

Original article

Influence of Dose and Route-Dependent Hepatotoxicity and Nephrotoxicity of Natural and Artificial Bee Venom (*Apis mellifera*) in Mice

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ABSTRACT

Keywords:*Apis Mellifera, Bee Venom, Hepatotoxicity, Nephrotoxicity, Mice, Histopathology, Toxicity.*

Bee venom (*Apis mellifera*) contains bioactive compounds such as melittin and phospholipase A₂, which have been reported to exert both therapeutic and toxic effects on various organs. This study aimed to evaluate the toxicity of natural and artificial bee venom on liver and kidney tissues in mice. Forty adult male albino mice were randomly assigned to five groups: control, natural bee venom (NBV) for 5 and 7 doses via stinging, and artificial bee venom (ArBV) for 5 and 7 doses via intraperitoneal injection. At the end of the exposure period, liver and kidney tissues were collected for histopathological analysis using hematoxylin and eosin (H&E) and Mallory's trichrome stains. Histopathological examination revealed dose- and duration-dependent changes in both liver and kidney tissues. In the liver, NBV and ArBV exposure caused congestion, inflammatory infiltration, Kupffer cell hypertrophy, hepatocellular activation, and focal degenerative changes, with more pronounced effects in seven-dose groups. In the kidneys, NBV induced proximal tubular degeneration and necrosis, whereas ArBV caused mild tubular alterations with inflammatory infiltration. No severe systemic toxicity or organ failure was observed at the administered doses. Both natural and artificial bee venom exhibit organ-specific toxic effects in mice, particularly at higher doses and prolonged exposure. The liver and kidneys are primary target organs, and the severity of histological alterations depends on dose, exposure duration, and administration route. These findings highlight the importance of careful dose selection in therapeutic applications of bee venom and underscore its dual role as a toxic and potentially therapeutic agent.

Introduction

Bee venom (*Apis mellifera*) is a complex biological mixture composed of peptides (e.g., melittin, apamin), enzymes (phospholipase A₂), and other small molecules that exhibit diverse biological activities, ranging from anti-inflammatory and antimicrobial effects to cytotoxicity and organ toxicity [1]. Recent research has highlighted that, while bee venom has therapeutic potential, its toxic effects on vital organs such as the kidney and liver remain a significant concern, especially at higher doses or prolonged exposure. In vivo toxicological studies in rodent models have demonstrated that bee venom can induce acute organ damage depending on dose and route of administration. For example, subcutaneous administration of high doses of bee venom in mice led to significant histopathological changes in major organs, indicating organ-specific toxicity [2]. Similarly, detailed biochemical and structural investigations showed that exposure to Algerian bee venom causes oxidative stress, renal functional alterations, and histological damage in kidney tissue, characterized by increases in serum markers associated with nephrotoxicity and histological degeneration of glomeruli and tubules [3].

Although most toxicological research has focused on the kidney as a primary target organ, there is growing evidence that bee venom also affects the liver. A recent study investigating the effect of *Apis mellifera* venom in mice with Ehrlich ascites carcinoma found changes in liver function enzymes and histopathological signs of hepatic injury, though co-treatment with bee venom altered some biochemical markers relative to untreated carcinoma controls [4]. Clinical evidence further supports this organ toxic potential in humans, where bee envenomation has been associated with transient hepatotoxicity and elevated liver enzymes following multiple stings [5]. In experimental rodent models, bee venom triggers dose-dependent oxidative stress and immune responses, contributing to organ injury. For example, venom exposure was associated with significant increases in reactive oxygen species (ROS), depletion of antioxidant defenses such as glutathione and catalase, and consequent histopathological damage in kidney tissue, demonstrating that

immune and oxidative mechanisms amplify toxicity and underscore the importance of controlled toxicity profiling in vivo [6]. In experimental models, apitoxin and melittin exhibited concentration-dependent cytotoxicity, significantly reducing the viability of endothelial cells at concentrations ≥ 5 $\mu\text{g/ml}$ and affecting smooth muscle cells at even lower levels, indicating that specific venom components drive organ-level toxicity [7]. Therefore, the current study aims to investigate in detail the toxicity of both natural and artificially processed *Apis mellifera* venom on liver and kidney tissues in mice, focusing on biochemical markers, oxidative stress parameters, and histopathological outcomes.

