DOI: https://doi.org/10.36602/jsba.2026.21.104

Histopathological Assessment of the Effects of *Ziziphus spina-christi* Leaf Extract on Renal and Hepatic Tissues in Male Rabbits

Sana A. A. Mohammed[1], Eda M. A. Alshailabi [2]*, Asma I. Ahmed[3], Fayrouz A. Khaled[4]
[1] Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya.
[2] Zoology Department, Omar Al-Mukhtar University, Faculty of Science, Libya.
[3]Libyan Academy for Postgraduate Studies, Biomedicine Department, Jabal Al-Akhdar Branch, Libya.
[4]Chemistry Department, Omar Al-Mukhtar University, Faculty of Science, Libya.

*Corresponding author email address: eda.muftah@omu.edu.ly

Submission date:25.2.2026 Acceptance date: 28. 5 .2026 Electronic publishing date: 6.6.2026

Abstract
The present study aimed to evaluate the histopathological effects of *Ziziphus spina-christi* leaf extract (ZSCLE) on liver and kidney tissues in male rabbits. Ten adult rabbits were divided into two groups: a control group receiving no treatment and a treatment group administered ZSCLE orally at a dose of 200 mg/kg/b.w. for six weeks. Histopathological examination of liver tissues in the treated group revealed dilated and congested central veins, portal area degeneration, inflammatory cell infiltration, fibrosis, and hemorrhage. Likewise, kidney sections exhibited glomerular degeneration, tubular necrosis, desquamation of tubular epithelium, damaged brush borders, and interstitial hemorrhage. These findings demonstrate that prolonged oral administration of ZSCLE at a dose of 200 mg/kg b. w. can induce substantial structural alterations in these organs, despite their reported antioxidant and anti-inflammatory properties. Further studies are recommended to determine safe therapeutic doses, evaluate biochemical markers, and elucidate the underlying mechanisms of action in these organs and beyond

Keywords: Histopathological changes; *Ziziphus spina-christi*; liver; kidney; rabbits.

Introduction:
Scientific study is increasingly focusing on the biological and ethnopharmacological foundations of herbal treatments, especially when assessing the effectiveness and safety of medicinal plants frequently employed in traditional medicine. Among these plants, *Ziziphus spina-christi* L. (ZSC), a member of the Rhamnaceae family, has a significant role in traditional Arab medicine. Its fruits and leaves have long been used to cure a variety of illnesses, such as oxidative stress-related diseases, gastrointestinal issues, and inflammatory conditions (Kadıoğlu *et al.,* 2016; El Maaiden *et al.,* 2020; Abdulrahman *et al.,* 2022).

ZSC leaves are abundant in a variety of bioactive components, including flavonoids, alkaloids, tannins, and saponins, which have been shown to have anti-inflammatory and antioxidant activities, according to phytochemical studies (Sakna *et al.,* 2022; Almeer *et al*., 2022; Sabir & Hussein, 2024). Moreover, these compounds are believed to play a crucial role in protecting biological tissues against cellular damage induced by oxidative and inflammatory stress, thereby contributing to the therapeutic potential of the plant (Dkhil *et al.,* 2018; Almeer *et al.,* 2022; Abdulrahman *et al.,* 2022; Khaled *et al.,* 2025).

Despite these reported beneficial properties, there is a growing need to critically evaluate the safety profile of plant extracts, particularly with respect to vital organs such as the liver and kidneys (Gonfa *et al.,* 2024; Suleiman & Alshailabi, 2025), where these organs are highly susceptible to xenobiotic-induced injury due to their central roles in the metabolism, detoxification, and excretion of both endogenous and exogenous compounds (Jaeschke *et al.,* 2012; Majeed *et al.,* 2020; Zhang *et al.,* 2022). Thus, previous studies have demonstrated divergent effects of *Ziziphus spina-christi* extracts (ZSCLE), ranging from protective to potentially harmful outcomes, depending on the type of extract, administered dose, and duration of exposure (Almeer *et al.,* 2019; Nuru *et al.,* 2024; Rabeh *et al.,* 2024). For instance, earlier investigations reported that aqueous or alcoholic extracts of ZSC fruits induced histopathological alterations in hepatic and renal tissues of experimental rats, particularly at higher doses, with observable architectural

disorganization in both organs (Ammari *et al.,* 2024; Nuru *et al.,* 2024). On the other hand, other studies documented the protective effects of ZSCLE, demonstrating attenuation of inflammatory responses and oxidative stress markers in various animal models of induced toxicity, suggesting potential therapeutic benefits under controlled conditions (Almeer *et al.,* 2018; Almeer *et al.,* 2019; Khedre *et al.,* 2025).

