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STUDIES ON VENOMS OF THE EGYPTIAN COBRA (*NAJA HAJA*), THE HORNED VIPER (*CERASTES CERASTES*), AND THE HONEY BEE (*APIS MELLIFERA*): COMPARISON SAFETY STUDY FOR 1/10 LD_{50}

By

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Abstract

Nowadays, whole world is interested in venoms of different origins. Snake and bee venom composed of many hundred different peptides, enzymes, toxins and inorganic ions with different biological and pharmaceutical effects. The present study assessed biochemical characterization of 1/10 LD_{50} by SDS page electrophoresis, total protein, and lethal dose (LD_{50}) of three types of venom [the Egyptian cobra (*Naja haja*), the horned viper (*Cerastes cerastes*) and honeybee (*Apis mellifera*)]. The bio-physiological effect of intraperitoneal injection of 1/10 LD_{50} dose of different 3 venoms was assessed *in vivo* using Swiss albino mice groups. *N. haja*, *C. cerastes*, and *A. mellifera*, CBC showed a significant decrease in both Hb concentration & RBCs, while platelets count showed highly significant increase. In WBCs both *N. haja* and *C. cerastes* showed significant increase, but BV decreased significantly. Biochemical analysis of mice sera injected with *C. cerastes* venom showed highest significant increase in liver ALT &AST) and kidney urea and creatinine, as well as cardiac enzyme creatinine phosphokinase (CPK) than cobra venom, but BV showed no significant change compared to controls. Histological examination showed altera-tions in liver, kidney & cardiac tissues of mice injected with both *N. haja* and *C. cerastes* while in BV showed normal kidney & cardiac tissues with minor changes in liver tissue outer region.

Keywords: Egyptian Cobra, *C. cerastes*, *A. mellifera*, Venom, Biochemical parameters, LD_{50}.

Introduction

The suitability of a medicinal product for human administration depended on intrinsic characteristics of its active components (Lehman *et al*, 2019). Natural products such as venoms are important source of pharmaceutical compounds (Kim *et al*, 2019). Many venoms and their components showed a potential antibacterial activity, as scorpion (Torres-Larios *et al*, 2002), ants (Sanad *et al*, 2002), spider (Benli and Yigit, 2008), snake (Samy *et al*, 2014), wasps venoms (Jalaei *et al*, 2014), and honey bee (Leandro *et al*, 2015).

Nevertheless, the stings in general were transient pain and redness at the site lasting a few hours (local reaction), and exaggerated swelling lasting a few days (large local reaction). The most dangerous immediate reaction is anaphylaxis, which was anaphylaxis potentially fatal (Abdel-Rahman *et al*, 2015).

Venomous snakes of phylum vertebrates, class of reptiles, superfamily Colubroidea consisted of several families, such as Colubridae, Viperidae, Lamprophiidae and Elapidae (Silva-de-Franc *et al*, 2019). The snake venom composition depended on a variety of factors, including snake family, genus and species, geographical location, typical prey type, age and size (Sanhajariya *et al*, 2018). Snake venoms is comprise a mixture of biologically active proteins and polypeptides (comprising approximately 90-95% venom load), along with other non-protein components including carbohydrates, lipids, amines, and inorganic salts.

Kalogeropoulos *et al.* (2019) classified the proteins and polypeptides into: 1- Enzymes e.g., phospholipase A2 (PLA2), metalloproteases (SVMP), serine proteases (SVSP), and L-amino acid oxidases (LAAO), and 2- Non-enzymatic substances e.g., three-finger toxins

(3FTx), kunitz peptides (KUN), and disintegrins (DIS). The biodiversity of venoms made them a rich unexplored source of bioactive compounds to cure disease that did not respond to available therapies (Jorge *et al*, 2011). They are advanced biochemical weapon exists in nature as anticancer (Tan *et al*, 2020).

Joubert and Taljaard (1978) found that protein abundances of phospholipases A_2 in cobra venom proteomes varied among cobra species, with significant colorimetric assay correlation between $svPLA_2$ enzymatic activities with the $svPLA_2$ protein abundances in venoms. High $svPLA_2$ activities were observed in the venoms of Asiatic spitting cobras (*Naja sputat-rix*, *N. sumatrana*) and moderate activities in Asiatic non-spitters (*Naja naja*, *N. atra*, & *N. kaouthia*), African spitters (subgenus *Afronaja*), and forest cobra (subgenus *Boulengerina*). African non-spitting cobras of subgenus *Uraeus* (*N. haje*, *N. annulifera*, *N. nivea*, & *N. senegalensis*) showed exceptionally low $svPLA_2$ enzymatic activities.

The Egyptian cobra (*Naja haja*) of family *Elapidae* is widely distributed in Africa and the Middle East (Gouda *et al*, 2017). Its venom is a viscous liquid with light yellow color consists of a mixture of proteins, peptides, and enzymes with a variety of biochemical and pharmacological functions. Main components include neurotoxins, cardiotoxins, phospholipase A2 (PLA2), cobra venom factor (CVF), nerve growth factor (NGF), and others (Wang and Qin, 2018).

