HISTOLOGICAL ALTERATIONS IN KIDNEY AND LIVER OF LABORATORY MICE FOLLOWING INTRAMUSCULAR INJECTION OF *MONTEVIPERA BORNMUELLERI* (WERNER, 1898) VENOM

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Abbreviations: LD50: Lethal Dose to kill 50% of the test population; IV: Intra venous; IP: Intra peritoneal; SC: Subcutaneous; IM: Intra muscular; C: Capillary; Bs: Bowman's space; Pt: Proximal tubule; Dt: Distal tubule; PLA2: Phospholipase A2; LAAO: L-amino acid oxidase

ABSTRACT

Kossaifi, E. Hraoui-Bloquet, S. Sadek, R. Fajloun, Z. Accary, C. and Hleihel. W. 2017. Histological alterations in Kidney and Liver of laboratory mice following intramuscular injection of *Montevipera bornmuelleri* (Werner, 1898) Venom. Lebanese Science Journal, 18(1): 122-135.

Histopathological changes after bites of the Montivipera bornmuelleri viper, endemic to high altitudes areas of Lebanon, have not yet been investigated. Our study focuses on histological changes and irreversible damages in the kidney and liver of white laboratory Balb/c mice that received an intramuscular injection of lyophilized venom diluted in saline solution (NaCl 0.9%). Different venom concentration doses ranging from 0.25 mg/kg to 15mg/kg in a total injection volume of 100µl was injected intramuscularly (IM) into 5 groups of mice. After dissection, observations showed no macroscopically identifiable damages in any of the organs studied. However, tissue samples from the liver and the kidney were obtained for histological studies at various time intervals following the venom injection. The histological study was carried out using the Bouin solution (fixing bath), followed by dehydration in alcohol. Paraffin-embedded sections were stained using hemalun-easine. Tissues from 6 control mice,

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which received only an injection of saline solution, were also examined. The common histological alterations observed microscopically in the liver and the kidney were: pycnotic nuclei, necrosis, vacuolization, cytoplasmic destruction, edema, hemorrhage and congestion of blood vessels. Moreover, inflammatory infiltration of lymphocytic cells was observed in the prevascular regions of both organs. The histological changes observed appeared within 1h and intensified with time. These changes were proportional to the dose of the venom injected into the laboratory mice. Our results on what happens in the kidney and liver of mice after injecting the venom of M. bornmuelleri had many similarities with the effect of venom of other vipers (e. g. Daboia palestinea, Bitis ssp, Bothrops moujenis etc…) observed by others authors.

Keywords**:** *Montevipera bornmuelleri*, lyophilized venom, intramuscular injections, liver, kidney.

INTRODUCTION

M. bornmuelleri is endemic to Lebanon (Werner, 1938) and only found in Lebanese mountains at altitudes above 1800m (Hraoui-Bloquet *et al.* 2002). It is now listed as endangered by IUCN (2006) based on its limited and patchy geographic range estimated to be less than 20 000 km^2. Moreover, its population is highly fragmented in the mountains of Lebanon. Studies concerning *M. bornmuelleri* are very rare. An ecological study with preliminary biochemical characterization of the viper's venom was conducted by Hraoui-Bloquet *et al.* in 2012. Other recent pioneer studies concerning proteomic analysis and biological properties of the *M. bornmuelleri* venom were conducted (Rima *et al*., 2013; Accary *et al*., 2014a; Accary *et al.*, 2014b; Accary *et al.*, 2015).

Lethality determinations were reported for *Montivipera (Vipera) bornmuelleri* and compared to *Montivipera* (*Vipera) latifii* by Weinstein and Minton (1984). Immunoelectophoretic profiles indicated a close antigenic relation between venoms of these species with *Daboia palestinae* and *Bitis spp.*

