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Effect of crude venom of *Echis carinatus sochureki* snake on hematological parameters of male and female rats

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ABSTRACT

The present study aimed to investigate the effect of crude venom of *Echis carinatus sochureki* snake on hematological 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. Animals killed within 24 hours. Results indicated a significant decrease ($P \leq 0.05$) in red blood cell count (RBC)), packed cell volume (PCV), mean corpuscular volume (MCV), total white blood cell count (WBC), lymphocytes, acidophils, neutrophils and monocytes count in male and female rats of second and third groups compared with the control group. The results showed non-significant differences ($p \leq 0.05$) in mean corpuscular hemoglobin (MCH), mean concentration corpuscular hemoglobin (MCHC) and basophils count between the three groups (first, second and third) in male and female rats. Also, it showed a significant increase ($P \leq 0.05$) in the number of platelets in second and third groups of female rats compared with the control group, while the number of platelets in male rats increased significantly in third group compared with the first and second groups.

Keywords: *Echis carinatus sochureki*, Blood 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. 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]. Life; helped the lack of agriculture, water scarcity and desertification conditions, to spread. This type of snakes is found in different substrates including; sand, rocks, soft soil and in scrublands. It is often found hiding under loose rocks, this species is mostly crepuscular and nocturnal, although there have been reports of activity during daylight hours [5].

E. carinatus venoms are complex mixtures; mainly it has proteins, which have enzymatic activities. Protein and peptides make 90 to 95 percent of the dry weight of venom. The enzymes form the large ratio from the snake venom including acetyl cholinesterase, L-amino acid oxidase, metalloproteinase, Phosphodiesterases and serine protease [6]. In addition to that snake venoms contain inorganic cations such as sodium, calcium, potassium, magnesium and small amounts of zinc, nickel, cobalt, iron, manganese. Zinc is necessary for anti-cholinesterase activity; calcium is required for activation of enzyme like Phospholipase. Some snake venoms also contain carbohydrate, lipid, biogenic amines, and free amino acids [7]. Envenoming resulting from snake bites remains the

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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 [8]. Generally envenoming by *Echis* snake vipers is responsible for several clinical complications of severe systemic and local pathology [2].

Bleeding is a major manifestation of viper bite and may occur from multiple sites including gums, nose, gastrointestinal tract, urinary tract, injection sites and even as multiple petechiae and purpurae over skin [9].

One of the major targets of snake venom is the somatic nervous system, in articular the skeletal neuromuscular junction. Inhibition of neurotransmission at this site results in the paralysis of bulbar and ocular muscles, as well as paralysis of respiratory muscles,5,6 the latter often resulting in death. Therefore, much research has been directed at increasing our understanding of the action of snake venoms and isolated toxins at the neuromuscular junction [8].

For example, hemorrhagic snake venom is composed of a variety of bioactive proteins such as phospholipase A2, metalloproteases, and disintegrins, which disrupt surrounding tissues. Loss of contact with extracellular matrix or basement membrane proteins may initiate ubstrate dependent cell death programming, involving intracellular ROS elevation. Because metallothioneins have the capacity to bind up to seven zinc atoms, they have been implicated in zinc trafficking, where changes in intracellular redox potential promote the release of zinc [6].

According to wide-spread of *Echis carinatus sochureki* (Said Dakhil Snake) which caused death of some people, the present study aimed to investigate the effect of crude venom on hematological parameters of male and female rats .

MATERIALS AND METHODS

Venom collection: The venom was obtained from *Echis carinatus sochureki*. Snakes were kept in a serpent arum at Department of Biology, College of Science, University of Thi-Qar, 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 collected from adult snakes, reconstituted in saline solution prior to use.

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 rat diet and water *ad lbitum* . 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* .

The blood parameters were measured by using coltter in the laboratory of Hussain hospital in Thi-Qar, Iraq. The blood samples were collected by EDTA tubes, and analyzed to determine of hematological parameters such as a red blood cell count (RBC), the packed cell volume (PCV) and the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and the total of white blood cells (WBC) by using an automatic hematological assay analyzer (Nihon Kohden corporation, Japan). Blood smears were also stained with giemsa for differential WBC count [10].

Statistical Analysis: 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 $P \leq 0.05$.

RESULTS

The effect of crude venom of *Echis carinatus sochureki* on hematological parameters of male rats exposed to two doses of crude venom are presented in table (1), the results showed a significant decrease ($P \leq 0.05$) in red blood cell count (RBC)), packed cell volume (PCV), mean corpuscular volume (MCV) in second and third groups compared with the control group. The results showed non-significant differences ($p \leq 0.05$) in mean corpuscular hemoglobin (MCH), mean concentration corpuscular hemoglobin (MCHC) between the three groups (first, second and third), also it showed a significant increase ($P \leq 0.05$) in the number of PLT third group compared with the control and second groups.

