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Original Research Article

SOME BIOCHEMICAL PARAMETERS OF MALE RATS TREATED WITH PROTEASE EXTRACTED FROM VENOM OF *ECHIS CARINATUS* SNAKE

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Abstract:
This work aimed to study the effect of extracted protease from *Echis carinatus* venom on some biochemical parameters of male rats which including total protein, albumin, MDA, total cholesterol, triglycerides, HDL, LDL, VLDL, alanine amino transferase(ALT) and aspartate amino transferase (AST) in male rats . Isolation and purification of protease from *Echis carinatus* venom were carried out by using combination of gel filtration and ion exchange chromatography. After that, three doses were used (0.05, 0.1 , 0.2) ml of purified protease and the animals were injected (I.P) for 14 days as one dose daily. The results showed that purified protease of *Echis carinatus* venom caused a significant decrease ($P< 0.05$) in serum total protein, albumin and HDL while MDA, the total cholesterol, triglycerides, LDL, VLDL, ALT and AST increased significantly($P<0.05$).

Kew words: *Echis carinatus*, Protease, Biochemical parameters.

Introduction:

Echis carinatus snake belongs to Viperidae family. It is found in Middle East, Central Asia and Indian Sub-continent (Slowinski, 2000). Also, it is found in south Iraq in Said Dakheel village (Afrasiab *et al*., 2012) with subspecies *sochureki*. Therefore, it is called Said Dakheel snake. It has got many names Saw- Scald viper Indian - Saw Scald viper and little viper (Mallow *et al*., 2003). *Echis carinatus* is deadly snake and it is the smallest member of the big four snake (Whitaker, 1990). Its body contains of three parts: head, mid body and sub caudals. The length of *Echis carinatus* ranges between 38 and 80cm, but usually no more than 60 cm. The top of head has a whitish cruciform or trident pattern, while the belly is whitish to pinkish (Boulenger, 1890). The venom of *Echis carinatus* is rich in serine proteases and metalloproteases, which reduces most clotting

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factors including (VIII, V, II, and XIII). Also, it affects blood coagulation that in turn causes hemostatic defect (Ali *et al*., 2004). At addition, *Echis carinatus* venom affects the cardiovascular, the central nervous systems and tissues (Gawade, 2000). Fatal dose of Echis *carinatus* venom is 80 mg (4.6 mg injected at the time of bite much less as compare to fatal dose) (Reid, 1982). The clinical symptoms of Echis *carinatus* envenoming are swelling, tissue necrosis and bleeding (Bawaskar & Bawaskar, 2002). Paralysis, renal damage and injury at the bite site (Gutierrez *et al*., 2005). Proteases are a large group of enzymes, they are also known as (proteinases or proteolytic enzymes) (Barrett & McDonald, 1986). They belong to the class of enzymes called (hydrolases) which catalytic to break down peptide bounds of protein to liberate the amino acids (Seife, 1997). Proteases are controlled on biological processes in all living organisms (Lopez & Overall, 2002). The essential roles of proteases are in cell behavior and survival and death of all organisms. The changes in proteolytic systems are under multiple pathologic conditions such as cancer and cardiovascular diseases. Therefore, many proteases are major focus of attention for the pharmaceutical industry as discover drug to diagnostic and prognostic biomarkers (Turk, 2006). Proteases are found in a wide diversity of sources such as plants, animals and microorganisms (Kenny, 1999). Microorganisms represent an excellent source of enzymes owing to their broad biochemical diversity and their susceptibility to genetic manipulation and easy control on the process product (Aboul-Soud*et al*., 2011).

Echis carinatus sochureki snake (Said Dakheel Snake) caused the death of many people in Said Dakheel village, Thi-Qar province, south of Iraq, therefore, the present study aimed to study the effect of protease extracted from venom of this snake in some biochemical parameters of male rats.

Materials and Methods:

Source of venom

This snake collected from said Dakheel village, Thi-Qar governorate, Iraq. It kept in special laboratory in Biology department, Science College, Thi-Qar University, Iraq. The crude venom of *Echis carinatus* was obtained in right conditions such as temperature and moisture. After that, the venom was dried by using lyophilization and its weight was 7.4 mg.

Isolation and purification of protease

Protease was extracted from lyophilized crude venom of Echis *carinatus* according to (Ghorbanpur *et al*., 2009).

Protease assay

The protease activity was estimated by the method described by (Brock *et al*., 1982; Tsuchida *et al*., 1986) .

