Original Article

Specific Antivenom Ability in Neutralizing Hepatic and Renal Changes 24 Hours after *Latrodectus dahli* Envenomation

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Abstract

Background: Latrodectism, a syndrome caused by *Latrodectus* genus, is one of the clinical problems that occur predominantly in north east of Iran. Nowadays antivenom therapy has become the most useful treatment for animal bites; however there is still a controversy about route and time of antivenom administration in spider bite. The aim of the present study was to determine the efficacy of specific antivenom in neutralizing hepatic and renal symptoms 24 h after *Latrodectus dahli* envenomation.

Methods: We selected a group of male New Zealand white rabbits, weighing 2±0.3 kg. The *L. dahli* venom (0.5 mg/kg) was injected subcutaneously. Specific antivenom (2.5 ml, I.V) was injected 24 h following venom injection. Blood sampling was performed before and 24 h after venom injection, as well within 24, 48 and 72 h after antivenom administration. Serum levels of (aspartate amino transferase (AST) alanine amino transferase (ALT), alkaline phosphatase (ALP), urea, bilirubin, creatinine and albumin were determined in all the sam.

Results: *Latrodectus dahli* venom caused significant increase ($P< 0.05$) in all foresaid serum parameters. Antivenom reversed the AST, ALP, creatinine, urea and bilirubin to normal levels, but failed about ALT level, also non-significant decrease was observed in albumin levels.

Conclusion: Antivenom administration 24 h after venom injection can greatly reverse symptoms caused by venom. Future studies in human beings should be conducted to assess the protection against the specific-*Latrodectus* antivenom.

Keywords: *L. dahli*, Liver, Kidney, Venom, Antivenom

Introduction

Arachnid bites are one of the most important clinical problems in tropical and subtropical countries (Almeida et al. 2009, Lima et al. 2009). Although the most spiders are venomous, but just a few are really dangerous for people (Vassilevski et al. 2009). Latrodectism is a syndrome caused by one of the most dangerous spiders in the world named *Latrodectus*. Black widow spider is a common name for this genus due to intrinsic behavior of females that kill their mates after mating (Bettini and Maroli 1978).

Among 30 species that distributed worldwide, only 4 species are reported as inhabitant of Iran. The clinical problems of latrodectism are common in north east part of Iran (Afshari et al. 2009, Shahi et al. 2011). Black widow spider venom contains a neurotoxic compound, α- Latrotoxin, that affect motor end plates in neuromuscular junction and presynaptic neurons in synapse region. α-Latrotoxin cause great secretion of neurotransmitters at central nervous system and neuromuscular junction. The toxin by interaction with specific receptors that called neurexin, a dependent calcium receptor, caused formation of cation nonspecific channels in the presynaptic membrane, as well Latro-

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philin, α- Latrotoxin independent calcium receptor, by activation of PLC and DAG caused release internal calcium from endoplasmic reticulum. In fact, both receptors increase exocytosis level in the presynaptic membrane (Henkel and Sankaranarayanan 1999, Ushkaryov et al. 2008, Silva and Ushkaryov 2010).

Previous reports (Maretic and Stani 1954, Levine et al. 2010) indicate that venom of spiders belonging to *Latrodectus* genus can cause cardiovascular changes including blood vessels spasm, arterial hypertension, tachycardia, bradycardia and change in the S-T segment with a prolongation of the Q-T interval. Same reports also showed urinary changes including oliguria and a high specific gravity of urine, with albuminuria, which are typical of patients with latrodectism. Other reports (Prior and Park 2004, Lima et al. 2009) also indicate priapism, hazy vision, diarrhoea, dyspnea, tachypnea and pressure in the chest of patients bitten by this spider.

The treatment of latrodectism by various drugs and compounds like calcium gluconate, methocarbamol, benzodiazepines and narcotics have been reported, but in fact, many of them provides only temporary relief. Nowadays use of antibodies and antibody fragments are considered as the most effective treatment in animal bites (Allen and Norris 1991, Guti´errez et al. 2003). However there is a substantial controversy about the efficacy, safety and antivenom administration route. Previously antivenoms were injected intramuscularly, but according to the data published in recent decades (Isbister et al. 2007a, Isbister 2007b) intramuscular route is not efficient, so intravenous route was proposed. However further researches are required to obtain doses, time and route of antivenom administration. On the other hand determination of antivenom ability to reverse complications of black widow needs more investigations.

In this study we attempted to assess the effects of intravenous antivenom injection in neutralization of renal and hepatic symptoms causing by *L. dahli* venom.

Materials and Methods

Venom

Crude spider venom was provided by Department of Venomous Animals and Antivenom Production, Razi Vaccine and Serum Research Institute, Karaj, Iran. *Latrodectus dahli* were dissected out, and a pair of glands was collected into ice cold phosphate buffered saline (PBS). The glands were washed in PBS in order to remove possible contaminants, and venom was harvested in PBS by gentle compressing of the glands. The suspension was clarified by centrifugation at 8000 rpm, and the venom was stored at -20 °C until use.