Materials and Methods

Bee venom

Foraging honeybees, *Apis mellifera* (order Hymenoptera, family Apidae), were obtained from naturally maintained colonies at the Faculty of Agriculture, University of Tripoli. The bees were collected in the morning, placed in ventilated glass containers, and used on the same day to sting mice every three days over periods of either 15 days (five treatments) or 21 days (seven treatments). Each forager bee carries roughly 150 μg of venom [8] and can deliver between 30 and 100 μg through its stinger [9]. Purified, lyophilized venom from *Apis mellifera* was provided by Al-Hart CAM clinic, which sourced it from VACSERA in Egypt. The freeze-dried venom was reconstituted in 0.9% saline to prepare the required concentrations for the experiment and stored at 4°C until use. It was administered intraperitoneally (i.p.) at a dose of 23 μg per kilogram of body weight, in a volume of 200 μl , once every three days for either 15 days (five doses) or 21 days (seven doses). The doses used in this study were determined by extrapolating from those applied in human treatments by traditional practitioners in certain complementary and alternative medicine clinics in Libya.

Experimental Animals

This experimental study was conducted using forty adult male albino mice, aged 6–8 weeks and weighing 25–30 g. The animals were raised in the animal house of the Zoology Department, Faculty of Science, University of Tripoli. Mice were housed under standard laboratory conditions with free access to a standard pellet diet and water ad libitum. All experimental procedures were performed in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Experimental Design

The research was conducted on 40 mice, which were randomly allocated into five experimental groups (n = 8 mice per group):

- Group 1 (Normal control): Mice were injected intraperitoneally (i.p.) with 0.9% isotonic saline at a dose of (23 $\mu\text{g/kg}$ body weight in a dose volume of 200 μL) and served as the normal control group.
- Group 2 "NBV" (Natural Bee Venom – 5 doses): Each mouse was subjected to one natural honeybee sting every third day for 15 days.
- Group 3 "NBV" (Natural Bee Venom – 7 doses): Each mouse was subjected to one natural honeybee sting every third day for 21 days.
- Group 4 "ArBV" (Artificial Bee Venom – 5 doses): Mice received intraperitoneal injections of artificial bee venom at a dose of (23 $\mu\text{g/kg}$ body weight in a dose volume of 200 μL of diluted bee venom) once every third day for 15 days.
- Group 5 "ArBV" (Artificial Bee Venom – 7 doses): Mice received intraperitoneal injections of artificial bee venom at a dose of (23 $\mu\text{g/kg}$ body weight in a dose volume of 200 μL of diluted bee venom) once every third day for 21 days.

Histopathological Examination

The mice were sacrificed, and liver and kidney tissues were carefully excised from all experimental and control groups. Tissue samples were immediately fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax [10]. Sections of approximately 5 μm thickness were prepared using a rotary microtome. Following deparaffinization, tissue sections were stained with hematoxylin and eosin (H&E) for general histological evaluation. Stained sections were examined under a light microscope for histopathological changes [11].

Results

General Observations

During the experimental period, mice were exposed to either natural bee venom (NBV) via direct stinging or artificial bee venom (ArBV) via intraperitoneal injection. A limited number of animals died during the course of treatment and were replaced to maintain the predetermined group size.

Histopathological Findings of the Liver

Control Group

Histological examination of liver sections from the control group (Group 1) revealed normal hepatic architecture. The liver parenchyma was organized into well-defined hepatic lobules with cords of polygonal hepatocytes radiating from the central vein toward the periphery. Hepatocytes exhibited acidophilic cytoplasm and centrally located, rounded nuclei with one or two prominent nucleoli. Hepatic cords were separated by blood sinusoids lined with endothelial and Kupffer cells (Fig. 1).

