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Saudi Journal of Health Research and Practice

المجلة السعودية للبحوث والممارسة الصحية

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Historical background and Objectives

The *Saudi Journal of Health Research and Practice (SJHRP)* was established to address the growing demand for a platform dedicated to publishing high-quality, evidence-based health research in Saudi Arabia and beyond. As healthcare in the region undergoes rapid transformation in line with Vision 2030, the journal aims to bridge the gap between clinical practice and research. The goal is to empower healthcare professionals, educators, and researchers by providing a venue for sharing innovative findings and insights that address local and global health challenges.

By amplifying regional voices, the SJHRP aspires to contribute to the global health dialogue while ensuring that the unique perspectives and priorities of the region are represented.

Inaugural Context

The launch of SJHRP comes at a pivotal moment in Saudi Arabia's healthcare evolution, closely aligned with Vision 2030's objectives of healthcare transformation and research innovation. The journal aims to support the development of evidence-based policies and practices by providing a platform for cutting-edge research. This issue is a celebration of the collaborative spirit and intellectual growth science, emphasizing the role of research in driving sustainable health outcomes.

Theme of the Issue

The inaugural issue of the SJHRP centers around **"Advancing Healthcare Research in Saudi Arabia"**, showcasing a variety of articles that explore groundbreaking studies, training innovations, and critical reviews. These include studies on nephrotoxicity mitigation, gynecological ultrasonography training, program evaluation in clinical nutrition, Antiphospholipid Syndrome, and advancements in neonatal hyperbilirubinemia management. This thematic focus highlights the journal's commitment to evidence-based healthcare and its alignment with national priorities

Message from Leadership
A Message from the President

On behalf of Jazan University, I am proud to introduce the inaugural issue of the *Saudi Journal of Health Research and Practice (SJHRP)*. This journal is a testament to our university's unwavering commitment to advancing scientific research and addressing critical healthcare challenges.

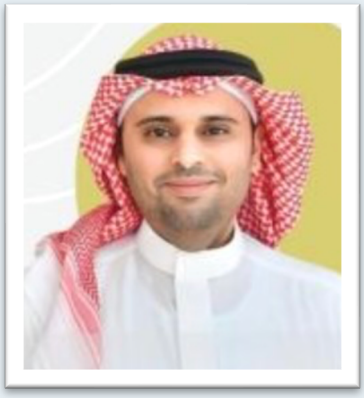
The SJHRP represents a milestone in our vision to foster innovation, collaboration, and excellence in health research. By creating this platform, we aim to promote high-quality research that not only serves the needs of our region but also contributes to the global body of scientific knowledge.

I would like to express my heartfelt gratitude to the editorial board, authors, and reviewers whose dedication and expertise have made this inaugural issue possible. I also extend my sincere thanks to our faculty and leadership for their continuous support in driving research excellence at Jazan University.

We look forward to seeing this journal grow and thrive as a source of inspiration and impact in the field of healthcare research and practice.

Professor Mohammed Aburasain
President, Jazan University

A Message from the Vice President for Postgraduate and Research Affairs



It is with great pleasure that I welcome you to the first issue of the *Saudi Journal of Health Research and Practice (SJHRP)*, a new and significant initiative by Jazan University to promote excellence in health research and evidence-based practice.

This journal reflects our dedication to advancing research that addresses pressing healthcare issues and improving the quality of life for communities, both locally and globally. By supporting interdisciplinary collaboration and fostering innovation, SJHRP serves as a vital platform for researchers and practitioners to share their insights and findings.

I would like to acknowledge the hard work and commitment of the journal's editorial board, the authors who have contributed to this issue, and the reviewers who have ensured the quality of the publications. Special thanks go to the leadership of Jazan University for their unwavering support in making this journal a reality.

As we move forward, I am confident that SJHRP will become a leading voice in healthcare research, setting new benchmarks for quality and impact.

Dr. Abdulkarim Muraea
Vice President for Postgraduate and Research Affairs

Editorial Message

Welcome to the First Issue of the Saudi Journal of Health Research and Practice

It is with great pride and excitement that I present to you the inaugural issue of the *Saudi Journal of Health Research and Practice (SJHRP)*. This journal marks a significant milestone in Jazan University's mission to advance scientific research and foster innovation in healthcare.

As healthcare evolves, the need for high-quality research and evidence-based practice becomes ever more critical. Our journal is dedicated to serving as a platform for the dissemination of impactful research that addresses current and emerging challenges in healthcare. By publishing articles that are both locally relevant and globally significant, we aim to contribute to the advancement of medical science and improve patient outcomes.

In this first issue, you will find a diverse range of topics, from experimental pharmacological studies to medical education and clinical case reports. This diversity reflects the journal's interdisciplinary scope and commitment to supporting researchers from various healthcare fields.

I would like to express my gratitude to the authors who entrusted us with their work, the reviewers who provided valuable insights, and the editorial board who dedicated their time and expertise to making this issue a reality. A special thanks to the leadership of Jazan University for their unwavering support in bringing this vision to life.

We look forward to building upon this foundation and establishing SJHRP as a reputable journal that inspires innovation and collaboration in health research and practice.

Sincerely,

Professor Hussein M. Ageely
Editor-in-Chief
Saudi Journal of Health Research and Practice

About the Journal

The *Saudi Journal of Health Research and Practice (SJHRP)* is a peer-reviewed, open-access journal dedicated to publishing high-quality research that advances healthcare and evidence-based practice. The journal serves as a platform for researchers, clinicians, and academics to share knowledge and insights across a wide range of healthcare disciplines.

Journal Mission:

- To promote innovation and excellence in health research.
- To provide a platform for interdisciplinary collaboration.
- To address pressing health challenges both locally and globally.

Scope:

This journal covers all topics related to all aspects of health issues and healthcare research. Basic medical research with clear clinical implications will also be considered. Research fields of interest include but are not limited to:

- Public Health and Epidemiology
- Health Promotion and Disease Prevention
- Clinical Medicine Across All Specialties (including all clinical medical , dental and other clinical specialties and subspecialities)
- Pharmaceutical Research and Development
- Biomedical Sciences and Technology
- Mental Health and Behavioural Science
- Environmental and Occupational Health
- Quality of Care and Patient Safety
- Health Informatics and Digital Transformation
- Health Education and Behavioral Science
- Health Economics and Policy Research

Key Features:

- **Open Access:** Ensures free and unrestricted access to research for all readers.
- **Double-Anonymous Peer Review:** Guarantees a rigorous and unbiased review process.
- **Interdisciplinary Focus:** Publishes research from a variety of healthcare fields to encourage collaboration and innovation.

For more information, visit the journal's website at:
https://journals.jazanu.edu.sa/ojs/index.php/SJHR/index.

Announcements and Key Features

Announcements:

- SJHRP is currently accepting submissions for its next issue. Researchers are encouraged to submit their work via the journal's online portal.
- Plans for indexing SJHRP in major databases such as Scopus and PubMed are underway.

Key Features:

- **Open Access:** All published articles are freely accessible to readers worldwide.
- **Double-Anonymous Peer Review:** Ensures impartial and rigorous evaluation of submissions.
- **High Standards:** Committed to publishing impactful, evidence-based research.

Acknowledgments

The launch of the *Saudi Journal of Health Research and Practice (SJHRP)* is the result of the collaborative efforts and unwavering support of numerous individuals and institutions.

We extend our deepest gratitude to:

- **H.E. Professor Mohammed Aburasain**, President of Jazan University, for his visionary leadership.
- **Dr. Abdulkarim Muraea**, Vice President for Postgraduate and Research Affairs, for his guidance and dedication to advancing research at the university.
- **The Journal's Editorial Office**, for their tireless efforts in managing submissions and ensuring the journal's quality.
- **The Editorial Board Members**, whose expertise and commitment have been instrumental in shaping this journal.

Your contributions have made this milestone possible, and we look forward to your continued support as we advance together.

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Original Article

Barbeya Oleoides Leaves Extract Mitigates Acetaminophen- Induced Nephrotoxicity by Reducing Oxidative Stress and Inflammation in a Rat Model

Abdulmajeed M. Jali [1]*, Mohammed Firoz Alam [1], Sulaiman Sayyar [1], Faroq Kamli [1], Ali Zarban [1], Ali Hanbashi [1]

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ABSTRACT Acetaminophen is widely used as an analgesic and antipyretic, but its potential for nephrotoxicity, particularly in overdose situations, remains a significant concern. Identifying agents that can mitigate this nephrotoxicity is crucial for kidney protection. Barbeya oleoides Schweinfurth (BOL), known for its antioxidant and anti-inflammatory properties, may offer protective effects against acetaminophen-induced kidney injury. This study investigates the nephroprotective potential of an ethanolic extract from BOL leaves against acetaminophen-induced nephrotoxicity. Five groups of rats (n = 6 per group) were treated orally for seven days: Group 1 received a placebo solution (vehicle), Group 2 received a single acetaminophen dose (2 g/kg) on day 5, Groups 3 and 4 received BOL at doses of 100 or 200 mg/kg, respectively, along with acetaminophen on day 5, and Group 5 received only BOL (200 mg/kg). Acetaminophen significantly increased the level of the kidney function biomarkers blood urea nitrogen (BUN), triglycerides (TG), uric acid (UA), and creatinine, indicating renal dysfunction. It also elevated pro-inflammatory cytokines, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), highlighting an inflammatory response. Furthermore, a notable reduction in antioxidant enzyme levels, namely glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), was observed, signifying oxidative stress and impaired antioxidant defense. Histopathological examination showed disruption of the glomerular basement membrane, confirming renal tissue damage. BOL co-treatment restored kidney function biomarkers and antioxidant levels, reduced cytokine levels, and preserved renal tissue structure suggesting improved oxidative balance. In conclusion, BOL leaves extract demonstrated significant nephroprotective effects against acetaminophen-induced nephrotoxicity by reducing oxidative stress and inflammation, highlighting its potential as an adjunct therapy to prevent acetaminophen-induced kidney injury.

Keywords: Acetaminophen; Nephrotoxicity; Antioxidants; Antiinflammation; Nephroprotective; Barbeya Oleoides Schweinfurth.

INTRODUCTION

The kidneys are crucial in sustaining body homeostasis and regulating multiple physiological processes. They are involved in blood pressure management, acid-base balance, erythropoiesis, and elimination of metabolic waste products [1,2]. In addition to their essential role in drug clearance, recent advances in research have revealed that they are involved in drug metabolism for certain medications such as morphine, and acetaminophen [3–5]. Therefore, maintaining normal kidney functions while consuming medications is essential.

Acetaminophen (APAP) was discovered and introduced to the market in the late nineteenth century. Since then, it has been available as an over-the-counter medication and one of the most commonly utilized analgesics. It is broadly used by the elderly, adults, kids and infants. Also, it is considered a first-line treatment for fever, acute and many chronic pain cases and pregnancy [6]. Nevertheless, the risk of overdosing on APAP persists due to its widespread availability and easy access. Alarmingly, APAP overdose is one primary cause of hospital admission for hepatotoxicity and nephrotoxicity [7]. Although APAP has been used for over a century as an analgesic, its mechanism of action is still poorly understood. An early study by Flower and Vane (1972) showed that APAP analgesic activity is mediated by inhibiting central prostaglandin E2 synthesis [8]. Several in-vivo and in-vitro investigations followed and pointed out that APAP interacts with a central cyclooxygenase enzyme that is responsible for prostaglandin E2 synthesis [6], which was then confirmed in 2002 by Simmons' laboratory [9]. This central cyclooxygenase enzyme was named COX 3.

Research conducted over four decades ago demonstrated that APAP primarily metabolizes through phase II reactions specifically via sulfation and glucuronidation. However, a minor oxidation metabolic pathway of APAP is also involved. Microsomal cytochrome P450 (CYP) enzymes oxidize APAP to form the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [10–13]. Upon interaction with hepatic or extrahepatic tissues, NAPQI causes cellular damage and eventually cell death [14–16]. Numerous investigations have demonstrated that NAPQI substantially contributes to the APAP toxic effects on body organs such as the kidney [10]. Results of these studies showed that NAPQI metabolic pathways such as prostaglandin endoperoxide, N-deacetylase [17–21], renal CYP-450 microsomal enzymes [22–24] and glutathione S-conjugate [17] contribute to APAP's renal toxicity. These pathways are activated by consuming large doses or chronic use of APAP and concurrent alcoholism, causing nephrotoxicity consequent to hepatotoxicity [10]. Nonetheless, a study by Trumper et al., 1992 showed that a highly toxic dose of APAP causes renal toxicity with or without concurrent hepatoxicity [25]. Regardless of the activated metabolic pathway, renal toxicity is one major side effect of APAP. Thus, since APAP is easily accessible and widely used by individuals, it is crucial to develop medications that could serve as therapeutics or prophylactics against APAP-induced renal toxicity.

Given the potential for nephrotoxicity associated with APAP, developing protective agents is essential. Various antioxidant-rich plants, such as Nigella Sative [47], Carica papaya [54] and Cinnamomum zeylanicum L. [55] Curcuma longa [70], and Camellia sinensis [71], have demonstrated nephroprotective effects by reducing oxidative stress and inflammation. Although *Barbeya Oleoides Schweinfurth* (BOL) shares common antioxidant properties with these plants, it remains underexplored for its potential against APAP-induced kidney damage.

BOL is a medicinal plant that is native to North Africa, Ethiopia, Somalia, and the Arabian Peninsula (Saudi Arabia and Yemen) [26]. Leaves, stems and roots of BOL have been empirically used to treat fever, infectious diseases, skin disorders, and inflammatory-related disorders [27,28]. This could be due to BOL wide range of bioactive compounds with several pharmacological properties (Figure 1). For instance, BOL's leaves contain flavonoids and phenols, which are known for their antioxidant effects [29]. Moreover, it has been demonstrated that BOL extracts can mitigate inflammation by decreasing the inflammatory mediators' levels [28]. In his characterization study of BOL, Al-Oqail showed that different parts of BOL, including leaves, exhibit antimicrobial and antispasmodic activities [29]. Recently, Khoja et al., 2021 reported that extracts from BOL leaves may offer therapeutic benefits for type 2 diabetes mellitus by inhibiting the enzymes α-glucosidase and α-amylase. This inhibitory effect was primarily attributed to the presence of polyphenolic compounds within the extract, known for their significant bioactive properties [72]. These properties have sparked interest in investigating the nephroprotective effects of BOL's leaves. This interest stems from the potential of BOL leaves' bioactive compounds to counter the associated alterations in kidney injury such as oxidative stress and inflammation. The availability of diverse pharmacotherapy options to prevent harmful effects on the kidney is highly beneficial for individuals. Nevertheless, to our knowledge, no previous literature has studied the nephroprotective effects of BOL leaves extract against kidney injury caused by APAP. Therefore, this study aims to evaluate the potential nephroprotective effects of extract from BOL's leaves by exploring its anti-inflammatory, antioxidant, and other possible protective mechanisms against APAP-induced kidney injury in rats.

List of abberviations

Full name	Abbreviation
Acetaminophen	APAP
Barbeya oleoides	BOL
Blood Urea Nitrogen	BUN
Catalase	CAT
Glutathione	GSH
Interleukin-1 beta	IL-1β
Lipid Peroxidation	LPO
Malondialdehyde	MDA
Superoxide Dismutase	SOD
Triglycerides	TG
Tumor Necrosis Factor-alpha	TNF-α
Uric Acid	UA
Acetaminophen	APAP
cytochrome P450	CYP-450
N-acetyl-p-benzoquinone imine	NAPQI

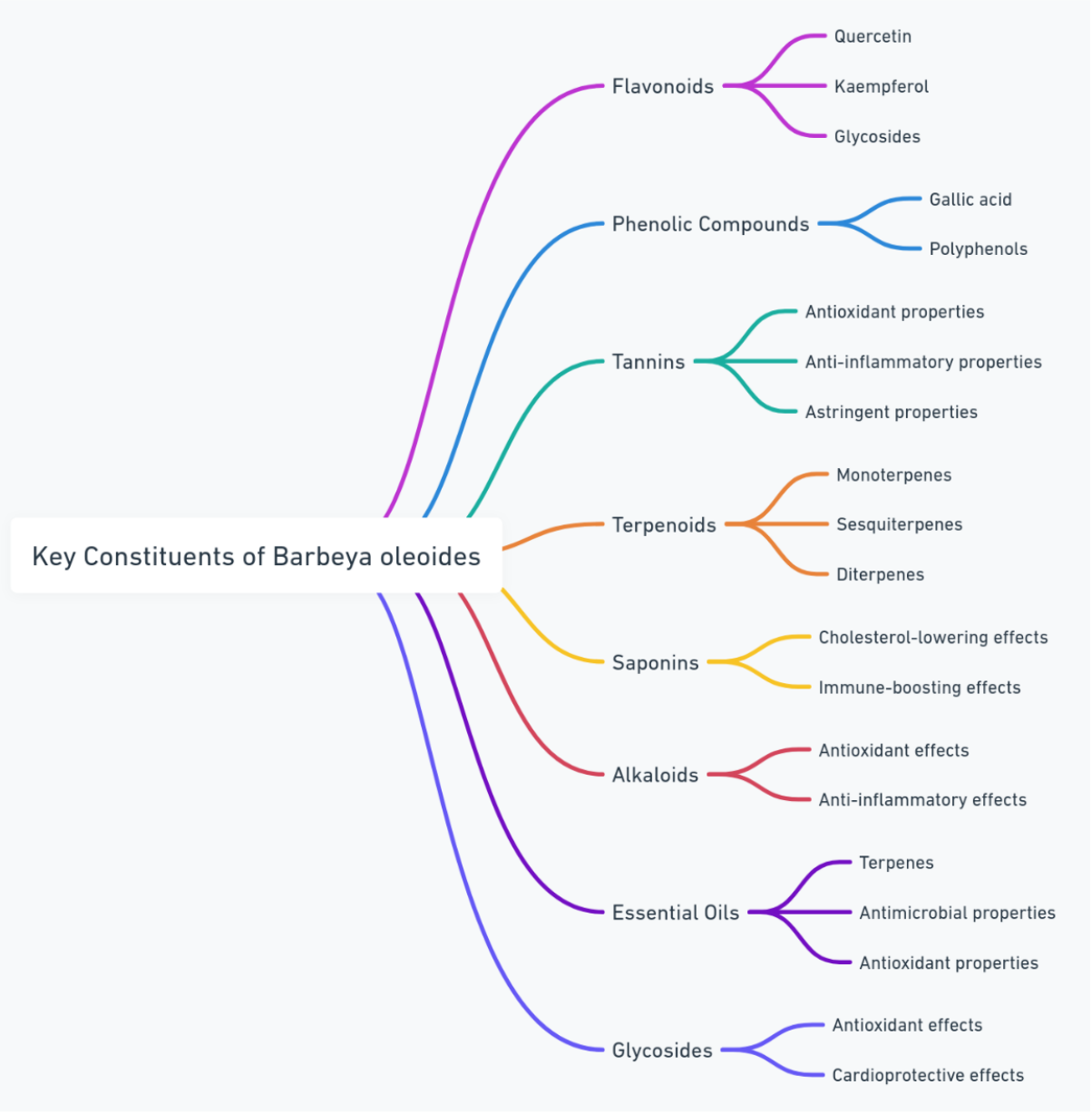

Figure 1. Key constituents of Barbeya oleoides and their associated biological activities. This figure illustrates various phytochemicals found in Barbeya oleoides, including flavonoids [30] (e.g., quercetin, kaempferol, glycosides), phenolic compounds [31] (e.g., gallic acid, polyphenols), tannins [31], terpenoids [32] (e.g., monoterpenes, sesquiterpenes, diterpenes), saponins [33], alkaloids [34], essential oils [32], and glycosides [30]. The content of this figure has been adapted from several previous publications, with specific adaptations to integrate and highlight the therapeutic relevance of each constituent.

MATERIALS AND METHODS

1.1. Drugs and Chemicals

D6908 M-13 DNA Isolation Maxi Kit Extraction kits were bought from Solarbio, Tongzhou Dist, Beijing in China. Ethidium bromide, 6X DNA loading dye, TBE buffer and agarose were used for DNA sample preparation, gel formation and electrophoresis applications. Sodium phosphate dibasic and potassium phosphate monobasic solutions were used to prepare phosphate buffer for sample preparation. Dintrobenzoic acid, sulfosalicylic acid and Phosphate buffer were used for Spectrophotometer applications. All the ingredients were sourced from Sigma Aldrich USA.

1.2. Plant Collection and Preparation

Plant Collection: *Barberiya oleiadi* leaves were collected from the Fifa mountains, located in the Jazan territory, southwest of Saudi Arabia. Extraction Process: Collected plants rinsed with water and then placed in a sheltered area to dry. The resulting powder (500g) was extracted using percolation with 95% ethanol for 72 hours. The ethanol was eliminated using a rotary evaporator to extract the pure crude stock. These plant extracts were further used in this experiment.

1.3. Animal Model and Study Design

Male Wistar albino rats weighing 150-180 g were used for this study. The rodents were maintained in an ideal laboratory condition, with unrestricted water access and a daily pellet meal. This project was ethically approved by the ethical committee of Jazan University with reference number REC-45/07/943. The study design and animal numbers were planned using the principles of the 3Rs (Replacement, Reduction, and Refinement) and the Experimental Design Assistant (EDA) tool. The EDA ensured that the minimum number of animals required for robust and reproducible results was used. Five groups, each comprising six rats, were randomly assigned 30 male Wistar albino rats. Group 1 received vehicle orally (p.o.) only for 7 days, serving as the control group; group 2 received a single dose of APAP 2 g/kg p.o. on the fifth day. The toxic APAP dose was previously established in our laboratory [49]. Groups 3 and 4 were given BOL 100 or 200 mg/kg p.o.daily for seven days. A single oral dose of APAP 2 g/kg was given only once on the fifth day one hour after BOL doses in both groups. In group 5 200 mg/kg of BOL was administered p.o. once daily for seven days. On the eighth day, rats were sedated and blood was drawn for biochemical analysis. After that, animals were sacrificed to isolate the kidney for further biochemical and histopathological assay (Figure 2).

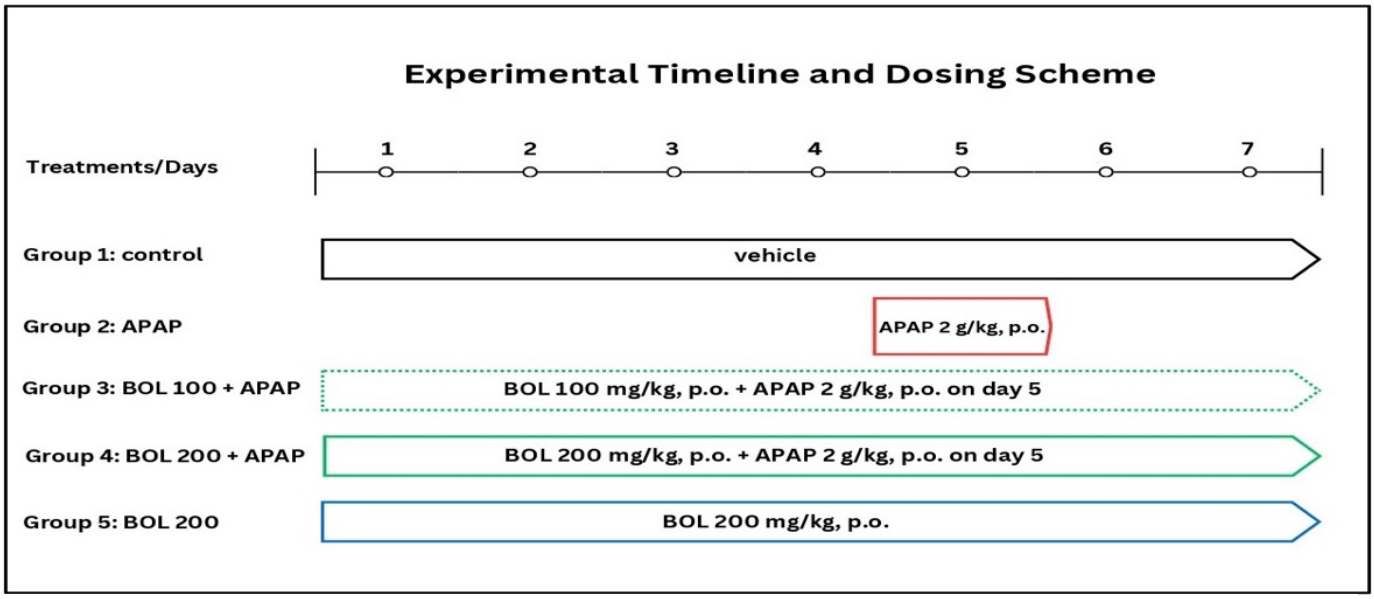

Figure 2. Experimental Timeline and Dosing Scheme: The figure illustrates the experimental timeline and dosing scheme for five groups of rats over seven days. Each group's treatment regimen is represented by color-coded arrows indicating the treatments administered.

1.4. Sample Preparation

The collected blood samples were allowed to coagulate at room temperature without being disturbed for 30 minutes. After that, the sample was centrifuged at 3000 rpm for 10 minutes at 4°C. Finally, the upper layer called Serum was collected for kidney function tests.

Kidney sample preparation was performed as established protocol from our laboratory by Alam et al., 2022 [35]. Briefly, 10% of the kidney homogenate was prepared by homogenizing it in a phosphate-buffered solution (0.1 M and pH 7.4) for oxidative stress markers (GSH, CAT, SOD, and MDA) test. Subsequently, the homogenized material was centrifugated at 800× g for 15 minutes at 4°C to isolate the supernatant. This supernatant is further used for MDA, CAT, and SOD test. The remaining homogenate was subjected to centrifugation at 10,500× g for 15 minutes at 4 °C to get the PMS for the GSH test.

1.5. Blood Serum Test

To evaluate kidney function, Crescent diagnostic testing kits were used to measure uric acid, creatinine, blood urea nitrogen (BUN), and triglycerides. Each marker was analyzed following the manufacturer's standard protocol. Briefly, blood samples were collected and centrifuged at 3,000 × g for 10 minutes to separate the serum. The serum was then processed using the respective kits for each marker, following the detailed step-by-step instructions provided by the manufacturer. The absorbance of each sample was measured at specific wavelengths recommended by the Crescent diagnostic kit using a spectrophotometer, ensuring consistency and accuracy in the readings. All tests were conducted in duplicate to enhance the reliability of the results.

1.6. Protein assay: Lowry et al. (1951) estimated the protein content of each sample [36].

1.7. Antioxidant Assays:

1.7.1. Malondialdehyde (MDA)

The method described by Islam et al. (2002) [37] was employed to measure lipid peroxidation. Specifically, 0.5 mL of supernatant-1 was placed in a metabolic shaker and incubated at 37 °C for 1 hour, while an identical 0.5 mL sample of supernatant-1 was maintained at 0 °C for the same duration as a control. Following the 1-hour incubation period, 0.5 mL of 5% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) were added to each sample. The mixture was then centrifuged at 4,000 × g for 10 minutes. The resulting supernatant was collected and heated in a water bath for 10 minutes, during which a pink color developed, indicating the presence of malondialdehyde (MDA). The absorbance of the sample was measured at 535 nm, and the MDA content was expressed as the amount of MDA formed per hour per gram of protein.

