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BIOCHEMICAL PARAMETERS OF MALE AND FEMALE RATS TREATED WITH CRUDE VENOM OF *ECHIS CARINATUS SOCHUREKI* SNAKE

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ABSTRACT

The present study aimed to investigate the effect of crude venom of *Echis carinatus sochureki* snake on biochemical parameters of rats. Adult male and female rats divided into three groups for each sex (6 for each group), the first group injected with normal saline (0.9%Nacl) as a control group, the second group injected with (0.04ml/kg/day) of crude venom for once time, and the third group injected with (0.08ml/kg/day) of crude venom for once time. The results indicated the levels of glucose, Creatinine, ALT, AST and HDL were increased significantly in male and female rats , In contrast, the injection of crude venom of the viper *Echis carinatus* induced a significant decrease in cholesterol, triglycerides, total serum protein, albumin, , ALP, LDL and VLDL in male and female within 24 hr. after injection Viper *Echis carinatus* crude venom caused hepatic and renal dysfunction in envenomated rats.

KEYWORDS: *Echis carinatus sochureki*, biochemical parameters, Crude venom, Rats.

INTRODUCTION

Saw Scaled Viper are cold-blooded vertebrates and some species possess dangerous venoms.[1] one of the class Viperidae is one of the types of poisonous snakes that are found in parts of the Middle East, Central Asia, especially the Indian. *Echis carinatus* of four types of dangerous snakes in India. The most recent taxonomy based on phylogeny based on four mitochondrial fragments re-classified the genus into four main groups: the *E. carinatus, E. coloratus, E. ocellatus and E. pyramidum,* of which, the *E. carinatus* is mainly distributed in the Indian Subcontinent and Central Asia, Pakistan and Sri Lanka, as well as in parts of

Nigeria[2], are also distributed widely in many parts of tropical Africa, Ethiopia and the Arab countries, Egypt, Bangladesh, southern Afghanistan, western Pakistan, Iran, Oman and Southern Iraq, where there are in Iraq in an area called Said Dakhil 15 km southeast of the city of Nasiriyah.[3,4] Snake venoms are complex mixtures of pharmacologically active proteins and polypeptides. They play an important role in incapacitating and immobilizing, as well as in digesting prey.[5] Thus toxins have evolved to specifically target various critical points in the physiological systems of prey animals. These factors inhibit blood coagulation by different mechanisms, Venom proteins from Viperidae affect blood clotting factors by starting the clotting process and then blocking formation of large clots. Also,they cause damaging the artery walls leading to non-stop perfuse bleeding.[6] Envenoming resulting from snake bites remains the most neglected public health issues in many countries, particularly in poor rural communities living in the tropics. *E. c. sochureki* causes numerous deadly bites especially in Asia.[7] Generally envenoming by Echis snake vipers is responsible for several clinical complications of severe systemic and local pathology. There are some attempts to use *Echis carinatus* venom in the manufacture of drugs. One of these drugs is called ecarin. It is the primary reagent in the ecarin clotting time (ECt) test, which is used to monitor anticoagulation during treatment with hirudin.[8] In snake venom, the effect of proteins is multifunction including the blood coagulation, regulation of blood pressure and cleave some plasma proteins of victims.[9] The present study aimed to study the effects of crude venom of the Echis carinatus sochureki (Said Dakhil Snake) venom and study its effects in Biochemical parameters in rats over a period of time after venom injection.

MATERIALS AND METHODS

Venom collection

The venom was obtained from E. carinatus sochureki. Snakes were kept in a serpent arum at the Biology Department/ Science College / Thi- Qar University, after being collected from Said Dakhil by a skilled professional hunter. The snakes were kept in a glass cage 50 * 50 cm, heat was provided from a 100 W lamp for a daily period of 9 h. Water was always available. Venom was milked from adult snakes, reconstituted in saline solution prior to use.

Effect crude venom on biochemical parameters in serum of rats

Experimental animals

Male and female rats aged between (8-10) weeks and weighted (250-300) g were obtained from the animal house Biology Department, Sciences College, Thi-Qar University, Iraq.

They are housed in a room at constant temperature of (20-22˚C) with 12 h light/dark cycles and fed a standard laboratory the animals supply with water and food (*ad libitum*) during the experiment.. The rats divided into three groups each group included six animals (n = 6) and were as follows.

1-The first group (control), treated I.P. with a signal dose of (0.5 ml/animal /day) of normal for saline (0.9 % Nacl).

2- The second group, treated I.P. with a signal dose of (0.04 ml / kg/ day) crude venom of *Echis carinatus sochureki* .

3- The third group, treated I.P. with a signal dose of (0.08 ml / kg/ day) crude venom *Echis carinatus sochureki*.

Serum analysis

The experiment continues for 24 hour and by two different dose throughout day. At the end of the experiment period 24 hour the animals were killed, blood was collected from each animal into plain centrifuge tubes, at room temperature for clotting. Serum was separated by centrifugation at 3000g for 30 min and analyzed, for the concentration of total protein, albumin, urea, Creatinine, glucose, cholesterol and triglycerides determination.

