



RESEARCH ARTICLE - MEDICAL TECHNIQUES

Histopathological Study of Bee Venom with Different Concentrations in Laboratory Mice

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Article Info.	Abstract
<p><i>Article history:</i></p> <p>Received 06 September 2021</p> <p>Accepted 20 December 2021</p> <p>Publishing 31 December 2021</p>	<p>Bee venom (BV) of <i>Apis mellifer</i> L has recently been utilized as a traditional medicine for treating a variety of medical conditions. When mice were given different concentrations of bee venom, it was found to be effective in repairing some histopathological changes. Then, when applying BV as a treatment material for some disorders, as indicated by the amount of damage to the liver and kidney tissues. The animals were randomly divided into 7 groups. Six of which were treated with BV extraction, and one group was designated as the control group, was treated with distilled water. Mice were injected intraperitoneally by 0.2ml of BV extraction in concentrations (1000, 750, 500, 350, 300, and 250) µg/ml. In some cases, histopathological studies revealed mild to moderate alterations in the liver and renal tissues, characterized by congestion, acute cell swelling, and focal coagulated necrosis. Atherosclerotic changes in the aorta and some arteries were found in two groups. Whereas several mild degenerative changes were observed in hepatic cells of one group. In conclusion, bee venom administered to groups in different concentrations revealed hepatic and renal complications at histological investigations of hematoxylin and eosin-stained sections of liver and kidney section.</p>

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Keywords: Kidney; Liver; Melittin; Histopathology; Bee Venom

1. Introduction

Since the late 19th century, bee venom was studied as a potential therapeutic due to its biomolecules. [1]. BV has been classified as neurotoxic, hemolytic, digestive, hemorrhagic, and allogeneic in respect of its biological effects. [2]. BV is a natural toxin made up of a complex and effective mixture of compounds created by honey bees to defend them against a number of predators. BV healing is a treatment that involves injecting live bee stings into the patient's skin. BV has been proven useful in the treatment of a variety of diseases and their consequences, including arthritis, pain, and malignant tumors [3]. In addition to other components, the BV contains peptides such as melittin, secapin, apamin, adolapin, tertiapin, the mast-cell-degranulating (MCD) peptide, enzymes such as (phospholipase A2, hyaluronidase, acid phosphomonoesterase, and lysophospholipase), and active amines such as (histamine, dop [4]. For self-defense, honey bees store their venom in sacs as apitoxin, [5]. Greeks, Chinese, and Egyptians used bee stings or injections of purified and diluted BV to treat a variety of diseases, including osteoarthritis, rheumatoid arthritis, fatigue, and skin problems. [6-8]. In addition to having a variety of pharmaceutical properties include analgesic [9], anticancer [10], antibacterial [11,12], antifungal [13], antiviral [14], neuroprotective [15], and the treatment of many skin conditions [16]. The melittin peptide makes up roughly 50-60% of the dry weight of the bee and is one of the most toxic compounds, as well as being one of the most active and effective molecules in the medical and therapeutic fields. [17]. While the second component of BV dries weight, enzyme PLA 2, has a biological effectiveness of 10-12 percent (1). MCD, on the other hand, contains 22 amino acids at a rate of 1-3 percent of BV. It

Nomenclature & symbols			
BV	Bee Venom	MCD	Mast Cell Degranulating
API	Api toxin	HX E stain	Hematoxylin & Eosin stain
G	Glomeruli	R	Renal Tubules
Ct	Collecting tubule	Dt	Convolutated tubules
Tn	The thin segment	H	hemorrhage
Ts	thick segment	AKI	Acute Kidney injury
PGF 2 α	prostaglandin f2 alpha	TNF- α	Tumor necrosis factor alpha
PARP	poly (ADP-ribose) polymerase	TGF- β_1	transforming growth factor

also has potent anti-inflammatory properties [18]. Apamin is a neurotoxic peptide made up of 18 amino acids with the ability to inhibit the Ca²⁺ activated K⁺ channel. [19]. Secapin has three biological properties that are appealing: anti-fibrinolytic, anti-elastolytic, and antimicrobial [20]. Apart from that, Adolapin is a polypeptide with anti-inflammatory and analgesic properties [21]. Finally, hyaluronidase is identified as a spreading component that aids BV penetration [22].

