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Effects of an Acute Envenomation of *Echis coloratus* on Some Tissues Enzymes Activities of Male Rats

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The biological effect of an acute LD_{50} dose of the carpet viper, *Echis coloratus* crude venom on Liver Total protein, Triglycerides with alanine transaminase (ALT), aspartate transaminase, (AST) and gamma glutamyl transferase (GGT), enzyme activity, Kidney creatinine, total protein concentration and Alkaline phosphatase (ALP) in the liver, abdominal muscles and cerebrum brain were measured. The effect of the LD_{50} dose was monitored over a period of seven days, with time interval of 1,3,6,12,24,72 hours. Regarding tissue enzymes activities tested show fluctuation with time, with tendency to regain normal control level after 12 hours. The 12 to 24 hours seems to be crucial for the process of physiological recovery, in spite of the irreversible damage and tissue distraction. It is concluded that the process of physiological adaptation and recovery from the lethal destructive venom effect seems to stabilized after one week, leaving the animal alive with several biochemical altered metabolism and disturbed physiological profile.

Keywords: *Echis coloratus*, LD_{50}, Liver enzymes, Biochemical parameters.

The eastern carpet viper, *Echis coloratus* is one of the vipers inhabiting various region of Saudi Arabia. In Saudi Arabia, it is occurs in the north and eastern parts of the central region but predominates in rocky areas. It is also common throughout Africa and found in southern Palestine, Jordan and Egypt. However, it is aggressive and responsible for the majority of snake bites in Saudi Arabia. Their bites can cause serious health problems, disturbance in metabolism and even death (Al-Sadoon, 1989). The clinical symptoms of *E. coloratus* envenoming are pain, local swelling, skin discoloration, necrosis and bleeding (Bawaskar and Bawaskar, 2002). In addition, Paralysis, renal damage and injury at the bite (Gutierrez *et al*., 2005). Snake venoms contain a complex mix of components, with biologically active proteins and peptides comprising the vast majority (Casewell, *et al*., 2009). Viperidae venoms typically contain an abundance of protein-degrading enzymes, called proteases, that produce symptoms such as pain, strong local swelling and necrosis, blood loss from cardiovascular damage complicated by coagulopathy, and disruption of the blood clotting system (Kadhim *et al*., 2014). It has been reported that snakes from family Viperidae species showed moderate levels of proteinase, alkaline phosphomonoesterase, phosphodiesterase, arginine ester hydrolase, L-amino acid oxidase, hyaluronidase and a high nucleotidase and phospholipase A_2 activities.

There are studies showing the effects of various snake venoms on ALT, AST, ALP and GGT in rat indicate that venom increase the levels of these enzymes and damage of hepatocytes of liver (Al-Sadoon *et al*., 2013, Al-Saleh *et al*., 2002, Fahim, 1998, 2001, Mohammed *et al*., 1981 and Al-Jammaz *et al*., 1992).

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Vipers' venoms were reported to exhibit different toxic effects, due to the presence of lipolytic and proteolytic enzymes in their compositions (Tan *et al*., 1990). The ability of the venom to induce cytotoxicity (Bertke & Atkins, 1961), Nephrotoxicity (Ickowiz *et al*. , 1966), muscular dystrophy (Mohamed & Khaled, 1966), diverse immune response (Brando *et al* ,2000) , alteration in general metabolism and above all, inducing hyperglycemia (Abdel Raheem *et al*, 1985) ,also the contrary was reported, hypoglycemia (Abu-Sinna *et al*, 1993) .

There are relatively few studies on the long term effects of *E. coloratus* crude venom on clinical parameters (Al-Sadoon *et al*., 201). Report of biting does not cover the real picture of accidental envenomation, leaving the possibility of self-healing and recovery.

This study aims to determine the bio-physiological changes from the first hour of envenomation, with an acute LD_{50} dose of *Echis coloratus* crude venom extended to seven days monitoring the changes in some chosen biochemical parameters. This study could allow a good understanding of the early and late changes undergoing in different vital organs at the onset of envenomation and after a period of time physiologically enough to restore partially or completely the altered function resulting from the envenomation. Monitoring of the biochemical changes in some organs of envenomated rats after 1, 3, 6, 12 ,24 , 72 hours till a week after envenomation. One of our goals is to explore the biochemical changes which could occur leading to healing , recovery or physiological and cellular adaptation, and if the damage due to envenomation, is reversible or permanent.