Experimental animals

Six New Zealand male white rabbits weighing 2±0.3 kg were selected. All rabbits were maintained in quarantine for at least 3 days before the experiment. The temperature was controlled at 18–22 ˚C with food and water. Rabbits were anaesthetized with intramuscular injection of 0.5 ml ketamine and 0.5 ml xylazine in ratio 1:1 respectively. *Latrodectus dahli* venom (0.5 mg/kg) was injected subcutaneously. Blood sampling was carried out for all animals before and 24 h after venom injection as well 24 h, 48 h and 72 h after antivenom injection. Blood collected with EDTA (1 mg/ml of blood), and processed for in clinical signs and symptoms of all the animals were recorded during the experiment.

Biochemical Kits

For determination of the changes in aspartate amino transferase (AST) alanine amino transferase (ALT) and alkaline phosphatase (ALP) enzymes, as well bilirubin, urea, crea-

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tinine and albumin, analytical kits were purchased from Pars Azmoon Company (Iran). Other chemicals and reagents used in this study were analytical grade and purchased from Merck (Germany).

Methods

In this study *L. dahli*'s venom (0.5 mg/kg) was injected by subcutaneously route to the fore legs of rabbits. Blood sampling was performed before and 24 h after venom administration and later antivenom (2.5 ml with neutralization capacity of 500 LD 50/ml) was injected by I.V route. The blood collection was carried out again at 24, 48 and 72 h following antivenom injection. Experiment ended by scarifying rabbits for extraction of heart, liver and kidney tissues throughout the surgery. Blood collected in the tubes containing EDTA, were processed for biochemical studies. For this purpose blood samples were centrifuged at 2500×g for 10 min at 4 °C, then the serums were processed for biochemical studies.

This article was a part of project work in Razi Vaccine and Serum Research Institute which was approved by Ethics Committee of this institute.

Statistical analysis

Data were analyzed with statistical software SPSS 20.0. The comparison was between the data obtained before and after the venom injection as well as before and after antivenom injection. All the results were statistically analyzed using the Student "t" test. The results were considered to be statistically significant if ($P< 0.05$).

Results

The signs and symptoms of envenomation appeared within first few hours by redness, mild swelling as well as muscle cramps at bite site. Later the rabbits showed tremors and finally paralysis in legs. Difficulty in respiration was observed in most of the animals within 24 hours following venom injection.

Table 1 shows the changes in all parameters levels, before and after the venom injection.

Following venom injection (0.5 mg/kg, S.C), increasing in alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine and bilirubin levels were seen within 24 h, which were statistically highly significant ($P< 0.01$). Aspartate aminotransferase (AST) level within 24 h was also increased, that was extremely significant ($P< 0.001$). Although the rise in urea and albumin levels, 24 h after venom injection, were not more than 21.80 % and 16.21 %, respectively, but were significant ($P< 0.05$) too (Table 1).

Antivenom injection 24 h after venom, compared with pre-injection of the antivenom, leads to changes in the parameters levels within 24, 48 and 72 h. In comparison with before and 24 h after antivenom injection, decrease in ALP and ALT were statistically significant ($P< 0.05$), also a highly significant ($P< 0.01$) decrease was observed in AST level when compared with the level before antivenom injection. The level of creatinine at 24 h after antivenom injection, showed unexpectedly increase, which was highly significant ($P< 0.01$), however the bilirubin, urea and albumin levels showed non-significant decrease at 24 h (Table 2). Following antivenom injection within 48 h, the levels of all the parameters continued decrease in their levels which were statistically highly significant ($P< 0.01$) but unexpectedly the levels of ALT and creatinine did not decrease but rather increased; the rise were respectively significant ($P< 0.05$) and non-significant (Table 3). Within 72 h after antivenom injection, compared with pre-injection of the antivenom, AST and ALP levels were decreased, which statistical analysis showed that this decline were highly significant ($P< 0.01$), also extremely significant decrease ($P< 0.001$) were observed in bilirubin

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and urea levels. However decrease in albumin and creatinine levels, within 72 h compared with pre-injection of the antivenom, were non-significant. ALT level within 72 h did not decrease, rather increased up to 27 %, which was statistically non-significant (Table 4).