Therefore, the present study was designed to evaluate the histopathological alterations in the liver and kidney tissues of male rabbits following oral administration of ZSCLE at a fixed dose for six weeks. This assessment aims to provide detailed histology evidence that may contribute to a better understanding of the balance between potential therapeutic benefits and possible adverse effects of long-term ZSCLE use.

Model Description:

Experimental animals

Total of ten healthy adult male rabbits (*Oryctolagus cuniculus*), weighing between 1.5 and 2.0 kg, were used in this study. The animals were kept in typical laboratory conditions, which included a 12-hour light/dark cycle, an ambient temperature of 22–25°C, and unlimited access to water and a standard pellet diet.

Preparation of ZSC Leaf extract

ZSC fresh leaves were gathered, carefully cleaned under running water, and air-dried at room temperature in the shade. A mechanical grinder was used to reduce the dried leaves to a fine powder. 50g of the powdered leaves were extracted by soaking them in 500mL of 80% methanol at 4°C for 72 hours, with occasional shaking. Whatman No. 1 filter paper was used to filter the mixture, and a rotary evaporator was used to concentrate the filtrate under low pressure to produce a semisolid extract. Before use in experimental procedures, the resultant extract was stored at -20 °C (Almeer *et al*., 2019; Ahmed *et al.,* 2026).

Experimental Design

The institutional rules for the use and care of laboratory animals were followed in all experimental procedures.

- **Control group**: Rabbits received no pharmacological treatment and were orally administered distilled water throughout the experimental period.
- **Treated group**: Rabbits were orally administered ZSCLE at a dose of 200 mg/kg body weight once daily for 6 consecutive weeks using oral gavage (Michel *et al.,* 2011; Khaled *et al.,* 2025).

Throughout the study period, all animals were observed daily to monitor general health and well-being.The selected dose of 200 mg/kg was based on previous studies reporting dose-dependent biological and histopathological effects of ZSCLE in experimental models (Nuru *et al.,* 2024; Ahmed *et al.,* 2026). This dose was chosen to ensure a detectable tissue response while maintaining comparability with earlier toxicological studies.

Histopathological examination

At the end of the experimental period, the rabbits were humanely euthanized in accordance with ethical guidelines for animal experimentation (AVMA, 2020). Liver and kidney tissues were carefully excised, rinsed with normal saline to remove blood residues, and immediately fixed in 10% neutral buffered formalin for histopathological examination. Fixed tissue samples were processed using standard histological techniques, including dehydration, clearing, and paraffin embedding. Tissue sections approximately 5 μm thick were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E) for general histological assessment. The stained sections were examined under a light microscope to evaluate histopathological alterations in hepatic and renal tissues (Lillie, 1954). At least ten microscopic fields per section were examined to ensure a representative evaluation. Histopathological alterations were assessed descriptively and graded using a semi-quantitative scoring system expressed as absent (–), mild (+), moderate (++), and severe (+++), according to Alshailabi *et al.* (2021) and Moshaie-Nezhad *et al.* (2021). Histopathological evaluation was performed in a blinded manner to minimize observer bias. Given the descriptive nature of histopathological assessment and the relatively small sample size,

inferential statistical analysis was not applied, and data were reported using the semi-quantitative scoring approach.

Ethical approval

All experimental protocols were reviewed and approved by the Bioethics Committee of Al-Jabal Akhdar Branch (JCB) at its 13th meeting held on 21 August 2025. Ethical clearance was granted under reference number NBC: 004.A.25.22. All procedures involving animals were conducted in compliance with institutional ethical standards and international guidelines for the care and use of laboratory animals.

