J. King Saud Univ., Vol. 14, *Science* (2), pp. 81-89, pp. Riyadh (1422/2002)

Effect of Acute and Chronic Injections of *Bitis arietans* Crude Venom on Some Metabolic Aspects in Rats

Ibrahim. A. Al-Jammaz
Department of Biology, Teachers' College,
P.O.Box 4341,Riyadh 11491, Saudi Arabia

(Received 15/1/1421H.; accepted for publication 23/6/1422H.)

Abstract. The effect of acute and chronic intraperitoneal injections of *Bitis arietans* crude venom on serum glucose, total protein, and total lipid as well as the activities of AST, ALT, ALP and LDH enzymes in serum, brain, liver, kidney and muscle tissues were evaluated in rats. Hyperglycemia was developed in all envenomated rats and associated with depletion of glycogen in the liver. Kidney, muscle and brain glycogen contents were increased significantly in all envenomated rats. Total proteins were decreased in all organs tested. Similar results were obtained for total lipids, except in the liver where they increased. Serum AST, ALP and LDH activity levels were increased in all envenomated rats, but ALT level did not change. Furthermore, the snake venom produced variation in the enzyme activity levels in the organs investigated.

Introduction

Poisonous snakes are one of the most dangerous poisonous animals in the world. Their bites may be serious, depending on the amount of venom injected, the location of the bite, the size of the victim, the species of the snake and the amount of time elapsed between the bite and the injection of the right antivenin. Snake venom is a complex mixture of a number of toxins and /or proteinaceous enzymatic substances [1,2], which are capable of attacking the nervous system as well as the circulatory system or the vital organs depending on the species of snake. Viper snakes are widely distributed in Africa [2] and Arabian Peninsula [3]. There are at least four species of vipers in Saudi Arabia, including *Bitis arietans,* a species inhabiting the southwestern region, that is capable of causing fatal bites [3;7].Viper venoms were reported to be structurally and functionally different due to individual seasonal, and geographical variations [4-6]. However, common venom characteristics at the familial and generic levels were proven [1].

Although much work has been done on the physiological effects of the venom of other vipers especially on biochemical parameters in plasma of rats [8-9], little is known

about the possible physiological effects of *Bitis arietans* venom particularly on the function of some vital organs.

Therefore, the present study aims to investigate the effect of *B. arietans* venom on some biochemical components of the serum and changes in metabolism of some important metabolic components regarding the effect of toxicity duration on these changes.

Material and Methods

Bitis arietans venom

Crude venom was obtained from adult *B. arietans* snakes kept in a serpentarium at the Department of Zoology, College of Science, King Saud University every 2-3 months. 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 9h/day. Collected venom was 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. Animals were kept under standard experimental conditions and had free access to regular chow diet and drinking water. Food, but not water was withheld for 12 h prior to the start of experiments. The rats were divided at random into four groups as follows:

Group 1: 8 rats were given intraperitoneal(i.p.) injection of 0.5 ml isotonic physiological saline , served as control and killed by decapitation 1h after saline injection.

Group 2: 8 rats received single i.p. injection of LD_{50} (4 mg/kg body weight) of *B. arietans* crude venom and killed by decapitation 1h after injection.

Group 3: 8 rats received single i.p. injection of LD_{50} (4 mg/kg body weight) of *B. arietans* crude venom and killed by decapitation 3h after venom injection.

Group 4: 8 rats received chronic i.p. injections of LD_{10} of *B. arietans* crude venom for seven consecutive days and killed by decapitation 24h after the last injection.

