



Postoperative Hemorrhagic Complication after Elective Cesarean Section under Spinal Anesthesia in Patients receiving Long-Term Anticoagulant Therapy: Retrospective Study

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ABSTRACT

Background: This study was conducted on pregnant women which admitted to obstetrics section of Alkhoms Medical Center between July15; and October15, 2021. **Aims:** The study aimed to assess the severity of bleeding and identify the specific factors that contribute to the varying rates of hemorrhage reported in clinical cases.

Methods:

The study conducted on 60 pregnant women, information was obtained from registration book and files of patients including; medical problems that occurred after caesarean section particularly, bleeding, coagulation and coagulopathy and also anticoagulant used.

Results:

The majority of cases (96.7%) had bleeding during caesarean delivery, while 3% had not. The commonest medication used was Low Molecular weight heparin LMWH (35%), the commonest coagulation problem was deep vein thrombosis (50%), and the most anticoagulation used was during the antepartum period (66.7%).

Conclusion:

Research findings indicated that postpartum bleeding was prevalent among the patients, all of whom were using anticoagulants—primarily to treat DVT. However, most patients suffered obstetric hemorrhages linked to these drugs. The study concludes that because long-term anticoagulation can be fatal for pregnant women, strict medical supervision is vital to ensure patient safety.

Keywords: Hemorrhagic disorder, Anticoagulation, Cesarean Section, spinal anesthesia, complication

1. Introduction

Anesthesia refers to a medically induced, controlled, and temporary loss of sensation or consciousness. It can involve one or more of the following: analgesia (pain relief or prevention), muscle relaxation (paralysis), memory loss (amnesia), and unconsciousness [1]. For patients undergoing elective cesarean section, spinal anesthesia is generally the preferred technique because it carries fewer risks for both mother and fetus compared to general anesthesia [2]. Spinal anesthesia continues to be a cornerstone of modern anesthetic practice due to its reliability,





high patient satisfaction, and low incidence of complications. Advances in spinal needle design, particularly in diameter and tip configuration, have contributed to reducing complications such as post-dural puncture headaches (PDPHs) [3]. The first spinal analgesia was performed in 1885 by James Leonard Corning (1855–1923), a neurologist in New York, who accidentally pierced the dura mater while experimenting with cocaine on a dog's spinal nerves [4].

Most side effects of spinal anesthesia are mild, self-limiting, or easily managed, while severe complications—though rare—can result in permanent neurological damage or even death. These adverse events may occur immediately after administration or within 48 hours postoperatively. Common minor complications include mild hypotension, bradycardia, nausea and vomiting, transient neurological symptoms (such as lower back pain radiating to the legs), and PDPH [5]. Cesarean section is the most frequent surgical procedure in obstetrics and gynecology, accounting for about 30% of births worldwide. With rising cesarean delivery rates globally, the number of surgical interventions continues to increase. The choice of anesthesia depends on patient preference, obstetric indications, and the anesthesiologist's expertise [6]. Introduced in the late nineteenth century, cesarean section became a life-saving intervention for mothers and newborns facing serious pregnancy or childbirth complications [7]. Spinal anesthesia is traditionally considered the first choice for uncomplicated elective cesarean sections because it avoids airway manipulation, reduces aspiration risk, and is technically straightforward [5].

A growing number of surgical patients are on anticoagulant therapy preoperatively, which increases the risk of epidural hematoma during spinal anesthesia. It is therefore essential for anesthesiologists to anticipate this risk and adjust anesthetic plans accordingly [7]. Guidelines for managing patients on anticoagulation during spinal or epidural anesthesia emphasize avoiding concurrent medications that affect coagulation, considering technical challenges with regional blocks, and carefully timing block administration relative to thromboprophylaxis. If traumatic needle or catheter placement occurs, anticoagulation should be delayed for 24 hours postoperatively to reduce hematoma risk. For patients on high-dose anticoagulation, spinal anesthesia should be performed 10–12 hours after the last dose, while needle insertion should be postponed for at least 24 hours. If prophylactic anticoagulation is given two hours before surgery, spinal anesthesia should be avoided due to peak anticoagulant activity. The most feared complication in these patients is symptomatic spinal hematoma, which can cause severe neurological outcomes such as paralysis. Early diagnosis and preoperative neurological status are critical for prognosis after surgical decompression [8]. Managing pregnant patients on anticoagulation is particularly complex because of the added risk of spinal hematoma and the unpredictable timing of labor [10].

Obstetric hemorrhage remains a leading cause of maternal mortality. From an anesthesiologist's perspective, it is vital to assess coagulation changes during pregnancy and evaluate the risk of coagulation disorders [9]. Pregnancy often leads to physiological anemia and alterations in coagulation factors, shifting the balance toward hypercoagulability to prepare for delivery. Anesthesiologists must understand both normal physiological changes and pathological alterations caused by disease or medication to ensure safe regional anesthesia during labor and cesarean section [10]. Spinal hematoma after regional anesthesia has been linked to coagulation abnormalities in 68% of cases. Obstetric bleeding also contributes significantly to maternal mortality, necessitating careful evaluation of coagulation disorders, transfusion needs in postpartum hemorrhage, and pharmacological management in special circumstances such as intrauterine fetal death [9]. Normal pregnancy induces a hypercoagulable state that persists for up to eight weeks postpartum, increasing thrombotic risk three- to five-fold compared to





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non-pregnant women. Anticoagulant therapy is often required for prevention and treatment of coagulation disorders during pregnancy [9]. Common agents include unfractionated heparin, low molecular weight heparin, and aspirin, while warfarin is contraindicated in pregnancy. Adjustments in anticoagulation regimens aim to balance maternal safety with minimizing fetal risk [10]. Oral anticoagulants include warfarin and aspirin (the latter being an antiplatelet agent considered safe at low doses), while parenteral options include UFH, LMWH, and newer heparinoids, which are reserved for cases where UFH or LMWH are unsuitable [11]. The most significant adverse effect of anticoagulants is bleeding, with risk influenced by drug class, patient age, and comorbidities. Hemorrhage following cesarean section under spinal anesthesia is particularly dangerous [12].

Aims: This study aims to evaluate postoperative hemorrhagic complications after elective cesarean section under spinal anesthesia in pregnant women receiving long-term anticoagulant therapy.

2. Methods

This study was designed as a retrospective observational study based on review of hospital medical records. The study was conducted at Alkhoms Medical Center, Libya. It was carried out over a three-month period from July15to October15 2021. This study included 60 participants who met the inclusion criteria. Sample size was determined based on all eligible and available cases during the specified study period. Additionally, this number was considered statistically acceptable for retrospective observational studies of similar scope, particularly given the limited number of pregnant women receiving long-term anticoagulant therapy during pregnancy at the study center.

2.1 Participants

Data were collected through a comprehensive review of medical records of participants who underwent elective cesarean section under spinal anesthesia and received anticoagulant therapy during pregnancy, whether for prophylactic or therapeutic purposes.

The collected data included:

- Type and duration of anticoagulant therapy.
- Indications for anticoagulation.
- Postoperative hemorrhagic complications, including bleeding events, coagulopathy, and related adverse outcomes.

2.2 Data Collection Procedure

A structured data collection form was used as the primary tool for extracting relevant information from hospital records. The form included demographic characteristics, obstetric history, details of anticoagulant therapy, type of anesthesia, and postoperative clinical outcomes.

2.3 Inclusion Criteria

- Pregnant women undergoing elective cesarean section under spinal anesthesia.
- Receiving anticoagulant therapy during pregnancy.
- Availability of complete medical records including bleeding and coagulation data.

2.4 Exclusion Criteria

- Medical records of women not receiving anticoagulant therapy Known primary bleeding disorders.
- Emergency caesarean sections or procedures performed under general anesthesia.

2.5 Statistical Analysis

Statistical analysis was conducted using SPSS version 22. Descriptive statistics were used to summarize demographic and clinical characteristics. Continuous variables were presented as means and standard deviations, while categorical variables were expressed as





frequencies and percentages. The incidence of hemorrhage was described according to its timing, whether occurring intraoperative or postoperative. Given the high overall prevalence of hemorrhagic events and the limited number of non-hemorrhagic cases, the analysis primarily focused on descriptive reporting of outcomes.

2.6 Ethical Considerations

Official approval was obtained from the administration of Alkhoms Medical Center prior to data collection.

In addition, A strict confidentiality of personal and medical information was maintained. All data were used solely for research purposes and handled in accordance with established ethical principles for medical research.

3. Results

The present study included 60 participants who attended Alkhoms Medical Center between 15 July 2021 and 15 October 2021. The analysis focused on identifying the main indications for anticoagulant therapy during pregnancy and evaluating associated hemorrhagic complications during cesarean delivery.

3.1. Indications for Anticoagulant Therapy:

The most common indication for anticoagulation was deep vein thrombosis (DVT), accounting for 50% of cases. Placental related coagulation disorders represented 13.3%, while other medical indications collectively accounted for 36.7% of participants. For more details see Figure 1.

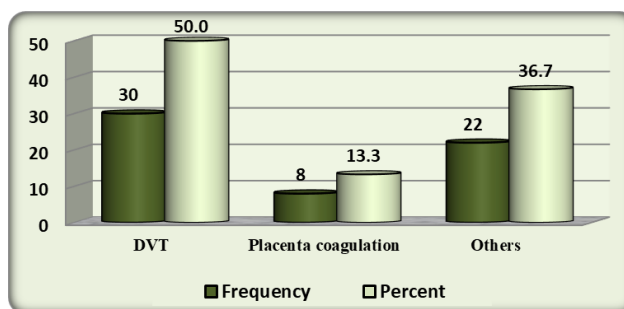


Figure 1: Repetitive Distribution and percentage for the indication of use anticoagulation

3.2. Types and Distribution of Anticoagulant Therapy:

The most commonly used anticoagulant was low molecular weight heparin (LMWH), including enoxaparin and related preparations (35%). This was followed by unfractionated heparin (31.7%). Aspirin was used in 30% of cases. Fraxiparine and warfarin were the least frequently prescribed, each accounting for 1.7%. For more details see Figure 2.

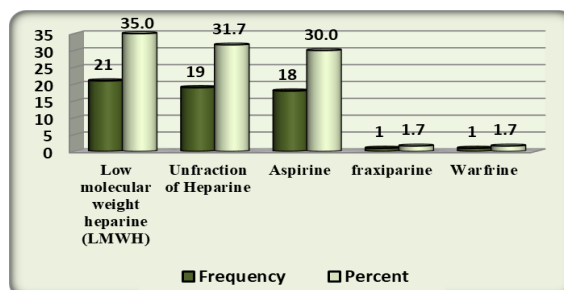


Figure 2: Repetitive Distribution and percentage for the anticoagulation.



3.3. Timing of Anticoagulant Use:

Regarding the timing of anticoagulation therapy, the majority of patients (66.7%) received treatment during the antepartum period only. This was followed by a combined antepartum and postpartum regimen in 30% of cases, while postpartum-only administration was the least frequent, accounting for only 3.3% of the study population. For more details see Figure 3.

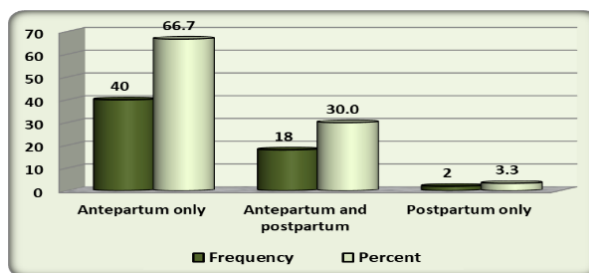


Figure 3: Repetitive Distribution and percentage for the Anticoagulation used.

3-4. Types of treatment and duration use anticoagulation:

Table (1): The Statistical description of the anticoagulants, the type of treatment and how often it is used:

Name of anticoagulation	For how many taken medication					Total
	Therapeutic dose	Prophylactic dose	days	months	years	
Unfractionated Heparin	8	10	0	16	2	18
Low molecular weight heparin (LMWH)	15	7	0	21	1	22
Aspirin	5	14	1	18	0	19
Fraxiparine	0	1	1	0	0	1

From the data presented in Table 1, LMWH was the most frequently used anticoagulant, predominantly in therapeutic doses. Unfractionated heparin was also common, while aspirin was mainly prescribed prophylactically. Fraxiparine and warfarin were rarely used.

3-5. Hemorrhagic complication:

Among the study sample, 96.7% experienced significant hemorrhage during cesarean delivery, while the remaining 3.3% did not encounter bleeding complications. The high rate of hemorrhage was mainly related to the high-risk profile of the participants, particularly the use of long-term anticoagulant therapy, in addition to obstetric and surgical factors that may have contributed to increased blood loss. For more details see Figure 4.

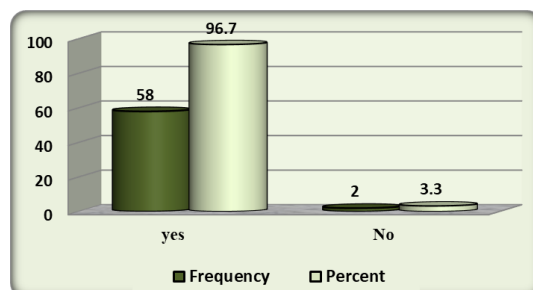


Figure 4: Repetitive distribution and percentage of bleeding complications among patients undergoing cesarean section.

4. Discussion

Anticoagulation therapy during pregnancy, particularly in women undergoing cesarean section under spinal anesthesia, represents a clinical challenge due to the need to balance the prevention of thromboembolic events against the risk of postoperative hemorrhage. Limited evidence exists regarding surgical bleeding risks in anticoagulated pregnant women, and previous studies have reported increased rates of perioperative bleeding complications among patients receiving long-term anticoagulant therapy.

In the present study, 60 pregnant women who received anticoagulation therapy and underwent cesarean delivery under spinal anesthesia were evaluated. Postoperative bleeding was observed in 58 participants (96.7%), whereas only 2 participants (3.3%) did not experience bleeding. This high frequency of hemorrhagic events highlights the significant association between anticoagulant exposure and bleeding risk during delivery. These findings are consistent with Kearsley et al., [13], who reported that patients receiving long-term oral anticoagulants experienced higher rates of moderate to severe postoperative bleeding compared with controls. This supports the concept that prolonged anticoagulation increases the likelihood of hemorrhagic complications during surgical delivery.

Regarding the indication for anticoagulation, DVT was the most common indication in the current study (50%). This differs slightly from other reports where VTE was reported as the most frequent indication for anticoagulation during pregnancy (approximately 21% in some studies) [15]. The variation may be attributed to differences in study populations and diagnostic criteria.

In terms of medication patterns, LMWH was the most commonly used anticoagulant (35%), followed by unfractionated heparin (31.67%). These findings are partially consistent with previous studies reporting that LMWH is the most frequently prescribed anticoagulant during the antepartum period [14]. However, in contrast to other reports where unfractionated heparin was more commonly used postpartum [16], the current study showed a higher overall use of LMWH. This may reflect institutional prescribing preferences and clinical protocols.

Most anticoagulation therapy in the present study was administered during the antepartum period (66.7%), while combined antepartum and postpartum use accounted for 30%, and postpartum-only use was minimal (3.3%). These results align with established clinical practice, where anticoagulation is primarily required during pregnancy to prevent thromboembolic complications [17].

The findings indicate a high frequency of postoperative bleeding among pregnant women receiving anticoagulation therapy, particularly low



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molecular weight heparin and unfractionated heparin. The observed pattern is consistent with previously published studies reporting an increased risk of hemorrhagic complications in anticoagulated obstetric patients. These results underscore the importance of individualized risk assessment, appropriate dose adjustment, and careful perioperative monitoring to balance bleeding risk and thromboembolic prevention.

Strengths and Limitations

Strengths:

This study addresses a clinically relevant and underexplored population—pregnant women receiving long-term anticoagulant therapy—providing valuable real-world data. Inclusion of all eligible cases during the specified period minimized selection bias and reflects routine clinical practice.

Limitations:

The relatively small sample size (n = 60) may limit statistical power and generalizability. The retrospective single-center design increases the risk of information bias, incomplete records, and residual confounding. Additionally, the limited number of pregnant women receiving long-term anticoagulant therapy restricted the ability to perform detailed subgroup analyses.

5. Conclusion

The study demonstrates that anticoagulation therapy in high-risk pregnant women undergoing cesarean delivery under spinal anesthesia is accompanied by a substantial frequency of postoperative bleeding. Rather than establishing causality, the findings reflect an observed pattern of hemorrhagic events in a population characterized by thrombotic risk and exposure to anticoagulant treatment. These results emphasize the clinical importance of individualized risk assessment, appropriate dose adjustment, and vigilant perioperative monitoring to minimize bleeding complications while maintaining effective thromboembolic prophylaxis.

Interpretation of these findings should consider the limitations of the study, including its retrospective design, single-center setting, and relatively small sample size. Further prospective, larger-scale studies are required to better define risk determinants and optimize management strategies in this population.

Recommendations:

Based on the findings, anticoagulation therapy in pregnant women should be prescribed according to individualized thrombotic and bleeding risk assessment, with appropriate dose adjustment and close perioperative monitoring to minimize hemorrhagic complications. Careful planning of temporary interruption and timely resumption around surgical procedures is essential to balance thrombosis prevention with bleeding risk. In cases of significant bleeding, rapid recognition and prompt hemostatic management are necessary to reduce maternal morbidity and related complications.

ETHICAL APPROVAL

The study was approved by the institutional ethics committee and conducted in accordance with institutional ethical regulations. Patient confidentiality was strictly maintained.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

All authors contributed to study design, data collection, analysis, and manuscript preparation.

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Sterigmatocystin an emerging mycotoxin—a comprehensive review

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ABSTRACT

Background: Sterigmatocystin (STC) is an indoor air and food mycotoxin contaminant, which is formed by several fungal genera, most notably *Aspergillus* species. It is considered a potential factor in causing esophageal, gastric, and lung malignancies in humans. It is grouped as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC). STC shares structural and biosynthetic similarities with aflatoxin B1, underscoring its toxicological relevance. Global climate change has expanded the occurrence of STC beyond tropical regions into temperate zones, raising significant food safety concerns. **Amis:** This review consolidates current knowledge on STC occurrence in food, feed, and indoor air, its biosynthesis, methods of isolation and detection, biological activities, and toxicological effects.

Methods: The literature review was conducted using two major scientific databases, PubMed and Google Scholar. The keyword “sterigmatocystin” was applied to retrieve relevant publications, and articles were selected for citation based on the significance of the information they provided.

Results: Analytical advances such as LC-MS/MS, High-Resolution Mass Spectrometry (HRMS), and biosensor technologies have improved detection sensitivity, while biological detoxification strategies using lactic acid bacteria show promise in mitigating contamination. Despite these developments, gaps remain in understanding STC’s toxicological mechanisms, Circulation through nutritional pathways, and regulatory thresholds.

Conclusion: This review underscores the importance of continued investigation into the health hazards posed by sterigmatocystin and highlights the need for effective preventive measures. Overall, it distinguishes itself by offering a more comprehensive, current, and integrative perspective, weaving together the chemistry, biology, toxicology, and public health implications of STC into a unified analysis.

Keywords: *secondary metabolites, food poisoning, air pollution, human carcinogen*

1. Introduction

For over a century, mold genera such as *Aspergillus*, *Penicillium*, and *Talaromyces* have served as valuable sources for isolating diverse





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bioactive compounds [1]. According to Bladt et al [1], although an estimated three million fungal species exist worldwide, only about 100,000 have been formally identified. This highlights the vast, untapped potential of fungi to yield novel and promising bioactive molecules. *Aspergillus* is a genus of filamentous fungi first described nearly 300 years ago, now comprising more than 250 recognized species. While many *Aspergillus* species are valued for their secondary metabolites widely applied in medicine and industry, others are pathogenic and produce toxins harmful to humans, birds, and animals [2]. Mycotoxins are highly toxic secondary metabolites predominantly produced by fungi belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium* [2]. These pathogenic fungi and their toxins are especially problematic in tropical and subtropical regions. However, due to global warming and climate change, their presence and associated risks have increasingly extended into temperate zones [3].

Sterigmatocystin (STC) is recognized as an emerging mycotoxin and a natural carcinogen. The International Agency for Research on Cancer (IARC) has classified it as a Group 2B substance, meaning it is possibly carcinogenic to humans. [4,5]. Belonging to the polyketide class of mycotoxins, STC shares structural similarities with aflatoxin B1. It is mainly produced by *A. versicolor*, though other *Aspergillus* species such as *A. flavus*, *A. parasiticus*, and *A. nidulans* also contribute [6]. In contrast, aflatoxin B1 (AFB1) is primarily associated with *A. flavus* and *A. parasiticus*. Both toxins arise from the same biosynthetic pathways (the alternative oxidase (AOX) and cytochrome C oxidase (COX) pathways); however, literature indicates that AFB1 exerts more severe effects on living organisms compared to STC. Despite this, available knowledge on the biological effects of sterigmatocystin remains quite restricted [7]. Most of the existing evidence comes from in vitro experiments or animal studies, while human data are minimal. Overall, this review distinguishes itself by presenting a more comprehensive, updated, and integrative account, linking STC's chemistry, biology, toxicology, and public health relevance within a single framework.

Aims:

Owing to global climate change and the appearance of pathogenic moulds and their mycotoxins in the countries located in the temperate zone, there is a massive amount data on sterigmatocystin occurrence, and toxicology, but there is no comprehensive review that collects and summarizes the updated aspects of sterigmatocystin. The aim of this study is to provide an updated and comprehensive review of the occurrence, chemistry, isolation, detection, biology and toxicology of the emerging mycotoxin sterigmatocystin.

2. Methods:

The literature review was conducted using two major scientific databases, PubMed and Google Scholar. The keyword “sterigmatocystin” was applied to retrieve relevant publications, and articles were selected for citation based on the significance of the information they provided.

3. Results:

Occurrence of sterigmatocystin (STC):

Natural sources of Sterigmatocystin:

STC is an emerging mycotoxin produced by several fungi, notably *Aspergillus* species and genera like *Chaetomium*, *Emericella*, *Penicillium*, and *Bipolaris*. STC production is confirmed in several *Versicolores* strains, including *A. versicolor* and related species, while *A. sydowii* is typically excluded [6,8,9]. Nevertheless, some investigations have indicated that *A. sydowii* may also synthesize STC [5].

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Furthermore, Géry et al [5] reported for the first time that *A. tabacinus* is capable of sterigmatocystin biosynthesis.

Site of production of STC within the mold's colony:

Ámon et al [10] reported that sterigmatocystin is synthesized during the sexual reproduction of *Aspergillus nidulans* as a means of protecting its reproductive structures. Their findings showed that STC production occurs specifically in hyphae located adjacent to hülle cells.

Sterigmatocystin presence in food and feed:

As presented in Table 1, Molds are a major cause of post-harvest diseases in fruits and vegetables, leading to the loss of approximately 20 - 25% of harvested produce in both developed and developing nations. In the United States alone, mycotoxin contamination is estimated to result in annual economic losses of about 1 billion USD [11]. Mycotoxin contamination in cereals like rice, wheat, and maize has created health risks for people and animals in recent years. During the post-harvest stage, fungal infestation can give rise to sterigmatocystin (STC) and aflatoxins in cereal grains [6]. Beyond animal feed, STC has been consistently detected in food items including bread, corn, grains, spices, coffee beans, soybeans, cheese, and pistachios, with STC-producing fungi frequently isolated from these commodities [9]. In a study conducted in China, Zhao et al [12] analyzed 126 tea samples using optimized procedures and found STC in 17 samples, with concentrations ranging from 0.13 to 4.48 µg/kg. While Veršilovskis et al [9] and Zhao et al [12] confirmed the occurrence of STC in food, Dabelić et al [13] reported that STC is relatively rare, appearing only at low concentrations in plants used in traditional Chinese medicine, cereals, spices, beer, and cheese.

Current knowledge on the fate of sterigmatocystin (STC) and its metabolites during food processing is still limited, with most investigations concentrating on its metabolic conversion in animals [6]. In a related study, Pietri et al [4] reported the presence of STC in 101 grated cheese samples obtained from retail outlets in Northern Italy, with concentrations ranging from <LOD to 6.87 µg/kg. Nevertheless, STC occurrence in cheese is generally rare, as aflatoxin-producing fungi are seldom associated with such products. Furthermore, no evidence has yet been documented regarding the transfer of STC or its metabolites into other animal-derived foods, including eggs and meat [11].

Due to the limited characterization of sterigmatocystin (STC), European authorities have advised the systematic collection of reliable data regarding its presence in food and feed. For food products, a limit of quantification (LOQ) below 1.5 µg/kg has been suggested, while the lack of sufficient data currently prevents the establishment of feed limits. In addition, the creation of certified reference materials and the implementation of proficiency testing have been recommended to enhance analytical reliability [11].

Table 1. Summary of STC detection in various foodstuffs

Source	Occurrence of STC	Concentration of STC	Notes / Citations
Cereal grains (rice, rye, barley, wheat, oats, maize)	Contamination during post-harvest	Not specified	STC + aflatoxins detected [6]
Bread, corn, grains, spices, coffee beans, soybeans, cheese,	Regular presence	Not specified	STC-producing fungi isolated [9]





Source	Occurrence of STC	Concentration of STC	Notes / Citations
pistachios.			
Tea samples (China, Zhao et al)	Detected in 17/126 samples	0.13 – 4.48 µg/kg	[12]
Plants used in traditional Chinese medicine, cereals, spices, beer, and cheese.	Rare occurrence	Low concentrations	[13]
Grated cheese (Italy, Pietri et al)	Detected in 101 samples	LOD – 6.87 µg/kg	[4]
Cheese (general occurrence)	Rare	Not specified	Aflatoxin-producing fungi are uncommon [11]
Animal-derived foods (eggs, meat)	No documented transmission	—	[11]

Sterigmatocystin presence in indoor-air:

Indoor air pollution is considered one of the most significant health hazards, particularly in developed countries where people spend much of their time in transport systems or enclosed buildings. Fungal genera such as *Aspergillus*, *Penicillium*, and *Cladosporium* are frequently identified as major indoor contaminants, contributing to health issues including mycotoxin production, allergic reactions, itching, and the worsening of asthma symptoms. These fungi are also linked to sick-building syndrome, which presents with non-specific complaints such as dizziness, fatigue, irritability, poor concentration, and headaches, along with respiratory and dermatological symptoms like a runny nose, nasal itching, eye irritation, dry throat, breathing difficulties, and skin dryness or rash [5].

According to Dabelić et al [13], sterigmatocystin has been detected in the air of water-damaged buildings and industrial environments. Its presence in indoor air is associated with mycelial fragments and spores of producing fungi, particularly strains within the *Aspergillus* series *Versicolores* [5]. While several studies suggest that these strains may cause respiratory or skin-related symptoms, the direct relationship between sterigmatocystin exposure and such health effects remains insufficiently explored.

Biosynthesis of sterigmatocystin:

Sterigmatocystin (STC), a potent mycotoxin and natural carcinogen, exhibits structural similarity to aflatoxin B1, as both molecules contain xanthone and furan ring systems (Figure 1 and 2). In *Aspergillus flavus* and *A. parasiticus*, the primary producers of aflatoxin B1, STC follows the same biosynthetic pathway as aflatoxins, functioning as a late-stage intermediate in the aflatoxin B1 pathway [7][14][19]. Furthermore, Molnár et al [15] demonstrated that in *Aspergillus nidulans*, STC biosynthesis proceeds via both the alternative oxidase (AOX) and cytochrome C oxidase (COX) pathways.

Unlike the previously mentioned studies, Pardo and Esteve-Turrillas [25] noted that sterigmatocystin (STC) acts as a precursor to aflatoxins, sharing the same polyketide-derived biosynthetic pathway, which underscores its toxicological relevance. Within this pathway,



STC serves as an intermediate in the formation of aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1) (Figure 1). In aflatoxin-producing fungi, STC is quickly converted into O-methylsterigmatocystin (OMST), the direct precursor of AFB1 and AFG1. Consequently, STC rarely accumulates; however, species such as *Aspergillus nidulans* and *A. versicolor* appear unable to carry out this conversion into OMST.

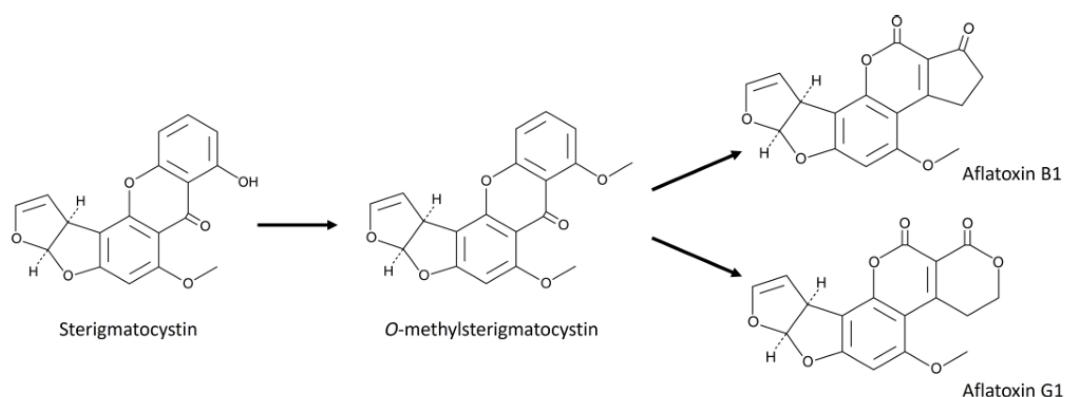


Figure 1: Sterigmatocystin and its derivative O-methylsterigmatocystin undergo conversion within the biosynthetic pathway, resulting in aflatoxin B1 and G1 [25]

Methods of isolation and detection of Sterigmatocystin:

As summarized in Table (2) and Figure (2), several analytical methods were used to detect the presence of STC in foodstuffs. Michael and Rodricks [17] described a qualitative and quantitative method for detecting sterigmatocystin (STC) in grain samples. Initially, the samples were extracted using acetonitrile: water (9:1) with potassium chloride (KCl). The extract was then partitioned first with hexane and subsequently with chloroform (CHCl_3), followed, when necessary, by silica gel column chromatography. This process yielded an extract containing STC. Its presence was assessed by comparing fluorescence intensity on TLC plates with that of a pure STC standard, and visualization was enhanced using an AlCl_3 spray reagent. STC was further isolated on preparative TLC plates, and its identity was confirmed through the formation of acetate derivatives and acid-treated derivatives.

Kubosaki et al [8] demonstrated that single nucleotide polymorphism (SNP)-based PCR amplification, employing a high-discrimination DNA polymerase (HiDi DNA polymerase), is a reliable and robust screening approach for identifying STC-producing fungal strains.

Pardo and Esteve-Turrillas [25] reviewed various analytical methods for detecting sterigmatocystin (STC) in food. Among these, liquid chromatography (LC) is the most commonly applied technique due to its strong resolution capacity, often paired with detectors such as diode array (DAD), fluorescence (FD), or mass spectrometry (MS). LC-MS/MS provides superior sensitivity and selectivity, enabling trace-level detection of STC and simultaneous analysis of multiple mycotoxins across diverse food matrices. High-resolution mass spectrometry (HRMS), including LC-HRMS/MS with Orbitrap or QTOF systems, further enhances accuracy by allowing unambiguous identification.

Immunoassays like ELISA offer a rapid and cost-effective screening option, though they are generally less specific and sensitive compared to LC-MS/MS. More recently, innovative biosensors, such as graphene oxide-aptamer FRET-based aptasensors, have been developed, enabling one-step, highly selective detection of STC in foods like chili and pepper with minimal interference.



Table 2. Advantages and limitations of STC's analysis methods

Technique	Principle	Advantages	Limitations	Citation
TLC-based extraction (Michael & Rodricks)	TLC with fluorescence, STC standard. visualization with $AlCl_3$ spray reagent	Simple, cost-effective, qualitative & quantitative	Lower sensitivity, labor-intensive, requires derivatization	[17]
SNP-PCR (Kubosaki et al)	SNP-based PCR amplification using HiDi DNA polymerase to identify STC-producing fungal strains	Reliable, robust, highly specific for strain screening	Limited to fungal strain detection, not direct toxin quantification	[8]
Liquid Chromatography (LC)	Reverse-phase C18 columns with DAD, FD, or MS detectors	High resolution, widely used	Requires instrumentation, moderate sensitivity	[25]
LC-MS/MS	LC coupled with tandem MS for trace-level detection and multiresidue analysis	Highest sensitivity & selectivity, detects multiple mycotoxins simultaneously	Expensive, requires skilled operation	[25]
High-Resolution MS (HRMS)	LC-HRMS/MS with Orbitrap or QTOF detectors for unambiguous identification & non-targeted screening	Extreme selectivity, detects emerging/unknown compounds	High cost, complex data analysis	[25]
ELISA (Immunoassay)	Antibody-based detection of STC and other	Rapid, cost-effective, high-throughput	Less specific & sensitive, matrix interferences	[25]





Technique	Principle	Advantages	Limitations	Citation
	mycotoxins	screening		
Graphene oxide-aptamer FRET biosensor	Aptamer-based fluorescence resonance energy transfer for one-step STC detection	Highly selective, minimal interference, innovative	Still emerging, with limited availability	[25]

Biological activities of sterigmatocystin:

Inhibition of *Plasmodium falciparum*:

Fungi have long been acknowledged as reliable sources of antimalarial agents, with numerous metabolites demonstrating significant anti-plasmodial properties [18]. In their study, Niu et al [18] successfully isolated sterigmatocystin (STC) from *Penicillium janthinellum*. Their findings revealed that STC inhibited the growth of *Plasmodium falciparum* in blood with an IC_{50} of 34 μ M and reduced the infectivity of sexual-stage parasites in mosquitoes with an IC_{50} of 48 μ M. Notably, STC exhibited no acute toxicity toward human kidney cells at concentrations up to 64 μ M. These results suggest that STC could represent a promising lead compound for malaria control and serve as a valuable marker for exploring the molecular mechanisms of malaria transmission.

Biological detoxification and binding of sterigmatocystin on Lactobacilli

Lactic acid bacteria (LAB) are recognized as effective agents for biological detoxification, acting as a natural barrier against the entry of mycotoxins into the food chain. Their protective mechanisms involve suppressing the growth of mycotoxin-producing fungi, binding toxins to their cell surfaces, and, in rare cases, degrading the toxins. LAB occur naturally in both humans and animals. Several investigations have shown that certain LAB strains can bind mycotoxins such as aflatoxin B1 (AFB1) to their surfaces [3]. In the study by Kosztik et al [3], it was demonstrated that mycotoxin exposure did not hinder bacterial growth, and notably, the binding capacity of Lactobacillus strains for sterigmatocystin (STC) was approximately twice that observed for aflatoxin B1.

Binding of sterigmatocystin to human serum albumin:

Sterigmatocystin is a precursor of aflatoxin B1 biosynthesis. As they share the same biosynthetic pathway, the toxic effects of these two mycotoxins are similar.

The affinity of xenobiotics to human albumin plays an important role in determining the magnitude of biological effects of these bioactive molecules on human tissues.

The binding affinity of (camptothecin, warfarin, and naproxen) to albumin was not significantly affected by STC. On human serum albumin (HSA), STC binds to the Heme site (FA1). The acute toxicity of STC is relatively low. Although, acute exposure to STC can cause hepatocellular necrosis in certain species. It is nephrotoxic in rats and monkeys. Also, in vitro studies suggested that STC has genotoxic effects [16].

Effects of STC on common Crap liver:

Cereals are frequently incorporated into fishmeal for aquaculture feeds. However, the presence of filamentous fungi in cereals [9] has





contributed to increased mycotoxin contamination in such diets. The liver is the primary target organ of aflatoxin B1 (AFB1) toxicity, whereas sterigmatocystin (STC) can affect both the liver and kidneys [20]. In the study conducted by Kövesi et al [21], the short-term effects of dietary exposure to AFB1 (100 µg/kg feed), STC (1000 µg/kg feed), and their combination (100 µg AFB1 + 1000 µg STC/kg feed) were evaluated in one-year-old common carp (*Cyprinus carpio*) by examining lipid peroxidation and the glutathione redox system at both biochemical and gene expression levels. The results indicated that STC markedly reduced glutathione (GSH) levels, while glutathione peroxidase activity remained unchanged. In contrast, AFB1 or the combined treatment (AFB1+STC) produced weaker effects compared to STC alone. The authors concluded that the individual actions of AFB1 and STC on different parameters may interact synergistically or antagonistically under multi-toxin exposure.

Sterigmatocystin toxicology:

In London, in 1962, almost 100,000 turkeys died in an unfamiliar veterinary disaster. The death of turkeys was linked to the contamination of peanut meals with aflatoxin, “turkey X disease”. Since that time, research in the field of mycotoxins has become an important scientific issue. Nowadays, mycotoxicosis and mycotoxin contaminations are considered as main health problems in the third world. For a long time, the countries located in the tropical region have been fighting the risk of mycotoxins [11].

In humans, the toxic impact of sterigmatocystin (STC) appears to be less pronounced than that of aflatoxins; however, its significance cannot be disregarded [11]. Contrary to the observations of Ráduly et al [11] and Géry et al [5], in vivo studies have shown that STC can induce toxic effects in several animal species, including mice, rats, pigs, fish, chickens, monkeys, and ruminants, depending on the species, exposure frequency, and route of administration. In one trial, sheep fed the highest dose of STC (16 mg/kg feed, approximately 0.3 mg/kg body weight per day) exhibited no observable signs of toxicity. Therefore, the precise toxicity profile of STC in livestock remains uncertain [11].

Since the toxicity of STC on humans and livestock is not clear, an illustration of the mechanism’s toxicity, through which it exerts its toxic effects on humans and animals, was needed.

Sterigmatocystin mechanism of toxicity:

As previously noted in this review, sterigmatocystin (STC) has been designated by the IARC as a Group 2B possible human carcinogen [4], [5], due to its demonstrated tumor-inducing capacity in diverse cell culture systems and animal models. Nevertheless, internationally harmonized regulations specifying maximum allowable concentrations of STC in food remain inadequately established [11]. Díaz Neito et al [22] reported that STC exhibits relatively low acute oral toxicity, with LD₅₀ values ranging from 120 to 166 mg/kg body weight. Documented findings include malignant and premalignant changes—such as angiosarcomas and hepatocellular carcinomas—occurring in brown fat following oral administration of STC (Figure 2). Although STC is both genotoxic and carcinogenic, its potency is approximately three orders of magnitude lower than that of aflatoxin B1. In hepatic and pulmonary tissues, several CYP enzymes metabolize STC into multiple hydroxy derivatives, which are subsequently excreted via urine and bile [11].

As revised by Ráduly et al, [11], the reactive epoxy-adducts of STC can covalently bind to DNA and generate the STC-N₇-guanine adducts. These derivatives are proposed to be responsible for the mutagenicity of STC. In addition, Pfeiffer et al, [23] proposed that the hydroxylation of the aromatic ring in the STC parent structure creates a catechol. The created derivative could produce mutagenicity; when it reacts with DNA. In comparison to the epoxide derivative, catechol was formed as a major metabolite in the liver microsomes of humans



and rats.

Recently, the mechanism of toxicity by which STC can induce human oesophageal and gastric cancers has been illustrated. STC can induce DNA double-strand disruptions in humans immortalized bronchial epithelial cell line. This effect may lead to adenocarcinomas. In addition, an *in vivo* experiment in a rat model also confirmed these findings [11].

The presence of STC in the dust of indoor settings may result in inhalation of these mycotoxins by the inhabitants [5]. *In vivo* experiment on rats carried out by Jakšić et al, [24], the rats were exposed to air contaminated with STC in concentrations (0.3 mg STC/kg of lung weight). They clarified that, STC in naturally occurring concentrations may induce DNA-single strand breaks in rat lungs with insignificant cytotoxicity.

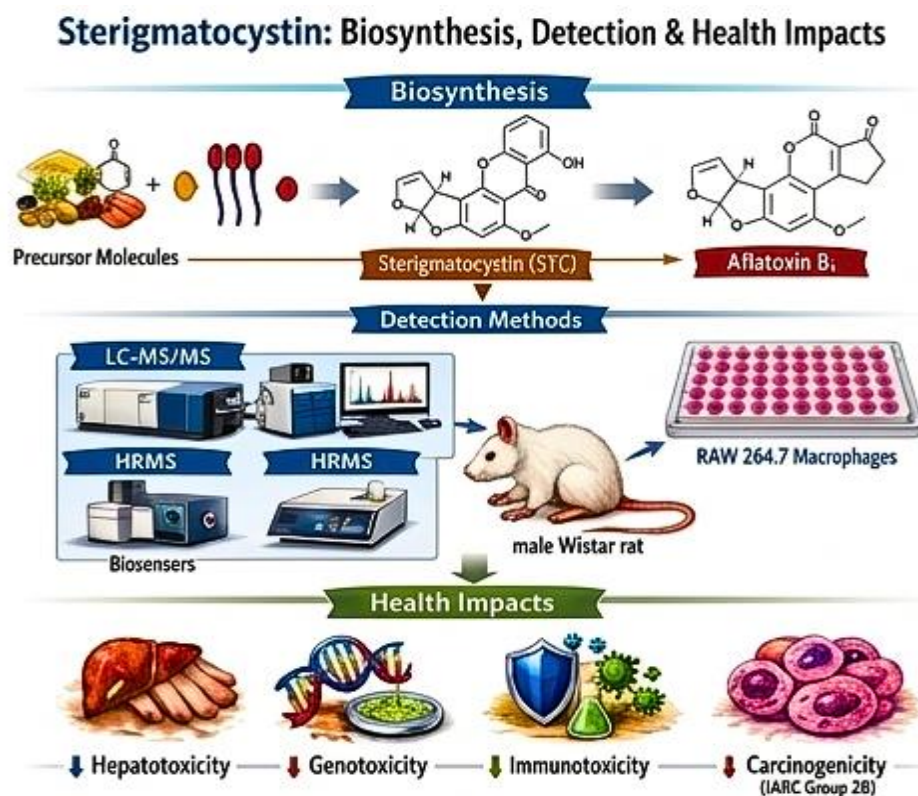


Figure 2: Sterigmatocystin: Biosynthesis, Detection and Health Impacts

Preventive strategy of sterigmatocystin poisoning:

To our knowledge, there is no specific treatment of sterigmatocystin poisoning, although a well-documented case of human poisoning with aflatoxin B₁ (structurally similar to STC) was reported by Ráduly et al, [11]. They stated that “A young woman attempted suicide using purified aflatoxin B₁ (AFB₁), ingesting 5.5 mg over two days, followed six months later by a cumulative dose of 35 mg within two weeks. Despite these exposures, successive diagnostic evaluations, including liver imaging (X-ray and ultrasound) as well as urine and blood analyses, revealed no pathological findings over the years. The absence of clinical symptoms or physiological abnormalities was attributed



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to her robust physical health and adequate nutritional status”.