Damages caused in tissues from the snake envenomation depend on the type and amount of venom injected, as well as on the species because not all venoms have the same composition. Those damages rest also in the susceptibility of the tissue studied for a particular component of the venom (kamiguti et *al.*, 2000). Interestingly, viper venom affects the kidney tissues more frequently than other snakes. Renal alterations are also very common following viper bites (Sheriff, 1983). These alterations are more prominent when the venom contains hemotoxins and vasculotoxins. A histological study demonstrated that alterations/damages/outcomes in renal tissues are very common after viper bites (Soe *et al.*, 1993). The changes have been observed in all renal structures. Biochemical and clinico-pathological changes were induced by *Bungarus caeruleus* venom in the rat model (Kiran *et al.*, 2004). It was shown that changes observed in the kidney after the administration of snake venom include degeneration, necrosis and regeneration of renal tubular epithelial cells, interstitial edema, cellular infiltration, arteritis, thrombophlebitis, congestion, infarction and cortical necrosis (Kiran *et al*. 2004, Stiprija *et al.* 2006). Histological and functional renal alterations, caused by *Bothrops moujeni* snake venom in rats,asserted the lymphatic contribution to the systemic absorption of venom toxins from the tissues (Boer-Lima *t al*. 1999). Therefore, studies of potential effects of snake venoms on the structure and function of lymphatic vessels, might have implications for the pathogenesis of edema and the absorption of venom by the tissues (Gutierrez, *et al.* 2003).

Changes in the liver following snake bites were reported in studies conducted by De Silva *et al.* (1992) and Kumaranayake (1971). Both studies observed congestion, petechial haemorrhages, microvescicular fatty change, hydropic degeneration,and necrosis of hepatocytes. The above-mentioned observations are in close accordance with the findings reported for *Vipera berus* and *Daboia (Vipera) palaestinae* (Tu, 1977).

Jarrar (2011) studied the histological alterations and biochemical changes in the liver of sheep following *Echis colora* envenomation. This venom elevated glucose, , aspartate amino transferase (AST), alanine aminotransferase (ALT), triglyceride and total bilirubin, while cholesterol was reduced. The histological alterations detected, were pyknosis, karyorrhexis, cytoplasmic vacuolation, necrosis, fatty changes, hepatocytes atrophy, sinusoidal dilatation, kupffer cell activation, amyloidosis, portal vein thrombosis, partial glycogen depletion and hepatic architecture distortion. These findings revealed that *E. Colora* venom produced biochemical changes and histological alterations in the liver, which may have a severe effect on the functions of this organ.

Nanayakkara*etal*. (2009) described the histological changes in liver, kidney and brain tissues following intramuscular administration of the venom of *Bungarus ceylonicus and Bungarus caeruleus.* Histopathological changes relative to congestion, inflammation and necrosis were also observed microscopically in these tissues. These changes were proportionate to the dose of venom.

In this study, we aim to identify the histological changes in liver and kidney tissues following an intramuscular injection of *M. bornmuelleri* venom, at different concentrations, into white mice (Balb/c).

MATERIALS AND METHODS

Venom

Venom was extracted from 6 adult specimens of *M. bornmuelleri* caught in Jabal Sannine and Jabal Makmel (two mountains located in Lebanon). Stock solution* of the venom were prepared immediately before the experiments. This was done using 25 mg of fresh venom in 5 ml of a phosphate buffered saline solution at pH 7.

The LD50 of *M. bornmuelleri* previously determined by Weinstein S. and Minton S. (1984) corresponds to 0.6 mg/kg in intra venous (I.V.) injection and 1.9 mg/ kg in intra peritoneal (I.P.) injection and 6.2 mg/kg in subcutaneous (S.C.) injection. Here, we adopted the LD50 obtained by intramuscular (I.M.) injection of the fresh, rather than lyophilized venom and determined to be 5.39 mg/ kg for mice weighing between 25 and 30 g (Abi-Rizk *et al*., 2017).

Animals and treatments

Six groups of white laboratory mice (Balb/c), 2 to 3 months old, weighing 25-30 gr were used. Aliquots of 0.2 ml of the venom at different concentrations (Table 1) were injected intramuscularly (I.M.) into the left thighs of mice. The intensity of venom used was based on

the LD50 (5.39 mg/kg) values for *M. borrnmuelleri.* A control group (or group No 1) was injected with 0.2 ml of phosphate buffered saline solution at pH 7.

