Medical Technology Journal of Applied Science (MTJAS)

Volume 2, Issue 1, 2026, Page No: 44 - 56
Website: https://mtjas.com.ly/index.php/mtjas/index

Protective and Therapeutic Effects of Libyan Sidr Honey on Aspartame-Induced Nephrotoxicity in Adult Albino Rats: A Histopathological Study

Nadia A. Al-Shukri[1], Fathia M. Senossi[2], Hafedah N. Elsharef[3], Hanan S. Elsharia[4], Osama M. El-barasi[5], Samah A. K. Abdallah[6], Rayya G. Alsultan[7], Noura A. A. Alhissade[8], Hala Y. M. Hassan[9], Mohammed A. Abusharya[10], Samia M. Efkeren[11], Sana A. A. Mohammed[12], Eda M. A. Alshailabi[13]*

[1] Zoology Department, Faculty of Science, Al-Asmarya Islamic University, Libya & Libyan Centre for Sustainable Development Research.
[2] Zoology Department, College of Arts and Sciences, AL-Marj, Benghazi University, Libya & Libyan Centre for Sustainable Development Research.
[3] Libyan Authority for Scientific Research. Libya & Libyan Centre for Sustainable Development Research.
[4] Zoology Department, College of Arts and Sciences Seluq, Benghazi University, Libya & Libyan Centre for Sustainable Development Research.
[5] Zoology Department, Faculty of Science, University of Derna, Libya & Libyan Centre for Sustainable Development Research.
[6, 11, 13] Zoology Department, Faculty of Science, Omar Al-Mukhtar University, Libya & Libyan Centre for Sustainable Development Research.
[7] Biology Department, Education College for Girls. Mosul University, Iraq & Libyan Centre for Sustainable Development Research.
[8] Microbiology and Immunology Department, Faculty of Pharmacy, University of Derna, Libya & Libyan Centre for Sustainable Development Research.
[9] Biomedical Engineering Department, Faculty of Engineering, Wadi Alshatti University, Libya & Libyan Centre for Sustainable Development Research.
[10] Biology Department, Faculty of Science, University of Elmergib, Libya & Libyan Centre for Sustainable Development Research.
[12] Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Libya & Libyan Centre for Sustainable Development Research.

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

Received: February, 01, 2026	Accepted: February, 25, 2026	Published: February 26, 2026

Abstract:

Aspartame (ASP) is a widely used artificial sweetener that may induce renal structural alterations following chronic exposure, potentially through oxidative stress–mediated mechanisms. Sidr honey is a natural antioxidant-rich product that may provide protective effects against chemically induced organ damage. In this study, thirty adult female albino rats were randomly allocated into five groups (n = 5): control, Libyan Sidr honey (LSH, 100 mg/kg/body weight/day), ASP (75 mg/kg/body weight/day), protective (LSH for two weeks followed by ASP for four weeks), and combination (LSH and ASP concurrently for four weeks). All treatments were administered orally. At the end of the experiment, serum urea, creatinine, calcium, and uric acid were analyzed, and kidney tissues were examined histologically using a semi-quantitative scoring system. ASP induced marked renal histopathological alterations, including glomerular shrinkage, tubular epithelial degeneration, inflammatory cell infiltration, and vascular congestion. However, no significant changes were observed in serum urea, creatinine, or uric acid levels ($p > 0.05$). A modest but significant reduction in serum calcium was detected in the combination group ($p < 0.05$).

Both pretreatment and concurrent LSH administration significantly ameliorated ASP-induced renal damage and preserved normal renal architecture. These findings suggest that LSH may exert protective and therapeutic effects against ASP-induced nephrotoxicity.

Keywords: Aspartame; Libyan Sidr Honey; Nephrotoxicity; Histopathology; Albino Rats.

Introduction

Aspartame (L-alpha-aspartyl-L-phenylalanine methyl ester; ASP) is one of the most widely used artificial sweeteners worldwide, commonly incorporated into a variety of food and beverage products as a low-calorie sugar substitute (Magnuson *et al.,* 2007; Alsaeh *et al.,* 2025). Although generally considered safe at recommended dietary levels, emerging experimental evidence indicates that chronic or high-dose consumption may induce adverse effects, including oxidative stress, inflammation, and organ toxicity, with the kidney being particularly vulnerable (Alsaeh *et al.,* 2025; Omozee & Timothy, 2025). The kidney's susceptibility to chemical-induced injury is largely due to its central roles in filtration, excretion, and metabolic processing (Elduob *et al.,* 2023; Omozee & Timothy, 2025). Experimental studies have shown that ASP-induced nephrotoxicity is associated with structural and functional alterations, including tubular degeneration, glomerular shrinkage, interstitial inflammatory infiltration, and vascular congestion. Biochemically, these changes are reflected in elevated serum urea and creatinine levels (Azez *et al.,* 2023; Omozee & Timothy, 2025). Oxidative stress, resulting from the generation of reactive oxygen species (ROS) and lipid peroxidation (LPO), is considered a primary mechanism underlying these renal alterations (Azez *et al.,* 2023). Natural antioxidants have attracted considerable attention as potential protective agents against chemically induced organ damage (Alshailabi, 2016; Elshama *et al.,* 2018). Honey, particularly Sidr honey (*Ziziphus spina-christi*), is rich in flavonoids, phenolic acids, vitamins, enzymes, and other bioactive components that collectively confer antioxidant, anti-inflammatory, and tissue-regenerative properties (Gajger *et al.,* 2025). These bioactive compounds can scavenge free radicals, reduce LPO, and enhance cellular repair, as documented in studies evaluating honey's protective effects against chemical-induced hepatic and cardiac injuries (Alshailabi *et al.,* 2023; Alshailabi *et al.,* 2024). Despite accumulating evidence on the beneficial effects of honey, limited studies have specifically investigated the protective and therapeutic potential of LSH against ASP-induced nephrotoxicity. Therefore, the present study was designed to assess the effects of LSH on renal structure and function in adult female albino rats exposed to ASP. The study employed a dual approach, including evaluation of biochemical markers of kidney function and detailed histopathological assessment, to provide comprehensive insight into the potential prophylactic and therapeutic roles of LSH in mitigating ASP-induced renal damage.

Material and methods

Chemicals

LSH was obtained from the Natural Food and Honey Production Company, Benghazi, Libya. Before administration, LSH was freshly diluted in distilled water to obtain the required concentration. The honey was stored in dark, airtight containers at room temperature to preserve its bioactive compounds until use.