The horned viper *C. cerastes gasperetti* (Gasperetti's sand viper) of family *Viperidae* is the most common snake in Saudi Arabia (Al-Sadoon *et al*, 2013a), as well as Egypt, Jordan and Saudi Arabia (Al-Sadoon, 2013b). Like the majority of *Viperidae* venoms, contains a cocktail of proteins that interact with platelets and sub-endothelium, some of which act by a catalysis mechanism mainly represented by nucleotidases, phospholipase A2 (Matsui *et al*, 2000), metalloproteinase and serine proteinases (Ameziani *et al*, 2020). Also, venom of *A. mellifera* is a natural toxin produced by honey bee that has at least 28 different active compounds with a distinguished health benefits, such as phospholipases, hyaluronidase, phosphatase, α- glucosidase, serotonin, histamine, dopamine, noradrenaline, and adrenaline, as well as melittin, apamin, and mast cell degranulating peptide (Hamza *et al*, 2019). BV was used as traditional treatment from ancient time for diseases such as arthritis, rheumatism, herpes…etc. (Ubaid and Ubaid, 2020), rheumatism, pain, rheumatoid arthritis and osteoarthritis (Berman *et al*, 2000), inhibit mammary carcinoma cell proliferation (Oršolić *et al*, 2003), antimicrobial activity on some Gram-negative bacteria (Park *et al*, 2013). BV has a high biological activity and toxicity and is the best form for potential pharmacological importance (Abdel-Monsef *et al*, 2020). Melittin the main BV component contained about 50% of the dry weight of venom, with medical effect on various cancer cells, growth inhibition, inducing caspase-dependent pathway, necrosis and lysis as well anti-inflammatory and antimicrobial effect (Zarrinnahad *et al*, 2018).

The present study aimed to evaluate the safety dose (1/10 LD_{50}) of *N. haja*, *C. cerastes* and *A. mellifera* venoms in envenomed mice as to hematological, biochemical and histopathological changes

Materials and Methods

Venoms: Studied lyophilized crude venoms (the Egyptian cobra (*N. haje*), *C. cerastes* and honey bee venom (*A. mellifera*) were obtained from the Egyptian organization of biological products and vaccines (VACSERA). Venoms were stored in dark condition at 4˚C till used.

Protein Determination: Protein content was determined (Bradford, 1976). Venoms and bovine serum albumin (BSA) were dissolved in distilled water (1mg/ml), 5 different concentrations between 0 & 20µg/ml were used as the standard and measured by spectrophotometer (Perkin Elmer, USA) at wave length 595λ.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): Protein profile of quality of cobra (*N. haja*), *C. cerastes* and BV (*A. mellifera*) were determined by SDS-PAGE 10% (Laemmli, 1970). Venom samples were run at 20µg/100µl in each lane with 5µl of Genedirex B-ultra standard pre-stained ladder protein for estimation of molecular masses. Electrophoresis was set at 100 volts (Bio-rad system, serial no 277BR, USA). Gels were then stained with 0.1% Coomassie Brilliant Blue R-250 after electrophoresis with shacking for 1hr then de-stain to clear background for determination of protein bands. All chemicals are used in this test from Bio-Rad, USA.

Determination of LD_{50} of (*N. haja, C. cerastes and A. mellifera)* venoms: LD_{50} of crude venoms was determined (Reed and Muench, 1938) in mice within 24 hr after venom injection intraperitoneally (I.P.) using male albino Swiss mice (6-10wk-old & 20-25gm). Mice were housed in standard condition at about 25˚C with a 12hr light-dark cycle and fed with normal diet and water *ad libitum*. Experiments were approved by the Ethical authorities (CU-1-S-57-17 approved by Institutional Animal Care and use committee CU-IACUC in Cairo University). LD_{50} of venom was determined by intraperitoneal injection of different doses in mice. When the differences in accumulated lethality percentages at 5 dose levels were significant, LD_{50} was calculated (Reed and Muench, 1938) by the following equation:

Log. LD_{50} = Log dose next below 50% + (Log increasing factor) × (proportionate distance)

Proportionate distance = (% mortality next above - % mortality next below) / (% mortality next above + % mortality next below 50%).

In vivo safety of venoms (*N. haje, C. cerastes & A. mellifera*): Venoms were dissolved in saline; animals were divided into 4 groups of 10 mice each. G1: served as control mice. G2: I.P. injected with Egyptian cobra (0.106µg/g body weight/ mouse), G3: I.P. injected with *C. cerastes* (0.535µg/g body weight), and G4: I.P. injected with *A. mellifera* BV (9.02µg/g body weight/ mouse). Injections were repeated 3 times as 24hr interval.

All mice were bled by retro-orbital vein and blood samples were divided into two volumes: EDTA blood samples were analyzed for CBC and sera were separated by centrifugation at 3000rpm for 15 min. and were kept at -20^{o}C until used for biochemical analysis.

