Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Research Paper

Effects of Egyptian Cobra (Naja Haje) Venom on Post Mortem Changes and Some Biochemical Parameters in Rats

Forensic Science

KEYWORDS : Naja haje, post mortem changes, biochemical parameters, rats

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ABSTRACT

Snakebite envenomation is one of the most important health problems all over the world. These envenomations are responsible for lethality as well as tissue damage and dysfunction. The effects of envenomation on post mortem changes are not well investigated. The aim of this study was to explore the possible influences of Egyptian cobra (Naja haje) on the postmortem events. A total of 40 male Sprague-Dawley rats were divided into two groups: a control group was injected IM with phosphate buffer saline and envenomated group was injected with venom at a dose of 0.1 µg/gm.b.wt. (4 time the LD50). Groups were subdivided into four subgroups of five animals each. Blood samples were obtained soon after death while, tissue samples were obtained at 0, 2, 4 or 6hs after death. Our results revealed that N. haje venom had altered post mortem changes including the early changes (post mortem cooling, hypostasis, rigor mortis and secondary flaccidity) as well as hematological and biochemical parameters. All organs were deteriorated by the cobra venom.

INTRODUCTION

Snakes are cold-blooded vertebrates, and some species possess dangerous venoms. 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 and Laraba-Djebari, 2013 and Tohamy et al., 2014). Cobra snakes are widely distributed all over the world especially in Africa and the Middle East, they are belonging to the Elapidae family. In Egypt, Egyptian cobra Naja haje (N. haje) spreads in Nile Valley and Delta, Fayoum and Western Desert. Cobra envenoming is known to produce damage of many organs, causing death in case of severe envenoming (Cher et al., 2005). N. haje is one of the most dangerous snake species in the world that provokes a high number of deaths (Li et al., 2004). N. haje cobra venom contains a mixture of many different proteins, including a variety of enzymes (proteases and phospholipases), non-enzymatic polypeptide toxins (neurotoxins and cardiotoxins) along with other substances (Binh et al., 2010).

The toxicity of the venoms of Naja species has been attributed to the presence of cardiotoxins or other cytotoxins (cytotoxin P4) and nigexine (basic phospholipase A2) (Chwetzoff et al., 1989). Liver is considered as one of the most affected organs by cobra venom, many reports showing that snake venoms causing damage of the hepatocytes with an increasing of liver integrity serum parameters (Fu et al.,1997, Omran et al.,1997, Rahmy and Hemmaid, 2000 and Al-Sadoon et al., 2012).

Desert cobra, Walterinnesia aegyptia, was reported to alter total proteins in serum, abdominal muscles and brain; it also impaired hepatic and renal functions and inducing various pathological changes. Serum, myocardial and total proteins, glucose and to alter liver (Al-Jammaz et al., 1992 and Al-Jammaz et al., 1994 and Al-Sadoon et al.,2013). There was observed reduction in serum total albumin, uric acid, cholesterol and phosphorus along with calcium levels and disturbances in serum electrolyte levels (Al-Jammaz, 2001).

Mukhopadhyay et al., (2010) reported that The fatal bites by Naja naja (Indian Cobra) showed serious histo-pathologicl changes in kidneys including Intense medullary congestion and Cortical hemorrhage with tubular and cortical.

Many literatures discussed the toxic effects of cobra bites on alive animal but, to our knowledge, there are no studies investigated the effects of Cobra envenomation on the post mortem changes. The aim of this study was to explore the possible influences of Egyptian cobra N. haje on the postmortem events in male Sprague-Dawley rats.

2. MATERIALS AND METHODS

2.1. Venom collection and preparation

Venom was obtained from an adult venomous elapid snakes (Naja Haje Cobra). The snakes were stimulated for milking and collecting venom, and then the fresh venom was lyophilized and preserved at –20°C until use. At use, the venom was dissolved in phosphate buffered saline (PBS), pH 7.2, to obtain the dose (0.1 µg /gm.b.wt.).

