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The Effects of *Walterinnesia aegyptia* Venom on the Serum and Tissue Metabolites and on Some Enzyme Activities in Albino Rats. III-Effects on Lipid Metabolism and Two Dehydrogenases

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Abstract. An LD 50 (0.2 mg/kg) dose of *Walterinnesia aegyptia* venom to albino rats causes a significant decrease in total serum lipids, triacylglycerols and total cholseterol, with a decline in both glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) activities, possibly due to lipolytic and specific inhibitors present in the venom. Accumulation of total lipids was observed in the liver, kidneys, heart and brain of envenomated animals with a subsequent insignificant decrease in total cholesterol and triacylglycerols, except in the liver, where the decrease was significant. This suggests mobilization of lipids from peripheral tissues to these organs accompanied by release of other components of total lipids such as phospholipids and fatty acids. Deficiency in the pentose phosphate pathway (PPP), disturbance in the oxidoreduction system and altered energy metabolism were also detected. Variations in enzyme activities were also seen with the G6PDH level which generally elevated in the all organs studied, except in brain. The level of LDH declined in liver and kidney, but increased in heart and brain. This may suggest prevalence of anaerobic conditions in such organs as a result of venom detoxication.

Introduction

Since the venom of snakes of the family Elapidae contains enzymes that can split tissue lipids [1-3] and can alter the NAD^+ specific dehydrogenases [4;5], the possible role of lipids in the mechanism of action of these venoms could be considerable. Moreover, it has been suggested that the venom catalytic, hydrolytic and lipolytic enzymes of the Elapidae could have a role in the mode of action of the venom of these snakes [6].

Walterinnesia aegyptia is the only representative of the Elapidae in the Central Region of Saudi Arabia [7]. In the present study, an attempt is made to investigate

the effects of the venom of this snake on lipids and dehydrogenases in the serum and various organs of albino rats.

Materials and Methods

Experimental animals

Male (Wistar) albino rats (200 -250 g) were used in two groups, a test group inoculated ip each with an LD50 of 0.2 mg/Kg venom in 0.2 ml of normal saline, and a control group in which each rat received 0.2 ml of saline ip. The LD 50 was calculated from a dose mortality curve set up for *W. aegyptia* venom. Rats survived envenomation for 2hr, as well as the controls were killed by a sudden blade stroke immediately dissected and the brain, liver, kidney and heart were quickly frozen at -20 C° for later analysis. Blood samples were collected in EDTA-coated clean centrifuge tubes and were allowed to stand for 20 min at 25 C° for serum separation. The serum was then separated by centrifugation at 600 g. for 15 m.

Venom

The venom used was obtained from *W. aegyptia* snakes kept in the Serpentarium at the Zoology Department, College of Science, King Saud University. These snakes had been collected from the Central Region of Saudi Arabia by a skilled professional hunter and were kept in large tanks with sand subterranuim. Heat was provided from a 100 Watt lamp for a daily period of 9 hr. The snakes were fed on laboratory-bred mice, each being given a mouse every 10-14 days. Water was provided *adlibitum*. The venom was milked from adult snakes by its voluntary injection into a receptacle through a rubber membrane. The venom collected was dried and reconstituted in normal saline solution prior to envenomation.

Methodology

A. 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 600 g., 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 bytest-combination kits purschased from Boehringer-Mannheim-GmbH using a sulfophosphovanillin reaction.

B. Triacylglycerol extraction from tissue was carried out by mixing 0.25 - 1 g of fresh tissue with 1.5 g gum arabic and 10 ml albumin solution (6% w/v), then warmed until melting. The mixture was homogenized, when still warm, for 2 m. and rinsed with 5 ml albumin solution. After cooling to room temperature, 0.02 ml of the clear solution was used for determination of triacyglcycerols by kits from Boehringer - Mannheim.

