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Physiological Effect of LD_{50} of *Walterinnesia aegyptia* Crude Venom on Rat Metabolism Over Various Periods of Time

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Abstract: Serum physiological parameters are important in assessing the vital organ functions following envenomation. The effects of LD_{50} of the crude venom members of the snake family Elapidae, *Walterinnesia aegyptia* on the serum physiological parameters of white rats have been evaluated over various periods of time. The snake venom has induced a reduction in serum total protein, total albumin, uric acid, cholesterol, phosphorus, and calcium levels, as well as, the activities of LDH and AST. Serum urea, total bilirubin, creatinine, glucose, and triglycerides levels, as well as, the activities of ALT, ALP, AMY and AST were significantly increased in some of the envenomated rat groups. Thus, it appeared that *W. aegyptia* crude venom can cause time dependent disturbances of some of the vital organs of envenomated rats.

Key words: Snake, venom, Saudi Arabia

Introduction

Snakes of the family Elapidae are widely distributed in Central Asia, South, Southeast Africa and the Middle East. They are represented in Saudi Arabia by two species: *W. aegyptia* in the central (Al-Sadoon, 1989) and *Naja naje* in the southwest region (Gasperetti, 1988). Their venom consists of a variety of proteins and is considered as neurotoxic (Warrell *et al.*, 1989), cardiotoxic (Lee *et al.*, 1968) or nephrotoxic (Raab and Kaiser, 1966) and can cause several metabolic disorders (Mohamed *et al.*, 1964; 1965). Nevertheless, the effects of the venom of *W. aegyptia* were not sufficiently covered in the available literature. Thus, it is of special interest to examine the possible effects of LD_{50} of the crude venom on the blood biochemical parameters of white rats over various periods of time.

Materials and Methods

***Walterinnesia aegyptia* venom:** Crude venom was obtained from *W. aegyptia* 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, 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 tap water always available. Food, but not water, was withheld for 12 hr prior to decapitation and collecting the blood samples. The animals were divided into seven groups of 10-32 rats in each group (Tables 1-4). The first group (group 1, n=24) was designated as control and was injected i.p. with physiological saline (0.5ml) each. Rats of the other groups (groups 2-7, n=8 in each group) were each injected with i.p. LD_{50} dose of *W. aegyptia* venom in 0.5ml physiological saline (Al-Jammaz, 1995) and were killed by decapitation at 4,8,12,24,48 and 72h, respectively after envenomation. Blood was collected from each animal into plain centrifuge tubes, allowed to clot for 1h at room temperature (25 ± 2 °C) 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). Data are presented as means ± S.E. and are statistically analyzed using ANOVA. All experiments had been performed on January through March 2001, in the Department of Biology, Faculty of Teachers, Riyadh, Saudi Arabia.

Results

Walterinnesia aegyptia LD_{50} crude venom has produced significant ($P<0.001$) reduction in serum albumin, uric acid and total bilirubin levels in rats. However, the reduction in total bilirubin level has occurred in rats of groups 2 and 3 only, but has significantly ($P<0.001$) increased in the other groups

Table 1: The effects of a single i.p. injection of *Walterinnesia aegyptia* crude venom on the serum total proteins, albumin, urea, creatinine and uric acid levels of rats 4, 8, 12, 24, 48 hr and 72 hr after envenomation.

Experimental groups	Measured parameters					
	Total proteins (g/L)	Total bilirubin (μmol/L)	Albumin (g/L)	Urea (mmol/L)	Creatinine (μmol/L)	Uric acid (μmmol/L)
Group 1(controls, n=24)	67.77 ±0.07	1.29 ±0.07	42.01 ±1.51	5.45 ±0.17	37.21 ±2.46	152.3 ±6.91
Group 2(4hr) (n=8)	64.08 ±0.30*	0.34 ±0.06**	39.11 ±1.01	5.74 ±0.29	44.60 ±1.95*	123.1 ±13.6*
Group 3(8hr) (n=8)	63.50 ±1.51**	0.79 ±0.1**	36.20 ±0.54**	4.30 ±0.22**	46.33 ±2.01**	98.41 ±3.27**
Group 4 (12hr) (n=8)	63.98 ±0.89**	2.10 ±0.23**	33.29 ±1.10**	7.81 ±0.30**	59.10 ±2.18**	105.0 ±6.79**
Group 5 (24hr) (n=8)	61.13 ±2.84**	1.90 ±0.25**	30.20 ±1.4**	6.58 ±0.42**	42.89 ±3.10	112.7 ±7.29**
Group 6(48 hr) (n=8)	54.22 ±3.62**	1.82 ±0.31**	26.63 ±1.65**	7.32 ±0.43**	38.17 ±2.87	118.0 ±8.76**
Group 7 (72hr) (n=8)	60.33 ±1.04**	1.70 ±0.04**	30.70 ±0.32**	7.72 ±0.44**	43.00 ±0.82	104.8 ±8.16**

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.