Hematological examination: EDTA blood samples were used for estimation of quantitative hemoglobin using Diamond Diagnostics kit (Franco, 1984). **E**rythrocytes count: Hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured (Cheesbrough, 2006), and platelets & leukocytes count were measured (Becton, 1996).

Biochemical examinations: kidney functions were investigated by quantitative estimation of creatinine and urea using commercial kit from Diamond Diagnostics, Egypt (Kaplan, 1984). Liver functions (ALT & AST) were estimated using Biosystems S.A. kit (Gella *et al*, 1985), and serum creatinine phosphokinase using Biosystems S.A. kit (CPK total) as cardiac function (Schumann *et al*, 2010).

Histological studies: Mice (4/each group) were anesthetized by Isoflurane 3-4%, and livers, kidneys and hearts were collected and fixed in 10% neutral buffered formalin, washed in tap water, dehydrated in ethanol (70, 80, 90 & 100%), and cleared in xylene and embedded in paraffin. Tissues 4-5µ thickness were deparaffinized, and stained with H & E for examination (Bancroft *et al*, 1996).

Statistical analysis: Data were collected and tabulated as mean ± standard error of me-an, by using Microsoft Excel 2007. T-test values were done using program SPSS (version 17).

Results

Difference in venoms' protein composition was among *N. haja*, *C. cerastes* & *A. mellife*

ra. About 11 protein bands between 4 & 100 kDa were in *N. haja* venom, as compared to 8 protein bands in both *C. cerastes* and *A. mellifera* (Tab. 1).

Table 1: Different protein composition of *N. haja, C. cerastes, and A. mellifera venoms*:

Band name	*N. haja*	*C. cerastes*	*A. mellifera*
PLA2 protein (14 kDa)	Yes	Yes	Yes
Zinc-metalloproteiase (25 kDa)	Yes	Yes	No
serine proteases (60 kDa)	Yes	Yes	No
L-amino acid oxidase (LAAO) (69 kDa)	Yes	Yes	No
Three finger toxins (3FTxs) (6 kDa)	Yes	No	No
cobra venom factor γ (31-36.5 kDa)	Yes	No	No
acid phosphatase enzyme (57 kDa)	No	No	Yes
hyalurodinase enzyme (18.4 kDa)	yes	yes	Yes
Protease inhibitors (8-10 kDa)	No	No	Yes
Melittin (2.8 kDa)	No	No	Yes

Table 2: LD_{50} determinations of (*N. haja, C. Cerastes* and *A. mellifera* venoms)

LD_{50}	*N. haja*	*C. cerastes*	*A. mellifera*
µg/ Kg body weight of mouse	10.6	53.5	902

Hematological parameters: Mice envenomed showed hematological parameters changes (Tab. 3). RBCs showed a significant decrease in all groups compared to control and highly significant increase ($P \leq 0.001$) in MCV, MCH, MCHC, platelets count. WBCs count in *N. haja* showed non-significant difference ($P > 0.05$) while recorded significant increase in *C. cerastes* venom but, *A. mellifera* venom group showed a high significant decrease ($P \leq 0.001$). Hb in *N. haja* group showed high significant increase ($P \leq 0.001$) but in *C. cerastes* and *A. mellifera* showed non-significant difference ($P > 0.05$) compared to control.

Table 3: Complete blood picture (HB, HCT%, MCV, MCH, MCHC, RBCs count, WBCs & platelets count) of mice groups.

Type of Treatment	Control	*N. haja*		*C. cerastes*		*A. mellifera*	
Parameters	m±S	m±SE	%change	m±SE	%change	m±SE	%change
RBCs ($\times 10^6$/ cmm)	6.9 ± 0.041	6.12±0.01**	11.3%↓	5.79±0.01**	16.1%↓	5.93±0.02**	14.1%↓
Hb (g/dl)	11.1 ± 0.028	12.4±0.051 ***	11.7%↑	11±0.03*	0.9%↓	11.07± 0.015*	0.27%↓
PCV %	34.45±0.05	33.82±0.05*	1.83%↑	33.13±0.43*	3.9%↑	31.4±0.13***	8.85%↓
MCV fl	49.9± 0.05	55.3±0.51***	10.8%↑	57.2±0.05***	14.6%↑	52.93±0.26***	6.07%↑
MCH pg	16.37±0.14	20.29±0.03***	24.7%↑	19±0.05***	16.63%↑	18.66±0.053***	14.5%↑
MCHC g/dL	32.2±0.08	36.7±0.026***	13.48%↑	33.2±0.05***	3.1%↑	35.29±0.06***	9.6%↑
Platelets ($\times 10^3$/cmm)	387±2.58	765±5.59***	97.7%↑	537 ±4.54***	51.7%↑	448±3.36***	15.8%↑
WBCs ($\times 10^3$/cmm)	6.93±0.02	6.85±0.027*	1.15%↓	7.2 ±0.03**	3.9%↑	4.55±0.02***	39.1%↓

$P \leq 0.001$ = highly significant (***), $P \leq 0.05$ = significant (**), $P > 0.05$ = non-significant (*), P compared to control.