MATERIALS AND METHODS

Venom collection and preparation

The venom was obtained from the eastern carpet viper, *Echis coloratus.* Snakes were kept in serpentarum at the Zoology Dept., College of Science, King Saud University, after being collected from central region of Saudi Arabia by skilled professional hunter. The snakes were kept in large tanks, heat was provided from a 100 watt lamp for a daily' period of 9 hours. Water was always available. Venoms was milked from adult snakes, lyophilized and reconstituted in saline solution prior to use in this investigation.

Determination of LD_{50} Dose

The LD_{50} values was determined according to the study of Sun (1963) and obtained from a dose mortality curves set up especially for venom LD_{50} was calculated as the aggregate dose at which the weight 50% of the animals survived, according to the following equation:

LD50= (Dos (min)-(sum (Dose (diff) x Average mortality)/K. Where, K is the number of groups. Only surviving animals were anesthetized with pentobarbital (60 mg/kg) body envenomation, dead animals were neglected. The LD_{50} of *E. coloratus* venom was found to be 0.175 mg/kg of rats' body weight

Experimental design

Forty adult male albino rats (*Rattus norvegicus*) weighing 200-250 g were obtained from the Central Animal House of the Faculty of Pharmacy at King Saud University. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH publication No. 85-23. revised in 1985). The study protocol was approved by the Animal Ethics Committee of the Zoology Department, College of Science, King Saud University. All animals were allowed to acclimatize in metal cages inside a well-ventilated room for 2 weeks prior to the experiment. Animals were maintained under standard laboratory conditions (temperature 23°C, relative humidity 60-70% and a 12-h light/dark cycle) and were fed a diet of standard commercial pellets and water *ad libitum*.

Animals were equally distributed in two experimental groups. Group I (5 animal), is a control group that was intraperitoneally (i.p.) injected with physiological saline (0.2 ml); and group II (35 animals), treated with LD_{50} (0.175 mg/kg, i.p.) of the crude *E. coloratus* venom. ALP enzyme activity in the liver, abdominal muscles and cerebrum brain were measured at time intervals of 1, 3, 6, 12, 24, 72 h and 7 days post envenomation in both the control and treated animals. After scarification, treated animals were immediately anesthetized for twenty minutes and dissected; the liver, abdominal muscles and brain were removed, washed in ice-

cold saline, patted dry and weighed. About 100 mg of tissue from the liver, abdominal muscles and cerebrum brain were collected and homogenized in chilled 0.1 M Tris–HCl buffer using Potter–Elvehjem Teflon homogenizer. The homogenates were used for biochemical investigation. Enzyme assay tissues were homogenized in ice cold Tris buffer bath (pH = 7.7) for AST or (pH = 7.4) for ALT and in phosphate buffer (pH = 7.5) for ALP and GGT. Determination of the enzymes activity were made according to the recommendations of the Scandinavian Committee on Enzymes (SCE) using Kits from Sera-Pack (Ames Division, Miles Ltd. England) according to the manufacturer's instructions. Total protein concentration was determined according to the method of Lowry *et al.* (1951).

Statistical analysis

The comparison between the control (group I) and envenomated group (group II) at different time intervals was done using a Student T-test. The data are presented as means ± S.E. and statistically analyzed using SPSS 10 (IBM, USA). Significance was set at the level of $P < 0.05$ or $P<0.001$ vs control.

RESULTS AND DISCUSSION

Envenoming by (Viperidae: Echis) species lead to a combination of systemic and local haemorrhagic symptomatologies and up to 20% mortality rates without antivenom treatment (Casewell *et al*., 2009).