Melittin, also known as API m4 because of its allergenicity, is a cationic, linear-helical polypeptide with 26 amino acid residues that is water soluble and amphipathic, with a molecular weight of 2846.5 Da. The sequence of amino acids is GIGAVLKVLTGLPALISWIKRKRQQ, and the chemical formula is C₁₃₁H₂₂₉N₃₉O₃₁, with a hydrophobic N-terminus and hydrophilic C-terminus properties. One of the most important properties of melittin is its nonspecific cytolytic activity, which might be useful for biological membrane holes, and its capacity to attract anion lipid membranes attributed to its hydrophobic section and positive charge [23].

The study aimed to detect the level of damage in liver and kidney tissues after using BV as a treatment for some diseases by using histopathological examinations.

2. Materials and Methods

2.1. Animals and methods

One hundred seventy-five white mice weigh 30-35 gm, were used in this study. So, all procedures of animal care and experiments were done in the house with standard conditions of illumination and ventilation. They were allowed free access to standard laboratory feed and water. This experiment was carried out at the laboratories of the Ministry of Science and Technology.

2.2. Experimental design

Mice were randomly divided into seven groups distributed to 25 animals in each group:

Group I (control group): 25 mice were injected intraperitoneally with 0.2 ml distilled water. Treatment groups were intraperitoneally injected with 0.2 ml of bee, with the following concentrations, group II 100 0 μ g/ml, group III 750 μ g/ml, group IV 500 μ g/ml, group V 350 μ g/ml, group VI 300 μ g/ml, and group VII 250 μ g/ml.

Lyophilized bee venom was used in different concentrations in this experiment.

The API toxin collection of honey bee venom was collected from Kut province, Iraq, in consecutive seasons during the period 2016-2018, Venom is collected from *Apis mellifera*, which are Africanized honey bees (AHBs) with ages ranging from 30 to 40 days. Venom passes through extraction twice a week using electrical simulation, as well as isolation and distillation of melittin using a phase liquid chromatography (RP-HPLC) procedure (University of Baghdad Faculty of agriculture apiaries).

2.3. Histological study

One week after the mice were injected intraperitoneally, mice were sacrificed by either inhalation and abdominally dissected. Body organs (liver and kidney) were dissected from all animals immediately after death, thoroughly washed with formal saline, and fixed for at least 24 hours in 10% neutral-buffered formalin. All specimens were washed for half an hour in tap water, dehydrated in ascending grades of alcohol (70, 90, % absolute), cleared in xylene, and then impeded in paraffin wax. For histopathological examination, serial sections of 3-7 micron thickness were cut and stained with Haematoxylin and Eosin. [25].

3. Results and Discussion

Renal parenchyma, glomerulus development, and kidney tubules were apparent in hematoxylin and eosin-stained sections of the kidney in the control group. Sections of the kidney in the control group (Fig. 1, 2, and 3) exhibited a normal appearance of the renal cortex and medulla, as well as minor figures of vacillation in several renal tubes. The renal medulla, on the other hand, appeared normal. (Fig. 9, and 10).

Sections of the renal cortex revealed severe nephrosis which characterized by sever cortical hemorrhage, tubular dilation and degeneration of tubules. Other parts of the renal medulla indicated extensive vascular degeneration and necrosis of most renal tubules, while others revealed fibrosis of the renal interstitium. (Fig. 4, 5, 6, 7, and 8). The groups given varied dosages of bee venom showed renal corpuscle atrophy and shrinkage, a decrease in glomerular cellularity, disorganization, and kidney tubule degradation.

The liver section of the control group exhibited normal hepatic structure with a normal hepatic appearance on histopathological inspection of hematoxylin and eosin-stained sections. (Fig. 11). While in bee venom groups, specifically group II, the major hepatic characterized by modest hepatocyte enlargement (Fig. 12) and vascular congestion (either in the central vein (C.V) or in the portal vein) associated by focal mononeuclear

cell (MNC) aggregation, constituted of several types of inflammatory cells (Fig. 13). Also, the results showed perivascular lymphocytic aggregation (Fig. 14), in addition, a few sections revealed several apoptotic hepatocytes within hepatic parenchyma (Fig. 15). The effects of bee venom range from mild to severe degenerative and necrotic alterations, with indications of nuclear pyknosis in many hepatocytes (Fig. 16), as well as necrotic hepatic cords with multiple apoptotic hepatocytes (Fig. 17).