Biochemical parameters: All three venoms showed a high significant increase ($P \leq 0.001$) in the levels of liver enzymes ALT and AST, while in kidney functions the urea levels both in mice injected with *N. haja* or *C. cerastes* venom showed high significant increase ($P \leq 0.001$) while no significant difference ($P > 0.05$) in *A. mellifera* animal group, in creatinine level showed no significant difference ($P > 0.05$) in all groups. CPK (cardiac enzyme) level was significantly increased ($P \leq 0.001$) in both *N. haja* and *C. cerastes* venom groups without significant difference in *A. mellifera* compared to control (Tab. 4).

Table 4: Biochemical analysis of mice injected 1/10 LD_{50} dose of *N. haja, C. cerastes & A. mellifera* venoms compared to control.

Type of Treatment	Control	*N. haja*		*C. cerastes*		*A. mellifera*	
Parameters	m±SE	m±SE	%change	m±SE	%change	m±SE	%change
AST (U/L)	47± 1.64	135±0.316***	119%↑	103±0.836***	187.2%↑	62±2.57***	32%↑
ALT (U/L)	27± 1.05	42±0.83***	47%↑	54± 0.84***	89.5%↑	36.5±1.47***	28.1%↑
Creatinine (mg/dl)	0.53±1.05	0.63±0.015*	18.8%↑	0.57±0.029*	7.5%↑	0.5±0.03*	6%↑
Urea (mg/dl)	35.9±0.21	50.3±0.08***	40%↑	46.4±0.13***	29.2%↑	38.7±0.56*	7.8%↑
CPK U/L	70±0.26	100.8±0.9***	44%↑	112±0.71***	60%↑	76±1.41*	8.5%↑

Histopathological studies: Kidney of control group showed a normal histological structure of Malpighian corpuscles with its glomerulus and normal inter bowman space Figure (2a) showed proximal tubules (PT) and distal tubules (DT). In mice group treated with *N. haja* showed abnormal, irregular and cleavage of glomerulus with RBCs, and a mild degeneration in renal tubules was detected with slightly wider bowman space (Fig. 2b). The *C. cerastes* injected group showing severe congestion in the cortical blood vessels (Fig. 2c). The *A. mellifera* showed no histopathological alteration (Fig. 2d).

Liver tissue pictures of control group revealed normal histological structure and hepatocytes, centrally located nucleus arranged around central vein and blood sinusoid with kupffer cell (Fig.3a). Meanwhile, liver sections of mice from *N. haja*-injected group showed minor cytoplasmic vacuolization of hepatocytes with macro vascular fatty degeneration and minor focal hepatic hemorrhage (Fig. 3b). However, mice liver from *C. cerastes*-injected group showed that congestion of hepatoportal blood vessel with cytoplasmic vacuolization of hepatocytes with macro vascular fatty degeneration, minor focal hepatic hemorrhage and minor necrotic cell present (Fig. 3c). While liver from *A. mellifera* injec-ted mice showed normal histological structure surrounding normal signs and articature with no histopathological alteration, cells with normal signs and articature, no congestion in the portal vein (Fig.3d).

Heart muscle of control mice group showed normal histological structure (Fig. 4a). Meanwhile both mice groups treated with of *N. haja* or *C. cerastes* venoms showed marked dilation and congestion of myocardial blood vessel with minor intermuscular hemorrhage in addition to slightly degenerated myocytes in *C. cerastes* group (Fig. 4b&4c), cardiac tissue in mice treated with *A. mellifera* venom showed no histopathological alteration, and cells with normal signs and caricature (H&E) 40x

Discussion

Snake venoms represent an essentially a bioactive compound that may cure disease which do not respond to currently available therapies (Jorge *et al,* 2011), also through ancient times BV products of honey bee's colony used in traditional medicine to treat diseases (Ubaid and Ubaid, 2020). This included autoimmune diseases, antiviral, cancer (Wehbe *et al,* 2019), antimicrobial effect (Park *et al,* 2013). But, the therapeutic use of venoms was problematic due to their low bioavailability via the oral route, poor permeability, metabolic inactivation, the danger of proteolysis or enzymatic degradation, binding to plasma protein and finally, toxicity (Craik *et al,* 2013). Such limitations were overcome by using biocompatible carriers to enhance bioavailability (Lax and Meenan 2012; Maia *et al,* 2014), encapsulation in biodegradable polymers or venum detoxification (Bocian and Hus, 2020).

