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Research Article

PHYSIOLOGICAL EFFECTS OF EGYPTIAN COBRA VENOM ON ALBINO MICE AND ITS ANTIBACTERIAL EFFECT

Lamiaa S. El-Din Shaker[1]., Osama A. Abbas[2]., Ola N. Sayed[3]., Dyaa M. Fawzi[4]., Alaa G. Mohamed[4] and Naglaa El-Sayed R. Ismael[4]

[1]VACSERA, Applied Research Department, Pilot plant unit, Agouza, Giza
[2]Radioisotopes Department, Egyptian Atomic Energy Authority
[3]Department of Chemistry, Faculty of Science, Fayoum University
[4]Department of Zoology, Faculty of science, Fayoum University

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ABSTRACT

Snake venoms are complex mixtures, composed of many hundred different peptides, enzymes, toxins and inorganic ions with a wide spectrum of biological activities. The present study was aimed to evaluate anti-bacterial effect of the crude venom of the Egyptian cobra (*Naja haje*) at dose of mice median lethal dose (LD_{50}) on some strains of bacteria and the bio-physiological effect of subcutaneously injecting two sub-lethal doses ($^1/_2$ LD_{50} & $^1/_{10}$ LD_{50}) venom on Swiss albino mice. The *E. coli* was found to be high resistance to the venom in both the minimum inhibitory concentration (MIC) and disc diffusion, while the venom affected on *S. typhi* and *S. aureus*, it showing zones of inhibition in disc diffusion and MIC. The sera biochemical parameters of injected animals with the Egyptian cobra venom with two different doses showed high significant increase in liver and cardiac enzymes in addition to kidney function tests (urea and creatinine) compared to control group. The mice complete blood pictures (CBC) showed in both of the two doses a significant decrease in hemoglobin (Hb) concentration and platelets count, while red blood cells (RBCs) and white blood cells (WBCs) count revealed highly significant decrease. Histological examination showed alterations in liver, kidney and heart tissues of mice injected with two doses of venom. Also, the skin tissue showed dermal oedema with few inflammatory cells.

INTRODUCTION

The Egyptian cobra (*Naja haje*) is considered the most dangerous species of snakes in the world because it resulted in big number of mortalities (Shuting *et al.,* 2004). *N. haje* cobra is among many venomous snake species that belongs to family Elapidae. *Naja haje* exists in Fayoum, Nile Valley, Delta and Western Mediterranean Coastal Desert (Cher *et al.,* 2005). The Egyptian cobra venom is considered as a rich source of many cytotoxins and neurotoxins. Also, the (*Naja haje*) venom contains polypeptides of low molecular weight that have low toxicity and immuno-chemical properties (Dkhil *et al.,* 2014). Venoms of snakes include large diversity of biological active proteins which cause many pathological effects. They have useful value can be exploited in pharmacological research for producing new drugs (Harvey & Robertson 2004 & Koh, *et al.,* 2006). The effect of toxic components of snake venoms differs from species to other. The difference in venom composition causes variations in pathology and lethality induced by the venom (Casewell *et al.,* 2014). Venom of *Naja* snakes species toxicity is because of the cardiotoxins, cytotoxins, and phospholipase A2 presence (Chwetzoff *et al.,* 1989). Worldwide increase in resistance of bacteria for the use of antibiotics and the undesirable side effects associated with it has become a serious public health problem (Norrby *et al.,* 2005; Choudhury *et al.,* 2012; Echols 2012; Ghafur 2013). This resistance to conventional antibiotic has prompted an intensive search for new therapeutic agents from diverse sources, including of animal origin (Zasloff, 2002). Only a few researches have been made on the antimicrobial activities of snake venoms (Samy *et al.,* 2006). Among these studies Florea *et al,* (2016) reported the antibacterial activity of the crude venom of *Naja haje.* Many antimicrobial peptides existed in different venoms of many animals are linked to defense mechanisms (Gallo *et al.,* 2002). Talan *et al.* 1991 studied the action of snake venom antimicrobial peptides on clinical

Corresponding author:* **Lamiaa S. El-Din Shaker
VACSERA, Applied Research Department, Pilot plant unit, Agouza, Giza

bacterial strains. Venoms from 30 different snake species were tested using disk diffusion test for antibacterial activity (Stiles *et al.,* 1991). *N. haje* crude venom induced hepatic injury causing many histopathological changes and an elevation in total Bilirubin (T. Bil), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), ɣ-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) (Al-Quraishy *et al.,* 2014). Snake venom proteolytic enzymes cause local changes in cells' permeability leading to edema, blistering, bruising, and local necrosis (Williams *et al.,* 2010 & Warrell *et al.,* 2013).

MATERIALS AND METHODS

Venom: Lyophilized crude venom of the Egyptian cobra (*Naja haje*) was obtained from the Egyptian organization of biological products and vaccines (VACSERA). LD_{50} was calculated according to Reed and Muench, 1938.

Bacteria: *Escherichia coli*, *Staphylococcus aureus and Salmonella typhi* were kindly obtained from American Type Culture Collection (ATCC). A loopful from the overnight growth of each bacterium was transferred to 150 ml Tryptone Soya Broth (TSB) medium and incubated at 37°C with vigorous shaking (150 rpm) for 18 h. The minimum inhibitory concentration (MIC) of the crude venom was determined by using 0.5 McFarland Standards for each bacterium (McFarland 1907). A 100 µl of each strain was pipette into each well of a microtiter plate and a serial dilution ($^1/_2 - {}^1/_{64}$) 100 µl from 40 mg venom /1 ml of venom was added in plate from well number 5 to 12. Well number 1 & 2 was negative control and 100 µl of antibiotic was pipette into well number 3 & 4 as positive control. The plates were incubated at 37°C for overnight and finally read on ELISA reader at wave length 600 nm (Andrews 2001). Disc diffusion was estimated the zone of inhibition was indicative the degree of antibacterial effect of the venom (Bauer *et al.,* 1966). The surface of plates was uniformly covered with the 0.5 McFarland bacterial growth for *S. aureus*, *S. typhi* and *E. coli*, then sterile filter paper discs were impregnated in venom, also disc of antibiotic like erythromycin, Ampicillin as positive control. The plate was incubated at 37°C for 18 hr. and examined for the presence of zone of inhibition for bacterial growth around the disc.