Serum and tissue analysis

Blood samples were collected from each rat into plain centrifuge tubes, left to coagulate for 1h 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

glucose, total proteins and total lipid and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH). Samples of liver, kidney, brain and abdominal muscle were quickly removed and frozen at –20°C until use. Serum glucose and glycogen content of tissues were measured by the Anthrone method [11]. Estimation of total proteins was carried out according to the method of Lowry *et al.* [12]. Tissue lipids were extracted by homogenization of 0.25 - 1 g of fresh tissue in 10 ml chloroform-methanol (2:1) mixture. The organic layer was separated by centrifugation for 5 min at 600 g and then washed twice with saline solution. After evaporation of the organic layer in a boiling water bath, it was reconstituted in 1 ml chloroform-methanol mixture, of which 0.05 ml was used for total lipids measurement using test- combination kits purchased from Boehringer-Mannheim, Germany applying GmbH using a sulfophosphovanillin reaction. Samples of the different organs tested (0.25-1.0g), were homogenized in ice-cold phosphate buffer. All parameters in serum and tissues (except total lipids) were determined using Kits purchased from Sera-Pack (Ames division, Miles Ltd., England) using RA-50 Clinical Chemistry Analyzer (Miles Inc., Germany).

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 $P<0.001$ versus control.

Results

The effects of *B. arietans* crude venom on the serum and tissues biochemical parameters of rats are shown in Table 1. Hyperglycemia was observed in all envenomated rats. Serum total lipids concentrations were significantly decreased ($P<0.001$) in all treated rats, whereas serum total proteins level was significantly decreased ($P<0.001$) in rats of group 3. Glycogen level was significantly increased ($P<0.001$) in brain tissues of rats of group 4 and in kidney and muscle tissues in all envenomated rats. In contrast, glycogen level was significantly decreased ($P<0.001$) in liver in all envenomated animals compared to the controls. The total proteins level of the tissues measured was significantly decreased ($P<0.001$) in all envenomated rats. The snake venom also reduced brain total lipids concentration significantly ($P<0.001$) in rats of groups 3 and 4 as well as in the kidney and muscle tissues in all envenomated rats. However, liver total lipids were significantly increased ($P<0.001$) in all envenomated rats.

The activity of serum and tissues enzyme was variable following rats envenomation (Table 2). The activity of AST was significantly increased ($P<0.001$) in rats of groups 2 and 3 in serum, liver and kidney. However, the activity of AST was significantly decreased ($P<0.001$) in brain tissue in all envenomated rats and liver and kidney tissues

in rats of groups 4, and in muscles of groups 2 and 3. ALT level of activity in serum did not change. However, the activity of ALT enzyme was significantly decreased ($P<0.001$) in brain of group 2 and liver of all envenomated rats. In addition, the activity of ALT enzyme has significantly increased ($P<0.001$) in kidney in all envenomated rats and in muscles of rats in groups 2 and 3 compared to the controls. The activity of ALP enzyme was significantly increased ($P<0.001$) in serum and kidney in all envenomated rats. In contrast, the ALP activity was significantly decreased ($P<0.001$) in brain of groups 2 and 3 and muscle of group 3.The activity of LDH enzyme was significantly increased ($P<0.001$) in serum, brain, liver, kidney and muscle tissues in envenomated rats compared to the controls, except for those of brain in group 4 and liver in groups 2 and 3 where the levels of enzyme have decreased significantly ($P<0.001$).

Table 1. Effect of acute and chronic i.p. injections of *Bitis arietans* crude venom on glucose, glycogen, total proteins and total lipids level in serum and tissues of rats after envenomation