Protein determination

Protein concentration was measured according to (Lowry *et al*., 195) using BSA as a standard.

Effect of protease on biochemical parameters of male rats

The study was carried out on (24) mature male rats *Rattus norvegicus* weighting between (180- 230) g were procured from Biology dept., Science college, Thi-Qar university, Iraq. The animals were housed in a well-ventilated 12hrs light and 12hrs dark cycles. The animals were divided into four equal groups, every group consisted (6) rats. The first group was injected (I.P.) with (0.1 ml/ animal) from normal saline (0.9% Nacl), the second, third and fourth groups injected (I.P.) with (0.05, 0.1, and 0.2 ml/ animal) from protease respectively. The animals were injected for 14 days as one dose daily. After 14 days of treatment, the animals were sacrificed subsequently, the blood samples were collected by cardiac puncture, and 5ml of blood samples were collected from heart and kept in tubes without EDTA and centrifugation at 3000 rpm for 15min to separate the serum. Total protein, albumin, MDA, total cholesterol, triglycerides, HDL, LDL, VLDL, AST and ALT were measured.

Statistical analyses were done utilizing the computer data processing (SPSS, version 14). A probability value ($P < 0.05$) was considered to be statistically significant.

Results

The results showed a significant increase (P<0.05) in serum MDA of male rats treated with purified protease of Echis *carinatus* venom at dose (0.2) ml compared with the control group. Also, the male rats treated with purified protease at dose (0.2) ml showed a significant increase (P< 0.05) compared with second and third groups.

The results showed a significant decrease (P<0.05) in serum total protein of the male rats treated with purified protease of Echis *carinatus* venom at doses (0.05, 0.1, 0.2) ml compared with the control group. Also, the results showed a significant decrease (P<0.05) in serum albumin of the male rats treated with purified protease of *Echis carinatus* venom at doses (0.1, 0.2) ml compared with the control group. While the male rats treated with purified protease at dose (0.05) ml showed non-significant difference compared with the control group.

Table 1. Effect of the purified protease of *Echis carinatus* venom on MDA, total protein, albumin of male rats

Animal groups	MDA (nmole/ml) Mean ± S.E.	Total Protein (g/100ml) Mean ± S.E.	alb. (g/100ml) Mean± S.E.
First group	6.41±1.1	7.85±0.13	4.11 ± 0.24
Second group	6.94±1.08	6.23 ± 0.14	4.23 ± 0.24
Third group	7.58±1.1	5.37±0.13	3.49 ± 0.24
Fourth group	9.93±1.08	4.14±0.14	3.27 ± 0.24
R.L.S.D.	2.32	0.25	0.49

Values are means ± S.E.
Different letters refer to significant differences (p<0.05).
Same letters refer to non-significant differences (p<0.05).

A significant increase (P<0.05) in serum total cholesterol of male rats treated with purified protease of *Echis carinatus* venom at doses (0.1, 0.2) ml compared with the control group, while the male rats treated with purified protease at dose (0.05) ml showed non-significant differences compared with the control group. Also, the results showed significant increase (P< 0.05) in serum triglyceride of male rats treated with purified protease of *Echis carinatus* venom at doses(0.05, 0.1, 0.2) ml compared with the control group. The male rats treated with purified protease at dose (0.2) ml showed a significant increase (P< 0.05) compared with the groups treated with purified protease at doses (0.05, 0.1) ml. On the other hand, the results showed a significant decrease (P<0.05) in serum HDL of male rats treated with purified protease of Echis *carinatus* venom at doses (0.05, 0.1, 0.2) ml compared with the control group. The male rats treated with purified protease at dose (0.2) ml showed a significant decrease (P<0.05) compared with the male rats treated with purified protease at doses (0.05, 0.1) ml. The male rats treated with purified protease at dose (0.1) ml showed a significant decrease (P<0.05) compared with the male rats treated with purified protease at dose (0.05)ml . However, the results showed a significant increase (P<0.05) in serum LDL of male rats treated with purified protease of Echis *carinatus* venom at doses (0.1,0.2) ml compared with the control group. The male rats treated with purified protease at dose (0.2) ml showed a significant increase (P< 0.05) compared with the male rats treated with purified protease at doses (0.05,0.1)ml . Also, the male rats treated with purified protease at dose (0.1)ml showed a significant increase (P< 0.05) compared with the male rats treated with purified protease at dose (0.05) ml. While the male rats treated with purified protease at dose (0.05) ml did not show a significant increase (P<0.05) compared with the control group. The results showed a

significant increase (P< 0.05) in serum VLDL of male rats treated with purified protease of Echis *carinatus* venom at doses (0.05, 0.1, and 0.2) ml compared with the control group. Also, the fourth group treated at dose (0.2) ml showed a significant increase (P< 0.05) compared with second and third groups treated at doses (0.05, 0.1) ml. However, the male rats treated with purified protease at dose (0.1) ml showed a significant increase (P<0.05) compared with the male rats treated with purified protease at dose (0.05) ml

