RECOGNIZED BY:

HIGHER EDUCATION COMMISSION OF PAKISTAN

INDEXING

Crossref

Google Scholar

Dimensions

ICMJE
INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

OCLC WorldCat®

EuroPub
Directory of Academic and Scientific Journals

LOCKSS

ASIAN Research Index

BASE

OPEN ARCHIVES

Scilit

World Health Organization
Regional Office for the Eastern Mediterranean

IMEMR
INDEX MEDICIUS
FOR THE EASTERN MEDITERRANEAN REGION

ASCI
Asian Science Citation Index

CAS
A division of the American Chemical Society

Aims and Scope

Futuristic Biotechnology (FBT) is a quarterly journal that publishes broad-spectrum publications with close connection to experimental activity in Biological and Biotechnology fields. FBT is intended for exploring the molecular mechanisms that support key biological processes in the fields of biochemistry, cellular biosciences, molecular biology, plant biotechnology, genetic engineering, nanotechnology, and bioinformatics. Furthermore, it also covers topics related to immunology, antibody production, protein purification studies, primer synthesis, DNA sequencing, production of transgenic animal models, insect resistant crop varieties and edible and ornamental plant varieties.

Types of Articles

- Research Papers
- Short Communications
- Review and Mini-reviews
- Commentaries
- Perspectives and Opinion
- Meta Analysis
- Case Reports
- Case Studies
- Case Control Studies

Reviews on recent progress in biotechnology are commissioned by the editors. The purpose of the Futuristic Biotechnology is to publish scientific and technical research papers to bring attention of International Researchers, Scientists, Academicians, and Health Care Professionals towards recent advancements in food sciences. The articles are collected in the form of reviews, original studies, clinical studies among others. It may serve as a global platform for scientists in relevant fields to connect and share ideas mutually. This journal is open to all the research professionals whose work fall within our scope. Submissions are welcome and may be submitted here.

editor@fbtjournal.com

Title
The title of the paper should provide a concise statement of the contents of the paper. A good title is very important and will attract readers and facilitate retrieval by online searches, thereby helping to maximize citations. The title should include topical keywords and allude to the interesting conclusions of the paper. A title that emphasizes the main conclusions, or poses a question, has more impact than one that just describes the nature of the study.

Running Head
Running head should be added in the header along with the page numbers.

Type of Article
Research Article/ Case Report/ Review Article/ Opinion/ Short Communication/ Mini Review/ Letter to Editor.

Running Title: A short version of the paper title.
Keywords: The major keywords used in the article have to be mentioned.
Authors
List here all author names, Author[1], Author[2] and Author[3]
[1]Author department, University, Country
[2]Author department, University, Country
[3]Author department, University, Country

***Corresponding Author**
Author name, Affiliation, Department Name, University Name, Address, City, State, Country, E-mail.

Abstract
Abstract should include a brief content of the article. It should be structured and not more than 250 words. It should include following sub headings: Objective, Methods, Results, Conclusions.

Abbreviations
If there are any abbreviations in the article they have to be mentioned.

INTRODUCTION
Provide a context or background for the study (i.e., the nature of the problem and its significance). State the specific purpose or research objective or hypothesis tested by the study or observation; the research objective is often more sharply focused when stated as a question. Both the main and secondary objectives should be made clear, and any pre-specified subgroup analyses should be described. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

METHODS
The Methods section should include only information that was available at the time the or plan of the protocol. All information gathered during the conduct of study should be included in the method section.
Study Design, Inclusion / Exclusion Criteria, Data Collection Procedure and Statistical Analysis.

RESULTS
Present your results in logical sequence in the text, tables and illustrations, giving the main or most important findings first.

Do not repeat data that is already present in tables and illustrations. emphasize or summarize only important observations. When data are summarized in the Results section, give numeric results not only as derivatives (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical methods used to analyze them. Table font should be 10 and caption should be above the table and below the figure.

Data should not be duplicated in both figures and tables. The maximum limit of tables and figures should not exceed more than 4. Mention the findings of the study in paragraph, while mentioning figure and table number in text in sequential order.

TABLE

Table should not be copy pasted or in picture form.

DISCUSSION

Discuss your findings by comparing your results with other literature.

REFERENCES

References should not be less than 20.
In text references should be in number style. For Example [1].
Follow the Pubmed Referencing style.
Provide the DOI link.

Example

Cook NR, Rosner BA, Hankinson SE, Colditz GA. Mammographic screening and risk factors for breast cancer. American Journal of Epidemiology. 2009 Dec;170(11):1422-32. doi: 10.1093/aje/kwp304.

If there are more than six authors, write *et al*. after the first six names.

CONCLUSION(S)

Conclusion should elucidate how the results communicate to the theory presented as the basis of the study and provide a concise explanation of the allegation of the findings.

ACKNOWLEDGEMENT

Provide the list of individuals who contributed in the work and grant details where applicable.

Plagiarism policy

Similarity index should be less than 19, and less than 5 from individual sources.

Authorship Letter

Signed authorship letter by all authors including there current department, University, City, Country, Email.

Declaration Form

Signed declaration form submit by corresponding author.

The submission of article should include: manuscript according to journal guidelines, authorship letter, declaration form. It should be submitted to the following email id: editor@fbtjournal.com

FUTURISTIC BIOTECHNOLOGY

ISSN (E) 2959-0981
ISSN (P) 2959-0973

EDITORAL BOARD

ADVISORY BOARD

VOL. 05 ISSUE. 02

ISSN (E) 2959-0981
ISSN (P) 2959-0973

FUTURISTIC BIOTECHNOLOGY

VOL. 05 ISSUE. 02

EDITORIAL BOARD (INTERNATIONAL)

EDITORIAL BOARD (NATIONAL)

FUTURISTIC BIOTECHNOLOGY

ISSN (E) 2959-0981
ISSN (P) 2959-0973

TABLE OF CONTENTS

VOL. 05 ISSUE. 02

FUTURISTIC BIOTECHNOLOGY

ISSN (E) 2959-0981
ISSN (P) 2959-0973

TABLE OF CONTENTS

VOL. 05 ISSUE. 02

DOI: https://doi.org/10.54393/fbt.v5i2.176

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

STEM Education Unites a Divided World

Aditya Mojumdar[1]

[1]University of Victoria, Canada
amojumdar@uvic.ca

ARTICLE INFO

How to Cite:

Mojumdar, A. (2025). STEM Education Unites a Divided World. Futuristic Biotechnology, 5(2), 01. https://doi.org/10.54393/fbt.v5i2.176

The political tensions and the clash of cultures separate the countries as never before. However, in one field, there is hope for peace in the world of STEM education. The world speaks the same language in mathematics, physics, and engineering. A chemical reaction in Seoul will do the same in São Paulo. This common base opens areas of cooperation that are beyond borders and ideologies.

Common problem-solving styles are taught in STEM education. When learning to solve a problem about robotics competition or climate research together, students in different countries find the things they have in common and forget about the differences in culture. This unity may be illustrated by the International Mathematical Olympiad, which has been operating since 1959, when students of more than 100 nations compete with one another using the same mathematical principles in spite of a huge diversity in their backgrounds.

This unifying effect has research to back it up. Research by the Organization for Economic Co-operation and Development in 2019 revealed that nations with robust STEM research collaborations internationally displayed a 23 percent rise in scientific advancements and innovation.

Somehow, big obstacles exist anyway. The digital divide is generated due to a lack of equality in education- advanced nations are equipped with the latest facilities, while basic equipment is not available in poor states. Such inequality only tends to deepen the already existing rifts instead of reversing them. The barriers also come in the form of cultural resistance. Other societies will not embrace principles that contradict traditional beliefs and may therefore reduce the integration of STEM.

These are aggravated by gender barriers. In the report published by UNESCO in 2020, it was revealed that women make up a mere 28 percent of the researchers working in STEM areas, and participation remains even lower in studies in countries with social limits. This marginalization is the waste of human talent and division.

Revolutionary technologies democratize STEM education globally. Virtual reality enables equal laboratory experiences from Bangladesh to Boston. European Space Agency simulators connect 50,000 students across 30 countries. Cloud platforms like GitHub host 200 million student projects worldwide. AI tutors operate in 46 languages, personalizing learning while maintaining scientific accuracy.

The STEM education establishes world harmony to global standards of scientific realities, where diplomacy cannot make a mark. This possibility lies in the COVID-19 vaccine partnership and the International Space Station. To succeed, we should eradicate inequalities, make investments, and break down the barriers. We have a choice to make: we can use STEM as a unifier, or the rifts in the world can get even wider.

REFERENCES

[1] Marrero ME, Gunning AM, Germain-Williams T. What is STEM Education?. Global Education Review. 2014 Nov; 1(4).

[2] Dong Y, Ma H, Shen Z, Wang K. A Century of Science: Globalization of Scientific Collaborations, Citations, and Innovations. Inproceedings Of The 23rd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining 2017 Aug; 1437-1446. doi: 10.1145/3097983.3098016

DOI: https://doi.org/10.54393/fbt.v5i2.167

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Review Article

OPEN ACCESS

DNA Vaccines Against Foot-and-Mouth Disease: A Novel Biotechnological Strategy

Suliman Khan[1], Muhammad Tauseef Ahmad[2*], Bilal Abbas[3], Ehsan Kakar[4], Adeel Nasir[5], Muhammad Fayaz Taj[6], Muhammad Asad Raza[4], Abdul Samad[7], Ahmed Raza[4] and Sanwal Abbas[4]

[1]Centre for Biotechnology and Microbiology, University of Swat, Swat, Pakistan
[2]Department of Livestock and Dairy Development, Government of Punjab, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Biomedical Research Center, South China College of Life Sciences, Fujian Normal University, China
[4]Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan
[5]Faculty of Veterinary and Animal Sciences, Arid Agriculture University, Rawalpindi, Pakistan
[6]Faculty of Veterinary and Animal Sciences, Sindh Agriculture University Tandojam, Pakistan
[7]Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat, Pakistan

ARTICLE INFO

Keywords:
Foot-and-Mouth Disease, DNA Vaccines, Immunological Response, Humoral Immunity

How to Cite:
Khan, S., Ahmad, M. T., Abbas, B., Kakar, E., Nasir, A., Taj, M. F., Raza, M. A., Samad, A., Raza, A., & Abbas, S. (2025). DNA Vaccines Against Foot-and-Mouth Disease: A Novel Biotechnological Strategy: DNA Vaccines Against Foot-and-Mouth Disease. Futuristic Biotechnology, 5(2), 02-10. https://doi.org/10.54393/fbt.v5i2.167

***Corresponding Author:**
Muhammad Tauseef Ahmad
Department of Livestock and Dairy Development, Government of Punjab, University of Veterinary and Animal Sciences, Lahore, Pakistan
vet.tauseef@gmail.com

Received Date: 7th April, 2025
Revised Date: 25th May, 2025
Acceptance Date: 10th June, 2025
Published Date: 30th June, 2025

ABSTRACT

This review article points out the Foot-and-Mouth Disease (FMD) as a major global animal disease of serious economic importance and limitations of conventional inactivated whole-virus vaccines. It gives DNA vaccine technology as a potential and safer way of achieving immunity by expressing microbial antigens in host cells and thereby eliciting both humoral and cellular immunity responses. The principles and mechanisms of the DNA vaccines are described in the article: antigen presentation, activation of T- T-cells, as well as the functions of adjuvants, the delivery methods, and electroporation, gene gun, and nanoparticles in the increasing of their efficacy. In addition, it summarizes the advancement in the FMD DNA vaccines against diverse viral proteins such as VP1 and the P1 polyprotein, reviews conducted experimental studies and studies in efficacy trials on animal models and the targeted livestock animal.

INTRODUCTION

Foot-and-mouth disease (FMD) is a paramount animal disease and zoonotic with serious, highly contagious, and economically ruinous viral illness that affects millions of cloven-hoofed animals all over the world (cattle, pigs, deer, goats, and sheep) [1]. Animal disease pathogen, first identified as a virus in the 16th century, and the very first animal disease pathogen identified [2]. The disease not only deteriorates the commercial values of livestock by loss of weight and milk output, but also results in enormous economic loss to livestock producers and industry [3]. Involving fever and manifestation of vesicular lesions of the mouth and feet, widespread patterns and rapid transmission of FMD as well as the presence of seven serotypes of the foot-and-mouth disease virus (FMDV) pose challenges to the control [4]. For several decades now, inactivated whole-virus vaccination has been the most helpful approach to the prevention and control of FMD. Nevertheless, even though, after more than 70 years, there is a vaccine at our disposal, FMD is an endemic disease in the world. There are a few drawbacks associated

DOI: https://doi.org/10.54393/fbt.v5i2.167

with these traditional vaccines, such as a lack of induction of long-term protection, restricted antigen coverage, and a lack of ability to curb infection [5-7]. The advances in biotechnology have opened new doors for synthesizing specific proteins through microorganisms, and the DNA vaccines were a breakthrough made in that regard [8,9].

Despite decades of reliance on inactivated whole-virus vaccines, Foot-and-Mouth Disease (FMD) continues to persist in many endemic regions, highlighting the need for more effective and long-lasting immunization strategies. Although DNA vaccines have emerged as a promising biotechnological alternative, existing literature remains fragmented regarding their immunogenicity, delivery optimization, and large-scale applicability in target livestock species. Furthermore, limited synthesis of current advancements in adjuvant strategies, delivery systems, and efficacy outcomes creates a gap in understanding their translational potential. Therefore, a comprehensive evaluation of DNA vaccine development and performance against FMDV is warranted.

FMD: A Global Threat

FMD is one of the major animal diseases that is linked to a severe and highly contagious, economically devastating viral infection of numerous cloven-hoofed animals [10]. Epidemics may cause significant monetary losses and disrupt the production of animals and animal products. The high rate and varied nature of the FMD virus require intervention measures to avert the destructive effects [11].

Etiology and Pathogenesis of FMD Virus (FMDV)

FMD is caused by the FMDV. FMDV is the prototype of the genus Aphthovirus in the family Picornaviridae [12]. It is a virus that consists of RNA with an icosahedral capsid. FMDV is known for the existence of seven serotypes that are reported to be immunologically distinct: A, O, C, SAT1, 2 & 3, and Asia1 [13]. There is also a great genetic and antigenic variation of strains within each serotype. Historically, the first animal disease pathogen to be found as a virus was FMDV [14-15]. The children of the disease may be higher in young animals with occasional mortality as a result of myocardial degeneration. FMDV is also able to establish a carrier state, especially in cases of cattle, in which persistently infected animals can shed the virus [16].

Transmission and Economic Impact

FMDV can be transmitted through various routes. These include direct contact with infected animals, as well as indirect contact via contaminated materials such as agricultural tools, vehicles, and animal products [17]. Airborne transmission over short distances can also occur. Factors such as animal movement, trade, community grazing, and insufficient surveillance contribute to the spread of the disease [18]. This contamination can then lead to new infections through direct contact with aerosols, indirect contact with contaminated materials, or ingestion of contaminated products (Figure 1).

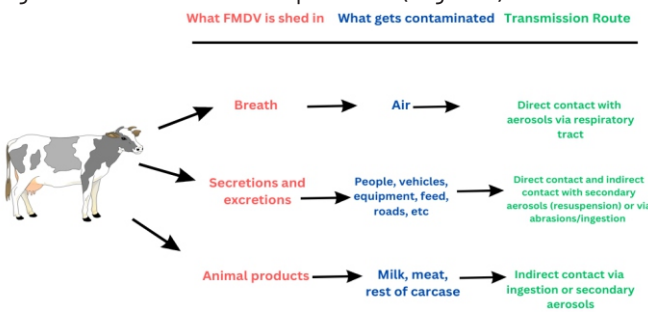

Figure 1: The Pathways of FMDV Spread from Infected Animals Through Breath, Secretions/Excretions, and Animal Products, Leading to Contamination and Subsequent Infection Via Direct or Indirect Contact

FMD poses a major economic threat to agriculture worldwide. Outbreaks result in direct losses due to reduced weight gain, decreased milk production, and mortality, especially in young animals [19]. Affected countries often face trade embargoes, leading to significant financial losses for livestock producers and the industry as a whole [20]. The 1997 outbreak in Taiwan, caused by a CHY topotype virus, resulted in the slaughter of over 4 million pigs and over 6 billion U.S. dollars in financial losses. Control and eradication efforts also incur substantial costs [21]. Vaccination with inactivated whole-virus vaccines has been the most widely used method for prevention and control for over 70 years [22]. In India, a nationwide FMD Control Programme (FMD-CP) using an indigenously produced killed trivalent vaccine has shown encouraging results in reducing disease incidence. However, conventional vaccines have limitations such as failure to induce long-term protection, narrow antigenic coverage, and inability to prevent infection [23].

DNA Vaccines: Principles and Mechanisms

DNA vaccines are a modern approach utilizing engineered plasmid DNA encoding specific antigens under a strong promoter to elicit immunity [24-26]. Upon delivery (e.g., direct injection or gene gun [27], the plasmid enters host cells, where the antigen gene is transcribed and translated [28, 29]. Immunogenicity arises from the plasmid DNA's inherent adjuvant properties, initially attributed to CpG motifs and more recently linked to TLR-independent TBK1 signalling triggered by its double-stranded structure [30-32], absence of live pathogens eliminating infection risk, focused immune response on the target antigen (without inducing anti-DNA antibodies) [33], rapid and cost-effective production via standard molecular biology, ease of modification for different targets, enhanced antigen presentation due to in vivo expression and potential post-translational modifications [34-35], and the ability to polarize T-cell responses [36].

Development of DNA Vaccines Against FMD

The development of DNA vaccines has emerged as a

DOI: https://doi.org/10.54393/fbt.v5i2.167

promising and safer alternative to traditional inactivated vaccines for FMD [37]. A lot of efforts have been aimed at targeting several viral proteins for the development of FMD DNA vaccines, especially on structural proteins [38]. VP1, the most abundant capsid protein of FMDV, is also the most intensively studied one, as it has proven to be immunogenic [39]. Using one of the approaches, the VP1 gene was engineered into the Hepatitis B virus (HBV) core gene to produce a chimeric core-VP1 VLP that, with a DNA construct, produced significantly high immune responses and protection as compared to a standard VP1 DNA construct [40]. Another important target is the P1 polyprotein that is cleaved to yield the structural proteins: VP0, VP3, and VP1 [41]. DNA vaccines containing the P1 region, have been effective in triggering an immune response and protection in pigs [42]. Combination of B- and T-cell epitopes has also been examined in DNA vaccine designs and has shown protection in mice even without specific antibodies at the challenge level [43]. DNA vaccines for FMD are typically plasmid-based [44]. A common promoter used in DNA vaccine studies is the cytomegalovirus (CMV) immediate-early promoter. [45]. Some plasmids have been designed to express not only the antigen but also immunoregulatory molecules like cytokines (e.g., interleukins, interferons, GM-CSF) or co-stimulatory molecules as genetic adjuvants to boost the immune response [46]. The FMDV Vaccine Development Strategies illustrate various approaches, including whole inactivated, modified live attenuated, virus-like particles, recombinant viral vectors, DNA, and peptides [47].

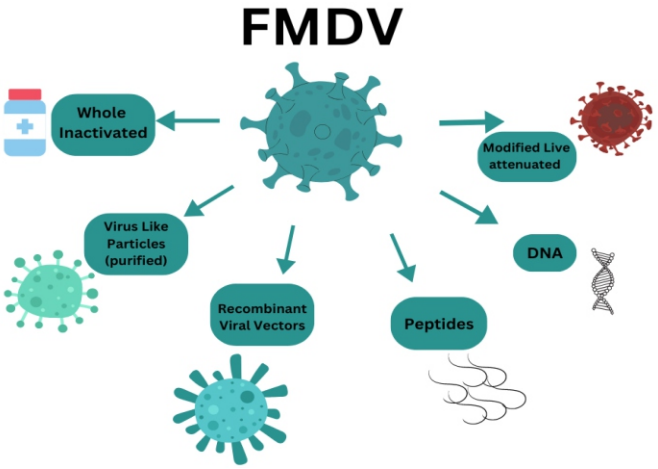

Figure 2: Various Approaches Including Whole Inactivated, Modified Live Attenuated, Virus-Like Particles, Recombinant Viral Vectors, DNA, and Peptides

Delivery Methods (Electroporation, Gene Gun, Nanoparticles)

The efficacy of DNA vaccines can be influenced significantly by the method of delivery, which affects the uptake of plasmid DNA by host cells [47]. Several approaches have been explored to enhance DNA delivery and immunogenicity for FMD vaccines. Electroporation involves the application of brief electrical pulses to the injection site, which transiently increases cell membrane permeability, facilitating DNA uptake [48]. This method increases the transfection efficiency of DNA vaccines in vivo, provoking a better immune response to FMDV. Gene gun (biolistics) involves a mortar-like tool to shoot the gold micro particles coated with DNA right into the skin with the help of the gas pressure [49]. This approach attacks the antigen-presenting cells in the skin and has been used for FMD DNA vaccination to show the development of the immune responses. Nanoparticles like mannosylated chitosan nanoparticles and calcium phosphate nanoparticles have been examined for use as the delivery method for the FMDV DNA vaccines [50]. These nanoparticles can prevent the degradation of DNA and can increase cellular uptake and improve the immunological values and protection for viral challenge. For instance, mannosylated chitosan nanoparticles loaded with FMDV VP1-Ompa DNA vaccine indicated a promising immunological evaluation in guinea pigs [51].

Immunological Effects Caused by FMD DNA Vaccines

The DNA vaccines for FMD target both humoral and cellular immune responses with the delivery of genetic materials encoding the antigens of FMDV into host's cells. These vaccines utilize host's cells for production of viral proteins that are processed and presented to the immune response [52].

Humoral and Cellular Immunity

FMD DNA vaccines can produce neutralizing antibody responses, which are known to be the primary immunity against FMDV. The major capsid protein VP1 protein that is frequently targeted to induce these antibodies. For instance, a DNA construct of a chimeric core-VP1 virus-like particle caused far greater amounts of antigen-specific IgG production and neutralizing antibodies in mice than a regular VP1 DNA construct [53]. Other than humoral immunity, cell-mediated immunity (CMI) can be stimulated by FMD DNA vaccines [54]. The endogenously expressed antigens are presented via both MHC class I and class II pathways, leading to the activation of these T cell subsets. Studies have shown that FMD DNA vaccines can induce FMDV-specific T cell proliferation and CTL responses [55].

T-Cell Activation and Memory Response

Upon uptake of the DNA vaccine by host cells, including antigen-presenting cells (APCs) like dendritic cells, the encoded antigen is expressed and processed [56, 57]. FMD DNA vaccination can lead to the generation of memory T cells, contributing to long-term immunity [58].

Role of Adjuvants and Co-Stimulatory Molecules

The immunogenicity of FMD DNA vaccines can be

DOI: https://doi.org/10.54393/fbt.v5i2.167

significantly enhanced by the use of adjuvants and co-stimulatory molecules. Due to the sometimes lower immunogenicity of DNA vaccines compared to traditional vaccines, various strategies have been employed to boost the immune response [59]. Genetic adjuvants, such as cytokines and chemokines, can be co-expressed from the same plasmid or delivered separately. Several cytokines have shown promise in enhancing FMD DNA vaccine efficacy. IL-6 has been shown to advance the cellular immune response and promote the maturation of dendritic cells [60]. IL-15 has been shown to enhance cellular and mucosal immune responses and the level of IFN-γ induced by FMD DNA vaccines [61]. Intranasal administration of FMDV DNA vaccine with IL-15 as an adjuvant induced enhanced CMI. IL-18 can increase the immunogenicity of DNA vaccines. Co-administration of bovine IL-18 with a DNA vaccine gave a protective immune response in cattle. GM-CSF as an adjuvant with a DNA vaccine encoding P1-2A induced robust FMDV-specific and neutralizing antibodies in swine [62, 63]. Delivery methods can also act as adjuvants by improving DNA uptake and transfection efficiency. Electroporation and gene gun delivery have been shown to enhance immune responses against FMDV DNA vaccines. Nanoparticles, such as mannosylated chitosan nanoparticles, have demonstrated substantial improvements in immunological parameters [64]. Cationic PLGA micro particles used to coat DNA vaccines have also resulted in long-term immune responses.

Experimental Studies and Efficacy Trials

Experimental studies and efficacy trials are crucial steps in the development of foot-and-mouth disease (FMD) vaccines to evaluate their immunogenicity and protective potential before widespread use. These studies involve rigorous testing in animal models, followed by trials in target livestock species.

Experimental Studies

Mice and guinea pigs are often used as initial small animal models to assess the immunogenicity of FMD vaccine candidates, including DNA vaccines, subunit vaccines, and empty capsid vaccines [65]. For instance, mice have been used to analyze the immune response induced by DNA constructs encoding FMDV antigens. Guinea pigs have been used to evaluate the immunological parameters of nanoparticle-based DNA vaccines. However, it's important to note that results in these models may not always directly correlate with those in natural hosts like cattle and swine. These studies typically measure humoral immune responses, such as the induction of neutralizing antibodies [66]. Assays like plaque-reduction assays are used to detect specific neutralizing antibodies against FMDV in serum samples. Cellular immune responses, including T cell proliferation, cytotoxic T lymphocyte (CTL) responses, and cytokine production (e.g., IFN-γ), are also evaluated [67]. For example, one study compared the immune responses induced by a chimeric core-VP1 DNA vaccine and a regular VP1 DNA construct in mice by assessing IgG production, T cell proliferation, CTL response, and cytokine production. Before in vivo testing, studies might involve in vitro experiments to assess antigen expression in transfected cells. For example, the expression levels of DNA vaccine constructs were studied in HeLa cells and HEK and CHO cells [68].

Efficacy Trials

Efficacy trials in cattle and swine, the natural hosts of FMDV, assess a vaccine's protective ability against viral challenge. Serum neutralizing antibody levels often correlate with protection [69]. Vaccinated animals are challenged with live FMDV, and protection is evaluated by monitoring clinical signs (e.g., vesicular lesions) and sometimes sub-clinical infection and viral persistence [70-73], DNA vaccines in swine). Overall, efficacy testing progresses from small animal models to target species, rigorously evaluating clinical protection and ideally, sterile immunity [74]. Measuring humoral and cellular responses, are critical, as is considering scalability for industrial production [75].

Limitations and Future Prospects

This review is limited by its reliance on currently available experimental and preclinical studies, with relatively fewer large-scale field trials evaluating DNA vaccine efficacy in natural host species. Challenges such as suboptimal immunogenicity, delivery efficiency, scalability, regulatory approval, and cost-effectiveness continue to restrict widespread application. Future research should focus on enhancing plasmid design, incorporating novel molecular adjuvants, improving delivery platforms such as nanoparticle-based systems and electroporation, and conducting robust field-based efficacy trials. Addressing these aspects will be critical for translating DNA vaccine technology into practical and commercially viable solutions for FMD control.

CONCLUSION

In conclusion, this review article highlights FMD as a significant global threat to animal agriculture, underscoring the limitations of traditional inactivated whole-virus vaccines despite their long history of use. It presents DNA vaccine technology as a novel and promising biotechnological strategy that offers several advantages, including enhanced safety profiles and the potential to induce both humoral and cellular immune responses by expressing microbial antigens within host cells. The article details the principles, mechanisms of action, and various

DOI: https://doi.org/10.54393/fbt.v5i2.167

approaches to enhance the efficacy of FMD DNA vaccines, such as targeting specific viral proteins like VP1 and the P1 polyprotein, utilising adjuvants and advanced delivery methods like electroporation and nanoparticles. While acknowledging the progress made in experimental studies and efficacy trials, the review also recognises the challenges and limitations that currently hinder the widespread application of DNA vaccines against FMD, paving the way for future research and development efforts to overcome these obstacles and realise the full potential of this innovative approach.

Authors' Contribution

Conceptualization: SK, TA
Methodology: BA, EK
Formal analysis: AN, MFT
Writing and Drafting: AS, AR, SA
Review and Editing: AS, AR, SA, AN, MFT, BA, EK

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Suchowski M, Eschbaumer M, Teifke JP, Ulrich R. After Nasopharyngeal Infection, Foot-and-Mouth Disease Virus Serotype A RNA Is Shed in Bovine Milk without Associated Mastitis. Journal of Veterinary Diagnostic Investigation. 2021 Sep; 33(5): 997-1001. doi: 10.1177/10406387211022467.

[2] Ghonaim AH, Rouby SR, Hopo MG, Li W. Foot-and-Mouth Disease: A Persistent Challenge for the Livestock Industry. In Veterinary Virology of Domestic and Pet Animals. Cham: Springer Nature Switzerland. 2024 Dec: 1-19. doi: 10.1007/978-3-031-54690-7_31-1.

[3] Lu Z, Yu S, Wang W, Chen W, Wang X, Wu K, Li X, Fan S, Ding H, Yi L, Chen J. Development of Foot-And-Mouth Disease Vaccines in Recent Years. Vaccines. 2022 Oct; 10(11): 1817. doi: 10.3390/vaccines10111817.

[4] Kenubih A. Foot and Mouth Disease Vaccine Development and Challenges in Inducing Long-Lasting Immunity: Trends and Current Perspectives. Veterinary Medicine: Research and Reports. 2021 Sep: 205-15. doi: 10.2147/VMRR.S319761.

[5] Fang CY and Liu CC. Novel Strategies for the Development of Hand, Foot, and Mouth Disease Vaccines and Antiviral Therapies. Expert Opinion on Drug Discovery. 2022 Jan; 17(1): 27-39. doi: 10.1080/17460441.2021.1965987.

[6] Haynie T. Recombinant DNA Vaccine Design as A Potential Strategy Against Bovine Foot and Mouth Disease Virus (FMDV). Louisiana State University and Agricultural and Mechanical College. 2023.

[7] Puckette M, Clark BA, Barrera J, Neilan JG, Rasmussen MV. Evaluation of DNA Vaccine Candidates Against Foot-And-Mouth Disease Virus in Cattle. Vaccines. 2023 Feb; 11(2): 386. doi: 10.3390/vaccines11020386.

[8] Elrashedy A, Nayel M, Salama A, Zaghawa A, El-Shabasy RM, Hasan ME. Foot-and-Mouth Disease: Genomic and Proteomic Structure, Antigenic Sites, Serotype Relationships, Immune Evasion, Recent Vaccine Development Strategies, and Future Perspectives. Veterinary Research. 2025 Apr; 56(1): 78. doi: 10.1186/s13567-025-01485-0.

[9] Lu Y, Dong H, Li J, Li L, Wang M, Liu H *et al.* Enhanced Protective Immune Response of Foot-and-Mouth Disease Vaccine Through DNA-Loaded Virus-Like Particles. Microbial Pathogenesis. 2020 Jun; 143: 104130. doi: 10.1016/j.micpath.2020.104130.

[10] Belsham GJ, Kristensen T, Jackson T. Foot-and-Mouth Disease Virus: Prospects for Using Knowledge of Virus Biology to Improve Control of This Continuing Global Threat. Virus Research. 2020 May; 281: 197909. doi: 10.1016/j.virusres.2020.197909.

[11] Aslam M and Alkheraije KA. The Prevalence of Foot-and-Mouth Disease in Asia. Frontiers in Veterinary Science. 2023 Jun; 10: 1201578. doi: 10.3389/fvets.2023.1201578.

[12] Azeem A, Rashid I, Hassan MM, Asad M , Kaukab G, Tehseen A *et al.* 10. A Review on Foot and Mouth Disease in Dairy Animals, Etiology, Pathogenesis and Clinical Findings. Pure and Applied Biology. 2020 Feb; 9(1): 821-32. doi: 10.19045/bspab.2020.90088.

[13] Sukoco H, Wahyuni S, Utami S, Cahyani AP, Andanawari S, Siswanto FM. Foot and Mouth Disease (FMD): Etiology, Pathogenesis, Prevention and Control in Even or Split Hoofed Livestock. Jurnal Sain Peternakan Indonesia. 2023 Dec; 18(4): 268-73. doi: 10.31186/jspi.id.18.4.268-273.

[14] Zhang Z, Waters R, Li Y. Pathogenesis of Non-Epithelial Foot-and-Mouth Disease in Neonatal Animals. Veterinary Microbiology. 2021 Mar; 254: 108961. doi: 10.1016/j.vetmic.2020.108961.

[15] Raoof HS. Foot and Mouth Disease (FMD). Assiut Veterinary Medical Journal. 2024 Jul; 70(182): 1-8. doi: 10.21608/avmj.2024.274826.1232.

[16] Shimelis E, Tesfaye S, Tadesse F. Diagnostic Overview of Foot-and-Mouth Disease Viruses

DOI: https://doi.org/10.54393/fbt.v5i2.167

(FMDV). South Asian Research Journal of Agriculture and Fisheries. 2023; 5(4): 36-44. doi: 10.36346/sarjaf.2023.v05i04.002.

[17] Marschik T, Kopacka I, Stockreiter S, Schmoll F, Hiesel J, Höflechner-Pöltl A *et al.* The Epidemiological and Economic Impact of a Potential Foot-and-Mouth Disease Outbreak in Austria. Frontiers in Veterinary Science. 2021 Jan; 7: 594753. doi: 10.3389/fvets.2020.594753.

[18] He DM, Qian KX, Shen GF, Li YN, Zhang ZF, Su ZL *et al.* Stable Expression of Foot-and-Mouth Disease Virus Protein VP1 Fused with Cholera Toxin B Subunit in the Potato (Solanum Tuberosum). Colloids and Surfaces B: Bio-interfaces. 2007 Apr; 55(2): 159-63. doi: 10.1016/j.colsurfb.2006.11.043.

[19] Abdelkarim OF, Rehman SU, Samad A, Khan IU, Akram MW, Sayeed MA *et al.* Biotechnological Approaches to Discovery of Drugs for Veterinary Use: Discovery of Drugs for Veterinary Use. Futuristic Biotechnology. 2024 Dec: 21-8. doi: 10.54393/fbt.v4i04.150.

[20] Zewdie G, Akalu M, Tolossa W, Belay H, Deresse G, Zekarias M *et al.* A Review of Foot-and-Mouth Disease in Ethiopia: Epidemiological Aspects, Economic Implications, and Control Strategies. Virology Journal. 2023 Dec; 20(1): 299. doi: 10.1186/s12985-023-02263-0.

[21] Yadav MP, Singh RK, Malik YS. Epidemiological Perspective in Managing Viral Diseases in Animals. Recent Advances in Animal Virology. 2019: 381-407. doi: 10.1007/978-981-13-9073-9_20.

[22] Barrera J, Schutta C, Pisano M, Grubman MJ, Brake DA, Miller T *et al.* Use of ENABL® Adjuvant to Increase the Potency of an Adenovirus-Vectored Foot-and-Mouth Disease Virus Serotype a Subunit Vaccine. Vaccine. 2018 Feb; 36(8): 1078-84. doi: 10.1016/j.vaccine.2018.01.026.

[23] Robinson L, Knight-Jones TJ, Charleston B, Rodriguez LL, Gay CG, Sumption KJ *et al.* Global Foot-and-Mouth Disease Research Update and Gap Analysis: 3-Vaccines. Transboundary and Emerging Diseases. 2016 Jun; 63: 30-41. doi: 10.1111/tbed.12521.

[24] Kozak M and Hu J. DNA Vaccines: Their Formulations, Engineering and Delivery. Vaccines. 2024 Jan; 12(1): 71. doi: 10.3390/vaccines12010071.

[25] Qin F, Xia F, Chen H, Cui B, Feng Y, Zhang P, Chen J, Luo M. A Guide to Nucleic Acid Vaccines in the Prevention and Treatment of Infectious Diseases and Cancers: From Basic Principles to Current Applications. Frontiers in Cell and Developmental Biology. 2021 May; 9: 633776. doi: 10.3389/fcell.2021.633776.

[26] Koonin EV, Krupovic M, Agol VI. The Baltimore Classification of Viruses 50 Years Later: How Does It Stand in the Light of Virus Evolution? Microbiology and Molecular Biology Reviews. 2021 Aug; 85(3): 10-128. doi: 10.1128/MMBR.00053-21.

[27] Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Dempsey DM *et al.* Changes to Virus Taxonomy and the Statutes Ratified by the International Committee on Taxonomy of Viruses (2020). Archives of Virology. 2020 Aug; 165: 2737–2748. doi: 10.1007/s00705-020-04752-x.

[28] Harryvan TJ, de Lange S, Hawinkels LJ, Verdegaal EM. The ABCs of Antigen Presentation by Stromal Non-Professional Antigen-Presenting Cells. International Journal of Molecular Sciences. 2021 Dec; 23(1): 137. doi: 10.3390/ijms23010137.

[29] Baljon JJ and Wilson JT. Bioinspired Vaccines to Enhance MHC Class-I Antigen Cross-Presentation. Current Opinion in Immunology. 2022 Aug; 77: 102215. doi: 10.1016/j.coi.2022.102215.

[30] Webster RG and Robinson HL. DNA Vaccines: A Review of Developments. Bio-Drugs. 1997 Oct; 8: 273-92. doi: 10.2165/00063030-199708040-00004.

[31] Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K *et al.* A DNA Vaccine Induces SARS Coronavirus Neutralization and Protective Immunity in Mice. Nature. 2004 Apr; 428(6982): 561-4. doi: 10.1038/nature02463.

[32] McLaws M, Ahmadi BV, Condoleo R, Limon G, Kamata A, Arshed M *et al.* Risk of foot-and-mouth disease SAT2 introduction and spread in countries in the Near East and West Eurasia: FAO Qualitative Risk Assessment, October 2023. Food and Agriculture Organization. 2023 Oct.

[33] Wells TJ, Esposito T, Henderson IR, Labzin LI. Mechanisms of Antibody-Dependent Enhancement of Infectious Disease. Nature Reviews Immunology. 2025 Jan; 25(1): 6-21. doi: 10.1038/s41577-024-01067-9.

[34] Mort M, Convery I, Baxter J, Bailey C. Psychosocial Effects of the 2001 UK Foot and Mouth Disease Epidemic in A Rural Population: Qualitative Diary-Based Study. British Medical Journal. 2005 Nov; 331(7527): 1234. doi: 10.1136/bmj.38603.375856.68.

[35] Kim JY, Park SY, Jin JS, Kim D, Park JH, Park SH *et al.* Efficacy of Binary Ethylenimine in the Inactivation of Foot-and-Mouth Disease Virus for Vaccine Production in South Korea. Pathogens. 2023 May; 12(6): 760. doi: 10.3390/pathogens12060760.

[36] Boshart M, Weber F, Jahn G, Dorsch-H K, Fleckenstein B, Schaffner W. A Very Strong Enhancer Is Located Upstream of an Immediate Early Gene of

DOI: https://doi.org/10.54393/fbt.v5i2.167

Human Cytomegalovirus. Cell. 1985 Jun; 41(2): 521-30. doi: 10.1016/S0092-8674(85)80025-8.

[37] Ivanov G, Klement E, Gelman B, Elnekave E, Karniely S. Foot and Mouth Disease Viruses Are Recurrently Introduced to Israel and Spread by Extensively Reared Sheep and Cattle: Insights from A Whole-Genome Sequence Analysis. Virology. 2024 Feb; 590: 109950. doi: 10.1016/j.virol.2023.109950.

[38] Zhang X, Zhang Y, Li H, Liu L. Hand-Foot-And-Mouth Disease-Associated Enterovirus and the Development of Multivalent HFMD Vaccines. International Journal of Molecular Sciences. 2022 Dec; 24(1): 169. doi: 10.3390/ijms24010169.

[39] Li K, Wang C, Yang F, Cao W, Zhu Z, Zheng H. Virus-Host Interactions in Foot-and-Mouth Disease Virus Infection. Frontiers in Immunology. 2021 Feb; 12:571509. doi: 10.3389/fimmu.2021.571509.

[40] Aberle JH, Aberle SW, Allison SL, Stiasny K, Ecker M, Mandl CW *et al.* A DNA Immunization Model Study with Constructs Expressing the Tick-Borne Encephalitis Virus Envelope Protein E in Different Physical Forms. The Journal of Immunology. 1999 Dec; 163(12): 6756-61. doi: 10.4049/jimmunol.163.12.6756.

[41] Tobing TM, Rantam FA, Widiyatno TV, Tacharina MR, Rahmahani J, Triakoso N *et al.* Inactivation of an Indonesian Isolate of Foot-and-Mouth Disease Virus Using Formaldehyde. Veterinary World. 2024 Jun; 17(6): 1190. doi: 10.14202/vetworld.2024.1190-1195.

[42] Mikhalishin DV, Mikhalishin VV, Gochmuradov YM, El'kina YS. Inactivation of Foot and Mouth Disease Virus for Vaccine Production. Veterinary Science Today. 2023; 12(2):164. doi: 10.29326/2304-196X-2023-12-2-164-170.

[43] Yadav S, Delgado AH, Hagerman AD, Bertram MR, Moreno-Torres KI, Stenfeldt C *et al.* Epidemiologic and Economic Considerations Regarding Persistently Infected Cattle During Vaccinate-to-Live Strategies for Control of Foot-and-Mouth Disease in FMD-Free Regions. Frontiers in Veterinary Science. 2022 Oct; 9: 1026592. doi: 10.3389/fvets.2022.1026592.