TABLE 1

Different Concentrations and Volumes Injected in Mice by IM of the Venom Dilutions of *M. bornmuelleri* Venom

Group Numbers	Solution injected	Injected volume	Individual Number	Action time
1	Phosphate buffered saline	0.2ml	6 (control)	24h
2	Stock solution*	0.2 ml	16	1h-3h-5h-24h
3	8 mg/kg	0.2 ml	16	1h-3h-5h-24h
4	4 mg/kg	0.2 ml	16	1h-3h-5h-24h
5	1.6 mg/kg	0.2 ml	16	1h-3h-5h-24h
6	0.8 mg/kg	0.2 ml	16	1h-3h-5h-24h

Stock solution* see paragraph venom in Materials and Methods

Experimental protocol

After 1h, 3h, 5h and 24h following the administration of venom to the mice, livers and kidneys were removed and fixed in Bouin solution for 24 h. The tissues were then washed witch tap water, dehydrated in a graded ethanol series (70%, 90% and 100%) and embedded in paraffin at 60 ºC.

Histopathologic analysis

5 µm thick paraffin sections were prepared and stained with hematoxylin and eosin and then assessed under light microscopy. Histological changes were scored according to a predetermined system given in table 2.

Results

Some mice died directly after injection of the venom while others died sometime before dissection (table 2). These mices were eliminated from the study.

TABLE 2

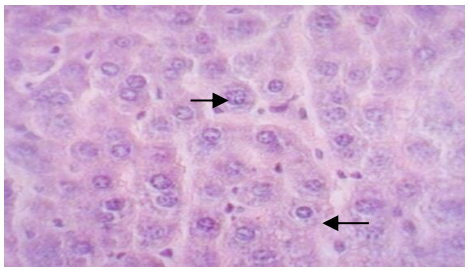
Venom Concentrations, Number of Mice Injected and Number of Dead Mice Observed during the Experiments

Serial numbers	Solution concentrations	Number of mice injected (IM)	Number of dead mice and surviving time
S1 & S6 ST3 & ST6	Stock solution*	**16**	2 mice (just after solution injection), 2 mice (30 min after solution injection)
ST1 S2 & S4	dilution 1/5 : 8 mg/kg	**16**	1 mice (just after solution injection) 2 mice (15 min after solution injection)
S7 & S11	dilution 1/25 : 1.60 mg /kg	**16**	2 mice (just after solution injection)

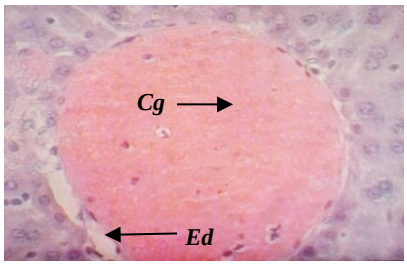
All envenomated mice presented the following visual observations/symptoms in the region of the injection: hemorrhage, edema, dermonecrosis and myonecrosis reaction after intramuscular (I.M.) of the venom. Microscopic observations of histological sections of livers and kidneys from all envenomated mice presented many common histopathological changes like necrosis, pyknotic nuclei, edema, congestion of blood vessels, tearing of the vessel walls, bleeding and inflammations (inflammatory infiltrates consisted of lymphocytes). Histopathological changes, observed due to the administration of this venom, appeared within 1h in liver and in kidney.

Histopathology of liver: Changes observed in histological tissues sections

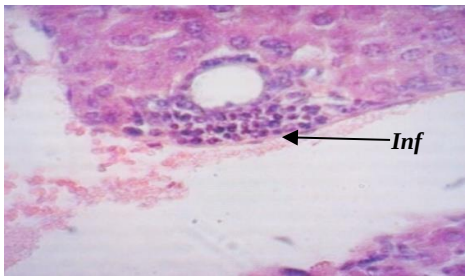
Histopathological changes namely vessel congestion, sinusoidal dilatation, inflammation, necrosis, suffering damage (degradation or degeneration) and depletion of hepatocytes, hepatic architecture distortion and edema were microscopically observed in the liver tissues. These findings revealed that *M. bornmuelleri* venom altered the histology of the liver of the envenomated mice. Accordingly, these alterations might severely affect the functions of this organ. They were acutely observed in livers of mice that received the stock solution or the dose concentration of 8 mg/mice kg. Moreover, congestion of the portal vein was seen in almost all liver tissues from 1h to 24 h post-injection (Fig.1B).