1.7.2. Glutathione (GSH)

To test for glutathione (GSH), the sulfhydryl reagent DTNB) is used to react with GSH to form a yellow color TNB. The method outlined by Jollow et al., 1974 [38] were used to analyse the GSH. There was a mix of 0.5 mL PMS and 0.5 mL of 4% SSA and incubated at 4 °C for 1hr. After that, it was spun at 3,000 rpm for 15 minutes at 4 °C. At the end, 0.5 mL of the supernatant, 2 mL of PB, and 0.5 mL of DTNB were mixed to make 3 mL, and further samples were analysed at 412 nm.

1.7.3. Catalase (CAT)

Claiborne methods were employed to ascertain the catalase (CAT) [39]. The variation in absorbance was quantified at 240 nm. The catalase units were expressed in nanomole H2O2 consumed per minute per milligram of protein.

1.7.4. Superoxide Dismutase (SOD)

Marklund methods were employed to determine the activity of superoxide dismutase (SOD) at a 580 nm wavelength [40]. The SOD unit was expressed in units per mg of protein, with 1 unit representing a 50% reduction in pyrogallol autoxidation.

1.8. Inflammatory Cytokine (Elisa Assay for IL-1β)

The cytokine IL-1β was quantified using the protocol of the cytokine assay kit from MyBioSource, Inc. The measurement was performed using an ELISA microplate reader (Bio-Tech ELX800) at a 450 nm wavelength.

1.9. Inflammatory Cytokine (ELISA Assay for TNF-α)

The Rat TNFα -ELISAKit (ab236712) measured serum TNF-α protein following the manufacturer instructions. After adding samples or standards to 96-wells, an antibody mixture was added. The bores were rinsed after incubation to remove unwanted protein. A second confirmatory solution catalyzed the reaction and turned it blue. The blue tint turned yellow when a stop solution stopped the process. The amount of bound analyte determines the signal intensity at 450 nm.

1.10. Renal Histopathology

The isolated tissue samples of the kidney were washed with an ice-cold normal saline solution containing 0.9% and then fixed in 10% formalin. Further dehydration was performed, and kidney tissue was implanted in liquid paraffin before being molded into slabs. The blocks were then split into 3 to 5 µm thick segments using a microtome. After staining with H&E, the samples were viewed under a 40x microscope. The injury score ranged from 0 to 4, with 1 indicating a slight injury, 2 indicating a mild injury, and 3 and 4 indicating a severe injury. The injury score considers observations like tubular necrosis, vacuolization, and glomerulus membrane degradation [41].

1.11. Statistical Evaluation

The results were examined by GraphPad Prism 9.0. One-way ANOVA was employed to compare the means across multiple groups to evaluate the overall treatment effects, as it is suitable for analyzing data from experiments involving more than two groups. Post-hoc analysis was performed using the Tukey-Kramer test, which ensures accurate pairwise comparisons between groups, particularly when group sizes are equal. The significance level was set at $p < 0.05$ to ensure robust and reliable results. All data are presented as Mean ± SEM, with six replicates per group, ensuring precise and consistent reporting of variability within the dataset.

RESULTS

2.1. Effect of BOL on Kidney Function Markers

APAP significantly elevated ($p < 0.0001$) the tested kidney function biomarkers levels (BUN, uric acid, TG and creatinine) as opposed to the control (Figure 3). Both doses of BOL (100 and 200 mg/kg) significantly reduced ($p < 0.0001$) the APAP-induced increase in the levels of the tested parameters. Similar to the control group, BOL alone (200 mg/kg) displayed no changes in the tested kidney function marker levels.

2.2. Effect of BOL on Malondialdehyde (MDA)

APAP considerably increased ($p < 0.0001$) the MDA level as opposed to the control (Figure 4). BOL (100 mg/kg) decreased the MDA level in contrast to APAP ($p < 0.001$). Likewise, the higher dose of BOL (200 mg/kg) produced an even greater decrease in the MDA level ($p < 0.0001$). Like the control group, BOL did not affect the MDA level.

2.3. Effect of BOL on Antioxidants (GSH, CAT, and SOD)

As shown in Table 1, APAP noticeably reduced ($p < 0.0001$) the tested antioxidant enzyme levels opposite to the control. BOL (100 mg/kg) partially dampened the APAP-induced reduction in GSH ($p < 0.05$) and SOD ($p < 0.05$) levels and showed a more significant reduction in CAT levels ($p < 0.001$) in comparison to the APAP group. More profoundly, the higher dose of BOL (200 mg/kg) almost diminished the APAP-induced reduction in GSH, CAT ($p < 0.0001$), and SOD ($p < 0.001$) levels as opposed to the APAP group. BOL alone did not cause any changes in the level of the tested antioxidants ($p > 0.05$).

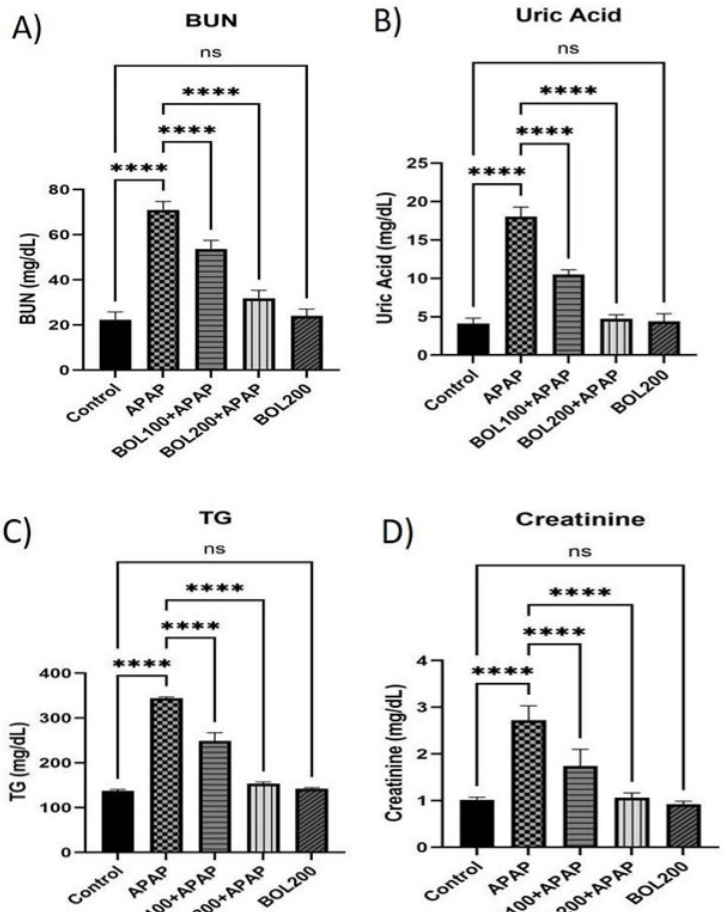

Figure 3. Effect of BOL on kidney function markers; A) Blood Nitrogen Urea (BUN), B) Uric Acid, C) triglycerides (TG) and D) Creatinine. Values are presented as Mean ± SEM (n = 6). **** $p < 0.0001$ (APAP vs. Control, BOL100 +APAP vs. APAP, and BOL200 + APAP vs. APAP) and ^{ns} $p > 0.05$ (BOL200 vs. Control) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test.

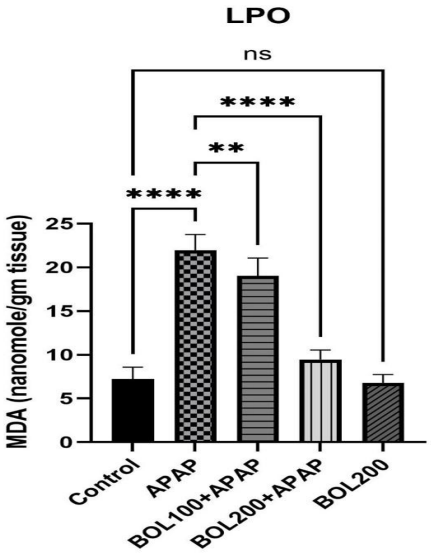

Figure 4. Effect of BOL on lipid peroxidation (LPO). Values are presented as Mean ± SEM (n = 6). **** p < 0.0001 (APAP vs. Control and BOL200 + APAP vs. APAP), ** p < 0.001 (BOL100 + APAP vs. APAP) and ns p > 0.05 (BOL200 vs. Control) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test.

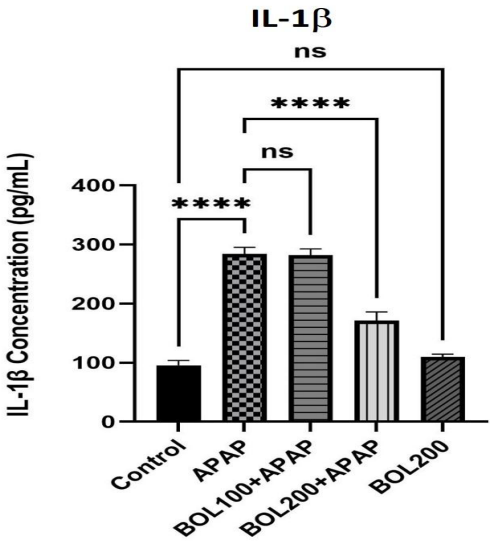

Figure 5. Effect of BOL The of IL-1β Concentration. Values are presented as Mean ± SEM (*n* = 6). **** *p* < 0.0001 (APAP vs. Control and BOL200 + APAP vs. APAP), and ns *p* > 0.05 (BOL100 + APAP vs. APAP and BOL200 vs. Control) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test.

2.4. Effect of BOL on the IL-1β Concentration

IL-1β concentration was increased by APAP treatment (p < 0.0001) (Figure 5). BOL (100 mg/kg) did not inhibit APAP-induced IL-1β increased concentration (p > 0.05). However, BOL (200 mg/kg) substantially reduced IL-1 β concentration in the kidney following APAP treatment (p < 0.0001) compared to the APAP group. Also, no significant alteration was noticed with BOL alone comparable to the control (p > 0.05).

2.5. Effect of BOL on Tumor Necrosis Factor (TNF-α) Concentration

The levels of TNF-α were elevated by APAP relative to the control group (p < 0.0001). BOL (100 mg/kg) yielded a partial but significant decrease in TNF-α levels (p < 0.001) in contrast to the APAP group (Figure 6). However, BOL (200 mg/kg) produced a remarkable decline in TNF-α levels as opposed to the APAP group (p < 0.0001). No significant difference was observed with BOL alone comparable to the control (p > 0.05).

Table 1. Effect of BOL on The Levels of Antioxidant Enzymes.

Test/Group	Control	APAP	BOL100+APAP	BOL200+APAP	BOL200
GSH (DTNB Conjugate Formed/mg Protein)	16.60±1.35	8.05±0.96***	9.72±1.05*	15.89±2.23***	17.19±1.97ns
CAT nmol of H_2O_2 consumed/min/ mg / protein	13.84±2.20	6.61±0.78***	9.76±0.59**	12.19±1.46***	14.14±1.79ns
SOD (nmol epinephrine protected from oxidation/min/ mg protein)	39.256±2.64	20.18±1.75***	19.075±2.51*	38.40±2.33**	40.72±2.88ns

Table 1. Values are presented as Mean ± SEM (*n* = 6) *** *p* < 0.0001 (APAP vs. Control) (BOL200 + APAP vs. APAP), ** *p* < 0.001 (BOL100 + APAP vs. APAP and BOL200 + APAP vs. APAP), * *p* <0.05 (BOL100 + APAP vs. APAP), ^{ns}p > 0.05 (BOL200 vs. Control) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test.

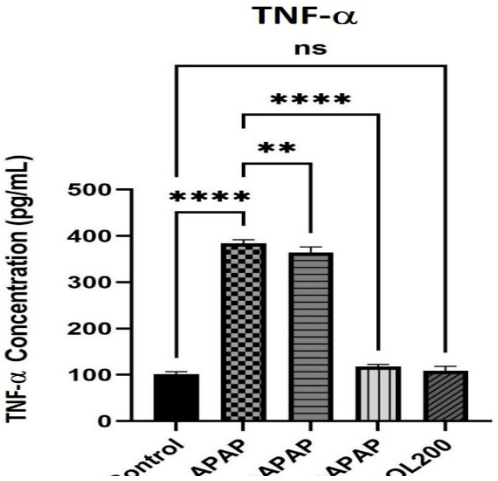

Figure 6. Effect of BOL on tumor necrosis factor (TNF-α) concentration. Values are presented as Mean ± SEM (n = 6). **** $p < 0.0001$ (APAP vs Control and BOL200+ APAP vs APAP), ** $p < 0.001$ (BOL100 + APAP vs. APAP) and ns $p > 0.05$ (BOL200 vs. Control) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test.

2.6. Effect of BOL on Renal Histology

The control and the BOL-treated groups illustrated a regular kidney architecture with healthy morphology and proper glomerular structure without any pathological lesions; the injury score was 0 (Figure 7 A & 7 E). On the contrary, the APAP group demonstrated a degenerative glomerular basement membrane as indicated by abnormal morphology with damaged glomerular intact and tubular cells; an injury score of 3 was given to the APAP group (Figure 7 B). In contrast, treatment with BOL (100 mg/kg) partially mitigated the degenerative changes in the kidney caused by APAP treatment and improved morphology and glomerular structure of the kidney, and the injury score was 2 (Figure 7 C). BOL (200 mg/kg) remarkably restored the glomerular intact and normal morphology; the injury score was 1 (Figure 7 D). The quantification of injury scores is shown in Figure 7 F.

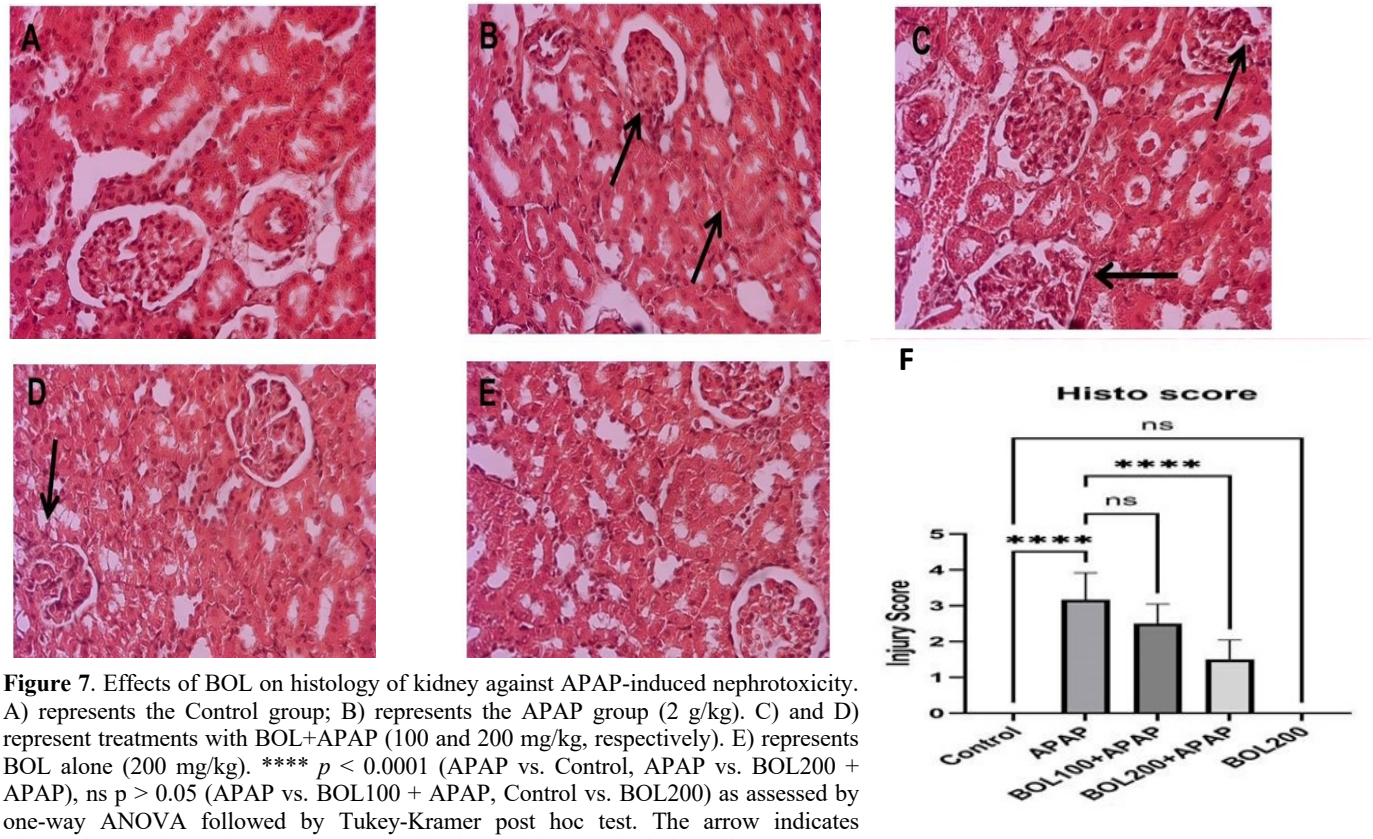

Figure 7. Effects of BOL on histology of kidney against APAP-induced nephrotoxicity. A) represents the Control group; B) represents the APAP group (2 g/kg). C) and D) represent treatments with BOL+APAP (100 and 200 mg/kg, respectively). E) represents BOL alone (200 mg/kg). **** $p < 0.0001$ (APAP vs. Control, APAP vs. BOL200 + APAP), ns $p > 0.05$ (APAP vs. BOL100 + APAP, Control vs. BOL200) as assessed by one-way ANOVA followed by Tukey-Kramer post hoc test. The arrow indicates glomerular membrane degeneration, vacuolization and damaged tubular cells.

DISCUSSION

Despite its broad use as an analgesic and antipyretic, overdosing on APAP or even chronic use presents a substantial risk of nephrotoxicity [16]. One plausible way of causing nephrotoxicity is the generation of toxic metabolite NAPQI [10]. Upon kidney injury, certain indicators are influenced such as kidney function biomarkers, antioxidant enzymes, inflammatory mediators and protein expression, as a response to the damage caused to the renal tissue [42–44]. BOL, originated in North Africa and the Arab Peninsula, is a medicinal plant used for several purposes such as fever, infections and inflammatory-related disorders mainly due to its bioactive constituents [29]. The properties of these bioactive compounds could aid in alleviating APAP's nephrotoxicity by decreasing inflammation, oxidative stress and rebalancing kidney function biomarkers [27–29]. The current study focused on exploring BOL leaves' protection against APAP-developed kidney damage in rats by studying their impact on inflammation, oxidative stress, and DNA fragmentation.

BUN, uric acid, TG and creatinine are critical indicators of kidney health status. Kidney injury resulting from a medication or disease alters the level of these indicators where elevated levels denote impaired kidney function, reflecting the severity of the renal damage [45,46]. In our study, APAP increased the level of the tested indicators suggesting comprised kidney function (Figure 3). This is consistent with previous studies that showed similar [47–49] or lower [50–52] toxic doses of APAP cause kidney damage. On the contrary, both doses of BOL attenuated the APAP-induced elevation in the level of the tested biomarkers. The antioxidant properties of the flavonoids and phenols found in BOL's leaves may be responsible for this beneficial effect. [29]. Consistently, multiple studies have investigated various medicinal plants with similar bioactive compounds that demonstrated their antioxidant effects in reducing elevated kidney function biomarkers induced by APAP. Such plants include curcumin [53], Nigella Sative [47], Carica papaya [54], and Cinnamomum zeylanicum L. [55]. It has been established that APAP oxidative stress dampens kidney performance leading to elevated serum levels of particular biomarkers, mainly as a result of impaired renal glomerular filtration [7]. Thus, counteracting oxidative stress can aid in restoring proper kidney function.

Antioxidant enzymes such as GSH, SOD and CAT are crucial in protecting renal integrity, hence, maintaining proper kidney functions. These antioxidants act like scavengers for reactive oxygen species (ROS) such as superoxide ($O2•−$) or free radicals such as hydrogen peroxide ($H2O2$). Those could emerge from a variety of sources encompassing endogenous and exogenous factors. Diseases and medications are considered exogenous sources of ROS. Elevated levels of ROS and free radicals cause oxidative stress, which damages cells and contributes to numerous diseases. Antioxidant enzymes collectively work to eventually convert ROS and free radicals into water and oxygen, thereby minimizing oxidative stress and preventing cellular damage [7,56]. As mentioned earlier, upon APAP metabolism via CYP enzymes, NAPQI, a highly reactive metabolite, is generated heavily, particularly when overdosed. This reduces the antioxidant enzymes and saturation of the glutathione system, which then produces ROS and free radicals [7]. Thus, we tested the ability of BOL leaves to restore antioxidant enzyme levels. Consistent with previous studies, APAP reduced GSH, CAT and SOD (Table 1) [25,48,50,51,53,57]. Our study revealed that BOL-treated groups restored GSH, CAT and SOD levels although the higher dose showed a greater magnitude of restoring the antioxidant enzyme levels. Additionally, lipid peroxidation is one hallmark of cellular oxidative stress. Once this stress is initiated, the contents of membrane lipids begin to degrade, and MDA leaks out of the cell membrane as a sign of lipid peroxidation [7]. Hence, we investigated the effect of BOL's leaves on MDA levels. Figure 4 illustrates that APAP raised MDA levels similar to previous studies [48,50,53]. Conversely, BOL dampened APAP-induced MDA levels with the higher dose showing a greater magnitude effect. The antioxidant property of BOL might be the reason for producing such an effect [29]. Natural plants with similar bioactive compounds have shown similar ROS neutralization effects in the kidney, such as resveratrol [58], quercetin [50,59], and thymoquinone [41]. Our findings are consistent with previous studies in which natural antioxidants dampened APAP–induced renal toxicity.

Innate immunity responds to various types of injuries, including renal injury, by initiating an inflammatory response. When oxidative stress and cellular injury occur, inflammatory cytokines, such as TNF-α and IL-1β, are produced by immune cells [7,42]. Within the renal tissue, TNF-α is produced by the renal tubular and IL-1β is produced by the renal epithelial. Both cytokines are key in physiological processes that initiate and extend inflammation and activate proinflammatory pathways. These processes include activation of NF-κB and MAPK signaling pathways, apoptotic induction, and leukocyte infiltration. Several studies have established that an overdose of APAP increased either serum levels or gene expression of both cytokines [7]. In alignment with previous research, our study showed that APAP elevated serum concentration of IL-1β and TNF-α (Figures 5 and 6). Notably, BOL reduced APAP-induced high levels of both mediators, although the low dose did not affect IL-1β levels. The current result suggests that one mechanism for BOL's leaves extract to reduce kidney injury is by modulating the TNF-α and IL-1β inflammatory mediators. Previous studies investigating natural antioxidants have reported similar findings in which they

decreased APAP-induced high levels of inflammatory mediators [49,60].

A subsequent consequence of oxidative stress is DNA damage that can be assessed by detecting DNA fragmentation [61]. Our study shows that APAP treatment did not cause DNA smearing and displayed a similar DNA laddering pattern to the respective treatments, denoting no DNA damage occurred (Supplement Figure 1). One possible explanation for this is the APAP treatment period. The main objective of the current study was to establish the nephroprotective effects of BOL against APAP; thus, an acute APAP treatment protocol was adopted in which a single high-toxic dose of APAP (2 g/kg) was used. Previous studies that used a similar APAP dosing regimen protocol in rats did not report DNA damage [48,49].

In contrast to lipid peroxidation and other oxidative stress markers, DNA damage induced by APAP might necessitate a longer exposure duration [7]. Y. Wang et al., 2015 displayed that a 70-day treatment with 400 mg/kg APAP caused DNA damage in mice liver [62]. Moreover, studies that used lower toxic doses of APAP (700-750 mg/kg) for 14 days showed DNA damage in rats' kidneys [63,64]. Another justification is that APAP-induced kidney damage occurs more slowly compared to liver damage. In-vivo and in-vitro analyses reported that tissue necrosis, as a sign of DNA damage, is faster and more evident in the liver than in the kidney [65–6]). Instead, a study by Das et al., 2010 reported that a single dose of 2 g/kg APAP can cause DNA damage in mice [68]. The genetic differences between rats and mice could explain this. Previous studies have shown that rats are more resistant to APAP toxicity in the kidney and liver than mice [66,69]. Furthermore, medications that directly target DNA, such as cisplatin, exhibit faster and more noticeable renal DNA damage [44]. Regardless of the controversies, there is a general agreement that renal DNA damage caused by APAP is affected by factors such as the dose, route of administration, and treatment period of APAP and the genetic differences between rodents [7].

The histopathological alterations induced by APAP and the protective benefits of BOL's leaves are shown in Figure 7. All the impacts caused by APAP, such as disturbance of the glomerular basement membrane, vacuolization, and tubular necrosis, were dampened by BOL's leaves, particularly with the higher dose showing more significant enhancement of renal tissue integrity. This result supports the current biochemical investigations. In accordance, several previous studies have presented similar findings with extracts from medicinal plants that possess similar properties to those of BOL [48–50,53,63].

The nephroprotective effects of BOL against APAP are believed to involve multiple interrelated mechanisms: Antioxidant Defense: BOL enhances the kidney's antioxidant defenses by upregulating key enzymes such as GSH, CAT, and SOD. These enzymes work synergistically to neutralize ROS generated by APAP metabolism, thereby reducing oxidative stress and preventing cellular damage in renal tissues. Anti-inflammatory Action: BOL downregulates pro-inflammatory cytokines, particularly TNF-α and IL-1β. By inhibiting the inflammatory pathways, BOL reduces inflammation and subsequent damage to renal tissues, preserving their structural and functional integrity. Mitochondrial Protection: BOL may protect mitochondrial function by preventing mitochondrial dysfunction induced by APAP. This protection involves maintaining mitochondrial membrane potential, reducing the release of cytochrome c, and inhibiting the activation of the intrinsic apoptotic pathway, thus preventing renal cell death. Enhancement of Detoxification Pathways: BOL may promote the detoxification of APAP by enhancing the conjugation of toxic metabolites with GSH, thus facilitating their safe excretion. This reduces the accumulation of harmful metabolites like NAPQI, minimizing their nephrotoxic effects. Tissue Regeneration: BOL may also support the regeneration and repair of damaged renal tissues by promoting cellular proliferation and inhibiting apoptosis in kidney cells, thereby aiding the recovery of kidney function.

