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Effect of antivenom of *Echis carinatus* snake on sex hormones, immunological and sperm parameters of male rats

Sami J. Al-Maliki [1], Khalid G. Al-Fartosi [2], Baida R. Ali[1*]
1.Department of Biology, College of Education for pure Sciences, University of Basrah, Iraq
2.Department of Biology, College of Science, University of Thi -Qar, Iraq

ABSTRACT

The present study aimed to investigate the effect of antivenom of *Echis carinatus sochureki* snake on sex hormones, immunological and sperm parameters of male rats. Adult male rats divided into three groups (6 for each group), the first group injected (I.P.) with normal saline (0.9%Nacl) as a control group, the second group injected 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. The results indicated a significant increase ($P<0.05$) in the level of FSH, LH and testosterone in second and third groups compared with the first group. IgG, IgM and IgA increased significantly in male and female rats of second and third groups compared with the control group within 24 hr. The injection of antivenom of the *Echis carinatus* induced a significant decrease in sperm countof second and third groups compared with the control group, and

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*Corresponding Author Email: khalidalfartosi@yahoo.com
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INTRODUCTION

Echis carinatus commonly called the saw-scaled viper is a venomous viper species found in parts of the Middle East and Central Asia, and especially the Indian subcontinent. The occurrence of local and systemic snake bite related symptoms is directly related to the toxicity of the venom. 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 (Afrasiab *et al*.,2012; Lakhotia *et al*., 2014)[1,2].

The venom of *Echiscarinatus* (Saw-scaled viper) is a venomous snake belonging to the family viperidae, these vipers have an extensive geographical distribution , envenoming by *E. carinatus* is the leading cause of death and morbidity in Africa due to snakebite (Sun *et al* ., 2009; Rama *et al.*, 2010)[3,4].

Antivenom antibodies present in immunized humans or animals may down regulate the antibody response produced in subsequent contacts with the venom, This down regulation might influence the production of antivenom antibodies, thus implying that different immunization schedules may affect the extent of antivenom antibody production in immunized animals (Heyman, 2003; Brady, 2005)[5,6]. Antivenom produced in Australia is the only specific treatment for envenomation by these venomous snakes. The decision to use antivenom should be based on the patient's history, examination and pathologic findings, and the type of antivenom used will depend on geographic, clinical and pathologic factors (malasit *et al*., 1986)[7]. Antivenom is only indicated if there is clear evidence of systemic envenomation, and it should be given by the intravenous route. As antivenoms are animal products, acute and delayed adverse reactions (anaphylaxis and serum sickness) can occur and premedication should be considered. The patient should be monitored for their response to antivenom and multiple doses may be required. Other treatments such as clotting factor replacement, supplemental oxygen and, sometimes, mechanical ventilation may be indicated (Pradeep and Kumar, 2011)[8].

Victims of snake bites are often subjected to cutaneous or conjunctival hypersensitivity testing before being given antivenom. The incidence and severity of early reactions was the same whether antivenom was given by intravenous injection over 10 minutes or diluted and given as an intravenous infusion over 30 minutes. Although antivenom activated complement in vitro, there was no evidence of complement activation or formation of immune complexes in patients bitten by snakes who were treated with antivenom, whether or not they developed early reactions (WHO, 2010)[9]. Higher doses of antivenom might induce the complement activation and formation of immune complexes (aggregates) that have been observed during

the clinically more severe reactions associated with homologous immunoglobulin treatment (Chanhome *et al*., 2002)[10].

MATERIALS AND METHOD

Experimental animals

The present study aimed to investigate the effect of antivenom of *Echis carinatus sochureki.* Male rats aged (10-12) weeks, and weighting (250-300) gm were used in study. The rats obtained from 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 for each six animals (n = 6) for each group as following:-

The first group (control) injected I.P. with two times of (0.5 ml/animal/day) of normal saline (0.9 % Nacl).

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

The third group, injected I.P. with two times of (0. 5 ml / kg/ day) of antivenom *Echis carinatus sochureki* .

The antivenom was obtained from Thi- Qar health office, 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. Blood samples and serum analysis.

Serum analysis

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 measurements of FSH, LH and testosterone level. Also IgG, IgM and IgA measured.

Sperm count

Method of Vega *et al.* (1988)[11] was used in sperm count by taking the right epididymis of rat and cut into very small parts in one milliliter of Phosphate buffered saline (PBS, PH = 7.2).

Sperm malformations

Wyrobek and Bruce (1975)[12] method was used for the purpose of the study of malformations of sperm by taking the left epididymis and cut into very small pieces in a Petri dish contain on 5 ml of Phosphate buffered saline (PBS).

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 AND DISCUSSION

The results indicated a significant increase (P≤0.05) in FSH and LH levels in the second and third groups compared with the control group. Also, there was a significant increase (P≤0.05) of testosterone level in the third group, compared with the control and second groups, while the results showed non-significant difference in testosterone level between the second and control groups(table1).