ASP was procured from Sigma-Aldrich Corporation (St. Louis, MO, USA). For each administration, ASP was freshly dissolved in distilled water to ensure stability and precise dosing.

Experimental Animals

Thirty healthy adult female albino rats, weighing between 100 and 200 g, were used in this study. The animals were obtained from the Department of Zoology's animal house at the University of Derna, Libya. They were housed in well-ventilated cages under controlled laboratory conditions and maintained on a 12-hour light/dark cycle, with ambient environmental parameters kept constant throughout the experimental period. The rats were allowed a ten-day acclimatization period before the start of the experiment to ensure physiological adaptation and minimize stress-related effects. Throughout the study, the animals had ad libitum access to water and were fed a standard laboratory diet composed of 21.27% protein, 2.83% fat, and 2.46% fiber.

Experimental Design

After acclimatization, rats were randomly assigned to five experimental groups (n = 5 per group):

- **Group 1 (Control):** Received distilled water orally once daily for four weeks.
- **Group 2 (LSH):** Received LSH at 100 mg/kg body weight/day orally for four weeks (Alshailabi *et al.,* 2024).
- **Group 3 (ASP):** Received ASP at 75 mg/kg body weight/day orally for four weeks (Iyyaswamy & Rathinasamy, 2012).
- **Group 4 (Protective, LSH → ASP):** Rats were pretreated with LSH (100 mg/kg/body weight/day) orally for two weeks, followed by ASP (75 mg/kg/body weight/day) orally for four weeks. This group was designed to assess the prophylactic effect of LSH.
- **Group 5 (Combination, LSH+ASP):** Rats received LSH (100 mg/kg/body weight/day) concurrently with ASP (75 mg/kg/body weight/day) orally for four weeks to evaluate the therapeutic effect during ongoing exposure.

All treatments were administered once daily using a stainless-steel gastric gavage needle. Dosing volumes were adjusted according to each animal's body weight to ensure accuracy.

Sample Collection and Tissue Processing

At the end of the experimental period, the rats were euthanized, and both kidneys were carefully excised. The tissues were rinsed with ice-cold saline to remove any residual blood and subsequently fixed in 10% neutral buffered formalin for 48 hours. Standard histological processing procedures were followed, including dehydration through ascending grades of ethanol, clearing in xylene, and embedding in paraffin wax. Paraffin-embedded kidney tissue sections were cut at 5 µm using a rotary microtome, mounted on clean glass slides, and stained with Hematoxylin and Eosin (H&E) for histopathological evaluation of renal architecture (Bancroft & Gamble, 2008).

For each animal, three non-consecutive tissue sections were examined, and at least ten randomly selected microscopic fields per section were analyzed to ensure representative evaluation (Haghighat *et al.,* 2022). Renal histopathological alterations, including glomerular and tubular degeneration, interstitial hemorrhage, vascular congestion, and inflammatory cell infiltration, were assessed using a semi-quantitative scoring system. Lesions were graded descriptively as absent (–), mild (+), moderate (++), or severe (+++) to facilitate standardized comparison among experimental groups (Moshaie-Nezhad *et al.,* 2021; Abdalally *et al.,* 2026). Since the scoring system was based on ordinal qualitative grading, histopathological findings were presented descriptively without numerical statistical analysis.

Biochemical Assessment

Serum creatinine was determined using a kinetic test without deproteinization according to the method of Newman and Price (1999) with DiaSys reagent kits. Serum urea was measured using the urease-GLDH enzymatic UV method following Thomas (1998) and with DiaSys reagent kits. The determination of uric acid was performed using a modified method of Fossati *et al*. (1980). Serum calcium levels were measured using the method of Anderegg *et al.* (1954).

Statistical Analysis

Data from serum biochemical measurements (urea, creatinine, calcium, and uric acid) were expressed as mean ± standard error (Mean ± SE). Since the data were not normally distributed, differences among the experimental groups were analyzed using the Kruskal–Wallis non-parametric test. When the Kruskal–Walli's test indicated a statistically significant difference ($p < 0.05$), pairwise comparisons between groups were performed using the Mann–Whitney U test to identify which groups differed. Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA).

Results

Biochemical Findings

The renal biochemical parameters and electrolyte levels among the experimental groups are summarized in Table 1.

Serum urea levels showed no statistically significant differences among all groups ($p > 0.05$), with all groups assigned the same statistical letter (A). The control group recorded 24.94 ± 6.77 mg/dL, and the ASP group recorded 24.50 ± 5.80 mg/dL. Similarly, the LSH, protective, and combination groups showed comparable

values (24.80 ± 5.54, 30.68 ± 5.09, and 27.80 ± 3.83 mg/dL, respectively), indicating that neither ASP nor LSH significantly affected serum urea levels.

Serum creatinine levels also did not differ significantly among the groups ($p > 0.05$). The mean values for the control, ASP, LSH, protective, and combination groups were 0.574 ± 0.061, 0.614 ± 0.083, 0.578 ± 0.097, 0.548 ± 0.044, and 0.574 ± 0.060 mg/dL, respectively. All groups were classified under the same statistical letter (A), indicating no significant differences.

Serum calcium levels showed slight but statistically significant differences ($p < 0.05$). The ASP, LSH, and protective groups recorded similar values (10.72 ± 0.44, 10.94 ± 0.49, and 10.99 ± 0.99 mg/dL, respectively) and were classified as group A. The control group (10.09 ± 0.52 mg/dL) was categorized as AB, indicating intermediate significance. The combination group showed a significantly lower calcium level (9.36 ± 0.83 mg/dL; group B), suggesting a modest reduction compared with some other groups.

Serum uric acid levels did not show statistically significant differences among the experimental groups ($p > 0.05$). The control group recorded 3.18 ± 0.90 mg/dL, while the ASP, LSH, protective, and combination groups recorded 2.68 ± 0.36, 2.69 ± 0.58, 3.02 ± 0.84, and 1.81 ± 0.99 mg/dL, respectively. All groups shared the same statistical letter (A).

Finally, Under the conditions of the present study, ASP administration, either alone or in combination with LSH, did not induce significant alterations in most renal biochemical parameters. The only notable change was a modest but statistically significant reduction in serum calcium levels in the combination group. These findings suggest that renal function remained largely preserved throughout the experimental period.

Table (1): Effect of ASP and/or LSH on renal biochemical parameters in experimental groups (Mean ± SEM).