Injection of animals: Male Swiss Albino mice used in this work, weighing 18-20 grams from three to five weeks in age were selected from the experimental animal house of the Egyptian organization of biological products and vaccines (VACSERA). Animals were kept under hygienic conditions with a 12 hr light-dark cycle and were allowed free access to food and water *ad libitum* for at least one week prior to the experiment.

The animals were divided into three groups containing ten mice each. The first group served as control. The second group, mice were injected subcutaneously with a dose of $^1/_{10}$ LD_{50} of venom (0.212 µg/gm body weight of mouse). The third group, mice were injected subcutaneously with a dose of $^1/_2$ LD_{50} of venom (1.06 µg /gm body weight of mouse). Blood samples were collected from all mice groups after 3 days of injection. EDTA blood samples were analyzed for complete blood picture and sera were separated by centrifugation at 3000 rpm for 15 min. The labeled sera samples were kept at -70°c until used.

Biochemical examinations: The blood samples were used for estimation of quantitative Creatinine using commercial kit from Diamond Diagnostics (Murray, 1984), serum urea was determined using kit from Diamond Diagnostics (Kaplan *et al.,* 1984), activity of serum ALT and AST by Biosystems S.A. kit (Gella *et al.,* 1985), activity of serum Creatinine phosphokinase using Biosystems S.A. kit (CPK total) according to (Schumann *et al.,* 2010) and determination of Lactate Dehydrogenase activity by using Biosystems S.A. kit (LDH) (Sociedad Española de Química Clínica, 1989).

Hematological examination: EDTA blood samples were used for estimation of quantitative hemoglobin using Diamond Diagnostics kit (Franco, 1984). Manual Erythrocytes count: Hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured according to Cheesbrough, 2006. Manual platelets count and manual leukocytes count (WBCs) were measured according to Becton-Dickinson (1996), on the other hand differential leukocytes count was measured according to Leishman, (1901).

Statistical analysis: All numerical values were expressed as mean ± standard error of mean. They were calculated by the use of Microsoft Excel 2007 and t-test values was performed using the statistical program SPSS version 17 and $P \leq 0.001$ is considered highly significant, $P \leq 0.05$ is considered significant and $P > 0.05$ is considered non-significant, *P* versus control.

Histological studies: Specimens from livers, hearts, skin, and kidneys were collected and fixed in neutral buffered formalin 10%, washed in tap water overnight and exposed to ascending concentrations of ethanol (70, 80, 90 and 100%) for dehydration, cleared in Xylene and embedded in paraffin. Sections of the tissues (4-5 µ thick) were prepared and stained with Hematoxylin and Eosin for subsequent histopathological examination (Bancroft *et al.,* 1996).

RESULTS

The minimum inhibitory concentrations (MIC)

Table 1 The minimum inhibitory concentration of the serial dilution ($^1/_2 - {}^1/_{64}$) 0f 40 mg venom /1 ml saline in Egyptian cobra venom against the three strains of bacteria.

Cobra Venom	*E. coli*	*S. aureus*	*S. typhi*
MIC	10 mg/ml	18.5 µg/ml	62.5 µg/ml

As represented in table (1), the minimum inhibitory concentration of the Egyptian cobra venom against *E. coli* was 10 mg/ml which indicates the resistance of *E.* coli strain against cobra venom, MIC against *S. aureus* was 18.5 µg/ml which indicates the effectiveness of the cobra venom against *S. aureus* strain, and MIC against *S. typhi* was 62.5 µg/ml which indicates the effectiveness of the cobra venom against *S. typhi* strain.

Disc diffusion

The disc diffusion method showed the susceptibility of the organism to antibiotics and venom which showed zone of inhibition like *S. aureus* and *S. typhi*, while *E. coli* showed resistance for venom. This indicated that Egyptian cobra venom have an antibacterial activity against some strains of bacteria as an antibiotic.

Table 2 The inhibition zones diameters of used antibiotics and cobra venom against the bacterial strains.

Bacterial strain	Ampicillin	Erythromycin	Cobra venom
S. aureus	25mm	10mm	10mm
S. typhi	25mm	8mm	10mm
E. coli	22mm	12mm	resistant

Fig 1 Effect of Ampicillin (AMP), Erythromycin (E) and the Cobra venom on the *Salmonella typhi* bacterial growth.

Fig (1) Showed highly effective AMP (Ampicillin) antibiotic produced a wide ring of inhibition zone (25 mm), while the Cobra venom was produced intermediate inhibition zone (10 mm) and the E (Erythromycin) antibiotic produced low of inhibition zone (8 mm)on the *Salmonella typhi* bacterial growth.

Fig (2) showed the highly effective of AMP (Ampicillin) antibiotic produced a wide ring of inhibition zone (25 mm), while the Cobra venom produced intermediate of inhibition zone (10 mm) like the E (Erythromycin) antibiotic with inhibition zone (10 mm) on the *Staphylococcus aureus* bacterial growth.

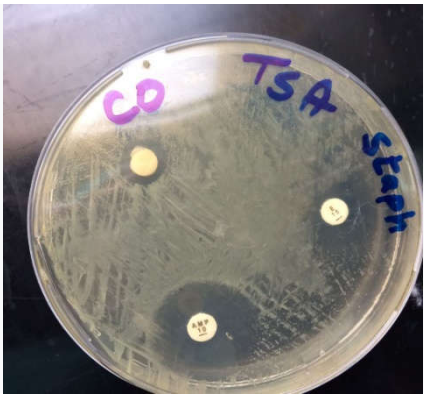

Fig 2 Effect of Ampicillin (AMP), Erythromycin (E) and the Cobra venom on the *Staphylcoccus aureus* bacterial growth.

Fig 3 Effect of Ampicillin (AMP), Erythromycin (E) and the Cobra venom on the *Escherichia coli* bacterial growth.

Table 3 the levels of serum urea, creatinine concentration, ALT, AST, CK-total and LDH activities in all studied groups and control group.