The available research indicates that antivenom exposure is clearly associated with the following: increased levels of FSH and LH. The pathogenesis of renal lesions is complex involving both the direct action of antivenom on the kidney and the inflammatory effects due to the release of various endogenous cytokines and mediators, stimulates hypothalamus-pituitary and immune axes to increase adrenocorticotrophic hormone, corticosteroid(Queen *et al*., 2006)[13].

Table 1: Effect of antivenom on sex hormones of male rats

Parameters Groups	FSH	LH	Testosterone
First group (control) Physiological Saline 0.5ml	1.90 ± 0.19[b]	0.80 ± 0.06[b]	1.12 ± 0.13[b]
Second group Antivenom (0.25)	2.32 ± 0.35 [a]	0.84 ± 0.02 [a]	1.17 ± 0.22[b]
Third group Antivenom (0. 5)	3.17 ± 1.00[a]	0.85 ± 0.07 [a]	2.17 ± 0.72[a]
LSD	1.17	0.01	0.44

□□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).

Results recorded a significant increase (P≤0.05) in IgG in the second and third groups compared with the control group, while not shown any significant difference between the second and third groups. Also, there was a significant decrease in the second group compared with the third group. Also there was a significant increase (P≤0.05) in IgM and IgA in the second and third groups compared with the control group(table 2).

Although antivenom activated complement in vitro, there was no evidence of complement activation or formation of immune complexes in male rats, whether or not they developed early reactions (WHO, 2010)[9.] Higher doses of antivenom might induce the complement activation and formation of immune complexes (aggregates) that have been observed during the clinically more severe reactions associated with homologous immunoglobulin treatment (Chanhome *et al*., 2002)[10]

Higher doses of antivenom might induce the complement activation and formation of immune complexes (aggregates) that have been observed during the clinically more severe

reactions associated with homologous immunoglobulin treatment (Chanhome *et al*., 2002; WHO, 2010)[9.10].

The antivenom was sufficiently antigenic to generate a good immune response in the animals, the immune response was mature and the antibodies produced were essentially immunoglobulins G from the secondary response (Choo Hock *et al.*,2012)[14]. Polyspecific antivenom obtained from a horse immunised with both venoms gives better protection. In some cases, it also appears that a pool of antivenoms may act synergistically to induce the optimum immunological response(Kalyan *et al.*, 2010)[15].

The human humoral response to the antivenom is involved in most of the adverse reactions caused by the antivenom, so it is desirable to keep it to a minimum (Morais and Massaldi, 2009)[16]. This probably means that the presence of antivenom antibodies is not sufficient to produce serum sickness. The correct ratio of antivenom antibodies and antivenom is essential to produce immunocomplexes, This means that probably exist a correlation between amount of injected antivenom and antibody response(Caron *et al* ., 2009)[17].

When giving the antidote male laboratory rats was observed rise in the level of immune globulin because it works as an immune as the natural protein and what is a multi-saccharide and this immune globulin producing cellular mediated immune responses (Charles *et al* ., 2001)[18].These results strongly suggest that one mechanism of the cellular immune response after injury in a variety of tissues is primarily mediated by neutrophils and macrophages, and is responsible for clearance of tissue debris and wound healing (Fairweather, 2007)[19].

Table 2: Effect of the antivenom on immunological parameters of male rats

Parameters **Groups**	**IgG**	**IgM**	**IgA**
First group (control) Physiological Saline 0.5ml	3207.50 ± 48.06 [b]	56.00 ± 2.29 [c]	51.50 ± 1.95[c]
Second group Antivenom (0.25)	3228.33 ± 101.03 [a]	59.33 ± 2.59 [b]	68.83 ± 3.18[b]
Third group Antivenom (0. 5)	3407.67 ± 14.12 [a]	69.50 ± 1.82 [a]	79.33 ± 5.110[a]
LSD	181.52	2.91	3.64

□□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 sperm count of the second and third groups compared with the control group. Also, there was a significant decrease in sperm malformations of the second and third groups compared with the control group, while, there wasnon-significant difference in the second compared with the third groups(table 3).

In the present study, antivenom significantly increased sperm motility and viability in both experimental groups as compared with the control group. Also, significantly decreased sperm malformations. In according with these results, have demonstrated that antivenom treatment increased the activities of testicular antioxidants enzyme and restore sperm count and motility of rats, the present study showed an increase serum testosterone levels and accumulations of sperm in the lumen of seminiferous tubules (Arash *et al.*, 2009)[20].

Antidote reduces the formation of free radicals, which reduces the breaking down fatty acids saturated and proteins in these membranes leading a rise in the sperm count and decease sperm malformations (Chanhome *et al.*, 2002)[10].

Table 3: Effect of antivenom on sperm of male rats

Parameters / Groups	The sperm count *10^4	Sperm malformations
First group (control) Physiological Saline 0.5ml	459.17 ± 10.10^{c}	22.33 ± 2.01^{a}
Second group Antivenom (0.25)	2041.67 ± 223.02^{b}	18.93 ± 0.84^{b}
Third group Antivenom (0. 5)	4128.33 ± 277.43^{a}	19.30 ± 0.89^{bc}
LSD	256.46	0.42

□□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).

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