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Physiological Effects of LD_{50} of *Echis coloratus* Crude Venom on Rat at Different Time Intervals

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Abstract. Variations in physiological parameters of rat serum following snake venom administration can be used in assessing the functions of vital organs of envenomated subjects. In the present study the effects of LD_{50} of *Echis coloratus* crude venom on the serum physiological parameters of white rats were investigated over various periods of time. The snake venom has induced a reduction of serum total proteins, albumin, uric acid, phosphorus, calcium and sodium levels, as well as, the activity of LDH. Serum urea, total bilirubin, creatinine, glucose, triglycerides, and iron levels, as well as the activities of ALT, ALP, AMY and AST have shown significant increment significantly in some of the envenomated rats. Hence, *E. coloratus* crude venom seems to disturb the function of some vital organs of rat, such as liver, heart, kidney and skeletal muscles. Such disturbances appear to have variable durations.

Introduction

Vipers are widely distributed in many parts of the world, and in Saudi Arabia they are represented by several species some of them are *Echis coloratus* and *Echis carintus* [1]. Their bites can cause serious health problems, disturbance in metabolism and even death [1-6].

Few studies were done on the metabolic effects of viper venom in man [1] and following short periods of time of venom injection in rats [6,7]. Such effects were still apparent 24 h after envenomation, yet their full duration is to be determined. Hence, in the present study, an attempt is being made to evaluate the physiological effects of *E. coloratus* crude venom on the biochemical parameters of rats serum over various periods of time.

Material and Methods

***Echis coloratus* venom**

Crude venom was obtained from *E. coloratus* snakes kept in the serpentarium at the Department of Zoology, College of Science, King Saud University. The snakes were originally collected from the southwest region of Saudi Arabia and were kept in large tanks and water was provided *ad libitum*. They were fed on laboratory bred mice every 10-14 days. Heat was provided from a 100 W lamp for a daily period of 9 h. Venom was milked from adult snakes, freeze, dried and reconstituted in saline solution prior to use.

Experimental animals and methodology

Male, Sprague-Dewely rats (200-250g), were used. They were fed commercially obtained regular rat chow, and water was provided *ad lib*. Food, but not water was withheld for 12 h prior to decapitation and blood sample collection. The animals were divided into seven groups of 10-32 rats in each group (Tables 1-4).The first group was designated as control and each rat was injected 0.5ml of physiological saline i.p. Each rat of the other groups (Group2-7) was injected i.p. with venom (LD_{50} 1.1 mg/kg) in 0.5ml physiological saline [7].The rats of group 2-7 were killed by decapitation 4,8,12,24,48 and 72 h following envenomation, respectively (Tables 1-4) . Blood was collected from each animal into plain centrifuge tubes, allowed to clot for 1 hr at room temperature (25ºC ±2) and the serum was collected by centrifugation. Serum total proteins, total bilirubin, albumin, urea, creatinine, uric acid, glucose, cholesterol, triglycerides, sodium, potassium, magnesium, phosphorus, calcium and iron and the activity of the enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactic dehydrogenase (LDH) and amylase (AMY) were determined using Chemistry Analyzer (Dimension, RXL , USA) and RA-50 Clinical Chemistry Analyzer (Miles Inc., Germany).The data are presented as means ± S.E. and were statistically analyzed using the analysis of variance ANOVA test.

Results

Echis coloratus LD_{50} crude venom produced significant ($P<0.001$) increase in serum total bilirubin levels of all envenomated rats and so were the increases in serum total urea but the increases in rats of groups 3,4 and 5 were only significant at the level of $P<0.05$ (Table 1). In addition, the venom produced significant ($P<0.001$) increase in serum creatinine levels in group 3,4 and 5 only (Table 1). On the other hand, it caused significant reductions in serum total proteins, albumin and uric acid . The reductions in serum albumin were significant ($P<0.001$) 8h after envenomation and onwards. The uric acid levels were significant 12h after venom administration and onwards, but those in serum total proteins were only significant in group 2,3 and 4 and returned to normal level in rats of group 5,6 and 7 (Table 1).

There was general increase in the levels of serum glucose, cholesterol and triglycerides of envenomated rats.Increases were significant ($P<0.001$) in all tested rats, except the cholesterol level of group 4 and the glucose level in group 4,5 and 6, but only in those of group 6 and 7 in case of triglycerides level (Table 2).