Groups	ALT U/L	AST U/L	Urea mg/dL	Creatinine mg/dL	CPK total (U/L)	LDH (U/L)
Control	26.81 ± 0.534	38.5 ± 1.70	47.8 ± 1.54	0.631 ± 0.0368	588.1 ± 7.05	555.8 ± 7.15
N=10	1.68	5.38	4.89	0.116	22.2	22.6
	41.9 ± 1.479	236.2 ± 6.59	71.7 ± 2.47	1.58 ± 0.033	987.0 ± 2.42	4875.9 ± 19.8
$^{1}/_{10}$ LD_{50}	4.67	2.085	7.83	0.106	7.67	62.7
N=10	0.000	0.000	0.000	0.000	0.000	0.000
	56.2	513.5	50.0	150.3	67.8	777.2
	75.8 ± 2.66	264.1 ± 1.98	94.4 ± 2.35	1.92 ± 0.11	1208.8 ± 33.7	5239.9 ± 28.1
½ LD_{50}	8.43	6.26	7.44	0.348	106.8	89.1
N=10	0.000	0.000	0.000	0.000	0.000	0.000
	182.7	585.9	97.4	204.2	105.5	842.7

Data represented as mean ± Standard Error, Standard deviation, % Change and $P \leq 0.001$ is considered highly significant, $P \leq 0.05$ is considered significant, $P > 0.05$ is considered non-significant, ***P*** versus control.

Table 4 complete blood count including (HB, HCT%, MCV, MCH, MCHC, RBCs count, , and Platelets count) of tested groups and control group.

Groups	Parameter	Hb g/dL	HCT %	MCV fL	MCH pg	MCHC g/L	RBCs x 10^{12}/L	Platelets x 10^{9}/L
Control	Mean ± Std. Err.	11.16 ± 0.197	37.0 ± 1.28	48.7 ± 1.79	14.7 ± 0.45	30.6 ± 1.60	7.6 ± 0.16	231.5 ± 9.06
N=10	Std. Dev.	0.62	4.0	5.67	1.45	5.08	0.53	28.6
	Mean ± Std. Err.	10.16 ± 0.247	33.2 ± 0.78	57.4 ± 0.97	17.5 ± 0.29	30.5 ± 0.11	5.79 ± 0.17	338.5 ± 11.02
1/10 LD50	Std. Dev.	0.78	2.47	3.09	0.94	0.36	0.56	34.8
N=10	P	0.005	0.021	0.001	0.000	0.952	0.000	0.000
	% change	-8.96	-10.2	17.8	19.0	-0.32	-23.8	46.2
	Mean ± Std. Err.	8.47 ± 0.27	27.7 ± 0.96	58.5 ± 1.52	18.2 ± 0.36	29.0 ± 0.20	3.94 ± 0.24	358.9 ± 7.99
½ LD50	Std. Dev.	0.86	3.06	4.82	1.15	0.64	0.78	25.2
N=10	P	0.000	0.000	0.001	0.000	0.351	0.000	0.000
	% change	-24.10	-25.1	20.1	23.8	-5.22	-48.1	55.03

Data represented as mean ± Standard Error, Standard deviation, % Change and $P \leq 0.001$ is considered highly significant, $P \leq 0.05$ is considered significant, $P > 0.05$ is considered non-significant, ***P*** versus control.

Table 5 WBCs count and Differential count of tested groups and control group.

Groups	Parameter	WBCs x 10^9/L	Lymph. %	Seg. Neutro. %	Band Neutro. %	Mono. %	Eosin. %
Control	Mean ± Std. Err.	12.9 ± 0.189	61.5 ± 1.36	29.5 ± 1.48	1.5 ± 0.34	5.4 ± 0.54	2.1± 0.34
N=10	Std. Dev.	0.59	4.3	4.69	1.08	1.71	1.1
	Mean ± Std. Err.	9.76 ± 0.24	70.1 ± 2.36	20.9 ± 2.37	1.9 ± 0.348	5.6 ± 0.37	1.5 ± 0.22
1/10 LD50	Std. Dev.	0.78	7.48	7.51	1.10	1.17	0.70
N=10	P	0.000	0.007	0.008	0.423	0.768	0.167
	% change	-24.3	13.9	-29.1	26.6	3.70	-28.5
	Mean ± Std. Err.	7.9 ± 0.34	71.9 ± 1.58	19.2 ± 1.43	2.1 ± 0.31	6.2 ± 0.46	1.5 ± 0.22
½ LD50	Std. Dev.	1.08	4.9	4.54	0.99	1.47	0.70
N=10	P	0.000	0.000	0.000	0.213	0.278	0.167
	% change	-38.7	16.9	-34.9	40.0	14.8	-28.5

Data represented as mean ± Standard Error, Standard deviation, % Change and $P \leq 0.001$ is considered highly significant, $P \leq 0.05$ is considered significant, $P > 0.05$ is considered non-significant, ***P*** versus control.

Fig (3) showed highly effective of AMP (Ampicillin) antibiotic produced a wide ring of inhibition zone (22 mm), the E (Erythromycin) antibiotic produced low inhibition zone of (12 mm) while the Cobra venom produced no inhibition zone (resistant) on the *Escherichia coli* bacterial growth.

Chemical parameters

The biochemical parameters of injected animals treating with the Egyptian cobra venom with two different doses showed high significant increase in liver, kidney and cardiac enzymes compared to control group.

Hematological parameters

The CBC with the two doses showed significant decrease in Hb and high significant decrease in RBCs count but, HCT and MCHC showed non-significant decrease while, MCV and MCH as well showed high significant increase compared to control. Also, Platelets count showed high significant increase with both doses compared to control. The WBCs count showed high significant decrease with both doses compared to control. Differential count showed non-significant change in all cells except the group treated with ½ LD_{50} dose.

Histological findings

Liver

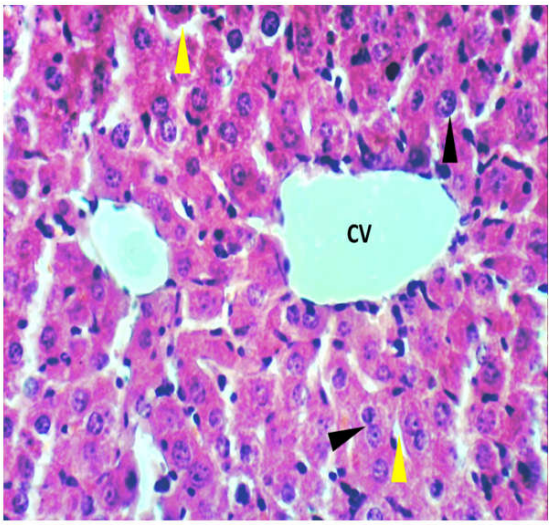

Fig 4 Light micrograph of mouse liver from control group (untreated) showing the normal histological structure of hepatic lobule with central vein (CV), strands of hepatocytes (black arrow heads) and sinusoids (yellow arrow heads) (H & E X 400).

