Effects of single doses of *Bitis arietans* crude venom on serum biochemical parameters in rats

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ABSTRACT:

The acute effect of single intraperitoneal injections of 2 mg/kg and 4mg/kg body weight of *Bitis arietans* crude venom on serum enzyme and other constituents was evaluated in rats .The snake venom caused within 24hr of the dosing a reduction in the concentration of total serum protein, albumin, uric acid, potassium, magnesium , phosphorus and calcium levels, as well as in the activity of alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase and lactic dehydrogenase. The levels of glucose, cholesterol, urea and creatinine were elevated and the activity of alanine aminotransferase. Serum sodium levels significantly increased in envenomated rats. Serum iron and triglycerides levels and the activity of amylase did not change. *B. arietans* crude venom caused hepatic, renal and cardiac dysfunction in envenomated rats.

Key words : Viper snake, *Bitis arientans* venom, biological parameters, rats.

INTRODUCTION:

Viper snakes are widely distributed snakes in Africa (Marsh et al., 1997) and Arabian Peninsula (Tilbury et al., 1987). There are at least four species of vipers in Saudi Arabia, including *Bitis arietans,* a species that is capable of causing fatal bites (Tilbury et al., 1987). Several studies have been made on the metabolic , cardiovascular and haematological effects of viper venoms on man and rats (Tilbury et al., 1897; Abu-Sinna et al., 1993; Abdul-Nabi et al.,1997; Fahim 1998 ; Al-Jammaz et al., 1999). In view of the paucity of information on the effects of *B. arietans* crude venom, the present study was planned to investigate the effects of the snake crude venom on the serum biochemical parameters of rats .

MATERIAL AND METHODS:

Bitis arietans venom

Crude venom was obtained from *B. arietans* snakes kept in a serpentarium at the Department of Zoology, College of Science, King Saud University. The snakes were collected from the southwestern regions of Saudi Arabia, kept in large tanks, fed on laboratory bred mice every 10 -14 days and water was provided *ad libitum*. Heat was provided from a 100 W lamp for a daily period of 9hr/day. Venom was milked from adult snakes every 2-3 months, lyophilized, stored in a desiccator at 4 °C in the dark and reconstituted in saline solution prior to use.

Study design

Thirty-two male albino Sprague-Dawely rats weighing 200 - 250g were used. The rats were obtained from the College of Pharmacy, King Saud University, Riyadh, kept under standard experimental conditions and had free access to regular chow diet and drinking water. Food, but not water was withheld for 12 hr. prior to the start of experiments. The rats were divided at random into three groups as follows:

Group 1: 12 rats were given intraperitoneal(i.p.) injection of 0.5 ml physiological saline (0.9 % NaCl) and served as control. Group 2: 8 rats received single i.p. injections of low dose (2 mg/kg body weight) of *B. arietans* crude venom .Group 3: 12 rats received single i.p. injections of medium dose (4 mg/kg body weight) of *B. arietans* crude venom. All rats in groups 1, 2 and 3 were killed by decapitation 24 hr. after venom injection.

Serum analysis

Blood was collected from each rat into plain centrifuge tubes, left for 1hr at room temperature (25°C±2) and serum was separated by centrifugation at 600g for 15 min and analyzed , without delay , for the concentration of total protein, albumin, urea, creatinine, uric acid, glucose, cholesterol, triglycerides, magnesium, phosphorus, calcium and iron.The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT), lactic dehydrogenase (LDH) and amylase (AMY) were determined using Kits purchased from Sera-Pack (Ames division, Miles Ltd. England)

Chemistry Analyzer (Dimension, RXL, USA) or RA-50 Clinical Chemistry Analyzer (Miles Inc., Germany). The concentrations of serum sodium and potassium were determined using a flame photometer (NOVA 1, Massachusetts, USA).

Statistical analysis

The data are presented as means ± S.E. and statistically analyzed using ANOVA test. Significance was set at the level of $P < 0.05$ or $P<0.001$ vs control.