The present study revealed that the injection of an acute dose of LD_{50} of *E. coloratus* crude venom causes a significant increase ($P<0.001$) in liver protein content after 3,6,12,24 hours (Fig.1A),but decrease to control level at the seventh last day of the experiment. Rahmy, (2000) observed that the sublethal dose of *N. haje* venom caused alterations in liver total protein following it's intramuscularly injection. Significant depletion of abdominal muscle total protein was noticed at the first hour, followed by a significant increase at the 6th hour. From the 12 hour to the end of the experiment a sever highly significant decrease ($P<0.001$) was observed in Abdominal muscle total protein (Fig.3A). Brain total protein was found to significantly increase ($P<0.05$) after 72 hr and at the seventh day (Fig.4A). This result is in agreement with other studies (Al-Sadoon *et al* 2013). Changes in organs total protein content could be explained on the basis of the venom components which contain cytotoxins, neurotoxics, myotoxins and different enzymes such as phospholipases A_2. The disturbance in protein synthesis in the hepatocytes could be due to cellular damage .The venom directly affect the Abdominal muscles, as a step of its digestion process and to a lesser extent the brain ,as nervous system is known to be more resistance to toxicity. The continuity of protein degradation in abdominal muscle studied could not be attributed only to the proteolytic venom effect as its concentration decline with time, but it could be also a direct effect of insulin deficiency noted after envenomation, as mentioned before, and causing the diabetogenic effect. Also it could be possible that the venom altered the gluconeognesis mechanism - especially in liver and kidney - favoring the usage of the key amino acids and resulting in the augmentation of serum glucose level (Fahim, 2001). Delayed accumulation of brain total protein may indicate neuronal plastic changes following an acute stress (Baubet *et al.*, 1996).

Regarding the liver Triglycerides, the acute dose of LD50 of *E. coloratus* caused highly significantly increase in the first hour ($P<0.001$), then steadily decline to reach a level below control value after 24 hr where the decrease was significant ($P<0.05$), then increase to reach the control level at the seventh day (Fig.1B). Accumulation of Triglycerides in liver could be plausibly attributed to their mobilization from adipose tissue as proposed by EL-Asmar *et al.*, (1979) and EL-Jammaz *et al.*, (1992) using different venoms. However, the inhibition of the lipolytic action of the venom may develop as a result of the possible presence of an enzyme inhibitor in the venom (Middleton and Phillips, 1964) which seems to be dominant when large doses of the venom have been applied. This might explain the present fluctuation observation in liver Triglycerides content.

On the other hand, kidney creatinine level fluctuated with a tendency to increase with time. It recorded its lower significant level after one hour and its highly significant increase after 24hr ($P<0.001$), Creatinine level was still significantly high, then decrease to control level at the seventh day (Fig.2).The rise in serum creatinine levels

indicates impairment renal function and nephrotoxicity. Similar observations were reported in rats following administration with *Naja haje* venom (Omran *et al*., 1997). Acute renal damage together with glomerular, tubular and vascular lesions following snake bites have been reported (Tilbury *et al.*, 1987). In addition, increased vascular permeability and hemorrhages in various other vital organs, in general and in the kidneys in particular, as has been observed by Meier &Stocker (1991) in the majority of snake envenomation. The elevated creatinine levels in kidneys of rats could be attributed to renal infarction (Mohamed *et al*., 1974). The possible lytic activity of the venom on kidney nephrons cannot be neglected. However, this impairment of renal function is developed with time, generally venom act to fit a triexponential equation characteristic of a three-compartment open pharmacokinetic model comprising a central compartment 'blood', a rapidly equilibrating 'shallow' tissue compartment and a slowly equilibrating 'deep' tissue compartment. The tissue distribution of the venom showed the highest uptake in the kidney (Ismail *et al*., 1996).

Meanwhile, liver AST, was significantly increased (P<0.05) at the first six hours, after that it steadily decrease reaching its lowest significant level after 72hr, attaining the control level at the end of the time experiment (Fig.1D). The liver ALT maintained a significant increased level from the third to the 72 hours (P<0.05), dropping to control level at the seventh last day of the experiment (Fig.1C). Mohamed *et al*., (1981) reported that *N. haje* venom induced a significant increase in the liver AST activity and that the increase may be due to destruction of hepatic cellular organelles and liberation of the enzymes intracellularly, as those enzyme activities fluctuate following the damage to liver, myocardial and skeletal muscles. On the other hand, liver ALT activity was found to fluctuate with venom administrations ,this could be explained according to Felig (1975) who suggested that the glucose-alanine cycle in which pyruvate produced from glucose is transaminated to alanine via ALT enzyme and transported to liver to be reconverted to glucose by gluconeogenesis to enhance the hyperglycemic phenomenon observed after envenomation. The possible utilization of ALT in this mechanism could be the reason for its fluctuation observed in our study. Similar observation was reported by (Al-Sadoon *et al*., 2013; Addi and Naser, 1999; Murray *et al*., 1988 and porth, 1990).