The result showed a significant decrease ($p \leq 0.05$) in the total white blood cell count (WBC),

lymphocytes, acidophils, neutrophils and monocytes count of male rats treatment with crude venom groups (second and third) compared with the control group, while there was non-significant difference in the basophiles count compared with the control group (table 2) .

The effect of crude venom of *Echis carinatus sochureki* on hematological parameters of female rats exposed to two doses of crude venom are presented in table (3), the results showed a significant decrease (P≤0.05) in red blood cell count (RBC)), packed cell volume (PCV), mean corpuscular volume (MCV) of female rats treatment with crude venom groups (second and third) compared with the control group.

The results showed non-significant differences (p≤ 0.05) in mean corpuscular hemoglobin (MCH), mean concentration corpuscular hemoglobin (MCHC) between the three groups (first, second and third), Also it showed a significant increase (P≤0.05) in the number of platelets (PLT) in second and third groups compared with the control group.

The result showed a significant decrease (p≤ 0.05) in the total white blood cell count (WBC), lymphocytes, acidophiles, neutrophils and monocytes count of female rats in second and third groups compared with the control group, while there was non-significant difference in the basophiles count compared with the control group (table4)

Table **(**1) Effect of *Echis carinatus sochureki* venom in RBC count, blood index and platelet of male rats

Parameter	The first group **Control**	**The second group 0.04**	**The third group 0.08**	**L.S.D**
RBC (10^6/mm^3)	8.23 ± 0.25[a]	7.55 ± 0.23 [a]	6.74 ± 0.95 [b]	0.69
PCV%	48.88 ± 1.24 [a]	43.57 ± 1.19 [b]	40.13 ± 5.92 [b]	4.23
MCV(µm3)	62.93 ± 2.29 [a]	57.08 ± 2.04 [b]	55.75 ± 1.20[b]	2.26
MCH (pg)	18.08 ± 0.65 [a]	17.01 ± 0.59 [a]	16.01 ± 1028 [a]	1.06
MCHC (g/dL)	30.80 ± 0.22 [a]	31.48 ± 0.94 [a]	31.55 ± 0.55 [a]	0.76
PLT ($\times10^3$/mm^3)	110.0 ± 16.01 [b]	135.58 ± 12.18 [b]	390.83 ± 39.21 [a]	33.34

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

Table (2) Effect of *Echis carinatus sochureki* venom in total and differential WBC of male rats

Parameter	The first group **Control**	**The second group 0.04**	**The third group 0.08**	**L.S.D**
WBC (10^3/mm^3)	11.21 ± 0.59[a]	5.64 ± 0.51 [b]	3.51 ± 1.08 [b]	2.32
Neut %	29.31 ± 1.18[a]	10.56 ± 7.43 [b]	2.10 ± 0.72 [b]	13.15
Lym %	83.50 ± 2.83[a]	69.58 ± 8.81 [b]	66.97 ± 0.94[b]	3.09
Mono %	15.09 ± 6.85[a]	9.86 ± 2.16 [b]	4.03 ± 0.92[c]	4.96
Eosi %	3.23 ± 0.66[a]	1.99 ± 0.46 [b]	1.51 ± 0.40[b]	0.61
Baso %	0.36 ± 0.14[a]	0.03 ± 0.02[b]	0.02 ± 0.00[b]	0.06

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

Table **(**3): Effect of *Echis carinatus sochureki* venom in RBC count, blood index and platelet of female rats

Parameter	The first group **Control**	**The second group 0.04**	**The third group 0.08**	**L.S.D**
RBC (10^6/mm^3)	6.99 ± 0.09^{a}	4.74 ± 0.49^{bc}	4.05 ± 0.89^{c}	0.73
PCV%	38.93 ± 0.46 ^{a}	27.02 ± 3.29^{b}	22.0 ± 4.95^{c}	4.26
MCV(µm3)	55.65 ± 1.42 ^{a}	55.46 ± 1.07^{ab}	54.13 ± 0.96^{b}	1.45
MCH (pg)	18.16 ± 0.14 ^{a}	17.55 ± 0.22 ^{a}	17.19± 0.29 ^{a}	0.28
MCHC (g/dL)	32.64 ± 0.43 ^{a}	32.75 ± 1.27 ^{a}	33.70 ± 0.75 ^{a}	1.100
PLT (×103/mm3)	136.80 ± 16.81^{c}	164.50 ± 13.20^{b}	505.71 ± 21.15 ^{a}	21.51

Table **(**4) Effect of *Echis carinatus sochureki* venom in total and differential WBC of female rats