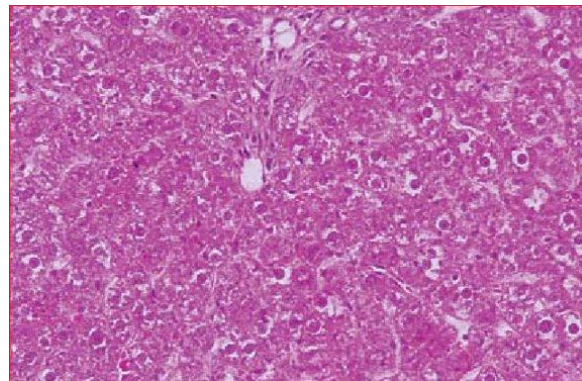


Fig. 1. Section of liver showing normal hepatocytes with usual arrangements of hepatic cords and sinusoids (Control) X400

Natural Bee Venom (NBV)

Liver sections from mice exposed to natural bee venom for five doses showed mild histopathological alterations. Some hepatic lobules exhibited mild congestion of hepatic blood vessels and sinusoids (Fig. 2A), while the majority of hepatocytes appeared morphologically normal. However, focal degenerative changes were observed in a limited number of hepatocytes. Mild portal and interstitial infiltration of inflammatory cells—predominantly lymphocytes, plasma cells, eosinophils, and macrophages—was evident (Fig. 2C, 2E). In addition, hypertrophy and increased prominence of Kupffer cells were frequently observed (Fig. 2G). In contrast, liver sections from mice treated with natural bee venom for seven doses demonstrated more pronounced histological changes compared with the five-dose group. Moderate congestion of hepatic sinusoids and blood vessels was evident (Fig. 2B). Moderate portal and perivascular aggregation of inflammatory cells, mainly lymphocytes, plasma cells, eosinophils, and macrophages, was observed (Fig. 2D). Moderate interstitial inflammatory cell infiltration was also noted (Fig. 2F). Also, mild biliary hyperplastic reaction was observed (Fig. 2H). Despite these changes, most hepatocytes exhibited morphological features comparable to those observed in the control and five-dose groups.

