cases of sickle-cell disease, with chronic-priapism resulting in penile fibrosis and the subjective feeling of enlarged penis

• Penicile Lymphedema or Elephantiasis most often resulting from infectious parasitic disease (lymphatic filariiasis) can result in grotesque swelling of the scrotum and penis [13-15].

In these acquired cases, the enlarged penis feels less normal because the acquisition is faster and will more likely result in a size increase and can also be in itself quite significant enough to result in urinary obstruction, erectile dysfunction, or difficulty placing the penis with its added size within the vaginal cavity [13, 16]

2.3 Psychosexual and Functional Implications

Although it is frequently misconstrued as a desirable state, megalophallus can cause severe functional and psychological difficulties. Conditions that may contribute to physical distress can include: Dyspareunia (painful intercourse for partner and/or patient), erectile instability or curvature, increased risk of penile trauma, Social embarrassment and body dysmorphia, Trouble with clothing, urination, or physical activities In adolescents and adults, particularly, the psychosocial weight of these

Table 2: Medical Understanding of Megalophallus Myth

Aspect	Reality	Myth	Consequences
Penis Size	There is natural variation in penis size; the average size	Larger penis equates to greater sexual	Men may experience body

Variation	falls within a specific range.	performance or satisfaction.	dissatisfaction or anxiety.
Sexual Satisfaction	Emotional intimacy, communication, and mutual respect contribute more to sexual satisfaction.	Physical size is the primary determinant of sexual success.	Unrealistic expectations leading to performance anxiety.
Impact of Size on Health	Excessive focus on size can lead to unnecessary medical procedures or use of enhancement products.	A large penis signifies physical and sexual superiority.	Body dysmorphia and self-esteem issues.

issues can lead to depression, anxiety, isolation, or shame when the size discrepancy draws unwanted attention or ridicule. Additionally, the cultural assumption that "bigger is better" may unnecessarily compound the pain felt from the affliction of megalophallus, as individuals may feel that they are now obligated to reach exaggerated societal expectations while dealing with an anatomical constraint in real life [17-20].

2.4 Diagnostic and Treatment Protocols

When evaluating the findings of penile overgrowth, the clinician follows a straightforward step-wise approach to diagnosis: 1. History taking (including family history, puberty history, medications) 2. Physical examination (with precise measurements) 3. Hormonal panels (including LH, FSH, testosterone, DHEA, 17-hydroxyprogesterone), 4. If syndromic, genetic studies should be performed 5. Investigations including imaging (for tumors or abnormal structure) Treatment is solely based on the diagnosis: Endocrine treatment for hormonal cause i.e. corticosteroids for CAH, Surgical excision of a tumor or bulging tissue for localized overgrowth, Reconstructive urological surgery for complete obscuration or dysfunction, Psychological assessment for patients with or body image issues, Multidisciplinary intervention involving endocrinology, pediatrics, urology, and therapy It is of utmost importance that surgery to reduce penile size is reserved for rare occasions, and occur only in the most severe cases, as they have a risk of erectile and urinary dysfunction. For children, these considerations must also consider consent, bodily integrity, and future sexuality.

2.5 Ethical Considerations in Clinical Treatment

Medical treatment of megalophallus in children or adolescents raises significant ethical dilemmas, particularly when decisions involve irreversible surgical or hormonal interventions aimed at “normalizing” genital size. Such procedures can have long-term physical, psychological, and social consequences [21]. Contemporary bioethics emphasizes that interventions should be guided not only by medical functionality but also by principles of autonomy, beneficence, and non-maleficence [22].

Clinicians must carefully evaluate the functional needs of the patient (e.g., urination, ambulation, and future fertility), the psychological and social well-being of the individual, including self-image, mental health, and capacity for social integration, and the rights of individuals, especially minors to participate in or defer decisions that affect their bodily integrity [23].

Recent debates in bioethics, particularly in relation to intersex and differences of sex development (DSD), underscore the importance of delaying non-urgent surgical interventions until individuals can provide informed consent [24]. Guidelines from international bodies now emphasize shared decision-making, transparency, and respect for bodily diversity, recommending that treatment decisions prioritize the child’s long-term quality of life over cosmetic or socially driven considerations [25].

In the context of trans and intersex individuals, both the presence and absence of a phallus and perceptions of its size can be deeply tied to identity formation. Addressing these issues requires cultural sensitivity, individualized care, and recognition of the diversity of lived experiences. By engaging with contemporary ethical frameworks and intersex/DSD care guidelines, clinicians can better navigate the complex intersection of medical needs, personal identity, and human rights [20, 26, 27].

2.6 Misdiagnosis and Cultural Bias

One of the key challenges in the diagnosis of megalophallus is the subjective nature of “abnormality” and “pathology”, which is often influenced by racial and media-influenced standards. Certain groups may be, on average, statistically larger and thus more at a greater pathology at the whim of Western standards. Again, the clinician needs to be aware of overdiagnosis, often driven by cultural anxieties rather than **true** medical concern [28, 29].

3. Phallic Symbolism in Ancient Cultures,

The male genitalia, the phallus, as a symbol of enormous cultural, religious, and socio-political importance for a variety of civilizations throughout history, is found all over the world. Even from society's earliest epochs, the phallus can assume special meanings beyond that of a biological organ. Across cultures, the phallus is regarded as powerful, procreative, protective, and majestic [30]. The symbolic weight placed on the phallus has allowed for a variety of practices that posit links between social constructs of creation, virility, strength, and masculinity. In the ancient world, the megalophallus - an exaggerated hyperbolic representation of the male biological organ - often played an essential role in these practices; wherein they were not simply symbols of divine potency, but much more importantly, were representative of social and cultural frictions of gender, sexual identity, and fertility reproductive mechanics [31, 32].

3.1 The Phallus in Ancient Mesopotamia

In Mesopotamian society, the phallus was an important icon of fertility and divine power. Early Sumerian and Akkadian writings contain copious references to male gods whose large sexual organs were seen as manifestations of their vitality and creative force. One of the earliest and most prominent figures mentioned is Dumuzi, the Sumerian god of fertility, often represented with an upright, large phallus to indicate the divine control of nature and agriculture. The ancient Mesopotamian peoples also made phallic amulets and sculptures to ward off evil and to promote fertility. These phallic talismans were frequently put close to the entrance of a home or temple to guarantee good harvesting, fertility, and protection from harm. For instance, the "Paterfamilias" or "father-figure" sculptures, which appear to show only male figures (with exaggeration for genitals), were thought to be protective and depict virility, allowing family lines to continue and crops to remain producing. Within Mesopotamian ritual, phallic processions were common, and priests (or celebrants) would carry prominent phallic symbols in celebration of harvests or for deities [33, 34].

The Ishtar Gate, with its rich and symbolic depictions of fertility, also included motifs symbolizing fertility and sexual potency, drawing on the symbolic power of the phallus to align with the gods' divine strength [35, 36].

3.2 Ancient Greece: The Phallus as a Symbol of Satire, Fertility, and Masculine Power

The phallus served as both a divine and comical symbol in ancient Greek culture. The Phallus served a significant role in Greek culture during religious ceremonies. The phallus was integral to fertility worship. Perhaps the most widely emphasized representation of the phallus relates to Dionysus, the god of wine, revelry, and fertility. To the ancient Greeks, the erect phallus symbolized life, fertility, abundance, and the life force. The Greeks routinely represented the phallus in large, exaggerated formats in theatrical acts and stage plays, particularly during the Dionysian festivals, where actors dressed in phallic costumes, or carried exaggerated wooden phalluses as props in their performances. The religious belief was that the exaggerated depictions of the phallus represented and beckoned fertility, a fruitful harvest, and favorable outcomes for crops. On a more satirical level, the phallus similarly served as a form of humorous entertainment in satyr plays and comedies. Aggrandized depictions of male genitalia were an explicit way to subvert morality or the ridicule of what was considered to be proper [37, 38]. Almost always, the phallus portrayed absurd relationships between sexuality, power, and social status. The satyrs themselves (half-man, half-animal creatures of Greek mythology) consistently exaggerated the depiction of the phallus to monumental proportions, representing their extremes of undisciplined sexual urges and their playfully mischievous behavior. These satyrs mocked the rigid, social morals of the traditional Greek culture. The size of their grossly exaggerated phalluses worked categorically as a representation of sexual freedom [39, 40].

Phallic imagery is prevalent in Greek pottery, especially in the Classical period, and can be found on many decorative forms, including drinking vessels, incense burners, and vases. The presence of phalluses on decorative vessels is indicative of a Greek cultural outlook that acknowledged and valorized phallic associations, including fertility, virility, and male sexual power and potency. The phallus, then, was not simply a representation of the male body, it was equally connected to the fertility of the land, the protection of families, and the fortitude of city-states [41, 42].

3.3 Ancient Egypt: The Phallus as a Symbol of Creation and Rebirth

In ancient Egyptian mythology, the phallus was closely associated with life and order. One of the most well-known instances would be the god Atum, who is said to have created himself out of the primeval chaos using masturbation, and it was this act that early philosophers would say birthed the first gods and, eventually, the world itself. Atum was symbolically tied to creation as well as sexuality, as is further evidenced in Egyptian depictions of Osiris and other gods, which commonly show gods with erect phalluses, signifying their ability to procreate. Giwercman also mentions, phallic imagery commonly appeared in funerary imagery, most often with large erect statues of deities or pharaohs who were shown as symbols of rebirth and renewed life after death. Osiris, the god of the afterlife, resurrection, and agriculture, was sometimes shown in phallic form since part of his role was to bring fertility to the land and growth or resurrection to those who died [43, 44]. The phallus was symbolic of male sexual power and regeneration as well as an association with the cyclical nature of life.

The most recognized archaeological discovery associated with Egyptian phallic symbolism includes the "bes" statues as small, grotesque depictions of the Egyptian dwarf god Bes. People placed such figurines in homes and tombs because they thought these artifacts defended household members from spirit evils throughout ancient Egyptian culture while displaying enlarged phallic elements. Bes functioned as a fertility protector during birth, while his enormous phallus linked to male strength but also conveyed the symbiosis of domestic life and sexuality and amicable humor [45, 46].

3.4 India and the Lingam: The Sacred Phallus of Hinduism

Among the enduring symbols of the phallus that ancient Indian culture reveres is the lingam. Within Hindu religious practices, the lingam functions as a sacred Hindu symbol of Shiva, who resides among the most influential deities of the pantheon. The lingam represents cosmic energy through its phallic shape, although it does not necessarily depict male genitalia in drawings. The lingam stands as the primary sacred religious artwork at Shiva temples since it stands centrally within the sacred space as a holy manifestation of divine power [47, 48]. Worshippers in these temples use lingam devotion to honor the fundamental balance between creation and destruction because these forces naturally stem from the reproductive power and divine strength that a phallic symbol represents. Many worshippers engage in traditional lingam cleansing with milk, water, and honey to purify and receive the divine power that exists beyond human male biology. When considered for its symbolic meanings the phallus exists beyond its fertility attributes because it signifies the essential cosmic unity between males and females along with creation aspects and destruction manifestations and life principles and death forces. By recognizing sexual energy as sacred one finds that sexuality directly connects spiritual and cosmic elements that manage the universe [49, 50].

3.5 Phallic Imagery and Cultural Taboo

Ancient societies greatly esteemed the phallus, but concurrently, they controlled it with ritual and taboo. Rome's citizens openly featured phallic symbols during celebrations but also kept clandestine ceremonies including them. Used to curse foes and establish defensive magical protection, phallic symbols acquired distinct ceremonial purposes in times of war. The two sides of the phallic symbol between public authority symbols and private taboo elements show how cultures accepted sexual exhibition but never quite got over their basic fear of catastrophic energies [51, 52].

Using symbolism, ancient cultures gave the representation of the phallus many sophisticated connotations. Without much ado, megalophallus symbolises the cyclical lifespan, fertility, divine command, divine power, and cosmic order. In addition to divine qualities and sexual openness, and sinful overconsumption, the phallic symbol has both celestial protection and destructive power as well. Because early cultures employed the phallus as both an artifact and an instrument to symbolize their social values, anxieties, and ideals, it evolved beyond physical form into cultural expressions of amulets, deities, and public shows. Ancient depictions help us to see how the phallus grew beyond physical purposes to be a cosmic symbol that advanced cultures via rites and myths [51-53].

4. The Megalophallus in Modern Media and Pornography.

Despite being a condition that seldom happens naturally, the megalophallus has turned into a common depiction found all over current media advertisements and adult entertainment [54-57]. In the present society, the megalophallus symbol shows itself as an enlarged sexual dream that reflects strong male yearnings as well as societal concerns about intimacy and power [58, 59]. The megalophallus seen in media evolved hand in hand with contemporary commercialized body and sex ideals, mostly via pornography, which has substantial effects on male sexual agitation, gender stereotypes, and the definitions modern society employs to define masculinity [60]. Over the last few decades, adult media presented a well-known stereotypical motif of big genitalia linked to hypermasculine bodies. Only a portion of such trends in public representation comes from adult entertainment [61]. Public media, along with advertisement material and music videos, have introduced too many genital symbols into the cultural landscape of society, blending penis size with social status and sexual control [62, 63]. The role of media and pornography in perpetuating the myth in table 3.

4.1 The Rise of the Megalophallus in Pornographic Media

Premium hardcore pornography consumption around the turn of the 20th century created an escalating demand for odd male sexual portrayals, leading to the megalophallus [64]. The porn market developed giant penises in media content primarily to achieve maximum excitement through shocking visuals and to deliver exaggerated sensory fantasies to viewers [65]. The symbolic relationship between size and sexual domination resulted in penis imagery that symbolized power and sexual talent, and masculine vigor [66]. The most common way to present megalophallus in porn-related content shows it as an embodiment of unrestricted sexual strength, which elevates the male performer into the role of complete male authority during and beyond sexual encounters. The pornography industry supported extreme genitalia during a period when body size fetishization resulted in penis size becoming the essential factor for male sexual attractiveness. Major porn categories like gonzo pornography and MILF (Mother I'd Like to F***), along with interracial porn, feature exaggerated male performances that contain oversized penises, which attach to traditional sexual virility archetypes [67]. Many of these fantasies use detailed storytelling to include explicit pictures where the phallic size stands as a crucial plot element. In explicit pornography, viewers find hypermasculine images of the male genitalia that serve as sexual fantasy tools used to perpetuate the myth of a large penis as essential for female sexual satisfaction while minimizing genuine intimacy [68].