[44] Yang Y, Yuan H, Zhang Y, Luan J, Wang H. Progress in African Swine Fever Vector Vaccine Development. International Journal of Molecular Sciences. 2025 Jan; 26(3): 921. doi: 10.3390/ijms26030921.

[45] Bhatt M, Mohapatra JK, Pandey LK, Mohanty NN, Das B, Prusty BR *et al.* Mutational Analysis of Foot and Mouth Disease Virus Nonstructural Polyprotein 3AB-Coding Region to Design A Negative Marker Virus. Virus Research. 2018 Jan; 243: 36-43. doi: 10.1016/j.virusres.2017.10.010.

[46] Dong H, Liu P, Bai M, Wang K, Feng R, Zhu D *et al.* Structural and Molecular Basis for Foot-and-Mouth Disease Virus Neutralization by Two Potent Protective Antibodies. Protein and Cell. 2022 Jun; 13(6): 446-53. doi: 10.1007/s13238-021-00828-9.

[47] Singh RK, Sharma GK, Mahajan S, Dhama K, Basagoudanavar SH, Hosamani M *et al.* Foot-and-Mouth Disease Virus: Immunobiology, Advances in Vaccines and Vaccination Strategies Addressing Vaccine Failures—An Indian Perspective. Vaccines. 2019 Sep; 7(3): 90. doi: 10.3390/vaccines7030090.

[48] Qiu Y, Abila R, Rodtian P, King DP, Knowles NJ, Ngo LT *et al.* Emergence of an Exotic Strain of Serotype O Foot-and-Mouth Disease Virus O/ME-SA/Ind-2001d in South-East Asia in 2015. Transboundary and Emerging Diseases. 2018 Feb; 65(1): e104-12. doi: 10.1111/tbed.12687.

[49] Gubbins S, Paton DJ, Dekker A, Ludi AB, Wilsden G, Browning CF *et al.* Predicting Cross-Protection Against Foot-and-Mouth Disease Virus Strains by Serology After Vaccination. Frontiers in Veterinary Science. 2022 Dec; 9: 1027006. doi: 10.3389/fvets.2022.1027006.

[50] Urge B and Dawo F. Outbreak Study of Foot and Mouth Disease in Relation to Vaccination and Vaccine Efficacy Assessment in Central Ethiopia. Biomedical Journal of Scientific and Technical Research. 2021; 35(2): 27573-81. doi: 10.26717/BJSTR.2021.35.005685.

[51] Ren H, Jin Z, Pei X, Li M, Wang Y, Zhang J. Assessment of Immunization Procedures for Foot-And-Mouth Disease in Large-Scale Pig Farms in China Based on Actual Data and Dynamics. Animal Diseases. 2022 Feb 18; 2(1):3. doi: 10.1186/s44149-021-00035-3.

[52] Mignaqui AC, Ruiz V, Durocher Y, Wigdorovitz A. Advances in Novel Vaccines for Foot and Mouth Disease: Focus on Recombinant Empty Capsids. Critical Reviews in Biotechnology. 2019 Apr; 39(3): 306-20. doi: 10.1080/07388551.2018.1554619.

[53] Jin H, Xiao W, Xiao C, Yu Y, Kang Y, Du X *et al.* Protective Immune Responses Against Foot-and-Mouth Disease Virus by Vaccination with A DNA Vaccine Expressing Virus-Like Particles. Viral Immunology. 2007 Sep; 20(3): 429-40. doi: 10.1089/vim.2007.0031.

[54] Barnett PV, Geale DW, Clarke G, Davis J, Kasari TR. A Review of OIE Country Status Recovery Using Vaccinate-to-Live Versus Vaccinate-to-Die Foot-and-Mouth Disease Response Policies I: Benefits of Higher Potency Vaccines and Associated NSP DIVA Test Systems in Post-Outbreak Surveillance. Transboundary and Emerging

DOI: https://doi.org/10.54393/fbt.v5i2.167

Diseases. 2015 Aug; 62(4): 367-87. doi: 10.1111/tbed.12166.

[55] Berryman S, Feenstra F, Asfor A, Coco-Martin J, Jackson T, Tuthill TJ. Foot-and-Mouth Disease Vaccines by Design; Production of Capsid-Modified Foot-and-Mouth Disease Viruses with Improved Cell Culture Growth. Vaccines. 2025 Mar; 13(3): 281. doi: 10.3390/vaccines13030281.

[56] Anil KU, Sreenivasa BP, Mohapatra JK, Hosamani M, Kumar R, Venkataramanan R. Sequence Analysis of Capsid Coding Region of Foot-And-Mouth Disease Virus Type a Vaccine Strain During Serial Passages in BHK-21 Adherent and Suspension Cells. Biologicals. 2012 Nov; 40(6): 426-30. doi: 10.1016/j.biologicals.2012.08.002.

[57] Capozzo AV, Vosloo W, De los Santos T, Pérez AM, Pérez-Filgueira M. Foot-and-Mouth Disease Epidemiology, Vaccines and Vaccination: Moving Forward. Frontiers in Veterinary Science. 2023 Jun 20; 10: 1231005. doi: 10.3389/fvets.2023.1231005.

[58] Wubshet AK, Werid GM, Teklue T, Zhou L, Bayasgalan C, Tserendorj A *et al.* Foot and Mouth Disease Vaccine Efficacy in Africa: A Systematic Review and Meta-Analysis. Frontiers in Veterinary Science. 2024 Jun; 11: 1360256. doi: 10.3389/fvets.2024.1360256.

[59] Rasmussen P, Shaw AP, Jemberu WT, Knight-Jones T, Conrady B, Apenteng OO *et al.* Economic Losses Due to Foot-and-Mouth Disease (FMD) in Ethiopian Cattle. Preventive Veterinary Medicine. 2024 Sep; 230: 106276. doi: 10.1016/j.prevetmed.2024.106276.

[60] Clarke JD, Duyvesteyn HM, Perez-Martin E, Latišenko U, Porta C, Humphreys KV *et al.* A Broadly Reactive Ultralong Bovine Antibody That Can Determine the Integrity of Foot-and-Mouth Disease Virus Capsids. Journal of General Virology. 2024 Oct; 105(10): 002032. doi: 10.1099/jgv.0.002032.

[61] Biswal JK, Subramaniam S, Ranjan R, Sharma GK, Misri J, Pattnaik B. Marker Vaccine Potential of Foot-and-Mouth Disease Virus with Large Deletion in the Non-Structural Proteins 3A and 3B. Biologicals. 2015 Nov; 43(6): 504-11. doi: 10.1016/j.biologicals.2015.07.004.

[62] Lalzampuia H, Elango S, Biswal JK, Krishnaswamy N, Selvan RT, Saravanan P *et al.* Infection and Protection Responses of Deletion Mutants of Non-Structural Proteins of Foot-and-Mouth Disease Virus Serotype Asia1 in Guinea Pigs. Applied Microbiology and Biotechnology. 2022 Jan: 1-4. doi: 10.1007/s00253-021-11692-2.

[63] Bouazzaoui A, Abdellatif AA, Al-Allaf FA, Bogari NM, Al-Dehlawi S, Qari SH. Strategies for Vaccination: Conventional Vaccine Approaches Versus New-Generation Strategies in Combination with Adjuvants. Pharmaceutics. 2021 Jan 22;13(2):140. doi: 10.3390/pharmaceutics13020140.

[64] Song X, Li Y, Wu H, Qiu HJ, Sun Y. T-Cell Epitope-Based Vaccines: A Promising Strategy for Prevention of Infectious Diseases. Vaccines. 2024 Oct; 12(10): 1181. doi: 10.3390/vaccines12101181.

[65] Tang H, Wang H, Yang L, Chen H, Kong L, Xin X. Immunogenicity Analysis of the E. Coli Expressed Structural Protein VP1 of Persistent Infection Foot-and-Mouth Disease Virus. Virology. 2023 Feb; 579: 111-8. doi: 10.1016/j.virol.2023.01.004.

[66] Forner M, Cañas-Arranz R, Defaus S, de León P, Rodríguez-Pulido M, Ganges L *et al.* Peptide-Based Vaccines: Foot-and-Mouth Disease Virus, A Paradigm in Animal Health. Vaccines. 2021 May; 9(5): 477. doi: 10.3390/vaccines9050477.

[67] Arjmand B, Alavi-Moghadam S, Khorsand G, Sarvari M, Arjmand R, Rezaei-Tavirani M *et al.* Cell-Based Vaccines: Frontiers in Medical Technology for Cancer Treatment. Regenerative Engineering and Translational Medicine. 2024 Mar: 1-20. doi: 10.1007/s40883-024-00338-0.

[68] Kuhn JH. Virus Taxonomy. Encyclopedia of Virology. 2021 Mar: 28. doi: 10.1016/B978-0-12-809633-8.21231-4.

[69] Gorbalenya AE and Lauber C. Bioinformatics of Virus Taxonomy: Foundations and Tools for Developing Sequence-Based Hierarchical Classification. Current Opinion in Virology. 2022 Feb; 52: 48-56. doi: 10.1016/j.coviro.2021.11.003.

[70] Dash HR, Shrivastava P, Das S. Principles and Practices of DNA Analysis: A Laboratory Manual for Forensic DNA Typing. Humana Press. 2020. doi: 10.1007/978-1-0716-0274-4.

[71] Zaher MR, El-Husseiny MH, Hagag NM, El-Amir AM, El Zowalaty ME, Tammam RH. A Novel Immuno-informatic Approach for Design and Evaluation of Heptavalent Multiepitope Foot-and-Mouth Disease Virus Vaccine. BioMed Central Veterinary Research. 2025 Mar; 21(1): 152. doi: 10.1186/s12917-025-04509-1.

[72] Akbarzadegan M, Mahravani H, Piravar Z. Investigation of the Relationship between Virus Neutralization Test and ELISA with Challenge Test in Evaluation of FMD Vaccine Potency. Iranian Journal of Virology. 2023 Jul; 17(1): 57-62.

[73] Motamedi-Sedeh F, Hosseini SD, Mahravani H, Behgar M, Shawrang P, Mohammadi M. Immune Responses to Foot-and-Mouth Disease Vaccines in A Guinea-Pig Model. Sustainable Animal Production and Health. 2021: 44.

DOI: https://doi.org/10.54393/fbt.v5i2.167

[74] Mignaqui AC, Ruiz V, Perret S, St-Laurent G, Singh Chahal P, Transfiguracion J *et al.* Transient Gene Expression in Serum-Free Suspension-Growing Mammalian Cells for the Production of Foot-and-Mouth Disease Virus Empty Capsids. PLOS ONE. 2013 Aug; 8(8): e72800. doi: 10.1371/journal.pone.0072800.

[75] Capon TR, Garner MG, Tapsuwan S, Roche S, Breed AC, Liu S *et al.* A Simulation Study of the Use of Vaccination to Control Foot-and-Mouth Disease Outbreaks Across Australia. Frontiers in Veterinary Science. 2021 Aug; 8: 648003. doi: 10.3389/fvets.2021.648003.

DOI: https://doi.org/10.54393/fbt.v5i2.170

FUTURISTIC BIOTECHNOLOGY

https://fbtjournal.com/index.php/fbt

ISSN (E): 2959-0981, (P): 2959-0973

Vol 05 Issue 02, (April-June, 2025)

Review Article

OPEN ACCESS

Recent Advances in 3D Bioprinting and Biofabrication

Fariha Javaid[1*], Malik Hammad Ul Hassan[3], Eman Naveed Butt[2], Fariha Shafique[2], Abdul Hassan Khan[4] and Rabia Pervaiz[2]

[1]School of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan
[2]Department of Biotechnology, University of Central Punjab, Lahore, Pakistan
[3]Department of Science, The City College of New York, New York, United States
[4]Department of Pharmaceutics, Bahauddin Zakariya University, Multan, Pakistan

ARTICLE INFO

Keywords:

3Dimensional Bioprinting, Biofabrication, Regenerative Medicine, Tissue Engineering

How to Cite:

Javaid, F., Hassan, M. H. U., Butt, E. N., Shafique, F., Khan, A. H., & Pervaiz, R. (2025). Recent Advances in 3D Bioprinting and Biofabrication: Advances in 3D Bioprinting and Biofabrication. Futuristic Biotechnology, 5(2), 11-17. https://doi.org/10.54393/fbt.v5i2.170

***Corresponding Author:**

Fariha Javaid
School of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan
javaid.fariha@hotmail.com

Received Date: 21st April, 2025
Revised Date: 24th May, 2025
Acceptance Date: 12th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Biomedical technology has gone beyond the limit due to the 3D bioprinting and biofabrication, to create a new regenerative medicine. To explore the advancements in biomedical technology through 3D bioprinting and biofabrication, with a focus on their applications in regenerative medicine and the development of functional tissue and organ constructs. This paper reviewed key bioprinting technologies, bioink components, and advanced biofabrication strategies including nanomaterials and organoid-based methods. The review highlights tissue engineering potential and challenges in biofabrication, emphasizing emerging solutions like 4D bioprinting, organ-on-chip systems, and AI integration. Translating bioprinting advances into clinical therapies demands interdisciplinary collaboration and integration of emerging technologies to overcome current barriers.

INTRODUCTION

The exponential growth of medical technology has pushed and pulled the industry into the development of tools and applications not previously available before. 3D bioprinting and biofabrication are the stand-out sectors, which unlike other medical procedures, have the potential to upend the patients' health care system by driving solutions to the most challenging problems, i.e., the scarcity of organs, damage to the tissues, and finally, the drug production bottleneck [1]. 3D bioprinting is an advanced additive manufacturing technique that precisely layers bioinks comprising living cells and biomaterials to create functional, customized biological structures [2]. At the same time, the technological process of biofabrication encompasses those ways and means that lead to the production of cells and tissues. For instance, the use of cells that can replicate themselves, scaffold-based fabrication, and the use of organ-on-chip systems are some of the methods by which something like biofabrication takes place. The main objective of the biofabrication process is to engineer the living systems in an ordered way and within the construct in a way suitable for the particular requirements such as restoration, replacement, or enhancement [3]. 3D bioprinting was first reported in the literature in the 2000s when the technology was used to deposit cells precisely. Currently, the field is characterized by a good trend and is driven by the combination of many technologies and associated sciences such as materials science, microengineering,

DOI: https://doi.org/10.54393/fbt.v5i2.170

cellular biology, and digital design. At present, 3D bioprinting is not only a concept, it is a dynamic component of the process of regenerative medicine and pharmaceutics, the clinical safety testing of some products, and particularly space biology, with its RandD cycle [4]. The impressive steps that have been made in biomedicine are largely indebted to breakthroughs in fields such as 3D bioprinting and biofabrication that have been made in materials science, robotics, Computer Aided Design (CAD) and Artificial Intelligence (AI) that support it. The new trends depict the fact that the systems may become complex and that the human factor is no longer needed except in the case of some systems, as things that were once just figments of the imagination have now become possible such as multi-material bioprinters that are capable of creating tissues with numerous cell types as well as vascular networks and AI algorithms that manage to make the printer at the same time optimise the parameters of the print and maintain in real-time cell viability and the fidelity of the structure [5]. As technology becomes more sophisticated and printer and fabrication materials advance, it reveals to us that we are one step nearer to the big accomplishment: changing the situation where we only need the machines instead of the organs. Although there are still quite a number of issues in the spheres of science, technology, ethics, and legislation, the trend is unstoppable. 3D bioprinting and biofabrication is the area that not only changes how doctors treat patients but also changes the understanding of life [3]. Among the core technologies used in 3D bioprinting are different printing procedures, materials used, and their different application scopes. Inkjet bioprinting, laser-assisted bioprinting, microextrusion bioprinting, and stereolithography-based bioprinting are the four most prominent of them. Each technology greatly affects the formation and structure of living cells and biomaterials in the 3D biological constructions [5]. Inkjet bioprinting is one of the oldest methods in bioprinting, and it is referred to as a biological application. It uses the action of ejecting bioink droplets directly to a substrate [6]. On the other hand, Laser-Assisted Bioprinting (LAB) is a method that mimics the working principle of an inkjet printer [7]. The high cell viability that LAB continues to offer is due to its gentle, non-contact nature. However, LAB is very complex and expensive and it must be under a strict alignment and calibration regime. The need for laser-based equipment is limiting for its availability and expandability, particularly in the case of large-volume tissue fabrication. Even so, LAB plays an irreplaceable part in scientific research that requires extreme precision to get the bioinks moving and is very delicate and sensitive, such as the printing of vascular networks or neural tissues [8, 9]. The stereolithography (SLA)-based bioprinting technique also offers very precise printing. In this instance, the process of photopolymerization is instrumental in the solidification of the bioink through the use of light, typically ultraviolet (UV) or visible light. geometries [10]. For example in Digital Light Processing (DLP), the method displays the complete image of a single layer of the image at once to quicken the process, by forming an image of the layer with directed light. Comparison of different types of bioprinter is shown in table 1 [11].

Despite significant advancements in 3D bioprinting and biofabrication, critical challenges remain in achieving functional, clinically translatable tissue constructs. Limitations such as insufficient vascularization, inadequate multicellular integration, and bioink constraints continue to hinder the reliable production of complex tissues and organs. Moreover, the integration of emerging technologies like 4D bioprinting and AI-driven optimization is still in its early stages, leaving a gap in translating laboratory innovations into practical regenerative medicine applications. This study aims to address these gaps by exploring the latest strategies and technologies that enhance bioprinting fidelity, tissue complexity, and functional relevance.

Table 1: Types of Advanced Bioprinting Techniques

Technology	Resolution	Bioink Viscosity	Cell Viability	Cost	Best For
Inkjet	High	Low	Moderate	Low	Patterning, Drug Screening
Laser-Assisted	Very High	High	High	High	Vascular Structures, Precise Tissues
Micro-extrusion	Moderate	High	Moderate	Moderate	Large, Load-Bearing Tissues
Stereo-lithography	Very High	Low-Medium	Moderate-High	Moderate-High	Microstructures, Scaffold Fabrication

Bioinks: The Living "Ink" Behind Bioprinting

In the world of 3D bioprinting, bioinks are the essential players having a central part in converting digitalized print designs into living, functional biological materials [12, 13].

Non-Natural vs. Synthetic Bioinks

The classification of bioinks may be done in a general way into natural and synthetic types, and each of these types has its advantages as well as problems [14].

Table 2: Comparison of Natural Bioinks and Synthetic Bioinks

Property	Natural Bioinks	Synthetic Bioinks
Source	Biologically derived (e.g., collagen, gelatin)	Engineered materials (e.g., PEG, PLGA)
Biocompatibility	High	Variable (require functionalization)
Mechanical Strength	Low	High (tunable)
Cell adhesion	Excellent	Poor without modifications
Reproducibility	Low (batch variability)	High
Customized potential	Limited	High

New Trends in Bioink Development

The field of bioink development has been experiencing rapid advancements with a number of novel tendencies

DOI: https://doi.org/10.54393/fbt.v5i2.170

that can elevate the standards of not just technical but also biological beauty [15].

Biofabrication Strategies and Techniques

Biofabrication is a process that uses cells, biomaterials, and bioactive molecules to create a biologically functional product in a more precise way [16]. Illustration of components of bioink is represented in Figure 1.

Scaffold-Based vs. Scaffold-Free Approaches

The first and most significant choice one has to make in biofabrication is whether they should proceed with scaffold-based or scaffold-free methods. First, we can speak about the former which are the scaffold-based methods and imply using materials that are bioabsorbable and which act as the structure that the cells can adhere to and which also controls the growth and establishes the tissue [15, 16].

Self-Assembly and Cell-Sheet Engineering

The method is a great scaffold-free process which involves the use of cells that are able to organize themselves into specific forms just by the help of cell-to-cell communication and mechanical interactions [17, 18]. Cell-sheet engineering, on the other hand, is a highly clever approach to the task of forming 3D tissue [19].

Layer-by-Layer Fabrication

Layer-By-Layer (LbL) fabrication is the basis of 3D bioprinting and implies the successive application of cell-laden bioinks or biomaterials to produce tissue constructs from the ground up [20].

Organoid and Spheroid-Based Methods

Organoid and spheroid-based biofabrication, which is now just emerging, represents a change in paradigm, moving to more biomimetic and functionally relevant tissue models [21, 22].

Vascularization and Multicellular Complexity

The most difficult problem in 3D bioprinting and biofabrication which people are struggling to meet is the perfect vascularization and the inclusion of several cell types in a single tissue [23].

Challenges in Vascular Network Printing

Human vascular system is extremely complex and comprises large arteries, small arterioles, and microcapillaries that penetrate each tissue [24]. Furthermore, the vascular channels that are printed should be easily connected to the vascular system of the patient after the implant [25].

Bioprinting Vessels and Microcapillaries

For all the challenges presented here, the world of researchers has come up with a variety of approaches which can be considered breakthroughs in the sphere of the bioprinting of blood vessels and biological microorganisms [23]. The other ways that are available include the use of coaxial extrusion in which bioink containing endothelial cells is co-extruded with a protecting layer to form directly tubular tissue structures that are vasculature [26].

Integrating Multiple Cell Types and Tissues

Biological tissues, as part of their nature, are heterogeneous because they contain different cell types that perform specific functions [27].

Integration of 4D Bioprinting

3D bioprinting is an area of research that is constantly developing. Today, scientists are looking into the next step in the bioprinting process, which is 4D bioprinting [28].

What is 4D Bioprinting?

4D bioprinting describes the production of vibrant constructs that can change their 3D status according to a timing schedule when having contact with a selected group of items such as temperature, pH, light, water, or enzymatic energy [29].

Time-Responsive and Stimuli-Sensitive Bioinks

The driving force of 4D bioprinting is the materials that are time responsive to the triggers bioinks. These are intelligent materials to where some sort of external factors apply for a specific attribute of the substance to be modified. This one alters mechanically, while another one swells, contracts etc. [6]. Some 4D bioinks are also designed in such a way that they can communicate with the cells in a biologically smart way, where they may release the growth factors or change stiffness as cells divide and differentiate [19].

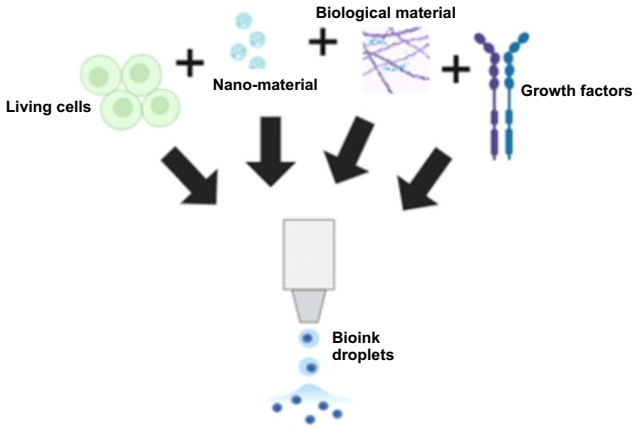

Figure 1: Breakdown of Components of Bioink

Applications in Dynamic Tissue Structures

4D bioprinting has a huge array of potential applications, especially in areas where innovative, smart, interactive tissues are required [30]. Furthermore, the new application of 4D bioprinted tissues is seen in organ-on-chip technologies and drug screening platforms, where the tissues can be stimulated dynamically to mimic temperature changes, come up with predetermined responses and maintain the self of the tissue under pressure, i.e., barrier integrity [31].

Microfluidics and Organ-on-Chip Technology

Microfluidics and organ-on-chip technology are the driving

DOI: https://doi.org/10.54393/fbt.v5i2.170

forces behind the convergence of bioengineering, medicine, and bioprinting[32].

Role in Drug Testing and Disease Modeling

One of the biggest breakthrough in the application of organ-on-chip devices is their role in the reduction or replacement of animal experiments conducted in the development of new drugs [33]. Furthermore, these research tools provide a great potential for the study of complex and highly prevalent diseases, such as cancer, neurodegeneration, and cardiovascular diseases, in a more controlled and adjustable environment[34].

Combining 3D Bioprinting with Microfluidic Chips

The congruence of 3D bioprinting with microfluidic systems demonstrates a quantum leap in the field of biofabrication [35]. One scenario is the bio-printing of endothelial cells within micro-scale channels, which not only enables the creation of a realistic vascular network but also avoids the growth of non-targeted tissues[36].

Realistic Physiological Environments

Organ-on-chip systems have the aim of imitating the mechanical stimulation that is found in organs besides static tissue models, and those examples are included, stretching (Physiologically speaking, lungs), electrical stimulation (Physiologically speaking, the heart), and cyclic pressure (Physiologically speaking, vascular systems)[28].

Artificial Intelligence and Computational Modeling

Artificial Intelligence (AI) and computational modeling are rapidly changing the face of bioprinting, thus offering predictive analytics, automation, and real-time adaptiveness. With 3D and 4D bioprinting technologies reaching a certain level of sophistication, the necessity to handle, interpret, and optimize the vast amount of data in the biofabrication process also increases. AI is actually a certain bioprinting process that provides it with the possibility to quickly, rightly, and precisely carry out the tasks[37].

AI for Optimizing Printability and Cell Viability

Bioprinting is about correlation of material properties, biological parameters, and environmental conditions to actualize the concept[38]. What's more, profound learning software is enabled to instantly examine the imaging data as soon as printing is in progress, and regulate the proper alignment and structure of layers[39].

Predictive Tissue Growth Models

Simulation of the experimental condition reveals how cells will go through the stages of proliferation, differentiation, migration, and organization from simple tissue constructions to mature structures[40].

Artificial Intelligence for Real-Time Error Correction

In the highest level of bioprinting technology, AI also takes part in the building of the systems. The AI-driven system integrates into the machine to track the printing process to monitor the process in real-time [41]. The feedback from high-resolution cameras and sensors gets processed by neural networks which will automatically identify any deviations from the desired printed images [42]. This live feedback loop also not only enhances the success chances of the printing but also assures the reproducibility of the bioprinted tissues' high quality[43].

Challenges and future prospects

Despite the significant impact that 3D bioprinting and biofabrication can have on the industry, the field still faces a lot of difficulties, which impede the full-fledged clinical translation[20]. The factors that are currently limiting the print resolution in the technologies, together with the problems in the attachment of the smaller vessels and the creation of the larger tissue with the vascular hierarchy, are now beyond the scope of the solution [44]. The other part of the issue comes from a situation of the need for a bioink that addresses multiple cell types in a spatial manner as they are in the natural tissues but is also at least a step further in terms of the processing of the biosystem[45]. In addition to the above, the ability of 4D bioprinting where the constructs exhibit self-adaptive and regenerative behavior when subjected to environmental changes will create new opportunities in the biomedical field[46].

Limitations and Future prospects

Current 3D bioprinting and biofabrication approaches face limitations including restricted print resolution, challenges in replicating natural tissue heterogeneity, and limited long-term biocompatibility. Future prospects lie in integrating advanced bioinks, multi-cellular and vascularized constructs, and smart, stimuli-responsive 4D bioprinting systems. Coupling these with AI-driven modeling and organ-on-chip platforms may overcome existing barriers, enabling patient-specific tissue engineering and accelerating clinical translation.

CONCLUSION

3D bioprinting and biofabrication have significantly advanced regenerative medicine by addressing critical healthcare challenges like organ shortages and tissue damage. Despite progress, hurdles remain in vascularization, multicellular integration, and long-term biocompatibility. Emerging technologies like 4D bioprinting, organ-on-chip systems, and AI are paving the way for smarter, patient-specific tissue engineering solutions.

DOI: https://doi.org/10.54393/fbt.v5i2.170

Authors' Contribution

Conceptualization: FJ
Methodology: MHUH
Formal analysis: ENB
Writing and Drafting: FS, AHK, RP
Review and Editing: FS, AHK, RP, ENB, MHUH, FJ

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] George AH, George AS, Baskar T, Shahul A. 3D printed organs: a new frontier in medical technology. Partners Universal International Innovation Journal. 2023 Jun; 1(3): 187-208. doi: 10.5281/zenodo.8076965.

[2] Al Hashimi N and Vijayavenkataraman S. Toxicity Aspects and Ethical Issues of Bioprinting. 3D Bioprinting from Lab to Industry. 2024 Aug: 251-71. doi: 10.1002/9781119894407.ch8.

[3] Alzoubi L, Aljabali AA, Tambuwala MM. Empowering precision medicine: the impact of 3D printing on personalized therapeutic. Aaps Pharmscitech. 2023 Nov; 24(8): 228. doi: 10.1208/s12249-023-02682-w.

[4] Askari M, Naniz MA, Kouhi M, Saberi A, Zolfagharian A, Bodaghi M. Recent progress in extrusion 3D bioprinting of hydrogel biomaterials for tissue regeneration: a comprehensive review with focus on advanced fabrication techniques. Biomaterials Science. 2021 Oct; 9(3): 535-73. doi: 10.1039/D0BM00973C.

[5] Aziz MF. Mapping the Ethical and Regulatory Issues of 3D Bioprinting Using Biomaterials in a Low-and Middle-Income Nation: Malaysian Perspectives. InSustainable Material for Biomedical Engineering Application. 2023 Aug: 467-482. doi: 10.1007/978-981-99-2267-3_22.

[6] Bektas CK, Luo J, Conley B, Le KP, Lee KB. 3D Bioprinting Approaches for Enhancing Stem Cell-Based Neural Tissue Regeneration. Acta Biomaterialia. 2025 Jan. doi: 10.1016/j.actbio.2025.01.006.

[7] Bian L. Functional hydrogel bioink, a key challenge of 3D cellular bioprinting. APL bioengineering. 2020 Sep; 4(3). doi: 10.1063/5.0018548.

[8] Boopathi S and Kumar P. Advanced bioprinting processes using additive manufacturing technologies: Revolutionizing tissue engineering. 3D Printing Technologies: Digital Manufacturing, Artificial Intelligence, Industry. 2024 Jan; 4(95): 1627-57. doi: 10.1515/9783111215112-005.

[9] Budharaju H, Sundaramurthi D, Sethuraman S. Embedded 3D bioprinting-An emerging strategy to fabricate biomimetic & large vascularized tissue constructs. Bioactive Materials. 2024 Feb; 32: 356-84. doi: 10.1016/j.bioactmat.2023.10.012.

[10] Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV *et al.* RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Research. 2021 Jan; 49(D1): D437-51. doi: 10.1093/nar/gkaa1038.

[11] Chandra DK, Reis RL, Kundu SC, Kumar A, Mahapatra C. Nanomaterials-Based Hybrid Bioink Platforms in Advancing 3D Bioprinting Technologies for Regenerative Medicine. ACS Biomaterials Science & Engineering. 2024 Jun; 10(7): 4145-74. doi: 10.1021/acsbiomaterials.4c00166.

[12] Ciocca M, Febo C, Gentile G, Orlando A, Massoumi F, Altana A *et al.* 3D-Bioprinted Light-Sensitive Cell Scaffold Based on Alginate-Conjugated Polymer Nanoparticles for Biophotonics Applications. BioNanoScience. 2025 Jun; 15(2): 1-8. doi: 10.1007/s12668-025-01863-0.

[13] Costa JB, Silva-Correia J, Reis RL, Oliveira JM. Deep learning in bioengineering and biofabrication: A powerful technology boosting translation from research to clinics. Journal of 3D Printing in Medicine. 2021 Dec; 5(4): 191-211. doi: 10.2217/3dp-2021-0007.

[14] Crook JM and Tomaskovic-Crook E. Bioprinting 3D human induced pluripotent stem cell constructs for multilineage tissue engineering and modeling. 3D Bioprinting: Principles and Protocols. 2020: 251-8. doi: 10.1007/978-1-0716-0520-2_17.

[15] Dai Y, Wang P, Mishra A, You K, Zong Y, Lu WF et al. 3D Bioprinting and Artificial Intelligence Assisted Biofabrication of Personalized Oral Soft Tissue Constructs. Advanced Healthcare Materials. 2024 Dec: e2402727. doi: 10.1002/adhm.2402727.

[16] Ding Z, Tang N, Huang J, Cao X, Wu S. Global hotspots and emerging trends in 3D bioprinting research. Frontiers in Bioengineering and Biotechnology. 2023 May; 11: 1169893. doi: 10.3389/fbioe.2023.1169893.

[17] Debnath S, Agrawal A, Jain N, Chatterjee K, Player DJ. Collagen as a bio-ink for 3D printing: a critical review. Journal of Materials Chemistry B. 2025 Jan. doi:

DOI: https://doi.org/10.54393/fbt.v5i2.170

10.1039/D4TB01060D.
[18] Fang Y, Guo Y, Liu T, Xu R, Mao S, Mo X *et al.* Advances in 3D bioprinting. Chinese Journal of Mechanical Engineering: Additive Manufacturing Frontiers. 2022 Mar; 1(1): 100011. doi: 10.1016/j.cjmeam.2022.100011.
[19] Garcia-Garcia LA and Rodriguez-Salvador M. Uncovering 3D bioprinting research trends: A keyword network mapping analysis. International Journal of Bioprinting. 2018 Jul; 4(2): 147. doi: 10.18063/ijb.v4i2.147.
[20] Ghilan A, Chiriac AP, Nita LE, Rusu AG, Neamtu I, Chiriac VM. Trends in 3D printing processes for biomedical field: opportunities and challenges. Journal of Polymers and the Environment. 2020 May;28:1345-67. doi: 10.1007/s10924-020-01722-x.
[21] Gopinathan J and Noh I. Recent trends in bioinks for 3D printing. Biomaterials Research. 2018 Apr; 22(1): 11. doi: 10.1186/s40824-018-0122-1.
[22] Gu Y, Zhang L, Du X, Fan Z, Wang L, Sun W *et al.* Reversible physical crosslinking strategy with optimal temperature for 3D bioprinting of human chondrocyte-laden gelatin methacryloyl bioink. Journal of Biomaterials Applications. 2018 Nov; 33(5): 609-18. doi: 10.1177/0885328218805864.
[23] Guida L, Cavallaro M, Levi M. Advancements in high-resolution 3D bioprinting: exploring technological trends, bioinks and achieved resolutions. Bioprinting. 2024 Nov: e00376. doi: 10.1016/j.bprint.2024.e00376.
[24] Gungor-Ozkerim PS, Inci I, Zhang YS, Khademhosseini A, Dokmeci MR. Bioinks for 3D bioprinting: an overview. Biomaterials Science. 2018 May; 6(5): 915-46. doi: 10.1039/C7BM00765E.
[25] Gugulothu SB, Asthana S, Homer-Vanniasinkam S, Chatterjee K. Trends in photopolymerizable bioinks for 3D bioprinting of tumor models. JACS Au. 2023 Aug; 3(8): 2086-106. doi: 10.1021/jacsau.3c00281.
[26] Gomes Gama JF, Dias EA, Aguiar Coelho RM, Chagas AM, Aguiar Coelho Nt J, Alves LA. Development and implementation of a significantly low-cost 3D bioprinter using recycled scrap material. Frontiers in Bioengineering and Biotechnology. 2023 Apr; 11: 1108396. doi: 10.3389/fbioe.2023.1108396.
[27] Bharadwaj T and Verma D. Open source bioprinters: Revolutionizing the accessibility of biofabrication. Bioprinting. 2021 Aug; 23: e00155. doi: 10.1016/j.bprint.2021.e00155.
[28] Lindner N and Blaeser A. Scalable biofabrication: A perspective on the current state and future potentials of process automation in 3D-bioprinting applications. Frontiers in Bioengineering and Biotechnology. 2022 May; 10: 855042. doi: 10.3389/fbioe.2022.855042.
[29] Yang Q, Gao B, Xu F. Recent advances in 4D bioprinting. Biotechnology Journal. 2020 Jan; 15(1): 1900086. doi: 10.1002/biot.201900086.
[30] Harley WS, Li CC, Toombs J, O'Connell CD, Taylor HK, Heath DE *et al.* Advances in biofabrication techniques towards functional bioprinted heterogeneous engineered tissues: a comprehensive review. Bioprinting. 2021 Aug; 23: e00147. doi: 10.1016/j.bprint.2021.e00147.
[31] Hölzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A. Bioink properties before, during and after 3D bioprinting. Biofabrication. 2016 Sep; 8(3): 032002. doi: 10.1088/1758-5090/8/3/032002.
[32] Filippi M, Mekkattu M, Katzschmann RK. Sustainable biofabrication: from bioprinting to AI-driven predictive methods. Trends in Biotechnology. 2024 Jul. doi: 10.1016/j.tibtech.2024.07.002.
[33] Fan C, Basharat Z, Mah K, Wei CR. Computational approach for drug discovery against Gardnerella vaginalis in quest for safer and effective treatments for bacterial vaginosis. Scientific Reports. 2024 Jul; 14(1): 17437. doi: 10.1038/s41598-024-68443-2.
[34] Byrne R, Carrico A, Lettieri M, Rajan AK, Forster RJ, Cumba LR. Bioinks and biofabrication techniques for biosensors development: A review. Materials Today Bio. 2024 Aug: 101185. doi: 10.1016/j.mtbio.2024.101185.
[35] Kawecki F and L'Heureux N. Current biofabrication methods for vascular tissue engineering and an introduction to biological textiles. Biofabrication. 2023 Mar; 15(2): 022004. Doi: 10.1088/1758-5090/acbf7a.
[36] Mirzaei M, Okoro OV, Nie L, Petri DF, Shavandi A. Protein-based 3D biofabrication of biomaterials. Bioengineering. 2021 Apr; 8(4): 48. doi: 10.3390/bioengineering8040048.
[37] Naorem RS, Pangabam BD, Bora SS, Fekete C, Teli AB. Immunoinformatics Design of a Multiepitope Vaccine (MEV) Targeting Streptococcus mutans: A Novel Computational Approach. Pathogens. 2024 Oct; 13(10): 916. doi: 10.3390/pathogens13100916.
[38] Ahankari SS, Subhedar AR, Bhadauria SS, Dufresne A. Nanocellulose in food packaging: A review. Carbohydrate Polymers. 2021 Mar; 255: 117479. doi: 10.1016/j.carbpol.2020.117479.
[39] Chand R, Kamei KI, Vijayavenkataraman S. Advances in microfluidic bioprinting for multi-material multi-cellular tissue constructs. Cell Engineering Connect. 2025 Feb; 1(1): 1-0. doi: 10.69709/CellEngC.2024.111335.

DOI: https://doi.org/10.54393/fbt.v5i2.170

[40] Moroni L, Tabury K, Stenuit H, Grimm D, Baatout S, Mironov V. What can biofabrication do for space and what can space do for biofabrication?. Trends in Biotechnology. 2022 Apr; 40(4): 398-411. doi: 10.1016/j.tibtech.2021.08.008.

[41] Zhou C, Liu C, Liao Z, Pang Y, Sun W. AI for biofabrication. Biofabrication. 2024 Nov; 17(1): 012004. doi: 10.1088/1758-5090/ad8966.

[42] Santos-Beato P, Midha S, Pitsillides AA, Miller A, Torii R, Kalaskar DM. Biofabrication of the osteochondral unit and its applications: Current and future directions for 3D bioprinting. Journal of Tissue Engineering. 2022 Nov; 13: 20417314221133480. doi: 10.1177/20417314221133480.

[43] Yeo M, Sarkar A, Singh YP, Derman ID, Datta P, Ozbolat IT. Synergistic coupling between 3D bioprinting and vascularization strategies. Biofabrication. 2023 Nov; 16(1): 012003. doi: 10.1088/1758-5090/ad0b3f.

[44] Wu C, Xu Y, Fang J, Li Q. Machine learning in biomaterials, biomechanics/mechanobiology, and biofabrication: State of the art and perspective. Archives of Computational Methods in Engineering. 2024 Sep; 31(7): 3699-765. doi: 10.1007/s11831-024-10100-y.

[45] Wu Y, Yang X, Gupta D, Alioglu MA, Qin M, Ozbolat V *et al.* Dissecting the Interplay Mechanism among Process Parameters toward the Biofabrication of High Quality Shapes in Embedded Bioprinting. Advanced Functional Materials. 2024 May; 34(21): 2313088. doi: 10.1002/adfm.202313088.

[46] Woodfield TB, Moroni L, Miller JS. Biophysics of biofabrication. APL Bioengineering. 2021 Sep; 5(3). doi: 10.1063/5.0057459.

