Bee Venom Causes Oxidative Stress, Biochemical and Histopathological Changes in the Kidney of Mice

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Summary

Accidents with venomous bees are a serious worldwide health concern. Since the kidney has been reported as the main venom-target organ, the present study was undertaken to investigate the *in vivo* nephrotoxic effect of Algerian bee venom (ABV) (*Apis mellifera intermissa*) collected in the middle east of Algeria. A preliminary study was performed on ABV to identify the ABV using SDS-PAGE analysis and to determine the *in vivo* intraperitoneal median lethal dose (LD_{50}) using the Probit analysis test. *In vivo* nephrotoxic effect was assessed through the determination of physiological and kidney biochemical markers in mice intraperitoneally injected with ABV at doses of 0.76 (D1); 1.14 (D2) and 2.29 mg/kg body weight (bwt) (D3), corresponding respectively to $LD_{50}/15$, $LD_{50}/10$, and $LD_{50}/5$ (i.p. LD_{50}=11.48 mg/kg bwt) for seven consecutive days. Results revealed a marked decrease in body weight gain and food intake, and an increase in absolute and relative kidney weights in ABV D2 and D3 treated mice compared with controls. Furthermore, ABV D2 and D3 resulted in a significant increase in serum creatinine, urea, and uric acid. ABV-induced oxidative stress was evidenced by a significant increase in kidney MDA level, and a significant depletion in kidney GSH level, and catalase activity. Meanwhile, no marked changes in the above-mentioned parameters were noticed in ABV D1. Accordingly, the adverse nephrotoxic effect of ABV was proved by the dose-dependent kidney histological changes. In summary, the results of the present study evidence that ABV at doses of 1.14 (D2) and 2.28 mg/kg body weight (bwt) can cause marked changes in kidney biochemical and major antioxidant markers, and histological architecture.

Key words

Bee venom • Kidney failure • Oxidative stress • Nephrotoxicity • Mice

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Introduction

Algerian bees form a group belonging to the African evolutionary Moroccan and Guinean, including subspecies, namely *Apis mellifera intermissa* and *Apis mellifera sahariensis* [1,2]. Bee venom (ABV) is a bee natural product, rich in a mixture of pharmacologically active molecules, including peptides (melittin and apamin) and enzymes (phospholipase A2, hyaluronidase, and histidine decarboxylase) [3]. It has traditionally been used to treat a variety of health conditions such as

arthritis and rheumatic pains, cancerous tumors, and skin diseases [4,5], however, the severe nephrotoxic effects limit its uses [6]. Moreover, bee venom is the main weapon used to protect the bee kingdom against a wide range of enemies, including predators, arthropods, and vertebrates [7]. Whilst, the massive bee swarm attacks with hundreds or thousands of stings may cause acute allergic reactions ended by severe health issues [8-10], in addition, to increased accidental death cases [11]. In the envenomed body, the bee venom circulates to the soft organs, and excretion mainly through urine making the kidney a venom-exposed organ [12]. Indeed, the kidney is a target organ for bee venom exposure [13], and as a result, an increased risk of kidney injury occurrence has been associated with venom exposure [6]. As reported [14,15], bee venom *in vivo* nephrotoxicity can develop acute renal failure (ARF) associated with acute tubular necrosis (ATN) [14,15]. In this context, the occurrence of ATN has been previously reported in rats (body weight 100 g) injected intravenously with 0.4 µl/100 g [11], and 1.5 µl/100 g body weight [13] of Africanized bee venom. However, the detailed mechanism of kidney injury associated with acute tubular necrosis (ATN) is poorly elucidated, as well as up to now, the *in vivo* nephrotoxicity of bee venom collected from Algerian regions (*Apis mellifera intermissa*) has not been yet investigated. Thus, the present study was conducted to investigate the induction of ATN in Algerian bee venom intraperitoneally injected mice.

Materials and Methods

Collection of bee venom

Algerian bee venom (ABV) lyophilized powder was obtained from honeybees, *Apis mellifera intermissa,* in the region of M'Sila city (Northeast Algeria), according to a previously described method [13]. The ABV was produced by Mr. Belhamdi Oussama head of the "*NehlaTech*" beekeepers' Start-up company (Algeria).