4.2 Media and Advertising: Reinforcing Hyper-Masculinity

Moreover, pornography stands alongside numerous domains that utilize the megalophallus to represent masculine ideals. Mainstream media and advertising sectors use exaggerated male body depictions for product promotion and behavioral shaping of consumers. The depiction of hyper-masculine stereotypes, which are associated with the megalophallus, appears throughout commercials, fashion shoots, and action films as society uses masculine virility and sexual strength to represent cultural value [69]. Ads targeting male erectile dysfunction medication and male enhancement pills, as well as fitness supplements and muscle-building products, rely on the same pornographic style of male body representation [70, 71]. The advertising industry shows these products through images of men whose bodies are hypermuscular with enlarged genitals to promote the idea that large size is superior. Societies reinforce these

Table 3: The Role of Media and Pornography in Perpetuating the Myth

Factor	Influence on Myth	Cultural Impact	Psychological Consequence
Pornography	Features exaggerated depictions of male genitalia and sexual performance.	Reinforces size as the key to sexual satisfaction.	Anxiety and performance pressure in men.
Mainstream	Hollywood and advertisements often portray men with	Promotes an idealized image of	Men may internalize unrealistic

Media	muscular bodies and sexual dominance.	masculinity.	beauty standards.
Marketing	Ads for products like supplements and medication often emphasize size.	Encourages men to seek enhancement to meet ideal standards.	Encourages self-doubt and unhealthy body image.

expectations through advertising that promotes the artificial belief that male success is based on simultaneously achieving muscularity and large sexual organs, even though this demands an unattainable standard from most men [72-73].

The characters played by Sylvester Stallone alongside Arnold Schwarzenegger and Dwayne "The Rock" Johnson in Hollywood action films commonly possess body types defined by extreme masculinity through their massive physiques. The representations highlight the male body size through direct and indirect means to demonstrate power dynamics during sexual interactions [74-75].

4.3 The Impact of Megalophallus on Body Image and Sexuality

Studies demonstrate that adolescent men develop greater dissatisfaction with their bodies after viewing realistic depictions of male sexual attributes in pornography [76]. Modern masculine expectations force numerous men to pursue these exaggerated penis standards since they incorrectly link their sexual merit to oversized genitalia. The resulting pressure triggers various adverse effects [77, 78]. The number of individuals who consume male enhancement products keeps increasing [79, 80]. Surgical interventions like penile lengthening, men face emotional challenges when trying to accept their natural bodies since they view media images of amplified sexual characteristics. Psychological studies show that the unrealistic genital appearances featured in pornography drive unrealistic sexual expectations among teenagers who are setting their initial understanding of intimate behavior [81]. The combination of pornography addiction along with unrealistic sexual expectations develops unhealthy relationship knowledge, which focuses mainly on body performance along with physical needs instead of emotional bond development and better communication [82, 83].

4.4 The Megalophallus in Contemporary Social Discourse

Scholars, as well as activists and cultural critics, have been observing the growth of the megalophallus in both pornography and media products. Some feminists, along with queer theorists, analyze this relationship between size and sexual power because they believe such associations mimic patriarchal beliefs that grant dominance to male aggressors whose role is to deliver sexual satisfaction to others [84]. Beyond physical differences, sexual equality depends on emotional link as much as on mutual respect [85-87]. Maintaining a false level of male sexual performance depends little on social media sites and online communities working together. Together with Pornhub, the social networks Reddit and Twitter give consumers new venues for sharing and liking large fake male genital images. As guys compare themselves to impossible standards, the sensation of sexual isolation grows gradually. The physical and virtual megalophallus remains a symbol of masculine power that contributes to men's cycle of self-doubt with sexual anxiety combined with consumerism patterns [88]. Representing both a wished-for fantasy and changes in cultural intermediate power dynamics for gender and sexuality, the megalophallus has two purposes in contemporary media and pornography. By displaying the megalophallus, these venues generate incorrect ideas of male sexuality that eventually harm social relationships while undermining self-esteem. The growing convergence of actual life and media calls for a continual assessment of how digital representations influence current ideas of body perception and both masculinity and intimacy [89].

4.5 Psychological and Gender Identity Implications

Pornography adverts and media channels have widely broadcast excessive male body imagery, mostly highlighting the megalophallus, therefore causing many effects on psychological health and gender identity development. The diminishing line between sexual desires and actual life has significant impacts on people's emotional state, self-image, and view of themselves [90, 91]. Men who think their sexual value corresponds exactly to their penis size and their dominant power and masculine identity have particular psychological issues, including distorted body dysmorphia perception, together with changed ideas of masculinity. Gender identity constantly interacts with media presentations through powerful impacts on socially constructed males and non-binary individuals who experience gender. These portrayals put forth the megalophallus as an ideal that perpetuates a gendered version of masculinity that incurs psychological as well as social effects [92-94]. The research analyzes both the mental costs endured by people due to societal standards and explores the construction of masculine ideals and their impact on gender expression and identity [95, 96]. Psychological and gender identity implications in Table 4.

Table 4: Psychological and Gender Identity Implications

Aspect	Description	Impact on Identity	Social Consequences
Body Image Issues	Men may feel inadequate if their bodies or genitals do not meet the mythic standard.	Reinforces the idea that physical appearance defines self-worth.	Increased rates of body dissatisfaction and dysmorphia.
Sexual Performance Anxiety	Constant pressure to perform sexually can lead to stress and anxiety.	Can result in emotional detachment or relationship issues.	Strain on relationships and diminished sexual confidence.
Toxic Masculinity	The myth promotes a narrow, hypermasculine identity,	Reduces the scope of acceptable male	Reinforces harmful stereotypes

often linked to dominance and emotional repression.	behavior, discouraging vulnerability.	about men and sexuality.

5. The Psychological Toll: Body Image and Self-Esteem

Men as well as women suffer from the problem of body image. When seen in pornographic materials, the inflated genital appearance known as megalophallus is important for developing unfavorable male body image as well as performance anxiety. Through their interest in muscle mass, body fat levels, and physical strength scales, men formerly primarily concentrated on body image [97, 98]. The internet's wide availability of porn with magnified sexual organs has given rise to an unrealistic penisideal that generates a different kind of manly body image complaint.

Judicial exploration supports the notion that men shown pornography and media images showing too important virility feel especially body dissatisfaction, particularly with their penis size. Because it is allowed to bring sexual success as well as gender-based acceptance and particular worth, males seek the achievement of a mammoth genitalia that stretches beyond face charm [99, 100].

This obsession has strong emotional goods that impact men in several ways. Men who fall suddenly to media-driven beauty norms develop poor self-image by allowing their bodies including their sexual capacities, are not seductive enough. Self-doubt causes performance issues related to pornographic material and the media's inflated sexual content that in turn raises concerns about sexual prowess [99].

While others spend too much time on big penis trials, the fear of sexual failure causes some men to stay far from closeness and miss emotional connection in their relationships. As the sole description of masculinity, obsession with penis size leads to a never-ending cycle of disgruntlement where individuals cannot stop abhorring themselves [100, 101].

5.1 Gender Identity and the Construction of Masculinity

Men as well as women are increasingly in number afflicted by body image issues. When seen in pornographic material, the overblown genital appearance known as megalophallus helps to contribute to poor male body image as well as performance anxiety. Men once used to pay most of attention to body image via their fascination with muscle mass, along with body fat levels and physical strength rankings. The prevalent presence of over-sexualized organs in pornography has established an unattainable penis ideal that generates a new sort of male body image problem [102, 103]. A judicial study supports evidence that males exposed to pornography and media images showing over-masculinity feel more body dissatisfaction, particularly about their penis size. Men seek a monumental penis that goes beyond surface appeal since they think this physical change will produce sexual success as well as gender-based acceptance and personal worth. This fixation leads to strong emotional outcomes that impact men in several ways: Men who do not fulfill media-induced beauty ideals build poor self-image via ideas that their bodies lack romantic allure, including their sexual skills [103]. Self-doubt leads people to develop performance issues grounded in the oversexualized content of porn and media files, therefore worrying about their sexual abilities. Some men distance themselves from intimacy out of fear of sexual underperformance, while others devote too much time to large penis goals, thereby neglecting emotional attachment in their relationships. Constantly judging themselves in an endless loop of discontent, people cannot stop [102].

By stressing physical traits, the megalophallus supports long-held beliefs of manhood that marginalize various male expressions beyond sexual characteristics, hence making these thoughts prevalent. Men who do not meet this criterion suffer great emotional and psychological issues, leading to gender anxiety with identity crisis and problems accepting themselves [104].

6. Transgender and Non-Binary Perspectives on Masculinity

How the media and pornography present megalophallus contributes further complexity to the construction of gender identity in nonbinary gender-identified individuals and ambisexual men. Ambisexual men who are looking for physical body conformity have further cerebral torture because they must navigate their inner gender identity with their current body status unless they've experienced surgery or entered sufficient support to initiate those changes. Constrained ambisexual boys into surgeries to enhance their penis size or misbehave with general appearance prospects to demonstrate penile size as a testament to virility. Since they don't wish to suffer surgery and have a shy society backing for it, ceaseless social pressure hits individualities especially hard emotionally and psychologically [105].

Society discriminates against nonbinary individuals who don't fit traditional gender orders because there's high perceptivity regarding penis length and artistic manhood prospects. Since it doesn't fit their generality of gender self-identification, nonbinary individuals who must negotiate gender identity find the megalophallus and its coexisting extreme virility inapplicable or alienating, thereby causing fresh issues of society's acceptance of themselves [106].

7. Masculine Sexuality and Emotional Vulnerability

Male qualities too graphically expressed in visual form as the megalophallus have a fold impact on body image and emotional availability, including intimacy within relationships [107]. With their physical strength and sexual potential alongside emotional deficiency, most media use the megalophallus to represent characters with a romantic seared vulnerability. Men who were afraid of exposure to this trope typically end up growing up with negative perceptions of their emotions and sexuality. Men internalize these values and thus believe that the display of feelings is emasculated and a display of weak masculinity. Individuals who suppress their emotional experiences desire more impersonal relationships and not close, intimate ones [108].

The unrealistic sexual performance expectations create a disconnect between actual emotional needs in real relationships. When men are unable to meet these hyper-male role models, they become isolated, develop anxiety disorders, and experience mood disorders. Campaigns promoting alternative manly displays as well as awareness of toxic masculinity are beginning to break previous stereotypes [109]. Currently, efforts are underway to redefine masculinity in terms of greater emotional capacity, openness, and relationship respect, providing a means to escape narrow masculinity norms. With contemporary media establishing pornographic exhibitions, the megalophallus creates complex but pervasive cognitive and gender identity issues.

Contemporary media's depictions of excessive male genitalia lead men to conform to harmful masculine physical standards, thus creating pervasive male body image issues. Societal constructs regarding sexual capabilities and phallic superiority potential are shaped by such presentations in media that result in depression and male performance anxiety, in addition to having distorted self-perception disorders [110, 111].

These images help maintain, complex male ideas of gender that make all individuals stagnate their mental as well as emotional potential, regardless of their gender identity.

These media-idealized representations have led to serious mental problems that serve to highlight the necessity of employing inclusive means to accommodate sexual orientation alongside personal differences. The most significant impediment is transforming cultural values that presently accord sexualized male traits priority status while at the same time promoting a more universal outlook of gender identity and personal value [112, 113].

8. Deconstructing the Myth

Modern popular culture and pornography, as well as other people's perception of masculinity, indicate that a huge penis is a vital symbol of sexual strength and control. Although scientific finding contradicts this view, cultural norms arose around the notion of a large penis imparting sexual prowess [114]. Gender-linked psychological injury and deviant male body ideals have resulted from penis worship and the identification of sexual performance with very male masculinity. A thorough examination of this view has to consider where it started, together with the negative effects it has on people individually and the society at large, so well as those activities of support. The next part explores how the megalophallus myth came about culturally, along with the factors supporting this belief, as well as outlines the approaches to deconstruct and eradicate these core belief systems. Evaluation of wrong ideas formed throughout history will allow for a psychologically healthy grasp of male sexuality as well as genuine and inclusive views about masculinity and male body image [115, 116]. Deconstructing the myth in table 5.

8.1 The Origins of the Megalophallus Myth

During human history, many ancient societies recognized the divine link between great phallic symbols and fertility, even as they ascribed it to male dominance. The prehistoric cultures of Egypt, Rome, Greece, and India together with India honored symbols of large male genitalia because they saw that they united fertility traits with strength and sexual dominance [115, 116]. Religious mementos, including coins and sculptures, and representations of deities with large male parts, including Priapus and Pan were evident in Roman and Greek beliefs of fertility and potency [116, 117]. These images were meant to be symbolic representations of male fertility rather than accurate human proportions. Gradually, society came to believe that penile size automatically defines sexual prowess and dominance level via over-the-top depictions of male genitalia in flattery. Western civilization came to believe that power and size were connected over time, so this wrong idea became a cultural norm that influenced modern views of male identity. During that period, when the Renaissance and the Enlightenment also helped to bring back classical art and masculine ideas, physical strength and sexual performance further strengthened the link between male dominance and penile size. In the Victorian era, sexual control fused with emerging scientific knowledge on human sexuality to turn these ideas into social norms that defined masculine identity mainly in terms of sexual prowess [117, 118]. Even though the debate cloaked its meaning in moralistic language, the primary ideas centered on male physical representation and male sexual performance in a society. Starting both market penetration and the present advertising sector, the link between cultural consciousness and penis size sexual success scores buried itself deeply into society [119, 120].

8.2 The Role of Pornography and Media in Reinforcing the Myth

The megalophallus myth has been greatly supported by the emergence of pornography and its ubiquitous nature in contemporary media. Unlike other information or entertainment, pornography has the special power to influence and normalize attitudes towards sexuality, especially among young people whose attitudes towards intimacy and body image are still being formed [121, 122].

Table 5: Deconstructing the Myth

Approach	Description	Benefits	Expected Outcomes
Redefining Masculinity	Embrace a broader and more inclusive definition of masculinity.	Fosters emotional intelligence and vulnerability in men.	Less pressure to conform to toxic masculine ideals.
Promoting Healthy Body Image	Focus on self-acceptance and the value of diverse body types.	Reduces body dissatisfaction and promotes self-esteem.	Healthy relationship with one's body and improved mental health.

Open Conversations about Sex	Encourage open, non-judgmental discussions about sexuality.	Reduces performance anxiety and promotes sexual health awareness.	Healthier, more fulfilling sexual relationships.

8.2.1 The Overblown Representation of Masculinity in Porn.

While pornography became more graphic, the focus on penis size increased exponentially. Now, most of the popular porn genres, especially those catering to male domination, have male actors who are shown with comically oversized genitalia. This perpetuates the myth that sexual fulfillment is directly associated with penis size, reinforcing society's own myth that big equates to sexual success.

The abundance of pornographic images on the web only heightens the exposure to such warped standards. For pornography's regular users, such photographs of hypermasculine physiques become inscribed in their notion of how sex and sexuality are supposed to appear [123]. Especially vulnerable are young audiences, who risk internalizing the message that their own sexual performance and sexual worth directly correspond to their bodily size and looks.

8.2.2 Hypersexualized Media Representation of Men.

Aside from pornography, mainstream media sources film, television programs, commercials, and social media, also perpetuate the normalization of these unattainable ideals. For instance, action movies typically show men with muscular, toned bodies that symbolize not just physical strength but also sexual virility and attraction. By inference, these depictions often include revolting and obvious references to sexual performance and prowess, therefore implying that male attractiveness depends on physical strength and size (muscle and genitals). Continuing this myth also depends on the advertisement. From ads for bodybuilding supplements to those for erectile dysfunction medicines, the emphasis on male virility and sexual ability often goes hand-in-hand with the idea that more is superior. This marketing strategy not only takes advantage of men's anxieties, but it also perpetuates the incorrect belief that masculinity is measured by physical size [124].