Statistical Analysis

In order to compare between the control and envenomated group, a Student's t-test was used. The data are presented as means ± S.E. and statistically analyzed using SPSS (version 14). Significance was set at the level of $P < 0.05$.

RESULT

The results showed a significant increases ($P<0.05$) of the means serum glucose, urea and Creatinine levels of male rats in second and third groups compared with the control group , while significant decreases ($P<0.05$) of their means of serum total protein content and albumin after of male rats in second and third groups compared with the control group (table 1). The results showed a significant decreases ($P<0.05$) of their means off cholesterol, triglycerides and HDL of male rats in second and third groups compared with the control group, while significant increases ($P<0.05$) of the means serum LDL and VLDL levels of male rats in second and third groups compared with the control group (table 2). The a significant increases ($P<0.05$) of their means of ALP, ALT and AST of male rats in second and third groups compared with the control group (table 3). The effect of crude venom of *Echis carinatus sochureki* on biochemical parameters of female rats exposed to two doses of

crude venom are presented in (table 4), the results showed a significant increases (P<0.05) of the means serum glucose, urea and Creatinine levels of female rats treatment with crude venom groups (third) compared with the control group, while significant decreases (P<0.05) of their means of serum total protein content and albumin of female rats treatment with crude venom groups (second and third) compared with the control group. The results a significant decreases (P<0.05) of their means of cholesterol, triglycerides and HDL of female rats in second and third groups compared with the control group, while significant increases (P<0.05) of the means serum LDL and VLDL levels of female rats in second and third groups compared with the control group (table 5). The results show a significant increases (P<0.05) of their means of ALP, ALT and AST of female rats in second and third groups compared with the control group (table 6).

Table (1): shows the effect of crude venom on the level of serum glucose, urea, Creatinine, albumin and total protein in male laboratory rats

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
Glucose	85.80 ± 40.78^{c}	160.20 ± 27.08^{b}	225.40 ± 28.80^{a}	38.95
Urea	37.60 ± 1.98^{c}	78.80 ± 14.15^{b}	122.20 ± 40.17^{a}	29.24
Creatinine	0.43 ± 0.03^{b}	1.00 ± 0.27^{a}	1.17 ± 0.15^{a}	0.21
Albumin	3.82 ± 0.24^{a}	2.67 ± 0.13^{b}	2.43 ± 0.29^{b}	0.27
Total Protien g/dL	7.14 ± 0.29 ^{a}	5.44 ± 0.29 ^{b}	4.05 ± 0.35^{c}	o.37

- Values are means ± S.E.
- Different letters refer to a significant difference (p≤ 0.05).
- Same letters refer to no a significant differences (p≤ 0.05).

Table (2): shows the effect of crude venom on the level of serum cholesterol, triglycerides, HDL, LDL, VLDL in female laboratory rats

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
cholesterol	75.60 ± 3.48^{a}	63.60 ± 9.17^{b}	61.40 ± 1.76^{b}	6.84
triglycerides	99.80 ± 7.34^{a}	95.0 ± 7.38^{a}	64.80 ± 3.38^{b}	7.51
HDL	25.40 ± 0.55^{a}	23.80 ± 2.83^{b}	8.80 ± 1.58^{c}	2.26
VLDL	12.96 ± 0.68^{b}	18.05 ± 1.48^{b}	19.96 ± 1.47^{a}	1.58
LDL	23.04 ± 1.98^{b}	25.77 ± 7.81^{b}	46.84 ± 4.08^{a}	6.50

- Values are means ± S.E.
- Different letters refer to a significant difference (p≤ 0.05).
- Same letters refer to no a significant differences (p≤ 0.05).

Table (3) Enzyme values in serum of male laboratory rats 24hr post-administration of Echis carinatus crude venom by I . p. route

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
ALP U/l	145.80 ± 21.69^{c}	242.20 ± 18.62^{b}	334.20 ± 62.41^{a}	41.08
ALT IU/L	41.60 ± 5.80^{b}	47.40 ± 0.84^{b}	95.80 ± 34.37^{a}	20.91
AST IU/L	225.80 ± 17.72^{b}	233.0 ± 26.12^{b}	464.80 ± 96.06^{a}	69.35

- Values are means ± S.E.
- Different letters refer to a significant difference (p≤ 0.05).
- Same letters refer to no a significant differences (p≤ 0.05).

Table (4) shows the effect of crude venom on the level of serum glucose, urea, Creatinine, albumin and total protein in female laboratory rats

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
Glucose	95.67 ± 15.64^{c}	134.83 ± 19.01^{b}	185.33 ± 15.15^{a}	19.82
Urea	43.67 ± 3.94^{b}	71.17 ± 24.03^{a}	97.50 ± 29.87^{a}	26.08
Creatinine	0.51 ± 0.05^{c}	0.91 ± 0.21^{b}	2.03 ± 0.45^{a}	0.34
Albumin	4.03 ± 0.25^{a}	2.48 ± 0.19^{b}	1.95 ± 0.20^{c}	0.27
Total Protien g/dL	6.33 ± 0.31^{a}	3.85 ± 0.35^{b}	2.73 ± 0.31^{c}	0.39

- Values are means ± S.E.
- Different letters refer to a significant difference (p≤ 0.05).
- Same letters refer to no a significant differences (p≤ 0.05).