(Wide Table 1)

Table 2. The effects of a single i.p. injection of *Echis coloratus* crude venom on the serum glucose, cholesterol and triglycerides levels of rats levels of rats 4,8,12,24,48 hr and 72 hr after envenomation

Experimental groups	Measured parameters		
	Glucose mmol/L	Cholesterol mmol/L	Triglycerides mmol/L
Group 1(controls,n=32)	5.49±0.17	1.59±0.05	0.73±0.34
Group (4hr) 2 (n=10)	5.41±0.25	0.74±0.05**	0.74±0.02
Group (8hr) 3 (n=10)	5.31±0.41	1.05±0.05**	0.75±0.09
Group (12hr) 4 (n=10)	6.77±0.30**	1.53±0.10	0.99±0.04
Group (24hr)5 (n=10)	7.49±0.25**	2.10±0.05**	0.96±0.02
Group (48hr) 6 (n=10)	8.21±0.22**	0.83±0.05**	1.22±0.07**
Group (72hr) 7 (n=10)	5.14±0.30	0.96±0.05**	1.16±0.7**

Results are presented as mean ± S.E. (n)=number of animals per group.
*P<0.05; ** P<0.001 when compared to the control rats.

Serum enzyme activities were variable following rat envenomation. The activities of ALT, ALP and AMY were significantly (P<0.001) increased in all tested rats. The LDH levels were significantly lower (*P<0.05-0.001)* in rats of groups 4,5 and 6, but AST level was significantly higher in group 2 and 3 (Table 3).

Table 3. The effects of a single i.p. injection of *Echis coloratus* crude venom crude venom on the activities of alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactic dehydrogenase (LDH) and amylase (AMY) in the serum of rats 4,8,12,24,48 hr and 72 hr after envenomation

Experimental groups	Measured parameters				
	ALT U/L	AST U/L	ALP U/L	LDH U/L	AMY U/L
Group 1 (controls, Nn=32)	58.00±2.3	277.3±11.57	188.5±10.50	2044±77.8	838.6±37.6
Group (4hr) 2 (n=10)	109.9±6.4**	471.9±11.4**	249.9±12.3**	2305.3±117	1397±44.3**
Group (8hr) 3 (n=10)	115.6±5.1**	505.6±52.2**	253.1±17.7**	1989.2±126	1092.3±45.2**
Group (12hr) 4 (n=10)	107.7±6.7**	313.2±22.3	347.5±26.8**	1673.6±88.0*	1651.1±61.3**
Group (24hr) 5 (n=10)	144.7±3.1**	297.6±21.4	427.5±21.5**	1695.8±73.9*	1784.7±55.3**
Group (48hr) 6 (n=10)	256.7±10.2**	228.2±24.1	441.1±35.5**	1247.9±98.3**	1989.6±45.9**
Group (72hr) 7 (n=10)	265.7±10.3**	242.6±14.1	508.4±38.9**	2194±139	1714.7±60.1**

Results are presented as mean ± S.E. (n)=number of animals per group.
*P<0.05; ** P<0.001 when compared to the control rats.

There were no changes in levels of the serum potassium or magnesium. Serum sodium level was significantly (P<0.05)lower in group 2, and so was the level of serum calcium in group 6 and 7. Significant reductions were also observed in the serum phosphorus levels of group 4,5, 6 and 7. However, the serum iron level rose significantly (*P<0.001)* in group 2,3 and 4, then returned to normal in group 5,6 and 7 only (Table 4).

(Table No. 4)

Discussion

Variations in serum physiological parameters can be used as biomarkers for monitoring the functions of vital organs of envenomated victims. Most of our knowledge on this mater has come from data collected few hours after envenomation [6,7]. However, several of the toxicological effects of snake venoms might appear several hours later. Hence, the present study was undertaken to evaluate those effects over various periods of time.

The reduction in total serum proteins and albumin and the rise in total serum bilirubin in envenomated rats are in accordance with observations of other investigators in this field [1-5; 8;9]. The observed effects upon those parameters might suggest that the snake venom could have disturbed proteins synthesis in hepatocytes due to cellular damage together with haemorrhges in vital organs leading to protein loss. Acute renal damage together with glomerular, tubular, interstitial and vascular lesions have been reported following various snake bite[1;10-14]. Moreover, haemorrhages in vital organs, together with increased vascular permeability were observed in the majority of viper and pit viper envenomation [15]. 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. Similar observations were reported following various viper envenomation of rats [4;16;17].

