

**STUDY THE IMPACT OF ANTIVENOM OF *Echis carinatus*
SNAKE ON BLOOD PARAMETER, ENZYMES AND SEX
HORMONES PARAMETERS IN WISTER ALBINO MALE
RATS**

<https://doi.org/10.32792/utq/utj/vol13/1/4>

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ABSTRACT

In the current study the authors aimed to investigate the effect of anti-venom of *Echis carinatus* on hematological and hormones parameters as well as explore its influence on liver enzymes of male rats. Mature adult rats were divided into three groups 6 animals for each group. IP injected method was used. First group (Control group) injected with normal saline (0.9%Nacl), Two group injected with (0.25ml/kg) of anti-venom for two times, and the third group injected with (0.5ml/kg) of antivenom for two times. Animals killed within 24 h.

The outcome of the current study showed that there are statistically different in RBC, MCH, MCHC, PCV, MCV, total WBC, lymphocytes, monocytes and granulocytes among second and third groups compared with control group ($P \leq 0.05$). In addition, the results show a significant increasing the number of platelets and liver enzymes ALT, AST, ALP in the highly-treated group comparing with the control. Also, level of FSH, LH and testosterone likewise increased in the treated groups comparing with control. Anti-venom reduced sperm count in male rats.

Keywords: Antivenom, blood parameters, liver enzymes, sex hormone, rats.

Introduction

Saw scale vipers or *Echis carinatus* are prevalence in the semi-arid deserts of Western Rajasthan with predominant nocturnal habitus. Poisons and death from snake bites remains as public health issues in many countries, particularly in poor rural societies living in the tropics. *Echis carinatus sochureki* is known by deadly bites especially in Asia (Kochar *et al.*, 2007).

Many local tissue influenced by snake venom and lead to alternation in these tissues including haemorrhage, necrosis and edema which may extend to tissue loss or organs impairment (Gutierrez, 1995). The effect of venom would be developed very quickly which making the action of specific anti-venom is very complex, especially if there are any delay in the treatment facilities (Onyema *et al.*, 2013).

Organic compound mixtures are the main components of the Snake venom (Marsh and Williams 2005; León *et al.*, 2011). Many of these compounds produce a variety of pathophysiological effects. Viperid snake venom are rich with cysteine secretory proteins, L-amino oxidases (LOA), peptides, growth factors, C-type lectins, phospholipase A2s, phosphatidylcholine 2-acylhydrolases and snake venom metalloproteinase (SVMP) (León *et al.*, 2011).

There have been various attempts by different research groups to elucidate the complex pathological mechanisms induced by snake venom PLA2s, 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 (*Naja Naja*). Anti-venom is made by immunizing mammals such as horse, goat, rabbit with snake venom and the specific immunoglobins are isolated from the blood (Goswami *et al.*, 2014).

Sometimes lyophilized polyvalent anti-snake venom 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). This study provides the possibility to describe and clarify a series of details on the information about and some questions which have remained unanswered about using some anti-venom as vaccine against snakes.

Materials and Methods

Experimental animals: *Wister Albion* male rats at 8 to 10 weeks and at weight ranged from 250 to 300 g were gained from the animal house Biology Department, Science College, Thi-Qar University, Iraq. The animals were kept at a room temperature of (20-22°C) with 12 h light/dark cycles and fed a standard laboratory rat diet and water *ad libitum*. In the beginning of the experiment, rats randomly distributed into three groups. Each group has sex animals. The three groups received through I.P. injection as following; Control group (A) injected of (0.5 ml/animal) of normal saline (0.9 % NaCl), the treatment groups include second group (B) which injected with (0.25 ml / kg) of anti-venom, third group (C) injected within (0.5 ml / kg) of Anti-venom.

The anti-venom of *Echis carinatus sochureki* which has been used in

this experiment was acquired from the department of health of Thi-Qar, Iraq. It is produced by Razi vaccine and serum research institute, Islamic republic of Iran. Each group get two injections; two hours' period between first and second injections.

24 hours from the first injection, animals were scarified and blood samples were collected through cardiac puncture. The blood samples divided in to two parts the first part was isolated in EDTA tubes for hematological parameters and second parts of blood collected into plain centrifuge tubes at room temperature for clotting. Serum was separated by centrifugation at 3000g for 30 min and utilized for liver enzymes and hormones measurements.