Table (5) shows the effect of crude venom on the level of serum cholesterol, triglycerides, HDL, LDL, VLDL in female laboratory rats

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
cholesterol	66.50 ± 4.98^{a}	57.67 ± 13.08^{b}	35.0 ± 6.87^{c}	10.72
triglycerides	112.0 ± 21.50^{a}	74.50 ± 6.82^{b}	48.67 ± 9.91^{c}	17.74
HDL	10.20 ± 1.92^{a}	9.67 ± 2.27^{ab}	7.50 ± 1.21^{b}	2.20
vLDL	8.07 ± 1.75^{c}	14.95 ± 1.44^{b}	22.40 ± 4.30^{a}	3.48
LDL	9.60 ± 5.70^{c}	24.35 ± 2.73^{b}	47.27 ± 11.66^{a}	7.55

- Values are means ± S.E.
- Different letters refer to a significant difference (p≤ 0.05).
- Same letters refer to no a significant differences (p≤ 0.05).

Table (6) Enzyme values in serum of female laboratory rats 24hr post-administration of Echis carinatus crude venom by i.p. route

Parameter	The first group Control	The second group 0.04	The third group 0.08	L.S.D
ALP U/l	58.17 ± 9.13^{c}	101.33 ± 37.76^{b}	166.20 ± 15.37^{a}	28.65
ALT IU/L	25.67 ± 4.21^{c}	40.20 ± 6.52^{bc}	60.67 ± 21.80^{a}	15.87
AST IU/L	146.50 ± 17.58^{b}	168.17 ± 14.58^{b}	290.0 ± 130.64^{a}	90.95

- Values are means ± S.E.
- Different letters refer to a significant difference ($p \leq 0.05$).
- Same letters refer to no a significant differences ($p \leq 0.05$).

DISCUSSION

Several works dealing with the effects of snake venoms in blood cells, marrow cells and in cells from other organs of animals, like muscle, liver and kidney, showed varying results, depending on the experimental concentrations, exposure time, site of injection, and type of toxin.[10, 11] The increases in serum glucose levels could be attributed to the effects of the venom on glycogen metabolism in the hepatocytes, muscle fibers and medullary catecholamines that timulate glycogenolysis and gluconeogenesis in those tissues.[12]

Such increased vascular permeability, together with, renal damage would further aggravate the accompanying hypoproteinemia and hypoalbuminaemia. Furthermore, the rise in serum urea and Creatinine associated with the reduction of serum uric acid level observed, in the present study, supports the proposed impairment of renal function.[13]

The liver is a major producer for most of serum proteins and its total level in the blood is a main liver function test. It is established that liver is the main source of plasma albumin. Decrease in plasma albumin is mainly due to the diminishing 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.[14] Proteases play an important role in biotechnological industry because of their usefulness as biochemical reagents or in the manufacture numerous products.[15]

The decrease in total cholesterol level may be resulted from protease effect on the cell membranes. They contain the ratio of cholesterol. The cell membranes release cholesterol when they damage.[16]

Variations in serum physiological parameters can be used as biomarkers for monitoring the functions of vital organs of envenomed victims. The reduction in total serum cholesterol, triglycerides, HDL and the rise in total serum Creatinine in envenomed rats are in accordance with observations of other investigators in this field.[17] The observed effects upon those parameters might suggest that the snake venom could have escaped into circulation and disturbed protein synthesis in hepatocytes in vital organs leading to protein loss. Similar observations were reported following various viper envenomation of rats.[18]

In the present study the rise in serum urea and Creatinine levels indicates impairment of renal function. Similar observations were reported in rats following dministration of various viper venoms.[12] High levels Creatinine indicate several disturbances in the kidney.[19] The venom lowers triglycerides due to the work of the poison on the lipoproteins in the plasma membrane and taken triglycerides of adipose tissue.[20]

In the present study, the elevated activity of ALT, AST might indicate liver and other vital organ damage brought about by the venom. Such findings are in agreement with those reported for.[21] The reduction of other enzyme activities could be due to renal damage as well as to the inhibition of their activities caused by the venom.

When the enzymes of liver increase in serum, the damage causes in cytoplasm membrane and mitochondria membrane. This activity raises very clear until if the few cells are damaged because the liver cells contain high concentration of AST and ALT.[23] In the present study , the elevated activity of ALT might indicate liver and other vital organ damage brought about by protease.[24]

The present study showed a decrease the level of cholesterol Such findings are in agreement with those reported for.[25] the hyperthyroidism thyroid accompanied by a decrease in fat and fatty proteins such as cholesterol levels because of the increase taken by the liver.

On the other hand, the injection of protease caused a significant rise in serum AST and ALT in rats which were accompanied with brutal injuries and necrosis of hepatocytes as well as a nephrotoxic action of the venom as reported by the reference.[26]

Protease breaks down the LDL receptors in liver cells causing increase in LDL level because conversion of VLDL to LDL.[17]

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