Parameter (mg/dL)	Control	LSH	ASP	Protective	Combination
Urea	24.94 ± 6.77 A	24.80 ± 5.54 A	24.50 ± 5.80 A	30.68 ± 5.09 A	27.80 ± 3.83 A
Creatinine	0.574 ± 0.061 A	0.578 ± .097 A	0.614 ± 0.083 A	0.548 ± 0.044 A	0.574 ± 0.060 A
Ca^{2+}	10.09 ± 0.52 AB	10.94 ± 0.49 A	10.72 ± 0.44 A	10.99 ± 0.99 A	9.36 ± 0.83 B
Uric acid	3.18 ± 0.90 A	2.69 ± 0.58 A	2.68 ± 0.36 A	3.02 ± 0.84 A	1.81 ± 0.99 A

Histopathological Findings of the Kidney

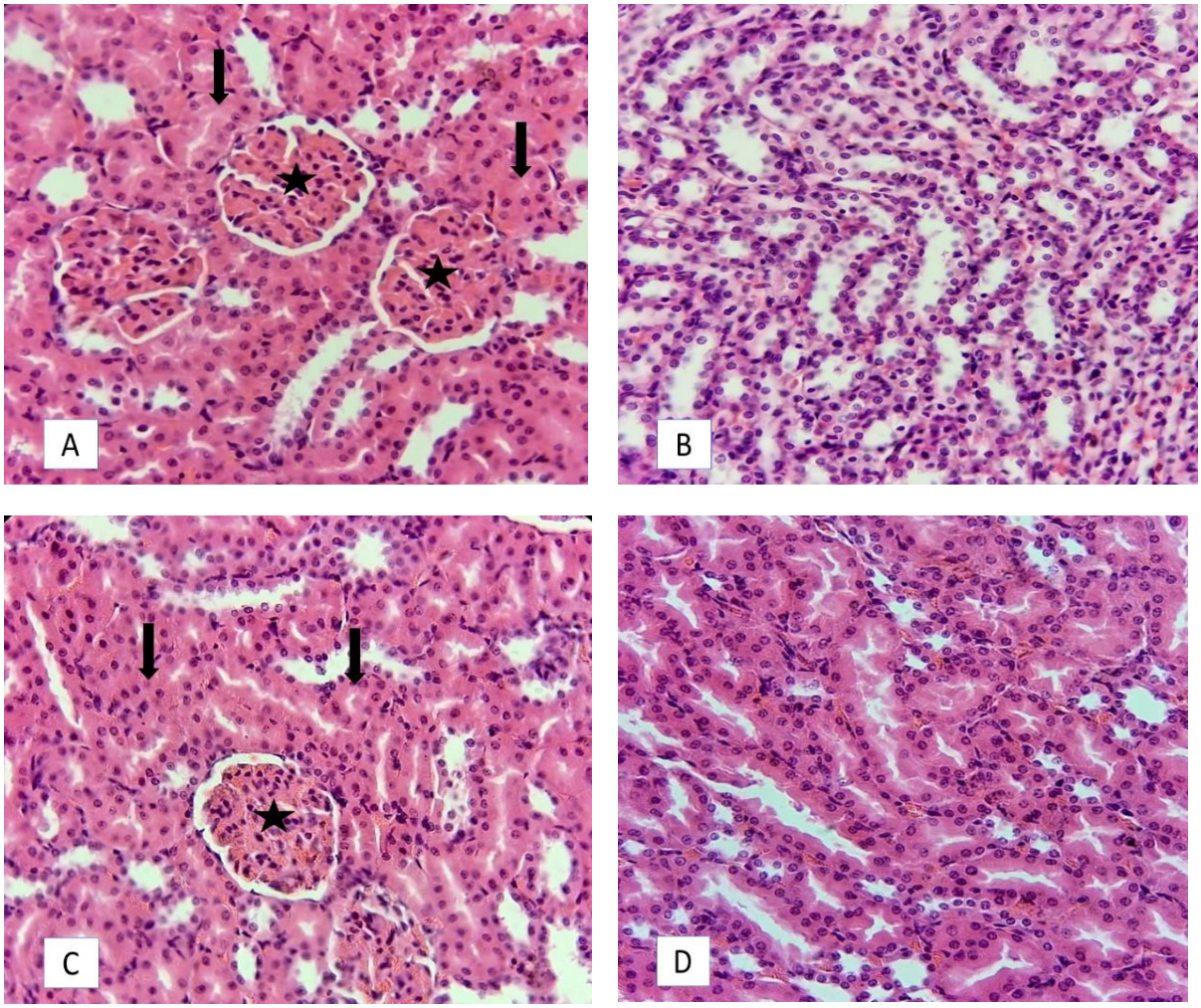
Microscopic examination of kidney sections from the control group revealed normal renal architecture, characterized by intact renal cortex and medulla. The renal cortex showed normal renal corpuscles, well-defined Bowman's space, and normal renal tubules without any pathological alterations (Figure 1A). The renal medulla also exhibited normal collecting tubules and preserved structural organization (Figure 1B). Similarly, kidney sections from the LSH-treated group demonstrated normal renal histological features comparable to those of the control group. The renal cortex displayed normal glomeruli, intact Bowman's space, and well-preserved renal tubules (Figure 1C), while the renal medulla showed normal collecting tubules without any observable histopathological abnormalities (Figure 1D).