Table 1. Effects of *Latrodectus dahli* venom at 24 hours on rabbit's serum ALT, AST, ALP, bilirubin, creatinine, urea, albumin (mean±SEM)

Parameters	Before venom	After venom (500µg/kg; S.C)	% Change	P value
ALT (U/l)	48.61 ± 4.6	92.58± 10.49	+ 90.7	P< 0.01
AST (U/l)	54.35 ± 6.01	169.81± 15.31	+ 213.81	P<0.001
ALP (U/L)	49.01± 4.62	199± 23.28	+306.04	P< 0.01
Bilirubin (mmol/l)	0.67 ± 0.087	0.93± 0.1	+ 38.80	P< 0.01
Creatinine (mg/dl)	0.81 5± 0.08	1.12± 0.23	+ 37.42	P< 0.01
Urea (mg/dl)	27.09 ± 1.92	33± 4.08	+ 21.80	P<0.05
Albumin (gr/dl)	25.95 ± 3.1	30.1± 4.1	+ 16.21	P<0.05

% changes: Changes between before and 24 hours after venom administration.

Table 2. Effects of *Latrodectus dahli* antivenom at 24 hours on rabbits serum ALT, AST, ALP, bilirubin, creatinine, urea, albumin (mean±SEM)

Parameters	After venom	24 h antivenom (2.5ml, IV.)	% Change	P value
ALT (U/l)	92.58±10.49	78.26±8.63	- 14	P< 0.05
AST (U/l)	169.81±15.31	114±10.77	- 32.86	P< 0.01
ALP	199± 23.28	88.5±22.75	-55.52	P<0.05
Bilirubin (mmol/l)	0.93±0.1	0.87± 0.087	- 6.45	N.S.
Creatinine (mg/dl)	1.12±0.23	1.4±0.67	+ 25	P< 0.01
Urea (mg/dl)	33±4.08	32±3.56	- 3.03	N.S.
Albumin (gr/dl)	30.1±4.1	29.15±4.75	- 3.15	N.S.

N.S: Not significant.
% changes: Changes between before and 24 hours after antivenom administration.

Table 3. Effects of *Latrodectus dahli* antivenom at 48 hours on rabbits serum ALT, AST, ALP, bilirubin, creatinine, urea, albumin (mean±SEM)

Parameters	After venom	48 h antivenom (2.5ml, I.V.)	% Change	P value
ALT (U/l)	92.58± 10.49	145±15.20	+ 58	P<0.05
AST (U/l)	169.81± 15.31	89±11.30	- 47.58	P<0.01
ALP	199±23.28	67.88±8.78	- 65.88	P<0.01
Bilirubin (mmol/l)	0.93± 0.1	0.74±0.06	- 20.43	P<0.01
Creatinine (mg/dl)	1.12± 0.23	1.23±0.188	+ 9.82	N.S
Urea (mg/dl)	33± 4.08	27.56±3.2	- 16.48	P<0.01
Albumin (gr/dl)	30.1± 4.1	25.5±3.98	- 15.28	N.S

N.S: Not significant.
% changes: Changes between before and 48 hours after antivenom administration.

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Table 4. Effects of *Latrodectus dahli* antivenom at 72 hours on rabbits serum ALT, AST, ALP, Bilirubin, Creatinine, Urea, Albumin (mean ± SEM)

Parameters	After venom	72 h antivenom (2.5 ml, I.V.)	% change	P value
ALT (U/l)	92.58±10.49	116.83±27.98	+27	N.S.
AST (U/l)	169.81±15.31	88±9.72	- 48.17	P<0.01
ALP	199±23.28	58±8.71	-70.85	P<0.01
Bilirubin (mmol/l)	0.93±0.1	0.65±0.05	-30.10	P<0.001
Creatinine (mg/dl)	1.12±0.23	0.9±0.18	-19.64	N.S.
Urea (mg/dl)	33±4.08	24±2.87	-27.27	P< 0.001
Albumin (gr/dl)	30.1±4.1	25.2±3.2	-16	N.S

N.S: Not significant.
% changes: Changes between before and 72 hours after antivenom administration.