DOI: https://doi.org/10.54393/fbt.v5i2.177

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Review Article

OPEN ACCESS

Potential of Plant Bioactive Compounds for the Treatment of Cancer

Hammad Ahmed Abbasi[1,2*], Muhammad Atif[3], Muhammad Naveed Anjum[1], Jawad Ahmad[4], Abdul Wahab[5] and Syed Ashmam Ali Shah[6]

[1]Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan
[2]Department Molecular Biology and Genetics, Erzurum Technical University, Turkey
[3]Center of Applied Molecular Biology, University of the Punjab, Lahore, Pakistan
[4]Institute of Biotechnology and Genetic Engineering, University of Agriculture, Peshawar, Pakistan
[5]Institute of Kidney Diseases, Peshawar, Pakistan
[6]Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

ARTICLE INFO

Keywords:
Alkaloids, MAPK signaling, Apoptosis, Phytochemicals, Oncogenic Pathways

How to Cite:
Abbasi, H. A., Atif, M., Anjum, M. N., Ahmad, J., Wahab, A., & Shah, S. A. A. (2025). Potential of Plant Bioactive Compounds for the Treatment of Cancer: Bioactive Compounds for the Treatment of Cancer.Futuristic Biotechnology, 5(2), 18–30. https://doi.org/10.54393/fbt.v5i2.177

***Corresponding Author:**
Hammad Ahmed Abbasi
Department Molecular Biology and Genetics, Erzurum Technical University, Turkey
hammadahmedabbasi403@gmail.com

Received Date: 8th May, 2025
Revised Date: 20th June, 2025
Acceptance Date: 25th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Cancer remains one of the leading causes of mortality worldwide. Despite recent advances, current chemotherapeutic options often have undesirable side effects, and the development of resistance limits their long-term effectiveness. The botanical kingdom contains a vast repository of phytochemicals with varying biological activities. This review examines the anticancer potential of various classes of plant bioactive compounds. Specific alkaloids like berberine demonstrate remarkable apoptosis induction through mitochondrial stress and caspase activation in numerous cancer cell lines. Curcumin modulates multiple oncogenic pathways, including PI3K/Akt, Wnt/β-catenin, and MAPK signaling. Resveratrol elicits favorable anti-tumor responses through intrinsic apoptosis, autophagy stimulation, and antiangiogenic effects. Promising preclinical studies have elucidated the underlying molecular mechanisms by which bioactive components such as quercetin, genistein, and epigallocatechin gallate exert chemopreventive effects. While intensive research is still required, progress in standardizing extracts, isolating marker compounds, and clinical testing validates nature's treasure as a source for novel anticancer options. Future studies should focus on overcoming translational barriers to move these promising compounds from bench to bedside.

INTRODUCTION

Epidemiology of Cancer

Cancer is a pathological condition caused by abnormal cell division and is a major contributor to global fatalities. Based on the data, it can be inferred that the emerging figures indicate a significant loss of lives, with an estimated 10 million fatalities occurring in the year 2020. Furthermore, globally, it is seen that a substantial number of individuals, ranging from 19 to 20 million, receive a cancer diagnosis every year. [1]. The specific factor responsible for cancer development varies among individuals and is contingent upon the type of cancer and geographical location. It is crucial to establish appropriate treatment strategies for each case. The rise in cancer incidence rates is believed to be linked to changes in the environment, including climate, resulting from industrialization, as well as lifestyle choices related to living and dietary habits[2].

Molecular Basis of Cancer Development

While there are many potential causes of cancer, the most widely held theory holds that oncogene and tumor suppressor gene mutations occur sequentially, making the development of cancer an extremely complicated illness

DOI: https://doi.org/10.54393/fbt.v5i2.177

[3]. Oncogenes are mutant genes with cancer-causing potential. Proto-oncogene is a term used for oncogenes that have a role in controlling normal cell division before they become mutated. When a proto-oncogene undergoes a mutation, it becomes an oncogene and drives unchecked cell division and proliferation, setting the stage for the development of cancer. Tumor suppressive genes, which are also present in normal cells but work to prevent cancer from developing, are crucial to the cell's normal growth and differentiation. There is a large group of genes called tumor suppressor genes that all share a single property: they all prevent neoplasia from developing in the body. For a cancer cell to multiply and survive, both copies of a tumor-suppressor gene must be dormant. When tumor-suppressor genes are missing or turned ineffective by mutations, cancer develops. Genes like p53 and retinoblastoma 1 (RB1) are examples of tumor-suppressor genes [4]. DNA damage is a regular occurrence caused by both internal (endogenous) and external (exogenous) factors. Different DNA repair mechanisms identify and eliminate the damage. When DNA damage is left unaddressed, checkpoints in the cell cycle can impede its progression or trigger cellular senescence or apoptosis. Mistaken restoration or replicative bypass of lesions can lead to genetic alterations and abnormalities in chromosomes. When alterations occur in tumor suppressor genes or oncogenes, cells can transform into cancerous cells [5].

Limitations of Conventional Cancer Therapies

Conventional cancer treatment approaches include surgical intervention, radiation therapy, and chemotherapy. The utilization of chemotherapy has been linked to the recurrence of cancer, the formation of resistance, the imposition of significant pressure on patients, and the development of numerous serious side effects [6, 7]. During Radiotherapy, the radiation exposure has been observed to lead to elevated levels of discomfort, anxiety, and depression among patients who are undergoing this treatment. While it is seen that the occurrence of these issues tends to diminish following the completion of RT, a notable proportion of patients continue to exhibit psychological consequences after their treatment [8]. Radiation exposure elicits a psychological impact that triggers a cascade of reactions to repair the tissues that are damaged. The reaction commences with the activation of when the damage occurs in DNA, as a result, it activates the mitotic cell death, cellular senescence, and apoptosis. Subsequently, a continuous cytokine cascade ensues, leading to the induction of inflammation and collagen deposition. Late adverse effects, specifically, tend to be persistent and frequently exhibit a progressive pattern, resulting in a decline after their treatment [9].

Despite extensive research on plant-derived bioactive compounds, their clinical translation into standardized anticancer therapies remains limited. Most available evidence is derived from in vitro and preclinical studies, with comparatively fewer well-designed clinical trials validating safety, dosage optimization, and long-term efficacy. Additionally, challenges such as poor bioavailability, pharmacokinetic variability, and lack of targeted delivery systems create a significant gap between experimental findings and clinical application. Therefore, a comprehensive evaluation of molecular mechanisms alongside translational limitations is essential to bridge the gap between bench research and bedside implementation.

Role of Phytochemicals in Cancer Treatment

To combat the aforementioned adverse effects associated with alternative cancer treatment modalities, it is plausible that plant extracts possess the capability to mitigate these risks. The botanical realm encompasses a wide array of plants that exhibit significant chemical diversity and possess versatile chemical properties, with the majority exhibiting favorable toxicity profiles. Hence, herb formulations, crude plant extracts, and dietary sources are widely employed as primary reservoirs of phytochemicals for cancer therapeutics. Undoubtedly, they have demonstrated unparalleled benefits in mitigating the adverse effects of anticancer drugs. Over 3000 plant species have been investigated for anticancer properties, and numerous phytochemicals have shown potential either as standalone agents or in synergy with standard therapies. Notably, compounds such as berberine and camptothecin have demonstrated strong anticancer activities. Additionally, broader classes such as polyphenols [e.g., resveratrol, quercetin] and terpenoids e.g., taxol, artemisinin have also received attention due to their antioxidant, anti-inflammatory, and immune-modulating properties [10]. Their integration into cancer therapies underscores their biocompatibility, target-specific activity, and potential to enhance therapeutic outcomes.

Plant-Derived Compounds in Cancer

The plants that are a good source of natural compounds and compounds which have the potential to fight against cancerous cells and which have more phytonutrients are medicinal plants, many of which have been traditionally applied for cancer treatment and are widely identified for their broad-spectrum treatment potential against a range of diseases. A substantial proportion of chemopreventive agents are derived from botanical sources, largely due to their favorable safety profiles and minimal adverse effects. Over the past several decades, plant-derived bioactive molecules such as flavonoids, polyphenols, alkaloids, and sesquiterpenes have been extensively studied and applied in anticancer strategies [11]. The following are some of the major plant extracts used in cancer treatment.

DOI: https://doi.org/10.54393/fbt.v5i2.177

Alkaloids

Alkaloids are a diverse class of naturally occurring compounds characterized by a cyclic structure that contains at least one nitrogen atom, which distinguishes them from other classes of phytochemicals. Alkaloids are mostly manufactured from amino acids and their precursors, including ornithine, arginine, lysine are most common, then they also have phenylalanine, tryptophan, and tryptophan. Widely distributed throughout the plant kingdom, alkaloids are especially abundant in families such as Leguminosae, Menispermaceae, Ranunculaceae, Loganiaceae, and Papaveraceae. They are of considerable significance for the discovery of drugs due to their potent biological activities.Numerous alkaloid compounds are obtained from mostly the herbs and medicinal plants have been investigated and shown to exhibit strong antiproliferative and anticancer properties in both in vitro and in vivo studies.Currently, several plant-derived alkaloids have received FDA approval and are commercially available as anticancer agents [12, 13].

Berberine

Berberine constitutes yet another isoquinoline alkaloid of natural provenance, sourced from numerous botanical families, including Papaveraceae, Berberidaceae, and Ranunculaceae. This bioactive compound can be extracted from diverse plant species, prominently including Goldenseal, Barberry, Coptis, tree turmeric, and Oregon grape [14]. Berberine [BBR] has exhibited many effects on cancer cells, encompassing the modification of the cell cycle, induction of apoptosis, stimulation of autophagy, and influence on the tumor microenvironment. In light of the current emphasis on tumor immunotherapy and the considerable expense associated with immune-suppressing medications, BBR, a cost-effective Chinese traditional medicine exhibiting notable immunomodulatory characteristics, exhibits potential as a viable contender for extensive clinical application in the field of immunotherapy [15]. The promotion of apoptosis through the activation of caspases has been demonstrated by BBR. BBR has the potential to cause cell death in leukemia and hepatoma cells by upregulating the expression of caspase-8 and caspase-9 while simultaneously downregulating the expression of bcl-2 through the activation of caspase-3 [16](Figure 1)(Table 1).

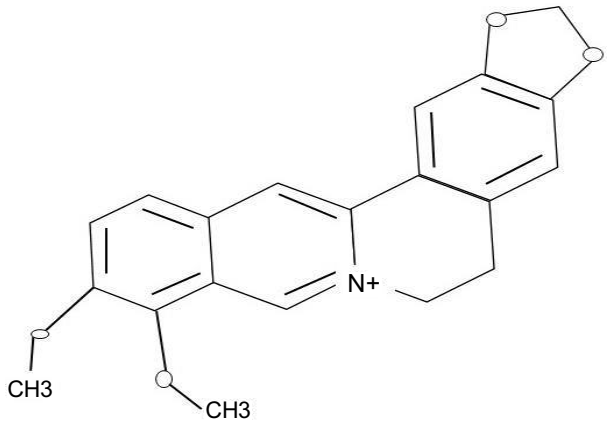

Figure 1: Structural Formula of Berberine

The main role play to control and regulate the apoptosis by controlling membrane permeability and initiating downstream signaling cascades is done by mitochondria. Through many studies, it is proven that external stimuli increase mitochondrial membrane permeability, resulting in the activation of caspase-dependent pathways that lead to programmed cell death. Notably, berberine (BBR) has been reported to enhance apoptosis by promoting the acetylation of FOXO1/3a, thereby reinforcing its role as a key regulator of mitochondria-dependent apoptosis [17]. Additionally, BBR uses a variety of signaling pathways to cause autophagy in tumor cells. The AMPK/mTOR/ULK1 axis, which affects JNK phosphorylation, is the precise target in glioblastoma. This modification stimulates Beclin-1 release in hepatocellular carcinoma and aids in the dissociation of the Bcl-2/Beclin-1 complex in breast cancer cells. The ULK1/mTOR signaling pathway is also activated by BBR by improving the interaction, which mediates this mechanism. Given that autophagy plays a part in drug resistance, cancer development, and apoptosis evasion, BBR's capacity to activate autophagic processes points to its substantial therapeutic promise in oncology [18]. Tissue and organ degradation brought on by unchecked cell proliferation and the invasive expansion of tumor cells accounts for a major portion of cancer-associated mortality. The breast cancer proliferation and spreading are controlled and prevented by targeting the ephrin-B2, and simultaneously downregulate the production of metalloproteinases MMP-2 and MMP-9 [19]. The MMPs are downregulated as a result of the suppression of the COX-2/PGE2-JAK2/STAT3 signaling cascade [20]. Additionally, by reducing the expression of Snail-1, a transcription factor crucial to the epithelial-mesenchymal transition, BBR prevents metastasis. Together, our results highlight how BBR modulates cancer-related signaling pathways and regulatory proteins to have anti-cancer effects [18, 20, 21]. Apart from its anti-metastatic and cytotoxic qualities, BBR has demonstrated potential in tumor immunotherapy. TNF-α and IL-1, BBR functions as a dopamine receptor

DOI: https://doi.org/10.54393/fbt.v5i2.177

antagonist and improves the immunological profile in the tumor microenvironment. Additionally, it suppresses CD4+ T cell proliferation and, by blocking STAT 1 phosphorylation, lowers the production of indoleamine 2,3-dioxygenase (IDO1) triggered by interferon-gamma (IFN-γ). The therapeutic promise of BBR in cancer immunotherapy techniques is supported by these immunomodulatory actions [18] (Table 1).

Table 1: Key Alkaloids, Anti-Tumor Activities, and Underlying Mechanisms

Compounds	Class	Anticancer Mechanisms	Plant Sources	References
Berberine	Alkaloid	Apoptosis via mitochondrial stress, caspase activation	Goldenseal, barberry, Japanese goldthread	[18, 21, 23]
Camptothecin	Alkaloid	Topo I inhibition, DNA damage, and autophagy	Camptotheca acuminata tree	[24, 25, 26, 27]

It induces apoptosis by activation of p53, Bax, and caspases, and inhibits anti-apoptotic proteins like Bcl-2. BBR regulates cell cycle progression by affecting cyclins and CDKs, leading to cell cycle arrest. It promotes autophagy through AMPK/mTOR and Beclin 1 pathways and suppresses invasion and migration by downregulating MMP-2/9 and Snail-1 (Figure 2).

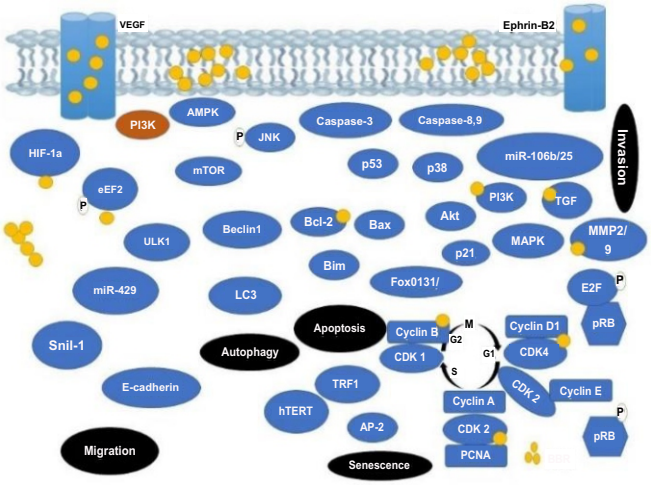

Figure 2: Berberine [BBR] exerts anticancer effects by modulating multiple signaling pathways.

Camptothecin [CPT]

Camptothecin [CPT] stands as a formidable contender among anticancer compounds. CPT belongs to the class of compounds that have a distinctive planar pentacyclic ring structure [27]. Camptothecin (CPT) is synthesized in numerous angiosperm plant species across various taxonomic groups [26]. Camptothecin (CPT) has demonstrated significant anticancer effects by selectively inhibiting intracellular topoisomerase I. The potential of CPT in therapeutic applications is restricted by various issues, such as the instability of its lactone ring and its insolubility in water. These limitations result in reduced oral solubility of the medicine and hinder its bioavailability in blood plasma. Different analogues of CPT are employed in the therapeutic management of lung cancer, ovarian, and colon [28]. The clinical trials of CPT in 1970 resulted, investigations into its mechanism of action have remained a subject of ongoing research. The initial discoveries about the mechanism of action of the CPT were made by Drs. Marshall and Susan Horwitz, who work as a collaborative team at Albert Einstein College of Medicine, with other researchers. The findings of their research demonstrated that CPT can impede the synthesis of DNA and RNA, particularly ribosomal RNA, while also causing DNA damage. The researchers made observations indicating that CPT exhibits its most efficacy during the S-phase of the cell cycle. Additionally, they hypothesized that the involvement of the DNA replication fork is likely in the mechanism behind cell death produced by CPT. Subsequent investigations have revealed that CPT effectively halts the progression of the cell cycle at both the S and G2 phases, which are crucial for the manifestation of CPT's cytotoxic effects [29]. Research findings have demonstrated that the analogue of CPT FL118 exhibits a specific inhibitory effect on the production of certain antiapoptotic proteins, namely survivin, Mcl-1, XIAP, and cIAP2. It was determined that FL118's inhibition of these proteins is not influenced by the state of the tumor suppressor p53, regardless of whether it is wild type, mutant, or null. This characteristic of FL118 holds significant importance, as the majority, if not all, of DNA-damaging medicines exhibit ineffectiveness in cases where p53 is mutated or absent [29, 30]. A camptothecin (CPT) analog in one of the analyses shows the potential to induce apoptosis in acute myeloid leukemia cell lines such as NB4 and U937. This apoptotic response is marked by the activation of caspase-3 and a disruption in mitochondrial membrane potential. The underlying mechanism involves the rapid activation of protein kinase C delta (PKCδ) following exposure to NSC606985. Notably, the pro-apoptotic effect of this compound can be completely abrogated by co-treatment with rottlerin, a selective PKCδ inhibitor [31]. In the NB4 cell line, a commonly used in vitro model of acute promyelocytic leukemia (APL), NSC706985 exhibited a pronounced inhibitory effect on cell proliferation. This restraint manifested with dependency on both time and concentration. Likewise, there was a marked reduction in cellular viability. It is noteworthy that this suppressive impact was evident even at exceedingly low concentrations [24]. CT-2106 is another very important chemical entity, the conjugation of camptothecin (CPT) with poly-L-glutamate. This conjugation is proposed to enhance the stability of the active lactone form of CPT and augment its aqueous solubility. Additionally, there was a

DOI: https://doi.org/10.54393/fbt.v5i2.177

hypothesis suggesting that the inclusion of the poly-L-glutamate component could potentially improve the transportation of CPT to tumor sites by capitalizing on the improved permeability and retention phenomenon. Nevertheless, the results of a clinical phase I trial indicated that the pharmacokinetic characteristics of the conjugated CPT did not exhibit substantial benefits in comparison to the unconjugated CPT [32]. Chimmitecan, a lipophilic derivative of camptothecin (CPT), has demonstrated significant cytotoxicity by effectively reducing the catalytic activity of Topoisomerase 1 (top1) and forming stable covalent complexes between Top1 and DNA. These effects are equivalent to the impact observed with top1 inhibition [33] (Table 1).

Indole Alkaloids

The heterocyclic aromatic chemical indole (C_8H_7N) is characterized by its weakly basic characteristics. Its fused structure, which consists of a pyrrole ring and a benzene ring, allows 10 pi electrons to delocalize over the entire molecule. Numerous well-known plant groups, such as the Apocynaceae, Rubiaceae, Nyssaceae, and Loganiaceae, have been found to contain indole-based alkaloids [35]. According to Muranaka and Saito [2010], these indole alkaloids are an important class of naturally occurring substances with strong pharmacological significance. Clinically, a range of indole alkaloids and their synthetic analogs are used to treat a number of cancers, including breast cancer, small-cell lung cancer, malignant lymphoma, and acute leukemia [35-37]. When it comes to signal transduction, mitogen-activated protein kinases (MAPKs) are crucial mediators that convert extracellular inputs into a variety of intracellular reactions. Among these pathways, the MAPK cascade is essential for transmitting signals from membrane receptors to intracellular targets, which has a significant impact on autophagy and other cellular functions. The three main MAPK branches-ERK1/2, JNK, and p38/MAPK-have been thoroughly studied in the control of cell destiny and homeostasis [36, 38]. Chinese medicinal plant Evodiae fructus causes human glioblastoma cells to undergo both autophagy and death. Reduced cytosolic calcium levels, alteration of the mitochondrial membrane potential, and increased apoptosis after calcium channel blocking are all indicators that evodiamine's pro-apoptotic mechanism includes the calcium signaling pathways. These results imply that the activation of a calcium-dependent JNK pathway is how evodiamine-induced autophagy functions [39]. Another indole-based alkaloid that was separated from Murraya Koenigii is isomahanine, which has been shown to have a variety of biological functions. Studies using the multidrug-resistant oral squamous cell carcinoma cell line CLS-354/DX have demonstrated that this chemical concurrently induces autophagy and apoptosis. These effects are linked to P38/MAPK signaling pathway activation and are most likely caused by stress reaction in the endoplasmic reticulum (ER) [40]. In mammalian systems, Beclin-1, which is encoded by a gene on chromosome 17q21, is an essential autophagy regulator. This 450-amino-acid, 60 kDa protein forms multi-protein complexes to carry out its autophagic actions. Its regulation mechanism includes interaction with Bcl-2 protein family members, including Bcl-2 and Bcl-XL, which bind to Beclin-1's BH3 domain and stop it from associating with the PI3KC3 complexes, so inhibiting autophagy [4, 42].

Taxol

Taxoids are a class of cyclic diterpenoids distinguished by their characteristic taxadiene core. These compounds have attracted considerable interest due to their strong anticancer properties, with paclitaxel-commonly referred to as Taxol, being the most well-known representative. Paclitaxel is predominantly obtained from the bark of yew trees [genus Taxus], which are slow-growing species. Paclitaxel has become one of the most widely employed chemotherapy drugs for treating a range of cancers [43, 44]. The pioneering research conducted by the Horwitz laboratory revealed the potent cytotoxic effects of Taxol, demonstrating its ability to inhibit HeLa cell proliferation at nanomolar concentrations. While cells exposed to the drug progressed through a normal S phase, Taxol induced a cell cycle arrest specifically at metaphase. The most remarkable and distinctive property of Taxol was its ability to enhance the polymerization of stable microtubules, a mechanism that underlies its antimitotic activity [45]. Beyond its clinical application in oncology, paclitaxel plays a crucial role in cell biology research. In unperturbed cells, the proper bipolar attachment of sister chromatids generates tension at kinetochores, facilitating the stabilization of kinetochore-microtubule interactions. This process, often referred to as sister chromatid exchange, ensures accurate chromosome segregation. However, paclitaxel treatment disrupts this dynamic by reducing the tension on kinetochores that maintain bipolar attachment. As a result, paclitaxel serves as a valuable experimental tool for inducing mitotic arrest and investigating the interplay between tension and attachment in the activation of the mitotic checkpoint [46].

Polyphenols

Polyphenolic compounds are one of the most diverse classes of secondary plant metabolites. They have several qualities that are beneficial to human health, such as antioxidant, anti-inflammatory, and antineoplastic actions. Previous research has demonstrated that it inhibits the growth of a wide variety of malignancies, making it an effective anticancer agent.The limited bioavailability of polyphenolic compounds is one of the issues that arises while using these kinds of substances.

DOI: https://doi.org/10.54393/fbt.v5i2.177

Upon ingestion, polyphenols must first be absorbed before undergoing biotransformation into their bioactive forms. Typically, following consumption, polyphenols undergo enzymatic hydrolysis of their carbohydrate moieties [if present], resulting in the release of aglycones. These aglycones then traverse the epithelial cells of the small intestine primarily through passive diffusion, facilitating their subsequent metabolism and systemic distribution. This happens after polyphenols have been broken down into their parts. Polyphenols exhibit antioxidant, anti-inflammatory, antiproliferative, pro-apoptotic, and anti-angiogenic properties. These compounds can modulate various molecular signaling pathways implicated in cancer development and progression, including the PI3K/Akt, MAPK, NF-κB, and Wnt/β-catenin pathways. For example, quercetin has been shown to suppress tumor growth in colon and breast cancer models by inducing apoptosis and cell cycle arrest, while EGCG from green tea can inhibit metastasis and reduce angiogenesis in prostate and lung cancers. If polyphenolic compounds are unable to be absorbed in this district, they will make their way to the colon, where the microbiota will metabolize them. Therefore, it is expected that an alteration in the gut microbiota will lead to a reduction in the amount of polyphenols that are absorbed by the human body, which will hurt human health. Nanomization has emerged as a prominent strategy among various formulation techniques aimed at enhancing the bioavailability of polyphenolic compounds. This approach utilizes diverse polymers and nanostructured carriers to develop polyphenolic compounds as potential anticancer agents, thereby improving their therapeutic efficacy. PLGA nanoparticles are comprised of polymers that can break down naturally. This polymer is made up of lactic acid and glycolic acid, both of which are acids that the body can digest [47-49].

Flavonoids

Flavonoids represent a diverse class of naturally occurring polyphenolic compounds characterized by the presence of a flavan core structure. They are structurally characterized by a 15-carbon skeleton consisting of two aromatic rings connected by a three-carbon bridge, forming a closed pyran ring C. This basic structure, known as the flavan nucleus, underlines the diversity of flavonoids, which are broadly classified into subgroups: flavones e.g., luteolin, apigenin, flavonols e.g., quercetin, kaempferol), flavanones e.g., naringenin, isoflavones e.g., genistein anthocyanins, and chalcones. They are abundantly distributed in a wide range of dietary sources, including fruits, vegetables, and plant-based beverages, rendering them one of the most prevalent groups of phytochemicals in the human diet. Flavonoids are widely famous for their broad spectrum of biological activities, notably their antioxidant, anticancer, anti-inflammatory, and antimutagenic properties. Due to their significant presence in commonly consumed foods and drinks, such as tea, wine, and various fruits and vegetables-they are considered promising candidates for therapeutic intervention in numerous diseases, including cancer. From a pharmacological perspective, flavonoids exhibit multifaceted anticancer properties by interfering with key pathways involved in carcinogenesis. These include antioxidant activity, which reduces oxidative stress and prevents DNA damage; anti-inflammatory effects, achieved through inhibition of NF-κB and COX-2 signaling; and induction of apoptosis by intrinsic and extrinsic pathways by modulating proteins such as Bcl-2, Bax, and caspases. Cell cycle arrest, particularly at G1 or G2/M phases, through modulation of cyclins and CDKs. Inhibition of angiogenesis, often by downregulating VEGF expression. Flavonoids exert anticancer effects by targeting multiple hallmarks of tumor development and progression, including the inhibition of invasion, metastasis, and angiogenesis, modulation shown in Figure [50, 51]. Flavonoids can't be used as an anticancer treatment because of things like their low bioavailability, poor stability and solubility, ineffective targeted delivery, and chemoresistance. Some famous examples of flavonoids and how they work are discussed below (Figure 3).

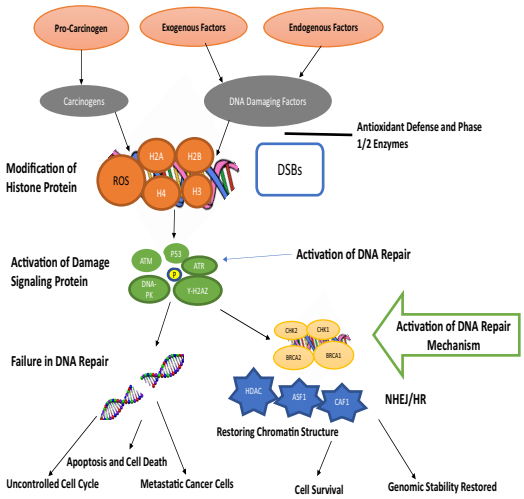

Figure 3: Cellular Mechanisms of Flavonoids on DNA Damage and Repair

Quercetin

Quercetin (Quer) is a widely distributed pentahydroxyflavone, predominantly present in many fruits and vegetables [52]. Querc holds significant importance, particularly due to its potential as an anticancer agent. The variable method of action is responsible for exhibiting varying efficacies, specificities, and targets in different types of malignancies [53]. Quercetin has been observed to impede the multiplication of cancer cells by the induction of apoptosis, hence diminishing the growth of various types of malignancies. The overexpression of cyclin-

DOI: https://doi.org/10.54393/fbt.v5i2.177

dependent kinase 6 [CDK6] has been closely linked to the onset and progression of several cancer types. Recent studies have highlighted quercetin as an effective natural inhibitor of CDK6. In a comparative analysis of various phytochemicals, including gallic acid, ferulic acid, caffeic acid, rosmarinic acid, capsaicin, tocopherol, limonene, and quercetin-quercetin showed the most pronounced inhibitory effect on CDK6 activity [54]. Research indicates that quercetin promotes apoptosis predominantly through the intrinsic [mitochondrial] pathway. This involves the activation of caspase-3 and caspase-9, the release of cytochrome c into the cytoplasm, and the subsequent cleavage of poly [ADP-ribose] polymerase [PARP]. These apoptotic events have been consistently observed in a variety of human cancer cell lines, including MCF-7 (breast cancer), CNE2 and HK1 (nasopharyngeal carcinoma), HL-60 (leukemia), HPBALL (thymic lymphoma), and SCC-9 (oral squamous carcinoma). A key feature of this pathway is the disruption of mitochondrial membrane potential, which facilitates caspase activation and PARP cleavage initiated by quercetin [55; 54]. Quercetin's lipophilic nature enables it to cross cell membranes and modulate multiple intracellular signaling pathways involved in chemoprevention. It has been reported to inhibit cytochrome P450 (CYP) enzymes, which are essential for drug metabolism. Additionally, quercetin downregulates the expression of metastasis-associated proteins, particularly matrix metalloproteinases (MMPs), thereby impairing metastatic potential. Since angiogenesis supports tumor dissemination, quercetin's anti-angiogenic properties-evident through its suppression of neovascularization in the tumor microenvironment, further contribute to its anticancer effects. In the group of rats subjected to quercetin treatment, there was a notable reduction in the serum levels of inflammatory markers such as IL-1β, IL-1, IL-6, IL-8, and TNF-α, when compared to rats that were administered ethanol. However, it is noteworthy that IL-10 exhibited a distinct increase in the quercetin-treated group [56] (Table 2).

Genistein

The chemical name for genistein is 4',5,7-trihydroxyisoflavone. Genistein is considered the most basic isoflavonoid chemical within the Leguminosae family in terms of its biosynthesis. This compound serves as a pivotal intermediary in the production of intricate isoflavonoids, which play significant roles in either facilitating or impeding interactions between plants and microorganisms. The primary sources of genistein include legumes such as beans, alfalfa, peas, and soybeans [56]. Genistein exerts its anticancer effects through the modulation of a wide array of molecular signaling pathways. These include the regulation of microRNAs and the modulation of key proteins associated with apoptosis, transcription regulation, tumor suppression, kinase activity, growth factor signaling, and receptor functions [57]. A substantial body of evidence supports genistein's ability to trigger apoptosis by influencing the expression of proteins central to cell death mechanisms. In human cervical cancer (HeLa) cells, genistein enhances apoptosis by increasing the activity of caspase 9 and caspase 3. In addition, studies on LoVo and HT-29 colon cancer cell lines have shown that genistein induces cell death by inhibiting the NF-κB signaling cascade and altering the balance of apoptotic regulators-specifically, downregulating the anti-apoptotic protein Bcl-2 and upregulating the pro-apoptotic protein Bax. The pro-apoptotic effects of genistein in HT-29 cells have also been linked to this influence on the caspase-3 and p38 MAPK pathways, reinforcing its therapeutic potential as an anticancer compound, as depicted in Figure 4 [58, 59]. The process of cancer metastasis has been observed to be contingent upon increased expression levels of matrix metalloproteinases (MMPs). A research study conducted on nude mice demonstrated that genistein exerts inhibitory effects on the metastasis of salivary adenoid cystic carcinoma (ACC) cells. This suppression was associated with downregulating vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) expression. Furthermore, additional findings suggest that genistein effectively inhibits the migration of MAT-LyLu and AT-2 rat prostate cancer cells, further supporting its potential as an antimetastatic agent [59]. GEN downregulates the TLR4/MYD88/ NF-κB and EGFR/VEGFR pathways, thereby suppressing inflammatory mediators (e.g., TNF-α, STAT3, JAK) and transcription factors (NF-κB, C-Jun, ATF2), which leads to decreased expression of pro-inflammatory cytokines and matrix metalloproteinases [MMP-2, MMP-9] (Figure 4).

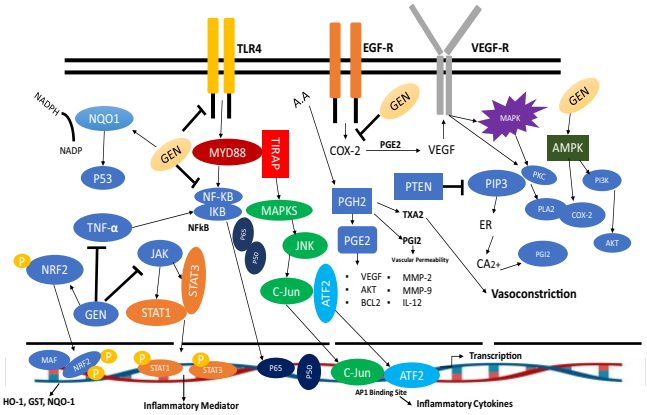

Figure 4: Schematic Diagram Illustrating the Inhibitory Effect of Genistein (GEN) on Key Signaling Pathways Involved in Cancer Metastasis, Inflammation, and Angiogenesis. Epigallocatechin Gallate (EGCG)

DOI: https://doi.org/10.54393/fbt.v5i2.177

Epigallocatechin-3-gallate (EGCG) is widely recognized as the most extensively investigated compound within the flavanol category. EGCG, a polyphenolic compound resulting from the esterification of epigallocatechin and gallic acid, is the predominant catechin with antioxidant properties found in green tea. Green tea and epigallocatechin gallate (EGCG) have shown promise as potential candidates for the prevention and control of various health conditions, including cancer, obesity, diabetes mellitus, cardiovascular illnesses, neurological diseases, and liver diseases. This is attributed to their notable antioxidant, anti-inflammatory, and anti-fibrogenic capabilities [60, 61]. DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) are enzymatic proteins that play a crucial role in the process of transcriptional gene silencing. On the other hand, histone acetyl transferases [HATs] are responsible for positively regulating gene expression. Multiple studies have demonstrated the involvement of EGCG in regulating epigenetic mechanisms, particularly through its influence on the expression and activity of DNMTs, HDACs, and HATs in various types of tumors. Epigallocatechin gallate (EGCG) exhibits binding affinity towards DNA methyltransferase (DNMT) and effectively hinders its enzymatic activity by competitive inhibition, as evidenced by a measured inhibition constant (Ki) of 6.89 μM. Consequently, this molecular interaction leads to the reactivation of genes that have been silenced through methylation in prostate cancer cells. The binding of EGCG to DNMT3B and HDAC1 was supported by molecular modeling and docking studies [62] (Table 2).

Table 2: Key Flavonoids, Anti-Tumor Activities, and Underlying Mechanisms

Compounds	Class	Anticancer Mechanisms	Plant Sources	References
Quercetin	Flavonoid	Apoptosis, cell cycle arrest, and anti-inflammatory	Fruits, vegetables, tea	[52, 56, 57, 68]
Genistein	Flavonoid	Apoptosis, autophagy, anti-anti-angiogenesis	Soybeans	[59, 63]
Apigenin	Flavonoid	Apoptosis, cell cycle arrest, anti-angiogenic	Parsley, thyme, chamomile	[64-68]
EGCG	Flavonoid	Apoptosis, auto -phagy, epigenetic modulation	Green tea	[60-62]

Curcumin

Curcumin, also known as diferuloylmethane, has the molecular formula C21H2006. The hydrophobic polyphenol in question is derived from the rhizome of perennial herbs of the Curcuma genus, which belongs to the Zingiberaceae family. *Curcuma longa* is a notable species in this group [69, 70]. Curcumin, a bioactive compound derived from *Curcuma longa*, has demonstrated considerable potential in inhibiting the progression of various cancer types. It effectively modulates critical processes involved in tumor development, including cell proliferation, invasion, angiogenesis, and metastasis. The anticancer activity of curcumin is primarily attributed to its regulatory influence on numerous cellular signaling pathways. Notably, curcumin has been reported to suppress the expression of oncogenic microRNAs (miRNAs) while enhancing the levels of tumor-suppressive miRNAs, thereby contributing to its therapeutic efficacy. Through its simultaneous modulation of these pathways, curcumin serves as a potent regulator of tumor progression and enhances the therapeutic response in cancer treatment strategies [71]. When growth factors and cytokines bind to receptors, downstream signaling pathways like PI3K/Akt, JAK/STAT, and MAPK are activated. These pathways affect cell survival (Table 3), (Figure 5).

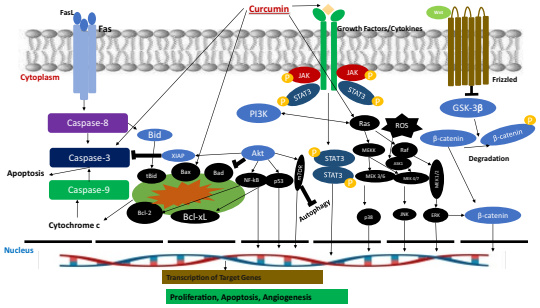

Figure 5: Signal Transduction Mechanisms Controlled by Curcumin Regulate Cancer Progression

Table 3: Key Non-Flavonoids, Anti-Tumor Activities, and Underlying Mechanisms

Compounds	Class	Anticancer Mechanisms	Plant Sources	References
Resveratrol	Non-flavonoid	Apoptosis, auto-phagy, anti- anti -angiogenesis	Grapes, berries	[72-74]
Curcumin	Non-flavonoid	Modulates PI3K/Akt, Wnt, NF-kB pathways	Turmeric	[70, 71,74]

Rhein

Rhein (1,8-dihydroxy-3-carboxyanthraquinone) is a naturally occurring anthraquinone derivative primarily found in plants belonging to the Polygonaceae family, notably *Rheum* [rhubarb] and *Reynoutria japonica* (Japanese knotweed). It has been extensively used in traditional Chinese medicine, largely due to its potent anticancer properties. Against HepG2 and Huh7, rhein has shown significant cytotoxic effects in liver cancer cell lines. Treatment with Rhein resulted in dose- and time-dependent alterations in cell morphology. Additionally, Rhein was found to induce double-stranded DNA breaks in these cells, further confirming its pro-apoptotic activity. A marked reduction in mitochondrial membrane potential (MMP) was also observed, suggesting early apoptotic events and compromised cellular integrity. One of the key mechanisms underlying Rhein's apoptotic effects is the

DOI: https://doi.org/10.54393/fbt.v5i2.177

elevation of intracellular reactive oxygen species (ROS). The ROS generated in response to Rhein exposure contributes to the initiation of apoptosis by activating redox-sensitive signaling pathways. Specifically, the JNK/Jun/caspase-3 cascade is activated, promoting programmed cell death. Furthermore, it activates multiple caspases, including caspase-1, -3, -8, -9, and -12, indicating a broad engagement of both intrinsic and extrinsic apoptotic pathways. Rhein also induces cell cycle arrest at the S-phase by downregulating cyclin A1, cyclin E1, cyclin D1, and CDK2. These results highlight Rhein's potential as a promising anticancer agent [75] (Table 4).

Table 4: Key Anthraquinones, Anti-Tumor Activities, and Underlying Mechanisms

Compounds	Class	Anticancer Mechanisms	Plant Sources	References
Emodin	Anthraquinone	ROS generation, JNK activation, and autophagy	Rhubarb, buckthorn	[77,78]
Rhein	Anthraquinone	Apoptosis via ROS,JNK/caspase signaling	Chinese rhubarb	[20; 75]

Limitations and Future Prospects

This review is limited by its reliance on published experimental and preclinical studies, as large-scale clinical validation of many phytochemicals remains insufficient. Variability in extraction methods, compound standardization, bioavailability issues, and potential drug-herb interactions further complicate therapeutic translation. Future research should prioritize well-structured clinical trials, nanotechnology-based delivery systems, molecular docking validation, and combination therapy strategies to enhance therapeutic precision and efficacy. Integrating phytochemicals with personalized medicine and targeted oncogenic pathway modulation may unlock their full potential as adjunct or standalone anticancer agents.

CONCLUSION

The pursuit of effective cancer therapies remains one of the most pressing challenges in modern medicine, necessitating innovative and multidisciplinary approaches. Among the strategies under investigation, the use of plant-derived extracts has emerged as a promising frontier that integrates traditional medicinal practices with contemporary biomedical research. This review explores the diverse and potent anticancer potential of various plant extracts, highlighting their multifaceted mechanisms of action. Plant-based compounds have been shown to exert anticancer effects through several pathways, including modulation of cell signaling, inhibition of enzymes crucial for tumor progression, and direct induction of apoptosis. These mechanisms often operate synergistically, enhancing therapeutic efficacy. Additionally, many plant extracts possess antioxidant and anti-inflammatory properties, further supporting their role in both cancer prevention and treatment. While plant extracts are not a universal solution to cancer, they significantly broaden the spectrum of available therapeutic options and offer renewed hope to patients worldwide.

Authors' Contribution

Conceptualization: HAA, MNA
Methodology: MA, JA
Formal analysis: JA
Writing and Drafting: HAA, AW, SAAS
Review and Editing: HAA, AW, SAAS, MNA, MA, JA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Mayor S. Covid-19: Eight In 10 Wuhan Residents with Antibodies Had No Symptoms, Finds Study. British Medical Journal. 2021. doi: 10.1136/bmj.n778.