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Table 2. Effect of the purified protease of *Echis carinatus* venom on lipid profile of male rats

Animal groups	Total cholesterol (mg/dl) Mean±S.E.	Triglyceride (mg/dl) Mean± S.E.	HDL (mg/dl) Mean±S.E.	LDL (mg/dl) Mean±S.E.	VLDL (mg/dl) Mean±S.E.
First group	58.18±3.91	32.93±2.25	21.72±0.51	29.88±4.08	6.59±0.45
Second group	63.77±4.00	43.02±2.30	19.05±0.51	36.11±4.07	8.61±0.50
Third group	77.44±3.91	56.37±2.26	15.13±0.53	51.03±4.10	11.27±0.46
Fourth group	117.37±4.00	74.34±2.25	12.84±0.50	89.66±4.07	14.86±0.50
R.L.S.D.	7.08	4.07	0.93	7.37	0.82

Values are means ± S.E.

Different letters refer to significant differences (p<0.05).

Same letters refer to non-significant differences (p<0.05).

AST increased significantly in male rats treated with (0.05, 0.1, and 0.2) ml of purified protease compared with the control group. Also, the results showed a significant increase in ALT level of male rats treated with ((0.05, 0.1, 0.2) ml of purified protease compared with the control group.

Table 3. Effect of the purified protease of *Echis carinatus* venom on AST & ALT levels in male rats

Animal groups	AST activity (U/L) Mean ± S.E.	ALT activity (U/L) Mean ± S.E.
First group	9.91±0.74	6.61±0.90
Second group	13.51±0.74	8.53±0.91
Third group	17.33±0.74	11.96±0.91
Four group	21.61±0.74	18.06±0.90
R.L.S.D.	1.34	1.64

Values are means ± S.E.

Different letters refer to significant differences (p<0.05).

Same letters refer to non-significant differences (p<0.05).

Discussion:

It is well known that viper bites cause toxic effects on victims due to the presence of lipolytic and proteolytic enzymes in their venoms (Tan &Ponndurai, 1990). The present study revealed that the injection of the purified protease from *Echis carinatus* venom causes a increase in serum MDA is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acids peroxidation and arachidonic acid metabolism (Yahya*et al*., 1996). Increase in the accumulation of MDA in cells can result into cellular degradation, some biochemical charges and even cell death (Winrow *et al*., 1993). On the other hand, the reduction of total protein level may result in disturbances in renal function and hemorrhages in some internal organs (Al-Jammaz *et al*., 1999). It might be assumed that the reduced level of serum

albumin in the present study could be due to disturbances in renal functions as well as hemorrhages in some internal organs due to the toxic action of various snake venoms (Marsh *et al*., 1997). Albumin concentration is low with causing damage in liver or kidney. Also, protease attacks endothelial cells in the walls of blood capillarity causing small molecule albumin comparison with the blood proteins size. Protease has penetrated in blood stream or the concentration is low because of amino acids absorbance in gut (Riepe *et al*., 1980). Decrease in albumin concentration is mainly due to the diminished of its synthesis in hepatic cells, accompanied by losses of large amounts of albumin into the urine and gastrointestinal tract due to damage kidney and intestinal mucosa (West, 1985). Such changes in total protein and albumin reflect hepatocellular injury and disturbed amino acid metabolism (Gomes *et al*.,1999; Yousef *et al*., 2006).

The increases in serum cholesterol and triglyceride concentration in injected rats observed in the present study could be due to the hepatocytes damage rendering them unable to phosphorylate the increase amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues (El-Asmar *et al*., 1979). Furthermore,(Meier & Stocker, 1991) suggested that, these disturbances might be due to acute nephropathy. On the other hand, the increase in serum total cholesterol level may be resulted of protease effect on stimulation of adrenal cortex leading to aldosterone secretion (Mohamed *et al*.,1980) . Several studies have been made on the metabolic, cardiovascular and hematological effects on the experimental animals (Tilbury *et al*., 1987; Abdul-Nabi *et al*., 1997). Increase in total cholesterol level and decrease in HDL-cholesterol level indicate that protease has the ability to change cholesterol profile , that may be by inducing oxidation of cholesterol (Yalcin *et al*., 1989 ; Kelly *et al*., 1999).