RESULTS:

The effects of *B. arietans* crude venom on the serum biochemical parameters of rats are presented in Table 1. Serum albumin concentration of rats in group 2 receiving the low dose of the venom (2 mg/kg), did not change while significant decreases (*P<0.05*) were observed in rats of group 3. Total serum protein and uric acid concentrations significantly decreased (*P<0.001*) in all envenomated rats .The concentrations of both urea and creatinine were higher (*P<0.001)* in group 3 than group 2 and control rats. Serum glucose concentration was higher (*P<0.05*) in group 3 and cholesterol level was (*P<0.05*) in group 2 than control (group 1). However, serum triglycerides and iron levels did not change, but sodium level was higher (*P<0.01*) in groups 2 and 3 than the controls (group 1).On the other hand, the concentrations of potassium, magnesium, phosphorus and calcium were lower (*P<0.05* and *P<0.001*) in groups 2 and 3 than in the controls(group 1), Table 1.

The activity of serum ALT was higher (*P<0.05* and *P<0.001*) in rats of group 2 and group 3.However, activities of AST and LDH significantly reduced (*P<0.001*) in rats of group 2 and group 3. ALP and GGT activities significantly decreased (*P<0.05* and *P<0.001*) only in rats of group 3 but, AMY activity did not change compared to the control rats (Table 2).

DISCUSSION:

It is well known that viper bites cause toxic effects on victims due to the presence of lipolytic and proteolytic enzymes in their venoms (Tan & Ponndurai 1990). The present study extends our previous findings (Al-Jammaz et al., 1998) that *B. arietans* crude venom causes alterations of rat metabolism. Furthermore, the study has also shown some important changes in biochemical parameters .

The reduction in serum total proteins, albumin and uric acid in envenomated rats was reported in laboratory animals exposed to viper snake venoms by various investigators (Abdul-Nabi et al., 1997; Fahim 1998; Al-Jammaz et al., 1998 ; 1999), However, the precise mechanisms whereby the venoms cause reduction of these parameters are not fully known . It might be assumed that the reduced levels of these serum constituents could be due to disturbances in renal function as well as haemorrhages in some internal organs. In fact, increased vascular permeability and haemorrhages in vital organs due to the toxic action of various snake venoms were described by Meier and Stocker 1991; March et al., 1997. Several workers reported acute renal failure characterized by vascular lesions and tubular necrosis in the renal cortex following various snake bites (Tilbury et al., 1987).

In the present work, the rise in serum urea and creatinine levels indicates impairment of renal function. Similar observations were reported in rats following administration of various viper venoms (Rahmy et al., 1995; Omran et al., 1997; Abdel-Nabi et al., 1997).

Bitis arietans venom caused an increase in serum glucose level in the envenomated rats. Snake venoms including that of *B. arietans* were found to produce hyperglyceamia in rats (Mohamed et al., 1980; Abdul-Nabi et al., 1993,1997; Fahim 1998; Al-Jammaz et al., 1999). The increases in serum glucose levels could be attributed to the effects of the venom on glycogen metabolism in the hepatocytes, muscle fibers and medullary catecholamines that stimulate glycogenolysis and gluconeogenesis in those tissues (Abdul-Nabi et al., 1997; March et al., 1997).

The increases in serum cholesterol levels in envenomated rats observed in the present study could be due to the hepatocytes damage rendering them unable to phosphorylate the increasing amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues (El-Asmar *et al.,* 1979).

In the present study, *B. arietans* venom caused a rise in serum sodium, together with reductions in serum potassium, magnesium, phosphorus and calcium in the envenomated rats. Such disturbances of serum electrolytes were reported in rats following various snake venom injections (Mohamed et al., 1964; Al-Jammaz 1992). Meier and Stocker (1991) suggested that these disturbances might be due to acute nephropathy following viper bites and Mohammed et al (1980) speculate that this effect was brought about by stimulation of adrenal cortex leading to aldosterone secretion. Moreover, such changes in solute levels could be due to the damage in skeletal and myocardial muscles caused by the venom.

In the present work, the elevated activity of ALT might indicate liver and other vital organ damage brought about by the venom. Such findings are in agreement with those reported for *B. arietans* venoms (Mohamed et al., 1981; Fahim 1998).The reduction of other enzyme activities could be due to renal damage as well as to the inhibition of their activities caused by the venom as had been suggested by Mohamed et al., 1981.

Measurements of clinical chemistry parameters following *B. arietans* crude venom injection clearly demonstrate disturbances of vital organs, especially liver, renal and muscles. Such disturbances appeared to continue for at least 24hr after envenomation, regardless of the dose used.

