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Effects of Different Time Protocols Treatment on Coagulation Parameters of Sheep Injected With *Hemiscorpius lepturus* Venom

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Abstract

Background: *Hemiscorpius lepturus* is one of the most dangerous scorpion species, endemic in Khuzestan province. Its venom has hemolytic activity and can lead to hemoglobinuria and death.
Objectives: The aim of this study was to determine the effects of three different time treatment protocols on changes of coagulation parameters in sheep received *Hemiscorpius lepturus* venom.
Materials and Methods: Sixteen healthy male lambs were divided randomly into four groups. *Hemiscorpius lepturus* venom (0.01 mg/kg of body weight) injected subcutaneously. Blood samples were taken at 30 minutes before and 1, 6, 12, 24, 48, 96 and 168 hours after injection. The sheep of group 1 were treated only by injection of *Hemiscorpius lepturus* venom. In groups 2, 3 and 4 (Experimental groups), treatment of animals began at 1, 24 and 48 hours respectively after the venom injection. In addition to intravenous injection of 5 mL antivenom, supportive therapy was performed as well. Prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen were measured by commercial kit and platelet (PLT) count was performed by hematology analyzer. All results were statistically analyzed using two way repeated measures analysis of variance.
Results: In the studied groups, the effect of time on PT and platelets counts was significant ($P < 0.05$); however, it did not have a significant effect on the amounts of PTT and fibrinogen. No significant statistic difference was found regarding four factors measured between different treatment groups. Interaction of time and treatment groups had significant effect only on the platelet counts ($P < 0.05$).
Conclusions: *Hemiscorpius lepturus* venom in sheep can cause severe changes in some of the coagulation factors. This should be considered as part of the pathogenesis of scorpion sting and seriously in the treatment protocol of scorpion sting victims.

Keywords: Venom, Antivenom, Coagulation Parameters, Sheep, *Hemiscorpius lepturus*

1. Background

Scorpion sting is a major public health problem in tropical and subtropical areas (1). According to available statistics, for each person killed by a poisonous snake, there are 10 who are killed by a poisonous scorpion annually (2). The highest rate of death caused by scorpion sting in Iran is in Khuzestan Province and is mostly caused by *Hemiscorpius lepturus* venom (3). Studies have shown the presence of three toxins of Hemitoxin, Hemicalcin and Heminecrolysin in Hemiscorpius lepturus Venom (4). The results of a study on mice confirmed that *Hemiscorpius lepturus* venom can reduce red blood cell count and hematocrit value (5). Sting of this scorpion might cause hemolysis, renal failure, disturbance in the central nervous system and heart disorders (6, 7), and complications that ultimately cause death of the person bitten. The treatment strategy for the bitten in addition to the use of polyvalent antivenom (which is suitable for the treatment of six common Iranian scorpion stings Androctonus crassicauda, Buthotus saulcyi, Buthotus schach, Odontobuthus doriae, Mesobuthus eupeus and *Hemiscorpius lepturus*) is based on supportive therapy (8, 9). Although there are many oral reports of scorpion stings in farm animals, there are only few scientific studies conducted in this field. According to previous studies, fatal *Hemiscorpius lepturus* venom can have the same clinical effects on sheep similar as human beings (9).

Since the results of studies on sheep, as an animal model for human use is accepted by the scientific community (10), the results of this study may help to determine hemostatic aspect of victims bitten by *Hemiscorpius lepturus* venom.

2. Objectives

The aim of this study was to determine the effects of three different time treatment protocols on changes of coagulation parameters in sheep that received *Hemiscorpius lepturus* venom. Although the effects of toxin components on hemostatic system are not well understood, there are severe physiologic changes in the system.

3. Materials and Methods

3.1. Scorpion Venom and Antivenom

Lyophilized *Hemiscorpius lepturus* venom in the form of powder was prepared from Razi vaccine and serum research institute, Karaj, Iran. Polyvalent antivenom (5 mL ampoules) was also prepared from the same institute.

3.2. Experimental Animals

In this study, sixteen native healthy male lambs of 6 months of age with an average weight of 23 kg were divided randomly into four groups (4 in each). Before the study, they were given at least one week period for adaptation. During the study, animals were fed with alfalfa, oats, wheat bran and straw.

3.3. Preparation of the Venom

Specific amount of lyophilized *Hemiscorpius lepturus* venom (0.01 mg per kg of body weight) was dissolved in distilled water and injected subcutaneously under the skin in the groin area. Blood samples were taken at -30 minutes, 1, 6, 12, 24, 48, 96 and 168 hours after injection (if the animals survived).

3.4. Grouping the Animals

Group 1 (Control group): The sheep of this group were only injected with *Hemiscorpius lepturus* venom.

Groups 2, 3 and 4 (Experimental groups): in these three groups, treatment of animals began at 1, 24 and 48 hours respectively after the venom injection. In addition to intravenous injection of 5 mL of antivenom, the treatment protocols included dexaphenylarthrite (phenylbutazone and dexamethasone-Lure Cedex, France) and cyanoferin (Iron and Vit B12-NASR pharma, Iran). Fluid therapy was also used to the animals of test groups.

3.5. Evaluation Samples

The citrated plasma was removed after centrifugation at 3,000 rpm for 10 minutes and immediately analyzed. Prothrombin time and activated partial thromboplastin time were measured by Pacific Hemostasis Thromboplastin-D kit and APTT-XL (Fischer Diagnostics®, USA), respectively. Fibrinogen was measured by a clauss assay using commercially available enzymatic kits (Mahsa Yaran, Tehran, Iran) and platelet count was performed by BC-2800Vet hematology analyzer (Mindray, China).