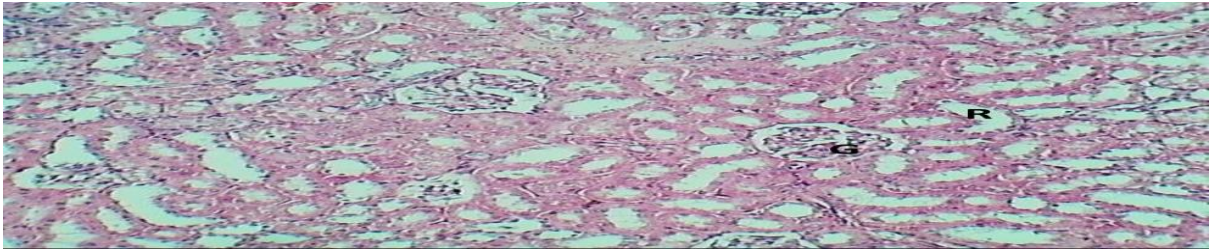


Fig. 1 Section of kidney (Control group) shows: normal appearance of nephrons glomeruli (G) and renal tubules (R), H and E stain

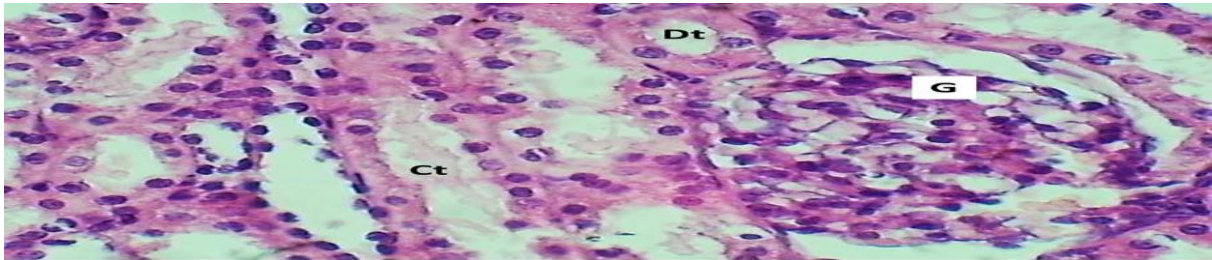


Fig. 2 Section of renal cortex (Control group) shows: normal appearance of glomeruli (G), collecting tubule (Ct), distal convoluted tubules (Dt), H and E stain. 400x.

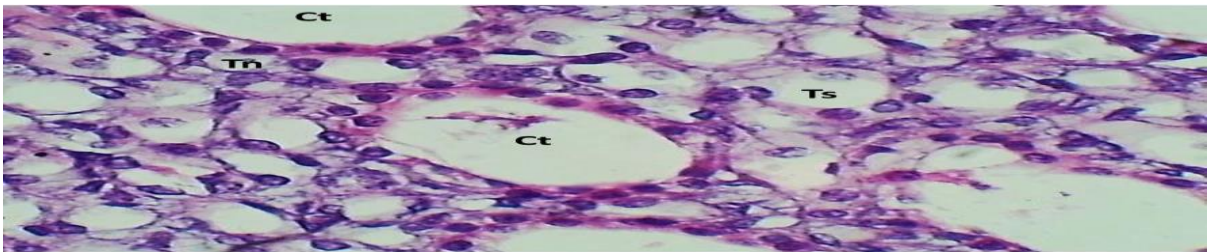


Fig. 3 Section of renal medulla (control group) shows: normal collecting tubule (Ct), thick segment (Ts) and thin segment (Tn) of loop of henle, H and E stain, 400x