Table (1) : Changes in serum constituents of rats 24hr after administration of *B. arietans* crude venom by i.p. route.

Parameters and Units	Experimental groups and Doses (n)		
	Group 1 (n = 12)	Group 2 (2mg/kg) (n = 8)	Group 3(4mg/kg) (n = 12)
Total proteins g/L	67.35±0.80	62.48±0.37	63.83±0.34
Albumin g/L	35.49±0.50	34.98±0.36	33.64±0.31*
Urea mmol/L	5.78±0.17	5.89±0.33	7.21±0.41**
Creatinine µmmol/L	32.50±1.53	34.57±1.27	40.09±2.96**
Uric acid µmmol/L	145.2±3.04	82.10±1.70**	62.85±2.15**
Glucose mmol/L	4.93±0.29	5.65±0.23	6.28±0.25*
Cholesterol mmol/L	1.48±0.06	1.87±0.07*	1.59±0.05
Triglycerides mmol/L	0.614±0.05	0.620±0.04	0.633±0.05
Sodium mmol/L	143.6±0.42	146.3±0.64**	147.9±0.60**
Potassium mmol/L	6.62±0.08	5.24±0.18**	5.46±0.12**
Magnesium mmol/L	1.00±0.01	0.86±0.01*	0.81±0.01**
Phosphorus mmol/L	2.96±0.01	2.42±0.01**	2.16±0.01**
Calcium mmol/L	2.65±0.01	2.44±0.01**	2.53±0.01*
Iron µmmol/L	33.5±0.71	34.3±0.72	35.5±0.86

(n)=number of animals per groups.
Results are presented as mean ± S.E. *P<0.05; ** P<0.001 V.S controls

Table (2) : Enzyme values in serum of rats 24hr post-administration of *B. arietans* crude venom by i.p. route.

Parameters and Units	Experimental groups and Doses (n)		
	Group 1 (n = 12)	Group 2 (2mg/kg) (n = 8)	Group 3(4mg/kg) (n = 12)
ALT (U/L)	27.06±0.52	29.18±0.83*	30.45±1.34
AST (U/L)	376.9±7.47	144.9±2.79**	154.7±2.31**
ALP (U/L)	193±2.49	176±4.42	139.1±3.55**
GGT (U/L)	4.22±0.7	3.63±0.11	2.75±0.9*
LDH (U/L)	1867±95.18	601.3±63.88**	533±70.01*8
AMY (U/L)	845.1±32.59	780.9±20.53	781±30.57

(n)=number of animals per groups.
Results are presented as mean ± S.E. *P<0.05; ** P<0.001 V.S controls

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دراسة تأثير جرعات من سم الأفعى النفاثة *B. arietans* على مكونات المصل في الجرذان

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الملخص :

تعتبر الأفعى النفاثة أحد الأفاعي التي تعيش في الجزيرة العربية ، ولازالت معلومتنا عن تأثير سمومها على وظائف أعضاء الجسم الرئيسة قليلة سواءً على الإنسان أو الحيوان.

ولهذا فقدتم إجراء هذه ا لدراسة على الجرذان باستخدام جرعتين (٢مغم/كغم، ٤غم/كغم من وزن الجسم) وأظهرت الدراسة انخفاضًا في مكونات الدم من البروتين الكلي والألبومين وحامض اليوريك ، وكذلك على مكونات الدم من أيونات البوتاسيوم والفوسفات و والكالسيوم . :كما أثرت جرعات السم بانخفاض نشاط عدد من الأنزيمات الهامة مثل إنزيم T,GGT,AMY,AST,LDH

كما أن السم أدى إلى ارتفاع السكر والكولسترول وثلاثي الغلسريد والبولينا و الكريتينين وأيونات الصوديوم ، بالإضافة إلى نشاط الأنزيم ALT .

إن هذه النتائج تدل دلالة واضحة على مدى تأثير سم الأفعى النفاثة على الأعضاء الهامة في الجسم مثل الكبد والكلى والقلب والعضلات بعد اللدغ في الإنسان ، وأن هذه التأثيرات عادة تستمر لفترة تزيد عن ٢٤ساعة.