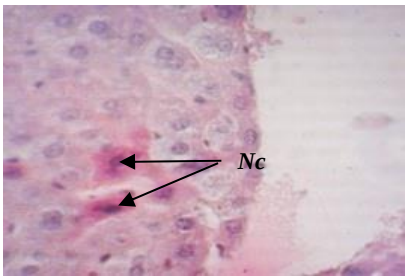
A: healthy liver section showing the hepatocytes in good shape (→)



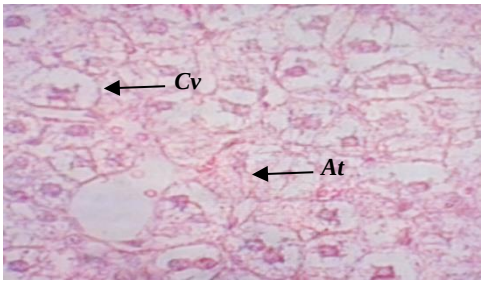
B: liver section after 1h of venom injection showing congestion (→ ***Cg***) and an edema (→ ***Ed***) of the portal vein.



C: liver section after 1h of venom injection showing the inflammatory infiltration (⟶***Inf***)



D: liver section after 1h of venom injection showing the necrosis hepatocytes (⟶ ***Nc***)



E: liver section after 1h of venom injection showing the diseased hepatocytes with cytoplasm vacuolation (⟶ Cv) and hepatocyte atrophy (⟶***At***)

Figure 1. Five pictures taken after staining and microscopic observations of five liver sections. A: section of the liver of healthy mice not injected by the venom; B, C, D and E: sections of the livers of mice after 1 hour of IM injection of *Montivipera bronmuelleri* venom showing congestion, edema, inflammatory infiltration, necrosis hepatocytes and diseased hepatocytes respectively.

Inflammatory infiltration (Figure 1D) was seen in the perivascular region of the liver. Inflammatory infiltrates were confined to the periportal and perivenular regions of the liver, and appeared in tissues observed from 1h to 24h post-injection following the administration of the venom (Table 3). Liver necrosis (Figure 1E) was a consistent finding following the venom injection. Karyopycnosis of some hepatocytes was evident namely in the necrotic hepatocytes. A variable degree of hepatocyte cytoplasmic vacuolation was detected (Figure 1E), where its severity was associated with necrosis. Hepatocytes suffering and atrophy were also observed. It is noteworthy that edema was observed in all cases of envenomated mice.

These sections were compared to those from control mice that were not injected with the *M. bornmuelleri* venom.

TABLE 3

Histopathological Changes Observed in Liver Tissues after IM Injection of Different Doses of *M. bornmuelleri* Venom (Stock Solution; 1/5=8mg/kg; 1/10=4mg/kg; 1/25=1.6mg/kg; 1/50=0.8mg/kg).

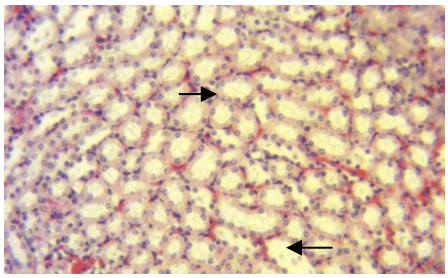
Number of samples	Doses (mg/Kg)	Time	congestion	Inflammation	edema	necrosis	Hemorrhage	Suffering cells
3	Control	24h	-	-	-	-	-	-
6	Saline solution	24h	2 +	1 +	-	-	-	-
12	Stock solution	1h	12 ++	10 +++	12 +	12 +++	10 ++	12 +++
13	8 mg/kg	1h - 5h	13 ++	9 ++	11 +	12 +++	9 ++	13 ++
16	4 mg/kg	1h - 5h	14 ++	12 +	16 ++	16 +	10 +	16 ++
14	1.6mg/kg	1h - 24h	12 +	12 +	12 +	14 +	8 +	14 +
16	0.8mg/kg	1h - 24h	13 +	13 +	13 +	13+	13+	13+

The sign (+) Indicates the Presence of the Anomaly/Histopathology.
+ = Moderate; ++ = High; +++ = Very high; - = Absent

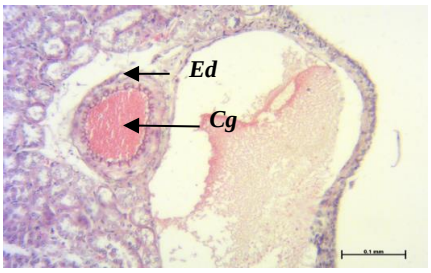
Histopathology of kidney: Changes observed in the tissues sections of the Kidney