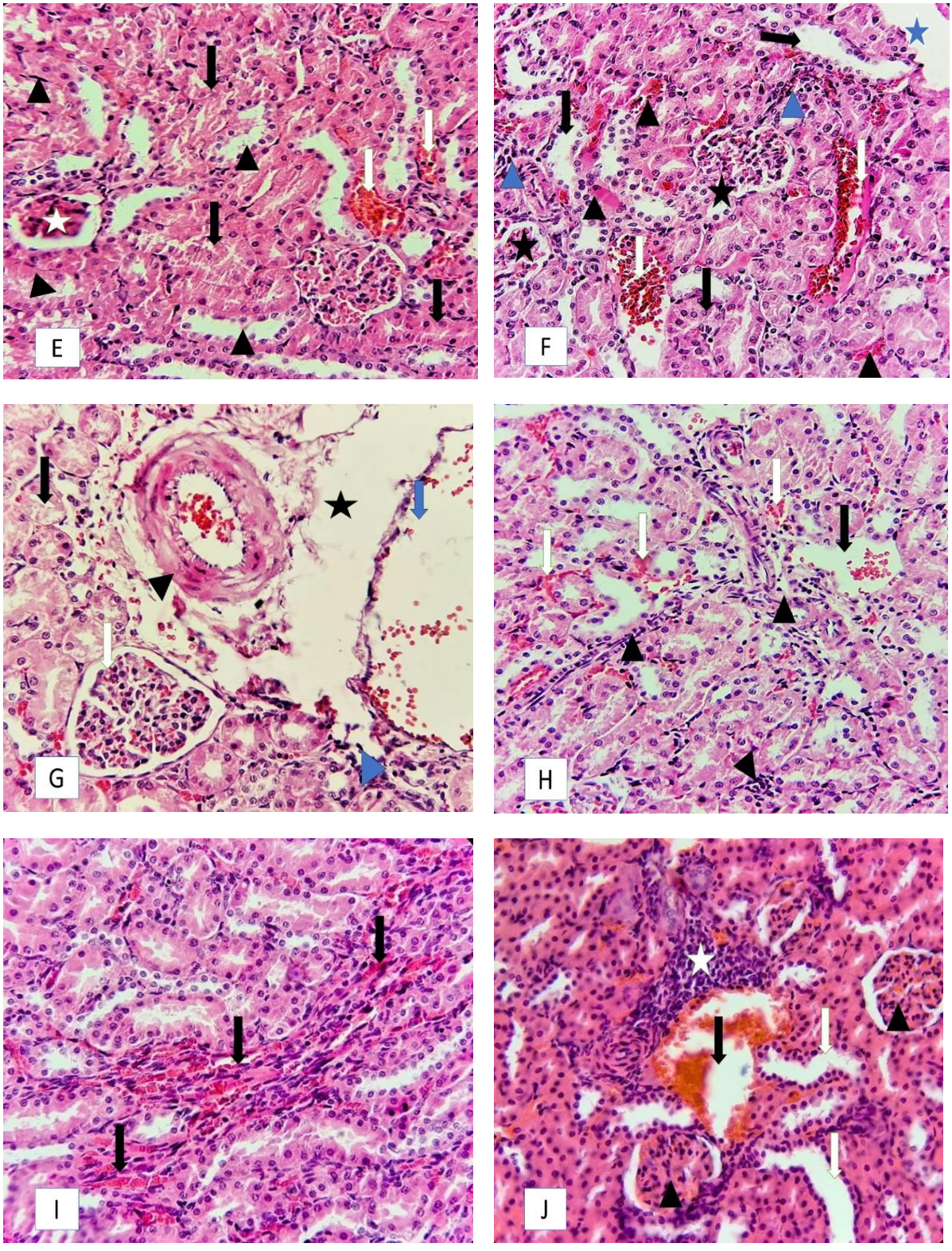
In contrast, the ASP-treated group exhibited severe histopathological alterations in both the renal cortex and medulla. The renal cortex revealed marked glomerular atrophy and degeneration, accompanied by shrinkage and widening of Bowman's space, vascular congestion, hemorrhage, and structural disruption of renal tissue (Figure 1E). Additional cortical changes included tubular degeneration, tubular dilation, interstitial inflammatory cell infiltration, necrotic areas, and congestion and dilation of blood vessels (Figure 1F). In some sections, extensive tissue damage was evident, including large cavitary areas, distorted renal tubules, glomerular degeneration, vascular dilation, arterial wall thickening, and widespread interstitial inflammatory infiltration (Figure 1G).

The renal medulla also demonstrated degenerative changes, including tubular epithelial cell degeneration and necrosis, medullary hemorrhage, vascular dilation, and inflammatory cell infiltration (Figure 1H). Furthermore, syncytial-like tubular structures with poorly defined morphology and associated hemorrhage were observed (Figure 1I).

Kidney sections from the protective-treated group showed noticeable histological improvement compared with the ASP-treated group. The renal cortex exhibited relatively preserved renal corpuscles and Bowman's space, with mild vascular congestion and limited interstitial inflammatory infiltration (Figure 1J). Improved tubular architecture was also observed, although mild degeneration of tubular epithelial cells remained present in some areas (Figure 1K). The renal medulla showed partial restoration of collecting tubule structure, with only mild epithelial degeneration and minimal hemorrhagic areas (Figure 1L). Similarly, the combination-treated group demonstrated marked improvement in renal histological structure. The renal tubules and corpuscles appeared largely normal, with only mild vascular congestion, minimal interstitial hemorrhage, and limited tubular epithelial degeneration (Figure 1M). The renal medulla also showed improved tubular organization, with only slight degeneration of some tubular epithelial cells (Figure 1N).

Overall, ASP administration induced severe renal histopathological damage, whereas treatment with LSH alone showed no adverse effects. Furthermore, protective and combination treatments markedly attenuated ASP-induced renal damage and preserved renal structural integrity to a considerable extent.