Discussion

In the present study the effects of *L. dahli* venom within 24 h on various serum biochemical parameters were determined which showed a significant rise in liver and kidney function tests. So it seems that *L. dahli* venom in less than 24 h, exerts its destructive effects on foresaid organs. Clinical manifestations of black widow spider envenomation indicated that the nervous system is the primary target of alpha-latrotoxin, as by activation of sympathetic nervous system cause intense release of neurotransmitters (Vetter and Isbister 2007. ALT is considered as the principal liver marker enzymes that frequently used as an indicator of hepatocyte damage (Barraviera et al. 1995a, França et al. 2009). Aspartate aminotransferase (AST) has a wide distribution, so that can be found in many organs such as heart, kidneys, liver and skeletal muscles (Huang et al. 2006). Significant increase in ALT and AST levels observed 24 hours after venom injection, indicating liver damage caused by the venom of *L. dahli*. However the results showed that antivenom administration can reverse AST levels but it was failed to return ALT level to initial state (before venom injection) within 72 hours. In clinical trials, increasing in ALT level represents severe hepatic injury (Huang et al. 2006). It seems that black widow spider venom exerts its destructive effects, in less than 24 hours, in liver tissue. Marzan and Maretic investigated the pathological effects of black widow spider venom on liver, they confirm the results of this study. According to their observations, after 30 minutes to 6 hours of venom injection, hepatic cells swollen and gradually massive hyperaemia appears, pericapillary edema after 10 hours, necrosis and lobular necrosis were seen respectively after 12 and 24 hours of venom injection (Maretic 1953, Marzan 1955). The AST to ALT ratio appears to be a useful index for distinguishing liver disease and according to this report, if this ratio is less than 1 (>1) represents mild liver injury, but if this ratio is more than 1(<1) indicates severe liver damage (Siddiqi et al. 2007). In our study, considering the ratio of AST/ALT, 24 hours after venom injection, severe damages appeared in the liver, which lasted 24 hours after antidote injection; but within 48 and 72 hours after antivenom injection, the ratio dropped, that indicates the antivenom is able to reduce the severity of the injury. Since 1995 several authors (Barraviera et al. 1995b, Voronov et al. 1999) have described in envenomations the systemic inflammatory response syndrome.

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This causes transient liver abnormalities with increased enzymes and decreased production of albumin and fibrinogen. This acute phase reaction could have also contributed to the changes observed in this study.

Other indicators of liver health are measurements of alkaline phosphatase and bilirubin levels in blood. Alkaline phosphatase increased significantly, following venom injection, however, it seems that antivenom was able to restore the amount of enzyme to the primary level (before venom injection). High levels of bilirubin can be caused by liver dysfunction, which happens when it is damaged (Marzan 1955). Results of present study showed that, 24 hours after venom injection, bilirubin level was increased dramatically, this fact was also present by other researchers (Maretic 1953, Marzan 1955). Although antivenom has little effect after 24 hours, but within 48 and 72 hours could improve bilirubin levels significantly.

The kidney, because of its extensive blood supply network, is one of the most vulnerable organs to toxin injury by either hemodynamic alterations which lead to renal ischemia or by direct kidney injury (Sitprija and Sitprija 2012). Determination amount of creatinine, urea and albumin in blood serum were basic parameters of kidney health. Twenty four hours after venom injection, creatinine, albumin and urea levels were increased significantly, demonstrating venom effects on kidney tissues. This fact also has been approved by other researchers. Marzan and Maretic in a histological studies have shown that, after 10–20 hours of black widow spider venom injection, degeneration of tubular epithelium with necrosis (within 24 hours) were seen in kidney tissue (Maretic 1953, Marzan 1955).

Increase in albumin level, can be due of dehydration that occurs through intense sweating and increased saliva (Walker et al. 1990). Body dehydration by profuse sweating and increasing saliva are quite compatible to the results of Maretic and Prior, but increasing albumin level, is contrary to their findings (Maretic and Stani 1954, Prior and Park 2004). Increasing urea level, 24 hours after venom injection, is reported by Maretic and Prior and it seems, that is due to dehydration or impaired renal functions (Maretic and Stani 1954, Prior and Park 2004). Antivenom injection could decrease significantly urea and creatinine levels, within 72 hours and was able in reverse albumin levels to initial state.

The α-LTX after entering the circulation, through activating L-type calcium channels, which are abundant on vascular smooth muscle cells, results in calcium influx which subsequently leads to vasoconstriction and hypertension (Sitprija and Sitprija 2012). As well as, increasing catecholamine levels followed by venom injection, causes vasoconstriction through interaction with alpha adrenoceptors in proximal and distal tubules, and causes renin secretion through interaction with beta adrenoceptors of juxtaglomerular cells of kidney. Increasing renin secretion from kidneys will result in enhancement of angiotensin II production. Followed by these events, it seems renal blood flow decreases that can lead to a significant reduction in glomerular filtration rate and induces tissue ischemia (Schrier 1974). On the other hand, according to immunological studies of Herberth in 2005, some latrophilin receptor (α-Latrotoxin independent calcium receptor) genes are expressed in various mammalian tissues, so that highest expression of latrophilin II can be found in placenta, lungs, liver and mammary glands tissues, respectively (Herberth et al. 2005). Thus it seems that black widow spider venom can induce its effects by either directly and indirectly mechanisms. The present study provides evidence that *latrodectus* venom with impressing sympathetic nervous system, increasing blood catecholamines, hypertension and maybe direct interaction with its recep-

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tors on tissues can induce hepatotoxicity and nephrotoxicity effects in rabbits.

Conclusion

The *L. dahli* venom can cause kidney and liver function changes within 24 h which are reversed to normal using specific antivenom even 24 h after envenomation. However more investigations are needed to validating route and time of black widow spider specific antivenom administration. Future studies in human beings should be conducted to assess the protection against the specific-*Latrodectus* antivenom.

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