Parameter	The first group **Control**	**The second group 0.04**	**The third group 0.08**	**L.S.D**
WBC (10^3/mm^3)	9.68 ± 0.39^{a}	4.60 ± 0.54^{b}	3.41 ± 0.66^{c}	0.67
Neut %	47.23 ± 4.52^{a}	27.73 ± 6.35^{b}	24.44 ± 6.29^{b}	7.16
Lym %	65.17 ± 6.01^{a}	60.04 ± 6.08^{a}	39.18 ± 5011^{b}	7.12
Mono %	5.95 ± 1.10^{a}	4.11 ± 2.36^{ab}	3.40 ± 1.07^{b}	2.01
Eosi %	3.34 ± 0.81^{a}	3.01 ± 0.96^{ab}	2.11 ± 0.53^{b}	0.96
Baso %	0.25 ± 0.04^{a}	0.01 ± 0.02^{a}	0.16 ± 0.04^{a}	0.24

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

DISCUSSION

The results of the current study showed a reduction in blood parameters of male rats treated with crud venom extracted out of *Echis carinatus sochureki* snake, venom have an impact on blood components. The decrease of those blood parameters may be attributed to effect of venom on the membranes of blood cells, the blood ones, as other cells, are surrounded by plasma membranes made of a high rate of phospholipids and the phosphatidyl choline is the most important phospholipids [9]. The phospholipids A_2 are the target tissue for the effectiveness of the venom, indicated that increasing the level of the compound Lysophosphotidyl acid (LPA) dues to decomposition of the Phosphotidylinosetol (PI) on the surface of the red blood cells as a part of phospholipids that form membranes, the level of the compound (LPA) increases in the cases of hard inflammation resulting from increasing the concentration of venom and this compound leads to a damage against the crossing the substances through the blood cells membranes causing a swelling of the blood cells then bursting them [11]. Causes for a decrease RBCs could be exposure to extreme physiological stress such as envenomation [12]. Both RBC and platelets counts fluctuated during the experiment indicating that the process of clotting arose to resist bleeding

or haemorrhage and then declined parallel to RBCs. Thrombocytosis is seen in many inflammatory disorders, as well as in acute or chronic blood loss and haemolytic cases. On the other hand, Thrombocytopenia could occur as result of chronic infections and liver disease [11]. Protease degrades the fourth collagen completely and causes damage capillary productive the blood components leak including RBC, Noting the venom of *Echis carinatus* causes coagulation including intravascular by the components of *Echis carinatus* venom before coagulation as serine protease and metalloprotease which catalyze the start of coagulation [13].

However, there is a reducing number of WBC_s and the occurrence cases of internal bleeding. Protease attacks proteins found on surface cell membrane and consequently the cell dies [14]. The decrease is in the number of WBC and RBC , it preforms to production the free radicals by treatment protease which acts oxidation of lipids membrane , the membrane contains unsaturated fatty acids [15].

The present results showed that envenomation causes a significant increase in the MCV. This increase reached a maximum after 24 h. This might be explained in term of RBCs trying to carry the largest amount of hemoglobin as a mechanism to counteract hypoxia caused by the venom initial.

The crude venom is preferred Phosphatidyl choline as a controlled substance, that is found mostly in cell membranes of the blood cells, therefore, it leads to hemolysis of the cell membranes and blood cells [16]. In addition to the lysolecithin which results from hemolysis of lecithin by venom and causes the hemolysis of red blood cells then reduction of its number which is reflected in the reduction of hemoglobin concentration and inside other cellular components, this reduction of blood parameters dues to the effect of venom in directly on the production of harmones in charge of production of blood cells [17]. On the other hand that venom damages the cell mitochondria as well as the blood platelets, red blood cells, white blood cells, skeletal muscles, the endings of outside nerve, it also causes dissolution of blood through the decomposition of phospholipids cell membranes of the red and white blood cells [18].[19] showed that the people will have anemia and reduction of the number of the white blood cells if they have a snakes stinging, it is due to the influence of the venom in the viper venom on the membranes of the red and white blood cells then these cells will become fragile and cannot stand against the routine stress resulting from the blood circulation, or may rupture before that, causing the hemolytic anemia which may occur from the stress and inflammation or snakes and spider toxics [20].

The reduction of the total number of white blood cells and the percentage of the lymphocytes and neutrophils, may be due to the act ion of the venom to crack and destruction the membranes of the white blood cells as a result of its high relation ability with the two phospholipids layers of the cell membranes, thus it leads to a bursting of the cells membranes, therefore , they cannot to support its immune functions and have inability to respond to various cases that require their presence [21]. The high increasing in the total number of blood platelets in the groups treated to venom, especially at the high dose, may be due to the damaging of the blood vessels, increased bleeding, attacking the tissues and crashing its components and causes a bleeding of the blood vessels and ulceration of tissues, so the number of the platelets will increases to prevent blood loss, perhaps the reason is to found in enzyme effect on the endocrine glands, causing an imbalance in the secretion of the hormones in charge of formation of platelets [22].

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