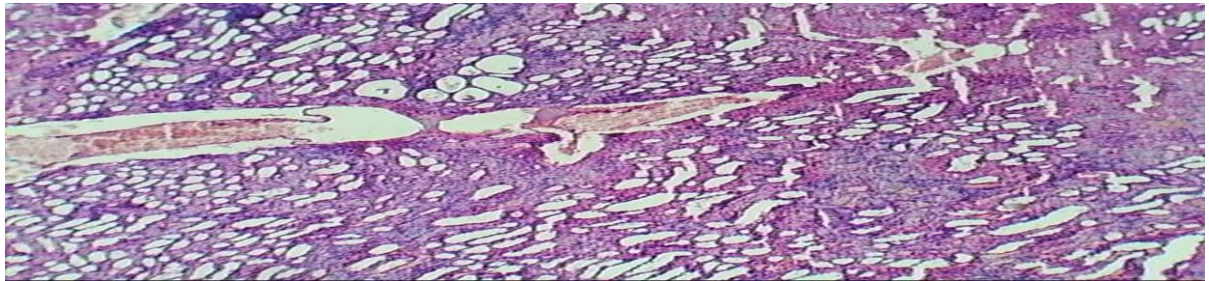


Fig. 4 Section of renal cortex (BV group) shows: sever cortical hemorrhage (H), tubular dilation (Arrows) & degeneration of tubules (Asterisks), H and E stain, 40x

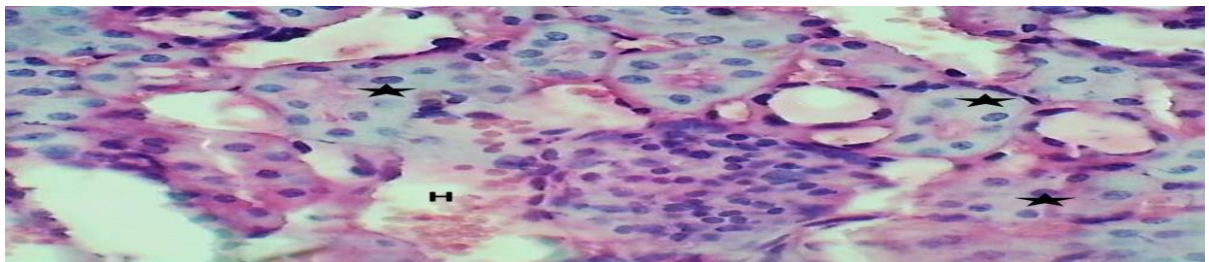


Fig. 5 Section of renal cortex (BV group) shows: sever cloudy swelling of renal of tubules (Asterisks), H and E stain, 400x

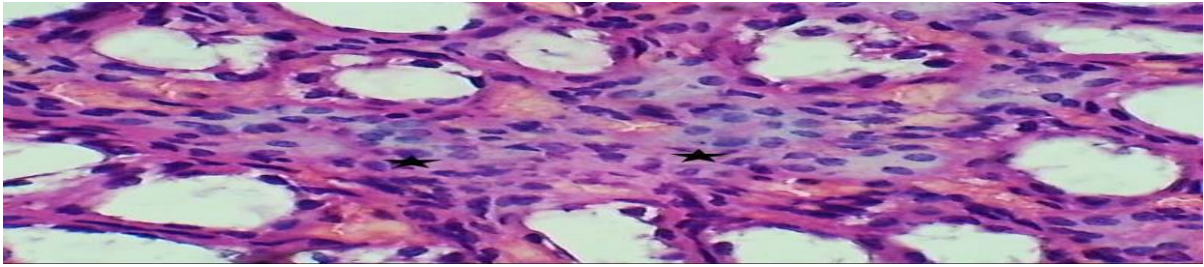


Fig. 6 Section of renal medulla (BV group) shows: fibrosis of renal interstitium (Asterisks). H and E stain. 400x.

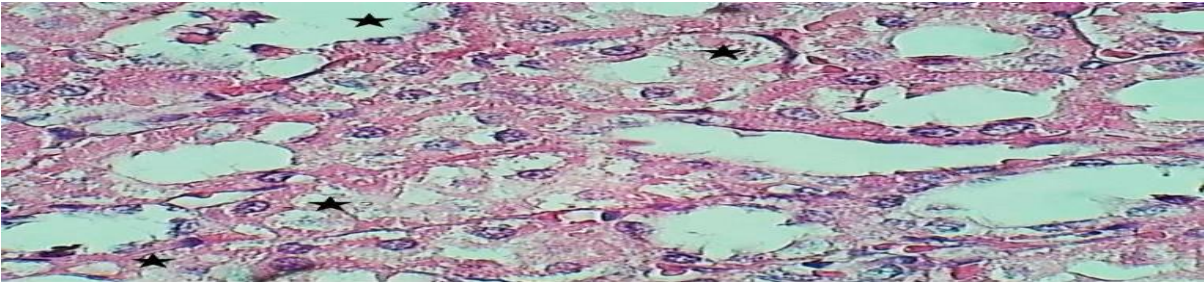


Fig. 7 Section of renal cortex (BV group) manifested by severe vacuolar degeneration of renal tubules (Asterisks), H and E stain, 400x