In the present study, SDS analysis showed the composition of venom at different protein bands, the three venoms characterized by the presence of phospholipase A_2 (PLA_2) which agreed with Palm *et al.* (2013) reported that PLA2 used as antibacterial, also confers protective immunity as it induces T helper type 2 (Th2) cell- type response and group 2 innate lymphoid cell activation. But, Al-Quraishy *et al.* (2014) and Yeh *et al.* (2021) reported that potent toxic effect of non-enzymatic fraction of cytotoxins and phospholipase A2 enzyme lead to these extensive necrotic observations in tissues of injected groups by the formation of pores and disturbing the phospholipid structure of lipid bilayer, in addition to the toxic effect of 3FTxs in *N. haja* venom. *N. ha-ja* and *C. cerastes* contributes with zinc metallooproteiase protein that is responsible of induced local and systemic bleeding after bites affecting various organs (heart, liver, lungs, intestines and brain), so they are called hemorragins (Wong *et al,* 2016). Also, the SDS ana-

lysis revealed Serine proteases and L- amino acid oxidase (LAAO) this agreed Sani *et al.* (2019) reported that they used for the development of more effective chemotherapeutic and other antitumor agents.

At the present study, some hematological alterations were observed in tested animals, RBCs showed significant decrease in all the groups compared to control. In *A. mellifera*-injected group, the same decrease in RBCs reported by Prajapati and Upadhyay (2018) who reported that Melitins was interacts with the RBC membrane inducing biochemical changes and disorders in lipids proteins matrix both in hydrophobic core of the lipid bilayer causing the RBCs rupture. Bhaumik (2013) showed that viper snake venoms are known to induce spontaneous hemorrhage secondary to microvascular damage, coagulopathy and platelet dysfunction, together with cardiovascular shock. The coagulopathy and hemorrhage are caused by venom components with proteolytic actions. Riaz *et al.* (2015) reported that the hemolytic effect of phospholipase A_2 and hyaluronidase that found in all venom groups and the Hb parameter in *N. haja* injected group showed high significant increase ($P \leq 0.001$) due to hemolytic effect of venom, these result similar with the present results, while the injected groups with *C. cerastes* or *A. mellifera* showed non-significant effect. The erythrocytes indices as MCV, MCH, MCHC showed highly significant increase indicating that the erythrocytes were trying to carry the maximum amount of Hb as a part of the homeostatic mechanisms to overcome the envenomation effects like (Al-Sadoon *et al,* 2012).

In the current study, platelets count induced highly significant increase in all the treated groups, this agreed with Riaz *et al.* (2015) who reported that platelets play an important role in coagulation, and thrombocytosis may indicate that clotting process was initiated in response to intravascular hemorrhages and bleeding.

WBCs egress from reservoir of bone marrow to blood under varies factors as stress, fever, trauma or a medical agent administration as reported in (Thrall *et al,* 2012). The present study revealed that the injected group with *N. haja* venom induced non-significant decrease in WBCs count which compatible with Riaz *et al.* (2015) study, he recorded that the number of leukocytes was decreased at 1hr post-envenomation then return gradually to its normal stat after 2hr. In *C. cerastes* injected groups the WBCs count showed significant increase which agreed with Corneille *et al.* (2006) reported the increase in WBCs count as a result of their toxic effect of the *C. cerastes* venom (Bankhurst *et al,* 2005). However, the BV of *A. mellifera* injected group showed highly significant decrease in total number of WBCs. This agreed with Mohammed and Hassan (2019) who reported that the highly significant decrease in the total number of WBCs was due to immunosuppressive effect of BV

In the present study, measurement of enzyme activity in serum was of importance to assess the condition of the liver and other organs. Normally serum transaminase levels were low, but after extensive tissue injury, these enzymes were liberated into the serum and the levels of serum transaminase increased following the damage of skeletal muscles, myocardial muscles and liver (El Missiry *et al.* 2010). Hepatoprotective activity was evaluated by measuring hepatotoxicity biomarkers (ALT, & AST) in the sera (Hashem *et al,* 2019).

In the present study, the activity of both ALT & AST showed high significant elevation in both *N. haja* and *C. cerastes* injected groups. This agreed with Al-Quraishy *et al.* (2014) who reported the increase in both liver enzymes after the envenomation of *N. haje*. Also, Tohamy *et al.* (2014) reported an increase in both ALT & AST enzymes in animals treated with 1/2 LD_{50} of *N. haje*, regardless of the differences in injecting route, dose, species and time post-injection. Another study showed el-

evation in the ALT & AST levels after snake envenomation, indicating that liver was primary target organ of venom (Shabaan *et al,* 2018). This was confirmed by some alterations in the liver histological features of injected groups as minor cytoplasmic vacuolization of hypatocytes with macro vascular fatty degeneration and minor focal hepatic hemorrhage (Shaker *et al,* 2018). While, this safe dose of BV (*A. mellifera*) injection recorded nearly normal results in both physiological and histological parameters. Uthawarapong *et al.* (2019) reported that at high dose of BV caused the vascular degenerative hepatocytes, prominent apoptotic cells and scattered areas of necrosis.