As far liver ALP enzyme activity, it maintained a level close the control value till the 12 and 24 hr ,where it significantly increase (P<0.05) to decline after that ,reaching the control level at the seventh last day of the experiment time (Fig.1E).On the other hand, Abdominal muscle ALP enzyme activity level revealed a trend similar to liver ALP, where its level significantly increase (P<0.05) at the 12 and 24 hr then decline after that to reach the control level at the seventh last day of the experiment time (Fig.3B). Brain ALP enzyme activity level remained unchanged till the 6hr where it showed a significant decrease (P<0.05), then rise to control level after 12hr and remain within control level till the end of the experiment (Fig.4B). ALP enzyme is found mainly in the bile ducts of the liver. Its increase can indicate obstructive or

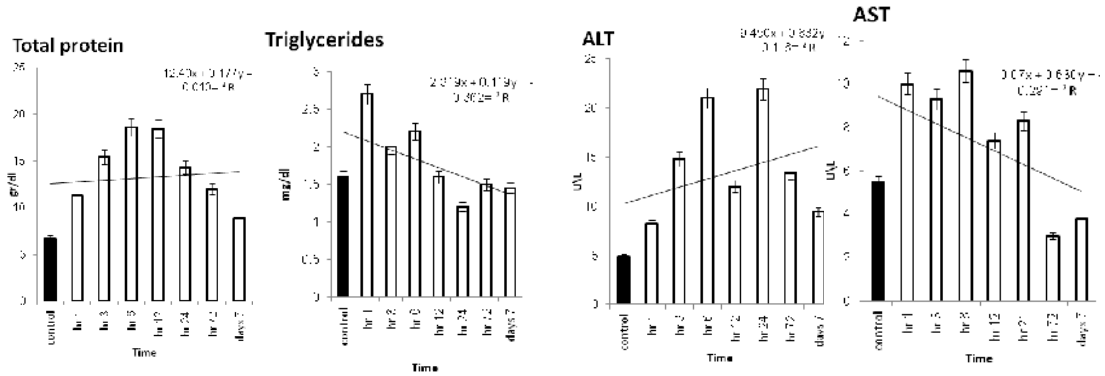
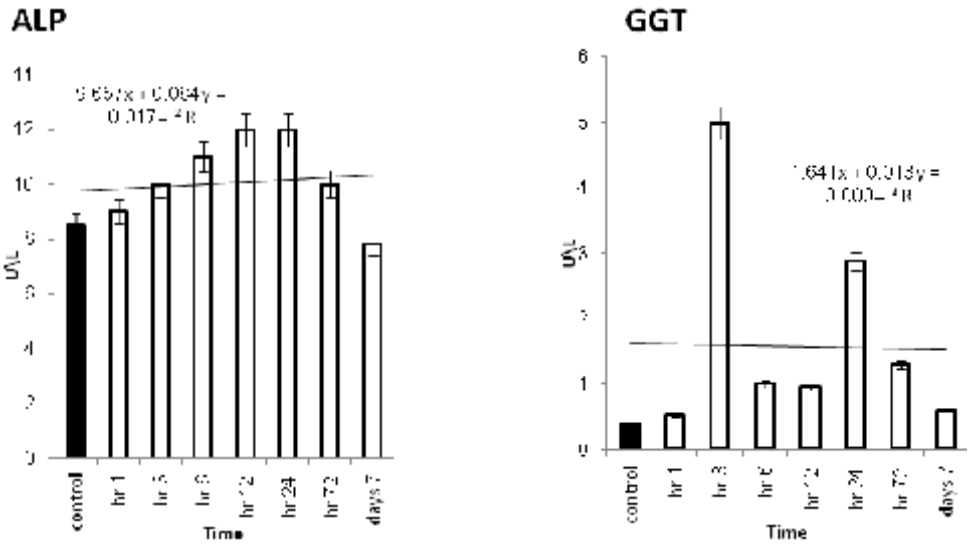


Fig. 1(A-D). Effect of an acute dose of Ld_{50} of *Echis coloratus* crude venom on Liver Total protein(1A), Triglycerides(1B), Alanine aminotransferase (ALT) (1C) and Aspartate aminotransferase (AST)(1D) over a period of 7 days.