C. Total cholesterol was extracted by mixing 0.25 - 1 g of fresh tissue with 1 g of sea sand and 10 ml freshly prepared methanolic hydroxide solution (10 mol/L). The mixture was heated for 25 min under a reflux condenser. The supernatant was transferred into another flask and the residue was boiled twice with 5 ml isopropanol each time for 5 min. After filtration, the filtrate was added to the former supernatant and 0.02 ml of the clear solution was used for the cholesterol assay by using kits purchased from Boehniger - Mannhein.

The extraction methods used, were carried out according to methods supplied with the Boehringer Mannheim kits for biochemical analysis. Statistical analysis was carried out using a two-tailed t-test. The differences were considered to be statistically significant at $P < 0.05$.

Enzyme assay

Portions of tissues from different organs (0.25-1g) were homogenized in ice cold Triethanolamine buffer (pH = 7.6) for the determination of glucose-6-posphate dehydrogenase (G6PDH) enzyme activity or in phosphate buffer (pH = 7.5) for the determination of lactate dehydrogenase (LDH) enzyme activity. The mixtures were homogenized by a motor homogenizer for 5 min. in an ice cold bath. The determination of G6PDH in the clear homogenate was done using a UV method by a kit from Boehringer-Mannhein and that the LDH was carried out by a colorimetric kit from BioMerieux, France.

Results

Assay of serum total lipids, triacylglycerols and total cholesterol of animals envenomated with LD50 *W. aegyptia* venom revealed a highly significant decrease compared with control, ($P < 0.001$) (Table 1). Moreover, G6PDH and LDH activities were also inhibited (Table 2).

However, liver, kidney and heart total lipids content increased significantly ($P < 0.05$) in envonomated rats compared to the controls with significant decrease in brain lipids. Insignificant changes ($P > 0.05$) were noticed in triacylglycerol and total cholsterol in organs other than liver, but the triacylglycerol increased significantly ($P < 0.05$) in the liver while cholesterol significantly decreased (Table 1).

The enzyme activity of G6PDH is seen to increase significantly ($P < 0.05$), in kidney and heart, but no significant ($P > 0.05$) change was observed in its level in both the liver and the brain. As for the LDH enzyme level, it was found to be inhibited in the liver and kidneys but is significantly ($P < 0.05$) elevated in the heart and the brain of envenomated animals (Table 2).

Table 1. **Effect of i.p. envenomation with LD 50 dose of *Walterinnesia aegyptia* on total lipids, triacylglycerol, and total cholesterol content of serum, liver, kidney, heart and brain of male albino rats**

	Total lipids			Triacylglycerol			T. cholesterol		
	Control Mean ± S.D.	LD 50 Mean ± S.D.	% Diff.	Control Mean ± S.D.	LD 50 Mean ± S.D.	% Diff.	Control Mean ± S.D.	LD 50 Mean ± S.D.	% Diff.
Serum (m/100ml)	n = 8 463.4±40.	256.38±34.93**	-44.68	91.96±2.90	40.38±13.2**	-56.00	104.29±4.00	65.32 ± 13.45**	-37.36
Liver●	n = 10 39.22±15	n = 10 56.17±9.94**	+43.21	n = 10 20.15±4.33	27.55±2.1**	+36.72	n = 10 9.11±1.02	n = 10 7.69±0.9*	-15.58
Kidney●	n = 7 47.59±11.85	n = 7 63±11.31*	+32.38	n = 8 30.33±7.47	n = 8 24.63±6.33	-18.79	n = 9 17.18±2.83	n = 10 15.68±4.23	-8.73
Heart●	n = 6 30.86±2.57	n = 6 43.25±10.13*	+40.41	n = 6 22.49±9.15	n = 6 14.86±2.6	-33.92	n = 10 2.45±0.97	n = 10 2.09±0.64	-14.96
Brain●	n = 7 96.7±10	n = 7 80.3±5.65*	-15.2	n = 7 5.3±2.27	n = 7 5.87±2.5	+10.75	n = 9 18.58±2.36	n = 9 20.28±4	+9.4

* = Significant at $P < 0.05$ ** = highly significant at $P < 0.001$ ● = mg/g tissue wt.