Until now, there is limited data on the treatment of mycotoxin poisoning and it is still not specific. Therefore, the best strategy for preventing of mycotoxin poisonings is to block the access of mycotoxins to the food and feed chain. E.g. monitoring of the pre-harvest and post-harvest practices that ensure the mycotoxin-free agricultural products [11].

4. Conclusion

STC is an emerging food safety hazard due to its carcinogenicity and broad occurrence in crops, foods, and indoor settings. Although structurally related to aflatoxin B1, STC remains less studied, with limited data on its toxicological mechanisms, metabolism, and long-term effects in humans and livestock. Current evidence highlights its genotoxicity, hepatotoxicity, and nephrotoxicity, as well as its potential role in malaria control and biological interactions with lactic acid bacteria. Advances in analytical methodologies have enhanced detection capabilities, yet regulatory frameworks remain incomplete, particularly for feed. Preventive strategies should prioritize monitoring pre- and post-harvest practices, improve analytical standards, and expand toxicological studies. Ultimately, STC must be considered a critical mycotoxin requiring further investigation to safeguard food security and human health.

ABBREVIATIONS AND ACRONYMS

STC: sterigmatocystin, **IARC:** International Agency for Research on Cancer, **LC-MS/MS:** Liquid Chromatography/Mass Spectroscopy, **HRMS:** High-Resolution Mass Spectrometry, **AFB1:** aflatoxin B1, **AFG1:** aflatoxin G1, **HAS:** human serum albumin, **AOX:** alternative oxidase, **COX:** cytochrome C oxidase, **LOD:** Limit of Detection, **LOQ:** Limit of Quantification, **OMST:** O-methylsterigmatocystin, **HiDi DNA polymerase:** high-discrimination DNA polymerase, **SNP:** single nucleotide polymorphism, **DAD:** diode array, **FD:** Fluorescence Detector, **MS:** Mass Spectrometry, **QTOF:** Quadrupole Time-of-Flight Mass Spectrometry, **ELISA:** Enzyme-Linked Immunosorbent Assay, **FRET:** Förster Resonance Energy Transfer, **IC₅₀:** Half Maximal Inhibitory Concentration, **LAB:** Lactic acid bacteria, **HAS:** human serum albumin, **GSH:** glutathione, **CYP:** Cytochrome P450 superfamily.

ETHICAL APPROVAL

Not Applicable

CONFLICT OF INTEREST

The authors affirm that neither financial interests nor personal relationships compromised the integrity of this work.

AUTHORS' CONTRIBUTIONS

JS: conceptualization of the study, **MSF:** conceptualization, review. **MEOA:** literature review, writing original draft, review and editing.

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Determining the concentrations of some essential elements in powdered infant formula in the Al-Khums region of Libya

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ABSTRACT

Background: Milk is a rich source of protein, fats, and essential minerals, and is considered an ideal food for humans. For infants, breast milk is usually the sole source of nutrition during the first four months of life. The best way to feed an infant is through exclusive breastfeeding for the first six months, followed by breastfeeding with the introduction of complementary foods after six months. In pharmacies, parents have complete faith in the nutritional claims printed on infant formula cans. **Amis:** This study aimed to verify whether the contents of the cans matched these claims by determining the concentrations of certain essential elements in five brands of powdered infant formula sold in pharmacies in Al-Khums, Libya in 2025, and their compliance with international standards.

Methods: Subjected five brands to rigorous testing, going beyond visual inspection to include the use of stringent analytical techniques. These techniques ranged from drying and burning to assess physical stability to flame spectroscopy and titration to determine mineral content.

Results: Despite the stable physical structure, with a moisture content of 4.90% and an ash content of 2.90%, the electrolyte analysis revealed a concerning imbalance. A severe and persistent potassium deficiency was observed, accompanied by sharply elevated sodium levels exceeding acceptable limits.

Conclusion: Although calcium and iron levels remained within acceptable ranges, this electrolyte imbalance poses a hidden risk to the infants' kidney health, necessitating immediate regulatory intervention and an update to laboratory testing procedures.

Keywords: food safety, mineral content, electrolytes, label compliance, and powdered infant formula (PIF)

1. Introduction

Infant formula is not merely a food; it is a complete biological support system during the most rapid growth phase of human life. Because of this crucial role, international organizations such as ESPGHAN and the Codex Alimentarius Commission have established strict, non-





negotiable standards [1, 2]. Since these regulations demand absolute precision, any tampering or labeling error becomes a serious public health issue [3]. Maintaining quality means mastering the balance between moisture and ash content. Excess water in the powder allows bacteria to grow and leads to Maillard reactions, which spoil the product [4]. Ash content, on the other hand, acts as a mirror to the integrity of the minerals when cow's milk is modified for human consumption [5]. While the legally permissible margin of error is between 10% and 20% [6]. Exceeding this limit is a clear indication of quality control failure. Potassium (K) deficiency can lead to heart and muscle problems [7]. While excess sodium (Na) puts a baby's fragile kidneys under severe stress [8]. Recognizing that the accuracy of calcium (Ca) and iron (Fe) ratios is just as important for building bones and blood [9]. This study examined five brands in Al-Khums city in 2025 to see how well their reality matched their claims.

Aims:

This study aimed to determine the concentrations of some essential elements in five types of powdered infant formula sold in pharmacies in Al-Khums city, Libya in 2025 and their compliance with international standards.

2. Methods

2.1. Sample Collection and Preparation

The field study by collecting samples from various pharmacies in Al Khums, Libya at the end of 2025. Five infant formula brands, one sample of each formula brand (intended for infants aged 0-6 months), were randomly selected. Data for each sample were meticulously documented. Relevant information including: brand name and expiration date, were recorded before transferring the samples to a dry, Sealed environment for laboratory analysis. All containers were verified to be factory-sealed to ensure accuracy.

Table 1: Brand names and country of origin of the selected infant formula samples.

Sample. No	Brand Name	Country of Origin
1	Mamilac	Poland
2	Milucky	Belgium
3	Cutebaby	Denmark
4	Babelac	France
5	Bevamil	Holland

2.2. Methods of Analysis

The tests were conducted in the Inorganic Chemistry Laboratory, Department of Chemistry, Faculty of Science. The researchers followed the strict scientific recommendations of the International Organization for Accreditation of Chemicals (AOAC International, 2020).

Statistical Analysis: the analysis of each sample was performed in triplicate. The results were analyzed using Microsoft Excel and the results obtained were presented as the mean values \pm standard deviation.

Determination of moisture content (%): We adopted the oven drying method, subjecting the samples to temperatures up to ($105^{\circ}\text{C} \pm 2^{\circ}\text{C}$) until the weight stabilized, to accurately measure the amount of material lost [10].

Determination of ash content (%): Our study used the dry incineration technique; the organic materials were completely thermally oxidized inside a muffle furnace at a temperature approaching 550°C for approximately four hours until the residue was carbon free and appeared grayish-white [11].





Measurement of mineral salts (sodium and potassium): The present study employed a Flame Photometer (Model 410, Sherwood Scientific Ltd, Cambridge, UK), A technique that relies on the excitation of alkali metal atoms by the heat of a flame, then measuring the intensity of the emitted light, which is directly proportional to the concentration of the element within the sample [12].

Determination of calcium (Ca): We applied the titration method with the EDTA complex, which is a volumetric analysis process characterized by the neutralization of magnesium ion interference at a high pH, resulting in the formation of a stable complex between calcium and EDTA. To visually determine the endpoint, the murexide reagent (ammonium purpurate) was used, which provides conclusive colorimetric evidence of the reaction's completion. The color transition from pink-red to deep violet indicates the successful complexation of calcium ions with EDTA [13].

Determination of iron (Fe): The process begins by reducing iron to its divalent form (Fe^{2+}), then reacting it with an organic reagent (such as 1,10-phenanthroline) to form a highly colored compound. By comparing the absorption of this compound at 510 nm with a standard curve prepared from a high-purity material such as Mohr's salt, we were able to determine the amount of iron with an accuracy of up to mg/100 g using visible and ultraviolet spectroscopy [14].

3. Results and Discussion

The tables presented summarize the results of laboratory analysis of the five powdered infant formula samples, compared to the values stated on their labels (mg/100g).

Table 2: Physicochemical Parameters (Moisture and Ash %)

Sample	Moisture (%)	Ash (%)
1	4.90 ± 0.08	2.86 ± 0.04
2	4.85 ± 0.07	2.91 ± 0.05
3	2.80 ± 0.04	2.94 ± 0.03
4	1.87 ± 0.03	2.90 ± 0.05
5	2.83 ± 0.05	2.88 ± 0.04



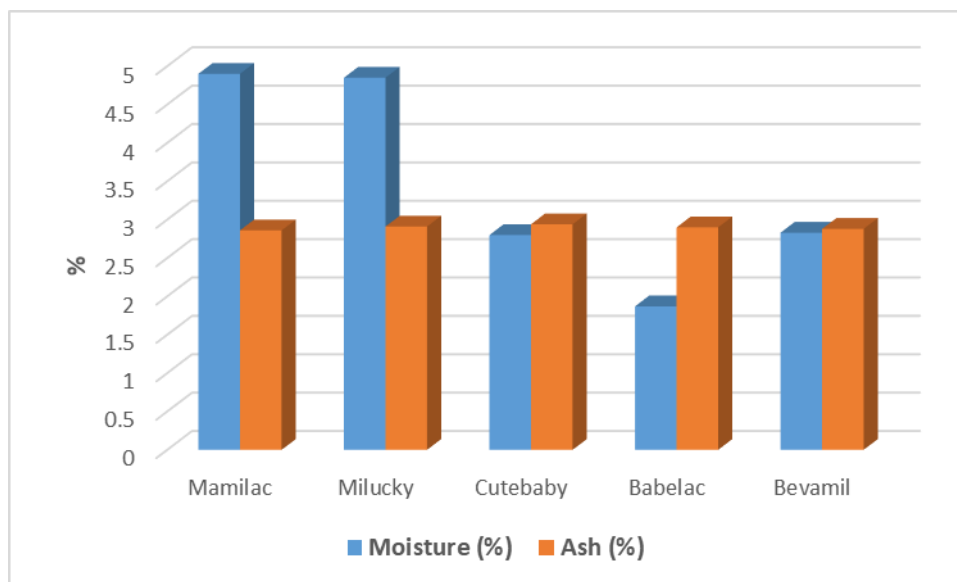


Figure 1: Physical and chemical parameters (moisture and ash %).

Table 3: Comparison of measured and named concentrations of some minerals in (mg/100g).

Sample	Na (In Sample)	Na (On Label)	K (In Sample)	K (On Label)	Fe (In Sample)	Fe (On Label)	Ca (In Sample)	Ca (On Label)
1	176.9±1.2	150	343.82 ±2.5	500	4.75 ±0.15	5.1	387.4 ±3.1	370
2	147.5±1.1	158	320.89 ±2.1	511	3.56 ±0.12	3.3	407 ±3.5	425
3	196.7±1.5	150	369.03 ±2.8	520	4.27 ±0.18	4.2	410 ±2.9	380
4	167.2±1.0	159	334.65 ±2.3	517	3.26 ±0.11	3.9	442 ±3.8	448
5	186.0±1.3	185	311.73 ±2.0	500	4.75 ±0.14	4.2	500.5 ±4.2	485

Note: All mineral concentrations are expressed (mg/100g).

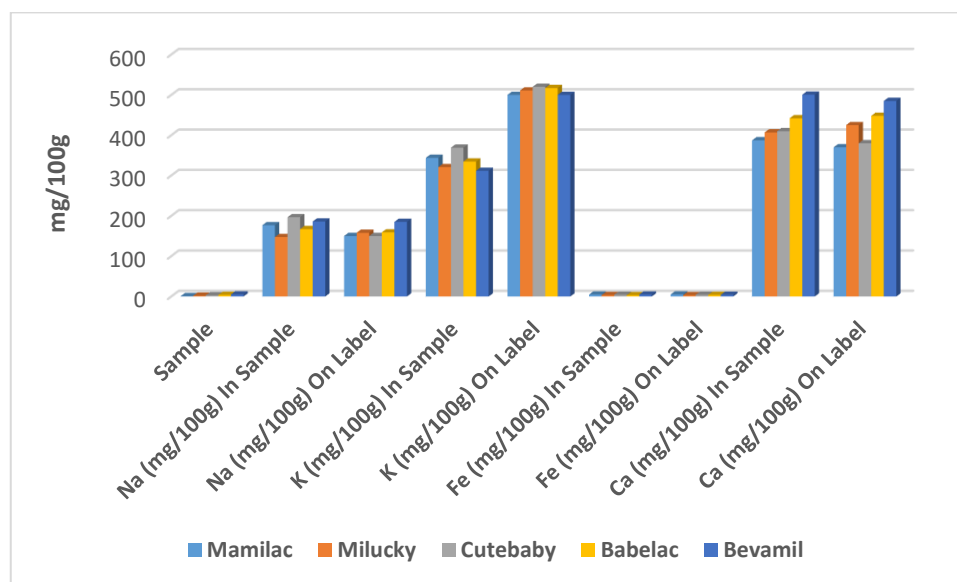


Figure 2: Comparison of measured and named concentrations of some minerals in (mg/100g).

Chemical and Physical Substances

Moisture measurements revealed a range between 1.87% and 4.90%, results generally consistent with international standards requiring moisture below 5.0% [1]. However, Table (2) shows that the first two samples (4.90% and 4.85%) are just above the critical limit. This means that any storage in a high-humidity environment could cause these percentages to exceed the permissible limit, accelerating quality deterioration and shortening the product's shelf life [4]. The ash content (2.86% to 2.94%) indicates stability and consistency in the mineral composition and manufacturing processes across different brands [11].

Mineral Content

We observed a discrepancy between the measured minerals and those declared in Figure (2) and Table(3) .

A. Potassium (K): Potassium is the leading intracellular element responsible for nerve impulse transmission and muscle contraction [15]. Therefore, any severe deficiency negatively impacts the infant's electrolyte balance, exposing them to health risks such as hypokalemia [7]. Potassium content of infant formulas set in Codex Alimentarius range between 60 to 160 mg/100 kcal about 399.6 to 1065.6 mg/100g. Alarmingly, all five samples showed a clear deficiency, exceeding the acceptable margin of variation (10%-20%) [6]. the scales fell to (311.73 to 369.03 mg/100g), while the labels indicated levels between 500 and 520 mg/100g.

These findings are consistent with Almeida et al [16]. who evaluated mineral compliance in infant formulas using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). In their study, potassium levels ranged from 394 to 1018 mg/100g, yet they reported significant cases of non-compliance where laboratory values were lower than label claims, these discrepancies, similar to the low levels found in this study, suggest potential issues in mineral stability during storage."

B. Sodium (Na): Elevated sodium levels directly increase the renal salt load on the infant's delicate systems, raising the likelihood of



dehydration due to hypernatremia [8]. Sodium content of infant formulas set in Codex Alimentarius range between 20-60 mg/100 kcal about 133.2 to 399.6mg/100g. Our studies have shown that the sodium content often exceeds the figures printed on the cans [16]. this was clearly evident in samples (1) and (3) with a significant difference. This consistent increase points to the accuracy of the mineral fortification processes during manufacturing [17].

C. Iron (Fe) and Calcium (Ca): In contrast to its counterparts, the iron and calcium contents showed remarkable consistency. The iron concentrations were consistent with the stated values, indicating the product's ability to fulfill its role in preventing anemia [9]. Iron contents of infant formulas set in Codex Alimentarius range between 0.3 to 1.3 mg/100 kcal about 1.99 to 8.66 mg/100g. The results, as shown in Figure 2 and Table 3, proved that the calcium values showed only slight differences from the labeled values, all within commercially permissible limits. Calcium contents of infant formulas set in Codex Alimentarius range between 50 to 140 mg/100 kcal about 333 to 932.4 mg/100g. Sample No. 5, which recorded 500.5 mg compared to the mentioned 485 mg, demonstrates the ability of manufacturers to accurately control calcium levels, calcium being an important element for bone growth in children.

4. Conclusion

Given the critical importance of infant formula as the sole source of nutrition, international organizations have established stringent standards for its composition. Our independent evaluation confirmed the effectiveness of quality control measures regarding chemical and physical stability. However, our study revealed significant variations in essential minerals, particularly a marked increase in sodium (Na) and a general deficiency in potassium (K). This dietary pattern poses a direct and serious threat to the body's mineral balance and kidney health in infants, necessitating immediate regulatory measures to ensure that products in Libya comply with international standards. Furthermore, it is crucial to emphasize that storage conditions, transportation methods, and the precision of analytical instrumentation play a pivotal role in the final quality and nutritional integrity of the milk.

ETHICAL STATEMENT:

Not Applicable.

CONFLICT OF INTEREST

There is no conflict of interest to be declared.

AUTHORS' CONTRIBUTIONS

R.E. and E.A.: organising, conducting research, composing the first draft, and editing and rewriting it. S.E.: Text formatting, editing, and revision techniques. H.A., H.B., and E.S.: Research, resources, and lab work.

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Molecular Docking Analysis of Bioactive Compounds from *Lepidium sativum* as Potential Breast Cancer Inhibitors Using AutoDockVina

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ABSTRACT

Breast cancer is a complicated and multidimensional disease that has emerged as one of the world's top causes of death for women, which emphasises the significance of developing novel and efficient treatments. Molecular docking has become an essential part of drug discovery and facilitating the identification of possibilities of therapeutic agents, as it predicts the binding affinities of the therapeutic agents to the target proteins. A molecular docking analysis of 10 bioactive compounds of *Lepidium sativum* with the target protein of tyrosine kinase (Protein Data Bank (PDB) ID: 1M17) was done using AutoDock Vina to identify potential breast cancer inhibitors. The top 4 compounds identified that displayed high binding affinity to the target breast cancer protein were stigmasterol (-9.6 kcal/mol), 1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea (-8.4 kcal/mol), semilepidinside (-7.7 kcal/mol), and delta-tocopherol (-7.4 kcal/mol). Through detailed analysis of binding poses, the molecular interaction reveals that these compounds exhibited different interactions such as hydrogen bonding and hydrophobic interactions with the essential amino acids in the binding pocket. These results propose the identified phytochemicals in *Lepidium sativum* as possible potential candidates as breast cancer inhibitors. Nevertheless, additional in vitro and in vivo experiments are required to confirm those computational results and investigate their therapeutic possibilities.

Keywords; Breast cancer, Molecular docking, Lepidiumsativum, drug discovery

1. Introduction

Breast cancer ranks among the most frequently diagnosed cancers globally and is a primary contributor to morbidity and mortality. GLOBOCAN 2020 reported around 2.3 million new cases globally, underscoring its extensive prevalence. It represents a substantial public health challenge across many demographics and constitutes about 25% of all cancer cases in women, rendering it the most often diagnosed cancer among females. (1). The death rates are significantly high, approximately 88% in the areas with socioeconomic transitions (Melanesia, Western Africa, and the Caribbean) rather than the fully developed ones, such as Australia, Western Europe, and Northern America. The early detection and prevention approaches, using screening programmes, are critical to minimising the occurrence of breast cancer and enhancing treatment results. The Breast Health Global Initiative (BHGI) is the organization that spearheads the attempts to formulate international standards in managing breast cancer (2). The rates vary according to geographical area and are higher in the developed countries





countries, where access to screening programs and healthcare resources is more prevalent. Although breast cancer mostly occurs in women, 1 percent of the cases are in males. The risk of the disease is correlated with the ages: the diagnoses begin to be more common after 50 years old, but younger women and even adolescents can be occasionally diagnosed with the disease. Certain health care interventions will be improved by an understanding of these epidemiological trends (3). Breast cancer risk factors can be divided into non-modifiable (e.g., genetic mutation: BRCA1, BRCA2), gender, age, and personal cancer history; and modifiable (e.g., obesity, alcohol, and hormone replacement therapy (HRT)). These risk factors are identified, which helps in the effective prevention and risk assessment. Several molecular subtypes of breast cancer that are different in their profile include hormone receptor-positive (HR+), epidermal growth factor receptor tyrosine kinase domain (EGFR TKD), and triple-negative breast cancer (TNBC). Each subtype informs specific treatment strategies and has varying prognostic outcomes (4,5) which means that understanding these differences is crucial for tailoring individualised treatment plans for patients with breast cancer. *Lepidiumsativum* (garden cress) is an annual herb that belongs to the *Brassicaceae* family and is known to have medicinal effects, particularly in areas such as South Asia. Garden cress has traditionally been used in the management of various conditions, including bronchitis, asthma, and rheumatism, and is similarly valued as a diuretic, digestive aid, and mild laxative due to its high content of phytochemicals such as alkaloids, glucosinolates, and flavonoids (6). In India, Cassia cress seeds, leaves, and roots are similarly utilized in the management of health conditions, where they serve as anti-inflammatory agents, galactagogues, and blood purifiers (7). *Lepidiumsativum* has demonstrated anticancer, antimicrobial, antioxidant, anti-asthmatic and diuretic effects. Research has shown that it has potential in causing cancer cell apoptosis and combating bacterial infections, in addition to eliminating free radical scavenging (8–10). Molecular docking is an important tool in drug discovery that allows the interaction between the ligand and the receptor to be simulated in predicting the binding affinities and drugs working mechanisms. Recent developments such as reverse molecular docking give more insights into drug-target interactions that can prove to be more efficient in undertaking pharmacological evaluations (11,12). AutoDockVina (13) is the latest update version of a molecular docking and virtual screening application, with reliable precision in predicting ligand–receptor interactions. AutoDockVina is significantly faster than its predecessor, AutoDock 4, with reported speed improvements of up to two orders of magnitude (approximately 100-fold), depending on the system and computational settings. AutoDockVina is more precise when predicting binding modes, as confirmed by the training set on which it was tested first on AutoDock 4. It uses multi-core systems to use Multithreading capabilities to increase speed. AutoDockVina makes the user experience easier by taking care of grid map computations and sorting its results automatically (14, 15). The study aims to utilize *in silico* molecular docking techniques and apply them to identify and evaluate potential inhibitors obtained from the *Lepidium sativum* plant against EGFR TKD, followed by interaction analysis to identify promising lead compounds for breast cancer therapy.

2. Materials and Methods

2.1 Protein preparation

The three-dimensional crystal structure of the Epidermal Growth Factor Receptor tyrosine kinase domain (EGFR TKD) (PDB ID: 1M17) (16) was retrieved from the RCSB Protein Data Bank. The protein structure was prepared using AutoDockTools (ADT) (ADT) (17) as shown in Figure 1. The protein structure was prepared using AutoDockTools (ADT) v1.5.6. All non-essential water molecules and co-crystallized ligands were removed. Polar hydrogen atoms were added, and Gasteiger charges were computed. The prepared protein structure was saved in the PDBQT format for subsequent docking analysis. Chain B was selected for this study.



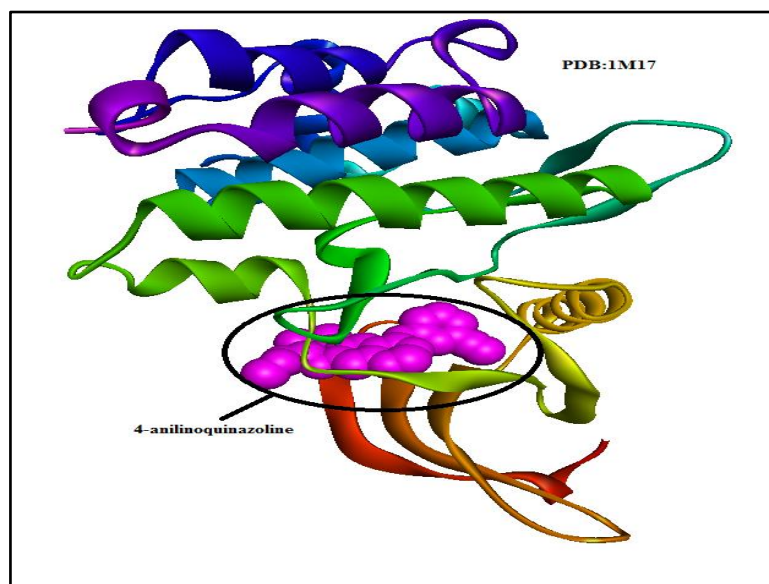


Figure 1. Represent the 3D structure of the tyrosine kinase (Protein Data Bank (PDB) ID: 1M17)

2.2 Ligand preparation

The 2D structures of ten bioactive compounds previously identified in *Lepidium sativum* were obtained from the PubChem database. The structures were converted to 3D format, and their energies were minimized using appropriate computational chemistry tools. The prepared ligands were then saved in PDBQT format, with rotatable bonds defined to allow conformational flexibility during docking.

Ten bioactive compounds were identified in *Lepidium sativum* and their 3D structures were from the PubChem (18) database in SDF format and then converted to PDB to be used in molecular docking using Discovery studio visualiser. The energy was minimised using Avogadro software, and the rotatable bonds are defined to allow conformational flexibility during docking.

2.3 Molecular docking and Grid Generation

Molecular docking simulations were performed using AutoDockVina v1.2.3. A grid box was defined to encompass the active site of the 1M17 protein, ensuring that the ligand search space was centred on the known binding pocket. The grid box was set with dimensions of $22.5 \times 22.5 \times 22.5$ Å and centred at coordinates $x = -23.368$, $y = 29.808$, and $z = 7.596$. Vina's search algorithm explored various ligand conformations, and the pose with the lowest free energy of binding (FEB) was selected as the most probable binding mode.

2.4. Docking Protocol Validation

To validate the accuracy and reliability of the docking protocol, the co-crystallized ligand (4-anilinoquinazoline) was extracted from the complex (PDB) ID: 1M17 and re-docked into the protein's active site. The root-mean-square deviation (RMSD) between the predicted pose and the original crystallographic pose was calculated. An RMSD value below 2.0 Å is generally considered an indicator of a successful and

reliable docking protocol.

2.5. Drug-Likeness Analysis

The top-ranked compounds were evaluated for their drug-like properties based on Lipinski's Rule of Five. This rule assesses physicochemical properties critical for oral bioavailability, including molecular weight ($MW < 500$ g/mol), logarithm of the partition coefficient ($xLogP < 5$), number of hydrogen bond donors ($HBD < 5$), and number of hydrogen bond acceptors ($HBA < 10$).

3. Results and Discussion

3.1 Validation of docking procedure

Before conducting molecular docking, we validated the docking protocol by re-docking the co-crystallized 4-anilinoquinazoline into the active site of the target protein (Protein Data Bank (PDB) ID: 1M17) within an acceptable range. In this validation, the pose of the 4-anilinoquinazoline exhibited a similar orientation to the crystallographic pose (RMSD = 0.82 Å), and the binding affinity was -7.2 kcal/mol. Consequently, these results affirm the reliability of the protocol employed, indicating that the docking software can be trusted to accurately reproduce the anticipated binding mode of the co-crystallized ligand as shown in Figure 2.

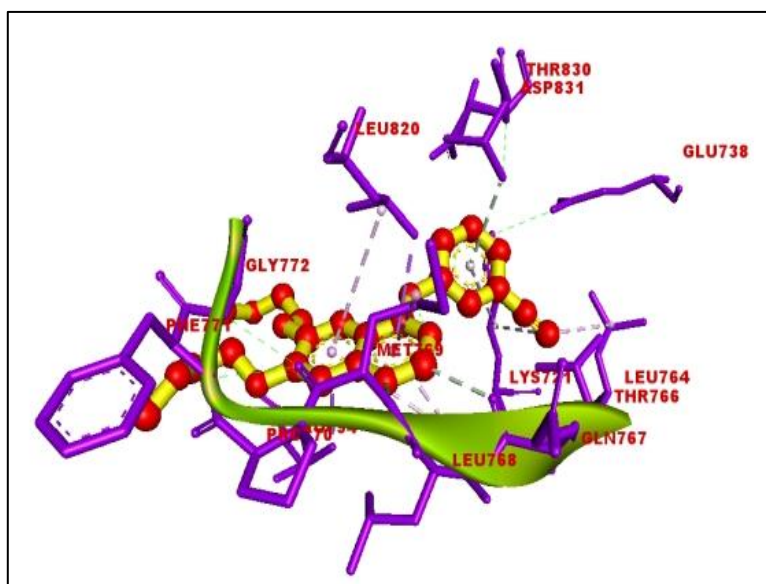


Figure 2. The Superimposition between the docked conformation (yellow) and the crystal structure (Red) in the binding pocket of 1M17 protein.

3.2 Molecular Docking of *Lepidium sativum* Bioactive Compounds

In the initial phase, we gathered a set of 10 compounds from *Lepidium sativum*, as documented in the literature (19,20) as shown in Table 1. Molecular docking using AutoDock Vina was conducted of these compounds against the target protein, and their arrangement was based on the free energy of binding (FEB). Subsequently, we selected the top four compounds that displayed the lowest binding energy.

**Table 1. Name and structure of ten selected compounds from *Lepidiumsativum* plant**

NO	Compounds	AutoDock 4.2 (kcal/mol)
1	stigmasterol	-9.6
2	1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea	-8.4
3	Semilepidinoside A	-7.7
4	Delta tochpherol	-7.4
5	Butylatedhydroxytoluene	-6.9
6	methyl glucosinolate	-6.1
7	Oleic acid	-5.3
8	Eicosenoic acid	-5.2
9	Benzyl cyanide	-5.0
10	Arachidic acid	-4.8

3.3 Free Energy of Binding and Ranking of Docked Compounds

As shown in Figure 3, the 2D structure of the top four bioactive compounds ranked by AutoDockVina scores, these molecules exhibited the lowest FEB among the other compounds in the protein-ligand complex; subsequently, they were applied in the docking calculation. The docking simulation results showed that all the 20 compounds displayed FEB in the range -9.6 to -4.8 kcal/mol. Therefore, the compounds that showed the lowest FEB were considered the best, as shown in Table 2. Therefore, the top 4 ranked compounds were suggested as the most suitable candidates. Stigmasterol, 1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea, semilepidinoside and Delta-tochpherol displayed a minimum FEB of -9.6, -8.4, and -7.7 and 7.4 kcal/mol by AutoDockVina.

Table 2. Molecular properties of the 5 compounds obtained from *Lepidiumsativum* plant

NO	Compounds	xlogP	H-bond donors	H-bond acceptors	MW(g/mol)	Rotatable bonds
1	stigmasterol	8.32	1	1	420.45	6
2	1,3-dibenzyl-1,3-bis[(1R)- 1-benzyl-2-hydroxy- ethyl]urea	5.40	2	3	508.65	14
3	Semilepidinoside A	-0.43	5	7	336.34	5
4	Delta tochpherol	9.97	2	1	402.65	12



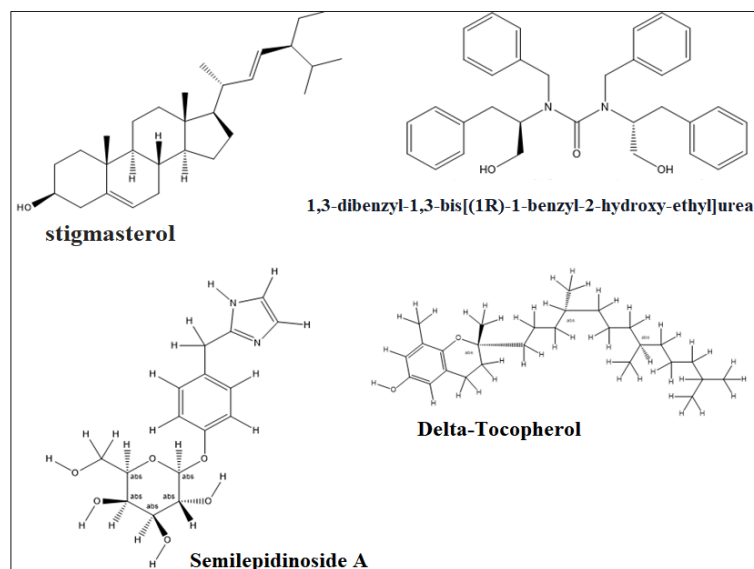


Figure 3. four compounds from *Lepidiumsativum* Stigmasterol , 1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea, Semilepidinoside A and Delta tochpherol.

3.4 Drug Likeness and Lipinski's Rule of Five Analysis

To further assess their drug-likeness properties, we applied Lipinski's Rule of Five (21), which considers specific molecular properties in comparison to approved drugs. This rule dictates that potential drug candidates should not exceed one violation of the following criteria: $\text{ALogP} < 5$, molecular weight < 500 , number of hydrogen bond donors (HBD) < 5 , number of hydrogen bond acceptors (HBA) < 10 , and rotatable bonds < 10 . Notably, all three selected compounds show some violation from the acceptable range defined by the Lipinski rule, as shown in Table 2. This rule is used to determine the drug-likeness properties should be no more than one violation of the following criteria: $\text{xLogP} < 5$, molecular weight < 500 , number of HBD < 5 , number of HBA < 10 , and rotatable bond < 10 . As can be seen from Table 2, the three compounds fall within the acceptable range of the Lipinski rule; the stigmasterol compound violates only one rule, 1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea compounds violates the rule of 5 in three rules, semilepidinside compound was ompounds violate the rule of 5 , Delta-tochpherol compound violates two rules.

3.5 Molecular Interaction Analysis of Top Ranked Compounds

3.5.1 Interaction Profile of Stigmasterol

The compound stigmasterol exhibited various interactions as follows: Five hydrogen bonds were formed, with four of them involving amino acids Ser144, Cys145, Gly143, Leu141, and the oxygen atom O2. The fifth hydrogen bond was established between Arg188 and another oxygen atom (O₂) on the compound. Three Pi-Alkyl bonds were observed, with amino acids His41, Cys145, and Pro168 forming bonds with the benzene ring and methyl group, respectively. A Pi-Sulphur bond was exhibited by His163 with a benzene ring on the compound.

Hydrophobic interactions occurred with amino acids Thr190, Pro168, Gln189, Glu166, Arg188, Met165, Asn142, and His41 at the binding pocket. Van der Waals interactions were noticed between Thr190, Gln192, Glu166, and Asn142, and the carbon atoms C-1, C-5, C-11, and C-14 of the compound. A carbon-hydrogen bond was formed between the amino acid Gln189 and the benzene ring as shown in Figure 4,5, Table 3).

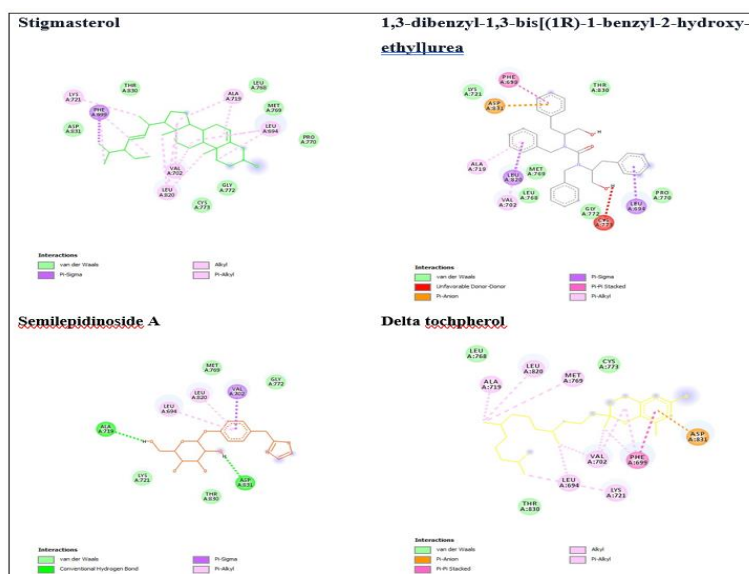


Figure 4. 2D of Binding modes of the selected compounds at breast cancer binding site.

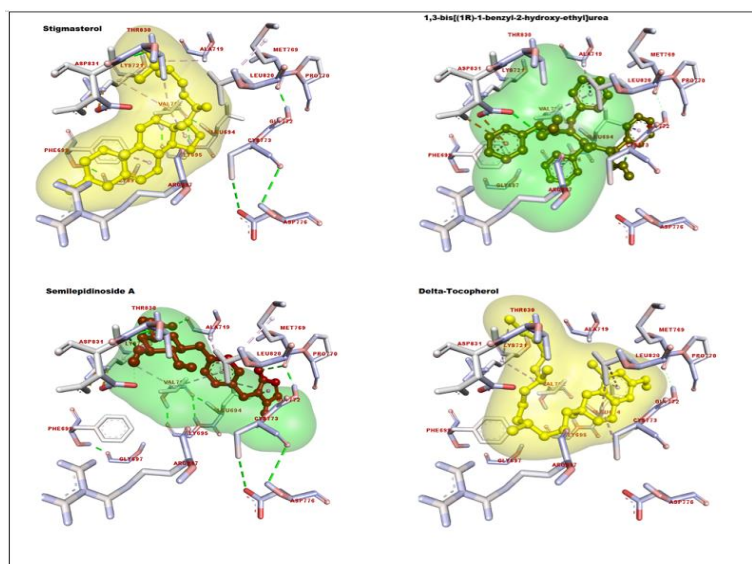


Figure 5. 3D of Binding modes of the selected compounds at breast cancer binding site



Table 3. Binding interactions details of the Potential ten compounds docked into active site

No	Ligands	AutoDockVina (kcal/mol)	Residue	Type of interactions
1	Stigmasterol	-9.6	Cys773, Gly772, Pro770, Met769, Leu768, Thr830, Asp831 Phe699 Leu820, Val207, Leu649 Ala719, Lys721	Van der waals Pi-Sigma Alkyl Pi-Alkyl
2	1,3-dibenzy	-8.4	Leu768, Gly772, pro770, Thr830 Lys721 Leu694, Leu820 Ala719, Val702 Phe699 Asp831 Cys773	Van der waals Pi-sigma Pi-Alkyl Pi-Pi stacked Pi-anion Unfavorable donerdoner
3	Semilepidinside	-7.7	Ala719, Asp831 Lys721, Thr830, Gly772, Met769 Val702 Leu820, Leu694	Conventional Hydrogen bond Van der waals Pi-sigma Pi-Alkyl
4	Delta-tocopherol	-7.4	Leu694, Lys721, Val702 Thr830, cys773, Leu768 Asp831 Phe699, Met769, Leu694, Ala719	Alkyl Van der waals Pi-anion Pi-pi stacked Pi-alkhyl

3.5.2 Interaction Profile of 1,3 Dibenzy 1,3 Bis[(1R) 1 Benzyl 2 Hydroxy Ethyl]urea

The compound 1,3-dibenzy 1,3-bis[(1R)-1-benzyl-2-hydroxy-ethyl]urea was found to exhibit the following interactions: Van der Waals interactions with the amino acids Leu768, Gly772, Pro770, Thr830, Lys721, and Asp831. Pi-sigma bonds formed interactions between the benzene ring and Leu694 and Leu820. Alkyl and Pi-Alkyl bonds interacting with Ala719 and Val702. Pi-Pi stacking interactions observed between the benzene ring and Phe699. Pi-anion interactions involving the benzene ring and Asp831. Unfavourable donor-donor bonds between the amino acid Cys773 and the alcohol group are shown in Figure 4,5, Table 3).

3.5.3 Interaction Profile of Semilepidinside

The compound Semilepidinside displayed the following interactions: Conventional Hydrogen bond interactions between the alcohol group and Ala719 and Asp831. Van der Waals interactions with the amino acids Lys721, Thr830, Gly772, Met769. Pi-sigma interactions between the benzene ring and Val702. Pi-Alkyl bonds are formed with the benzene ring and Leu820, Leu694 as shown in (Figure 4,5, Table 3).

3.5.4 Interaction Profile of Delta Tocopherol

Additionally, the compound delta-tocopherol demonstrated the following interactions: alkyl and Pi-Alkyl bonds formed with Leu694, Lys721, Val702, Met769, Leu694, and Ala719. Pi-anion interactions between the benzene ring and Asp831. Pi-pi stacking interactions





observed between the benzene ring and the amino acids Phe699. Van der Waals interactions also formed with the amino acids Thr830, Cys773, and Leu768.

3.6 Implications of Molecular Docking in Natural Product Based Drug Discovery

Molecular docking is considered one of the recent procedures used in drug discovery among numerous sources, especially natural products like medicinal plants. It has been shown to have a major role in the discovery of possible therapeutic agents against different diseases as demonstrated in numerous studies. Molecular docking was used in this research to evaluate bioactive compounds in *Lepidium sativum* as potential breast cancer target inhibitors. The objective of this work aims at discovering promising compounds that have a high binding affinity to the target protein. The results obtained suggest that the applied methodology is consistent with the study objective.

4. Conclusion

In the current study, molecular docking and molecular interaction analysis were effectively used in search of potential inhibitors of the 1M17 protein, a key target in breast cancer treatment. The four compounds of stegmasterol, 1,3-dibenzyl-1,3-bis[(1R)-1-benzyl-2-hydroxyethylurea, semilepidinside, and delta-tocopherol were found to have high affinity with the 1M17 binding pocket. Free energy of binding (FEB) was their strong binding affinity (-9.6, -8.4, -7.7, and -7.4 kcal/mol). The selected compounds exhibited different positive interactions, such as hydrogen bonds, hydrophobic and pi-connected interactions, and van der Waals forces with the key amino acid residues at the binding pocket. The most promising docking hits had gone past the Lipinski rule of five and probably become oral active drug. Molecular docking could be used to drastically reduce the cost of the drug synthesis and production. Some of the ingredients of *Lepidium sativum* have the potential to be used as a cancer inhibitor at the molecular level of breast cancer. The significance of this finding is that it underscores the importance of utilizing molecular docking as an initial tool in identifying drugs derived from natural product. The compounds can thus only undergo further *in vitro* and *in vivo* studies to confirm anticancer activity, safety and pharmacokinetic behaviour before being considered potential therapeutic agents.

ETHICAL STATEMENT:

This research has been done completely through computational methods and publicly accessible data. The software tools that are in use are all freely accessible to use with academic and non-commercial purposes. In this work did not involve human or animal subjects hence no ethical approval was needed.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

A.A.A.A. conceived and designed the study, supervised, extracted and analyzed the data, and finalized the manuscript. A.A.A., R.E.A., Z.M.I., N.E.A., and H.M.R. conducted the molecular docking experiments and wrote the initial draft of the manuscript. All authors read and approved the final manuscript.