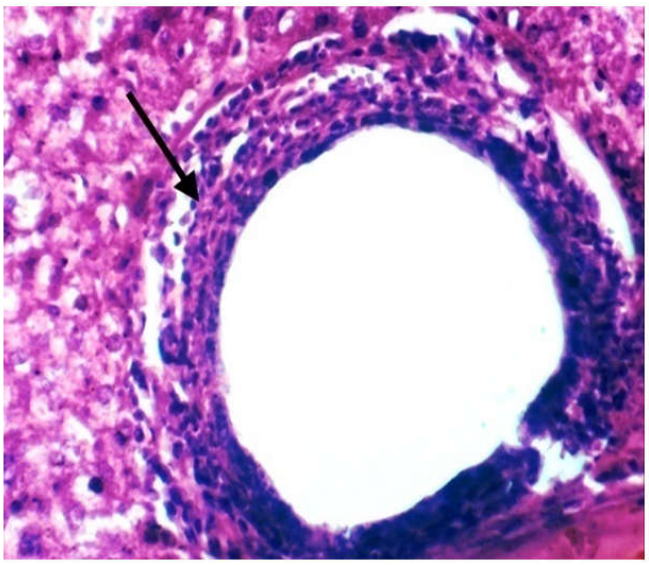

Fig 5 Light micrograph of mouse liver from group treated (envenomated) with $^1/_{10}$ LD_{50} of cobra venom showing cystic dilatation of bile duct and cholangitis (H & E X 400).

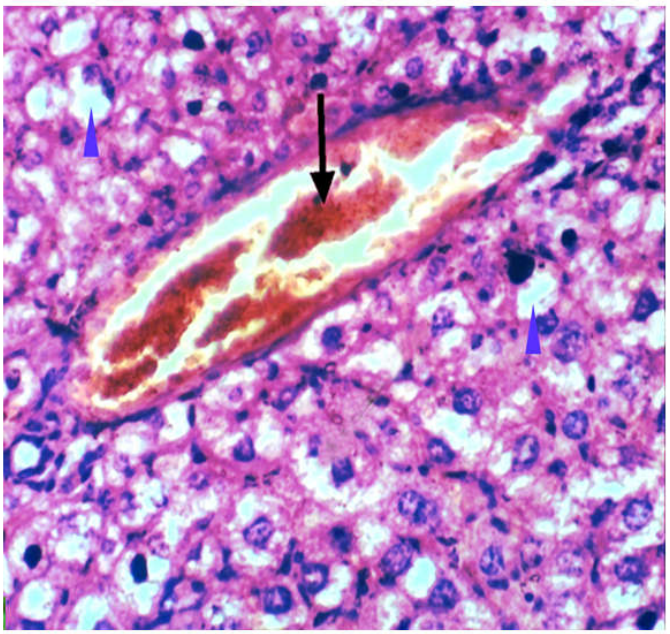

Fig 6 Light micrograph of mouse liver from group treated (envenomated) with $^1/_{10}$ LD_{50} of cobra venom showing congestion of hepatoportal blood vessel (black arrow) and cytoplasmic vacuolization of hepatocytes (blue arrow heads) (H & E X 400).

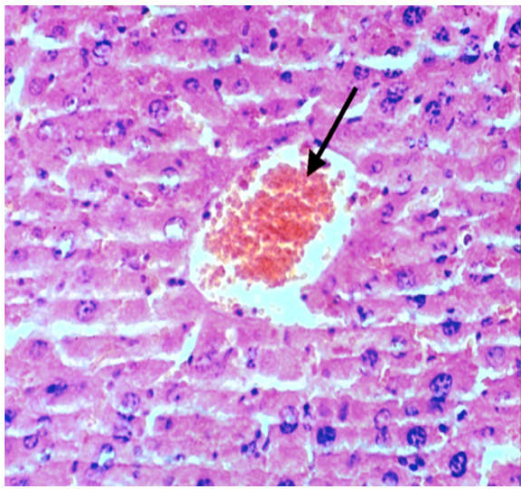

Fig 7 Light micrograph of mouse liver from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing congestion of central vein (H & E X 400).

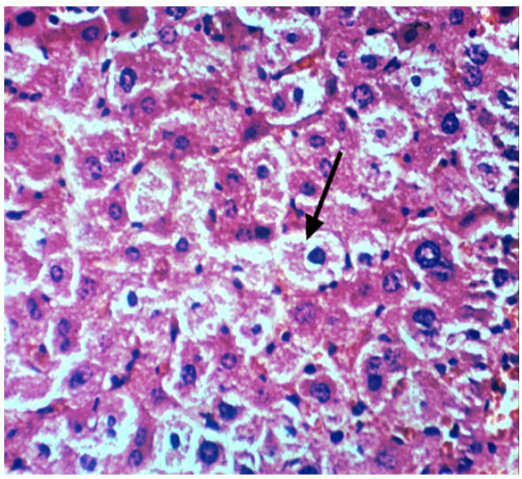

Fig 8 Light micrograph of mouse liver from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing ballooning degeneration of hepatocytes (H & E X 400).

Kidney

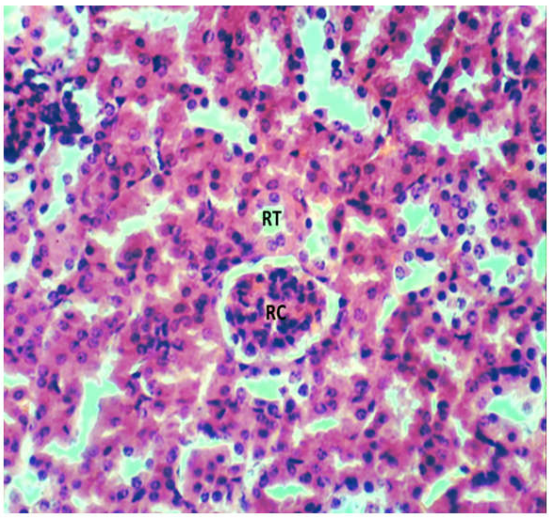

Fig 9 Light micrograph of mouse kidney from control group (untreated) showing the normal histological structure of renal parenchyma, renal tubules (RT) and renal corpuscles (RC) (H & E X 400).