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

Experimental groups	Measured Parameters		
	Glucose mmol/L	Cholesterol mmol/L	Triglycerides mmol/L
Group 1(controls, n=24)	5.56±0.16	1.58±0.04	0.82±0.05
Group 2(4hr) (n=8)	5.35±0.29	1.38±0.12	0.97±0.01
Group 3(8hr) (n=8)	6.38±0.46*	1.29±0.06	0.77±0.07
Group 4 (12hr) (n=8)	6.60±0.35**	1.29±0.05	0.94±0.05
Group 5 (24hr) (n=8)	6.84±0.45**	1.38±0.13	0.83±0.06
Group 6(48 hr) (n=8)	5.98±0.24	0.52±0.05**	0.95±0.13
Group 7 (72hr) (n=8)	5.97±0.08	0.67±0.06**	1.12±0.06**

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.

Table 3: The effects of a single i.p. injection of *Walterinnesia aegyptia* 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 and 72hr after envenomation.

Experimental groups	Measured Parameters				
	ALT U/L	AST U/L	ALP U/L	LDH U/L	AMY U/L
Group 1(controls, n=24)	57.35±2.25	275.70±10.77	177.3±8.07	2016.0±59.3	880.7±37.62
Group 2(4hr) (n=8)	76.50±4.19**	336.7±24.19**	261.3±21.99**	1524.3±79.68**	930.7±50.60**
Group 3(8hr) (n=8)	78.17±2.06**	254.2±6.19	527.0±64.63**	1597.3±144.4**	1091.2±42.3**
Group 4 (12hr) (n=8)	94.00±6.16**	312.5±18.52	689.5±43.50**	1683.5±96.6**	1635.7±51.27**
Group 5 (24hr) (n=8)	80.29±7.07**	300.3±790	296.0±6.47**	1855.4±65.4**	1632.7±60.4**
Group 6(48 hr) (n=8)	135.6±12.33**	156.0±18.55**	305.3±13.4**	1393.4±146.9**	1631.8±58.99**
Group 7 (72hr) (n=8)	87.83±6.59**	177.5±14.00**	396.8±28.2**	1911.8±100	1667.0±72.1**

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.

Table 4: The effects of a single i.p. injection of *Walterinnesia aegyptia* crude venom on the sodium , potassium , magnesium, phosphorus, calcium and iron levels in the serum of rats 4, 8, 12, 24, 48 and 72hr after envenomation.

Experimental groups	Measured Parameters					
	Sodium mmol/L	Potassium mmol/L	Magnesium mmol/L	Phosphorus mmol/L	Calcium mmol/L	Iron µmol/L
Group 1(controls, n=24)	142.0±0.8	5.40±0.07	0.96±0.06	3.27±0.08	2.60±.02	38.80±2.55
Group 2(4hr) (n=8)	140.0±1.36	6.66±0.31**	1.18±0.03**	3.25±0.09	2.64±0.02	33.81±2.90
Group 3(8hr) (n=8)	143.8±1.08	6.06±0.15**	0.96±0.01	3.09±0.10	2.66±0.04	30.89±3.54
Group 4 (12hr) (n=8)	145.7±1.98	5.57±0.26	1.05±0.03	2.47±0.09**	2.44±0.03**	28.63±2.18
Group 5 (24hr) (n=8)	144.2±1.24	5.77±0.18	0.93±0.02	2.69±0.18**	2.47±0.05**	34.50±2.99
Group 6(48 hr) (n=8)	147.7±1.98	5.77±0.26	0.92±0.02	2.33±0.19**	2.10±0.12**	38.22±5.75
Group 7 (72hr) (n=8)	140.0±0.57	5.53±0.10	0.90±0.02	3.15±0.11	2.53±0.02	29.55±3.83

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.

of envenomated rats, together with the serum urea level as compared to controls. Serum creatinine level has also increased in all envenomated rats, but it was significant in rats of groups 2, 3 and 4 (Table 1).

Serum glucose levels have significantly (P<0.01-0.001) increased in rats of groups 3, 4 and 5 as compared to control. However, the effects on the serum cholesterol levels were variable (Table 2).

Serum enzyme activities were variable following the rat envenomation. The activities of ALT, ALP and AMY have significantly increased in all envenomated rats, that of AST was significantly higher in rats of group 2 before becoming significantly lower than that of the controls with time in groups 6 and 7. LDH activity was significantly lower than that of the controls, before starting to rise with time in rats of group 7 (Table 3).