[2] Bahrami H, Tafrihi M. Global Trends of Cancer: The Role of Diet, Lifestyle, and Environmental Factors. Cancer Innovation.2023Aug;2(4):290–301.doi: 10.1002/cai2.76.

[3] Heng HH, Stevens JB, Bremer SW, Liu G, Abdallah BY, Ye CJ. Evolutionary Mechanisms and Diversity in Cancer.Advances In Cancer Research.2011Jan;112:217–53. doi: 10.1016/B978-0-12-387688-1.00008-9.

[4] Lin PM, Lee HM, Huang CI, Tai TS, Chen JH, Chen CI *et al.* Synergistic Antiproliferative Effect of Ribociclib (LEE011) And 5-Fluorouracil on Human Colorectal Cancer.Anticancer Research.2020Nov;40(11):6265–71. doi: 10.21873/anticanres.14647.

[5] Torgovnick A, Schumacher B. DNA Repair Mechanisms in Cancer Development and Therapy.Frontiers In Genetics.2015Apr;6:157.doi:10.3389/fgene.2015.00157.

[6] Greenwell M, Rahman PK. Medicinal Plants: Their Use in Anticancer Treatment. International Journal of Pharmaceutical Sciences and Research.2015Oct;6(10): 4103.

[7] Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S *et al.* Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy. Molecules.2021Feb;26(4):1109.doi:10.3390/molecules26041109.

DOI: https://doi.org/10.54393/fbt.v5i2.177

[8] Dhillon H, Elsner K, Naehrig D, Halkett G. Reduced Patient Anxiety as a Result of Radiation Therapist-Led Psychosocial Support: A Systematic Review.2017

[9] Rübe CE, Raid S, Palm J, Rübe C. Radiation-Induced Brain Injury: Age Dependency of Neurocognitive Dysfunction Following Radiotherapy.Cancers.2023 May; 15(11): 2999. doi: 10.3390/cancers15112999.

[10] Mandal MK, Mohammad M, Parvin SI, Islam MM, Gazi HA. A Short Review on Anticancer Phytochemicals. Pharmacognosy Reviews.2023Jan;17(33).doi:10.5530/097627870236.

[11] Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK *et al*. Medicinal Plants and Cancer Chemoprevention.Current Drug Metabolism.2008 Sep; 9(7): 581–91. doi: 10.2174/138920008785821657.

[12] Mondal A, Gandhi A, Fimognari C, Atanasov AG, Bishayee A. Alkaloids for Cancer Prevention and Therapy: Current Progress and Future Perspectives. European Journal of Pharmacology.2019Sep;858:172472. doi: 10.1016/j.ejphar.2019.172472.

[13] El-Sayed NS, Jureka AS, Edwards MR, Lohan S, Williams CG, Keiser PT *et al*. Synthesis and Antiviral Activity of Fatty Acyl Conjugates of Remdesivir Against Severe Acute Respiratory Syndrome Coronavirus 2 And Ebola Virus. European Journal of Medicinal Chemistry.2021Dec;226:113862.doi:10.1016/j.ejmech.2021.113862.

[14] Joubert F, Guerrero-Moreno A, Fakih D, Reboussin E, Gaveriaux-Ruff C, Acosta MC *et al*. Topical Treatment with A Mu Opioid Receptor Agonist Alleviates Corneal Allodynia and Corneal Nerve Sensitization in Mice. Biomedicine & Pharmacotherapy.2020Dec;132:110794. doi: 10.1016/j.biopha.2020.110794.

[15] Ren JL, Zhang AH, Wang XJ. Traditional Chinese Medicine For COVID-19 Treatment. Pharmacological Research.2020Mar;155:104743.doi:10.1016/j.phrs.2020.104743.

[16] Almatroodi SA, Alsahli MA, Rahmani AH. Berberine: An Important Emphasis on Its Anticancer Effects Through Modulation of Various Cell Signaling Pathways.Molecules.2022Sep;27(18):5889.doi:10.3390/molecules27185889.

[17] De Oliveira AL, Comar JF, de Sá-Nakanishi AB, Peralta RM, Bracht A.The Action of P-Synephrine on Hepatic Carbohydrate Metabolism and Respiration Occurs Via Both Ca2+-Mobilization and cAMP Production. Molecular And Cellular Biochemistry.2014Mar;388(1):135–47.doi:10.1007/s11010-013-1905-2.

[18] Wang J, Qi Q, Feng Z, Zhang X, Huang B, Chen A *et al*. Berberine Induces Autophagy in Glioblastoma by Targeting the AMPK/mTOR/ULK1-Pathway.Oncotarget.2016Aug;7(41):66944.doi:10.18632/oncotarget.11396.

[19] Fernández-Caballero JA, Alvarez M, Chueca N, Pérez AB, García F.The Cobas® HCV GT Is A New Tool That Accurately Identifies Hepatitis C Virus Genotypes for Clinical Practice. PLoS One.2017 Apr; 12(4): e0175564. doi: 10.1371/journal.pone.0175564.

[20] Liu X, Ji Q, Ye N, Sui H, Zhou L, Zhu H *et al*. Berberine Inhibits Invasion and Metastasis of Colorectal Cancer Cells Via COX-2/PGE2 Mediated JAK2/STAT3 Signaling Pathway.PLoS One.2015May;10(5):e0123478.doi:10.1371/journal.pone.0123478.

[21] Xue P, Yang X, Liu Y, Xiong C, Ruan J. A Novel Compound RY10-4 Downregulates P-Glycoprotein Expression and Reverses Multidrug-Resistant Phenotype in Human Breast Cancer MCF-7/ADR Cells. Biomedicine & pharmacotherapy.2014;68(8):1049–56. doi: 10.1016/j.biopha.2014.10.004.

[22] Wang Z, Hou X, Wang Y, Xu A, Cao W, Liao M, Zhang R, Tang J. Ubiquitination of Non-Lysine Residues in the Retroviral Integrase.Biochemical and Biophysical Research Communications.2017;494(1–2):57–62.doi:10.1016/j.bbrc.2017.10.086.

[23] Samadi P, Sarvarian P, Gholipour E, Asenjan KS, Aghebati-Maleki L, Motavalli R, *et al*. Berberine: A Novel Therapeutic Strategy for Cancer.IUBMBlife.2020; 72(10): 2065–79. doi: 10.1002/iub.2350.

[24] Gogol EP, Luecken U, Bork T, Capaldi RA. Molecular Architecture of Escherichia coli F1 Adenosinetriphosphatase.Biochemistry.1989;28(11):4709–16.doi:10.1021/bi00437a030.

[25] Harrison C. Opening the Door to an Epigenetic Target. Nature Reviews Drug Discovery.2012;11(9):672–3.doi:10.1038/nrd3827.

[26] Zhang H, Shen X, Sun S, Li Y, Wang S, Wei J, *et al*. Integrated Transcriptome and Proteome Analysis Provides New Insights into Camptothecin Biosynthesis and Regulation in Camptotheca Acuminata.Physiologia Plantarum.2023;175(3):e13916. doi: 10.1111/ppl.13916.

[27] Venditto VJ, Simanek EE. Cancer Therapies Utilizing the Camptothecins: A Review of The In Vivo Literature.Molecular Pharmaceutics.2010;7(2):307–49. doi: 10.1021/mp900243b.

[28] Kamle M, Pandhi S, Mishra S, Barua S, Kurian A, Mahato DK, *et al*. Camptothecin and Its Derivatives: Advancements, Mechanisms, and Clinical Potential in Cancer Therapy. Medical Oncology.2024;41(11):263. doi: 10.1007/s12032-024-02527-x.

[29] Ling X, Liu X, Zhong K, Smith N, Prey J, Li F. FL118, A Novel Camptothecin Analogue, Overcomes Irinotecan and Topotecan Resistance in Human Tumor Xenograft Models.American Journal of Translational Research. 2015; 7(10): 1765.

DOI: https://doi.org/10.54393/fbt.v5i2.177

[30] Ma J, Huang C, Yao X, Shi C, Sun L, Yuan L, *et al.* Inhibition of Hepatitis B Virus and Induction of Hepatoma Cell Apoptosis by ASGPR-Directed Delivery of shRNAs..2012

[31] Wang S, Zheng Y, Yu Y, Xia L, Chen GQ, Yang YZ, Wang LS. Phosphorylation of β-Actin by Protein Kinase C-Delta in Camptothecin Analog-Induced Leukemic Cell Apoptosis. Acta Pharmacologica Sinica.2008;29(1): 135–42. doi: 10.1111/j.1745-7254.2008.00753.x.

[32] Homsi J, Simon GR, Garrett C, Alberts DS, Weiss GJ, Springett G, *et al.* Phase I Trial of Poly-L-Glutamate Camptothecin (CT-2106) Administered Weekly in Patients with Advanced Solid Malignancies.Clinical Cancer Research.2007;13(19):5855–61.doi:10.1158/1078-0432.CCR-06-2821.

[33] DeVita VT, Eggermont AM, Hellman S, Kerr DJ. Clinical Cancer Research: The Past, Present, and the Future. Nature Reviews Clinical Oncology.2014;11(11):663–9. doi: 10.1038/nrclinonc 2014.153.

[34] Tilaoui M, Ait Mouse H, Zyad A. Update and New Insights on Future Cancer Drug Candidates from Plant-Based Alkaloids. Frontiers in pharmacology. 2021; 12: 719694. doi: 10.3389/fphar.2021.719694.

[35] Xu D, Xu Z. Indole Alkaloids with Potential Anticancer Activity. Current Topics in Medicinal Chemistry. 2020;20(21):1938–49.doi:10.2174/1568026620666200622150325.

[36] Nagata Y, Yoshida M, Kitazawa H, Araki E, Gomyo T. Improvements in Seasonal Allergic Disease with Lactobacillus plantarum No.14.Bioscience, Biotechnology, and Biochemistry.2010;74(9):1869–77. doi:10.1271/bbb.100270.

[37] Zhang W, Ren X, Zhang L, Chen J. Preparation and Performance of Thickened Liquids for Patients with Konjac Glucomannan-Mediated Dysphagia. Molecules.2022;27(7):2194.doi:10.3390/molecules27072194.

[38] Iovanna J, Dusetti N. Speeding Towards Individualized Treatment for Pancreatic Cancer by Taking An Alternative Road. Cancer Letters.2017; 410: 63–7. doi: 10.1016/j.canlet.2017.09.016.

[39] Koturbash I, Melnyk S, James SJ, Beland FA, Pogribny IP. Role of Epigenetic and miR-22 and miR-29b Alterations in the Downregulation of Mat1a and Mthfr Genes in Early Preneoplastic Livers in Rats Induced By 2-Acetylaminofluorene.Molecular Carcinogenesis .2013;52(4):318–27.doi:10.1002/mc.2 1861.

[40] Li F, Li Q, Shi X, Guo Y. Maslinic Acid Inhibits Impairment of Endothelial Functions Induced by High Glucose in HAEC Cells Through Improving Insulin Signaling and Oxidative Stress.Biomedicine and Pharmacotherapy.2017;95:904–13.doi:10.1016/j.biopha.2017.09.001.

[41] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of Autophagy And Inhibition of Tumorigenesis by Beclin 1.Nature.1999; 402(6762): 672–6. doi: 10.1038/45257.

[42] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, *et al.* Bcl-2 Antiapoptotic Proteins Inhibit Beclin 1-Dependent Autophagy.Cell.2005;122(6): 927–39. doi: 10.1016/j.cell.2005.07.002.

[43] Zhang D, Yang R, Wang S, Dong Z. Paclitaxel: New Uses for An Old Drug. Drug design, Development and Therapy. 2014; 8: 279–84. doi: 10.2147/DDDT.S56801.

[44] Weaver BA. How Taxol/Paclitaxel Kills Cancer Cells. Molecular biology of the cell.2014;25(18):2677–81.doi: 10.1091/mbc.e14-04-0916.

[45] Wani MC, Horwitz SB. Nature as A Remarkable Chemist: A Personal Story af the Discovery and Development of Taxol. Anti-cancer drugs.2014;25(5): 482–7. doi: 10.1097/CAD.0000000000000063.

[46] Waters JC, Chen RH, Murray AW, Salmon ED. Localization of Mad2 to Kinetochores Depends on Microtubule Attachment, Not Tension. The Journal of Cell Biology.1998;141(5):1181–91.doi:10.1083/jcb.141.5.1181.

[47] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-Based Nanoparticles: An Overview of Biomedical Applications. Journal of Controlled Release.2012;161(2):505–22.doi:10.1016/j.jconrel.2012.01.043.

[48] Davatgaran-Taghipour Y, Masoomzadeh S, Farzaei MH, Bahramsoltani R, Karimi-Soureh Z, *et al.* Polyphenol Nanoformulations for Cancer Therapy: Experimental Evidence and Clinical Perspective. International Journal of Nanomedicine.2017;12:2689–702.doi: 10.2147/IJN.S131973.

[49] Anand S, Bharadvaja N. Potential Benefits of Nutraceuticals for Oxidative Stress Management. Revista brasileira de farmacognosia.2022;32(2): 211–20. doi: 10.1007/s43450-022-00246-w.

[50] George VC, Dellaire G, Rupasinghe HV. Plant Flavonoids in Cancer Chemoprevention: Role in Genome Stability.The Journal of Nutritional Biochemistry.2017;45:1–4.doi:10.1016/j.jnutbio.2016.11.007.

[51] Kokot I, Piwowar A, Jędryka M, Kratz EM. Is There a Balance in Oxidative-Antioxidant Status in Blood Serum of Patients with Advanced Endometriosis? Antioxidants.2021;10(7):1097.doi:10.3390/antiox10071097.

[52] Erdman Jr JW, Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, *et al.* Flavonoids and Heart Health: Proceedings of The ILSI North America Flavonoids Workshop, May 31–June 1, 2005, Washington, DC. The Journal of Nutrition.2007;137(3):718S–737S.doi:

DOI: https://doi.org/10.54393/fbt.v5i2.177

10.1093/jn/137.3.718S.

[53] Rather RA, Bhagat M. Quercetin as an Innovative Therapeutic Tool for Cancer Chemoprevention: Molecular Mechanisms and Implications in Human Health. Cancer Medicine.2020;9(24):9181–9192.doi: 10.1002/cam4.1411.

[54] Kashyap D, Garg VK, Tuli HS, Yerer MB, Sak K, Sharma AK, *et al.* Fisetin and Quercetin: Promising Flavonoids with Chemopreventive Potential.Biomolecules .2019;9(5):174.doi:10.3390/biom905 0174.

[55] Thomsen AR, Mortensen A, Breinholt VM, Lindecrona RH, Peñalvo JL, Sorensen IK. Influence of Prevastein®, An Isoflavone-Rich Soy Product, On Mammary Gland Development and Tumorigenesis in Tg. NK (MMTV/c-neu) Mice. Nutrition And Cancer.2005;52(2):176–188 .doi:10.1207/s15327914nc52 02_8.

[56] Zhang R, Letham DS, Willcocks DA. Movement to Bark and Metabolism of Xylem Cytokinins in Stems of Lupinus Angustifolius. Phytochemistry.2002;60(5): 483–488. doi: 10.1016/S0031-9422(02)00085-7.

[57] Rasheed S, Rehman K, Shahid M, Suhail S, Akash MS. Therapeutic Potentials of Genistein: New Insights and Perspectives.Journal Of Food Biochemistry.2022;46 (9):e14228.doi:10.1111/jfbc.14228.

[58] Nalli M, Puxeddu M, La Regina G, Gianni S, Silvestri R. Emerging Therapeutic Agents for Colorectal Cancer. Molecules.2021;26(24):7463.doi:10.3390/molecules 26247463.

[59] Tuli HS, Tuorkey MJ, Thakral F, Sak K, Kumar M, Sharma AK, *et al.* Molecular Mechanisms of Action of Genistein in Cancer: Recent Advances. Frontiers in Pharmacology.2019;10:1336.doi:10.3389/fphar.2019. 01336.

[60] Chen Y, Chen Y, Shi Z, Jin Y, Sun H, Xie F, *et al.* Biosynthesis and Signal Transduction Of ABA, JA, And BRs In Response to Drought Stress of Kentucky Bluegrass. International Journal of Molecular Sciences.2019;20(6):1289.doi:10.3390/ijms200612 89.

[61] Li D, Cao D, Cui Y, Sun Y, Jiang J, Cao X. The Potential of Epigallocatechin Gallate in the Chemoprevention and Therapy of Hepatocellular Carcinoma. Frontiers in Pharmacology.2023;14:1201085.doi:10.3389/fphar .2023.1201085.

[62] Negri A, Naponelli V, Rizzi F, Bettuzzi S. Molecular Targets of Epigallocatechin-Gallate (EGCG): A Special Focus on Signal Transduction and Cancer. Nutrients. 2018; 10(12): 1936. doi: 10.3390/nu10121936.

[63] Desmet C, Warzée B, Gosset P, Mélotte D, Rongvaux A, Gillet L, *et al.* Pro-Inflammatory Properties of Thiazolidinediones. *Biochemical Pharmacology*. 2005; 69(2): 255–265. doi: 10.1016/j.bcp.2004.09.017.

[64] Singh A, Singh J, Parween G, Khator R, Monga V. A Comprehensive Review of Apigenin a Dietary Flavonoid: Biological Sources, Nutraceutical Prospects, Chemistry and Pharmacological Insights and Health Benefits. Critical Reviews in Food Science and Nutrition.2024;doi:10.1080/10408398.2024.23 90550.

[65] Sharma A, Ghani A, Sak K, Tuli HS, Sharma AK, Setzer WN, *et al.* Probing into Therapeutic Anti-Cancer Potential of Apigenin: Recent Trends and Future Directions. Recent Patents on Inflammation & Allergy Drug Discovery.2019;13(2):124–133.doi:10.2174/1872 213X13666190816160240.

[66] Shankar E, Goel A, Gupta K, Gupta S. Plant Flavone Apigenin: An Emerging Anticancer Agent.Current *Pharmacology Reports*.2017;3(6):423–446.doi:10.10 07/s40495-017-0113-2.

[67] Rahmani AH, Alsahli MA, Almatroudi A, Almogbel MA, Khan AA, Anwar S, *et al.* The Potential Role of Apigenin in Cancer Prevention and Treatment.Molecules.2022 ;27(18):6051.doi:10.3390/molecules 27186051.

[68] Zhao H, Zhu X, Luo Y, Liu S, Wu W, Zhang L, *et al.* LINC01816 Promotes the Migration, Invasion, And Epithelial-Mesenchymal Transition of Thyroid Carcinoma Cells by Sponging miR-34c-5p and Regulating CRABP2 Expression Levels.*Oncology Reports*. 2021; 45(5): 81. doi: 10.3892/or.2021.8032.

[69] Rigotto G, Montini B, Mattiolo A, Lazzari N, Piano MA, Remondini D, *et al.* Mechanisms Involved in the Promoting Activity of Fibroblasts In HTLV-1-Mediated Lymphomagenesis: Insights into the Plasticity of Lymphomatous Cells. International Journal of Molecular Sciences.2021;22(19):10562.doi:10.3390/ ijms 221910562.

[70] Alwabli AS, Alattas SG, Alhebshi AM, Mohammed N, Zabermawi FK, Azhar EI, *et al.* Pharmacological Modulation of Flavivirus Methyltransferase Activity of Dengue Virus (DEV). Indian Journal Of Pharmaceutical Education and Research.2019;53(3):S433–S436.doi: 10.5530/ijper.53.3s.116.

[71] Bartels AK, Gottert S, Desel C, Schäfer M, Krossa S, Scheidig AJ, Gotzinger J, Lorenzen I. KDEL Receptor 1 Contributes to Cell Surface Association of Protein Disulfide Isomerases.

[72] Konuray G, Erginkaya Z. Potential Use of *Bacillus Coagulans* in the Food Industry.Foods.2018Jun13; 7(6): 92. doi: 10.3390/foods7060092.

[73] Elshaer M, Chen Y, Wang XJ, Tang X. Resveratrol: An Overview of Its Anti-Cancer Mechanisms.*Life Sciences*.2018Aug15;207:340–9.doi:10.1016/j.lfs.2018 .06.028.

[74] Zaravinos A, Volanis D, Lambrou GI, Delakas D, *Spandidos DA*. Role of the Angiogenic Components, VEGFA, FGF2, OPN, and RHOC, In Urothelial Cell Carcinoma of the Urinary Bladder. Oncology Reports.

DOI: https://doi.org/10.54393/fbt.v5i2.177

2012 Oct; 28(4): 1159–66. doi: 10.3892/or.2012.1948.

[75] Sun PH, Chen G, Mason M, Jiang WG, Ye L. Dual Roles of Protein Tyrosine Phosphatase Kappa In Coordinating Angiogenesis Induced by Pro-Angiogenic Factors. International Journal of Oncology.2017Feb;50(4):1127–35.doi:10.3892/ijo. 2017.3884.

[76] Shi P, Huang Z, Chen G. Rhein Induces Apoptosis and Cell Cycle Arrest in Human Hepatocellular Carcinoma BEL-7402 Cells. The American Journal of Chinese Medicine.2008;36(04):805–13.doi:10.1142/S0192415X08006259.

[77] Adnan M, Rasul A, Hussain G, Shah MA, Sarfraz I, Nageen B, *et al.* Physcion and Physcion 8-O-β-D-Glucopyranoside: Natural Anthraquinones with Potential Anticancer Activities. Current Drug Targets.2021Mar;22(5):488–504.doi:10.2174/1389450121999201013154542.

[78] Chen YC, Shen SC, Lee WR, Hsu FL, Lin HY, Ko CH, *et al.* Emodin Induces Apoptosis in Human Promyeloleukemic HL-60 Cells Accompanied by Activation of Caspase 3 Cascade but Independent of Reactive Oxygen Species Production. Biochemical Pharmacology.2002Dec;64(12):1713–24.doi:10.1016/S0006-2952(02)01386-2.

DOI: https://doi.org/10.54393/fbt.v5i2.183

FUTURISTIC BIOTECHNOLOGY

https://fbtjournal.com/index.php/fbt

ISSN (E): 2959-0981, (P): 2959-0973

Vol 05 Issue 02, (April-June, 2025)

Review Article

OPEN ACCESS

Exploring Thermostable Lipases: Molecular Innovations and Expanding Industrial Horizons

Tehmina Bashir[1*], Alamgir[2], Syeda Amna Batool[3], Tahir Hussain[4], Washdev[5] and Iram Rafique[6]

[1]Department of Botany, Government College University, Lahore, Pakistan
[2]Faculty of Allied Health Sciences, Sohail University, Karachi, Pakistan
[3]Department of Botany, University of Narowal, Narowal, Pakistan
[4]Faculty of Allied Health Sciences, Hamdard Institute of Management Sciences, Karachi, Pakistan
[5]Chughtai Laboratory, Karachi, Pakistan
[6]Faculty of Allied Health Sciences, Karachi Institute of Nursing Allied Health Sciences, Karachi, Pakistan

ARTICLE INFO

Keywords:
Thermo-Stable Lipases, Commercial Uses, Characterization, Enzyme Engineering, Biocatalysts

How to Cite:
Bashir, T., Alamgir, ., Batool, S. A., Hussain, T., Washdev, ., & Rafique, I. (2025). Exploring Thermostable Lipases: Molecular Innovations and Expanding Industrial Horizons: Exploring Thermostable Lipases: Molecular Innovations . Futuristic Biotechnology, 5(2), 31-38. https://doi.org/10.54393/fbt.v5i2.183

***Corresponding Author:**
Tehmina Bashir
Department of Botany, Government College University, Lahore, Pakistan
tehminabashir25@gmail.com

Received Date: 23rd April, 2025
Revised Date: 20th June, 2025
Acceptance Date: 25th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Thermostable lipases are crucial biocatalysts valued for their stability and functionality in both aqueous and non-aqueous environments, enabling efficient catalysis at elevated temperatures. These enzymes, derived from both wild-type and genetically engineered strains, exhibit unique properties that make them indispensable across diverse industrial sectors. Despite their potential, challenges remain in optimizing their production, purification, and characterization to meet specific application requirements ranging from highly purified pharmaceutical formulations to less refined industrial uses. This review consolidates recent advances in the isolation, engineering, and detailed characterization of thermostable lipases, highlighting their substrate specificity, catalytic efficiency, enantioselectivity, and tolerance to harsh conditions. Emphasis is placed on emerging molecular innovations and metagenomic approaches for discovering novel enzymes with enhanced industrial applicability. By bridging fundamental insights with practical applications, this overview aims to guide future research and development efforts in harnessing thermostable lipases for expanding biotechnological horizons.

INTRODUCTION

Thermostable lipases are extensively used in various biocatalytic reactions due to their excellent performance at elevated temperatures. Operating at high temperatures offers several advantages, including increased product formation rates with minimal diffusional limitations, improved conversion efficiency, enhanced solubility of hydrophobic substrates, greater molecular mobility, and a reduced risk of microbial contamination [1]. Lipases (EC 3.1.1.3, triacylglycerol acylhydrolases) rank among the most widely employed enzymes in organic synthesis and biotechnological processes. Although lipases from extremophiles exhibit activity across diverse pH ranges, their stability often declines above 70°C. Typically, microbial lipases show optimal activity between 30°C and 65°C. Extremophilic organisms inhabiting various environments have been identified as sources of a broad spectrum of thermostable lipases, whose stability under limited water conditions enhances their industrial applicability [2, 3]. Many thermostable lipases have been characterized from moderate extremophiles, particularly

DOI: https://doi.org/10.54393/fbt.v5i2.183

from the genus Bacillus, such as Bacillus sp. J-33 [4], *Lactobacillus acidophilus* [5], *Bacillus thermoleovorans* ID1 [6], and *Pseudomonas aeruginosa* species [7]. Advances in genomics and protein engineering continue to deepen our understanding of thermostable lipases. These enzymes maintain exceptional stability in both aqueous and non-aqueous environments at elevated temperatures, facilitating higher reaction rates, reducing medium viscosity, and minimizing microbial contamination [1, 2]. Recent studies highlight structural features that contribute to their enhanced properties, including robust hydrogen bonding networks, increased hydrophobic interactions, and optimized surface loops—traits commonly observed in enzymes from extremophiles. For example, semi-rational design strategies have been used to improve thermostability, as demonstrated in novel esterase structures [8]. The exploration for novel thermostable lipases increasingly employs metagenomic approaches, enabling the discovery of enzymes from unculturable microorganisms in diverse extreme environments, such as medicinal wastewater [9, 10]. This has expanded opportunities to isolate wild strains with unique features and to engineer recombinant strains tailored to biotechnological needs. The intrinsic heat resistance of proteins in extremophiles arises from multiple molecular interactions that stabilize their structure, including strengthened ionic networks, enhanced hydrophobic contacts, increased hydrogen bonding, the presence of disulfide bridges, shortened surface loops, and the capping of N- and C-termini. Moreover, thermophilic enzymes exhibit a densely packed and rigid framework that supports stability at high temperatures but often compromises catalytic activity at lower temperatures. This reduced activity at moderate temperatures limits their application in processes where fine chemicals remain stable only under such conditions. Additionally, these biocatalysts can be sensitive to adverse factors like organic solvents, ionic strength variations, extreme pH, high pressure, and temperature fluctuations, which may challenge their economic viability in industrial processes [11]. Thermal Stability (T Max), Optimum pH, and half-life ($t_{1/2}$) of thermostable lipases from various naturally sourced microorganisms, illustrating their diverse stability profiles at different temperatures, are shown in Table 1.

Table 1: Thermal Stability, Optimum pH, and Half-Life of Thermostable Lipases From Various Naturally Sourced Microorganisms

Source	T max°C	pH optimum	Stability	References
Bacillaceae sp. RSJ1	50°C	8-9	$t_{1/2}$ = 150, 90, 55, 7 and 45 min at 60°C,65°C, 71°C and 76°C, respectively	[11]
Pseudomonas spp.	90°C	11	$t_{1/2}$ = 13 hr at 90°C	[12]
Bacillus thermoleovorans ID-1	70-75°C	7.5	$t_{1/2}$ = 30 min at 70°C and 1 hr at 60°C	[13]
Bacillus strain A30-1	60°C	6-9	$t_{1/2}$ = 8 hr at 75°C	[14]
AneurinIbacillus thermoaerophilus strain HZ	65°C	7	$t_{1/2}$ = 3 hr 10 min and 1 hr 20 min at 65°C and 70°C, respectively	[15]
Burkholderia multivorans V2	45-50°C	8	$t_{1/2}$ = ten and 50 minutes at fifty to sixty degrees Celsius, respectively	[16]
Burkholderia spp.	60°C	8.5	$t_{1/2}$ = 2 and 0.5 hr at 50°C and 60°C, respectively	[17]
Bacillus subtilis NS 8	60°C	7	$t_{1/2}$ = 4 hr 33 min at 60°C, 52 min at 71°C and ~42 min at 80°C	[18]
Bacillus coagulans MTCC-6375	45°C	8.5	$t_{1/2}$ = 4 hr 33 min at 60°C, 51 min at 70°C and ~42 min at 80°C	[19]
Geobacillus thermodenitrificans IBRL-nra	65°C	7	$t_{1/2}$ = 20 min at 55°C. 65°C 7 $t_{1/2}$ = 8 hr at 60°C, 16 hr at 65°C – 75°C	[20]

Optimum pH and temperature conditions for catalytic activity of thermostable lipases from various micro-organisms are shown in Table 2.

Table 2: Optimum pH and Temperature Conditions for Catalytic Activity of Thermostable Lipases from Various Microorganisms

Microorganism	Enzyme Optimum pH	Enzyme Optimum Temperature (°C)	References
Bacillaceae sp. RSJ-1	8-9	50°C	[11]
Bacillaceae acidocaldarius	-	68 to 70°C	[21]
Bacillaceae strain J33	8	60°C	[22]
Bacillus thermocatenulatus BTL2	9	60-70°C	[23]
Geobacillus Stearothermophilus	-	65°C	[24]
Geobacillus Stearothermophilus	8	70°C	[25]
Pseudomonas spp.	15	90°C	[26]
Pseudomonas sp.	9.7	60°C	[27]
Pyrobaculum calidifontis	-	95°C	[28]
Pyrococcus furiosus	-	99°C	[29]

DOI: https://doi.org/10.54393/fbt.v5i2.183

Pyrococcus horikoshii	7	95°C	[30]
Pyrococcus horikoshii	5.9	90°C	[31]
Staphylococcus aureus	5-12	55°C	[32]
Rhizopus chinensis	9	30-50°C	[33]
Thermoanaerobacter thermohydrosulfuricus	7-8	75-90°C	[34]
Staphylococcus xylosus	5-12	65-70°C	[35]
Bacillus Subtilis	10	55 to 79°C	[36]
Bacillus pumilus Rk31	7	40-70°C	[11]

Despite extensive studies on lipases from mesophilic and thermophilic sources, a consolidated understanding of the molecular determinants governing thermostability, catalytic efficiency, and industrial adaptability remains fragmented. Many reported thermostable lipases demonstrate promising activity under laboratory conditions; however, challenges persist in translating these enzymes into cost-effective, large-scale industrial applications due to limitations in production yield, solvent tolerance, and long-term operational stability. Furthermore, comparative analyses integrating structural insights, metagenomic discoveries, and enzyme engineering strategies are still limited. Therefore, a comprehensive review bridging molecular innovations with expanding industrial demands is essential to guide future biotechnological advancements.

Industrial Use of Thermostable Lipases

The application of substance catalysts doesn't just include many drawbacks linked to it; however as well provides an increase to several unwanted byproducts together with poisonous effluents, while at the same time, substrate uniqueness, bio-degradability, and lots of these kinds of positive aspects related to biocatalysts provide them with an advantage over the usage of catalysts in the chemical industrial sectors [37]. Thermostable enzymes can take on higher temperatures, therefore endowing a long half-life to the bio-catalyst. These types of enzymes may also put up with elevated levels of substrates as well as be protected from chemical denaturants. Their potential to perform different reactions at a high reaction rate as a result of a rise in substrate diffusion coefficient, as well as lower viscosity at elevated temperature ranges, make them a well-liked option over the sources of mesophiles [11]. The bio-technological possibilities related to lipases cause them to become a good biocatalyst within the world of many bio-technological and industrial sectors.

Application of Thermostable Lipases in the Food Industry

Lipases play a large role in the food industry in where they are widely used for manufacturing as well as customization of fats and oils (since they develop an essential element of food) to produce healthier food items. Lipases modify the lipids by causing the alteration within the placement of the important fatty acids in several glycerides [8]. The uniqueness related to lipases ensures they are a perfect option for oleochemical industrial sectors for the manufacturing of several higher value-added products, such as fats in human milk alternatives, the obroma oil counterparts, along with other specific prepared lipids [38]. Since absolutely no method provides such specificities, this amazing trait of thermostable lipases might be focused on industrial and commercial advancements.

Problem/Need

Lipases from *Thermomyces lanuginosus* happen to be identified by producing different transesterification and esterification reactions. Lipases from *Thermomyces lanuginosus* are recognized to perform the development of glycerides to be able to create healthier kinds of margarine [39]. Lipases seemed to be involved with the growth and development of several favouring agents. Thermostable lipases from several microbes have been employed to result in the oil customization, milk fat manufacturing similar to lipids, production of margarine making use of glycerides, and so on. Modification of fats and oils to produce healthier food products and speciality lipids requires enzymes with high specificity. Chemical catalysis often lacks positional specificity, generates unwanted byproducts, and cannot tailor fatty acid composition effectively.

How Thermostable Lipases Solve It

Thermostable lipases catalyze selective transesterification and esterification reactions, enabling precise alteration of fatty acid positions in glycerides at elevated temperatures, improving stability and reaction rates. This allows the creation of customized lipids, flavor development, and healthier fat substitutes [40].

Examples with Relevant Performance Data

Lipases from Thermomyces lanuginosus are recognized for producing modified glycerides used to make healthier margarine and for flavor generation. Beyond classic uses, thermostable lipases assist in synthesizing novel compounds such as mono- and di-acyl esters of glyceryl caffeate with enhanced antioxidant properties [3]. Their effectiveness at elevated temperatures (up to 70°C) supports industrial scalability.

Future Directions

Ongoing industrial innovation aims to improve thermostability and substrate specificity through enzyme engineering [41]. These advances will broaden the application scope in food biotechnology, enabling the production of novel functional lipids, customized fats for nutrition, and improved process efficiency.

DOI: https://doi.org/10.54393/fbt.v5i2.183

Detergent Sector and Thermostable Lipases

The cleaning agent market is among the major marketplaces for enzymes, along with a huge development that takes place to generate the newest enzymes, which include proteases, lipases, and amylases, along with greater as well as significantly better potential. The foremost commercially essential use of Thermostable lipases is within the cleaning agent business, where it's included with the laundry detergent for boosting the second activity [42]. Enzyme revenue in '95 was approximately thirty million dollars, with cleaning agent enzymes creating 30 per cent. The production of laundry detergents is approximately 13 billion tons per year, and the number of lipases is approximately 1,000. One of the primary problems in the laundry sector is the excretion of the adsorbed lipids through the components that are normally constructed of long-chain, water-insoluble triacylglycerols. These lipids might be eliminated by using cleaners containing lipolytic enzymes, which break them down into complementary fatty acids, diacylglycerols, and monoacylglycerols [43]. The First commercialized lipase used in cleaning agents was lipase TM, which Novo Nordisk introduced in the early nineteen nineties.

Problem/Need

Effective removal of water-insoluble, long-chain triacylglycerol lipid stains under harsh alkaline, high-temperature laundry conditions remains a challenge [44]. Conventional detergents lack sufficient enzymatic components to fully degrade these lipids, impacting cleaning performance and fabric care.

How Thermostable Lipases Solve It

Thermostable lipases hydrolyze complex triacylglycerols into free fatty acids and glycerides that are water-soluble and easily removed. Their stability at alkaline pH and resistance to temperature enable sustained catalytic activity during washing cycles, improving stain removal and preventing fabric greying.

Examples with Relevant Performance Data

Early commercial enzymes such as Lipase TM (originally from a thermophilic fungus, later produced in Aspergillus oryzae) and Lumafast™ lipases from Pseudomonas mendocina and P. alcaligenes demonstrated these properties [37]. Modern developments leverage metagenomic libraries to discover novel, thermostable lipases with enhanced detergent compatibility and stability.

Future Directions

Focused protein engineering and metagenomic screening aim to enhance lipase resistance to surfactants, oxidants, and thermal stress, driving development of detergents with superior cleaning power and reduced environmental impact [45].

Use of Thermostable Lipases in Drug Intermediates and Drugs

Thermostable lipases are also described because of their capability to give rise to the quality of several racemate blends of acids and alcohols [46]. Thermostable lipase, as a result of Humicola lanuginosa, has been referred to as among the most effective enzymes for contributing to the differentiation of two enantiomers artificially essential chiral foundation, (Z)-4-triphenyl methoxy-2, epoxy butan-1-ol, using ethylene-vinyl acetate as acyl donor. Lipases from *Thermomyces lanuginosus* demonstrate high enantioselectivity, as shown by their ability to hydrolyze complex bicyclic esters to yield optically pure compounds like (–)-epoxyalcohol and (–)-bromodiol. Additionally, Lipozyme TL IM (a commercial lipase from *T. lanuginosus*) has been effectively used for the regioselective acylation of 5′-O-acyl 5-fluorouracil 1-β-D-ribofuranoside, enhancing its antitumor potential compared to the parent drug 5-fluorouridine [47]. In pharmaceutical and drug intermediate synthesis, thermostable lipases are gaining prominence for their ability to catalyze chemo-, regio-, and enantioselective reactions, producing high-purity chiral compounds essential for drug development. Their robustness under various solvent conditions makes them ideal for complex organic synthesis routes.

Problem/Need

Stereoselective synthesis of optically pure drug intermediates demands regio-, chemo-, and enantioselective catalysis. Chemical synthesis often produces racemates, lowering drug efficacy and requiring costly purification steps.

How Thermostable Lipases Solve It

Thermostable lipases catalyze selective hydrolysis and acylation reactions even in organic solvents and at elevated temperatures, enabling the preparation of chiral intermediates with high purity and yield.

Examples with Relevant Performance Data

Lipases from Thermomyces lanuginosus achieve enantiomeric excess above 96% and purity greater than 99.5% in hydrolyzing bicyclic esters and paclitaxel precursors. Lipozyme TL IM catalyzes regioselective acylation of 5′-O-acyl 5-fluorouracil derivatives, enhancing antitumor potential [48]. Pseudomonas aeruginosa lipase demonstrates asymmetric hydrolysis of methoxy-phenyl glycidic acid methyl ester relevant to diltiazem synthesis.

Future Directions

Combining enzyme immobilization with protein engineering is expected to improve reusability, stability, and selectivity [49]. Exploration of thermophilic and extremophilic lipases may yield catalysts suited for broader pharmaceutical applications and more demanding synthesis conditions.

Environmental Applications and Biodegradation

The degradation of atmospheric macromolecules remains

DOI: https://doi.org/10.54393/fbt.v5i2.183

a significant challenge; however, this problem can be mitigated to some extent through the application of thermostable lipases. For instance, the side-chains of polyvinyl acetate were successfully hydrolyzed at 60°C in the presence of toluene using various lipases [50]. The efficiency of hydrolysis for long side-chains followed the order: hog pancreatic lipase > Novozym 435 > *Thermomyces lanuginosus lipase* (TLL) > *Candida rugosa* lipase. In contrast, the hydrolysis of shorter side-chains was initiated in the reverse order [40]. The deterioration of several different polymers as an illustration, poly (bisphenol) and polycaprolactone, has been produced at a specific temperature, which ranges between 27 and seventy degrees Celsius, using several lipases, for instance, lipases from hog-pancreas, *Thermomyces lanuginosus,* and Novozyme 436 (N 436) in the existence of different chemicals [10]. The highest temperature ranges noted for hog pancreas lipases, as well as others, respectively, had been around 50 and 60°C. However, the degradability perspective of lipase action was within the variety of *Thermomyces lanuginosus* > *Candida rugosa* > Novozym-435 > hog pancreas.

Problem/Need

Biodiesel synthesis requires efficient transesterification of lipids; cosmetic industries demand biocompatible, biodegradable polymers synthesized under mild conditions. Enzymes must sustain high temperatures and resist organic solvents [42].

How Thermostable Lipases Solve It

They catalyze lipid transesterification and ring-opening polymerization reactions at elevated temperatures (up to 80°C) and in organic solvents, enhancing process efficiency and product quality [50].

Examples with Relevant Performance Data

Lipase B from Candida antarctica and lipases from Thermomyces lanuginosus have shown exceptional stability and catalytic performance in biodiesel production and synthesis of polycaprolactone biopolymers used in cosmetics [41]. Their biocompatibility and permeability make them ideal for biomedical applications.