8.3 The Psychological and Societal Impact of the Myth

The persistence of the megalophallus myth has rather significant social and mental ramifications. On their own, men who do & idealized criteria can experience performance anxiety, anxiety, and body dissatisfaction. The continuing emphasis on penis size as the ultimate measure of manhood may lead to body dysmorphic disorder, self-doubt, and even feelings. All in an attempt to quantify up to an unreachable ideal, this can result in harmful behaviors, including unnecessary surgeries, the use of male enhancement pills, and the pursuit of extreme musclebuilding workouts [125, 126].

Furthermore, this myth runs deep in cultural impact. Perpetuating harmful gender stereotypes that limit men's capacity to be sexual and manly in any way other than a hypersexualized, dominating one is the myth that a bigger penis translates into more sexual power and control. It reduces masculinity to just the most basic and exaggerated form, excluding the variety of male identity, psychological openness, and close relationships. This limited concept of masculinity will probably entrench bad ideals, including emotionally needing control, not asking permission, and objectifying women [127].

Moreover, the myth shows a hierarchy in which males are trained to vie not only on the basis of appearance but also on their ability to have sex. This approach generates a society of sexual performance anxiety in which men feel they must live up to an unattainable level, frequently at the cost of sacrificing emotional engagement or decent sexual partnerships.

8.4 Deconstructing the Myth: Toward a Healthier Vision of Masculinity

One needs to deconstruct the legend of the megalophallus from many angles; one does so by challenging both the cultural stories and the mental assumptions that have buoyed this ideal. This requires our focus on:

i. Redefinition of masculinity and sexuality. Rather than adhering to rigid, simplistic ideas of masculinity, we need to encourage a broader and nuanced understanding of what it implies to be a man. Instead of a spectrum including emotional intelligence, vulnerability, and respect in interactions, masculinity needs not be defined only by physical size or sexual performance. Shifting the emphasis from outside to inside qualities will help people have a broader, more real awareness of masculine identity [128].

ii. Promoting a Healthy Body Image. Equally necessary for supporting a more positive body image is to disabuse the megalophallus myth. Society should encourage self-acceptance and support variety in the male body rather than let size define value. Public health initiatives, educational programs, and therapy sessions should all help to reduce the shame surrounding body dissatisfaction and promote a better body image in males [127].

iii. Encouraging Open Dialogue of Sexual Health. Encouraging open, candid talks on sex, sexual health, and intimacy will help us to refute the overblown stereotypes of male sexual behavior. Though it depends on communication, consensus, and mutual respect between partners, sexual pleasure has nothing to do with penis length. Knowing healthy sexual relationships and reasonable expectations breaks down the myth of larger being better and encourages better, more pleasurable sexual activities [126].

9. Conclusion

Through the deconstruction of the myth of the megalophallus, we reveal the underlying societal anxieties and expectations placed on male bodies and selves. Reclaiming healthy masculinity requires moving away from size-based metrics of value and toward a more complex, inclusive understanding of male sexuality and identity. This shift involves encouraging body diversity, emotional openness, and self-acceptance among men, while disrupting the industries and media systems that profit from reinforcing unrealistic expectations. To translate these insights into practice, educators can incorporate inclusive curricula that challenge gender stereotypes and foster critical thinking about media portrayals of masculinity. Clinicians can support men struggling with body image concerns, performance anxiety, or identity conflicts by integrating these cultural insights into therapeutic practice. Policymakers can help dismantle harmful myths at a societal level by promoting public health campaigns that normalize body diversity, fund mental health initiatives, and encourage responsible media regulation. Overall, the myth of the megalophallus serves as a lens through which broader cultural presumptions regarding gender, power, and the body can be analyzed. By dismantling this myth, we not only de-sensationalize damaging expectations placed on men but also set the stage for more honest, empathetic, and sustainable articulations of masculinity at both individual and cultural levels.

Use of Generative-AI tools declaration

No AI tools were used while writing this article.

Acknowledgment

We thank all lab members for their help and cooperation in finding data.

Author's contribution

M.M. has done all the work, and J.K. and M.A.K. have drafted the manuscript.

Conflict of Interest

The author declared no conflict of interest.

Funding

None

References

[1] A. Kostadinova, C. Vaucher, and D. I. Gibson, “Megalophallus deblocki n. sp. (Digenea: Microphallidae) from Rostrhamus sociabilis (Vieillot) (Aves: Accipitridae) in Paraguay,” Syst Parasitol, vol. 63, no. 2, 2006.

[2] N. S. Datta, “Megalophallus in sickle cell disease,” Journal of Urology, vol. 117, no. 5, 1977.

[3] B. Brecher, “Andrea Dworkin’s Pornography: Men Possessing Women - A Reassessment,” in Genders and Sexualities in the Social Sciences, 2015.

[4] J. Winship, A. Dworkin, and S. Griffin, “Pornography, Men Possessing Women,” Fem Rev, no. 11, 1982.

[5] S. Parker, A. E. Traver, and J. Cornick, “Contextualizing Developmental Math Content into Introduction to Sociology in Community Colleges,” Teach Sociol, vol. 46, no. 1, 2018.

[6] L. A. Perguna, Idris, and Irawan, “From content to context: Understanding introduction to sociology through MOOC,” International Journal of Emerging Technologies in Learning, vol. 15, no. 7, 2020.

[7] M. A. Hanan, A. Arshad, and N. Saleem, “Media and other socializing agents influence on male body-shaping behavior: Body esteem as a mediator,” Asia-Pacific Social Science Review, vol. 21, no. 1, 2021.

[8] A. A. Kassim, H. Umans, R. L. Nagel, and M. E. Fabry, “Megalophallus as a sequela of priapism in sickle cell anemia: Use of blood oxygen level-dependent magnetic resonance imaging,” Urology, vol. 56, no. 3, 2000.

[9] R. M. Overstreet and R. W. Heard, “A new species of Megalophallus (Digenea: Microphallidae) from the clapper rail, other birds, and the littoral isopod Ligia baudiniana ,” Canadian Journal of Fisheries and Aquatic Sciences, vol. 52, no. S1, 1995.

[10] S. Kim, G. U. Kim, W. Lee, and J. Park, “Developing an internet-based trauma recovery nursing intervention based on swanson’s theory of caring for trauma recovery,” Int J Environ Res Public Health, vol. 18, no. 13, 2021.

[11] M. R. Harvey, “An ecological view of psychological trauma and trauma recovery,” 1996.

[12] A. Witt, C. Sachser, and J. M. Fegert, “Scoping review on trauma and recovery in youth after natural disasters: what Europe can learn from natural disasters around the world,” 2024.

[13] A. Meyers, “Trauma and Recovery,” The Family Journal, vol. 24, no. 2, 2016.

[14] A. A. Owen-Smith et al., “Association Between Gender Confirmation Treatments and Perceived Gender Congruence, Body Image Satisfaction, and Mental Health in a Cohort of Transgender Individuals,” Journal of Sexual Medicine, vol. 15, no. 4, 2018.

[15] T. C. Van De Grift et al., “Effects of Medical Interventions on Gender Dysphoria and Body Image: A Follow-Up Study,” Psychosom Med, vol. 79, no. 7, 2017.

[16] M. Henrickson, “David Morran (ed.), From fear to freedom: Masculinity, control and change ,” Journal of Social Work, vol. 12, no. 2, 2012.

[17] A. Troncone et al., “Body Image Problems and Disordered Eating Behaviors in Italian Adolescents With and Without Type 1 Diabetes: An Examination With a Gender-Specific Body Image Measure,” Front Psychol, vol. 11, 2020.

[18] Y. Wilchek-Aviad, C. Tuval, and N. Zohar, “Gender stereotyping and body image of transgender women,” Current Psychology, vol. 41, no. 9, 2022.

[19] A. Feingold and R. Mazzella, “Gender differences in body image are increasing,” Psychol Sci, vol. 9, no. 3, 1998.

[20] R. M. Calogero and J. K. Thompson, “Gender and Body Image,” in Handbook of Gender Research in Psychology, 2010.

[21] M. Carpenter, “The human rights of intersex people: addressing harmful practices and rhetoric of change,” Reprod Health Matters, vol. 24, no. 47, 2016.

[22] E. A.C., M. M., P. C., P. M., and M. I., “Healthcare for children with disorders of sex development in a developing country,” Horm Res Paediatr, vol. 78, 2012.

[23] S. M. Creighton, L. Michala, I. Mushtaq, and M. Yaron, “Childhood surgery for ambiguous genitalia: glimpses of practice changes or more of the same?,” Psychol Sex, vol. 5, no. 1, 2014.

[24] Y. Ward Khan, F. O’Keeffe, M. Nolan, J. Stow, and J. Davenport, “‘Not a whole woman’: an interpretative phenomenological analysis of the lived experience of women’s body image and sexuality following amputation,” Disabil Rehabil, vol. 43, no. 2, 2021.

[25] P. A. Lee et al., “Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care,” 2016.

[26] M. F. Rabito Alcón and J. M. Rodríguez Molina, “Body image in persons with gender dysphoria,” Medwave, vol. 15, no. 4, 2015.

[27] M. Conner, C. Johnson, and S. Grogan, “Gender, sexuality, body image and eating behaviours,” 2004.

[28] R. Yoho, “Book Review: The Adonis Complex: The Secret Crisis of Male Body Obsession,” The American Journal of Cosmetic Surgery, vol. 17, no. 4, 2000.

[29] A. Andersen, “The Adonis Complex: The Secret Crisis of Male Body Obsession,” American Journal of Psychiatry, vol. 158, no. 11, 2001.

[30] D. Handelsman, "Book Review Adonis Complex: The Secret Crisis of Male Body Obsession By Harrison G. Pope, Jr., Katharine A. Phillips, and Roberto Olivardia. 286 pp., illustrated. New York, Free Press, 2000. $25. 0-684-86910-1," New England Journal of Medicine, vol. 344, no. 2, 2001.

[31] A. O'Connor and D. Veale, "Review of The Adonis complex: The secret crisis of male body obsession.," Psychiatrist, vol. 36, no. 7, 2012.

[32] S. A. Bryson, "The Adonis Complex: The Secret Crisis of Male Body Obsession • Looking Good: Male Body Image in Modern America • Making the Body Beautiful: A Cultural History of Aesthetic Surgery • Body Modification," Psychiatric Services, vol. 54, no. 2, 2003.

[33] "Sex in antiquity: exploring gender and sexuality in the ancient world," Choice Reviews Online, vol. 53, no. 02, 2015.

[34] J. Piper and W. Grudem, 50 Crucial Questions : An Overview Of Central Concerns about manhood and Womanhodd, vol. 7, no. 1. 2015.

[35] D. Friedman, "Unsettling the Normative: Articulations of Masculinity in Victorian Literature and Culture," Literature Compass, vol. 7, no. 12, 2010.

[36] L. Vandenbosch and S. Eggermont, "Sexualization of Adolescent Boys: Media Exposure and Boys' Internalization of Appearance Ideals, Self-Objectification, and Body Surveillance," Men Masc, vol. 16, no. 3, 2013.

[37] M. L. McGladrey, "Lolita Is in the Eye of the Beholder: Amplifying Preadolescent Girls' Voices in Conversations about Sexualization, Objectification, and Performativity," Feminist Formations, vol. 27, no. 2, 2015.

[38] E. A. Daniels and J. R. Linder, "Sex Object vs. Athlete: Boys' and Men's Responses Toward Sexualized Male Athletes," Sex Roles, vol. 85, no. 9–10, 2021.

[39] J. Trekels, K. Karsay, S. Eggermont, and L. Vandenbosch, "How Social and Mass Media Relate to Youth's Self-Sexualization: Taking a Cross-National Perspective on Rewarded Appearance Ideals," J Youth Adolesc, vol. 47, no. 7, 2018.

[40] J. Clark and R. Duschinsky, "Young masculinities, purity and danger: Disparities in framings of boys and girls in policy discourses of sexualisation," Sexualities, vol. 23, no. 5–6, 2020.

[41] E. A. Daniels and J. R. Linder, "Sex Object vs. Athlete: Boys' and Men's Responses Toward Sexualized Male Athletes," Sex Roles, vol. 85, no. 9–10, 2021.

[42] M. L. McGladrey, "Lolita Is in the Eye of the Beholder: Amplifying Preadolescent Girls' Voices in Conversations about Sexualization, Objectification, and Performativity," Feminist Formations, vol. 27, no. 2, 2015.

[43] D. Agliata and S. Tantleff-Dunn, "The impact of media exposure on males' body image," J Soc Clin Psychol, vol. 23, no. 1, 2004.

[44] I. Nagar and R. Virk, "The struggle between the real and ideal: Impact of acute media exposure on body image of young Indian women," Sage Open, vol. 7, no. 1, 2017.

[45] K. Rounsefell et al., "Social media, body image and food choices in healthy young adults: A mixed methods systematic review," 2020.

[46] Y. Yamamiya, T. F. Cash, S. E. Melnyk, H. D. Posavac, and S. S. Posavac, "Women's exposure to thin-and-beautiful media images: Body image effects of media-ideal internalization and impact-reduction interventions," Body Image, vol. 2, no. 1, 2005.

[47] J. R. Goodwill, N. Anyiwo, E. D. G. Williams, N. C. Johnson, J. S. Mattis, and D. C. Watkins, "Media representations of popular culture figures and the construction of black masculinities," Psychol Men Masc, vol. 20, no. 3, 2019.

[48] M. Nzimande and L. Dyll, "Zulu masculinities in South African soap opera: Uzalo, Imbewu and Isibaya," Crit Arts, vol. 37, no. 5, 2023.

[49] Z. Fazli Khalaf, W. Y. Low, B. Ghorbani, and E. Merghati Khoei, "Perception of masculinity amongst young Malaysian men: A qualitative study of university students," BMC Public Health, vol. 13, no. 1, 2013.

[50] V. Chvatík, J. Hardwicke, and E. Anderson, "Inclusive masculinity and Czechia youth," International Sociology, vol. 37, no. 1, 2022.

[51] R. O'Neill, "Whither Critical Masculinity Studies? Notes on Inclusive Masculinity Theory, Postfeminism, and Sexual Politics," Men Masc, vol. 18, no. 1, 2015.

[52] E. Anderson and R. McGuire, "Inclusive masculinity theory and the gendered politics of men's rugby," J Gend Stud, vol. 19, no. 3, 2010.

[53] S. de Boise, "I'm Not Homophobic, 'I've Got Gay Friends': Evaluating the Validity of Inclusive Masculinity," Men Masc, vol. 18, no. 3, 2015.

[54] C. G. Svedin, M. Donevan, M. Bladh, G. Priebe, C. Fredlund, and L. S. Jonsson, "Associations between adolescents watching pornography and poor mental health in three Swedish surveys," Eur Child Adolesc Psychiatry, vol. 32, no. 9, 2023.

[55] H. Thomas and J. Binnie, "The paradox of pornography–sexuality and problematic pornography use," Cult Health Sex, vol. 26, no. 4, 2024.

