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Effect of LD_{50} Dose of *Echis coloratus* Venom on Serum and Tissue Metabolites and Some Enzymes of Male Albino Rats

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Abstract. A single LD_{50} dose of *Echis coloratus* venom - A Saudi viper - was found to cause sever hyperglycemia with decrease serum total lipids and unchanged serum total protein. These changes were simultaneous, with significant elevation in AST, LDH and intact ALT serum enzymes level. The envenomation also caused significant accumulation of glycogen in liver and kidney, but muscle glycogen was unaffected. Total lipid content of liver was found to be significantly higher than control, while a dramatic decrease was observed in both kidney and muscle . In addition, total protein was significantly decreased in liver, kidney and muscle. Regarding tissue enzymes activities, AST level was significantly decreased in all organs studied, while ALT level was significantly decreased in liver and muscle and significantly increased in kidney. LDH enzyme activity was noted to augment significantly in liver and muscle, with significant decline in its level in kidney. It is concluded that the snake venom may cause disturbance in the metabolic and enzymatic pathways of different vital organs manifested by the hyperglycemia and elevated AST and LDH serum enzymes levels.

Introduction

Viper's venoms are reported to exhibit different toxic effects due to the presence of lipolytic and proteolytic enzymes in their composition [1]. The ability of the venom to induce cytotoxicity [2], nephrotoxicity [3], muscular dystrophy [4] alteration in general metabolism [5] and above all inducing hyperglycemia [6] or even the contrary, hypoglycemia [7] are common manifestations of envenomation.

For testing the effect of snake venom on metabolism , *Echis coloratus*, a viper inhabiting the Saudi fauna [8], was chosen for such a purpose. Serum, liver, kidney and abdominal muscle of male albino rats were the organs in which parameters such as glucose (in serum), glycogen, total lipids and total proteins were measured in response to an LD_{50} of the venom. Also some clinically important enzymes, as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were estimated in plasma and the same organ.

Material and Methods

Experimental animals

Male albino rats, Wistar (200-250g), were used. Food but not water was withheld for 12 hr prior to experiment. Animals were divided into two groups where the first was designated as a control and received i.p.physiological saline (0.2ml) dose. The other group was injected a sublethal (LD_{50}) i.p. dose of *E. coloratus* venom (0.2 ml saline solution containing 1.1 mg crude venom /1000g body weight), and sacrificed after 2hr.

The crud venom was obtained from *E.coloratus* snakes kept in serpentarum at the Department of Zoology, College of Science, King Saud University, these were collected from southwest regions of Saudi Arabia. The snakes were kept in large tanks and water was always available .The animals were fed on laboratory bred mice every 10-14 day. Heat was provided from a 100 W lamp for a daily period of 9 hr. Venom was milked from adult snakes, dried and reconstituted in saline solution prior to envenomation.

Methodology

All experimental animals were killed by decapitation and blood was collected into centrifuge tubes coated with EDTA and centrifuged at 600g for 15 min. Plasma was divided into several aliquots for glucose, total proteins total lipids, AST, ALT and LDH analysis. Portions of brain, liver and kidney were quickly removed and frozen at $-20\,^{\circ}C$ till analysis day. Plasma glucose and glycogen content of tissues were measured by Anthronc method [9]. Estimation of total protein was carried out according to the method of Lowry *et al.* [10]. Tissue lipids were extracted by homogenization of 0.25 - 1 g fresh tissue in 10 ml of chloroform methanol (2: 1) mixture, the organic layer was separated by centrifugation for 5 min at 600g, 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 by test-combination kits purchased from Boehringer-Mannheim GmbH using a sulfophosphovanillin reaction.

Thc portions of tissues (0.25-1g) were homogenized in ice cold bath of Tris buffer (pH = 7.7) for AST or (pH = 7.4) for ALT, and in Phosphate buffer (pH = 7.5) for LDH using a motor homogenizer for 5 min. Enzyme activity determinations were carried out

according to the recommendations of Scandinavian Committee on Enzymes (SCE) and using kits purchased from Sera-Pack (Ames division, Miles Ltd. England) for AST & ALT, while determination of LDH activity was carried out by kit from Bio Merieux, France.

The results are presented as means ± S.E.M. Data were analyzed by Student's *t*-test and *P*-values of 0.05 or less were considered statistically significant.

Results

Table1 shows that a dose of LD_{50} crude *E. coloratus* venom caused a highly significant increase in plasma glucose and decrease in total lipid levels, while no significant change was observed in the total protein level. Meanwhile, serum AST and LDH enzyme levels were significantly elevated after envenomation, with no change in ALT enzyme level.