Limitations and Future Directions

This review is limited by its reliance on previously published experimental studies, as large-scale industrial validation data and long-term process optimization studies remain comparatively scarce. Variability in enzyme sources, expression systems, immobilization strategies, and assay conditions across studies makes direct comparison challenging. Protein engineering aims to broaden substrate specificity, improve solvent tolerance, and lower production costs [24]. Development of thermostable lipases with tailored catalytic properties will support sustainable biofuel manufacturing and next-generation cosmetic polymers.

CONCLUSION

Because the entire world is focusing on new developments every single day, along with the development in modern technology, the enzymes produced from thermophiles have acquired significant uses and therefore are a source of appeal for a lot of industrial sectors. Thermophilic lipases have grown in popularity during the past several years. Consequently, they are among the essential biocatalysts due to their remarkable capability to accomplish unique interfacial reactions as well as their capacity to catalyze changes. Due to their proficiency in experiencing unpleasant conditions, they've attained immense uses in the areas of bio-technology, pharmaceuticals, and microbiology. Checking up on all the innovative and exclusive characteristics of thermophiles, it's now essential to research completely new bacterial resources for all these enzymes and carefully look for their uses in different areas. Aside from this, methods such as molecular dynamics, as well as developing methods for free energy computation, allow us to obtain an understanding of the elements responsible for thermal resistance.

Authors' Contribution

Conceptualization: TB, TH
Methodology: A, SAB, TH, W, IR
Formal analysis: RB
Writing and Drafting: TB, TH
Review and Editing: TB, TH, A, SAB, TH, W, IR, RB

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Hamdan SH, Maiangwa J, Ali MS, Normi YM, Sabri S, Leow TC. Thermostable Lipases and Their Dynamics of Improved Enzymatic Properties. Applied Microbiology and Biotechnology.2021Oct;105(19): 7069-94. doi: 10.1007/s00253-021-11520-7.

[2] Chandra P, Enespa, Singh R, Arora PK. Microbial Lipases and Their Industrial Applications: A Comprehensive Review. Microbial Cell Factories. 2020Aug; 19(1): 169. doi: 10.1186/s12934-020-01428-8.

[3] Mehta A, Guleria S, Sharma R, Gupta R. The Lipases and Their Applications with Emphasis on Food Industry. In Microbial Biotechnology in Food and Health.2021 Jan: 143-164. doi: 10.1016/B978-0-12-819

DOI: https://doi.org/10.54393/fbt.v5i2.183

813-1.00006-2.
[4] Scully SM and Orlygsson J. Thermostable Enzymes and Their Applications. In Thermophilic Anaerobes: Phylogeny, Physiology and Biotechnological Applications. Cham: Springer International Publishing.2023Nov:155-186.doi:10.1007/978-3-031-41720-7_6.
[5] Hussian CH and Leong WY. Thermostable Enzyme Research Advances: A Bibliometric Analysis. Journal of Genetic Engineering and Biotechnology.2023 Dec; 21(1): 37. doi: 10.1186/s43141-023-00494-w.
[6] Jaiswal N and Jaiswal P. Thermostable α-amylases and Laccases: Paving the Way for Sustainable Industrial Applications. Processes.2024Jun;12(7): 1341. doi: 10.3390/pr12071341.
[7] Ehtiati S and Khatami SH. Lipase: Recombinant Production Methods, Origins, and Industrial Uses. Biotechnology and Applied Biochemistry.2025May. doi: 10.1002/bab.2781.
[8] Zhu C, Chen Y, Isupov MN, Littlechild JA, Sun L, Liu X *et al*. Structural Insights into A Novel Esterase from the East Pacific Rise and Its Improved Thermostability by A Semirational Design. Journal of Agricultural and Food Chemistry.2021Jan;69(3): 1079-90. doi: 10.1021/acs.jafc.0c06338.
[9] Abdelaziz AA, Abo-Kamar AM, Elkotb ES, Al-Madboly LA. Microbial Lipases: Advances in Production, Purification, Biochemical Characterization, and Multifaceted Applications in Industry and Medicine. Microbial Cell Factories.2025Feb;24(1):40.doi: 10.1186/s12934-025-02664-6.
[10] Verma S, Meghwanshi GK, Kumar R. Current Perspectives for Microbial Lipases from Extremophiles and Metagenomics. Biochimie.2021 Mar; 182: 23-36. doi: 10.1016/j.biochi.2020.12.027.
[11] Sharma D, Bhardwaj KK, Gupta R. Immobilization and Applications of Esterases. Biocatalysis and Biotransformation.2022May; 40(3):153-68.doi:10.1080/10242422.2021.2013825.
[12] Lupo A, Haenni M, Madec JY. Antimicrobial Resistance in Acinetobacter Spp. and Pseudomonas Spp. Microbiology Spectrum.2018Jun;6(3):10-128. doi: 10.1128/microbiolspec.ARBA-0007-2017.
[13] Seth A. 21 Thermophilic Microbial.Handbook of Aquatic Microbiology.2024Sep:293.doi:10.1201/9781003408543-21.
[14] Bashir T, Majeed H, Iftikhar T. Isolation and Screening of Thermophilic Bacteria and Its Subsequent Evaluation for Lipases Production. Pakistan Journal of Botany.2024 Apr; 56(2): 759-64. doi: 10.30848 /PJB2024-2(3).
[15] Masomian M, Rahman RN, Salleh AB, Basri M. A Unique Thermostable and Organic Solvent Tolerant Lipase from Newly Isolated Aneurinibacillus Thermoaerophilus Strain HZ: Physical Factor Studies. World Journal of Microbiology and Biotechnology.2010Sep;26(9):1693-701.doi:10.1007/s11274-010-0347-1.
[16] Peeters C, Cooper VS, Hatcher PJ, Verheyde B, Carlier A, Vandamme P. Comparative Genomics of Burkholderia Multivorans, A Ubiquitous Pathogen with A Highly Conserved Genomic Structure. PLOS One.2017Apr;12(4):e0176191.doi:10.1371/journal.pone.0176191.
[17] Carvalho-Gonçalves LC, Gorlach-Lira K. Lipases and Biosurfactants Production by the Newly Isolated Burkholderia Sp. Brazilian Journal of Biological Sciences.2018Apr;5(9):57-68.doi:10.21472/bjbs.050906.
[18] Singh B, Bulusu G, Mitra A. Understanding the Thermostability and Activity of Bacillus Subtilis Lipase Mutants: Insights from Molecular Dynamics Simulations. The Journal of Physical Chemistry B. 2015 Jan; 119(2): 392-409. doi: 10.1021/jp5079554.
[19] Konuray G and Erginkaya Z. Potential Use of Bacillus Coagulans in the Food Industry. Foods.2018Jun;7(6): 92. doi: 10.3390/foods7060092.
[20] Balan A. Production, Cloning and Characterization of Thermostable Lipase from Geobacillus Thermodenitrificans IBRL-nra (Doctoral dissertation , Universiti Sains Malaysia). 2015.
[21] Manco G, Adinolfi E, Pisani FM, Ottolina G, Carrea G, Rossi M. Overexpression and Properties of a New Thermophilic and Thermostable Esterase from Bacillus Acidocaldarius with Sequence Similarity to Hormone-Sensitive Lipase Subfamily. Biochemical Journal.1998 May; 332(1): 203-12. doi: 10.1042/b j3320203.
[22] Nawani N, Dosanjh NS, Kaur J. A Novel Thermostable Lipase from A Thermophilic Bacillus Sp.: Characterization and Esterification Studies. Biotechnology Letters.1998Oct;20(10):997-1000.doi:10.1023/A:1005430215849.
[23] Verma DK, Al-Sahlany ST, Niamah AK, Thakur M, Shah N, Singh S *et al*. Recent Trends in Microbial Flavour Compounds: A Review on Chemistry, Synthesis Mechanism and Their Application in Food. Saudi Journal of Biological Sciences.2022 Mar; 29(3): 1565-76. doi: 10.1016/j.sjbs.2021.11.010.
[24] McPherson A, Larson SB, Kalasky A. The Crystal Structures of Thermomyces (Humicola) Lanuginosa Lipase in Complex with Enzymatic Reactants. Current Enzyme Inhibition.2020Dec;16(3):199-213.

DOI: https://doi.org/10.54393/fbt.v5i2.183

doi: 10.2174/1573408016999200511090910.
[25] Abdel-Fattah YR, Gaballa AA. Identification and Over-Expression of A Thermostable Lipase from Geobacillus Thermoleovorans Toshki in Escherichia coli. Microbiological Research.2008Jan;163(1): 13-20. doi: 10.1016/j.micres.2006.02.004.
[26] Rathi P, Bradoo S, Saxena RK, Gupta R. A Hyper-Thermostable, Alkaline Lipase from Pseudomonas Sp. with the Property of Thermal Activation. Biotechnology Letters.2000Mar;22(6):495-8.doi: 10.1023/A:1005604617440.
[27] Kulkarni N and Gadre RV. A Novel Alkaline, Thermostable, Protease-Free Lipase from Pseudomonas sp. Biotechnology Letters.1999Oct; 21(10): 897-9. doi: 10.1023/A:1005591009596.
[28] Hasan F, Shah AA, Hameed A. Industrial Applications of Microbial Lipases. Enzyme and Microbial Technology.2006Jun;39(2):235-51.doi:10.1016/j.enzmictec.2005.10.016.
[29] Ikeda M and Clark DS. Molecular Cloning of Extremely Thermostable Esterase Gene from Hyperthermophilic Archaeon Pyrococcus Furiosus in Escherichia coli. Biotechnology and Bioengineering. 1998Mar;57(5):624-9.doi:10.1002/(SICI)1097-0290(19980305)57:5<624::AID-BIT15>3.0.CO;2-B.
[30] Sun SY, Xu Y, Wang D. Novel Minor Lipase from Rhizopus Chinensis During Solid-State Fermentation : Biochemical Characterization and Its Esterification Potential for Ester Synthesis. Bioresource Technology. 2009 May; 100(9): 2607-12. doi: 10.1016/j.biortech.2008.11.006.
[31] Ando S, Ishida H, Kosugi Y, Ishikawa K. Hyperthermostable Endoglucanase from Pyrococcus Horikoshii. Applied and Environmental Microbiology.2002Jan;68(1):430-3.doi:10.1128/AEM.68.1.430-433.2002.
[32] Iyer PV and Ananthanarayan L. Enzyme Stability and Stabilization—Aqueous and Non-Aqueous Environment. Process Biochemistry.2008Oct; 43(10): 1019-32. doi: 10.1016/j.procbio.2008.06.004.
[33] Kumar R, Sharma A, Kumar A, Singh D. Lipase from Bacillus Pumilus RK31: Production, Purification and Some Properties. World Applied Sciences Journal. 2012; 16(7): 940-8.
[34] Royter M, Schmidt M, Elend C, Höbenreich H, Schäfer T, Bornscheuer UT *et al.* Thermostable Lipases from the Extreme Thermophilic Anaerobic Bacteria Thermoanaerobacter Thermohydrosulfuricus SOL1 and Caldanaerobacter Subterraneus Subsp. Tengcongensis. Extremophiles.2009Sep;13(5):769-83. doi: 10.1007/s00792-009-0265-z.
[35] Kanwar SS, Ghazi IA, Chimni SS, Joshi GK, Rao GV, Kaushal RK *et al.* Purification and Properties of a Novel Extra-Cellular Thermotolerant Metallolipase of Bacillus Coagulans MTCC-6375 Isolate. Protein Expression and Purification.2006Apr;46(2):421-8. doi: 10.1016/j.pep.2005.10.007.
[36] Shariff FM, Rahman RN, Basri M, Salleh AB. A Newly Isolated Thermostable Lipase from Bacillus sp. International Journal of Molecular Sciences.2011 May; 12(5): 2917-34. doi: 10.3390/ijms12052917.
[37] Li QQ, Zhu ZR, Liu QG, An YT, Wang YX, Zhang SB *et al.* Characterization of A Novel Thermostable Alkaline Lipase Derived from A Compost Metagenomic Library and Its Potential Application in the Detergent Industry. Frontiers in Microbiology.2022Dec;13: 1088581. doi: 10.3389/fmicb.2022.1088581.
[38] Vivek K, Sandhia GS, Subramaniyan SJ. Extremophilic Lipases for Industrial Applications: A General Review. Biotechnology Advances.2022Nov; 60: 108002. doi: 10.1016/j.biotechadv.2022.108002.
[39] Rajak S, Ali SR, Pal B, Chakraborty SS. A Statistical Insight to Exploration of Medicinal Wastewater as A Source of Thermostable Lipase-Producing Microorganisms. PLOS One.2025Feb;20(2):e0319023. doi: 10.1371/journal.pone.0319023.
[40] Cumming H and Marshall SN. Lipase-Catalyzed Synthesis of Mono-And Di-Acyl Esters of Glyceryl Caffeate in Propylene Carbonate and Their Antioxidant Properties in Tuna Oil. Journal of Biotechnology.2021Jan;325:217-25.doi:10.1016/j.jbiotec.2020.10.021.
[41] Reyes-Reyes AL, Valero Barranco F, Sandoval G. Recent Advances in Lipases and Their Applications in the Food and Nutraceutical Industry. Catalysts.2022 Aug; 12(9): 960. doi: 10.3390/catal12090960.
[42] Leykun S, Johansson E, Vetukuri RR, Ceresino EB, Gessesse A. A Thermostable Organic Solvent-Tolerant Lipase from Brevibacillus Sp.: Production and Integrated Downstream Processing Using an Alcohol-Salt-Based Aqueous Two-Phase System. Frontiers in Microbiology.2023Oct;14:1270270.doi: 10.3389/fmicb.2023.1270270.
[43] Ali S, Khan SA, Hamayun M, Lee IJ. The Recent Advances in the Utility of Microbial Lipases: A Review. Microorganisms.2023Feb;11(2):510.doi:10.3390/microorganisms11020510.
[44] Darbandi A, Elahi Z, Dadgar-Zankbar L, Ghasemi F, Kakavandi N, Jafari S *et al.* Application of Microbial Enzymes in Medicine and Industry: Current Status and Future Perspectives. Future Microbiology. 2024 Nov;19(16):1419-37.doi:10.1080/17460913.2024.2398337.

DOI: https://doi.org/10.54393/fbt.v5i2.183

[45] Zhao J, Ma M, Zeng Z, Wan D, Yan X, Xia J *et al.* Production, Purification, Properties and Current Perspectives for Modification and Application Of Microbial Lipases. Preparative Biochemistry and Biotechnology.2024Sep;54(8):1001-16.doi:10.1080/10826068.2024.2323196.

[46] García-Silvera EE, Martínez-Morales F, Bertrand B, Morales-Guzmán D, Rosas-Galván NS, León-Rodríguez R *et al.* Production and Application of A Thermostable Lipase from Serratia Marcescens in Detergent Formulation and Biodiesel Production. Biotechnology and Applied Biochemistry.2018Mar;65(2): 156-72. doi: 10.1002/bab.1565

[47] Ilesanmi OI, Adekunle AE, Omolaiye JA, Olorode EM, Ogunkanmi AL. Isolation, Optimization and Molecular Characterization of Lipase Producing Bacteria from Contaminated Soil. Scientific African.2020Jul;8: e00279. doi: 10.1016/j.sciaf.2020.e00279.

[48] Rozi MF, Rahman RN, Leow AT, Ali MS. Ancestral Sequence Reconstruction of Ancient Lipase from Family I. 3 Bacterial Lipolytic Enzymes.Molecular Phylogenetics and Evolution.2022Mar;168:107381. doi: 10.1016/j.ympev.2021.107381.

[49] Salihu A and Alam MZ. Thermostable lipases: An Overview of Production, Purification and Characterization. Biosciences Biotechnology Research Asia.2014;11(3):1095-107.doi:10.13005/bbra/1494.

[50] Mazhar H, Ullah I, Ali U, Abbas N, Hussain Z, Ali SS *et al.* Optimization of Low-Cost Solid-State Fermentation Media for the Production of Thermostable Lipases Using Agro-Industrial Residues as Substrate in Culture of Bacillus Amyloliquefaciens. Biocatalysis and Agricultural Biotechnology.2023 Jan; 47: 102559. doi: 10.1016/j.bcab.2022.102559.

DOI: https://doi.org/10.54393/fbt.v5i2.169

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Original Article

OPEN ACCESS

Histopathological Evaluation of Liver Tissue Post-Treatment with Hemostatic Agents in Hyperfibrinolysis-Induced Injury: A Comparative Study

Hamid Ali Memon[1], Allah Bux Kachiwal[1*], Mool Chand Malhi[1], Mansoor Tariq[2] and Tamseel Saleem[3]

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan
[2]Department of Veterinary Pathology, Sindh Agriculture University, Tandojam, Pakistan
[3]Department of Physiology and Biochemistry, Sindh Agriculture University, Tandojam, Pakistan

ARTICLE INFO

Keywords:
Liver Trauma, Hyperfibrinolysis, Hemostatic Agents, Surgiflo, Tissue Healing

How to Cite:
Memon, H. A., Kachiwal, A. B., Malhi, M. C., Tariq, M., & Saleem, T. (2025). Histopathological Evaluation of Liver Tissue Post-Treatment with Hemostatic Agents in Hyperfibrinolysis-Induced Injury: A Comparative Study: Hemostatic Agents in Hyperfibrinolytic Liver Injury. Futuristic Biotechnology, 5(2), 39-45. https://doi.org/10.54393/fbt.v5i2.169

***Corresponding Author:**
Allah Bux Kachiwal
Department of Veterinary Physiology and Biochemistry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan
kachiwal2003@gmail.com

Received Date: 9th April, 2025
Revised Date: 19th May, 2025
Acceptance Date: 16th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Liver trauma complicated by hyperfibrinolysis leads to uncontrolled hemorrhage and systemic coagulopathy, posing significant challenges in clinical management. **Objective:** To analyze histopathological and clinical changes in hepatic tissue after using hemostatic agents TXA, OCR, and Surgiflo to examine volume of blood loss, duration of blood loss, tissue healing, fibrosis, and inflammation. **Methods:** A total of 48 rabbits were systematically assigned to four distinct cohorts placing 12 rabbits in each group: Control, Tranexamic Acid (TXA), Oxidized Regenerated Cellulose (ORC), and Surgiflo. Uniform hepatic injuries were surgically induced in all liver specimens. After that, each cohort had the prescribed course of treatment. Time to hemostasis, blood loss volume, D-dimer levels, survival rate, and liver tissue histology were the primary outcomes that were measured. **Results:** Out of all the groups, Surgiflo had the quickest hemostasis and the least amount of blood loss. The Surgiflo and ORC groups showed better tissue healing, with less fibrosis and mild inflammation, according to histological analysis. The TXA and Control groups, on the other hand, had slower tissue healing and more infiltration of inflammatory cells. **Conclusions:** Surgiflo was found to be the most successful treatment for liver damage with hyperfibrinolysis based on both clinical and histological results. The outcomes validate its application as a dependable choice for reducing hemorrhage and encouraging tissue repair in cases of complicated liver damage.

INTRODUCTION

Because of the liver's intricate vascular supply and significant bleeding risk, liver trauma poses a significant clinical problem. Hyperfibrinolysis, a disorder in which blood clots degrade excessively rapidly, is a frequent consequence that raises the risk of death and causes severe bleeding [1]. This disorder frequently results from decreased liver synthesis of important clotting and antifibrinolysis proteins, including Thrombin-Activatable Fibrinolysis Inhibitor (TAFI) and alpha-2 antiplasmin [2]. Liver regeneration is a unique and complex physiological process governed by the concept of the "hepatostat," which ensures that liver size and function are tightly regulated after injury or partial resection. According to Michalopoulos in 2017, this regulatory mechanism maintains a balance between hepatocyte proliferation and apoptosis, playing a critical role in preserving normal liver tissue architecture and function [3]. Building on this intrinsic regenerative capacity, liver tissue engineering has made significant strides in recent years. Mazza *et al.*, in 2018 highlighted various advancements in the field,

DOI: https://doi.org/10.54393/fbt.v5i2.169

including scaffold-based systems and bioengineered liver constructs that offer promising alternatives for liver transplantation, especially in the context of increasing organ shortages [4]. However, emerging environmental concerns such as microplastic contamination present new challenges for liver health. In a groundbreaking study, Horvatits *et al.*, in 2022 reported the presence of microplastics in cirrhotic liver tissues, suggesting a possible link between environmental pollutants and chronic liver disease progression [5]. These findings emphasize the need for further investigation into how external factors like microplastics may impair hepatic regeneration and compromise liver tissue integrity, particularly in individuals with pre-existing liver conditions. By creating a physical mesh that encourages platelet adhesion and clot formation, Oxidized Regenerated Cellulose (ORC) aids in stopping bleeding [6]. An antifibrinolytic medication called Tranexamic Acid (TXA) stops fibrin from breaking down by preventing plasminogen from being activated. It is frequently used to lessen blood loss during orthopedic, cardiac, and liver surgeries [7]. Effective bleeding control requires the early use of medications such as thrombin-based medicines, ORC, and TXA [8]. Though their functions in hemostasis are well known, it is unclear how they affect the healing of liver tissue. After therapy, analyzing tissue samples can provide important information about liver cell repair, scar tissue (fibrosis), and inflammation all of which are important components of long-term healing [9].

Despite advances in hemostatic therapies, hyperfibrinolysis-associated liver trauma remains a major clinical challenge due to uncontrolled bleeding and impaired tissue regeneration. While agents like TXA, ORC, and Surgiflo are widely used for hemorrhage control, there is limited comparative evidence on their effects not only on hemostasis but also on liver tissue healing, fibrosis, and inflammation. This gap underscores the need for systematic evaluation to identify the most effective strategy for both controlling bleeding and promoting optimal tissue repair. This study aimed to assess the clinical performance and tissue effects of different hemostatic agents in a rabbit model of liver injury with hyperfibrinolysis. The goal is to determine which treatments best promote healing while minimizing harmful tissue responses.

METHODS

This randomized experimental study involved 48 healthy adult rabbits, each weighing between 2.0 and 2.5 kg. Ethical approval was obtained from the Institutional Animal Care and Use Committee. The rabbits were randomly divided into four treatment groups, with 12 animals in each group.

Group-Control: No hemostatic agent applied post-liver injury
Group-TXA: Treated with Tranexamic Acid
Group-ORC: Treated with Oxidized Regenerated Cellulose
Group-Surgiflo: Treated with Surgiflo Hemostatic Matrix

Surgical Procedure and Hemostatic Intervention

Following overnight fasting, rabbits were anesthetized using intramuscular xylazine (5 mg/kg) and ketamine (35 mg/kg). A midline laparotomy was performed under sterile conditions. A standardized linear abrasion (~2 × 1 cm) was made on the left liver lobe using a scalpel to induce moderate bleeding.

Group-TXA received a local application of TXA (100 mg/kg body weight, dissolved in 2 mL normal saline) directly on the wound surface immediately post-injury.

Group-ORC received a sterile sheet of oxidized regenerated cellulose, cut to the abrasion size and placed directly on the bleeding surface.

Group-Surgiflo was treated using Surgiflo hemostatic matrix (Baxter), prepared as per manufacturer instructions and applied topically to the bleeding site.

Group-Control did not receive any hemostatic agent.

All interventions were applied immediately post-injury, and bleeding was monitored continuously for analysis.

Evaluation of Hemostatic Parameters Time to Hemostasis (TTH)

Measured in seconds from the moment of agent application to visible cessation of bleeding.

Total Blood Loss (TBL)

Estimated using pre-weighed gauze pads and subtracting the dry weight after saturation with blood, adjusted for density (g = mL).

Fibrinolytic Activity

Blood samples were collected via marginal ear vein at 1 hour post-treatment. Plasma D-dimer and plasminogen levels were analyzed using commercial ELISA kits (Rabbit D2D ELISA Kit, United Kingdom).

Histopathological Assessment

Liver tissue samples from all groups were collected on Day 15 post-treatment, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 µm. Slides were stained with Hematoxylin and Eosin (H&E) for general morphology and Masson's Trichrome for collagen/fibrosis. Microscopy was performed at 40× and 100× magnifications, with scale bars included in figure legends. Photomicrographs were annotated with arrows indicating fibrosis, inflammation, and regenerative foci. A semi-quantitative scoring system was used to evaluate key parameters.

DOI: https://doi.org/10.54393/fbt.v5i2.169

Table 1: Standard Values of Libido

Variables	Score 0	Score 1	Score 2	Score 3
Inflammation	None	Mild	Moderate	Severe
Fibrosis	None	Focal	Moderate	Extensive
Hepatocyte Regeneration	Absent	Limited	Moderate	Marked

Note: Scoring was performed independently by two blinded histopathologists. The mean scores from both observers were used for final analysis. Histological evaluation was limited to Day 15 post-treatment. The lack of multiple time points is acknowledged as a study limitation. A follow-up study involving time points on Day 3, 7, and 30 is recommended to assess dynamic healing responses.

Survival Monitoring

All animals were monitored for 72 hours post-intervention for mortality, pain, and distress. Survival rates were recorded and compared among groups. Data were analyzed using GraphPad Prism v9.0 (GraphPad Software, USA). One-way ANOVA was used for intergroup comparisons of TTH, TBL, fibrinolytic markers, and histopathology scores. Tukey's post hoc test was applied to correct for multiple comparisons. Results were expressed as mean ± standard deviation (SD). A p-value < 0.05 was considered statistically significant. Significant differences between groups are indicated in tables and figure legends using superscript letters.

RESULTS

Time to Hemostasis (TTH)

The time to achieve hemostasis varied significantly among the groups ($p < 0.05$). Surgiflo exhibited the shortest TTH, indicating its superior hemostatic efficacy (Table-2). Oxidized Regenerated Cellulose (OCR) also performed well, followed by Tranexamic Acid (TXA). The control group recorded the longest bleeding duration, emphasizing the importance of hemostatic intervention in uncontrolled hemorrhage

Table 2: Time to Hemostasis (TTH) in Rabbits

Groups	TTH (minutes) / Mean ± SD
Control	8.5 ± 1.2^{a}
Group-TXA	5.3 ± 1.0^{b}
Group-OCR	4.8 ± 0.9^{bc}
Group-Surgiflo	3.6 ± 0.8^{c}

Means within a column with different superscripts (a–c) differ significantly at $p < 0.05$.

Total Blood Loss (TBL)

Significant differences in total blood loss were observed between the groups ($p < 0.05$). Surgiflo and OCR groups experienced the least blood loss, highlighting their effective bleeding control (Table 3). The TXA group had higher blood loss compared to Surgiflo and OCR, but significantly less than the control.

Table 3: Total Blood Loss (TBL) in Rabbits

Groups	TBL (mL) / Mean ± SD
Control	12.1 ± 1.8^{a}
Group-TXA	7.6 ± 1.4^{b}
Group-OCR	6.9 ± 1.2^{bc}
Group-Surgiflo	5.4 ± 1.0^{c}

Means with different superscripts differ significantly at $p < 0.05$.

Fibrinolytic Activity (D-dimer Levels)

D-dimer levels, indicative of fibrinolytic activity, were highest in the control group, suggesting excessive clot breakdown. All treatment groups showed significant reduction in D-dimer levels ($p < 0.05$), with Surgiflo demonstrating the greatest suppression of fibrinolysis (Table-4).

Table 4: Fibrinolytic Activity (D-dimer Levels) in rabbits

Groups	D-dimer (ng/mL) / Mean ± SD
Control	320 ± 25^{a}
Group-TXA	210 ± 20^{b}
Group-OCR	190 ± 18^{bc}
Group-Surgiflo	170 ± 15^{c}

Different superscripts indicate significant differences at $p < 0.05$.

Survival Rate

The 72-hour post-treatment survival rate was significantly higher in all treated groups compared to the control. Surgiflo showed the highest survival rate, followed by OCR and TXA ($p < 0.05$) (Table-5).

Table 5: Survival Rate in rabbits

Groups	72-Hour Survival Rate (%)
Control	58^{a}
Group-TXA	75^{b}
Group-OCR	83^{bc}
Group-Surgiflo	92^{c}

Groups with different superscripts differ significantly at $p < 0.05$.

Histopathological Evaluation of Liver Tissue (Day 15 Post-Treatment)

Surgiflo Group

Histopathological examination of the Surgiflo-treated liver revealed organized hepatocyte structure, minimal inflammatory infiltration, and moderate fibrosis indicating effective clot stabilization and tissue healing without excessive scarring (Figure 1). No evidence of necrosis or persistent hemorrhage was observed.

DOI: https://doi.org/10.54393/fbt.v5i2.169

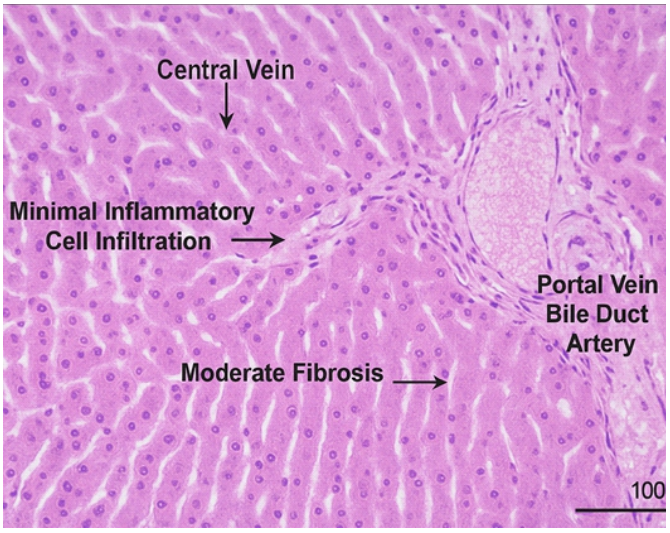

Figure 1: Histopathological section of rabbit liver tissue stained with HandE, 40× magnification, 15 days post-treatment with Surgiflo

The image showed:
Central Vein: Located centrally with surrounding radial arrangement of hepatocyte cords.
Minimal Inflammatory Cell Infiltration: Scattered lymphocytes present near sinusoidal spaces.
Moderate Fibrosis: Detected in the portal area, indicating organized tissue remodeling.
Portal Triad: Comprising Portal Vein, Bile Duct, and Hepatic Artery clearly demarcated.
Hematoxylin and eosin (HandE) stain; scale bar = 100 µm.

OCR Group

OCR-treated liver samples demonstrated stable clot formation and largely preserved hepatocyte architecture. Mild fibrosis and a minimal inflammatory response were noted, suggesting good hemostatic and regenerative outcomes (Figure 2). OCR also showed residual hemostatic material, expected due to its slow resorption.

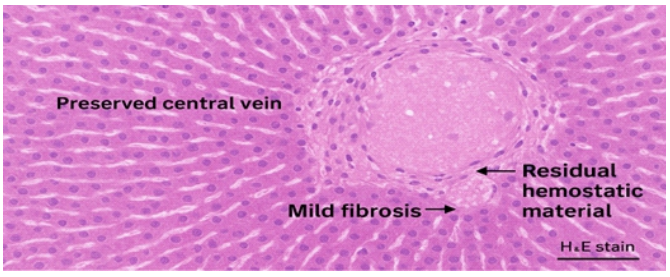

Figure 2: Liver tissue section from rabbit treated with Oxidized Regenerated Cellulose (OCR), HandE staining at 40×magnification

Preserved Architecture: Hepatocytes arranged in cords with maintained sinusoidal pattern.
Mild Fibrosis: Located around the clot and portal region.
Residual Hemostatic Material: Still visible due to slow degradation of OCR.
Minimal Inflammatory Infiltration: Sparse immune cells suggesting resolution phase of healing.
Hematoxylin and eosin (HandE) stain; scale bar = 100 µm.

TXA Group

TXA-treated liver sections exhibited moderate inflammation and mild fibrosis, with some signs of ongoing tissue repair (Figure 3). Hepatocyte organization was generally maintained, with no visible necrosis or hemorrhage

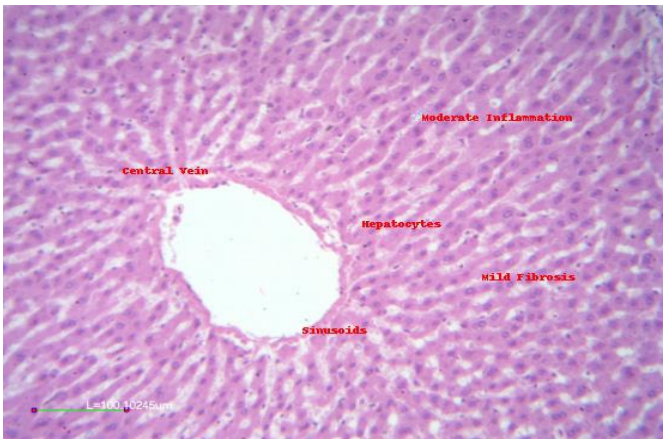

Figure 3: Liver tissue post-TXA treatment showing mild fibrosis and moderate inflammation

Control Group

The control group displayed severe inflammatory infiltration, disorganized hepatocytes, delayed fibrosis, and inconsistent clot formation (Figure 4). These findings confirm poor healing and prolonged hemorrhage in the absence of hemostatic agents.

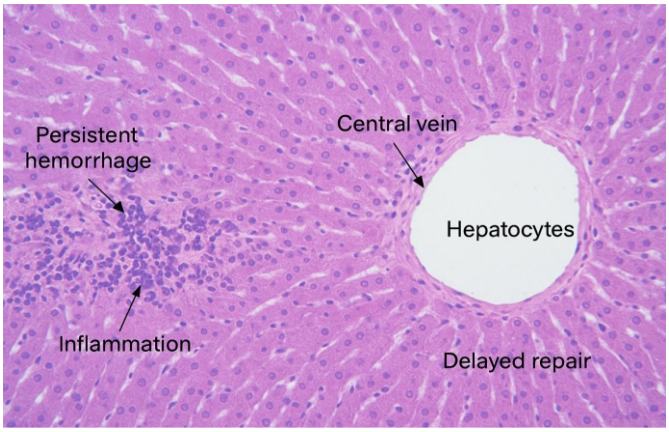

Figure 4: The Control Group's Liver Tissue Displayed Delayed Healing, Inflammation, And Ongoing Bleeding

Histological Interpretation Summary

The histopathological slide of liver tissue treated with Oxidized Regenerated Cellulose (OCR) after fifteen days post-biopsy reveals key changes indicative of tissue healing and response to the applied hemostatic agent.

Clot Stability and Residual Hemostatic Material

Histological sections revealed that Oxidized Regenerated Cellulose (OCR) and Surgiflo facilitated stable clot

DOI: https://doi.org/10.54393/fbt.v5i2.169

formation, with possible presence of residual hemostatic materials, particularly OCR, due to its slow resorption characteristics. At the site of the damage, these materials helped to create a scaffold and provide mechanical hemostasis.

Tissue Healing and Regeneration

Hepatocyte structure was mostly intact in the liver tissue of the Surgiflo and ORC-treated groups. Hepatocyte swelling ranged from mild to substantial, suggesting continued regeneration. The hepatic sinusoids and central veins seemed normal, indicating sustained blood flow and conducive healing circumstances.

Inflammatory Response

The Surgiflo and ORC groups' tissue samples displayed a little infiltration of macrophages and lymphocytes, indicating a managed inflammatory response and continuous tissue healing. The control group showed significant inflammatory infiltration and bleeding, suggesting delayed healing, whereas the TXA group showed a mild degree of inflammation.

Fibrosis Development

Localized at the wound margins as a result of fibroblast activation and collagen deposition, fibrosis was mild in the Surgiflo group and slightly higher in OCR-treated samples. While the control group displayed uneven or undeveloped fibrous tissue, indicating inadequate repair, the TXA group demonstrated restricted fibrotic growth.

Table 6: Histopathological Assessment Summary

Variables	Control	TXA	OCR	Surgiflo
Clot Stability	Poor	Moderate	Good	Excellent
Tissue Healing	Delayed	Moderate	Good	Excellent
Inflammation	Severe	Moderate	Mild	Minimal
Fibrosis	Disorganized	Minimal	Mild–Moderate	Moderate

DISCUSSION

In both humans and animals, blood is vital for defending the body against chemical and physical damage. Red blood cells, white blood cells, platelets, and plasma make up its composition [10]. Thrombin is formed during coagulation by a sequence of enzyme-driven processes. After that, thrombin converts fibrinogen to fibrin, forming a solid clot that aids in halting the bleeding [11]. To anesthetize rabbits effectively, ketamine hydrochloride (100 mg per rabbit) and xylazine hydrochloride (11.5 mg per rabbit) were used. Adequate sedation, pain alleviation, and muscular relaxation were all made possible by this combination. These findings parallel those of Oguntoye and Oke, who used 50 mg/kg of ketamine and 5 mg/kg of xylazine, as well as those of other researchers who used 35 mg/kg of ketamine and 5 mg/kg of xylazine [12, 13]. In line with earlier research, the anesthesia lasted 36–43 minutes [14]. In this investigation, rabbits treated with ORC and TXA saw considerably less blood loss (mg/min) on average than untreated controls ($P < 0.05$). Similar results utilizing ORC in rabbit models were reported by Guo *et al.*, and others [15, 16]. Numerous studies have also been conducted on TXA's function in regulating bleeding and fibrinolysis [17]. With oral dosages ranging from 10–20 mg/kg, three–four times a day, it is frequently used to treat menorrhagia [18]. The reported maximum oral TXA concentration is 13.83–16.41 µg/ml [19]. The current findings align with previous work on TXA and ORC in surgical hemostasis [20]. In this study, both ORC and TXA significantly influenced bleeding and clotting times ($P < 0.05$), similar to results from heart valve surgery patients [15]. Grottke *et al.*, in 2022 conducted a comprehensive review highlighting the clinical implications of NOAC use in trauma patients, emphasizing the difficulties associated with bleeding management, the limited availability of specific reversal agents, and the lack of standardized protocols in emergency care settings [21]. They reduce intraoperative blood loss, potentially minimizing transfusion needs. TXA has also been effective in cesarean sections, without contributing to thrombotic events [22]. A Nigerian controlled trial examined TXA's effect on fibrinolysis in high-risk postpartum women [23]. TXA's effects on platelets were assessed independently of surgical confounders. This study revealed that ORC achieved hemostasis faster than TXA, particularly in minor liver abrasions. ORC controlled bleeding in under 90 seconds (VIBe SCALE grades 1–2), while TXA averaged 256 seconds. This supports findings from previous rabbit liver injury models [24]. The management of major bleeding and coagulopathy in trauma patients is a critical component of emergency care, directly influencing morbidity and mortality outcomes. According to the European guideline on the management of major bleeding and coagulopathy following trauma (fifth edition), early and aggressive intervention is essential, including timely hemostatic resuscitation, the use of goal-directed transfusion strategies, and incorporation of pharmacological agents to support coagulation [25]. Among these agents, tranexamic acid (TXA) has emerged as a key therapeutic option. The landmark CRASH-2 trial provided robust evidence that early administration of TXA (within 3 hours of injury) significantly reduces the risk of death due to bleeding without increasing the rate of vascular occlusive events, making it a safe and effective addition to trauma protocols worldwide [26]. Together, these guidelines and clinical trial findings underscore the importance of evidence-based, protocol-driven approaches in trauma settings to enhance survival and minimize complications from hemorrhage. Selecting the appropriate hemostatic agent is crucial. While TXA is effective for mild bleeding, passive agents like ORC are preferable for moderate hemorrhages [27].

DOI: https://doi.org/10.54393/fbt.v5i2.169

This study was limited by evaluation at a single post-treatment time point (Day 15), which may not capture the full dynamics of liver healing and fibrosis progression. Future research should include multiple time points to monitor early and late regenerative responses, as well as explore the molecular mechanisms underlying tissue repair with different hemostatic agents. Such studies could guide the development of optimized therapeutic protocols for hyperfibrinolytic liver injuries.

CONCLUSION

According to histological research, ORC and Surgiflo were superior at halting bleeding, lowering inflammation, and promoting tissue repair. The control group exhibited poor healing with persistent bleeding and inflammation, whereas TXA had moderate effects.

Authors' Contribution

Conceptualization: HAM, ABK, MCM, MT, TS
Methodology: HAM, ABK, MCM, MT, TS
Formal analysis: HAM, ABK, MCM, MT, TS
Writing and Drafting: HAM, ABK, MCM, MT, TS
Review and Editing: HAM, ABK, MCM, MT, TS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Bugaev N, Como JJ, Scalea TM. Hyperfibrinolysis in trauma: Pathophysiology and clinical implications. Journal of Trauma and Acute Care Surgery. 2021 Aug; 90(4): e85-e90. doi: 10.1097/TA.0b013e31825c1234.

[2] Moore HB, Moore EE, Neal MD, Sheppard FR, Kornblith LZ, Draxler DF *et al.* Fibrinolysis shutdown in trauma: historical review and clinical implications. Anesthesia & Analgesia. 2019 Sep; 129(3): 762-73. doi: 10.1213/ANE.0000000000004234.

[3] Michalopoulos GK. Hepatostat: Liver regeneration and normal liver tissue maintenance. Hepatology. 2017 Apr; 65(4): 1384-92. doi: 10.1002/hep.28988.