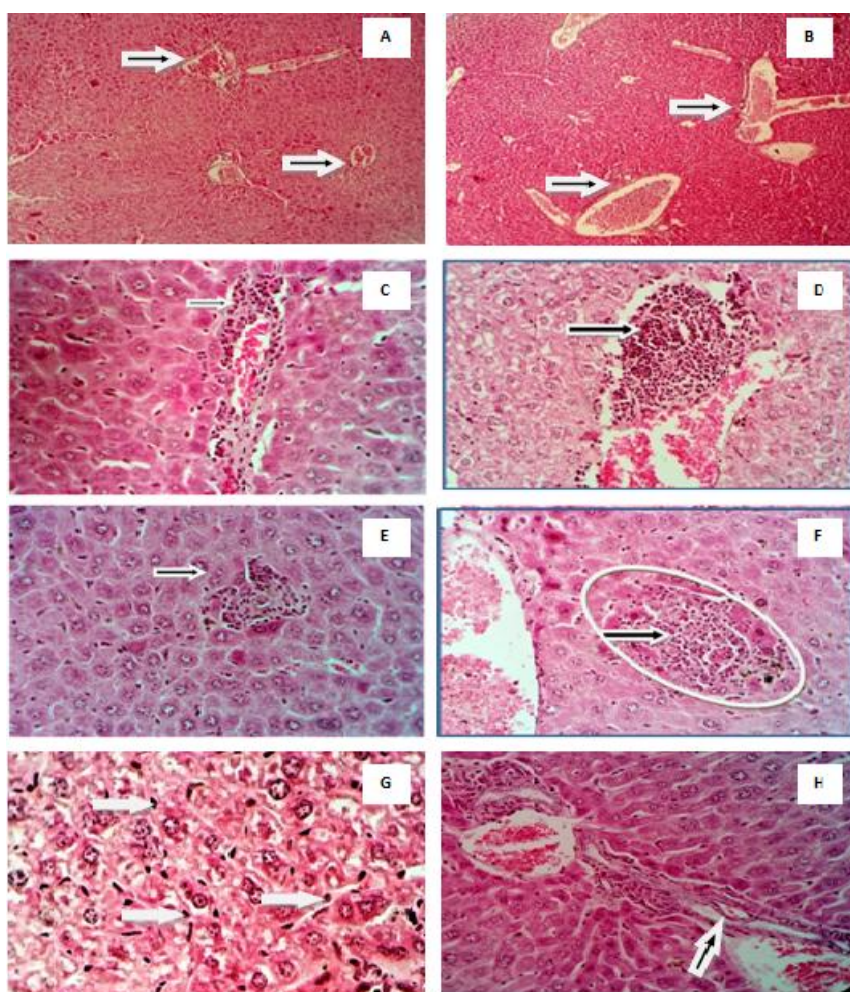


Fig. 2. Liver of mice stinging with natural BV: A; for five days, showing mild congestion of hepatic blood vessels and sinusoids (arrows). B; for seven days showing moderate congestion of hepatic blood vessels and sinusoids, H&E. X100. C; for five days, showing mild portal aggregation of inflammatory cells, mainly lymphocytes, plasma cells, eosinophils, and macrophages. X 400 D; for seven days, showing moderate portal aggregation of inflammatory cells, mainly lymphocytes, plasma cells, eosinophils, and macrophages (arrow). H&E. X 200. E; for five days showing mild interstitial infiltration and aggregation of inflammatory cells, mainly lymphocytes, plasma cells, eosinophils, and macrophages (arrows). F; showing moderate interstitial aggregation of inflammatory cells, mainly lymphocytes, plasma cells, eosinophils, and macrophages (Circle & arrow). G; for five days showing hypertrophy of the Kuffer's cells (arrows). H; showing mild biliary hyperplastic reaction (arrow) H&E. X400

Artificial Bee Venom (ArBV)

Liver sections from mice injected intraperitoneally with artificial bee venom for five doses revealed activation of a moderate number of hepatocytes. These cells appeared enlarged, with vesicular nuclei and occasional binucleation, suggesting increased mitotic activity. A small number of hepatocytes exhibited cloudy swelling and hydropic degeneration (Fig. 3A, 3B). Mild congestion of hepatic blood vessels and sinusoids was also observed, accompanied by hepatocellular activation (Fig. 3C). Following seven doses of artificial bee venom, liver sections showed continued hepatocellular activation, together with mild portal, perivascular, and interstitial infiltration of inflammatory cells, particularly lymphocytes, plasma cells, and macrophages (Fig. 3D, 3E). Hypertrophy of Kupffer cells was a consistent finding in this group (Fig. 3F). Additionally, a limited number of hepatocytes exhibited cloudy swelling, hydropic degeneration, and focal microsteatosis. In some cases, degenerative changes and microsteatosis were more pronounced, with focal areas of necrosis and hemorrhage observed in isolated sections. Mild bile duct proliferation was occasionally detected.