Results and Discussion:

Histopathological findings of the liver

Microscopic examination of liver sections from the control group revealed a typical lobular architecture (Figure1). Hepatocytes were arranged in anastomosing cords radiating from the central vein and separated by intact hepatic sinusoids. The hepatocytes appeared polyhedral with acidophilic cytoplasm and large, centrally located vesicular nuclei. In contrast, liver sections from rabbits treated with ZSCLE for six weeks showed various histopathological alterations. These included focal hepatocellular degeneration and dilatation with congestion of the central vein (Figure 2). Deteriorating changes were observed in the portal zones, characterized by expanded and congested portal veins and thickened walls of the portal vein and hepatic artery (Figure 3). Inflammatory cell infiltration associated with enlarged and congested central veins was observed in several sections (Figure 4). Other sections showed marked dilatation and congestion of the central vein with wall thickening and surrounding fibrotic zones (Figure 5). Hemorrhagic parts were also observed in association with inflammatory cell infiltration in some sections (Figure 6).

Histopathological findings of the kidney

Kidney sections from the control group exhibited normal histological architecture of both the cortex and medulla. The glomeruli showed regular basement membranes, mesangial cellularity, and matrix, with no evidence of shrinkage or swelling. Renal tubules were lined by cuboidal epithelial cells with a typical luminal appearance (figures 7 and 8). Conversely, kidney sections from rabbits treated with ZSCLE for six weeks revealed noticeable histopathological changes compared with the control group. These changes included degeneration of renal corpuscles, vacuolar degeneration and necrosis of the tubular epithelium, and shedding of renal tubular epithelial cells, accompanied by damage to the brush limits (Figure 9). In addition, hemorrhage within the interstitial tissue and medullary tubules was observed, along with shedding of the medullary tubular epithelium (Figure 10).

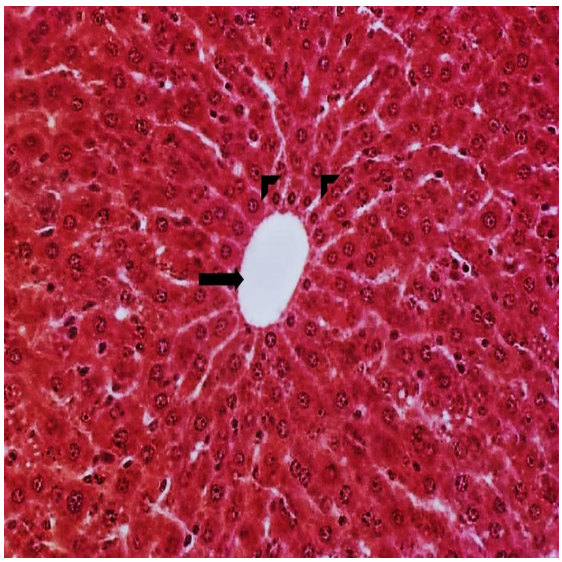

Figure 1: The Liver section from control rabbits showing normal hepatic lobular architecture with a central vein (arrow) surrounded by radially arranged hepatocytes (arrowheads) and intervening sinusoids (H&E stain, 400×).

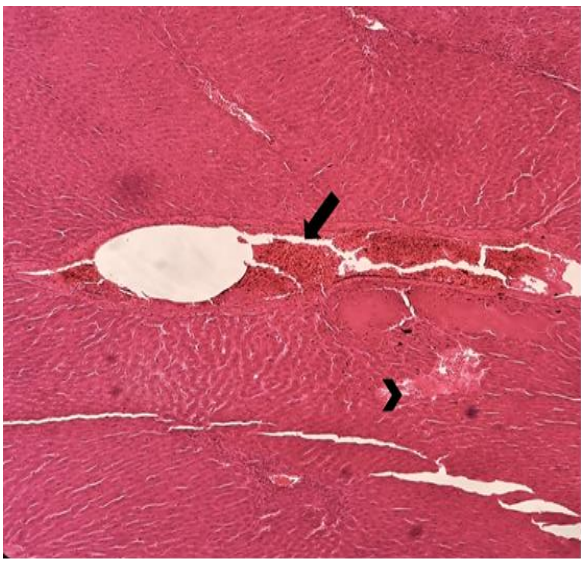

Figure 2: The liver from ZSCLE rabbits displays an eroded zone (head arrow), enlarged, congested central vein (arrow) (H & E stain, 100×).