E
F
G
H
I
J

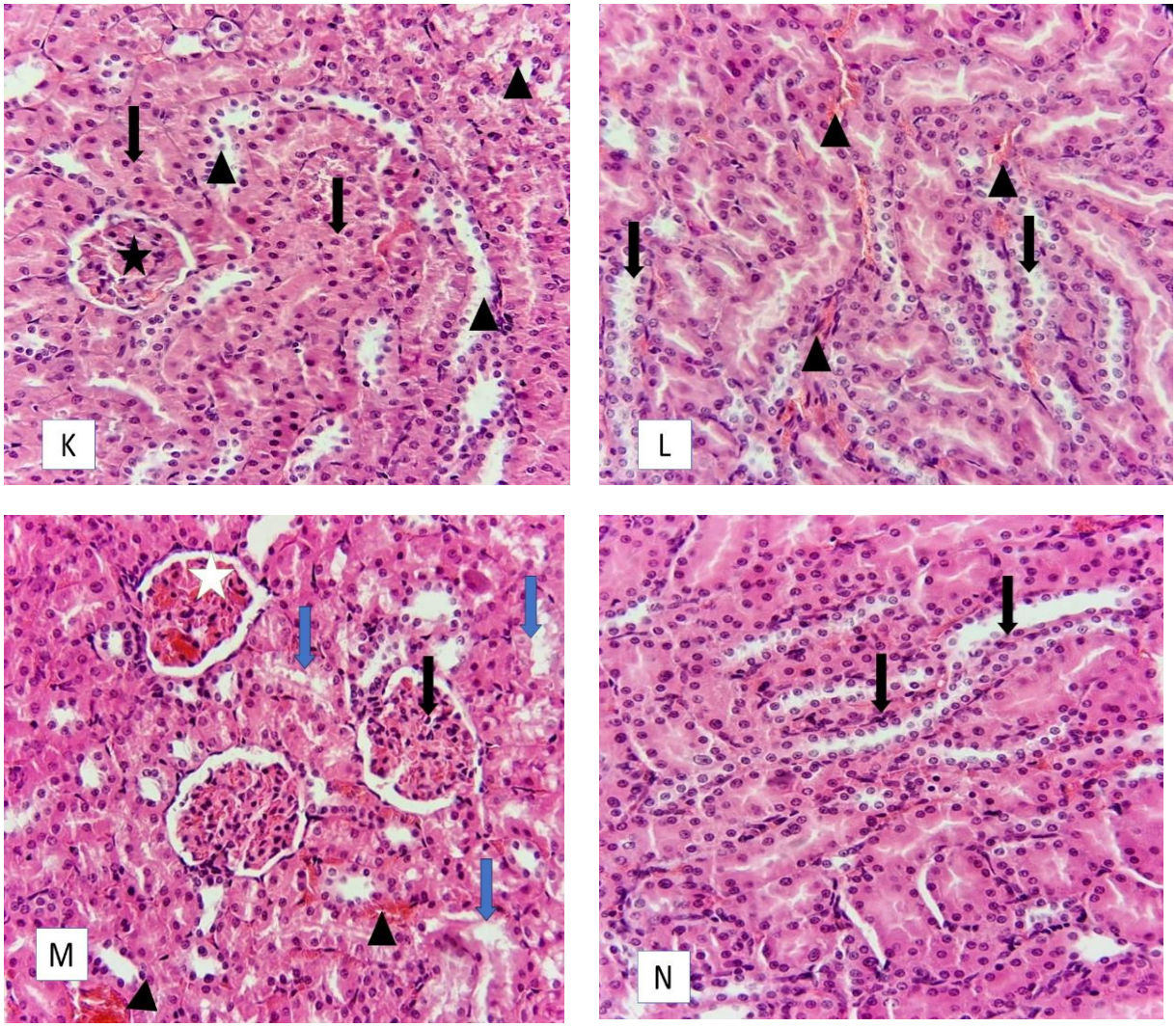


Figure 1. Histopathological lesions in the rat kidney (x400; H&E).

A: Kidney section of control rats displayed a normal renal cortex with renal tubules (arrows), normal renal corpuscles, and a normal Bowman's space (stars).
B: Panel a kidney of control rats displayed a normal renal medulla with normal collecting tubules.
C: Kidney section of LSH rats displayed a normal renal cortex with renal tubules (arrows), normal renal corpuscles, and a normal Bowman's space (star).
D: Kidney section of LSH rats displayed a normal renal medulla with normal collecting tubules.
E: Kidney sections of ASP-treated rats' renal cortex display glomerular atrophy and degeneration (arrowheads), with shrunken and distension of the renal glomerulus capsular space (white star), hemorrhage/congestion (white arrows), and damaged renal cortex (arrows).
F: Kidney sections of ASP-treated rats' renal cortex display glomerular/tubule renal degeneration (stars), dilation of renal tubules (arrows), interstitial cell infiltration (blue arrowheads), renal necrosis (blue star), interstitial hemorrhage (black heads), and congestion, and dilatation of blood vessels (white arrows).
G: Kidney sections of ASP-treated rats' renal cortex display a huge cavity with fragmented areas (star), distorted renal tubules, as well as degenerating cells exhibiting hyalinized cytoplasm and scattered cells (arrow), a highly dilated vessel (blue arrow), glomerular degeneration (white arrow), interlobular arterial thickness (black arrowhead), and interstitial cell infiltration (blue arrowhead).
H: Kidney section of ASP-treated rats' renal medulla displayed scattered degeneration and necrosis of renal tubular epithelial cells with medullary hemorrhage (white arrows), dilatation of blood vessels (black arrow), and interstitial cell infiltration (arrowheads).
I: Kidney section of ASP-treated rats' renal medulla displayed areas of syncytium of ill-defined tubule with medullary hemorrhage (arrows).

J: Kidney sections of protective-treated rats' renal cortex display mild improvement in the structure of renal tubules with normal renal corpuscles, and a normal Bowman's space (arrowheads), congestion, and dilatation of blood vessels (arrow), and interstitial cell infiltration (white star).
K: Kidney sections of protective-treated rats' renal cortex display improvement in the structure of renal tubules (arrows), with normal renal corpuscles, and a normal Bowman's space (star), and mild degeneration of renal tubular epithelium (arrowheads).
L: Kidney section of protective-treated rats' renal medulla displayed mild improvement in the structure of the collecting tubules, with scattered degeneration of some renal tubular epithelial cells (arrows), and a few medullary hemorrhages (arrowheads).
M: Kidney section of combination-treated rats' renal medulla displayed improvement in the structure of renal tubules with normal renal corpuscles, and a normal Bowman's space (arrows), as well as congestion of blood vessels in a few renal corpuscles (white star), little interstitial hemorrhage (arrowheads), and degeneration of a few renal tubular epithelial cells (blue arrows).
N: Kidney section of combination-treated rats' renal medulla displayed improvement in the structure of the collecting tubules, with little degeneration of some renal tubular epithelial cells (arrows).

Histopathological Evaluation and Scoring Results
Semi-quantitative histopathological scoring of renal tissue revealed marked differences among the experimental groups (Table 2; Figure 2). The control and LSH-treated groups exhibited normal renal architecture, with lesion scores graded as absent (–) for all evaluated parameters, indicating preserved renal structural integrity without detectable histopathological alterations. In contrast, the ASP-treated group showed severe renal histopathological damage, with lesion scores graded as severe (+++) for glomerular degeneration, tubular degeneration, interstitial hemorrhage, vascular congestion, and inflammatory cell infiltration. These findings indicate extensive disruption of renal structural integrity following ASP administration.

The protective-treated group demonstrated marked histological improvement compared with the ASP-treated group. Most lesions were graded as mild (+), with limited tubular degeneration, mild vascular congestion, and minimal inflammatory cell infiltration, indicating partial restoration of renal tissue structure. Similarly, the combination-treated group showed significant attenuation of ASP-induced renal damage. Lesion scores ranged from absent (–) to mild (+), with nearly normal glomerular and tubular architecture and only minimal residual histopathological alterations.

Overall, these findings confirm that ASP administration induced severe renal histopathological damage, whereas protective and combination treatments effectively mitigated renal injury and preserved renal tissue integrity.

Table 2. Semi-quantitative scoring of renal histopathological alterations in different experimental groups.