The state of hyperglycemia, which was seen 12h after the venom injection, could be due to pancreatic insufficiency in the release of insulin, release of tissue and medullary catecholamines and inhibition of glucose uptake by skeletal muscles [9;18;19].

The variations in serum cholesterol and the rise in triglycerides observed after 48 and 72 h in the present study could be due to the damage of hepatocytes by the venom making them unable to phsphorylate the large amounts of fatty acids, together with the destruction of cell membranes of other tissues [20].The results also suggest that those variations could be time dependent.

Measurements of serum enzyme activities are important in assessing the state of the liver, heart and other organs; and fluctuations indicate damage of those organs [21]. In this respect, severe heptocellular injuries, necrosis of hepatocytes and kidney have been suggested to be the result of the significant rise in serum ALT, AST and ALP levels of rats after *Echis carinatus* venom injection [22].In addition, Tiets [23,p.25], reported that although kidney, heart and skeletal muscles have significant amount of ALT, liver damage is more likely to be the source of the elevation of serum ALT levels because it is more specific to liver cells. Moreover, increased of serum AST levels as a result of Elapidae neurotoxins and cardiotoxins effects were found to induce severe myonecrosis

and fatal myocardial injury [24].The increased in serum ALP activity in snake envenomated rats might be attributed to the destruction of liver cells [8]. In the present study, the elevated activity of ALT, AST, ALP and AMY, together with the reduction of that of LDH indicate damage of the liver, heart and other organs brought about by the venom. Such findings are in agreement with previous reports on venoms of other snake species [4;5;25-27].

Although solutes account for only 4-5 percent of the non-organic matter in the human body, they play a significant role in body fluid balance. However, little is known of the effects of snake venoms on electrolyte levels either in man or rat. In the present study, *E. coloratus* venom has produced a moderate reduction in serum sodium in rats following 4h of envenomation and moderate to significant reduction in phosphorus and calcium levels in other envenomated rats. However, serum iron levels have significantly increased in the rats 4-12h after envenomation. Such disturbances of serum electrolytes have been reported on other snake venoms in experimental animals [8;28] and might be due to acute renal failure and glomerular tubule damage [15], or possibly through direct stimulation of the adrenal cortex leading to aldosterone secretion [25]. Moreover, such changes in solute levels could be due to the damage in skeletal and myocardial muscles caused by the venom.

Thus, the present study has showed that *E. coloratus* crude venom caused various biological changes in most of the blood parameters. Those changes suggest disturbances in the functions of the liver, heart, kidneys and other tissues and they seem to last for various periods of time.

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التأثيرات الفسيولوجية للجرعة نصف المميتة لسم أفعى السجاد الشرقي *Echis coloratus* على الجرذان البيضاء خلال فترات زمنية مختلفة

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(قدّم للنشر في ١٤٢٢/١/١٥هـ؛ وقبل للنشر في ١٤٢٣/١/٥هـ)

ملخص البحث. يمكن اعتبار التغيرات الفسيولوجية والأحيائية لمكونات المصل في الجرذان ،أحد الطرق المهمة لتقويم وظائف أعضاء الجسم. فبالرغم من أن معظم الدراسات التي لدينا في الوقت الحاضر قد أجريت بعد ساعات قليلة من حقن السم، فإن الدراسة الحالية قد أجريت على تأثير السم على مكونات المصل ولفترات مختلفة.

وقد أظهرت الدراسة أن سم الأفعى سبّب انخفاض تركيز كل من البروتين الكلي، ألألبيومين، حمض البوليك، الفوسفات، الكالسيوم، الصوديوم وكذلك نشاط الأنزيم LDH. كما أظهرت الدراسة ارتفاع مكونات الدم من البولينا ، البولوربين الكلي، الكرياتيين، الجليكوز، ثلاثي الجلسريد والحديد، هذا بالإضافة إلى نشاط كل من إنزيم AST، ALP، ALT، وAMY . وقد استمرت هذه التغييرات لأوقات مختلفة.

إن هذه التغييرات تدل على أن سم الأفعى أحدث تأثيرات مختلفة على وظائف عدد من الأعضاء المهمة في الجسم مثل الكبد، القلب، الكلى ،والعضلات.