EDTA blood tube used for measuring 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). All the tests done in laboratory of Hussain hospital in Thi-Qar province, Iraq. blood smears were also stained with giemsa for differential WBC count according (Dacie and Lewis, 1984).

Serum isolated from blood used to estimate the concentration of ALT, ALS and ALP enzymes. It is also being used for FSH, LH, and testosterone determinations. All the enzymes and hormones measurement likewise done in laboratory of Hussain hospital in Thi-Qar province, Iraq under supervision of very professional doctors there and after changing some instrument setting for animal's blood measurements.

During animal's dissection, right epididymis was harvested and cut into very small pieces and kept in petri dish contain 1 ml of phosphate buffered saline (PBS). Number of sperm counted based on method described somewhere (Vega et al., 1988).

Additionally, Left epididymis collected and cut to small slices and saved in dish counting 5ml of PBS for sperm malformations measurement as pronounced in Wyrobek and Bruce paper in 1975.

Statistical analysis

The data that present in the current study is calculated in SAS 9.3 or Graph Pad prism 6. The statistics different which stated in the present study

University of Thi-Qar Journal Vol.13 No.1 Mar 2018

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were analyzed utilizing tow way ANOVA followed by Bonferroni. A p-value of <0.05

Result

The outcome of anti-venom introduced in tow doses to healthy male rats showed some influence on hematological parameters that represent by a no significant change in RBC in group A (7.42 ± 0.25) comparing to animals treated with 0.25ml/kg group B (8.42 ± 0.63) and group treated with 0.5ml/kg group C (8.87 ± 0.31), HCT and MCV showed significant increase in group B (45.58 ± 3.43), (55.93 ± 0.07) and C (58.13 ± 1.08) (57.12 ± 1.45) comparing with group A (42.17 ± 0.84) (52.67 ± 1.95) respectively ($P \leq 0.05$). MCH level elevated in group C (17.02 ± 0.36) comparing to group A (15.55 ± 0.21). The results also showed that there was no a significant change in the MCHC Figure (1A) ($P \leq 0.05$), PLTs' level significantly increased in the third group (C) (1184.33 ± 53.79) comparing with the control A (890.00 ± 154.05) and second groups (B) (987.17 ± 93.01) Figure (1B).

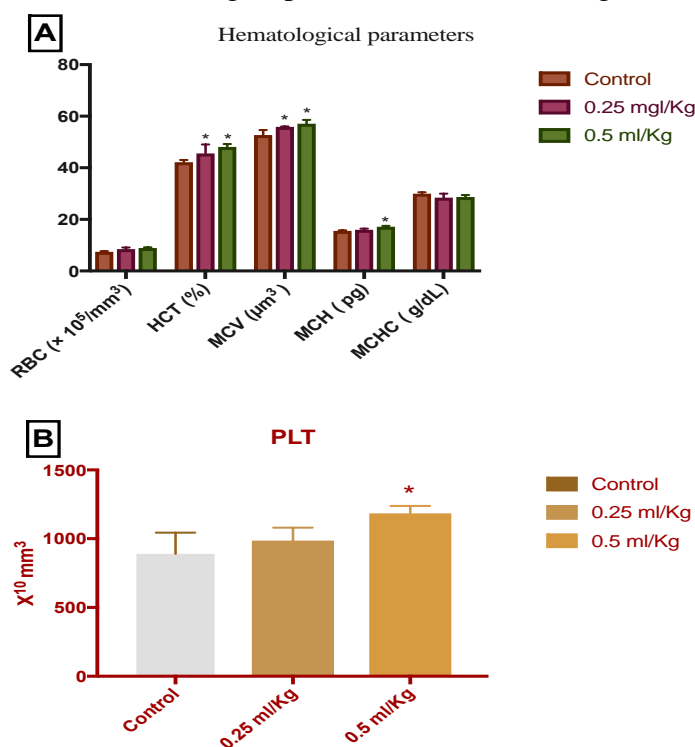


Figure 1: effect of anti-venom on hematology parameters. The anti-venom showed significant effect on HCT and MCV while there are no different in

RBC and MCHC (A) PLT displayed significant increase at 0.5ml/Kg only (B) ($P \leq 0.05$).