In the current study, *N. haja* injected group was found to induce high significant increase in the blood urea concentration. Kidney functions were assessed by determination of the levels of urea, uric acid, and creatinine in plasma (Amra *et al,* 2018). Acute kidney injury (AKI) was a common complication after snake bite envenomation from members of the family Viperidae (Aye *et al,* 2017). Besides**,** Abdel-Aty *et al.* (2018) reported that the viper venom causes various complications as renal abnormalities. This agreed with the present results that *N. haja* and *C. cerastes* injected groups showed an infiltration cleavage of glomerulus with RBCs, slightly degenerated renal tubules, venom atrophy of glomerular tuft and high significant increase in the concentration of blood urea. Shaker *et al.* (2018) reported that 1/10 LD_{50} of *N. haja* venom showed atrophy of glomerular tuft and congestion of renal blood vessel, also the present result showed no significant difference of both kidney function and normal histological structure in *A. mellifera* venom group.

Creatinine phosphokinase (CPK) is an enzyme present in tissue and in energy-demanding cells, such as skeletal and cardiac muscles (Nogueira *et al,* 2019). Clinically, it assayed in blood tests was an enzymatic index of cellular damage (Salama *et al,* 2018). In the present study, both *N. haja* and *C. cerastes* injected groups showed significant increase which agreed with Oukkache *et al.* (2012) who estimated the CPK concentration 3hr after induction of envenomation for both venoms. *C. cerastes* caused a threefold increase of CPK serum concentration, a variation that depended on the venom dose. Also, due to the presence of myotoxic PLA2 and other cardio-toxins that were salient features of *N. haja* venom responsible for the cellular necrosis and cytotoxicity (Yingprasertchai *et al,* 2003). These results were in harmony with the present histological findings of both *N. haja* and *C. cerastes* injected group. They showed marked dilation and congestion of myocardial blood vessels with minor intermuscular hemorrhage in addition to slightly degenerated myocytes in *C. cerastes* injected groups. This agreed with Shabaan *et al.* (2018) and Shaker *et al,* (2018) they reported marked dilation and congestion of myocardial blood vessel Also, Al- Mamun *et al.* (2015) found that snake venom induced variable degrees of histopathological alterations in cardiac tissues according to injected dose. In the present study, *A. mellifera* injected group showed non-significant increase in CPK enzyme, which attenuated activities in *A. mellifera* group were possibly linked with its antioxidant properties of Melittin that protected and solidified the cell membranes from oxidative stress-initiated damage, which agreed with Sadek *et al.* (2019).

Conclusion

The safety study on the dose 1/10 LD_{50} for Egyptian cobra *(N. haje,* the horned viper (*C. cerastes*) and honey BV (*A. mellifera*) crude venoms showed that *A. mellifera* is more safe to be used as natural crude therapeutic drug for many diseases, while *N. haja* and *C. cerastes* venom induced minor significant effect on pathophysiological and pathohistological alterations in liver, kidney and heart tissues that correlate with the rate of enzymatic and bio-

logical activities of the venom, that they need further studies (investigations) to use as safe natural drugs.

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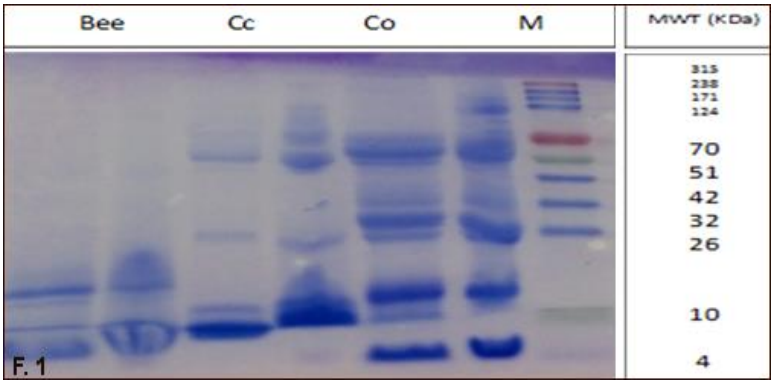
Explanation of figures