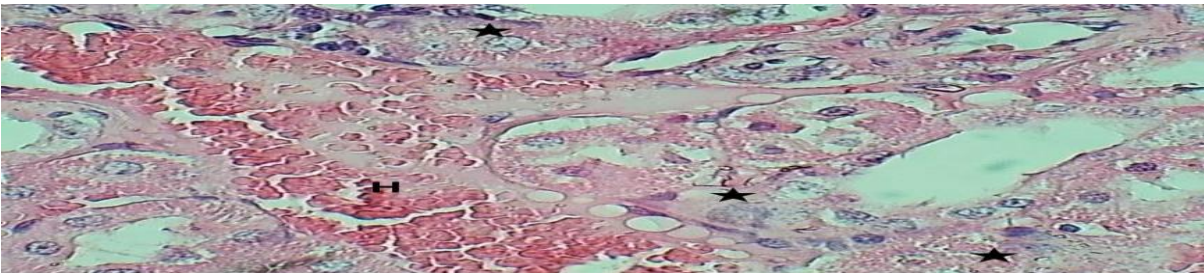


Fig. 8 Section of renal cortex (BV group) manifested by severe hemorrhage (H), necrosis of renal tubules (Asterisks), H and E stain. 400x

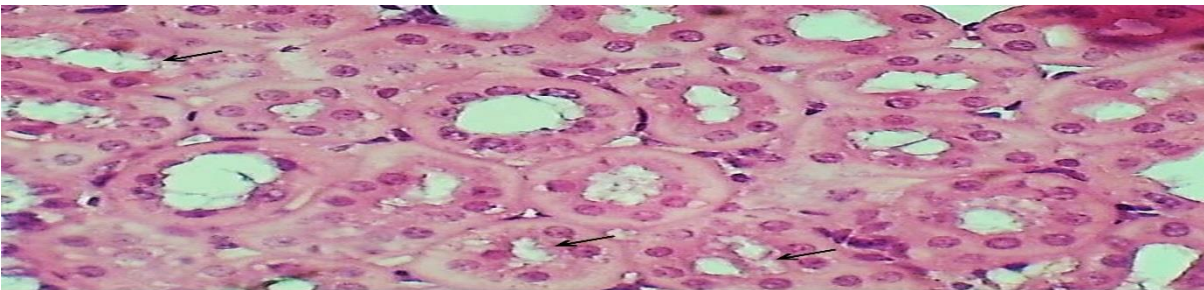


Fig. 9 Section of renal cortex (Control group) shows: normal appearance of most renal tubule with mild vacuolation of some tubules (arrows), H and E stain, 400x

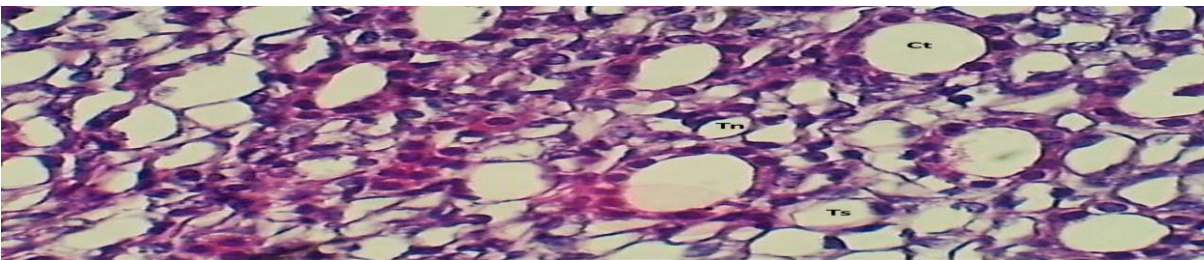


Fig. 10 Section of renal medulla (Control group) shows: normal appearance of collecting tubule (Ct), thick segment (Ts) & Thin segment (Tn) of loop of henle, H and E stain, 400x