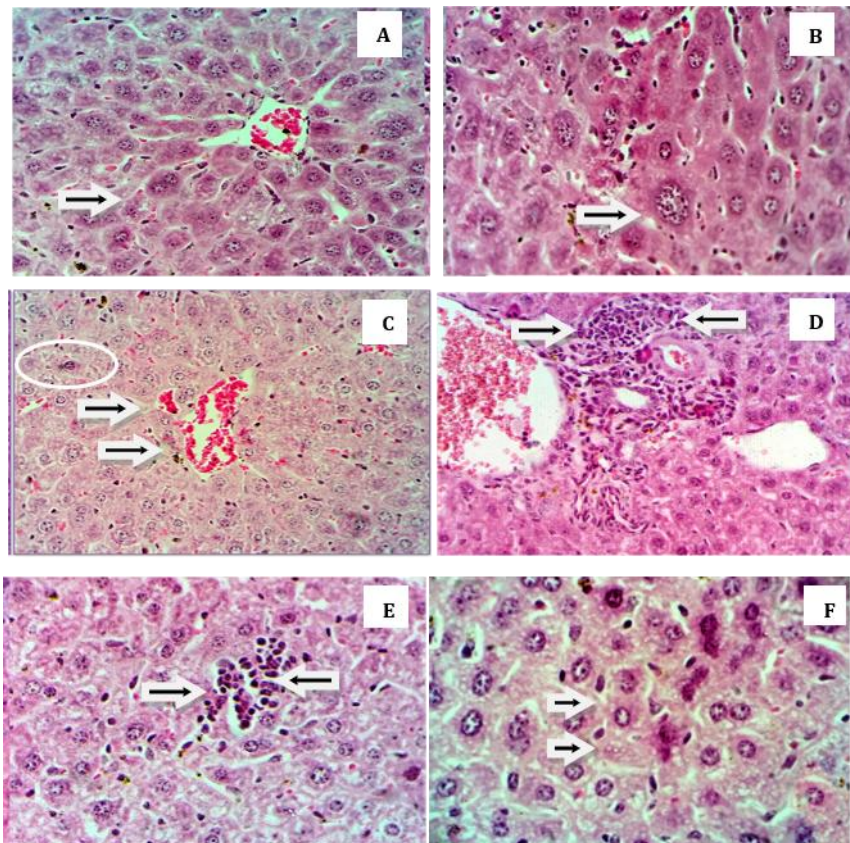


Fig. 3. Liver of mice injected ip with artificial BV for five days, showing A and B; showing activation of a moderate number of hepatocytes, which appeared large or very large (megahepatocytes) with vesicular nuclei and mitotic activities (duple nuclei) (arrows). H&EX400. C; showing mildly congested hepatic blood vessels and sinusoids (arrow). Activated hepatocytes are also seen (circle and arrow). H&E X200. D and E; Liver of mice injected ip with artificial BV for seven days, showing portal, perivascular, and interstitial infiltrations and aggregations of round cells, particularly lymphocytes, plasma cells, and macrophages (arrows). H&E X400. F; showing hypertrophied Kuffer's cells (arrow). H&E X1200

Histopathological Findings of the Kidney

Control Group

Kidney sections from the control group (Group 1) exhibited normal renal histological architecture. The renal cortex contained intact renal corpuscles composed of glomeruli surrounded by Bowman's spaces, along with proximal and distal convoluted tubules separated by minimal interstitial tissue. Proximal convoluted tubules were lined by acidophilic cuboidal epithelium with a well-defined apical brush border and narrow lumen, while distal convoluted tubules exhibited wider lumina and less prominent brush borders (Fig. 4).

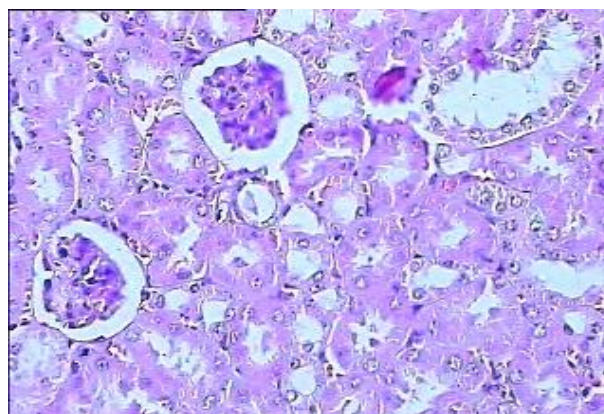


Fig. 4. Sections of the kidney of the control group showing normal renal histological architecture features; the cortex consisted of usual renal tubules, renal corpuscles, and minimal interstitial tissue in between H&E. X 200

Natural Bee Venom (NBV)

Kidney sections from mice treated with natural bee venom for the shorter exposure period (five doses) showed marked degenerative and necrotic changes, particularly in the proximal convoluted tubules. These tubules exhibited cytoplasmic vacuolation, loss of brush border integrity, and disruption of epithelial lining. Renal blood vessels were congested, and some renal tubules lost their characteristic histological appearance. In contrast, the structure of distal convoluted tubules and collecting ducts was relatively preserved (Fig. 5A). In animals treated with natural bee venom for the longer exposure period (seven doses), most renal tubules appeared morphologically comparable to those of the control group. Preservation of near-normal architecture was evident in many proximal convoluted tubules, with restoration of the brush border and epithelial integrity (Fig. 5B).