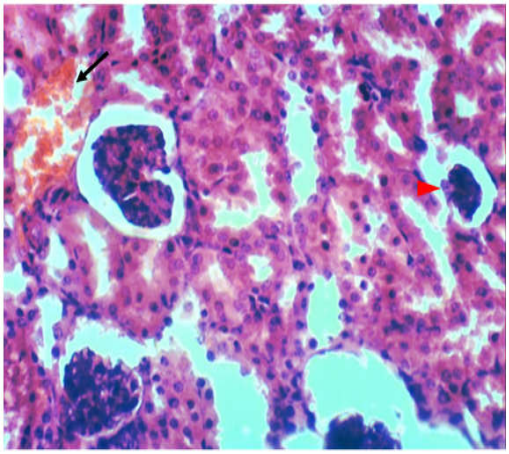

Fig 10 Light micrograph of mouse kidney cortex from group treated (envenomated) with $^{1}/_{10}$ LD_{50} of cobra venom showing atrophy of glomerular tuft (red arrow head) and congestion of renal blood vessel (black arrow) (H & E X 400).

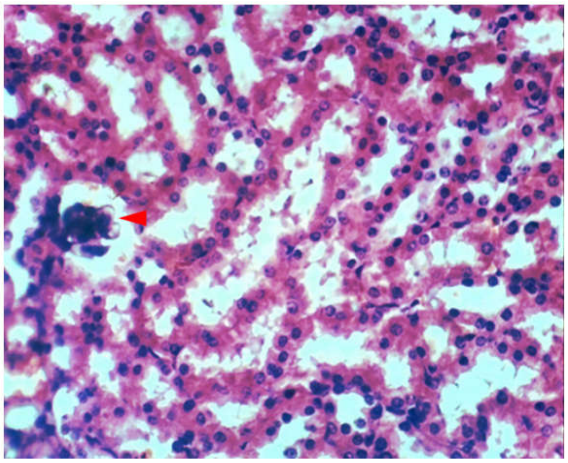

Fig 11 Light micrograph of mouse kidney cortex from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing atrophy of glomerular tuft (H & E X 400).

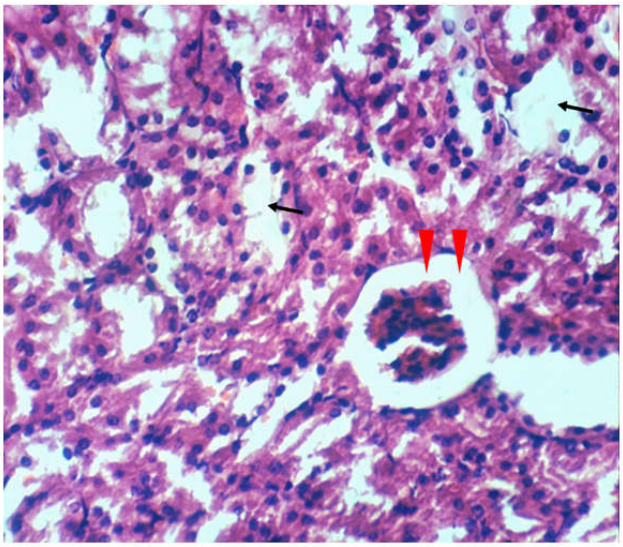

Fig 12 Light micrograph of mouse kidney cortex from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing vacuolation of renal tubular epithelium (black arrows) and slight distension of Bowman's space (red arrow heads) (H & E X 400).

Heart

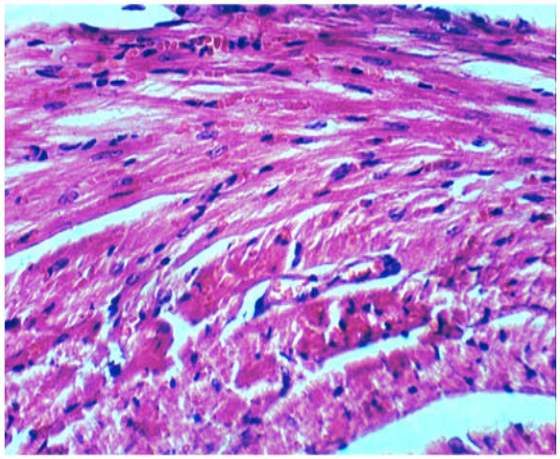

Fig 13 Light micrograph of mouse heart from control group (untreated) showing normal cardiac myocytes (H & E X 400).

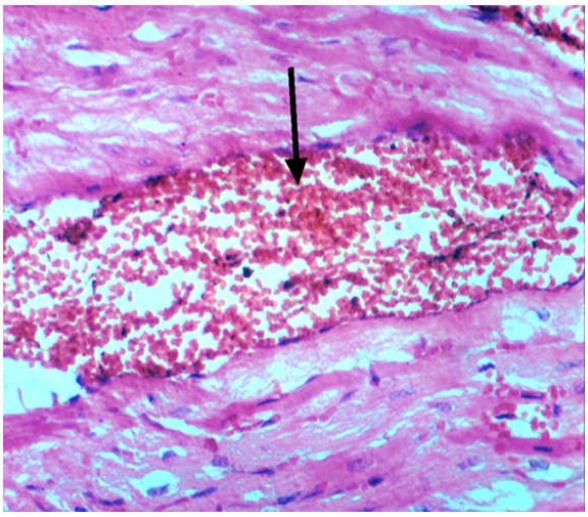

Fig 14 Light micrograph of mouse heart from group treated (envenomated) with $^{1}/_{10}$ LD_{50} of cobra venom showing marked dilatation and congestion of myocardial blood vessel (H & E X 400).

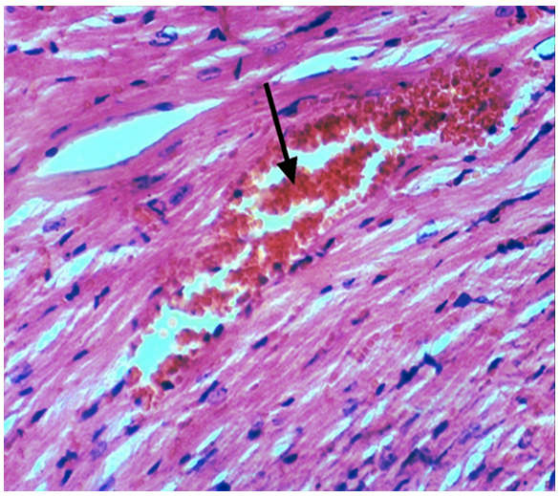

Fig 15 Light micrograph of mouse heart from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing dilatation and congestion of myocardial blood vessel (H & E X 400).