UV-Absorption Spectrum

Crude Algerian bee venom in a concentration of 1mg/ml in saline solution (0.9 % NaCl) was recorded on a spectrophotometer, using a range of UV wavelengths from 220 to 360 nm. Absorption spectra were automatically registered and the absorbance was obtained using saline solution (0.9 % NaCl) as blank.

SDS-page electrophoresis of ABV

The whole bee venom proteins were separated by the routine procedure of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described [16]. In brief, the used Tris-Tricine buffer was prepared by dissolving 14.11 g Tris HCL and 21.3 g Tricine (Cat. No. 5704-04-1, USA) in 100 ml distilled water. The gel was prepared by mixing acrylamide/Bis-acrylamide (30 % solution) (Sigma-Aldrich, Louis, USA), SDS (10 %) (Merck, Cat. No. 8220500100), ammonium persulphate (10 %) (MerckKGaA, Cat. No. 7727-54-0, Germany) and N,N,N', N'-Tetramethyl ethylenediamine (TEMED) (Merck, Cat. No. 110-18-9). The SDS-PAGE gel electrophoresis was run on BIO-RAD Mini PROTEAN©Tetra Cell tetra-gel hand casting system (California, USA). Bee venom samples were loaded at 4 % stacking and 15 % separating gel under room temperature, a current of 50-60 mA, and approximately for 2 h. The gel slabs were stained with a mixture solution formed by Coomassie brilliant blue, glacial acetic acid, and methanol. Samples were dissolved in 0.9 % saline to get a concentration of 3 mg/ml, and then 10 µl of the sample solution was loaded onto the gel.

Animals and treatments

Adult male *Swiss* mice weighing 28-32 g were obtained from the Pasteur Institute of Algiers (Northeast Algeria) and kept in the animal house of Annaba University, under a controlled temperature environment and natural photoperiod (12 h dark/12 h light). Animals received a standard diet and were allowed free access to tap water. The use of animals respected the Algerian Guidelines for Laboratory Animal Care and Use, which has been approved by the Ethical Committee of the Algerian Ministry of Higher Education and Scientific Research under the ethical number PRFU D01N01EN210120220001.

The acute toxicity of ABV was preliminarily studied based on the determination of the LD_{50} value in mice as described elsewhere [17]. Here, forty-nine mice were equally divided into control and seven groups intraperitoneally injected with 200 µl of ABV in six different doses (mg/kg body weight (bwt)); 2.5; 4.5; 6.5; 10, 14, 17, and 21 mg/kg body weight. The Control group was daily injected via the intraperitoneal route with 200 µl of 0.9 % NaCl. The lethality of mice was recorded 48 h after injection.

To assess the oxidative kidney biochemical injury,

thirty-two male *Swiss* mice were equally divided into a control group receiving intraperitoneally 0.9 % saline, and three ABV treated groups receiving, respectively 2.3; 1.14, and 0.76 mg/kg body weight (bwt) corresponding respectively to 1/5; 1/10 and 1/15 of the determined LD_{50} of ABV (LD_{50}, i.p.=11.48 mg/kg bwt) for 7 consecutive days. Accordingly, the LD_{50} value of venoms of other bee species was previously used to determine the acute toxicity [32]. The body weight of control and treated animals were recorded every day up to the 7th day. At the end of the experimentation, mice were sacrificed by cervical decapitation and blood samples were immediately collected in anticoagulant-free tubes, and centrifuged at 2500× g for 15 min at 4 °C. For the histopathological evaluation, the kidneys were carefully extracted, rinsed with a 0.9 % NaCl ice solution, weighed, and then one kidney was stored at -20 °C for the oxidative markers' evaluation, and the other one was fixed in formalin until the histological use.

Oxidative stress evaluation

The oxidant parameter malondialdehyde (MDA), an end product of lipid peroxidation, oxidative marker, and the major antioxidant parameters, including total glutathione (GSH) content, and catalase activity were determined in kidney tissue homogenate, which was prepared by using a homogenizer in presence of a phosphate buffer solution. MDA level was determined using Uchiyama and Mihara method [19], and the result was displayed in nanomolar/mg protein. GSH level was determined as previously described [18], and catalase (CAT) activity was spectrophotometrically (wavelength of 240 nm) determined as described elsewhere [20]. In addition, the level of proteins in kidney homogenates was determined as previously reported [21].