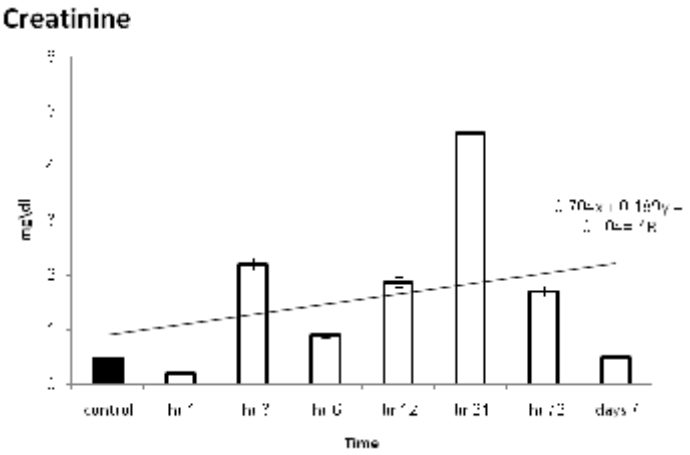
cholestatic liver disease, where bile is not properly transported from the liver because of bile duct obstruction. Liver ALP activity elevation with *E. coloratus* venom, offer additional evidence of liver lesion, acute hepatitis, irreversible hepatic necrosis of hepatocytes, permanent liver damage and disturbed phosphorylation process (Ueno & Rosenberg, 1996). Brain ALP enzyme activity was not deeply altered as the nervous system could be more resistant to Viperidae venom which is mainly hemolytic targeting circulatory fluid and organs. Biardi *et al.*, (2006) mentioned that rattlesnake venom showed mild neuro impact on California ground squirrel, *Spermophilus beecheyi*

While liver GGT enzyme activity level remains highly and significantly elevated from the third hour (P<0.001) till 72 hr, then decrease to the control level at the last day of the experiment (Fig.1F). *Bitis arietans* and *Echis coloratus* crude venom was mentioned to have the same effect on GGT rat levels (Al-Jammaz 2001&2002). GGT is produced in many tissues as well as the liver. It



Where*is significant at < 0.05 and highly significant **at < 0.001 .

Figure 1(E-F):Effect of an acute dose of LD_{50} of *Echis coloratus* crude venom onLiver enzymes activitiesAlkaline phosphatase ALP(1E)andGamma Glutamyl Transferase GGT (1F)over a period of 7 days.



Where*is significant at < 0.05 and highly significant **at < 0.001.

Fig. 2. Effect of an acute dose of Ld_{50} of *Echis coloratus* crude venom on Kidney Creatinine

increase in lesions which cause intrahepatic or extrahepatic obstruction of bile ducts, including parenchymatous liver diseases with a major cholestatic hepatitis. These pathological changes reflect altered metabolic processes since most enzymes are present in cells at much higher concentration than in plasma and normal plasma enzyme level reflects the balance between the release of enzyme during ordinary cell turnover and their metabolism and excretion. An increase occurs in certain enzymes, as alkaline phosphatases, ALP and GGT which are possible marker for cell damage and degeneration as reported by Mohamed *et al*. (1978) using LD_{50} of *N. haje* venom, while its decrease indicates a reduced synthesis, direct inhibition or failure to excrete also