Skin

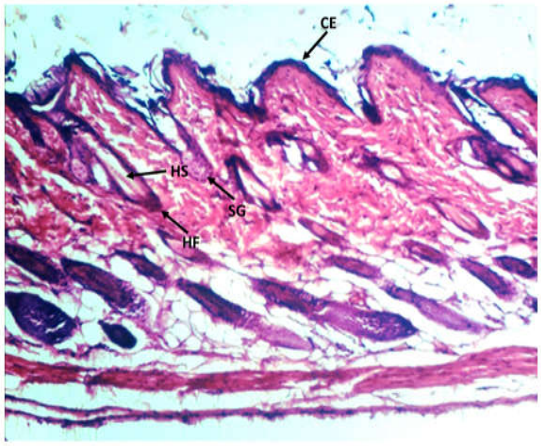

Fig 16 Light micrograph of mouse skin from control group (untreated) showing no histopathological changes (H & E X 100). Note normal histology with normal covering epithelium (CE), hair shaft (HS), hair follicle (HF), and sebaceous gland (SG) (H & E X 400).

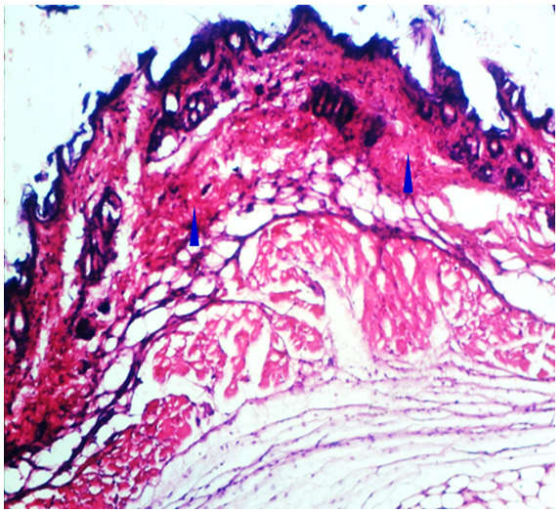

Fig 17 Light micrograph of mouse skin from group treated (envenomated) with $^{1}/_{10}$ LD_{50} of cobra venom showing dermal oedema (blue arrow heads) (H & E X 100).

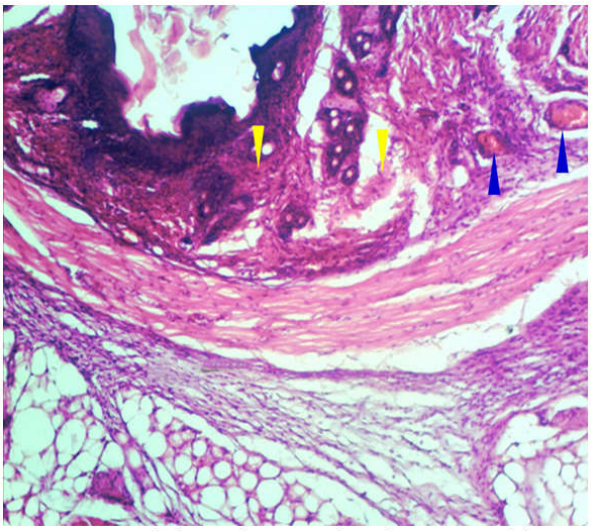

Fig 18 Light micrograph of mouse skin from group treated (envenomated) with $^{1}/_{10}$ LD_{50} of cobra venom showing focal necrosis (blue arrow heads) and dermal oedema with few inflammatory cells infiltration (yellow arrow heads) (H & E X 100).

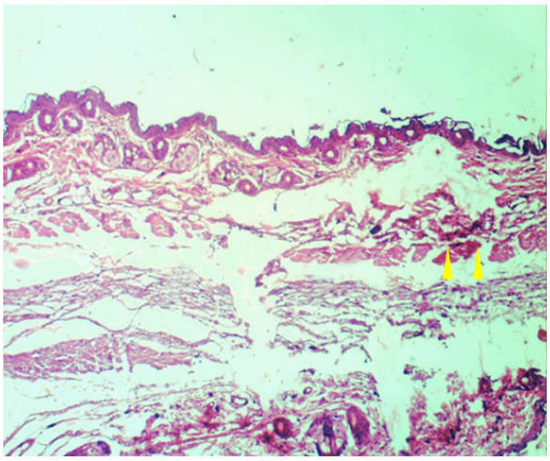

Fig 19 Light micrograph of mouse skin from group treated (envenomated) with $^{1}/_{2}$ LD_{50} of cobra venom showing slight dermal oedema with a few inflammatory cells infiltration (yellow arrow heads) (H & E X 100).

DISCUSSION

Worldwide increase in resistance of bacteria for the use of antibiotics and the undesirable side effects associated with it has become a serious public health problem (Norrby *et al.,* 2005; Choudhury *et al.,* 2012; Echols, 2012; Ghafur, 2013). Snake venoms represent an essentially unexplored source of bioactive compounds that may cure disease which do not respond to currently available therapies (Jorge *et al.,* 2011). Snake venoms had antibacterial activity comparable to antibiotics, with a directly proportional relationship of venom concentration whilst cobra venoms appear to be relatively more efficient as antimicrobial agents than viper venoms (Al-Asmari *et al.,* 2015). In order to evaluate the antibacterial activity of crude venom of the Egyptian cobra (*Naja haje*) the disc diffusion method with concentration of LD_{50} was used showed noticeable antibacterial effect, the inhibition effect of venom on bacteria was observed. The Egyptian cobra venom affected on the growth of both Gram positive *S. aureus* with moderate minimum inhibitory concentration (MIC) and the Gram negative *S. typhi* with moderate MIC, while the Gram negative *E. coli* showed resistance against the venom with high resistance MIC. Resistance of the Gram negative bacteria had been attributed to the outer membrane of the bacteria formed of lipopolysaccharides (LPS) which affected the uptake of antimicrobial peptides (Devine & Hancock, 2002). These results are in agreement with Florea *et al.* (2016) reported that Crude fresh venom of *Naja haje* inhibited the growth of Gram-positive *S. aureus,* showing a dose-dependent manner, while crude venom has no effect on Gram-negative *E. coli.* As well as Hakim & Reza (2015) who demonstrated that the venom of *Naja naja* from Bangladesh showed antibacterial activity to some extent against *E. coli*. and *B. thuringiensis*. The investigation of Sudarshan & Dhananjaya (2016) documented the effect of *Naja naja* venom phospholipase A2 venom which inhibited more effectively the gram positive bacteria like *S. aureus and B. subtilis*, when compared to gram negative bacteria like *E. coli*, *V. cholerae*, *K. pneumoniae* and *S. paratyphi*. Also, Zoriasatein *et al.* (2018) reported that animal venoms have been in the center of attention because their derivative compounds have shown antimicrobial properties. The antibacterial effect of peptide derivatives of Naja naja snake against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa was studied, Results showed that the peptide derived from Naja snakes' venom has an antibacterial effect on gram-positive and gram-negative bacteria.