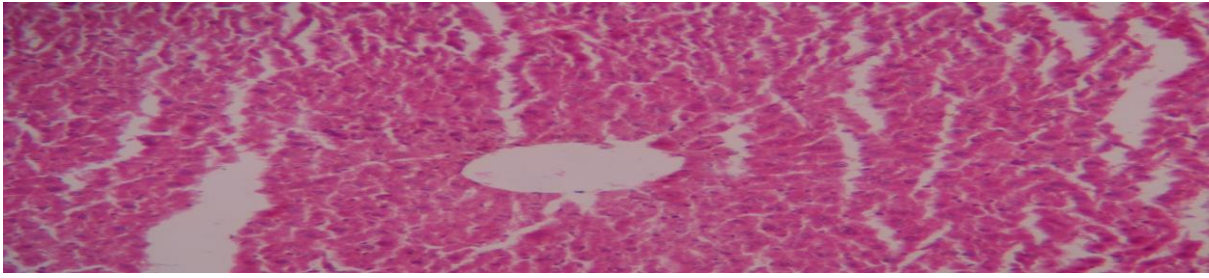


Fig. 11 Section control group showed normal hepatic structural details with normal hepatic appearance, H and E stain, 400x

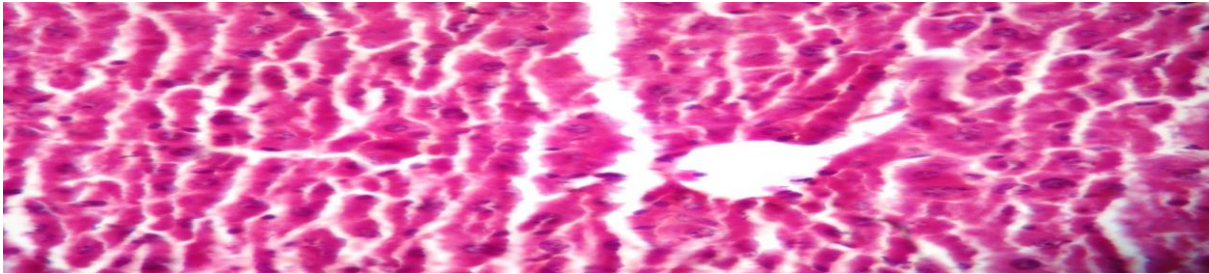


Fig. 12 Section bee venom groups, the main hepatic characterized by slight swelling of hepatocyte with vascular congestion, H and E stain, 400x

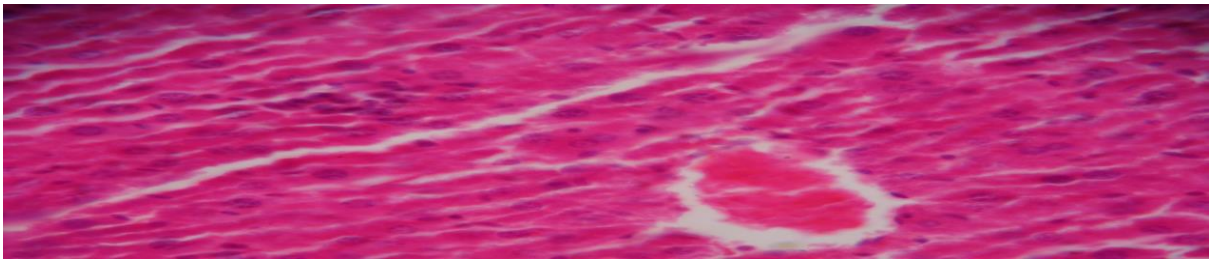


Fig. 13 Focal MNCs aggregation composed of various types inflammatory cells, H and E stain, 400x

