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EFFECT OF ANTIVENOM OF *Echis carinatus* SNAKE ON HEMATOLOGICAL PARAMETERS AND LIVER ENZYMES OF MALE AND FEMALE RATS

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ABSTRACT

The present study aimed to investigate the effect antivenom of *Echis carinatus* on hematological parameters and liver enzymes of rats. Adult male and female rats divided into three groups for each sex (6 for each group), the first group injected (i.p.) with normal saline (0.9%Nacl) as a control group, the second group injected (i.p.) with(0.25ml/kg/day) of antivenom for two times, and the third group injected with (0.5ml/kg/day) of antivenom for two times. Animals killed within 24 hours. Results indicated a significant increase (P≤0.05) in redblood cell count (RBC), meancorpuscular hemoglobin (MCH), mean concentration corpuscular hemoglobin (MCHC) packed cell volume (PCV), mean corpuscular volume (MCV), total (WBC), lymphocytes, monocytes and granulocytes in male and female rats of second andthird groups compared with the control group. Also, it showed a significant increase(P≤0.05) in the number of platelets in third group of male and female rats compared with the first andsecond groups.Also, it showed a significant increase(P≤0.05) in the liver enzymes ALT, AST and ALP in second and third groups of male rats compared with the control group. There was significant increase(P≤0.05) in the liver enzymes ALT, AST in third group compared with the second andcontrolgroup, while showed ALPsignificant increase(P≤0.05) in third compare with the second and control groups.

KEYWORDS: Antivenom, blood parameters, liver enzymes, rats.

INTRODUCTION

Saw scale vipers or *Echis carinatus* are commonly found in the semi-arid deserts of Western Rajasthan with predominant nocturnal habitus.Envenoming resulting from snake bites remains the mostneglected public health issues in many countries, particularlyin poor rural communities living in the tropics. *Echiscarinatus sochureki* causes numerous deadly bites especially in Asia (Kochar *et al.*, 2007).

Following a viperian envenomation various local tissue alterations occur such as haemorrhage, edema and myonecrosis leading to tissue loss or organ dysfunction **(**Gutierrez, 1995). Within 15 minutes of entry into the blood stream goose pimples appear on the skin of the snakebite victim, signifying the onset of action of the secondary metabolite and vanish within seconds(Onyeama *et al.*, 2013*)*.

Snake venom is a complex mixture of organic compounds (Marsh and Williams 2005; León*et al.*, 2011). Many of these compounds produce a variety of pathophysiologicaleffects including local tissue damage and/or systemic effects in theaffected individual (León*et al.*, 2011).

Snake venoms from *Colubridae*(*sensulato*), *Elapidae*, and *Viperidae*families are rich sources of phospholipase A2s (PLA2s,phosphatidylcholine 2-acylhydrolases), there seems to be no clear relationship between the amount of enzymatic activity (lipid hydrolysis) , There have been various attempts by different research groups to elucidate the complex pathological mechanisms induced by snake venomPLA2s, particularly regarding their ability to block neuromuscular transmission and induce acute muscle damage, two activities responsible for key pathological events in humans following snakebite envenoming (Sardar *et al.*, 2014).

Antivenom snake immunoglobulins (antivenoms) are the only specific treatment for envenoming by snakebites. Antivenoms can prevent or reverse most of the snakebite envenomings effects, and play a crucial role in minimizing mortality and morbidity. Antivenom is created by milking venom from a relevant snake, spider, insect, or fish. The venom is then diluted and injected into a horse, sheep, rabbit, or goat. The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecules which can then be harvested from the animal's blood and used to treat envenomation (Theakston*et al.*, 2003).

The killed snake was identified as *Echis carinatus* by the initial attending medical officer. At the local hospital, management consisted of antibiotics, anti-tetanus prophylaxis, analgesics and 10 vials of polyvalent anti snake venom (ASV). ASV was repeated on the next two days. The only available treatment against snake bite is the usage of anti-venom. The first anti-venom was developed by Alberte Calmette against the Indian cobra (NajaNaja). Anti-venom is made by immunizing mammals such as horse, goat, rabbit with particular snake venom and the specific immunoglobins are isolated from the blood (Goswami *et al*., 2014).

Sometimes lyophilized polyvalent anti-snakevenom may cause anaphylactic reactions (Sai *et al.*,2008).The subject animal will undergo an immune response to the venom, producing antibodies against the venom's active molecule which can then be harvested from the animal's blood and used to treat envenomation. Ant venom is classified into two types. Monovalent ant venom when they are effective against a given species venom. Polyvalent when they are effective against a range of species (Paul *et al* ., 2011).Anti-venom should be stored at a temperature within the range that assures stability, as found by stability tests. This is particularly critical for liquid formulations, which usually require storage at between 2 and 8 °C (Silva *et al*., 2011).

MATERIALS AND METHODS

Experimental animals: In the present study male and female rats aged(8-10) weeks and weighting (250-300)g were obtained from the animal house Biology Department, Science College, Thi-Qar University, Iraq. They were 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), injected I.P. with two times of (0.5 ml/animal /day) of normal saline (0.9 % Nacl).