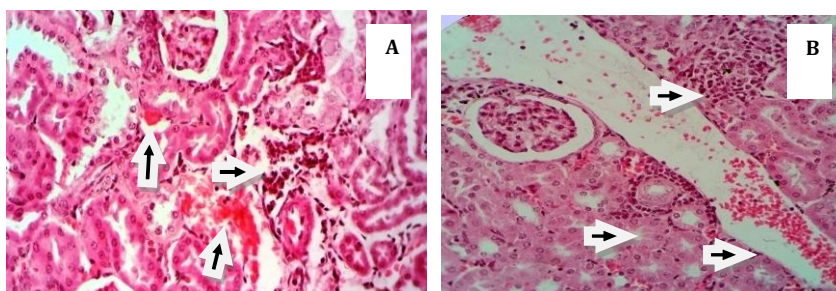


Fig. 5. Kidney of albino mice stinging with natural BV A; for five days, showing mild congestion of renal blood vessels and capillaries, perivascular and interstitial aggregation of plasma cells and lymphocytes (arrows). H&E. X 200. B: for seven days showing moderate congestion of renal blood vessels and capillaries, perivascular and interstitial round cells aggregation (arrows). H&EX 200

Artificial Bee Venom (ArBV)

Renal tissue from albino mice, following five weeks of intraperitoneal artificial bee venom administration, exhibited mild interstitial inflammation characterized by lymphocytic and plasmacytic infiltration, alongside macrophage aggregation (Fig. 6A). Seven weeks of systemic artificial bee venom administration in albino mice, resulted in moderate perivascular infiltration of inflammatory cells (Fig. 6B). Pathophysiological of the renal tissue exhibited signs of tubular atrophy alongside widespread cellular edema and vacuolar degeneration. Also, sporadic distribution of glomerular atrophy was noted. (Fig. 6C, 6D).

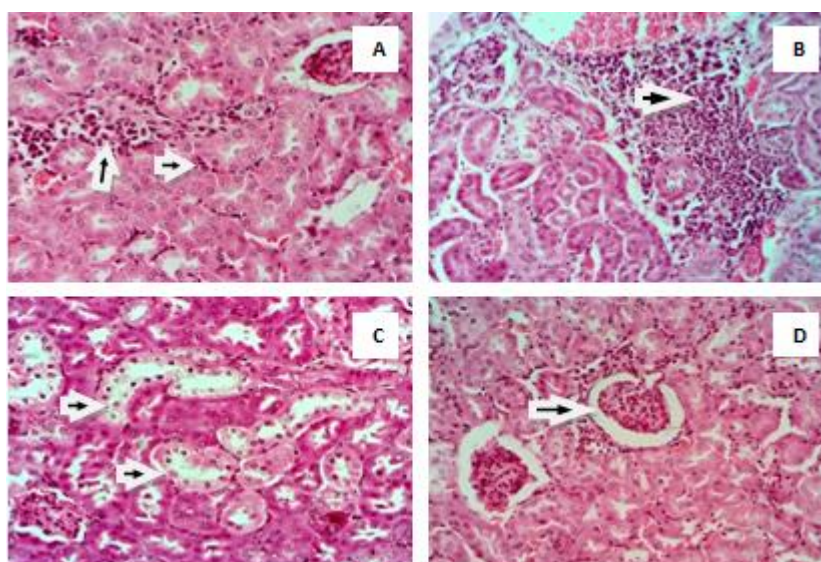


Fig. 6. Kidney of albino mice injected ip with artificial BV for five days, showing A; showing mild interstitial infiltration and aggregation of plasma cells, lymphocytes, and macrophages. (arrows). H&E. X 200. B: albino mice injected ip with artificial BV for seven days, showing moderate perivascular aggregation of lymphocytes and plasma cells (arrow). H&E. X200, C; showing tubular degeneration, mostly cloudy swelling, and hydropic degeneration. (arrows). H&E. X200. D, showing atrophy of some glomeruli. (arrow). H&E. X 200.