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Insulin Resistance and Glycaemic Markers in a Sample of Non-Alcoholic Fatty Liver Disease Libyan Patients

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disorder worldwide, mainly associated with obesity and insulin resistance. **Aims:** This study aimed to evaluate the association between NAFLD and insulin resistance. **Methods:** A comparative cross-sectional study was conducted at Zliten Medical Centre (ZMC) from February to August 2025. It included 85 participants (55 with NAFLD and 30 healthy controls). Age, gender, Body mass index (BMI), fasting blood glucose (FBG), HbA1c (glycated hemoglobin), and serum insulin were measured. Insulin resistance was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index. **Results:** Patients with NAFLD had significantly higher BMI, FBG, HbA1c, serum insulin, and HOMA-IR compared to controls. Mann–Whitney U tests showed significant differences in HOMA-IR, HbA1c. Receiver Operator curve (ROC) analysis indicated excellent diagnostic accuracy for insulin and HOMA-IR (AUC) (Area under curve) ≈ 0.88). Regression analysis revealed that insulin and HOMA-IR were positively associated with NAFLD risk. The linear regression analysis revealed that among metabolic parameters in NAFLD group, HbA1c had a significant association with HOMA-IR, while BMI showed weak or non-significant relationships; the logistic regression model showed an excellent accuracy (AUC = 0.998), indicating strong prediction ability for NAFLD compared to previous studies. **Conclusion:** Insulin resistance plays a pivotal role in fatty liver development, that could be concluded from elevated insulin, HOMA-IR, and impaired glycaemic control. higher BMI, further highlighting the underlying metabolic abnormalities. Insulin and HOMA-IR may be used as clinical markers for identifying high-risk individuals. Early diagnosis and lifestyle modification targeting insulin resistance are recommended.

Key words: NAFLD, insulin resistance, HOMA-IR, Libya

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), also known as hepatic steatosis, is a common chronic liver disease characterized by fat deposition within the liver, which may lead to insulin resistance and other metabolic disturbances (1). Understanding this relationship between NAFLD and insulin resistance is important for early diagnosis, prevention, and management of related metabolic disorders.

NAFLD can progress to non-alcoholic steatohepatitis (NASH), which may advance to cirrhosis and hepatocellular carcinoma (2). Metabolic syndrome—comprising obesity, insulin resistance, dyslipidaemia, and hypertension—is closely associated with NAFLD pathogenesis (3).

The hyperinsulinemic-euglycemic clamp is the gold standard for measuring of insulin resistance (4), but, surrogate indices such as Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI) are widely





used in large-scale studies due to their practicality (5). Insulin resistance impairs hepatic glucose suppression and peripheral glucose uptake, leading to hyperglycaemia and hyperinsulinaemia.

Hepatic steatosis is defined by triglyceride accumulation in more than 5% of hepatocytes or >55 mg/g of liver tissue (6). Progression to NASH involves inflammation, hepatocyte injury, and fibrosis, which may result in cirrhosis and increased hepatocellular carcinoma risk (7). Although liver biopsy still considered as confirmed diagnostic tool for the disease, its invasiveness makes it undesirable. Histological examination can provide additional insight into the degree of steatosis and its correlation with insulin resistance markers (8).

Due to the increasing prevalence of NAFLD and its correlation with insulin resistance, investigations and understanding of this relationship is required for early diagnosis and guiding effective preventive and therapeutic strategies.

1.1. Aims:

The aim of this comparative cross-sectional study was to evaluate the prevalence of insulin resistance in patients diagnosed with NAFLD. The research focused on identifying glycemic alterations in patients with NAFLD compared to healthy individuals, aiming to understand the potential role of insulin resistance and glycemic parameters in disease progression.

2. Methods

2.1 Study Participants

The study was carried out at Zliten Medical Hospital and included 55 patients with NAFLD who attended the hospital in the period from February to June 2025. All cases underwent a comprehensive assessment which included clinical history taking, physical examination, and liver ultrasonography to evaluate age, gender, and disease status

The control group of 30 healthy individuals, free from liver disease based on physical examination, was recruited and followed the same study protocol.

2.2.1 Inclusion Criteria

The study enrolled adults aged 18 to 65 years with a confirmed diagnosis of hepatic steatosis via abdominal ultrasonography. Participants were required to have no prior history of chronic liver diseases other than NAFLD to ensure the specificity of the metabolic findings. Finally, all subjects provided verbal informed consent prior to their participation in the clinical evaluation

2.2.2 Exclusion Criteria

Participants were excluded if they have other chronic liver conditions, such as viral hepatitis, autoimmune hepatitis, or cirrhosis. Individuals currently using medications that alter lipid metabolism or insulin level, such as statins, metformin, antidiabetic agents, or hydrocortisone therapy, were also excluded. Moreover, the study excluded any case with pregnancy, lactation, and the presence of severe comorbidities, such as malignancy or chronic kidney disease.

3. Materials

3.1 Equipment and Apparatus

The equipment used in this study are listed in Table1.





Table 1. Equipment and Apparatus

Equipment	Company (origin)
Cobas c 311 (biochemical analysis & HbA1C)	Roche - Switzerland
Labofuge-400	Roche - Switzerland
Pipette-200-500 µL	Heraeus - Germany
InBody 770	Eppendorf - Germany
Digital height and weight scale	Asmid - Turkey
Tubes, EDTA tubes, cotton, alcohol	

3.2 Criteria for NAFLD Diagnosis

The cases with hepatic steatosis were identified using abdominal ultrasound reports provided by the attending physicians. Participants were referred to the outpatient department (OPD) after consultation with either a surgical or internal medicine specialist. Cases were defined as individuals with ultrasonographic evidence of steatosis consistent with NAFLD. To ensure the diagnosis of disease, patients with secondary causes of liver disease—including significant alcohol consumption, viral hepatitis, or drug-induced steatosis—were excluded from the study.

3.3 Determination of BMI

Weight and height were measured by a digital InBody device or a physical scale during blood sample collection.

3.4 Specimen collection

Specimen were collected from all participants using standardized techniques appropriate for each biochemical analysis.

3.5 Insulin Testing and Analysis

Serum insulin was quantified using ECLIA on the Cobas e 411 analyzer following the manufacturer's protocol.

3.6 Glycemic Analysis

Fasting blood glucose, and HbA1C were measured using the Cobas c311 analyzer according to manufacturer instructions.

3.7 Statistical Analysis

The data collected for this study was analysed using SPSS software (version 21). Statistical analysis is imperative for the summarisation and interpretation of data, the evaluation of differences between groups, and the examination of potential correlations among study variables.

A descriptive statistical analysis was conducted for the purpose of obtaining a general description of the study population. The mean, median, and standard deviation of the continuous variables, such as clinical and biochemical measures, were computed for each of the study groups and of the total study cohort. The utilisation of this approach facilitates the estimation of both the central tendency and the variability of the data.

The frequency distributions and percentages were utilised to ascertain the representation of male and female participants in the study, given that categorical variables, such as gender, were employed. These descriptive statistics formed the foundation for the subsequent application of comparative and inferential statistics.

The Shapiro test was employed to evaluate the normality of data distribution, given that the total number of participants was less than 100 (n





= 85). The subsequent analyses employed non-parametric tests due to the indication that the data were not normally distributed.

To compare continuous variables between groups, the Mann-Whitney U test was employed. The comparison of means was evaluated using the chi-squared test, and the coefficient of rank correlation between variables was calculated using Spearman's method. For all statistical tests, a p-value of < 0.05 was considered statistically significant.

The utilisation of inferential statistics has been employed in the context of diagnostic checks, encompassing the evaluation of receiver operating characteristic (ROC) curves.

Furthermore, regression evaluation determined the strength and path of the relationship between observed variables. Predictive linear regression was applied to assess the accuracy of the data in predicting future cases. P-value of equal or less than 0.05 was deemed to be significant

4.Results

4.1 Descriptive Analysis of Data

The study sample was predominantly female, with females making up 88.2% of the total population. In terms of health status; females were over-represented in the healthy category (83.3 %).

4.1.1 Healthy Participants

The descriptive statistics of the healthy participants are summarized as follows.

Table 2. statistical description of healthy participants

Parameter	Mean	Median	Standard deviation
Age	34.13	34.13	9.85
BMI	25.50	25.30	3.49
FBG	98.36	98.65	8.99
HbA1C	5.08	5.09	0.25
HOMA_IR	2.53	2.30	1.27

4.1.2 NAFLD Participants

The descriptive statistics of NAFLD participants are summarized as follows.

Table 3 statistical description of NAFLD participants

Parameter	Mean	Median	SD
Age	35.56	35.00	11.94
BMI	43.39	43.98	9.66
FBG	104.61	97.0	24.98
HbA1C	5.67	5.50	0.71
HOMA_IR	5.95	5.51	6.06





4.1.3 All Participants

For overall study population, the descriptive statistics are summarized as follows.

Table 4 statistical description of all cases

Parameter	Mean	Median	Standard deviation
Age	35.05	35.00	11.21
BMI	37.08	36.58	11.75
FBG	102.40	97.40	20.93
HbA1C	5.46	5.30	0.65
HOMA_IR	5.39	4.06	5.36

4.2 Relationship Between Age and Disease Status

Chi-square test of independence was conducted. The distribution of cases within the three age groups revealed that the youngest group had a higher proportion of healthy individuals, whereas the oldest group had more diseased cases. The middle-aged group showed a nearly balanced distribution. Chi-square test gave a non-significant association between age group and disease status ($P = 0.230$).

4.3 Correlation of HOMA-IR with BMI

The statistics indicate a moderate positive correlation between BMI (mean = 37.08 ± 11.75 kg/m²) and HOMA-IR, suggesting that greater obesity is associated with higher insulin resistance. As shown in Table 5.

Table 5. Correlation of HOMA-IR with BMI.

Parameter	Sig (2-tailed)	Correlation coefficient
BMI	<0.001*	0.510

*Correlation is significant at the 0.01 level (2-tailed)

4.4 Comparison Between NAFLD and Control Groups

Significant differences between NAFLD and control groups were observed for HOMA-IR, Insulin, HbA1c and BMI, whereas FBG expressed non-significant difference between the two groups. As show in Table 6.

Table 6. Comparison Between NAFLD and Control Groups.

Parameter	Sig (P)
BMI	<0.001
HOMA-IR	<0.001
Insulin	<0.001
HbA1C	<0.001
FBG	0.46





4.5 Receiver-Operating Characteristic (ROC) Analysis

One of the most well-known applications of ROC curve analysis is its use as a simple graphical tool for displaying the accuracy of a medical NAFLD. In this study, the predictive power of insulin, biochemical, demographic and anthropometric parameters in diagnosis of disease was evaluated. The sample included 85 cases, 55 were classified as positive cases and 30 as negative cases.

4.5.1 HOMA-IR and Insulin

HOMA-IR and insulin demonstrated high diagnostic performance with AUC values of 0.87 and 0.89, respectively. Both showed 80.0% sensitivity and specificity above 83.2%, indicating strong discriminative ability between NAFLD and healthy participants.

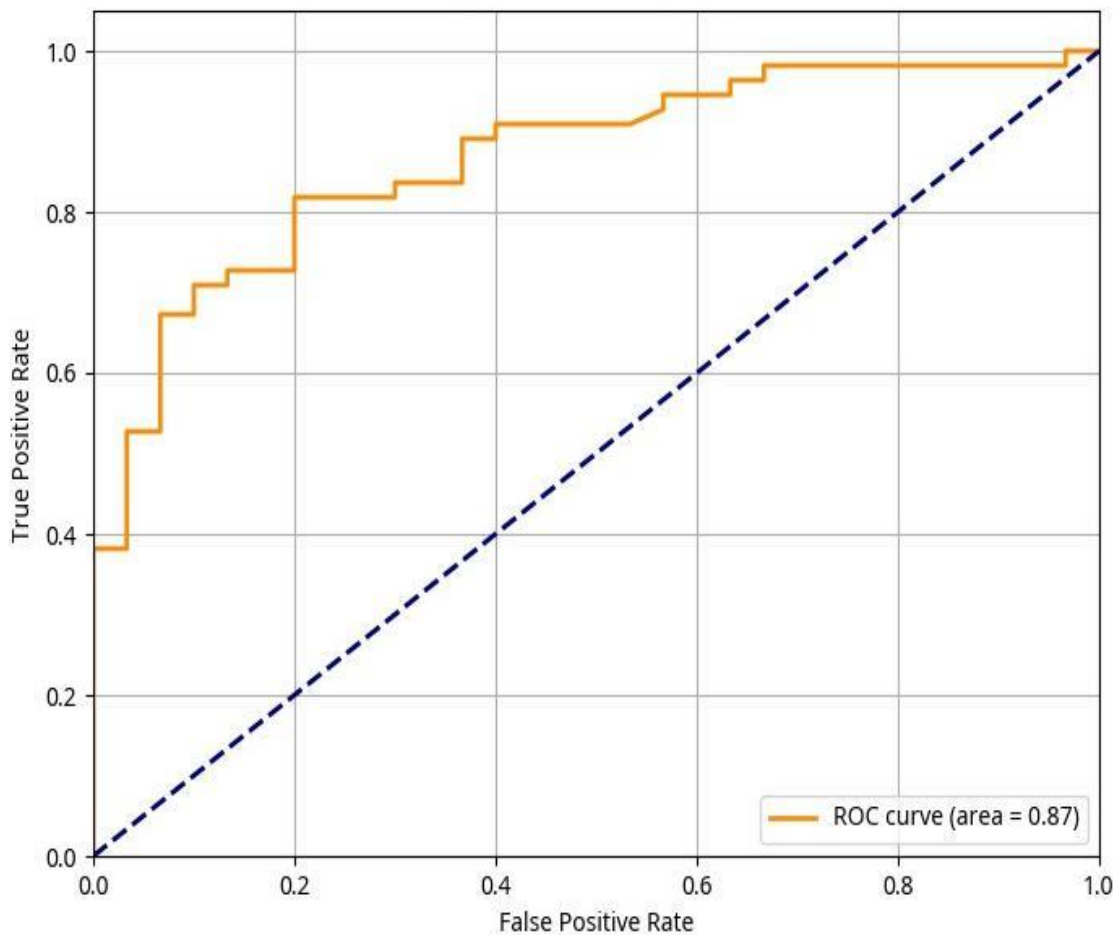


Figure 1. Receiver Operating characteristic (ROC) curve for HOMA-IR

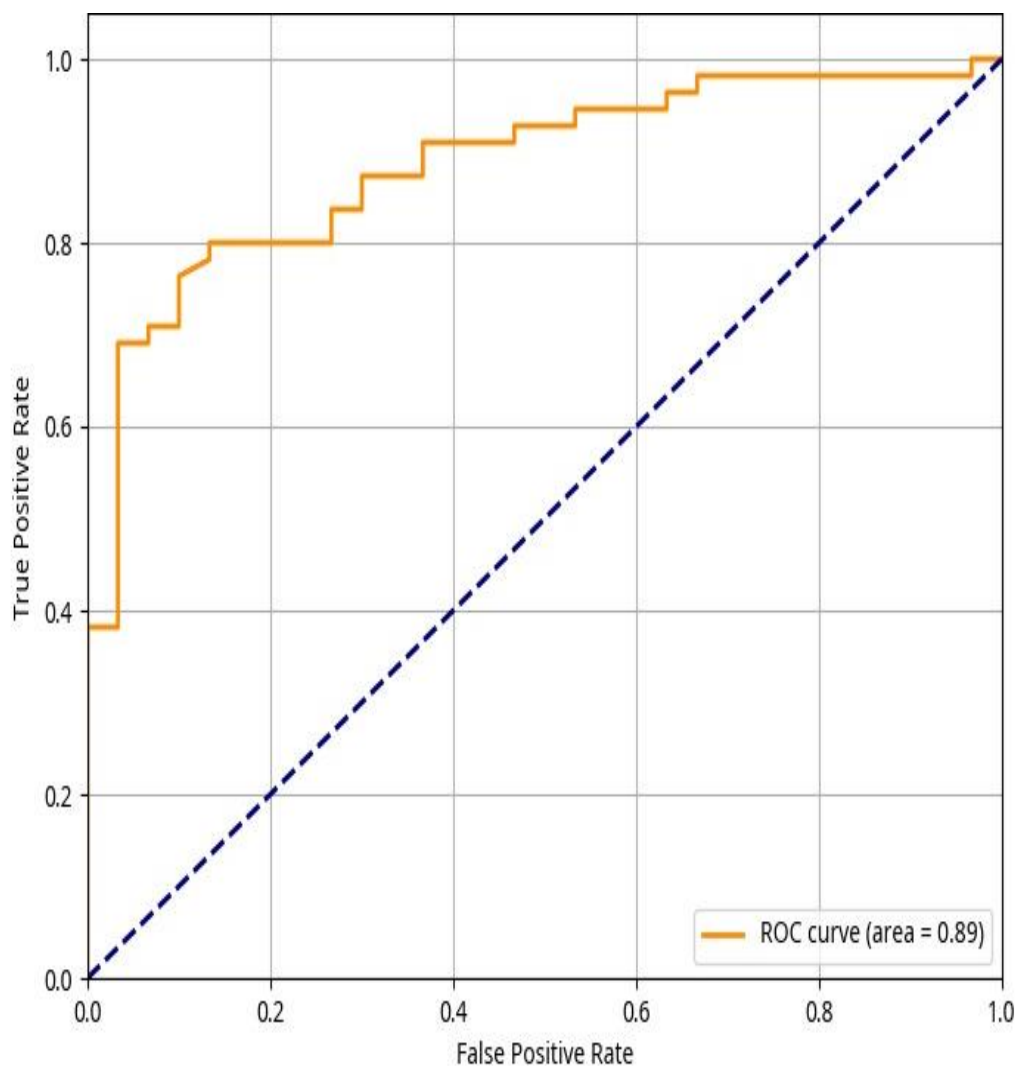


Figure 2. Receiver Operating characteristic (ROC) curve for Insulin

4.5.2 The predictive value of anthropometric parameters and age

The ROC analysis revealed that BMI (AUC = 0.97) and weight (AUC = 0.96) had an excellent ability to discriminate between outcomes, showing high sensitivity and specificity. On other hand, height (AUC = 0.54) and age (AUC = 0.51) showed poor predictive performance, suggesting that they are unreliable markers when considered in differentiation. These results suggest that, in this population, anthropometric measures reflecting body mass and adiposity, particularly BMI and weight, are strong predictors, whereas age and height have low predictive value. As shown in figures (3.-6) below.

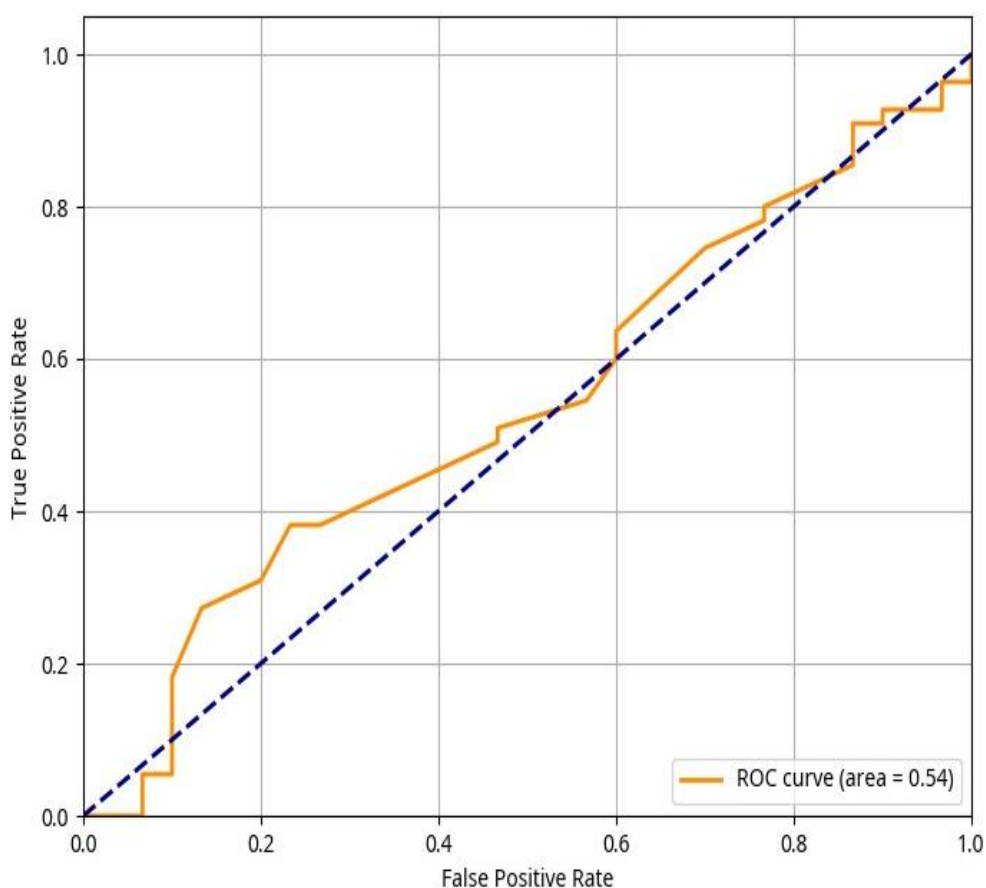


Figure 3. Receiver Operating characteristic (ROC) curve for height



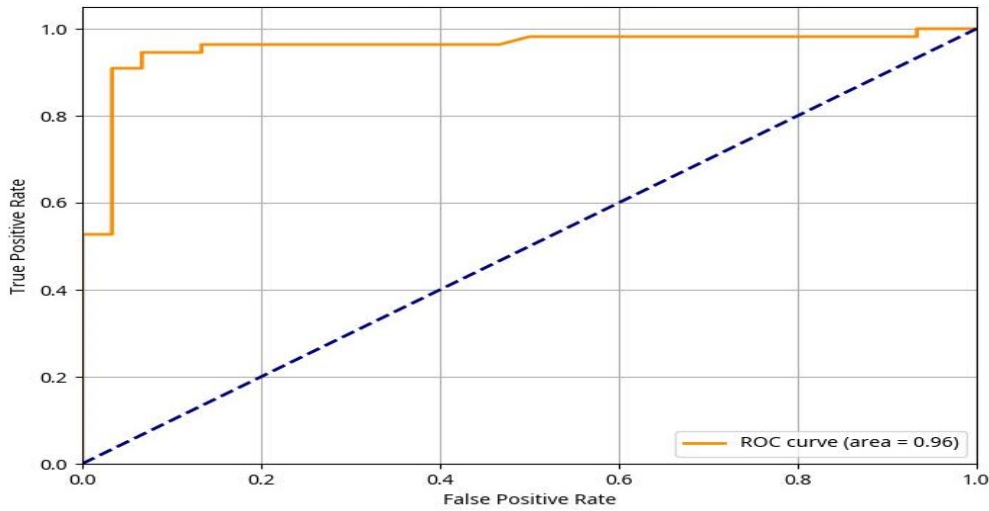


Figure 4. Receiver Operating characteristic (ROC) curve for Weight

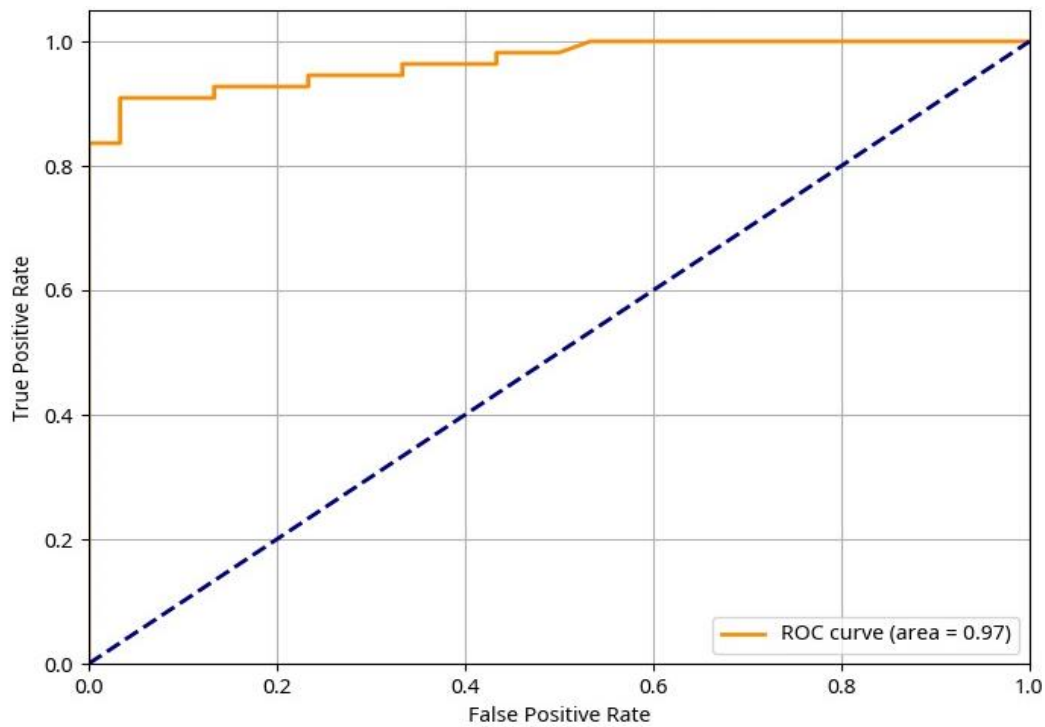




Figure 5. Receiver Operating characteristic (ROC) curve for BMI

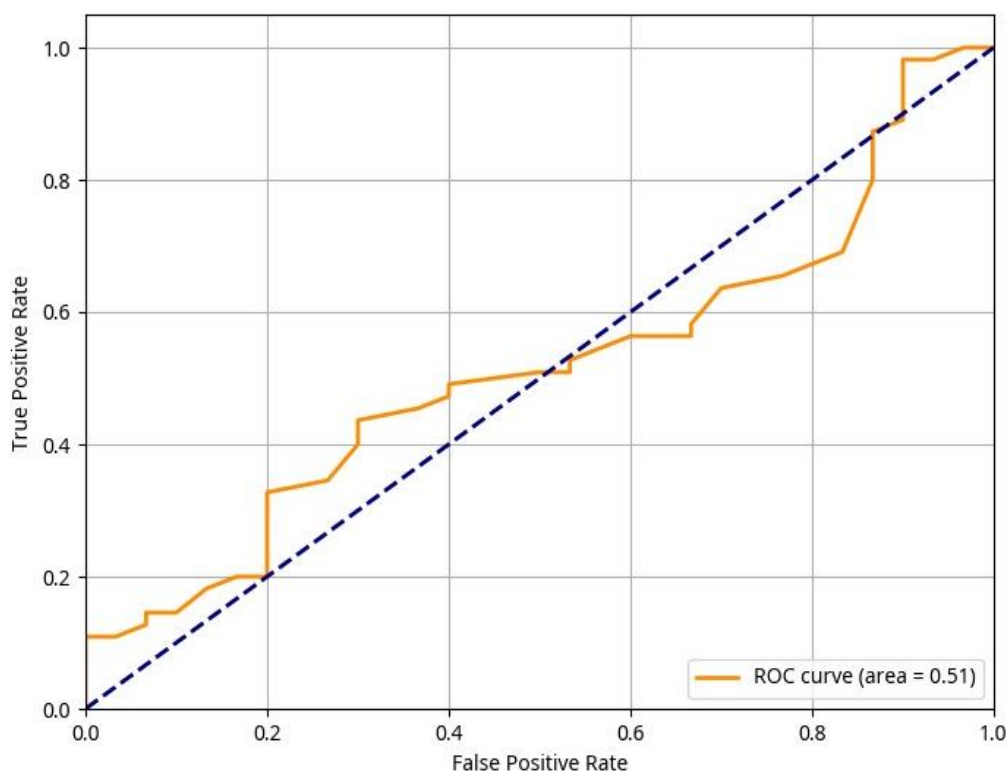


Figure 6. Receiver Operating characteristic (ROC) curve for age

4.5.3 Glycemic Parameters

HbA1c demonstrated good diagnostic ability (AUC = 0.82, sensitivity 63.6%, specificity 90.0%), whereas FBG showed poor discriminative ability (AUC = 0.55, sensitivity 34.6%, specificity 86.7%). This indicates that HbA1c was more reliable than FBG in identifying true positive and true negative cases. As shown in figures 7 & 8.



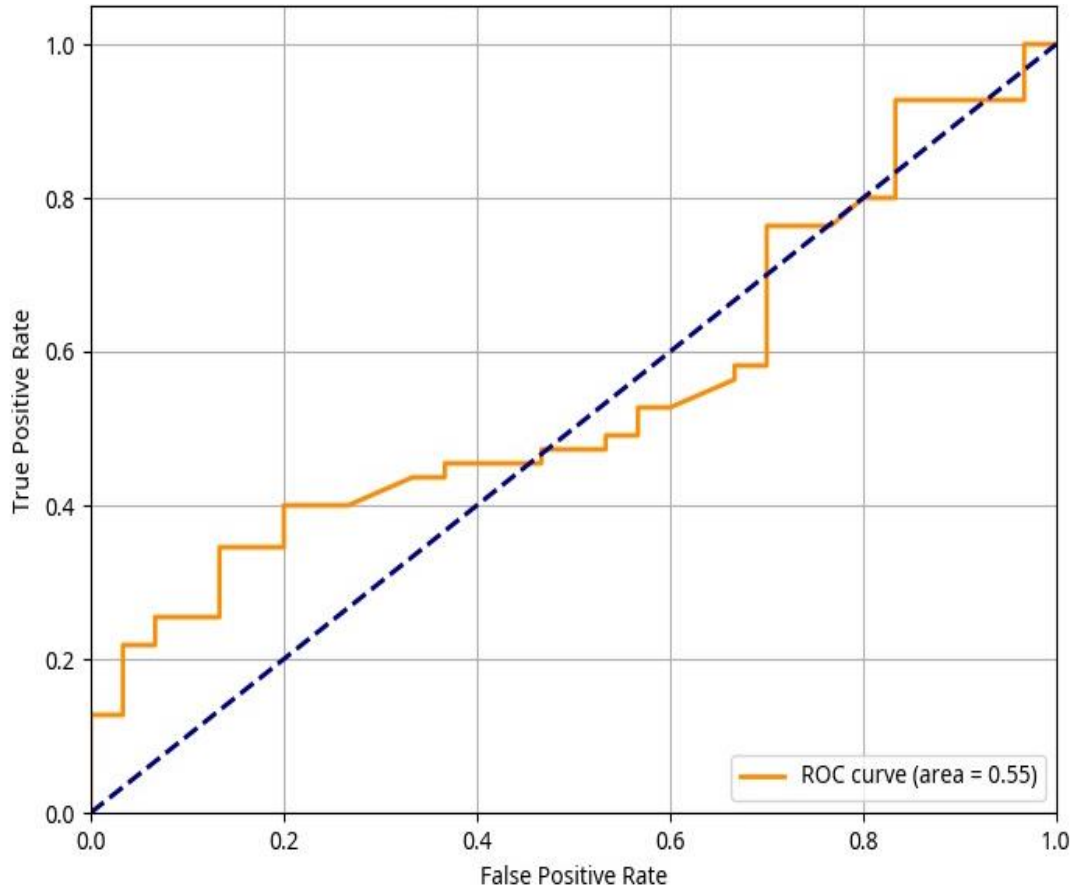


Figure 7. Receiver Operating characteristic (ROC) curve for FBG



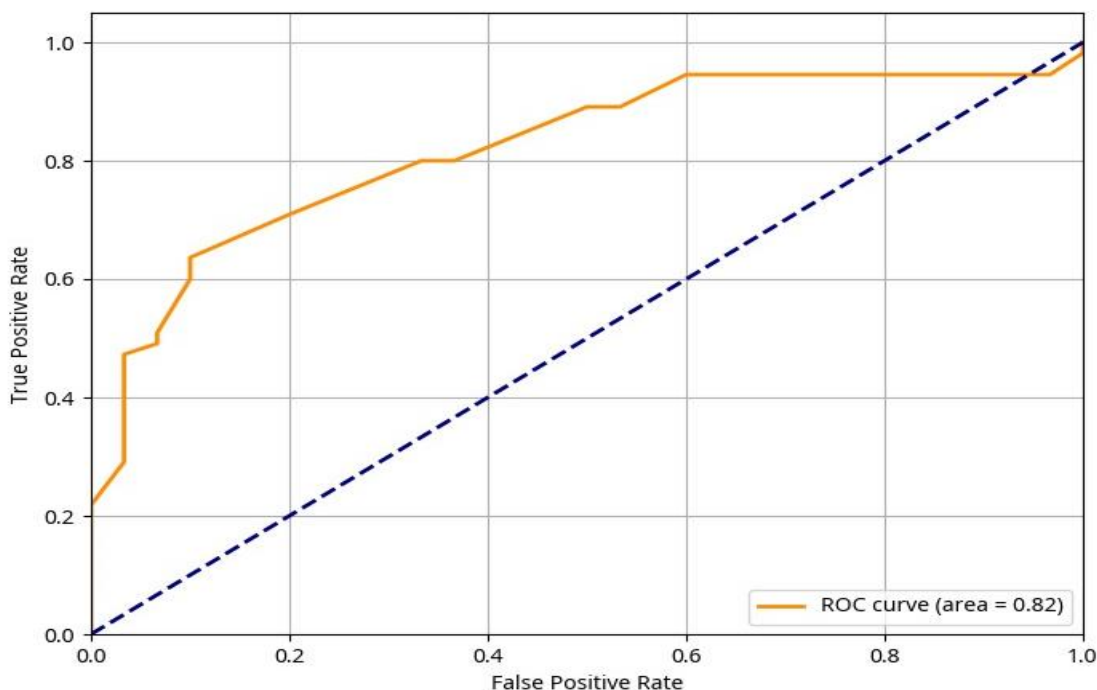


Figure 8. Receiver Operating characteristic (ROC) curve for HbA1c.

4.6 Simple Linear Regression

Linear regression analyses indicated that HbA1c was positively associated with HOMA-IR ($R^2 = 0.078$, $P = 0.039$).

In the present study, no significant correlation was found between body mass index (BMI) and insulin resistance (HOMA-IR) ($R = 0.021$, $p = 0.881$). The correlation coefficient was very close to zero, indicating the absence of a linear relationship between these two variables. Furthermore, the P-value was well above the conventional threshold for statistical significance ($p < 0.05$), suggesting that BMI did not significantly predict HOMA-IR levels in this study population.

This result indicates that, within our data, variations in BMI were not associated with reliable alterations in insulin resistance. The regression line ($y = 6.39 + 0.01x$) gave a nearly flat slope (0.01), further emphasizing the lack of a clear effect. See figure 9.

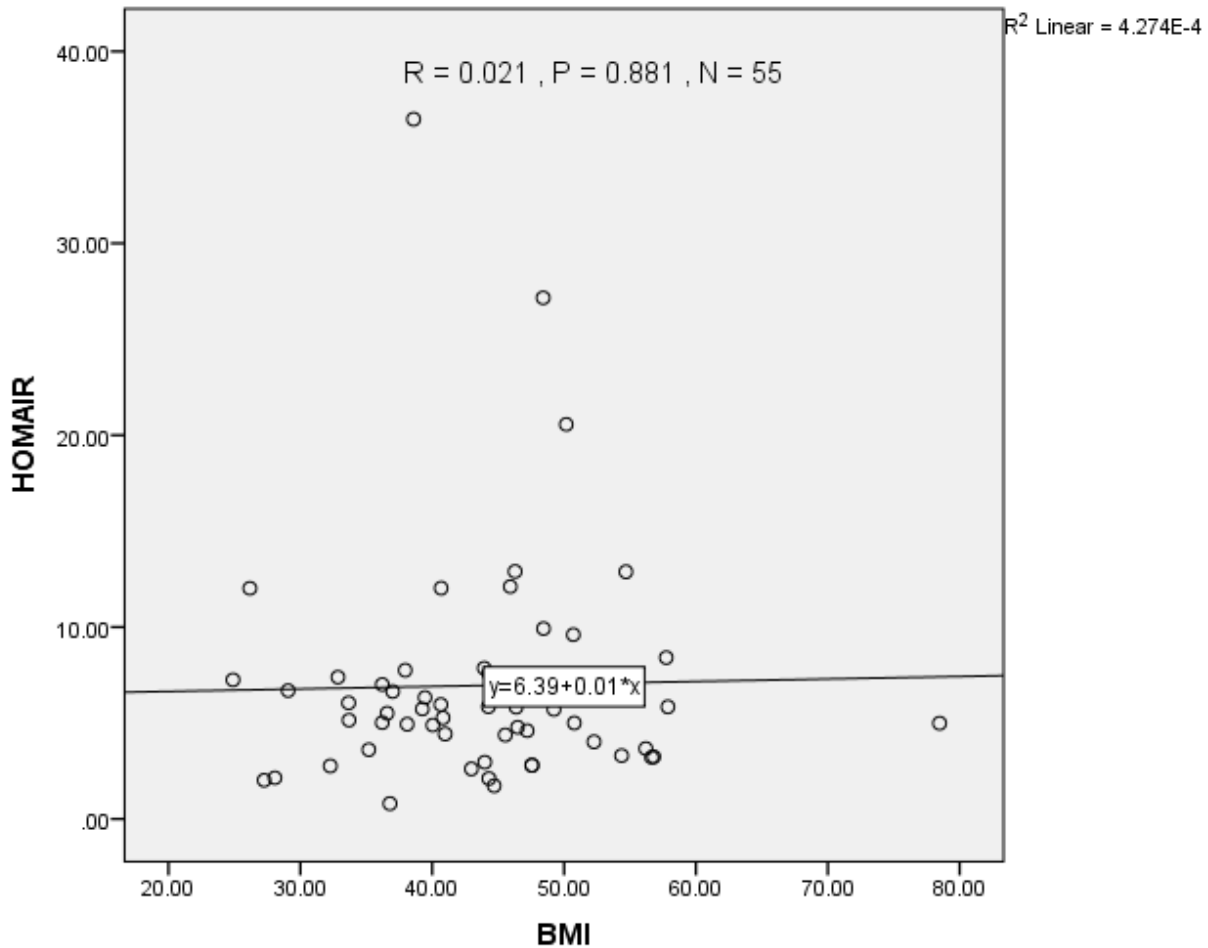


Figure 9. Linear regression between insulin and BMI.





Linear regression analysis demonstrated a statistically significant positive association between HbA1c and insulin resistance (HOMA-IR) ($R^2 = 0.078$, $P = 0.039$) (Figure 10). This indicates that higher HbA1c levels are associated with a slight increase in HOMA-IR, with HbA1c explaining approximately 7.8% of the variance in insulin resistance.

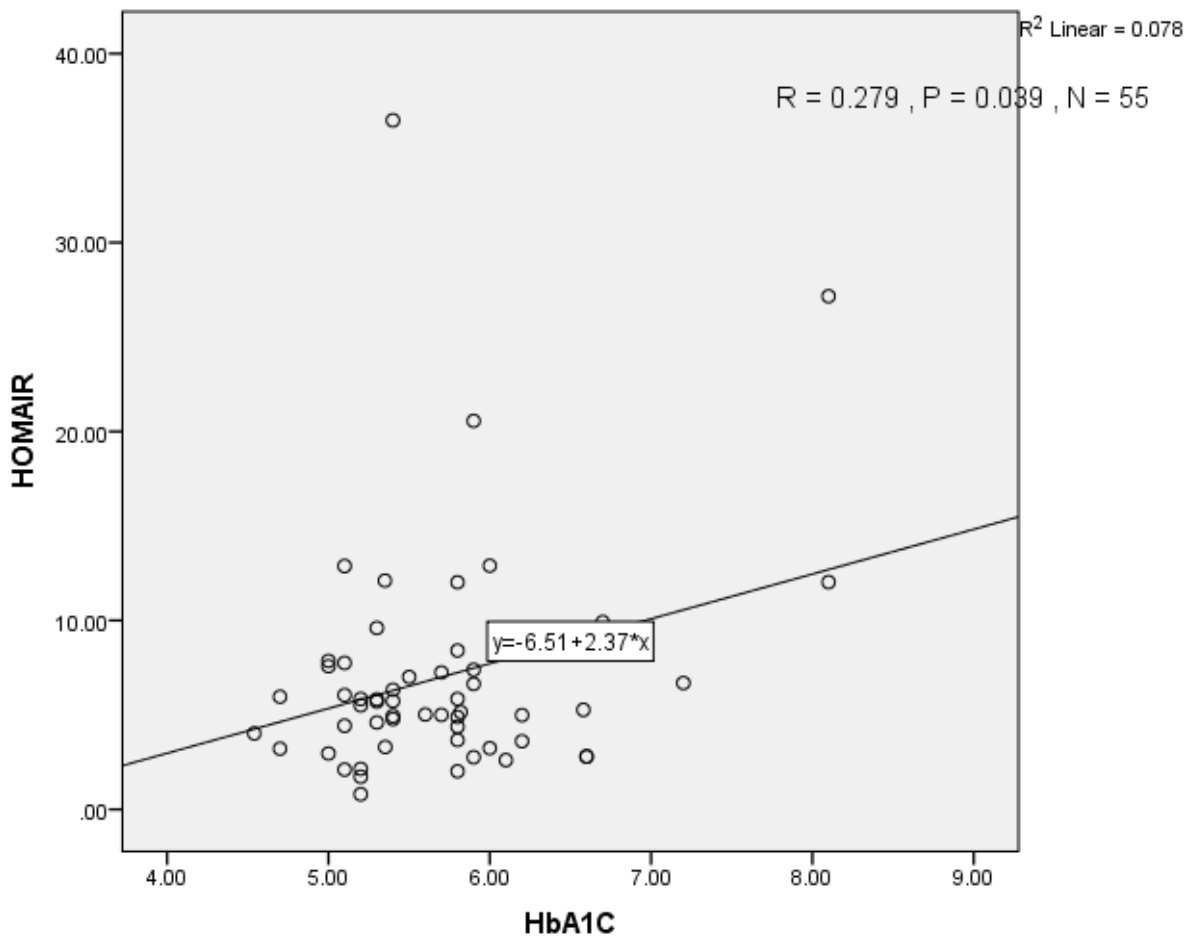


Figure 10. Linear regression between insulin and HbA1c.

4.7 Binary Logistic Regression Analysis

Binary logistic regression indicated that insulin and HOMA-IR were strong predictors for NAFLD ($AUC > 0.8$). Insulin had the strongest positive association with NAFLD ($B = 0.389$), while HbA1c also had positive coefficients. HOMA-IR had a negative coefficient ($B = -0.600$), potentially due to collinearity.



In the multivariate logistic regression analysis, BMI was considered one of the predictor variables that demonstrated a statistically significant relationship with NAFLD prior to adjustment but its significance reduced when adjusted for insulin-related measures.

The model showed 97.6% of cases, with 96.7% accuracy for controls and 98.2% for NAFLD cases. ROC analysis of the predictive power showed an AUC of 0.998, indicating near-perfect discriminating ability.

5. Discussion

This study analyzed the metabolic and demographic parameters of NAFLD patients compared to healthy controls, spotting on insulin resistance, lipid parameters, and demographic trends. The results provide details into the relations between obesity, insulin resistance, and demographic parameters in NAFLD patients, with implications for clinical screening and preventive strategies.

Although the mean age was slightly higher in NAFLD patients compared to controls, age-related trends indicated that the prevalence of NAFLD increased in participants over 40 years. This finding supports previous epidemiological data suggesting that advancing age is associated with increased metabolic dysfunction and higher NAFLD risk (9).

5.1. Comparison Between NAFLD and Controls

NAFLD patients demonstrated significantly higher HOMA-IR, HbA1c compared to controls, FBG expressed non-significant differences between both groups. These results corroborate the established links between NAFLD and glycemic parameters (9).