2- The second group, injected I.P. with two times of (0.25 ml / kg/ day) of antivenom of *Echis carinatus sochureki* .

3- The third group, injected I.P. with two times of(0.5 ml / kg/ day) of antivenom of *Echis carinatus sochureki*.

The antivenom was obtained from the department of health of Thi- Qar, Iraq. The antivenom was used in the present study produce by Razi vaccine and serum research institute, Islamic republic of Iran. The first injection was used at 9:00 AM, and the second injection was used after two hours.

After 24 hours from the first injection, The blood parameters were measured by using coulter in the laboratory of Hussain hospital in Thi-Qar province, Iraq. 4 ml of blood samples were collected and divided in to two parts the first part was 2 ml byEDTA 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 (Dacie and Lewis, 1984)and the second part was 2ml 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 ALT, ALS and ALP determination.

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 $P \leq 0.05$.

RESULT

The effect of antivenom of *Echis carinatus sochureki* on hematological parameters of male rats exposed to two doses of antivenom were presented in (table 1), the results showed a significant increase ($P \leq 0.05$) of RBC, HCT in the second and third groups compared with the control group. Results showed a significant increase ($P \leq 0.05$) in MCV and MCH in the third group compared with the control and second groups, also, there was a significant decrease ($P \leq 0.05$) in the MCHC in the second group compared to the first and the third groups, also there was a significant increase ($P \leq 0.05$) in the PLT in the third group compared with the control and second groups.

Table (1): Effect of antivenom on some hematological parameters of male rats

Parameters / groups	RBC ($\times 10^5$/mm^3)	HCT (%)	MCV (μm^3)	MCH (pg)	MCHC (g/dL)	PLT ($\times 10^3$mm3)
First group (control) Physiological Solution 0.5ml	7.42 ± 0.25^b	42.17 ± 0.84^b	52.67 ± 1.95^c	15.55 ± 0.21^b	29.97 ± 0.52^a	890.00 ± 154.05^b
Second group Antivenom(0.25ml)	8.49 ± 0.63^a	45.58 ± 3.43^a	55.93 ± 0.07^b	15.98 ± 0.45^b	28.43 ± 1.52^b	987.17 ± 93.01^b
Third group Antivenom(0. 5ml)	8.87 ± 0.31^a	48.13 ± 1.08^a	57.12 ± 1.45^a	17.02 ± 0.36^a	28.68 ± 0.77^a	1184.33 ± 53.79^a
L.S.D	0.54	2.66	1.82	0.44	1.28	100.26

Values are means ± S.E.

Different letters refer to a significant difference at (p≤ 0.05).
Same letters refer to non-significant differences at (p≤ 0.05).

The results showed a significant increase (P≤0.05) in total WBC, Lymphocytes and monocytes in the third group compared with the control and second groups of male rats, also there was a significant decrease (P≤0.05) in granulocytes cell in the second and third groups compared to the control group, while there was a significant increase (P≤0.05) in the second group compared with the third group (table 2).

Table (2): Effect of antivenom on total and differential WBC of male rats

Parameters / groups	Total WBC ($\times10^3$/mm^3)	Lymphocytes (%)	Monocytes (%)	Granulocytes
First group (control) Physiological Solution 0.5ml	8.82 ± 1.26^{b}	63.08 ±2.74	3.32 ± 0.70^{bc}	30. 97 ± 1.75^{a}
Second group Antivenom(0.25ml)	8.12 ±0.62^{b}	64.23 ± 1.91^{b}	3.82± 0.29^{b}	28.10 ± 2.98^{b}
Third group Antivenom(0. 5ml)	12.22 ± 1.44^{a}	68.57 ± 3.36^{a}	4.80 ± 0.29^{a}	25.15 ± 3.04^{c}
L.S.D	1.45	3.41	0.59	0.54

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

The effect of antivenom of *Echis carinatus sochureki* on hematological parameters of female rats exposed to two doses of antivenom was presented in (table 3),The results showed a significant increase (P≤0.05) in RBC and HCT in the second and third groups compared with the control group. Also, there was a significant increase in MCV in the third group compared with the control and the second groups. There was a significant increase in MCH, MCHC, PLT in the third group compared with the first and the second groups.

Table (3): Effect of antivenom on some hematological parameters of female rats

Parameters / groups	RBC ($\times 10^5$/mm^3)	HCT (%)	MCV (µm^3)	MCH (pg)	MCHC (g/dL)	PLT ($\times10^3$mm3)
First group (control) Physiological Solution 0.5ml	6.95 ± 0.63^{b}	40.67 ±3.77^{b}	58.52 ± 0.41^{c}	17.98 ± 0.36^{c}	30.17 ±2.77^{b}	977.17 ± 180.44^{b}
Second group Antivenom(0.25ml)	8.10 ± 0.51^{a}	48.65 ±3.38^{a}	60.07 ± 0.96^{b}	18.13 ± 0.13^{bc}	31.67 ±1.91^{b}	1152.33 ±52.62^{b}
Third group Antivenom(0. 5ml)	8.56 ±0.23^{a}	50.83 ± 1.56^{a}	63.17 ± 0.95^{a}	19.07 ± 0.28^{a}	33.33 ± 1.71^{a}	1477.00 ± 180.88^{a}
L.S.D	0.58	2.64	0.97	0.33	1.59	178.90

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

The results showed a significant increase (P≤0.05) in total WBC in the third group compared with the control and second groups of female rats. Also there was showed significant increase (P≤0.05) in lymphocytes and monocytes in the third group compared with the control and second groups. There was a significant decrease (P≤0.05) in granulocyte in the second and third groups compared with the control group (table 4).