2.2. Animals and experimental design

A total of 40 male Sprague-Dawley rats (140-150 g) obtained from animal Health Research Institute and kept one weak for acclimatization. Rats were divided into two groups: a control group was injected IM with PBS and envenomated group was injected with venom at dose of 0.1 µg/gm.b.wt. (4 times the LD50, Rahmy and Hemmaid, 2001). Groups were subdivided into four subgroups of five animals each. Blood was collected soon after death by cardiac puncture and serum was prepared. Tissue samples were obtained at 0, 2, 4 or 6 hrs after death. Rate of cooling, progress of rigor mortis and color of hypostasis were recorded. The present experiment was approved by the Committee of Ethics in Faculty of veterinary medicine, Suez Canal University.

2.3. Histological Examination

The following organs were collected: skeletal and cardiac muscles, kidneys, liver and lungs then fixed in 10% buffered formalin. After standard processing of the tissue, 5µm sections were prepared and stained with H&E. The slides then studied under light microscope.

2.4. Biochemical Analysis

After blood collection, serum was separated by centrifugation and CRP was estimated by a commercial kits (GenWay Biotech Inc., San Diego, USA) (Tietz, 1995). ATP and lactic acid were determined in cardiac and skeletal muscles after 2, 4, or 6 hours intervals. ATP was evaluated using the ATP assay kits (abcam®, Cambridge, UK) while, lactic acid was assessed using the lactate assay kits (BioVision, Inc. USA). In line with every post-mortem examination, the vitreous humor was obtained using a 20-gauge needle. The vitreous humor was gently withdrawn by syringe, and each sample was placed in a separate 13-ml sample tube (polypropylene container, screw top). To prevent degradation, the samples were stored at -18 °C until used to evaluate Na, Cl

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and K electrolytes levels. Na, Cl and K were estimated according to the method described by (Wu, 2006) using BQ kits (BQ Kits, Inc. San Diego, USA).

2.5. Statistical Analysis

Data of normal distribution were expressed as means ± S.D.M. The values obtained were analyzed initially using Bartlett's test for homogeneity of variance. In case of homogeneity, Student's t-test was used. In case of heterogenic variance, the nonparametric Kruskal-Wallis test was used to compare between envenomated and control groups. In all cases, the considered statistical significance level was $p \leq 0.05$. Data analysis was carried out using the software program;Systat.SigmaPlot.v11.0-NULL.

3. RESULTS

3.1. Early post mortem changes:

Soon after death, body temperature was fallen faster in envenomated animals than in control (approximately 33°C and 36°C in envenomated and control rats, respectively) while, it was the same at 3 and 6 hours after death (around 29°C and 27°C at 3 and 6 hrs, respectively) in both envenomated and control groups. Hypostasis was not clear in control groups while, it was faint violet in groups that administered the N. haje venom especially at 4 hours after death (Figure 1).

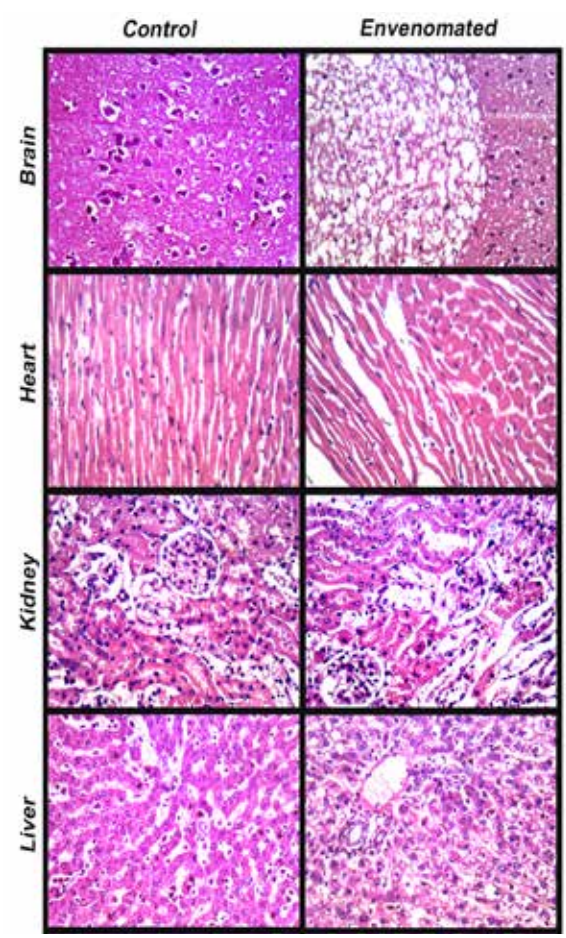
fig. 1: Degree of hypostasis in envenomated animals compared with control at 4 hrs after death.