Assessing biochemical renal function

The serum biochemical markers of kidney function including urea, creatinine, and uric acid were spectrophotometrically assayed by using commercially available kits provided by SpinReact, Spain.

Histopathological evaluation

The extracted kidneys were subjected to histopathological examination according to the routine histological method [22]. Kidney specimens were fixed in 10 % paraformaldehyde for at least 24 h, sectioned into 5 µm slices, washed, and embedded in paraffin. Histological slices were stained with hematoxylin-eosin and examined under a light microscope.

Statistical analysis

Results are provided as mean ± S.E.M. Pairwise comparisons between groups were tested for significant differences by one-way ANOVA, using GraphPad Prism where $p<0.05$ was considered significant.

Results

Characterization of ABV

As shown in Figure 1A, ABV exhibited low absorbance at 220 to 270 nm, followed by higher absorbance at 280 nm, then a gradual decrease between 280 and 300 nm, and finally, no absorbance from 300 nm. In Figure 1B, SDS-gel electrophoresis was conducted to qualify the venom proteins, and the gel image was compared with marker proteins. Also, the major bands were less than 10 kDa.

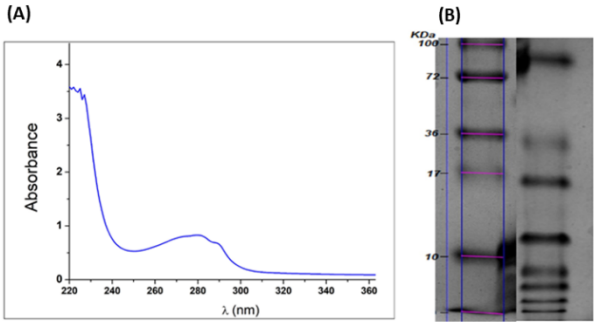

Fig. 1. (**A**): Absorption spectra of crude Algerian bee venom in saline solution (0.9 % NaCl). (**B**): SDS-PAGE electrophoresis of the bee venom. Marker proteins between 10 and 100 kDa (1); Algerian bee venom was used at concentration of 3 mg/ml (dissolved in 0.9 % NaCl).

Lethality result

As indicated in Figure 2, the mean lethal dose (LD_{50}) of Algerian bee venom following intraperitoneal injection was found as 11.48 mg/kg body weight (bwt) based on probit regression analysis.

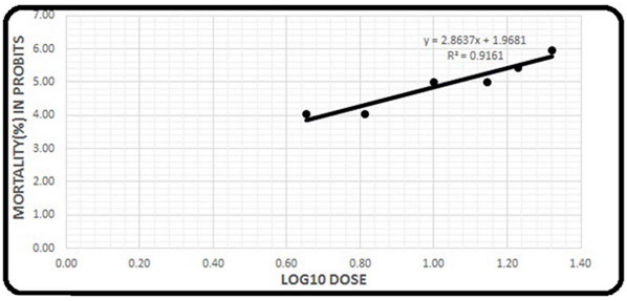

Fig. 2. Plot of log-doses versus probits of ABV administered intraperitoneally to male mice (11.48 mg/kg bwt). Mean Lethal Dose (LD_{50}) value was calculated from the linear equation.

Table 1. Changes in growth, food and water intake and kidney weights in control and treated mice.

Groups Parameters	Control	ABV D1	ABV D2	ABV D3
Initial body weight (g)	29.16±1.09	31.67±1.11	32.02±1.32	31.01±1.06
Food intake (g)	4.69±1.13	4.71±0.73	3.93±1.07*, #	3.78±1.11*
Body weight gain (%)	1.72±0.37	1.69±0.73	0.66±0.19**, #	0.33±0.11**, #
Kidney index (%)	1.19±0.34	1.21±0.77	1.98±1.1**, #	2.34±1.21***, #

Values are mean ± SEM (n=7). Values with superscripts are statistically different p value * p<0.05, and ** p<0.01; all treated groups versus control group. # p<0.05 and ## p<0.01; ABV (Algerian bee venom) (D2) and (D3) versus ABV (D1). ABV: Algerian bee venom; LD_{50}: Mean Lethal Dose. D1, D2 and D3 are respectively, 0.76 ($1/15^{th}$ LD_{50}); 2.28 ($1/5^{th}$ LD_{50}), and 1.14 ($1/10^{th}$ LD_{50}) mg of ABV/kg body weight (bwt).