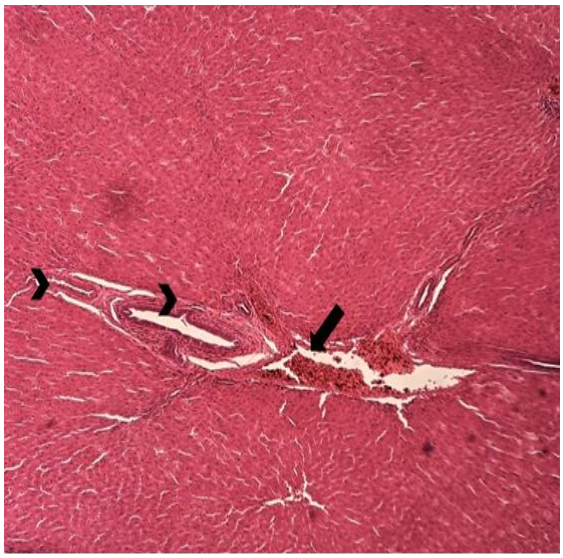

Figure 3: The liver section from ZSCLE rabbits showing degeneration of the portal zone with enlarged and congested portal zone (arrow), thickening of the wall of the portal vessels (head arrows) (H & E stain, 100×).

Figure 4: The liver section of ZSCLE rabbits showing enlarged, congested central vein (arrow), and inflammatory cell infiltration (head arrow) (H & E stain, 100×).

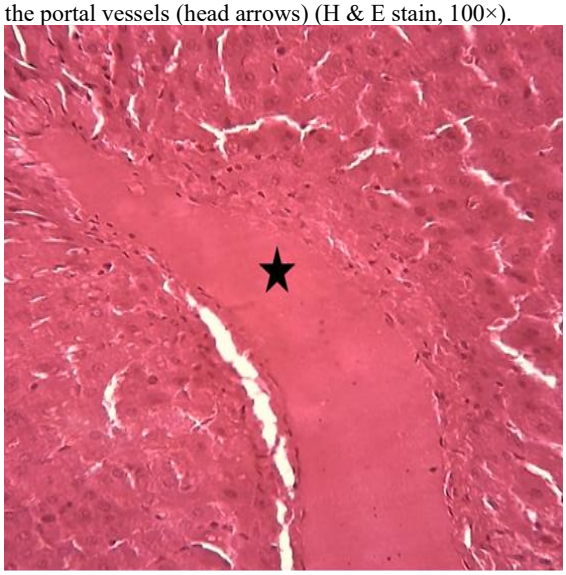

Figure 5: The liver section of ZSCLE rabbits displays an enlarged, congested, and congealed wall of the central vein (star), enclosed by a fibrotic zone (H & E stain, 400×).

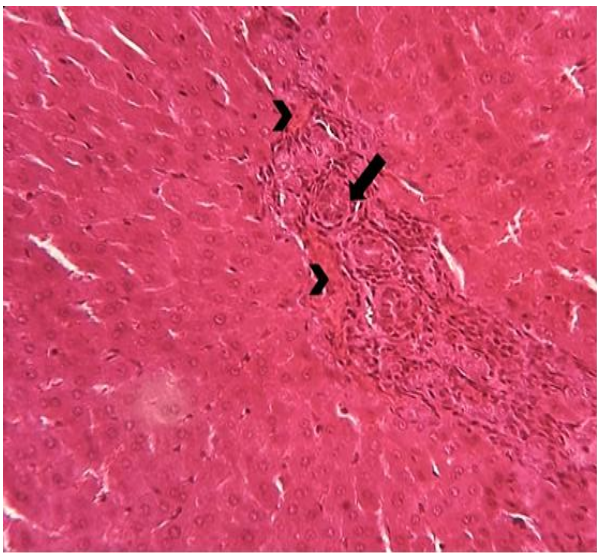

Figure 6: The liver section of ZSCLE rabbits displays inflammatory cell infiltration (arrow), and haemorrhage (head arrow) (H & E stain, 400×).