Histopathological alteration	Control	LSH	ASP	Protective	Combination
Glomerular atrophy and degeneration	–	–	+++	+	+
Bowman's space dilatation	–	–	+++	+	+
Tubular degeneration	–	–	+++	+	+
Tubular dilatation	–	–	+++	+	+
Interstitial inflammatory cell infiltration	–	–	+++	+	+
Interstitial hemorrhage	–	–	+++	+	+
Vascular congestion and dilatation	–	–	+++	+	+
Renal necrosis	–	–	+++	+	–
Medullary tubular epithelial degeneration	–	–	+++	+	+
Medullary hemorrhage	–	–	++	+	–
Structural distortion/cavitation	–	–	+++	–	–

- Severity of renal histopathological lesions was graded as absent (–), mild (+), moderate (++), and severe (+++). ASP induced severe renal damage, whereas LSH and protective treatments markedly reduced lesion severity.

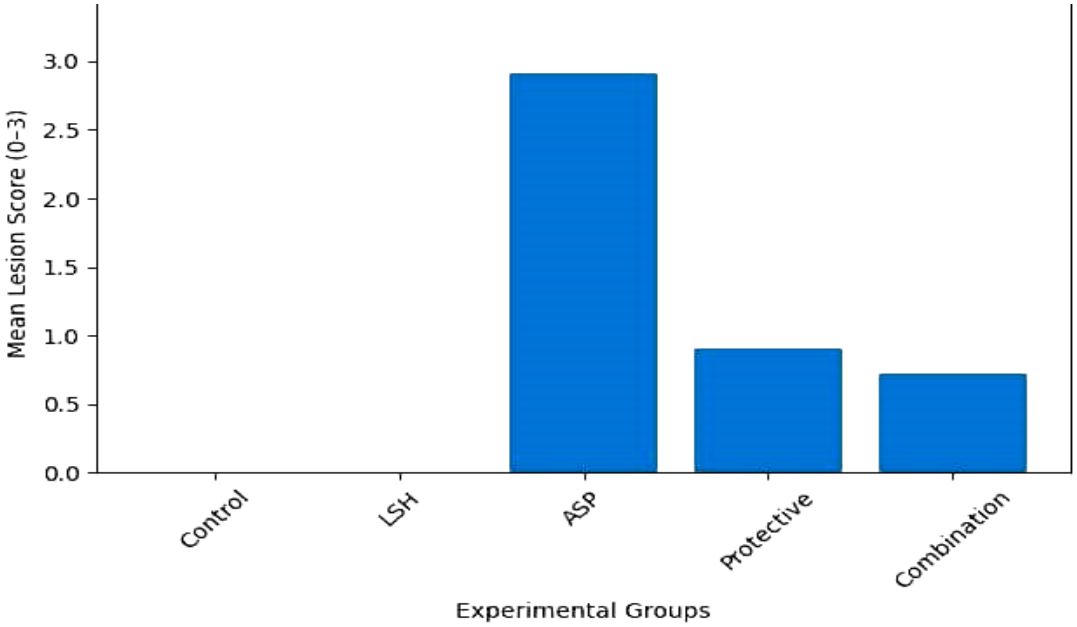


Figure 2. Semi-quantitative histopathological scoring of renal lesions in different experimental groups.

Discussion

The present study investigated the nephrotoxic effects of ASP and the potential protective and therapeutic role of LSH through biochemical and histopathological assessments. The findings demonstrated that ASP administration induced marked renal histopathological damage, whereas LSH exerted significant protective and restorative effects on renal tissue architecture.

Effects of ASP on Renal Biochemical Parameters

Despite the pronounced histopathological alterations observed in the ASP-treated group, serum urea, creatinine, and uric acid levels did not show statistically significant changes compared with the control group. Similar findings have been reported in experimental studies demonstrating that aspartame administration induces marked renal histopathological damage, including inflammatory cell infiltration, tubular epithelial degeneration, and vascular congestion, even when biochemical parameters remain within normal or nonsignificant ranges (Allam *et al.,* 2019; Azez *et al.,* 2023). These observations suggest that structural renal injury may precede detectable functional impairment, highlighting the sensitivity of histopathological assessment in detecting early-stage nephrotoxicity (Stevens & Levey, 2005; Allam *et al.,* 2019; Hall & Hall, 2020). According to established renal physiology, serum creatinine levels typically do not increase until approximately 50% or more of nephron function is lost, due to compensatory hyperfiltration and functional reserve of the remaining nephrons (Hall & Hall, 2020). Therefore, histopathological evaluation remains a more sensitive approach for detecting early nephrotoxic effects.

Serum creatinine and urea are widely used indicators of glomerular filtration rate and overall renal function (Abdalally *et al.,* 2021). However, significant elevations in these biomarkers typically occur only after substantial nephron loss or advanced renal functional impairment, due to the compensatory hyperfiltration capacity of the remaining functional nephrons (Stevens & Levey, 2005; Hall & Hall, 2020). Therefore, the absence of significant biochemical alterations in the present study does not exclude renal injury but rather suggests an early or subclinical stage of nephrotoxicity, which is consistent with the pronounced histopathological alterations observed in renal tissue (Allam *et al.,* 2019; Azez *et al.,* 2023).

Interestingly, serum calcium levels showed a modest but statistically significant reduction in the combination-treated group. This finding may be related to alterations in renal tubular calcium handling or interactions between ASP metabolism and mineral homeostasis. The kidneys play a central role in maintaining calcium homeostasis through glomerular filtration and tubular reabsorption, primarily in the proximal and distal nephron segments, and disturbances in these processes can lead to mild hypocalcemia (Blaine *et al.,* 2014; Bonny *et al.,* 2008). However, further studies are needed to elucidate the precise mechanisms by which ASP and combined treatments influence calcium handling in the kidney.

Histopathological Evidence of ASP-Induced Nephrotoxicity

Histopathological examination revealed severe renal damage in ASP-treated rats, including glomerular atrophy, tubular degeneration, interstitial inflammatory infiltration, vascular congestion, hemorrhage, and necrosis. These findings are consistent with previous experimental studies demonstrating that ASP and its metabolites can induce oxidative stress, inflammation, and cellular injury in renal tissues (Mourad, 2011; Allam *et al.,* 2019; Omozee & Tijesunimi, 2025).