Physiological results

Mortality

No mortality has been observed in all treated animals.

Growth indices

As indicated in Table 1, body weight gain decreased significantly in ABV D2 and D3 when compared with the control group ($p<0.01$), and ABV D1 treated mice ($p<0.05$), and similarly the food intake decreased significantly ($p<0.05$) in ABV D2 and ABV D3 as compared with control and ABV D1 groups. Further, the kidney relative weight was significantly increased ($p<0.01$) in ABV D2 and D3 compared with control, and significantly higher in ABV D2 ($p<0.05$) and D3 ($p<0.01$) compared with ABV D1 treated mice. Further, the food intake was significantly decreased in ABV D2 and ABV D3 ($p<0.05$) compared with control, and in ABV D2 and D3 ($p<0.05$) compared with ABV D1, showing no significant change. No significant change in water consumption was noticed in treated mice compared with controls.

Biochemical results

A significant increase was noticed in serum level of creatinine ($p<0.001$), uric acid ($p<0.01$) in ABV D2 and ABV D3 groups, and urea in ABV D2 ($p<0.01$) and ABV D3 ($p<0.001$) groups as compared with the control group. No significant change was found in ABV D1-treated mice compared with controls. Compared with ABV D1, the serum level of creatinine, and urea increased significantly in ABV D2 ($p<0.01$) and ABV D3 ($p<0.001$), and similarly, the serum uric acid increased significantly ($p<0.01$) in ABV D2 and ABV D3 treated groups (Fig. 3).

Oxidative stress results

As shown in Figure 4, the content of MDA, an end product of lipid peroxidation, in kidney tissue increased significantly ($p<0.001$) in ABV D2 and ABV D3 treated mice as compared with controls. Similarly, this oxidant parameter increased significantly in ABV D2 ($p<0.01$) and ABV D3 ($p<0.001$) when compared with ABV D1 treated mice. Whilst, the content of total glutathione decreased significantly in treatment with ABV D2 ($p<0.01$) and ABV D3 ($p<0.001$) compared with control mice, and ($p<0.01$) when compared with ABV D1. Also, a significant decrease in the enzymatic activity of catalase in treatment with ABV D2 ($p<0.05$) and ABV D3 ($p<0.001$) as compared with control, and ($p<0.01$) in comparison with ABV D1 treated mice. No significant variations in the above-mentioned parameters were noticed in the ABV D1 group compared with the control group.

Histopathological changes

Kidney sections from control and ABV D1 treated mice showed a similar normal architecture of glomeruli and renal tubules, while the kidneys from mice treated with the other doses of ABV showed marked dose-dependent histological abnormalities in the kidney evidenced by degeneration of glomeruli, tubular necrosis in approximately 60 % in ABV D2 and 80 % in ABV D3 of the examined tissue areas, and presence of inflammatory cells, including neutrophils and lymphocytes (Fig. 5).

Discussion

The present work is the first study investigating Algerian bee venom (ABV) induced kidney failure in mice. In this study, the highest absorption of ABV at