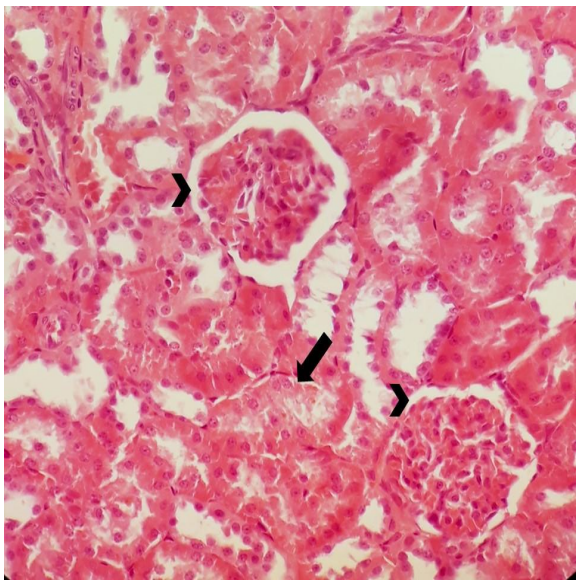

Figure 7: The kidney section from control rabbits displays regular architecture of renal cortex, regular renal tubules (arrow), and Bowman's capsule (head arrow), with standard Bowman's interstitial (H & E stain, 400×).

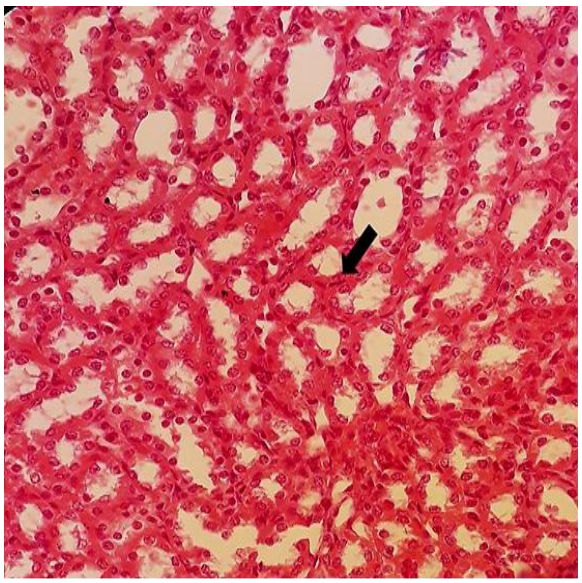

Figure 8: The kidney section from control rabbits displays the normal architecture of the renal medulla. Collecting tubules are lined by simple cuboidal epithelial cells with centrally located round nuclei (arrow) (H&E stain, 400×).

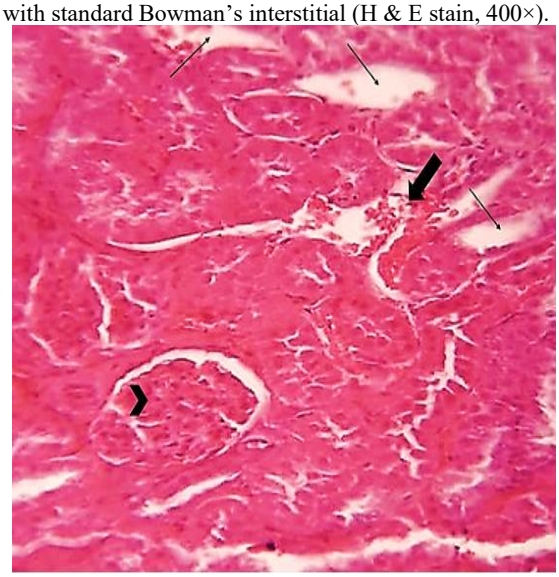

Figure 9: The kidney section from ZSCLE rabbits displays degeneration of renal corpuscles (head arrow), marked vacuolar degeneration and necrosis of tubular epithelium (thin arrows), with shedding of renal tubular epithelium with damage to the brush limits of the cell, and hemorrhage within interstitial tissue (thick arrows) (H & E stain, 400×).