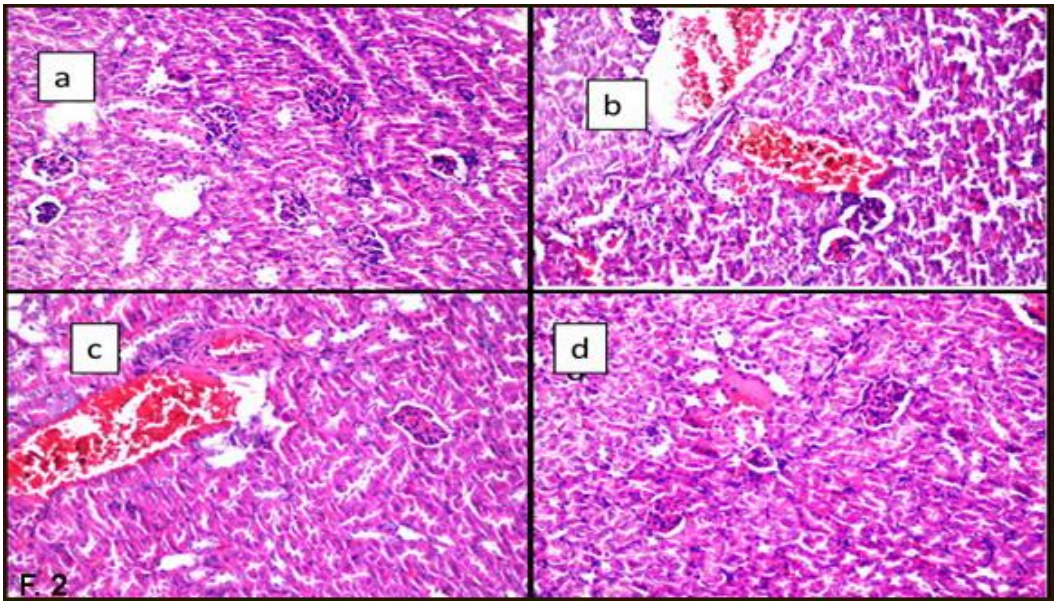
Fig. 1: SDS polyacrylamide gel for evaluation of 3 venoms M: marker, Co: Egyptian Cobra (*N. haja*) venom, CC: *C. cerastes*, Bee: *A. mellifera*.

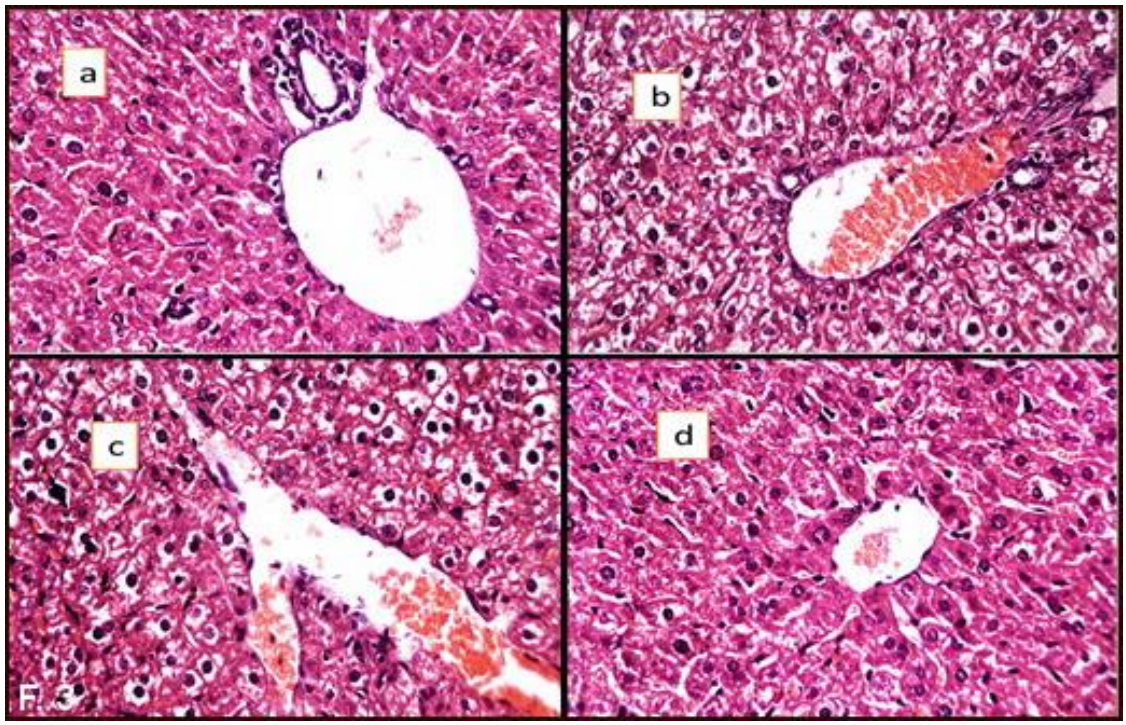
Fig. 2: Light micrograph of mouse kidney a: control mice b &c: mice treated with 10.6 and 53.5 µg /Kg body weight mouse 1/10 LD_{50} of *N. haja* & *C. cerastes* showed infiltrated cleavage of glomerulus with (RBCs), and slightly degenerated renal tubules (PT&DT), venom atrophy of glomerular tuft. d: mice treated with 0.902 mg/Kg body weight mouse 1/10 LD_{50} of *A. mellifera* venom showed normal histology (H&E x40).