The markedly elevated BMI observed among NAFLD patients highlights the central role of obesity in disease pathogenesis. The strong correlation between BMI and HOMA-IR suggests that excess adiposity contributes to insulin resistance, which in turn promotes hepatic lipid accumulation. These findings are consistent with previous studies demonstrating that obesity, particularly visceral adiposity, is a major driver of NAFLD development and progression (3).

5.2. Insulin Resistance as a Central Marker

ROC analysis demonstrated excellent diagnostic performance for HOMA-IR and fasting insulin in distinguishing NAFLD patients from controls, with high sensitivity and specificity. These findings align with prior studies highlighting insulin resistance as a pivotal pathogenic factor in NAFLD. These differences likely reflect population-specific metabolic profiles and the interplay with insulin resistance (9).

5.3. Glycemic Predictors

HbA1c outperformed fasting glucose in predicting NAFLD (AUC = 0.82 vs. 0.55), reflecting its utility as a marker of long-term glycemic control and insulin resistance (10).

5.4. Regression Analysis and Predictive Model

HbA1c was significantly associated with HOMA-IR ($R^2 = 0.078$, $P = 0.039$), reinforcing the importance of glycemic dysregulation in NAFLD pathogenesis. Logistic regression including insulin, BMI, cholesterol, HbA1c, and HOMA-IR achieved 97.6% classification accuracy and an AUC of 0.998, outperforming previously reported predictive models (11).

Study Limitations

Waist circumference was not assessed, limiting evaluation of central obesity. In addition, cross-sectional design prevents causal inference. Moreover, single time-point measurement of insulin and lipids limits assessment of dynamic changes and treatment response; sensitivity may vary by disease severity.





6. Conclusion

The study underscores the central role of insulin resistance and obesity in NAFLD risk and provides evidence for their use in early screening. HbA1c, is particularly useful as non-invasive indicators. These results targeted metabolic and lifestyle interventions for NAFLD prevention and management

This study demonstrated a strong association between (NAFLD and insulin resistance. Overall, these findings support the concept that NAFLD is not solely a liver disorder but a systemic metabolic disease in which insulin resistance plays a central role.

Recommendations

Routine insulin measurement could be benefit in early detection. Standardized imaging methods are recommended for consistent staging and follow-up. Optimal control of obesity and diabetes must be done. A multidisciplinary cooperation (dietitians, endocrinologists, radiologists) is recommended to improve patient outcome.

Future Directions

Future research should involve larger multicenter and longitudinal studies to clarify disease progression. Advanced statistical analyses are needed to explore the mediating role of insulin resistance and the modifying effects of comorbidities such as diabetes and Polycystic Ovary Syndrome (PCOS). Incorporating biomarkers of De novo Lipogenesis (DNL), adipokines, and genetic variants (e.g., Patatin-Like Phospholipase Domain-Containing Protein 3(PNPLA3), Transmembrane 6 Superfamily Member 2 (TM6SF2) may improve mechanistic understanding. Interventional trials evaluating pharmacologic and lifestyle therapies are warranted.

ETHICAL STATEMENT:

The study was conducted in accordance with guidelines established by the World Health Organization (WHO) and the Libyan Ministry of Health. Approval was granted by the Misurata Libyan Academy, the Zliten Medical Center (ZMC), and a selected private laboratory (AlQalb Laboratory). All cases participated in the research received a comprehensive detail about the study objectives, procedures, and benefits. Verbal consent was collected from each participant before starting the data collection. All information about the cases was kept confidential and used solely for research purposes.

CONFLICT OF INTEREST

There is no conflict of interest to be declared

AUTHORS' CONTRIBUTIONS

SA developed the concept and structure of the review. EA led the literature search and drafted the initial manuscript, provided critical revisions.

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Insulin Resistance and Vitamin D Deficiency in Relation to Body Mass Index

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ABSTRACT

Background: Vitamin D deficiency and obesity are worldwide health issues associated with metabolic diseases. **Aims:** This study examined the association between vitamin D insufficiency and insulin resistance (IR) and body mass index (BMI) in Zliten, Libya, to assess whether BMI modulates the association between vitamin D and IR. **Methods:** A cross-sectional study of 60 individuals (18-60 years) was conducted. Serum 25(OH)D, fasting glucose, and insulin were measured using automated chemiluminescence. IR was calculated via HOMA-IR. **Results:** Participants showed a mean BMI of 40.28 (morbid obesity), vitamin D of 21.04 ng/mL (deficiency), and HOMA-IR of 4.97. While regression analysis showed weak overall predictors, Spearman's correlation analysis revealed a significant negative correlation between vitamin D and HOMA-IR ($p=0.031$). **Conclusion:** Vitamin D and BMI were not strong sole predictors of IR in this morbidly obese cohort, although a significant inverse association between vitamin D and IR was observed.

Keywords: Vitamin D deficiency, Insulin resistance, Body mass index, HOMA-IR, Obesity

. Introduction1

Vitamin D, previously known for its role in bone and mineral metabolism, has emerged as a key regulator of metabolic health. Growing evidence suggests that vitamin D deficiency is linked to an increased risk of metabolic disorders, including polycystic ovarian syndrome (PCOS), metabolic syndrome, and type 2 diabetes mellitus (T2DM) [1]. Clinical and epidemiological studies have shown an inverse relationship between serum 25-hydroxyvitamin D [25(OH)D] levels and insulin resistance. Insulin resistance (HOMA-IR) is assessed using the Homeostatic Model Assessment, suggesting that adequate vitamin D levels may improve insulin sensitivity and glucose homeostasis [2]. The proposed biological mechanisms underlying this relationship are multifaceted. Vitamin D is thought to influence metabolic function by modulating pro-inflammatory cytokine production [3]. Furthermore, the vitamin D receptor (VDR) is expressed in pancreatic β -cells, suggesting a direct role in regulating insulin secretion and cellular survival. Research shows that VDR overexpression in β -cells ameliorates diabetes, while vitamin D enhances insulin secretion by increasing calcium influx and upregulating voltage-gated calcium channels [4]. Vitamin D also promotes the insulin signaling cascade in skeletal muscle, the liver, and adipose tissue, notably by reducing oxidative stress and upregulating the SIRT1/AMPK/GLUT4 pathway [5].





Obesity adds a layer of complexity to this metabolic interplay. It is widely acknowledged that excessive adiposity predisposes individuals to vitamin D deficiency. This is primarily due to vitamin D being partitioned into fat stores, which reduces the fraction available for metabolic processes [6]. Additionally, the chronic low-grade inflammation associated with obesity, characterized by complex interactions among proinflammatory cytokines, chemokines, and adipokines, exacerbates insulin resistance (IR), especially in people with a high body mass index (BMI) [7,9].

Despite these global insights, localized data from Libyan populations, particularly regarding the specific metabolic trends in the Zliten region, remain scarce. This study aims to evaluate the association between vitamin D status, BMI, and IR among adults in Zliten, Libya, to provide essential clinical insights and support evidence-based metabolic interventions in the region.

2. Materials and Methods

2.1 Study Design and Participants

The current study was conducted as a cross-sectional observational study in the clinical setting of Alpha Medical Laboratory in Zliten, Libya. The study included 60 participants aged between 12 and 60 years. Exclusion criteria included individuals with known chronic diseases affecting vitamin D metabolism (such as severe renal or liver disease), those using vitamin D supplements, and patients on medications that alter insulin sensitivity.

2.2 Ethical Consideration

The study followed the ethical guidelines outlined in the Declaration of Helsinki. The Faculty of Pharmacy's Institutional Review Board granted ethical approval (Alasmarya Islamic University, Ref. No. PH14:2024). All participants provided informed consent before their inclusion in the study.

2.3 Anthropometric Measurements

The BMI was calculated for all participants using the standard formula: $\text{Weight (kg)} / \text{Height (m)}^2$. To ensure a rigorous analysis of the metabolic trends, participants were categorized based on the World Health Organization (WHO) guidelines into the following four distinct groups: underweight BMI $< 18.5 \text{ kg/m}^2$; normal weight BMI $18.5\text{-}24.9 \text{ kg/m}^2$; overweight BMI $25.0\text{-}29.9 \text{ kg/m}^2$; and obese BMI $\geq 30.0 \text{ kg/m}^2$.

2.4 Laboratory Procedures

Fasting blood samples were collected from all participants after an 8–12-hour overnight fast. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured in nanograms per milliliter (ng/mL) using the Mindray CL-1200i automated chemiluminescence immunoassay (CLIA) analyzer.

Fasting serum insulin levels were quantified in micro-international units per milliliter ($\mu\text{IU/mL}$), and fasting glucose levels were measured in milligrams per deciliter (mg/dL), both using the Mindray CL-900i system.

2.5 Calculation of IR

The HOMA-IR index, calculated as the product of fasting insulin ($\mu\text{IU/mL}$) and fasting glucose (mg/dL) divided by 405, was used to assess insulin resistance [10]. This constant ensures the appropriate conversion for the clinical units used in the analysis.





2.6 Statistical Analysis

For the statistical framework of this study, IBM SPSS Statistics v. 26.0 was used. The distributional characteristics of the variables were scrutinized for normality using the Shapiro-Wilk test, ensuring the validity of subsequent parametric or nonparametric analyses. Because the data did not follow a normal distribution (Shapiro-Wilk $p < 0.05$), the median and interquartile range (IQR; 25th-75th percentiles) were used to report continuous variables. To further describe the distribution, skewness and kurtosis were calculated from the standardized third and fourth central moments, respectively. The threshold for statistical significance was set at $p < 0.05$, using two-tailed tests for all comparative analyses.

3. Results

3.1. Descriptive Characteristics

Table 1 shows the baseline clinical and biochemical parameters of all 60 participants. In strict adherence to the distributional diagnostics, all parameters are expressed as median (IQR).

The cohort exhibited a high prevalence of obesity, with a median BMI of 36.3 kg/m² (IQR: 30.1–44.8). Biochemical analysis of vitamin D status revealed a significant deficiency burden, as indicated by a median serum 25(OH)D level of 18.0 ng/mL (IQR: 12.0–26.0). Consistent with these findings, the median HOMA-IR was 5.0 (IQR: 3.0–7.0), markedly exceeding the clinical cut-off of 2.5, confirming the presence of established IR in the study population.

Notably, distributional diagnostics confirmed a pronounced positive skewness for serum Vitamin D (Skewness = 4.12; Kurtosis = 21.3). These coefficients, calculated using the standardized moment method in SPSS, indicate pronounced clustering of values within the deficient range, further supporting the superiority of the median over the mean for providing a more robust representative value for this metabolic cohort.

Table (1): Descriptive Characteristics of the Study Population (N=60)

<i>Variable</i>	<i>Median (IQR)</i>	<i>Range (Min – Max)</i>	<i>Skewness</i>	<i>Kurtosis</i>
BMI (kg/m²)	36.3 (30.1- 44.8)	16.0 – 96.0	1.34	2.51
Vitamin D (ng/mL)	18.0 (12.0-26.0)	4.0 – 96.1	4.12	21.3
HOMA-IR	5.0 (3.0-7.0)	1.6 – 15.0	1.48	1.83

3.2. Correlation and Regression Analysis

The Shapiro-Wilk test indicated a non-normal distribution ($p < 0.05$). The correlations between the variables were evaluated using Spearman's rank correlation coefficient (ρ). (Table 2).

A statistically significant negative correlation was identified between serum Vitamin D levels and HOMA-IR ($\rho = -0.278$, $p = 0.031$), suggesting that lower Vitamin D levels were linked with increased IR. In contrast, the relationship between BMI and HOMA-IR ($\rho = 0.231$,





$p = 0.076$) and the association between BMI and Vitamin D ($\rho = 0.053$, $p = 0.688$) did not reach statistical significance. Additionally, multiple linear regression analysis revealed that the combination of BMI and Vitamin D accounted for only 6.9% of the variance in IR ($R^2 = 0.069$, $p > 0.05$).

Table (2): Summary of Correlation Analysis (Spearman's rho)

Variable Pair	Spearman (ρ)	P-value	Significance
Vitamin D vs. HOMA-IR	-0.278	0.031*	Significance
BMI vs. HOMA-IR	0.231	0.076	Non-significant
BMI vs. Vitamin D	0.053	0.688	Non-significant

* Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

The present study investigated the relationship among vitamin D status, obesity, and IR in a specific Libyan population. Our findings demonstrate a significant inverse correlation between serum Vitamin D levels and HOMA-IR ($r = -0.278$, $p = 0.031$). This supports the concept that Vitamin D insufficiency is a major contributor to impaired glucose metabolism. This negative association aligns with global research suggesting that Vitamin D serves a diverse function in regulating systemic insulin sensitivity [1, 2, 13].

A notable finding in this study was that despite the high prevalence of obesity (Median BMI = 36.3 kg/m²), BMI was not a statistically significant predictor of IR ($p = 0.076$). This observation underscores the limitations of BMI as a metabolic marker, as it fails to differentiate between subcutaneous fat and visceral adiposity. Visceral fat is more immunologically active, secreting pro-inflammatory cytokines like Tumor Necrosis Factor- and Interleukin-6, which directly impair insulin signaling pathways [7- 9]. In this cross-sectional study, the lack of significant correlation suggests that Vitamin D status might serve as a more sensitive and independent indicator of metabolic risk than total body mass alone.

The high variability and severe vitamin D deficiency (Median = 18.0 ng/mL, Skewness = 4.12) observed in this study could be attributed to several physiological factors. In obese individuals, Vitamin D, being a lipophilic molecule, is often sequestered within the expanded adipose tissue volume, significantly reducing its bioavailability in the systemic circulation [4]. Additionally, the inverse relationship between serum Vitamin D and markers of adiposity suggests that metabolic dysfunction is compounded by chronic low-grade inflammation, further exacerbating the deficiency state [3, 7].





The results have major clinical and public health implications for Zliten and the wider Libyan region. They suggest that routine Vitamin D screening and targeted supplementation could serve as a cost-effective strategy to reduce IR and lower the risk of Type 2 Diabetes Mellitus. This is particularly relevant for individuals for whom achieving sustainable weight loss is clinically challenging.

5. Conclusion

In conclusion, this study shows a significant inverse association between serum Vitamin D levels and IR among our sample. Although the study population had a high prevalence of morbid obesity, BMI alone was not a statistically significant predictor of IR. These findings suggest that Vitamin D status may play a more pivotal role in glucose homeostasis than total body mass index in this specific population.

The clinical implications of these results are substantial, highlighting the importance of Vitamin D screening as an essential component of metabolic health evaluation. Given the widespread shortage reported, vitamin D supplementation may provide a cost-effective and accessible strategy to increase insulin sensitivity and reduce the incidence of Type 2 Diabetes Mellitus in the region, particularly when weight control is difficult.

Future recommendations: Further longitudinal studies with larger sample sizes are needed to clarify causal relationships and to account for confounding factors such as dietary habits, seasonal sun exposure, and genetic variation in the vitamin D receptor in the Libyan population.

ETHICAL STATEMENT

The Ethics Committee of the Faculty of Pharmacy at Alasmarya Islamic University authorized the study protocol (Approval No. **PH14:2025**).

CONFLICT OF INTEREST

The writers disclose no conflict of interest.

AUTHORS' CONTRIBUTIONS

N.A.A: Supervision and design. A.M.A, A.S.S, H.M.A, S.A.A: Data collection and analysis. M.S.A: Writing and formatting.

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Evaluation of Some Active Components of Palm Leaves (*Phoenix dactylifera*) Ethanol and Aqueous Extracts and Study of Their Antibacterial Effects

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ABSTRACT

Introduction: Date palm (*Phoenix dactylifera* L) leaves are a widely available natural resource in Libya. Previous studies have demonstrated that various parts of the date palm (*Phoenix dactylifera* L) contain bioactive secondary metabolites with potential therapeutic applications, including antibacterial, antioxidant, and anti-inflammatory effects.

Aims: This study investigated the bioactive secondary metabolites present in three commonly cultivated date palm (*Phoenix dactylifera* L) leaf varieties in Libya: Tabuni, Hamuri, and Ammi. The aim was to conduct a phytochemical screening and evaluate the antibacterial activity of selected Libyan date palm leaf extracts.

Methods: Phytochemical screening was performed to identify the active constituents in the palm leaves. This was followed by quantitative assessment of specific compounds (moisture and Ash content) and evaluation of their biological efficacy against bacterial isolates including *Staphylococcus aureus* and *Escherichia coli* using the zone of inhibition method.

Results: Qualitative phytochemical analysis of ethanol and aqueous extracts showed that the leaves are rich in active compounds such as carbohydrates, proteins, phenols, flavonoids, tannins, steroids, terpenes, and terpenoids, while resins and alkaloids were not found in any of the dissolving extracts. The study also found that the leaves contained a moderate percentage of moisture and ash. The highest moisture percentage was recorded in Ammi leaves (9.96%), which is very close to Hamuri leaves (9.03%). On the other hand, the lowest percentage was recorded in Tabuni leaves at 6.45%. The highest ash content was found in Ammi leaves at 5.9% and the lowest in Tabuni leaves at 3.825%. Hamuri leaves had an ash content of 4.7%. The alcoholic extract of palm leaves showed good antibacterial activity against *Staphylococcus aureus* at all concentrations, with the largest growth inhibitory zones observed at a concentration of 100 mg/ml for all samples. The only exception was related to the Tabuni extract at the concentration of 12.5 mg/ml. In contrast, the results showed insensitivity of *Escherichia coli* to the extracts of the study.

Conclusion: Palm leaf extracts show antibacterial activity; more studies are needed to quantify this activity. The Hamuri and Ammi extracts demonstrated superior antibacterial activity compared to Tabuni, especially against *Staphylococcus aureus*, with phytochemical richness varying among varieties. The lack of activity against *Escherichia coli* is consistent with the greater resistance of Gram-negative bacteria due to their outer membrane structure.

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Keywords: *Date palm leaves, Moisture content, Phytochemicals and Antibacterial Activity*

1. Introduction

The date palm (*Phoenix dactylifera* L), one of the oldest fruit trees in the Arab world, is widely grown for its edible fruit [1]. In Libya, date palms are grown in the gardens of the southern and northern region for shading fruit plants. Many pharmaceutical companies are interested in developing plant-based treatments, mainly due to the growing belief that Green Medicine is more reliable and safer than expensive synthetic drugs that can have adverse side effects [2]. According to a World Health Organization (WHO) report, eighty percent of the world's population currently receives primary health care through herbal medicine [3]. Of the 25 most popular drugs sold in the world, approximately 42% are natural or derived from herbal products [4]. From ancient times to the present, most peoples and civilizations have relied entirely or largely on herbal therapy. Medicinal plants represent a source of enormous economic value throughout the world. Our country possesses a rich botanical diversity, with a wide variety of plant species growing in different regions. Plant phenolics present in fruits and vegetables have received considerable attention due to their potential antioxidant activity [5]. Crude extracts of palm leaves have been chemically analyzed for the presence of phytochemicals that may be responsible for their medicinal use in traditional medicine to control diabetes, hyperlipidemia and treat bronchopneumonia [6,7]. There have been many studies on palm leaves, especially in Arab countries such as Iraq, Algeria and Saudi Arabia [8-11]. However, this study focuses on three types (Tabuni, Hamuri and Ammi) which are widely used in Libya and have not been previously studied. Specifically, this study aims to evaluate the antibacterial activity of Libyan palm against specific bacterial strains, as well as to determine its antioxidant potential using standardized in vitro assays. By combining phytochemical profiling with biological evaluation, this work seeks to provide scientific evidence supporting the medicinal use of Libyan palm leaves in treating microbial infections.

2. Materials and Methods

2.1. Plant samples collection:

Plant leaf samples (Tabuni, Hamuri, and Ammi) were collected from city of Misurata in northern Libya during the period from November to May (2022-2023). The samples were preserved by [air-drying in the shade / refrigeration at 4°C / immediate processing] until analysis. The plant samples were identified in the Department of Botany, while the bacterial strains were identified in the Department of Microbiology, Faculty of Science, Misurata University.

2.2 Basic preparations:

Leaves were used for each type of palm used in the study. They were cleaned of dirt and insects, then washed well with water and dried in air away from moisture and sunlight at room temperature for 15 days. The plant material was then finely ground into powder using an electric mill (Silver Crest brand, Germany) and stored in dark, tightly sealed bottles away from light, moisture and heat until used. In this study, the soaking (solid-liquid) extraction method was used. Ten grams of dry material was soaked in 100 ml of solvent (water or ethanol) for 72 hours at laboratory temperature with continuous stirring with an electric stirrer. The extracts were then filtered using filter paper. The extracts were collected and the solvents were evaporated on a rotary evaporator at 40°C. The final product obtained was a crude extract, which was stored at a temperature gradient of 7°C until use [12].





2.3 Phytochemical detection of active components of palm leaves:

Chemical testing of the palm leaf extract was carried out using standard procedures to identify the components as follows:

2.3.1 Tannin Detection:

The extract (0.5 g) was dissolved in 10 ml of distilled water, then the mixture was filtered. Four drops (0.3 ml) of a 1% ferric chloride solution were added to 2 ml of the filtrate. The appearance of a blue-black, green, or blue-green precipitate was considered evidence of the presence of tannins [13].

2.3.2 Steroid Detection:

Acetic acid (2 ml) was added to 0.2 g of the extract and cooled with ice, then concentrated H_2SO_4 was carefully added. A color change from purple to blue or bluish-green indicated the presence of an asterol ring [14].

2.3.3 Detection of terpenoids:

A quantity of 100 mg of the extract was dissolved in ethanol, after which 1 ml of acetic anhydride was added, followed by the careful addition of concentrated sulfuric acid. Color change from pink to purple was considered indicative of the presence of terpenoids [14].

2.3.4 Detection of saponins:

One gram of extract was boiled with 5 ml of distilled water and then filtered. Distilled water (3 ml) was added to the filtrate, and the mixture was shaken vigorously for approximately 5 minutes. Subsequently, 5 ml of silver nitrate solution was added to 5 ml of the extract in a test tube, and the tube was placed in a boiling water bath for 5 minutes. The appearance of a silver mirror on the inner walls of the test tube indicated the presence of saponins. In a separate test, 1-3 ml of mercuric chloride solution was added to 5 ml of the extract; the formation of a white precipitate served as a good indicator of the presence of saponins [15].

2.3.5 Detection of flavonoids:

Add 3 ml of extract and a few drops of NaOH solution to the test tube. The formation of an intense yellow color, which became colorless with the addition of a few drops of dilute HCl, indicated the presence of flavonoids [16].

2.3.6 Detection of alkaloids:

Ten grs of the extract were boiled in 50 ml of water acidified with 4% HCl, filtered and 0.5 ml of the supernatant was mixed with Mayer's reagent in a watch glass. A white precipitate indicates the presence of alkaloids [17].

2.3.7 Phenol detection:

To 2 ml of the test solution, 0.5 ml of $FeCl_3$ solution (w/v) was added, the formation of an intense color indicated the presence of phenols [17].

2.3.8 Resin detection

Five mgs of raw extract were dissolved in ml of acetone, treated with 5 ml of 4% hydrochloric acid. The presence of resins is indicated by the appearance of transparent turbidity in the solution [14].

2.3.9 Carbohydrates Molisch Test: One ml of extract, 3 drops of Molisch reagent and 2 ml of H_2SO_4 concentrate were added to the test

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tube, carefully keeping the test tube slightly bent. The formation of a purple ring at the junction indicated the presence of glycosides [16].

2.3.10 Ninhydrin test:

To 5 ml of extract, 2 drops of freshly prepared 0.2% Ninhydrin reagent were added and heated. The appearance of a blue colour indicates the presence of amino acids [16].

2.3.11 Detection of glycosides

Kilani-Keller test:

A mixture of glacial acetic acid (2 ml) with two drops of a 2% FeCl₃ solution is added to 5-2 mg of crude plant extract, then treated with 1 ml of concentrated sulfuric acid H₂SO₄. A brown ring forms between the layers with the appearance of purple and green solution in the layers, indicating the presence of cardiac glycosides [16].

2.4. Quantitative assessment:

2.4.1 Moisture

Two g were dried at 105°C for 3 hours in an oven until constant weight. After this, it was cooled in a desiccator, cooled in a desiccator-formator for 15 minutes. The percentage of moisture content was calculated using the following equation:

$$\% \text{ Moisture} = \frac{\text{Weight 1} - \text{Weight 2}}{\text{Weight 1}} \times 100$$

Where; Wt1: weight (g) of the plant sample before drying and wt2: weight (g) of the plant sample after drying [18].

2.4.2 Ash content

Exactly 2g of air-dried leaves were placed in a quartz crucible and heated at 550°C for 3 hours in a muffle furnace to constant weight, the percentage of ash content was calculated using the equation:

$$\% \text{ Ash} = \frac{\text{Mass of ash (g)}}{\text{Mass of dry sample (g)}} \times 100 \text{ [18].}$$

2.5 Estimation of biological effectiveness:

2.5.1 Antimicrobial efficacy study:

The effectiveness of plant extracts against some types of pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*) was evaluated to find out their ability to inhibit and stop the growth of these bacteria, all raw alcoholic extracts (ethanol) were prepared by dissolving them in DMSO solution (it does not affect the extracts nor the microbial growth) to obtain the desired concentration in our study (100- 75- 50 – 25- 12.5%). The bacteria suspensions were adjusted compared to a 0.5 MacFarland stander solution and using cotton swabs to spread on Miller Hinton Agar plates. By using sterile flamed cork borer, wells with diameter of 6mm were made in inoculation agar plates. 50µl of the different concentration were transfer into the wells, and the plates were then incubated at 37°C for 24 hours. The effective of the extract was determined by measuring the diameter of the inhibition zone around the wells compared to DMSO used as negative control [19].

3. Results

As shown in Table 1, ethanol and aqueous extracts of the studied leaves contained flavonoids, phenols, carbohydrates, saponins, proteins, terpenoids, tannins, and glycosides, while resins and alkaloids were absent in all extracts. Crude ethanol and aqueous extracts were



generally rich in terpenes, tannins, flavonoids, carbohydrates, glycosides, and phenolic compounds. However, the Hamuri ethanol extract showed relatively low phenol content compared to Tabuni and Ammi, which were rich in phenols. Saponins were more abundant in Ammi extracts than in Hamuri. These phytochemical findings confirm the presence of therapeutically important secondary metabolites (flavonoids, phenols, tannins, terpenoids) in the studied leaf extracts.

Table (1): Chemical Compounds in Extracts of Three Plant Species Using Different Solvents.

Functional group	Detector	Hamuri Solvent		Tabuni solvent		Ammi solvent	
		Ethanol	Water	Ethanol	Water	Ethanol	Water
Carbohydrates	Molish	++	+++	+	+++	+++	+++
Squirrels	Ninhydrin	-	+++	-	+++	-	+++
Phenols	Folin-Ciocalteu	+	+++	+++	+++	+++	+++
Tannins	FeCl ₃	++	+++	+	+++	++	+++
Flavonoids	Alkaline	++	+++	++	+++	+	+++
Alkaloids	Mayer	-	-	-	-	-	-
Saponins	foaming	+	++	++	+++	+++	++
Glycosides	Keller-Killani	+++	+++	+++	+++	++	++
Resins	/	-	-	-	-	-	-
Steroids	Liebermann-Burkhard	+++	+++	+++	+++	+++	+++
Terpenoids	Salkoski	+++	+++	+++	+++	+++	+++

The concentration was indicated as (+++) obvious change, (++) relative change, (+) very slight change, and (-) no change

Moisture content ranged from 6.45% (Tabuni) to 9.96% (Ammi), while ash content ranged from 3.82% (Tabuni) to 5.90% (Ammi). Tabuni consistently demonstrated the lowest values for both parameters, suggesting comparatively lower inorganic residue and better dry stability, whereas Ammi exhibited the highest levels of both moisture and Ash content as shows in Figure 1 and Figure 2.

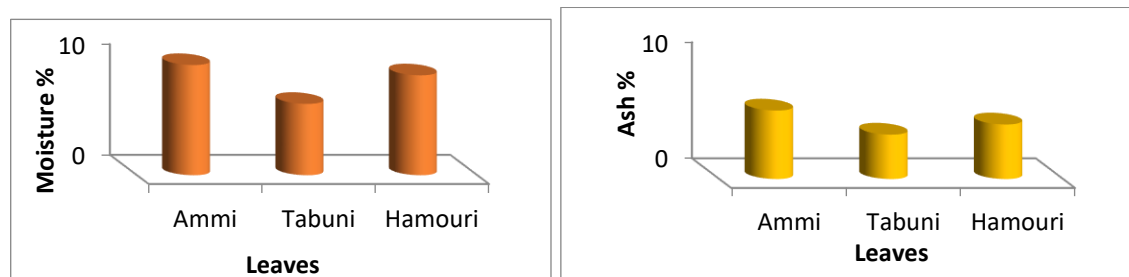


Figure 1. Percentage (%) of moisture in different palm leaves **Figure 2: Percentage (%) of Ash in different palm leaves**

The results obtained and shown in the table (Table.3) and the following figures (3-7) showed that the extract has an inhibitory effect on bacterial growth. S-aureus and did not show an inhibitory effect on E-coli growth, as the area of inhibition was measured for each type of bacteria.

Table 3: Inhibitory area against *Staphylococcus aureus*

The focus(mm) Type of leaves	Concentration of <i>Staphylococcus aureus</i>				
	100	50	25	12.5	6.25
Hamouri	16	13.5	12.5	10.5	9
Common	15.5	14	12.5	10.5	9.5
Tabuni	12.5	10.5	9.5	-	-

where (-) indicates no inhibition

In Table3 the three extracts showed concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones decreasing as concentrations decreased. Hamuri extract recorded the highest inhibition at 100 concentration (mean 16 mm) and was the only extract that maintained clear inhibition even at the lowest concentration of 6.25 (mean 9 mm), making it the most effective and broadest in range. Common extract showed very similar results to Hamuri at concentrations 50, 25, and 12.5 (13.5, 12.5, and 10.5 mm respectively). At concentration 6.25, it was slightly better than Hamuri (9.5 mm vs. 9 mm). Tabuni extract was clearly the weakest, with the highest inhibition at concentration 100 not exceeding 12.5 mm, and its effect completely disappeared at concentrations of 12.5 and below (no inhibition). Figures 3 and 4 illustrate the concentration-dependent antibacterial activity of Tabuni extract and Common extract, respectively, against *Staphylococcus aureus*.

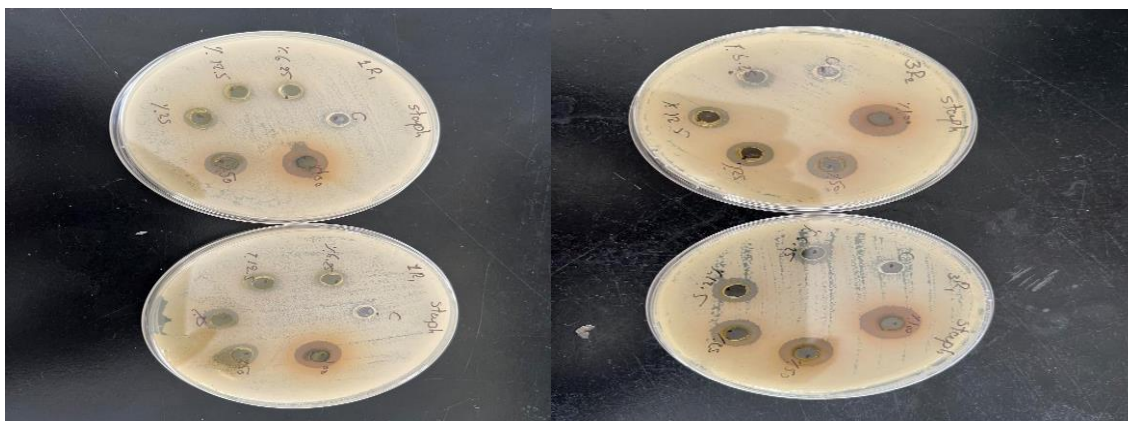


Figure 4 Zones of inhibition resulting from a range of concentrations of common extract showing its antibacterial effect on *staphylococci*

Figure 3 Zones of inhibition resulting from a range of concentrations of Tabuni extract showing its antibacterial effect on *staphylococci*

Figures 5 and 6 demonstrate the antibacterial activity of Hamuri extract against two different bacterial species: the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*.

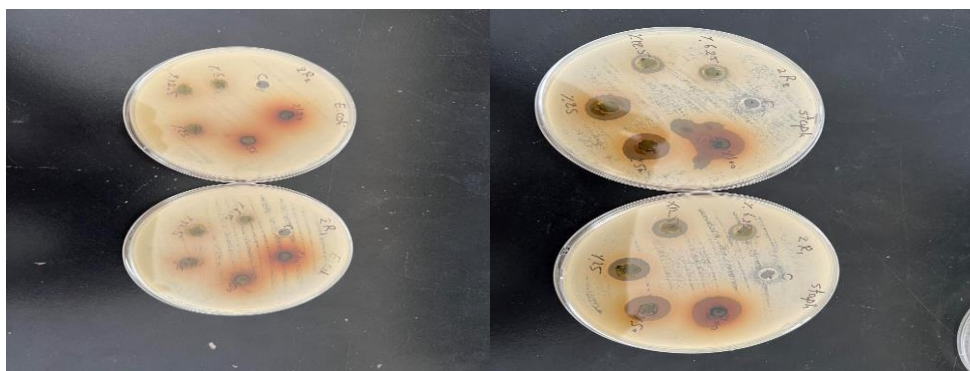


Figure 6: Zones of inhibition resulting from a range of concentrations of Hamuri extract showing antibacterial effect on *Escherichia coli*

Figure 5: Zones of inhibition resulting from a range of concentrations of Hamuri extract showing its antibacterial effect on *staphylococci*.

Figure 7 illustrates the concentration-dependent antibacterial activity of Common extract and Tabuni extract against the Gram-negative bacterium *Escherichia coli*.



Figure 7: Zones of inhibition resulting from a range of concentrations of common extract (R3) and Tabuni extract (R1) show antibacterial effect on *E. coli*.

4. Discussion

This study investigates the phytochemical composition and antibacterial activity of different date palm leaf extracts. Understanding these properties is important because date palm leaves are a widely available natural resource that may contain bioactive compounds with potential medical applications, including antioxidant, analgesic, antispasmodic, and bactericidal effects. Identifying which types of leaves possess the most effective antibacterial activity against pathogens such as *Staphylococcus aureus* can support the development of natural, plant-based therapeutic agents. A group of previous studies have confirmed the presence of various phytochemical compounds in date palm leaves. One study [16] discovered that date palm leaves contain flavonoids, phenols, saponins, and steroids. Another study on the same



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plant [20] showed that three types of palm leaves contain carbohydrates, steroids, and tannins. The present study is consistent with another investigation [21] in confirming that date palms contain flavonoids, phenols, steroids, saponins, and tannins, but disagrees with the presence of alkaloids. An additional study [22] found in palm leaf extracts the presence of various active substances, including tannins, flavonoids, saponins, phenols, carbohydrates, and terpenoids, which clarified the absence of steroids and the presence of alkaloids, differing from the results of this study. Our research has shown that date palm leaves do not contain alkaloids or resins, a difference that may be attributed to the influence of environmental conditions. Tannins, which are a group of phenolic compounds, are known to form irreversible complexes with proline-rich protein [23,24], resulting in inhibition of cellular protein synthesis [25]. Tannins react with proteins to produce a typical tanning effect, which is important for the treatment of inflamed or ulcerated tissues. Herbs containing tannins have an astringent effect and are used to treat intestinal disorders such as diarrhea and dysentery [26] when administered to animals, hence their widespread use in medicine for drug development [26]. They also have analgesic, antispasmodic, and bactericidal effects [27]. In addition, this study showed the presence of flavonoids in the form of phenolic compounds. The main group of compounds that act as primary antioxidants or free radical scavengers used for medical purposes, such as catechol, hydroquinone, and resorcinol, are phenolic salicylates, used as analgesics and antipyretics. Table (2) shows that the highest moisture percentage was recorded at the Aami plant (9.96%), which is very close to the humidity percentage at the Hamuri plant (9.03%). These results are consistent with the moisture percentage obtained in a study [28] which estimated humidity at 9%. They also correspond to the average moisture content reported in another study on areca palm leaves [29] of 9.35%. On the other hand, the lowest percentage was recorded at the Tabuni plant (6.45%), which is similar to the calculated moisture percentage in a study [30] where moisture was recorded at 7%. The highest ash content was found at the Aami plant (5.9%), and the lowest at the Tabuni plant (3.825%). The Hamuri plant had an ash content of 4.7%, which is very close to the value of 4.26% estimated in a study [31]. Another study [32] found lower values, with an ash content of about 2.9%. This difference can be explained by the different environments in which the plants grow.

The effect of the ethanol extract on some types of pathogenic bacteria was studied. The effect of the ethanol extract on some types of pathogenic bacteria was studied. The results obtained and shown in the table (Table.3) and the following figures showed that the extract has an inhibitory effect on bacterial growth. *S. aureus* and did not show an inhibitory effect on *E. coli* growth, as the area of inhibition was measured for each type of bacteria. From the results obtained in the table (Table 3), we note the presence of inhibitory activity of the three extracts against bacteria. *S. aureus* and the lack of inhibition against *E. coli* bacteria, as it was observed after 24 hours of incubation at a temperature of 37 degrees, a variation in the effect of the extracts at different concentrations, and this effect is evident in the appearance of a transparent area surrounded by the pits containing the extracts, which is called the inhibition zone, and this is confirmed by measurements of the diameters of the inhibition zones. The largest inhibition diameter was found in the case of bacteria. *S. aureus* was found in the Hamuri plant (16 mm), followed by the common plant (15.5 mm), then the Tabuni plant (12.5 mm) with the least effect, and did not have any effect on *E. coli* bacteria in any of the three extracts. The results of the current study are also consistent with a study by [22]. In another study conducted by [30]. For the same type of plant, Hamuri showed similar results, and the result was (14.5 mm) for *S. aureus* bacteria and did not show results against *E. coli* bacteria, which is not consistent with this study, which showed inhibition of the same bacteria. The current study is also consistent with the study [33]. In the absence of inhibition of the leaf extract against *E. coli* and also did not agree on the presence of inhibition of *S. aureus* bacteria. The study [34] showed, results are close to the results of the study, as there is an inhibitory effect on bacteria. *S. aureus* leaf extract and its ineffectiveness against *E. coli* of the same plant.





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5. Conclusion

Phytochemical screening of palm leaf extract revealed the presence of alkaloids, flavonoids, phenols, sterols, tannins, terpenoids, as well as carbohydrates and proteins. Moisture and ash were also assessed. The phytochemicals contained in palm leaf extract may be used for pharmacological purposes. Palm leaf extract shows efficacy against *Staphylococcus aureus* in a dose-dependent manner but ineffective against *Escherichia coli*, *Staphylococcus aureus* indicating a selective antibacterial action.

ETHICAL APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Author A designed the study and performed the phytochemical screening. Author B wrote the manuscript and revised it critically. Author C conducted the antibacterial activity tests and analyzed the data. All authors reviewed and approved the final version.

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Clinical Evaluation of Pethidine in Vaginal Labour: A Prospective Cohort Study at Soba and Saad Abo Alella University Hospitals

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ABSTRACT

Background: Labour is divided into three stages. Stage one encompasses cervical dilation from 0–10 cm, stage two involves foetal descent with perineal distension and activation of somatic nociceptive pathways, and stage three concludes with placental delivery. Pethidine, a narcotic opioid analgesic classified as a pregnancy category C drug, is widely used for intrapartum pain management. **Aim:** To evaluate the impact of pethidine on pain severity, cervical dilation, uterine contractions, and delivery outcomes in vaginal labour.

Methods: A prospective cohort study was conducted at Saad Abo Alella University Hospital and Soba University Hospital, Khartoum. A total of 385 women were enrolled, comprising 317 controls and 68 who received pethidine. Pain scores, contraction frequency, duration of the second stage of labour, and mode of delivery were systematically recorded and analyzed.

Results: Pethidine administration significantly reduced pain severity ($p < 0.05$). Contraction frequency increased from 2 contractions per 10 minutes (56%) before administration to 3 contractions per 10 minutes (74%) afterward. However, caesarean section rates were higher in the pethidine group (21%) compared with controls (11.7%). The second stage of labour was prolonged in the pethidine group, with 36.8% lasting 21–45 minutes, versus 36.3% lasting 8–20 minutes in the control group.

Conclusion: Pethidine provides effective analgesia and enhances uterine contractility during labour. Nevertheless, its use is associated with prolonged stage two and increased caesarean section rates. These findings highlight the need for cautious clinical application and further investigation into optimizing opioid use in obstetric practice.

Keywords: Pethidine, vaginal delivery, labour pain, uterine contractions, caesarean section

1. Introduction

Labour pain follows distinct neurophysiological pathways across the three stages of labour. The first stage comprises the latent phase (0–4 cm cervical dilation) and the active phase, which continues until full dilation (10 cm). Pain during this stage is primarily associated with regular uterine contractions and progressive cervical stretching. Transmission occurs via the hypogastric plexus toward the spinal cord through the lumbar paravertebral sympathetic chain. Nociceptive impulses enter the dorsal roots of T10–L1 and ascend through the spinothalamic tracts to higher centres.

In the second stage, near the completion of cervical dilation, descent of the foetal head produces perineal distension and activates somatic nociceptive pathways. These impulses are transmitted predominantly through the pudendal nerves, originating from the sacral roots S2–S4.

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According to the American College of Obstetricians and Gynaecologists (ACOG), a prolonged second stage is defined as exceeding 3 hours with regional anaesthesia or 2 hours without anaesthesia. The third stage of labour is characterized by the interval between foetal delivery and expulsion of the placenta and foetal membranes, typically completed within 30 minutes (1).

Narcotic analgesics are pregnancy category C drugs, except oxycodone which is category B, and are not advised in pregnancy unless maternal benefit outweighs foetal risk. Pethidine is a synthetic opioid structurally distinct from morphine, used for acute pain. It has less effect on uterine smooth muscle than morphine and is the opioid most frequently employed in obstetrics (2).

Pethidine has faster onset and shorter duration than morphine salts. It may be administered epidural, intrathecal, intravenous, or intranasal. About 60–80% binds to plasma proteins. It is metabolized in the liver and excreted by the kidney. Pethidine crosses the placenta and is detectable in breast milk (2).

The use of narcotic analgesics such as pethidine during labour remains controversial. Although pethidine is administered for analgesia or to enhance uterine contractions, clinical evidence indicates that it may paradoxically delay labour and provide limited pain relief. This creates a clinical dilemma, particularly as pethidine is classified as a pregnancy category C drug with potential neonatal respiratory effects (3).

Despite its widespread use, the dual impact of pethidine on pain relief and labour progression continues to be debated, necessitating comprehensive evaluation.