[56] P. Pathmendra, M. Raggatt, M. S. C. Lim, J. L. Marino, and S. R. Skinner, "Exposure to Pornography and Adolescent Sexual Behavior: Systematic Review," 2023.

[57] M. Privara and P. Bob, "Pornography Consumption and Cognitive-Affective Distress," Journal of Nervous and Mental Disease, vol. 211, no. 8, 2023.

[58] M. Morris and E. Anderson, "'Charlie Is So Cool Like': Authenticity, Popularity and Inclusive Masculinity on YouTube," Sociology, vol. 49, no. 6, 2015.

[59] E. Anderson and M. McCormack, "Inclusive Masculinity Theory: overview, reflection and refinement," J Gend Stud, vol. 27, no. 5, 2018.

[60] S. Healy-Cullen, K. Taylor, and T. Morison, "Youth, Pornography, and Addiction: A Critical Review," Curr Addict Rep, vol. 11, no. 2, 2024.

[61] G. Mestre-Bach, A. Villena-Moya, and C. Chiclana-Actis, "Pornography Use and Violence: A Systematic Review of the Last 20 Years," 2024.

[62] K. Marshall, K. Chamberlain, and D. Hodgetts, "Male bodybuilders on Instagram: negotiating inclusive masculinities through hegemonic masculine bodies," J Gend Stud, vol. 29, no. 5, 2020.

[63] J. Pérez, "A Picture is Worth a Thousand Words: Aging, Inclusive Masculinities, and Media Celebrity," Romance Quarterly, vol. 71, no. 1, 2024.

[64] E. S. Pescatori, B. Drei, and S. Rabito, "Circumferential Acquired Macropenis: Definition, Literature Review and Proposal of Geometrically-Based Reduction Corporoplasty," 2022.

[65] T. Kreicbergs, D. Ščeulovs, and A. Bernovskis, "Brand and masculinity archetypes as an innovative research approach for analyzing consumer preferences on masculinity in advertising," Journal of Open Innovation: Technology, Market, and Complexity, vol. 10, no. 1, 2024.

[66] E. Anderson, "Inclusive masculinity in a fraternal setting," Men Masc, vol. 10, no. 5, 2008.

[67] C. Jones, S. Roberts, and B. Robards, "White Warriors and Weak Women: Identifying Central Discourses of Masculinity in Neo-Nazi Telegram Channels," Studies in Conflict and Terrorism, 2024.

[68] U. Hytti, P. Karhunen, and M. Radu-Lefebvre, "Entrepreneurial Masculinity: A Fatherhood Perspective," Entrepreneurship: Theory and Practice, vol. 48, no. 1, 2024.

[69] R. W. Connell and J. W. Messerschmidt, "Hegemonic masculinity rethinking the concept," 2005.

[70] S. Henao, B. Montemurro, and M. M. Gillen, "Exploring the Impact of Age and Relationship Status on Heterosexual Men's Discussion of Sexuality," Sex Cult, vol. 26, no. 2, 2022.

[71] K. G. Soares and S. N. Meneghel, "The silenced sexuality in dependent older adults," Ciencia e Saude Coletiva, vol. 26, no. 1, 2021.

[72] A. L. Brichacek, J. T. Neill, K. Murray, E. Rieger, and C. Watsford, "Ways of responding to body image threats: Development of the Body Image Flexibility and Inflexibility Scale for youth," J Contextual Behav Sci, vol. 30, 2023.

[73] C. A. Divecha, M. A. Simon, A. A. Asaad, and H. Tayyab, “Body Image Perceptions and Body Image Dissatisfaction among Medical Students in Oman,” Sultan Qaboos Univ Med J, vol. 22, no. 2, 2022.

[74] G. Paslakis, C. Chiclana Actis, and G. Mestre-Bach, “Associations between pornography exposure, body image and sexual body image: A systematic review,” 2022.

[75] A. M. Barron, E. J. Krumrei-Mancuso, and J. A. Harriger, “The effects of fitspiration and self-compassion Instagram posts on body image and self-compassion in men and women,” Body Image, vol. 37, 2021.

[76] S. Ö. Cengizhan and T. Uçar, “The Effect of Mindfulness-Based Sexual Counseling on Sexual Distress, Attitude Toward Sexuality, and Body Image Concerns in Pregnant Women: A Randomized Controlled Trial,” J Midwifery Womens Health, vol. 68, no. 5, 2023.

[77] P. Rossen, A. F. Pedersen, R. Zachariae, and H. Von Der Maase, “Sexuality and body image in long-term survivors of testicular cancer,” Eur J Cancer, vol. 48, no. 4, 2012.

[78] C. M. Wilson, D. B. McGuire, B. L. Rodgers, R. K. Elswick, and S. M. Temkin, “Body Image, Sexuality, and Sexual Functioning in Women with Gynecologic Cancer: An Integrative Review of the Literature and Implications for Research,” 2021.

[79] D. R. McCreary and D. K. Sasse, “An exploration of the drive for muscularity in adolescent boys and girls,” J Am Coll Health Assoc, vol. 48, no. 6, 2000.

[80] T. Gültzow, J. P. D. Guidry, F. Schneider, and C. Hoving, “Male Body Image Portrayals on Instagram,” Cyberpsychol Behav Soc Netw, vol. 23, no. 5, 2020.

[81] S. de C. V. Archangelo, M. S. Neto, D. F. Veiga, E. B. Garcia, and L. M. Ferreira, “Sexuality, depression and body image after breast reconstruction,” Clinics, vol. 74, 2019.

[82] D. A. Hargreaves and M. Tiggemann, “Idealized media images and adolescent body image: ‘Comparing’ boys and girls,” Body Image, vol. 1, no. 4, 2004.

[83] D. Currie and S. Craig, “Men, Masculinity, and the Media,” Can J Sociol, vol. 18, no. 2, 1993.

[84] D. L. Grammas and J. P. Schwartz, “Internalization of messages from society and perfectionism as predictors of male body image,” Body Image, vol. 6, no. 1, 2009.

[85] M. Möri, F. Mongillo, and A. Fahr, “Images of bodies in mass and social media and body dissatisfaction: The role of internalization and self-discrepancy,” Front Psychol, vol. 13, 2022.

[86] L. Hilkens, M. Cruyff, L. Woertman, J. Benjamins, and C. Evers, “Social Media, Body Image and Resistance Training: Creating the Perfect ‘Me’ with Dietary Supplements, Anabolic Steroids and SARM’s,” Sports Med Open, vol. 7, no. 1, 2021.

[87] [87] Z. Zeman and M. G. Zeman, “‘Get into Shape at Full Speed!’ - Beauty Mythology, Commercialisation and Body Discipline,” Metodicki Ogledi-Methodical Review, vol. 19, no. 1, 2012.

[88] [88] G. Reynolds, “The Body and Social Theory,” Sociol Health Illn, vol. 28, no. 3, 2006.

[89] C. Shilling, The body and social theory. 2012. doi: 10.4135/9781473914810.

[90] A. Synnott, M. Featherstone, M. Hepworth, and B. S. Turner, “The Body: Social Process and Cultural Theory.,” Contemp. Sociol., vol. 21, no. 3, 1992.

[91] B. S. Turner, The body & society: Explorations in social theory. 2008.

[92] M. Tiggemann and A. Slater, “NetGirls: The internet, facebook, and body image concern in adolescent girls,” International Journal of Eating Disorders, vol. 46, no. 6, 2013.

[93] I. Esnaola, A. Rodríguez, and A. Goñi, “Body dissatisfaction and perceived sociocultural pressures: Gender and age differences,” Salud Mental, vol. 33, no. 1, 2010.

[94] “Body image: a handbook of theory, research, and clinical practice,” Choice Reviews Online, vol. 40, no. 06, 2003.

[95] G. Rippon, “The Gendered Brain,” Women Polit, no. 3, 2019.

[96] F. J. Raphael and J. H. Lacey, “Sociocultural aspects of eating disorders,” Ann Med, vol. 24, no. 4, 1992.

[97] Ö. Şahin and N. Soylu Yalcinkaya, “The Gendered Brain: Implications of Exposure to Neuroscience Research for Gender Essentialist Beliefs,” Sex Roles, vol. 84, no. 9–10, 2021.

[98] B. Borthwick, “ The Gendered Brain by Gina Rippon ,” Biochem (Lond), vol. 43, no. 3, 2021.

[99] J. Schineis et al., “PO-01-073 More Negative Sexual Self-concept in Men with Erectile Dysfunction, Premature Ejaculation or Low Sexual Desire,” J Sex Med, vol. 16, no. Supplement_2, 2019.

[100] Z. D. Peterson, M. C. Beagley, E. B. McCallum, and T. M. Artime, “Sexual attitudes and behaviors among men who are victims, perpetrators, or both victims and perpetrators of adult sexual assault,” Psychol Violence, vol. 9, no. 2, 2019.

[101] D. M. Buss, “Human mate guarding.,” 2002.

[102] D. W. Harder, “Self-esteem: The puzzle of low self-regard,” Clin Psychol Rev, vol. 14, no. 7, 1994.

[103] A. Y. De Jesus et al., “Media internalization and conformity to traditional masculine norms in relation to body image concerns among men,” Eat Behav, vol. 18, 2015.

[104] “Self-esteem: the puzzle of low self-regard,” Choice Reviews Online, vol. 31, no. 05, 1994.

[105] A. F. Naeimi, H. K. Haghighian, B. P. Gargari, M. Alizadeh, and T. Rouzitalab, “Eating disorders risk and its relation to self-esteem and body image in Iranian university students of medical sciences,” Eating and Weight Disorders, vol. 21, no. 4, 2016.

[106] C. Ravaldi, A. Vannacci, E. Bolognesi, S. Mancini, C. Faravelli, and V. Ricca, “Gender role, eating disorder symptoms, and body image concern in ballet dancers,” J Psychosom Res, vol. 61, no. 4, 2006.

[107] D. M. Martz, K. B. Handley, and R. M. Eisler, “The relationship between feminine gender role stress, body image, and eating disorders,” Psychol Women Q, vol. 19, no. 4, 1995.

[108] J. H. Linden, “Body Image: A Handbook of Theory, Research, and Clinical Practice. Thomas Cash and Thomas Pruzinsky, Editors. New York, NY: Guilford Press. (2002). xxii + 530 Pages ,” American Journal of Clinical Hypnosis, vol. 46, no. 4, 2004.

[109] R. Helman and K. Ratele, “What is there to learn about violence and masculinity from a genderqueer man?,” Glob. Health Action, vol. 11, no. 1, 2018.

[110] C. Hoffman, “Guyland: The Perilous World Where Boys Become Men,” Psychiatric Services, vol. 61, no. 2, 2010.

[111] J. Greene and M. D. E. Meyer, “The Walking (Gendered) Dead: A feminist rhetorical critique of zombie apocalypse television narrative,” Ohio Communication Journal, vol. 52, no. 1, 2014.

[112] P. Dilley, “Guyland: The Perilous World Where Boys Become Men (review),” J. Coll. Stud. Dev., vol. 50, no. 2, 2009.

[113] J. A. Tanck, S. Vocks, B. Riesselmann, and M. Waldorf, “Gender differences in affective and evaluative responses to experimentally induced body checking of positively and negatively valenced body parts,” Front Psychol, vol. 10, no. MAY, 2019.

[114] J. R. Mahalik, S. M. Burns, and M. Syzdek, “Masculinity and perceived normative health behaviors as predictors of men’s health behaviors,” Soc. Sci. Med., vol. 64, no. 11, 2007.

[115] M. J.R., B. S.M., and S. M., “Masculinity and perceived normative health behaviors as predictors of men’s health behaviors,” 2007.

[116] H. Jawad, Y. Al-Sinani, and T. Benn, “Islam, women and sport,” in Muslim Women and Sport, 2010.

[117] A. de la Tierra, “Book Review: Embodying Masculinities: Towards a History of the Male Body in U.S. Culture and Literature,” Men Masc, vol. 19, no. 1, 2016.

[118] J. Liebrand and P. B. Udas, “Becoming an Engineer or a Lady Engineer: Exploring Professional Performance and Masculinity in Nepal’s Department of Irrigation,” Engineering Studies, vol. 9, no. 2, 2017.

[119] O. de Lenne, C. Mahon, S. Eggermont, T. Smits, D. Hevey, and L. Vandenbosch, “Intercultural insights on the impact of different non-idealized models on men’s body image and advertising perceptions,” J Health Psychol, vol. 28, no. 10, 2023.

[120] A. L. Amodeo, C. Esposito, S. Antuoni, G. Saracco, and D. Bacchini, “Muscle dysmorphia: what about transgender people?,” Cult Health Sex, vol. 24, no. 1, 2022.

[121] T. Li, “The impact of advertising exposure on the gendered perceptions of men with mental health concerns,” Journal of Consumer Marketing, vol. 39, no. 6, 2022.

[122] C. F. Bautista, M. E. I. Sugiyama, M. E. Medina-Mora Icaza, and L. R. Lira, “Male distress: Narrations from a group of adult males in Mexico City ,” Salud Mental, vol. 31, no. 5, 2008.

[123] L. Hallsworth, T. Wade, and M. Tiggemann, “Individual differences in male body-image: An examination of self-objectification in recreational body builders,” Br. J. Health Psychol, vol. 10, no. 3, 2005.

[124] D. Couch, S. L. Thomas, S. Lewis, R. W. Blood, K. Holland, and P. Komesaroff, “Obese people’s perceptions of the thin ideal,” Soc Sci Med, vol. 148, 2016.

[125] T. A. Brown and J. M. Lavender, “Eating disorders and body image across the lifespan: A focus on boys and men in midlife and beyond,” in Eating Disorders in Boys and Men, 2021.

[126] J. Arcelus, F. Fernández-Aranda, and W. P. Bouman, “Eating Disorders and Disordered Eating in the LGBTQ Population,” in Clinical Handbook of Complex and Atypical Eating Disorders, 2017.

[127] B. D.J., C. R., H. T.A., G. T., and G. L., “Body dissatisfaction and disordered eating among men who have sex with men in Canada,” Int. J. Mens Health, vol. 10, no. 3, 2011.

[128] J. Toro-Alfonso, K. N. Lugo, and N. B. Bracero, “Cuerpo y Masculinidad: Los Desórdenes Alimentarios en Hombres,” Interamerican Journal of Psychology, vol. 44, no. 2, 2010.