On the other hand, the results from current study exhibited a significant increase in total WBC (8.82 ± 1.26), Lymphocytes (63.08 ± 2.74) in animals treated with 0.05ml/Kg group C (12.22 ± 1.44), (68.57 ± 3.36) and there is no alternation in these parameters among animals treated with second groups of male rats, also there was a significant decrease in granulocytes cell in the second and third groups compared to the control group. Monocytes (3.32 ± 0.70), (4.82 ± 0.29), (4.80 ± 0.29) values doesn't effected by the anti-venom treatments figure (2).

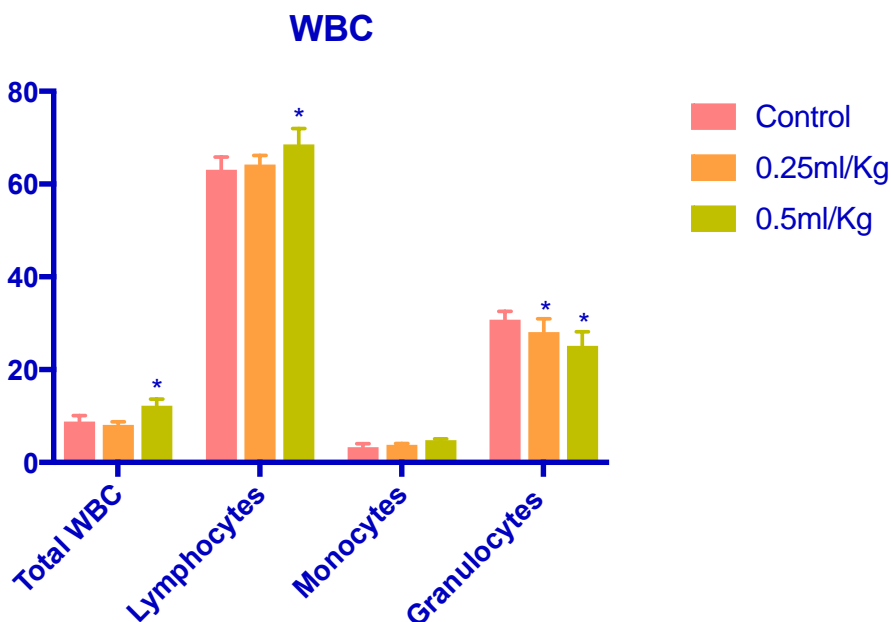


Figure 2: Effect of anti-venom on WBCs. The anti-venom showed significant increase in total WBC and lymphocytes. Reduction in granulocytes displayed significant at 0.5ml/Kg only (B) ($P \leq 0.05$).

The effect of anti-venom of *Echis carinatus sochureki* on liver enzyme's included ALT, AST, ALP exhibited that no influence can be seen in ALT (37.24 ± 2.01) value comparing with group B (37.05 ± 2.05) and group

C (42.30 ± 2.37). AST (160.81 ± 9.22) analysis showed that significant increase in at 0.25ml/Kg anti-venom treatment (190.75 ± 17.610) as well as at 0.5ml/Kg (216.11 ± 25.44). ALP level (118.59 ± 16.58) also measured in the current study to illustration that there is significant increase at group B (149.33 ± 16.58) and even higher at group C (195.80 ± 4.11) see figure (3) ($P \leq 0.05$).