In the present work, The increase in AST level may be a result of liver damage or symptoms similar to hepatitis , liver cirrhosis and muscular dystrophy . This result is in agreement with other investigators (Mohamed *et al*. ,1981 ; Porth *et al*.,1990). The elevated activity of ALT might indicate liver and other vital organ damage brought about by protease. Such finding are in agreement with those reported for *B. arietans* venoms (Mohamed *et al*., 1981). Measurements of clinical chemistry parameters following purified protease of *Echis carinatus* injection clearly demonstrate disturbances of vital organs, especially liver, renal and muscles.

References

- Aboul-Soud, M.A.; Foda, M.S.; Kahil, T.; Asar, A.R.; El-Desoky, G.E.; Al- Othman, A. M.; Al- Othman, Z.A. and Giesy, J.P. (2011) . Purification and biochemical characterization of alkaline protease from an Egyptionbiopesticide – producing Bacillussphaericus strain . *African Journal of Microbiology Research*., 5(28): 5076-5084.
- Abdel- Nabi, I.M.; Raffat, A. and El-Shamy, H.I. (1997) . Biological effects of intraperitoneal injection of rats with the venom of the snake *Echiscarinatus*Egypt. *J. Zool*., 29: 195-205.
- Afrasiab, R.S.; AL-Ganabi, I.M.; AL-Fartosi, K. (2012) . Snake species new or rare to the herpetofauna of Iraq. *J. HERPETOZOA*., 24:(3/4), p: 179.
- Ali, G.; KaK, M.; Kumar, M.; Bali, S.K.; Tak, S.I.; and Hassan, G. (2004) . Acute renal failure following *Echis carinatus* (Saw Scaled viper) envenomation . *Indian Journal of Nephrology*., 14: 177-181.
- Al –Jammaz, I.; Al –Sadoon, M.K. and Fahim, A. (1999) . Effect of LD50 dose of Echis coloratus venom on serum and tissue metabolites and some enzyme of male albino rats. *J. King Saud Univ.* sceience , (2): 61- 68 .
- Barett, A.J.; McDonald, J.K.(1986). Proteases, proteinase and peptidase., *J. Biochem* ., 237 - 935 .
- Bawaskar, H.S. and Bawaskar, P.H. (2002) . Profile of snake bite envenoming in

western Maharashta , India . *Transactions Royal Soc. Trop. Med. Hyg.*, 96: 79 - 84 .