Table 1. Effect of i.p. envenomation with LD_{50} dose of *E. coloratus* venom on serum glucose, total protein, total lipids, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) enzymes activities of male albino rats

Para-meter	Glucose mg/ml	Total lipids mg/ml	Total protein mg/ml	AST U/L	ALT U/L	LDH U/L
Control	1.03±0.032	5.19 ± 0.22	59.5 ± 1.2	79.39±2.95	40.79±3.87	242.9±13.47
LD_{50}	1.46±0.044**	2.56 ± 0.12**	58 ± 2.1	115.5±8.08*	43.86±1.41	477.9±36.57**

Results are presented as mean ± S.E.M. (n=8)

* P<0.05; ** P<0.001 when compared to the control rats before envenomation.

The accumulation of glycogen and total lipids recorded in liver was highly significant, with significant decrease in its total protein content after envenomation (Table 2). However, LDH enzyme level was significantly elevated, as compared to control, after envenomation with concomitant significant decline in both ALT and AST enzyme levels (Table 3). On the other hand, kidney glycogen content was found to increase significantly accompanied with significant decrease in its total lipids, and total proteins after envenomation. Meanwhile, kidney ALT level was significantly elevated, but AST and LDH levels were significantly decreased in the same organ (Table 3).

Table 2. Effect of i.p. envenomation with LD_{50} dose of *E. coloratus* venom on glycogen, total lipids and total protein contents of liver,kidney and abdominal muscle of male albino rats

Parameter	Glycogen		Total lipids		Total protein	
	Control	LD_{50}	Control	LD_{50}	Control	LD_{50}
Liver (mg/g tw)	16.97±2.06	171.46±28.19**	25.96±2.23	37.94±1.59**	159.69±3.0*	132.65±3.9**
Kidney (mg/g tw)	2.76± 0.3	3.80 ± 0.3*	88.12±9.18	50.56±9.09*	190.0± .88	156.7 ± 10.8*
Muscle (mg/g tw)	1.19± 0.3	1.29 ± 0.24	81.94±10.1	5.79±7.06**	409.85±15.0	341.11±13.31*

Results are presented as mean ± S.E.M. (n=8)
*P<0.05; ** P<0.001 when compared to the control rats before envenomation.

Table 3. Effect of i.p. envenomation with LD_{50} dose of *E. coloratus* venom on Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) enzymes activities of liver, kidney and muscle of male albino rats

Parameter	AST		ALT		LDH	
	Control	LD_{50}	Control	LD_{50}	Control	LD_{50}
Liver U/mg prt/m	0.241± .031	0.116+.005*	0.469± 027	0.262±0.019**	0.599±0.058	1.56±0.1**
Kidney U/mg prt/m	0.872±0.103	0.478±0.101*	1.72 ± 0.02	1.87± 0.034*	0.827±0.04	0.149±0.01**
Muscle U/mg prt/m	0.88 ± 0.05	0.44± 0.01**	0.55±0.003	0.38 ± 0.03**	6.72± 0.53	9.92±1.08*

Results are presented as mean ± S.E.M. (n=7)
* P<0.05; ** P<0.001 when compared to the control rats before envenomation.

Regarding muscle glycogen content, it was found unaffected by the envenomation, while a dramatic highly significant decrease than the control was observed in muscle total protein and total lipids (Table 2). These changes in muscle metabolites were accompanied with a significant decrease in AST and ALT , and a significant increase in LDH enzyme levels of the envenomed rats (Table 3).

Discussion

Changes in the enzymatic activities of serum and mammalian tissues could be one of the mechanisms by which venomous snakes produce their harm. The venom may either act by activating or inhibiting enzyme activities in the cell [11], or destruction of the cell organelles with liberation of particular enzymes [12-14].

The parallel increase in both serum AST and LDH indicate the occurrence of myocardial infarction in the present study, as it is known that their simultaneous increase is diagnostic of destruction of heart disease [15,16], liver cirrhosis and erythrocytes [14-16]. Mohamed *et al.* [17] noticed the same increase in serum AST, ALT and LDH levels after using the venom of *Bitis arietans* and *Bitis gabonica*. In addition to that the increase in serum enzyme levels observed in the present study is in agreement with those finding by others [14;18] in viper snakes *Echis carinatus* and *Cerastes cerastes*. Snake venoms are known to cause hyperglycemia in experimental animals [14; 19-23]. Many suggestions were offered to clarify phenomenon such as: central and peripheral adrenergic mechanism [24], B-receptor activation [25], release of tissue and medullary catecholamines [13;26], inhibition of glucose uptake by skeletal muscle [20], inhibition of insulin release [28, pp.98-110], stimulation of glucagon secretion [28], glycogenolysis and/or retarded glucose utilization by peripheral tissues [21].