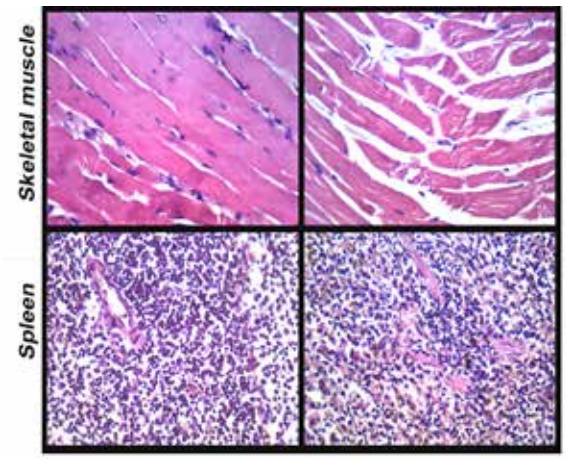
Rigor mortis was started about 1.5 hrs after death in all groups, but its progression and strength of the rigidity were more powerful in the envenomated groups compared with the control. Moreover, secondary flaccidity and putrefaction were occurred faster in rats that injected with the venom than the normally died rats (started around 4.5 hrs after death in envenomated groups and almost 6 hrs after death in control rats), At about 36 hrs intervals the rats were completely decayed.

3.2. Histological findings:

Brain, heart, kidney, liver, skeletal muscles and spleen were examined histologically at different intervals (0, 2, 4, and 6 hrs). At 0 time, in control rats: the organs were almost normal except moderate edema in heart, moderate hydropic degeneration in liver and few hemosiderin pigments in spleen. In envenomated rats: brain showed moderate focal degeneration with focal interstitial edema and degenerated neurons, heart showed minimal focal degeneration of the myofibrils with moderate interstitial edema, liver showed focal inflammatory infiltrate within portal areas and moderate sinusoidal dilatation with bile pigments, spleen: showed preserved white and red pulp with scattered hemosidren pigments indicating mild hemolytic process, kidney and skeletal muscle were almost normal. At 2 hrs interval, the situation was almost same as at 0 time. At 4 hrs, in envenomated group brain showed marked degeneration of the fibrillary matrix with focal interstitial edema and degenerated neurons, heart showed moderate degeneration of the myofibrils with marked interstitial edema, liver revealed mild inflammatory infiltrate within portal areas, bile pigments accumulation with partially disturbed architecture and marked hydropic degeneration of hepatocytes and spleen showed decreased white and expanded red pulp with many hemosiderin pigments while, in the control group all organs were normal except mild edema in heart and few hemosiderin pigments in spleen. The situation was worth at 6 hrs after death in envenomated rats, brain showed marked degeneration of the fibrillary matrix with focal interstitial edema and degenerated neurons and increased glial activity, heart showed moderate degeneration of the myofibrils with moderate interstitial edema, kidney showed distorted architecture with moderate degeneration of the tubular epithelium and glomeruli beside of moderate edema, liver exhibited minimal inflammatory infiltrate within portal areas, bile pigments accumulation with partially disturbed architecture and marked hydropic degeneration of hepatocytes, there were moderate interstitial edema and moderate degeneration in the skeletal muscles, and spleen showed decreased white and markedly expanded red pulp with many hemosiderin pigments indicating marked hemolytic process. In the control rats there were mild degenerative changes in all examined organs, heart exhibited mild edema and there were few pigments in spleen. The histopathological alternations in the organs of envenomated animals, compared with that of control animals (at 6 hrs after death) are shown in Fig. 2.

Fig. 2: Effects of *Naja haje* cobra venom on different organs at 6hrs after death compared with control. Sections were stained with Hematoxylin & Eosin stain (400× magnification).