Histopathological changes namely congestion, inflammation and necrosis were observed microscopically in the tissues of the kidney. Peritubular capillary and glomerular congestion were also seen in most tissue samples of the kidney (Figure 2B). On the other hand, inflammatory infiltration, mainly lymphatic (Figure 2C), was found in the perivascular regions of the kidney. Marked changes and severe morphologic disturbances were observed in the glomeruli after venom injection. Histological examinations of the kidney showed wide Bowman's spaces, filled with debris of cells, and damaged glomeruli (Figure 2D and 2E).

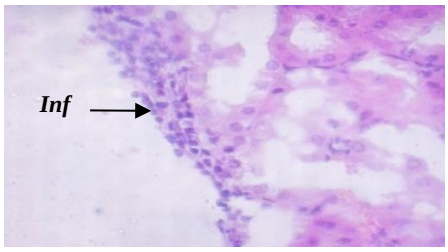
Severe morphologic disturbances or degenerations, especially in the distal tubules, were unveiled. Degenerative changes were also seen in the proximal tubules after envenomation. These changes consisted of the loss of the proximal brush border, the cytoplasmic vacuolation, or in some tubules the degeneration and desquamation of the necrotic cells (Figure 2F). Cell debris and casts resulting from necrosis were observed in the collecting ducts and in the lumen of the proximal and distal tubules (Figure 2F). The nuclei of the various proximal tubular cells often showed pyknosis with clumped chromatin material. In some tubules, the renal epithelium was completely necrotic, whereas the basal membrane was either intact or disrupted by tubular necrosis (Fig.2F). The distal tubules, nephron loops, and collecting ducts had swollen lumens. In some areas, there were disruptions of both tubular walls (Figure 2F) and peritubular capillaries walls, indicating hemorrhage (Figure 2D). Additionally, hyaline casts, red blood cells and sloughed cellular debris filled the lumens. Necrotic and damaged areas were present in tubules of the cortico-medullary regions. All these damaged tissues were compared to healthy kidney section of mice not injected by the *M. bornmuelleri* venom (Figure 2A).



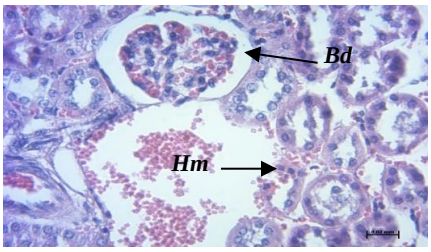
A: healthy kidney section showing the good quality of epithelial cells of different tubules (→)



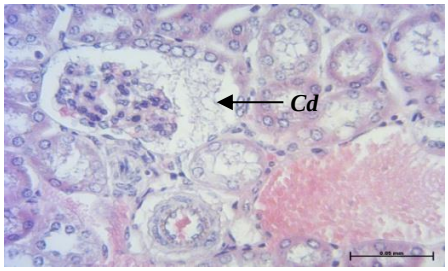
B: kidney section after 1h of venom injection showing congestion (→ ***Cg***) and an edema (→ ***Ed***) at the cortical area



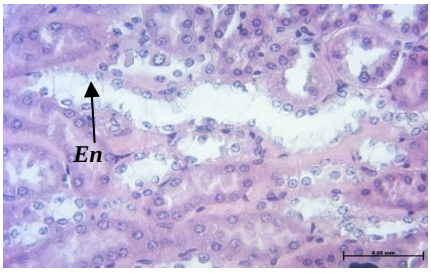
C: kidney section after 1h of venom injection showing the inflammatory infiltration (→ ***Inf***)



D: kidney section after 1h of venom injection showing the Bowman's spaces dilated (→ ***Bd***) and disruption of capillary wall with hemorrhage (→ ***Hm)***



E: kidney section after 1h of venom injection showing the cell debris in the dilated lumen of the Bowman's space (→***Cd***) and damage of its basal membrane



F: kidney section after 1h of venom injection showingrenal epithelium damage of the epithelium and basal membrane of the distal tubule (→ ***En***).