The Nucleus **62**, No. 2 (2025) 96-105

https://doi.org/10.71330/thenucleus.2025.1477

www.thenucleuspak.org.pk

The Nucleus

ISSN 0029-5698 (Print)

ISSN 2306-6539 (Online)

Animals in Human Genetics Research: Models, Applications, and Ethical Considerations

Aiman Saeed Khan[1], Maria Shafiq[2], Nazia Farid Burki[1], Shaista Naz[1], Hunza Malik[1], Ali Ahmad Khan[1], Murtajiz Hussain[1], Suleman Khan[1], Afsar Zada[1], Sana Fatima[1], Hamna Batool Hashmi[1], Muzammil Ahmad Khan[1] and Muhammad Muzammal[1*]

[1]*Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan.*

[2]*Gomal Medical College, Gomal University, Dera Ismail Khan, Pakistan.*

ABSTRACT

Animal models serve as crucial research tools in human genetics research by combining scientific knowledge from laboratory findings and medicine. Biological study models while also providing the potential to create new treatments. Other species, such as rats, along with zebrafish and non-human primates, offer unique advantages when used in combination with mice in genetic studies, despite mice have been the conventional leaders due to their small size and ease of genetic modification. Rats offer better abilities in scientific studies due to their larger body size, which facilitates extensive experimental tests. These tests enable investigations of complex diseases, such as neurodegenerative disorders, cardiovascular diseases, and metabolic disorders. CRISPR-Cas9 gene editing tools have expanded the diversity of rat models, enhancing their value as more accurate disease research models. Using animals in human genetic research poses several challenges to be addressed. The three primary issues that the debate on the use of animals in scientific research is based on include the differences in biology with the various species and the ethical issues, and the expenditure of keeping the animals as models of research. The ethical factors applied in all the animal studies presented in this review have followed the established ethical principles, such as the 3Rs (Replacement, Reduction, and Refinement), to ensure humane treatment and scientific responsibility. The use of animal models, especially rats, remains significant in a holistic study on human genetic disorders and the advancement of innovative treatment procedures. The particular aim of this review is to offer a comprehensive description of the nature, merits, and demerits of the regularly used animal models in human genetic studies to help researchers in choosing the best models to use in their studies.

Keywords: *CRISPR-Cas9, Animals, Rat model, Ethical, Zebra fish*

1. Introduction

The development of human genetics over the past several decades has occurred at a rapid pace, driven by CRISPR-Cas9 gene editing technology and advancements in next-generation sequencing and bioinformatics tools. Advances in technology have allowed scientists to decode genetic sequences from the human genome and identify the causes of certain diseases, which, in turn, have resulted in the development of specific medical therapies. Scientists have made significant progress in their study, but different essential questions about gene function and heredity, significant improvement in their research, but essential questions about gene function, hereditary patterns, and disease processes still await resolution [1]. Human subject testing in direct experiments is generally always unviable both ethically and practically, thus necessitating alternative models of research [2]. Biomedical science has relied significantly on animals as research surrogates for centuries, and their role in genetic studies has grown exponentially over modern history. Experiments on animal models, such as those conducted by Gregor Mendel using peas, and current applications of transgenic mice have provided vital information regarding genetic principles that operate across species, including humans [3-5]. Mice work perfectly well for studying intricate genetic diseases since they share 99% genetic homology with human protein sequences. Genetic studies on zebrafish and fruit flies are greatly advantaged by their rapid production cycles and transparent embryonic exposure, as well as their well-mapped genomes, which facilitate large-scale genetic testing operations [6-9]. Translational medicine heavily relies on animal models, as they offer invaluable support in the creation of medical applications. The process of scientific approval requires gene therapies and genetic medications to go through testing in animals as an initial step towards determining their safety record and efficacy before conducting human trials [10-16]. Experimental treatments for Duchenne muscular dystrophy in canine and cystic fibrosis in pigs have yielded results that progress toward clinical trials for evaluation. Medical advancements are largely reliant on these animal experiments, as their absence would cause significant complications for patients seeking treatment alternatives [17].

Genetic experimentation with animals generates conflicting debates between researchers and scientists [14], [18-20]. Experiments on animals have sparked constant debates regarding their ethical impact on animal suffering, alongside the moral value of animal species, and the threats of unforeseen outcomes due to genetic alterations [19-27]. The "3Rs" regulatory framework, founded on Replacement and Reduction, and Refinement, endeavors to minimize the use of animals, but animals continue to encounter constant ethical challenges. New advances in technology that incorporate organoids, along with AI simulations and in vitro techniques, raise questions about the role of animals in genetic research [3]. The review discusses how animal models are critical to human genetics research, particularly in clinical and biotechnological applications, while evaluating their ethical implications [28, 29].

Both scientific benefits and ethical considerations enable us to make more informed choices regarding the connection between animal research and human genetic advancement.

*Corresponding author: mustafamuzammal@gu.edu.pk

2. Major Animal Models in Human Genetics

2.1. Mouse (Mus musculus)

The genetic similarity between mice and humans, at approximately 85%, combined with the availability of highly effective genetic tools such as transgenic and knockout technologies, as well as CRISPR-Cas9, makes them the primary mammalian model for research [30]. Numerous studies for cancer and neurodegenerative diseases, as well as cardiovascular disease and immune system studies, have relied extensively on their utility [31]. Mouse embryonic stem cells allow scientists to perform sophisticated genetic procedures by coupling conditional knockouts with humanized disease models to study rare diseases. The little rodent family Mus musculus plays a critical role in human genetics research by allowing scientists to expand their understanding of genetic function, as well as disease representation and therapeutic progress [32-35]. Mice are an important model organism for biomedical research primarily due to their resemblance to human DNA and their short reproductive cycle and well-defined genomic sequence. Mice play critical research roles in genetic studies due to their capacity to facilitate gene function analysis. The integration of transgenic techniques and gene knockout technologies allows researchers to study how various genes influence normal and disease-related physiological processes [36].

The CRISPR-Cas9 gene editing tool, designed for controlled genome manipulation, provides researchers with improved tools to investigate disease-associated genetic mutations in mice. Mice research has played a crucial role in the investigation of genetic characteristics responsible for causing cancer, diabetes, neurodegenerative, and cardiovascular diseases [37]. Researchers develop mouse models that mimic human genetic diseases to investigate disease progression and evaluate therapeutic possibilities in the laboratory. Alzheimer's disease research using mouse models has yielded critical insight into amyloid plaque development and tau protein activities, thereby contributing to drug discovery [31, 36, 38].

Epigenetics, as well as gene-environment interaction research, is supported by research that has been done using mice [39]. Research using mice has demonstrated that environmental exposures, such as diet, in addition to mental stresses and toxins, alter genetic expression patterns that lead to many complicated health issues. Research findings have direct implications for individual patient care practices, as well as population health intervention strategies [38].

2.1.1. Advantages of Using Mice in Genetics Research

Experiments using mice as model organisms possess a number of beneficial traits because these animals have almost 85% gene similarity with human beings. Studies on genetic inheritance become more effective due to the mice reproduction rate in addition to their short lifespan. Scientists gain the advantage of a completed mouse genome because it facilitates both comparisons with other genomes as well as precise functional analysis [40, 41]. Scientists can carry out targeted studies of gene function through their access to various genetic research models, including knockout, knock-in, and conditional gene expression models [31]. The cost and maintenance aspects involved in mouse research make them better than large mammalian models, thereby allowing extensive genetic study programs. The mouse model remains crucial for augmenting human genetics research, as it generates a wealth of information regarding gene functions, disease processes, and insights into treatment discovery. The significant contributions of mice outweigh their limitations since their research advances humanity despite differences between human and mouse physiological and immunological reactions [42, 43]. The advancement of genetic engineering technologies and computational modelling systems enhances the use of mouse models in biomedical research. Researchers leverage the benefits of mouse models to transfer genetic information from the laboratory to clinical applications, resulting in improved human health outcomes [44, 45]. A rundown of Mus musculus (Mouse) characteristics for human genetics research appears in Table 1. Basics of human genetics utilize Mus musculus to improve many study fields, as indicated in the ensuing table.

Table 1. Summarizing key aspects of Mus musculus (Mouse) in human genetics research.

Aspect	Mouse (Mus musculus)
Genetic Similarity to Humans	~85% of the mouse genome is similar to the human genome
Role in Neurological Research	Models for neurodegenerative diseases (e.g., Alzheimer's, Parkinson's), neurological disorders (e.g., autism, schizophrenia), and brain development
Reproductive Research	Used in studies on fertility, pregnancy, embryonic development, and stem cell research
Infectious Disease Research	Models for a variety of diseases (e.g., influenza, tuberculosis, HIV/AIDS) and immune responses
Metabolic and Cardiovascular Research	Used to model obesity, diabetes, heart disease, and atherosclerosis
Genetic Manipulation	Well-established genetic tools (e.g., knock-out, knock-in, CRISPR-Cas9) for creating specific gene mutations
Developmental Biology	Studies on early development, organogenesis, and genetic pathways governing development
Cost and Complexity	Relatively inexpensive, short lifespan (2-3 years), and well-established protocols for research
Ethical Concerns	Ethical considerations are present but less intense than for primates due to smaller cognitive capacity
Behavioral Studies	Used for studies of anxiety, social behavior, memory, and cognition

2.2. Rat (Rattus norvegicus)

Rats are a typical model for various scientific studies on cardiovascular health and brain activity, along with behavioral tests. Science at an increased complexity is achieved through rats' larger size compared to mice. Genetic manipulation of rats advances through recent improvements in genome editing technology [46]. The coupling of cognitive capacities and socially engaged behaviors in rats renders them desirable research subjects for addiction and metabolic disease and neurophysiology research because these behaviors closely mimic human behavioral patterns. Since the advent of biological and genetic research, Rats (Rattus norvegicus) have demonstrated their worth as potent model organisms [47].

The union of thoroughly documented behavioral traits with in-depth physiological data and their augmented sizes compared to mice makes rats ideal subjects for sophisticated medical disease analyses. New CRISPR-Cas9 genome editing tools have boosted the status of rats as experimental animals for neurobiological research and studies of cardiovascular disease and metabolic disorders, and patterns of drug responses. The use of rats in human genetics studies comes under scrutiny regarding their advantages and limitations, and potential advancements within this discipline in this review [48]. Studies of human genetics, in large part, rely on animal models for investigating genetic mechanisms and developing therapeutic options, as well as research into disease mechanisms. Rats are better than mice as a mammalian model for certain research purposes due to their superior performance characteristics. The use of rats enhances translational application-based research because they have a physiological relationship with humans that primarily influences cardiovascular and neurological functions [49]. The assessment centers on rat participation in human genetics and their utilitarian applications, along with the challenges that researchers encounter when using these subjects.

2.2.1. Genetic and Physiological Advantages of Rats

Disease modeling research is aided by rat subjects since their cardiovascular system exhibits close similarities to human physiology, while their renal system and metabolic processes also show similarities with human biology. Rats, due to their large body size, enable precise surgical treatments and pharmaceutical applications, making them ideal for complex neuroscience and cardiovascular clinical studies [47-49]. Rats have sophisticated social behavioral patterns and cognitive functions, which make them the best model of research for schizophrenia and depression studies when examining addiction [50-52]. Genetic tools in the past worked better in mice, but with modern genome-editing techniques, gene modification was possible in rats, which made them increasingly popular.

2.2.2. Applications of Rats in Human Genetics Research

Scientists widely employ rats as biological models to study human psychiatric diseases in combination with neurodegenerative disorders. Investigation of genetically modified rats has provided critical data on the mechanisms by which genetics influence cognitive capacities, social behavior, and memory. Scientists apply rat models for studying both amyloid-beta accumulations and loss of dopaminergic neurons as the major characteristics of these degenerative brain conditions [43, 53, 54]. Thus, rats are used as research models to examine how neurobiology and genetics collectively generate substance abuse disorders. Suggested studies using rats as essential models for examining conditions such as hypertension, along with diabetes and obesity, are based on the fact that these conditions have significant genetic influences. Their greater vasculature and metabolism enable i.e. Hypertension Research: Spontaneously hypertensive rat (SHR) models assist scientists in understanding the genetics of blood pressure control, Diabetes and Insulin Resistance: Genetic rat models like the Zucker diabetic rat are instrumental in learning about Type 2 diabetes and metabolic syndrome and Atherosclerosis and Heart Disease: Rats are used as models to study lipid metabolism and genetic susceptibility to cardiovascular disease. Rats serve significantly in pharmacogenetics and toxicology studies since they possess similar drug metabolic processes as humans, thereby prompting scientists to identify genetic variations in drug response that aid in the formulation of personalized treatment measures [55, 56]. Scientists utilize rats to comprehend hereditary drug reaction susceptibility, hence creating drug use guidelines for human populations. Even though there are rat cancer models, they still provide researchers with vital information regarding how genetic factors, as well as epigenetic factors, influence tumor development. Studies conducted using rats investigate breast cancer susceptibility and determine the impact of hormones on tumor growth. Test subjects of genetically altered rats give researchers vital information regarding genetic changes that lead to gastrointestinal cancers.

2.2.3. Limitations and Challenges of Using Rats in Genetics Research

The advent of CRISPR-based genome editing tools has introduced a vast improvement in genetically available tools for rats, significantly expanding their previously limited supplies. Longer gestation periods and higher resource requirements in rats translate to increased costs for large-scale genetic examinations. While numerous inbred stocks have been established for mice, genetically altered rats are still expanding their available models. The biological similarity of humans and rats does not always ensure that findings from research will translate directly into human health conditions [43, 54]. CRISPR gene editing technology, along with other innovations, will increase the application of rats to human genetic research. Researchers struggle to employ some promising disease-modelling approaches, such as creating rats with humanized genes and immune systems. The integration of genomic techniques along with transcriptomic techniques, along with epigenomic techniques allows investigators to investigate intricate traits and disorders in rat subjects. Sophisticated behavioral test techniques combined with artificial intelligence-based testing offer an avenue to ascertain the genetic origins of neuropsychiatric illnesses [43]. Stem cell therapy and organ regeneration investigation occur through genetic modification research conducted on rats. Rats continue to be a valuable research model because they enable scientists to acquire information regarding neurogenetics as well as cardiovascular diseases and metabolism, and pharmacogenetics. Biomedical research will benefit from improved outcomes through advances in gene-editing technology and multi-omics system solutions, which will enhance research potential [46, 48]. Rats will continue to play their vital role in the transfer of genetic findings to clinical application through technical and ethical advancement. The table below outlines Rattus norvegicus (Rat) behavior in human genetics research (see Table 2).

Table 2. Summarizing the key aspects of Rattus norvegicus (Rat) in human genetics research.