3.3. Hematological results:

There were significant increase in red blood cell (RBCs) count, hemoglobin (HB) level and hematocrit (HCT) value in envenomated animals compared with control at p≤0.05 (table 1).

3.4. Biochemical analysis

C-reactive protein (CRP) was significantly increased soon after death in rats that injected with the venom in comparison with the control rats (p≤0.05) as showed in (table 1). Electrolytes concentrations in vitreous humor did not affected with the N. haje venom at any of the time intervals (table 2). Adenosine triphosphate (ATP) level was significantly decreased in envenomated animals' skeletal muscles at 0, 2 and 4 hours after death while, it was decreased significantly in cardiac muscles immediately after death, in relation to control levels (p≤0.05). Lactic acid concentration in skeletal muscles of the envenomated animals was significantly higher than the control at 2 and 4 hours (p≤0.05). Lactic acid was nearly the same in cardiac muscles of both envenomated and control groups. ATP and lactic acid results are expressed in (table 3).

Table (1): Blood and serum parameters in control and envenomated groups

Parameter	Groups	
	Control	Envenomated
RBCs (10^6/ul)	6.47 ± 0.65	8.90 ± 1.10*
HGB g/dl	11.4 ± 0.91	15.7 ± 1.67*
HCT %	37.3 ± 2.72	50.2 ± 5.00*
MCV fL	58.0 ± 1.61	56.7 ± 3.26
WBCs (10^3/ul)	7.87 ± 1.39	9.00 ± 3.17
PLT (10^3/ul)	381 ± 85.5	620 ± 177
CRP mg/L	7.14 ± 0.59	16.3 ± 2.23*

Data are expressed as means ± SDM of five rats per group. Red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), white blood cells (WBCs), platelets (PLT) and C-reactive protein (CRP).
*** Significantly different from sham group using Student's t-test at P ≤ 0.05.**

Table (2): Electrolyte concentrations in vitreous humor of control and envenomated groups

Electrolyte	Groups							
	Control				Envenomated			
	0 hrs	2 hrs	4 hrs	6 hrs	0 hrs	2 hrs	4 hrs	6 hrs
Na mmol/L	150 ± 11.1	143 ± 9.84	145 ± 11.6	149 ± 10.0	157 ± 15.7	148 ± 9.50	152 ± 11.0	154 ± 12.3
K mmol/L	6.09 ± 0.74	5.33 ± 0.60	5.41 ± 0.52	5.26 ± 1.02	6.27 ± 0.61	5.76 ± 0.85	5.69 ± 0.54	5.72 ± 0.90
Cl mmol/L	132 ± 11.8	112 ± 8.51	108 ± 9.00	125 ± 11.7	128 ± 16.5	124 ± 10.7	125 ± 9.85	134 ± 10.5

Data are expressed as means ± SDM of five rats per group. Sodium (Na), potassium (K) and chloride (Cl)
*** Significantly different from sham group using Student's t-test at P ≤ 0.05.**

Table (3): ATP and lactic acid concentrations in skeletal and cardiac muscles of control and envenomated groups

	Parameter	Groups							
		Control				Envenomated			
		0 hrs	2 hrs	4 hrs	6 hrs	0 hrs	2 hrs	4 hrs	6 hrs
Skeletal muscles	ATP nmol/gm	1.85 ± 0.09	1.81 ± 0.25	1.66 ± 0.14	2.03 ± 0.19	1.50 ± 0.14*	1.28 ± 0.06*	1.32 ± 0.09*	1.90 ± 0.18
	Lactic acid nmol/gm	1.81 ± 0.09	1.23 ± 0.13	1.50 ± 0.11	1.77 ± 0.10	1.66 ± 0.13	1.80 ± 0.10*	1.73 ± 0.09*	1.72 ± 0.10
Cardiac muscles	ATP nmol/gm	3.93 ± 0.57	2.91± 0.32	2.24 ± 0.42	2.57 ± 0.55	2.79 ± 0.40*	2.36 ± 0.35	1.88 ± 0.11	1.80 ± 0.50
	Lactic acid nmol/gm	1.76 ± 0.10	1.56 ± 0.11	1.83 ± 0.09	1.95 ± 0.24	2.01 ± 0.17	1.66 ± 0.11	1.88 ± 0.22	2.15 ± 0.26