Histopathological Changes

Overall, exposure to both natural and artificial bee venom induced dose- and duration-dependent histopathological alterations in liver and kidney tissues. Natural bee venom primarily induced inflammatory and vascular changes, whereas artificial bee venom was associated with hepatocellular activation, degenerative changes, and focal necrosis. Renal tissues were particularly susceptible to venom-induced injury, especially at shorter exposure durations.

Discussion

In the current study, both natural bee venom (NBV) administered by repeated stinging and artificial bee venom (ArBV) delivered intraperitoneally produced observable histopathological changes in the liver and kidney of mice, though the severity and character of these changes varied with dose and route of administration. These findings align with and differ from various recent animal studies exploring the toxicological and biological effects of *Apis mellifera* venom and its main bioactive components.

Our histological observations demonstrated mild to moderate hepatic congestion, inflammatory infiltration, Kupffer cell hypertrophy, hepatocellular activation, and focal degenerative changes, especially with prolonged exposure to NBV and ArBV. These morphological alterations suggest inflammatory responses and cellular stress in liver tissue that increase with dose and duration. These outcomes are consistent with acute toxicity studies reporting dose-dependent effects of bee venom on organ histology. For example, Liu et al. showed acute toxicity signs in multiple organs of mice at higher doses of whole bee venom, including behavior and survival changes, indicating that bee venom can impact systemic physiology when administered above a certain threshold (e.g., LD₅₀ ~ 119.7 mg/kg) [12]. However, some recent studies suggest that bee venom may also exhibit protective or modulatory effects on liver tissue under specific pathological conditions. For instance, in a mouse model of Ehrlich ascites carcinoma, *Apis mellifera* venom co-treatment modestly improved hepatic histology and biochemical markers compared to tumor-only groups, indicating that the context of venom exposure (e.g., disease model versus toxic model) influences outcomes. Similarly, broader pharmacological reviews highlight that bee venom components like melittin and PLA₂ possess anti-inflammatory and immunomodulatory activities that can interact with liver signaling pathways, potentially reducing inflammation and oxidative stress in some disease settings [13,14]. The divergent findings may be explained by differences in dose, duration, administration route, and the physiological context (e.g., healthy vs disease models). High or repeated doses, as used in toxicity assessments, tend to promote inflammation and cell injury, while controlled lower doses in pathological models may modulate immune responses and reduce some forms of damage. Additionally, differences in the extraction, composition, and purity of venom could alter its effects on hepatic cells.

Kidney histopathology in our study revealed degenerative and necrotic changes with NBV and activated hepatocellular responses with ArBV, suggesting the kidney is an important target organ following systemic exposure. The kidney's vulnerability is supported by studies showing that bee venom toxicity can lead to oxidative stress, biochemical disruption, and structural damage in renal tissues. Dahdouh et al. reported that intraperitoneal Algerian bee venom induced significant kidney injury in mice at moderate and high doses, evidenced by increased serum urea, creatinine, depleted antioxidants, lipid peroxidation, and tubular necrosis, whereas low doses produced no marked changes. This dose-dependent nephrotoxicity parallels the trend observed in our NBV groups, especially with extended exposure [15]. Yet, some investigations illustrate potential protective roles of bee venom and its constituents against kidney injury in specific pathological models. For example, studies have shown that bee venom reduces gentamicin-induced nephrotoxicity by enhancing antioxidant defenses and reducing inflammation, and melittin has been reported to promote macrophage polarization, favoring renal recovery in cisplatin-induced acute kidney injury models [16]. These contrasting results suggest that venom's net effect on kidney tissue depends on biological context and dose. While high systemic exposure tends toward toxicity and inflammation, controlled or therapeutic dosing can activate protective pathways, including modulation of immune responses and redox balance [17]. An additional factor might be the role of major venom components, such as melittin and phospholipase A₂, which have complex interactions with cell membranes and immune signaling. Reviews of bee venom pharmacology indicate that such components can regulate cytokines and immune pathways influencing inflammation and tissue injury – mechanisms that, depending on context, may be either detrimental or beneficial.