Even though our present study depicts promising protective effects of extract from BOL's leaves against APAP-induced kidney injury, some limitations should be addressed to guide future studies for a better understanding of BOL's nephroprotective mechanism. For example, although the animal model is informative, it may not fully represent human physiological responses. Further studies involving human cell lines or clinical trials would be necessary to confirm the efficacy and safety of BOL in human subjects. Another limitation is that an additional characterization of the ethanolic extract could identify the bioactive components responsible for BOL's effects in the current study. This may include testing different extraction methods or refining purification methods to isolate individual compounds. Moreover, examining BOL's leaves' extract against the long-term treatment of APAP and testing apoptosis induction by measuring the expression of markers such as caspase-3, caspase-9, and NF-κB could provide the extent of the protective mechanism. Further research should also explore varying doses of BOL in both acute and chronic exposure studies to assess potential side effects.

CONCLUSIONS

In conclusion, the ethanolic extract from BOL's leaves shows potential nephroprotective effects against APAP-induced nephrotoxicity. The findings suggest that this protective effect is attained by modulating oxidative stress by restoring normal levels of kidney function biomarkers and antioxidant system status and mitigating inflammation and histopathological kidney injury. BOL leaves extract appears

to be a natural antioxidant that could be used as an adjunct agent to reduce the negative impact of APAP on the kidney while preserving APAP analgesic effectiveness.

INFORMED CONSENT STATEMENT

Not applicable

DATA AVAILABILITY STATEMENT

The authors declare that data is available inside the article.

SUPPLEMENTARY MATERIALS

Figure S1: Effect of BOL on DNA fragmentation.

AUTHOR CONTRIBUTIONS:

Conceptualization, writing—original draft preparation, project administration, funding acquisition A.M.J.; methodology, software, writing—review and editing, formal analysis, data curation, M.F.A.; methodology, data curation, S.S.; writing—review and editing, validation, F.K.; writing—review and editing, resources A.Z.; visualization, conceptualization A.H.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Finco DR. Kidney Function. Clinical Biochemistry of Domestic Animals. 1997 Jan 1;441–84.

[2] Ogobuiro, I. & Tuma, F. Physiology, Renal. StatPearls (2023).

[3] Bertram G. K. Basic & Clinical Pharmacology Fourteenth Edition a LANGE medical book [Internet]. 2017 [cited 2024 Jun 14]. Available from: https://www.academia.edu/42339154/Basic_and_Clinical_Pharmacology_Fourteenth_Edition_a_LANGE_medical_book

[4] Gibson TP. Renal Disease and Drug Metabolism: An Overview. American Journal of Kidney Diseases [Internet]. 1986 Jul 1 [cited 2024 Jun 14];8(1):7–17. Available from: http://www.ajkd.org/article/S0272638686801482/fulltext

[5] Rang HP, Ritter J, Flower RJ, Henderson G. Rang & Dale's Pharmacology (8th ed.). Pharmacology [Internet]. 2016 [cited 2024 Jun 14];630–1. Available from: https://search.worldcat.org/title/904420215

[6] Ayoub SS. Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. Temperature: Multidisciplinary Biomedical Journal [Internet]. 2021 [cited 2024 Apr 5];8(4):351. Available from: /pmc/articles/PMC8654482/

[7] Wang X, Wu Q, Liu A, Anadón A, Rodríguez JL, Martínez-Larrañaga MR, et al. Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug Metab Rev [Internet]. 2017 Oct 2 [cited 2024 Jul 6];49(4):395–437. Available from: https://www.tandfonline.com/doi/abs/10.1080/03602532.2017.1354014

[8] Flower RJ, Vane JR. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature [Internet]. 1972 [cited 2024 Apr 16];240(5381):410–1. Available from: https://pubmed.ncbi.nlm.nih.gov/4564318/

[9] Chandrasekharan N V., Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A [Internet]. 2002 Oct 15 [cited 2024 Apr 16];99(21):13926–31. Available from: https://pubmed.ncbi.nlm.nih.gov/12242329/

[10] Bessems JGM, Vermeulen NPE. Paracetamol (Acetaminophen)-Induced Toxicity: Molecular and Biochemical Mechanisms, Analogues and Protective Approaches. Crit Rev Toxicol [Internet]. 2001 [cited 2024 Apr 16];31(1):55–138. Available from: https://www.tandfonline.com/doi/abs/10.1080/20014091111677

[11] Howie D, Adriaenssens PI, Prescott LF. Paracetamol metabolism following overdosage: application of high performance liquid chromatography. J Pharm Pharmacol [Internet]. 1977 [cited 2024 Apr 17];29(4):235–7. Available from: https://pubmed.ncbi.nlm.nih.gov/17674/

[12] Knox JH, Jurand J. Determination of paracetamol and its metabolites in urine by high-performance liquid chromatography using reversed-phase bonded supports. J Chromatogr A. 1977 Nov 11;142(C):651–70.

[13] Prescott L. Kinetics and metabolism of paracetamol and phenacetin. Br J Clin Pharmacol [Internet]. 1980 [cited 2024 Apr 17];10 Suppl 2(Suppl 2):291S-298S. Available from: https://pubmed.ncbi.nlm.nih.gov/7002186/

[14] Khandkar MA, Parmar D V., Das M, Katyare SS. Is activation of lysosomal enzymes responsible for paracetamol-induced hepatotoxicity and nephrotoxicity? J Pharm Pharmacol [Internet]. 1996 [cited 2024 Apr 17];48(4):437–40. Available from: https://pubmed.ncbi.nlm.nih.gov/8794998/

[15] Lorz C, Justo P, Sanz AB, Egido J, Ortíz A. Role of Bcl-xL in paracetamol-induced tubular epithelial cell death. Kidney Int [Internet]. 2005 [cited 2024 Apr 17];67(2):592–601. Available from: https://pubmed.ncbi.nlm.nih.gov/15673306/

[16] Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. J Med Toxicol [Internet]. 2008 [cited

2024 Apr 16];4(1):2–6. Available from: https://link.springer.com/article/10.1007/BF03160941
[17] Emeigh Hart SG, Wyand DS, Khairallah EA, Cohen SD. Acetaminophen nephrotoxicity in the CD-1 mouse. II. Protection by probenecid and AT-125 without diminution of renal covalent binding. Toxicol Appl Pharmacol [Internet]. 1996 [cited 2024 Apr 18];136(1):161–9. Available from: https://pubmed.ncbi.nlm.nih.gov/8560470/
[18] Larsson R, Ross D, Berlin T, Olsson LI, Moldéus P. Prostaglandin synthase catalyzed metabolic activation of p-phenetidine and acetaminophen by microsomes isolated from rabbit and human kidney. Journal of Pharmacology and Experimental Therapeutics. 1985;235(2).
[19] Mohandas J, Duggin GG, Horvath JS, Tiller DJ. Metabolic oxidation of acetaminophen (Paracetamol) mediated by cytochrome P-450 mixed-function oxidase and prostaglandin endoperoxide synthetase in rabbit kidney. Toxicol Appl Pharmacol. 1981;61(2):252–9.
[20] Mugford CA, Tarloff JB. The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. Toxicol Lett. 1997 Sep 19;93(1):15–22.
[21] Newton JF, Bailie MB, Hook JB. Acetaminophen nephrotoxicity in the rat. Renal metabolic activation in vitro. Toxicol Appl Pharmacol [Internet]. 1983 Sep 30 [cited 2024 Apr 18];70(3):433–44. Available from: https://pubmed.ncbi.nlm.nih.gov/6636173/
[22] Bartolone JB, Beierschmitt WP, Birge RB, Hart SGE, Wyand S, Cohen SD, et al. Selective acetaminophen metabolite binding to hepatic and extrahepatic proteins: an in vivo and in vitro analysis. Toxicol Appl Pharmacol [Internet]. 1989 Jun 15 [cited 2024 Apr 18];99(2):240–9. Available from: https://pubmed.ncbi.nlm.nih.gov/2734789/
[23] Emeigh Hart SG, Beierschmitt WP, Bartolone JB, Wyand DS, Khairallah EA, Cohen SD. Evidence against deacetylation and for cytochrome P450-mediated activation in acetaminophen-induced nephrotoxicity in the CD-1 mouse. Toxicol Appl Pharmacol [Internet]. 1991 [cited 2024 Apr 18];107(1):1–15. Available from: https://pubmed.ncbi.nlm.nih.gov/1987650/
[24] Hoivik DJ, Fisher RL, Brendel K, Gandolfi AJ, Khairallah EA, Cohen SD. Protein arylation precedes acetaminophen toxicity in a dynamic organ slice culture of mouse kidney. Fundamental and Applied Toxicology [Internet]. 1996 [cited 2024 Apr 18];34(1):99–104. Available from: https://pubmed.ncbi.nlm.nih.gov/8937897/
[25] Trumper L, Girardi G, Elías MM. Acetaminophen nephrotoxicity in male Wistar rats. Arch Toxicol [Internet]. 1992 Feb [cited 2024 Jul 6];66(2):107–11. Available from: https://pubmed.ncbi.nlm.nih.gov/1605724/
[26] Chaudhary SA. Vol. II. Ministry of Agriculture and Water, Riyadh, 342-354. - References - Scientific Research Publishing. 2001 [cited 2024 May 14]. p. 342–54 Flora of the Kingdom of the Saudi Arabia. Available from: https://www.scirp.org/reference/ReferencesPapers?ReferenceID=1823368
[27] Ahmed B, Al-Rehaily AJ, Mossa JS. Barbeyol: A New Phenolic Indane Type Component from Barbeya oleoides. Zeitschrift fur Naturforschung - Section C Journal of Biosciences [Internet]. 2002 Feb 1 [cited 2024 May 14];57(1–2):17–20. Available from: https://www.degruyter.com/document/doi/10.1515/znc-2002-1-203/html
[28] Yeşilada E, Üstün O, Sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1α, interleukin-1β and tumor necrosis factor α. J Ethnopharmacol. 1997 Sep 1;58(1):59–73.
[29] Al-Oqail MM, Al-Rehaily AJ, Hassan WHB, Ibrahim TA, Ahmad MS, Ebada SS, et al. New flavonol glycosides from Barbeya oleoides Schweinfurth. Food Chem. 2012 Jun 15;132(4):2081–8.
[30] Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev [Internet]. 1998 [cited 2024 Aug 10];56(11):317–33. Available from: https://pubmed.ncbi.nlm.nih.gov/9838798/
[31] Hagerman AE, Butler LG. Choosing appropriate methods and standards for assaying tannin. J Chem Ecol [Internet]. 1989 Jun [cited 2024 Aug 10];15(6):1795–810. Available from: https://pubmed.ncbi.nlm.nih.gov/24272183/
[32] Langenheim JH. Higher plant terpenoids: A phytocentric overview of their ecological roles. J Chem Ecol [Internet]. 1994 Jun [cited 2024 Aug 10];20(6):1223–80. Available from: https://pubmed.ncbi.nlm.nih.gov/24242340/
[33] Hostettmann K, Marston A, Ndjoko K, Wolfender JL. The Potential of African Plants as a Source of Drugs. Curr Org Chem [Internet]. 2005 Mar 25 [cited 2024 Aug 10];4(10):973–1010. Available from: https://www.eurekaselect.com/article/10634
[34] Wink M. Annual plant reviews volume 40, Biochemistry of plant Secondary Metabolites. Biochemistry of Plant Secondary Metabolism: Second Edition [Internet]. 2010 Mar 26 [cited 2024 Aug 10];40:1–445. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9781444320503
[35] Alam MF, Alshahrani S, Alamer EA, Alhazmi MA, Anwer T, Khan G, et al. Nephroprotective effects of 4-4(hydroxyl-3 methoxyphenyl)-2-butane against sodium tellurite induced acute kidney dysfunction by attenuating oxidative stress and inflammatory cytokines in rats. Arabian Journal of Chemistry. 2022 Jun 1;15(6):103857.
[36] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951 Nov;193(1):265–75.
[37] Islam F, Zia S, Sayeed I, Zafar KS, Ahmad AS. Selenium-induced alteration of lipids, lipid peroxidation, and thiol group in circadian rhythm centers of rat. Biol Trace Elem Res [Internet]. 2002 Dec [cited 2024 Jun

14];90(1–3):203–14. Available from: https://pubmed.ncbi.nlm.nih.gov/12666835/
[38] Jollow D, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology [Internet]. 1974 [cited 2024 Jun 14];11(3):151–69. Available from: https://pubmed.ncbi.nlm.nih.gov/4831804/
[39] Claiborne A. Catalase activity. In Handbook of Methods for Oxygen Radical Research. Greenwald RA,, Ed., editors. Boca Raton, FL, USA,: CRC Press; 1985. 283–284 p.
[40] Marklund S, biochemistry GME journal of, 1974 undefined. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. journal of biochemistry [Internet]. 1974 [cited 2024 Jun 14];47:469–74. Available from: https://www.researchgate.net/profile/Surendra-Katyare/post/How-can-i-calculate-SOD-MDA-GSH-Catalase-GPx-for-EX-vivo/attachment/5d35d0b03843b0b9825bcf74/AS%3A783481357467649%401563807920310/download/Marklund+and+Marklund.pdf
[41] Qadri MM, Alam MF, Khired ZA, Alaqi RO, Khardali AA, Alasmari MM, et al. Thymoquinone Ameliorates Carfilzomib-Induced Renal Impairment by Modulating Oxidative Stress Markers, Inflammatory/Apoptotic Mediators, and Augmenting Nrf2 in Rats. International Journal of Molecular Sciences 2023, Vol 24, Page 10621 [Internet]. 2023 Jun 25 [cited 2024 Jun 14];24(13):10621. Available from: https://www.mdpi.com/1422-0067/24/13/10621/htm
[42] Akcay A, Edelstein CL, Nguyen Q. Mediators of Inflammation in Acute Kidney Injury. Mediators Inflamm [Internet]. 2009 Jan 1 [cited 2024 Jul 4];2009(1):137072. Available from: https://onlinelibrary.wiley.com/doi/full/10.1155/2009/137072
[43] Gyurászová M, Gurecká R, Bábíčková J, Tóthová Ľ. Oxidative Stress in the Pathophysiology of Kidney Disease: Implications for Noninvasive Monitoring and Identification of Biomarkers. Oxid Med Cell Longev [Internet]. 2020 [cited 2024 Jul 4];2020. Available from: /pmc/articles/PMC7007944/
[44] Wang P, Ouyang J, Jia Z, Zhang A, Yang Y. Roles of DNA damage in renal tubular epithelial cells injury. Front Physiol [Internet]. 2023 [cited 2024 Jul 4];14. Available from: /pmc/articles/PMC10117683/
[45] Bishop ML, Schoeff LE, Fody EP. Clinical Chemistry: Principles, Tehnique, Correlations. 2013;299.
[46] Hosten AO. BUN and Creatinine. Clinical Methods: The History, Physical, and Laboratory Examinations [Internet]. 1990 [cited 2024 Jul 4]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK305/
[47] Canayakin D, Bayir Y, Kilic Baygutalp N, Sezen Karaoglan E, Atmaca HT, Kocak Ozgeris FB, et al. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharm Biol [Internet]. 2016 Oct 2 [cited 2024 Jul 6];54(10):2082–91. Available from: https://www.tandfonline.com/doi/abs/10.3109/13880209.2016.1145701
[48] Haidara MA, Al-Hashem F, El Karib AO, Zaki MS, Kamar SS, El-Bidawy MH, et al. Inhibition of Paracetamol-Induced Acute Kidney Damage in Rats Using a Combination of Resveratrol and Quercetin. International Journal of Morphology [Internet]. 2019 Dec 1 [cited 2024 Jul 5];37(4):1422–8. Available from: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0717-95022019000401422&lng=es&nrm=iso&tlng=en
[49] Alshahrani S, Ashafaq M, Hussain S, Mohammed M, Sultan M, Jali AM, et al. Renoprotective effects of cinnamon oil against APAP-Induced nephrotoxicity by ameliorating oxidative stress, apoptosis and inflammation in rats. Saudi Pharmaceutical Journal : SPJ [Internet]. 2021 Feb 1 [cited 2024 Jul 16];29(2):194. Available from: /pmc/articles/PMC7910143/
[50] Bayoumy NM. Quercetin Protects Against Acetaminophen-Induced Acute Nephrotoxicity Associated with the Inhibition of Biomarkers of Acute Kidney Injury in Rats. International Journal of Morphology [Internet]. 2020 Aug 1 [cited 2024 Jul 5];38(4):876–81. Available from: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0717-95022020000400876&lng=es&nrm=iso&tlng=en
[51] Bektur NE, Sahin E, Baycu C, Unver G. Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. http://dx.doi.org/101177/0748233713502841 [Internet]. 2013 Nov 5 [cited 2024 Jul 5];32(4):589–600. Available from: https://journals.sagepub.com/doi/abs/10.1177/0748233713502841
[52] Gopi KS, Reddy AG, Jyothi K, Kumar BA. Acetaminophen-induced Hepato-and nephrotoxicity and amelioration by silymarin and Terminalia chebula in rats. Toxicol Int. 2010 Dec;17(2):64–6.
[53] Cekmen M, Ilbey YO, Ozbek E, Simsek A, Somay A, Ersoz C. Curcumin prevents oxidative renal damage induced by acetaminophen in rats. Food and Chemical Toxicology. 2009 Jul 1;47(7):1480–4.
[54] Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. Afr Health Sci [Internet]. 2015 [cited 2024 Jul 6];15(2):598. Available from: /pmc/articles/PMC4480493/
[55] Hussain Z, Khan JA, Arshad A, Asif P, Rashid H, Arshad MI. Protective effects of Cinnamomum zeylanicum L. (Darchini) in acetaminophen-induced oxidative stress,

hepatotoxicity and nephrotoxicity in mouse model. Biomedicine & Pharmacotherapy. 2019 Jan 1;109:2285–92.
[56] Hong YA, Park CW. Catalytic Antioxidants in the Kidney. Antioxidants [Internet]. 2021 Jan 1 [cited 2024 Jul 8];10(1):1–22. Available from: /pmc/articles/PMC7831323/
[57] Abdul Hamid Z, Budin SB, Wen Jie N, Hamid A, Husain K, Mohamed J. Nephroprotective effects of Zingiber zerumbet Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. J Zhejiang Univ Sci B [Internet]. 2012 Mar [cited 2024 Jul 16];13(3):176. Available from: /pmc/articles/PMC3296068/
[58] Rashid H, Jali A, Akhter MS, Abdi SAH. Molecular Mechanisms of Oxidative Stress in Acute Kidney Injury: Targeting the Loci by Resveratrol. International Journal of Molecular Sciences 2024, Vol 25, Page 3 [Internet]. 2023 Dec 19 [cited 2024 Jul 6];25(1):3. Available from: https://www.mdpi.com/1422-0067/25/1/3/htm
[59] Yang H, Song Y, Liang YN, Li R. Quercetin Treatment Improves Renal Function and Protects the Kidney in a Rat Model of Adenine-Induced Chronic Kidney Disease. Med Sci Monit [Internet]. 2018 Jul 10 [cited 2024 Jul 6];24:4760–6. Available from: https://pubmed.ncbi.nlm.nih.gov/29987270/
[60] Alqahtani QH, Fadda LM, Alhusaini AM, Hasan IH, Ali HM. Involvement of Nrf2, JAK and COX pathways in acetaminophen-induced nephropathy: Role of some antioxidants. Saudi Pharmaceutical Journal : SPJ [Internet]. 2023 Oct 1 [cited 2024 Jul 16];31(10):101752. Available from: /pmc/articles/PMC10480313/
[61] Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol [Internet]. 2004 [cited 2024 Jul 17];26(3):249–61. Available from: https://pubmed.ncbi.nlm.nih.gov/15004294/
[62] Wang Y, Li D, Cheng N, Gao H, Xue X, Cao W, et al. Antioxidant and hepatoprotective activity of vitex honey against paracetamol induced liver damage in mice. Food Funct [Internet]. 2015 Jul 1 [cited 2024 Jul 16];6(7):2339–49. Available from: https://pubmed.ncbi.nlm.nih.gov/26084988/
[63] Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. Ren Fail [Internet]. 2016 Jul 2 [cited 2024 Jul 17];38(6):1007–20. Available from: https://www.tandfonline.com/doi/abs/10.3109/0886022X.2016.1163998
[64] Ahmad ST, Arjumand W, Nafees S, Seth A, Ali N, Rashid S, et al. Hesperidin alleviates acetaminophen induced toxicity in wistar rats by abrogation of oxidative stress, apoptosis and inflammation. Toxicol Lett [Internet]. 2012 Jan 25 [cited 2024 Jul 17];208(2):149–61. Available from: https://www.researchgate.net/publication/51810673_Hesperidin_alleviates_acetaminophen_induced_toxicity_in_Wistar_rats_by_abrogation_of_oxidative_stress_apoptosis_and_inflammation
[65]. Vrbová M, Roušarová E, Brůčková L, Česla P, Roušar T. Characterization of acetaminophen toxicity in human kidney HK-2 cells. Physiol Res. 2016;65(4):627–35.
[66] Mudge GH, Gemborys MW, Duggin GG. Covalent binding of metabolites of acetaminophen to kidney protein and depletion of renal glutathione. Journal of Pharmacology and Experimental Therapeutics. 1978;206(1).
[67] McMurtry RJ, Snodgrass WR, Mitchell JR. Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. Toxicol Appl Pharmacol. 1978 Oct 1;46(1):87–100.
[68] Das J, Ghosh J, Manna P, Sil PC. Taurine protects acetaminophen-induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation. Toxicology [Internet]. 2010 Feb [cited 2024 Jul 17];269(1):24–34. Available from: https://pubmed.ncbi.nlm.nih.gov/20067817/
[69] Hongslo JK, Smith C V., Brunborg G, SØderlund EJ, Holme JøA. Genotoxicity of paracetamol in mice and rats. Mutagenesis [Internet]. 1994 Mar 1 [cited 2024 Jul 17];9(2):93–100. Available from: https://dx.doi.org/10.1093/mutage/9.2.93
[70] Topcu-Tarladacalisir, Y., Sapmaz-Metin, M. & Karaca, T. Curcumin counteracts cisplatin-induced nephrotoxicity by preventing renal tubular cell apoptosis. Ren Fail 38, 1741–1748 (2016).
[71] Ibrahim D, A., Albadani R, N. Evaluation of the Potential Nephroprotective and Antimicrobial Effect of Camellia sinensis Leaves versus Hibiscus sabdariffa (In Vivo and In Vitro Studies). Adv Pharmacol Sci. 2014;2014:389834. doi:10.1155/2014/389834
[72] Khojah, A. A. et al. Barbeya oleoides Leaves Extracts: In Vitro Carbohydrate Digestive Enzymes Inhibition and Phytochemical Characterization. Molecules 2021, Vol. 26, Page 6229 26, 6229 (2021).

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Review Article

Therapeutic Efficacy of Zinc Sulfate in Treating Neonatal Hyperbilirubinemia – A Review of The Recent Evidence

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ABSTRACT: Bilirubin levels above 5 mg/dL (86 µmol/L) in newborns may indicate hyperbilirubinemia, which sometimes requires hospitalization. In these patients, physical jaundice peaks between days 2 and 6 following birth. Hemolysis, infections, premature delivery, exclusive breastfeeding, and hereditary predispositions are common causes of elevated bilirubin levels. This review aims to evaluate the causes and mechanisms of neonatal hyperbilirubinemia and identify risk factors and therapies, with a focus on assessing zinc sulfate's safety and efficacy as a phototherapy adjuvant in treating hyperbilirubinemia. The mechanism by which phototherapy and zinc reduce enterohepatic circulation of bilirubin was also analyzed. Zinc sulfate supplementation markedly lowers blood bilirubin levels by enhancing its removal via reduced enterohepatic circulation. When combined with phototherapy, zinc sulfate was more effective than phototherapy alone. Clinical trials showed significant bilirubin reductions within 12–72 hours of zinc administration, shortening treatment duration. Zinc sulfate combined with phototherapy shows potential in treating hyperbilirubinemia, improving bilirubin reduction, and lowering neurotoxicity risk. However, zinc alone is inadequate. Further experimental studies are needed to confirm these findings and establish a standardized treatment protocol.

Keywords: Zinc Sulfate, Neonatal, Bilirubin, Treatment.

INTRODUCTION

Jaundice is the predominant cause for readmission following discharge from the maternity ward and is recognized as a clinical indication for emergencies in infants. [1] Indirect bilirubin accumulation in cell membranes leads to persistent neuronal injury.[2] The primary objective in preventing bilirubin encephalopathy and its long-term consequences is the identification and management of hyperbilirubinemia in newborns. Unconjugated hyperbilirubinemia in newborns has been managed using various expensive, time-intensive, and occasionally hazardous interventions, including phototherapy and blood exchange transfusion. [1, 3] Therefore, developing new therapeutic strategies is essential for reducing elevated serum bilirubin levels. Inhibition of enterohepatic circulation may reduce unconjugated bilirubin levels, potentially serving as a therapeutic approach to mitigate bilirubin neurotoxicity. [4] Zinc salts induce the precipitation of unconjugated bilirubin in the colon, inhibiting the enterohepatic circulation of bilirubin. [3, 5] Previous studies have shown that the administration of zinc salts, whether acutely or chronically, can reduce serum bilirubin levels by inhibiting the indirect bilirubin cycle within the enterohepatic system. [1, 3, 5] Oral administration of zinc sulfate results in increased bilirubin excretion and decreased serum levels. [5] Zinc has also been shown to inhibit the enzyme heme oxygenase [3, 6]. Consequently, zinc administration significantly reduced levels of carbon monoxide (CO) and bilirubin 6 hours post-treatment. Zinc salts inhibit heme oxygenase enzymes, suppressing jaundice development. [7]

As a result, clinical trials have been undertaken to assess the impact of zinc supplementation in neonates suffering from hyperbilirubinemia. [1, 8] Recently, potentially encouraging results have been reported regarding the effectiveness of zinc supplementation, both alone and in conjunction with phototherapy, for managing neonatal hyperbilirubinemia.

Thus, this review aims to investigate the current evidence that explains the etiology and pathogenesis of newborn hyperbilirubinemia, identify associated risk factors, explore various treatment modalities, assess the efficacy of zinc sulfate as a therapeutic option, and examine potential side effects.

MATERIALS AND METHODS

A literature search, from the years 2010 to 2024, was performed across seven distinct medical electronic databases: PubMed, PsycINFO, Google Scholar, Scopus, MEDLINE, and ProQuest. The associated research involved the use of individual and/or combinations of terms including bilirubin encephalopathy, hyperbilirubinemia, newborns, infantile, unconjugated, phototherapy, blood transfusion, enterohepatic circulation, zinc salts, placebo-controlled, cross-sectional, clinical trial, randomized, qualitative or

quantitative, empirical, and survey. Relevant papers were selected following a screening of references, titles, keywords, abstracts, and full texts, by two independent reviewers, utilizing a pre-piloted form. The selection primarily focused on variables related to infantile hyperbilirubinemia, phototherapy, and zinc. The literature selected comprised 45 full-length articles, with the remainder encompassing reports, guidelines, theses, and literature reviews.