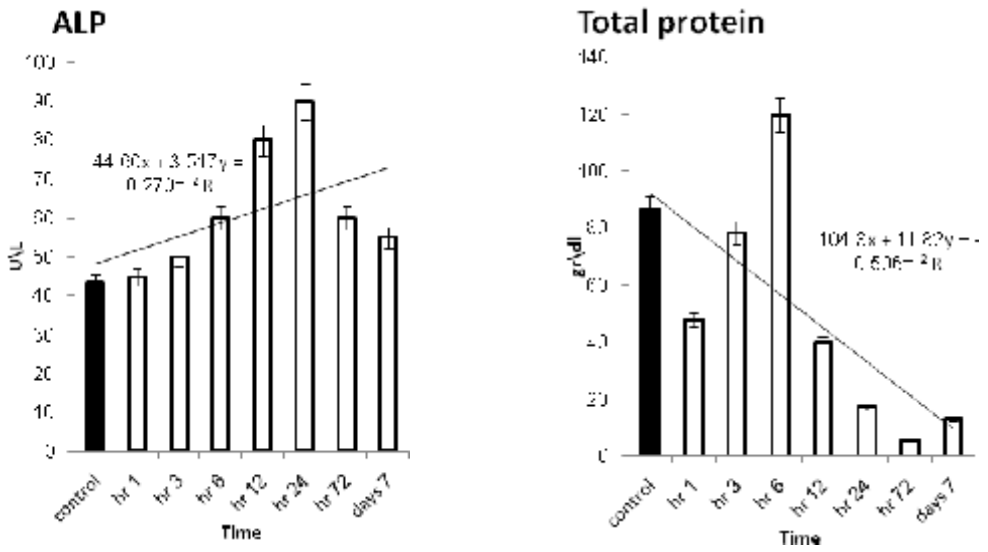
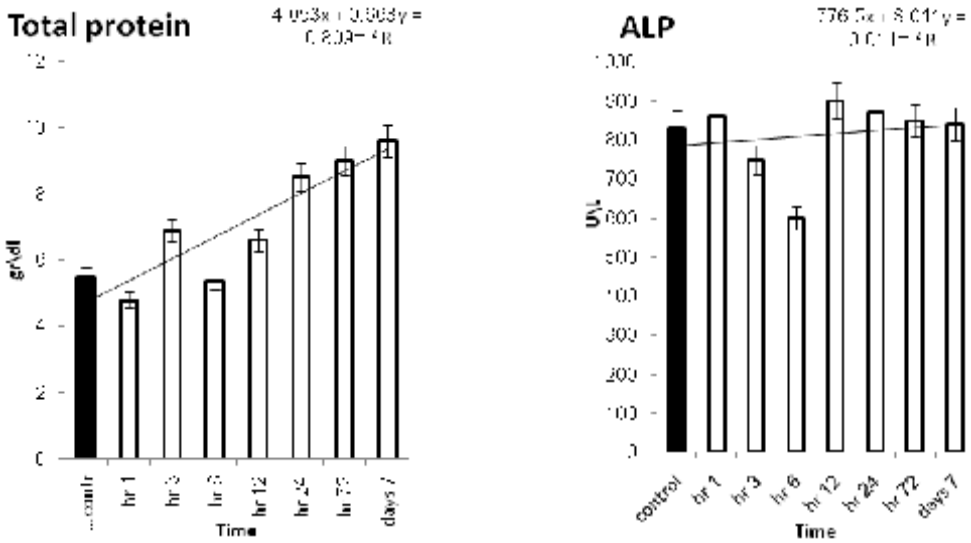


Fig. 3. (A-B) : Effect of an acute dose of Ld_{50} of *Echis coloratus* crude venom onAbdominal Total protein(3A) and Alkaline phosphatase ALP(3B)



Where*is significant at < 0.05 and highly significant **at < 0.001 .

Fig. 4 (A-B). Effect of an acute dose of Ld_{50} of *Echiscoloratus* crude venom onBrain Total protein (4A) and Alkaline phosphatase ALP (4B).

due to cell damage by the toxic venom (Mohamed *et al*., 1981).