Serum & organs	Groups	Parameters		
		Glucose	Total protein	Total lipids
Serum (mg/dL)	Group 1	101.9±2.70	6.60±0.20	5.61±0.40
	Group 2	148.7±9.4**	6.11±0.40	2.41±0.25**
	Group 3	124.1±8.9**	5.60±0.19**	2.10±0.20**
	Group 4	130.4±5.9**	6.46±0.18	2.95±0.40**
Brain (mg/g tissue)		Glycogen	Total protein	Total lipids
	Group 1	1.60±0.15	121.8±2.94	39.88±2.5
	Group 2	1.13±0.12	100.1±1.96**	37.17±2.1
	Group 3	1.83±0.17	96.9±4.2**	11.72±0.50**
	Group 4	3.93±0.24**	85.47±5.2**	10.19±0.28**
Liver (mg/g tissue)	Group 1	12.54±0.50	146.3±7.30	13.54±1.10
	Group 2	5.95±0.18**	132.2±3.1*	18.27±1.60**
	Group 3	7.09±0.12**	127.6±2.1**	23.97±0.90**
	Group 4	3.40±0.20**	84.50±3.40**	18.92±0.15**
Kidneys (mg/g tissue)	Group 1	1.16±0.07	111.1±4.40	22.43±0.89
	Group 2	3.36±0.26**	45.55±1.26**	19.64±2.70
	Group 3	2.05±0.16**	52.79±1.9**	15.79±1.07**
	Group 4	2.33±0.34**	18.70±1.2**	9.82±1.5**
Muscles (mg/g tissue)	Group 1	2.29±0.11	285.9±40.2	7.65±0.53
	Group 2	3.66±0.27**	158.4±10.7**	6.15±0.83**
	Group 3	3.13±0.10**	123.2±7.4**	2.40±0.36**
	Group 4	3.44±0.20**	144.2±2.35**	1.80±0.10**

Results are presented as mean ± S.E. *$P<0.05$; ** $P<0.001$ vs. controls

Table 2. **Effect of acute and chronic i.p. injections of *Bitis arietans* crude venom on the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT),alkaline phosphatase (ALP) and lactic dehydrogenase (LDH) in the serum and tissues of rats after envenomation**

Enzyme activity in :	Groups	Parameters			
		AST	ALT	ALP	LDH
Serum (U/L)	Group 1	70.4±2.76	27.1±0.52	193.2±2.51	563.2±30
	Group 2	87.12±4.9*	25.86±2.62	294.3±12.4**	702.9±50*
	Group 3	121.6±5.1**	29.08±1.30	228.5±13.9*	800.2±60**
	Group 4	70.2±9.3	27.37±2.30	240.3±20.9*	660.3±60*
Brain (U/mg protein per m)	Group 1	0.794±0.03	0.446±0.02	2.23±0.14	1.19±0.03
	Group 2	0.573±0.02*	0.312±0.02**	1.57±0.11**	4.53±0.09**
	Group 3	0.408±0.01**	0.450±0.01	1.13±0.10**	9.63±0.08**
	Group 4	0.345±0.03**	0.453±0.03	2.20±0.20	3.26±0.01*
Liver (U/mg protein per m)	Group 1	0.195±0.01	0.237±0.01	21.12±1.3	1.20±0.02
	Group 2	0.753±0.15**	0.119±0.02**	12.48±0.8**	0.700±0.01**
	Group 3	0.571±0.03**	0.062±0.001**	7.17±0.20**	0.592±0.01**
	Group 4	0.020±0.001**	0.043±0.001**	4.34±0.16**	1.30±0.03
Kidneys (U/mg protein per m)	Group 1	0.056±0.001	0.090±0.01	19.64±1.3	0.300±0.05
	Group 2	0.299±0.03**	0.423±0.02**	38.27±2.8**	2.67±0.5**
	Group 3	0.250±0.02*	0.536±0.03**	45.50±4.2**	17.60±1.5**
	Group 4	0.027±0.001**	0.389±0.01**	49.50±5.8**	12.30±1.6**
Muscles (U/mg protein per m)	Group 1	1.35±0.05	0.063±0.01	1.70±0.15	3.99±0.32
	Group 2	0.791±0.02**	0.387±0.01**	1.60±0.10	15.3±2.10**
	Group 3	0.518±0.02**	0.325±0.01**	0.730±0.05**	13.85±1.30**
	Group 4	1.40±0.10	0.070±0.01	1.73±0.03	16.90±2.3**

Results are presented as mean ± S.E. *P<0.05; ** P<0.001 vs. controls

Discussion

Measurement of biochemical parameters in plasma and vital organs, such as liver, brain, kidney and muscle, is of great importance in the assessment of the pathophysiological state of snake bite victims. In spite of that, most studies were mainly concentrated on the effect of snake venoms on plasma biochemical parameters (8-10). The vital organs were rarely evaluated.