Data are expressed as means ± SDM of five rats per group. Adenosine triphosphate (ATP).
*** Significantly different from sham group using Student's t-test at P ≤ 0.05.**

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4. Discussion

Snakebite envenomation is one of the most important, although neglected, health problems all over the world, particularly in Africa, Asia, and Latin America (Gutiérrez et al., 2006, WHO, 2007 and Alirol et al., 2010). In addition to lethality, these envenomations are induced prominent tissue damage leading to tissue loss and dysfunction. Many literatures (Tan, 1991, Omran et al.,1997, Chafer, 2004, Cher et al., 2005, Sejrsen and Nielsen, 2006, Fung et al., 2009, Al- Sadoon et al., 2013 and tohamy et al., 2014) discussed the toxic effects of the snake venom but how the snake venom alters the post mortem events is not fully known. Our study focused on the effects of the egyptian cobra Naja haje on the post mortem changes.

The early changes after death (post mortem cooling, hypostasis and rigor mortis) were faster and more pronounced in case of envenomation.

Our histopathological findings showed that most of the organs were deteriorated by the venom, and these results were in agreement with the results of (Mohamed et al., 1978 and tohamy et al., 2014) which indicated that Naja haje envenomation resulted in a severe inflammatory response of the liver, as indicated by cytoplasmic vacuolation and degeneration of hepatocytes. Also, some kidney tubules were vacuolated with shrunken glomeruli. (Tan, 1991, Cher et al., 2005and Fung et al., 2009) reported that injection of sub-lethal dose of Malayan cobra (Naja sputatrix) venom induced alterations in heart, brain, kidney, liver and lung.

Hematological results were consistent with Chafer, (2004) and Sejrsen and Nielsen, (2006) that reported Polycythemia caused by venom as a result of kidney or liver failure or an imbalance in the hemoglobin composition leading to a decrease in plasma volume and thus increasing the amount of hematocrit. This lead in turn to a case of hemoconcentration, and that Hb increase could be attributed to a physiological mechanism attempting to restore the normal blood composition and counteract hypoxia caused by the venom initial . (Ferreiraet al., 2005) stated that snake venom produces a prominent local edema which is responsible for significant fluid loss and can be contributed to tissue injury and ischemia.

C-reactive protein (CRP) was significantly increased soon after death in rats that injected with the venom in comparison with the control rats, and this is matching with the previous studies as the serum level of C-reactive protein (CRP) increase during inflammation, infection and tissue damage caused by venom, in response to stimulation by cytokines, mainly interleukin-6 that released from macrophages as a defense mechanism against the venom (Pepys and Hirschfield, 2003).

Our results revealed that, ATP was decreased in skeletal and cardiac muscles after envenomation with Naja haje cobra while lactic acid was increased compared with the control. These results are in harmony with (Fahim, 2001, El-Refael and Sarkar, 2009; Evangelista et al., 2010). It is known that snake venom causes muscle contraction, and this may explain the rapid depletion of ATP. Moreover, venom frequently contains ATPase, which promotes the hydrolysis of ATP (Anthony, 1991). Cintra-Francischinelli et al., (2010) mentioned that after snake bite the toxin-damaged muscle fibers liberate ATP into the extracellular environment. Snake venoms contain proteins with different toxicological functions and special pharmacological effects that cause cellular damage. The venom directly affects body muscles cause protein degradation due to its proteolytic effect (El-Refael and Sarkar, 2009). Lactic acid was produced from the damaged muscles and as a byproduct of anaerobic metabolism after death.

4. CONCLUSION

This is a first-hand investigation showing the effect of cobra envenomation on the post mortem changes, we can summarize that Egyptian cobra Naja haje had altered post mortem events including early post mortem changes as well as hematological, pathological and biochemical parameters.

5. ACKNOWLEDGEMENT

The authors are grateful to Dr. Mohamed A. Abdel Rahman, Zoology Department, Faculty of Science, Suez Canal University for his assistance in the collection and lyophilization of the venom.