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REFERENCES

1. Al-Sadoon, M.K. Survey of the reptilian fauna of the Kingdom of Saudi Arabia. I. The snake fauna of the Central Region, *J. King Saudi Univ. 1A, Science.*, 1989; **1**: 53-69.
2. Bawaskar, H. S., Bawaskar, P. H. Profile of snake bite envenoming in western Moharshta, India. Transactions Royal SOC. Trop. *Med. Hyg.*, 2002; **96**: 79-84.
3. Gutierrez, J. M., Rucavado, A., Escalant T., Diaz, C. Hemorrhage induced by snake venom metalloproteinases biochemical and biophysical mechanisms involved in microvessel damage. *Toxicon.*, 2005; **45** (8): 997-1011.
4. Casewell,N. R., Harrison,R. A., Wüster,W., Wagstaff, S. C. Comparative venom gland transcriptome surveys of the saw-scaled vipers (Viperidae: Echis) reveal substantial intra-family gene diversity and novel venom transcripts. *BMC Genomics*. 2009; **10**: 564.
5. Kadhim, A. S., Awda M. A., Al-Fartosi K. G. Some biochemical parameters male rats treated with protease extracted from venom of Echis carinatus snake. *Journal of Harmonized research in pharmacy.*, 2014; **3**(2): 23-29.
6. Al-Sadoon, M. K., Orabi, G. M., Bader, G. Toxic Effects of Crude Venom of a Desert Cobra, Walterinnesia aegyptia, on Liver, Abdominal Muscles and Brain of Male Albino Rats. *Pakistan J. Zool.*, 2013; 1359-1366.
7. Al-Saleh S. S., Ghneim H. K., Haddad H. Y., Khan S. U. Separation and purification of Echis coloratus venom and some biological and biochemical effects of the proteins. *Cell Biochem Funct.*, 2002; **20**(2): 153-62.
8. Fahim, A. Biochemical effects of crude venoms of two elapidae from saudi fauna on male albino rats. *J. Egypt. Ger. Soc. Zool.*, 2001; **36**: 165-175.
9. Mohamed, A. H., Fouad, S., El-Assar, S., Salem, A. M., Abdel Aal, A., Amr, H., Zahran, F., Abbas, N. Effect of several snake venoms on serum and tissue transaminases, alkaline phosphatase and Lactate dehydrogenase. *Toxicon.*, 1981; **19**: 605-609.
10. Al-Jammaz, I., Al-Sadoon, M.K., Attia, M.A., Fahim, A. 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.*, 1992; **30**: 207-222.
11. Tan, N. Tan, N H., Ponnudurai, G. A comparative study of the biological properties of venoms from snakes of the genus vipera (True adders) *Comp. biochem. physiol.*, 1990; **96**(4) 683- 688.
12. Bertke, E. M., Atkins, J. H. Effect of Centrunoides Sculputuratus venom upon rat tissues. A histopathological study. *Toxicon.*, 1961;**2**: 205-218.
13. Ickowiz, M., Shulov, A., Naor, D. The effect of vipera palestina venom on the thymus, lymph nodes and kidneys. *Toxicon.*, 1966; **3**: 305.
14. Mohamed, A.H., Khaled, L.Z. Effect of the venom of Cerastes cerastes on nerve tissue and skeletal muscle. *Toxicon.*, 1966; **3**: 223.
15. Brando, C., Marcinkiewicz, C., Goldman, B., McLane, M.A., Niewiarowski S. EC3, a heterodimeric disintegrin from Echis carinatus, inhibits human and murine alpha4 integrin and attenuates lymphocyte infiltration of Langerhans islets in pancreas and salivary glands in nonobese diabetic mice. *Biochem Biophys Res .Commun.*, 2002; **7**(2): 413-7.
16. Abdel-Raheem, K., Dehlaw, G., EL-Mossallamy, N., Mahmoud, I. Influence of Viper (Cerastes vipera) Venom on metabolism in mice. *Proc. Zool. Soc. A.R.Egypt.*, 1985; **9**: 57 - 66.
17. Abu-Sinna, G.; AL-Zahaby, A.S.; Abd EL-Aal, A.; Abd EL-baset, A., Soliman, N.A. The Effect of the viper cerastes cerastes cerastes venom and venom fractions on carbohydrate metabolism. *Toxicon.*, 1993; **31** (6): 791 - 801.
18. Al-Sadoon, M.K., Fahim A. Possible recovery from an acute envenomation in male rats with LD50 of Echis coloratus crude venom: I-A seven days hematological follow-up study. *Saudi Journal of Biological Sciences.*, 2012; **19**: 221-227.
19. Sun, R. A forthright and practical method for comprehensive calculation of LD50. *Learned J. Pharmacy* (Chinese)., 1963; **10**(2):65.
20. Lowry, O., Rosenbraugh, N., Farr, L., Rondall, R. Protein measurement with the Folin-phenol reagent. *J. biol. Chem.*, 1951; **183**: 265-275.
21. Rahmy,T.R., Hemmaid, K.Z. Histological and histochemical alterations in the liver following intramuscular injection with a sublethal dose of the Egyptian cobra venom. *J. Nat Toxins.*, 2000;

9: 121-132.