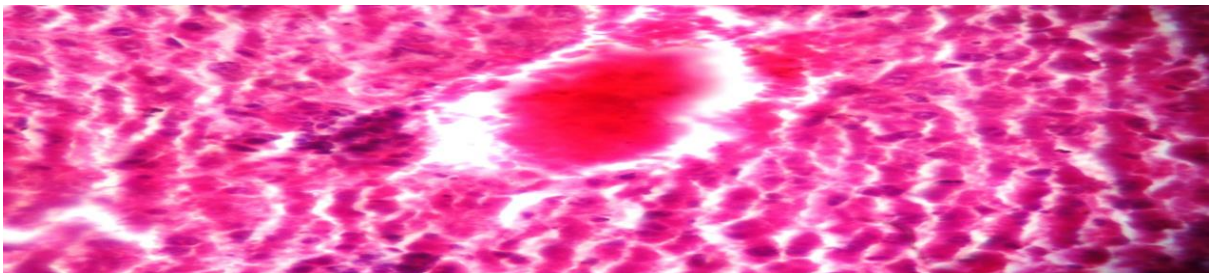


Fig. 14 Perivascular lymphocytic aggregation, H and E stain, 400x

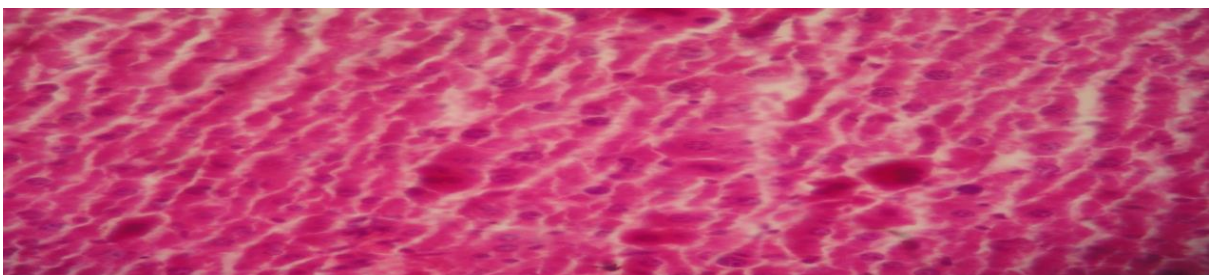


Fig. 15 Several apoptotic hepatocytes within hepatic parenchyma, H and E stain, 400x

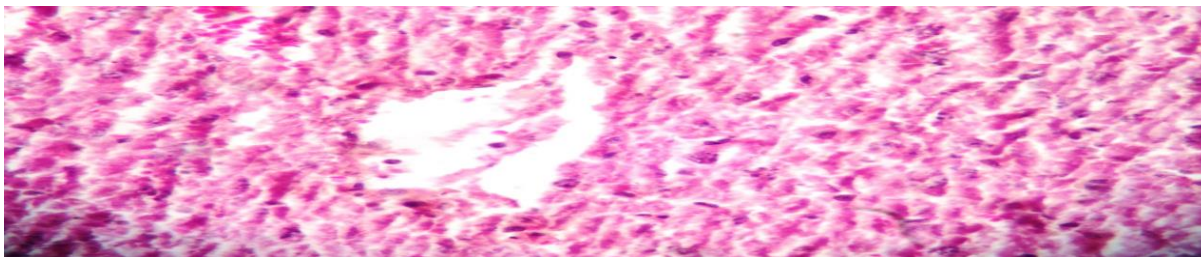


Fig. 16 Moderate to severe degenerative and necrotic changes with evidence of nuclear pyknosis of many hepatocytes, H and E stain, 400x

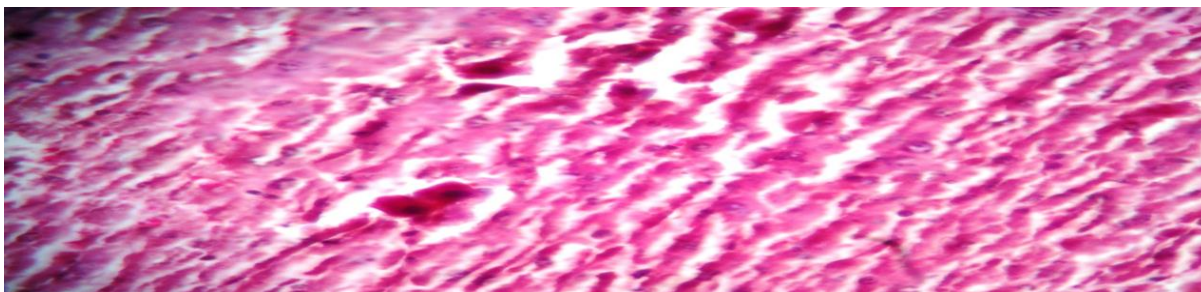


Fig. 17 Necrotic hepatic cords with numerous of apoptotic hepatocytes, H and E stain, 400x