In the present study, hyperglycemia state observed due to *B. arietans* envenomation is not surprising as most viper venomous were reported to induce hyperglycemic effect [9; 10; 13]. An interesting observation in the present work is that, hyperglycemia effect was pronounced in both acute and chronic dose studies and associated with depletion of

glycogen in liver as well as disturbance in the metabolism of glycogen in the other organs.The mechanism by which venom induce hyperglycemia is not clearly defined. However, many suggestions have been reported such as inhibition of glucose uptake by liver [14], release of tissue and medullary catecholamines [15] and inhibition of insulin release [16; 17]. Furthermore, the increases in serum glucose level could be attributed to the effect of the venom on glycogen metabolism in hepatocytes, muscle fibers and medullary catecholamines which stimulate glycogenolysis and gluconeogenesis in those tissues [2; 10] .The accumulation of glycogen in brain, kidney and muscle could be a response to the increased blood sugar as a result of the enhancement of glucose uptake by these tissues.

The reduction in serum total proteins and tissues was reported in laboratory animals exposed to viper snake venoms by various investigators [10; 13; 18, 19]. However, the precise mechanisms whereby the venoms cause reduction of total proteins are not fully known. It might be assumed that the reduced levels of serum total proteins and tissues 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 [2; 20]. Several workers reported acute renal failure characterized by vascular lesions and tubular necrosis in the renal cortex following various snake bites [3].

The present study revealed that serum total lipids, brain, kidney and muscles decreased. Whereas liver content increased after both acute and chronic *B. arietans* venom injections. This result suggests that the snake venom may mobilize lipids from adipose and other tissues. Lipolytic enzymes, which are present in many snake venoms, could split tissue lipid with the liberation of free fatty acids [21]. This idea of mobilization of lipids from tissues, as well as the destruction of cell membrane of other tissues, were supported in the present study by the decrease in total lipids content of the brain, kidney and muscle following the venom injection in treated rats. Furthermore, venom injection could cause 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 [22].

The data obtained in the present study also showed that *B. arietans* venom affect the activity of important enzymes in serum and tissues of the envenomated rats. Serum AST and ALT activity levels are low in normal conditions. After tissue destruction both enzymes are liberated into blood and increased after the damage of skeletal muscle, myocardial muscle and liver [23]. In the present study, the changes in the activity of AST and ALT enzymes level in serum and tissues could be due the damage brought upon those tissues by the venom. This explanation could be supported also by the increased activity of LDH level in serum, brain, kidney and muscle. It has been reported that simultaneous increase of AST and LDH in serum reflects a diagnostic heart damage [24,pp.137-215]. Furthermore, the increased level of AST in serum could be due hepatic damage or muscular dystrophy [25, pp.719-730]. This finding is in agreement with others

[23], who suggested that the increase of serum AST and LDH following *B. arietans* and *B. gabonica* envenomation might be a result of tissue destruction caused by the venoms, which led to the release of these enzymes from liver, kidney and heart.The increased ALP enzyme activity level in serum and other organs tested (except the Kidney) in the acute and chronic studies could be attributed to the effect of the venom in hepatocytes and myocardial muscles. In fact, elevated ALP enzyme activity is known to occur in congestive heart failure and osteoblastic sarcoma [23]. From the results obtained in the present study and the previous investigations, it is quit clear that acute and chronic *B. arietans* crude venom injections clearly demonstrate disturbances of vital organs, especially liver and heart.

References

[1] Frobert, Y., Creminon, C., Cousin, X., Remy, M.H., Chatel, J.M., Bon, C. and Grassi, J. "Acetylcholinesterases from Elapidae venoms: biochemical, Immunological and Enzymatic Characterization". *Biochm. Biophys. Acta.*, 1339 (1997), 253-267

[2] Marsh, N., Gattullo, D., Pagliaro, P. and Losano G. "The Gaboon Viper, and Bitis Gabonica: Hemorrhagic, Metabolic, Cardiovascular and Clinical Effects of the Venom". *Lif. Sci.*, 61, No. 8 (1997), 763-769.