Operational definitions: Hyperbilirubinemia is defined as an unusually high blood bilirubin concentration. [3] Bilirubin is a yellow pigment produced during the breakdown of red blood cells. [1, 4] Elevated bilirubin levels in the blood result in jaundice, marked by yellow discoloration of the skin, mucous membranes, and sclera. This condition is particularly common among newborns and toddlers. [1, 5] Kernicterus is an extremely unusual and potentially deadly consequence of untreated acute hyperbilirubinemia. [3] It causes damage to the brain from bilirubin that manages to cross the blood–brain barrier. [3] When severe jaundice occurs as a result of blood type incompatibility like Rh or ABO incompatibility, intravenous immunoglobulin (IVIG) is administered to help reduce the breakdown of red blood cells. [6] Notably, newborns with jaundice may benefit from phototherapy treatment that uses blue light to degrade bilirubin in the skin, thus reducing the elevated bilirubin levels. [8]

Etiology of Neonatal Hyperbilirubinemia

Many circumstances can cause increased plasma bilirubin concentrations in the neonatal period, such as preterm delivery, exclusive breast feeding, infections (cutaneous and pulmonary), hemolysis (blood type incompatibility), internal bleeding (cranial hematoma), hypoxia, acidosis, hypoglycemia, and genetic variables. [3, 9] These are just a few of the factors that might contribute to these difficulties. The probability of severe infantile hyperbilirubinemia increases with the number of risk factors. [10] These variables pertain to the following three associated factors:

1) Fetal factors: Researchers have found a negative correlation between gestational age and jaundice occurrence in newborns. [10] A low birth-weight percentage is more prevalent. In terms of race, the incidence is higher among East Asians and Americans compared to Africans. [11] The likelihood of severe jaundice in newborns is higher among male infants than female infants; [3, 5] a higher incidence in high-altitude populations is noted [10]. Infants with siblings are more likely to experience jaundice, primarily due to genetics and familial risk. [8] When babies are born with chromosomal abnormalities or polymorphisms, they often have problems with glucose 6-phosphatase dehydrogenase (G-6-PD), an important enzyme, and another genetic hemolytic anemia (7). Additionally, these newborns are more likely to possess proteins and enzymes that are essential for the metabolism of bilirubin. Breastfeeding is either more common or insufficient for newborns. There are several aspects of the mechanisms that underline these behaviors that are not completely understood. Differentiating between breastfed and formula-fed children can be challenging with currently available formulas. [12, 13] Pathogens such as herpes simplex virus (HSV), cytomegalovirus (CMV), *Toxoplasma gondii*, and rubella virus can be ingested during the peripartum period, transmitted from mother to infant through the placenta, or through direct contact with the mother postpartum [10, 12]. The body is more likely to contain certain medications like vitamin K, chloramphenicol, and streptomycin, which decrease the sensitivity of albumin to bilirubin [8]. Other medicines, like dexamethasone and phenobarbital, raise the production of the enzyme uridine diphosphoglucuronide acid glucuronyl transferase (UDPGG), which lowers the risk of jaundice in newborns. [14]

2) Maternal Disease: Mothers with diabetes give birth to approximately 6% of icteric infants. [15] According to various studies, newborns of diabetic mothers account for 1%–17% of cases of neonatal jaundice. [16] Jaundice in neonates born to diabetic mothers can be caused by several factors, including preterm birth, polycythemia, macrosomia, and an increased enterohepatic cycle. [15] Pregnant women with high blood pressure are at risk for newborn jaundice. The most frequent maternal condition associated with newborn jaundice (4.7%–19%) is hypertension. [12, 15] Infants born prematurely are more prone to jaundice due to their immature livers and elevated red blood cell counts. Therefore, maternal hypertension plays a significant role in preterm deliveries. [17]

3) Pregnancy Problems or Complications: Premature birth and delivery are two maternal issues that influence the prevalence of jaundice in newborns. A study [18] found that prematurity causes 30% of newborn jaundice occurrences. Gestational age correlates with the maturity of the uridine diphosphoglucuronide acid glucuronyl transferase (UDPGT) enzyme, and enzyme activity was only at one-third among infants born at 32 weeks of gestation. [13] Consequently, premature infants are more likely to develop jaundice and complications. Conversely, postponement of the production of milk and weakening of the baby's suckling impulse result in a reduction in the consumption of calories, leading to dehydration and an increase in bilirubin's enterohepatic flow, which increases the amount of bilirubin. [19] Premature rupture of the membranes (PROM) is another maternal risk factor, with 1.7%–4.8% of newborn with jaundice in earlier studies having a history of PROM during pregnancy. [20] ABO blood grouping incompatibility, which arises from the mother's O blood group and the neonate's A or B blood group, is another risk factor for newborn jaundice. [3, 10] The most common risk factors for early neonatal jaundice are RH incompatibility, preterm birth, cephalohematoma, and G6PD enzyme deficiency. Additionally, there is a strong link between the occurrence of jaundice and ABO incompatibility. [19] One of the characteristics that puts neonates at risk of jaundice is the mode of delivery. However, debate persists over which mode of delivery will

reduce jaundice. According to various studies, 40% of jaundiced neonates were delivered via caesarean section, and the remaining 16.7%–75% were born normally. [10] Maternal vaginal hemorrhage during childbirth is an additional issue associated with renal impairment. Damage sustained during delivery may result in bleeding from other organs, cephalohematomas, and subcutaneous hemorrhage. Newborns who reabsorb this blood experience more acute jaundice. [21]

Pathogenesis: Hemoglobin is released when mononuclear macrophages in the bloodstream identify and phagocytose ageing blood cells. The liberated hemoglobin is broken down into two main parts: heme and globin. Heme oxygenase transforms heme into biliverdin, which is further broken down into bilirubin by biliverdin reductase. Healthy individuals produce most of their daily bilirubin through this pathway. This phase produces unconjugated bilirubin. The liver receives free bilirubin in the form of a bilirubin–albumin complex, following coupling with plasma albumin. Hepatocytes subsequently absorb bilirubin after its isolation from albumin. Hepatocytes then transport this, coupling it with glucuronic acid in the endoplasmic reticulum to generate direct bilirubin. [23] It combines with ligandins (Y and Z proteins) in this process. The highly water-soluble bile then contains conjugated bilirubin released by hepatocytes and drains into the lumen of the small intestine. Intestinal flora breaks down and lowers conjugated bilirubin to create bilinogen, with the majority excreted in stool. Intestinal mucosal cells take up a small amount and reabsorb it into circulation. Only a small portion (less than 10%) enters the bloodstream, travels through the renal system, and is eliminated in urine. The majority (90%), together with the bile, is reabsorbed and enters the intestinal cavity as kininogen, creating a bilinogen enterohepatic circulation. [7]

Factors contributing to hyperbilirubinemia: There are very few microbes in a newborn's stomach, and bilirubin entering the smaller intestine is excreted directly in the stool rather than being metabolized by bacteria. [24] Excess plasma bilirubin can accumulate if the liver is unable to absorb and/or bind bilirubin properly or if bilirubin cannot be excreted from the body appropriately (for example, biliary atresia or hepatitis) during the neonatal period. [25] Consequently, aberrant liver processing and/or restricted excretion cause(s) elevated bilirubin levels, which may be important contributing factors to newborn hyperbilirubinemia. [26] Neonatal hyperbilirubinemia can also result from genetic mutations in the gene encoding uridine diphosphate glucuronic acid transferase 1A, congenital glucose-6-phosphate dehydrogenase deficiency, hypoxia, infection, and factors related to pregnancy, such as early delivery and use of oxytocin. [27]

Neurotoxicity: Free bilirubin can cross the blood–brain barrier because it is not water-soluble. [5] Unfortunately, neonates have a low capacity to absorb, conjugate, and eliminate bilirubin, especially premature infants whose blood–brain barrier is still developing. [17] Excessive levels of free bilirubin can cause kernicterus, acute bilirubin encephalopathy, severe neurotoxicity, and other conditions possibly leading to long-term, permanent cerebral injury. [5] These conditions can occur when excessive amounts of free bilirubin enter the globus pallidus after crossing the blood–brain barrier, cerebellum, thalamus, hippocampus, and other areas of the brain. Children with kernicterus frequently experience long-term neurological consequences that can be fatal, including paralysis, seizures, deafness, speech problems, and motor dysfunction. [28]

Clinical presentation of hyperbilirubinemia in neonates: The most prominent sign of hyperbilirubinemia in neonates is jaundice – a yellowish discoloration of the skin and sclera. Jaundice usually starts on the face and head and progresses to the arms, legs, chest, and abdomen as bilirubin levels rise. [29] Some neonates with marked hyperbilirubinemia may exhibit poor feeding, lethargy, or irritability. [30] Although less common, neonates with conjugated hyperbilirubinemia (obstructive jaundice) pass dark-colored urine and have pale or clay-colored stools. [31] As bilirubin levels rise, neonates may become more lethargic, which can be an early sign of bilirubin toxicity. [32]

Complications of hyperbilirubinemia in neonates: Kernicterus is a rare complication; however, it is the most serious complication of untreated severe unconjugated hyperbilirubinemia. When extremely high concentrations of unconjugated bilirubin penetrate the blood–brain barrier, bilirubin deposition occurs in brain tissues. Kernicterus can lead to permanent neurological damage, resulting in intellectual difficulties, hearing loss, vision issues, cerebral palsy, and even death. [33] High levels of bilirubin can cause a wide range of neurological issues, ranging from mild problems to severe kernicterus [34]. This is called bilirubin-induced neurological dysfunction (BIND). In cases where hyperbilirubinemia is due to hemolysis (e.g., ABO or Rh incompatibility), there may be additional complications such as anemia, hepatosplenomegaly, and more severe jaundice. [30] Chronic bilirubin encephalopathy is due to the long-term effect of the kernicterus, characterized by persistent and often permanent neurological deficits, such as movement disorders (e.g., dental enamel hypoplasia, auditory impairment, upward gaze palsy, and athetoid cerebral palsy). [32]

Treatment of Neonatal Hyperbilirubinemia: It is imperative to detect free serum bilirubin levels early and take appropriate action to prevent severe harm to the neurological system. [35] In clinical practice, albumin, [36] intravenous immunoglobulin, [37] phototherapy, [1, 3, 4, 16] liver enzyme inducers, [38] and blood exchange therapy are the primary treatments for neonatal hyperbilirubinemia.

Phototherapy: Phototherapy is the primary treatment for neonatal unconjugated hyperbilirubinemia, a condition characterized by elevated bilirubin levels in the blood. It involves exposing the infant to specific wavelengths of light, usually in the blue spectrum (430–490 nm), that convert bilirubin into water-soluble isomers. These isomers can be

easily excreted from the body, reducing the risk of bilirubin-induced neurological damage. Phototherapy is indicated when bilirubin levels exceed the age-specific threshold for treatment, often determined by the infant's gestational age and other risk factors.[39]

The advantages of phototherapy include its effectiveness in lowering bilirubin levels, non-invasiveness, and ability to avoid more invasive treatments like exchange transfusion. However, it has some disadvantages, such as the need for continuous monitoring, possible disruption of maternal–infant bonding, and risks of dehydration or overheating. Rare complications can include skin rashes, bronze baby syndrome, and eye damage if the baby's eyes are not adequately protected. Despite this, phototherapy remains the cornerstone of neonatal jaundice management. [1, 20, 40]

Moreover, certain photo-oxidation events occur, resulting in colorless polar molecules that replace free bilirubin. Bile or urine can eliminate these soluble water compounds from the body, reducing the amount of unconjugated plasma bilirubin and preventing the development of bilirubin-induced encephalopathy. [41]

Hydration and Feeding: Adequate hydration help maintain renal excretion of bilirubin, preventing dehydration, which can worsen jaundice and increase the risk of kernicterus.

Frequent feeding promotes bowel movements, aiding bilirubin elimination through the gastrointestinal tract. It also stimulates gut motility and reduces enterohepatic circulation of bilirubin. Proper hydration and feeding are crucial for managing jaundice, reducing the need for more invasive treatments.

Intravenous Immunoglobulin:

Definition: Intravenous immunoglobulin is a plasma-derived product containing IgG antibodies that is used to modulate immune responses. Indications: IVIG is used in severe unconjugated hyperbilirubinemia due to hemolytic diseases (e.g., Rh/ABO incompatibility) to prevent kernicterus.

Mechanism: It works by blocking Fc receptors on macrophages, reducing hemolysis and bilirubin production.

Benefits: IVIG decreases the need for exchange transfusion, acts rapidly, and is generally safe. Limitations: It is expensive and requires intravenous administration. Complications: Common side effects include mild fever and headache, while rare complications include anaphylaxis and thrombosis. IVIGs are typically used alongside phototherapy when bilirubin levels rise rapidly. [6]

Exchange transfusion therapy:

Definition: This is a medical procedure that partially or completely replaces a patient's blood with donor blood to remove toxins and abnormal cells or correct severe imbalances.

Mechanism: Small amounts of the patient's blood are sequentially removed and replaced with donor blood, ensuring circulatory stability. Indications: Severe hyperbilirubinemia unresponsive to phototherapy, hemolytic disease of the newborn (e.g., Rh or ABO incompatibility), severe anemia with hyperbilirubinemia, metabolic disorders (e.g., severe acidosis or hyperammonemia), severe sepsis, or disseminated intravascular coagulation (DIC) in neonates.

Advantages: Rapidly reduces bilirubin levels, preventing kernicterus, removes antibodies, sensitizes red blood cells in hemolytic conditions, and corrects severe anemia and other blood abnormalities. Disadvantages: Technically complex and requires expertise and preparation. Complications: Immediate: Hypocalcemia, hypoglycemia, electrolyte imbalances, arrhythmias, coagulopathy, and infection. Rare: Delayed anemia, thrombocytopenia, or rebound hyperbilirubinemia. The serious side effects of exchange transfusion therapy include necrotizing enterocolitis, sepsis, embolism, and even death. [3, 4]

Zinc Sulfate: Zinc sulfate and medication therapy are linked to both low healthcare costs and high compliance. Researchers have discovered the involvement of clofibrate, phenobarbital, bile salts, penicillamine, and zinc compounds in various mechanisms such as enzymatic inhibition, stimulation of hepatic clearance, and production inhibition. [42, 43] However, no widely accepted therapeutic approach has emerged thus far. Zinc is a vital trace element that plays numerous biological roles in a wide range of enzymes and "Zn-finger" proteins based on its structural and catalytic roles [44]. Zinc sulfate can stop bilirubin from moving through the liver and into the colon by inducing unconjugated bilirubin precipitation by the colon. [26] Newborns can develop jaundice in case of insufficient zinc, which is necessary for producing Z and Y proteins that break down bilirubin. [26, 45] Research has shown that patients with hyperbilirubinemia have lower Zn levels. [46] Zinc sulfate shows promise in in vitro and in vivo settings in jaundice. [20, 47] Its ability to lower enterohepatic circulation in hyperbilirubinemia depends on its ability to enter the distal intestine, where it undergoes reabsorption into the blood. [48] From a different perspective, oral administration of zinc can improve the effectiveness of phototherapy and shorten its duration. However, phototherapy is still the best method to treat hyperbilirubinemia since zinc supplements do not work on their own. This is supported by the fact that oral zinc supplements are seen as an extra treatment. [7] Furthermore, zinc consumption by mothers reduces the frequency and severity of treatment needed in case idiopathic hyperbilirubinemia in neonates; it also reduces the severity of hyperbilirubinemia. [49] The relationship between zinc levels and neonatal hyperbilirubinemia is relevant to zinc's capacity to enter the gut, where bilirubin is absorbed into the bloodstream, and reduces the enterohepatic cycle of bilirubin. The enterohepatic cycle of bilirubin is inhibited by zinc sulfate by binding to unconjugated bilirubin. [49]

DISCUSSION OF THE RECENT EVIDENCE

Zinc sulfate's effect on total serum bilirubin (TSB) and indirect serum bilirubin (IDSB): Hashemian et al. [48] found that zinc supplementation reduced total bilirubin levels by 23.11% from baseline (22.5 mg/dL) after 12 hours;

these further decreased by 20.23% at 24 hours, 25.36% at 48 hours, and 16.5% at 72 hours, indicating an overall decrease of 61.77% (Table 1). Mandlecha et al. [50], reported that the mean (SD) TSB levels in the zinc group decreased by 23.52% at 24 hours (baseline 15.3 mg/dL), 42.73% between 24 and 48 hours, and 23.88% between 48 and 72 hours, indicating a total decrease of 66.67% from baseline to the end of 3-day treatment. Waheed et al. [51] evaluated the effect of phototherapy in combination with zinc supplementation and observed a reduction of TSB level by 13.93% after 24 hours, against a reduction of 15.74% in the group without phototherapy. Overall reduction was greater in the group without phototherapy (32.72%) than in the group administered phototherapy in conjunction with zinc supplementation (28.08%). However, there was no significant difference between the groups ($p = 0.20$). Ibrahim et al. [7] reported a 16.78% reduction in TSB levels after 12 hours, a reduction of 30.03% after 24 hours, and a further reduction at discharge, amounting to a total decrease of 46.05% from baseline (16.98 mg/dL).

In contrast, Khoshnevisasl et al. [52] reported that the mean bilirubin level at admission was reduced by a total of 49.70% from baseline to the 5th day of zinc supplementation. Hamed et al. [53] reported a total of 58.53% reduction in bilirubin level after 84 hours post-zinc treatment. Meanwhile, Faal et al. [7] demonstrated that the bilirubin level changes in the experimental six hours after intervention were -1.45 ± 3.23 and -0.49 ± 0.37 ($p = 0.024$), respectively. The changes of 24 and 48 hours postintervention were -3.26 ± 2.78 and -1.89 ± 1.20 ($p = 0.017$) in the experimental group. Table 1 presents the mean direct serum bilirubin and the total serum bilirubin levels observed in the abovementioned studies. Figure 1 illustrates the percentage decrease in total serum bilirubin (TSB) levels across multiple studies at different time intervals. The highest reduction in TSB levels has generally been observed at 48 hours, with another supporting study reporting a reduction of 42.73% [50].

Studies	Year of study	Type of study	Control or baseline	6 hours	12 hours	24 hours	48 hours	72 hours	Discharge/ last day
Hashemian S et al. [48]	2017	Double-Blind, Placebo-Controlled, RCT	22.5±2.31	-	17.3 ±1.62	13.8±1.98	10.3±2.09	8.6±2.11	-
Waheed A et al [51]	2019	Quasi Experimental Study	16.94±3.04 (PT + ZS)	-	-	14.58±3.50	-	12.06±3.36	-
			17.02±3.24 (PT)	-	-	14.34±3.30	-	11.45±2.35	-
Faal G et al [5]	2020	RCT (DB)	12.2±71.67	11.2±26.62	-	9.2±45.83	-	-	-
Ibrahim E et al [7]	2020	RCT	16.98±0.86	-	14.13±0.93	11.88±0.89	-	-	9.16±0.47
Khoshnevisasl P et al. [52]	2020	RCT (DB)	16.98±3.33	-	-	11.95±2.35	9.4± 1.79	-	8.64±0.96
Hamed AM et al. [8]	2022	prospective clinical trial	16.84±0.83	-	-	13.4±1.11	10.7±1.17	8.87±0.64	7.0
Mandlecha TH et al [50]	2023	RCT (DB)	15.3±2.85	-	-	11.7±4.46	6.7±4.77	5.1 ± 3.95	-

Table 1: Summary of the research articles on the effect of zinc sulfate supplementation on direct serum bilirubin (DSB) and total serum bilirubin (TSB) (mg/dL)

- DSB – direct serum bilirubin; TSB – total serum bilirubin; PT – phototherapy; ZS – zinc sulphate; RCT – randomized controlled trial; DB – double Blind
- All values are expressed as milligrams per deciliter (mg/dL)

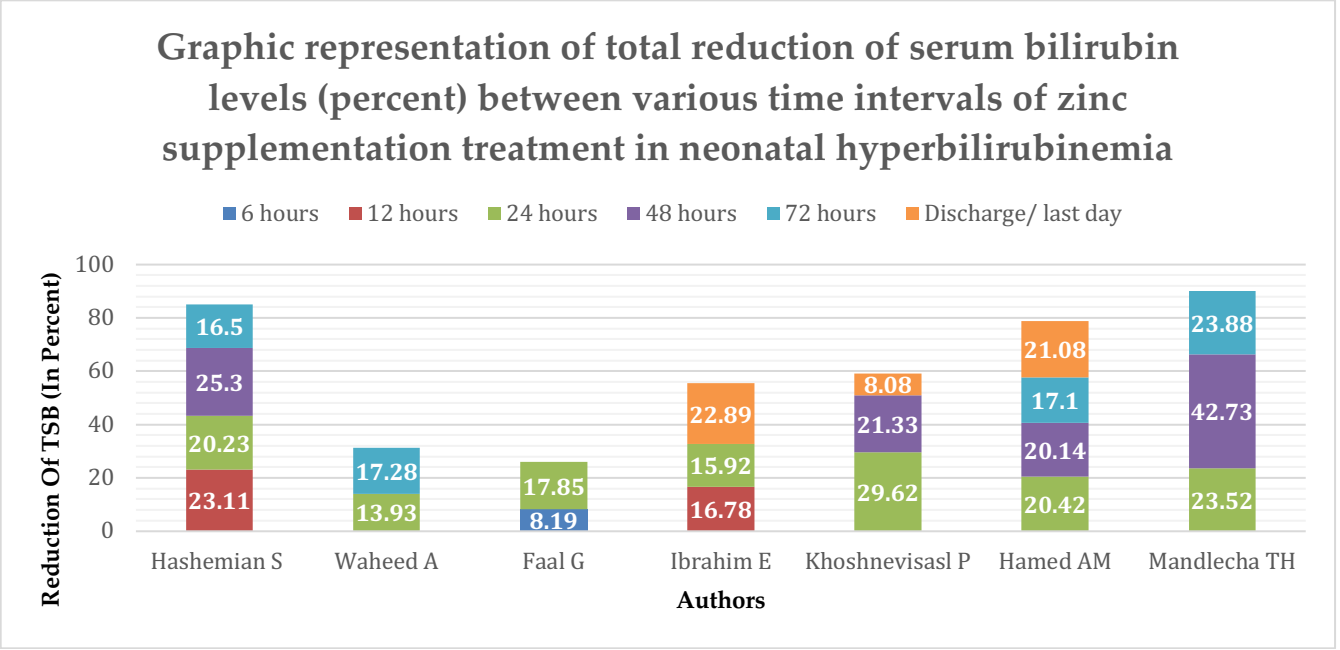

Figure 1: Graphical representation of the reduction in total serum bilirubin levels (in percent) in various studies, at different time intervals

Adverse effect of zinc sulfate supplementation: Mandlecha et al. [50] reported that zinc sulfate supplementation produced minimal or no adverse effects in patients with hyperbilirubinemia. Ibrahim et al. [7] consistently demonstrated that there was no discernible negative impact associated with zinc supplementation, as vomiting occurred in only one case with no incidence of diarrhea. In addition, Hamed et al. [8] identified adverse effects in two (8.0%) cases of hyperbilirubinemia with zinc sulfate supplementation as an adjuvant to phototherapy. Moreover, Faal et al. [5] found no significant adverse effects of zinc sulfate supplementation.

Critical appraisal: All randomized controlled trials considered for this review fulfilled the qualitative requirements, except for the study by Waheed et al. [51], which was a quasi-experimental design, leading to selection bias. The study by Hashemian et al. [48] fulfills the qualitative requirement of a clinical trial. The study was also conducted in a high resource setting. However, the trial had a smaller sample size and did not determine the effects of other dosages and formulations for either phototherapy and zinc supplementation that could be more or less effective in such treatments. The study used a dose of 10 mg/day for zinc sulfate (syrup), while other doses could either prolong the treatment period or be more or less effective. It has been shown that 5 mg zinc sulfate can reduce serum bilirubin levels [5]. The dose of zinc sulfate used has been thought to be associated with lack of effect of zinc sulfate [54]. The presence of potential adverse reactions to phototherapy and the zinc supplement for any included participant was not disclosed. The potential effects of other drugs in overcoming any adverse effect could alter the duration, course, and effect of the intervening drug. Similar issues were also found in other clinical trials that have reported the effects of zinc sulfate in patients with hyperbilirubinemia [7, 8, 50, 51, 52]. Among all included studies, Khoshnevisasl et al. had the largest sample size but lacked determination of the effects of other doses, differentiation of low- and high-risk groups, and assessment of other associated disorders with hyperbilirubinemia. Faal et al. [5], despite a high-resource setting, had a smaller sample of 60 neonates. Neonates with higher gestational age may respond to zinc sulfate and phototherapy differently, which may also be considered a limitation of these studies. Whether the newborn participants in these studies were high, or low risk groups may also be considered as limitations since high-risk newborns are bound to be treated proactively with zinc sulfate at admission [5]. Neonates that have associated disorders due to hyperbilirubinemia also need to be considered for further trials. Furthermore, whether zinc sulfate can be used as a preventive or prophylactic treatment option should also be investigated in future studies. Studies conducted under low-resource settings [8, 51] demonstrate notable challenges, such as limited access to advanced neonatal care, which impacts the outcome.

CONCLUSIONS

In summary, an analysis of research on maternal risk factors for newborn jaundice has demonstrated a significant influence of maternal risk variables on the prevalence of neonatal jaundice. Maternal age, maternal hypertension, gestational diabetes, cesarean section, birth order, PROM, preterm delivery, low birth weight, frequency of exclusive breastfeeding, and weight loss in the neonate were the risk

variables. Thus, frequent and efficient care is required during pregnancy, including education about the negative effects of certain herbal medicines, adequate nutrition for the mother, a normal birth, and monitoring of the unborn child in the event of difficulties such as PROM. The major risk factors for hyperbilirubinemia during pregnancy are high blood pressure, gestational diabetes mellitus, and pre-eclampsia, and these need to be monitored for carefully to prevent preterm birth and neonatal sepsis.

We also suggest that oral zinc sulfate alone is ineffective for treating hyperbilirubinemia in newborns. However, its combination with phototherapy causes a reduction in bilirubin levels, with minimal or no adverse effects, in patients with hyperbilirubinemia. Owing to several important limitations, such as the high risk of bias in certain trials included herein and the possibility of publication bias resulting from linguistic prejudice, these conclusions should be considered cautiously.

INFORMED CONSENT STATEMENT

Not applicable. This study is a review article and does not involve any human or animal participants requiring ethical approval.

DATA AVAILABILITY STATEMENT

This study is a review article and does not involve the generation or analysis of new data. All data supporting the reported results are derived from previously published studies, which are appropriately cited in the reference list.