Table 2. **Effect of i.p. envenomation with LD 50 dose of *Walterinnesia aegyptia* on glucose-6-phosphate dehydrogenase(G6PDH)* and lactate dehydrogenase (LDH)** enzymes activity of serum, liver, kidney, heart and brain of male albino rats**

	G6PDH			LDH		
	Control Mean ± S.D	LD 50 Mean ± S.D	% Diff.	Control Mean ± S.D	LD 50 Mean ± S.D	% Diff.
Serum	n = 6 6.75±2.78	3.59±0.779*	-45.48	n = 6 425.34±194.6	303.48±128.9	-28.65
Liver	n = 7 2.06±0.3	n = 6 2.3±0.28	+11.5	n = 6 221.9±75.64	n = 6 133.22±46.57*	-39.96
Kidney	n = 5 0.373±0.11	n = 5 0.496±0.098*	+32.94	n = 8 232.88±33.55	n = 8 172.72±36.61	-25.83
Heart	n = 5 0.5±0.157	n = 5 0.704±0.079*	+40.73	n = 7 966.27±417.81	n = 7 177.3±174.99*	+83.93
Brain	n = 5 2±0.002	n = 5 1.83±0.16	-8.23	n = 7 550.82±32.98	n = 7 1005±212.7**	+82.45

* = significant at $P < 0.05$

** = highly significant at $P < 0.001$

G6PDH expressed as mU/ml in serum and as mU/mg prt in other organs

LDH expressed as U/L in serum and as U/mg prt in other organs

Discussion

The decreases observed in the serum total lipids, triacylglycerols and total cholsterol indicate that plama lipids are possible targets for the *W. aegyptia* venom, probably due to the lipolytic action of the enzymes found in the Elapidae as phospholipase A_2 [8]. A decrease in serum total cholesterol was observed as a result of scorpion envenomation [9], and *Cerastes cerastes* venom was also reported to cause degradation of serum lipoproteins [10]. As for the accumulation of total lipids in the organs studied, this could plausibly be attributed to their mobiliztion from adipose tissue to the major metabolic organs. Similar observations have been made on another species of the Elapidae, *Naje - nigricolis* [11]. The increased lipid content in the cardiac muscle observed in the present study is consistent with observations in envenomated patients [12].

In general, the accumulation of lipids in liver, kidney and heart could be a result of increased lipogenesis in the presence of the venom, lack of esterification of fatty acids by the envenomated tissues, and as a consequence, lack of their secretion [14], and increase of availability and absorption of lipids from the medium due to the activity of the phospholipases present in the venom as they attack different portions of total lipids such as cholesterol and triacylglycerols, with subsequent liberation of free fatty acids [15].

It should be taken in consideration that the lipolysis and lipogenesis activities take place simultaneously, but in the presence of venom, the metabolic mechanisms and lipids turnovers could deviate according to the vital needs of the different organs and to save their metabolic energy in such a way that seems contradictory from the first point of view.

However, the inhibition of the lipolytic action of the venom may develop as a result of the possible presence of an enzyme inhibitor in the venom [16] which seems to be dominant when large doses of the venom have been applied. This might be indicated from the present observation of decreased serum lipids content, as well as in the kidney, heart and brain triacylglycerol and total cholesterol contents. This could be supported by the observation of Braganca *et al.* [17] of a polypeptide inhibitor specific for phospholipase A in cobra venom.

The known neurotoxic effects of the venom of the Elapidae [13] could be a possible explanation for the decreased lipid content in brain, which might indicate functional alteration.

Meanwhile, the inhibition observed in the present study in he levels of activities of serum G6PDH and LDH enzymes could well be due to the action of non-specific proteolytic enzymes present in the venom [18], but not due to the specific inhibitors

present in the venom. This might be supported by the demonstration of such non-specific inhibitors in scorpion venom [19], as well as in the venom of *Bitis arientans* [20; 21].