Aim: Determining whether the benefits of pethidine in vaginal delivery outweigh its risks, especially in contexts where it remains commonly employed. Previous research has often focused on isolated aspects—either supporting its analgesic role or emphasizing its adverse effects—without integrating overall clinical outcomes. This prospective cohort study, conducted at Soba University Hospital and Saad Abo Alella University Hospital, Sudan, in 2022, aims to address this gap by systematically evaluating the clinical importance of pethidine in vaginal labour, including its effective dose, route of administration, and justification for continued use.

2. Methods and materials

Study Design and Setting: This study was conducted as a hospital-based prospective cohort at Soba University Hospital and Saad Abo Alella University Hospital, Khartoum, Sudan. The investigation spanned six months, from June to December 2022, during which eligible participants were consecutively enrolled and systematically assessed according to predefined objectives.

Study Population: The study population included pregnant women who delivered vaginally, as well as those initially attempting vaginal delivery but subsequently converted to caesarean section. Inclusion criteria were singleton term pregnancies, while women with pre-existing obstetric complications were excluded.

Sample Size and Sampling Methods: A total of 385 participants were included. Sample size was determined using the formula:

$$n = \frac{z^2 pq}{e^2}$$

Where e represents the desired level of precision, p the estimated population proportion, and $q = 1 - p$. This calculation yielded a sample size of 385 women, distributed into 317 controls and 68 received pethidine.

Data Collection and Analysis Data were collected using a structured questionnaire designed to capture relevant clinical and demographic information. The dataset was entered and organized with Microsoft Excel (version 10) and analyzed using SPSS (version 21). Descriptive statistics, including frequency distributions, were applied, and associations between variables were evaluated using independent chi-square tests to ensure clarity and systematic reporting. P value of 0.05 was considered significant.





Ethical Considerations Participation was voluntary, and patients were free to accept or decline inclusion in the study. Ethical approval was obtained from institutional review boards, and confidentiality was maintained throughout.

3. Results

Pain severity

Pain severity was assessed in Group A using the dimensional pain intensity scale both before and after pethidine administration. The findings revealed that 31% of women experienced moderate pain prior to receiving pethidine, whereas after administration, 30% reported only mild pain. Severe pain (10%) was reduced to 0% after pethidine. The reduction in pain severity was statistically significant ($p < 0.05$). These results are illustrated in Figures 1 and 2.

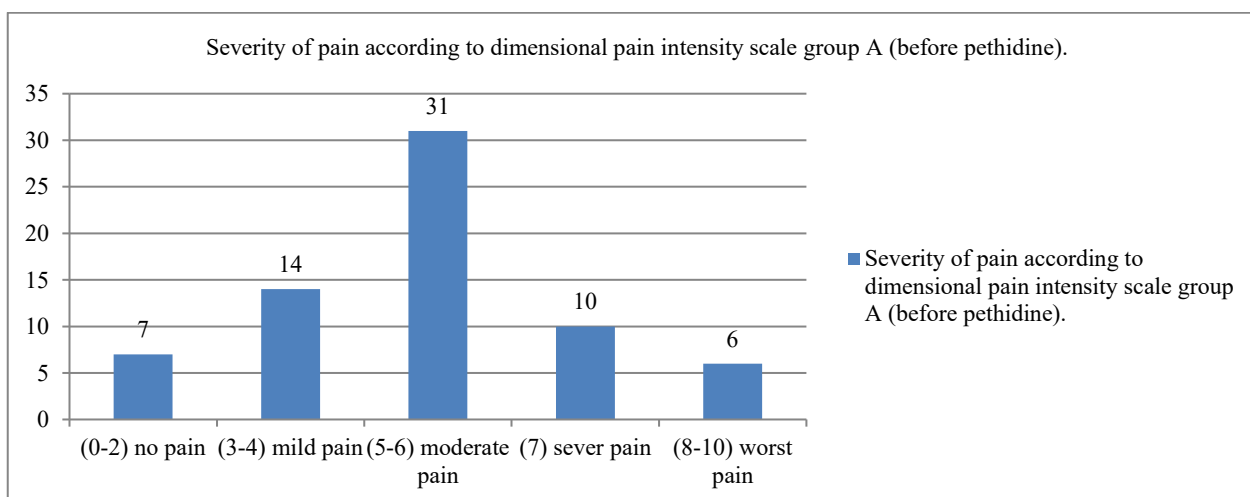


Figure1. Severity of pain according to dimensional pain intensity scale group A (before pethidine)

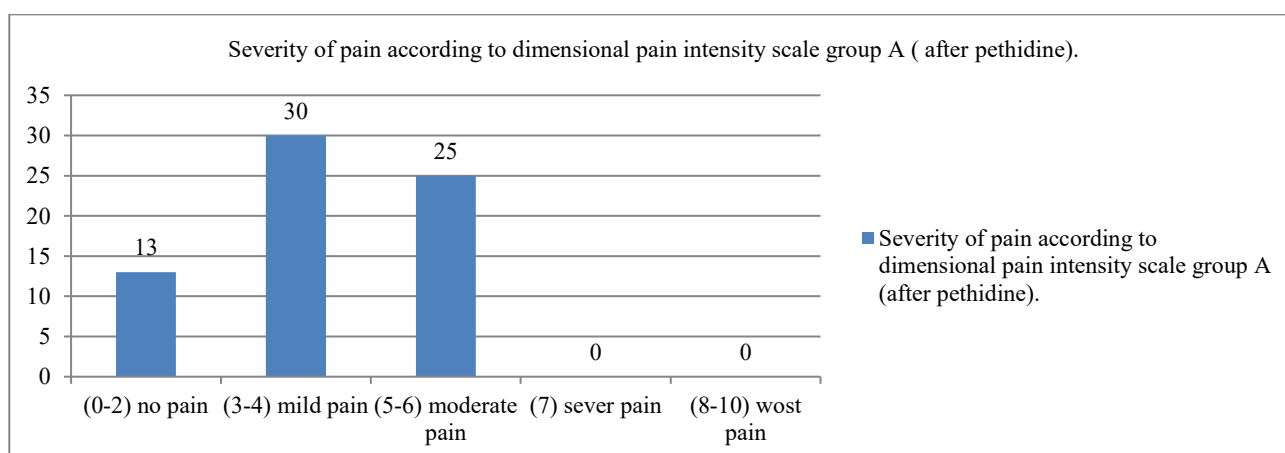


Figure2. Severity of pain according to dimensional pain intensity scale group A (after pethidine)



The frequency of uterine contractions

Was assessed per 10-minute interval in both study groups in the control group (Group B), 56% of women experienced two contractions within 10 minutes. In the contrast, Group A showed an increase from 56% (two contractions per 10 minutes before pethidine) to 74% (three contractions per 10 minutes after pethidine). The difference in contraction frequency between groups was statistically significant ($p = 0.002$). See Figure 3 & 4 and Table 1.

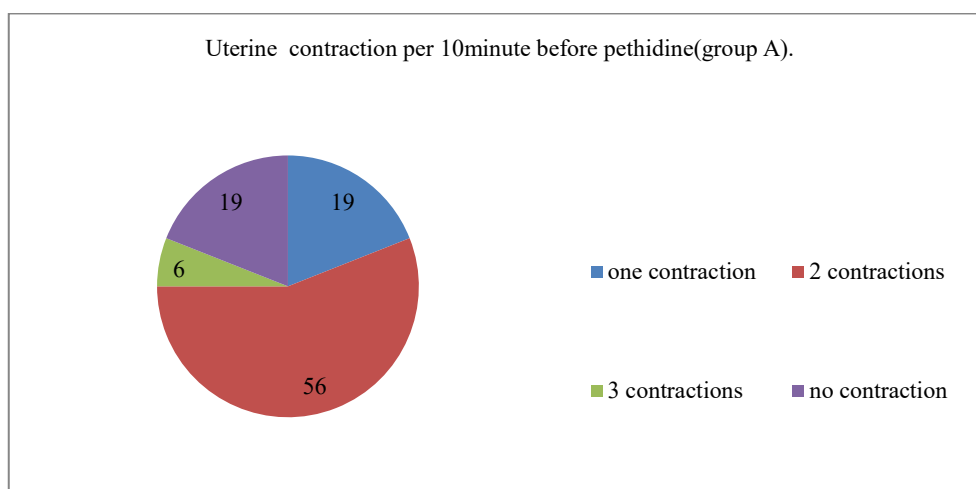


Figure 3 Uterine contraction per 10-minute (before pethidine) groups A

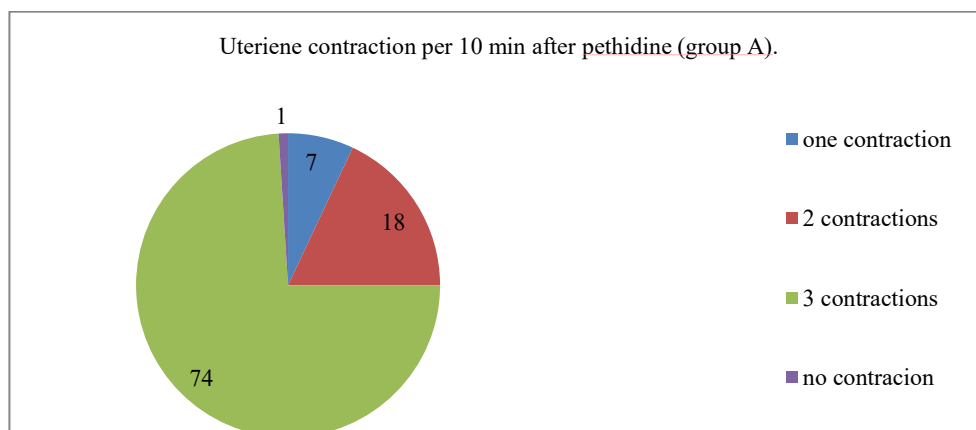


Figure 4 Uterine contraction per 10-minute (after pethidine) group A.

**Table1. Uterine contraction per 10-minute (control) group B**

Group	Uterine contraction per 10-minute (control)group B	Frequency	Percent
Control group	1/10	35	11.0
	2/10	51	16.1
	3/10	214	67.5
	Non	17	5.4
	Total	317	100.0
Pethidine group	Non	68	100.0

Interventions required

Interventions such as oxytocin administration and artificial rupture of membranes (ARM) were compared between groups. In Group B, 83% of women required both oxytocin and ARM, while in Group A, 79% required oxytocin before pethidine and 19% required oxytocin after pethidine. This indicates that pethidine reduced the reliance on combined interventions compared to control.

Table 2 comparison between Interventions needed for group A and B (oxytocin before pethidine, oxytocin after pethidine or oxytocin and artificial rupture membrane (ARM) before pethidine).

Group	Intervention (oxytocin before pethidine oxytocin after pethidine or oxytocin and ARM before pethidine)	Frequency	Percent
Control group	Oxytocin	2	0.6
	Oxytocin and ARM	264	83.3
	Non	51	16.1
	Total	317	100.0
Pethidine group	Oxytocin before pethidine	54	79.4
	Oxytocin after pethidine	13	19.1
	Oxytocin and ARM before pethidine	1	1.5
	Total	68	100.0

Caesarean section outcomes

Conversion to caesarean section was more frequent in Group A (21%) compared to Group B (11.7%). This suggests that while pethidine reduced pain and enhanced contractions, it was associated with a higher rate of caesarean section.

Evaluation of the First Stage of Labour Progression

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The duration of the first stage of labour, defined by cervical dilatation up to 10 cm, typically ranges between two to three hours. However, in some cases from both study groups, progression to the third stage of labour was not achieved, necessitating conversion to caesarean section. This highlights variability in labour outcomes and underscores the importance of monitoring stage one duration as a determinant of delivery mode. The results indicated that 36% of pregnant women experienced a stage one, duration of at least 8 minutes, with 12% subsequently converted to caesarean section (Table 3), while 37% of women had stage one lasting at least 21 minutes, with 31% requiring conversion to caesarean section (Table 4).

Table 3 length of the stage one in group B and cervical dilation.

Group	length of the stage one in group B and cervical dilation	Frequency	Percent
Group B	3-7 min	33	10.4
	8 - 20 min	115	36.3
	21 - 45 min	96	30.3
	46 - 100 min	34	10.7
	> 100 min	2	0.6
	Converted to caesarean section	37	11.7
	Total	317	100.0
Group A	Non	68	100.0

Table 4. length of the stage one in group A and cervical dilation

Group	Length of the stage one in group A and cervical dilation.	Frequency	Percent
Group B	Non	317	100.0
Group A	8 - 20 min	17	25.0
	21 - 45 min	25	36.8
	46 - 100 min	4	5.9
	> 100 min	1	1.5
	Converted to caesarean section	21	30.9
	Total	68	100.0

Gravidity Status of Participants

In this study, pregnant women from both groups were categorized according to their gravidity status into primigravida (women





experiencing pregnancy for the first time) and multigravida (women with more than one pregnancy). The results showed that 62% of participants in Group A were classified as primigravida, whereas 61% of participants in Group B were classified as multigravida. (Figure 5)

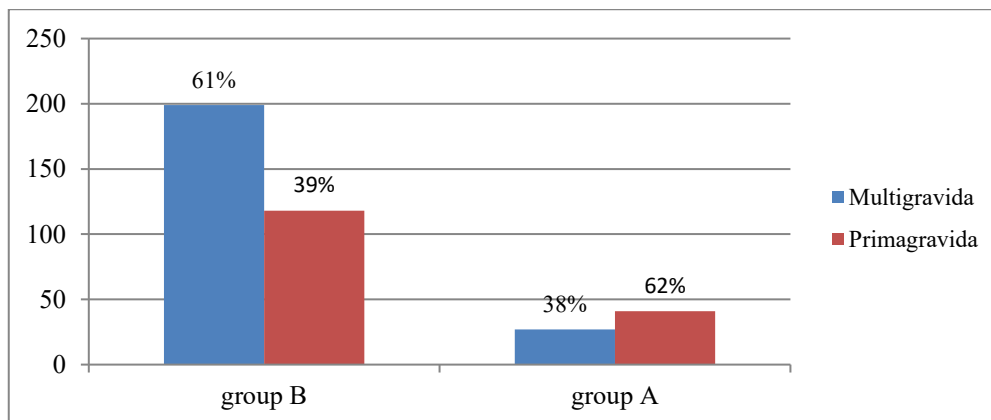


Figure5. Comparison between group A and group B in number of gravida, primigravida or multigravida

Indication of pethidine use:

Pethidine been used in Saad Abo Ella and Soba Teaching Hospital mostly to reduce the labour pain. The results showed that 95%of pregnant women taken pethidine for induction of labour and 5% take it to relief the labour pain.

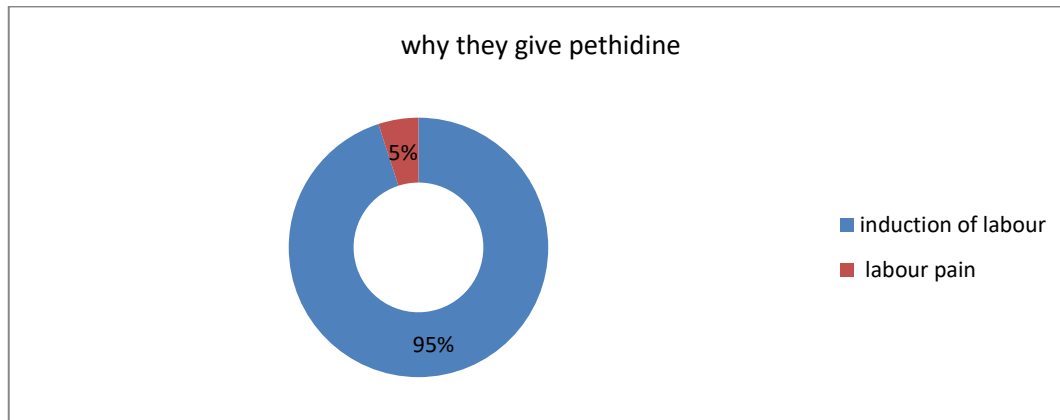


Figure 6 Indication of pethidine for been used in labour.

Evaluation of Pethidine Dose in Relation to Gravidity

In Group A, participants were further subdivided into two subgroups: the first subgroup received 50 mg of intramuscular pethidine, while the second subgroup received 100 mg of intramuscular pethidine, in order to determine the effective dose in relation to primigravida and multigravida. The results were statistically significant (P value=0.002), indicating that the administered dose of pethidine influenced both primigravida and multigravida participants. A dose of 50 mg produced effective outcomes in both groups; however, primigravida women demonstrated a higher overall consumption of pethidine compared to multigravida women. (Table 5)

**Table5. Correlation between the Dose of pethidine and the number of gravida (Primigravida or Multigravida)**

Dose of pethidine	Number of gravida		Total
	Multigravida	Primigravida	
Group A dose 50 mg	16	22	38
	4.2%	5.7%	9.9%
Group A dose 100 mg	11	19	30
	2.9%	4.9%	7.8%
Group B	199	118	317
	51.7%	30.6%	82.3%
Total	226	159	385
	58.7%	41.3%	100.0%

Correlation between Pethidine Indication and Gravidity Status

Data were collected according to the clinical indications for pethidine use among both primigravida and multigravida participants. The women were categorized into two groups based on indication: those who received pethidine for labour induction and those who received pethidine for labour pain management.

The analysis revealed a highly significant association (P value = 0.0001), confirming that pethidine administration influenced delivery outcomes in relation to gravidity status. Specifically, primigravida women were more likely to consume pethidine for labour induction, whereas multigravida women predominantly received pethidine for pain management. (Table 6)

Table 6 Correlation between Indication of pethidine used for labour and Number of gravida: Primigravida or Multigravida.

Introduction of pethidine	primigravida or multigravida		Total
	multigravida	primigravida	
induction of labour	23	41	64
	6.0%	10.6%	16.6%
labour pain	4	0	4
	1.0%	0.0%	1.0%
Non	199	118	317





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Introduction of pethidine	primigravida or multigravida		Total
	multigravida	primigravida	
	51.7%	30.6%	82.3%
Total	226	159	385
	58.7%	41.3%	100.0%

Association between First Stage Labour Duration and Gravidity Status in Group A

The duration of the first stage of labour was documented for both primigravida and multigravida participants in Group A to allow comparison between the two categories. The analysis revealed a statistically significant difference (P value = 0.027), indicating that the length of stage one was affected by gravidity status, with notable variation in time consumption between primigravida and multigravida women. (Table 7)

Table 7. Correlation between the length of stage one and cervical dilation in (Group A) and Number of gravid (primigravida or multigravida).

length of the stage one and cervical dilation (Group A)	primigravida or multigravida	
	Multigravida	Primigravida
8 - 20 min	9	8
	2.3%	2.1%
21 - 45 min	10	15
	2.6%	3.9%
46 - 100 min	0	4
	0.0%	1.0%
> 100 min	1	0
	0.3%	0.0%
Non	206	132
	53.5%	34.3%
Total	226	159
	58.7%	41.3%





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4. Discussion

In this study, 385 pregnant women (both primigravida and multigravida) planning vaginal delivery were enrolled and divided into two groups. Group A (pethidine group) comprised 68 women who received intramuscular pethidine; of these, 31% required conversion to caesarean section. Group B (control group) included 317 women, among whom 12% underwent caesarean section due to prolonged first stage of labour without identifiable cause.

Intramuscular administration was the most frequently employed route for pethidine, consistent with the findings of Michelle H.Y. et al, who demonstrated that intramuscular pethidine provided superior analgesia compared with placebo during labour. These observations support its continued use as a simple and cost-effective therapeutic option for managing labour pain, particularly in facilities where epidural analgesia is not readily available. However, the analgesic effect of a 100 mg dose of pethidine was modest, indicating limited efficacy in achieving optimal pain control.

In accordance with Onyeoji al, the addition of 50 mg of pethidine to bupivacaine for epidural analgesia significantly prolonged the mean duration of analgesia. Conversely, Olofsson et al reported that labour pain is not responsive to systemically administered morphine or pethidine, which primarily induce sedation rather than effective analgesia. Given the well- documented adverse neonatal effects, systemic pethidine should therefore be used with caution. In the present study, pethidine reduced pain severity in Group A, with cases of severe pain decreasing from 10% to 0%, and moderate pain declining from 31% to 25% following administration.

The increased caesarean section rate observed in the pethidine group may be attributed to prolonged stage one labour, consistent with the findings of Thomson et al. A limitation of this study is the absence of neonatal outcome assessment. Cho et al likewise reported that pethidine delayed labour progression while providing only modest analgesic benefit.

The findings of this study demonstrated that pethidine influenced uterine contractions, with maximum contraction frequency increasing from 56% to 74% following administration. Kamyabi al, explained that severe pain and anxiety elevate catecholamine release, resulting in ineffective contractions and prolonged labour. By lowering catecholamine levels, pethidine may enhance contraction efficiency, although this effect remains debated.

In contrast, Thomson A.M. et al, reported a positive correlation between the amount of pethidine administered during the first stage of labour and the length of both the first ($F = 0.5687$, $P = 0.0001$, $CI = 0.33-0.74$) and second stages ($R = 0.3204$, $P = 0.037$, $CI = 0.03-0.56$). In the present study, women in the pethidine group required longer time in stage one, with 37% of Group A consuming 21–45 minutes compared with 30% in Group B. These results suggest that the addition of pethidine further prolonged the first stage of labour.

In this study, 95% of pregnant women received pethidine for labour pain and 5% for induction of labour at doses ranging from 50–100 mg. Gallen B.F. et al, reported that doses between 100–400 mg significantly shortened labour duration in primiparae, with an average dose of 300 mg. Adequate analgesia without amnesia was achieved in 72% of patients, while satisfactory amnesia was observed in 61% when pethidine was combined with a barbiturate. No adverse maternal or neonatal effects were documented in their cohort. Roby and Schumann (1943) administered intramuscular pethidine at 100 mg in combination with scopolamine at the onset of labour pains, producing satisfactory amnesia in 85% of cases. However, 14% of neonates required resuscitation, highlighting potential risks associated with this regimen.

In this study, pethidine influenced uterine contractions in Group A, with maximum contraction frequency increasing from 56% before administration to 74% after. Kamyabi al, noted that uterine contractions at the end of pregnancy facilitate cervical dilatation and foetal





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expulsion, yet they remain a painful and distressing experience for mothers. The obstetrician's objective is to minimize both the duration of labour and the intensity of pain through careful management of labour phases. In cases of severe pain resulting from uterine contractions, narcotic agents such as pethidine (meperidine) are commonly indicated.

Pain and anxiety can alter labour progression by stimulating catecholamine and cortisol release. Noradrenaline enhances uterine contractions, whereas cortisol and adrenaline reduce them. Excessive pain and anxiety often elevate adrenaline and cortisol, leading to diminished contraction efficiency and prolonged labour. In some cases, contractions become severe and frequent but fail to produce cervical progress. Pethidine reduces circulating catecholamine levels, thereby potentially accelerating delivery. Considering its effects on cervical smooth muscle and consistency, alongside prior evidence of its analgesic role, pethidine appears to relieve labour pain while maintaining contraction activity.

5. Conclusion

This study demonstrated that pethidine played a role in reducing labour pain and lowering the need for additional interventions, such as oxytocin administration and artificial rupture of membranes (ARM), particularly when administered in low doses. Intramuscular administration was the most frequent route, especially among primigravida women, and was associated with an increase in uterine contraction frequency. At the same time, the findings indicate that pethidine may contribute to prolongation of the first stage of labour and a higher rate of caesarean section. These results highlight both the potential benefits and limitations of pethidine use in labour, underscoring the need for careful clinical judgment in its application.

5.1. Recommendation

Further research is recommended to explore alternative routes of pethidine administration; as such adjustments may provide improved clinical outcomes. Optimizing administration methods could contribute to reducing the incidence of caesarean section and enhancing the overall safety and effectiveness of labour management

5.2. Limitations

A limitation of this study is that higher doses are often required with intramuscular administration, whereas intravenous or epidural routes may achieve effective analgesia with lower doses and faster onset of action. Such alternative approaches could potentially reduce the incidence of caesarean section and improve overall labour outcomes.

ETHICAL STATEMENT

The Ethics Committee of the Faculty of Pharmacy at Alasmarya Islamic University approved the study protocol (Approval No. PH03:2022).

CONFLICT OF INTEREST

Authors disclose no conflict of interest.

AUTHORS' CONTRIBUTIONS

R.A: Study design : W.A Data collection and analysis. Both authors shared writing and formatting.

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Study of the Physicochemical Properties of Different Hair and Body Shampoo Brands Available in the Local Market of Al Khums, Libya

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ABSTRACT

Background: Shampoos, for both hair and body, are essential agents for removing sebum and environmental contaminants. Recent research has focused on the evolving requirements of these formulations, emphasizing their composition, functional roles, and evaluative criteria. **Aims:** The aim of this study was to perform a comparative physicochemical analysis of hair care and body care shampoo formulations, searching for physical or chemical differences to justify the varied use. The goal of this study was to assess the physicochemical characteristics of hair and body shampoo samples consistent with those observed differences.

Methods: Ten randomly selected commercial brands by chosen different brands that was more trading by Libyan people (five hair shampoos and five body washes from various manufacturers) were analyzed. The evaluation included surface tension, foam height, pH, Total Dissolved Solids (TDS), Refractive Index (R.I.), Brix%, and moisture content.

Results: The data exhibited notable differences between the two product categories. Body shampoos generally displayed lower surface tension values, which suggests a higher efficiency of the surfactant systems in reducing interfacial tension compared to hair formulations. Furthermore, body shampoos demonstrated higher foam heights, aligning with consumer sensory expectations for skin cleansing. Conversely, some hair shampoos produced lower foam volumes, which may be attributed to the presence of conditioning agents added to protect the hair shaft. Hair shampoos also tended to be more acidic, a property beneficial for maintaining cuticle integrity. Both formulations displayed high TDS levels, indicating the consistent use of electrolytes as rheology modifiers to achieve desired viscosity.

Conclusion: Overall, the differences observed in both product types were likely greater due to intentional differences made for the sake of specific functional biologically needs (skin health v. hair health) and sensory performance.

Keywords: shampoo, surface tension, pH, Total Dissolved Solids TDS





1. Introduction

In the realm of physics and chemistry applied to consumer goods, everyday cleansing agents have grown far more sophisticated due to advances in personal care formulations, take shampoos and body washes as prime examples. What was once a straightforward substance for removing dirt and oil has now become a multifunctional system. Looking back, a quality shampoo was simply expected to perform three basic tasks, lift away grime from hair fibers, restore surface reflectivity (shine), and keep strands easy to comb or style. Now, shampoo is a subjective term that can describe an even larger variety of formulations and functions, so there is no longer one standardized definition to represent a shampoo. Thus, there has become an acceptable expectation of basic functional criteria for the consumer, which would be an acceptable product. These basic functional criteria would be the ability to clean the secretions of the sebaceous gland, remove environmental pollution, and remove residuals from the previously applied hair product (e.g., polymeric materials). After cleaning, there should not be a negative impact on the condition of the hair after rinsing regarding management or combing, wet, or dry, act as a carrier to deposit beneficial substances, which are relatively non-irritating, non-harmful to the scalp or the eye [1]. The amount of foam a product produces is not directly linked to how well it cleans [2], the ability to generate a considerable amount of foam is still viewed as a very important component in consumer acceptance as, from pure psychology terms, consumers like foaming formulas. Some formulations may lead undesirable effects such as excessive dryness of the hair, or irritation to the eye or corneal opacification in some formulations, indicating that conditioning and formulation approaches are clearly important [1]. The primary function of these types of products is to utilize surfactants to remove oils and soil (Sebum) [3]. While the primary ingredients may be similar for hair shampoo and body wash, they are formulated with careful physicochemical properties to meet their individual functional and biological purposes. Hair shampoo has the objective of keeping the cuticle scales intact while cleansing the scalp. Hair shampoos are formulated with a low (pH) (acidic) [4], consumers often associate abundant lather with superior cleaning power. Therefore, shampoo manufacturers typically add surfactants that produce rich foam. Shampoos are also formulated to have a slightly acidic pH (between 3.5 and 5.5). This helps close the hair cuticle and gives hair a smoother look. In contrast, body washes should be formulated with a pH close to that of the skin's natural acid mantle, in order to protect the skin's barrier function [2]. It is important to measure the physicochemical properties of these products in order to consider their functional properties. While rheological properties, influenced by Total Dissolved Solids (TDS) and additional electrolytes, govern physical stability and viscosity, surface tension is a more straightforward measure of surfactant effectiveness [5,6].

2. Material and Methods

Sample Collection

To ensure a representative assessment of products available in the local market, ten commercially available formulations comprising five hair shampoos and five body shampoos were randomly purchased from selected pharmacies in Al Khums City, Libya. Pharmacies were specifically chosen as procurement points to guarantee that the samples had been stored under standardized professional conditions (controlled temperature and humidity), thereby ensuring the integrity of their physicochemical properties. The samples were coded as 1–5 for hair shampoos and 6–10 for body washes show at in Table (1).





Table (1): Specification of the Selected Hair and Body Shampoo Samples

Sample No.	Sample Category	Brand Name
1	Hair Shampoo	Dove
2	Hair Shampoo	Vaseline
3	Hair Shampoo	Pantene
4	Hair Shampoo	Lifebuoy
5	Hair Shampoo	Clear
6	Body Shampoo	Fa
7	Body Shampoo	Agrado
8	Body Shampoo	Genera
9	Body Shampoo	Felce Azzurra
10	Body Shampoo	Neutro Derma

Moisture Content Analysis

The moisture content (total volatile matter) of the samples was determined using the atmospheric oven-drying method in accordance [7]. Standards. Approximately 2.0 g of each shampoo and body wash sample was accurately weighed into a pre-dried porcelain crucible. The samples were then placed in a digital thermostatic oven maintained at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Drying continued until a constant weight was achieved, indicating the complete removal of volatile components. To ensure statistical reliability and precision, all measurements were conducted in triplicate ($n=3$). The moisture percentage was calculated based on the weight loss relative to the initial sample mass.

Sample Preparation

Standard test solutions for each sample were prepared using distilled water at a uniform concentration of 10% (w/v). These solutions served as the basis for all subsequent physicochemical evaluations.

Surface Tension Measurement

The surface tension was determined using the Drop Weight Method (Stalagmometer). This technique involves measuring the weight or counting the number of drops for a fixed volume of the sample solution compared to distilled water as a reference standard [5]. The data calculated by following equation (1)

$$R_2 = R_1 \times \frac{n_1(w_1-w_3)}{n_2(w_1-w_2)} \dots \dots \dots (1)$$





W_1 is weight of empty beaker.

W_2 is weight of beaker with distilled water.

W_3 is weight of beaker with shampoo solution.

n_1 is number of drops of distilled water.

n_2 is number of drops of shampoo solution

R_1 is surface tension of distilled water.

R_2 is surface tension of shampoo solution.

Foaming Capacity Test

Foaming ability was evaluated using the Cylinder Shaken Method [2]. A predetermined volume of the 10% shampoo solution was transferred into a graduated cylinder. The cylinder was shaken vigorously for 30 seconds (approximately 20 uniform shakes) to generate foam. The initial foam height (cm) was recorded immediately to assess foaming capacity, while stability was observed over a fixed duration.

pH and TDS Quantification

pH Assessment: To evaluate the biological compatibility of the products with skin and hair, the pH of the prepared solutions was measured using a calibrated digital pH meter [8]. Total Dissolved Solids (TDS): The concentration of electrolytes (salts), which serve as rheology modifiers, was quantified using a digital conductivity meter. Results were recorded in parts per million (ppm) to reflect the ionic strength of each formulation [6].

Refractive Index and Brix Measurement

The Refractive Index (R.I.) was measured using a refractometer, calibrated with distilled water, to assess the purity and concentration of dissolved solids. A drop of each sample was placed on the prism, and the R.I. was read directly. Subsequently, the Brix percentage (Brix %), representing the total weight of non-volatile dissolved solids, was derived from the R.I. values to characterize the formulation density [3].

3. Results and Discussion

The quality and efficacy of personal care products are fundamentally dependent on the correct balance of their physicochemical properties. Knowledge of these properties is essential to ensure functionality and consumer safety.

Table (2): Comparison of Moisture Content in Hair and Body Shampoos

Sample No.	Sample Type	Sample Color	Moisture Content (M.C) %
1	Hair Shampoo	Creamy White	66.54 ±0.45
2	Hair Shampoo	Orange	61.19 ± 0.32
3	Hair Shampoo	Creamy White	58.76 ± 0.51
4	Hair Shampoo	Sky Blue	59.71 ± 0.28





Sample No.	Sample Type	Sample Color	Moisture Content (M.C) %
5	Hair Shampoo	Pink	50.40 ± 0.58
6	Body Shampoo	Pink	80.60 ± 0.35
7	Body Shampoo	Sky Blue	78.74 ± 0.42
8	Body Shampoo	Sky Blue	88.69 ± 0.25
9	Body Shampoo	Faint Green	84.63 ± 0.38
10	Body Shampoo	Creamy White	85.01 ± 0.47

Note: Values are presented as Mean ± Standard Deviation (SD) for triplicate measurements (n=3).

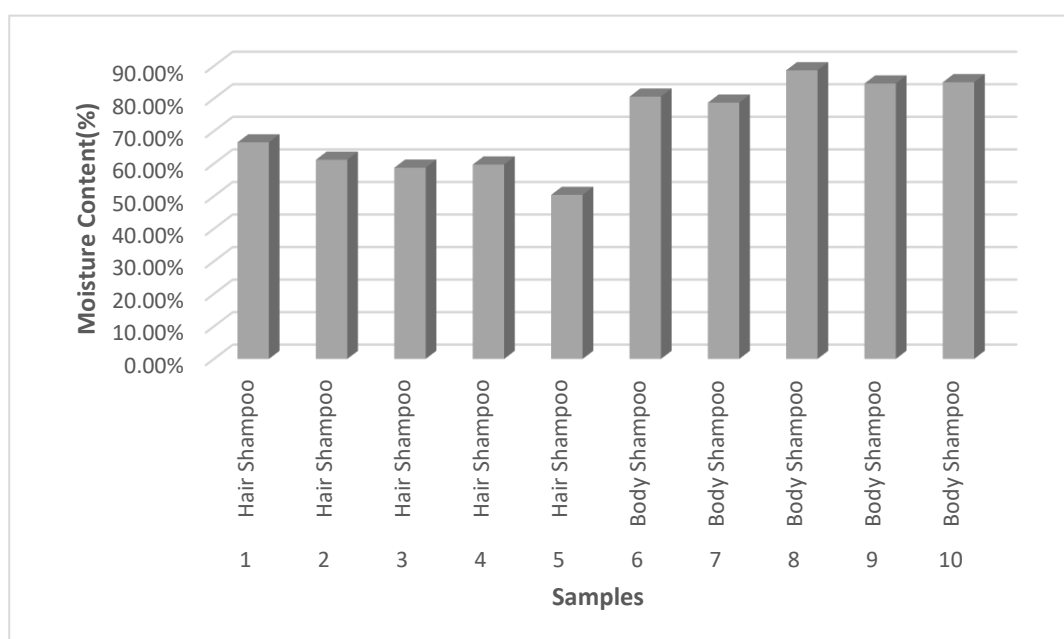


Figure (1): Comparison of Moisture Content in Hair and Body Shampoos

Moisture Content (MC) is the complementary category of Total Solids concentration. The results displayed in Table 2 clearly indicate that hair shampoos (Samples 1–5) contained moisture contents (50.40%–66.54%) that were consistently lower than those of body shampoos (Samples 6–10) (78.74%–88.69%). This observation is significant, as it indicates that hair products are more commonly formulated with a higher concentration of thickeners and conditioning agents (such as silicones and polymers). These non-volatile solids are essential for enhancing hair manageability and adjusting the viscosity of the formulation [3]. The reasoning behind this compositional difference lies in





the fact that hair shampoos require a higher viscosity to ensure the product remains stable and easy to apply.

Table (3): Comparative Analysis of Physicochemical Properties of Hair and Body Shampoos

Sample No.	Sample Type	pH	Conductivity ($\mu\text{S}/\text{cm}$)	TDS (mg/L)	Salinity (ppm)	Refractive Index (R.I.)	Brix (%)
1	Hair Shampoo	5.45	1675	1006	1694	1.3371	2.9
2	Hair Shampoo	5.88	1179	725	1191	1.3373	3.0
3	Hair Shampoo	6.70	1553	870	1578	1.3373	3.0
4	Hair Shampoo	6.83	1406	979	1634	1.3373	3.0
5	Hair Shampoo	6.63	1517	909	1511	1.3373	3.0
6	Body Shampoo	5.30	1200	643	1071	1.3372	2.9
7	Body Shampoo	6.68	2120	1278	2130	1.3372	2.9
8	Body Shampoo	6.25	1664	977	1699	1.3372	2.9
9	Body Shampoo	5.73	2410	1457	2430	1.3372	2.9
10	Body Shampoo	6.26	2740	1810	2620	1.3372	2.9

The measured pH range (5.30 to 6.83) for all samples demonstrates overall compatibility with the biological acid mantle. Notably, hair shampoos are formulated at a slightly acidic pH to ensure hair cuticle protection and minimize fiber swelling [9]. Analysis of Total Dissolved Solids (TDS) revealed a clear distinction between the two categories; body shampoos (Samples 6–10) exhibited higher and more varied TDS values, peaking at 1810 mg/L, compared to hair shampoos which reached up to 1006 mg/L. These elevated levels in body washes are attributed to the strategic addition of electrolytes primarily Sodium Chloride (NaCl), which are essential for optimizing viscosity and achieving the desired properties in surfactant-based formulations [5, 6]. Finally, the consistency of the Refractive Index (1.3371 – 1.3373) confirms rigorous quality control and ensures the visual clarity required for commercial products [10].



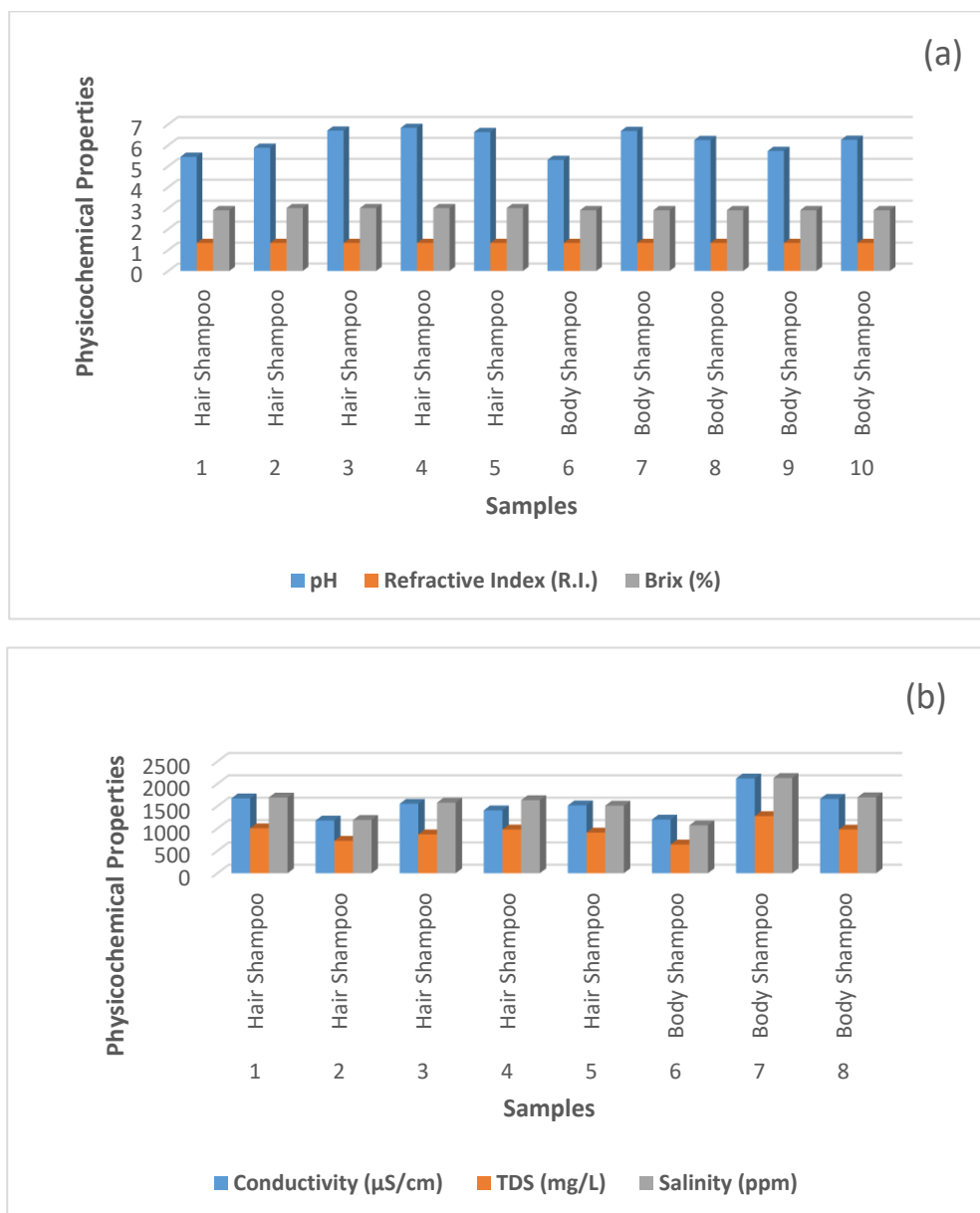


Figure (2a&b): Comparative Analysis of Physicochemical Properties of Hair and Body Shampoos





Table (4): Surface Tension Values for Hair and Body Shampoos Compared to Distilled Water

$R_1 = \text{constant} = 72.88 \text{ dynes/cm}$		$n_1 = \text{constant} = 412 \text{ drop}$		$w_1 = \text{constant} = 110 \text{ g}$	
Sample No.	Sample Type	w_2 (g)	w_3 (g)	n_2 (drop)	R_2 (dyne/cm)
1	Hair Shampoo	18.32	20.22	1100	29.87
2	Hair Shampoo	18.68	19.98	676	46.70
3	Hair Shampoo	18.61	20.22	1080	29.93
4	Hair Shampoo	18.57	20.38	1000	32.52
5	Hair Shampoo	18.16	19.81	905	35.82
Average	Hair Shampoos	18.47	20.12	932	35.00
6	Body Shampoo	18.12	19.42	1080	29.43
7	Body Shampoo	18.70	20.15	1130	28.20
8	Body Shampoo	18.17	19.41	1050	30.17
9	Body Shampoo	18.42	20.34	1050	31.28
10	Body Shampoo	18.58	19.96	1060	28.32
Average	Body Shampoos	18.60	19.86	1070	29.48

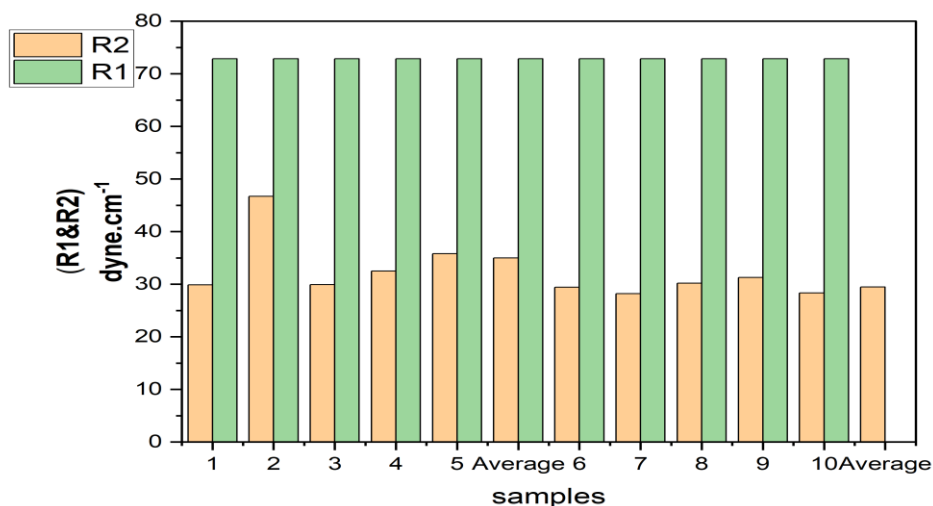


Figure (3): Surface Tension Values for Hair and Body Shampoos Compared to Distilled Water





All samples in Figure 3 demonstrated a marked reduction in surface tension compared to distilled water (72.88 dyne/cm). The average surface tension for hair shampoos (35.00 dyne/cm) was notably higher than that of body shampoos (29.48 dyne/cm). This numerical difference suggests that body wash formulations may incorporate a higher concentration or a more potent mixture of surfactants. Conversely, the presence of conditioning agents in hair shampoos such as oils and polymers may slightly interfere with the surfactants' orientation at the air-water interface, thereby resulting in relatively higher surface tension values compared to body wash products.