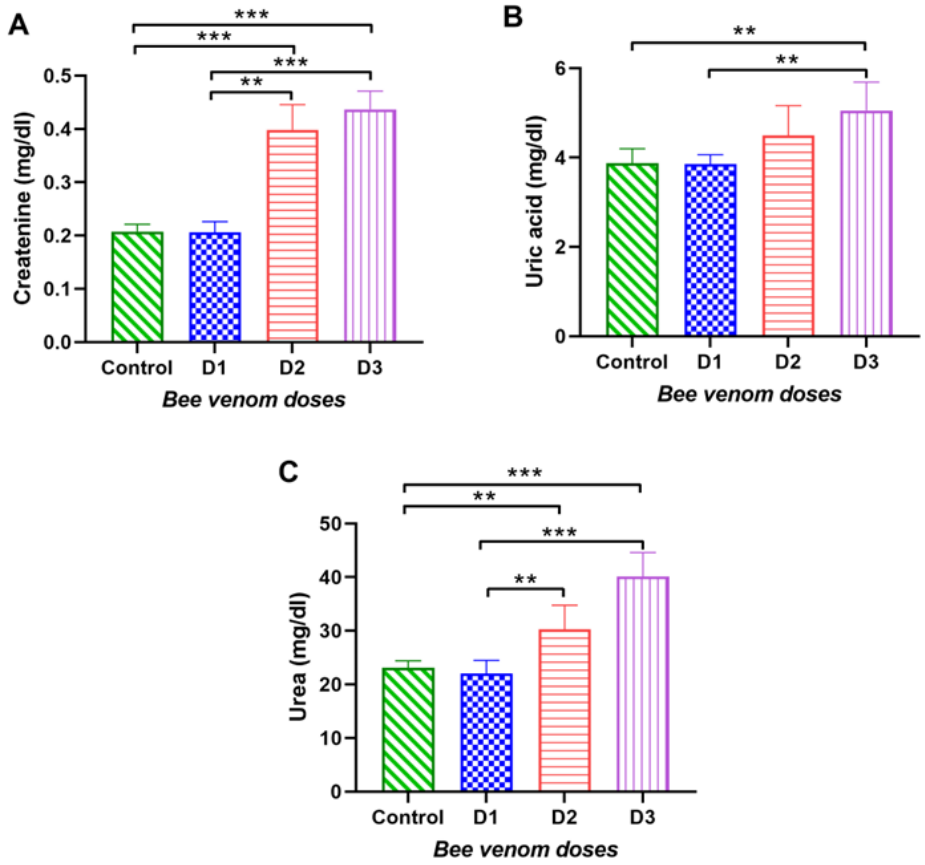

Fig. 3. Changes in serum creatinine, uric acid and urea in control and ABV treated mice. ABV: Algerian bee venom; Mean Lethal Dose (LD_{50}). D1, D2 and D3 are respectively, 0.76 ($1/15^{th}$ LD_{50}); 2.28 ($1/5^{th}$ LD_{50}), and 1.14 ($1/10^{th}$ LD_{50}) mg of ABV/kg body weight (bwt).