Snake venom is a complex mixture composed of different substances, such as toxins, enzymes, growth factors activators and inhibitors with a variety of biological activities that cause multiple metabolic disorders, changing cellular and enzymatic activities in animals as well as releasing many pharmacological substances (Al-Sadoon *et al.,* 2013, Cherifi *et al.,* 2013 and Tohamy *et al.,* 2014). One of the most dangerous species of snakes in the world is *Naja haje* cobra as it causes large number of deaths (Shuting *et al.,* 2004). Venom of *Naja haje* cobra comprises many different proteins mixture, including many enzymes (proteases and phospholipases), non-enzymatic polypeptide toxins (neurotoxins and cardio toxins) along with other components (Binh *et al.,* 2010). Major signs and symptoms of cobra envenomation are edema, necrosis, pain, respiratory paralysis, vomiting, headache, hypotension, cardiac arrest, coagulopathies, bleeding wounds, hematuria, mucus discharge, proteinuria, and increased creatinine and urea levels along with altered consciousness (Asad *et al.,* 2012). This study was established also to evaluate some physiological effects of injecting two different sub-lethal doses $^{1}/_{2}$ LD_{50} and $^{1}/_{10}$ LD_{50} of crude venom on Albino mice by estimating some chemical parameters, hematological parameters and some histological examinations.

ALT and AST is the commonly used as clinical biomarker in evaluating hepatic injury. The levels of ALT and AST enzymes are important in evaluating the degree of liver inflammation and hepatic cells necrosis that increase the hepatic cells permeability resulting in the release of these enzymes in blood circulation (Abdel Moniem *et al.,* 2013). The activity of both enzymes (ALT& AST) in this study showed high significant elevation in both groups treated with two doses $^{1}/_{2}$ LD_{50} & $^{1}/_{10}$ LD_{50} as compared to control group. The group treated with $^{1}/_{2}$ LD_{50} of venom showed more increase in enzymes levels than group treated with $^{1}/_{10}$ LD_{50} of venom. The increase in (AST and ALT) levels agreed with many previous studies such Asad *et al.* (2014) & Riaz *et al.* (2015) who demonstrated that *Naja naja karachiensis* venom caused significant increase in ALT and AST levels. As well as the study of Al-Quraishy *et al.* (2014) reported the increase in both liver enzymes after the envenomation of *Naja haje*. The study of Tohamy *et al.* (2014) also reported an increase in both ALT and AST enzymes in animals treated with $^{1}/_{2}$ LD_{50} of *Naja haje*. James *et al.* (2013) also reported an increase of both enzymes in animals treated with venom of *naja nigricollis.* Regardless of the differences in routes, dose, species and time post-injection, all the studies showed elevation in ALT and AST levels after snake envenomation, indicating that liver is the primary target organ of venom. This was confirmed by some alterations in the liver histological structure of tested animals' tissues in both groups in the present study.

Liver from group treated with $^{1}/_{10}$ LD_{50} of cobra venom showed cystic dilatation of bile duct, cholangitis, congestion of hepatoportal blood vessel and cytoplasmic vacuolization of hepatocytes. Liver from group treated with $^{1}/_{2}$ LD_{50} of cobra venom showed congestion of central vein and ballooning degeneration of hepatocytes. The alterations in liver structure

of envenomated animals in our study are confirmed with the elevation of serum liver enzymes.

These findings agreed with studies revealed that *Naja haje* envenoming causes cellular swelling, cytoplasmic granulation and vacuolization in addition to intra hepatic hemorrhage, liver necrosis and activation and hyperplasia of the Kupffer cells (Rahmy & Hemmaid, 2000, Nanayakkara *et al.* 2009, Ghani *et al.* 2010, Tohamy *et al.* 2014 & Abdou & Ibrahim 2015) Snake venom has been responsible for harmful effects to the renal tissues (Schneemann *et al.,* 2004). In the current study the Egyptian cobra venom was found to induce high significant increase in the concentration of blood urea in both groups treated with two doses of venom compared to control group. The group treated with $^1/_2$ LD_{50} of venom showed more increasing in blood urea level than group treated with $^1/_{10}$ LD_{50} of venom. Also, the concentration of serum creatinine in both injected groups showed high significant elevation compared to control group. The group treated with $^1/_2$ LD_{50} of venom showed more increasing in serum creatinine level than group treated with $^1/_{10}$ LD_{50} of venom. This agreed with the study of Asad *et al.* (2014) which reported that *Naja naja* venom was found to make severe damage in the kidney by significant rise in serum urea and creatinine levels. The findings were supported by studies found the increase in the urea and creatinine levels in animals treated with $^1/_2$ LD_{50} of *Naja haje* (Tohamy *et al.* 2014, Dkhil *et al.* 2014, & Riaz *et al.* 2015). histological investigations in kidney of animals treated subcuatneously with cobra venom revealed that kidney cortex from group treatedwith $^1/_{10}$ LD_{50} of venom showed atrophy of glomerular tuft and congestion of renal blood vessel. Kidney cortex from group treated with $^1/_2$ LD_{50} of venom showed atrophy of glomerular tuft, vacuolation of renal tubular epithelium and slight distension of Bowman's space. In accordance different types of lesions (glomerular, tubular, interstitial or vascular) were also recorded by Tohamy *et al.* (2014) & Schneemann *et al.* (2004). Severe histopathological changes observed by Hemmaid (2010) after 4 h of Egyptian cobra (*Naja haje*) crude venom injection.