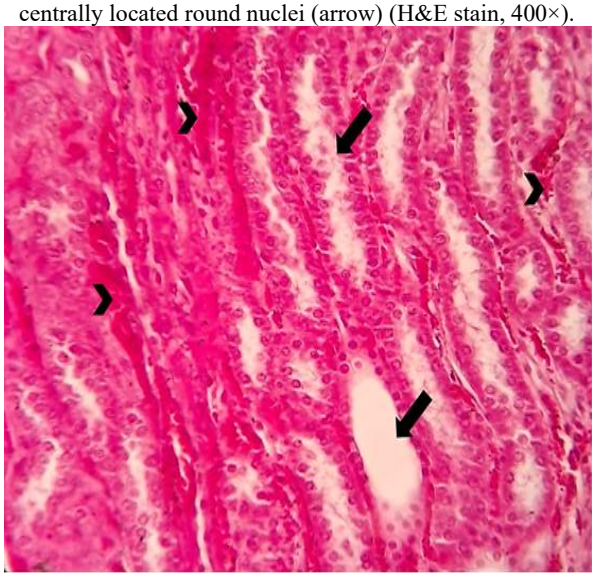

Figure 10: The kidney section of ZSCLE rabbits displays shedding of medulla tubular epithelium with damage to the brush limits of the cell (arrows), and severe hemorrhage in medulla tubules (head arrows) (H & E stain, 400×).

Provides a semi-quantitative summary of histopathological findings in liver and kidney tissues from control and ZSCLE-treated rabbits (Table 1). No detectable histological alterations were recorded in the control group. In the treated group, mild to moderate changes were observed in both organs. Hepatic findings primarily involved vascular and portal alterations with inflammatory cell presence, while renal changes were mainly related to glomerular and tubular structures. These observations are limited to the experimental conditions of the present study.

Table 1. Semi-quantitative Histopathological Scoring of Liver and Kidney Tissues in Control and ZSCLE-Treated Rabbits

Tissue	Histopathological Parameter	Control Group	ZSCLE-Treated Group
Liver	Central vein congestion	–	++
	Portal vein congestion	–	++
	Portal area degeneration	–	++
	Inflammatory cell infiltration	–	++
	Fibrosis	–	+
	Hemorrhage	–	+
Kidney	Glomerular degeneration	–	++
	Tubular epithelial degeneration	–	++
	Tubular necrosis	–	++
	Epithelial cell shedding	–	++
	Brush border damage	–	+
	Interstitial hemorrhage	–	+
	Medullary tubular epithelial shedding	–	++

Scoring system: – = absent, + = mild, ++ = moderate, +++ = severe

The present study demonstrated that chronic oral administration of ZSCLE at a dose of 200 mg/kg induced marked histopathological alterations in both hepatic and renal tissues of male rabbits. Hepatic examination revealed pronounced vascular congestion, degeneration of the portal areas, inflammatory cell infiltration, fibrotic changes, and hemorrhage, indicating significant liver injury. These findings are in agreement with previous experimental studies reporting disruption of hepatic architecture and inflammatory responses following high-dose or prolonged administration of ZSCLE in rodent models (Rabeh *et al.,* 2024; Nuru *et al.,* 2024). The observed hepatic alterations may be attributed to the dual biological nature of ZSC bioactive constituents. These compounds, including flavonoids and phenolic acids, exhibit antioxidant and anti-inflammatory effects at moderate doses. However, at higher concentrations, they may act as pro-oxidants (Valko, 2022). In models of chemically induced hepatotoxicity, ZSCLE have demonstrated hepatoprotective effects through attenuation of oxidative stress and modulation of apoptotic pathways (Khedre *et al.,* 2025); however, in healthy tissues, prolonged exposure appears to predispose to vascular disturbances, inflammatory reactions, and fibrogenesis (Elduob *et al.,* 2023; Nuru *et al.,* 2024). Similarly, renal histopathological evaluation revealed severe structural damage characterized by glomerular degeneration, tubular necrosis, desquamation of tubular epithelium, disruption of brush borders, and interstitial hemorrhage, reflecting significant nephrotoxicity. These renal lesions are consistent with findings reported in experimental models following high-dose or long-term ZSC administration (Rabeh *et al.,* 2024; Nuru *et al.,* 2024). The renal damage observed in the present study is likely mediated by sustained oxidative stress and inflammatory processes triggered by prolonged exposure, emphasizing the dose- and duration-dependent nature of ZSC extract effects. These findings highlight the importance of dose regulation, as plant-derived compounds may exert beneficial or toxic effects depending on exposure conditions (Valko, 2022). In contrast, several studies have reported nephroprotective effects of ZSC extract at controlled doses in models of induced renal injury, attributed to enhanced antioxidant enzyme activity and reduced reactive oxygen species (Almeer *et al.,* 2019; Abdulrahman *et al.,* 2022). Collectively, the histopathological findings suggest that the bioactive constituents of ZSC, including flavonoids, phenolic acids, and saponins, may activate pro-inflammatory and fibrogenic signaling pathways, potentially involving NF-KB activation and cytokine-mediated mechanisms, when administered at high doses or for prolonged periods, ultimately leading to tissue injury in vital organs (Al-Khayri *et al.,* 2022; Olędzka & Czerwińska, 2023). Moreover, the semi-quantitative scoring results presented in Table 1 further support the microscopic findings, demonstrating moderate (++) severity for most hepatic and renal alterations, while control animals exhibited completely normal histological architecture. This consistency between qualitative and semi-quantitative assessments strengthens the reliability of the present findings and highlights the usefulness of histopathological scoring systems in toxicology studies (Alshailabi *et al.*, 2021; Moshaie-Nezhad *et al.,* 2021; Elduob *et al.,* 2023).