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CONFLICTS OF INTEREST

None

REFERENCES

[1]. Goodarzi R, Saadat SH, Janbozorgi F, et al. Zinc level alternation after phototherapy in neonates with hyperbilirubinemia. Iran J Pediatr. 2023;11(1). https://doi.org/10.5812/ijp.4146

[2]. Gazzin S, Jayanti S, Tiribelli C. Models of bilirubin neurological damage: lessons learned and new challenges. Pediatric Research. 2023 Jun;93(7):1838-45. https://doi.org/10.1038/s41390-022-02351-x

[3]. Satrom KM, Farouk ZL, Slusher TM. Management challenges in the treatment of severe hyperbilirubinemia in low-and middle-income countries: Encouraging advancements, remaining gaps, and future opportunities. Frontiers in Pediatrics. 2023 Feb 13;11:1001141. https://doi.org/10.3389/fped.2023.1001141

[4]. Haghdoost-Yazdi H. Neurotoxicity induced by biliverdin and bilirubin. InNatural Molecules in Neuroprotection and Neurotoxicity 2024 Jan 1 (pp. 997-1019). Academic Press. https://doi.org/10.1016/B978-0-443-23763-8.00076-2

[5]. Faal G, Khatib Masjedi H, Sharifzadeh G, et al. Efficacy of zinc sulfate on indirect hyperbilirubinemia in premature infants admitted to neonatal intensive care unit: a double-blind, randomized clinical trial. BMC Pediatrics. 2020;20(1):130. https://doi.org/10.1186/s12887-020-02025-9

[6]. Cariaco Y, Almeida MP, Araujo EC, Briceño MP, Durán-Rodriguez AT, Franco RR, Espindola FS, Silva NM. Inhibition of Heme Oxygenase-1 by Zinc Protoporphyrin IX Improves Adverse Pregnancy Outcomes in Malaria During Early Gestation. Frontiers in Immunology. 2022 May 10;13:879158. https://doi.org/10.3389/fimmu.2022.879158

[7]. Ibrahim E, Mahmoud E, El-Samannody M, Ibrahim;, et al. Effect of zinc supplementation on serum bilirubin level in term neonates undergoing phototherapy. Int J Med Arts. 2020;2(4):736-40. https://doi.org/10.21608/IJMA.2020.31551.1131

[8]. Hamed AM, Ismael AH, Ragab MS. Comparison between oral zinc and agar with phototherapy in the treatment of neonatal jaundice: A prospective clinical trial study. Annals of Neonatology Journal. 2022 Jul 1;4(2):204-16. https://doi.org/10.21608/anj.2022.124661.1050

[9]. Das S, van Landeghem FK. Clinico pathological spectrum of bilirubin encephalopathy/kernicterus.Diagnostics.2019;9(1):24. https://doi.org/10.3390/diagnostics9010024

[10]. Mojtahedi SY, Izadi A, Seirafi G, et al. Risk Factors Associated with Neonatal Jaundice: A Cross-Sectional Study from Iran. Open Access Maced J Med Sci. 2018;6(8):1387-93. https://doi.org/10.3889/oamjms.2018.319

[11]. Iwuala C, Taylor SC. Structural and functional differences in skin of colour. Clin Exp Dermatol. 2022;47(2):247-50. https://doi.org/10.1111/ced.14892

[12]. Kumral A, Ozkan H, Duman N, et al. Breast milk jaundice correlates with high levels of epidermal growth factor. Pediatr Res. 2009;66(2):218-21. https://doi.org/10.1203/PDR.0b013e3181ac4a30.

[13]. Memon N, Weinberger BI, Hegyi T, et al. Inherited disorders of bilirubin clearance. Pediatr Res. 2016;79(3):378-86. https://doi.org/10.1038/pr.2015.247

[14]. Hansen TW. Core concepts: bilirubin metabolism. NeoReviews. 2010 Jun 1;11(6):e316-22. https://doi.org/10.1542/neo.11-6-e316

[15]. Voona MM. A study of complications in infants of Diabetic mothers. Medical and Research Publication. 2023. https://doi.org/10.21275/SR24722140302

[16]. Mohammad KI, Al–Shdefat M, Halasa S, Joseph R, Alafi M, ALBashtawy M, Alkhawaldeh A, Abdalrahim A, Malak M, Creedy D, Gamble J. Maternal and neonatal

factors associated with neonatal jaundice in Jordan: a case-control study. British Journal of Midwifery. 2024 Mar 2;32(3):126-34.
https://doi.org/10.12968/bjom.2024.32.3.126
[17]. Gleason CA, Juul SE. Avery's Diseases of the Newborn. Tenth Edition ed. Seattle, Washington: Elsevier, Health, Sciences;2017. https://doi.org/https://doi.org/10.1016/C2013-0-00320-9
[18]. Tessema M, Mekonnen H, Alemu T, Godie Y, Teklehaimanot WZ, Mengstie LA. Magnitude and its associated factors of neonatal jaundice among neonates admitted to the neonatal intensive care unit of Dessie Town public hospitals, Amhara region, Ethiopia, 2020: a multicenter cross-sectional study. Frontiers in Pediatrics. 2024 Jan 26; 12:1288604. https://doi.org/10.3389/fped.2024.1288604
[19]. Gao C, Guo Y, Huang M, et al. Breast Milk Constituents and the Development of Breast Milk Jaundice in Neonates: A Systematic Review. Nutrients. 2023;15(10). https://doi.org/10.3390/nu15102261
[20]. Boskabadi H, Zakeri Hamidi M, Goudarzi M. Investigating the effect of maternal risk factors in incidence of neonatal jaundice. Iran J Obstet Gynecol Infertil. 2012;15(34):1-6. https://doi.org/10.22038/IJOGI.2013.273
[21]. Isa HM, AlBuainain NY, Bunajem FY, et al. Neonatal and Maternal Risk Factors for Indirect Hyperbilirubinemia: A Cross-Sectional Study from Bahrain. Int J Pediatr. 2022; 2022:5199423. https://doi.org/10.1155/2022/5199423
[22]. Ayalew T, Molla A, Kefale B, et al. Factors associated with neonatal jaundice among neonates admitted at referral hospitals in northeast Ethiopia: a facility-based unmatched case-control study. BMC Pregnancy Childbirth. 2024;24(1):150. https://doi.org/10.1186/s12884-024-06352-y
[23]. Mitra S, Rennie J. Neonatal jaundice: aetiology, diagnosis and treatment. Br J Hosp Med. 2017;78(12):699-704. https://doi.org/10.12968/hmed.2017.78.12.699
[24]. Su H, Yang S, Chen S, Chen X, Guo M, Zhu L, Xu W, Liu H. What Happens in the Gut during the Formation of Neonatal Jaundice—Underhand Manipulation of Gut Microbiota? International Journal of Molecular Sciences. 2024 Aug 6;25(16):8582. https://doi.org/10.3390/ijms25168582
[25]. Roy-Chowdhury N, Wang X, Roy-Chowdhury J. Bile pigment metabolism and its disorders. InEmery and Rimoin's Principles and Practice of Medical Genetics and Genomics 2020 Jan 1 (pp. 507-553). Academic Press. https://doi.org/10.3390/ijms25168582
[26]. Hansen TW, Wong RJ, Stevenson DK. Molecular physiology and pathophysiology of bilirubin handling by the blood, liver, intestine, and brain in the newborn. Physiol Rev. 2020;100(3):1291-346. https://doi.org/10.1152/physrev.00004.2019
[27]. Zhou Y, Lee L, Ng S, et al. UGT1A1 haplotype mutation among Asians in Singapore. Neonatology. 2009;96(3):150-5. https://doi.org/10.1159/000209851
[28]. Dean E. Neonatal jaundice. Nurs Stand. 2016;30(44):15. https://doi.org/10.7748/ns.30.44.15.s17
[29]. Maisels MJ, McDonagh AF. Phototherapy for Neonatal Jaundice. N Engl J Med. 2008 Feb 28;358(9):920-8. DOI: [10.1056/NEJMra070860](https://doi.org/10.1056/NEJMra070860).
[30]. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004 Jul;114(1):297-316. DOI: [10.1542/peds.114.1.297](https://doi.org/10.1542/peds.114.1.297).
[31]. Bhutani VK, Johnson LH, Keren R. Diagnosis and management of hyperbilirubinemia in the term neonate: for a safer first week. Pediatr Clin North Am. 2004 Jun;51(4):843-61. DOI: [10.1016/j.pcl.2004.03.011] https://doi.org/10.1016/j.pcl.2004.03.011
[32]. Shapiro SM. Definition of the clinical spectrum of kernicterus and bilirubin-induced neurologic dysfunction (BIND). J Perinatol. 2005 Jul;25(1):54-9. DOI: [10.1038/sj.jp.7211276] (https://doi.org/10.1038/sj.jp.7211276).
[33]. Watchko JF. Bilirubin-Induced Neurotoxicity in the Preterm Neonate. Clin Perinatol. 2016 Jun;43(2):297-311. DOI: [10.1016/j.clp.2016.01.009] (https://doi.org/10.1016/j.clp.2016.01.009).
[34]. Zhou S, Wu X, Ma A, et al. Analysis of therapeutic effect of intermittent and continuous phototherapy on neonatal hemolytic jaundice. Exp Ther Med. 2019;17(5):4007-12. https://doi.org/10.3892/etm.2019.7432
[35]. Qian S, Kumar P, Testai FD. Bilirubin encephalopathy. Current Neurology and Neuroscience Reports. 2022 Jul;22(7):343-53. https://doi.org/10.1007/s11910-022-01204-8
[36]. Hegyi T, Kleinfeld A. Neonatal hyperbilirubinemia and the role of unbound bilirubin. The Journal of Maternal-Fetal & Neonatal Medicine. 2022 Dec 12;35(25):9201-7. https://doi.org/10.1080/14767058.2021.2021177
[37]. Rasiah S, Jegathesan T, Campbell DM, Shah PS, Sgro MD. Intravenous immunoglobulin G therapy for neonatal hyperbilirubinemia. Pediatric Research. 2023 Dec;94(6):2092-7. https://doi.org/10.1038/s41390-023-02712-0
[38]. van der Schoor LW, Verkade HJ, Bertolini A, de Wit S, Mennillo E, Rettenmeier E, Weber AA, Havinga R, Valášková P, Jašprová J, Struik D. Potential of therapeutic bile acids in the treatment of neonatal Hyperbilirubinemia. Scientific reports. 2021 May 27;11(1):11107. https://doi.org/10.1038/s41598-021-90687-5
[39]. Wang J, Guo G, Li A, Cai WQ, Wang X. Challenges of phototherapy for neonatal hyperbilirubinemia. Experimental and therapeutic medicine. 2021 Mar 1;21(3):1-. https://doi.org/10.3892/etm.2021.9662
[40]. Tham EH, Loo EXL, Goh A, et al. Phototherapy for neonatal hyperbilirubinemia and childhood eczema, rhinitis

and wheeze. Pediatr Neonatol. 2019;60(1):28-34. https://doi.org/10.1016/j.pedneo.2018.03.004
[41]. Faulhaber FR, Procianoy RS, Silveira RC. Side effects of phototherapy on neonates. Am J Perinatol. 2019;36(03):252-7. https://doi.org/10.1055/s-0038-1667379
[42]. Ashraf M, Ahmadshah F, Habibullah E, et al. Prophylactic effect of clofibrate on hyperbilirubinemia in very low birth weight twins. British Journal of PharmaceuticalResearch.2014;4(7):818-25. https://doi.org/10.4103/ijpvm.IJPVM_407_20
[43]. Nouri SA, Zarkesh M. Recent advances in adjuvant pharmacotherapy for neonatal indirect hyperbilirubinemia: A narrative review. Journal of Comprehensive Pediatrics. 2023 Dec 31(In Press). https://doi.org/10.5812/compreped-136461
[44]. Costa MI, Sarmento-Ribeiro AB, Gonçalves AC. Zinc: from biological functions to therapeutic potential. International Journal of Molecular Sciences. 2023 Mar 2;24(5):4822. https://doi.org/10.3390/ijms24054822
[45]. Elfarargy MS, Al-Ashmawy GM, Abu-Risha SE, Khattab H. Zinc supplementation in preterm neonates with jaundice: is it beneficial? Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2021 Oct 1;21(10):1929-34. https://doi.org/10.2174/1871530321666201223110241
[46]. Ali SR, Abdel-aal M, Elsamanoudy M, et al. Serum zinc level in neonates with indirect hyperbilirubinemia. International Journal of Medical Arts. 2020;2(1):217-22. https://doi.org/10.21608/IJMA.2019.18377.1038
[47]. Al-Matary A, AlGhamdi A, Alenaze B, Mandili RA, Alhawsawi DA, Magzoub D, Abu-Zaid A. Therapeutic benefits of zinc sulfate on neonatal hyperbilirubinemia. World Journal of Pediatrics. 2022 Apr;18(4):300-4. http://dx.doi.org/10.1007/s12519-022-00532-6
[48]. Hashemian S, Mohammadzadeh A, Farhat A, et al. The Therapeutic Effect of Zinc Sulfate on Neonatal Hyper-bilirubinemia. Iran J Neonatol. 2017;8(2):10-5. https://doi.org/10.22038/ijn.2016.7777
[49]. Boskabadi H, Maamouri G, Zakerihamidi M, Vatanchi AM, Sokhtanloo M, Mousavi MS, Ghahremani S, Bagheri F. Comparison of hyperbilirubinemia incidence between the newborns of zinc-taking and non-zinc-taking mothers during the third trimester of pregnancy. Caspian Journal of Internal Medicine. 2021;12(4):521. https://doi.org/10.22088/cjim.12.4.52
[50]. Mandlecha TH, Mundada SM, Gire PK, et al. Effect of Oral Zinc Supplementation on Serum Bilirubin Levels in Term Neonates With Hyperbilirubinemia Undergoing Phototherapy: A Double-blind Randomized Controlled Trial. Indian Pediatr. 2023;60(12):991-5. https://doi.org/10.1007/s13312-023-3061-4
[51]. Waheed A, Shirazi IH, Mustafa A, et al. Therapeutic effects of zinc sulphate in reduction of neonatal hyperbilirubinemia: an experimental study. Pak J Med Sci. 2019;30(2):36-9. https://doi.org/10.1007/s12519-022-00532-6
[52]. Khoshnevisasl P, Sadeghzadeh M, Kamali K, et al. Effect of Zinc on Hyperbilirubinemia of Newborns, a Randomized Double Blinded Clinical Trial. Curr Health Sci J. 2020;46(3):250-4. https://doi.org/10.12865/chsj.46.03.06
[53]. Stokowski LA. Fundamentals of phototherapy for neonatal jaundice. Adv Neonatal Care. 2011;11(6):303-12. https://doi.org/10.1016/j.adnc.2006.08.004
[54]. Kumar A, Bagri NK, Basu S, Asthana RK. Zinc supplementation for neonatal hyperbilirubinemia: a randomized controlled trial. Indian Pediatr. 2014;51(5): 375–8.

Saudi Journal of Health Research and Practice

Original Article

Evaluation of Training for Undergraduate Medical Students in Gynecological Ultrasonography Skills

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ABSTRACT The integration of ultrasound training into various clinical courses during undergraduate medical education is slowly expanding. In this study, we evaluated an ultrasound training program to enhance the integration of ultrasound education into the curricula of different medical colleges. We conducted a facility-based, cross-sectional study in which 202 fifth-year medical students participated in a course of eight ultrasound training sessions provided by four instructors over a 2-week period. Performance assessments comprised subjective pre- and post-training evaluation surveys completed by the students, along with an objective assessment completed by the trainers at the end of the training sessions. The Wilcoxon matched-pairs signed-rank test was used to evaluate the differences between the responses to the pre- and post-training survey questions, with values of $p<0.05$ considered significant. All tests revealed significantly better evaluation scores in the post-training survey. The objective assessment confirmed the trainers' satisfaction with the students' performance. Some medical colleges express concern regarding insufficient time and limited resources to incorporate ultrasound training into their educational programs. The present study provides a straightforward and effective training approach within the available faculty resources and curricular time.

Keywords: medical education, ultrasound education, ultrasound training, and undergraduate medical education.

INTRODUCTION

Recent years have seen a substantial growth in the use of ultrasound in clinical practice for image-guided therapies and bedside diagnostics across various medical and surgical fields [1,2]. For example, ultrasound has effectively replaced the stethoscope in the fields of cardiology [3], obstetrics [4], and gastroenterology [5]. Moreover, the application of ultrasonography is expanding into other fields, such as internal medicine, emergency medicine, surgery, anesthesia, and critical care [6,7]. The advent of portable and handheld ultrasound devices has facilitated the rapid development of focused ultrasonography, enabling doctors to conduct and evaluate imaging assessments at the patient's bedside [8]. To accommodate this increasing use, there is an immediate necessity to cultivate advanced ultrasound proficiency among professionals across different specialties [9]. In response to this demand, several colleges, universities, and accreditation bodies have initiated the implementation of ultrasound competency criteria for medical students and residents. Numerous medical colleges have incorporated ultrasound into all 4 years of their medical training programs [10,11], thereby empowering students to develop the skills and knowledge required to become leaders and educators in ultrasonography [12–14]. Several studies have indicated that even 8 weeks of ultrasound teaching can significantly enhance the diagnostic capabilities of medical students and residents [15–17]. Nevertheless, other studies have shown that institutions have been slow to add ultrasound training to their medical programs because of limited course schedules, high equipment costs, and a shortage of qualified instructors [18–20]. Furthermore, the growing demands of medical school courses and the significant costs and time required for training make it difficult for medical schools to include ultrasound training [21,22]. In a study conducted in Saudi Arabia, Hendi concluded that undergraduate medical students were able to gain adequate skills and satisfaction in ultrasound procedures following a short training course [23]. Thus, certain institutions have structured their education programs to enhance relevant instruction in anatomy, physiology, and physical examinations [24]. This has led to enhanced proficiency and self-assurance among students regarding the use of ultrasonography. Despite these advances, there remains a need to establish precise guidelines for evidence-based practices in undergraduate ultrasonography education [25,26]. At Jazan University, we started to integrate obstetrics and gynecological ultrasound teaching into our curricular modules in 2017. However, we have not assessed the impact of this training on students' confidence and proficiency in conducting ultrasound examinations. In the present study, we aimed to evaluate the effect of ultrasonography training during curricular clinical courses on students' proficiency in the performance of ultrasound examinations.

MATERIALS AND METHODS

Setting, subjects, and data collection

The Faculty of Medicine at Jazan University, a prominent public university established in 2006, is located in Jazan City, Jazan Region, in southern Saudi Arabia. This city, which is situated along the Red Sea coast, is known for its rich culture and scenic landscapes. We conducted the present study as part of an obstetrics and gynecology course covering fifth-year medical students. The study included 202 students, both male and female, who were regular students in the College of Medicine and enrolled in the course at the time of the study. All materials were taken from the curricular course of obstetrics and gynecology at Jazan University, which was adapted from the ISUOG basic training for gynecological ultrasound [27,28]. A previous study found that a few weeks of ultrasound training was effective for acquiring both theoretical knowledge and practical skills [28]. Another study revealed that a 2-week ultrasound training program significantly enhanced the skills of medical students [29]. Thus, we designed a 2-week course of ultrasound training sessions for all 202 students who participated in the study and completed their gynecology course in the 2022–2023 academic year. The training program began with a lecture on the principles of ultrasound. A simulation skill laboratory is a well-equipped clinical laboratory that caters to the specific needs of a clinical specialty program. We conducted our training program in this type of skill laboratory under the guidance of four ultrasonography-trained faculty members. We divided the students into four groups and assigned each group a single ultrasound machine and two ultrasound training models (BPOB1220 and BPOB1210; Blue Phantom Company) featuring normal gynecological anatomy. One model included an adnexal mass, while the other included uterine fibroid pathology. The duration of the program was four sessions conducted on 2 days per week over a 2-week period. Before the first training session and after the final session, the participants were requested to complete the questionnaires. The pre- and post-training surveys both evaluated each participant's self-reported confidence level in their ability to perform various basic ultrasound skills (Appendix-1 and Appendix-2) using a 5-point Likert scale (1 = strongly disagree to 5 = strongly agree). The trainers concluded the final session by posing objective survey questions (checklist below) to evaluate and assess the students' real-time hands-on skills, which they quantified using objective numerical scores. After completing the program, the participants completed a post-training survey to share their ultrasound session experiences and evaluate its efficacy. Each session lasted 4 hours, beginning with a demonstration of how to operate the machine, introduce the probe into the phantom model, and perform the movements necessary to locate the pathology. Each participant was allowed approximately 20 minutes to perform all the listed sonographic skills. The trainers, who were faculty members and experts in teaching ultrasonography for many years with guaranteed validity, utilized a 22-point Likert scale checklist [28] to assess the students' performance in gynecological ultrasound following the training sessions. The questionnaire and structured checklist used in this study were validated by the Department of Gynecology and Medical Education Units 5 years previously. These products were used during structured ultrasound training to achieve the learning outcomes in the gynecology 2 course, including competence in basic skills, patient approach, preparing the necessary equipment, operating the ultrasound machine, focusing on the correct images, and diagnosing the pathology seen.

The checklist included the following skills:

Greet the patient.
Introduce yourself.
Explain the procedure to the patient.
Check that the required equipment is available.
Explain the need to have an empty bladder.
Explain the need for the lithotomy position on the examination table.
Press the button for a new patient on the machine.
Select the transvaginal probe and menu.
Put on gloves.
Place gel on the tip of the probe and attach the probe cover.
Check the orientation of the probe and screen.
Introduce the probe into the vagina.
Visualize the uterus on the longitudinal axis.
Conduct a panning movement to view the right and left sides of the uterus and adnexa on the longitudinal axis.
Correctly rotate the probe to view the uterus in a transverse section.
Move the probe correctly to observe the cervix and uterus transversely at different levels.
Conduct a panning movement to view the right and left sides of the uterus and adnexa in the transverse axis.
Freeze the image each time.
Measure the endometrial thickness and any fibroids observed.
Remove the probe correctly and dispose of the cover.
Give the patient a towel for cleaning and inform her that she has privacy to dress.
Mention what findings are necessary to document in the report.

INCLUSION CRITERIA

All 202 students enrolled in the obstetrics and gynecology course in 2022 were eligible for the study.

EXCLUSION CRITERIA

The study excluded any students who had previously received ultrasound training.

STATISTICAL ANALYSIS

The data were collected and transferred to a Microsoft Excel sheet. We performed statistical analyses using SPSS Statistics version 25 (IBM Corporation, Armonk, NY). Values of $p<0.05$ were considered to indicate statistical significance. We tested normality using the Shapiro–Wilk test and the Kolmogorov–Smirnov test. We performed a Wilcoxon matched-pairs signed-rank test to evaluate the differences between the responses to the pre- and post-training survey questions. We conducted a one-way ANOVA to analyze the inter-skill differences between students.

RESULTS

The primary aim of the study was to assess the feasibility of teaching ultrasound to medical students during their obstetrics and gynecology course. We included all 202 fifth-year medical students, with a male-to-female ratio of 1:1. We found that the median score after the training sessions was significantly higher than the median score before the sessions (p=0.000; Table 1) when we analyzed the sums of the subjective responses to the pre- and post-training surveys. Four panels (Figure 1)

Table 1. Overall confidence in performing ultrasound scan skills based on a comparison between the pre- and post-training survey self-assessment scores

Self-assessment score	Mean	SD	Median	Min	Max	*p*-value
Pre-training survey summed self-assessment score	47.1	18.5	**48.0**	18.0	90.0	**0.000***
Post-training survey summed self-assessment score	82	12.9	**89**	17	90	

*The *p*-value was obtained using the Wilcoxon matched-pairs test (*n*=202).

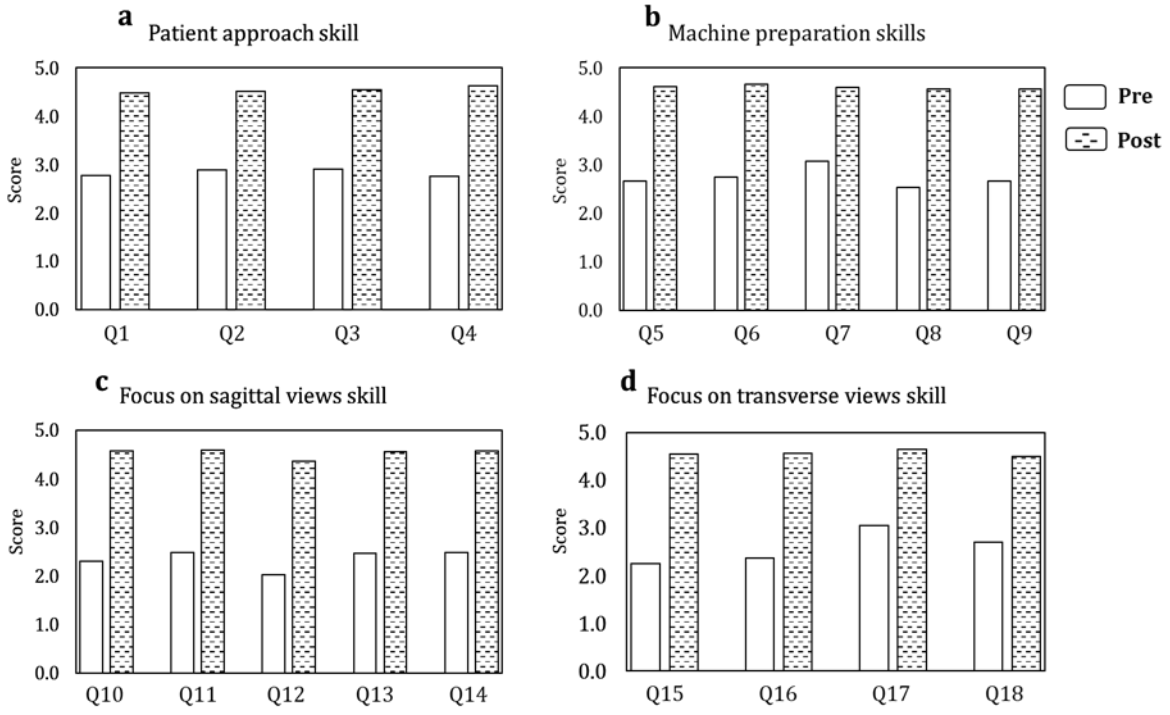

Figure 1: The unshaded bars represented the students' pre-training subjective levels of confidence in their ability to perform a series of ultrasound-related tasks independently, while the shaded bars displayed the scores in the post-session surveys.

were created to separately analyze the tasks in the surveys that evaluated specific types of ultrasound skills. Specifically, panel A assessed "patient approach skills" (Q1–4), panel B assessed "machine preparation skills" (Q5–9), panel C assessed "focus on sagittal views skills" (Q10–14), and panel D assessed "focus on transverse views skills" (Q15–18). Across all questions in the pre-training survey regarding confidence in completing the

covered ultrasound-related tasks independently, most of the students reported that they disagreed or strongly disagreed. For the same questions in the post-training survey, the students largely and somewhat strongly agreed that they could confidently complete all the covered ultrasound-related tasks independently. The trainers conducted comparisons between the students' scores assigned for each set of related skills. There was no significant difference in the performance of the students for the different parts of the training sessions (p=0.20 Table 2). After completing the training program, the items shown in Table 3

Table 2. One-way ANOVA of students' scores for each set of skills as assessed by the trainers (p=0.203482)

Skill	Count	Mean score (out of 5)	SD	Variance
Skill set 1 (Q1–4) Patient approach	202	4.71	0.61	0.17
Skill set 2 (Q5–9) Machine preparations	202	4.62	0.95	0.30
Skill set 3 (Q10–15) Focus on sagittal views	202	4.63	0.86	0.37
Skill set 4 (Q16–22) Focus on transverse views	202	4.71	0.72	0.36

SD: standard deviation

Table 3. Perceptions of students toward the training sessions

Perceptions of the students		Strongly disagree	Agree	Somewhat agree	Agree	Strongly agree
I feel that the ultrasound training sessions were enjoyable.	*N*	4	4	12	32	150
	%	2.0	2.0	5.9	15.8	74.3
I feel that the ultrasound training sessions effectively taught me overall basic tactile ultrasound skills.	*N*	4	2	14	35	147
	%	2.0	1.0	6.9	17.3	72.8
I feel that ultrasound training sessions like those I just completed are beneficial for medical students.	*N*	4	3	11	28	156
	%	2.0	1.5	5.4	13.9	77.2

provided the participants with an opportunity to share their perceptions about the ultrasound training sessions and their effectiveness. Most of the students strongly agreed that the ultrasound training sessions were enjoyable (74.3%) and effectively taught them the overall basic tactile ultrasound skills (72.8%). Most of the students also strongly agreed that such sessions are beneficial for medical students (77.2%).