22. Fahim, A. Biochemical effects of the viper, Bitis arietans, crude venom on albino rats. *Egypt. J. Zool.* 1998; **30**: 35-54.
23. EL-Asmar, M.F., Farag, R.M., Shoukry, S., EL-Shimi, I.R. Effect of scorpion (Leiurus quinquestriatus H&E) venom on lipid metabolism. *Toxicon.*, 1979; **17**: 279 - 283.
24. Middleton, E. Jr., Phillips, G.B. Distribution and Properties of Anaphylactic Induced Slow Reacting Substance and Histamine in Guinea Pigs. *J. Immunol.*, 1964; **93**: 220-228.
25. Omran, M.A., Abdel-Nabi, M.I. Changes in the arterial blood pressure, heart rate and normal ECG parameters of rat after envenomation with Egyptian cobra (Naja haje) venom. Hum. *Exp. Toxicol.*, 1997; (6):327-333.
26. Tilbury, R.C., Madkour, M.M, Saltissi, D., Suleiman, M. Acute renal failure following the bite of Burton's Carpet Viper Echis Coloratus Gunther in Saudi Arabia: Case Report Review. *Saudi Medical Journal.*, 1987; **8**: 87-95.
27. Meier, J., Stocker, K. Effect of snake venoms on homeostasis. *Toxicology.*, 1991; **21**(3): 171-182.
28. Mohamed, A.H., Nawar, N.N.Y., Fatma, A. Influence of hydrocortison microscopic changes produced by Naja nigricollis venom in kidney, liver and spleen. *Toxicon.*, 1974; **12**: 45-48.
29. Ismail, M., Aly M.H., Abd-Elsalam, M.A., Morad, A.M. A three-compartment open pharmacokinetic model can explain variable toxicities of cobra venoms and their alpha toxins. *Toxicon.*, 1996;**34(**9):1011-1026.
30. Mohamed, A.H., Fouad, S., EL-Assar, S., Salem, A.M., Abdel aal, A., Hassan, A., Zahran, F., Abbas, N. Effects of several snake venoms on serum and tissue transaminases, alkaline phosphatase and lactate dehydrogenase. *Toxicon.*, 1981;**19**: 605 - 609.
31. Felig, P. Amino acid metabolism in man. *Ann. Rev. Biochem.*, 1975; **44**: 933-955.
32. Assi, A.A., Nasser, H. An in vitro and in vivo study of some biological and biochemical effects of Sistrurus malarius barbouri venom. *Toxicology*., 1999: **20** (137): 81-94.
33. Murray, R.K., granner, D.K., Mayes, P. A., Rod Well, V. W. Chemical consitituents of blood and body fluids In: Harper's Biochemistry. 21th ed. (Pub.) Prentice-Hall int. Inc. 1988; 649-664.
34. Porth, C.M. Alterations in function of the hepatobilary system and Exocrine pancreas In: Pathophysiology concepts of altered health states, 3rd Ed. (pub.)J.B. Lippincott Comp. *Philadelphia*, 1990; 719-739.
35. Ueno, E., Rosenberg, P. Differential effects of snake venom phospholipase A2 neurotoxin (beta-bungarotoxin) and enzyme (Naja naja atra) on protein kinases. *Biochem. Pharmacol.*, 1996; **52**(8): 1287-1293.
36. Biardi, J. E., Chien, D. C., Coss, R. G. California ground squirrel (Spermophilus beecheyi) defenses against rattlesnake venom digestive and hemostatic toxins. I. *Journal of Chemical Ecology.*, 2006; **32**(1): 137-154.
37. Al-Jammaz, I. Effects of single doses of Bitis arietans crude venom on serum biochemical parameters in rats. *Sci. J. King. Faisal Univ.Saudi Arabia.*, 2001; **2**:103-112.
38. Al-Jammaz, I. Effects of three doses of Echis coloratus crude venom on rat metabolism" Dirast., 2002; 29(2).