[3] Tilbury, R.C., Madkour, M.M., Saltissi, D. and Suleiman, M. "Acute Renal Failure Following the Bite of Burton's Carpet Viper *Echis coloratus* Gunther in Saudi Arabia: Case Report Review". *Saudi Med. J.* 8 (1987), 87-95.

[4] Gubensek, F., Sket, D., Turk, V. and Lebez, D. "Fractionation of *Viper ammodytes* Venom and Seasonal Variation of Its Composition". *Toxicon*, 12 (1974), 167 - 171 .

[5] Jayanthi, G.P and Gowda, T.V. "Geographical Variation in India in the Composition and Lethal Potency of Russell's Viper (*Vipera russelli*) Venom". *Toxicon*, 26 (1988), 257-264 .

[6] Wuster, W., Golay, P. and Warrell, D.A. "Synopsis of Recent Development in Venomous Snake Systematics". *Toxicon*, 35 (1988), 319-340.

[7] Al-Sadoon, M.K., AL-Farraj, S.A. and Abdo, N.M. "Survey of the Reptilian Fauna of the Kingdom of Saudi Arabia. III: An Ecological Survey of the Lizard, Amphisbaenian and Snake Fauna of AL-Zulfi Area". *Bull. Maryland Herpetological Soc.*, 27 (1991), 1-22 .

[8] Abdel-Raheem, K., Dehlaw, G., EL-Mossallamy, N. and Mahmoud, I. "Influence of Viper (Cerastes Vipera) Venom on Metabolism in Mice". *Proc. Zool. Soc. A.R.Egypt.*, 9 (1985), 57- 66.

[9] Abu-Sinna, G., Al-Zahaby, A.S., Abd El-Aal, A., Abd El-baset, A. and Soliman, N.A. "The Effect of the Viper *Cerastes cerastes cerastes* Venom and Venom Fractions on Carbohydrate Metabolism". *Toxicon*, 31(1993), 791-801.

[10] Abdel-Nabi, I. M., Raafat, A. and El-Shamy, H. I. "Biological Effects of Intraperitoneal Injection of Rats with the Venom of the Snake *Echis carinatus*". *Egypt. J. Zool.*, 29 (1997), 195-205.

[11] Seiffer, S., Dayton, S., Novic, B. and Muntwyler, E. "Method for Glycogen Determination". *Arch. Biochem. Biophys.*, 25 (1951), 191-198.

[12] Lowry, O., Rosebrough, N.J., Farr, A.L. and Randall, R.J. "Protein Measurement with the Folin Phenol Reagent". *Journal of Biological Chemistry*, 193 (1951), 265-270.

[13] Al-Jammaz, I., Al-Sadoon, M.K. and Fahim, A. "Effect of LD_{50} Dose of *Echis coloratus* Venom on Serum and Tissue Metabolites and Some Enzyme of Male Albino Rats". *J. Science, King Saud Univ.*, 11 No. 2 (1999), 61-68.

[14] Mohamed, A.H., Beskhoroum, M.A. and EL-Damarawy, N.A. "Effect of Cobra Venom (*Naja haje*) on Blood Glucose and Phosphate and Plasma Insulin-like Activity in Dogs". *Toxicon*, 10 (1972), 385-391

[15] EL-Asmar, M.F., Soliman, S.F., Ismail, M. and Osman, O.H. "Glycemic Effect of Venom from the Scorpion (Bathus minax, L. Koch)". *Toxicon*, 12 (1974), 249-251.

[16] Johnson, D.G., Henry, D.P., Moss, J. and Williams, R.H. "Inhibition of Insulin Release by Scorpion Toxin in Rats Pancreatic Islets". *Diabetes*, 25 (1976), 198-201.

[17] Ismail, M., Elkhawad, A.O.A. and El-Seweidy, M. "Hyperglycemic Effect of Scorpion (*Androctonus amoreuxi*, Aud & Sav) Venom in Rabbits". *Congress of Pharmaceutical Sciences*, 15 (1977), 98-110.

[18] Fahim, A., "Biological Effects of the Viper *B. arietans*, Crude Venom on Albino Rats". *Egypt. J. Zool.*, 30 (1998), 35-54.