REFERENCE

Alirol E, Sharma SK, Bawaskar HS, Kuch U, Chappuis F (2010). Snake bite in South Asia: A review. PLoS Negl Trop Dis, 10.1371/journal.pntd.0000603. | Al-Jammaz, I., (2001). Effects of Walterinnesia aegyptia crude venom on rat metabolism over various periods of time Pakistan J. biol. Sci. 4:1429-1431. | Al-jammaz, I., Al-Sadoon, M.K., Attia, M.A. and Fahim, A. (1992). Effect of Walterinnesia aegyptia venom on serum, tissue metabolites and some enzyme activities in male albino rats. II. Enzyme activities. Ain Shams Sci. Bull. Egypt. 30: 207-222. | Al-Jammaz, I., Al-Sadoon, M.K., Fahim, A. and Attia, M.A. (1994). The effects of Walterinnesia aegyptia lll.Effects on lipid metabolism and two dehydrogenasesvenom on the serum and tissue metabolites and on some enzyme activities in albino rats. J. King Saud Univ. Saudi Arabia, 6: 207-215. | Al-Sadoon, M.K., Abdel Moneim, A.E., Diab, M.M., Bauomy, A.A., (2013). Hepatic and renal tissue damages induced by Cerastes cerastes gasperetti crude venom. Life Sci. J. 10: 191–197. | Al-Sadoon,M.; Fahim ,A. ; Safwat, F.; Gamal B . (2012). The effects of LD50 of Walterinnesia aegyptia crude venom on blood parameters of male rats. African Journal of Microbiology Research 6(3): 653-659 . | Al-Sadoon, M.; Fahim ,A. ; Safwat, F.; Gamal B . (2012). The effects of LD50 of Walterinnesia aegyptiacrude venom on blood parameters of male rats. African Journal of Microbiology Research 6(3): 653-659 | Al-Sadoon, M.K., Orabi, G.M. & Badr, G. (2013). Toxic Effects of Crude Venom of a Desert Cobra, Walterinnesia aegyptia, on Liver, Abdominal Muscles and Brain of Male Albino Rats. Pakistan J. Zool., vol. 45(5), pp. 1359-1366. | . | Anthony, Tu. (1991). Handbook of Natural Toxins: Reptile Venoms and Toxins. CRC press. p:86. | Binh ,DV.; Thanh, TT.;Chi ,PV. (2010) . Proteomic characterization of the thermostable toxins from Naja hajevenom. J. Venom. Anim. Toxins. Incl. Trop. Dis. , 16 (4) : 631-638. | Chafer AI (2004).Thrombocytosis. N. Engl. J. Med. 350: 1211. | Cher, C.D.; Armugam, A.; Zhu, Y.Z.; Jeyaseelan, K. (2005). Molecular basis of cardiotoxicity upon cobra envenomation . Cell Mol. Life Sci ., 62(1): 105-118. | Cher, C.D.N., Armugam, A., Zhu, Y.Z. & Jeyaseelan, K. (2005). Molecular basis of cytotoxicity upon cobra envenomation. Cellular and Molecular Life Sciences. 62: 105-118. | Cherifi F and Laraba-Djebari F. (2013). Isolated biomolecules of pharmacological interest in hemostasis from Cerastes cerastes venom. J. Venom. Anim. Toxins Incl. Trop. Dis. 19: 11 | Chwetzoff, S.; Tsunasawa, S.; Sakiyama, F.; Mènez, A. (1989).Nigexine, a phospholipase A2 from cobra venom with cytotoxic properties not related to esterase activity: Purification, amino acid sequence, and biological properties. J. Biol. Chem. 264(22):13289- 98. | Cintra-Francischinelli M, Caccin P, Chiavegato A, Pizzo P, Carmignoto G, Angulo Y, Lomonte B, Gutiérrez JM, Montecucco C. (2010). Bothrops snake myotoxins induce a large efflux of ATP and potassium with spreading of cell damage and pain. Proc Natl Acad Sci U S A. 107(32):14140-5. | Clinical Guide to Laboratory Tests". Edited by N.W. Tietz, 3rd Edition. W.B. Saunders Company, Philadelphia, PA. 19106 (1995). | EL-Refael, M.F. and Sarkar, N.H., (2009). Snake venom