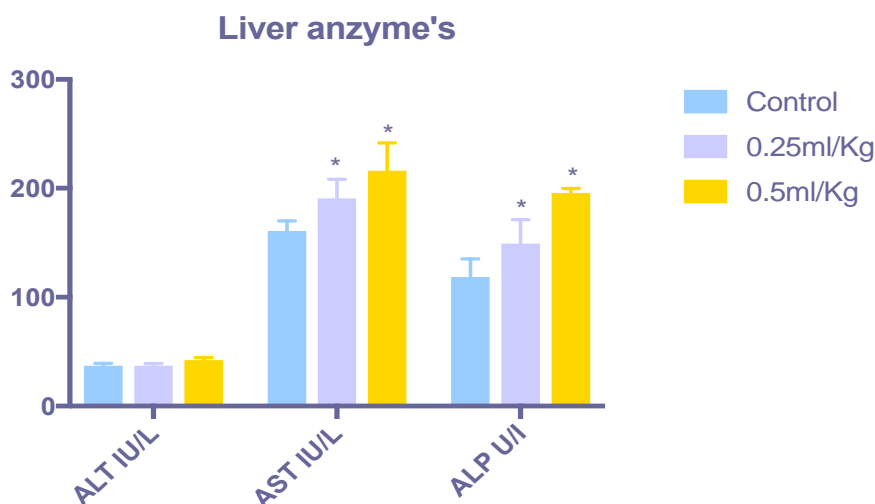


Figure 3: liver enzymes influenced after anti-venom introduced to male rates ($n=6$). Significant increase at 0.25ml/Kg and 0.5ml/Kg treatment in value of AST and ALP but there is no change in ALT level at any treatments. ($P \leq 0.05$)

Moreover, the results of present study indicated a significant raising in level of FSH hormone's (1.90 ± 0.19) comparing with 0.5ml/Kg (3.17 ± 1.00) but no at 0.25 ml/Kg anti-venom exposure (0.80 ± 0.06). LSH value did not effected with any treatments of anti-venom. While testosterone level (1.12 ± 0.13) showed no repetition at group B (1.17 ± 0.22) but in there is clear elevation at group C (2.17 ± 0.72) see figure (4) ($P \leq 0.05$).

In additions to sex hormones, anti-venom stimulus sperm count (459.17 ± 10.10) at 0.25 ml/Kg treatment (2041.67 ± 223.02) and at 0.5ml/Kg (4128.33 ± 277.43) figure (4). Anti-venom exposure inhibited sperm malformation and increased sperm motility and viability. Sperm

malformation was (2.01 ± 0.33) then increased with both experimental groups (0.84 ± 0.93) , (0.89 ± 0.30) respectively figure (5) ($P \leq 0.05$).

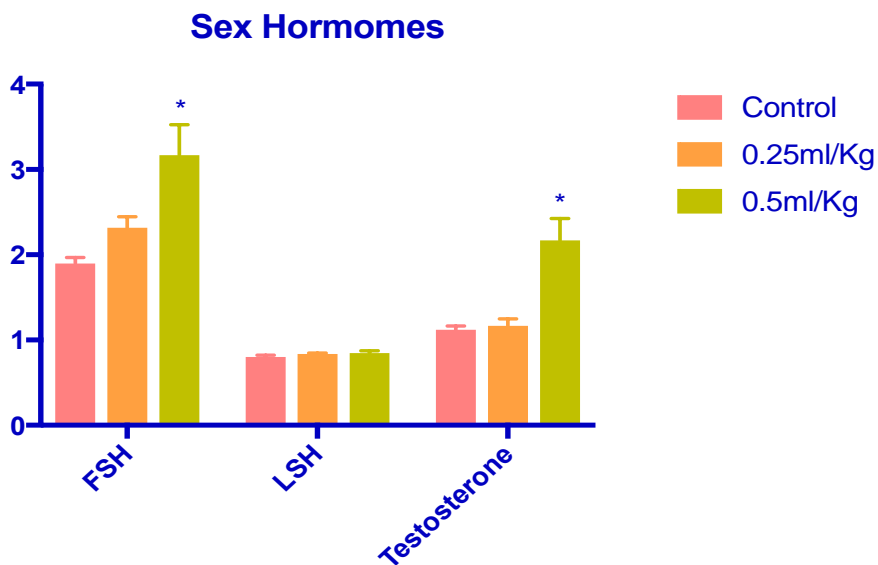


Figure 4: Anti-venom impact on male hormones level. It would be easily see the increase in level of FSH and Testosterone at 0.05 ml/Kg group only. No significant different with level of LSH. $P \leq 0.05$