Figure 2. Six pictures taken after staining and microscopic observations of six kidney sections. A: section of the kidney of healthy mice not injected by the venom; B, C, D, E and F: sections of the kidney of mice after 1 hour of IM injection of *M. bronmuelleri* venom showing congestion, edema, inflammatory infiltration, Bowman's space dilatation, necrosis epithelial cells of tubules respectively.

TABLE 4

Histopathological Changes Observed in Kidney Tissues after IM Injection of Different Doses of the M. Bornmuelleri Venom (Stock Solution; 1/5=8mg/kg; 1/10=4mg/kg; 1/25=1.6mg/kg; 1/50=0.8mg/kg).

Number of samples	Doses (mg/Kg)	Time	Mice with congestions	Inflammation	edema	hemorrhage	Glomeruli changes (C & Bs)	Necrosis &Suffering cells in (Pt & Dt)
3	Control	24h	-	-	-	-	-	-
6	Saline solution	24h	-	-	-	-	-	-
12	Stock solution	1h	12++	12++	12 ++	10+	12 +++	12+++
13	8mg/kg	1h - 5h	13++	13++	10 ++	8+	13 +++	13 +++
16	4mg/kg	1h - 5h	14++	13 ++	16 ++	10+	16 +	16+
14	1.6mg/kg	1h - 24h	10+	6 +	8+	-	8 +	10+
16	0.8mg/kg	1h - 24h	12+	-	-	-	-	-

The Sign (+) Indicates the Presence of Anomaly.
C: capillary, Bs: bowman's space; Pt: proximal tubule; Dt: distal tubule
+ = Moderate; ++ = High; +++ = Very high; - = Absent

CONCLUSION

Severity of the envenomation depends on several factors such as: the snake species, the bitten animal, the host animal size, the amount of venom injected (Latifi, 1995) because of the large variability in the venom components. Histological changes following*M. bornmuelleri*bites are not yet documented. We chose to evaluate the liver and kidney condition after IM injection of *M. bornmuelleri* venom since, they are the two first organs which are damaged in the body. The histopathological changes observed were of an acute toxic insult and the snake venom significantly affected the function of these organs (Nanayakkara*etal*., 2009). We have demonstrated that the administration of the *M. bornmuelleri* venom resulted in congestion, inflammation, edema, hemorrhage and necrosis in the liver and the kidney at all concentrations only 1 hour after the injection.

Lower molecular weight toxins in krait venom are rapidly absorbed and quickly distributed through the blood stream (De Silva and Ranasinghe, 1983). The first tissue changes were detected in the brain, kidney and liver 1 hour after injection. Accordingly, toxic changes would appear early in the target organs. Our results demonstrated that the *M. bornmuelleri* venom causes irreversible histological damages in the kidney and liver of mice 1 hour after injection. Histopathological changes caused by toxins present in the venom of *M. bornmuelleri that* resemble those observed by Nanayakkara *et al.,* (2009) who reported many changes like congestion, inflammation and necrosis in kidney and liver caused by the krait venom of *Bungarus.*

Viper venom, which is rich in phospholipases, causes intensive tissue damage in many organs. Phospholipases present in viper venom are toxic (Fry *et al.*, 2003). These enzymes

hydrolyze phospholipids in the cell membrane and disturb the cell membrane activity (Iwanaga and Suzuki, 1979). Here, we can consider that the histopathological manifestations, mainly inflammatory and necrotic in nature, could have occurred in response to the membrane damage caused by the action of these phospholoipases present in *M. bornmuelleri* venom. PLA2 enzymes induce edema and has been previously described in several snake venoms (Ali *et al*., 1999). Thus, edema was observed in all liver and kidney tissues of mice injected with different doses of *M. bornmuelleri* venom during our experiments. This can be explained perhaps by the presence of LAAO in the venom (Rima *et al*., 2013). The LAAO is widespread in nature, where snake venoms are apparently the richest sources. Several authors have reported that the LAAO was able to induce edema in the lung and is in some cases accompanied by slight bleeding (Stabeli*etal*., 2004; Izidoro*etal*, 2006). Other previous studies demonstrated that hemorrhage, hypotension, inflammation could be caused by serine-proteases, PLA2, metallo-proteases and lecitines of type C (Kardoug, 2002), all of which were detected in the *M. bornmuelleri* venom (Accary*etal*., 2014a).