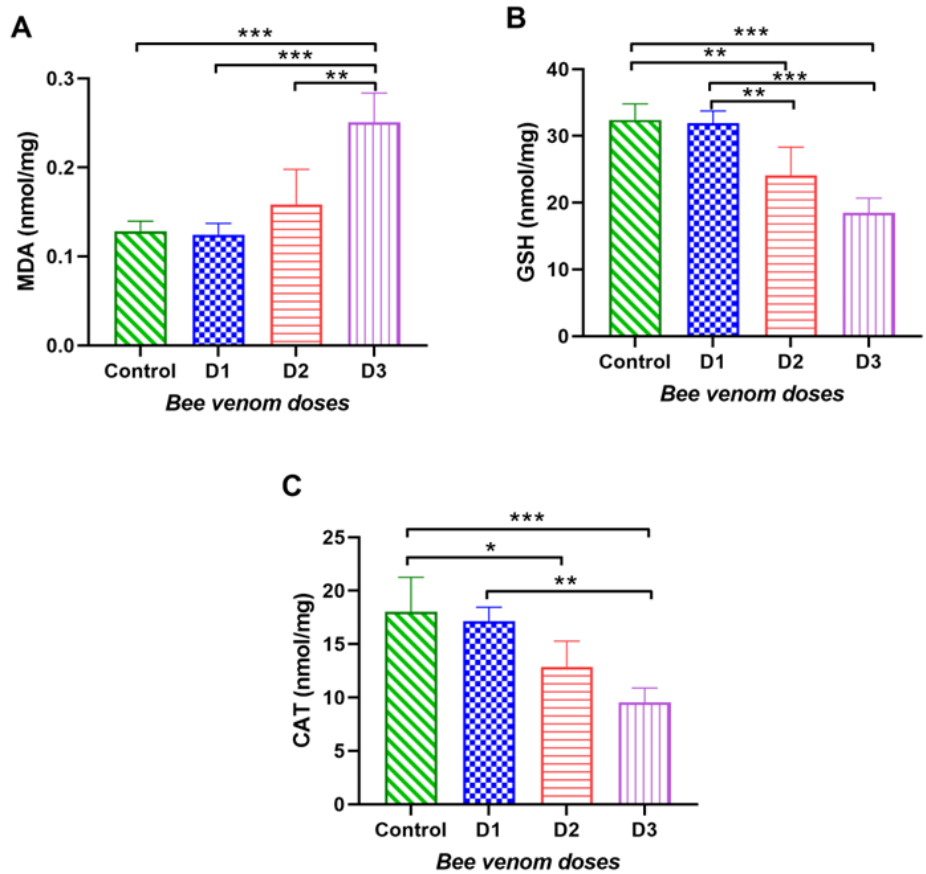

Fig. 4. Changes in total GSH and MDA contents and enzymatic activity of catalase (CAT) in control and ABV treated mice. ABV: Algerian bee venom; Mean Lethal Dose (LD_{50}). D1, D2 and D3 are respectively, 0.76 ($1/15^{th}$ LD_{50}); 2.28 ($1/5^{th}$ LD_{50}), and 1.14 ($1/10^{th}$ LD_{50}) mg of ABV/kg body weight (bwt).

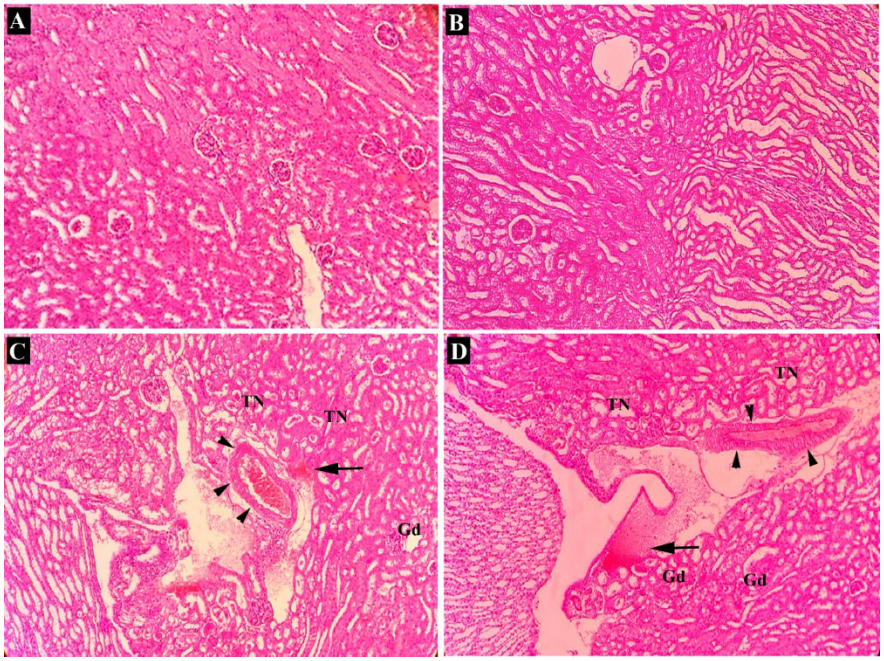

Fig. 5. H&E staining of renal tissue (100×). Control mice, showing normal renal histological structure and architecture, and those treated with ABV D2 (1.14 mg/kg bw), and ABV D3 (2.28 mg/kg bw), showing tubular necrosis (TN), and glomeruli degeneration (Gd), and inflammatory cells (arrow), arteriosclerosis (arrow-heads). Of note, Algerian bee venom ABV D1 (0.76 mg/kg bw) treatment show comparable tissue structure to that of control with no marked histological alterations. Scale bar: 50 µm.