G6PDH is a nicotinamide adenine dinucleotide phosphate (NADP) specific enzyme, which operates at the critical cross-roads of Embden - Meyerhof and pentose phosphate pathway (PPP) [22, p. 158]. It was found to be generally elevated in liver, kidney, heart and not in brain or serum after envenomation with *W. aegyptia* venom. The increased PPP may also contribute its pentoses to the synthesis of RNA promoting protein synthesis, as protein content was observed to be increased in a previous study using the same venom [23]. It is also possible that glucose is mobilized via PPP after venom poisoning, while the Krebs cycle is less operative. The inhibited level of the G6PDH enzyme in brain and serum could indicate the lack of use of phosphate as in these organs, which is a sign of toxicity, as the different oxidoreduction systems share a common pool of NAD(P)/NADP(P) ratio, thereby altering energy metabolism of the envenomated animal.

On the other hand, the inhibited LDH enzyme level in the liver and kidneys of envenomated rats could be further evidence for a disturbance in pyruvate oxidation, as well as in the Krebs cycle taking into consideration that these organs possess these main metabolic pathways. The elevated LDH enzyme level in the brain and the heart might suggest the prevalence of anaerobic conditions in such vital organs as a result of enhancing the metabolic cycle to restore energy loss following *W. aegyptia* envenomation, as has been observed in case of the venoms of both *D polylipis* and *N. haje* [24] which have been reported to induce the same effects on enzyme activities, as those observed in the present study.

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دراسة تأثيرات سُم الأفعى الصلّ على أيض البلازما والأنسجة والأنشطة الأنزيمية في الجرذ الأبيض
٣ ـ التأثيرات على أيض الدهون وأنزيمي الداى هيدروجينز

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(سُلِّمَ في ٢٩ محرم ١٤١٤هـ، وقُبل للنشر في ١٦ جمادي الثانية ١٤١٤هـ)

ملخص البحث. لقد تم في هذا البحث دراسة تأثيرات سُم الأفعى الصلّ التي تعيش في المنطقة الوسطى بالمملكة العربية السعودية. حيث تم إعطاء جرعة نصف مميتة (٢,٠ مغم/كغم) من السم إلى الجرّذان مما أدى إلى إحداث نقص معنوي للمجموع الكلي للدهون، وثلاثي الجلسروايد والمجموع الكلي للكولسترول في البلازما، كما صاحب ذلك انخفاض في تركيز كل من أنزيم الجلكوز ـ٦ـ فوسفات الداى هيدروجينز G6PDH) وأنزيم لكتات الداى هيدروجينز (LDH) وهذا التأثير ربما يرجع إلى تأثير مكونات السم المحلل للدهون والمثبط في الوقت نفسه.

ويمكن تفسير تراكم المجموع الكلي للدهون في هذه الدراسة في الكبد، والكلى، والقلب، والدماغ في الحيوانات المحقونة بالسم والمصاحب بانخفاض غير معنوي للمجموع الكلي لتركيز الكولسترول في هذه الأعضاء وكذلك الجلسروايد ما عدا الكبد، ويمكن تفسير ذلك إلى تحويل الدهون من الأنسجة الطرفية المختلفة إلى هذه الأعضاء مع تحرير كل من الأحماض الدهنية الحرة والدهون الفسفورية.

كما تم في هذه الدراسة ملاحظة حدوث عجز في مسار فوسفات السكريّات الخماسية (PPP) واختلال في جهاز الأكسده والاختزال وتغيير في أيض الطاقة وذلك من نتائج تركيز أنزيمي (LDH-G6PDH) حيث تم ملاحظة زيادة تركيز أنزيم (G6PDH) في جميع الأعضاء التي درست ما عدا الدماغ في حين انخفض تركيز أنزيم (LDH) في الكبد والكلى وارتفع تركيزه في القلب والدماغ مما يؤدي إلى الاعتقاد بسيادة التنفس اللاهوائي في هذه الأعضاء نتيجة لتأثير السم عليها.