DISCUSSION

General conclusions

The primary aim of this study was to assess and evaluate ultrasound training for medical students in an obstetrics and gynecology course. The results demonstrated a significant improvement in the confidence and perceived competence of fifth-year medical students at Jazan University in performing ultrasound-related tasks following a structured training program. The pre- and post-training program analysis revealed a marked increase in the median self-assessment scores, indicating that the training sessions were effective in enhancing the students' confidence in their ultrasound skills (p=0.000). These findings are consistent with a previous study conducted in Saudi Arabia, which found that medical students acquired sufficient skills and expressed satisfaction with their skills in using ultrasound [23]. Similarly, a 2-week point-of-care ultrasound training program was found to significantly enhance the skills of medical students [30], while trained students were observed to have better OSCE scores and self-reported confidence than their peers without such

training [31]. When examining specific types of ultrasound skills, the study found consistent improvements across all assessed areas: patient approach, machine preparation, focus on sagittal views, and focus on transverse views. Initially, most students reported low confidence in independently completing the ultrasound tasks. However, post-training, there was a significant shift toward higher confidence levels, with most students agreeing or strongly agreeing that they could perform these tasks independently. Therefore, medical students can acquire diagnostic-quality images across various body regions after only a brief training program early in their medical education. These findings are consistent with those of other medical programs that offer ultrasound training to students at an early stage [2,18].

Assessments

In the subjective data reported by the individual participants, the summed score for the pre-training self-assessment had a median of 48. Considering that the maximum score was 90, this low value indicates that the study subjects possessed unsatisfactory knowledge/practice prior to the training sessions. Meanwhile, the summed score after the training sessions increased to a median of 89. Thus, relative to the maximum score of 90, the students exhibited excellent knowledge/practice after completing the ultrasound training sessions. We grouped the survey questions that evaluated specific types of ultrasound skills together during the analysis. The results showed that the students largely agreed, and some strongly agreed, that they could confidently complete all the covered ultrasound-related tasks independently after completing the training sessions. These findings align with those in the study by Oteri et al. [1]. Interestingly, the one-way ANOVA comparing students' performance across different skill sets as assessed by trainers did not show significant differences (p=0.203482). This suggests that the training program was uniformly effective across all skill areas, providing a balanced and comprehensive enhancement of students' ultrasound capabilities (Table 2) Furthermore, the students' perceptions of the training sessions were overwhelmingly positive. A substantial majority strongly agreed that the sessions were enjoyable (74.3%) and effective for teaching basic tactile ultrasound skills (72.8%). An even higher percentage (77.2%) strongly agreed that such training sessions are beneficial for medical students. These findings highlight the perceived value and enjoyment of the training program, which could contribute to higher engagement and better learning outcomes. These findings are consistent with those reported by Mullen et al. [24]. The analysis by sex revealed some intriguing insights. Female students had higher pre-training self-assessment scores than their male counterparts (p=0.001), while male students had a higher median score than female students post-training (p=0.000). These findings suggest that, while female students started with higher confidence, male students experienced a greater relative increase in confidence following the training (Table 4). The trainer-assigned scores also showed a significant difference, with female students receiving higher scores than male students (p=0.000), indicating that female students performed better in the practical assessments (Table 5).

Table 4: Association between gender and self-assessment scores, Mann Whitney U test, n=202

Gender		N	Mean	SD	Median	P value
Pre-assessment sum score	Male	102	42.9	18.4	41	0.001*
	Female	100	51.4	17.6	54	
Post-assessment sum score	Male	102	85.0	12.5	90	0.000*
	Female	100	79.1	12.6	83	

Table 5: Association between gender and trainers score, Mann Whitney U test, n=202

Gender		N	Mean	SD	Median	P value
Trainers scores	Male	102	99.6	12.3	104.0	**0.000***
	Female	100	106.0	5.5	108.5	

These findings are like those in the study conducted by Hendi [23]. Our research highlights the impact of structured ultrasound training on enhancing the confidence and competence of medical students. The positive reception and significant skill improvements suggest that the incorporation of such training into the medical curriculum could be highly beneficial. This aligns with the study by Reem et al. [32], which demonstrated the feasibility of integrating a structured ultrasound training program into obstetrics and gynecology residency curricula. Future studies could explore the longitudinal outcomes to assess the long-term retention of skills and

confidence, as well as the impact on clinical practice. Future studies could also investigate the reasons behind the observed sex differences with the aim of tailoring training programs to address the specific needs of all students.

Study limitations

The present study has some potential limitations. The first limitation was the selection of participants. One could argue that the study evaluated students in their final years of medical college, and these students would possess advanced clinical knowledge and skills. Second, we were unable to provide any retention data for the study, which would have allowed us to assess how our training program influenced students' performance during their clerkships. Finally, we recognize that the students were engaged with various subjects within their course and had a demanding schedule filled with numerous activities related to their obstetrics and gynecology major module. Therefore, we cannot be certain whether any of the students had the opportunity to enhance their training with supplementary educational materials during the study.

Future directions

We aim to develop and evaluate new variations of condensed training programs before clinical clerkships for third- and fourth-year medical students. The programs will concentrate on evidence-based techniques for utilizing ultrasound to recognize anatomy and pathology while also refining specific and technical ultrasound skills. We further aim to promote medical education programs in medical colleges that are hesitant to incorporate ultrasound training into the pre-clerkship years. We also plan to examine the findings of the present study more deeply by establishing a skill retention study. This will involve performance comparisons between our study participants and a group of control participants for ultrasound-related tasks during clinical clerkships. Conducting similar evaluations after the students have entered residency would also be beneficial for assessing the retention of ultrasound skills and the performance of ultrasound scanning.

CONCLUSIONS

Medical colleges can integrate an ultrasound training program into their obstetrics and gynecology curricula, along with other specialty courses. By utilizing the resources available in university laboratories and hospitals, these programs can be efficiently completed on 4 days over a 2-week period, while incurring minimal expenses. It is possible for four faculty members to train 200 students using two ultrasound machines and two training models. The materials and models can be tailored to meet the students' requirements and the availability of session instructors within each specialty.

INFORMED CONSENT STATEMENT

Ethical approval was given on February 2023 from Standing Committee for Scientific Research - Jazan University (HAPO-10-Z-001).
Reference No.:REC-44/08/568. All student enrolled themselves in the course, so a verbal consent was taken before the training session started.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article. The de-identified datasets used during this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS:

Isameldin Elamin. Medani conceptualized and wrote the final draft of the article. Ahmed Altraifi wrote the initial draft of the article. Ahalm. Hakami provides research material and organized the data.
Ali. Khormi. provided research materials and organized data. Uma. Chourasia. provides research material and organized the data. Maha Murtada provided research materials and collected and organized data. Abeer Salihmethodology, and data analysis. Yasir Salih designed this study and wrote the initial draft of the article.
All authors have read and agreed to the published version of the manuscript

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS:

US: ultrasound
ISOUG: International Society of Ultrasound in Obstetrics and Gynecology
OSCE: objective structured clinical examination
ANOVA: statistical test
POCUS: Point-of-care ultrasound

REFERENCES

[1]. Oteri V, Occhipinti F, Gribaudo G, Marastoni F, Chisari E. Integration of ultrasound in medical School: Effects on Physical Examination Skills of Undergraduates. Med Sci Educ. 2020 Mar 5;30(1):417–27. DOI: 10.1007/s40670-020-00921-4
[2]. Nelson BP, Hojsak J, Dei Rossi E, Karani R, Narula J. Seeing Is Believing: Evaluating a Point-of-Care

Ultrasound Curriculum for 1st-Year Medical Students. Teach Learn Med [Internet]. 2017 Jan 2 [cited 2024 Oct 10];29(1):85–92. Available from: https://pubmed.ncbi.nlm.nih.gov/27191830/
[3]. Filipiak-Strzecka D, Lipiec P, Kasprzak JD. Handheld ultrasound in cardiology: Current perspective. Adv Clin Exp Med [Internet]. 2023 Mar 1 [cited 2024 Oct 11];32(3):267–74. Available from: https://pubmed.ncbi.nlm.nih.gov/36974343/
[4]. Leung KY. Applications of advanced ultrasound technology in obstetrics. Diagnostics [Internet]. 2021 Jul 1 [cited 2024 Oct 11];11(7):1217. Available from: https://www.mdpi.com/2075-4418/11/7/1217/htm
[5]. Maconi G, Hausken T, Dietrich CF, Pallotta N, Sporea I, Nurnberg D, et al. Gastrointestinal Ultrasound in Functional Disorders of the Gastrointestinal Tract - EFSUMB Consensus Statement. Ultrasound Int Open [Internet]. 2021 Apr 1 [cited 2024 Oct 11];7(1):E14–24. Available from: http://www.thieme-connect.de/products/ejournals/html/10.1055/a-1474-8013
[6]. Boccatonda A, D'Ardes D, Tallarico V, Vicari S, Bartoli E, Vidili G, et al. Gastrointestinal Ultrasound in Emergency Setting. J Clin Med [Internet]. 2023 Feb 1 [cited 2024 Oct 10];12(3):799. Available from: https://www.mdpi.com/2077-0383/12/3/799/htm
[7]. Hollerweger A, Maconi G, Ripolles T, Nylund K, Higginson A, Serra C, et al. Gastrointestinal Ultrasound (GIUS) in Intestinal Emergencies - An EFSUMB Position Paper. Ultraschall in der Medizin. 2020 Dec 1;41(6):646–57. DOI: 10.1055/a-1147-1295
[8]. Nuernberg D, Saftoiu A, Barreiros AP, Burmester E, Ivan ET, Clevert DA, et al. EFSUMB Recommendations for Gastrointestinal Ultrasound Part 3: Endorectal, Endoanal and Perineal Ultrasound. Ultrasound Int Open [Internet]. 2019 [cited 2024 Oct 11];5(1):E34–51. Available from: http://www.thieme-connect.com/products/ejournals/html/10.1055/a-0825-6708
[9]. Celebi N, Griewatz J, Malek NP, Krieg S, Kuehnl T, Muller R, et al. Development and implementation of a comprehensive ultrasound curriculum for undergraduate medical students - A feasibility study. BMC Med Educ [Internet]. 2019 May 28 [cited 2024 Oct 11];19(1):1–8. Available from: https://link.springer.com/articles/10.1186/s12909-019-1611-1
[10]. Díaz-Gómez JL, Mayo PH, Koenig SJ. Point-of-Care Ultrasonography. Ingelfinger JR, editor. New England Journal of Medicine [Internet]. 2021 Oct 21 [cited 2024 Oct 11];385(17):1593–602. Available from: https://www.nejm.org/doi/full/10.1056/NEJMra1916062
[11]. Nicholas E, Ly AA, Prince AM, Klawitter PF, Gaskin K, Prince LA. The Current Status of Ultrasound Education in United States Medical Schools. Journal of Ultrasound in Medicine. 2021 Nov 15;40(11):2459–65 DOI: 10.1002/jum.15633
[12]. Bacon DR, Cowles K, Thapa D, White A, Allen AJ, Doughton J, et al. Creating an ultrasound scholarly concentration program for medical students. Adv Med Educ Pract [Internet]. 2021 [cited 2024 Oct 11];12:1103–10. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=dame20
[13]. Allen AJ, White AB, Bacon DR, Dallaghan GLB, Jordan SG. Commentary on Ultrasound Instruction in Undergraduate Medical Education: Perspective from Two Students. Adv Med Educ Pract [Internet]. 2023 [cited 2024 Oct 11];14:1–7. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=dame20
[14]. Minardi J, Ressetar H, Foreman T, Craig K, Sharon M, Bassler J, et al. Longitudinal Ultrasound Curriculum Incorporation at West Virginia University School of Medicine: A Description and Graduating Students' Perceptions. Journal of Ultrasound in Medicine [Internet]. 2019 Jan 1 [cited 2024 Oct 11];38(1):63–72. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jum.14662
[15]. Prats M, Boone K, Gorgas D, Bahner D. Integrating ultrasound education and resources within undergraduate medical education in order to Bring Ultrasound Internationally for Long-term Development (BUILD). Med Ultrason [Internet]. 2022 [cited 2024 Oct 11];24(2):153–9. Available from: https://pubmed.ncbi.nlm.nih.gov/35045141/
[16]. Wong CK, Hai JJ, Chan KYE, Un KC, Zhou M, Huang D, et al. Point-of-care ultrasound augments physical examination learning by undergraduate medical students. Postgrad Med J [Internet]. 2021 Jan 1 [cited 2024 Oct 11];97(1143):10–5. Available from: https://dx.doi.org/10.1136/postgradmedj-2020-137773
[17]. [17] Glass C, Sarwal A, Zavitz J, Nitsche J, Joyner JN, Johnson LL, et al. Scoping review of implementing a longitudinal curriculum in undergraduate medical education: The wake forest experience. Ultrasound Journal [Internet]. 2021 Dec 1 [cited 2024 Oct 11];13(1):1–21. Available from: https://link.springer.com/articles/10.1186/s13089-021-00206-w
[18]. [18] Cevik AA, Cakal ED, Abu-Zidan F. Point-of-care Ultrasound Training During an Emergency Medicine Clerkship: A Prospective Study. Cureus [Internet]. 2019 Nov 11 [cited 2024 Oct 11];11(11). Available from: /pmc/articles/PMC6844539/
[19]. [19] Xantus G, Peczelák P, Hegyi K, Kanizsai P. [Bedside ultrasound in adult primary care]. Orv Hetil [Internet]. 2022 Dec 25 [cited 2024 Oct 11];163(52):2067–71. Available from: https://pubmed.ncbi.nlm.nih.gov/36566439/
[20]. [20] Dietrich CF, Hoffmann B, Abramowicz J, Badea R, Braden B, Cantisani V, et al. Medical Student

Ultrasound Education: A WFUMB Position Paper, Part I. Ultrasound Med Biol [Internet]. 2019 Feb 1 [cited 2024 Oct 11];45(2):271–81. Available from: http://www.umbjournal.org/article/S0301562918304174/fulltext
[21]. Russell FM, Zakeri B, Herbert A, Ferre RM, Leiser A, Wallach PM. The State of Point-of-Care Ultrasound Training in Undergraduate Medical Education: Findings from a National Survey. Academic Medicine [Internet]. 2022 May 1 [cited 2024 Oct 11];97(5):723–7. Available from: https://journals.lww.com/academicmedicine/fulltext/2022/05000/the_state_of_point_of_care_ultrasound_training_in.32.aspx
[22]. Ireson M, Warring S, Medina-Inojosa JR, O'malley MT, Pawlina W, Lachman N, et al. First year medical students, personal handheld ultrasound devices, and introduction of insonation in medical education. Ann Glob Health. 2019;85(1).
[23]. Hendi AM. Effectiveness of a Short Course on Undergraduate Medical Students' Acquisition of Basic Ultrasound Skills: Findings from a Saudi University. Saudi J Med Med Sci. 2022 Sep-Dec;10(3):253-258. doi: 10.4103/sjmms.sjmms_560_21. Epub 2022 Sep 7. PMID: 36247054; PMCID: PMC9555041.
[24]. Mullen A, Kim B, Puglisi J, Mason NL. An economical strategy for early medical education in ultrasound. BMC Med Educ [Internet]. 2018 Jul 18 [cited 2024 Oct 11];18(1):1–8. Available from: https://bmcmededuc.biomedcentral.com/articles/10.1186/s12909-018-1275-2
[25]. Johnson CD, Davison L, Graham EC, Sweeney EM, Johnson C. Ultrasound technology as a tool to teach basic concepts of physiology and anatomy in undergraduate and graduate courses: a systematic review. https://doi.org/101152/advan001992023 [Internet]. 2024 Sep 5 [cited 2024 Oct 11]; Available from: https://journals.physiology.org/doi/10.1152/advan.00199.2023
[26]. Edwards C, Tunny R, Allen H, Bowles D, Farley A, O'Hara S, et al. Sonographer training pathways - a discussion paper on curriculum design and implementation. International Journal of Work-Integrated Learning [Internet]. 2024 May 10 [cited 2024 Oct 11]; Available from: https://www.ijwil.org/
[27]. "Basic Training Guidelines." Accessed: Nov. 27, 2024. [Online]. Available: https://www.isuog.org/resource/isuog-education-committee-recommendations-for-basic-training-in-obstetric-and-gynecological-ultrasound-pdf.html
[28]. Jazan University Department of Obstetrics and Gyn, "Ultrasound Checklist," Jizan, Aug. 2019
[29]. J. Urbina, S. M. Monks, and S. B. Crawford, "Simulation in Ultrasound Training for Obstetrics and Gynecology: A Literature Review," Donald School Journal of Ultrasound in Obstetrics and Gynecology, vol. 15, no. 4, pp. 359–364, Dec. 2021, doi: 10.5005/jp-journals-10009-1816.
[30]. M. J. Taylor, A. Gittens, D. Beaubian, J. Grady, and M. K. Herbst, "Resident Instruction: Improving End-of-Year Medical Student Ultrasound Performance," Fam Med, vol. 56, no. 10, pp. 668–671, Oct. 2024, doi: 10.22454/FAMMED.2024.326354.
[31]. "STFM Journals." Accessed: Nov. 27, 2024. [Online]. Available: https://journals.stfm.org/
[32]. Abu-Rustum RS, Berwick M, Heft J. Successful Implementation of the AIUM Standardized 4-Year Residency Ultrasound Curriculum in Obstetrics and Gynecology: Lessons Learned and Way Forward. J Ultrasound Med. 2024 Jun;43(6):1109-1119. doi: 10.1002/jum.16439. Epub 2024 Mar 3. PMID: 38433458.

Appendix 1

Name: …… University number: …… Pre-training self-assessment

Steps	strongly disagree	disagree	Somewhat agree	Agree	strongly agree
1. I can explain the trans-vaginal scan procedure to the patient including the steps, indication and benefit from the scan then take consent.					
2. I can check for all the equipment required for the exam					
3. I feel confident that I can position the patient correctly on the examination table					
4. I know where to position the ultrasound machine in relation to the examination table					
5. I feel confident that I can press new patient on the machine and enter the patient data					
6. I feel confident that I can select the transvaginal probe on the machine					
7. I am sure how to put gel on the tip of the probe and put the probe cover (condom)					
8. I know how to check the orientation of the probe and screen					
9. I feel confident that I can introduce the probe into the vagina.					
10. I feel confident that I can visualize the uterus on longitudinal axis					
11. I feel confident that I can freeze the image					
12. I feel confident that I can measure the endometrial thickness					
13. I feel confident that I can do panning movement to view the right and left side of the uterus and adnexa in longitudinal axis.					
14. I feel confident that I can correctly rotate the probe to view the uterus in a transverse section					
15. I feel confident that I can move the probe correctly to see the cervix and uterus transversely at different levels.					
16. I feel confident that I can do panning movement to see the right and left sides of the uterus and adnexa on transverse axis					
17. I can remove the probe correctly and dispose the cover.					
18. I feel confident that I can document my findings and write the report					

Appendix-2
Name: ……University number: ……post-training self-assessment

Steps	strongly disagree	disagree	Somewhat agree	agree	strongly agree
1. I can explain the trans-vaginal scan procedure to the patient including the steps, indication and benefit from the scan then take consent.					
2. I can check for all the equipment required for the exam					
3. I feel confident that I can position the patient correctly on the examination table					
4. I know where to position the ultrasound machine in relation to the examination table					
5. I feel confident that I can press new patient on the machine and enter the patient data					
6. I feel confident that I can select the transvaginal probe on the machine					
7. I am sure how to put gel on the tip of the probe and put the probe cover (condom)					
8. I know how to check the orientation of the probe and screen					
9. I feel confident that I can introduce the probe into the vagina.					
10. I feel confident that I can visualize the uterus on longitudinal axis					
11. I feel confident that I can freeze the image					
12. I feel confident that I can measure the endometrial thickness					
13. I feel confident that I can do panning movement to view the right and left side of the uterus and adnexa in longitudinal axis.					
14. I feel confident that I can correctly rotate the probe to view the uterus in a transverse section					
15. I feel confident that I can move the probe correctly to see the cervix and uterus transversely at different levels.					
16. I feel confident that I can do panning movement to see the right and left sides of the uterus and adnexa on transverse axis					
17. I can remove the probe correctly and dispose the cover.					
18. I feel confident that I can document my findings and write the report					
19. I feel that the ultrasound training sessions were enjoyable					
20. I feel that the ultrasound training sessions effectively taught me overall basic tactile ultrasound skill					
21. I feel that ultrasound training sessions like those I just completed are beneficial for medical students.					

Saudi Journal of Health Research and Practice

Case Report

From Diagnosis to Treatment: Exploring Vascular Thrombosis in Antiphospholipid Syndrome Through a Case Report and the Literature1

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ABSTRACT Antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by recurrent arterial or venous thrombosis and pregnancy morbidity in the presence of antiphospholipid antibodies. Maintaining therapeutic anticoagulation is critical to prevent recurrent thrombotic events, particularly in high-risk individuals. Case Presentation: We present the case of a 33-year-old male patient with primary APS, a history of recurrent thrombotic events, and poor compliance with anticoagulation therapy who presented with sudden-onset chest pain and dyspnea. Imaging confirmed pulmonary embolism with a solitary pulmonary nodule and ground-glass opacities. Echocardiography revealed moderate right ventricular dilatation, a small pericardial effusion, and an inferior vena cava fibrin clot. Laboratory findings confirmed primary APS with positive lupus anticoagulant and elevated β2 glycoprotein I IgG. The patient was treated with low-molecular-weight heparin bridged to warfarin, achieving a therapeutic INR, and discharged on lifelong anticoagulation. Discussion: This case underscores the high risk of recurrent thrombosis in APS patients who discontinue anticoagulation therapy. It highlights the importance of a multidisciplinary approach to case management, patient education, and adherence to anticoagulation therapy. Additionally, current management strategies, challenges in risk stratification, and emerging therapies are discussed in this report, emphasizing the potential for rapid recurrence upon treatment discontinuation. Conclusion: Lifelong anticoagulation remains the cornerstone of APS management to prevent recurrent thrombotic events. Close monitoring, patient education, and a multidisciplinary approach are essential to optimize outcomes and prevent complications in APS patients.

Keywords: Antiphospholipid Syndrome; Antiphospholipid Antibodies; Lupus Anticoagulant; Anticardiolipin; B2 Glycoprotein I; Thrombosis.

INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder initially described by Graham Hughes and colleagues in the early 1980s. [1] It is characterized by recurrent arterial or venous thrombosis and/or pregnancy complications, such as fetal loss and stillbirth, in the presence of antiphospholipid (aPL) antibodies. The most common aPL antibodies associated with APS are lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β2 glycoprotein I (β2GPI). [2] This syndrome affects both sexes; however, it is more commonly recognized in women. APS accounts for a significant proportion of thrombotic events among young individuals without other known risk factors. [3] Unlike APS, catastrophic APS (CAPS) is characterized by multi-organ thrombosis within a short period, also known as a thrombotic storm. [4]

APS presents significant diagnostic and therapeutic challenges due to its heterogeneous manifestations, varying antibody profiles, and complex pathophysiological mechanisms. [5,6] The revised Sydney classification criteria remain the gold standard for diagnosing APS. A combination of clinical events (thrombosis or pregnancy morbidity) and persistently elevated aPL levels, detected at least 12 weeks apart, is required. [7] The management of APS involves a multidisciplinary approach, and the mainstay of treatment is anticoagulation with heparin, followed by warfarin. The current consensus is for lifelong anticoagulation as the risk of recurrence is high. [8]

CASE PRESENTATION

A 33-year-old male patient with a history of primary APS presented to the emergency department with sudden-onset

chest pain radiating to the back, exacerbated by movement and associated with exertional dyspnea. The patient was diagnosed with APS 2 years prior and had been on 15 mg warfarin daily but had poor follow-up compliance and discontinued his anticoagulation in the last 5 days. He reported easy fatigability when climbing stairs but denied photosensitivity, skin lesions, oral/genital ulcers, weight loss, sicca symptoms, or joint involvement. His medical history was significant for multiple thrombotic events, including bilateral deep vein thrombosis (DVT) of the lower limbs (8 years and 2 years ago), pulmonary embolism (PE) requiring ICU admission 4 years ago, and cardiac thrombus 2 years ago.

On examination, the patient was alert, conscious, and oriented. Vital signs showed a blood pressure of 134/94 mmHg, a mean arterial pressure of 103 mmHg, and oxygen saturation of 100% on room air. Arterial blood gas analysis indicated respiratory alkalosis. The laboratory findings are presented in Table 1.

Table 1: Laboratory findings of the patient.