280 nm may prove that the bee venom (BV) peptides formed by aromatic amino acids, in particular, melittin and phospholipase A2 (PLA2), the most abundant peptides (approximately 50-70 % in total), composed, respectively by tryptophan and phenylalanine [24]. In addition, peptide bonds present in bee venom components provide venom spectrophotometric absorption between 200 to 225 nm [25]. Furthermore, the separation of bee venom peptides using the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) technique has been well previously studied [26-29], where melittin and apamin, the main bee venom components, have molecular weights of less than 10 kDa. Also, another main bee venom component is the phospholipase whose molecular weight estimated by SDS PAGE was reported to be 14.5 kDa, and this evidences the monomeric structure of the enzyme [30]. Hence, the indicated molecular bands varying from 10 to 100 kDa (Fig. 1B) estimated by SDS-page electrophoresis proved that the peptide mixture was bee venom. On the other hand, the measured LD_{50} value referring to the acute toxicity of ABV was found to be 11.48 mg/kg body weight (bwt) of mice *via* intraperitoneal injection. This value is slightly lower than that reported for bee venom of Egypt (13.19 mg/kg bwt) [31], and quite higher than that of different regions of Argentina (LD_{50} BV: 7-11 mg/kg bwt) [32]. The variation in LD_{50} of BV is likely due to the geographical origin, seasonal changes, and bee feeding which may affect the venom content [33]. In addition, the chromatographic profiles of bee venom can differ depending on the venom collection method, either extraction of glandular venom or electrical stimulation [34]. In this study, the body weight gain and food intake decreased significantly in mice that received bee venom at D2 and D3 compared with control and BV D1. This result is similar to that previously reported [35,36], suggesting that the body weight change is likely related to the stress due to the treatment, as well as decreased food consumption. Whilst, the kidney weights were increased significantly in ABV D2 and ABV D3, and not significantly in ABV D1 which seemed to be not toxic. The increased organ weights can be correlated with acute renal tissue injury as evidenced by tubular necrosis and hypertrophy and glomerular degeneration [6]. Moreover, the study revealed a marked increase in the functional kidney markers, including serum urea, uric acid, and creatinine levels of ABV D2 and D3 as compared with those of control and ABV D1. The changes in these biochemical parameters have been previously reported in a study investigating bee venom envenomed horses and have been suggested as valuable indices of renal function impairment [37]. Overall, the renal failure associated with tissue necrosis and cell lysis results in increased levels of creatinine and urea [38], as well as the increased breakdown and alteration of nucleic acids may lead to increase uric acid production [39]. On top of that, ABV-induced kidney oxidative injury was evidenced by a significant increase in MDA content (the oxidant parameter), along with a significant decrease in GSH content, and catalase activity as compared with control mice. In this study, the antioxidant changes were dose-dependent in ABV D2 and ABV D3, except

ABV D1 treatment showed no marked effect on these antioxidant parameters. Similarly, previous studies have reported a high level of MDA and reduced GSH content [40], and catalase activity [41,42] in BV-treated rats. On the other hand, the plausible mechanism underlying BV toxicity is the induction of oxidative stress-mediated generation of reactive oxygen species (ROS) leading to damage and death of either normal or cancer cells [43], and hence BV was believed to neutralize the antioxidant effects [44]. The biochemical and antioxidant changes in ABV-treated rats were supported by the histopathological observations of kidney tissues as evidenced by glomerular degeneration, tubular necrosis, and tissue inflammation. These results are consistent with other previous studies investigating BV-induced kidney dysfunction [6,45]. In this context, a previous study reported a marked decrease in glomerular filtration rate (GFR) along with an increase in fractional excretion of sodium (FENa) and tubular necrosis ended by kidney dysfunction in intravenous injected Africanized bee venom in rats [11]. Thus, glomerulus and tubule cells were reported as the most susceptible renal tissues to venom toxicity because they are the main site of reabsorption and filtration of substances associated with intense metabolic activity [46,47].

Conclusions

The results revealed an oxidative kidney injury evidenced by significant changes in the main kidney biochemical and antioxidant markers, in addition to histological alterations showing degeneration of renal glomerulus and tubular necrosis found in mice, that received intraperitoneal injection of Algerian bee venom (ABV) at the selected doses of $1/5^{th}$ and $1/10^{th}$, excluding the dose $1/15^{th}$ of the determined LD_{50} (not toxic dose) corresponding respectively to 2.28; 1.14 and 0.76 mg/kg body weight. Conclusively, these findings provided valuable clinical information to obtain the final diagnosis of victims who received multiple bee stings.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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