The purified PLA2 reveals several biological effects including pro-inflammatory, antimicrobial, anticoagulant and hemolytic activities. Serine-proteases called SVMPS have been isolated primarily from the venoms of viperidae snakes. Therefore, these thrombin-like enzymes are widely distributed in venoms of several kinds of snakes. They affect many physiological processes. SVMPs being hemorrhagic, induce edema and increase vascular permeability (Rodrigues *etal*., 2001; Fernandes*etal*., 2006), thus contributing to the inflammatory response which is characteristic of envenomation by viperidae (Teixeira *et al*., 2003). This effect depends not only on the hydrolysis of the components of the basal membrane but also on the release of inflammatory mediators from the cells (Wei *et al*., 2003). SVMPs produce a substantial inflammatory leukocyte infiltration associated with an increase in the number of circulating leukocytes (Costa *et al.* ., 2002; Fernandes et al., 2006). Metallo-proteases Zinc-dependents are abundant in viperidea venom (Francischi etal., 2000*;* Markland and Swenson, 2013). Metallo-proteases SVMPs induce, in the injection region, hemorrhage, edema,necrosis of the muscle, dermonecrosis, and a substantial inflammatory reaction. All envenomated mice after IM injection of the *M. bornmuelleri* venom showed the same alterations (Abi-Rizk *et al.*, 2017).

The highest hemorrhagic activity is associated with SVMPs of the P-III class, that are the more active,such asVaH4 isolated from the venom of *Viperaammodytes* (Sajevic*et al.*, 2013) and the VLH2 isolated from the venom of Macrovipera (*Vipera) lebetina*(Hanz*etal*., 2010). SMVPs induced alterations in the endothelial cells and their basal membrane (Ohsaka*et al.*, 1973; Franceschi*et al.*, 2000; Rodrigues *etal*., 2000; Rucavado*et al.*1998; Gutierrez *et al.* 1995).

Nanayakkara*etal*., (2009), who studied the effect of elapid venom on mice kidney, described that the main route of excretion of toxins is via the kidney. This would explain the presence of congestion and inflammation in this organ. These two phenomena were observed in the kidney of mice after injection of *M. bornmuelleri* venom. However, absence of nephritic agents in elapid venom, vasculotoxins and haemotoxins would be the reason for the lack of necrosis in the kidneys. These two toxins present in viper venoms explain necrosis and hemorrhage observed in the kidney and liver of the mice treated with *M. bornmuelleri* venom.

Boer-Lima, (1999) described the histopathological alterations in rat kidney caused by *Bothropsmoojeni* snake venom. The injection of this venom led to acute tubular and glomerular

changes compatible with acute renal failure such as a decrease in the glomerular filtration rate and a sustained increase in tubular sodium rejection. Histological observations indicated morphological abnormalities such as tubular swelling and glomerular damage. These damages were largely resolved after 48h. More so, no resolved damages were observed in mice injected by *M. bornmuellei* venom. The presence of mitotic tubule cells showed that epithelial regeneration occured. Indeed, mitosis has been reported to coexist with areas of necrotic renal parenchyma (Lieberthal and Levinsky, 1992). We also observed the phenomena of mitosis in the kidney of mice envomated by *M. bornmuelleri* venom.

This study shows that the action of the venom of M. *bormuelleri* in small doses can cause very serious damage to vital organs such as the liver and kidney. The determination of the lethal dose shows that the toxicity of M. bornmuelleri venom is very high in comparison with other species of vipers (Abi-rizk et al., 2017). For high doses, greater than or equal to lethal degree (LD50) which is 5.39 mg / kg, the cellular damage observed is enormous in these two organs. All cells appear to be destroyed and nonfunctional. This irreversible damage is observed at the membrane, cytoplasmic and nuclear levels, causing the death of the animals injected. This venom also acts on the vascular system of these organs causing capillary endothelial ruptures and haemorrhages. The venom of *M. bornmueller* contains phospholipases, metalloproteases, transferases, etc… (Hraoui-Bloquet *et al*., 2012, Accary *et al*., 2014a)

This study would be of importance in the characterization of possible proteins in *M. bornmuelleri* venom, as well as in understanding their action in the process of development of specific anti-venom. In addition, a further comprehension of the toxicology of *M. bornmuelleri* is of local importance because of the spread of this species in Lebanon.

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