The results demonstrate degenerative alterations in the epithelial cell lining tubules, atrophy of certain glomeruli, and blood vessel congestion when the mice are given varied dosages of BV. In contrast, sections of the renal cortex in groups injected with various concentrations of BV and a control group (Fig. 1, 2, and 3) displayed acute nephritis, which is characterized by severe cortical hemorrhage, tubular dilatation, and cloudy swelling of renal tubules. Other parts of the renal medulla indicated extensive vascular degeneration and necrosis of most renal tubules, while others revealed fibrosis of the renal interstitium. (Fig. 4, 5, 6, 7, and 8). The sections of the kidneys of the control group (Fig. 1, 2, and 3) displayed a normal look of the renal cortex and medulla, and most sections revealed a tiny figure of vacuolation in certain renal tubules. The renal medulla, on the other hand, appeared normal (Fig. 9, and 10).

BV causes nephrotoxic acute tubular necrosis because it attacks in clusters. In patients who have been stung by bees, kidney damage can occur, including intravascular hemolysis, rhabdomyolysis, hypotension, and direct toxicity of the venom components to the renal tubules (26). In this kind of acute kidney injury AKI, arterial hypotension plays a significant role in the development of an ischemic renal lesion [27]. The oxidative effect may be responsible for the existence of histopathological abnormalities in the liver and kidneys following BV injection [28]. The liver secreted prostaglandins PGF₂ and thromboxane B₂, which are considered inflammatory vectors that increase the inflammatory response and induce tissue damage when melittin was added to the rat food [29]. The role of BV in causing changes in heme metabolism in hepatocytes is related to hepatic microsomal enzymes, where the activity of cytochrome P-450 has reduced the action of several related enzymes including ethyl morphine N-demethylase and benzopyrene hydroxylase. Within 70 minutes of injection, a dose of 0.5 mg/kg causes a significant decrease in glomerular filtration and blood flow in the renal tissue, particularly in the cortex and medulla. The histological changes, on the other hand, were indicated by significant tubular damage and myoglobin deposition 24 hours after the injection. According to the majority of similar investigations melittin causes tissue damage by secreting phospholipase A₂, which activates the inflammatory process depending on arachidonic acid metabolism. Other researchers have discovered that melittin plays a role in the secretion of corticosteroids from the adrenal cortex [30], which is supposed to inhibit the secretion of phospholipase A₂ and reduce the secretion of the inflammatory vector chain, as well as reduce inflammatory infiltrations and free radical secretion [31]. Multiple pathways can trigger apoptosis in hepatocytes, including pro-inflammatory cytokines and TNF- α [32]. The appropriate dose of BV is used to prevent hepatocyte apoptosis caused by ethanol via the mitochondrial mechanism [33].

As a result, BV protects hepatocytes from TNF- α induced apoptosis when combined with actinomycin-D. Low amounts of BV were then utilized to induce anti-apoptotic effects, which were linked to a decrease in the levels of caspase proteolytic fragments and PARP [34].

Park et al. [35] described that transforming growth factor (TGF)- decreased cell viability and promoted hepatocyte apoptosis, according to Park et al. Despite this, administering 10 mg/mL of BV to TGF- β 1-treated hepatocytes increased their viability in a safe manner. Lee and colleagues [36] also demonstrated that an appropriate dose of melittin had anti-apoptotic properties against TGF- β 1-induced hepatocyte damage via the mitochondrial pathway. Melittin protects mice from liver failure caused by D-galactosamine/LPS through modulating apoptosis and the inflammatory response [37]. Melittin inhibited hepatocyte apoptosis, reduced hepatic damage, and reduced inflammatory reactions in the liver. Melittin may be a viable option for preventing acute hepatic failure, according to this study.

4. Conclusion

Histopathological examinations of hematoxylin and eosin-stained sections of the liver and kidney section indicated hepatic and renal complications in groups that were administered bee venom in different doses.

Acknowledgement

I would like to thank all the colleagues working in the animal house at Al-Razi Center for medical and diagnostic kits production, at Ministry of Industry, as well as the employees of the biological detection department for mutagen and carcinogens in Environment and the Water Directorate of the Ministry of Science and Technology

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