Aspect	Rat (*Rattus norvegicus*)
Genetic Similarity to Humans	~90% of the rat genome is similar to the human genome
Role in Neurological Research	Models for neurodegenerative diseases (e.g., Parkinson's, Alzheimer's), brain injury, and psychiatric disorders (e.g., anxiety, depression)
Reproductive Research	Studies on reproductive health, gestation, embryonic development, and stem cell research
Infectious Disease Research	Models for diseases such as HIV, tuberculosis, and respiratory infections, and immune response studies
Metabolic and Cardiovascular Research	Used to study metabolic diseases (e.g., diabetes, obesity), heart disease, hypertension, and stroke
Genetic Manipulation	Advanced genetic tools for creating transgenic models (e.g., knock-out, knock-in)
Developmental Biology	Important in studying organ development, cellular differentiation, and genetic regulation of development
Cost and Complexity	Relatively inexpensive, medium lifespan (2-3 years), and widely used in research due to well-established protocols
Ethical Concerns	Ethical considerations exist but are generally less controversial than for primates, with acceptable standards in place for humane treatment
Behavioral Studies	Used for studying learning, memory, cognition, addiction, and social behavior

2.3. Zebrafish (Danio rerio)

Zebrafish have become crucial organisms for studying developmental genetics, as well as organogenesis and genetic diseases. Since their embryos are transparent and development is rapid, scientists find these organisms convenient to study gene expression as well as mutagenesis studies [57]. The drug screening process conducted using zebrafish models is especially advantageous for cardiovascular diseases and neurodegenerative disorders since these organisms breed in large numbers while producing genetically modified lines rapidly. Human genetics research is increasingly employing the zebrafish (Danio rerio) organism due to its numerous genes shared with humans, which develop rapidly and have clear, transparent embryos [58]. The combination of these unique qualities makes zebrafish a handy resource for studying gene functions while also allowing for abilities in studying diseases in a human-like fashion, along with testing potential therapeutic agents. Zebrafish act as critical organisms that will enable scientists to investigate the behavior of genes along with disease mechanisms. Genetic comparison identifies human zebrafish orthology, which occupies approximately 70% of genes, allowing researchers to study conserved genetic pathways that govern development and disease formation. High-throughput genetic screening within zebrafish labs will enable scientists to identify genes associated with cardiovascular diseases and neurodevelopmental disorders, and cancer conditions [57-59]. Developmental biology is one of the significant scientific advantages of employing zebrafish as a model organism. The study of organogenesis and the impact of genetic mutations on embryonic development becomes easy because zebrafish embryos are transparent throughout external development [60]. The findings of these experimental studies made scientists realize the nature of congenital disorders along birth defects more clearly. Zebrafish research yields valuable information regarding nervous system genetic disorders. The modeling of Parkinson's disease, Alzheimer's disease, and epilepsy in zebrafish allowed scientists to identify novel genetic factors along with possible drug targets. Studies on blood diseases get major impetus by employing zebrafish models that investigate leukemia and anemia. New drug discovery profits a lot from experimenting on zebrafish as model organisms. Zebrafish-based chemical screening helps scientists to screen thousands of compounds for therapeutic uses in a single experiment [61]. This has proven especially useful in identifying drugs for uncommon genetic disorders.

2.3.1. Advantages of Using Zebrafish in Genetics Research

The features that make zebrafish suitable for human genetics studies are that they have approximately 70% genetic similarity with humans, including genes associated with human diseases. Due to their transparent nature, zebrafish embryos allow scientists to view developmental dynamics as well as cell motions in real time [62]. Zebrafish embryos accelerate their development process such that major organs are formed within the first 24-48 hours, which facilitates faster genetic studies. The reproductive capacity of a single pair of zebrafish remains at high levels every week since they produce numerous embryos. The research tools that include CRISPR-Cas9 morpholino knockdown and transgenic methods provide accurate genetic modification techniques in zebrafish research [63, 64]. The expenses needed to maintain Zebrafish laboratory populations are less than what is required for mammalian models so researchers can carry out extensive research. Zebrafish are essential model animals for studying human genetics, with distinct advantages that enhance the utility of conventional mammalian models, such as mice. Progress in genetic research relies on studies using zebrafish, which facilitate better advances in developmental biology and disease modeling, and drug discovery. Zebrafish will play an even more prominent role in the study of human genetics and therapeutic discovery due to ongoing advances in genetic engineering technology [65].

Here's a table 3 summarizing the key aspects of *Danio rerio* (Zebrafish) in human genetics research.

This table outlines the key uses and characteristics of zebrafish in human genetics research.

Table 3. Summarizing the key aspects of *Danio rerio* (Zebrafish) in human genetics research.

Aspect	Zebrafish (*Danio rerio*)
Genetic Similarity to Humans	~70% of human genes have a counterpart in zebrafish; many conserved pathways
Role in Neurological Research	Used to study neurodevelopment, neurodegenerative diseases (e.g., Alzheimer's, Parkinson's), and brain function
Reproductive Research	Zebrafish are used in studies on early development, gene expression during development, and reproductive biology
Infectious Disease Research	Models for bacterial and viral infections, including tuberculosis, malaria, and Zika virus
Metabolic and Cardiovascular Research	Zebrafish models are used to study heart development, cardiovascular diseases, and metabolic disorders like diabetes and obesity
Genetic Manipulation	High efficiency in genetic manipulation using CRISPR/Cas9, transgenics, and gene knockdown techniques
Developmental Biology	Zebrafish embryos are transparent, making them ideal for live imaging studies of early development and organogenesis
Cost and Complexity	Inexpensive, short lifespan (3-4 months), and high reproductive output, making them ideal for large-scale genetic screens
Ethical Concerns	Ethical concerns are relatively low due to the small size and developmental similarities to humans, though guidelines still apply
Behavioral Studies	Used to study behavior, sensory processing, and learning, especially in relation to vision, movement, and social behavior

2.4. Fruit Fly (Drosophila melanogaster)

Fruit flies illustrate considerable importance in promoting our understanding of heredity processes alongside developmental processes and nervous system diseases, despite being far from human evolution [66, 67]. Fruit flies have short lives, but have clearly defined genomic information that allows researchers to utilize them economically for massive genetic studies. Research done on Drosophila acts as the epicenter for the identification of Parkinson's and Alzheimer's disease pathways, along with enlightening scientists regarding crucial epigenetic and chromatin transformation mechanisms [67, 68]. The genetics field of research depends on the workhorse organism of Drosophila melanogaster as an anchor organism throughout the past 100 years. In the early 20th century, Drosophila came into view in the work of Thomas Hunt Morgan, which later provided enormous amounts of knowledge related to the formation of genetic inheritance and disease simulation. Its fundamental body organization allows Drosophila to act as a perfect research model for human genetics research due to evolutionary genetic affinities towards human populations [68, 69].

2.4.1. Genetic Advantages of Drosophila melanogaster

Drosophila takes ten to twelve days to complete its life cycle, thereby facilitating rapid genetic studies between consecutive generations of offspring. One female Drosophila lays hundreds of eggs, which are sufficient for statistical data for research. The genetic study and mutation research is easier with Drosophila as it has only four pairs of chromosomes [70, 71]. The full sequence and thorough scientific analysis of the Drosophila genome facilitate the analysis of gene function. Transgenic, RNA interference (RNAi), and CRISPR-Cas9 genome editing are three gene modification methods available to scientists that enable them to make efficient methods of producing gene modifications. In his work on genetics in Drosophila, Morgan discovered sex inheritance functions that led to the development of the chromosome theory of inheritance [72, 73]. Scientists laid down connected genes and genetic recombination in their early experiments using Drosophila. Scientists using Drosophila discovered the Hox gene cluster, which delineated pivotal developmental pattern mechanisms later found to be present all over vertebrate bodies. Biologists' study of segmentation genes in Drosophila produced valuable information on human embryonic development [74, 75]. Over 75% of human disease-causing genes can be traced to similar counterparts in Drosophila. Drosophila serves as a model system for research in three prominent neurodegenerative disorders, like Alzheimer's and Parkinson's, and Huntington's diseases. Oncogenes and tumor suppressor genes, along with cell signaling pathways, have all benefited from Drosophila cancer clinical studies [76-78]. Drosophila circadian rhythm studies revealed genetic elements responsible for timing patterns that researchers later used to develop therapies for sleep disorders in humans. Research on Drosophila synaptic function and neurodegeneration trends has led to greater insight into mental illness and brain disease.

2.4.2. Limitations of Drosophila in Human Genetics

The lack of circulatory system activities, combined with adaptive immunity and multicellular organization, such as lungs and kidneys, hinders Drosophila from reproducing all human physiological activities [79, 80]. There are differences between flies and human beings regarding both control components and epigenetic control, even in cases of genomic conservation. Genetic findings in Drosophila need mammalian experiments for medical applications in human beings. Drosophila melanogaster remains one of the most important model organisms for human genetic research, despite certain limitations [81-83]. The union of rapid reproduction time and effective genetic design capacity, combined with intact biological pathway function, renders Drosophila melanogaster the ideal organism for fundamental genetic studies and research on human diseases. The ongoing advancement in DNA manipulation technology continues to expand the research potential of Drosophila, enabling it to maintain its crucial role in future biomedical research [84, 85]. The following table 4 gives an overview of key elements involved in Drosophila melanogaster (Fruit Fly) research for human genetics purposes. A comprehensive overview of the human genetics research role of Drosophila melanogaster is found within this table.

Table 4. Summarizing the key aspects of *Drosophila melanogaster* (Fruit Fly) in human genetics research.

Aspect	Fruit Fly (Drosophila melanogaster)
Genetic Similarity to Humans	~60% of human genes are conserved in fruit flies; many basic genetic pathways are shared
Role in Neurological Research	Used to study neurodegenerative diseases (e.g., Alzheimer's, Parkinson's), circadian rhythms, and synaptic function
Reproductive Research	Studies on sex determination, fertility, and genetic inheritance
Infectious Disease Research	Models for viral infections, such as dengue and Zika virus, and bacterial pathogenesis
Metabolic and Cardiovascular Research	Fruit flies are used to study metabolic pathways, obesity, and aging, as well as genes involved in cardiovascular diseases
Genetic Manipulation	High ease of genetic manipulation using RNAi, CRISPR-Cas9, and transgenic techniques for gene function studies
Developmental Biology	Key model for studying embryogenesis, gene regulation during development, and organogenesis
Cost and Complexity	Inexpensive, short lifespan (10-12 days), and large numbers of offspring, making them ideal for genetic screens and high-throughput studies
Ethical Concerns	Ethical concerns are minimal due to the simple anatomy and short life cycle of fruit flies
Behavioral Studies	Used to study learning, memory, sensory processing, and behavior in relation to genetics and neurobiology

2.5. Non-Human Primates (e.g., Macaques, Marmosets)

The shared basic link between non-human primates and humans in terms of physiology and genetics makes them tremendously valuable for research on complex brain functions and neurological disorders, and genomic intervention strategies. Due to ethical issues as well as costly maintenance, their use continues to remain strictly regulated [86]. Such modeling techniques drive medical research, particularly in infectious disease research to aid AIDS and the new coronavirus vaccine, autism spectrum studies, and organ transplantation research. Macaques, as well as marmosets, are also essential primates in the clinical research of human genetics. The evolution between the two primates is human like and therefore these primates are very useful in enabling scientists to study genetic disease, as well as the neurobiological functions and complex traits. The extension of human genetic correspondences in NHPs makes them important for translating laboratory knowledge into human therapy, compared to other model species [87].

2.5.1. Genetic Advantages of Non-Human Primates

The genetic composition of NHPs intersects with human DNA at the 93 to 98 percent level which results in more effective disease modeling outcomes [88]. Their brain structure along with their immune mechanisms and metabolic pattern closely resemble human neuroanatomy. The high-level learning capabilities along with social behavior and intellectual capability of NHPs, make them ideal subjects to study neurological as well as psychiatric disorders. Genome-editing technology CRISPR-Cas9 offers researchers the capability of creating genetically engineered primate disease models for human medicine studies. NHPs' developmental time frame parallels human development, which allows researchers to examine genetic disorders that emerge with age [89, 90].

2.5.2. Contributions to Human Genetics

The Neurological and Psychiatric Disorders (NHPs) have proven indispensable in studies of Alzheimer's, Parkinson's, and Huntington's diseases, as they possess a mature brain anatomy. Using the NHP models of autism spectrum disorders and schizophrenia, research helps to discover the core genetic factors behind the disorders [91, 92]. HIV/AIDS and tuberculosis, and malaria combined have received key insights from NHPs' studies. Through research on how primates cope with genetic predispositions in conjunction with their immune system reactions, scientists become better equipped to understand human disease resistance. Atherosclerosis research, as well as obesity and diabetes in primates, has revealed genetic elements at play in these diseases [89, 90]. Scientists utilize NHPs for medical research to examine how lipids function within the body as well as to research insulin resistance. The progress in research on infertility and in vitro fertilization, as well as fetal development, depends on NHP models. The investigation of primate embryonic stem cells yields crucial information for the development of novel medical treatment techniques.

2.5.3. Limitations of Non-Human Primates in Human Genetics

NHP-based research raises ethical issues related to animal well-being that need intense regulatory control. This type of research involves harvesting NHPs at a far greater expense and requires significantly extended experimental periods compared to both mouse and fruit fly models. The genetic and environmental features of Earth primates are very different from those in inbred rodent models so experimental outcomes have more variability. Scientists continue to advance genome editing methods in NHPs despite the techniques being challenging to employ and less efficient compared to rodent models [90]. Non-human primates are better than humans in human genetics studies due to their identical genetic backgrounds with human beings and complementary physiological functions. Researchers still find NHPs useful due to their extensive applications in the research of neurological diseases, as well as infectious and metabolic diseases in biomedical research. Genetic engineering advancements result in increased non-human primate applications in genetic studies, which guarantee their relevance to future medical findings despite some existing ethical and logistical challenges [89].

Table 5 highlights the differences and similarities between macaques and marmosets in the context of their use in human genetics research. The comparative role of animal models in human genetic research is illustrated in Figure 1.

Table 5. Summarizing the key aspects of non-human primates (e.g., Macaques, Marmosets) in human genetics research.

Aspect	Macaques	Marmosets
Genetic Similarity to Humans	~98% of genome shared with humans	~93% of genome shared with humans
Role in Neurological Research	Used extensively to model neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's)	Studied for cognitive and psychiatric disorders, including autism and schizophrenia
Reproductive Research	Models of infertility, IVF, and developmental genetics	Used for studies on reproductive health and stem cell research
Infectious Disease Research	HIV/AIDS, tuberculosis, and malaria modeling	Malaria, Zika virus, and immunology studies
Metabolic and Cardiovascular Research	Models for atherosclerosis, obesity, diabetes	Used to study insulin resistance, lipid metabolism
Ethical Concerns	High ethical scrutiny due to intelligence and social behaviors	Ethical concerns similar to macaques, but smaller size may reduce some concerns
Cost and Complexity	Expensive, long lifespan, and complex models	Smaller, shorter lifespan, more cost-effective for certain studies
Genetic Manipulation	Advanced genetic tools available (e.g., CRISPR)	Genetic modifications possible but less efficient than macaques
Behavioral Studies	Used for studies of cognition, social behaviors, and learning	Significant focus on social behavior and communication abilities

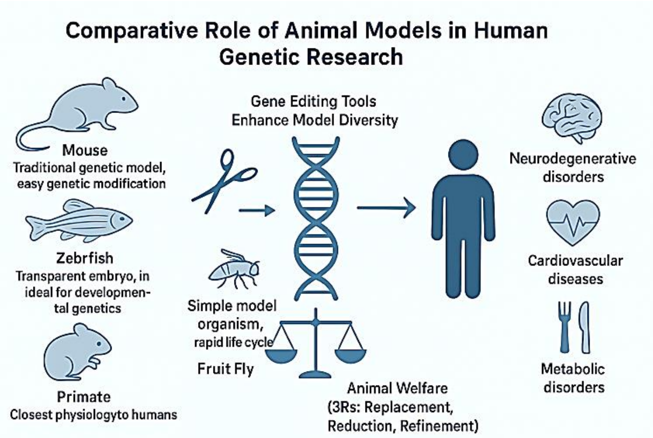


Fig. 1: Comparative role of animal models in human genetic research.