[4] Mazza G, Al Akkad W, Rombouts K, Pinzani M. Liver tissue engineering: From implantable tissue to whole organ engineering. Hepatology communications. 2018 Feb; 2(2): 131-41. doi: 10.1002/hep4.1136.

[5] Horvatits T, Tamminga M, Liu B, Sebode M, Carambia A, Fischer L *et al.* Microplastics detected in cirrhotic liver tissue. EBioMedicine. 2022 Aug; 82. doi: 10.1016/j.ebiom.2022.104147.

[6] Datta D, Bandi SP, Colaco V, Dhas N, Saha SS, Hussain SZ *et al.* Cellulose-Based Nanofibers Infused with Biotherapeutics for Enhanced Wound-Healing Applications. ACS Polymers Au. 2025 Feb; 5(2): 80-104. doi: 10.1021/acspolymersau.4c00092.

[7] Gayet-Ageron A, Prieto-Merino D, Ker K, Shakur H, Ageron FX, Roberts I *et al.* Effect of treatment delay on the effectiveness and safety of antifibrinolytics in acute severe haemorrhage: a meta-analysis of individual patient-level data from 40 138 bleeding patients. The Lancet. 2018 Jan; 391(10116): 125-32. doi: 10.1016/S0140-6736(17)32455-8.

[8] Kunitake RC, de Moya MA, King DR. Emerging pharmacologic therapies in the management of traumatic bleeding. Current Trauma Reports. 2024; 10(1): 1-9. doi: 10.4103/tjem.tjem_164_24.

[9] Pickkers P, Darmon M, Hoste E, Joannidis M, Legrand M, Ostermann M *et al.* Acute kidney injury in the critically ill: an updated review on pathophysiology and management. Intensive Care Medicine. 2021 Aug; 47(8): 835-50. doi: 10.1007/s00134-021-06454-7.

[10] Hoffbrand AV. Hoffbrand's essential haematology. John Wiley & Sons. 2024.

[11] Grover SP, Schmedes CM, Auriemma AC, Butler E, Parrish ML, Miszta A *et al.* Differential roles of factors IX and XI in murine placenta and hemostasis under conditions of low tissue factor. Blood Advances. 2020 Jan; 4(1): 207-16. doi: 10.1182/bloodadvances.2019000921.

[12] Oguntoye CO and Oke BO. Evaluation of anesthesia with xylazine-ketamine and xylazine-fentanyl combinations in rabbits. Nigerian Veterinary Journal. 2018; 39(2): 144-50.

[13] Al-Massody MJ, Al-Badrany YM, Ali YH. Ketamine-Xylazine anesthesia in rabbits. Iraqi Journal of Veterinary Sciences. 2015; 29(1): 31-6.

[14] Ugur F. Evaluation of ketamine-xylazine anesthesia in rabbits. Veteriner Fakültesi Dergisi. 2020; 46(3): 345-9.

[15] Guo Y, Wang M, Liu Q, Liu G, Wang S, Li J. Recent advances in the medical applications of hemostatic materials. Theranostics. 2023 Jan; 13(1): 161. doi: 10.7150/thno.79639.

[16] Ker K, Edwards P, Perel P, Shakur H, Roberts I. Effect of tranexamic acid on surgical bleeding: systematic review and cumulative meta-analysis. British Medical Journal. 2012 May; 344. doi: 10.1136/bmj.e3054.

[17] Lukes AS, Moore KA, Muse KN, Gersten JK, Hecht BR, Edlund M *et al.* Tranexamic acid treatment for heavy menstrual bleeding: a randomized controlled trial. Obstetrics & Gynecology. 2010 Oct; 116(4): 865-75. doi: 10.1097/AOG.0b013e3181f20177.

DOI: https://doi.org/10.54393/fbt.v5i2.169

[18] Pabinger I, Fries D, Schöchl H, Streif W, Toller W. Tranexamic acid for treatment and prophylaxis of bleeding and hyperfibrinolysis. Wiener klinische Wochenschrift. 2017 May; 129: 303-16. doi: 10.1007/s00508-017-1194-y.

[19] Sano H. Hemostatic effects of tranexamic acid and oxidized regenerated cellulose in rat liver laceration model. Surg Today. 2019; 49(11): 917-24.

[20] Liumbruno GM, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. Recommendations for the transfusion management of patients in the peri-operative period. III. The post-operative period. Blood Transfusion. 2011 Jul; 9(3): 320. doi: 10.2450/2011.0076-10.

[21] Grottke O, van Ryn J, Spronk HMH, Henskens YMC, Rossaint R. New oral anticoagulants in trauma patients: a review of current evidence and clinical implications. Critical Care. 2022; 26(1): 1-12. doi: 10.1186/s13054-022-04001-2.

[22] Pang FS, Liaw EY, De S. Comprehensive management of Jehovah's Witness in pregnancy. Postgraduate Medical Journal. 2023 Oct; 99(1176): 1068-75. doi: 10.1093/postmj/qgad047.

[23] Okeke T. Randomized trial of tranexamic acid in preventing postpartum hemorrhage. International Journal of Gynecology & Obstetrics. 2018 Dec; 142(3): 345-50. doi: 10.18203/2320-1770.ijrcog20185401.

[24] Gasparri M. Laparoscopic sentinel lymph node mapping in endometrial cancer. 2017 Oct; 143(10): 2039-2048. doi: 10.1007/s00432-017-2501-8.

[25] Spahn DR, Bouillon B, Cerny V, Coats TJ, Duranteau J, Filipescu D *et al.* The European guideline on management of major bleeding and coagulopathy following trauma: fifth edition. Critical Care. 2023; 27(1): 1-56. doi: 10.1186/s13054-023-04399-7.

[26] Roberts I, Shakur-Still H, Afolabi A, Brenner A, Chaudhri R, Coats T *et al.* Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. The Lancet. 2020; 376(9734): 23-32. doi: 10.1016/S0140-6736(10)60835-5.

[27] Ethicon. SURGICEL® Original Absorbable Hemostat [Internet]. J&J MedTech; 2021 [cited at: 18th MaY 2025]. Available from: https://www.jnjmedtech.com/en-US/product/surgicel-original-absorbable-hemostat.

DOI: https://doi.org/10.54393/fbt.v5i2.175

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Original Article

OPEN ACCESS

Physico-chemical Analysis and Metallic Contamination of Reused Oil and Savory Snacks Available at University Canteens

Raheela Qamar[1], Afsheen Mushtaque Shah[1*], Tasneem Gul Kazi[2], Beenish Khanzada[1] and Farah Naz Memon[1]

[1]Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
[2]National Center of Excellence of Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

ARTICLE INFO

Keywords:
Reused Oil, Savory Snacks, Public Health, Toxic Metals, Permissible Limits

How to Cite:
Qamar, R., Shah, A. M., Kazi, T. G., Khanzada, B., & Memon, F. N. (2025). Physico-chemical Analysis and Metallic Contamination of Reused Oil and Savory Snacks Available at University Canteens: Physico-chemical Analysis and Metallic Contamination of Reused Oil. Futuristic Biotechnology, 5(2), 46-51. https://doi .org/10.54393/fbt.v5i2.175

***Corresponding Author:**
Afsheen Mushtaque Shah
Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
afsheen.shah@usindh.edu.pk

Received Date: 23rd April, 2025
Revised Date: 10th June, 2025
Acceptance Date: 16th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Edible oils play a crucial role in our daily diet, providing energy, essential fatty acids, and vitamins. Frying is a common method for preparing many foods, but the deep-frying process can alter the composition of both oil and food. **Objectives:** To assess the potential health risks associated with the consumption of reused oil and savory snacks. **Methods:** A total of 25 oil samples, including Before Frying Oil (BFO) and After Frying Oil (AFO), as well as snack samples (samosa and pakora), were collected from the canteens of Sindh University, Jamshoro, Pakistan. Blood samples were collected from students studying at the main campus of Sindh University, Jamshoro, and control samples were also collected from individuals of the same age and gender. Elements like copper (Cu), iron (Fe), nickel (Ni), lead (Pb), zinc (Zn) and chromium (Cr) were analyzed from oil, blood and snacks samples by atomic absorption spectrometry. **Results:** Metals like Cu, Fe, Ni, Pb, Cr increased along with Zn decreased in AFO samples whereas all metals like Cu, Fe, Ni, Pb, and Cr were found increased and Zn decreased in blood of consumers, Cu, Fe, Ni, Pb, Cr decreased in Samosa and Zn increased in Savory samples. **Conclusions:** Used cooking oil is dangerous for human health. People should avoid buying contaminated food items, especially savory snacks, from open markets because they are harmful to health.

INTRODUCTION

Nowadays, the deep-fried snacks are being used at home, restaurants and workplaces, which mostly use canola oil for frying purposes. Many Asian countries prepare snacks in different ways; moreover, in local restaurants, shops and vendors do not observe proper hygiene and use oil for frying many times. Students don't have breakfast at home or hostel and use savory snacks [1]. South Asian countries, especially Pakistan, India, and Bangladesh, are developing countries, where University students belong to the middle socio-economic class, so they have less pocket money. Canteens of universities prepare savory snack items (samosa, pakora, roll, etc.) at very low prices, which are fried repeatedly in frying oil [2]. In different developing countries, especially Asian countries like Pakistan, India, China, and Thailand, shop vendors prepare snacks. These are ready-to-eat snack foods at low cost in university canteens. In Pakistan, generally, people use street vendor foods specially snacks daily. Street foods are prepared and even sold by local vendors at local markets, schools, parking lots, and universities, for direct consumption without further processing [3, 4]. In humans, ingestion of food contaminated with trace metals is the most common route of heavy metal (HM) exposure and can pose a serious health risk. Snacks are being used daily at schools and universities by students. Snacks' material or ingredients quality is usually not checked in local shops/Canteens.

DOI: https://doi.org/10.54393/fbt.v5i2.175

Moreover, the same oil is used many times by food vendors during the preparation of savory snacks [5]. These savory snacks are prepared in contaminated utensils, without poor sanitation, improper hygiene, improper cookware, inadequate storage conditions, and contaminated ingredients. High intake of snacks increases the risk of diseases; unhealthy eating habits lead to uncontrolled weight gain. Cooking oil deterioration is accelerated by the formation of degradation compounds such as free fatty acids and peroxides [6, 7]. The presence of trace metals in cooking oils depends on many factors: they might originate from the soil, fertilizers, plantations, presence of industry [8]. Metals may also be introduced during the contamination process through contact with packaging materials. The trace metals enhance the rate of oxidation of cooking oils by increasing the generation of free radicals from fatty acid hydro-peroxides [9, 10].

Despite the widespread consumption of deep-fried snacks by university students, limited studies in Pakistan have systematically assessed the extent of metallic contamination in reused cooking oils and associated health risks in consumers. Most previous research focuses on either oil quality or street food safety separately, leaving a gap in understanding the direct correlation between reused frying oil, toxic metal accumulation in snacks, and their impact on student health. This study addresses this gap by analyzing both physicochemical changes in oils and metal contamination in snacks and consumer blood samples. Metals were analyzed in 25 cooking oil samples (before and after frying), samosa and pakora samples collected from university canteens, and in blood serum from 30 student consumers and 30 non-consumer controls, to assess the potential health risks associated with the consumption of reused oil and savory snacks. This study aimed to determine the quality of oil and snacks used at University Canteens by the physicochemical properties and metals analysis.

METHODS

This cross-sectional study involved analysis of 25 cooking oil samples from the students' canteen of Sindh University, Jamshoro, Sindh, Pakistan, from May 2019 to July 2021. The study was started after ethical approval from the ethical committee at the Institute of Biochemistry, University of Sindh (Jamshoro) with a reference number IOB/48/2019. Informed consent was obtained from all students prior to the study. The sample sizes (25 oil samples, 30 consumers, and 30 non-consumers) were determined based on preliminary surveys of typical canteen operations and student consumption patterns. These numbers provided sufficient representation, while remaining logistically feasible, and consistent with similar studies on food contamination. The before/after frying comparison and consumer/non-consumer matching were designed to enhance the detection of meaningful differences. Samples were collected using purposive sampling. Twenty-five paired oil samples (before and after frying) and corresponding samosa and pakora samples were obtained from canteens that prepared and sold fried snacks on-site using unbranded oil. Canteens using branded or single-use oil were excluded. Blood samples were taken from 30 regular snack consumers and 30 matched non-consumers. A validated questionnaire (Cronbach's alpha=0.75) was filled out by students, consisting of questions about the habits of eating savory snacks. During the study, it was surveyed that all were using unbranded rapeseed cooking oil and the same cooking oil was used many times for frying snacks. Data were analyzed using SPSS-25.0. Metal concentrations and physicochemical parameters were compared using paired t-tests for before vs. after frying oil samples (BFO/AFO) and independent t-tests for consumer vs. non-consumer blood samples. Questionnaire data were analyzed using descriptive statistics and chi-square tests where appropriate. Normality was verified using Shapiro-Wilk tests; non-parametric alternatives (Mann-Whitney U, Wilcoxon signed-rank) have been used when assumptions were violated. Results with p-values <0.05 were considered statistically significant. Before studying, a survey was done for the collection of information from different canteens of the University for snack selection, mostly Samosa and Pakora were seen to be sold to students. Questionnaire responses were scored and analyzed using descriptive statistics, while metal concentration data were compared using independent t-tests with $p<0.05$ considered significant." Ultrapure water (Bucks, UK), Nitric acid (Merck, Darmstadt, Germany), Hydrogen peroxide (Merck), standards of Cu, Ni, Fe, Pb, Zn and Cr mg/L (Fluka-Kamica-Buchs, Switzerland). Glassware and plastic material were treated with 5M pure Nitric acid for 24 Hours. Nitric acid (65%) and Hydrogen Peroxide 30% (2:1 ratio) was used for CDM (Conventional digestion method). Standard solutions of Cu, Ni and Fe of 1000 mg/L were prepared by dilution of certified Standard solutions. Physicochemical Properties of Before Frying Oil (BFO) and After Frying Oil (AFO): Physical and chemical properties of before and after frying used oil were determined using already reported methods [11-14]. 5cc Blood samples were collected from 30 consumers and 30 non-consumer students, who were studying in different BS programs. The age range of students was from 17 to 25 years. Whole blood samples were collected and centrifuged at 4 °C for serum collection. Serum was used for the determination of toxic metals, i.e. Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb), Zinc (Zn) and Chromium (Cr). Each composite sample of snack was mixed and ground in a mortar and pestle, then kept at -4 Cº. Weighed 1.0 g of each mix composite sample of samosa and pakora samples (electronic balance machine, A and D

DOI: https://doi.org/10.54393/fbt.v5i2.175

company C0006). Then kept the samosa and pakora samples for ashing in a furnace. Ash content was measured as an indicator of total mineral residue, serving as a proxy for potential metal accumulation in snacks. 600°C, for 2 hours. Afterwards, the snacks were re-weighed [15]. Ash content was measured as an indicator of total mineral residue, serving as a proxy for potential metal accumulation in snacks. This study correctly weighed triplicate samples (0.5 g) of each reused cooking oil, snack samples (samosa, pakora) and blood samples into individual (100 ml) conical flasks. Two (2) mL of concentrated HNO_3 (65%) and H_2O_2 (30%) (2:1, v/v; analytical grade, Merck) were added to each flask, incubated for 10 min at room temperature, and heated at 80 °C until clear. Reagent blanks were run to validate digestion and control for background contamination. After evaporation of flask contents, the semi-dried mass was allowed to dissolve in 10 ml 0.2 M HNO3, and it was then filtered through filter paper (Whatman No. 42) and a final volume was made up to 10 ml in volumetric flasks with ultrapure water, which was designated as the stock solution. All samples were prepared in triplicate for reproducibility"[16]. Metals such as copper, nickel, lead, zinc, and chromium were analyzed using Electro Thermal Atomic Absorption Spectrometry, and specific Measurement conditions were followed using already reported methods [17, 18]. Moreover, iron concentration was detected via Flame Atomic Absorption Spectrometry using specific measurement conditions [19].

RESULTS

Questionnaire responses revealed some consumers reported health issues such as vomiting, acidity, headache, cough, obesity, chest pain, and self-reported diagnoses of cardiovascular or kidney disease. These findings are based on self-reported information and indicate possible associations rather than confirmed clinical causation. 25 oil samples from different canteens of Sindh University were subjected to physicochemical analysis. PH and saponification value were seen to decline in the oil samples after frying. Other parameters like specific gravity, viscosity and acid value increased in the case of after frying oil (AFO) and this increase was found to be statistically significant with a p value<0.050 (Table 1).

Table 1: Determination of Physical and Chemical Properties in BFO and AFO Samples

Sr. No.	Physico-chemical Parameters (n=25)	BFO (Mean ± SD)	AFO (Mean ± SD)	p-value
1	pH	5.15 ± 0.64[a], 6.8-4.71[b]	3.70 ± 0.42[a], 3.16-4.46[b]	0.230
2	Specific Gravity (g/ml)	0.58 ± 0.05[a], 0.51-0.76[b]	0.95 ± 0.01[a], 0.91-0.99[b]	0.040*
3	Viscosity (Pa/s)	1.62 ± 0.28[a], 1.6-3.9[b]	5.17 ± 1.66[a], 1.6-6.2[b]	0.010*
4	Acid Value (mg/KOH/g)	0.54 ± 0.18[a], 0.1-0.6[b]	1.40 ± 0.15[a], 1.2-1.9[b]	0.020*
5	Saponification Value (mg/KOH/g)	130 ± 7.79[a], 115-138[b]	96.4 ± 1.14[a], 91-98[b]	0.250

p<0.050* Statically Significant ('a indicates mean values and 'b' indicates minimum and maximum values)

Metal analysis in oil samples depicted that all metals, specifically Nickel, chromium and Lead, were significantly increased (p-value<0.050) in their content after frying oil samples. Whereas only the Zinc content was decreased in oils after frying (p-value>0.050) (Table 2).

Table 2: Determination of Metals in (BFO) and (AFO) Samples

Sr. No	Metals Concentration (mg/L)	BFO (Mean ± SD)	AFO (Mean ± SD)	p-value	Permissible Values of Metals (mg/L)
1	Copper	0.3 ± 0.01[a] 0.2 -0.9[b]	2.07 ± 0.3[a] 0.1-2.6[b]	0.041*	0.1
2	Iron	0.7 ± 0.2[a] 0.1-0.4[b]	1.24 ± 0.60[a] 2.1-2.6[b]	0.043*	54
3	Nickel	0.6 ± 0.1[a] 0.3-0.9[b]	2.43 ± 0.2[a] 1.2-2.7[b]	0.020*	0.2
4	Lead	1.21 ± 0.30[a] 1.2-1.9[b]	2.40 ± 0.54[a] 2.1-2.6[b]	0.040*	0.1 / 2
5	Zinc	0.8 ± 0.45[a] 0.07–0.05[b]	0.6 ± 0.55[a] 0.4-0.8[b]	0.350	0.60
6	Chromium	1.30 ± 0.83[a] 1.1-1.3[b]	2.93 ± 1.55[a] 2.1-2.7[b]	0.040*	1.30

p<0.005* Statically Significant (' a indicates mean values and 'b' indicates minimum and maximum values).

Metal analysis in blood samples of snack consumers (students) revealed that all the metal concentrations (except Zinc) were increased in consumers as compared to non-consumers (control), and this increase was statistically significant with p-value<0.050 (Table 3).

Table 3: Metal Concentration in Blood Samples

Sr. No.	Metals (mg/L)	Control Mean ± SD (n=30)	Consumers Mean ± SD (n=30)	p-value	Permissible Values of Metals (mg/L)
1	Copper	1.6 ± 0.06[a], 1.2-1.9[b]	2.7 ± 0.9[a], 2.9-3.7[b]	0.032*	0.59-1.4
2	Iron	690 ± 17[a], 590-680[b]	900 ± 105[a], 840-950[b]	0.001*	236-614
3	Nickel	1.3 ± 0.08[a], 1.2-1.9[b]	2.5 ± 0.8[a], 2.1-2.9[b]	0.021*	0.3-0.77
4	Lead	119 ± 12[a], 118-145[b]	160 ± 14[a], 178-190[b]	0.030*	20-100
5	Zinc	5.8 ± 1.3[a], 5.9-6.9[b]	5.4 ± 2.1[a], 5.1-5.8[b]	0.071	4.63-7.73

DOI: https://doi.org/10.54393/fbt.v5i2.175

6	Chromium	1.5 ± 0.03[a], 1.1-1.1[b]	2.72 ± 1.1[a], 2.1-2.6[b]	0.042*	0.4-1.2

p<0.050* Statically Significant (' a indicates mean values and 'b' indicates minimum and maximum values).

Findings show ash content percentages across different canteens. Pakoras consistently showed higher ash content (%) than samosas across all canteens, suggesting greater mineral residue retention in pakora samples. The highest ash percentage of samosas has been from the English canteen, while pakoras from the central canteen showed more ash content (Figure 1).

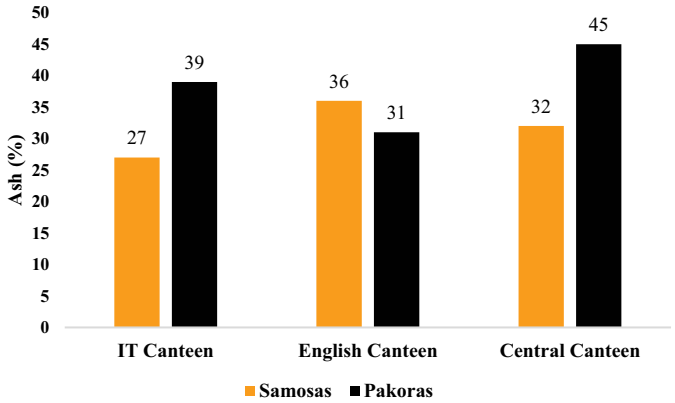

Figure 1: Ash Content (%) of Samosas and Pakoras from Different Canteens

When snacks sold at Sindh University canteens were analyzed for their metal content, it was seen that copper, iron and nickel concentrations were above the permissible limits in samosas whereas lead, zinc and chromium were found in normal ranges in both types of snacks (Table 4).

Table 4: Determination of Metals in Snacks

Sr. No	Metals (mg/kg)	Pakora (Mean ± SD)	Samosa (Mean ± SD)	Permissible Values of Metals (mg/Kg)
1	Copper	18.1 ± 1.31[a], 21-29[b]	40.2 ± 13[a], 27-39[b]	30
2	Iron	31 ± 2.5[a], 21-24[b]	80 ± 19[a], 77-90[b]	40-60
3	Nickel	1.39 ± 0.35[a], 1.4-1.9[b]	3.69 ± 1.32[a], 2.3-3.7[b]	1.5
4	Lead	1.09 ± 0.25[a], 1.2-1.8[b]	2.98 ± 0.57[a], 2.4-2.8[b]	2.5
5	Zinc	35 ± 1.94[a], 31-39[b]	25 ± 1.1[a], 20-29[b]	50.0
6	Chromium	19 ± 1.9[a], 14-19[b]	4.93 ± 1.55[a] 51-59[b]	20

p<0.050* Statically Significant. ('a indicates mean values and 'b' indicates minimum and maximum values)

DISCUSSION

Survey results from the questionnaire suggest that reused oil-based snacks can lead to various diseases. Therefore, consumers should opt for fewer fried items in order to save themselves from health complications. Specific gravity, viscosity and acid value tend to increase after more frying (Figure 1). It may be because during the frying process, chemical reactions like thermal reactions, polymerization, oxidation and hydrolysis take place. These reactions give rise to non-volatile, insoluble matter, which increases the viscosity and specific gravity, produces a darker colour and thickens the oil [19]. Our results are in agreement with previous studies, which also reported an increase in these parameters after frying [20, 21]. Acidic values and Saponification values in our investigation after frying oils samples 1.4 and 96.4 were in agreement with those reported by a previous study [22]. Moreover, previous researchers also reported increased concentrations of chromium and nickel after continuous frying of oils in snacks [23]. Our results are similar to previous studies, which also show that metal concentration increased above permissible limits as we subject oil to more frying [23]. The concentration of copper, iron, lead and zinc in our investigated samples of oils was found to be similar to that in previous studies [24]. Particularly, iron toxicity was revealed in consumers' blood after consumption of oily snacks from university canteens. Other metals like chromium, copper, lead and nickel were also not found in permissible limits in consumers' blood. The concentration level of Fe was found to be 690-900 mg/L in the blood of students studying at Sindh University, Jamshoro. Our findings, 600-888 mg/L, are in agreement with the results of previous studies [25]. Furthermore, an increase in copper, iron and nickel concentrations in samosas may be due to add more carbohydrate content due to the use of potato, cooking soda, white flour, salt and red chilli, etc. [26]. This increased metal content is very harmful to our health as it may lead to various disorders like cancer, diabetes, stomach ulcer, kidney disorder, etc. Pakora samples depicted an increase in ash content due to no other ingredient use, only besan in the pakora samples. Other studies have also reported more ash percentage in pakoras [27].

This study was limited by its relatively small sample size and focus on a single university, which may restrict the generalizability of the findings. Future research should expand to multiple institutions and diverse snack types, and consider long-term dietary exposure to toxic metals. Additionally, incorporating advanced analytical techniques could provide more detailed insights into the mechanisms of metal accumulation and aid in developing strategies to mitigate health risks associated with reused frying oils.

CONCLUSION

The present study demonstrates that repeated use of frying oil in snack preparation leads to significant increases in toxic metals (Cu, Fe, Ni, Pb, Cr) in both food products and the blood of consumers, with zinc levels showing a decline. Some detected concentrations

DOI: https://doi.org/10.54393/fbt.v5i2.175

exceeded permissible safety limits, indicating a potential risk of chronic metal exposure and associated health effects. Poor hygienic practices during preparation, handling, and storage further compound these risks. Public awareness, strict enforcement of food safety regulations, and discouragement of consuming snacks prepared in repeatedly heated oils are essential to safeguard public health.

Authors' Contribution

Conceptualization: AMS
Methodology: RQ, TGK, FNM
Formal analysis: TGK, BK, FNM
Writing and Drafting: AMS, TGK, BK
Review and Editing: AMS, TGK, BK, FNM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Valle C, Echeverría F, Chávez V, Valenzuela R, Bustamante A. Deep-Frying Impact on Food and Oil Chemical Composition: Strategies to Reduce Oil Absorption in the Final Product. Food Safety and Health.2024 Oct; 2(4): 414-28. doi: 10.1002/fsh3. 12056.

[2] Sousa S, de Morais IL, Albuquerque G, Gelormini M, Santos M, Filipović-Hadžiomeragić A *et al.* Nutritional Content of Street Food and Takeaway Food Purchased in Urban Bosnia and Herzegovina. Foods. 2021 Oct; 10(11): 2594. doi: 10.3390/foods10112594.

[3] Yulia C, Nikmawati EE, Widiaty I. Preliminary Study in Developing Traditional Street Foods as Nutrition Education Media for Indonesia Youth. Invotec XIII. 2017; 1: 1-7. doi: 10.17509/invotec.v13i1.6256.

[4] Girma Z, Temesgen K, Dubale A, Waritu NC, Jemal M, Wakeyo MU. Food Handling Practices Among Street Food Vendors and Their Associated Factors in the Town Administration of Gamo Zone, Southern Ethiopia. Frontiers in Food Science and Technology. 2025 Feb;5:1445910.doi:10.3389/frfst.2025.1445910.

[5] Verma R and Mishra S. Street Food Vendor Handling Practices and Bacterial Contamination of Ready-To-Eat Foods. Acta Scientific Agriculture.2023; 7(8): 50-61. doi: 10.31080/ASAG.2023.07.1288.

[6] Correa KL, de Carvalho-Guimarães FB, Mourão ES, Oliveira Santos HC, da Costa Sanches SC, Lamarão ML *et al.* Physicochemical and Nutritional Properties of Vegetable Oils from Brazil Diversity and Their Applications in the Food Industry. Foods.2024May; 13(10): 1565. doi: 10.3390/foods13101565.

[7] Mehany T, González-Sáiz JM, Pizarro C. Improving the Biostability of Extra Virgin Olive Oil with Olive Fruit Extract During Prolonged Deep Frying. Foods.2025 Jan; 14(2): 260. doi: 10.3390/foods14020260.

[8] Gholamibozanjani G, Leong SY, Oey I, Bremer P, Silcock P, Farid M. Heat and Mass Transfer Modeling to Predict Temperature Distribution During Potato Frying After Pre-Treatment with Pulsed Electric Field. Foods.2021Jul;10(8):1679.doi:10.3390/foods10081679.

[9] Abrante-Pascual S, Nieva-Echevarría B, Goicoechea-Oses E. Vegetable Oils and Their Use for Frying: A Review of Their Compositional Differences and Degradation. Foods.2024 Dec; 13(24): 4186. doi: 10.3390/foods13244186.

[10] Benmeziane F, Araba K, Belahcene A. Impact of Deep-Fat Frying Cycles on the Physicochemical Characteristics of Two Edible Vegetable Oils Marketed in Algeria. North African Journal of Food and Nutrition Research.2024Feb;8(17):21-31.doi:10.51745/najfnr.8.17.21-31.

[11] Isah AG, Faruk AA, Musa U, Garba UM, Alhassan M, Abdullahi UB *et al.* Oxidation Stability and Cold Flow Properties of Biodiesel Synthesized from Castor Oil: Influence of Alkaline Catalysts Type and Purification Techniques. Materials Today: Proceedings.2022 Jan; 57: 748-52. doi: 10.1016/j.matpr.2022.02.220.

[12] Nayak PK, Dash UM, Rayaguru K, Krishnan KR. Physio-Chemical Changes During Repeated Frying of Cooked Oil: A Review. Journal of Food Biochemistry. 2016 Jun;40(3):371-90.doi:10.3923/pjn.2013.647.650.

[13] Amos-Tautua BM and Onigbinde AO. Physicochemical Properties and Fatty Acid Profiles of Crude Oil Extracts from Three Vegetable Seeds. Pakistan Journal of Nutrition.2013Jul;12(7):647.doi:10.1016/j.arabjc.2014.05.025.

[14] Zahir E, Saeed R, Hameed MA, Yousuf A. Study of Physicochemical Properties of Edible Oil and Evaluation of Frying Oil Quality by Fourier Transform-Infrared (FT-IR) Spectroscopy.Arabian Journal of Chemistry.2017May;10:S3870-6.doi:10.18805/BKAP617.

[15] Kamble PA, Gore SB, Relekar SS, Telvekar PA, Rathod RH, Belsare SS *et al.* Development of Fish Cracker from Indian Major Carp, Labeo Catla. Bhartiya Krishi Anusandhan Patrika.2023;38(2):167-71.doi:10.1186/s12889-024-19695-0.

[16] Yohannes L, Feleke H, Melaku MS, Amare DE. Analysis of Heavy Metals and Minerals in Edible Vegetable Oils

DOI: https://doi.org/10.54393/fbt.v5i2.175

Produced and Marketed in Gondar City, Northwest Ethiopia. BioMed Central Public Health.2024Aug; 24(1): 2204. doi: 10.1590/s2175-97902022e21094.

[17] Aydinoglu S. Iron and Zinc Determination in Dietary Supplements by Flame Atomic Absorption Spectrophotometry.Brazilian Journal of Pharmaceutical Sciences.2022;58:e21094.doi:10.1515/chem-2018-0026.

[18] Prkić A, Politeo N, Giljanović J, Sokol V, Bošković P, Brkljača M *et al.* Survey of Content of Cadmium, Calcium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Mercury, Sodium and Zinc in Chamomile and Green Tea Leaves by Electrothermal or Flame Atomizer Atomic Absorption Spectrometry. Open Chemistry.2018Mar;16(1):228-37.doi:10.26656/fr.2017.6(5).608.

[19] NurSyahirah S and Rozzamri A. Effects of Frying on Fish, Fish Products and Frying Oil—A Review. Food Research. 2022 Oct; 6(5): 14-32. doi: 10.1007/s13197-022-05472-7.

[20] Zou M, Chen Y, Hu C, He D, Gao P. Physicochemical Properties of Rice Bran Blended Oil in Deep Frying by Principal Component Analysis. Journal of Food Science and Technology.2022 Nov; 59(11): 4187-97. doi: 10.9790/1959-0140110.

[21] Choudhary M and Grover K. Effect of Deep-Fat Frying on Physicochemical Properties of Rice Bran Oil Blends. Journal of Nursing and Health Sciences. 2013; 1(4): 1-0.

[22] Alajtal AI, Sherami FE, Elbagermi MA. Acid, Peroxide, Ester and Saponification Values for Some Vegetable Oils Before and After Frying. AASCIT Journal of Materials.2018; 4(2): 43-7. doi: 10.1016/j.jtemin. 2024 .100119.

[23] Ashong GW, Ababio BA, Kwaansa-Ansah EE. Evaluation of Trace Metals and Quality of Selected Brands of Vegetable Cooking Oils Available on the Ghanaian Market. Journal of Trace Elements and Minerals.2024 Jun; 8: 100119. doi: 10.1515/aep-2016-0029.

[24] Szyczewski P, Frankowski M, Zioła-Frankowska A, Siepak J, Szyczewski T, Piotrowski P. A Comparative Study of the Content of Heavy Metals in Oils: Linseed Oil, Rapeseed Oil And Soybean Oil in Technological Production Processes.Archives of Environmental Protection.2016.doi:10.1088/1742-6596/1123/1/012064.

[25] Nasir K, Khan M, Chandio TA, Bashir Y. Comparative Analysis of Essential and Toxic Elements in Water and Blood Samples of Kidney Patients in Quetta, Pakistan. In Journal of Physics: Conference Series. 2018 Nov; 1123(1): 012064. doi: 10.4314/jasem.v21i3.13.

[26] Dada EO, Ojo ON, Njoku KL, Akinola MO. Assessing the Levels of Pb, Cd, Zn and Cu in Biscuits and Home-Made Snacks Obtained from Vendors in Two Tertiary Institutions in Lagos, Nigeria. Journal of Applied Sciences and Environmental Management.2017Jul; 21(3): 521-4.

[27] Ahsan F, Sharif MK, Butt MS, Rauf A, Shariati MA, Imran M *et al*. Legumes and Leafy Vegetables Based Multi-Mix Pakoras To Alleviate Iron And Protein Deficiency Among School Aged Children. BioCell. 2019; 43(1).

DOI: https://doi.org/10.54393/fbt.v5i2.198

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Original Article

OPEN ACCESS

Detection and Quantification of Genetically Modified Organisms (GMOs) in Halal Food Products by qPCR Method— Utilization of GMO-Positive Cabbage Seeds

Syeda Areeba Nadeem[1*], Noor Fatima[1], Duaa Mughal[1], Aymen Arif[2], Shakil Ahmed[1] and Ishtiaq Ahmed Khan[1]

[1]International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
[2]Molecular Diagnostic and Research Laboratory, Isra University, Karachi, Pakistan

ARTICLE INFO

Keywords:
Genetically Modified Organism, Quantitative Polymerase Chain Reaction, NOS Terminator, Halal Authentication, Food Adulteration

How to Cite:
Nadeem, S. A., Fatima, N., Mughal, D., Arif, A., Ahmed, S., & Khan, I. A. (2025). Detection and Quantification of Genetically Modified Organisms (GMOs) in Halal Food Products by qPCR Method-Utilization of GMO-Positive Cabbage Seeds: GMOs in Halal Food Products by qPCR Method: GMO-Positive Cabbage Seeds. Futuristic Biotechnology, 5(2), 52-58. https://doi.org/10.54393/fbt.v5i2.198

***Corresponding Author:**
Syeda Areeba Nadeem
International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
areebanadeem992@gmail.com

Received Date: 15th April, 2025
Revised Date: 6th June, 2025
Acceptance Date: 11th June, 2025
Published Date: 30th June, 2025

ABSTRACT

Use of genetically modified organisms (GMOs) in international food production has resulted in religious and health issues, especially among Muslim consumers. There is a need to have good analyses that will guarantee the authenticity and safety of the halal food products. **Objectives:** To identify and determine the concentration of GM contamination in both raw and processed foodstuff samples using a sensitive quantitative polymerase chain reaction (qPCR) method. **Methods:** Certified Reference Material (CRM-BF410ep Soya Bean) was used to construct a qPCR standard curve by 10-fold serial dilution (0.1% 0.01% 0.001% 0.0001%). GMO-positive cabbage seeds were added to processed food (China noodles, mixed spices, rice protein) and unprocessed food (brown rice, basmati rice, IRRI-6 rice). The NOS terminator sequence has been measured using qPCR to amplify DNA by extracting it with a commercial kit, quantifying it, and subsequently analyzing the DNA sample. Efficiency and limits of detection. **Results:** NOS terminator sequence was easily identified in a concentration of 0.001% and the mean Ct values were similar to the CRM standard. The statistical analysis (p.05) showed that there is no significant differentiation between the CRM and spiked samples, which proves the accuracy and reproducibility of the method at low concentrations of DNA. **Conclusions:** The tested qPCR technique proved to be very sensitive in the detection of GM contamination in various food matrices at 0.001 percent. Conclusions: This method will aid in halal food authentication, and a reliable molecular tool will help in avoiding accidental intake of GM haram ingredients.

INTRODUCTION

Food laws and safety must be reviewed regularly due to their global significance and influence on public health. The massive cross-border commerce in food commodities presents serious issues for the global food business [1]. Due to related ethical and health issues, some religions forbid genetically modifying food. In order to create gene combinations that do not exist naturally, genetically modified organisms (GMOs) are living things that have had their genetic makeup changed by biotechnological methods that involve the insertion of genetic material from other species, such as bacteria, viruses, plants, or animals [2]. Products made from haram (prohibited) sources, such as those that have been genetically modified using non-halal ingredients, are not acceptable for ingestion from an Islamic standpoint [3]. Muslims view following the halal diet as a way to preserve their spirituality as well as a religious obligation. It is crucial to make sure that genetically modified (GM) products adhere to Islamic principles, especially when the genetic material comes from animals that are forbidden or unclean materials [4]. A key component of Islamic dietary regulation is avoiding haram foods, which are expressly prohibited in the Quran and

DOI: https://doi.org/10.54393/fbt.v5i2.198

Hadith [3]. "Food and beverages comprising results and/or by-products of GMOs or ingredients derived from non-halal sources are not halal," according to the Standards and Metrology Institute for Islamic Countries (SMIIC) [5]. Because of their high nutritional content, vegetables—especially those in the Brassicaceae family are vital dietary components. China, India, and Pakistan are the world's top producers of cauliflower and cabbage, and Poland is in the top 10 worldwide [6]. China and India accounted for roughly 72% of the world's 25.5 million tonnes of cabbage production in 2020. The growing frequency of GM crops, including soybean (47%), maize (32%), cotton (15%), and canola (5%), highlights the growing need for efficient detection technologies, even if genetically modified cabbage is not yet commercially available [7]. Although the majority of genetically modified meals now come from plants, goods made from genetically modified animals (GMAs) and microorganisms (GMMs) should soon be available on the market [8]. Global adoption of genetically modified crops for food and feed uses has been made easier by the quick development of recombinant DNA technology [9]. Due to its sensitivity and repeatability, Polymerase Chain Reaction (PCR) continues to be the most dependable analytical technique for detecting GMOs in both raw and processed foods [10]. Even in complicated food matrices, the precise determination of GM content is made possible by quantitative PCR (qPCR), a refined form of PCR [11]. Because of its exceptional sensitivity and accuracy in identifying even the smallest DNA amounts, qPCR was used for this investigation. Compared to qualitative approaches, the technique provides a more detailed assessment by quantifying the quantity of GM DNA in a sample. Genetic components like the nopaline synthase terminator (NOS terminator), which is derived from Agrobacterium tumefaciens, are frequently detected in order to identify GMOs. Assays based on nucleic acids are essential for verifying the existence of transgenic material [12]. In various dietary matrices, prior studies have shown LODs of 0.1% [13], 0.01% [14], 0.9% [15], and 5.0% [16]. By establishing detection at an unusually low threshold of 0.001%, the current study expands the analytical limitations of qPCR for halal food surveillance, building upon earlier discoveries.

Despite the global rise in genetically modified crops, reliable detection of GMOs in halal food products remains a challenge, particularly at very low concentrations. While previous studies have demonstrated qPCR detection of GMOs, most methods report limits of detection ranging from 0.01% to 0.1%, which may not be sufficient for strict halal certification. Moreover, there is limited research in Pakistan on validating sensitive qPCR methods for detecting GM contamination in complex processed and unprocessed food matrices. This study addresses this gap by evaluating an advanced qPCR approach to detect GMOs at unprecedentedly low levels in diverse food products. The study aims to enhance GMO detection reliability and sensitivity, achieving a limit of detection (LOD) as low as 0.001% and to establish the precision, sensitivity, and reproducibility of a qPCR assay for GMO detection in complex food matrices.