Mineral levels were also variable following rat envenomation. Serum sodium and potassium levels were higher in envenomated rats, but the potassium level was significantly (P<0.001) higher than the controls in rats of groups 2 and 3. Serum phosphorus, calcium and iron levels were lower in most of the envenomated rats, but the reductions in phosphorus and calcium levels were significant (P<0.001) in rats of groups 4, 5 and 6. Serum magnesium level varies from slight increases in rats of group 4, to significant (P<0.001) rises in rats of group 2 (Table 4).

Discussion

Measurements of serum physiological parameters in the rats following snake venom injection are of importance in the assessment of the functions of many vital organs. *W. aegyptia* is one of the snake family Elapidae that occurs in central Saudi Arabia. Its bite is relatively painless, but might swiftly cause death. There are relatively few studies on the effects of *W. aegyptia* crude venom on the metabolism, and the present study was undertaken to evaluate the effects of LD_{50} of *W. aegyptia* crude venom on the serum physiological parameters over various periods of time.

In present study, the reduction in serum total protein, albumin and total bilirubin levels in the envenomated rats could be attributed to the disturbance in protein synthesis in the hepatocytes due to cellular damage, together with hemorrhages in vital organs, which have led to protein loss. Such disturbances have been reported by various other investigators with snake venoms (Tilbury *et al.*, 1987; Abdel-Nabi *et al.*, 1993; Abdel-Nabi *et al.*, 1997; Marsh *et al.*, 1997; Al-Jammaz *et al.*, 1999). Furthermore, acute renal damage together with glomerular, tubular and vascular lesions following various snake bites have been reported (Sant and Purandare, 1972; Chugh *et al.*, 1975; Sitprija and Boonpucknaving, 1977; Aung-Khin, 1978; Sitprija *et al.*, 1982; Tilbury *et al.*, 1987). In addition, increased vascular permeability and hemorrhages in various other vital organs, in general and in the kidneys in particular, as has been observed by Meier and Stocker (1991) in the majority of snake envenomation, further aggravates the reduction in serum proteins.

In present study, the impairment of renal functions by the venom was evident since serum urea and creatinine levels were elevated and serum ureic acid level was reduced in envenomated rats. However, this impairment of renal function is developed with time, where at the first stages of

venom injections kidneys increased the loss of urea, and at latter stages where the development of renal cortical necrosis and tubular damage occurred and serum urea levels are elevated. The state of hyperglycemia has been reported with various snake venoms ((Tilbury *et al.*, 1987; Abdel-Nabi *et al.*, 1993; Abdel-Nabia *et al.*, 1997; March *et al.*, 1997; Fahim, 1998; Al-Jammaz *et al.*, 1999).

The reduction in serum cholesterol and the rise in triglyceride levels were seen 48-72hrs following envenomation of the rats. It could be due to the damage brought upon the hepatocytes by the venom making them unable to phosphorylate the huge amounts of fatty acids and the destruction of cell membrane of other tissues (El-Asmar *et al.*, 1979). In addition, the results also suggest that variation in serum cholesterol and triglyceride levels could be attributed to the mobilization of those two lipid compounds from the adipose tissues caused by the venom are time dependent .

Measurements of the serum enzyme activities are important in assessment of vital organs, and crude snakes venoms have been shown to affect the activities of several serum enzymes (Mohamed *et al.*, 1980; Al-Jammaz *et al.*, 1992; Abdel-Nabi *et al.*, 1997; Fahim, 1998). Those enzyme activities fluctuate following the damage to liver, myocardial and skeletal muscles (Mohamed *et al.*, 1981). In the present study, the general rise in the activities of ALT, ALP and AMY, the reduction in that ADH, as well as, the fluctuation of AST indicate the damage of liver, heart, and other organs brought about by the venom.

There are few studies available on the effects of snake venoms on serum electrolyte concentrations. Mohamed *et al.* (1964) have reported an initial decrease in blood sodium and initial increases in blood potassium following *W. aegyptia* envenomation. Similar observations were seen with venoms of both *W. aegyptia* and *E. coloratus* in rats (Al-Jammaz, 1995). In present study, *W. aegyptia* crude venom has produced slight decrease in sodium and significant increases in potassium levels of some of the envenomated rats, together with serum phosphorus, calcium and iron levels. Those disturbances of serum electrolyte levels might be due to acute renal failure and glomerular tubular damage. Furthermore, a direct stimulation of the adrenal cortex leading to aldosterone secretion (Mohamed *et al.*, 1980), as well as the damage in skeletal and myocardial muscles might have been caused by venom.

It appears that LD_{50} of *W. aegyptia* crude venom might have produced tissue distractions, especially in the liver, kidney, heart, and skeletal muscles and those changes appeared to be time dependent.

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