3. Applications of Animal Models in Human Genetics

i. Disease Modeling: Genetic diseases in animals can be used to model human diseases, indicating pathogenesis and treatments [93].

ii. Drug Development: Efficacy and safety testing of new drugs in animals precedes trials in humans [94].

iii. Gene Function Studies: Knockout and transgenic models enable the identification of the function of individual genes in disease and health [95].

iv. Regenerative Medicine: Research in animals gives rise to progress in stem cell therapy and organ transplantation [96].

v. Behavioral Genetics: Rodent models help elucidate genetic contributions to neuropsychiatric illnesses like autism and schizophrenia [97].

vi. Cancer Research: Mouse models of human tumor xenografts (patient-derived xenografts, or PDX) are employed for testing personalized cancer therapy [98].

vii. Cardiovascular and Metabolic Disease Research: Animal models play a key role in understanding diseases like diabetes, obesity, and hypertension, resulting in the creation of targeted interventions [99].

viii. Aging and Longevity Studies: The employment of short-lived organisms like nematodes (Caenorhabditis elegans) and fruit flies in aiding the identification of longevity genes and aging pathways [100].

4. Limitations and Challenges

Genetic Differences: Despite similarities, genetic and physiological differences between animals and humans may limit the direct application of findings.

Ethical Concerns: The application of animals for research purposes has ethical implications involving their welfare and the need for alternative models [101].

Cost and Maintenance: Large animal studies may be costly and resource-intensive. Reproducibility Issues: Lack of consistency in experimental conditions can influence reproducibility and the interpretation of findings [102].

Limited Success in Translating to Humans: Though encouraging results are reported in animal models, numerous drug candidates fail in human clinical trials because of species-specific reactions [103].

Alternative Models and Technologies: Technological breakthroughs in organoids, induced pluripotent stem cells (iPSCs), and computational models are expected to diminish dependency on animals in future research [104].

5. Ethical Considerations

Genetic Differences: Despite the similarities, genetic and physiological differences between animals and humans might limit the direct use of findings.

Ethical Concerns: The use of animals as research models raises ethical issues, including their potential suffering and the need for alternative models.

Cost and Maintenance: The large animal studies can be expensive and resource-consuming. Reproducibility Problems: The lack of consistency in experimental conditions may ruin reproducibility and findings interpretation.

Poor Track Record in Translating to Humans: Despite promising results reported in animal models, many drug candidates have failed in human clinical trials due to species-specific reactions.

Alternative Models and Technologies: Future research is anticipated to reduce the animal use in technological breakthroughs in organoids, induced pluripotent stem cells (iPSCs), and computational models. [88, 91, 105-109].

6. Future Directions

Genetic Engineering Progress: CRISPR-based genome editing in larger mammals could lead to more effective systems for treating complex human diseases. Humanized Animal Models: Animal models can be humanized by gene transfer, tissue transfer, or immune-cell transfer, which opens promising prospects of personalized medicine. Emerging Technologies: Systems of organoid culture technologies, artificial intelligence, and synthetic biology could reduce the use of animal models [110, 111].

7. Conclusion

Human genetics heavily depends on animal models as an essential study because animal models offer important information on the functioning of genes, mechanisms of diseases, and the possible approach to therapy. The future of biomedical research is being informed by advancements in alternative model construction in association with the innovations in genetic technologies, including CRISPR-Cas9 and genome editing. The developments will allow more accurate models that are human-relevant and will enhance the understanding of more complex diseases as well as expedite the translation application. Despite these improvements, there are still gaps in the research. Some of the limitations are that the current models have not fully replicated human disease phenotypes, experimental procedures are variable, and multi-omics data have not been sufficiently integrated in the models. There is a need to fill these gaps to enhance the predictive capabilities and translational relevance of animal models. The ethical consideration is also an important focus as it is necessary to follow some principles, including the principles of replacement, Reduction and Refinement (3Rs) to provide a humane treatment without violating the principles of scientific rigor. Research in the future is expected to combine computational models, organoids, and multi-species solutions and increase reproducibility, decrease the use of conventional animal models, and increase the opportunities to personalized medicine. In general, the further development of animal models alongside the technological progress, interest to gaps in research, and the ethical duty will lead to the considerable advance in the knowledge of human genetics, finding new therapeutic targets, and eventually to the better patient care. Continuous attempts to standardize protocols, integrate ethical models, and formulate alternative or more humanized models will be vital in ensuring the research is as far-reaching as it can be in the coming years.

Acknowledgment:

To all lab members helping in finding the data.

Conflict of interest: None

Authors' Contribution:

Aiman Saeed Khan, Maria Shafiq, Nazia Farid Burki, Shaista Naz, Afsar Zada, and Hunza Malik did data collection and drafting: Ali Ahmad Khan, Murtajiz Hussain, Suleman Khan, and Sana Fatima did editing and drafting: Muzammil Ahmad Khan did Literature review and English setting, and Muhammad Muzammal did supervision, drafting, and data collection.

Funding: None

Ethical Approval: None

References

[1] A. Domínguez-Oliva, I. Hernández-Ávalos, J. Martínez-Burnes, A. Olmos-Hernández, A. Verduzco-Mendoza, and D. Mota-Rojas, “The Importance of Animal Models in Biomedical Research: Current Insights and Applications,” 2023.

[2] S. Ahmad, M. Z. Ali, M. Muzammal, F. A. Mir, and M. A. Khan, “The molecular genetics of human appendicular skeleton,” 2022.

[3] R. C. Hubrecht and E. Carter, “The 3Rs and humane experimental technique: Implementing change,” Animals, vol. 9, no. 10, 2019.

[4] M. Muzammal, S. Ahmad, M. Z. Ali, and M. A. Khan, “Alopecia-mental retardation syndrome: Molecular genetics of a rare neuro-dermal disorder,” 2021.

[5] S. Fatima, N. Malkani, M. Muzammal, A. A. Khan, and M. Usama, “Stable Vesicle Production from Bacterial Total Lipid Extracts,” Abasyn Journal Life Sciences, vol. 4, no. 1, 2021.

[6] M. Muzammal, A. Firoz, H. M. Ali, A. Farid, M. A. Khan, and K. R. Hakeem, “Lumateperone Interact with S-Protein of Ebola Virus and TIM-1 of Human Cell Membrane: Insights from Computational Studies,” Applied Sciences (Switzerland), vol. 12, no. 17, 2022.

[7] M. Muzammal, M. A. Khan, M. Al Mohaini, A. J. Alsalman, M. A. Al Hawaj, and A. Farid, “In Silico Analysis of Honeybee Venom Protein Interaction with Wild Type and Mutant (A82V + P375S) Ebola Virus Spike Protein,” Biologics, vol. 2, no. 1, 2022.

[8] M. Hayat et al., “Formulation development and optimization of herbo synthetic gel: In vitro biological evaluation and in vivo wound healing studies,” Process Biochemistry, vol. 130, 2023.

[9] I. Ahmed et al., “Identification of Four Novel Candidate Genes for Non-syndromic Intellectual Disability in Pakistani Families,” Biochem Genet, vol. 62, no. 4, 2024.

[10] M. Muzammal et al., “The molecular genetics of UV-Sensitive syndrome: A rare dermal anomaly,” 2021.

[11] S. Fatima et al., “Crispr/Cas9 Endonucleases: A New Era of Genetic Engineering,” Abasyn Journal Life Sciences, vol. 4, no. 2, 2021.

[12] S. Fatima et al., “Composition and Function of Saliva: A review,” World J Pharm Pharm Sci, vol. 9, no. 6, 2020.

[13] M. Al Mohaini et al., “Pathological study of Pasteurella Multocida Recombinant Clone ABA392,” Pakistan Journal of Medical and Health Sciences, vol. 16, no. 2, 2022.

[14] M. Muzammal et al., “Whole exome sequencing coupled with in silico functional analysis identified NID1 as a novel candidate gene causing neuro-psychiatric disorder in a Pakistani family,” J Natl Sci Found, vol. 51, no. 4, 2024.

[15] M. Al Mohaini et al., “Enhancing Lipase Production of Bacillus salmalaya Strain 139SI Using Different Carbon Sources and Surfactants,” Appl Microbiol, vol. 2, no. 1, 2022.

[16] S. Ahmad et al., “Identification of GLI1 and KIAA0825 Variants in Two Families with Postaxial Polydactyly,” Genes (Basel), vol. 14, no. 4, 2023.

[17] W. Russell and R. Burch, "The Principles of Humane Experimental Technique by W.M.S. Russell and R.L. Burch," John Hopkins Bloomberg School of Public Health, 1959.

[18] M. Ayaz et al., "Genetic basis of ß-thalassemia in families of pashtun ethnicity in Dera Ismail Khan district of Khyber Pakhtun-Khwa province, Pakistan," Expert Rev Hematol, vol. 16, no. 9, 2023.

[19] M. Muzammal et al., "In silico Analysis of PRODH Mutations and their biological significance in disease etiology," Abasyn Journal Life Sciences, vol. 5, no. 1, 2022.

[20] H. Gul et al., "Homozygosity mapping coupled with whole-exome sequencing and protein modelling identified a novel missense mutation in GUCY2D in a consanguineous Pakistani family with Leber congenital amaurosis," J Genet, vol. 100, no. 2, 2021.

[21] M. Muzammal et al., "Exome sequence analysis in consanguineous Pakistani families inheriting Bardet-Biedle syndrome determined founder effect of mutation c.299delC (p. Ser100Leufs*24) in BBS9 gene," Mol Genet Genomic Med, vol. 7, no. 8, 2019.

[22] R. Ullah et al., "Exploitation of selected plant extracts as bio-control against fungal contaminants in animal feed," J King Saud Univ Sci, vol. 35, no. 5, 2023.

[23] M. Al Mohaini et al., "Screening of Anticancer and Immunomodulatory Properties of Recombinant pQE-HAS113 Clone Derived from Streptococcus Equi," Pakistan Journal of Medical and Health Sciences, vol. 16, no. 2, 2022.

[24] A. J. Alsalman et al., "Chitinase Activity by Chitin Degrading Strain (Bacillus Salmalaya) in Shrimp Waste," Int J Curr Res Rev, vol. 14, no. 11, 2022.

[25] K. A. Khan et al., "Preparation of Losartan Potassium Controlled Release Matrices and In-Vitro Investigation Using Rate Controlling Agents," Molecules, vol. 27, no. 3, 2022.

[26] M. Muzammal et al., "A novel protein truncating mutation in L2HGDH causes L-2-hydroxyglutaric aciduria in a consanguineous Pakistani family," Metab Brain Dis, vol. 37, no. 1, 2022.

[27] M. Muzammal et al., "In Silico Analysis of the L-2-Hydroxyglutarate Dehydrogenase Gene Mutations and Their Biological Impact on Disease Etiology," Genes (Basel), vol. 13, no. 4, 2022.

[28] M. Z. Ali et al., "In Silico Analysis Identified Putative Pathogenic Missense nsSNPs in Human SLITRK1 Gene," Genes (Basel), vol. 13, no. 4, 2022.

[29] R. Abid et al., "Pharmacological Properties of 4′, 5, 7-Trihydroxyflavone (Apigenin) and Its Impact on Cell Signaling Pathways," 2022.

[30] A. J. Alsalman et al., "Analysis and Characterization of Chitinase in Bacillus salmalaya Strain 139SI," Int J Curr Res Rev, vol. 14, no. 11, 2022.

[31] S. Kumar, L. L. Sandell, P. A. Trainor, F. Koentgen, and G. Duester, "Alcohol and aldehyde dehydrogenases: Retinoid metabolic effects in mouse knockout models," 2012.

[32] S. Hussain et al., "Mutation screening of multiple Pakistani MCPH families revealed novel and recurrent protein-truncating mutations of ASPM," Biotechnol Appl Biochem, vol. 69, no. 6, 2022.

[33] Y. N. Alhashem et al., "Protein Isolation and Separation Techniques of Pasteurella multocidavia One- and Two-Dimen-Sional Gel Electrophoresis," Int J Curr Res Rev, vol. 14, no. 12, 2022.

[34] M. A. Al Hawaj et al., "Biosurfactant Screening and Antibiotic Analysis of Bacillus salmalaya," Int J Curr Res Rev, vol. 14, no. 12, 2022.

[35] T. Lappalainen and D. G. MacArthur, "From variant to function in human disease genetics," *Science*, vol. 373, no. 6562, pp. 1464–1468, 2021.

[36] E. Yada, S. Wada, S. Yoshida, and T. Sasada, "Use of patient-derived xenograft mouse models in cancer research and treatment," 2018.

[37] H. T. Tzeng et al., "PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model," PLoS One, vol. 7, no. 6, 2012.

[38] I. P. Maly, V. Crotet, and M. Toranelli, "The so-called 'testis-specific aldehyde dehydrogenase' corresponds to type 2 retinaldehyde dehydrogenase in the mouse," Histochem Cell Biol, vol. 119, no. 2, 2003.

[39] A. Alzu'bi et al., "Acute Hepatic Injury Associated with Acute Administration of Synthetic Cannabinoid XLR-11 in Mouse Animal Model," Toxics, vol. 10, no. 11, 2022.

[40] J. C. Frank, B. H. Song, and Y. M. Lee, "Mice as an Animal Model for Japanese Encephalitis Virus Research: Mouse Susceptibility, Infection Route, and Viral Pathogenesis," 2023.

[41] Y. C. Chen et al., "Deletion of Carboxypeptidase E in b-Cells Disrupts Proinsulin Processing but Does Not Lead to Spontaneous Development of Diabetes in Mice," Diabetes, vol. 72, no. 9, 2023.

[42] Q. S. Zhang, Y. Heng, Z. Mou, J. Y. Huang, Y. H. Yuan, and N. H. Chen, "Reassessment of subacute MPTP-treated mice as animal model of Parkinson's disease," Acta Pharmacol Sin, vol. 38, no. 10, 2017.

[43] F. Alzoughool and M. B. Al-Zghoul, "Optimal therapeutic adropin dose intervention in mice and rat animal models: A systematic review," Vet World, vol. 14, no. 6, 2021.

[44] M. Bourin, B. Petit-Demoulière, B. Nic Dhonnchadha, and M. Hascöet, "Animal models of anxiety in mice," 2007.

[45] M. Gomes-Solecki, I. Santecchia, and C. Werts, "Animal models of leptospirosis: Of mice and hamsters," 2017.

[46] L. Olexová, T. Senko, P. Štefánik, A. Talarovičová, and L. Kršková, "Habituation of exploratory behaviour in VPA rats: Animal model of autism," Interdiscip Toxicol, vol. 6, no. 4, 2013.

[47] V. L. David et al., "Rat animal model of pectus excavatum," Life, vol. 10, no. 6, 2020.

[48] E. Benedikz, E. Kloskowska, and B. Winblad, "The rat as an animal model of Alzheimer's disease," 2009.

[49] C. D. Escabi, M. D. Frye, M. Trevino, and E. Lobarinas, "The rat animal model for noise-induced hearing loss," J Acoust Soc Am, vol. 146, no. 5, 2019.

[50] M. Salihoglu et al., "Ototoxicity of boric acid powder in a rat animal model," Braz J Otorhinolaryngol, vol. 84, no. 3, 2018.