ASP is metabolized into phenylalanine, aspartic acid, and methanol. Methanol is further converted into formaldehyde and formic acid, which are highly reactive and capable of inducing oxidative stress, LPO, and mitochondrial dysfunction (Humphries *et al.,* 2008; Mourad, 2011). Excessive production of ROS can damage cellular membranes, proteins, and DNA, ultimately resulting in tubular epithelial degeneration and glomerular injury, as observed in the present study (Allam *et al.,* 2019; Omozee & Tijesunimi, 2025). Moreover, recent reviews have highlighted the harmful effects of ASP as a food additive on multiple organs, including the kidney, emphasizing oxidative stress and inflammatory mechanisms as key mediators of tissue injury (Alsaeh *et al.,* 2025).

Furthermore, vascular congestion and interstitial inflammatory infiltration observed in ASP-treated rats may reflect endothelial dysfunction and inflammatory activation. Oxidative stress can activate inflammatory pathways, including nuclear factor kappa B (NF-κB), leading to cytokine release and recruitment of inflammatory cells, thereby exacerbating tissue injury (Abdalally *et al.,* 2021; Elduob *et al.,* 2023; Alsaeh *et al.,* 2025).

Protective and Therapeutic Effects of LSH

A key finding of the present study is the significant protective and therapeutic effect of LSH against ASP-induced renal damage. Both protective and combination treatment groups demonstrated marked improvement in renal histological architecture, with reduced tubular degeneration, decreased inflammatory infiltration, and preservation of glomerular structure. The renoprotective effect of LSH can be attributed to its rich content of bioactive compounds, including flavonoids, phenolic acids, enzymes, and antioxidant molecules (Alvarez-Suarez *et al.,* 2009; Alshailabi *et al.,* 2024). These compounds play a critical role in neutralizing ROS, reducing oxidative stress, and preventing cellular damage.

Honey has been shown to enhance antioxidant defense systems by increasing the activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Alshailabi *et al.,* 2023; Wilczyńska & Żak, 2024; Gajger *et al.,* 2025). This antioxidant action can protect renal tubular epithelial cells from oxidative damage and maintain cellular integrity. Additionally, honey possesses anti-inflammatory properties that can inhibit inflammatory pathways and reduce leukocyte infiltration into renal tissues. For example, experimental studies showed that honey feeding suppressed inflammation by reducing pro-inflammatory cytokine expression and leukocyte infiltration in kidney injury models, likely through modulation of NF-κB and related pathways. This anti-inflammatory effect likely contributed to the reduced interstitial inflammation observed in SH-treated groups (Hamad *et al.,* 2015; Navaei-Alipour *et al.,* 2021).

The observed improvement in renal histological structure following LSH treatment suggests both preventive and therapeutic potential. Pretreatment with LSH may enhance antioxidant defense before ASP exposure, while concurrent treatment may mitigate ongoing oxidative damage and promote tissue recovery. The discrepancy between severe histopathological alterations and relatively stable biochemical parameters observed in this study is consistent with the concept that structural damage may precede functional impairment. Early nephrotoxicity often manifests as cellular and tissue alterations before measurable changes occur in serum biomarkers such as creatinine and urea; thus, histopathological assessment remains a highly sensitive method for detecting early renal injury (Al-Naimi *et al.,* 2019).

Collectively, the findings of this study demonstrate that ASP induces significant renal histopathological damage, likely mediated through oxidative stress and inflammatory mechanisms. In contrast, LSH exhibited significant protective and therapeutic effects by preserving renal tissue structure and reducing pathological alterations. These results highlight the potential of LSH as a natural nephroprotective agent against chemically induced renal injury.

Conclusion

ASP administration induced significant histopathological alterations in renal tissue, including glomerular shrinkage, tubular degeneration, vascular congestion, and inflammatory infiltration, indicating its nephrotoxic potential. However, serum biochemical markers of renal function, including urea, creatinine, and uric acid, remained largely unchanged, suggesting that structural damage may precede detectable functional impairment. A modest reduction in serum calcium observed in the combination group may reflect early tubular functional alterations. Importantly, LSH demonstrated significant protective and therapeutic effects by preserving renal architecture and reducing tissue injury. These findings suggest that LSH may serve as a promising natural nephroprotective agent against chemically induced renal damage, likely due to its antioxidant and anti-inflammatory properties.

References

Abdalally, O. A., Alshailabi, E. M. A., & Ali, M. S. (2021). Effects of vitamin C on liver and kidney enzymes and some biochemical parameters against paracetamol induced hepato-nephrotoxicity in rats. *Sirte University Scientific Journal, 12*(2), 56–76.

Abdalally, O. A., Khalid, A. M., & Alshailabi, E. M. A. (2026). Histopathological study of N-acetyl-para-aminophenol-induced gastric alterations and ascorbic acid administration in albino rats. *The Derna Academy Journal of Applied Science, 5*(2), 143–151. https://doi.org/10.71147/853dyn19

Al-Naimi, M. S., Rasheed, H. A., Hussien, N. R., Al Kuraishy, H. M., & Al Gareeb, A. I. (2019). Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. *Journal of Advanced Pharmaceutical Technology & Research, 10*(3), 95–99. https://doi.org/10.4103/japtr.JAPTR_336_18

Alsaeh, F., Abusharya, M., Alsaeh, E., Baroud, N., & Alsharshary, A. (2025). The harmful effects of aspartame as a food additive on some body organs in laboratory animals: A review. *The North African Journal of Scientific Publishing, 3*(4), 36–43. https://doi.org/10.65414/najsp.v3i4.656

Alshailabi, E. M. A. (2016). Effects of omeprazole on healing of non-steroidal anti-inflammatory drug (NSAID)-induced peptic ulceration in rats and protective role of indole-3-carbinol. *Journal of Sciences and Humanities Studies, 12*, 174–188.

Alshailabi, E. M. A., Al-Zail, N. I., & Alsalhin, A. A. (2023). Effects of exposure to cigarette smoke on the cardiac tissues in male albino rats and the improvement role of Sidr honey. *Bani Waleed University Journal of Humanities and Applied Sciences, 8*(3), 261–270. https://doi.org/10.58916/jhas.v8i3.160

Alshailabi, E. M. A., Al-Zail, N. I., & Mohammed, W. E. (2024). The effects of Sidr honey on hepatotoxicity in rats exposed to sidestream cigarette smoke. *MINAR International Journal of Applied Sciences and Technology, 6*(3). http://dx.doi.org/10.47832/2717-8234.20.40

Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Bertoli, E., & Battino, M. (2009). Contribution of honey in nutrition and human health: A review. *Mediterranean Journal of Nutrition and Metabolism, 3*(1), 15–23. https://doi.org/10.3233/s12349-009-0051-6

Allam, W. A., Mahmoud, S. F., & Mahmoud, A. S. (2019). Protective effect of ascorbic acid and N-acetyl cysteine in aspartame induced nephrotoxicity in albino rats. *Egypt Journal of Forensic Sciences and Applied Toxicology, 19*(1), 107–128. https://doi.org/10.21608/ejfsat.2019.7078.1045

Anderegg, G., Flashka, H., Sallmann, R., & Schwartzenbach, G. (1954). Metallindikatoren VII. *Fasciculus, 37*, 113.