Table (5): Average Foam Height for Hair and Body Shampoos

Sample No.	Sample Type	Foam Height (cm)
1	Hair Shampoo	8
2	Hair Shampoo	15
3	Hair Shampoo	7.5
4	Hair Shampoo	12
5	Hair Shampoo	13
Average	Hair Shampoos	11.1
6	Body Shampoo	11
7	Body Shampoo	17
8	Body Shampoo	8
9	Body Shampoo	22
10	Body Shampoo	16
Average	Body Shampoos	14.8

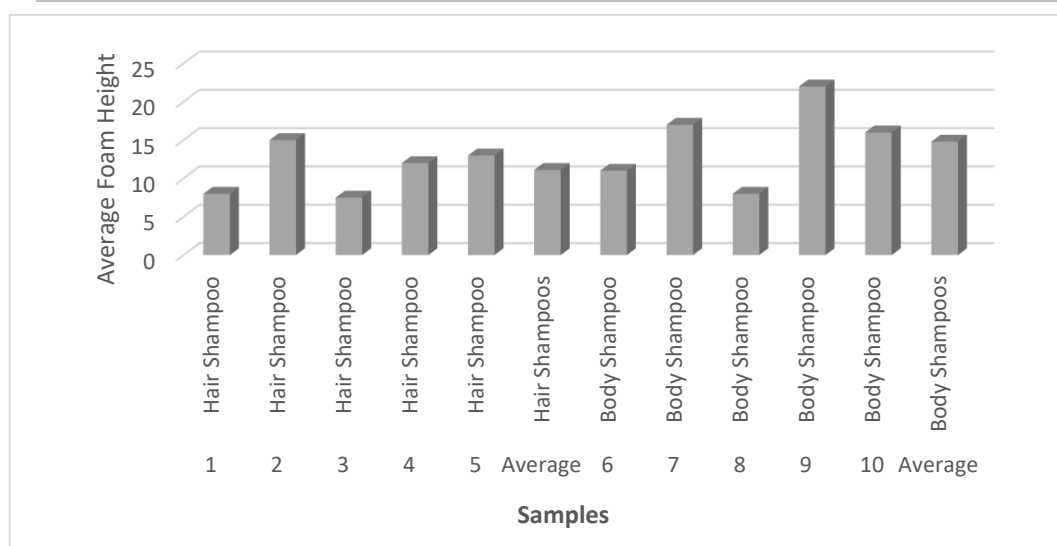


Figure (4): Average Foam Height for Hair and Body Shampoos





Table 5 and Figure 4 demonstrate that body shampoos yield a mean foam height (14.8 cm) that is consistently higher than the average for hair shampoos tested (11.1 cm). These results align with consumer sensory expectations, as cleansing efficacy is traditionally associated with a rich and voluminous lather [1]. The superior foaming performance of body washes is attributed to a synergistic blend of co-surfactants specifically formulated to enhance foam stability and volume. In contrast, the lower foam height observed in certain hair shampoos such as Sample 3 (7.5 cm) reflects a strategic formulation balance. This reduction in foaming power is often a consequence of incorporating conditioning agents, which are prioritized to improve hair manageability and provide fiber protection, even at the expense of maximum foam production.

4. Conclusion

Physicochemical analysis reveals distinct functional and compositional differences between body and hair shampoo formulations. Body shampoo met sensory criteria and exhibited high surfactant performance by generating more foam with lower surface tension. Hair shampoo was designed with a higher concentration of Total Solids (lower M.C) to contain the conditioning agents required for hair protection, along with an emphasis on acidic (PH) values for cuticle integrity. The high TDS values seen in both formulations underscore the key role of electrolytes in regulating the rheological properties (viscosity) of each formulation.

Recommendations

In order to fully understand how electrolyte levels, affect the stability and sensory properties of the final product, extensive investigations into about viscosity need to be conducted and compared to the chemical analysis, specifically TDS. Since the first foam height is insufficient for thoroughly evaluating the sensory performance of the product, Foam Stability (the foam remaining after a designated time) should be employed as an additional quality marker. Rather than relying on a purely gravimetric comparative approach, to improve the reliability of interpreting the surface tension and foaming results, the true concentration of total active surfactant for each sample should be determined. The true concentration of the total active surfactant for each sample should be measured rather than depending on simple gravimetric comparisons in order to improve the numerical analysis of both surface tension and foaming results. It is recommended that further testing is conducted to identify the surfactants that perform well at foaming and surface tension in the presence of high ratios of conditioning agents and oils. Body product manufacturers should confirm that the pH of the skin remains consistently in the safe range of approximately 5.5 during dilution and storage to be safety and optimal efficacy of the formulation.

ETHICAL STATEMENT

Not Applicable

CONFLICT OF INTEREST

No conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally.





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Biomarker Evaluation in Hemodialysis Patients at Al'assabiea Unit, Libya

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ABSTRACT

Background: Chronic kidney disease (CKD) is a progressive and irreversible condition associated with significant morbidity and mortality. Hemodialysis is widely used to remove metabolic waste products and maintain biochemical balance in patients with advanced renal failure. **Aims:** This study aimed to evaluate the effect of hemodialysis on key biochemical parameters in patients with chronic renal failure at the Al'assabiea Dialysis Unit, Libya.

Methods: A cross-sectional study was conducted including 10 patients with chronic renal failure undergoing regular hemodialysis and 10 healthy controls. Blood samples were collected before and after dialysis sessions. Biochemical parameters including urea, creatinine, glucose, uric acid, electrolytes, and hemoglobin were analyzed using standard laboratory methods. Statistical analysis was performed using SPSS, with $p < 0.05$ considered significant. The p-values were calculated using paired and independent t-tests as appropriate, comparing pre- vs. post-dialysis values within the patient group, and patient values vs. controls.

Results: Hemodialysis resulted in a statistically significant reduction in urea and creatinine levels ($p < 0.05$). Blood glucose levels increased significantly after dialysis ($p < 0.05$). Hemoglobin levels were lower in patients compared to controls. No significant changes were observed in sodium, potassium, or chloride levels.

Conclusion: Hemodialysis is effective in reducing uremic toxins such as urea and creatinine in CRF patients. However, the observed increase in blood glucose highlights the importance of routine monitoring during dialysis sessions. Addressing anemia and ensuring metabolic stability remain essential components of patient management.

Keywords: Chronic renal failure, urea, creatinine, hemodialysis, blood glucose

1. Introduction

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function lasting for more than three months with health implications [1]. It is characterized by a progressive and irreversible decline in renal function that may ultimately lead to end-stage renal





disease (ESRD) [2]. CKD represents a significant global health burden, contributing to high morbidity and mortality worldwide [3]. The global prevalence of CKD ranges from approximately 9% to 13%, with higher rates reported in low- and middle-income countries [3,4].

Several risk factors for CKD have been reported in the Libyan context [5]. Elevated levels of urea and creatinine are key indicators of renal dysfunction, reflecting impaired clearance of nitrogenous waste products [6]. Hemodialysis is a life-sustaining therapy used to remove these toxins and maintain metabolic balance in patients with severe renal failure [7].

Aims: This study aims to evaluate the effect of hemodialysis on key biochemical parameters, including urea, creatinine, glucose, electrolytes, and hemoglobin, in patients with chronic renal failure at the Al'assabia Dialysis Unit, Libya.

2. Methods: This cross-sectional study was conducted in March 2024 at the Al'assabia Dialysis Unit, Libya. Ten CRF patients (aged 21–65 years) undergoing regular hemodialysis and ten healthy controls were included. Blood samples were collected pre- and post-dialysis.

Biochemical parameters including urea, creatinine, glucose, and uric acid were measured using standard enzymatic methods. Electrolytes were analyzed using an automated analyzer. Hemoglobin was measured using the Drabkin method.

Normal reference ranges (as per laboratory standards) were as follows:

Hemoglobin: 13.0–17.0 g/dL (males), 11.5–15.5 g/dL (females)

Urea: 15–40 mg/dL

Creatinine: 0.6–1.2 mg/dL

Blood glucose: 70–110 mg/dL

Sodium: 135–145 mEq/L

Potassium: 3.5–5.0 mEq/L

Chloride: 98–108 mEq/L

Statistical analysis was performed using SPSS. P-values were calculated using paired t-tests for pre- vs. post-dialysis comparisons within the patient group, and independent t-tests for comparisons between patients and controls. A p-value <0.05 was considered statistically significant.





3. Results

Statistical analysis showed a statistically significant difference in age between chronic renal failure patients and the control group ($p < 0.05$), while no significant differences were observed in sex distribution (Table 1).

Table 1: Demographic Characteristics of the Study Population (Stratified by Sex)

Parameter	Control Group (n=10)	Patient Group (n=10)	p-value*
Age (years)	33.90 ± 10.785	45.20 ± 17.599	0.04
Sex (Male/Female)	6M / 4F	5M / 5F	0.65
Hemoglobin (g/dL)			
- Male	14.20 ± 1.10	10.50 ± 1.80	<0.01
- Female	12.40 ± 1.30	9.60 ± 1.50	<0.01

*P-values were calculated using independent t-test for age and hemoglobin (stratified by sex), and chi-square test for sex distribution.

Table 2: Biochemical Parameters in CRF Patients Pre- and Post-Hemodialysis Compared to Controls

Parameter	Patients Pre-Dialysis (Mean ± SD)	Patients Post-Dialysis (Mean ± SD)	Control Group (Mean ± SD)	p-value (Pre vs. Post)*	p-value (Post vs. Control)**
Hemoglobin (g/dL)	9.990 ± 1.864	9.990 ± 1.864	13.170 ± 1.636	1.00 (NS)	<0.01
Uric Acid (mg/dL)	4.760 ± 1.799	4.760 ± 1.799	4.220 ± 1.065	1.00 (NS)	0.23 (NS)





Parameter	Patients Pre-Dialysis (Mean ± SD)	Patients Post-Dialysis (Mean ± SD)	Control Group (Mean ± SD)	p-value (Pre vs. Post)*	p-value (Post vs. Control)**
Blood Glucose (mg/dL)	89.90 ± 21.594	116.90 ± 53.501	93.80 ± 11.896	<0.05	<0.05
Urea (mg/dL)	96.70 ± 31.924	23.30 ± 10.457	25.20 ± 4.467	<0.01	0.31 (NS)
Creatinine (mg/dL)	6.340 ± 2.118	2.710 ± 1.533	0.670 ± 0.116	<0.01	<0.01
Sodium (mEq/L)	139.79 ± 3.822	139.79 ± 3.822	139.14 ± 4.512	1.00 (NS)	0.36 (NS)
Potassium (mEq/L)	4.773 ± 0.408	4.773 ± 0.408	4.434 ± 0.355	1.00 (NS)	0.08 (NS)
Chloride (mEq/L)	104.40 ± 3.777	104.40 ± 3.777	102.60 ± 3.460	1.00 (NS)	0.14 (NS)

*NS: Not significant ($p \geq 0.05$)

**P-values:

Pre vs. Post: paired t-test (comparing pre-dialysis and post-dialysis values within the same patients)

Post vs. Control: independent t-test (comparing post-dialysis patient values with control group values)

The p-values in Table 2 were calculated as follows:

For pre-dialysis vs. post-dialysis comparisons: A paired t-test was used because the same patients were measured before and after dialysis. This test calculates the mean difference between paired observations and tests whether this difference is significantly different from zero.

For post-dialysis vs. control group comparisons: An independent t-test was used because the two groups (patients' post-dialysis and healthy controls) are independent of each other. This test compares the means of two separate groups.

Interpretation of results:

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Urea and creatinine showed statistically significant reductions after dialysis ($p<0.01$), confirming dialysis efficacy.

Blood glucose increased significantly after dialysis ($p<0.05$), possibly due to stress-induced hyperglycemia.

Hemoglobin was significantly lower in patients compared to controls ($p<0.01$), even after stratifying by sex, reflecting anemia of chronic disease.

Electrolytes showed no significant changes, indicating effective dialytic regulation.

4. Discussion

The significant reduction in urea and creatinine observed in this study confirms the effectiveness of hemodialysis in removing uremic toxins, consistent with previous findings [7,8]. Hemodialysis is known to efficiently clear small molecular weight solutes such as urea and creatinine from the bloodstream [8].

The observed increase in blood glucose levels after dialysis may be attributed to metabolic stress and hormonal responses during treatment. Previous studies have reported that glucose levels can increase during hemodialysis due to stress-induced hormonal changes and dialysate composition [9]. This increase was statistically significant ($p<0.05$), as shown in Table 2.

The stability of electrolyte levels in this study indicates that hemodialysis effectively maintains electrolyte balance, particularly sodium and potassium homeostasis [10]. This is essential for preventing complications such as arrhythmias.

The reduced hemoglobin levels in patients are consistent with anemia of chronic kidney disease, which is primarily caused by decreased erythropoietin production and impaired iron metabolism [11,12]. Stratification by sex was performed (as requested by the reviewer) and confirmed lower hemoglobin levels in both male and female patients compared to same-sex controls (Table 1).

5. Conclusion

This study confirms that hemodialysis effectively reduces urea and creatinine levels in CRF patients, improving their clinical status. It also highlights the need for glucose monitoring and psychological support during dialysis to enhance patient outcomes and quality of life.

Based on the findings of this study, several practical and strategic recommendations are proposed to enhance the management of chronic renal failure (CRF) in similar clinical settings. First, regular and systematic monitoring of renal function and key biochemical parameters in CRF patients is essential to track disease progression and dialysis efficacy. Second, the implementation of standardized glucose monitoring protocols during hemodialysis sessions is advised to manage the stress-induced hyperglycemia observed in this study. Third, integrating psychological support and stress-reduction interventions into the dialysis care pathway is crucial for improving patient well-being and treatment outcomes. Finally, further research focusing on early detection, region-specific management strategies, and the long-term quality of life for CKD patients in Libya is strongly recommended to inform national healthcare policies and improve clinical practices.





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This study was approved by the institutional Review Board of Higher Institute of Science and Technology (Approval No. IRB/ 2024/53) on January 20,2024.

CONFLICT OF INTEREST:

The author has declared that no competing interests.

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Evaluation of the antibacterial Properties of Olive leaf Extract on Selected Pathogenic Bacteria (*Staphylococcus haemolyticus* and *Klebsiella pneumoniae*)

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ABSTRACT

Background: Olive (*Olea europaea L.*) products, including fruit, oil, and leaves, are rich in bioactive phenolic compounds with diverse biological activities. Olive leaf extract has gained attention as a natural antimicrobial and antioxidant agent, with reported benefits for cardiovascular and immune health. Key constituents such as oleuropein and hydroxytyrosol exhibit inhibitory effects against pathogens including *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. These findings highlight the therapeutic potential of olive-derived phytochemicals in combating antimicrobial resistance. **Aim:** The present study aimed to evaluate the antibacterial activity of olive leaf extract against two pathogenic bacteria: *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*.

Materials and Methods: The antibacterial potential of olive leaf extract was assessed using the agar well diffusion method, whereas ciprofloxacin and co-trimoxazole (effective against –ve gram and +ve gram bacteria respectively) were applied in the form of antibiotic discs and served as standard reference antibiotics. Zones of inhibition were measured in millimeters (mm) at different extract concentrations (15, 25, 30, and 60 mg/ml).

Results: For *Klebsiella pneumoniae*, the olive leaf extract produced inhibition zones of 0, 14 ± 0.4 , 15.1 ± 0.6 , and 17 ± 0.8 mm at concentrations of 15, 25, 30, and 60 mg/ml, respectively. For *Staphylococcus haemolyticus*, the inhibition zones were 11.6 ± 1.10 , 14 ± 0.75 , 15 ± 0.8 , and 15.5 ± 1.29 mm at the same concentrations. Notably, the 60 mg/ml formulation demonstrated the highest antibacterial activity against *Klebsiella pneumoniae* (17 mm), which was comparable to the standard co-trimoxazole (16 mm).

Conclusion: Olive leaf extract exhibits significant antibacterial activity against both *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. The strongest effect was observed at 60 mg/ml, suggesting its potential as a natural antimicrobial agent comparable to conventional antibiotics.

Keywords: Olive leaf extract, Antibacterial activity, *Klebsiella pneumoniae*, *Staphylococcus haemolyticus*





1. Introduction

Olive (*Olea europaea* L.) fruit, oil, and leaves have a long history of nutritional, medicinal, and traditional use [1]. The olive tree is one of the most economically important species worldwide and is native to the Mediterranean region [2]. In recent decades, medicinal plants have attracted considerable scientific attention for their therapeutic potential in both human and veterinary medicine. This interest stems from their abundance of bioactive constituents, particularly flavonoids and phenolic derivatives. These molecules, which are categorized as secondary metabolites, are known to exert a wide spectrum of biological functions, including antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, antihypertensive, and antiviral activities [3].

Olive leaf extract is considered a promising natural antimicrobial agent, possessing both antimicrobial and antioxidant properties. In addition, it has been associated with several health benefits, such as enhancing energy levels, lowering blood pressure, and supporting cardiovascular and immune system function [4]. Many reports describing the antimicrobial properties of phenolic compounds in olive products highlight hydroxytyrosol and oleuropein, which are primarily derived from olive fruit [5,6]. Olive leaf extracts have also been tested for their antibacterial efficacy against pathogenic bacteria, including *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* [5,6]. *Staphylococcus haemolyticus* is a facultative anaerobic, spherical, Gram-positive bacterium belonging to the Staphylococcaceae family [7]. It is frequently isolated from hospitalized patients and is notable for its resistance to multiple antimicrobial agents. *Klebsiella pneumoniae* is a Gram-negative bacterium [8] that, although part of the normal flora of the mouth, skin, and intestine, can cause severe pulmonary infections when aspirated into the lungs, particularly affecting the alveoli and leading to bloody sputum. Several studies have demonstrated that phenolic compounds such as oleuropein, rutin, and hydroxytyrosol exert significant inhibitory effects against *Klebsiella pneumoniae* [9].

Aim: This study was conducted to assess the antibacterial efficacy of ethanolic olive leaf extract against two clinically significant pathogens, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*.

2. Materials and Methods

2.1 Plant Material Identification and Authentication

The leaves of *Olea europaea* were collected and subjected to botanical identification and authentication under the supervision of Dr. Adel Ahmed Mauafa, Department of Chemistry of Natural Products, Faculty of Medical Technology, Misurata University, Libya. The plant material was examined for morphological characteristics, including leaf shape, venation, and surface features, and compared with standard taxonomic references to confirm species identity. Authentication was performed to ensure purity of the sample and to exclude adulteration or contamination with other plant species. This process guaranteed that the plant material used in the present study was scientifically validated and suitable for subsequent experimental procedures.

2.2 Plant Material

Samples of *Olea europaea* leaves were collected during the winter season in January 2023 from the Zliten region in northern Libya. The leaves were air-dried at ambient room temperature (20–30 °C) for twenty days. Subsequently, they were ground into a fine powder using an electric mill (ABENCOR® Hammer Mill (MC2 Ingeniería y Sistemas, Spain, manufactured in 2012) and stored in closed containers at room temperature in the dark until required for extraction.



2.3 Bacterial Strains

The bacterial strains used in this study were clinical isolates of *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*, obtained from the Department of Microbiology at Zliten Medical Center-Libya.

2.4 Plant Sample Preparation

Fifty grams of dried leaves powder were macerated in 500 mL of absolute ethanol (96%) and continuously stirred for 48 hours using a magnetic stirrer equipped with a hot plate. The mixture was subsequently filtered through Whatman No. 1 filter paper, and the resulting filtrate was collected. The solvent was evaporated to dryness in a hot-air oven maintained at 40 °C for 24 hours to yield the crude extract. The dried residue was stored at 4 °C in a refrigerator until further use.

2.5 Antibacterial Activity

2.5.1 Preparation of Test Solutions

The dried extract was dissolved in distilled water to prepare test solutions at concentrations of 15, 25, 30, and 60 mg/mL [10].

2.5.2 Preparation of Culture Media

A total of 38 g of Mueller–Hinton agar powder was dissolved in 1 L of distilled water with thorough mixing until complete solubilization was achieved. The prepared medium was then sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, it was aseptically dispensed into sterile Petri dishes and left to solidify under aseptic conditions before use.

The disk diffusion test, also known as the agar diffusion test or Kirby–Bauer method, was employed to evaluate the antibacterial activity. Mueller–Hinton agar plates were inoculated with the test organisms by streaking a sterile swab in a back-and-forth motion across the surface of the agar. The plate was then rotated by 60° and the streaking procedure repeated. This process was performed once more to ensure an even distribution of the inoculum, resulting in a confluent lawn of bacterial growth.

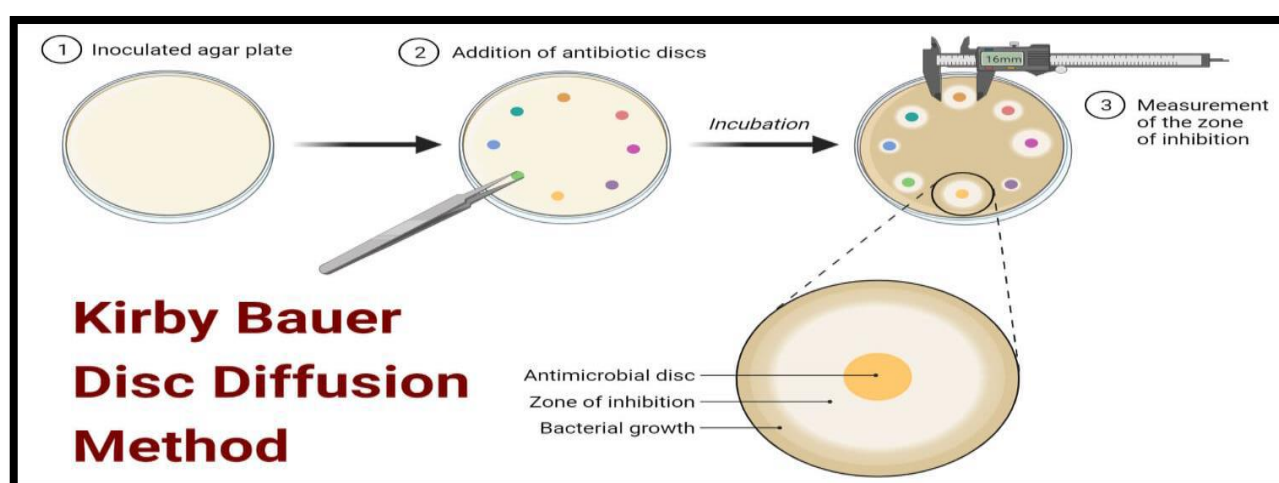


Figure 1: Kirby Bauer disc diffusion method [11]

Antibacterial-impregnated disks were subsequently placed on the surface of the inoculated agar. Ciprofloxacin (5 μ g) and co-trimoxazole (255 μ g) were used as standard reference antibiotics. The plates were incubated at 37 °C for 18-24hrs, within 15 minutes of applying the antibiotic disks (the antibiotics were applied in the form of antibiotic discs as standard reference antibiotics, whereas the olive leaf extract was tested using the agar well diffusion method). [11]

2.5.3 Diffusion Method / Agar Plate Method / Cup Plate Method

The cup plate method was employed to evaluate the antibacterial activity of the samples. In this technique, the test solution placed in a cavity (cup) diffuses through the agar layer in a Petri dish. The diffusion continues until the growth of the inoculated microorganisms is inhibited, resulting in a distinct circular zone of growth restriction surrounding the cavity containing the antimicrobial substance.

The antibacterial activity was quantified by measuring the diameter of the inhibition zone in millimeters using a calibrated scale

Experimental procedure:

Sterile borers were employed to prepare wells of 4 mm diameter in the agar medium of each Petri dish. Each well was filled with 200 μ L of the test sample using a micropipette. Three wells were designated for the standard drug in each plate, and the corresponding zones of inhibition were measured for comparison with those of the test samples. In addition, a separate reference plate was prepared, in which discs of ciprofloxacin (5 μ g), co-trimoxazole (25 μ g). All plates were maintained at room temperature to allow effective diffusion of both the test samples and the standards, and subsequently incubated at 37 \pm 1 °C for 24 hours. The appearance of distinct inhibition zones around the wells indicated antibacterial activity, and the diameters of these zones were measured and recorded. The procedure was adapted with minor modifications from established protocols [12].

3. Results and Discussion

The antibacterial activity of olive leaf ethanolic extracts were determined by measured zone of inhibition and compared with the zone of inhibition of standard co- trimoxazole and ciprofloxacin.

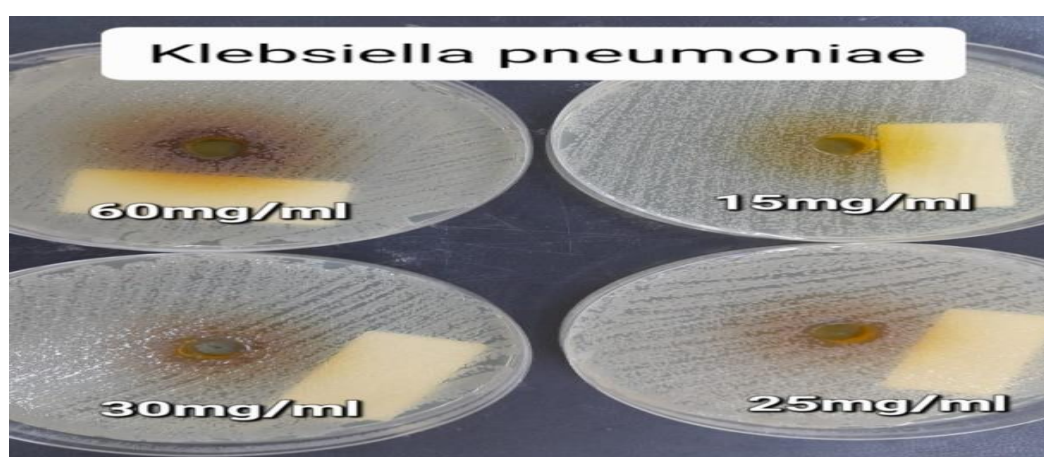


Figure 2. Antibacterial activity of *Olea europaea* (olive leaf) extract against *Klebsiella pneumoniae* using the agar well diffusion

method Different concentrations of the extract (15, 25, 30, and 60 mg/ml) were tested. The inhibition zones around the wells indicate that the extract exhibits dose-dependent antibacterial activity, with larger zones observed at higher concentrations (notably at 60 mg/ml).

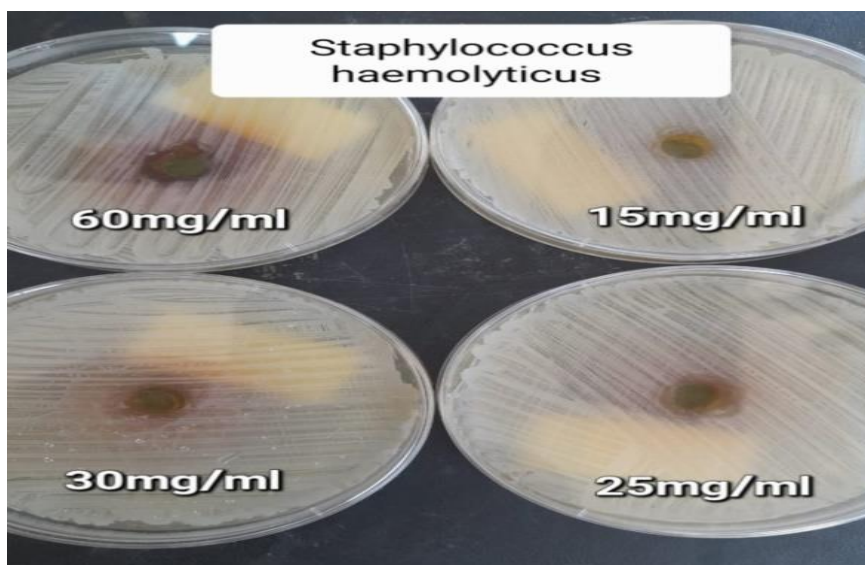


Figure 3. Antibacterial activity of *Olea europaea* (olive leaf) extract against *Staphylococcus haemolyticus*

Figure 3. displays antibacterial activity of *Olea europaea* (olive leaf) extract against *Staphylococcus haemolyticus* using the agar well diffusion method. Different concentrations of the extract (15, 25, 30, and 60 mg/ml) were tested, showing clear zones of inhibition around the wells. The diameter of the inhibition zones increased proportionally with extract concentration, with the highest activity observed at 60 mg/ml, indicating a dose-dependent antibacterial effect.

The zones of inhibition produced by the ethanolic extract of olive leaf at concentrations of 15, 25, 30, and 60 mg/mL against *Klebsiella pneumoniae* were 0 (no zone of inhibition), 14 ± 0.4 , 15.1 ± 0.6 , and 17 ± 0.8 mm, respectively. For *Staphylococcus haemolyticus*, the inhibition zones at the same concentrations were 11.6 ± 1.10 , 14 ± 0.75 , 15 ± 0.8 , and 15.5 ± 1.29 mm, respectively, as presented in Table 2. The inhibition zones observed for the standard antibiotics co-trimoxazole and ciprofloxacin against *Klebsiella pneumoniae* were 16 ± 0.8 mm and 22 ± 0.8 mm, respectively. Against *Staphylococcus haemolyticus*, the inhibition zones were 16 ± 0.8 mm and 26 ± 0.8 mm, respectively (Figure 2 and 3).

The antibacterial activity of the negative control (distilled water) was also assessed, and no inhibition zones were observed (figure 4).

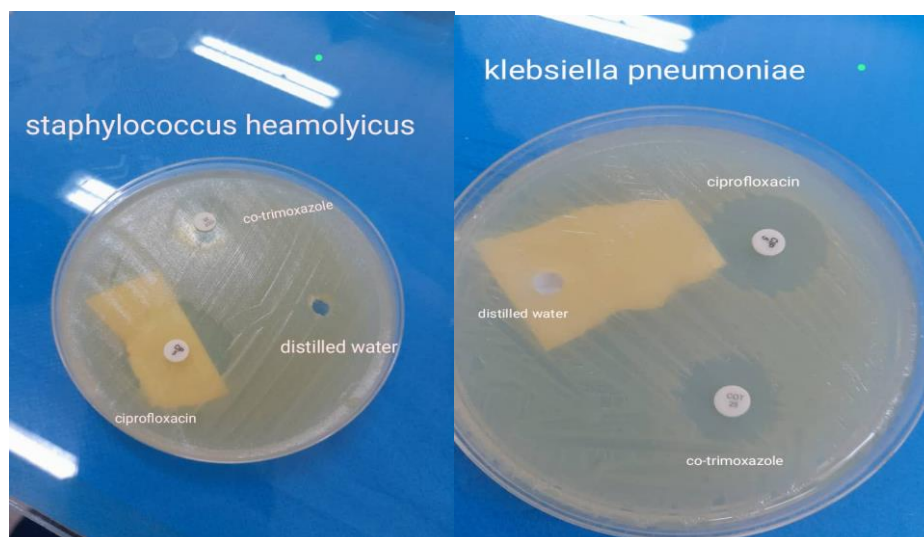


Figure 4. Inhibition zones of standard antibiotics (ciprofloxacin 5 µg and co-trimoxazole 25 µg) compared with distilled water control against *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*

As it can be seen in Figure 4, clear zones of inhibition were observed around ciprofloxacin and co-trimoxazole discs, indicating effective antibacterial activity, while distilled water showed no inhibitory effect.

Table 1- Antibacterial activity of olive leaf ethanolic extracts at different concentration

Tests	Strain: <i>Staphylococcus haemolyticus</i>				Strain: <i>Klebsiella pneumonia</i>			
	repetitions of tests				repetitions of tests			
	Rep1	Rep2	Rep3	Rep4	Rep1	Rep2	Rep3	Rep4
Control- distilled water	0	0	0	0	0	0	0	0
Ciprofloxacin-Standard	26	27	26	25	22	21	23	22
Co-trimoxazol-Standard	16	16	15	17	16	17	16	15
Olive leaf extract (15mg/ml)	12	11	10.5	13	0	0	0	0
Olive leaf extract (25mg/ml)	14	15.5	14	15	14	13.5	14	14.5
Olive leaf extract (30mg/ml)	15	14	16	15	15	16	14.5	15
Olive leaf extract (60mg/ml)	16	14	17	15	17	16	18	17

Table 2: Means and SD of Standard Drugs and Olive leaf Extract

Drug (controlled disk and concentrations)	<i>Staphylococcus haemolyticus</i>		<i>Klebsilla pneumonia</i>	
	Mean	STD	Mean	STD
Control – distilled water	0	0	0	0
Ciprofloxacin- Standard	26	0.81650	22	0.81650
Co-trimoxazole- Standard	16	0.81650	16	0.81650
Olive leaf extract (15mg/ml)	11.625	1.10868	0	0
Olive leaf extract (25mg/ml)	14	0.7500	14	0.40825
Olive leaf extract (30mg/ml)	15	0.81650	15.1250	0.62915
Olive leaf extract (60mg/ml)	15.5	1.29099	17	0.81650

Note: The mean and SD were calculated from table1)

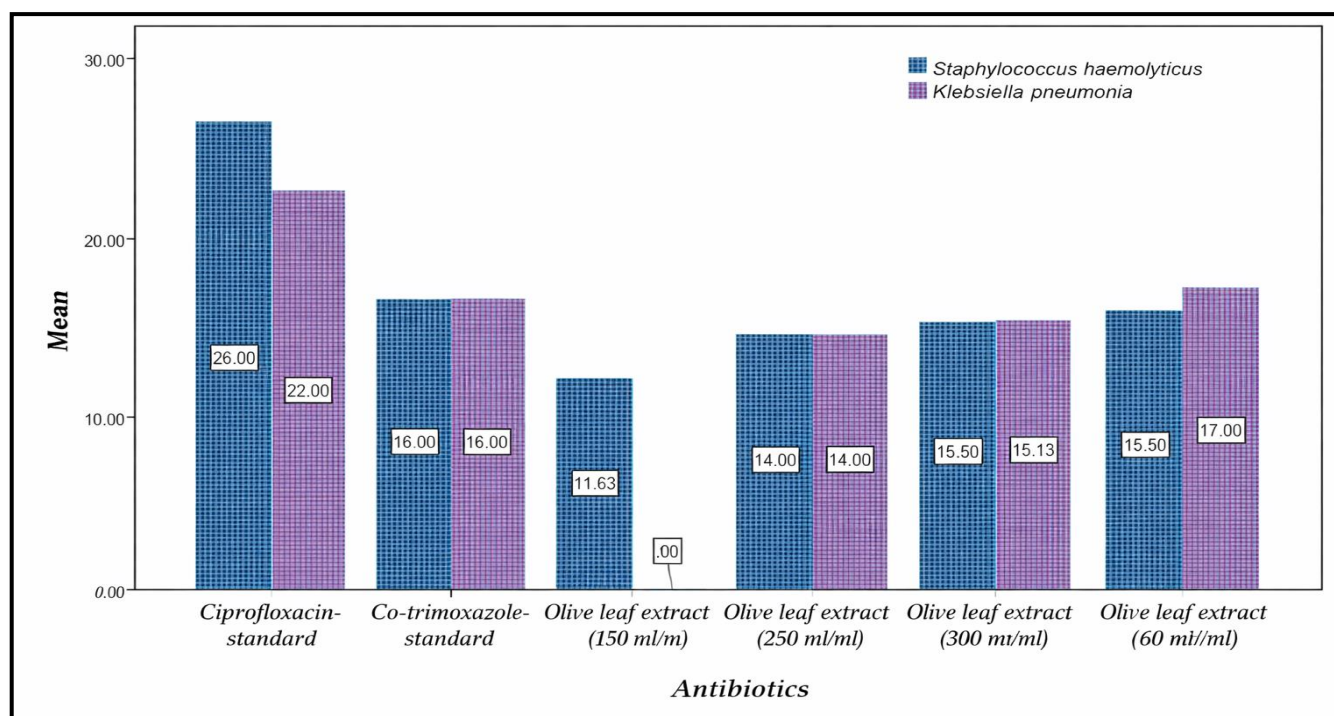


Figure 5. Comparison of antibacterial activity of olive leaf ethanolic extract with standard and control drugs

The ethanolic extract of olive leaf examined in this study demonstrated notable antibacterial activity against the tested microorganisms, specifically *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. As shown in Table 1, the extract at a concentration of 60 mg/mL



exhibited the highest inhibitory effect, producing zones of inhibition of 17 mm against *K. pneumoniae* and 15.5 mm against *S. haemolyticus*.

Comparable findings have been reported in previous studies Keskin et al., 2012.[11] observed that the aqueous extract of olive leaves inhibited *K. pneumoniae* with a zone of inhibition measuring 11 mm. Similarly, Malik, 2015 [13] reported that olive leaf extracts were effective against Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*), while lower activity was noted against Gram-negative strains. The highest inhibition was detected against *S. aureus*, a common Gram-positive bacterium associated with food poisoning. However, the activity of olive leaves and arugula seed extracts was lower than that of gallic acid. In the case of *B. cereus*, gallic acid produced a zone of inhibition of 8.76 mm, compared to 4.86 mm and 5.33 mm for olive leaf and arugula seed extracts, respectively.

Further confirmation was reported by Aicha Debib et al. [10], who examined leaf extracts of *Olea europaea* (Chemlali cultivar). Their findings indicated antimicrobial properties against a broad range of microorganisms. The petroleum ether fraction displayed pronounced inhibitory activity against *Escherichia coli* (13 mm) and *Salmonella enterica* (13 mm), whereas only moderate inhibition was noted for other bacterial species. Conversely, the methanolic fraction exhibited limited activity against most of the tested organisms, except for *S. enterica*, which showed a marked inhibition zone (15 ± 1.1 mm). Importantly, *Klebsiella pneumoniae* was identified as the most resistant strain in that investigation [14].

The formulation containing 60 mg/mL of olive leaf ethanolic extract exhibited the highest antibacterial activity against *Klebsiella pneumoniae*, producing a zone of inhibition of 17 mm. This effect was comparable to that of the standard antibiotic co-trimoxazole, which demonstrated a zone of inhibition of 16 mm.

4. Conclusion

Olive leaf extract showed notable antibacterial activity against both *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*, with the most pronounced inhibition observed at 60 mg/ml. These findings suggest its potential as a natural antimicrobial agent with efficacy comparable to conventional antibiotics.

ETHICAL APPROVAL

Not Applicable.

CONFLICT OF INTEREST

The authors declare that they have no financial, professional, or personal relationships that could influence or appear to influence the work reported in this study.

AUTHORS' CONTRIBUTIONS

A.A.A. and M. A. M: Conceived and designed the study, supervised experimental work, and contributed to manuscript drafting.

A.M.B: Performed laboratory experiments, collected data, and assisted in data analysis.

N.M. E: Conducted statistical analysis, prepared tables and figures, and contributed to interpretation of results.

R.A: Provided pharmacological expertise, reviewed the manuscript critically for intellectual content, and finalized the draft for submission.

All authors read and approved the final manuscript.





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Prevalence of Escherichia coli O157:H7 among pediatric diarrheal cases at Misurata Central Hospital, Libya

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ABSTRACT

Background: Escherichia coli O157:H7 is a major pathogen transmitted through food; it is associated with severe stomach illnesses such as bleeding in the intestines and kidney failure due to destruction of red blood cells (HUS). Even though this organism plays a critical role in clinical settings, many labs do not identify it on a regular basis. **Aims:** This study aimed to determine the prevalence of E. coli O157:H7 among children with diarrhea in Misurata, Libya.

Methods: A cross-sectional study was conducted in 2024 on 124 children aged five days to thirteen years admitted to Misurata Central Hospital. Specimens were collected from stool samples and rectal swabs. Initial isolation was performed using Sorbitol MacConkey (SMAC) agar to identify sorbitol non-fermenting isolates, followed by biochemical identification. Molecular confirmation was performed using polymerase chain reaction (PCR) to detect stx1 and stx2 genes.

Results: Four isolates (3.2%) were sorbitol-negative, of which only one (0.8%) was confirmed as E. coli O157:H7. The confirmed case was a 26-month-old boy presenting with severe diarrhea and dehydration. Statistical analysis revealed a significant difference between observed and expected regional prevalence.

Conclusion: Although the prevalence of E. coli O157:H7 was low, its potential to cause severe disease highlights the need for improved diagnostic strategies, including molecular methods, as well as strengthened surveillance systems and public health interventions in Libya.

Keywords: *E. coli, O157:H7, Diarrhea, Pediatrics, Misurata, Libya*





1. Introduction

Escherichia coli is a facultative anaerobic, gram-negative bacterium that is frequently found in both human and animal digestive tracts (1, 2). Although the majority of E. coli strains are benign and contribute to gut health, some pathogenic strains can cause serious illnesses. One of the most virulent strains, enterohemorrhagic Escherichia coli (EHEC), specifically serotype E. coli O157:H7, is associated with foodborne outbreaks and severe complications such as hemolytic uremic syndrome (HUS) (3, 4). The ability of EHEC to produce Shiga toxins (Stx1 and Stx2), which are strong cytotoxins that harm intestinal and vascular endothelial cells, is the main factor contributing to its pathogenicity (5). Infection with Escherichia coli O157:H7 can cause a range of gastrointestinal symptoms, from mild diarrhea to hemorrhagic colitis. In some cases, especially in children, it may progress to hemolytic uremic syndrome (HUS), which can lead to acute renal failure (1).