Table (4): Effect of antivenom on total and differential WBC of female rats

Parameters / groups	Total WBC ($\times10^3$/mm^3)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
First group (control) Physiological Solution 0.5ml	10.92 ± 0.89^{c}	66.38 ± 2.06^{c}	2.87 ± 0.08^{c}	30.07 ± 2.09^{a}
Second group Antivenom(0.25ml)	13.03 ± 1.54^{b}	70.50 ± 2.07^{b}	3.18 ± 0.32^{b}	21.32 ± 2.70^{c}
Third group Antivenom(0. 5ml)	16.50 ±2.11^{a}	75.50 ± 2.82^{a}	3.55 ± 0.27^{a}	26.63 ± 2.09^{b}
L.S.D	1.89	2.79	0.30	2.75

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

The results showed a significant increase (P≤0.05) in the liver enzyme ALT, AST, ALP in the third group compared with the first and second groups of male rats, while there was non-significant difference between the first and second groups (table 5).

Table (5): Effect of antivenom on liver enzymes of male rats

Parameters / groups	ALT IU/L	AST IU/L	ALP U/l
First group (control) Physiological Solution0.5ml	37.24±2.01[b]	160.81 ±9.22[c]	118.59 ±16.58[c]
Second group Antivenom(0.25ml)	37.05±2.05[b]	190.75 ±17.61[b]	149.33±21.87[b]
Third group Antivenom(0. 5ml)	42.30 ±2.37[a]	216.11 ±25.44[a]	195.80±4.11[a]
LSD	2.68	23.25	19.98

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

The results showed a significant increase (P≤0.05) in the liver enzymes ALT, AST, ALP in the third group compared with the control and second groups of female rats, while there was no significant difference between the first and second groups (table 6).

Table (6): Effect of antivenom on liver enzymes of female rats

Parameters / groups	ALT IU/L	AST IU/L	ALP U/l
First group (control) Physiological Solution0.5ml	38.35±2.08[b]	156.17 ± 8.85[c]	145.20± 11.46[c]
Second group Antivenom(0.25ml)	40.36 ± 2.84[b]	193.60 ± 17.01[b]	175.00 ± 19.22[b]
Third group Antivenom(0. 5ml)	60.41 ± 10.92[a]	228.93± 28.96[a]	201.16 ± 8.63[a]
LSD	9.37	27.90	21.57

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

DISCUSSION

Antisnake venom remains the specific antivenom for snake venom poisoning. They containimmunoglobulins, which frequently caused complement mediated side effects, and other proteins that cause serum sickness and occasionally, anaphylactic shock. Side effects of anti-venomanaphylactic reactions such as difficulty in breathing, reddening of skin, swelling of eyes and face, fever, pyrogen reaction probably due to the action of high concentrations of non-immunoglobulin proteins , inflammation of joints and enlargement of lymph gland (Paul *et al*, 2011; Deshpande *et al.*, 2014). Antivenom affects on the kidneys leading to increased secretion of erythropoietin, which increases the formation of blood cells(Paul *et al*., 2011). The increase MCV- MCH-MCHC related with the antivenom effects on the red blood cells which affected on this indicators (Kalyan *et al.*, 2010). The significant increase in blood platelet count related with the effect antivenom which represent one of the inflammatory responses concurrent with increase in the number white blood cells (Theakston *et al*., 2003).

The injected male and female rats with antivenom causes inflammation of the liver, kidney leading to increase in the total WBC as a means of defense (Kalyan *et al.*, 2010). The significant increase in total WBC have a natural reaction to the entry of foreign objects such as the antivenom by its injection into the body because the white blood cells are considered mainstay of the immune system, and this increase stimulates the bone marrow to produce new cells of WBC (Erdei *et al* ., 2004; Segura *et al* ., 2013). Also, the significant increase of total WBC, granulocytes and lymphocyte may be as result of effect of antivenom on the liver, kidney and other organs (De Francisco *et al* ., 2009).

The present study showed increase of ALT, AST and ALP in treatment groups. This increasing may be to the effect of antivenom on hepatocytes which causes the increasing of liver enzymes in the bloodstream (Deshpande *et al*., 2014). The increase of liver enzymes in serum considered a marker for the damage 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. In the present study, the elevated activity of ALT might indicate liver and other vital organ damage brought about by antivenom (Momoh *et al* ., 2012).

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