Echis carinatus venom was found to cause hyperglycemia with simultaneous loss of liver and muscle glycogen [19]. The pronounced hyperglycemia was not accompanied in our case, by liver or muscle glycogen depletion, but in the contrary, accumulation of glycogen was noted in liver and kidney. It is possible that the stressful situation following envenomation and the associated release of catecholamines and glucocorticoids [29,pp.1936-1985] would account for the reported hyperglycemia. Another explanation based on the lipolytic property of the venom could be the reason of the hyperglycemia associated with the increased glycogen content in liver, as the venom lypolitic enzymes provide gluconeogenic precursors leading to activation of the key gluconeogenic enzymes and enhancing *de novo* synthesis of glyconeogenic mechanism [20]. In fact the total protein content of hepatic and extra-hepatic tissues were observed to decline severely after envenomation in our experiment as protein could be a source of the gluconeogenic mechanism. Not only that, but also the transaminases enzymes in the organs studied were found to be decreased. This decrease could be explained by the proposition of Felig [30] who suggested that the glucose - alanine cycle, occurring in most vital organs and in which pyruvate, produced from glucose, is transaminated to alanine via ALT enzyme and transported to liver from other organs, to be reconverted to glucose by gluconeogenesis, and therefore enhancing the hyperglycemia observed after envenomation.

The possible utilization of ALT, as well as AST enzymes - where alanine and aspartate are known to be gluconeogenic amino acids [31,pp.271-280], in this mechanism could be the reason for their low levels observed in liver and muscle in this recent study. One more evidence confirming the possible occurrence of the above mechanisms came from the noted LDH enzyme activity. LDH is a glycolytic enzyme catalyzing the reversible oxidation of lactic to pyruvic acid [32,pp.158-164], its increase in liver and muscle indicates that the carbohydrate metabolic pathway was impaired that is, alternative gluconeognesis mechanisms may be taking place in the absence of the altered glycogenolysis, especially in extra - hepatic tissues as a direct or indirect effect of

the venom presence. The noticed depletion of total lipids observed in kidney and muscles with its simultaneous accumulation in liver could be attributed to their mobilization from extrahepatic and adipose tissue as proposed by El-Asmar *et al.* [33]. In fact this mobilization of lipids will provide an additional source for gluconeogenesis mechanism, regardless of the liver glycogen content.

In general, the accumulation of lipids in liver could be a result of increased lipogenesis in the presence of the venom, lack of esterification of fatty acids by the envenomed tissues, and as a consequence, lack of their secretion [34,pp.120-136], and increased availability and absorption of lipids from the medium. This is due to the activity of the phospholipases present in the venom as they attack different portions of total lipids with subsequent liberation of free fatty acids [33]. Therefore, we can conclude that the envenomation with *E. coloratus* leads to a generalized disturbance in the metabolic and enzymatic pathways of different vital organs manifested by the hyperglycemia and elevated AST and LDH serum enzymes levels.

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دراسة تأثير الجرعة نصف المميتة من سم حيَّة السجاد الشرقي على الأيض والنشاط الإنزيمي في مصل الدم وبعض أنسجة ذكور الجرذان البيضاء

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(استلم للنشر في ١٤١٨/١٠/٤هـ؛ وقبل للنشر في ١٤١٩/١١/١٢هـ)

ملخص البحث. تم في هذا البحث، دراسة تأثير الجرعة نصف المميتة من سم حيَّة السجاد الشرقي على الأيض والنشاط الإنزيمي في مصل الدم وبعض أنسجة ذكور الجرذان البيضاء. واتضح أن هذه الجرعة تسبب ارتفاعا معنويا في مستوى سكر الدم مصحوبا بانخفاض في مستوى الدهون الكلية في مصل الدم وبدون تغير يذكر في مستوى البروتينات الكلية. وكانت هذه التغيرات مصاحبة بارتفاع معنوي في مستويات إنزيمي AST و LDH في مصل الدم وبدون تغير في مستوى إنزيم ALT. وكذلك، أدت هذه الجرعة إلى ارتفاع معنوي في المحتوى الجليكوجيني في الكبد والكلية بينما لم يتغير المحتوى الجليكوجيني للعضلات. كما لوحظ ارتفاعا معنويا في محتوى الدهون الكلية في الكبد بينما انخفض مستواها في الكلية والعضلات. وتبين أن مستوى البروتينات الكلية انخفض انخفاضا معنويا في الكبد، الكلية والعضلات.

أما بالنسبة للنشاط الإنزيمي في الأنسجة، اتضح أن مستوى نشاط إنزيم AST انخفض معنويا في كل الأعضاء التي تمت دراستها، بينما انخفض مستوى نشاط إنزيم ALT معنويا في الكبد والعضلات وارتفع معنويا في الكلية. أما بالنسبة لمستوى نشاط إنزيم LDH فقد ارتفع معنويا في الكبد والعضلات مصحوبا بانخفاض معنوي في مستواه في الكلية.

أظهرت الدراسة أن سُمّ حيّة السجاد الشرقي قد يحدث تأثيرا على المسارات الأيضية والنشاط الإنزيمي لبعض الأعضاء المهمة في جسم الحيوان.