Although the relatively small sample size (n = 10) represents a limitation of the present study, the

consistency of histopathological alterations observed across multiple examined fields strengthens the reliability of the findings.

Conclusion:

The present study demonstrates that prolonged oral administration of ZSCLE at a dose of 200 mg/kg induces notable histopathological alterations in hepatic and renal tissues of male rabbits, indicating hepatorenal toxicity following chronic exposure. These findings suggest that despite its reported therapeutic properties, long-term use of ZSCLE at relatively high doses may pose potential risks to vital organs. Therefore, careful consideration of dose and duration is essential, and further studies are required to establish safe usage parameters. In addition, the assessment was limited to histopathological evaluation without parallel biochemical or molecular analyses. Future studies employing larger sample sizes and integrating biochemical and molecular biomarkers are recommended to further elucidate the mechanisms underlying the observed hepatorenal alterations.

Future studies should incorporate biochemical parameters (ALT, AST, creatinine, urea) and molecular markers to complement histopathological findings and provide a more comprehensive assessment.

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التقييم النسيجي المرضي لتأثيرات مستخلص أوراق نبات السدر (*Ziziphus spina-christi*) على أنسجة الكلى والكبد في ذكور الأرانب

سناء عبد اللطيف عياد محمد[1] ,عيدة مفتاح عبد الكريم الشيلابي[2]*,أسماء ادريس احمد[3], فيروز الزبير خالد[4]

الملخص

هدفت هذه الدراسة إلى تقييم التأثيرات النسيجية المرضية لمستخلص أوراق نبات السدر (*Ziziphus spina-christi*) على أنسجة الكبد والكلى لدى ذكور الأرانب. قُسِّمت عشرة أرانب بالغة إلى مجموعتين: مجموعة ضابطة لم تتلقَّ أي علاج، ومجموعة علاجية أُعطيت مستخلص السدر عن طريق الفم بجرعة 200 ملغم/كغم من وزن الجسم لمدة ستة أسابيع. كشف الفحص النسيجي المرضي لأنسجة الكبد في المجموعة المعالجة عن توسع واحتقان الأوردة المركزية، وتدهور منطقة البوابة، وتسلل الخلايا الالتهابية، والتليف، والنزيف. وبالمثل، أظهرت مقاطع الكلى تدهورًا كبيبيًا، ونخرًا أنبوبيًا، وتقشرًا في ظهارة الأنابيب، وتلفًا في الحواف الفرشاتية، ونزيفًا خلاليًا. تُشير هذه النتائج إلى أن الإعطاء الفموي المطوّل لمستخلص السدر بجرعة 200 ملغم/كغم من وزن الجسم يُمكن أن يُحدث تغييرات هيكلية كبيرة في هذه الأعضاء، على الرغم من خصائصه المضادة للأكسدة والالتهابات المعروفة. يوصى بإجراء المزيد من الدراسات لتحديد الجرعات العلاجية الآمنة، وتقييم المؤشرات الحيوية الكيميائية، وتوضيح آليات العمل الأساسية لهذه الأعضاء وغيرها من الأعضاء.

الكلمات المفتاحية: التغيرات النسيجية المرضية؛ نبات السدر؛ الكبد؛ الكلية؛ الأرانب.