[19] Al-Jammaz, I., Al-Ayed, M.I. and Al-Yahya, H. "Effect of Acute Envenomation with LD_{50} of *B. arietans*". *Am. Shams. Sci. Bull.*, 36 (1998), 207-222.

[20] Meier, J. and Stocker, K. "Effect of Snake Venoms on Homeostasis". *Toxicology*, 21, No. 3 (1991), 171-1820.

[21] Vasquez-Colon, L., Ziegler, F.D. and Elliot, W.B. "On the Mechanism of Fatty Acid Inhibition of Mitochondrial Metabolism". *Biochemistry*, 5 (1966), 1134-1142.

[22] El-Asmar, M.F., Farag, R.M., Shoukry, S. and El-Shimi, I.R. "Effect of Scorpion *Leiurus quinquestriatus* H & E Venom on Lipid Metabolism". *Toxicon*, 17 (1979), 279-283.

[23] Mohamed, A.H., Fouad, S. El-Assar, S., Salem, A.M., Abdel aal, A., Hassan, A., Zahran, F. and Abbas, N. "Effects of Several Snake Venoms on Serum and Tissue Transaminases, Alkaline Phosphatase and lactate Dehydrogenase". *Toxicon*, 19 (1981), 605-609.

[24] York, J.L. "Enzymes Classification Kinetics and Control". In: *Textbook of Biochemistry with Clinical Correlations*" New York: John Wiley & Sons Inc., 1982.

[25] Porth, C.M. "Alterations in Function of the Hepatobilary System and Exocrine Pancreas. In: *Pathophysiology Concepts of Altered Health States* (Ch. 41), 3rd ed. Philadelphia: J.B. Lippincott Comp., 1990.

دراسة تأثير الجرعة المفردة والمزمنة من سم الأفعى النفّاثة على بعض الوظائف الفسيولوجية في الجرذان

إبراهيم عبد الرحمن الجماز

قسم الأحياء ، كلية المعلمين بالرياض ، ص.ب ٤٣٤١ ، الرياض ١١٤٩١ ، المملكة العربية السعودية

(قدّم للنشر في ١٤٢١/١/١٥هـ ؛ وقبل للنشر في ١٤٢٢/٦/٢٣هـ)

ملخص البحث. تم في هذا البحث دراسة إعطاء الجرذان جرعة مفردة وجرعة مزمنة لمدة سبعة أيام من سم الأفعى النفّاثة وتأثيرهما على الجليكوجين ، الجليكوجين ، البروتين الكلي والدهون الكلية بالإضافة إلى عدد من الإنزيمات في مصل الدم وعيّنات من القلب ، الكلى ، الدماغ والعضلات وتقويمها. وأظهرت الدراسة أن سم الأفعى النفّاثة أدى إلى إصابة الحيوانات بارتفاع في السكر مع كلا الجرعتين ، بالإضافة إلى استنزاف الكبد من الجليكوجين ، بينما زاد محتوى كل من الكلى ، العضلات والدماغ من الجليكوجين في جميع الحيوانات المدروسة.

كذلك أدى السم إلى انخفاض التركيز الكلي للبروتين في جميع الأعضاء المدروسة ، وكذلك انخفاض التركيز الكلي للدهون ما عدا الكبد حيث زاد محتواها من الدهون الكلية. كذلك أدى السم إلى زيادة نشاط الإنزيمات LDH ، ALP و AST في جميع الحيوانات ما عدا إنزيم ALT لم يتغير تركيزه.

هذا بالإضافة إلى أن سم الأفعى النفّاثة أدى إلى إحداث تغيرات كبيرة ومتباينة في نشاط الإنزيمات المدروسة في جميع الأعضاء . ولذا فإنه يمكن تفسير هذه النتائج بقدرة تأثير السم على إحداث تأثيرات سمّية في الكبد ، القلب والعضلات بدرجة رئيسية ، وملاحظة ذلك دائمًا عند معالجة الأشخاص الملدوغين.