[51] R. Wuri, A. M. Rosdianto, and H. Goenawan, "UTILIZATION OF RATS AS BLUNT TRAUMA ANIMALS MODEL: A LITERATURE REVIEW," Indonesia Medicus Veterinus, vol. 10, no. 2, 2021.

[52] J. S. Aprioku, A. M. Amamina, and P. A. Nnabuenyi, "Mancozeb-induced hepatotoxicity: protective role of curcumin in rat animal model," Toxicol Res (Camb), vol. 12, no. 1, 2023.

[53] A. Mvoulana, R. Kachouri, and M. Akil, "Fully automated method for glaucoma screening using robust optic nerve head detection and unsupervised segmentation based cup-to-disc ratio computation in retinal fundus images," Computerized Medical Imaging and Graphics, vol. 77, 2019.

[54] M. Zandi, A. Dehghan, H. Malekzadeh, P. Janbaz, K. Ghadermazi, and P. Amini, "Introducing a protocol to create bisphosphonate-related osteonecrosis of the jaw in rat animal model," Journal of Cranio-Maxillofacial Surgery, vol. 44, no. 3, 2016.

[55] M. Sanati et al., "Impact of Gold Nanoparticles on Amyloid β-Induced Alzheimer's Disease in a Rat Animal Model: Involvement of STIM Proteins," ACS Chem Neurosci, vol. 10, no. 5, 2019.

[56] K. R. Foster, T. Wenseleers, and F. L. W. Ratnieks, "Kin selection is the key to altruism," 2006.

[57] N. Nowik, P. Podlasz, A. Jakimiuk, N. Kasica, W. Sienkiewicz, and J. Kaleczyc, "Zebrafish: An animal model for research in veterinary medicine," Pol J Vet Sci, vol. 18, no. 3, 2015.

[58] C. Chakraborty, A. R. Sharma, G. Sharma, and S. S. Lee, "Zebrafish: A complete animal model to enumerate the nanoparticle toxicity," J Nanobiotechnology, vol. 14, no. 1, 2016.

[59] J. Lachowicz, A. Szopa, K. Ignatiuk, K. Świąder, and A. Serefko, "Zebrafish as an Animal Model in Cannabinoid Research," 2023.

[60] T. Y. Choi, T. I. Choi, Y. R. Lee, S. K. Choe, and C. H. Kim, "Zebrafish as an animal model for biomedical research," 2021.

[61] L. Flinn, S. Bretaud, C. Lo, P. W. Ingham, and O. Bandmann, "Zebrafish as a new animal model for movement disorders," 2008.

[62] J. Lachowicz et al., "Zebrafish as an animal model for testing agents with antidepressant potential," 2021.

[63] Y. Niu, Y. Sun, Y. Liu, K. Du, X. Xu, and Y. Ding, "Using Zebrafish Animal Model to Study the Genetic Underpinning and Mechanism of Arrhythmogenic Cardiomyopathy," 2023.

[64] L. Maves, "Recent advances using zebrafish animal models for muscle disease drug discovery," 2014.

[65] H. J. Cho, W. S. Lee, J. Jeong, and J. S. Lee, "A review on the impacts of nanomaterials on neuromodulation and neurological dysfunction using a zebrafish animal model," 2022.

[66] R. S. P. Yadav, F. Ansari, N. Bera, C. Kent, and P. Agrawal, "Lessons from lonely flies: Molecular and neuronal mechanisms underlying social isolation," 2024.

[67] H. Li et al., "Fly Cell Atlas: A single-nucleus transcriptomic atlas of the adult fruit fly," Science (1979), vol. 375, no. 6584, 2022.

[68] M. V. Vatashchuk, M. M. Bayliak, V. V. Hurza, K. B. Storey, and V. I. Lushchak, "Metabolic Syndrome: Lessons from Rodent and Drosophila Models," 2022.

[69] T. Duarte et al., "The Drosophila melanogaster ACE2 ortholog genes are differently expressed in obesity/diabetes and aging models: Implications for COVID-19 pathology," Biochim Biophys Acta Mol Basis Dis, vol. 1868, no. 12, 2022.

[70] Nafisa Kusumawati and Ignatius Sudaryadi, "Effect of Feeding Kiwifruit (Actinidia deliciosa) Extract on Survival Rate and Reproductive Organ Morphology of Fruit Fly (Drosophila melanogaster Meigen, 1830) Ultraviolet Light Irradiation," Berkala Ilmiah Biologi, vol. 13, no. 3, 2022.

[71] S. P. Pain, "Signs of anger: Representation of agonistic behaviour in invertebrate cognition," Biosemiotics, vol. 2, no. 2, 2009.

[72] T. H. Morgan, "Sex limited inheritance in drosophila," Science (1979), vol. 32, no. 812, 1910.

[73] M. Vicedo, "The Chromosome Theory of Mendelian Inheritance: Explanation and realism in theory construction," *PSA: Proceedings of the Biennial Meeting of the Philosophy of Science Association*, vol. 1990, no. 1, pp. 179–191, 1990.

[74] W. Huber et al., "Orchestrating high-throughput genomic analysis with Bioconductor," Nat Methods, vol. 12, no. 2, 2015.

[75] F. S. Collins, M. Morgan, and A. Patrinos, "The Human Genome Project: Lessons from large-scale biology," 2003.

[76] J. Cabana-Domínguez et al., "The translational genetics of ADHD and related phenotypes in model organisms," 2023.

[77] J. Cheng, L. F. Hsu, Y. H. Juan, H. P. Liu, and W. Y. Lin, "Pathway-targeting gene matrix for Drosophila gene set enrichment analysis," PLoS One, vol. 16, no. 10, 2021.

[78] M. H. Chiang et al., "Drosophila Model for Studying Gut Microbiota in Behaviors and Neurodegenerative Diseases," 2022.

[79] O. Taghli-Lamallem, E. Plantié, and K. Jagla, "Drosophila in the heart of understanding cardiac diseases: Modeling channelopathies and cardiomyopathies in the fruitfly," 2016.

[80] Y. Grosjean et al., "An olfactory receptor for food-derived odours promotes male courtship in Drosophila," Nature, vol. 478, no. 7368, 2011.

[81] P. Ardin, F. Peng, M. Mangan, K. Lagogiannis, and B. Webb, "Using an Insect Mushroom Body Circuit to Encode Route Memory in Complex Natural Environments," PLoS Comput Biol, vol. 12, no. 2, 2016.

[82] H. Wagner, M. Egelhaaf, and C. Carr, "Model organisms and systems in neuroethology: one hundred years of history and a look into the future," 2024.

[83] E. E. Patton, D. L. Mitchell, and R. S. Nairn, "Genetic and environmental melanoma models in fish," 2010.

[84] E. Frasnelli, G. Vallortigara, and L. J. Rogers, "Left-right asymmetries of behaviour and nervous system in invertebrates," 2012.

[85] T. Prasad, S. Iyer, S. Chatterjee, and M. Kumar, "In vivo models to study neurogenesis and associated neurodevelopmental disorders-Microcephaly and autism spectrum disorder," WIREs Mechanisms of Disease, vol. 15, no. 4, 2023.

[86] N. Provençal et al., "The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells," Journal of Neuroscience, vol. 32, no. 44, 2012.

[87] K. J. Karczewski et al., "The mutational constraint spectrum quantified from variation in 141,456 humans," Nature, vol. 581, no. 7809, 2020.

[88] K. A. Phillips et al., "Why primate models matter," 2014.

[89] H. Zhao, Y. H. Jiang, and Y. Q. Zhang, "Modeling autism in non-human primates: Opportunities and challenges," 2018.

[90] J. Bailey, "Non-human primates in medical research and drug development: A critical review," 2005.

[91] A. M. Dettmer and S. J. Suomi, "Nonhuman primate models of neuropsychiatric disorders: Influences of early rearing, genetics, and epigenetics," ILAR J, vol. 55, no. 2, 2014.

[92] K. Godbout, J. Rousseau, and J. P. Tremblay, "Successful Correction by Prime Editing of a Mutation in the RYR1 Gene Responsible for a Myopathy," Cells, vol. 13, no. 1, 2024.

[93] M. Balls, "The principles of humane experimental technique: timeless insights and unheeded warnings," ALTEX, 2010.

[94] G. Griffin, "Book Review: The Principles of Humane Experimental Technique," Alternatives to Laboratory Animals, vol. 20, no. 4, 1992.

[95] A. Goldberg, "The principles of humane experimental technique: Is it relevant today?" ALTEX, 2010.

[96] G. Orlando et al., "Regenerative medicine as applied to general surgery," *Annals of Surgery*, vol. 255, no. 5, pp. 867–880, 2012.

[97] R. G. W. Kirk, "Recovering The Principles of Humane Experimental Technique: The 3Rs and the Human Essence of Animal Research," Sci Technol Human Values, vol. 43, no. 4, 2018.

[98] M. Balls, "It's Time to Reconsider the Principles of Humane Experimental Technique," ATLA Alternatives to Laboratory Animals, vol. 48, no. 1, 2020.

[99] R. G. W. Kirk, "Recovering The Principles of Humane Experimental Technique," Sci Technol Human Values, vol. 43, no. 4, 2018.

[100] Y. Gutfreund, "Harm-benefit analysis may not be the best approach to ensure minimal harms and maximal benefits of animal research—alternatives should be explored," 2020.

[101] B. J. Mohr et al., "Guidelines for the establishment and functioning of Animal Ethics Commitees (Institutional Animal Care and Use Committees) in Africa," Lab Anim, vol. 58, no. 1, 2024.

[102] E. T. Nsamba and M. L. Gupta, "Tubulin isotypes – functional insights from model organisms," 2022.

[103] Y. Du, Y. Liu, J. Hu, X. Peng, and Z. Liu, "CRISPR/Cas9 systems: Delivery technologies and biomedical applications," 2023.

[104] J. Pan et al., "The establishment and application of animal models for neurodegenerative diseases," 2023.

[105] C. Corrò, L. Novellasdemunt, and V. S. W. Li, "A brief history of organoids," Am J Physiol Cell Physiol, vol. 319, no. 1, 2020.

[106] G. Quadrato, J. Brown, and P. Arlotta, "The promises and challenges of human brain organoids as models of neuropsychiatric disease," 2016.

[107] Y. Liang, L. Zou, Y. Tian, S. Zhou, X. Chen, and C. Lin, "Dietary and metabolic risk of neuropsychiatric disorders: Insights from animal models," British Journal of Nutrition, vol. 126, no. 12, 2021.

[108] E. Finger et al., "Neurodevelopmental effects of genetic frontotemporal dementia in young adult mutation carriers," Brain, vol. 146, no. 5, 2023.

[109] L. T. Reiter, L. Potocki, S. Chien, M. Gribskov, and E. Bier, "A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster," Genome Res, vol. 11, no. 6, 2001.

[110] Z. Chen, F. Zhang, and H. Xu, "Human mitochondrial DNA diseases and Drosophila models," 2019.

[111] U. B. Pandey and C. D. Nichols, "Human disease models in drosophila melanogaster and the role of the fly in therapeutic drug discovery," Pharmacol Rev, vol. 63, no. 2, 2011.

Information for Authors

Submission: Manuscripts [in Word (.doc, .docx, .rtf)] should be submitted by one of the authors of the manuscript through the online submission system at www.thencleuspak.org.pk after registration of corresponding author. If for some technical reason on-line submission is not possible, then write an email describing the problem along with your phone no. at editorinchief@thenucleuspak.org.pk

Terms of Submission: Each submission to The Nucleus implies that the manuscript presents the results of original scientific research and has not been published nor has been submitted for publication elsewhere. The article is written in clear and Standard English. The reported research meets all applicable ethical standards and research integrity. The submitted manuscripts is screened for plagiarism during the editorial process.

Units of Measurement: should be presented simply and concisely using System International (SI) units.

Article Structure

Subdivision - numbered sections: The article should be divided into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Title: should be concise and informative. Avoid abbreviations and formulae where possible.

Author names and affiliations: Provide complete name of all the authors, their affiliation, complete postal addresses, contact numbers and e-mail addresses. Present the authors' affiliation addresses below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name. Clearly indicate the corresponding author by superscript*. Further when manuscript is under review process, as per policy of the journal, author cannot be addeded, deleted and sequence of author can't be altered.

Keywords: Provide a maximum of 6 keywords, These keywords will be used for indexing purposes.

Introduction Section: States the objectives of the work and provides an adequate background, avoiding a detailed literature survey or a summary of the results.

Experimental Section: should contain sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference, only relevant modifications should be described.

Theory/calculation S***ection:*** Should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results and Discussion: should provide the significance of the results of the work. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions: It should be presented in a short Conclusions section.

Acknowledgments: (if any) should be included at the very end of the paper before the references and may include supporting grants, presentations, and so forth.

References: We follow IEEE style for citations of references. Must be numbered consecutively and citations of references in text should be identified using numbers in square brackets (e.g., as discussed by Smith [9]; as discussed elsewhere [9, 10]). Reference to a publication:

[Ref number] Author's initials. Author's Surname, "Title of article," Title of journal abbreviated in Italics, vol. number, issue number, page numbers, Abbreviated Month Year.

[4] K.A. Nelson, R.J. Davis, D.R. Lutz, and W. Smith, "Optical generation of tunable ultrasonic waves," Journal of Applied Physics, vol. 53, no. 2, pp. 1144-1149, Feb., 2002.

For more details please see the link IEEE style for citations of different materials

Figures and Tables: Include all figures and tables in the word file of the manuscript. Figures and tables should not be submitted in separate files. If the article is accepted, authors may be asked to provide the source files of the figures. All figures should be cited in the paper in a consecutive order. In all figures, remove all unnecessary boxes, lines, marks. The resolution of all the figures must be at least 300 dpi. Tables should be cited consecutively in the text. Every table must have a descriptive title and if numerical measurements are given, the units should be included in the column heading. Vertical rules should not be used.

The Nucleus

An Open Access International Scientific Journal

ISSN : 0029-5698 (Print)
EISSN : 2306-6539 (Online)

Recognized by HEC in 'Y' Category

Call for Papers

Why Publish in The Nucleus?

- One of the Oldest Scientific Journals in Pakistan
- Regularly Published since 1964
- Published Both Electronically & in Paper Format
- Multidisciplinary
  - Natural Sciences
  - Applied Sciences
  - Engineering & Technology
  - Management Sciences
- Open Access
- Peer Reviewed*
- No Publication Charges
- High Visibility
- Electronic Submission
- Rapid On-line Publication (within three months)

Abstracted and Indexed in:

- Chemical Abstracts
- Biological Abstracts
- INIS Atom Index
- Bibliography of Agriculture (USA)
- The Institute of Electrical Engineers Publications
- Virology Abstracts (England)
- Pakistan Science Abstracts

For Further Information

Editorial Office The Nucleus
PINSTECH, 45650 Nilore
Islamabad. Pakistan
For Online
http://www.thenucleuspak.org.pk
E-mail
editorialoffice@thenucleuspak.org.pk

Why Perish when you can Publish in The Nucleus?

*Potential Reviewers are Invited to Submit their CV's Through E-mail