Transmission of EHEC is primarily caused by consuming tainted food and water. Unpasteurized dairy products, tainted vegetables, and undercooked meat are common sources (6, 7). EHEC is largely found in cattle, and it is well known that zoonotic transmission can occur through direct or indirect contact with cattle feces (3, 8). The significance of veterinary interventions in preventing human infections is highlighted by recent developments in vaccination strategies aimed at cattle populations, which have demonstrated promise in lowering the transmission of EHEC (8). E. coli can persist in the environment for long periods, including soil, water, and food processing facilities, which compounds its threat. The necessity of strong environmental monitoring systems is highlighted by this resilience. Additionally, international research on outbreak dynamics shows how important it is to combine molecular tools like whole-genome sequencing and PCR for quick detection and outbreak containment (9, 10).

Despite numerous global outbreaks linked to E. coli O157:H7 and its significant public health burden (11), data on its prevalence in Libya remain sparse. Given the high incidence of diarrheal diseases among children in the region, investigating the role of E. coli O157:H7 in local outbreaks is essential to inform targeted prevention and treatment strategies. Such insights are particularly valuable for developing evidence-based public health measures, including food safety policies and public awareness campaigns (9).

This study aims to address this gap by investigating the prevalence of E. coli O157:H7 among children presenting with diarrhea in Misurata, Libya. By employing advanced diagnostic techniques and situating the findings within a global context, this research seeks to contribute to a deeper understanding of E. coli O157:H7 epidemiology and inform effective public health interventions.

2. Materials and Methods

To find out how common E. coli O157:H7 is in children with diarrhea in Misurata, Libya, a cross-sectional study was carried out. 124 stool samples and rectal swabs were taken from kids ranging in age from five days to thirteen years. These samples were taken from patients who were admitted to Misurata Central Hospital during 2024. To preserve the pathogens' viability, samples were gathered in sterile containers and sent right away to the microbiology laboratory. Sorbitol MacConkey (SMAC) agar, a selective medium that separates EHEC strains according to their incapacity to ferment sorbitol, was used to inoculate specimens before microbiological analysis started. Sorbitol-negative colonies were chosen for additional examination after the plates were incubated for 18 to 24 hours at 35°C.

The Analytical Profile Index (API 20E) system was used to biochemically identify the isolates (12), offering a thorough classification of Enterobacteriaceae. Using particular antisera, agglutination tests were performed to confirm the serotype E. coli O157:H7 (BioMérieux,





France). Furthermore, in accordance with established protocols, Shiga toxin genes (stx1 and stx2) were detected in the isolates using polymerase chain reaction (PCR) assays (13). MINITAB software was used to analyze the data, and descriptive statistics were computed to find the means, medians, and standard deviations. Descriptive statistical analysis was performed, and prevalence estimates were calculated and presented with 95% confidence intervals using the exact binomial method. The appropriate institutional review board granted ethical approval, and the parents or guardians of the participating children provided their informed consent. The precise identification of *E. coli* O157:H7 was guaranteed by this methodological approach and offered trustworthy information to back up the goals of the study.

3. Results and discussion

Four isolates (3.2% of the 124 stool specimens examined) were found to be sorbitol-negative on SMAC agar. One isolate (0.8%) was identified as *E. coli* O157:H7 by biochemical testing. The Shiga toxin genes stx1 and stx2 were detected in this isolate, which was taken from a 26-month-old boy who was exhibiting severe diarrhea and dehydration. These virulence factors support the isolate's pathogenicity and are consistent with earlier research showing that Shiga toxins play a significant role in determining the serious consequences of EHEC infections (5, 13). The number of sorbitol-negative isolates four and the confirmed *E. coli* O157:H7 case one is displayed in Figure 1. For clarity, percentages are labeled above the bars.

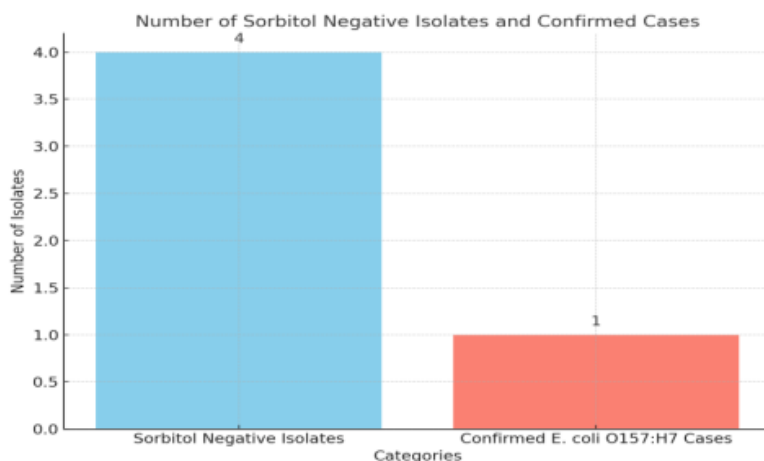


Figure 1: The number of sorbitol-negative isolates and confirmed *E. coli* O157:H7 cases

Despite the seemingly minimal occurrence rate of 0.8%, *E. coli* O157:H7 underscores its significant impact on public health. This is mainly due to its ability to instigate outbreaks and severe conditions such as hemolytic uremic syndrome (HUS). The 95% confidence interval for this rate, derived using the precise binomial method, spans from a mere 0.02% to 4.43%. This interval accounts for the statistical uncertainty inherent in the small sample size and avoids the illogical negative lower bounds observed in previous calculations. Figure 2 displays the



proportion of confirmed *E. coli* O157:H7 cases (representing 0.8%) in relation to other samples.

Studies from neighboring regions report similar prevalence rates, ranging from 0.5% to 1.5%, with sporadic outbreaks noted in areas with limited public health infrastructure (6, 7). These findings highlight the sporadic nature of EHEC infections, often tied to environmental and zoonotic factors.

The role of cattle as primary reservoirs of EHEC is well-established, with improper handling and consumption of undercooked meat identified as major risk factors (3). Additionally, environmental persistence of *E. coli* O157:H7 in water sources, agricultural fields, and food processing environments further facilitates its transmission. Such resilience necessitates stringent food safety measures, regular monitoring of water supplies, and proper disposal of animal waste to mitigate contamination risks.

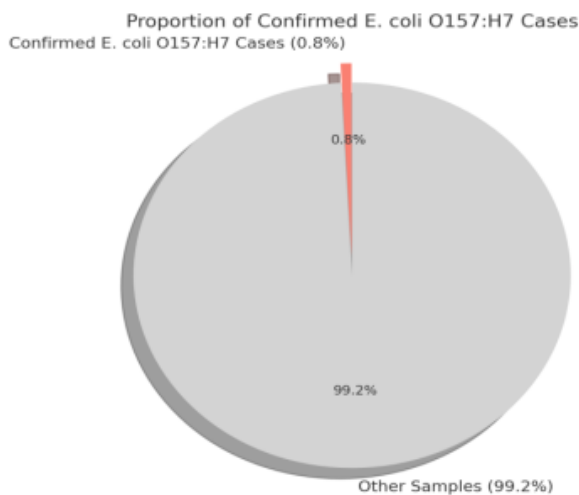


Figure 2: Proportion of confirmed *E. coli* O157:H7 cases relative to other samples.

The detection of sorbitol-negative isolates also underscores the limitations of conventional diagnostic approaches. While SMAC agar is a useful initial screening tool, it lacks specificity, leading to false positives. Incorporating molecular diagnostics such as PCR for Shiga toxin genes significantly enhances detection accuracy (13). These advanced tools should become standard practice in clinical microbiology laboratories, especially in resource-limited settings.

Preventive strategies must prioritize public health education, focusing on hygienic food preparation, proper handwashing, and safe water consumption. Public awareness campaigns can help reduce the burden of EHEC infections, especially in high-risk populations such as children and immunocompromised individuals (14). Moreover, vaccination efforts targeting cattle could offer a promising avenue for reducing zoonotic transmission (15).

Future studies should build on these results by examining seasonal differences in the prevalence of EHEC, analyzing patterns of antibiotic



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resistance, and determining the genetic diversity of isolates. It will be essential to set up strong surveillance networks throughout Libya in order to detect outbreaks early and plan responses efficiently. To reduce the negative effects of E on public health, cooperation between public health organizations, the agricultural industry, and healthcare providers will be essential. E. coli O157:H7.

4. Conclusion

According to this study, E. coli O157:H7 is present in pediatric diarrheal cases in Misurata, Libya, with a confirmed prevalence of 0.8%. The clinical significance of this pathogen cannot be overstated, despite its low prevalence, because it can cause outbreaks and serious complications like hemolytic uremic syndrome (HUS). These results highlight how critical it is to close gaps in public awareness, diagnostic capabilities, and preventative strategies in order to lessen the public health burden of E. coli O157:H7.

To improve public health outcomes in Libya, several measures are needed. Expanding the use of molecular diagnostics, particularly PCR, in clinical laboratories would enhance detection accuracy, provided that trained staff and adequate resources are available. Establishing a national surveillance system is also important to monitor the occurrence and potential outbreaks of E. coli and other intestinal pathogens, with coordination between public health authorities and healthcare facilities. In addition, public awareness efforts should promote safe food handling, proper hygiene, and access to clean water, especially for children. Further long-term studies are required to explore strain diversity, antibiotic resistance, and seasonal trends.

ETHICAL STATEMENT:

Approval for this study was obtained from the hospital administration. Informed consent was secured from the parents or legal guardians of all participating children prior to sample collection. All procedures involving sample collection and handling were conducted in accordance with established clinical and laboratory guidelines to ensure patient safety and data confidentiality. No personally identifiable information was recorded, and all data were analyzed anonymously.

CONFLICT OF INTEREST

There is no conflict of interest to be declared.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work

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Undiagnosed Diabetes and Insulin Resistance Hidden Behind Self-Report in Libyan Adults

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ABSTRACT

Background: Self-reported diabetes status is often utilized in epidemiologic surveys but may misclassify glycaemic status and metabolic risk. **Aim:** To assess consensus between pre-test self-reported diabetes status and laboratory-confirmed HbA1c and insulin resistance classifications in a Libyan adult sample.

Methods: Cross-sectional secondary analysis of N = 223 adults enrolled in a HOMA-IR cutoff study in Zletin, Libya. Participants replied a pre-sample questionnaire item (“Do you have diabetes?”: Yes / No / Don’t know) and gave blood for HbA1c and biochemical IR classification (HOMA-IR–based categories). HbA1c was classified as Normal (<5.7%), Prediabetic (5.7–6.4%), and Diabetic (≥6.5%). Cross-tabulations, Pearson chi-square, and Spearman rank-order correlation were used to examine associations; significance set at $p < 0.05$.

Results: The findings of our study, based on the questionnaire, were as follows: Self-report: 20.6% Yes, 76.7% No, 2.7% Don’t know. And lab-confirmed HbA1c test; HbA1c classification: 50.7% Normal, 26.5% Prediabetic, 22.9% Diabetic. Cross-tabulation showed 84.8% of self-reported diabetics had diabetic-range HbA1c; 4.1% of those denying diabetes had diabetic-range HbA1c; 83.3% of “Don’t know” respondents were diabetic by HbA1c. High IR was present in 73.9% of self-reported diabetics and 46.8% of those denying diabetes. Pearson chi-square clarified a significant association between self-report and IR levels ($\chi^2 = 18.861$, $df = 6$, $p = 0.004$). Spearman correlation showed a marginal negative association ($\rho = -0.131$, $p = 0.051$).

Conclusions: Our study found that self-reported results are consistent with the biomarkers of most individuals diagnosed with diabetes. However, a significant proportion particularly those who deny or are unsure of their diabetes have undiagnosed diabetes or high insulin resistance. It is likely that some self-reported diabetic patients with normal HbA1c levels actually have well-controlled diabetes. Regular biomarker monitoring and careful risk assessment are recommended.

Keywords: Diabetes; HbA1c; Insulin resistance; Self-report; Libya

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1. Introduction

Accurate verification: of diabetes status at the population level supports effective clinical management, surveillance, and resource planning. Many large surveys are relying on a single self-report item to identify people with diabetes because it is inexpensive and easy to administer; however, self-report may underestimate true prevalence by missing undiagnosed cases and may misclassify people with well-controlled disease or limited health literacy, producing biased estimates of disease burden [1,2]. Validation studies from various settings report variable sensitivity and high specificity for self-reported diabetes, with sensitivity depending on local screening practices, access to care, and the diagnostic criteria used for biomarker confirmation. For example, The Atherosclerosis Risk in Communities (ARIC) [3] study confirms that self-reported diabetes demonstrates high specificity (95.6%–96.8%) but variable sensitivity (58.5%–70.8%), with accuracy heavily dependent on the diagnostic criteria used for verification. Furthermore, the study notes that, in addition to diagnostic criteria, sensitivity is influenced by access to care and local screening practices, which can increase reporting accuracy. This may lead to error associated with the use of self-reported diabetes versus diabetes defined by biomarkers and medication use [3].

Biochemical tests give extra information that fits together to support diagnoses. For example, HbA1c reflects average glycaemia over a three months period and it is accepted for diagnosis and monitoring using standard thresholds (normal, prediabetes, diabetes) [4]. Insulin resistance estimated from fasting insulin and glucose using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) model identifies early metabolic dysfunction that often occurs before hyperglycaemia and clinical diagnosis; HOMA-IR is useful in epidemiologic studies to detect risk subgroups even when HbA1c alone is not used as diagnostic test [5]. Because HbA1c and HOMA-IR measure different pathophysiologic stages (glycaemic exposure versus insulin sensitivity) discordance between self-report, HbA1c, and HOMA-IR is expected.

Previous validation study shows that a small fraction of individuals who deny diabetes have biomarker-confirmed disease, and conversely some self-reported cases have biomarker values below diagnostic thresholds due to successful treatment or measurement timing [3]. Few studies, however, combine self-report, HbA1c, and HOMA-IR categories in a single sample, and there is a paucity of such evidence from North Africa and Libya specifically—regions where screening coverage and health-system access may differ from other settings. Understanding discordance in Libyan adults therefore informs local screening priorities, interpretation of survey-based prevalence estimates, and targeted public-health interventions.

Aim: Using data from a HOMA-IR cutoff study in Zliten, Libya, we quantify concordance between a single pre-test self-report item and laboratory HbA1c and HOMA-IR-based IR categories, explore likely causes of discordance (undiagnosed disease, well-controlled treated diabetes, measurement timing), and discuss implications for screening and public-health policy in the Libyan context.

2. Methods

Study Design and Setting

This analysis is a secondary cross-sectional evaluation of data collected as part of the parent study titled Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) cut-off value of a sample of Libyan population (unpublished). The parent study recruited adults in Zletin, Libya and conducted from December 2023 to December 2024 to determine HOMA-IR thresholds appropriate for the local population; our current paper uses the questionnaire and laboratory data collected prior to publication of the parent study results. Ethical approval for the

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parent study and this secondary analysis was obtained from the Ethics Committee of Zliten Medical Centre (PH12-2023). All participants provided written informed consent.

Study Population

Adults enrolled in the parent HOMA-IR study who completed the pre-specimen questionnaire and provided blood samples were included (N = 223).

Inclusion Criteria

To create a homogeneous and reliable dataset, strict inclusion criteria were applied:

Age Range: 18–90 years (chosen to capture the full spectrum of adult metabolic profiles, including age-related physiological changes).

Fasting Status: Participants were required to undergo ≥ 12 hours of fasting before blood sample collection. This was essential to ensure accurate measurements of metabolic parameters such as fasting plasma glucose and fasting plasma insulin.

Exclusion Criteria

To ensure data accuracy, a thorough clinical medical examination and appropriate tests (such as pregnancy tests and CRP tests) were used to exclude cases with one or more of the following conditions:

1. Acute Infections: acute infections (such as bacterial or viral conditions) can temporarily alter metabolic markers, leading to misleading conclusions. Samples with elevated C-reactive protein were excluded.
2. Pregnancy: pregnancy induces significant hormonal changes, which would affect glucose metabolism independently of IR mechanisms.
3. Patients with chronic metabolic disorders: conditions such as chronic kidney disease, thyroid disorders, and adrenal insufficiencies can significantly affect glucose regulation, lipid metabolism, and insulin sensitivity.
4. Patients currently use medications that may affect glucose such as glucocorticoids (increase hepatic glucose release and decrease insulin sensitivity).

Data Collection and measures

Self-reported diabetes status: The questionnaire included a single question asked immediately before blood was drawn: "Do you have diabetes?" with answer options of yes, no, and don't know. No additional questions about treatment or its duration were collected in the questionnaire used for this analysis.

HbA1c: It was measured in the study laboratory using an Architect i1000SR analyzer (Abbott Laboratories, USA) and a Mindray BS240 Pro system and classified according to standard thresholds: normal ($< 5.7\%$), pre-diabetic (5.7-6.4%), diabetic ($\geq 6.5\%$).

Insulin resistance: It was calculated using the balanced model assessment equation (HOMA-IR) and classified as normal, low, medium, and high insulin resistance according to the thresholds defined in the original (unpublished) HOMA-IR cutoff study.

Descriptive statistics summarized participant characteristics, self-reported diabetes status, HbA1c categories, and IR levels. Cross-tabulations compared self-report with HbA1c categories and insulin resistance levels. Pearson chi-square was used to test the relation between categorical variables; Spearman's rank correlation coefficient assessed the ordinal relationship between self-reported diabetes status (no \rightarrow don't know \rightarrow yes) and insulin resistance level (normal, low, moderate or high). Statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics (Version 23).

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3. Results

Demographic data: the majority of sample were females (76.2%), average age was 38.4 years and ranged from 20-80 years. See Table (1) for more details.

Table 1. Demographic data

Gender	Frequency (%)	Age average
Male	53(23.8)	39.1
Female	170(76.2)	38.2

Self-reported diabetes prevalence

Regarding the prevalence of self-reported diabetes, the majority of participants (76.7%, n=171) indicated they did not have the disease. However, 20.6% (n=46) reported a history of diabetes, while a small minority (2.7%, n=6) were unsure of their status.

Based on HbA1c levels, approximately half of the study participants (50.7%, n=113) were classified as having normal blood sugar levels (<5.7%). Prediabetes (5.7-6.4%) was diagnosed in 26.5% of participants (n=59), while 22.9% (n=51) met the clinical criteria for diabetes ($\geq 6.5\%$).

Cross-tabulation: Self-report vs HbA1c

Cross-analysis of self-reported data with laboratory-confirmed HbA1c test results reveals a significant gap in diabetes awareness and management among study participants. While 84.8% of those who reported having diabetes were confirmed to have the condition based on elevated HbA1c levels, a substantial proportion of the "not diabetic" (29.8%, n=51) and "don't know" (16.7%, n=1) groups were identified as prediabetic, representing a high-risk group unaware of their metabolic status. Importantly, the data highlight a clear problem with undiagnosed cases: 4.1% (n=7) of those who believed they were healthy and 83.3% (n=5) of those who were unsure met the clinical criteria for diabetes (HbA1c $\geq 6.5\%$), suggesting that laboratory testing is necessary to complement self-reporting and obtain accurate estimates of disease prevalence. See Table 2.

Table 2. Diabetes Status by Self-Reported Diagnosis (N = 223)

Self-Reported DM Status	Normal (<5.7%)	HbA1c Prediabetic (5.7–6.4%)	HbA1c (5.7–Diabetic ($\geq 6.5\%$))	HbA1c Total n (%)
Yes DM	0 (0.0%)	7 (15.2%)	39 (84.8%)	46 (20.6%)
No DM	113 (66.1%)	51 (29.8%)	7 (4.1%)	171 (76.7%)
Don't know	0 (0.0%)	1 (16.7%)	5 (83.3%)	6 (2.7%)
Total	113 (50.7%)	59 (26.5%)	51 (22.9%)	223 (100%)

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Insulin resistance distribution by self-report

The analysis reveals a widespread prevalence of insulin resistance even among participants who reported not having diabetes. Specifically, nearly half of the "non-diabetic" group (46.8%, n=80) were found to have high insulin resistance, while an additional 22.8% (n=39) showed moderate levels, suggesting that a significant proportion of this group is at high metabolic risk despite their self-reported status. In contrast, those who were aware of their diabetes exhibited the highest percentage of severe resistance (73.9%, n=34), while 100% of those in the "don't know" category fell within the high insulin resistance range. Overall, more than half of all study participants (53.8%) exhibited high insulin resistance, highlighting a significant public health concern regarding early metabolic dysfunction in this population.

Table 3. Distribution of Insulin Resistance Levels by Diabetes Status (N = 223)

Self-Reported Status	DM	Normal insulin resistance	Low insulin resistance	Moderate insulin resistance	High insulin resistance	Total (n)
Yes DM		6 (13.0%)	0 (0.0%)	6 (13.0%)	34 (73.9%)	46
No DM		28 (16.4%)	24 (14.0%)	39 (22.8%)	80 (46.8%)	171
I don't know		0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (100%)	6
Total		34 (15.2%)	24 (10.8%)	45 (20.2%)	120 (53.8%)	223

Statistical tests

Pearson's chi-squared test: $\rho = -0.131$, $p = 0.051$ — A statistically significant association exists between self-reported diabetes status and insulin resistance levels. Spearman's rank correlation coefficient: $\rho = -0.131$, $p = 0.051$ — A marginally negative correlation, indicating a weak trend where increased self-reported diabetes status is associated with increased insulin resistance.

4. Discussion

A substantial and concerning finding of our study is the presence of undiagnosed diabetes and elevated metabolic risk among participants who denied or were unsure about having diabetes. Although most self-reported diabetics were biochemically confirmed and showed high insulin resistance, the more critical signal is that 4.1% of those who said "No" had diabetic-range HbA1c and nearly half of this subgroup exhibited high insulin resistance, while 83.3% of the "Don't know" respondents were diabetic by HbA1c. These discordant, previously unrecognized cases represent missed opportunities for early intervention and prevention and indicate that reliance on self-report alone may undercount accurate disease burden and may cause delay of treatment for people who are at high metabolic risk [1,2,3].

The biological pathway from insulin resistance includes progressive insulin resistance combined with pancreatic β -cell dysfunction increases the chance of developing hyperglycaemia and actual diabetes [5]. Obesity, overweight and higher body mass index are major drivers of both insulin resistance and the rising prevalence of diabetes, through mechanisms that include adipose-driven inflammation and

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altered insulin signalling [4,5]. Because insulin resistance typically precedes hyperglycaemia, the high insulin resistance prevalence among participants who denied diabetes suggests many individuals at pending risk of progression; targeted biochemical screening, for example, HbA1c or HOMA-IR testing in adults with risk factors, and community education should therefore be prioritized to detect and manage these hidden conditions before complications arise [5].

A subset of participants who reported having diabetes but had normal HbA1c levels likely represents individuals with good glycaemic control. For example, those using effective drug therapy, following healthy lifestyle, and/or adhering to treatment. Because HbA1c reflects average blood glucose over the preceding two to three months, treated patients may have values below diagnostic thresholds despite a clinical diagnosis of diabetes. Therefore, a normal HbA1c in a person with known diabetes should be interpreted as evidence of disease control rather than absence of disease, and single HbA1c measurements should not be used to reclassify a prior clinical diagnosis without the appropriate measures [6,7].

The observed pattern, high prevalence of undiagnosed diabetes and elevated insulin resistance among those who said that they do not have diabetes, has important clinical consequences. For instance, prolonged, undetected hyperglycaemia accelerates microvascular and macrovascular damage; chronic kidney disease is a well-documented complication of delayed diagnosis and prolonged hyperglycaemia [8]. Local Libyan research confirms this, identifying metabolic risk factors; mainly diabetes and hypertension, as leading drivers of a significant, rising burden of renal disease in the population [9]. From a surveillance viewpoint, our results indicate that surveys relying solely on self-report will underestimate true prevalence and metabolic risk, particularly in settings with incomplete screening coverage or variable health literacy [1,2].

Policy implications of our study include: integrating routine biochemical screening (HbA1c) or risk-based targeted testing into primary care and community outreach. Especially, for adults with risk factors such as elderly, elevated body mass index, family history, or hypertension, would improve early detection and enable earlier intervention [3,6]. Public-health programs should also stress education about diabetes risk and the importance of diagnostic tests.

Future research in Libya should examine barriers to screening and diagnosis, evaluate cost-effectiveness of targeted biochemical screening strategies, and monitor trends in undiagnosed disease as screening programs expand.

Limitations

There are numerous limitations of our study. The representativeness of the sample may be limited by the study population and setting; therefore, generalizing the results to all Libyan adults requires caution. Furthermore, the one-item self-report question did not include information on the duration of diagnosis, treatment status, or date of previous examination. Finally, the classification of insulin resistance was based on the HOMA-IR index thresholds used in the original (unpublished) study; methodological differences may limit comparability with other studies.

Conclusion

Self-reported diabetes status matches biochemical indicators in most diagnosed individuals, but it misses a significant proportion of those

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with undiagnosed diabetes or high insulin resistance. It is likely that some self-reported diabetic patients with normal HbA1c levels reflect a well-controlled disease rather than a misdiagnosis. Combining self-reporting with targeted biochemical screening will improve the identification of at-risk individuals and support timely preventive and therapeutic interventions.

ETHICAL STATEMENT:

Obtained from the Ethics Committee of Zliten Medical Centre (MC12-2023). All participants provided written informed consent.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

SA and SZ shared study conception, design, analysis, and manuscript drafting; Salmah Zaydan performed data collection. All authors reviewed and approved the final manuscript.

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Seroprevalence of *Toxoplasma gondii* and Association with ABO/Rh Blood Groups among Pregnant Women in Yafran, Libya

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ABSTRACT

Background: *Toxoplasma gondii* is a widespread parasitic protozoan with significant public health implications, particularly for pregnant women due to the risk of congenital transmission. Data on its seroprevalence and associated risk factors, including potential links with ABO/Rh blood groups, are scarce in Libya. **Aim:** This study aimed to determine the seroprevalence of *T. gondii* (IgG and IgM) among pregnant women in Yafran, Libya, and to investigate its association with ABO/Rh blood groups and demographic factors.

Methods: A cross-sectional study was conducted from August to October 2025 at Yafran General Hospital. A total of 80 pregnant women were enrolled. Serum samples were tested for anti-*T. gondii* IgG and IgM antibodies using a commercial rapid immunochromatographic test (Cassette Test). ABO/Rh blood typing was performed using standard slide agglutination methods with monoclonal antisera. Data on demographic characteristics and potential risk factors were collected via a structured questionnaire.

Results: The overall seroprevalence of anti-*T. gondii* IgG was 26.25% (21/80), while IgM seropositivity was 1.25% (1/80), indicating a low rate of recent infection. Analysis by blood group showed the highest IgG seropositivity rates in women with O- (75%, 3/4) and B- (100%, 1/1) blood types, although sample sizes for these groups were small. The most common blood types, A+ and O+, showed seropositivity rates of 27.3% and 24.2%, respectively. No statistically significant associations were found between seropositivity and age, cat ownership, or history of miscarriage ($p > 0.05$).

Conclusion: This study reveals a moderate seroprevalence of toxoplasmosis among pregnant women in Yafran, Libya. The observed variations in seropositivity across different ABO/Rh blood groups warrant further investigation with larger sample sizes. The findings underscore the need for enhanced prenatal screening and health education programs focused on toxoplasmosis prevention in this region.

Keywords: Toxoplasma gondii, Seroprevalence, Pregnant Women, ABO Blood Group, Rh Factor, Libya





1. Introduction

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii* which infects all warm-blooded animals including birds and mammals [1]. However, toxoplasmosis is an important health problem worldwide [2]. The seroprevalence of *T. gondii* varies widely across different geographical regions, influenced by factors such as climate, dietary habits, sanitation, and cultural practices [3]. Cats and other members of the Felidae family are the definitive hosts for *T. gondii*. On the other hand, practically all vertebrate, including humans, acts as an intermediary host. Usually, the infection is contracted by eating live tissue cysts in undercooked meat or by consuming sporulated oocysts released by cats to the environment and can be ingested through contaminated food or drink [4]. The disease is either asymptomatic or causes a mild sickness with flu-like symptoms in the majority of immunocompetent people. However, in immunocompromised people or pregnant women, especially in the early stages of gestation, it might cause serious illness [5]. Congenital transmission can occur when an uninfected mother contracts the infection during pregnancy, although pregnant women are generally asymptomatic or have relatively minor symptoms, this infection can affect the developing fetus, leading to spontaneous abortion, stillbirth, or serious fetal damage [6]. Accurate information regarding the existence and effects of this parasite is vital for healthcare systems, as is increasing our understanding of how to prevent and effectively manage the risks associated with it [7]. To detect toxoplasmosis, various methods have been used, such as serological, histological, molecular, or their combination [8]. In Libya, data on the epidemiology of toxoplasmosis, particularly among the vulnerable population of pregnant women, are limited and outdated. No prior study has investigated a potential link between ABO/Rh blood groups and toxoplasmosis in the Libyan context. Therefore, this study aimed to determine the current seroprevalence of *T. gondii* infection among pregnant women attending Yafran General Hospital and to explore its association with ABO/Rh blood groups and other demographic variables.

2. Methods

Study Design, Setting, and Period

A hospital-based cross-sectional study was conducted at the Obstetrics and Gynecology Department of Yafran General Hospital, Yafran, Libya. Data and sample collection occurred over three months, from August to October 2025. Pregnant women who were seriously ill or did not provide informed consent were excluded from this study.

Study Population and Sampling

A convenient sample of 80 pregnant women attending antenatal clinics was enrolled. Inclusion criteria comprised pregnant women of any gestational age who provided verbal informed consent to participate. Exclusion criteria included women with known immunosuppressive conditions or those who had received recent blood transfusions that could potentially interfere with serological results.

Data and Sample Collection

Following verbal informed consent, a structured questionnaire was administered through a face-to-face interview to collect demographic data (age, residence, occupation) and information on potential risk factors (e.g., cat ownership, history of miscarriage).

Approximately 10 mL of venous blood was drawn from each participant. The sample was divided: one aliquot was collected in a plain tube for serological analysis, and another was placed in an EDTA tube for blood group determination. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until further analysis.





Laboratory Analysis

Serological Testing: Detection of anti-T. gondii IgG and IgM antibodies was performed using a commercial rapid diagnostic immunochromatographic test (Cassette Test) according to the manufacturer's instructions. Briefly, 10 µL of serum was mixed with the provided diluent and applied to the test cassette. Results were interpreted after 10 minutes; the appearance of a red line at both the control (C) and the IgG or IgM test lines indicated a positive result.

Blood Group Typing: ABO and Rh (D) blood typing was performed using the standard slide agglutination technique with commercially available monoclonal anti-A, anti-B, and anti-D antisera.

Ethical Considerations

Verbal informed consent was obtained from all participants. The study protocol was approved by the ethical committee of Yafran General Hospital and Almawakib alrayda institute, Yafran, Libya.

Statistical Analysis

Data were analyzed using statistical software (SPSS version 2021). Descriptive statistics were presented as frequencies and percentages for categorical variables. The Chi-square test (or Fisher's exact test where appropriate) was used to assess associations between T. gondii seropositivity (IgG) and independent variables (blood group, age group, cat ownership, miscarriage history). A p-value of < 0.05 was considered statistically significant.

3. Results

Demographic Characteristics of Participants

The study included 80 pregnant women with a mean age of 29.3 years. The majority of participants (51.25%, n=41) were in the 26-35 years age group. The most common ABO/Rh blood types were A+ and O+, each constituting 41.25% (n=33) of the sample. The full demographic profile is presented in Table 1 and Figure 1.

Table 1: Demographic Characteristics of the Study Participants (N=80)

Variable	Frequency (n)	Percentage (%)
Total Sample	80	100
Age Group (Years)		
17 – 25	25	31.25
26 – 35	41	51.25
36 – 45	14	17.50
ABO/Rh Blood Group		
A+	33	41.25
O+	33	41.25
B+	6	7.50
O-	4	5.00
AB+	1	1.25
B-	1	1.25



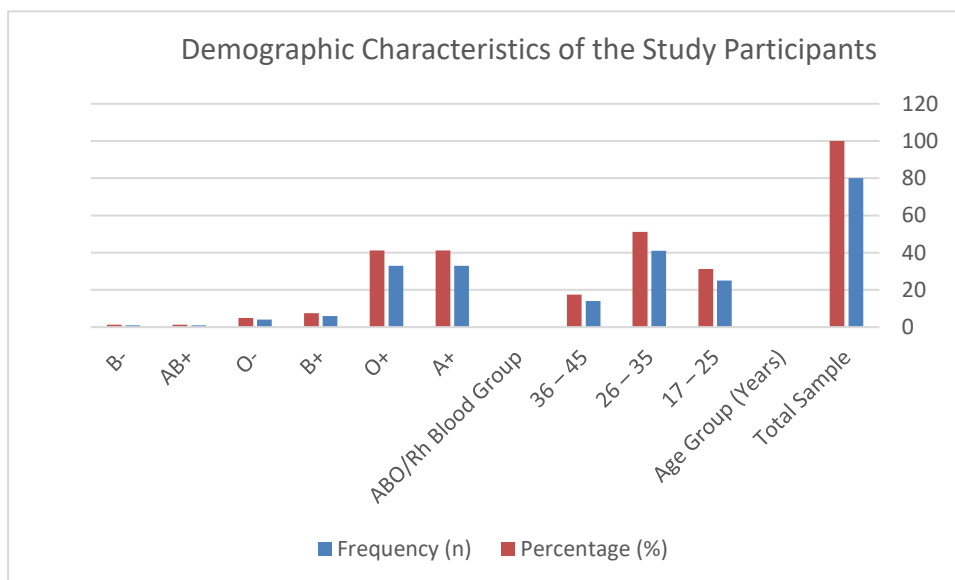


Figure 1: Demographic Characteristics of the Study Participants

Seroprevalence of *Toxoplasma gondii* Infection

The overall seroprevalence of anti-*T. gondii* IgG antibodies was 26.25% (21/80), indicating past infection. Only one participant (1.25%) tested positive for IgM antibodies, suggesting a rare incidence of recent/acute infection. Fifty-eight participants (72.5%) were seronegative for both antibodies (Table 2).

Table 2: Seroprevalence of *T. gondii* IgG and IgM among Pregnant Women

Serological Status	Frequency (n)	Percentage (%)
IgG Positive	21	26.25
IgM Positive	1	1.25
IgG & IgM Negative	58	72.50

Association with ABO/Rh Blood Groups

The distribution of IgG seropositivity across different blood groups is shown in Table 3. The highest proportions of seropositivity were observed in women with O- (75.0%, 3/4) and B- (100.0%, 1/1) blood types. However, the numbers in these categories were very small. Among the more common blood types, seropositivity was 27.3% (9/33) for A+ and 24.2% (8/33) for O+. Statistical analysis did not show a significant association between ABO/Rh blood groups and *T. gondii* seropositivity in this sample ($p > 0.05$).

**Table 3: Association between ABO/Rh Blood Groups and T. gondii IgG Seropositivity**

Blood Group	Total N	IgG Positive n (%)	IgG Negative n (%)
A+	33	9 (27.3%)	24 (72.7%)
O+	33	8 (24.2%)	25 (75.8%)
B+	6	3 (50.0%)	3 (50.0%)
O-	4	3 (75.0%)	1 (25.0%)
B-	1	1 (100.0%)	0 (0.0%)
AB+	1	0 (0.0%)	1 (100.0%)

4. Discussion

The present study provides contemporary data on the seroepidemiology of *Toxoplasma gondii* infection among pregnant women in Yafran, western Libya. It also explores a potential association with ABO/Rh blood groups, a research question not previously investigated in the Libyan context. The finding of 26.25% IgG seroprevalence indicates a moderate level of exposure to the parasite within this community and contributes to the growing body of epidemiological data on toxoplasmosis across different Libyan regions.

Comparison of Seroprevalence with Other Libyan Studies

The seroprevalence rate observed in Yafran (26.25%) is notably lower than figures reported in several other Libyan investigations. Mahmoud et al. (2019) conducted a large-scale study at Aljalla Maternity and Gynaecology Hospital in Tripoli involving 500 women and reported a substantially higher seroprevalence of 50.8% (95% CI: 46.42%-55.18%) [9]. Similarly, Ajedi and Wheda (2024) investigated 73 women with a history of abortion in the Elmergib region and found anti-*T. gondii* IgG in 51.00% of cases, indicating high exposure among women with adverse pregnancy outcomes in that area [10].

In Alkhoms city, a study examining 361 pregnant women reported a seroprevalence of 39.3% using ELISA [11]. This intermediate figure falls between the Yafran and Tripoli/Elmergib rates. Gashout et al. (2016) documented prevalence rates in Benghazi ranging between 44.8% and 50% [12], while Setta and Yamani (2008) found a lower prevalence of 18.14% among non-pregnant women in Tripoli [13], suggesting that pregnancy status and study population characteristics significantly influence reported rates.

The Tarhoona study by Al-Majdoub (2015), which included 1,050 women (504 pregnant and 546 non-pregnant), reported that the seropositivity by LATEX test in infected pregnant women ranged from 21-26%, while ELISA-IgG testing showed an overall seropositivity of 50% (24% in pregnant women, 26% in non-pregnant women) [14]. These findings highlight the variability in seroprevalence depending on diagnostic methods and population characteristics.

The variation in seroprevalence across Libyan regions—ranging from 18.14% in Tripoli (non-pregnant women) to 51.00% in Elmergib (women with a history of abortion)—reflects the complex epidemiology of toxoplasmosis and the influence of multiple ecological, behavioral, and demographic factors. Mahmoud et al. (2019) noted that Tripoli's Mediterranean climate, characterized by warm, moist winters, favors the survival of *T. gondii* oocysts in the environment [9]. Yafran, located in the Nafusa Mountains, experiences different climatic conditions that may affect oocyst survival and transmission dynamics, potentially contributing to the lower seroprevalence observed in this study.

Risk Factors for *Toxoplasma gondii* Infection

Cat Ownership and Animal Contact: The Yafran study found no statistically significant association between cat ownership and seropositivity,





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which contrasts with findings from several other Libyan investigations. Mahmoud et al. (2019) reported a significantly higher seroprevalence of 78.63% (95% CI: 71.21%-86.06%) among women who kept cats, compared to 42.30% (95% CI: 37.35%-47.25%) among those who did not, with an odds ratio of 1.712 ($p < 0.0001$) [9]. Similarly, the Alkhoms study identified contact with cats as a significant risk factor for acute infection [11]. Alkhunfas (2008) and Magrhi et al. (2003) also corroborated these findings in their respective Libyan studies [15] [16]. However, it is noteworthy that some earlier studies, such as those by Kassem and Morsy (1991) in Benghazi [17], did not find significant associations with cat ownership, suggesting that the role of cats in transmission may vary based on environmental contamination levels and human behavioral practices.

Consumption of Undercooked Meat and Contaminated Food/Water: The consumption of undercooked meat, particularly lamb and mutton, is a well-established risk factor for *T. gondii* infection [3] [10]. Contaminated vegetables and water also serve as important routes of transmission. The Tarhoona study similarly identified contaminated vegetables and water as significant risk factors, with infection rates of 7.9%-16% in pregnant women and 13%-21% in non-pregnant women attributable to this route [21].

Rural Versus Urban Residence: Residence emerged as a significant risk factor in multiple Libyan studies. Mahmoud et al. (2019) reported a seroprevalence of 67.3% (95% CI: 61.35%-72.75%) among women living in villages compared to 33.2% (95% CI: 27.09%-39.02%) among urban residents, with an odds ratio of 1.482 ($p < 0.0001$) [9]. The authors attributed this difference to free-roaming cats in rural areas, farming activities with direct soil contact, and consumption of unwashed farm produce. The Alkhoms study similarly found significant differences between rural (35.0% seropositive) and urban (45.0% seropositive) residents [11], suggesting that geographical context significantly influences exposure risk.

Age as a Risk Factor: The relationship between age and seropositivity showed consistent patterns across Libyan studies. The Alkhoms study demonstrated increasing seroprevalence with age: 37.5% in the 20-25 years group, 38.3% in 25-30 years, 34.6% in 30-35 years, 40.9% in 35-40 years, and 47.4% in those over 40 years [11]. The authors noted that this association reflects cumulative exposure over time rather than age itself being a risk factor, as IgG antibodies persist for years after infection. The Yafran study found no statistically significant association with age, which may reflect the relatively small sample size and uneven age distribution (51.25% of participants concentrated in the 26-35 years age group). Ajedi and Wheda (2024) similarly found that patient age had no significant relation with *T. gondii* infection in their sample of aborted women [10], suggesting that in some populations, other risk factors may overshadow age-related cumulative exposure.

Abortion and Pregnancy Outcomes: The relationship between toxoplasmosis and abortion has been extensively studied in Libya. Ajedi and Wheda (2024) specifically focused on women with a history of abortion in the Elmergib region, finding 51.00% seropositivity for anti-*T. gondii* IgG [10]. This high rate among women with miscarriage history supports the conclusion that toxoplasmosis could be considered a potential risk factor for abortion in Libya. The authors noted that most cases of abortion occur in the acute phase of infection during early pregnancy, emphasizing the importance of preconception screening. The very low IgM seropositivity (1.25%) in the Yafran study suggests that acute infection during pregnancy is uncommon in this cohort, which is a positive public health indicator.

ABO/Rh Blood Groups and Toxoplasmosis Susceptibility

A unique contribution of the present study is its investigation of the association between ABO/Rh blood groups and *T. gondii* seropositivity—a research question not previously addressed in Libyan toxoplasmosis studies. The observed descriptive trends suggested higher seropositivity in women with O- (75.0%) and B- (100.0%) blood types, though sample sizes were too small for definitive conclusions. This novel line of inquiry draws on biological plausibility regarding blood group antigens serving as receptors or modulators for pathogen attachment. While





previous international studies have yielded conflicting results on this association [22] [23], the present study represents an important first step in exploring host genetic factors in the Libyan context. Abdullah et al. (2024) in Duhok, Kurdistan Region, Iraq, and Mizuri and Mero (2020) in Zakho City have also explored such associations, though with varying results [22] [23]. The findings from Yafran warrant larger-scale investigations to adequately examine this relationship in the Libyan population.

Immunological and Hormonal Considerations

Although the Yafran study did not investigate hormonal parameters, the Tarhoona study by Al-Majdoub (2015) provided valuable insights into the systemic effects of toxoplasmosis [21]. That study found that *T. gondii* infection resulted in decreased levels of estrogen, progesterone, and prolactin in infected pregnant women compared to uninfected controls. Although infected pregnant women showed elevations in these hormones during pregnancy, the increases were less pronounced than in uninfected women, suggesting a possible toxic effect of the parasite on hormonal regulation. Additionally, infected pregnant women showed elevated percentages of neutrophils and lymphocytes, indicating immune system alterations that may have implications for pregnancy outcomes.