METHODS

Sample mixtures (SM) of China noodles, mixed spices, rice protein, brown rice, basmati rice, and IRRI-6 rice were tested in this study. The samples utilized in this study were obtained from the commercial market and were subjected to GMO testing at the Halal Lab of the Industrial Analytical Center, Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, from March 2023 to September 2023. For the GM-positive sample, cabbage seeds were used, which were the F1 hybrid obtained from the Department of Plant Protection, Karachi, Pakistan. An available positive sample for reference is GM cabbage seeds, which are positive for the target gene. The certified reference material (CRM-BF410ep Soya Bean) that contains 10% GMO-positive Soya matrix was utilized, likewise positive for standard curve preparation by diluting it to 0.1%,0.01%,0.001%, and 0.0001% and was purchased from Fapas65 Gresham St, London. The samples utilized in this study were obtained from the commercial market using a convenience sampling approach. This sampling strategy is appropriate for the method validation purpose of this study, which prioritizes assessing analytical performance across diverse matrices over achieving statistical representation of the broader food market. Written informed consent was taken. A total of six commercially available food products were selected to represent a diverse range of matrix complexities relevant to halal food testing. This included processed foods with potential PCR inhibitors like China noodles (high starch, potentially high oil), mixed spices (high polyphenols, polysaccharides), and rice protein (high protein), as well as unprocessed grains like brown rice, basmati rice, and IRRI-6 rice. The choice was specifically meant to test the DNA extraction and qPCR assay in different compositions, and in doing so, give a strong preliminary confirmation of the suitability of the method. The samples were homogenized individually and contaminated with GMO-positive cabbage seed and mixed with the 10% GM-positive sample that consisted of taking around 180 mg of China noodles and mixed spices, and contaminated with 20 mg of GMO-positive cabbage seed. SM 2 includes 10% of the GM-positive sample, which was prepared by taking approximately 180 mg of processed rice protein and raw brown rice, which was contaminated with 20 mg of GMO-positive cabbage seeds. SM 3 includes 10% of the GM-positive sample, which was prepared by taking

DOI: https://doi.org/10.54393/fbt.v5i2.198

approximately 180 mg of unprocessed IRRI 6 rice and basmati rice with the contamination of 20 mg of GMO-positive cabbage seeds. SM 4 includes a GM-negative sample, which was prepared by taking approximately 180 mg of processed China noodles and mixed spices with contamination of 20 mg of GMO-negative. As this study focuses on analytical method development and validation, a formal a priori sample size calculation was not performed. Instead, the experimental design and replication strategy were based on internationally accepted guidelines for the validation of qualitative and quantitative PCR methods, a relevant guideline here if desired, e.g., from ISO or MIQE. The robustness of the method was demonstrated through technical triplicates for each qPCR reaction to assess precision and biological replicates (independently prepared sample mixtures, SM1-SM5) to assess reproducibility across different matrices. The validation parameters were determined experimentally, which were the linearity (R 2), the amplification efficiency, coefficient of variation (CV%), limit of quantification (LOQ), and limit of detection (LOD). To statistically confirm the ability of the method to discriminate between the concentrations of GMOs, a one-way Analysis of Variance (ANOVA) was performed on the Ct values of CRM on its serial dilutions (0.1, 0.01, and 0.001%). This will ensure that the performance characteristics of the method are evaluated with a sufficient level of statistical rigor to implement the method. The findings showed that the qPCR test is able to differentiate accurately between these concentration levels by showing a significant difference between the mean Ct values of these dilutions ($F(2,6) = (\text{insert F-value})$, $p<0.001$). Approximately 180 mg of uncooked basmati rice and IRRI 6 rice were sprayed with 20mg of GMO-positive cabbage seeds to form the 10 percent GM-positive sample that is represented in SM 5. The extracted genomic DNA was obtained using the Kogenebiotech GMO Extraction Kit (Kogenebiotech, Geumcheon, Korea). In order to ensure that the accuracy is maintained, approximately 180 mg of each of the samples was carefully weighed and then used to prepare the 10% GM-positive sample. Controlled contamination was done using 20 mg of GMO-positive cabbage seeds introduced using an analytical balance of Mettler Toledo, Switzerland. A high-sensitivity analytical balance is necessary to determine the level of reliable results and obtain high levels of precision in weighing the samples. The sample homogenization was thereafter done using a Tissue Lyser II (QIAGEN, Germantown, MD, US). Besides, the samples were supplemented with 3mL of lysis buffer A,300 µL of lysis buffer B,10 µL of proteinase K, and 10 µL of RNAse. The extraction of the genomic DNA (gDNA) was performed according to the instructions of the manufacturer. Upon extraction, to achieve the same proportion, the DNA samples were diluted to a 10-fold serial dilution (lowest concentration being 0.0001%). The CRM was also extracted using the same protocol. The robustness of the experimental design is shown by the meticulous attention to detail in both the DNA extraction and sample preparation steps. The quality of the extracted gDNA was evaluated by 1% agarose gel electrophoresis. These procedures are essential for guaranteeing the precision and repeatability of the data, which eventually strengthens the validity of study conclusions. Using GMO CRM, 4 dilutions of 10-fold were prepared, i.e.0.1%,0.01%,0.001%, and 0.0001%. Sample mixtures SM1, SM2, SM3, SM4, and SM5were diluted at 10-folds, i.e. 0.1%, 0.01%,0.001%, and 0.0001%. Among all the dilutions, the 4th dilution (0.001%) was taken as a DNA template (sample) for further analysis, as the present research work aimed to quantify this concentration of DNA. Qubit Fluorometer 3.0 (Invitrogen Life Technologies, US) was used to evaluate the quality and concentration of DNA. QubitTM ds DNA HS standard 1 and QubitTM ds DNA HS standard S2 were produced using 189 µL of buffer, 1 µL of fluorescent dye, and 10 µL of each standard supplied with the Kit. To prepare the sample, 198 µL FOf buffer was dispensed into a tube containing 1 µL of dye (Qubit Assay Tubes, Thermo Fisher SCIENTIFIC). After adding 1 µL of the extracted DNA sample, the tubes were incubated at room temperature (25°C) for 1 minute before being placed into a Qubit fluorometer for observation. The amplification process using the PCR method was conducted in a final volume of 25 µL. 5 µL of template DNA, 12.5 µL of basic mix, and 7.5 µL of oligo mix made up this volume. The following approaches were used in a Thermal Cycler system (Rotor Gene Q, Qiagen Germany) to carry out the reactions: 45 cycles at 95°C for 10 minutes, 95°C for 10 minutes, and 60°C for 90 seconds. The data were assessed with the help of the QIAGEN rotor gene Q series program (version 2.3.1). Every sample mixture (SM1-SM5) was an independent biological replication that was generated independently in the same conditions, and each qPCR reaction was performed in technical triplicate to ensure analytical repeatability. In order to determine intra- and inter-assay precision, mean cycle threshold (Ct) and the corresponding standard deviations (SD) were calculated. Controls were employed in order to exclude any false positive or negative outcomes; these were borrowed from the PCR assay Kit. The 4 dilutions of 0.1 per cent,0.01 per cent, 0.001 per cent, and 0.0001 per cent of the GMO CRM (reference material) were amplified as standards. In addition to the 5 sample mixtures that had been diluted to 0.001% and 0.0001% to observe the GM event NOS terminator. Varying dilutions are employed with the aim of ensuring that this assay is sensitive enough to indicate the presence of GMO at an assortment of concentrations. All the data from qPCR were acquired in triplicate and evaluated as the mean and SD. The coefficient of variation

DOI: https://doi.org/10.54393/fbt.v5i2.198

(CV%) was computed in each dilution and was not more than 2, which proved a high degree of reproducibility. The results of CRM and spiked sample Ct values were compared using independent-sample t-tests, and no significant differences were detected ($p>0.05$). The fact that there was low variation between the replicates speaks in favor of both the precision and reliability of the assay.

RESULTS

To analyze genetically modified organisms (GMOs) in raw and processed food matrices at the lowest detectable concentration, this study applied quantitative polymerase chain reaction, or qPCR. This is the first formal effort that we have ever known in Pakistan to recognize GMO-positive cabbage seedlings. The control design of the study was based on the principles of analytical method validation. All analyses were performed in technical triplicate to ensure the accuracy of the results. The data are indicated in the form of mean and standard deviation (SD). The concentration and purity of DNA in the CRM and the samples of the test were determined using the Qubit Fluorometer 3.0 (Invitrogen, USA) at 430-495nm (blue) and 510-580nm (green). All samples had A260/280 absorbance ratios between 1.8 and 2.0, indicating little protein contamination and good DNA purity. The DNA yield derived from each sample combination was compiled in the findings. Quantifiable DNA was obtained from all extracted DNA samples at the desired 0.001% concentration, with quantities varying between 0.052 and 0.073 ng/µL. The DNA extraction and quantification procedures are very reproducible, as evidenced by the low standard deviations across triplicate assays (e.g., ± 0.004 to ± 0.008 ng/µL). This steady recovery from matrices that have been treated and those that have not shows how reliable the extraction procedure was (Table 1).

Table 1: DNA Concentration (ng/µl) Extracted from Sample Mixtures and CRM (Mean ± SD, n=3)

Dilution	SM1	SM2	SM3	SM4	SM5	CRM
Stock DNA	–	–	–	–	–	73.20 ± 0.15
0.1%	–	–	–	–	–	7.30 ± 0.12
0.01%	–	–	–	–	–	0.77 ± 0.09
0.001%	0.052 ± 0.006	± 0.004	0.064 ± 0.005	0.055 ± 0.007	0.073 ± 0.008	0.070 ± 0.005
0.0011%	Below Detection	Below Detection	Below Detection	Below Detection	Below Detection	Below Detection

SM1: China Noodles and Mixed Spices + Cabbage seeds (Positive) SM2: Rice protein and Brown Rice + Cabbage seeds (Positive) SM3: IRRI 6 Rice and Basmati rice + Cabbage seeds (Positive) SM4: China Noodles and Mixed Spices + Cabbage seeds (Negative) SM5: Basmati Rice and IRRI 6 Rice + Cabbage seeds (Negative) Certified Reference Material: ERM-BF410ep Soya Bean 10% GMO.

To improve detection reliability, qPCR was carried out employing both Cauliflower Mosaic Virus 35S (CaMV 35S) promoter targets and NOS terminator (T-NOS). To assess assay repeatability, each amplification was carried out in triplicate. Excellent linearity between concentration and cycle threshold (Ct) was confirmed by regression analysis of the CRM standard curve, which yielded a correlation coefficient (R2) of 0.992. Results are shown as mean Ct ± SD, and amplification efficiency was constant across replicates. Dual-marker reliability was confirmed by the amplification plots, which showed effective detection for both T-NOS and CaMV 35S targets in three of the five sample combinations (SM1–SM3) at the 0.001% level. Due to either matrix inhibitory effects or the lack of GM-positive material, two combinations (SM4 and SM5) did not exhibit any amplification (Table 2).

Table 2: Mean Ct (± SD, n=3) for CRM and Sample Mixtures at Different Dilutions

Dilutions	SM1 (Mean CT ± SD**)	SM2	SM3 (Mean CT ± SD)	SM4 (Mean CT ± SD)	SM5 (Mean CT ± SD)	CRM (Mean CT ± SD)
Stock DNA	–	–	–	–	–	21.69 ± 0.06
0.1%	–	–	–	–	–	26.71 ± 0.07
0.01%	–	–	–	–	–	30.67 ± 0.07
0.001%	33.72 ± 0.04	33.96 ± 0.67	33.69 ± 0.18	Not Detected	Not Detected	33.44 ± 0.13
0.0011%	No Amplification	No Amplification	No Amplification	No Amplification	No Amplification	No Amplification

SM1: China Noodles and Mixed Spices + Cabbage seeds (Positive) SM2: Rice protein and Brown Rice + Cabbage seeds (Positive) SM3: IRRI 6 Rice and Basmati rice + Cabbage seeds (Positive) SM4: China Noodles and Mixed Spices + Cabbage seeds (Negative) SM5: Basmati Rice and IRRI 6 Rice + Cabbage seeds (Negative) Certified Reference Material: ERM-BF410ep Soya Bean 10% GMO **SD = Standard Deviation.

Sm1 represents China noodles and mixed spices spiked with GMO-positive cabbage seeds; SM2 comprises rice protein and brown rice with GMO-positive contamination; SM3 includes IRRI-6 and basmati rice with GMO-positive cabbage seeds; SM4 depicts China noodles and mixed spices containing GMO-negative cabbage seeds; SM5 corresponds to basmati rice and IRRI-6 rice with GMO-negative cabbage seeds. The CRM (ERM-BF410ep Soya Bean, 10% GMO) served as the positive control for generating the standard curve (Figure 1).

DOI: https://doi.org/10.54393/fbt.v5i2.198

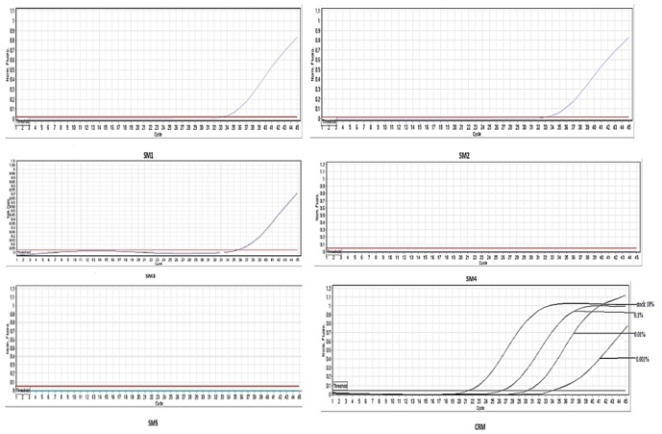

Figure 1: Amplification plots of sample mixtures and Certified Reference Material (CRM) analyzed by qPCR for GMO detection.

All CT data were analyzed using SPSS v25 (IBM, USA). Independent-sample *t*-tests were applied to compare mean Ct values between CRM and spiked samples. The *t*-test revealed no significant difference (*p* > 0.05), validating the reproducibility. Validating the reproducibility of detection across sample matrices. Mean ± SD and coefficient of variation (CV%) were within acceptable limits (<2%). The experimentally determined limit of detection (LOD) for both markers was 0.001%, while the limit of quantification (LOQ) was established at 0.01%, as verified through standard curve regression. The precision of the assay was statistically supported by the high R^2 value and low variability among triplicates. The precision of these limits was statistically verified by the high linearity of the standard curve (R^2 = 0.992), which confirms a precise and reliable relationship between the log of the DNA concentration and the Ct value across the tested range, including the LOD."

DISCUSSION

There are two important steps in the process of detecting the presence of genetically modified organisms (GMOs) in food products: the DNA should be extracted and purified, and the inserted genetic material should be amplified with the help of the polymerase chain reaction (PCR). The fact that food preparation can destroy DNA and break it down is irrelevant because even little pieces of DNA can be sufficient to conduct an accurate detection [17-19]. This necessitates the use of strong molecular tests to ensure consumer safety and to enforce the halal dietary laws, since this has been demonstrated by the increasing consumption of GMOs in food production. Other molecular techniques, such as quantitative PCR (qPCR), have proven to be a sensitive and accurate method of GM content detection and measurement. This study was able to identify the Agrobacterium tumefaciens nopaline synthase terminator (T-NOS) sequence in real-time qPCR with a concentration as low as 0.001% which illustrates the reliability of the method used in processed and unprocessed foods. The NOS terminator is a typical aspect of most transgenic plants, and an ideal target to be regularly screened by GMOs [20, 21]. Increased LODs occur typically in thermally processed foods due to DNA destruction, and similar studies done in other international countries have identified LOD of 0.001 -0.005 per cent in maize and soy-based matrices [22]. The fact that our results agree with those of past publications proves that, despite the differences in the food matrix structure, the achieved analytical sensitivity of our results is competitive on an international level. The low detection threshold that was achieved in this work and supports the quality of increased analytical sensitivity is facilitated by the use of Certified Reference Material (CRM) as a calibration reference. Statistical analysis showed that CRM and spiked samples were not different (p>0.05), which proves the accuracy and repeatability of the assay. Two out of five mixes of the samples (SM4 and SM5) failed to amplify at 0.001%, and this is likely to be caused by reduced DNA extraction efficiency or the effects of the matrix. Substances such as insecticides or polysaccharides, or residual lipids, may have inhibited the PCR amplification process. To minimize false negatives and enhance the test strength, these putative inhibitors stand to bring to notice the importance of the best extraction methods, purification steps, and internal amplification controls in future studies. The proper molecular detection is particularly important when it concerns the checking of halal food. Identification of GMOs is relevant to Muslim buyers because it ensures that they follow the Islamic regulation of diet, besides food safety. The clear labeling is achievable through the reliable screening techniques, such as qPCR, that enhance the confidence of consumers in food certification systems [23]. One of the most important requirements is to attain reproducible amplification results, which hinges on the quality of DNA. The A260/280 values in the 1.8-2.0 range corresponded to a decent DNA quality; however, the yield in this experiment could have been affected by the slight contamination or pesticides left in cabbage seeds. A260/280 ratios (1.8 to 2.0) and evident and intact agarose gel bands verified the purity of the DNA used to guarantee the integrity of the samples to be analyzed by trusted qPCR. Despite these challenges, the strength of the assay in various food matrices is evidenced by the fact that it was able to identify the assay in extremely low concentrations. Interrogation of assays with several additional transgenic components, including the CaMV 35S promoter or event-specific sequences, would add greater specificity and reduce the chances of false negatives, although this study focused on a single detection target (T-NOS) [24]. The sensitivity limits of different types of foods might be further optimized in the future with respect to other validation studies by comparing with either multiplex or digital PCR procedures and incorporating matrix-

DOI: https://doi.org/10.54393/fbt.v5i2.198

specific controls. GMOs are also being updated because newer versions might not have the classic marker genes, so the molecular testing methods should also be updated in accordance with the emergence of advanced gene-editing technologies, such as CRISPR-Cas9 [25]. The present research provides a reliable and reproducible paradigm to detect the presence of very low GMO contamination, even though the methodology has certain limitations, including the small size of the sample and the convenience-based sampling method. However, it must be said that the work contributes to another growing body of evidence that qPCR remains a reliable technique of GMO screening when used in culturally sensitive food systems such as halal certification. It can be seen that future research involving a greater sample size and detection targets will increase the accuracy and usefulness of qPCR in halal food safety monitoring and authentication. Since the samples were picked from conveniently available commercial sources, it is important to note that the sampling strategy applied was convenience-based. Thus, although the results on the sensitivity of detection were strong with the tested matrices, they cannot be directly translated to reflect the contamination levels or the effects of the matrices in the whole food market. Yet, this is in line with the main objective of this paper, which was to validate the method of analysis and not to conduct a wholesale survey of the market. It was aimed at showing the accuracy, sensitivity, and reproducibility of the method under controlled conditions of spiking.

A limitation of the current study is the use of a limited number of food matrices and controlled spiking with GMO-positive cabbage seeds, which may not fully represent real-world contamination scenarios. Future research could expand the range of processed and raw foods tested and include naturally occurring GM samples to further validate the method. Additionally, integrating multiplex qPCR or digital PCR could enhance detection sensitivity and allow simultaneous screening for multiple GMO events, supporting more comprehensive halal food authentication.

CONCLUSION

Considering food fraud and adulteration, the problem of consuming contaminated and dangerous food items was considered in the current study. It provides a reliable qPCR-based method of GM contamination quantification by placing GM-positive cabbage seeds in a range of food matrices. Nevertheless, it may be possible to measure even below the given limit by increasing the amount of DNA.

Authors' Contribution

Conceptualization: SAN, SA
Methodology: SAN, AA, IAK
Formal analysis: SAN
Writing and Drafting: NF, DM
Review and Editing: NF, DM, SAN, AA, IAK, SA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Akbar A, Shakeel M, Al-Amad S, Akbar A, Ali AK, Rahmeh R *et al.* A Simple and Sensitive NGS-Based Method for Pork Detection in Complex Food Samples. Arabian Journal of Chemistry. 2021 May; 14(5): 103124. doi: 10.1016/j.arabjc.2021.103124.

[2] Zhang C, Wohlhueter R, Zhang H. Genetically Modified Foods: A Critical Review of Their Promise and Problems. Food Science and Human Wellness. 2016 Sep; 5(3): 116-23. doi: 10.1016/j.fshw.2016.04.002.

[3] Moon GS and Shin WS. Establishment of Quantitative Analysis Method for Genetically Modified Maize Using a Reference Plasmid and Novel Primers. Preventive Nutrition and Food Science. 2012 Dec; 17(4): 274. doi: 10.3746/pnf.2012.17.4.274.

[4] Isa NM, Man S. "First Things First": Application of Islamic Principles of Priority in the Ethical Assessment of Genetically Modified Foods. Journal of Agricultural and Environmental Ethics. 2014 Oct; 27(5): 857-70. doi: 10.1007/s10806-014-9504-0.

[5] Latif MA. Halal International Standards and Certification. The Halal Food Handbook. 2020 Jan: 205-26. doi: 10.1002/9781118823026.ch14.

[6] Gerszberg A. Tissue Culture and Genetic Transformation of Cabbage (Brassica Oleracea Var. Capitata): An Overview. Planta. 2018 Nov; 248(5): 1037-48. doi: 10.1007/s00425-018-2961-3.

[7] Zayed A, Sheashea M, Kassem IA, Farag MA. Red and White Cabbages: An Updated Comparative Review of Bioactives, Extraction Methods, Processing Practices, and Health Benefits. Critical Reviews in Food Science and Nutrition. 2023 Sep; 63(24): 7025-42. doi: 10.1080/10408398.2022.2040416.

[8] Hanif M, Abbas W, Iqbal MA, Khawaja MS, Waheed M, Sarwar S *et al.* Status of GMOs in Pakistan: Need, Acceptability, Development and Regulation Status: A Comprehensive Review. Pak-Euro Journal of Medical

DOI: https://doi.org/10.54393/fbt.v5i2.198

and Life Sciences. 2023 Sep; 6(3): 313-26.

[9] Li Y, Xiao F, Liu B, Gao H, Wu Y, Li J *et al.* Rapid Screening of Genetically Modified Ingredients in Soybean and Cotton Processing by-Product and Waste Using Direct qPCR. Oil Crop Science. 2020 Jul; 5(3): 142-8. doi: .1016/j.ocsci.2020.07.006.

[10] Tortajada-Genaro LA, Maquieira A. Multiple Recombinase Polymerase Amplification and Low-Cost Array Technology for the Screening of Genetically Modified Organisms. Journal of Food Composition and Analysis. 2021 Oct; 103: 104083. doi: 10.1016/j.jfca.2021.104083.

[11] Hou Y, Chen S, Zheng Y, Zheng X, Lin JM. Droplet-Based Digital PCR (ddPCR) and Its Applications. TrAC Trends in Analytical Chemistry. 2023 Jan; 158: 116897. doi: 10.1016/j.trac.2022.116897.

[12] Košir AB, Demšar T, Štebih D, Žel J, Milavec M. Digital PCR as an Effective Tool for GMO Quantification in Complex Matrices. Food Chemistry. 2019 Oct; 294: 73-8. doi: 10.1016/j.foodchem.2019.05.029.

[13] Thirumalraj B, Rajkumar C, Chen SM, Veerakumar P, Perumal P, Liu SB. Carbon Aerogel Supported Palladium-Ruthenium Nanoparticles aor Electrochemical Sensing and Catalytic Reduction of Food Dye. Sensors and Actuators B: Chemical. 2018 Mar; 257: 48-59. doi: 10.1016/j.snb.2017.10.112.

[14] Park YH, Min DM, Kim YM, Kang SH. Quantitative Analysis of Two Genetically Modified Maize Lines by Real-Time PCR. Journal of Microbiology and Biotechnology. 2006 Jan; 16(2): 205-11.

[15] Lee D, La Mura M, Allnutt TR, Powell W. Detection of Genetically Modified Organisms (Gmos) Using Isothermal Amplification of Target DNA Sequences. BioMed Central Biotechnology. 2009 Feb; 9(1): 7. doi: 10.1186/1472-6750-9-7.

[16] Mandaci M, Çakir Ö, Turgut-Kara N, Meriç S, Ari Ş. Detection of Genetically Modified Organisms in Soy Products Sold in Turkish Market. Food Science and Technology. 2014; 34: 717-22. doi: 10.1590/1678-457X .6441.

[17] Harshitha R and Arunraj DR. Real Time Quantitative PCR: A Tool for Absolute and Relative Quantification. Biochemistry and Molecular Biology Education. 2021 Sep; 49(5): 800-12. doi: 10.1002/bmb.21552.

[18] Blakely GW. Mechanisms of Horizontal Gene Transfer and DNA Recombination. In Molecular Medical Microbiology. 2024 Jan: 309-324. doi: 10.1016/B978-0-12-818619-0.00043-5.

[19] Singh M, Pal D, Aminedi R, Singh AK. Multiplex Real-Time Loop-Mediated Isothermal Amplification (LAMP) Based on the Annealing Curve Analysis: Toward an On-Site Multiplex Detection of Transgenic Sequences in Seeds and Food Products. Journal of Agricultural and Food Chemistry. 2024 Jul; 72(31): 17658-65. doi: 10.1021/acs.jafc.4c01803.

[20] Hardegger M, Brodmann P, Herrmann A. Quantitative Detection of the 35S Promoter and the NOS Terminator Using Quantitative Competitive PCR. European Food Research and Technology. 1999 Jul; 209(2): 83-7. doi: 10.1007/s002170050462.

[21] Du Y, Chen F, Bu G, Zhang L. Distribution and Degradation of DNA from Non-Genetically and Genetically Modified Soybean (Roundup Ready): Impact of Soybean Protein Concentrate and Soybean Protein Isolate Preparation. Food Chemistry. 2021 Jan; 335: 127582. doi: 10.1016/j.foodchem.2020.127582.

[22] Chen X, Lu L, Xiong X, Xiong X, Liu Y. Development of A Real-Time PCR Assay for the Identification and Quantification of Bovine Ingredient in Processed Meat Products. Scientific Reports. 2020 Feb; 10(1): 2052. doi: 10.1038/s41598-020-59010-6.

[23] Ambali AR and Bakar AN. People's Awareness on Halal Foods and Products: Potential Issues for Policy-Makers. Procedia-Social and Behavioral Sciences. 2014 Mar; 121: 3-25. doi: 10.1016/j.sbspro.2014.01.1104.

[24] Li J, Gao H, Li Y, Zhai S, Xiao F, Wu G *et al.* The Development of a Series of Genomic DNA Reference Materials with Specific Copy Number Ratios for The Detection of Genetically Modified Maize DBN9936. Foods. 2024 Feb; 13(5): 747. doi: 10.3390/foods13050747.

[25] Zheng Y, Li Q, Ye M, Cehn A, Wang H. Applications of CRISPR/Cas9-Based Genome Editing in the Plant Biology. Turkish Journal of Botany. 2021; 45(4): 253-68. doi: 10.3906/bot-2103-50.

DOI: https://doi.org/10.54393/fbt.v5i2.174

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Original Article

OPEN ACCESS

Mathematical Modeling of DNA Yield Using Box-Behnken Design for Key Extraction Parameters

Saeeda Zia[1], Farwa Afzal[1], and Rashid Saif[2*]

[1]Department of Sciences and Humanities, National University of Computer and Emerging Sciences, Lahore, Pakistan
[2]Department of Biotechnology, Qarshi University, Lahore, Pakistan

ARTICLE INFO

Keywords:
DNA Yield Modeling, Response Surface Design, Mathematical Biology, Biomathematical Modeling

How to Cite:
Saif, R., Zia, S., & Afzal, F. (2025). Mathematical Modeling of DNA Yield Using Box-Behnken Design for Key Extraction Parameters: Box-Behnken Design for Optimizing DNA Extraction Yield. Futuristic Biotechnology, 5(2), 59-64. https://doi.org/10.54393/fbt.v5i2.174

***Corresponding Author:**
Rashid Saif
Department of Biotechnology, Qarshi University, Lahore, Pakistan
rashid.saif37@gmail.com

Received Date: 3rd April, 2025
Revised Date: 2nd June, 2025
Acceptance Date: 9th June, 2025
Published Date: 30th June, 2025

ABSTRACT

The current research was initiated to model an enzyme-assisted inorganic salt-based DNA extraction protocol to obtain maximum yield from peripheral whole blood samples. **Objectives:** DNA Key extraction parameters were considered for model optimization such as incubation temperature (54,56,58°C), time (8,10,12hrs.), and proteinase K enzyme concentrations (8,10,12µL). **Methods:** Box-Behnken Design (BBD) was employed to model and simulate the extraction process with three factors/variables and three levels each, which is one of the widely used functional approach of response surface methodology (RSM). With this selected design, RSM entails a mathematical model with experimental data to optimize DNA yield. **Results:** The best fit simulation settings yielded the maximum DNA at an incubation temperature of 56°C for 10hours with a proteinase K enzyme volume of 10µL. Utilizing these parameters, a yield of 300ng/µL was obtained. A confirmation run under these settings validated the prediction, with observed yields closely matching the model's estimates ($p<0.050$), indicating strong agreement between the model and experimental outcomes. **Conclusions:** Regression modeling identified the role of three key factors for optimal DNA yield. Additional extraction parameters can be integrated to develop a more robust and controlled model, ensuring efficient use of limited crime scene samples and reagents in forensic investigations.

INTRODUCTION

The DNA isolation of the specimens is a very important process in modern biomedical studies and diagnosis, and has wide-ranging applications that include genetic studies, as well as in identifying diseases. With the growing need to achieve efficiency and reliability in extraction methodologies, researchers are considering new methods of extraction using materials like magnetic nanoparticles and silica matrices as an overcoming of limitations inherent in traditional methods. This paper is concerned with the mathematical modeling of the response surface modeling (RSM) of Box-Behnken Design (BBD) of the entire whole-blood by inorganic salt-based DNA extraction, which is a basic traditional technique, to comprehend and optimize the parameters that can in turn enhance its economic viability in terms of time, reagents and cost saving [1-3]. DNA extraction procedures often use organic solvents, including phenol-chloroform-isoamyl alcohol (PCI), which is renowned due to its efficiency in getting high yields of DNA, but is very expensive. On the contrary, inorganic salt-based techniques make use of cheaper substances such as sodium chloride (NaCl). The optimization of extraction methods based on inorganic is, however, still a research topic. Regression, ANOVA, and the R2 values are mathematical models that are critical in explaining the complex interplay between inorganic compounds and biological elements and offer a logical structure of forecasting and controlling the results. This can be a groundbreaking strategy of DNA extraction,

DOI: https://doi.org/10.54393/fbt.v5i2.174

especially in low-resource environments, to facilitate the study of genetic medicine, forensics, and molecular diagnostics research in the third world developing nations [2, 4]. The use of inorganic DNA extraction techniques has a wide range of reagents and protocol parameters, such as incubation temperature, time period, and the most important enzyme concentration or proteinase K, which is the most important in increasing the yield of DNA in whole blood samples. Other statistical methods, like RSM, are used in this study to perform the optimization of DNA extraction parameters rigorously. The key goal is to identify conditions that will maximize the yield of DNA with the use of BBD surface design. This research plays an important role in the establishment of the optimum values of the minimum required incubation temperature, duration of time, and concentration of proteinase K enzyme to maximize the yield of DNA before experimentation [5, 6].

Despite the widespread use of DNA extraction methods in biomedical, forensic, and genetic research, traditional techniques often face challenges such as high cost, time consumption, and variability in yield. While enzyme-assisted inorganic salt-based extraction provides a low-cost alternative, the optimal combination of key parameters—incubation temperature, time, and proteinase K concentration—remains underexplored. Existing studies lack a systematic mathematical modeling approach to predict DNA yield under varying conditions, creating a gap in cost-effective, reproducible, and high-yield extraction strategies. This study aims to develop an integrated model that synthesizes principles from chemistry, biology, statistics, and mathematics to optimize key parameters.

METHODS

This study used Box-Behnken Design (BBD) to model and simulate the extraction process with three factors and three levels each. The study was conducted from August 2023 to July 2024 at Decode Genomics, Lahore, Pakistan and Ethical approval was obtained from the institutional review board (Ref. No. DG-adm-127). For our experiment, DNA was extracted from 200 µL of whole blood using a salt-based inorganic method. After lysis with TE buffer, SDS, and Proteinase K, 6M NaCl was added to pellet protein debris, incubating further on ice as well as centrifuging. DNA was precipitated in the supernatant using chilled isopropanol, which was monitored via gentle inversion. DNA pellets were washed sequentially three times with ethanol before a final harvest to remove impurities. Pure DNA was assayed using gel electrophoresis for intactness, as well as its concentration was determined [7, 8]. The following process variables and their ranges were used regarding the key DNA extraction reagents and chemicals (Table 1).

Table 1: Process variable labels and levels

Labels	Variables (units)	Levels		
		-1	0	1
A	Incubation Temperature (°C)	54	56	58
B	Incubation Time (Hour)	8	10	12
C	Proteinase-K Enzyme (µL)	8	10	12

These variables include levels, variables (incubation temperature (°C), incubation time (hours), and proteinase K enzyme (µL). Each variable is categorized into three levels: -1, 0, and 1. For temperature, the range spans from 54°C, 56°C, 58°C. Time varies from 8, 10, and 12 hours, while proteinase K enzyme volume ranges from 8, 10 and 12 µL. This table serves as a reference for understanding the experimental conditions and their corresponding parameter ranges, crucial for ensuring consistency and repeatability in the experiment's outcomes. BBD was used with Design Expert Software to design a DNA extraction optimization protocol. The software-designed experimental conditions that were simulated to run practical extraction trials as well as to simulate response surface to get a regression line (Table 2).

Table 2: Box-Behnken Experimental Design

Sr. No.	A	B	C	Yield
1	56.00	10.00	10.00	350
2	56.00	8.00	8.00	90
3	56.00	10.00	10.00	350
4	54.00	10.00	8.00	110
5	56.00	10.00	10.00	350
6	58.00	10.00	12.00	310
7	56.00	8.00	12.00	270
8	56.00	10.00	10.00	350
9	56.00	10.00	10.00	350
10	54.00	10.00	12.0	100
11	58.00	10.00	8.00	180
12	58.00	12.00	10.00	300
13	56.00	12.00	8.00	150
14	54.00	12.00	10.00	220
15	54.00	8.00	10.00	200
16	56.00	12.00	12.00	400
17	58.00	8.00	10.00	180

Through systematic exploration of these combinations, we conducted 17 experiments aimed at DNA yield from whole-blood samples followed by DNA quantification. Response Surface Methodology (RSM) is a statistical technique used in modeling and multi-variable process optimization. In our research work, we applied the RSM to optimize the DNA extraction protocol, considering the interactive effects of DNA concentration, incubation time, and incubation temperature on the yield. The technique allows the establishment of predictive models to identify the optimum conditions for efficient and reproducible DNA recovery [9, 10]. The BBD embedded into the RSM was

DOI: https://doi.org/10.54393/fbt.v5i2.174

applied to optimize DNA extraction conditions by the exploration of the linear and the quadratic effect of three variables. Three levels per parameter (–1, 0, and +1) decrease the number of run experiments economically in comparison with full-factorial experiments. In combination with the RSM, it enabled the building of a predictive model for the identification of the condition providing the optimum DNA recovery [11]. The second-order polynomial model used in response surface methodology (RSM) can be expressed as:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i=0}^{k} \sum_{j=2}^{k} \beta_{ij} X_i X_j + e_j \ (1).$$

After putting all the values in equation (1) and simplifying we get:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + 2\beta_{22} X_2^2 + 2\beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + 2\beta_{23} X_2 X_3 + e (2)$$

Now, by substituting the actual experimental factors: X_1=A (Incubation Temperature), X_2 =B (Incubation Time), X_3 =C (Proteinase K Volume) The model becomes:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + 2\beta_{22} B^2 + 2\beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + 2\beta_{23} BC + e \ (3)$$

Where Y is the predicted DNA yield $\left(\frac{ng}{\mu L}\right)$, β_0 is the intercept, and β_j , β_{jj} , β_{ij} are the linear, quadratic, and interaction coefficients, respectively and ε is the residual error term. A second order polynomial regression model was applied to correlate the response variable (DNA yield) and the independent variables: proteinase K enzyme concentration, time of incubation, and incubation temperature. The model applied in the Response Surface Methodology (RSM) was as follows: proteinase K enzyme concentration, time of incubation, and incubation temperature. The model applied in the Response Surface Methodology (RSM) was as follows: $Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{jj} X_i^2 + \sum \beta_{ij} X_i X_j$ Analysis of variance (ANOVA) was performed to test the significance and adequacy of the model. The model performance was tested based on the values of R^2, adjusted R^2, and predicted R^2 and also from lack-of-fit tests. All statistical analysis and model fitting were performed by Design Expert software (Stat-Ease Inc., USA). To authenticate the validity and predictive capacity of the constructed RSM model, a further experimental run was performed according to the optimal settings indicated by the model. The optimal levels of the concentration of the enzyme proteinase K, the time of incubation, and the temperature of incubation used for this purpose were derived from the numerical optimization function of the Design Expert software. The DNA extraction was performed according to the above-mentioned optimized parameters and the extracted DNA yield was validated against the corresponding value anticipated from the model to check the model's accuracy [12]. Model selection applied sequential sum of squares and model summary statistics. The quadratic model was the preferred model of fit because its p-value was significant, its R^2 and it's adjusted R^2 The values were reasonable, and it was able to capture linear and interaction effects between the variables that influenced DNA yield.

RESULTS

This equation represents the response function used for the estimation of DNA extraction yield from whole blood. The equation contains various independent variables, i.e., incubation temperature (A), incubation time (B), and Proteinase K Enzyme Concentration (C), with corresponding coefficients estimated from the experimental data. The equation follows the following form: $Y= 350.00 + 32.50A + 51.25B + 68.75C + 45.00AB +35.00AC +17.50BC - 98.75A^2 - 46.25B^2 - 76.25C^2$. Here, (Y) represents the DNA extraction yield in a ng/uL. The independent variables and the interaction effects between the variables show the influence of the variables on the extraction. The linear and nonlinear relationships between the variables and the extraction yield represented by the quadratic terms (such as A^2, B^2, and C^2) give insight about the potential nonlinear relationships. The above equation serves as the predictive model to predict DNA extraction yield from the specified experimental condition. The adequacy of the Box-Behnken model constructed was statistically validated, showing a very good fit with a high R^2 value of 0.975, an adjusted R^2 of 0.958, and a predicted R^2 of 0.922, while the lack-of-fit test value of 0.231 indicated an insignificant lack-of-fit, confirming that the model can be applied for the estimation of DNA yield under different extraction conditions. The relationship between incubation temperature, incubation time, and Proteinase K enzyme concentration and DNA yield was captured by the model, with linear, quadratic, and interaction terms indicating that the effects were not necessarily linear and allowing for the determination of optimal extraction conditions. Regression analysis showed that incubation temperature had a significant effect on DNA yield, with the response surface being concave and the optimal yield achieved at approximately 56°C; deviations to 54°C or 58°C resulted in decreased yield, reflecting the heat sensitivity of the enzymatic lysis reaction. Incubation time also significantly affected DNA yield, with a parabolic response surface and optimal yield at 10 hours, while shorter or longer durations reduced efficacy. Proteinase K volume exerted a significant influence, with the highest yield at 10 µL and lower or higher volumes leading to reduced yield due to incomplete lysis or over-digestion. Sequential sum of squares analysis and model summary statistics indicated that the quadratic model was the most appropriate for predicting DNA yield, as it was statistically significant, captured the relationships between variables effectively, and demonstrated a suitable fit with R^2=0.8751 and adjusted R^2=0.7146, while linear, 2FI, and cubic models were less suitable due to lack of explanatory power, aliasing, or inconsistencies between predicted and observed responses (Table 3).

DOI: https://doi.org/10.54393/fbt.v5i2.174

Table 3: Sequential Model Sum of Squares

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F	Remarks
Sequential Model-Sum of Squares for Total Yield						
Mean	$1.028 x10^{006}$	1.00	$1.028 x10^{006}$	–	–	–
Linear	67275.00	3.00	22425.00	2.43	0.1118	Suggested
2F1	14225.00	3.00	4741.67	0.45	0.7238	–
Quadratic	82336.76	3.00	27445.59	8.22	0.0108	Suggested
Cubic	23375.00	3.00	7791.67	6.366 x10007	<0.0001	Aliased
Residual	0.000	4.00	0.000	–	–	–
Total	$1.21 x10^{006}$	17.00	71470.59	–	–	–
Model Summary Statistic						
Source	Std. Deviation	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Linear	96.05	0.3594	0.2115	-0.0618	$1.988x10^{005}$	–
2F1	102.82	0.4353	0.0965	-0.7405	$3.258x10^{005}$	–
Quadratic	57.79	0.8751	0.7146	-0.9977	$3.740x10^{005}$	Suggested
Cubic	0.000	1.0000	1.00000	–	–	Aliased

ANOVA outcomes indicated the statistically significant fitted model ($p = 0.0180$), so the variation in DNA yield could be well explained using the selected factors. Incubation time (B) and Proteinase K volume (C) indicated significant effects ($p=0.0405$ and $p=0.0120$), respectively, while temperature (A) indicated a moderate effect ($p=0.1557$). Of particular interest was the statistical significance of the second-order terms A^2 and C^2, indicating non-linear behavior of the parameters. The interaction terms (AB, AC, BC) indicated statistical non-significance. The model indicated a large coefficient of determination ($R^2=0.8751$), showing significant agreement between the predictive and the observed responses. An adequate precision (AP=5.895) confirmed the signal-to-noise ratio of the model being satisfactory for the exploration of the design space (Table 4).