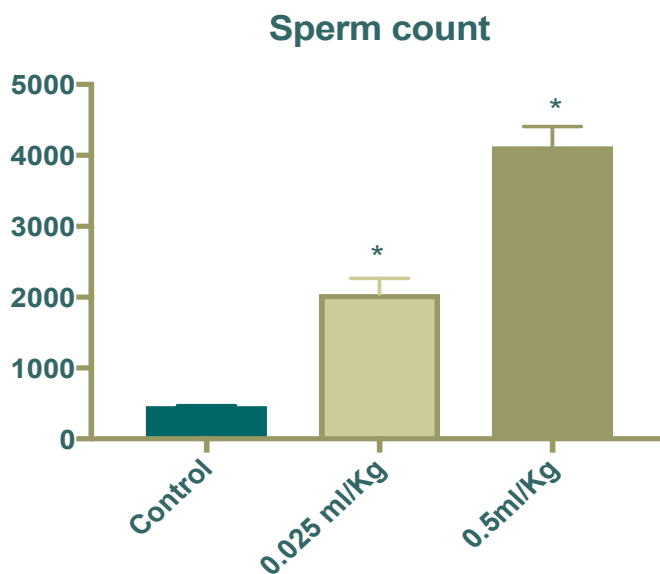


Figure 5: sperm count amplified after 0.25 and 0.5 ml/Kg treatment with anti-venom. $P \leq 0.05$

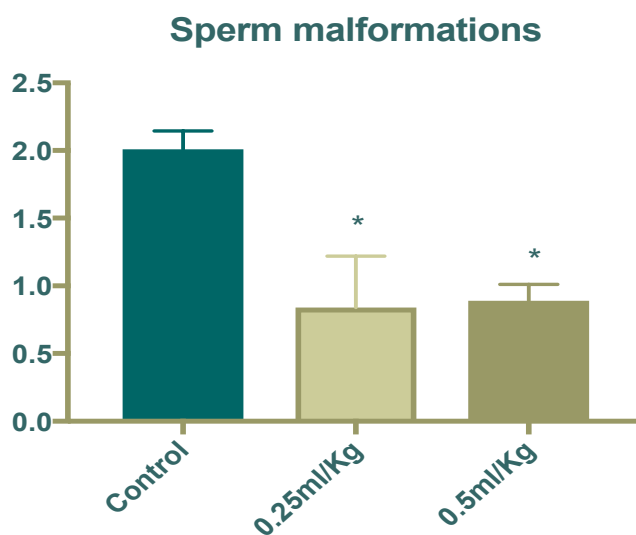


Figure 6: Anti-venom showed reduction in sperm malformations at the tow treatment groups comparing to the control. $P \leq 0.05$

Discussion

Lately, in the south of Iraq *Echis carinatus* snake bite can cause

significant morbidity and mortality therefore snakebite envenoming represents main public health problem concern. Anti-venom is foremost and main therapy for snake envenoming. Most of the anti-venom effectiveness tests is based animals study that usually calculate the efficacy of anti-venom against lethal dose (LD₅₀) and effective dose 50(ED₅₀) as well as venom consumption coagulopathy activity (Kalyan *et al.*, 2010). The current study carried out for the first time to measure the effect of new anti-venom, which produced by Razi vaccine and serum research institute in Islamic republic of Iran that export for south of Iraq, on healthy rats (no venom introduced to these animals).

Immunoglobulins consist in most of anti-venom that would cause complement mediated side effects. It has been proved that proteins founded in anti-venom may lead to serum sickness and rarely cause an anaphylactic shock. Non-immunoglobulin proteins also added in anti-venom which consider as the main reasons for the side effects which mostly represent by swelling of eyes and face, fever, breathing difficulties, reddening of skin, inflammation of joints and swelling of lymph gland (Paul *et al.*, 2011; Deshpande *et al.*, 2014). Anti-venom may consequences on the kidneys functions promote high level secretion of erythropoietin that in turn lead to formation of blood cells (Paul *et al.*, 2011). The results of the current study showed alternation in levels of HCT, MCV, MCH but not RBC or MCHC. It has been approved before that increasing level of MCV- MCH-MCHC related with the antivenom results of the red blood cells which impacted on these indicators (Kalyan *et al.*, 2010). The growth of PLT level from our study come with the agreement form pervious study that admitted that the significant increase in blood platelet count associated with the effect of anti-venom which characterize as inflammatory responses coexisting with upsurge in the number white blood cells (Theakston *et al.*, 2003).

Although Present study showed increasing level of total WBC and lymphocytes, there was reduction in level of granulocytes. A study carried out on male and female rats administrated that anti-venom injections triggered mainly liver and kidney inflammation which in turn would elevate total WBC as main defense system in the body (Kalyan *et al.*, 2010). In addition, high elevation in level total WBC after anti-venom injection is a natural reaction to the entry of alien to the body and this increase stimulates the bone marrow to yield a 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).