Al-Mamun *et al.* (2015) demonstrated that Injection of the venom *Bungarus caerulus* induced different degrees histopathological alterations in kidney tissues, included an inflammatory infiltration, vacuolation in renal tubules, glomerular and vessels congestion, shrinking of glomeruli, hemorrhage and necrosis of proximal tubules in renal tissue.
Cardiac injury, particularly systolic heart arrest, is one of the well-known toxicities related to *Naja naja* subspecies (Chethankumar and Srinivas, 2008). Cystolic enzymes (LDH and CK-MB) are sensitive indicators of myocyte injury (Nandave *et al.,* 2007). Its' increase is due to the presence of myotoxic PLA2 and other cardio-toxin(s) that are salient features of cobra venom and are responsible for cellular necrosis and cytotoxicity (Patel, *et al.,* 1997 & Yingprasertchai *et al.,* 2003). Subcutaneous injection of $1/_{10}$ LD_{50} *Naja haje* venom to mice in the current study resulted in elevation of the activities of LDH and CPK-total enzymes, which increased with dose $^1/_2$ LD_{50}. This finding is similar to those of Shaban & Hafez (2003) on *Naja haje* venom in rats. Asad *et al.,* (2014) reported that Pakistani cobra venom was found to release two cystolic enzymes (LDH and CK -MB). In Angaji *et al.* (2016) study, the levels of CPK and CK-MB showed a significant increase after envenomation of *Naja Naja Oxiana*.

Histological findings of heart taken from groups treated with $^1/_{10}$ LD_{50} & ½ LD_{50} of cobra venom showed marked dilatation and congestion of myocardial blood vessel. Abdou & Ibrahim, 2015 demonstrated focal degeneration of the myofibrils with moderate interstitial oedema in heart of rats envenomated with *Naja haje* venom at a dose of 4 times the $LD_{50.}$ Agarwal *et al.* (2007) reported cardiogenic edema after injection of *Bungarus coeruleus* venom. Also, Al-Mamun *et al.* (2015) found that the krait venom induced variable degrees histopathological alterations in cardiac tissues as heart-hemorrhage, multifocal areas of myocardial fiber and separation of the muscle fibers which appeared to contain separate myofibrils.

Histological alterations occurred in mouse skin from group treated with $^1/_{10}$ LD_{50} & ½ LD_{50} of cobra venom showed dermal oedema with few inflammatory cells infiltration and focal necrosis. Jiménez *et al.* (2008) reported that Metalloproteinase BaP1 from *Bothrops asper* induced rapid edema, hemorrhage, and blistering. BaP1 induced a drastic reduction in the microvessel density. This was followed by a rapid angiogenic response, leading to a partial revascularization. Skin damage was followed by inflammation and granulation tissue formation.

During this study some hematological parameter was estimated in order to evaluate the hematological alterations in tested animals. In this study cobra venom showed significant decrease in Hb and Hb % in animals treated subcutaneously with $^1/_{10}$ LD_{50} of venom while showed high significant decrease in Hb concentration and HCT in animals treated with ½ LD_{50} of venom compared with control group. Highly significant decrease in RBCs count in both groups treated with ½ LD_{50} & $^1/_{10}$ LD_{50} was observed. The decrease in erythrocyte count could be due to stress of envenomation, (Riaz *et al.* 2015) which was observed in this study. The increase in erythrocytes' indices, like MCV along with erythrocyte count and Hb content, indicates that erythrocytes were trying to carry maximum amount of Hb (Al-Sadoon & Fahim (2012), as a part of homeostatic mechanisms to cope with envenomation effects.

On the other hand, the venom was found to induce high significant increase in MCV and MCHC in both groups treated with ½ LD_{50} & $^1/_{10}$ LD_{50} of venom.

Malleswari *et al.* (2014) found that oral administration of *Naja naja* venom resulted in the decrease in Hb, RBC, HCT, MCV, MCH, MCHC. In the study of Abdou & Ibrahim (2015) there were significant increase in RBCs count, Hb level and HCT value in animals envenomated with *Naja haje* venom at a dose of 4 times the LD_{50}.

Riaz *et al.* (2015) reported that after 2 hours of envenomation with *Naja naja karachiensis* venom, all the erythrocytes' indices were higher and there was significant increase in RBCs count and Hb values. HCT, MCV, MCH and MCHC were also significantly increased after envenomation with *Naja naja karachiensis* venom.

During the study we observed high significant increase in platelets count in animals of both groups treated subcutaneously with the ½ LD_{50} & $^1/_{10}$ LD_{50} of venom after 6 hours of treating compared with control group. This agreed with Riaz *et al.* (2015) that reported platelets count was increased significantly during the time intervals and was highest at 2 hours post envenomation. Malleswari *et al.* (2014)

reported that oral administration of *Naja naja* venom resulted in the decrease in platelet count In our study, the results showed that cobra venom induced high significant decrease in WBCs count in animals of both groups treated subcutaneously with the ½ LD_{50} & $^{1}/_{10}$ LD_{50} of venom. In Riaz *et al.* (2015) study, the number of leukocytes was decreased at 1-hour post-envenomation and at 2 hours, leukocytes count was found to be significantly increased. In the study of Malleswari *et al.* (2014) oral administration of *Naja naja* venom resulted in an increase in WBC count which was not observed in the present study.

Leukopenia observed was probably an effect of peripheral destruction of cells in reticulo-endothelial system or liver impairment (Riaz *et al.,* 2015).

In Differential count of WBCs; monocytes, eosinophils and band neutrophils count in both treated groups showed non-significant change. Count of lymphocytes and segmented neutrophils of animals in group treated with $^{1}/_{10}$ LD_{50} of venom showed non-significant change, while count of lymphocytes showed high significant increase and segmented count showed high significant decrease in group injected with ½ LD_{50} of venom compared with control group.

In the study of Malleswari *et al.* (2014) the differential count showed a gradual decrease in neutrophils and an increase in lymphocytes and monocytes, eosinophils and basophils.

CONCLUSION

From this study it can be concluded that Egyptian cobra *(Naja haje)* crude venom has an antibacterial effect on some strains of bacteria. On the other hand, it induced significant pathophysiological and pathohistological alterations in skin, liver, kidney and heart tissues that correlate with the enzymatic and biological activities of the venom. So it cannot be used as natural therapeutic against bacterial diseases with the studied doses but further investigations on lower doses are required.

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