Table 4: ANOVA for Response

Source	Sum of Squares	DF	Mean Square	F-Value	p-Value
Model	$1.63x10^{005}$	9	18204.08	5.45	0.0180
A-Temperature	8450.00	1	8450.00	2.53	0.1557
B-Time	21012.50	1	21012.50	6.29	0.0405
C-Proteinase k	37812.50	1	37812.50	11.32	0.0120
AB	8100.00	1	8100.00	2.43	0.1633
AC	4900.00	1	4900.00	1.47	0.2651
BC	1225.00	1	1225.00	0.37	0.5638
A^2	41059.21	1	41059.21	12.30	0.0099
B^2	9006.58	1	9006.58	2.70	0.1445
C^2	24480.26	1	24480.26	7.33	0.0303
CV	23.50				
AP	5.895				
PRESS	3.740+005				
Mean	245.88				
R^2	0.8751				
Adj-R^2	0.7146				

The next graphs provide the combined diagnostic plots employed to check for the fitness of the fitted regression model. The normal probability plot of studentized residuals indicates that the residuals are normally distributed since they lie along a straight line. The predicted vs. actual plot shows a good correlation between observed and model-predicted values, with data points tightly scattered around the diagonal line, indicating the accuracy of the model. The residuals vs. predicted plot shows a random distribution of residuals, indicating homoscedasticity and the lack of systematic error. Both plots confirm that the regression model is statistically valid and accurate for prediction. (A) Normal probability plot. Blue circles closely follow the reference line, indicating normally distributed residuals. (B) Predicted vs. actual values. Green triangles lie near the diagonal, showing strong agreement between observed and predicted DNA yield. (C) Residuals vs. predicted values – Red squares are randomly scattered around zero, confirming homoscedasticity and model validity. Axes represent expected vs. actual residuals (A), actual vs. predicted yield (B), and predicted yield vs. residuals (C) (Figure 1).

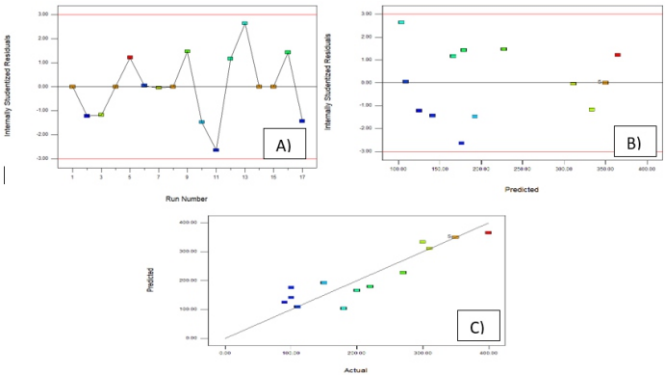

Figure 1: (A) Normal Probability Plot. (B) Predicted Vs. Actual Values. (C) Residuals Vs. Predicted. Axes Represent Expected Vs. Actual Residuals (A), Actual Vs. Predicted Yield (B), and Predicted Yield Vs. Residuals (C)

DOI: https://doi.org/10.54393/fbt.v5i2.174

A three-dimensional scatter plot was created to demonstrate the interaction effects of incubation temperature, incubation time, and Proteinase K enzyme concentration on DNA yield using the Box-Behnken experimental design. The plot visually shows a nonlinear response surface with the highest DNA yield (~350–400 ng/µL) at points closest to the center values of 56°C, 10 hours, and 10 µL, validating the regression model's prediction. Deviation from these optimal conditions resulted in a drop in yield, validating the occurrence of quadratic effects and that there is a very small range within which optimal extraction takes place. The visualization serves to reinforce that incubation time and enzyme concentration, specifically, need to be precisely balanced to preclude under- or over-digestion, both of which have a detrimental impact on yield. The even gradient of DNA concentration through the plotted surface gives a clear, intuitive view of the sensitivity of the extraction protocol to changes in each parameter. Generally, the plot reinforces the statistical results and verifies the robustness of the optimized conditions that were identified by the model (Figure 2).

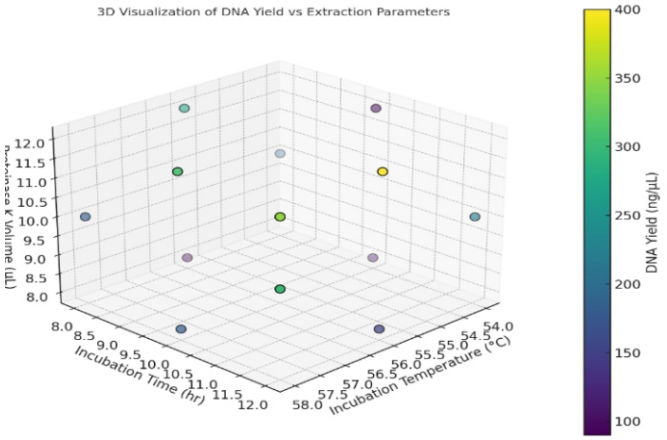

Figure 2: Three-Dimensional Scatter Plot

DISCUSSION

The optimization of the DNA extraction process by Box-Behnken design (BBD) showed that incubation temperature, incubation time, and Proteinase K enzyme concentration had a great nonlinear influence on the generation of the DNA, as had been previously reported [13]. The high-predictive quadratic regression model (R2=0.8751) illustrated that even minor alterations to the optimal conditions (56°C, 10 hours, 10 µL) had a major impact on the DNA recovery, which shows the sensitivity of enzymatic activity [14, 15]. The results are in line with the earlier optimization experiments with the use of factorial design and response surface methodology, which found the same quadratic behavior in the course of enzymatic extraction [16-18]. The concentration of proteinase K is critically important in determining extraction yield, due to the existence of two opposing conditions between total lysis and possible over-digestion [14, 16]. Comprehensively, the paper highlights the usefulness of response surface methodology (RSM) and Box-Behnken design in the effective determination of optimal conditions using a limited number of experimental programs to increase reproducibility and cost-efficiency [18, 19]. The improved salt-based extraction process was found to be appropriate in extracting high-quality DNA using a small amount of whole blood, and it is possible that it can be used in clinical diagnostics, forensic cases, and genetic studies [7, 20]. Even though the model system was chicken blood, the principles realized in this case can be applied in other species after validation. The model can be further improved in future research by adding some parameters to it, like buffer composition, storage conditions, and sample type.

Although the current model effectively identifies optimal extraction conditions for whole blood, it is limited to specific sample types and does not consider additional variables such as buffer composition, storage conditions, or variations across species. Future research could integrate these factors into an expanded model, enabling broader applicability, improved reproducibility, and the development of standardized protocols for clinical, forensic, and molecular biology applications.

CONCLUSION

An enzyme-based DNA extraction of whole blood was optimized by box-Behnken design and response surface methodology. The quadratic equation (R 2=0.8751) established optimal conditions (56 0 C, 10 hours, 10 0 -1) to give maximum yield. Reliability was also experimentally validated, and provides a low-cost, fast, and repeatable alternative to commercial kits with low sample volumes.

Authors' Contribution

Conceptualization: SZ, RS
Methodology: SZ, FA, RS
Formal analysis: SZ, FA, RS
Writing and Drafting: SZ, FA, RS
Review and Editing: SZ, FA, RS

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

DOI: https://doi.org/10.54393/fbt.v5i2.174

REFERENCES

[1] Dwivedi S, Purohit P, Misra R, Pareek P, Goel A, Khattri S, *et al.* Diseases and Molecular Diagnostics: A Step Closer to Precision Medicine. Indian Journal of Clinical Biochemistry. 2017 Oct; 32(4): 374–398. doi: 10.1007/s12291-017-0688-8.

[2] Neal CJ, Zbinden ZD, Douglas ME, Douglas MR. Reducing DNA Extraction Costs Through Factorial Design for the DNAdvance Kit. BMC Research Notes. 2024 Dec; 17(1): 397. doi: 10.1186/s13104-024-07063-5.

[3] Zhang S, Wang Y, Liu T, Li X, Wang M, Qiu Y. Optimization of the Extraction Process of Effective Components of Eleutherococcus senticosus Using Mathematical Models. Journal of Food Quality. 2024; 2024: 5538373. doi: 10.1155/2024/5538373.

[4] Crooke PS and Parl FF. A Mathematical Model for DNA Damage and Repair. Journal of Nucleic Acids. 2010; 2010: 352603. doi: 10.4061/2010/352603.

[5] Silva E, Rogez H, Larondelle Y. Optimization of Extraction of Phenolics from Inga edulis Leaves Using Response Surface Methodology. Separation and Purification Technology. 2007 Mar; 55(3): 381–387. doi: 10.1016/j.seppur.2007.01.008.

[6] Gulas-Wroblewski BE, Kairis RB, Gorchakov R, Wheless A, Murray KO, *et al.* Optimization of DNA Extraction from Field-Collected Mammalian Whole Blood on Filter Paper for Trypanosoma cruzi (Chagas Disease) Detection. Pathogens. 2021 Aug; 10(8): 1040. doi: 10.3390/pathogens10081040.

[7] Tang ZF and McMillen DR. An Inhibitor-Free, Versatile, Fast, and Cheap Precipitation-Based DNA Purification Method. PLOS ONE. 2025 Apr; 20(4): e0317658. doi: 10.1371/journal.pone.0317658.

[8] Lynton-Jenkins JG, Chaine AS, Russell AF, Bonneaud C. Parasite Detection and Quantification in Avian Blood Is Dependent on Storage Medium and Duration. Ecology and Evolution. 2023 Feb; 13(2): e9819. doi: 10.1002/ece3.9819.

[9] Khuri AI and Mukhopadhyay S. Response Surface Methodology. WIREs Computational Statistics. 2010 Mar; 2(2): 128–149. doi: 10.1002/wics.73.

[10] Djimtoingar SS, Derkyi NS, Kuranchie FA, Yankyera JK, *et al.* A Review of Response Surface Methodology for Biogas Process Optimization. Cogent Engineering. 2022 Dec; 9(1): 2115283. doi: 10.1080/23311916.2022.2115283.

[11] Gerzsenyi TB, Ilosvai ÁM, Kristály F, Daróczi L, Owen MC, Viskolcz B, *et al.* Investigation and Optimization of DNA Isolation Efficiency Using Ferrite-Based Magnetic Nanoparticles. Biotechnology Reports. 2025 Mar; 37: e00904. doi: 10.1016/j.btre.2025.e00904.

[12] Hussain MA, Ali A, Alsahli TG, Khan N, Sharif A, Haseeb MT, *et al.* Polysaccharide-Based Hydrogel from Seeds of Artemisia vulgaris: Extraction Optimization by Box–Behnken Design, pH-Responsiveness, and Sustained Drug Release. Gels. 2023 Jul; 9(7): 525. doi: 10.3390/gels9070525.

[13] Tan R, Zhou S, Sun M, Liu Y, Ni X, He J, Guo G, Liu K, *et al.* Modeling and Optimization of Culture Media for Recombinant Helicobacter pylori Vaccine Antigen HpaA. Frontiers in Bioengineering and Biotechnology. 2024 Dec; 12: 1499940. doi: 10.3389/fbioe.2024.1499940.

[14] Martínez-de la Puente J, Ruiz S, Soriguer R, Figuerola J, *et al.* Effect of Blood Meal Digestion and DNA Extraction Protocol on the Success of Blood Meal Source Determination in the Malaria Vector Anopheles atroparvus. Malaria Journal. 2013 Dec; 12(1): 109. doi: 10.1186/1475-2875-12-109.

[15] Afzal F, Zia S, Saif R. Mathematical Modeling Using Box–Behnken Response Surface Design for Optimal DNA Yield with Variable Parameters of Time, Temperature, and Proteinase K Concentration. bioRxiv. 2024 Jul; 2024-07. doi: 10.1101/2024.07.21.604461.

[16] Shahriar M, Haque MR, Kabir S, Dewan I, Bhuyian MA, *et al.* Effect of Proteinase K on Genomic DNA Extraction from Gram-Positive Strains. Stamford Journal of Pharmaceutical Sciences. 2011; 4(1): 53–57. doi: 10.3329/sjps.v4i1.8867.

[17] Van Biesen N, Cools P, Meyers E. Comparison and Optimization of DNA Extraction Methods for Human DNA from Dried Blood Spot Samples. Pediatric Reports. 2025 Mar; 17(2): 30. doi: 10.3390/pediatric17020030.

[18] Goemaere I, Punj D, Harizaj A, Woolston J, Thys S, Sterck K, *et al.* Response Surface Methodology to Efficiently Optimize Intracellular Delivery by Photoporation. International Journal of Molecular Sciences. 2023 Feb; 24(4): 3147. doi: 10.3390/ijms24043147.

[19] Yang JS, Dias FF, De Moura Bell JM. Optimizing Alkaline and Enzymatic Extraction of Black Bean Proteins: A Comparative Study of Kinetics, Functionality, and Nutritional Properties. Sustainable Food Technology. 2025; 3(1): 188–203. doi: 10.1039/D4FB00163J.

[20] Verma A, Manchel A, Melunis J, Hengstler JG, Vadigepalli R. From Seeing to Simulating: A Survey of Imaging Techniques and Spatially Resolved Data for Developing Multiscale Computational Models of Liver Regeneration. Frontiers in Systems Biology. 2022; 2: 917191. doi: 10.3389/fsysb.2022.917191.

DOI: https://doi.org/10.54393/fbt.v5i2.204

FUTURISTIC BIOTECHNOLOGY
https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Vol 05 Issue 02, (April-June, 2025)

Original Article

OPEN ACCESS

Impact of Plastic Residues on Soil Properties and Crop Productivity: A Comprehensive Research Study

Fadia[1*], Khalid Javed[1], Muhammad Azeem[2], Ali Ijaz[3] and Syed Ghulam Mohayud Din Hashmi[4]

[1]Glass and Ceramics, Pakistan Council of Scientific and Industrial Research, Lahore, Pakistan
[2]Department of Biological Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Department of Chemistry, Government College University, Lahore, Pakistan
[4]Department of Wildlife and Fisheries, University of Veterinary and Animal Sciences, Lahore, Pakistan

ARTICLE INFO

Keywords:
Plastic Residues, Microplastics, Soil Health, Soil Properties, Crop Productivity, Sustainable Agriculture, Food Security

How to Cite:
Fadia, ., Javed, K., Azeem, M., Ijaz, A., & Hashmi, S. G. M. D. (2025). Impact of Plastic Residues on Soil Properties and Crop Productivity: A Comprehensive Research Study: Impact of Plastic Residues on Soil Properties and Crop Productivity. Futuristic Biotechnology, 5(2), 65-70. https://doi.org/10.54393/fbt.v5i2.204

***Corresponding Author:**
Fadia
Glass and Ceramics, Pakistan Council of Scientific and Industrial Research, Lahore, Pakistan
fadia.shaheen@hotmail.com

Received Date: 5th May, 2025
Revised Date: 21st June, 2025
Acceptance Date: 27th June, 2025
Published Date: 30th June, 2025

ABSTRACT

The sources that have led to the development of plastic residues, microplastics, and macroplastics are plastic mulching, sewage sludge, compost, irrigation with contaminated water, and atmospheric deposition. These residues modify the physical, chemical, and biological characteristics of soil, eventually influencing crop yield. **Objectives:** To determine the impact of plastic residues on soil health and crop productivity through the evaluation of soil properties, microbial activity, and plant growth reactions to different levels of contamination. **Methods:** Soil and plant samples were taken in agricultural fields classified using the intensity of contamination. Density separation was used to extract microplastic particles, and FTIR and SEM were used to characterize the particles. Physical (bulk density, porosity, water-holding capacity), chemical (pH, organic matter, nutrient availability), and biological (microbial biomass, enzymatic activities, earthworm bioassays) soil parameters were examined. The performance of crops was determined in terms of germination, biomass, nutrient uptake, and yield. ANOVA and regression were statistically applied to analyze data. **Results:** Plastic debris interfered with the soil structure, decreased nutrient cycling, microbial activity, and suppressed crop development, causing drastic losses in production. Although plastic mulching originally improved the moisture content of soil and the control of weeds, the accumulation of the persistent residues over time produced adverse effects on soil fertility. **Conclusions:** Plastic wastes are dangerous in the long run to the soil ecosystems and agricultural productivity. The plastic pollution of the soil by plastics and the threat to food security require some urgency in the form of sustainable alternatives, better recycling, and stringent waste management policies.

INTRODUCTION

Previously hailed as groundbreaking substances in the contemporary industry and everyday experience, plastics have turned into one of the most widespread environmental contaminating substances of the 21st century. The world is producing more than 390 million tons of plastic each year, and the number is growing [1]. Although marine pollution from plastics has been well researched, land ecosystems, particularly farm soils, have only recently been identified as a significant source to absorb plastic residues. Macroplastics (>5 mm) and microplastics (<5 mm) are accumulated in soils. The latter is more threatening because it is persistent and associated with soil biogeochemical processes [2, 3]. The sources of plastic residues in agricultural soils include plastic mulching, sewage sludge, compost, use of contaminated water in irrigation, and atmospheric deposition [4-6]. Despite its positive effect on soil moisture preservation and the enhancement of the temperature, plastic mulching leads to the eventual breakdown of polyethylene and polypropylene film to slowly decaying residues [7]. These residues build up in the profile of soil that influences the productivity and the quality of the soil in the long run.

DOI: https://doi.org/10.54393/fbt.v5i2.204

Plastic pollution changes the physical, chemical, as well as biological characteristics of soil. Physically, it alters bulk density, porosity, and water-holding capacity, which affects infiltration and aeration negatively [8]. Microplastics are chemical carriers of heavy metals and organic pollutants, and they interfere with nutrient availability and soil pH [9]. Microbial communities, enzymatic processes, and soil fauna, including earthworms, are interfered with biologically and therefore make soil less fertile and stable in the ecosystem [10-12]. These interferences have dire consequences on crop productivity. Microplastics were found to inhibit seed germination, root growth, and uptake of nutrients, and eventually yield was decreased [13-15]. Despite the initial plastic mulches boosting production, the persistence of the plastic compounds in the soil causes degradation and a reduction of agricultural productivity in the long run. Hence, it is necessary to measure the effects of plastic residues on the health of the soil and crop performance to provide sustainable agricultural systems and food security worldwide [16-18]. Plastic in the soil of farms also has an adverse influence on the properties of soil, decreases the yield of crops, and changes the biological activity of the soil. The more the contamination of soils, the more harmful the effect on the plant and the well-being of the soil.

Despite the extensive use of plastics in agriculture, the long-term consequences of plastic residues on soil health and crop productivity remain poorly quantified. While previous studies have focused on either the physical or chemical impacts of microplastics, integrated analyses including biological interactions and crop responses are limited. Moreover, most research has been conducted under controlled conditions, leaving a gap in understanding the effects under real field scenarios with varying contamination levels. This study aims to address these gaps by assessing soil physical, chemical, and biological properties alongside crop performance under naturally occurring plastic contamination. This study aimed to determine the impact of plastic residues on the health of soil and the productivity of crops through the analysis of soil properties, the functions of microorganisms, and the response of plants to different levels of contamination.

METHODS

The researchers conducted the analytical comparative cross-sectional field study in agricultural fields (Mangal Mandi, Khot Haleem Khan, Qatal Garhi) that had recorded the history of the use of plastics and irrigation techniques [5, 6]. The study duration was from September 2024 to April 2025. The categories of contamination were divided into low, medium, and high regarding the time and extent of plastic mulching, the determination of the frequency of sewage or wastewater irrigation, and the initial quantification of plasticly deposited remnants. On the plastic mulching time, frequency of irrigation, and the initial quantity of microplastic, low and medium, and high contamination were determined. The density separation of the 0 -15cm soil samples produced low (less than 1 year, little irrigation, less than 200 particles/kg), medium (1-3 years, occasional irrigation, 200-500 particles/kg), and high (greater than 3 years, frequent irrigation, greater than 500 particles/kg) contamination. The wheat (*Triticum aestivum L*) and maize (*Zea mays L*) were examined. The local farmers cultivated crops with normal agronomic practices and collected samples when they reached maturity to determine biomass, uptake of nutrients, and yield at various levels of contamination. Low contamination was classified as fields where little or no mulch was used, whereas high contamination was where there were visible residues and which had been mulched over time. The categories each had three replicate plots (10 x 10 m) that were at a randomized block design, thus expressing naturally occurring field conditions as opposed to treatment being imposed. At 0-15 cm and 15-30 cm, soil samples were taken using a stainless-steel auger, and crop samples at the maturity stage were investigated in terms of biomass, nutrient value, and yield. NaCl and ZnCl2 density separation was used to extract microplastics present in soil samples between 0 and 15 cm, as well as microparticles in soil. The samples were oxidized by 30% H2O2 to eliminate organic matter, followed by FTIR to identify the polymer and SEM to determine the morphology of particles. Daily records were taken on an automatic weather station of temperature, precipitation, and relative humidity within 2 km of the site. Standard methods were used to determine the pH, organic matter, and available N, P, and K in soils, and Cd, Pb, and Zn were analyzed using atomic absorption spectrophotometry. Normal tests were conducted to establish and identify the contents of carbon in microbial biomass and enzymes (dehydrogenase, urease). The developed agronomic systems were utilized in establishing earthworm survival, crop germination, biomass, nutrient uptake, and yield. Data analysis was done in IBM SPSS Statistics version 26.0. ANOVA was used in the treatment effect evaluation, and Tukey HSD was used as a post-hoc test at $p \leq 0.05$. Based on the preliminary variability (CV<10%), the sample size (n=3 per level) was chosen to be large enough (пе 0.8). Under plastic contamination, the most important soil-plant interactions were identified using regression (PCA, cluster).

RESULTS

SEM micrographs revealed fragmentation, weathering, and surface cracks in particles, suggesting environmental aging and mechanical stress. Microplastic abundance ranged from 112 ± 8 items/kg soil at low-contamination sites to 987 ± 23 items/kg at high-contamination sites, indicating a clear gradient of pollution (Table 1).

DOI: https://doi.org/10.54393/fbt.v5i2.204

Table 1: Representative FTIR Absorption Peaks of Extracted Plastic Residues and Their Polymer Identification

Polymer Type	Characteristic Peaks (cm^{-1})	Functional Group	Detected in Soil (%)	Detected in Plant Root Samples (%)
Polyethylene (PE)	2915, 2848, 1465	C–H stretching /bending	42.3%	31.6%
Polypropylene (PP)	2950, 2870, 1376	C–H stretching/ deformation	28.5%	22.4%
Polystyrene (PS)	1601, 1492, 1452	Aromatic ring vibrations	12.7%	8.9%
PET	1715, 1240	C=O stretching, C–O stretching	9.6%	6.3%
Others (PVC, Nylon)	615, 1730, 3300	C–Cl, amide, N–H	6.9%	3.2%

Plastic residues were successfully extracted and characterized across all contamination levels. FTIR spectra confirmed the presence of polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET), with PE and PP being dominant (Figure 2).

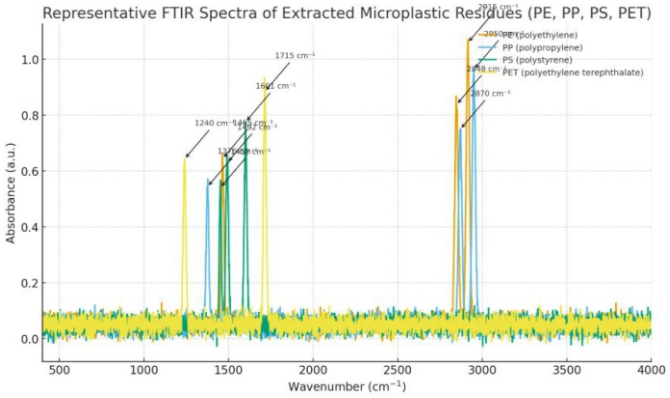

Figure 1: Representative FTIR Spectra of Extracted Microplastic Residues from Soil Samples Showing Diagnostic Peaks of PE, PP, PS, and PET

SEM micrographs of plastic residues were shown. Results show that (A) polyethylene fiber with erosion marks, (B) polypropylene fragment with sharp edges, (C) thin plastic film adhered to soil aggregates, and (D) microplastic particle attached to root hair surface (Figure 2).

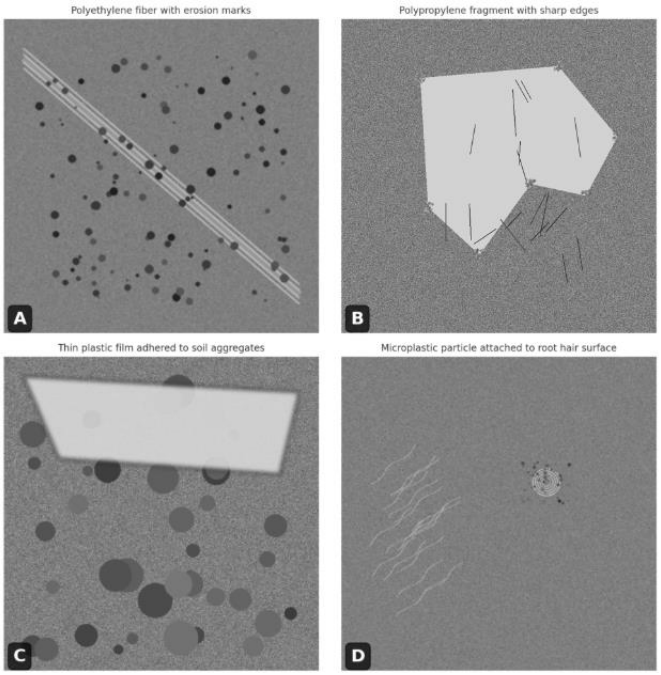

Figure 2: SEM Micrographs of Plastic Residues

Soil physical parameters showed significant ($p<0.05$) deterioration with increasing contamination. Bulk density increased. WHC decreased by 26.3 %, respectively. Regression analysis revealed a strong inverse relationship between WHC and microplastic abundance ($R^2 = 0.81$, $p<0.01$). Paired t-tests indicated significant ($p<0.05$) differences between the 0–15 cm and 15–30 cm soil depths, confirming greater accumulation and compaction in surface layers (Table 2).

Table 2: Morphological Features of Plastic Residues Under SEM

Particle Type	Size Range (µm)	Morphological Characteristics	Possible Source
Fibers	20–800	Elongated, thread-like, smooth to rough surfaces	Mulching films, textiles
Fragments	10–1000	Angular, irregular, with cracks/pits	Packaging plastics, films
Films	30–500	Thin, sheet-like, curled edges	Mulching, greenhouse covers
Spherical Particles	5–50	Smooth, bead-like	Industrial abrasives, personal care
Plant-Root Associated	2–200	Adhered/entangled within root hairs & biofilms	Secondary deposition

Chemical properties also varied significantly. Heavy metal concentrations (Cd, Pb, Zn) were 2–3 times higher, suggesting sorption of metals onto plastic surfaces (Table 3).

Table 3: The Significance of Soil Chemical Properties with Contamination Levels

Contamination Level	pH	SOM (%)	Available NPK (mg/kg)	Heavy Metals (mg/kg)
Low	7.1 ± 0.1	2.1 ± 0.2	N: 72, P: 18, K: 210	Cd: 0.12, Pb: 0.45, Zn: 1.1

DOI: https://doi.org/10.54393/fbt.v5i2.204

Medium	7.4 ± 0.2	1.7 ± 0.1	N: 58, P: 14, K: 182	Cd: 0.25, Pb: 0.77, Zn: 2.4
High	7.8 ± 0.2	1.3 ± 0.2	N: 46, P: 11, K: 154	Cd: 0.39, Pb: 1.12, Zn: 3.5

Biological indicators showed a marked decline ($p<0.01$) with increasing contamination. Earthworm survival dropped from 95 % to 42 %. Post-hoc Tukey analysis confirmed that all biological parameters differed significantly between contamination levels (Table 4).

Table 4: The Significance of Soil Biological Properties with Contamination Levels

Contamination Level	MBC (mg/kg)	Dehydrogenase (µg TPF/g/h)	Urease (µg NH4+/g/h)	Earthworm Survival (%)
Low	412 ± 12	52 ± 3	68 ± 4	95
Medium	276 ± 15	37 ± 2	49 ± 3	71
High	189 ± 10	24 ± 2	31 ± 3	42

Crop performance was directly affected. Germination rate declined. Nitrogen uptake decreased by 46 %. The reduction in yield components, including 1000-seed weight, indicated physiological stress induced by poor soil structure and nutrient limitation (Table 5).

Table 5: The Significance of Crop Performance with Contamination Levels

Contamination Level	Germination (%)	Biomass (g/plant)	Nutrient Uptake (mg/plant)	Grain Yield (g/plant)
Low	93 ± 2	18.4 ± 1.1	N: 28, P: 5.2, K: 21	42.1 ± 2.3
Medium	76 ± 3	13.7 ± 0.9	N: 19, P: 3.8, K: 15	31.4 ± 1.8
High	65 ± 4	10.8 ± 0.7	N: 15, P: 2.9, K: 11	27.0 ± 1.5

DISCUSSION

The results of the study are strong indications that plastic residues and microplastics in particular have tremendous and manifold effects on the soil characteristics and crop yield. This study combines soil physical, chemical, and biological measures with crop performance metrics to show the processes by which plastic residues reduce the sustainability of agriculture. The findings are congruent and relevant to the existing knowledge and crucial to the importance of discussing soil plastic contamination as a high-stakes environmental and agronomic issue. The fact that the bulk density goes up and the porosity and water-holding capacity (WHC) go down with the increase in contamination of the soil is evidence that plastic residues change the soil structure. Earlier research also indicates that microplastic particles have the potential to block soil pores, lowering aeration and hindering infiltration, thus lowering water retention [8, 11]. These mechanisms are verified by our findings and proved by the fact that these disruptions affect the soil aggregate stability, especially in high-contamination plots. The decrease in aggregate stability is a cause of concern in the form of greater susceptibility to erosion and loss of topsoil fertility. Moreover, depth-wise analysis revealed that the layer of 1530 cm had more compaction in contaminated soils, and it appeared that the plastic residues were transferred and concentrated vertically. This is in line with other studies by Corradini *et al.* who demonstrated that irrigation using polluted water stimulates the deeper penetration of plastics into soil horizons [5]. The chemical studies showed that the soils in polluted fields had very low amounts of organic matter and nutrients and high levels of heavy metals. Microplastics have already been found to absorb and carry toxic additives like phthalates, bisphenol A, and trace metals, which change the chemistry of soil and may mobilize pollutants [9, 16]. A decrease in both nitrogen and phosphorus availability is especially alarming, as these two elements are the key to crop yields. We found that we had a reduction of nitrogen by up to 36 percent in the high-contamination soils, which is in line with the results of Qi *et al.* who found that the nutrient cycling in microplastic-amended soils was reduced [14]. The fact that the electrical conductivity (EC) and alkalization of soils in the high-contamination area increase as well could be a sign of the leaching of salts and other additives used in plastics. These shifts in chemical fertility impair their nutrient uptake efficiency, directly influencing plant metabolism and growth. A decrease in microbial biomass carbon (MBC) and enzymatic activities (dehydrogenase, urease, and phosphatase) supports evidence that plastic residues have adverse impacts on soil microbial communities. The active roles of the soil microorganisms in decomposing organic matter and the cycling of nutrients are crucial, and their inhibition can have cascading effects on the soil fertility [9, 16]. A 50 + percent decrease in dehydrogenase activity of high-contaminated soils indicates that microbial respiration is being affected, whereas a reduction in the urease and phosphatase activities indicates that the nitrogen and phosphorus cycle, respectively, are disrupted. The results are in agreement with studies conducted by Fei *et al.* who established lower enzymatic activity in microplastic-contaminated soils [12]. Furthermore, the earthworm bioassays revealed good ecotoxicological impacts, and the survival of the earthworm was reduced to 42 percent in the presence of high contamination, and reproduction failure was observed. Earthworms play a crucial role in soil aeration, organic matter decomposition, and aggregation; therefore, their reduction has dire consequences on the soil ecosystem. Performance indices of crops, such as the rate of germination, biomass growth, nutrient absorption, and yield, were constantly lower in polluted soils. The high-contamination soils postponed and inhibited seed germination by 28% which supports the existing literature that microplastics physically hinder root emergence or chemically interfere with germination [13, 19]. Low biomass

DOI: https://doi.org/10.54393/fbt.v5i2.204

and root-to-shoot ratio also indicate the inhibition of root growth, which is likely a result of lower porosity and water content. This reduction in nutrient absorption and nitrogen in particular directly impacted photosynthetic efficiency and biomass development, leading to a decrease in yield of up to 36 percent. These are correlated to the findings of Gu *et al.* and Steinmetz *et al.* who showed the decrease in yield in wheat fields polluted with plastic debris. Notably, although plastic mulching has positively increased the yields by providing greater moisture retention [15, 20], our findings demonstrate that there are trade-offs in the long-term of continuous use of residues, which ultimately would tend to reduce the productivity. The joint evidence stresses the paradox of using plastic in agriculture. In the short term, it yields beneficial results in the form of mulching and packaging, but in the long term, the residual products of plastic use are detrimental to the fertility of the soil, biodiversity, and crop yields. Due to the rising world food demand, such negative impacts jeopardize agricultural sustainability and food security. Since soils are long-term plastic sinks, the threats of cumulative risks are especially troublesome. The plastic residues can also act against the attainment of sustainable agriculture targets, and in the absence of mitigation, plastic residues could only serve to worsen land degradation [16].

This study was limited to specific field sites and two major crops, which may restrict the generalizability of the findings to other soil types or cropping systems. Additionally, long-term impacts over multiple seasons were not examined. Future research should include multi-season monitoring, diverse crop systems, and the exploration of sustainable alternatives such as biodegradable mulches or improved plastic management strategies to mitigate soil contamination and ensure long-term agricultural productivity.

CONCLUSION

The effect of plastic residues in agricultural soils was high on increasing the bulk density of the soils, reducing the water-holding capacity of the soils, reducing nutrient availability, reducing the activity of the microorganisms, and hindering the germination of crops, biomass of crops, nutrient uptake, and yield. High contamination fields showed the worst results, and this gives an indication of the risks that the long-term plastic residues pose in the future on the health and crop yields of soils.

Authors' Contribution

Conceptualization: AI
Methodology: F, KJ, MA, AI, SGMDH
Formal analysis: AI, SGMDH
Writing and Drafting: F, MA, AI
Review and Editing: F, MA, AI, SGMD, KJ

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Geyer R. Production, Use, and Fate of Synthetic Polymers. In Plastic Waste and Recycling. 2020 Jan: 13-32. doi: 10.1016/B978-0-12-817880-5.00002-5.

[2] Zhang Z, Shi J, Yao X, Wang W, Zhang Z, Wu H. Comparative Evaluation of the Impacts of Different Microplastics on Greenhouse Gas Emissions, Microbial Community Structure, and Ecosystem Multifunctionality in Paddy Soil. Journal of Hazardous Materials. 2024 Dec; 480: 135958. doi: 10.1016/j.jhazmat.2024.135958.

[3] Li S, Ding F, Flury M, Wang Z, Xu L, Li S *et al.* Macro-and Microplastic Accumulation in Soil After 32 Years of Plastic Film Mulching. Environmental Pollution. 2022 May; 300: 118945. doi: 10.1016/j.envpol.2022.118945.

[4] Maraveas C, Kotzabasaki MI, Bartzanas T. Intelligent Technologies, Enzyme-Embedded and Microbial Degradation of Agricultural Plastics. Agri-Engineering. 2023 Jan; 5(1): 85-111. doi: 10.3390/agriengineering5010006.

[5] Corradini F, Meza P, Eguiluz R, Casado F, Huerta-Lwanga E, Geissen V. Evidence of Microplastic Accumulation in Agricultural Soils from Sewage Sludge Disposal. Science of the Total Environment. 2019 Jun; 671: 411-20. doi: 10.1016/j.scitotenv.2019.03.368.

[6] Weithmann N, Möller JN, Löder MG, Piehl S, Laforsch C, Freitag R. Organic Fertilizer as A Vehicle for the Entry of Microplastics into the Environment. Science Advances. 2018 Apr; 4(4): eaap8060. doi: 10.1126/sciadv.aap8060.

[7] Sintim HY and Flury M. Is Biodegradable Plastic Mulch the Solution to Agriculture's Plastic Problem? Environmental Science and Technology. 2017; 51(3): 1068–1069. doi: 10.1021/acs.est.6b06042.

[8] De Souza Machado AA, Lau CW, Till J, Kloas W, Lehmann A, Becker R *et al.* Impacts of Microplastics

DOI: https://doi.org/10.54393/fbt.v5i2.204

on the Soil Biophysical Environment. Environmental Science and Technology. 2018 Jul; 52(17): 9656-65. doi: 10.1021/acs.est.8b02212.

[9] Zheng X, Wu X, Zheng Q, Mai BX, Qiu R. Transfer of Microplastics in Terrestrial and Aquatic Food Webs: The Impact of E-Waste Debris and Ecological Traits. Environmental Science and Technology. 2022 Dec; 57(3): 1300-8. doi: 10.1021/acs.est.2c06473.

[10] Huerta Lwanga E, Gertsen H, Gooren H, Peters P, Salánki T, Van Der Ploeg M *et al.* Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris(Oligochaeta, Lumbricidae). Environmental Science and Technology. 2016 Mar; 50(5): 2685-91. doi: 10.1021/acs.est.5b05478.

[11] Boots B, Russell CW, Green DS. Effects of Microplastics in Soil Ecosystems: Above and Below Ground. Environmental Science and Technology. 2019 Sep; 53(19): 11496-506. doi: 10.1021/acs.est.9b03304.

[12] Fei Y, Huang S, Zhang H, Tong Y, Wen D, Xia X *et al.* Response of Soil Enzyme Activities and Bacterial Communities to the Accumulation of Microplastics in an Acid Cropped Soil. Science of the Total Environment. 2020 Mar; 707: 135634. doi: 10.1016/j.scitotenv.2019.135634.

[13] Bosker T, Bouwman LJ, Brun NR, Behrens P, Vijver MG. Microplastics Accumulate on Pores in Seed Capsule and Delay Germination and Root Growth of the Terrestrial Vascular Plant Lepidium Sativum. Chemosphere. 2019 Jul; 226: 774-81. doi: 10.1016/j.chemosphere.2019.03.163.

[14] Qi Y, Ossowicki A, Yang X, Lwanga EH, Dini-Andreote F, Geissen V *et al.* Effects of Plastic Mulch Film Residues on Wheat Rhizosphere and Soil Properties. Journal of Hazardous Materials. 2020 Apr; 387: 121711. doi: 10.1016/j.jhazmat.2019.121711.

[15] Gu X, Yin R, Cai W, Chen P, Cui K, Du Y et al. Residual Plastic Film Decreases Crop Yield and Water Use Efficiency Through Direct Negative Effects on Soil Physicochemical Properties and Root Growth. Science of the Total Environment. 2024 Oct; 946: 174204. doi: 10.1016/j.scitotenv.2024.174204.

[16] Rillig MC, Lehmann A, Ryo M, Bergmann J. Shaping Up: Toward Considering the Shape and Form of Pollutants. Environmental Science and Technology. 2019; 53(14). doi: 10.1021/acs.est.9b03520.

[17] Prata JC, Da Costa JP, Lopes I, Duarte AC, Rocha-Santos T. Environmental Exposure to Microplastics: An Overview on Possible Human Health Effects. Science of the Total Environment. 2020 Feb; 702: 134455. doi: 10.1016/j.scitotenv.2019.134455.

[18] Shi J, Wang Z, Peng Y, Zhang Z, Fan Z, Wang J *et al.* Microbes Drive Metabolism, Community Diversity, and Interactions in Response to Microplastic-Induced Nutrient Imbalance. Science of the Total Environment. 2023 Jun; 877: 162885. doi: 10.1016/j.scitotenv.2023.162885.

[19] Koskei K, Munyasya AN, Wang YB, Zhao ZY, Zhou R, Indoshi SN *et al.* Effects of Increased Plastic Film Residues on Soil Properties and Crop Productivity in Agro-Ecosystem. Journal of Hazardous Materials. 2021 Jul; 414: 125521. doi: 10.1016/j.jhazmat.2021.125521.

[20] Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Tröger J *et al.* Plastic Mulching in Agriculture. Trading Short-Term Agronomic Benefits for Long-Term Soil Degradation? Science of the Total Environment. 2016 Apr; 550: 690-705. doi: 10.1016/j.scitotenv.2016.01.153.