In this study, we administered that increase all the ALT, AST and ALP levels in treatment groups comparing to the control animals. It has been suggested that increasing in liver enzyme may be due to the impact of anti-venom on hepatocytes (Deshpande *et al.*, 2014). As consequence, damage cytoplasm membrane and mitochondria membrane of liver cells would be elevated liver enzymes in serum because liver cells contain high concentration of AST and ALT. Most of the earlier work come a long with results yield from the present study (Momoh *et al.*, 2012).

Our appeared clearly that there are increasing in level of male hormones which represented in the current study with FSH and testosterone at 0.5 mL comparing to the control. No significant alternation was noticed in level of LH hormone. This results partially agreed with available studies which indicated that anti-venom may associated with elevation of FSH and LH. It has been suggested that the influence of anti-venom would have direct impression on kidney and inflammatory action results from releasing varies kind of endogenous cytokines. Consequently, hypothalamus pituitary would be stimulated to release more adrenocorticotrophic hormone and corticosteroid (Queen *et al.*, 2006).

Anti-venom treatment approved to increase testicular antioxidants enzymes which accorded with current study (Arash *et al.*, 2009). Another study also proposed that anti-venom inters to the body is working effectively to reduce formation of reactive oxygen species (ROS) with reducing of fatty acids braked down in the member. This in turn will elevated sperm count and diminution of sperm malformations (Chanhome *et al.*, 2002).

In conclusion, the present study for the first time preformed on experimental animals to find out the impactions generated after anti-venom injection to healthy male rats. The outcome of the study exhibited increase in the blood parameter in respond to exogenous interring and alternation in liver enzyme after liver injury. Male hormones also respond to this sort of exogenous to show that new anti-venom, produced by Razi vaccine and serum research institute in Islamic republic of Iran as best treatment to venom of *Echis carinatus*, has multiple negative influence on healthy body needs to be taken care of after anti-venom injections. All the proposed impaction from

anti-venom should be followed up carefully after envenoming treatments. Prescription dosage also should be cautious based on the impact approved in the current study.

دراسة تأثير الترياق (مضاد السم) لأفعى ذات الحراشف المنشارية على المعايير
الدموية، انزيمات الكبد والهرمونات الجنسية
في ذكور الجرذان

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تهدف الدراسة الحالية لمعرفة تأثير الترياق على المعايير الدموية والهرمونية وكذلك تأثيره على انزيمات الكبد في ذكور الجرذان. قسمت الجرذان البالغة الى ثلاث مجاميع كل مجموعة 6 جرذان. استخدمت طريقة الحقن في منطقة الخلب. المجموعة الاولى (مجموعة السيطرة) والتي حقنت بالمحلول الفسيولوجي (0.9)، المجموعة الثانية حقنت ب (0.25 ملغم/كغم) من الترياق ولمرتين والمجموعة الثالثة حقنت ب (0.5 ملغم/ كغم) من الترياق ولمرتين ثم قتلت الحيوانات بعد 24 ساعة. اظهرت نتائج الدراسة اختلافا معنويا ($P \leq 0.05$) في كريات الدم الحمراء، معدل تركيز الهيموكلوبين، معدل كمية الهيموكلوبين، معدل حجم الخلايا، العدد الكلي لخلايا الدم البيض، الخلايا اللمفاوية، الخلايا الوحيدة والخلايا الحبيبية بين المجموعتين الثانية والثالثة مقارنة بمجموعة السيطرة. اضافة الى ذلك، اظهرت النتائج زيادة معنوية ($P \leq 0.05$) في عدد الصفائح الدموية وانزيمات الكبد ALT, AST, ALP في المجموعتين المعالجة بالترياق مقارنة مع مجموعة السيطرة. كما ازدادت كل من الهرمون المحفز للجريبات، الهرمون اللوتيني والتستوستيرون في المجموعتين المعالجة مقارنة مع مجموعة السيطرة. كما انخفض العدد الكلي للنف في ذكور الجرذان.

الكلمات المفتاحية: - الترياق، المعايير الدموية، انزيمات الكبد، الهرمونات الجنسية، الجرذان.

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