inhibits the growth of mouse mammary tumor cells in vitro and in vivo. Toxicon, 54: 33-41. | Evangelista, I.L., Martins, A.M., Nascimento, N.R., Havt, A, Evangelista, J.S., De Noroes, T.B., Toyama, M.H., Diz-Filho, E.B., Toyamadde, O., Fonteles, M.C. and Monteiro, H.S. (2010). Renal and cardiovascular effects of Bothrops marajoensis venom and phospholipase A2. Toxicon, 55:1061-1070. | FAHIM, A., (2001). Biochemical effects of crude venoms of two elapidae from saudi fauna on male albino rats. J. Egypt.Ger. Soc. Zool., 36: 165-175. | Ferreira J., Bastos M. F., Pelizon A. C., Peres C. M., Cavalcante D. P. and Sartori A. (2005). Assessment of the neutralizing potency of ovine antivenom in a swiss mice model of Bothrops jararaca envenoming. J. Venom. Anim. Toxins incl. Trop. Dis. 11 (3): 336-349. | Fu, Q.L.; Satyaswaroop, P.G.; Gowda, D.C. (1997). Tissue targeting and plasma membrane clearance of cobra venom factor in mice . BiochemBiophys. Res. Commun.,231(2): 316-320. | Fung, S.Y., Tan, N.H., Liew, S.H., Sim, S.M. and Aguiyi, J.C. (2009). The protective effects of Mucuna pruriens seed extract against histopathological changes induced by Malayan cobra(Naja sputatrix) venom in rats. Tropical Biomedicine. 26(1): 80–84. | Gutiérrez JM, Theakston RDG, Warrell DA (2006) Confronting the neglected problem of snake bite envenoming: The need for a global partnership. PLoS Med 3:e150. | Li ,S.; Wang, J.; Zhang, X.; Yan, R.; Wang, N.; Zhao, K .(2004) . Proteomic characterization of two snake venoms: Naja hajeatraand Agkistrodonhalys . Biochem J., 384(1):119-27. | Mohamed, A.H., Salah, A.M., Ahmed. S. and Behir ,S. (1978). Histopathological effects of Naja haje snake venom and a venom gland extract of the scorpion Buthusquinquestriatus on the liver, suprarenal gland and pancreas of mice. Toxicon, 16: 253-264. | Mukhopadhyay, P.P., Ghosh, S.& Ghosh, T.K. (2010). Pattern of Renal Pathology in Fatal Envenomation by Indian Cobra (Naja naja) . J Indian Acad Forensic Med, 32(2). | Omran, M A.; Abdel Nabi, M.; El Naggar, M .H. (1997). Serum Biochemical and Hormonal parameters for the toxic effects of Egyptian Corba (Najahage) envenomation. J. Nat. Toxins, 6:69-83. | Pepys MB, Hirschfield(2003). C-reactive protein: a critical update. J Clin Invest; 111: 1805-1812. | Rahmy TR., Hemmaid KZ. (2001). Prophylactic action of garlic on the histological and histochemical patterns of hepatic and gastric tissues in rats injected with a snake venom. J Nat Toxins. 10: 137-65. | Sejrsen KT, Nielsen MO (2006). In Ruminant physiology; Digestion metabolism and impact of nutrition on gene expression, immunology and stress. Wageningen Academic Publishers, NL: pp. 590. | Tan, N.H. (1991) The Biochemistry of venoms of some venomous snakes of Malaysia – A review. Tropical Biomedicine. 8: 91-103 | Tohamy, A.A., Mohamed, A.F., Abdel Moneim, A.E. & Diab, MS.M.. (2014). Biological effects of Naja haje crude venom on the hepatic and renal tissues of mice. Journal of King Saud University - Science. Volume 26(3): 205–212. | | World Health Organization (2007) Rabies and envenomings: A neglected public health issue (WHO, Geneva). | Wu, A.H.B., ed. Tietz clinical guide to laboratory tests, 4th edition, p. 880. | W.W. Saunders Company, St. Louis (2006). |