Azez, O. H., Baker, S. A., Abdullah, M. A., & Nabi, R. K. (2023). Histopathological and dose-dependent effects of aspartame toxicity on liver and kidney of rats. *Journal of Applied Veterinary Sciences, 8*(2), 16–22. https://dx.doi.org/10.21608/javs.2023.175196.1194

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques* (6th ed.). Churchill Livingstone.

Blaine, J., Chonchol, M., & Levi, M. (2014). Renal control of calcium, phosphate, and magnesium homeostasis. *Clinical Journal of the American Society of Nephrology, 10*(7), 1257–1272. https://doi.org/10.2215/CJN.09750913

Bonny, O., Rubin, A., Huang, C. L., Frawley, W. H., Pak, C. Y., & Moe, O. W. (2008). Mechanism of urinary calcium regulation by urinary magnesium and pH. *Journal of the American Society of Nephrology, 19*(8), 1530–1537. https://doi.org/10.1681/asn.2007091038

Elduob, R. E. A., Alshailabi, E. M. A., & Efkeren, S. M. (2023). The protective effects of rutin and stem cells against the kidney function changes induced by paracetamol in rats. *Sirte University Scientific Journal, 13*(2), 53–58. https://doi.org/10.37375/susj.v13i2.2501

Elshama, S., Abdalla, M. E., & Mohamed, A. M. (2018). Role of natural antioxidants in treatment of toxicity. *Journal of Toxicological Analysis, 1*(1), 3. https://doi.org/10.4172/jta.1000003

Fossati, P., Prencipe, L., & Berti, G. (1980). Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clinical Chemistry, 26*, 227.

Gajger, T. I., Dar, S. A., Ahmed, M. M. M., Aly, M. M., & Vlainić, J. (2025). Antioxidant capacity and therapeutic applications of honey: Health benefits, antimicrobial activity and food processing roles. *Antioxidants, 14*(8), 959. https://doi.org/10.3390/antiox14080959

Haghighat, M., Browning, L., Sirinukunwattana, K., Malacrino, S., Alham, N. K., Colling, R., Cui, Y., Rakha, E., Hamdy, F. C., Verrill, C., & Rittscher, J. (2022). Automated quality assessment of large digitised histology cohorts by artificial intelligence. *Scientific Reports, 12*, 5002. https://doi.org/10.1038/s41598-022-08351-5

Hall, J. E., & Hall, M. E. (2020). *Guyton and Hall textbook of medical physiology* (14th ed.). Elsevier.

Hamad, R., Jayakumar, C., Ranganathan, P., Mohamed, R., El-Hamamy, M. M. I., Dessouki, A. A., Ibrahim, A., & Ramesh, G. (2015). Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation. *Clinical and Experimental Pharmacology and Physiology, 42*(8), 843–848. https://doi.org/10.1111/1440-1681.12433

Humphries, P., Pretorius, E., & Naudé, H. (2008). Direct and indirect cellular effects of aspartame on the brain. *European Journal of Clinical Nutrition, 62*(4), 451–462. https://doi.org/10.1038/sj.ejcn.1602866

Iyyaswamy, A., & Rathinasamy, S. (2012). Effect of chronic exposure to aspartame on oxidative stress in the brain of albino rats. *Journal of Biosciences, 37*(4), 679–688. https://doi.org/10.1007/s12038-012-9236-0

Magnuson, B. A., Burdock, G. A., Doull, J., Kroes, R. M., Marsh, G. M., Pariza, M. W., Spencer, P. S., Waddell, W. J., Walker, R., & Williams, G. M. (2007). Aspartame: A safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Critical Reviews in Toxicology, 37*(8), 629–727. https://doi.org/10.1080/10408440701516184

Moshaie-Nezhad, P., Bahari, Z., Jangravi, Z., Zarei, S. M., & Iman, M. J. (2021). The effect of *Descurainia sophia* seed extract on nephrotoxicity markers induced by acetaminophen in mice. *Journal of Advanced Medical and Biomedical Research, 29*(134), 139–144. https://doi.org/10.30699/jambs.29.134.139

Mourad, I. M. (2011). Effect of aspartame on some oxidative stress parameters in liver and kidney of rats. *African Journal of Pharmacy and Pharmacology, 5*(6), 678–682. https://doi.org/10.5897/AJPP10.302

Navaei-Alipour, N., Mastali, M., Ferns, G. A., Saberi-Karimian, M., & Ghayour-Mobarhan, M. (2021). The effects of honey on pro- and anti-inflammatory cytokines: A narrative review. *Phytotherapy Research, 35*(7), 3690–3701. https://doi.org/10.1002/ptr.7066

Newman, D. J., & Price, C. P. (1999). Renal function and nitrogen metabolites. In C. A. Burtis & E. R. Ashwood (Eds.), *Textbook of Clinical Chemistry* (3rd ed., pp. 1204–1207). W. B. Saunders.

Omozee, E. B., & Timothy, O. T. (2025). Renal and oxidative consequences of prolonged aspartame and zero-sugar soda consumption in rat model. *Sokoto Journal of Medical Laboratory Science, 10*(3). https://dx.doi.org/10.4314/sokjmls.v10i3.21

Raslan, A. B. M., Tajoury, I. M., & FARAG, M. R. A. (2025). Malunion after Fixation of Intertrochanteeic Fracture by Proximal Cephalomedullry Nail Fixation. *Derna Academy Journal for Applied Sciences*, *5*(2), 133-142.

Stevens, L. A., & Levey, A. S. (2005). Measurement of kidney function. Medical Clinics of North America, 89(3), 457–473. https://doi.org/10.1016/j.mcna.2004.11.009

Thomas, L. (1998). *Clinical laboratory diagnostics* (1st ed.). Frankfurt: The-Books Verlagsgesellschaft.

Wilczyńska, A., & Żak, N. (2024). Polyphenols as the main compounds influencing the antioxidant effect of honey—A review. *International Journal of Molecular Sciences, 25*(19), 10606. https://doi.org/10.3390/ijms251910606