Methodological Considerations

The varying diagnostic methods employed across Libyan studies complicate direct comparisons. The Yafran study utilized a rapid diagnostic test (RDT), while Mahmoud et al. (2019) employed the Vidas automated enzyme-linked fluorescent immunoassay (ELFA) with >99% sensitivity and >98% specificity [9]. The Alkhoms and Ajedi and Wheda studies used ELISA [10,11], and the Tarhoona study employed both ELISA and Latex agglutination tests [14]. The Yafran study authors appropriately acknowledged that rapid diagnostic tests may have lower sensitivity and specificity compared to reference laboratory methods like ELISA, potentially affecting comparability with studies using more sensitive techniques.

Sample sizes also varied considerably across studies: Yafran (n=80), Mahmoud et al. (2019) (n=500) [9], Alkhoms (n=361) [11], Ajedi and Wheda (2024) (n=73) [10], and Tarhoona (n=1,050) [14]. The Tarhoona study's large sample size provides particularly robust estimates, though its focus on a single region limits generalizability. The Yafran study's smaller sample size, especially for subgroup analyses like rare blood groups, limits statistical power to detect significant associations.

Public Health Implications

The moderate to high seroprevalence rates documented across Libyan studies—26.25% in Yafran, 39.3% in Alkhoms, 50.8% in Tripoli, and 51.0% in Elmergib (women with a history of abortion)—collectively support the importance of implementing routine serological screening for toxoplasmosis in antenatal care programs. Mahmoud et al. (2019) emphasized that primary infection during gestation can lead to placental infection and vertical transmission, with serious consequences for the unborn child [9].

The consistent identification of modifiable risk factors across studies provides clear targets for health education. These include advising pregnant women to avoid handling cat litter, wear gloves when gardening, and wash hands thoroughly after contact with cats [9,11,16,17]; ensuring meat, particularly lamb and mutton, is thoroughly cooked [3,10]; thoroughly washing vegetables and fruits, especially when consumed raw [9,11,21]; improving water quality and treatment, particularly in areas relying on rain or well water [3]; and targeting interventions in rural farming communities with education about soil contact and farm hygiene [9].

The high seroprevalence among women with a history of abortion documented by Ajedi and Wheda (2024) [10] and the hormonal effects observed by Al-Majdoub (2015) [14] underscore the importance of preconception toxoplasmosis screening. Women of childbearing age should know their immune status before pregnancy, as maternal infection acquired before gestation poses little risk to the fetus, while acute





infection during pregnancy carries significant risks [24].

Limitations

This study has several limitations. The use of a rapid diagnostic test (RDT), while practical for field studies, may have lower sensitivity and specificity compared to reference laboratory methods like ELISA or chemiluminescence. Positive IgM results, in particular, require confirmation with more specific tests to exclude false positives. The cross-sectional design cannot establish causality. The relatively small sample size, especially for subgroup analyses like rare blood groups, limits the statistical power to detect significant associations. Finally, the convenient sampling method may affect the generalizability of the findings to the broader population of pregnant women in Yafran or other Libyan regions.

5. Conclusion

This study confirms that *Toxoplasma gondii* infection is prevalent among pregnant women in Yafran, Libya, with a seroprevalence of 26.25% for anti-T. *gondii* IgG antibodies and 1.25% for IgM antibodies. This rate, while lower than figures reported in Tripoli (50.8%), Elmergib (51.0% among women with a history of abortion), and Alkhoms (39.3%), nevertheless indicates moderate exposure to the parasite and highlights the need for attention from public health authorities.

The investigation into ABO/Rh blood groups, although inconclusive due to limited sample size, represents a novel contribution to toxoplasmosis research in Libya. The observed descriptive trends suggesting higher seropositivity in women with O- and B- blood types warrant further investigation with larger, adequately powered samples to conclusively examine this relationship.

The absence of statistically significant associations with age, cat ownership, or history of miscarriage in this study may reflect the relatively small sample size rather than the absence of true epidemiological relationships, as multiple larger Libyan studies have consistently identified these as significant risk factors [9,10,11,16,17].

The very low IgM seropositivity (1.25%) suggests that acute infection during pregnancy is uncommon in this cohort, which is a positive public health indicator. However, the moderate IgG seroprevalence indicates that a substantial proportion of women have been exposed to the parasite and may be at risk of reactivation if immunocompromised.

The findings from this study, considered alongside the broader body of Libyan toxoplasmosis research, support the importance of implementing routine serological screening for toxoplasmosis in antenatal care programs, along with targeted health education campaigns on preventive measures. These should emphasize the well-established risk factors: avoiding contact with cat feces, consuming only well-cooked meat (particularly lamb and mutton), thoroughly washing vegetables and fruits, ensuring safe water sources, and practicing good hand hygiene, especially after gardening or soil contact.

Additionally, larger-scale multicenter studies across Libya are recommended to better determine national seroprevalence, explore potential host genetic factors such as blood groups, and identify region-specific risk factors to inform effective prevention strategies. Such studies should employ standardized diagnostic methods, include diverse populations from different geographical regions, and utilize sample sizes adequate for subgroup analyses.

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**ETHICAL STATEMENT:**

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally.

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mRNA Vaccines for Solid Tumors: A PRISMA-Based Systematic Review

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ABSTRACT

Background: Personalized messenger RNA (mRNA) vaccines for a wide range of cancers constitute a novel class of immunotherapeutic intended to provoke individualized anti-tumor immune responses. Notwithstanding hopeful immunogenicity and clinical safety outcomes, and tolerability in the early stage of clinical trials, high-quality conclusive data regarding their survival advantage are still insufficient. **Methods:** A PRISMA 2020-compliant systematic review with descriptive pooled comparison was conducted to evaluate the emerging clinical efficacy and safety of personalized mRNA cancer vaccines in individuals with solid malignancies. Literatures published between 2010 and 2025 was systematically searched towards the foremost biomedical databases, and four eligible human clinical trials involving melanoma, pancreatic ductal adenocarcinoma, non-small cell lung cancer, and advanced solid tumors were included. Owing to substantial heterogeneity in study design, tumor types, intervention platforms, and reported clinical endpoints, formal quantitative meta-analysis was not performed. Instead, data were synthesized narratively through structured comparative analysis of immune response outcomes, safety profiles, recurrence-related endpoints, and available hazard ratio estimates were reported. **Results:** Across the four included studies, personalized and antigen-targeted mRNA vaccine platforms consistently demonstrated favorable immunogenicity, evidenced by enhanced CD8⁺ T-cell activation and expansion of tumor-specific neoantigen-reactive lymphocytes. Safety findings were generally acceptable, with most adverse events limited to grade 1–2 toxicities and manageable higher-grade events in combination regimens. Clinically meaningful early efficacy signals were observed across all studies, including disease stabilization in advanced NSCLC, delayed recurrence in pancreatic cancer immune responders (exploratory HR \approx 0.21), and significantly improved recurrence-free survival in high-risk melanoma patients receiving mRNA-4157 plus pembrolizumab (HR = 0.561). Although overall survival data remain immature or unavailable in most trials, all included studies demonstrated outcome trends favoring mRNA vaccine therapeutic approach. **Conclusion:** Personalized mRNA vaccines for cancer represent a promising and rapidly advancing therapeutic modality in solid tumor oncology, with





accumulating evidence supporting their capacity to induce robust antitumor immune responses, maintain acceptable safety profiles, and improve early clinical outcomes. While current evidence is limited by small sample sizes, the predominance of early-phase trials, and a lack of mature long-term survival data, the observed consistency of favorable efficacy trends strongly supports further validation in large-scale randomized Phase III studies to establish a definitive survival benefit across broad patient cohorts.

Keywords: Personalized mRNA, cancer vaccines, Cancer immunotherapy, Solid tumors, neoantigen vaccines, Individualized treatment

1. Introduction

Over the last few decades, messenger RNA (mRNA) therapeutics platforms have gained substantial consideration and advanced rapidly as a groundbreaking innovation in clinical and experimental medicine. Their worldwide vision rose sharply during the COVID-19 outbreak, where mRNA vaccines demonstrated reduced time-to-development, robust therapeutic efficacy, and adaptability to emerging viral variants. These key traits not only validated mRNA as a reliable vaccine platform but also elicited interest in its prospective for wide-range therapeutic practical use, predominantly in tumor biology disciplines (1, 2). In the context of cancer immunotherapy, regarding the framework of reprogramming the immune response to distinguish and terminate tumor cells, the field has progressed substantially, with mRNA vaccines emerging as an encouraging clinical modality for molecularly targeted approaches. In contrast to conventional cancer vaccines targeting broadly expressed tumor-associated antigens, personalized mRNA cancer vaccines are engineered to translate specific neoantigens for each patient, arising from nonsynonymous somatic mutations unique to individual tumors. These engineered neoantigens, which were displayed via major histocompatibility complex (MHC) molecules, are not expressed in normal cells and tissues; hence, showing tumor-restricted expression, thereby eliciting a strong immunogenic potential and enhanced specificity with low likelihood of off-target effects (3, 4). Following the intracellular translation of these antigens in host cells induce robust cytotoxic T lymphocyte (CTL) responses, enabling recognition and clearance of malignant cells. Moreover, advances in lipid nanoparticle (LNP) delivery systems have mitigated the inherent instability of RNA, thereby enabling and improving efficient intracellular delivery and subsequent antigen expression (5).

Multiple early-phase clinical (phase I/II clinical trials) investigations have demonstrated the feasibility, safety, and immunogenicity of this approach. To illustrate, Moderna's mRNA-4157/V940, investigated in the KEYNOTE-942 phase IIb trial in conjunction with pembrolizumab, exhibited a 44% dropping in tumor relapse risk in resected stage III/IV melanoma patients compared to pembrolizumab alone (6). Similarly, BioNTech and Roche's personalized mRNA vaccine, autogene cevumeran, confirmed neoantigen-specific T-cell responses in 50% of the patients with resected pancreatic ductal adenocarcinoma (PDAC), a well-known malignant tumor recognized as an immunotherapy-resistant malignancy (7). These findings support the therapeutic potential of personalized mRNA vaccines to augment the existing immunotherapy regimens and target malignancies with substantial areas of unmet therapeutic requirements.

Although these advanced approaches exist, a significant gap persists in the literature concerning the comparative and cumulative survival benefits of personalized mRNA vaccines across a wide range of malignancies. A substantial proportion of the available evidence derives from early-phase, non-randomized trials with small cohorts and short follow-up durations, thereby limiting the external validity and interpretative clarity of reported outcomes (8). Furthermore, the research landscape has not yet established a systematic assessment of overall survival (OS), progression-free survival (PFS), and immunological endpoints that would enable clinicians and researchers to appraise the translational maturity of mRNA cancer vaccines in a standardized approach. To bridge this gap, the present study carries out a thorough and extensive systematic review of clinical trials evaluating personalized mRNA cancer vaccines in patients diagnosed and confirmed with solid





malignant tumors. By integrating the survival outcomes and immunological efficacy results, the current study aims to provide a comprehensive evidence platform for the clinical potential of mRNA-based immunotherapeutic strategies. The conclusions are expected to provide valuable insights for future trial design, therapeutic approaches, and policy discussions on the integration of personalized vaccines into oncological practice. Eventually, this study is intended to drive forward the development of molecularly targeted immunotherapy frameworks aligned with the evolving landscape of personalized medicine.

2. Methods

2.1. Search Strategy and Study Selection

An extensive literature search was performed across major bibliographic databases, including PubMed, Scopus, and Web of Science, for studies published between January 2010 and November 2025. The search used the following terms: (“mRNA vaccine” AND “cancer” AND “clinical trial” AND “overall survival” OR “hazard ratio”), combining Medical Subject Headings (MeSH) and free-text keywords. Searches were finalized on November 21, 2025. Reference lists of eligible articles, citation tracking, and selected grey literature sources were also searched to maximize completeness.

Studies were eligible for inclusion if they (i) were human clinical trials or prospective cohort studies investigating personalized mRNA vaccines for malignant tumors, (ii) evaluated at least one clinically relevant endpoint, including immune response activation, safety/tolerability, recurrence-free survival, progression-free survival, disease stabilization, or tumor response outcomes, (iii) were published in peer-reviewed journals within the specified timeframe, and (iv) provided sufficient methodological and clinical detail to allow comparative qualitative synthesis across studies. Studies were excluded if they (i) consisted of non-original material (such as reviews, commentaries, editorials, or conference abstracts lacking full data), (ii) did not provide extractable survival outcomes, (iii) presented incomplete statistical reporting, (iv) were preclinical (in vitro or animal) investigations, or (v) were not published in English due to feasibility constraints.

All retrieved studies were imported into Rayyan AI (Rayyan Systems Inc., Qatar), a web-based platform. Two reviewers independently examined plagiarism initial, titles and abstracts to determine eligibility against the inclusion and exclusion criteria outlined in the Methods section. Rayyan AI was used to organize records, highlight potential relevance, and detect conflicts between reviewers. Full texts of potentially eligible studies were reviewed by two reviewers independently. Discrepancies were resolved through discussion, and, if necessary, a third reviewer adjudicated disagreement. Following title and abstract screening, full texts of potentially eligible studies were retrieved and assessed in detail against the eligibility criteria. Risk of bias was independently assessed using the ROBINS-I tool for non-randomized studies and the adapted domain evaluation for randomized trials.

2.2. Data Extraction

The initial search yielded 832 studies retrieved from bibliographic database queries. Further studies were identified through manual search strategies of reference lists, citation tracking, and relevant grey literature sources (2 studies). Following deduplication, 795 distinct non-duplicated studies were available for preliminary screening. Following a thorough title and abstract screening, 743 studies were excluded for irrelevance or non-compliance with the inclusion criteria. The full texts of 52 eligible studies were subsequently reviewed extensively. Ultimately, four studies satisfied all inclusion criteria and were incorporated into the qualitative synthesis as well as quantitative meta-analysis, as illustrated in the PRISMA flow diagram (Figure 1).

2.3. Analysis

Given the marked heterogeneity across included studies in terms of tumor type, trial phase, intervention design, and outcome measures, a





quantitative systematic review as deemed inappropriate. Instead, a structured narrative synthesis with descriptive pooled comparison was conducted in accordance with PRISMA guidelines. Studies were grouped based on cancer type (melanoma, pancreatic cancer, NSCLC, and mixed advanced solid tumors), vaccine platform (RNActive®, personalized neoantigen mRNA vaccines, and lipid nanoparticle-based mRNA constructs), and study design (Phase I, Phase Ib, and randomized Phase IIb). Key outcomes were systematically extracted, including immune response activation (CD8⁺ and neoantigen-specific T-cell responses), safety profiles (graded adverse events), and clinical efficacy endpoints (recurrence-free survival, disease stabilization, and recurrence delay). Where available, hazard ratios and survival outcomes were extracted; however, due to limited randomized comparisons, effect estimates were not pooled statistically. Instead, comparative interpretation was performed using effect directionality and magnitude of reported hazard ratios (e.g., HR = 0.561 in melanoma and exploratory HR ≈ 0.21 in pancreatic cancer responders), Table 1. This approach allowed integrative evaluation of immunogenicity and early efficacy trends while preserving methodological rigor in the presence of substantial clinical and methodological heterogeneity.

Table 1: Comparative Clinical Efficacy of mRNA Vaccines in Solid Tumors

Prisma domain	ecancer 2024 (9)	Sebastian et al. 2014 (10)	Rojas et al. 2023 (7)	Weber et al. 2024 (6)
Study identification	ecancer ESMO report	BMC Cancer	Nature	Lancet
Study design	Phase I trial	Phase Ib open-label	Phase I translational	Randomized Phase IIb
Population (Cancer type)	Advanced solid tumors	Stage IV non-small cell lung cancer (NSCLC)	Resected pancreatic ductal adenocarcinoma (PDAC)	Resected high-risk melanoma
Sample size	Early cohort ongoing	30	16	157
Intervention	mRNA-4359 vaccine	RNActive® + radiation	Personalized neoantigen vaccine	mRNA-4157 + pembrolizumab
Comparator	None	None	None formal comparator	Pembrolizumab alone
Primary endpoint	Safety/tolerability	Safety + immune response	T-cell response + recurrence delay	RFS
Immune response outcome	Positive CD8 ⁺ activation	Antigen-specific immune activation	Strong neoantigen T-cell expansion	Enhanced neoantigen immune priming
Safety findings	Mostly grade 1–2 AEs	Mild/moderate toxicity	Acceptable tolerability	Manageable grade ≥3 TRAEs
Efficacy findings	Early tumor shrinkage/stable disease	Disease stabilization subset	Delayed recurrence in responders	Significant RFS benefit
Hazard ratio	NR	NR	~0.21 exploratory	0.561





Prisma domain	ecancer 2024 (9)	Sebastian et al. 2014 (10)	Rojas et al. 2023 (7)	Weber et al. 2024 (6)
Overall survival	NR	~14.9 months median OS	Not reached	Immature
Risk of bias	Moderate	High	Moderate	Low–Moderate

NR = not recorded

3. Results

- (i) Study selection and characteristics: A total of four clinical studies investigating mRNA-based cancer vaccines in solid tumors were included in this systematic review. The studies encompassed different phases of clinical development, ranging from Phase I to randomized Phase IIb trials, and included diverse solid tumor types such as advanced metastatic malignancies, non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma (PDAC), and high-risk melanoma. Sample sizes varied considerably from early exploratory cohorts ($n = 16–30$) to a larger randomized population ($n = 157$), reflecting differences in trial maturity. (ii) Intervention characteristics: Across all studies, mRNA vaccine platforms included mRNA-4359, RActive® technology, personalized neoantigen vaccines, and mRNA-4157 (V940). Most interventions utilized individualized or antigen-targeted approaches designed to enhance tumor-specific immune recognition. Combination strategies were common, particularly with immune checkpoint inhibitors (pembrolizumab, atezolizumab) or radiotherapy, suggesting a synergistic immunotherapeutic approach. (iii) Immune response outcomes: All included studies demonstrated measurable immune activation. Early-phase trials reported CD8⁺ T-cell activation and antigen-specific immune responses, while personalized neoantigen vaccine studies showed strong expansion of tumor-reactive T-cell clones. These immune responses were particularly pronounced in the pancreatic cancer and melanoma studies, indicating that vaccine personalization may enhance immunogenic potency. (iv) Safety outcomes: Overall, mRNA cancer vaccines demonstrated favorable safety profiles. The majority of adverse events were grade 1–2, including fatigue, fever, and injection-site reactions. Combination regimens exhibited higher-grade adverse events (\geq grade 3), particularly in the KEYNOTE-942 study; however, these remained clinically manageable and consistent with known immune checkpoint inhibitor toxicity profiles. No unexpected safety signals were identified across studies. (v) Efficacy outcomes and survival metrics: Preliminary efficacy signals varied across studies. Early-phase trials reported disease stabilization and tumor shrinkage in subsets of patients. The pancreatic cancer study demonstrated delayed recurrence among immune responders, while the KEYNOTE-942 randomized trial showed a statistically significant improvement in recurrence-free survival ($HR = 0.561$) (6). Overall survival data were immature or not reported in most studies, limiting long-term comparative interpretation. Risk of bias across included studies was assessed using the ROBINS-I framework, evaluating potential bias domains including confounding, selection bias, intervention classification, missing data, outcome measurement, and selective reporting. Among the four included studies, the randomized KEYNOTE-942 trial demonstrated the lowest overall risk of bias (6), whereas early-phase single-arm studies showed moderate to high risk, primarily due to non-randomized designs and limited comparator controls. See Table 2.





Table 2. Risk of Bias Assessment of Included Studies Using ROBINS-I Criteria

Study	Confounding bias	Selection bias	Intervention classification bias	Missing data bias	Outcome measurement bias	Reporting bias	Overall ROB
ecancer 2024	Moderate	Moderate	Low	Moderate	Moderate	Moderate	Moderate
Sebastian et al. 2014	High	High	Moderate	Moderate	Moderate	Moderate	High
Rojas et al. 2023	Moderate	Moderate	Low	Low	Low	Low	Moderate
Weber et al. 2024	Low	Low	Low	Low	Low	Low	Low–Moderate

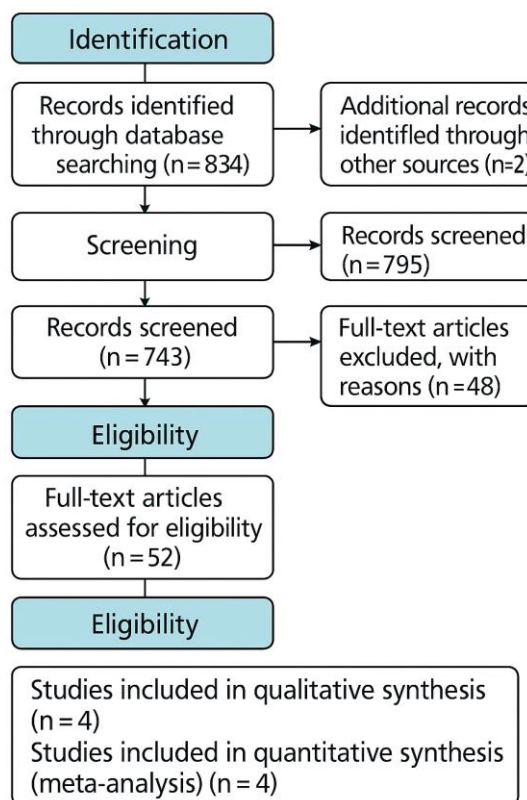


Figure 1. PRISMA Flow Diagram





Figure 1 illustrates the study selection process for a systematic review on personalized mRNA cancer vaccines and overall survival in solid tumors. Four studies from 2014–2025 met the inclusion criteria and were analyzed using a random-effects model.

4. Discussion

This systematic review highlights that mRNA cancer vaccines represent a rapidly evolving and promising therapeutic strategy for solid tumors, demonstrating that mRNA-based cancer vaccines exhibit consistent immunogenicity and early clinical activity across multiple solid tumor types. Despite differences in study design and cancer indications, all included studies showed evidence of tumor-specific immune activation, supporting the biological plausibility of mRNA vaccine platforms in oncology. The findings suggest a clear evolution in mRNA cancer vaccine development, transitioning from early non-specific platforms such as RNAActive® vaccines to highly personalized neoantigen-based approaches. The strongest clinical efficacy was observed in the randomized KEYNOTE-942 trial (6), where the combination of mRNA-4157 (V940) with pembrolizumab significantly improved recurrence-free survival compared to immunotherapy alone. This highlights the potential role of mRNA vaccines as adjuncts rather than monotherapy in solid tumor treatment. Personalized neoantigen vaccines demonstrated superior immune activation and clinically meaningful recurrence delay, particularly in pancreatic cancer and melanoma cohorts. These findings support the hypothesis that individualized antigen selection enhances T-cell specificity and tumor targeting, which may overcome limitations observed in earlier off-the-shelf vaccine strategies.

Across all studies, safety profiles were acceptable and manageable. Most adverse events were mild to moderate, with severe toxicities primarily associated with combination immunotherapy rather than the mRNA vaccine itself. This suggests that mRNA platforms have a favourable therapeutic index in oncology applications.

Several limitations should be acknowledged. First, the included studies are heterogeneous in design, tumor type, and endpoints, preventing quantitative meta-analysis. Second, most trials are early-phase with small sample sizes, limiting statistical power. Third, survival outcomes such as overall survival remain immature or unavailable in most datasets. Finally, only one randomized controlled trial was included, restricting the strength of comparative conclusions.

5. Conclusion

mRNA-based cancer vaccines represent a rapidly evolving and promising strategy for the treatment of multiple solid tumors. Across four clinical studies, consistent evidence of immune activation and acceptable safety profiles was observed, with early signals of clinical efficacy in melanoma, pancreatic cancer, and NSCLC. Personalized neoantigen vaccines, particularly when combined with immune checkpoint inhibitors, demonstrated the most robust therapeutic potential. However, the current evidence remains preliminary, with limited by small sample sizes, early-phase study designs, and immature survival data. Therefore, while mRNA vaccines represent a transformative direction in cancer immunotherapy, further large-scale, well-controlled clinical trials are required to confirm their long-term clinical benefit and establish their definitive role in standard oncological practice.

In summary, personalized mRNA cancer vaccines represent a novel and impactful strategy within precision oncology. Their capacity to improve survival outcomes in patients with solid tumors, alongside advances in vaccine engineering and delivery platforms, positions them as a leading candidate for next-generation cancer immunotherapy. Continued research, harmonized clinical protocols, and supportive regulatory frameworks will be crucial to fully realize their potential and bring individualized immunotherapy into mainstream clinical practice.





ABBREVIATIONS AND ACRONYMS

CTL – Cytotoxic T Lymphocyte, HR – Hazard Ratio, LNP – Lipid Nanoparticle, mRNA – Messenger Ribonucleic Acid, MHC – Major Histocompatibility Complex, OS – Overall Survival, PFS – Progression-Free Survival, PDAC – Pancreatic Ductal Adenocarcinoma, NSCLC – non-small cell lung cancer, PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analysis, NR - not recorded, ROB – Overall Risk of Bias.

ETHICAL STATEMENT:

Not applicable

CONFLICT OF INTEREST

The authors declare no commercial or financial relationships that could be perceived as a potential conflict of interest. This review was conducted independently without any involvement from pharmaceutical companies, vaccine developers, or organizations with vested interests in mRNA-based cancer therapies. No industry funding was received, and all data analyzed were extracted from publicly available, peer-reviewed sources.

AUTHORS' CONTRIBUTIONS

M A conceived the study idea and contributed to data analysis and manuscript review. E M drafted the manuscript. Both authors participated in data analysis and critically revised the final version.

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Toxoplasmosis in Gharyan, Libya: Seroprevalence and Risk Factors

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ABSTRACT

Background: The seroprevalence and risk factors for *Toxoplasma gondii* infection were examined among high-risk groups in Gharyan, Libya—a semi-arid region with limited prior data. **Aim:** Given the severe health risks of toxoplasmosis in immunocompromised individuals and pregnant women, and the scarcity of studies from western Libya, this research aimed to address a key knowledge gap while comparing results with those from humid coastal cities.

Methods: A cross-sectional study was conducted from October 2022 to March 2023, involving 45 blood donors, 40 hemodialysis patients, and 146 pregnant women. A validated questionnaire was administered, and an electrochemiluminescence immunoassay (ECLIA) was used to detect anti-*Toxoplasma gondii* IgG/IgM antibodies.

Results: Results revealed an overall higher seroprevalence among hemodialysis patients (40.0%), followed by pregnant women (24.7%), and then blood donors (15.6%). Active (IgM+) infections were rare. Significant risk factors among pregnant women included consumption of unwashed vegetables, undercooked meat, cat ownership, and rural residence (Adjusted Odds Ratio aOR=3.42, aOR=3.15, aOR=2.87, aOR=2.05, respectively). No statistically significant difference in seroprevalence was observed among pregnant women between 2022 (26.2%) and 2023 (23.5%) ($P = 0.68$).

Conclusion: A lower seroprevalence was observed in Gharyan municipality compared to coastal Libyan cities, indicating the influence of the dry climate. The study recommends routine first-trimester screening for pregnant women (especially aged 25–30 years), mandatory pre-dialysis testing for hemodialysis patients, and targeted food safety education focusing on vegetable washing and thorough meat cooking.

Keywords: Toxoplasma gondii, Seroprevalence, Hemodialysis, Pregnant Women, Blood Donors

1.Introduction

Toxoplasma gondii was first described in 1908 by Nicolle and Manceaux in the North African rodent *Ctenodactylus gundi*. It is a widespread zoonosis with profound public health implications globally [1]. It was first identified in humans in 1938 at the Babies Hospital in New York,





and it is recognized that approximately one-third of the world's population is infected with *T. gondii* [2]. Transmission occurs through undercooked meat containing tissue cysts, contaminated water or produce with oocysts, unpasteurized dairy products, and iatrogenic routes such as blood transfusion or organ transplantation [3]. Infection may be asymptomatic and self-limiting in immunocompetent individuals, while immunocompromised individuals, pregnant women, and fetuses are at risk of severe consequences including encephalitis, chorioretinitis, stillbirth, and congenital neurological sequelae [4]. The World Health Organization (WHO) classifies toxoplasmosis as a neglected tropical disease, due to its associated significant socioeconomic burdens, high diagnostic costs, the need for lifelong medical management of congenital cases, and productivity losses [5]. Seroprevalence defined as the proportion of individuals in a population who have detectable antibodies (IgG and/or IgM) in their blood serum, indicating past or current exposure to the pathogen. And exhibits distinct geographical variation, influenced by public health infrastructure, dietary practices, and climate. Tropical humid regions show seropositivity rates exceeding 60%, while arid regions show lower prevalence (10-25%) due to reduced environmental oocyst viability [6]. In North Africa, the epidemiological status of toxoplasmosis remains variable and dynamic. A recent meta-analysis encompassing countries of the Eastern Mediterranean Region found a pooled seroprevalence among pregnant women of 36.5% (95% confidence intervals (CI) : 32.6–40.4). In Libya specifically, recorded rates ranged from 26.9% in Al Bayda city to 50.8% in Tripoli [7]. This gradient correlates with climatic variations: coastal areas (high humidity, rainfall) promote oocyst formation and environmental persistence (>1 year), while the arid ecosystems in southern Libya likely suppress transmission [8, 9]. Risk factors identified in Libyan cohorts have included dietary practices such as consuming unwashed vegetables (Odds Ratio [OR]: 1.76), consuming raw/undercooked meat (OR: 1.75). Additionally, animal contact related to cat ownership (OR: 1.71) and soil exposure. Socioeconomic factors have also included rural residence (OR: 1.48) and limited disease awareness [7]. In contrast to data availability in coastal areas, western regions such as Gharyan municipality suffer from a scarcity of data on the parasite's seroprevalence. Gharyan's semi-arid climate (low rainfall, low humidity) theoretically limits oocyst survival, potentially creating a distinct transmission environment compared to coastal cities. Furthermore, no integrated studies have simultaneously examined high-risk groups—blood donors, hemodialysis patients, and pregnant women—within this region, obscuring targeted intervention needs.

1.1 Aims:

This study aims to determine immunoglobulin G IgG/ immunoglobulin M IgM seroprevalence of *T. gondii* among blood donors, hemodialysis patients, and pregnant women in Gharyan and identify region-specific risk factors using structured questionnaires assessing dietary habits, animal exposures, and socioeconomic variables. It further compares seroepidemiological profiles with Libyan coastal cities to evaluate climatic effects and develop evidence-based screening and prevention guidelines for healthcare facilities in arid regions. Otherwise, the study aimed to compare the seroprevalence of *T. gondii* between 2022 and 2023.

2. Methodology:

2.1 study design

A cross-sectional study was conducted from October 2022 to March 2023 at Gharyan Central Hospital and Al-Yusr Laboratory, Gharyan, Libya. This design enabled the simultaneous assessment of *T. gondii* seroprevalence and risk factors across three high-risk groups. The arid climate of Gharyan municipality (average humidity: 35%; annual rainfall: 150mm) was compared with the climate of Libyan coastal regions. A written informed consent was obtained from all participants.

2.2 study populations and sampling strategy





Three distinct groups were recruited. First, blood donors (n = 45): healthy male volunteers aged 20-50 years, confirmed negative for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) by standard screening protocols. Individuals with any history of immunosuppressive therapy were excluded. Second, hemodialysis patients (n = 40): patients diagnosed with End-Stage Renal Disease (ESRD) undergoing maintenance hemodialysis for at least three months (≥ 3 months). Third, pregnant women (n = 146): singleton pregnant individuals with gestational ages between 8 and 36 weeks, recruited during routine prenatal care visits at Al-Yusr Laboratory.

2.3 Data Collection and Procedures

A questionnaire adapted from Mahmoud et al. [10]. was prepared in Arabic and administered via interview during blood collection to minimize recall bias. It was later translated into English for manuscript preparation. The questionnaire was divided into two sections. First, the demographic characteristics section which included age, place of residence (urban/rural), and educational level. Second, the exposure risk section which focused on dietary habits (undercooked meat, unpasteurized dairy products, unwashed agricultural produce), animal contact (cat ownership, soil exposure through gardening/farming), medical history (prior blood transfusion, organ transplant), and clinical awareness (knowledge of toxoplasmosis transmission/prevention).

2.4 Biological Sample Collection and Serology

Participants were enrolled via systematic random sampling (every third eligible patient/donor on recruitment days). Approximately 5 ml of venous blood was drawn from each subject into a sterile plain tube. The sample was left at room temperature, centrifuged at 3000 rpm for 5 minutes, and the separated serum was stored at 4°C until analysis. Anti-*T. gondii* IgG/IgM antibodies were measured using an electrochemiluminescence immunoassay (ECLIA) on a Snibe Maglumi™ 800 analyzer (Snibe Diagnostics, Shenzhen, China).

2.5 Statistical Analysis:

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, New York). Descriptive statistics were used to calculate prevalence rates (seroprevalence) for each study group and the distribution of demographic (age, sex, education) and risk factors. Proportions were compared using Fisher's exact test to compare infection rates between blood donors (15.5%) vs. hemodialysis patients (40%) vs. pregnant women (24.6%) and subgroups (infected vs. non-infected based on risk factors). Risk factor analysis was conducted by calculating (OR) and (CI) with 95% to determine the strength of association between each risk factor and infection. Statistical significance was set at $P < 0.05$.

3.Results

Seroprevalence results for *T. gondii* were evaluated among the 231 participants, comprising 45 blood donors, 40 hemodialysis patients, and 146 pregnant women. The overall seroprevalence differed significantly between groups, with hemodialysis patients showing the highest infection rate.

3.1 Age-Specific Seroprevalence in Pregnant Women

Within the pregnant women group for *T. gondii* infections in year 2023 (n=85), notable variation in seroprevalence was observed across different age categories (Table 1). The highest infection rate was found in the 25-30 years' age group (37.0%, 10/27), which was statistically significant ($\chi^2=6.24$; $p=0.03$) compared to other age groups.



**Table 1: Antibody Distribution by Age Group among Pregnant Women in Gharyan, Libya (2023, n=85)**

Age Group (Years)	n	IgG+/IgM+	IgG+/IgM-	IgG-/IgM+	IgG-/IgM-	Seropositive n (%)
19-24	14	0	3	0	11	3 (21.4)
25-30	27	1	8	1	17	10 (37.0)*
31-36	22	1	2	0	19	3 (13.6)
37-42	15	0	3	0	12	3 (20.0)
43-48	7	0	1	0	6	1 (14.3)
Total	85	2	17	1	65	20 (23.5)

*Peak seropositivity in 25–30 age group ($\chi^2=6.24$; $p=0.03$)

3.2 Seroprevalence across Study Populations

As detailed in Table 2, seroprevalence was highest among hemodialysis patients at 40.0% (16/40), which was significantly higher than among blood donors at 15.6% (7/45) ($P < 0.001$). Among pregnant women, the overall seroprevalence was 24.7% (36/146). Upon analyzing the pregnant group by enrollment year, seroprevalence was 26.2% (16/61) in 2022 and 23.5% (20/85) in 2023, with no statistically significant difference between both years ($P = 0.68$).

Active infection, indicated by the presence of IgM antibodies, was uncommon across the study population. The overall IgM positivity rate was 1.4%, and these cases were detected exclusively within the pregnant women group.

Table 2: Seroprevalence of T. gondii IgG and IgM Antibodies among Blood Donors, Hemodialysis Patients, and Pregnant Women in Gharyan, Libya

Population	n	IgG+ (%)	IgM+ (%)	IgG+/IgM+ (%)	Overall Seropositive (%)
Blood Donors	45	7 (15.6)	0 (0.0)	0 (0.0)	7 (15.6)
Hemodialysis Patients	40	16 (40.0)	0 (0.0)	0 (0.0)	16 (40.0)
Pregnant Women (2023)	85	17 (20.0)	1 (1.2)	2 (2.4)	20 (23.5)





Population	n	IgG+ (%)	IgM+ (%)	IgG+/IgM+ (%)	Overall Seropositive (%)
Pregnant Women (2022)	61	12 (19.7)	0 (0.0)	4 (6.6)	16 (26.2)
Total Pregnant Women	146	29 (19.9)	1 (0.7)	6 (4.1)	36 (24.7)

Note: Total n for pregnant women = 146 (61 in 2022 + 85 in 2023). The overall seropositive percentage (24.7%) is calculated as 36/146.

3.3 Risk Factor Analysis for Seropositivity in Pregnant Women

Multivariate regression analysis was conducted to identify independent risk factors associated with *T. gondii* seropositivity among pregnant women (n=146). Results are summarized in Table 3

Table 3: Adjusted Odds Ratios for Seropositivity in Pregnant Women (n=146)

Risk Factor	aOR	95% CI	P-value
Consumption of unwashed vegetables	3.42	1.98–5.91	<0.001
Undercooked meat intake	3.15	1.82–5.45	<0.001
Cat ownership	2.87	1.63–5.05	<0.001
Rural residence	2.05	1.18–3.57	0.011
Soil contact	1.32	0.76–2.29	0.327

4. Discussion

4.1 Climatic Modulation of Seroprevalence

This study provides crucial insights into the seroepidemiology of *Toxoplasma gondii* in a semi-arid region of western Libya, revealing distinct patterns of infection prevalence and associated risk factors among high-risk groups.

The 24.7% seroprevalence among pregnant women in Gharyan aligns with findings from other dry Libyan cities such as Al Bayda (26.9%) and Sebha (25.9%) [8]. However, it is significantly lower than rates reported in humid coastal cities like Tripoli (50.8%; $\chi^2=22.1$, $p<0.001$) and Al Khums (39.3%; $\chi^2=7.9$, $p=0.005$) [7,9]. Based on the clear data gradient, the arid climate environment appears to suppress *T. gondii* transmission. The low humidity and scarce rainfall in Gharyan likely hinder oocyst formation and substantially reduce their environmental viability. This is consistent with global models showing a strong positive relationship between seroprevalence and rainfall [11].

4.2 Vulnerability of High-Risk Groups

The high seroprevalence of 40.0% among hemodialysis patients aligns with studies from other countries, such as Egypt, where seroprevalence among hemodialysis patients reached 44.6% [12]. This indicates that immunosuppression overrides the protective effect of the dry climate. The uremic state in end-stage renal disease patients impairs cellular immunity, which may facilitate reactivation of latent infection, a





phenomenon well-documented in immunosuppressed individuals [5]. This underscores the necessity for comprehensive serological screening for patients initiating hemodialysis, as recommended in other high-prevalence settings.

In contrast, blood donors in Gharyan showed lower seroprevalence (15.6%), comparable to healthy populations in other arid areas such as parts of Saudi Arabia [13]. Despite lower prevalence, the risk of transfusion-transmitted infection remains critical, as a single IgG-positive donor could infect multiple immunosuppressed recipients.

4.3 Age and Dietary Risk Dynamics

The peak seropositivity in pregnant women aged 25–30 years (37.0%) is a common observation in seroepidemiological studies, possibly reflecting increased domestic exposures and cumulative environmental risks over time. Similar age-specific peaks have been noted in studies from Brazil and France [14].

Our multivariate analysis highlighted dietary risks as predominant, with unwashed vegetables (aOR=3.42) and undercooked meat (aOR=3.15) showing the strongest associations. This aligns with global meta-analyses identifying undercooked meat consumption as a major risk factor [15]. Interestingly, while cat ownership was a significant risk (aOR=2.87), its effect was secondary to dietary habits. This contrasts with some temperate and humid region studies where cat or soil contact often shows higher attributable risk [16]. The explanation may lie in transmission ecology: in arid regions like Gharyan, high temperatures and low humidity rapidly inactivate oocysts [17]. Thus, while cat ownership remains a risk, ingestion of tissue cysts from improperly prepared food appears to become the primary transmission route, elevating the importance of dietary factors.

4.4 Temporal Stability of Seroprevalence

No statistically significant difference in seroprevalence was observed among pregnant women between 2022 (26.2%) and 2023 (23.5%) ($P = 0.68$). This temporal stability suggests that transmission dynamics in this arid region remain relatively constant over short time periods, reinforcing the role of stable environmental and behavioral factors rather than transient outbreaks.

4.5 Study Strengths and Limitations

A key advantage of this study lies in its comparative, multi-group design, which simultaneously captures infection dynamics across populations with varying immune competence and exposure risks. The use of a standardized ECLIA serological platform and a validated Arabic-language questionnaire strengthened data reliability and allowed for robust multivariate analysis. Additionally, the study contributes critical baseline data from a previously understudied region, enabling more nuanced comparisons with national and regional seroprevalence patterns.

However, several limitations must be acknowledged. The cross-sectional design precludes the establishment of causal relationships between risk factors and infection. The relatively small sample sizes for certain subgroups, particularly hemodialysis patients ($n=40$) and blood donors ($n=45$), may limit the statistical power and generalizability of subgroup comparisons. Furthermore, the study did not perform genotyping of *T. gondii* strains, which could have provided insights into transmission sources. Social desirability bias may also have influenced self-reported dietary and behavioral data, potentially underestimating certain risk exposures.





4.6 Benchmarking Against Regional Studies

Table 4: Benchmarking Against Regional Studies

City (Region)	Population	Seroprevalence (%)	Key Risk Factor (aOR)	Climate
Gharyan (Arid)	Pregnant women	24.7	Unwashed vegetables (3.42)	Low humidity
Tripoli (Coastal)	Pregnant women	50.8	Cat ownership (1.71)	High humidity
Alkhoms (Coastal)	Pregnant women	39.3	Rural residence (1.48)	Moderate humidity
Sabha (Desert)	Pregnant women	25.9*	Not reported	Arid

5. Conclusion

This first integrated seroepidemiological analysis of *Toxoplasma gondii* among blood donors, hemodialysis patients, and pregnant women in Gharyan confirms that the region's semi-arid climate is associated with lower seroprevalence (24.7% in pregnant women) compared to humid coastal Libyan cities (50.8% in Tripoli). Dietary practices—specifically consumption of unwashed vegetables (aOR=3.42) and undercooked meat (aOR=3.15)—emerged as the dominant modifiable risk factors, outweighing cat ownership and soil contact in this dry environment. No significant difference in seroprevalence was observed between 2022 and 2023.

Based on these findings, the study recommends: (1) routine first-trimester serological screening for all pregnant women, particularly those aged 25–30 years; (2) mandatory pre-dialysis and annual *T. gondii* testing for hemodialysis patients; and (3) targeted food safety education campaigns emphasizing thorough washing of produce and proper cooking of meat. Future research should incorporate molecular genotyping and larger sample sizes to further refine evidence-based prevention strategies in arid regions.

ETHICAL APPROVAL:

This study was approved by the institutional Review Board of Gharyian University (Approval No. IRB/2022/201) on June 15, 2022.

CONFLICT OF INTEREST:

The authors declare no conflict of interest

AUTHORS' CONTRIBUTION:

The first author contributed in theoretical paper writing; work on data design and collection, while the second author contributed in methodology, revising the manuscript as well as approved the paper for publication.





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