- Boulenger, G.A.(1890). The Fauna of British India, including Ceylon and Burma. Reptilia and Batrachia. Taylar &Francis, London, xviii,pp: 541.
- Brock, F.M.; Forsbery, C.W. and Buchanan, S.G. (1982). Proteolytic activity of rumen microorganisms and effect of proteinase inhibitors . *J. Appl. Environ. Microbiol.*, (44): 561- 569.
- El –Asmar, M.F.; Farag, R.M.; Shoukry, S. and El–Shimi, I.R. (1979) . Effect of scorpion Leivrusquinquestriatus H & E venom on lipid metabolism . *Toxicon* . 17 , 279 - 283.
- Gawade, S.P. (2000) . The photo dynamic action of UV sensitized methylene blue on the venom . *Anim. Toxins.*, 6: 271-278 .
- Ghorbanur, M.; ZareMirakabadi, A.; Zokaee, F.; Zolfagharian, H. and Rabiei, H. (2009). Purification and partial characterization of a coagulant serine protease from the venom of the Iranian snake Agkistrodonhalys . *J. venom Anim. Toxins incl. Trop Dis.*, 15 (3): 411 - 423.
- Gomes, J.; Dawodu, A.H.; LIoyd, O.; Revitt, D.M. and Anilal, S.V. (1999). Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus insecticides . *Hum Exp. Toxicol.*, 18(1): 33-37 .
- Gutierrez, J.M.; Rucavado, A.; Escalant, T.; Diaz, C. (2005). Hemorrhage induced by snake venom metalloproteinases biochemical and biophysical mechanisms involved in microvessel damage . *Toxicon.*, 45 (8) : 997- 1011.
- Kenny, A.J. (1999) . Introduction : Nomenclaiure and classes of peptidases . In Sterchi , E. E.and Stocker (eds), W.Proteolytic enzymes . Tools and targets Berlin : *Speringer – verlag*, PP. 1-8 .
- Lopez–Otin, C. and Overall, C.M. (2002) . Nat . Rev. Mol. Cell. Biol., 3: 509 - 519 .
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J.(1951). Protein measurement with the folin phenol reagent. *J. Biol.Chem.*, 193:265- 275.
- Mallow, D.; Ludwing, D.; Nilson, G. (2003). True vipers: Natural History and Toxicology of old world vipers Krieger publishing company, Malabar, Florida. Pp:359. IsB No 89464-877-2.
- Marsh, N.; Gattullo, D.; Pagliaro, P. and Losano, G.(1997).The Gaboon viper, and Bitisgabonic : hemorrhagic , metabolic , cardiovascular and clinical effects of the venom . *Lif. Sci.*, 61(8): 763-769.
- Meier, J. and Stocker, K. (1991). Effects of Snake Venoms on hemostasis . *Crit- Rev. Toxical.*, 21: 171-182.
- Mohamed, A.H.; Foaud, S.; Abbas, F.; Abdelaal, A.; Abd-El-Aal, A. Abd-El-Baset, A.; Abbas, N. and Zahran, F. (1980) . Metabolic Studies of the Egyption and allied African snake venoms . *Toxicon.*, 18: 381-383.
- Mohamed, A.H.; Foaud, S.; El- Assar, S.; Salem, A.M.; Abdelaal, A.; Amr, A.; Hassan, Zaharn, F. and Abbas, N.(1981). Effects of several snake venom on serum and tissue transaminases , alkaline phosphatase and lactate dehydrogenase . *Toxicon.*, 19: 605- 609.
- Reid, H.A. (1982). Animal poisons, in Manson Bahr PEC, Apted FIC. Eds. Manson's Trapical Diseases, 18th edition , London , Balliere Tilldall.
- Riepe, S.; Goldstein, J. and Alpers, D. (1980). Effect of Secreted Bacteroides proteases on Human Intestinal Brush Border Hydrolases . *The American Society for clinical Investigation . Inc*., 66: 314-322.
- Seife, C. (1997). Blunting nature'sswiss army knife . *Science*, 277 : 1602 -1603 .
- Slowinski, J. (2000) .Striking Beauties venomous snakes California . California Academy of sciences .
- Tan, N.H. and Ponnudurai, G. (1990). A comparative study of the biological properties of venoms from snakes of the genus vipera (True adders) .*Comp. Biochem. Physiol.*, 96(B) . 683-688.

- Tilbury, R.C.; Madkour, M.M.; Saltissi, D. and Suleiman, M. (1987) . Acute renal failure following the bite of Burton's carpet viper *Echis coloratus Gunther* in Saudi Arabic : case report review. *Saudi Med. J.*, 8: 87-95.
- Turk, B. (2006) .Nat , Rev. Drug Disco., 5 :785-799 .
- Tsuchida, O.; Yamagota, Y. and Ishizuka, J. (1986) . An alkaline proteinase of an alkalophilicBacillus SP. *CurrMicrobiol.*, 14 : 7-12 .
- West, J.B. (1985). Blood and the plasma proteins function and composition of blood . In: Best and Taylor's physiological basis of medical practical . 11th ed. , Wiliams and Wilkins , Baltimore. 334-340.
- Whitaker, Z. (1990). Snake man . *Penguin Books Ltd.* PP. 192, ISBNO 14-014308-4 .
- Winrow, V.R.; Winyard, P.G.; Morris, C.J. and Black, D.R. (1993) .Free radicals in inflammation : Second messengers and mediators of tissue destruction . *Br. Med. Bull*, 49: 506-522.
- Zahya, M.D.; Pinnsa, J.L.; Meinke, G.C. and Lung, C.C. (1996) .Antibodies agnist Malondialdehyde (MDA) in MLR/Ipr/Ipr Mice: Evidence for an outoimmune mechanism involving lipid peroxidation . *J Autoimmunity*,9: 3-9 .
- Yousef, M.I.; Awad, T.I. and Mohamed, E.H. (2006). Deltamethrin – induced oxidative damage and biochemical alterations in rats and its attenuation by vitamin E. *Toxicology*, 227(33): 240-247.