Laboratory Findings

Test	Result	Reference Range
INR	1.4	2.0-3.0
PTT	51 seconds	25-35 seconds
PT	29.7 seconds	11-13.5 seconds
WBC	3.2 x 10^9/L	4.5-11.0 x 10^9/L
Platelets	113 x 10^9/L	150-450 x 10^9/L
ESR	45 mm/hr	0-22 mm/hr
CRP	4.28 mg/L	<3.0 mg/L
D-dimer	12 µg/mL	<0.5 µg/mL
Troponin	41 ng/L	<14 ng/L

Regarding imaging studies, CT angiography demonstrated multiple filling defects in the right lower pulmonary artery and its branches, consistent with PE. A 1 cm solitary pulmonary nodule was noted in the right middle lobe, along with bilateral lower lobe ground-glass opacities (Figure 1A and 1B). Echocardiography showed normal left ventricular function (EF 55%), moderate right ventricular dilatation with normal function, and a small pericardial effusion. A small, flickering fibrin clot was observed in the inferior vena cava orifice. Venous duplex ultrasound of the lower limbs revealed chronic DVT in both common femoral veins and a new thrombus in the left popliteal vein (Figure 2A–C). Brain CT was unremarkable.

Immunological workup confirmed positive LA and elevated β2GPI IgG (79 U/ml), while autoimmune screening including ANA, anti-DNA, anti-SM, anti-SSA, and anti-SSB, was negative, supporting the diagnosis of primary APS. Overall, the clinical presentation and laboratory findings were consistent with primary APS, confirmed by positive LA and elevated β2GPI IgG, with no evidence of systemic lupus erythematosus (SLE).

The patient was initially treated with low-molecular-weight heparin (100 mg enoxaparin subcutaneously twice daily) alongside 15 mg warfarin orally. Once the INR reached the therapeutic range (2.32), heparin was discontinued, and the patient was maintained on 15 mg warfarin daily as a lifelong anticoagulation regimen to prevent future thrombotic events. Following multidisciplinary consultation with rheumatology and hematology, the diagnosis of primary APS was confirmed, and appropriate anticoagulation management was implemented. The patient's symptoms improved, and he was discharged with close follow-up appointments scheduled.

Close monitoring of anticoagulation therapy is essential, with regular INR checks to maintain the therapeutic range. The patient should be educated about the importance of medication adherence and the risks associated with discontinuing anticoagulation.

This case highlights the importance of maintaining therapeutic anticoagulation in patients with APS to prevent recurrent thrombotic events, as well as the need for close monitoring and multidisciplinary management.

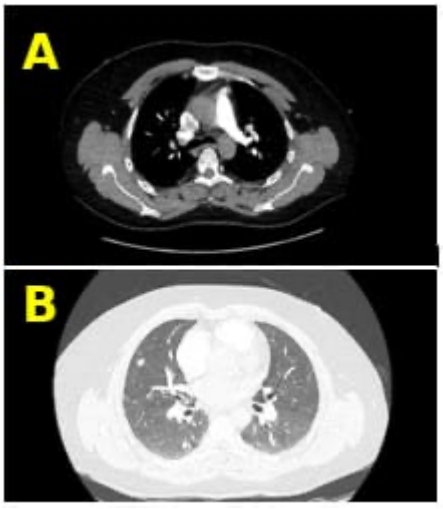

Figure 1:
(A) CT angiography of the chest reveals bilateral acute pulmonary embolism, characterized by linear filling defects in the segmental branches of the right and left lower pulmonary arteries.
(B) Additional findings include atelectatic bands and subpleural reticulations in both lower lobes, accompanied by a solitary pulmonary nodule measuring 8.5 mm in the right middle lobe.

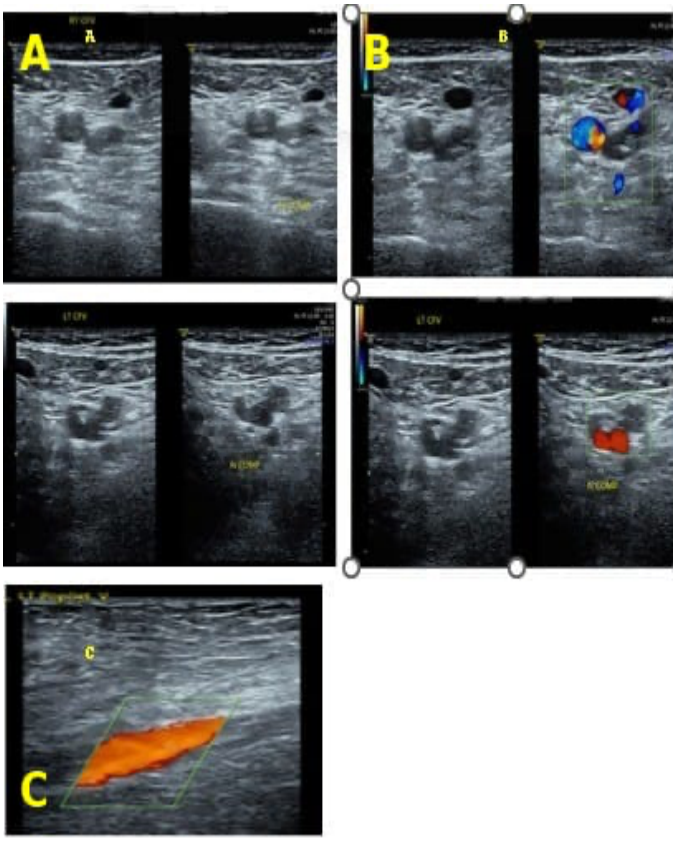

Figure 2:
Doppler ultrasound of the lower limbs demonstrates the following:
(A) Distension of both common femoral veins, containing echogenic thrombi that extend into the distal external iliac veins and partially occlude their lumens, consistent with acute DVT.
(B) A diffuse reduction in the caliber of both femoral veins, with weak flow observed on color Doppler imaging, indicative of chronic DVT.
(C) Presence of an echogenic thrombus within the left popliteal vein.

DISCUSSION

APS is an autoimmune disorder characterized by recurrent vascular thrombosis and pregnancy morbidity, often associated with the presence of aPL antibodies such as LA, aCL, and anti-β2GPI antibodies. These antibodies are central to the syndrome's pathogenesis and must be detectable in at least 2 instances, 12 weeks apart, to confirm diagnosis according to international criteria. [1,8] APS is recognized as a leading cause of acquired thrombophilia, and its vascular complications pose significant diagnostic and therapeutic challenges.[10]

APS can be idiopathic, termed primary APS, and diagnosed when there is no identifiable underlying condition (accounting for 53% to 59% of people with APS). [9] In contrast, secondary APS occurs in the context of other autoimmune diseases, most commonly SLE in 40% of cases (10).

Vascular thrombosis, a hallmark of APS, may manifest as arterial, venous, or small-vessel thrombosis. [11] The pathogenesis of vascular thrombosis in APS is multifactorial, involving both immune and non-immune mechanisms. aPL antibodies, including LA, aCL, and anti-β2GPI antibodies, play a central role in promoting thrombosis. [12] They bind to phospholipid-binding proteins on endothelial cells, platelets, and monocytes, leading to the upregulation of procoagulant and inflammatory pathways. [13,14] Genetic risk factors, such as coagulation factor mutations, increase the risk of antiphospholipid antibody-associated thrombosis. [10] Infections, particularly those caused by viruses like hepatitis C, HIV, COVID-19, Epstein-Barr virus, and Leptospira or bacteria like *Borrelia burgdorferi*, *Coxiella burnetii*, and *Treponema*, [11] have been identified as potential triggers for aPL antibody development. Certain medications, including hydralazine, chlorpromazine, procainamide, quinidine, and phenytoin have also been linked to the development of aPL antibodies.[12]

Vascular thrombosis in APS is characterized by a wide spectrum of clinical presentations depending on the affected vascular bed. Venous thromboembolism, particularly DVT, is the most common presentation, followed by PE. [13] Arterial thrombosis often leads to ischemic events in vital organs, including stroke, myocardial infarction, and limb ischemia.[14] Pregnancy complications, including recurrent pregnancy loss, intrauterine growth restriction, preeclampsia, and stillbirth, are attributed to placental thrombosis and vascular insufficiency. [15,16] Other clinical

presentations that may also occur in APS, classified as non-clinical criteria of APS, include pulmonary hypertension, acute respiratory distress syndrome, intra-alveolar hemorrhage, thrombocytopenia, livedo reticularis, heart valve lesions, epilepsy, leg ulcers, and amaurosis fugax. [17]

In our case, the patient presented with thrombocytopenia; the literature shows that patients with thrombocytopenia have a high risk of developing thrombosis Therefore, thrombocytopenia in APS patients might indicate more severe APS (including an increased risk of thrombosis). [18]

Additionally, our patient experienced recurrent thrombosis. The current PE, occurring shortly after discontinuing anticoagulation, emphasizes the high risk of thrombotic events in untreated APS patients. CAPS, a rare but severe variant, involves widespread small vessel thrombosis and multi-organ failure, necessitating prompt recognition and treatment. [19,20]

APS is diagnosed based on the revised Sapporo classification criteria (2006) and the updated ACR/EULAR classification (2023), both depending on clinical criteria (vascular thrombosis and pregnancy morbidity) and laboratory criteria (LA, aCL, and anti-β2GPI), followed by additional weighted criteria (macrovascular venous thromboembolism, macrovascular arterial thrombosis, and microvascular, obstetric, cardiac valve, and hematological factors). [21,22]

Risk stratification in APS is challenging due to heterogeneity in its clinical manifestations and antibody profiles. Triple positivity for LA, aCL, and anti-β2GPI antibodies is associated with the highest risk of thrombosis. [23] Imaging modalities, such as Doppler ultrasound and CT angiography, are essential in confirming clinical thrombosis. [24]

Management of vascular thrombosis in APS revolves around anticoagulation. Long-term anticoagulation with warfarin targeting an INR of 2–3 remains the cornerstone for the secondary prevention of venous thromboembolism. For arterial thrombosis, higher INR targets or combination therapy with antiplatelet agents may be considered. [25-27] Direct oral anticoagulants (DOACs) have shown variable efficacy in APS and are generally avoided in high-risk patients and those with triple-positive antibodies.[28]

Our case highlights several important aspects of APS management: 1- the critical importance of maintaining therapeutic anticoagulation in patients with APS to prevent recurrent thrombotic events; 2- the need for close monitoring and patient education to ensure compliance with anticoagulation therapy; 3- the value of a multidisciplinary approach involving rheumatology and hematology in managing complex APS cases; and 4- the potential for the rapid recurrence of thrombotic events upon discontinuation of anticoagulation therapy in APS patients.

In the case of treatment failure, if recurrent thrombotic events occur despite the target INR range of 2.0–3.0, alternative approaches, such as increasing the target INR to 3.1–4.0, or adding low-dose aspirin, low-molecular-weight heparin, or HCQ [29] may be tried. Moreover, immunomodulatory therapies, such as rituximab or intravenous immunoglobulin, are emerging as promising treatments, particularly in refractory or CAPS cases. [30] Adjunctive therapies, such as hydroxychloroquine and statins, have shown potential in mitigating endothelial activation and reducing recurrent thrombosis in APS, particularly in patients with concurrent SLE. [29,30] Emerging therapies targeting complement activation and cellular signaling pathways are also under investigation and may offer novel strategies to reduce thrombotic burden in APS. [31]. Inferior vena cava (IVC) filters can be a supplementary treatment for patients with recurrent thrombotic events, especially those who continue to experience issues despite anticoagulation therapy. However, their effectiveness in patients with APS remains uncertain.[32]

In a specific study involving 10 APS patients who had recurrent thromboembolism and received IVC filters, only 1 patient experienced a documented PE after the filter was placed. Notably, 5 out of the 10 patients died, and in 2 cases, PE could not be ruled out as a contributing factor in the sudden deaths. [33] This suggests that while IVC filters may reduce the risk of PE in some instances, their overall impact on mortality and thromboembolic complications in APS patients warrants further investigation. More extensive studies are needed to clarify the long-term efficacy and safety of IVC filters in this population. [34] For APS patients with chronic thromboembolic pulmonary hypertension, pulmonary endarterectomy (PEA) can be considered as a treatment option. However, it is important to note that PEA is a highly specialized surgical procedure and its use in APS patients should be carefully evaluated on a case-by-case basis.[35]

Recent studies have explored the role of DOACs as an alternative to warfarin, although their efficacy in APS remains under investigation. Additionally, research into the pathophysiological mechanisms underlying APS-associated thrombosis continues to evolve, identifying potential new therapeutic targets.[36]

With appropriate management, APS patients can achieve good long-term outcomes. However, the recurrence rates of thrombotic events remain high in suboptimally treated cases.[37] Factors influencing prognosis include the

presence of additional risk factors such as SLE, the triple positivity of antiphospholipid antibodies, and other comorbidities like hypertension, diabetes, and hyperlipidemia. Effective management requires a multidisciplinary approach, focusing on anticoagulation therapy, monitoring for potential complications, and addressing modifiable risk factors to reduce the likelihood of recurrence.[38] In pregnant patients, APS poses additional challenges, with a risk of recurrent miscarriages, preterm delivery, and other complications. However, the combination of low-dose aspirin and heparin has been shown to significantly improve pregnancy outcomes.[39] Ongoing research aims to refine treatment protocols, including exploring targeted therapies, to enhance patients' quality of life and reduce morbidity and mortality associated with APS.[40]

CONCLUSIONS

This case underscores the critical importance of maintaining therapeutic anticoagulation in patients with primary APS to prevent life-threatening thrombotic complications. The recurrence of thrombotic events in our patient, including pulmonary embolism, highlights the high risk associated with discontinued or inadequate anticoagulation therapy. Early diagnosis and a multidisciplinary approach, involving rheumatology, hematology, and patient education, are essential to optimize case management and improve outcomes. Overall, maintaining an effective management plan is crucial for enhancing the quality of life and prognosis of patients with APS.

INFORMED CONSENT STATEMENT

Informed consent was obtained from the patient involved in the study.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

SUPPLEMENTARY MATERIALS

Table 1 and Figures 1 and 2

AUTHOR CONTRIBUTIONS

Single author

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CONFLICTS OF INTEREST

The author declares no conflicts of interest.

REFERENCES

[1] Hughes GR. The anticardiolipin syndrome. Clin Exp Rheumatol. 1985;3(4):285-286.
[2] Chaturvedi S, McCrae KR. The antiphospholipid syndrome: still an enigma. Hematology Am Soc Hematol Educ Program. 2015;2015:53-60. doi:10.1182/asheducation-2015.1.53
[3] Pengo, V., Ruffatti, A., Legnani, C., et al. (2010). Incidence of a first thromboembolic event in asymptomatic carriers of high-risk antiphospholipid antibody profile: a multicenter prospective study. Blood, 116(3), 477-482.
[4] Prasad S, Xiong J, Embry E, Abdelghani L. Catastrophic Antiphospholipid Syndrome: A Life-Threatening Condition. Cureus. 2024;16(7):e64367. Published 2024 Jul 11. doi:10.7759/cureus.64367
[5] Oku, K., Amengual, O & ,.Atsumi, T. (2020). Antiphospholipid syndrome: Advances in the pathogenesis and management .Best Practice & Research Clinical Rheumatology.101472 ,(1)34 ,
[6] Vandevelde A, Devreese KMJ. Laboratory Diagnosis of Antiphospholipid Syndrome: Insights and Hindrances. J Clin Med. 2022;11(8):2164. Published 2022 Apr 13. doi:10.3390/jcm11082164
[7] Capecchi M, Abbattista M, Ciavarella A, Uhr M, Novembrino C, Martinelli I. Anticoagulant Therapy in Patients with Antiphospholipid Syndrome. Journal of Clinical Medicine. 2022; 11(23):6984. https://doi.org/10.3390/jcm11236984
[8] Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet. 2010;376(9751):1498-1509. doi:10.1016/S0140-6736(10)60709-X
[9] Ahn Y, Hawkins C, Pearson E, Kubler P. Diagnosis and management of antiphospholipid syndrome. Aust Prescr 2024;47:179-85.https://doi.org/10.18773/austprescr.2024.055
[10] Bustamante JG, Goyal A, Rout P, Singhal M. Antiphospholipid Syndrome. In: StatPearls. Treasure Island (FL): StatPearls Publishing; May 6, 2024.
[11] Shi M, Gao W, Jin Y, et al. Antiphospholipid Syndrome-Related Pulmonary Embolism: Clinical Characteristics and Early Recognition. Front Cardiovasc Med. 2022;9:872523. Published 2022 Jul 11. doi:10.3389/fcvm.2022.872523
[12] Ambati A, Zuo Y, Knight JS. An update on inflammation in antiphospholipid syndrome. Curr Opin Rheumatol. 2023;35(2):89-97. doi:10.1097/BOR.0000000000000926

[13] Willis R, Pierangeli SS. Pathophysiology of the antiphospholipid antibody syndrome. Auto Immun Highlights. 2011;2(2):35-52. Published 2011 Mar 24. doi:10.1007/s13317-011-0017-9
[14] D'Ippolito S, Barbaro G, Paciullo C, Tersigni C, Scambia G, Di Simone N. Antiphospholipid Syndrome in Pregnancy: New and Old Pathogenetic Mechanisms. Int J Mol Sci. 2023;24(4):3195. Published 2023 Feb 6. doi:10.3390/ijms24043195
[15] Satta R, Biondi G. Antiphospholipid syndrome and pregnancy. G Ital Dermatol Venereol. 2019;154(3):277-285. doi:10.23736/S0392-0488.18.06152-7
[16] Asherson RA, Cervera R. Unusual manifestations of the antiphospholipid syndrome. Clin Rev Allergy Immunol. 2003;25(1):61-78. doi:10.1385/CRIAI:25:1:61
[17] Atanassova PA. Antiphospholipid syndrome and vascular ischemic (occlusive) diseases: an overview. Yonsei Med J. 2007;48(6):901-926. doi:10.3349/ymj.2007.48.6.901
[18] Tomasello R, Giordano G, Romano F, et al. Immune Thrombocytopenia in Antiphospholipid Syndrome: Is It Primary or Secondary?. Biomedicines. 2021;9(9):1170. Published 2021 Sep 6. doi:10.3390/biomedicines9091170
[19] Jacobs L, Wauters N, Lablad Y, Morelle J, Taghavi M. Diagnosis and Management of Catastrophic Antiphospholipid Syndrome and the Potential Impact of the 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria. *Antibodies*. 2024; 13(1):21. https://doi.org/10.3390/antib13010021
[20] Erkan D, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: updated diagnostic algorithms. Autoimmun Rev. 2010;10(2):74-79. doi:10.1016/j.autrev.2010.08.005
[21] Barbhaiya M, Zuily S, Naden R, et al. The 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria. Arthritis Rheumatol. 2023;75(10):1687-1702. doi:10.1002/art.42624
[22] Mısırcı S, Ekin A, Yağız B, Coşkun BN, Dalkılıç E, Pehlivan Y. The Validation of the 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria in a Cohort from Turkey. *Diagnostics*. 2024; 14(19):2205. https://doi.org/10.3390/diagnostics14192205
[23] Sciascia, S., Baldovino, S., Schreiber, K. et al. Thrombotic risk assessment in antiphospholipid syndrome: the role of new antibody specificities and thrombin generation assay. Clin Mol Allergy 14, 6 (2016). https://doi.org/10.1186/s12948-016-0043-2
[24] Koniari, I., Siminelakis, S.N., Baikoussis, N.G. et al. Antiphospholipid syndrome; its implication in cardiovascular diseases: a review. J Cardiothorac Surg 5, 101 (2010). https://doi.org/10.1186/1749-8090-5-101
[25] Tumian NR, Hunt BJ. Clinical Management of Thrombotic Antiphospholipid Syndrome. *Journal of Clinical Medicine*. 2022; 11(3):735. https://doi.org/10.3390/jcm11030735
[26] Capecchi M, Abbattista M, Ciavarella A, Uhr M, Novembrino C, Martinelli I. Anticoagulant Therapy in Patients with Antiphospholipid Syndrome. *Journal of Clinical Medicine*. 2022; 11(23):6984. https://doi.org/10.3390/jcm11236984
[27] Les I, Ruiz-Irastorza G, Khamashta MA. Intensity and duration of anticoagulation therapy in antiphospholipid syndrome. Semin Thromb Hemost. 2012;38(4):339-347. doi:10.1055/s-0032-1304720
[28] Pastori D, Menichelli D, Cammisotto V, Pignatelli P. Use of Direct Oral Anticoagulants in Patients With Antiphospholipid Syndrome: A Systematic Review and Comparison of the International Guidelines. Front Cardiovasc Med. 2021;8:715878. Published 2021 Aug 3. doi:10.3389/fcvm.2021.715878
[29] Bala MM, Celinska-Lowenhoff M, Szot W, et al. Antiplatelet and anticoagulant agents for secondary prevention of stroke and other thromboembolic events in people with antiphospholipid syndrome. Cochrane Database Syst Rev. 2020;10(10):CD012169. Published 2020 Oct 12. doi:10.1002/14651858.CD012169.pub3
[30] Tumian NR, Hunt BJ. Clinical Management of Thrombotic Antiphospholipid Syndrome. Journal of Clinical Medicine. 2022; 11(3):735. https://doi.org/10.3390/jcm11030735
[31] Corban MT, Duarte-Garcia A, McBane RD, Matteson EL, Lerman LO, Lerman A. Antiphospholipid Syndrome: Role of Vascular Endothelial Cells and Implications for Risk Stratification and Targeted Therapeutics. J Am Coll Cardiol. 2017;69(18):2317-2330. doi:10.1016/j.jacc.2017.02.058
[32] .Baig S, Bert J, Gertner E. Safety of retrievable inferior vena cava filters in patients with the antiphospholipid syndrome. Eur J Rheumatol. 2018;5(2):100-103. doi:10.5152/eurjrheum.2018.17091
[33] Zifman E, Rotman-Pikielny P, Berlin T, Levy Y. Insertion of inferior vena cava filters in patients with the antiphospholipid syndrome. Semin Arthritis Rheum. 2009;38(6):472-477. doi:10.1016/j.semarthrit.2008.01.01
[34] Cherian J, Gertner E. Recurrent pulmonary embolism despite inferior vena cava filter placement in patients with the antiphospholipid syndrome. J Clin Rheumatol. 2005;11(1):56-58. doi:10.1097/01.rhu.0000152150.01274.1b
[35] Taş S, Antal A, Durusoy AF, et al. Pulmonary Endarterectomy in Patients with Antiphospholipid Syndrome-Associated Chronic Thromboembolic Pulmonary Hypertension. Anatol J Cardiol. 2022;26(5):394-400. doi:10.5152/AnatolJCardiol.2021.1138
[36] Hwang HG, Lee JH, Kim SA, et al. Direct Oral Anticoagulants in Antiphospholipid Syndrome-Associated Venous Thromboembolism: Real World Evidence. J

Korean Med Sci. 2024;39(36):e252. Published 2024 Sep 23. doi:10.3346/jkms.2024.39.e252
[37] Rodziewicz M, D'Cruz DP. An update on the management of antiphospholipid syndrome. Ther Adv Musculoskelet Dis. 2020;12:1759720X20910855. Published 2020 Apr 27. doi:10.1177/1759720X20910855
[38] Ahn Y, Hawkins C, Pearson E, Kubler P. Diagnosis and management of antiphospholipid syndrome. Aust Prescr. 2024;47:179-85. doi:10.18773/austprescr.2024.055
[39] Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment [published correction appears in Obstet Gynecol 2002 Dec;100(6):1361]. Obstet Gynecol. 2002;100(3):408-413. doi:10.1016/s0029-7844(02)02165-8
[40] Parepalli A, Sarode R, Kumar S, Nelakuditi M, Kumar MJ. Antiphospholipid Syndrome and Catastrophic Antiphospholipid Syndrome: A Comprehensive Review of Pathogenesis, Clinical Features, and Management Strategies. Cureus. 2024;16(8):e66555. Published 2024 Aug 10. doi:10.7759/cureus.66555

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Original Article

Evaluation of Program Learning Outcomes in the Clinical Nutrition Curriculum

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ABSTRACT Assessing program learning outcomes (PLOs) ensures academic curricula align with competencies needed for professional success, particularly in health fields. This study aims to evaluate the PLOs of the Clinical Nutrition (CLN) program at Jazan University. A mixed-method approach was adopted to assess the PLOs' validity, reliability, and achievement. Furthermore, content and construct validity were assessed using the content validity ratio (CVR) and exploratory factor analysis (EFA), respectively. Reliability was assessed using Cronbach's alpha, and aggregates were calculated for 2024 academic cohort examination scores to evaluate PLO achievement. The CVR of the 11 PLOs ranged from 0.5 to 0.9, indicating a strong alignment with educational objectives. EFA yielded a Kaiser–Meyer–Olkin (KMO) value of 0.92, affirming the construct validity. The Cronbach's alpha of 0.799 confirmed internal consistency among the PLOs. Aggregates of the examination scores showed high achievement (more than 85%) in all PLOs except S4 and S5 from the skills domain. In conclusion, this study underscores the significance of robust assessment practices in higher education programs, demonstrating that the PLOs of the CLN program effectively guide student learning and align with accreditation standards. Continuous evaluation and refinement of these outcomes are essential to maintain educational quality and ensure the graduates are well-equipped for professional challenges in the health sector.

Keywords: National Center for Academic Accreditation and Evaluation, Clinical Nutrition Program, Curriculum, Program Learning Outcomes, Validity, Reliability, Factor Analysis.