Mechanistically, bee venom's membrane-active peptides, like melittin, can disrupt phospholipid bilayers, leading to cell membrane destabilization and inflammatory signaling, processes that may underlie histopathological changes observed in liver and kidney tissues at higher exposure levels. The immune modulation effects observed in mice, where sublethal venom doses altered cytokine profiles, also support the notion that venom influences systemic inflammatory responses, which could mediate organ-level changes [18]. It is also important to note that differences in administration route (stinging vs intraperitoneal

injection) likely contributed to variations in distribution, peak concentration, and organ exposure. Intraperitoneal injection tends to produce more immediate systemic distribution, potentially explaining the pronounced histological changes observed with artificial venom [19]. Overall, our findings largely mirror dose-dependent toxic effects on liver and kidney tissues described in recent experimental reports, while also reflecting the complexity observed in other models where venom can exert protective or modulatory roles under certain conditions. The dual nature of bee venom — toxic at high doses but potentially therapeutic at low doses or in disease contexts — underscores the importance of careful dose selection and detailed mechanistic studies in future research.

This study evaluated the toxic effects of natural (NBV) and artificial bee venom (ArBV) on liver and kidney tissues in adult male albino mice. Histopathological analysis revealed dose- and duration-dependent alterations in both organs. In the liver, NBV and ArBV exposure led to congestion, inflammatory infiltration, Kupffer cell hypertrophy, hepatocellular activation, and focal degenerative changes, with more pronounced effects observed in seven-dose groups. In the kidneys, NBV induced proximal tubular degeneration and necrosis, while ArBV caused mild tubular alterations and inflammatory changes. These findings highlight that both forms of bee venom can exert organ-specific toxic effects, yet their severity depends on the route of administration, dose, and exposure period. Notably, while high doses and repeated exposures triggered toxicity, previous studies suggest that controlled doses may exhibit protective or immunomodulatory effects, indicating a dual role of bee venom in biological systems

Limitations

This study has several limitations that should be acknowledged. First, the sample size was relatively small, with only forty mice, and the exposure periods were short (five and seven days). Longer-term investigations are necessary to evaluate chronic toxicity and the potential for recovery. Second, the doses of natural and artificial bee venom were selected based on prior reports, but exact toxic thresholds were not fully established. Future work should explore dose-response relationships to better define safe and toxic ranges. Third, while histopathological changes were documented, the absence of complementary biochemical and molecular assays—such as liver enzyme activity, renal function markers, and oxidative stress indicators—limits the depth of interpretation regarding organ toxicity. Fourth, variability in venom composition presents another challenge. Natural bee venom may differ depending on bee species, diet, and environmental conditions, while artificial formulations may vary in purity and bioactive content. Standardization of venom sources and preparations is therefore essential for reproducibility. Finally, the study primarily focused on structural alterations, offering limited mechanistic insight. Further research should investigate molecular pathways, including oxidative stress responses, inflammatory cytokine signaling, and apoptosis, to provide a more comprehensive understanding of the toxicological effects of bee venom.

Conclusion

The study demonstrates that both natural and artificial bee venom exhibit hepatotoxic and nephrotoxic effects in mice, which are exacerbated by increased dose and prolonged exposure. Natural bee venom administered via stinging primarily caused renal tubular degeneration, whereas artificial bee venom administered intraperitoneally produced hepatocellular activation, mild liver necrosis, and inflammatory infiltration. These findings underscore the importance of careful dose selection and route of administration in potential therapeutic applications of bee venom. Additionally, the study supports the notion that bee venom's effects are context-dependent, with high doses leading to toxicity and controlled doses potentially exerting beneficial immunomodulatory or protective effects.

Ethical Approval

The experimental procedures in this study were performed according to the bioethical research guide established by the Libyan National Committee for Biosafety and Bioethics, which complies with the published guide “Principles of laboratory animal care”.

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