Fig. 3: Light micrograph of mouse liver a: control mice b: mice treated with 10.6 µg /Kg body weight mouse 1/10 LD_{50} of *N. haja* venom showed minor cytoplasmic vacuolization of hepatocytes with macro vascular fatty degeneration and minor focal hepatic hemorrhage c: mice treated with 53.5µg/Kg body weight mouse 1/10 LD_{50} of *C. cerastes* venom showed congestion of hepatoportal blood vessel with cytoplasmic vacuolization of hepatocytes with macro vascular fatty degeneration, minor focal hepatic hemorrhage and minor necrotic cell present. D: mice treated with 0.902 mg/Kg body weight mouse 1/10 LD_{50} of *A. mellifera* venom showed normal histological structure surrounding normal signs and articature with no histopathological alteration, cells with normal signs and articature, no congestion in the portal vein (H&E x40).

Fig. 4: Light micrograph of mouse cardiac tissue a: control mice, b: mice treated with 10.6 µg/Kg body weight mouse of *N. haja* venom showed marked dilation and congestion of myocardial blood vessel with minor intermuscular hemorrhage c: mice treated with and 53.5 µg/Kg body weight mouse of *C. cerastes* venom showed marked dilation and congestion of myocardial blood vessel with intermuscular hemorrhage and slightly degenerated myocytes d: mice treated with 0.902mg/Kg body weight mouse *A. mellifera* venom no histopathological alteration, cells with normal signs and articature (H&E x40).



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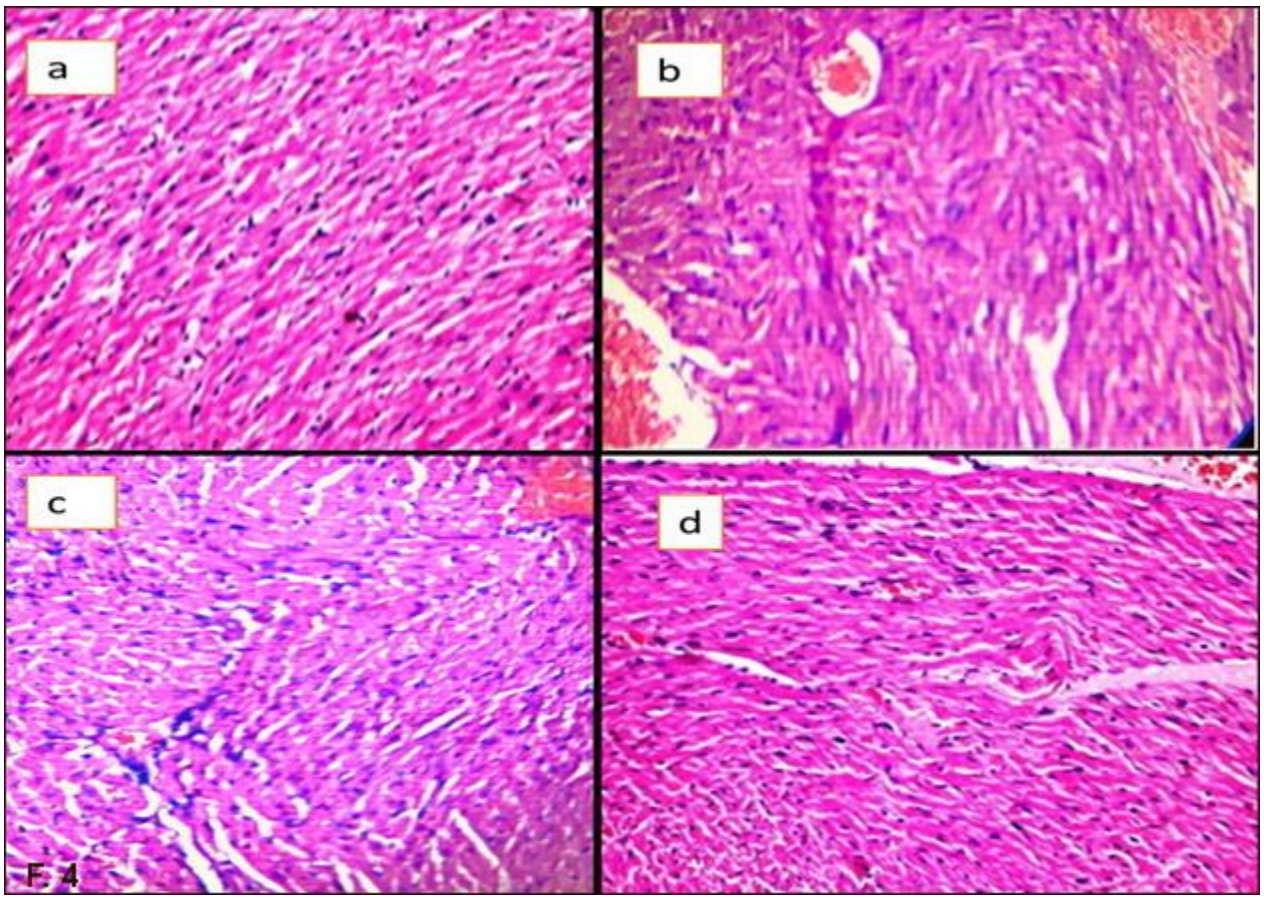
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