INTRODUCTION

The assessment of learning outcomes is a fundamental aspect of modern educational systems, particularly in higher education, where the alignment of curricula with expected competencies plays a pivotal role in ensuring the effectiveness of academic programs.[1] Learning outcomes guide not only curriculum development but also teaching practices and assessment strategies, providing a clear roadmap for both teaching staff and students.[2] The measurement of these outcomes is crucial for evaluating the quality of academic programs and ensuring that graduates are equipped with the knowledge and skills necessary for professional success.[3] In this context, program learning outcomes (PLOs) and course learning outcomes (CLOs) have become essential tools for measuring student progress, course effectiveness, and program quality.[4] PLOs refer to the broad competencies that students are expected to achieve by the end of an academic program. These outcomes encapsulate the knowledge, skills, and values that align with the program's overall objectives.[5] PLOs are often aligned with the mission and vision of the academic institution, reflecting the institution's broader educational goals and the needs of stakeholders, including employers, accreditation bodies, and the community.[6] On the other hand, CLOs are the specific knowledge, skills, and abilities that students are expected to develop upon completing an individual course.[7] Each course within a program contributes to the achievement of PLOs by developing certain competencies that are part of the program's overall learning framework.[8] The alignment between CLOs and PLOs is critical as it ensures that individual courses collectively contribute to the achievement of the program's overarching goals.[9] Assessing PLOs ensures that academic programs are held accountable to students, employers, and accreditation bodies.[10] It provides tangible evidence that the program is meeting its stated objectives and that the students are

acquiring the necessary knowledge and skills to excel in their chosen fields.[11] In health-related programs such as CLN, where the graduates are expected to perform in high-stakes environments, ensuring that learning outcomes are met is crucial for maintaining public trust and ensuring patient safety. In addition, the regular assessment of PLOs supports continuous improvement within academic programs. By evaluating the extent to which students are achieving PLOs, faculty members and administrators can identify areas of the curriculum that may need revision or enhancement.[12] This process helps maintain the relevance of the curriculum to the evolving demands of the profession and ensures that the students are receiving an education that is both current and comprehensive.[13] Moreover, the assessment of PLOs helps in fostering a student-centered approach to education. It provides the students with clear expectations about what they need to achieve and offers a framework for self-assessment and reflection. When the students understand the program's learning outcomes, they can take a more active role in their education, setting personal goals and seeking opportunities for improvement.[14] In terms of accreditation, academic programs must provide clear evidence that the students are achieving the learning outcomes essential for professional practice.[15] The effective assessment of PLOs provides this evidence, supporting the accreditation process and ensuring that the program maintains high standards of quality.[16] Different methods—such as exemplary, formative, and summative assessments, questionnaires, and interviews—can be used to assess the PLOs' achievement. These methods, in general, can be classified into two categories: direct and indirect approaches.[17] Direct assessment involves evaluating student performance based on tangible evidence of learning. Examples include exams, quizzes, practical exams, projects, presentations, and capstone experiences.[18] In the CLN program, direct assessment might take the form of clinical case studies, lab-based evaluations, and competency-based assessments where the students demonstrate their ability to apply theoretical knowledge to practical scenarios. These assessments provide concrete evidence of what students know and can do, making them a reliable method for evaluating PLOs.[19] Indirect assessment gathers information about student learning through perception-based methods such as surveys, self-assessments, exit interviews, and employer feedback.[20] While indirect assessments do not measure student performance directly, they offer valuable insights into the students' perceptions of their own learning and the program's effectiveness.[21] In the CLN program, for instance, surveys of alumni and employers could provide feedback on how well the program prepared the graduates for clinical practice. The effective assessment of PLOs often involves using a combination of direct and indirect techniques (Figure 1). This mixed-method approach ensures that the program can capture a comprehensive view of student learning, balancing objective measures of performance with subjective insights from students and stakeholders.[20,21]

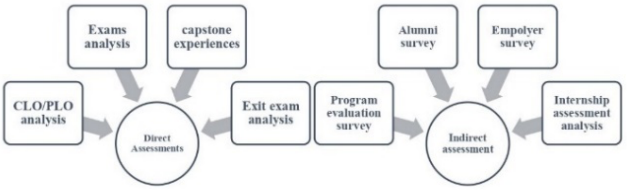

Figure 1: Assessment of Program Learning Outcomes

Outcomes-based assessment is a pedagogical approach that prioritizes the demonstration of specific competencies and skills over traditional forms of assessment.[22] This model shifts the focus from the content delivered to the actual learning outcomes achieved by the students. In the context of the CLN program at Jazan University, outcomes-based assessment aligns seamlessly with the program's objectives, emphasizing the application of knowledge and skills in real-world contexts. This approach necessitates the alignment of curriculum design, teaching strategies, and assessment methods with the PLOs and CLOs.[23] By clearly defining the expected outcomes, educators can create a cohesive learning experience that fosters student engagement and success. Outcomes-based assessment also facilitates the development of rubrics and standardized evaluation tools, providing clarity and consistency in grading practices.[24] The principles of assessment for PLOs are grounded in the concepts of validity, reliability, and fairness. Validity ensures that the assessments accurately measure what they intend to measure, while reliability refers to the consistency of assessment results over time and across different contexts.[25] Fairness entails providing all students with equitable opportunities to demonstrate their learning, regardless of their background or learning style.[26] By adhering to these principles, educators can create a robust assessment framework that not only promotes accountability but also fosters a supportive learning environment. In the CLN program at Jazan University, implementing these principles is crucial for developing a culture of continuous improvement, where the assessment results inform instructional practices and enhance student learning experiences. The primary aim of this study is to assess the learning outcomes of the CLN program at Jazan University, focusing on the effectiveness of PLOs in facilitating student learning and professional preparedness. Through a comprehensive evaluation of assessment practices, this research seeks to identify strengths and areas for improvement within the program, ultimately contributing to enhanced educational quality and student success.

MATERIALS AND METHODS

The CLN program, part of the College of Nursing and Health Sciences, collaborates closely with the Deanship of Academic Development to align with the accreditation practices of the National Center for Academic Accreditation and Evaluation (NCAAA). Each program is required to meet five standards, with PLOs being a key component of this assessment. PLOs articulate the knowledge and understanding, skills, values, autonomy, and responsibility that students should possess upon graduation. The bachelor's degree in CLN offered by the Department of Clinical Nutrition at Jazan University encompasses 11 PLOs (Table 1), which are developed in accordance with the Key Learning Outcomes for CLN Programs outlined in the Education & Training Evaluation Commission (ETEC) Manual 2023 as well as the Ministry of Education requirements. This structured approach ensures that the program meets national educational standards while preparing the graduates for professional practice.

Knowledge and Understanding	
K1	Describe the terminologies and principles of clinical nutrition practice, including nutritional instructions to optimize the patient care.
K2	Discuss the nutritional needs of an individual according to the current developments in nutrition & dietary standards.
K3	Demonstrate knowledge and understanding of nutritional education, health promotion, research and inquiry methodologies.
Skills	
S1	Develop critical thinking skills, analytical abilities, problem-solving and evidence-based approach to evaluate the nutritional well-being of individuals or populations.
S2	Demonstrate the ability to perform high-quality scientific research, community and inter- professional activities in the nutritional field by using advanced software's, techniques and tools.
S3	Apply Nutrition Care Process for critically ill patients by using nutritional knowledge and skills.
S4	Use counselling and educational advanced tools to facilitate behaviour change and enhance wellness for individuals and groups at community level.
S5	Communicate effectively, in an oral and written format, to multiple audiences and stakeholders.
Values, autonomy, and responsibility	
V1	Adhere to the code of ethics for healthcare professionals and values of clinical nutrition practice respective to the patient's culture and citizenship.
V2	Develop self-learning skills for nutritional management of chronic diseases.
V3	Ability to active participation in decision making, supervise a team, and performance management (Policies, Guidelines, and Standards in nutrition health care).

Table 1: Program Learning Outcomes of the Clinical Nutrition Program

This study proposed four indices—validity, reliability, sequencing equation model, and aggregates of final scores—to assess the PLOs by both qualitative and quantitative assessment methods. The qualitative assessment focused on the validity and reliability of PLOs, while the quantitative aspect employed two methods: sequencing equation model and aggregates of final scores. These methods provided a comprehensive analysis of the PLOs' alignment with program standards and outcomes.[27]

QUALITY OF PLO MEASUREMENT

Validity Assessment: The validity of the PLOs was evaluated through two sub-indices: content validity and construct validity. To assess content validity, feedback was solicited from program faculty members and other experts (employers, members of the advisory committee) in the clinical nutrition field. A self-designed questionnaire based on a Likert point scale was distributed, with response options ranging from "essential" and "useful but not necessary" to "not necessary." This method aimed to gather qualitative insights regarding the relevance of each PLO. The responses were analyzed using the Lawshe technique,[28] which quantifies content validity by calculating the content validity ratio (CVR) based on expert opinions. For construct validity, exploratory factor analysis (EFA) was conducted.[29, 30] Prior to performing EFA, the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were employed to assess the appropriateness of the data for factor analysis.[31] The KMO value indicates the degree of intercorrelation among the variables, while Bartlett's test checks whether the correlation matrix is significantly different from an identity matrix, suggesting that the data is suitable for factor analysis.

Reliability Assessment: The reliability of the PLOs was measured using Cronbach's alpha, which evaluates internal consistency among the respondents' feedback on the PLOs. A Cronbach's alpha value of 0.70 or higher is generally considered acceptable, indicating that the items in the questionnaire yield consistent results across the sample.

QUANTITY OF PLO MEASUREMENT

Sequencing Equation Model (SEM): Confirmatory factor analysis (CFA) is employed to assess the optimality and alignment of the PLOs,[32] ensuring that they adequately represent the educational objectives of the CLN program. This statistical method allows for the validation of relationships between the observed variables and their underlying latent constructs, thereby reinforcing the quantitative assessment of the PLOs.

Aggregate Method: Students' final examination scores for the academic year 2024 were utilized to evaluate the extent of achievement of the PLOs. This data serves as a quantitative measure of how well students meet the established PLOs.

RESULTS

In total, 24 responses were received during September 2024. Of these, 10 (41.7%) were teaching faculty members, and 14 (58.3%) were experts in the clinical nutrition field. This diverse participant pool enriched the validity and reliability of the feedback.

Content Validity: The overall content validity for the 11 PLOs ranged from 0.5 to 0.9. This indicates that the program

has successfully formulated the learning outcomes, ensuring they encompass the essential content.

Construct Validity: Exploratory factor analysis, Kaiser-Meyer-Olkin (KMO) test result 0.92 (>0.60) conforms that the sample adequacy and Bartlett's Test of Sphericity (BTS) significant P-value (<0.01) assured that the appropriateness of the factor analysis for the assessment of PLOs. The scree plot with an eigenvalue of more than 2 suggested that the three-factor structure solution (Figure 2) for the program 11 PLOs.

Internal Consistency and Reliability: The Cronbach's alpha index of 0.799 indicates that the 11 PLOs are reliable, with a high level of internal consistency.

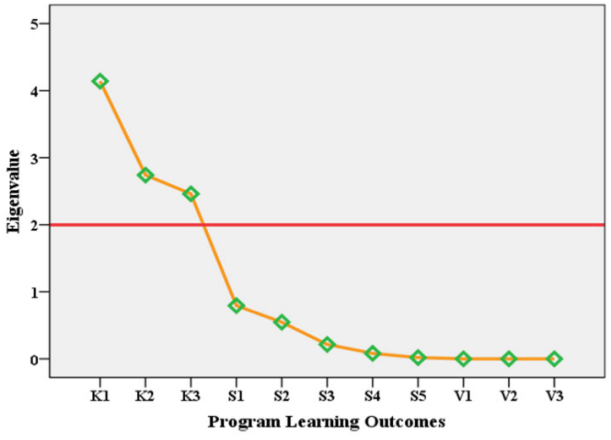

Figure 2: Scree Plot of Program 11 PLOs with Three-factor Solution

Sequence Equation Modal: The comparative fit index (CFI) of 0.912, with a non-significant chi-square value and a root mean square error of approximation (RMSEA) value of 0.050 (95% CI: 0.045–0.055, P < 0.01), indicates that the model has an excellent fit with the program 11 PLOs, with acceptable factor loading boundaries between 0.47 and 1.00. The PLOs K1, K2, and K3 had significant factor loadings (path coefficients) with knowledge and understanding domain (K1 = 1.00, K2 = 1.00; P < 0.01, and K3 = 0.47; P < 0.01), followed by S1, S2, S3, S4, and S5 with the skills domain (S1 = 0.97, S2 = 0.90; P < 0.01, S3 = 0.91; P < 0.01, S4 = 0.77; P < 0.01, and S5 = 0.70; P < 0.01). V1, V2, and V3 had significant factor loadings (path coefficients) with the values, autonomy, and responsibility domain (V1 = 1.00, V2 = 1.00; P < 0.01 and V3 = 0.67; P < 0.01). In summary, the skills and values, autonomy, and responsibility domains contributed more than the knowledge domain (knowledge = 0.09, skills = 0.28, and values = 0.61) in the overall student achievement of the educational objectives of the program, and there was no evidence of collinearity in the model (Figure 3). The factor structures remain intact, and all the PLOs had significant paths as well as correlations with the respective factor domains.

Aggregate Method: The students' cohort 2024 final examination scores were used for measuring the quantity of PLO achievement (Table 2). The target performance achievement of each PLO is set as 100% of the students getting 85% or above.

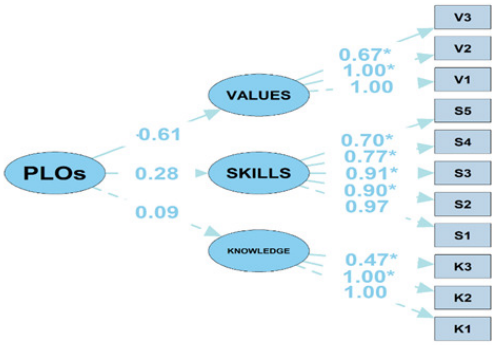

Figure 3: PLOs Structure by Structural Equation Modeling
PLOs: Program learning outcomes, KNOWLEDGE: Knowledge and understanding domain, SKILLS: Skills domain, VALUES: Values, autonomy, and responsibility domain and *Highly significant (P < 0.01).

Direct Assessment Method			
PLOs	Male	Female	Average
Knowledge and Understanding			
K1	80	90	85
K2	91	80	86
K3	89	89	89
Skills			
S1	85	85	85
S2	91	90	91
S3	90	90	90
S4	80	79	80
S5	80	80	80
Values, Autonomy, and Responsibility			
V1	87	84	86
V2	90	91	91
V3	87	84	86

Table 2: Aggregates of PLOs Attainment Results (%)

Figure 4 displays the overall performance of students across various assessments in 2024, indicating the percentage of students meeting the target benchmark of 85% or above for each PLO. The data reveals that, with the exception of PLOs S4 and S5, the students demonstrated strong proficiency, achieving the desired performance levels in their examinations.

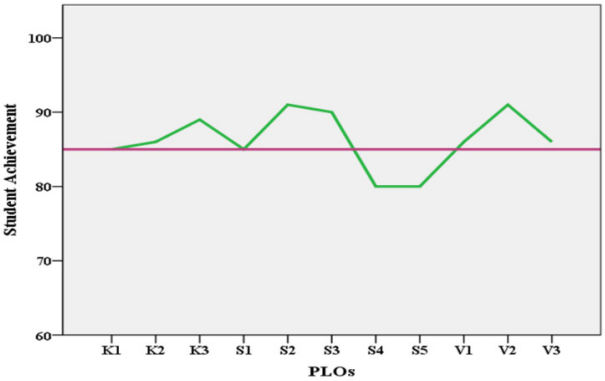

Figure 4: Students Achievement in all examinations for the academic year 2024

DISCUSSION

This study aimed to analyze PLOs within the CLN program at Jazan University. The findings from this study provide important insights into the validation of PLOs for this program. The diverse group of participants, including teaching faculty members and other clinical nutrition experts, ensures a broad representation of perspectives, enhancing the reliability of the results. The analysis of the content and construct validity, internal consistency, and assessment of PLO achievement presents a robust evaluation of the educational outcomes associated with the program.[33, 34] Along similar lines, the studies that emphasize the importance of continuous refinement of learning outcomes to ensure they remain relevant and comprehensive.[35, 36] This study employed EFA to assess the construct validity of the PLOs at the clinical nutrition department. This is consistent with the studies of Naglaa et al. (2022) and Chee-Peng (2024), which also employed similar statistical methods to validate educational constructs.[38, 39] The three-factor structure derived from the scree plot analysis aligns well with theoretical frameworks in educational assessment that suggest categorizing learning outcomes into knowledge and understanding, skills, values, autonomy, and responsibility.[40] The Cronbach's alpha index for the internal consistency of the PLOs signified a good level of reliability. This suggests that the PLOs are measuring a coherent construct, which is crucial for ensuring that the students are developing the intended competencies throughout their educational experience. A comparable study has reported similar findings, where high internal consistency was linked to effective learning outcomes and program quality.[41] In assessing the quantity of PLOs achievement, the results of this study indicate that the students performed well and achieved the target benchmarks of the PLOs except for S4 and S5 in the skills domain. This performance can be interpreted as a positive reflection of the teaching methodologies and curriculum implemented within the program. Notably, the high performance in values and autonomy outcomes reinforces the importance of instilling ethical and professional values in clinical practice, as outlined in the existing literature.[42]

The strengths of this study lie in its rigorous methodology, including a well-defined participant selection process and the use of established statistical techniques for validating PLOs. However, it is important to acknowledge the study's limitations. The sample size of 24, while adequate for preliminary analysis, may not be representative of the entire population of faculty members and experts in clinical nutrition. A larger, more diverse sample could provide more generalizable results. Additionally, the study relied on self-reported measures, which may introduce bias as the participants might have overestimated their competence or the program's effectiveness. Another limitation is the time frame of the data collection as it was conducted over only a month. This brief period may have influenced participant response rates and could limit the depth of insight obtained regarding the program's outcomes. Furthermore, while the assessment metrics for PLOs provide valuable quantitative data, qualitative insights from open-ended feedback could enhance the understanding of the participants' perspectives on the program's strengths and weaknesses. Lastly, while the study successfully demonstrated strong content and construct validity, it did not explore the longitudinal impact of PLOs on student performance over time. Incorporating a longitudinal approach could provide deeper insights into how well the PLOs prepare students for their professional roles post-graduation.

CONCLUSIONS

In conclusion, this study provides valuable insights into the validation of PLOs for the CLN program at Jazan University. The findings demonstrate that the program is effectively achieving its learning outcomes, particularly in knowledge and values, while highlighting areas for improvement in skills application. The analysis emphasizes the need for continuous evaluation and refinement of educational strategies to ensure that graduates are well-equipped to meet the demands of the clinical nutrition field. Future research should focus on expanding the sample size, incorporating qualitative assessments, and employing longitudinal studies to track the long-term effectiveness of the program. By addressing these areas, the CLN program can further enhance its educational outcomes and better prepare students for their professional roles.

AUTHOR CONTRIBUTIONS:

Z.H.H. was responsible for conceptualization, methodology, project administration, data curation, validation, formal analysis, writing the original draft, and review and editing of the manuscript. R.M.C assisted in data analysis. A.A.A contributed to the manuscript review. The authors have read and agreed to the published version of the manuscript.

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INFORMED CONSENT STATEMENT:

Not Applicable

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available. However, they can be obtained from the corresponding author upon request.

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CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

REFERENCES

[1] Kifle Mekonen Y, Anja Fitiavana R. Assessment of Learning Outcomes in Higher Education: Review of literature. Int J Res Publ. 2021;71(1). doi:10.47119/IJRP100711220211766

[2] Orr RB, Gormally C, Brickman P. A Road Map for Planning Course Transformation Using Learning Objectives. CBE Life Sci Educ. 2024;23(2):es4. doi:10.1187/cbe.23-06-0114

[3] Goss H. Student Learning Outcomes Assessment in Higher Education and in Academic Libraries: A Review of the Literature. J Acad Librariansh. 2022;48(2):102485. doi:10.1016/j.acalib.2021.102485

[4] Mendoza W, Ramírez GM, González C, Moreira F. Assessment of Curriculum Design by Learning Outcomes (LO). Educ Sci. 2022;12(8):541. doi:10.3390/educsci12080541

[5] Mahboob K, Ali SA, Laila U e. Investigating learning outcomes in engineering education with data mining. Comput Appl Eng Educ. 2020;28(6):1652-1670. doi:10.1002/cae.22345

[6] Goss H. Student Learning Outcomes Assessment in Higher Education and in Academic Libraries: A Review of the Literature. J Acad Librariansh. 2022;48(2):102485. doi:10.1016/j.acalib.2021.102485

[7] Rao NJ. Outcome-based Education: An Outline. High Educ Future. 2020;7(1):5-21. doi:10.1177/2347631119886418

[8] Ma NN, Aziz RC. Cultivating A Future-Ready Health Workforce: Aligning Bachelor of Medical and Health Sciences Program Outcomes with Malaysian Qualifications Framework for Advanced Competencies. 2023;15(2).

[9] Mahzari M, AlNahedh T, Ahmed AA, Al Rumyyan A, Shaban S, Magzoub ME. Practical Guide to Undergraduate Medical Curriculum Alignment and Mapping. Adv Med Educ Pract. 2023;14:1001-1012. doi:10.2147/AMEP.S424815

[10] Al-Shargabi MA. An Integrated Decision Support Model For Enhancing Continuous Improvement Of Academic Programs. Eng Technol Appl Sci Res. 2019;9(5):4835-4841. doi:10.48084/etasr.3079

[11] Bosch E, Spinath B. What evidence-based learning activities help students acquire knowledge, correct confidence in their own knowledge, and accurate self-assessment? Learn Individ Differ. 2023;108:102374. doi:10.1016/j.lindif.2023.102374

[12] Wei X, Saab N, Admiraal W. Assessment of cognitive, behavioral, and affective learning outcomes in massive open online courses: A systematic literature review. Comput Educ. 2021;163:104097. doi:10.1016/j.compedu.2020.104097

[13] Darling-Hammond L, Flook L, Cook-Harvey C, Barron B, Osher D. Implications for educational practice of the science of learning and development. Appl Dev Sci. 2020;24(2):97-140. doi:10.1080/10888691.2018.1537791

[14] D SP, Kumar R. Literature Review of The Learner Centered Teaching. Published online December 18, 2020. Accessed October 28, 2024. https://papers.ssrn.com/abstract=3996210

[15] Almurayh A, Saeed S, Aldhafferi N, Alqahtani A, Saqib M. Sustainable Education Quality Improvement Using Academic Accreditation: Findings from a University in Saudi Arabia. Sustainability. 2022;14(24):16968. doi:10.3390/su142416968

[16[Bougherira MR, Elasmar MH. Impact of academic accreditation on teaching and learning: faculty members' perceptions. J Furth High Educ. 2023;47(2):167-181. doi:10.1080/0309877X.2022.2102412

[17] Breslow LR. Methods of Measuring Learning Outcomes.

[18] Goel N, Deshmukh K, Patel BC, Chacko S. Tools and Rubrics for Assessment of Learning Outcomes. In: Assessment Tools for Mapping Learning Outcomes With Learning Objectives. IGI Global; 2021:211-254. doi:10.4018/978-1-7998-4784-7.ch013

[19] Maki PL. Assessing for Learning: Building a Sustainable Commitment Across the Institution. 2nd ed. Routledge; 2023. doi:10.4324/9781003443056

[20] Morris R, Perry T, Wardle L. Formative assessment and feedback for learning in higher education: A systematic review. Rev Educ. 2021;9(3):e3292. doi:10.1002/rev3.3292

[21] Carney EA, Zhang X, Charsha A, Taylor JN, Hoshaw JP. Formative Assessment Helps Students Learn over Time: Why Aren't We Paying More Attention to It? Intersect J Intersect Assess Learn. 2022;4(1). Accessed October 28, 2024. https://eric.ed.gov/?id=EJ1386053

[22] Iqbal S, Willis I, Almigbal TH, Aldahmash A, Rastam S. Outcome-based education: evaluation, implementation and faculty development. MedEdPublish. 2020;9:121. doi:10.15694/mep.2020.000121.1

[23] Tam M. Outcomes-based approach to quality assessment and curriculum improvement in higher education. Qual Assur Educ. 2014;22(2):158-168. doi:10.1108/QAE-09-2011-0059

[24] Qadir J, Shafi A, Al-Fuqaha A, et al. Outcome-Based Engineering Education: A Global Report of International OBE Accreditation and Assessment Practices. In: ; 2020. doi:10.18260/1-2--35020

[25] Aguayo-Hernández CH, Sánchez Guerrero A, Vázquez-Villegas P. The Learning Assessment Process in Higher Education: A Grounded Theory Approach. Educ Sci. 2024;14(9):984. doi:10.3390/educsci14090984

[26] Zlatkin-Troitschanskaia O, Schlax J, Jitomirski J, et al. Ethics and Fairness in Assessing Learning Outcomes in Higher Education. High Educ Policy. 2019;32(4):537-556. doi:10.1057/s41307-019-00149-x

[27] Clinical_Nutrition.pdf. Accessed November 3, 2024. https://etec.gov.sa/assets/sf/n/Clinical_Nutrition.pdf

[28] Romero Jeldres M, Díaz Costa E, Faouzi Nadim T. A review of Lawshe's method for calculating content validity in the social sciences. Front Educ. 2023;8. doi:10.3389/feduc.2023.1271335

[29] Sheehan J, Tessmer M. A Construct Validation of the Mental Models Learning Outcome Using Exploratory Factor Analysis.; 1997. Accessed November 3, 2024. https://eric.ed.gov/?id=ED409870

[30] Tavakol M, Wetzel A. Factor Analysis: a means for theory and instrument development in support of construct validity. Int J Med Educ. 2020;11:245. doi:10.5116/ijme.5f96.0f4a

[31] Shrestha N. Factor Analysis as a Tool for Survey Analysis. Am J Appl Math Stat. 2021;9(1):4-11. doi:10.12691/ajams-9-1-2

[32] Tungkunanan P. Learning Model of Undergraduate Students: Confirmatory Factor Analysis. Int J Instr. 2020;13(3):665-678.

[33] Alqahtani TM, Yusop FD, Halili SH. Content validity of the Constructivist Learning in Higher Education Settings (CLHES) scale in the context of the flipped classroom in higher education. Humanit Soc Sci Commun. 2023;10(1):268. doi:10.1057/s41599-023-01754-3

[34] Setiawan R, Wagiran W, Alsamiri Y. Construction of an instrument for evaluating the teaching process in higher education: Content and construct validity. REID Res Eval Educ. 2024;10(1):50-63. doi:10.21831/reid.v10i1.63483

[35] Dobbins K, Brooks S, Scott JJA, Rawlinson M, Norman RI. Understanding and enacting learning outcomes: the academic's perspective. Stud High Educ. 2016;41(7):1217-1235. doi:10.1080/03075079.2014.966668

[36] Wong BM, Headrick LA. Application of continuous quality improvement to medical education. Med Educ. 2021;55(1):72-81. doi:10.1111/medu.14351

[37] Structural validity and reliability of the return to work assessment scale among post stroke survivors - Peter. O. Ibikunle, Anthea Rhoda, Mario Smith, 2021. Accessed November 3, 2024. https://journals.sagepub.com/doi/full/10.3233/WOR-213528

[38] Youssef N, Alharbi H. Validity and reliability of the English version of the Student Evidence-Based Practice Questionnaire among Arabic-speaking undergraduate students at health sciences colleges: A cross-sectional study. Nurse Educ Today. 2022;118:105525. doi:10.1016/j.nedt.2022.105525

[39] Mason Seng CP, Huang CK, Lin TB. Development and Validation of a Questionnaire to Understand Students' Perceptions of Bilingual Education in Taiwan. Educ Sci. 2024;14(10):1126. doi:10.3390/educsci14101126

[40] Ardoin NM, Bowers AW, Gaillard E. Environmental education outcomes for conservation: A systematic review. Biol Conserv. 2020;241:108224. doi:10.1016/j.biocon.2019.108224

[41] Hsu LL, Hsieh SI. Development and psychometric evaluation of the competency inventory for nursing students: A learning outcome perspective. Nurse Educ Today. 2013;33(5):492-497. doi:10.1016/j.nedt.2012.05.028

[42] Poorchangizi B, Borhani F, Abbaszadeh A, Mirzaee M, Farokhzadian J. The importance of professional values from nursing students' perspective. BMC Nurs. 2019;18(1):26. doi:10.1186/s12912-019-0351-1

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