3.6. Statistical Analysis

Data was analyzed using statistical software SPSS 16.0 (Chicago, IL, USA). The measurements were expressed as means $\pm$ SEM. All the results were statistically analyzed using two way repeated measures analysis of variance to compare mean values obtained before the venom injection and the values after venom injection. The results were considered statistically significant if $P < 0.05$.

4. Results

In groups 1, 3 and 4, all twelve sheep showed hemoglobinuria in 12 to 24 hours after venom injection, while in group 2 hemoglobinuria was not seen.

In Tables 1 - 4 changes of the amount of PT, PTT, fibrinogen and platelet levels at different times have been noticed. Statistical analysis showed that in the studied groups the effect of time on PT and platelets counts was significant ($P < 0.05$); however, it did not have a significant effect on the amounts of PTT and fibrinogen. No significant statistical difference found regarding four factors measured between different treatment groups. Interaction of time and treatment groups had a significant effect only on platelet counts ($P < 0.05$).

5. Discussion

It was previously believed that complications of scorpion sting occur due to the release of significant amounts of neurotransmitter and during autonomic storm, but recent studies show the presence of hemotoxin, nephrotoxin and hepatotoxin in *Hemiscorpius lepturus* venom (11). According to some research, scorpion venom has at least ten protein components (12). Obviously, this diversity of materials would have different effects on various organs.

Almost all researches published on *Hemiscorpius lepturus* are limited to humans and laboratory animals. For example, an in vitro study showed that antivenom against this scorpion venom is useful in inhibiting hemolysis produced by the venom, but the duration of protection

Table 1. Effect of Different Time Protocols Treatment on Fibrinogen of Sheep Injected With *Hemiscorpius lepturus* Venom[a,b]

Group	T0	T1	T2	T3	T4	T5	T6	T7
1	246.75 ± 53.60	279.25 ± 71.86	265.00 ± 35.13	236.75 ± 85.73	249.25 ± 81.50	312.75 ± 79.17	230.00 ± 22.17	Sheep died
2	116.50 ± 28.61	111.00 ± 30.25	146.50 ± 34.25	131.50 ± 30.19	210.00 ± 18.23	174.25 ± 46.53	131.00 ± 19.34	108.00 ± 18.86
3	109.50 ± 16.53	104.25 ± 13.68	130.50 ± 20.02	155.25 ± 23.40	171.75 ±23.59	225.00 ± 26.60	235.00 ± 00	294.00 ± 8.50
4	180.50 ± 33.51	167.25 ± 29.16	121.50 ± 15.97	127.50 ± 17.06	136.50 ±3.61	229.25 ± 38.25	269.50 ± 21.08	346.50 ± 00

[a]T0: 30 minutes before venom injection, T1: 1 hour after venom injection, T2: 6 hours after venom injection, T3: 12 hours after venom injection, T4: 24 hours after venom injection, T5: 48 hours after venom injected, T6: 96 hours after venom injection, T7: 168 hours after venom injected.
[b]Data are shown as mean ± SD.

Table 2. Effect of Different Time Protocols Treatment on Prothrombin Time (PT) of Sheep Injected With *Hemiscorpius lepturus* Venom[a,b]

Group	T0	T1	T2	T3	T4	T5	T6	T7
1	14.25 ± 3.61	15.25 ± 3.72	15.75 ± 3.75	15.50 ± 3.70	16.50 ± 5.97	15.00 ± 4.91	18.00 ± 0.00	Sheep died
2	14.25 ± 3.59	14.75 ± 2.49	16.50 ± 2.90	17.75 ± 2.80	13.75 ± 1.43	16.25 ± 3.61	14.25 ± 1.65	11.00 ± 1.08
3	11.50 ± 0.64	19.75 ± 7.11	22.00 ± 8.37	24.25 ± 9.28	24.50± 9.61	25.00 ± 7.42	21.25 ± 6.42	21.75 ± 4.75
4	11.50 ± 0.28	14.00 ± 2.00	14.75 ± 1.75	16.25 ± 1.25	14.75 ± 1.88	25.25 ± 3.49	30.00 ± 00	33.00 ± 00

[a]T0: 30 min before venom injection, T1: 1 hour after venom injection, T2: 6 hours after venom injection, T3: 12 hours after venom injection, T4: 24 hours after venom injection, T5: 48 hours after venom injected, T6: 96 hours after venom injection, T7: 168 hours after venom injected.
[b]Data are shown as mean ± SD.

Table 3. Effect of Different Time Treatment Protocols on Activated Partial Prothrombin Time (APPT) of Sheep Injected With *Hemiscorpius lepturus* Venom[a,b]

Time Group	T0	T1	T2	T3	T4	T5	T6	T7
1	24.75 ± 5.40	23.00 ± 6.25	24.00 ± 8.15	32.50 ± 10.50	40.25 ± 10.93	16.00 ± 7.71	21.00 ± 0.00	Sheep died
2	21.75 ± 2.95	22.75 ± 3.75	17.25 ± 3.03	20.00 ± 2.16	15.50 ± 2.32	22.50 ± 3.27	25.25 ± 4.40	25.25 ± 7.29
3	27.50 ± 6.83	22.50 ± 3.94	24.75 ± 8.78	30.25 ± 8.43	33.25 ± 8.34	35.75 ± 6.83	33.25 ± 9.41	34.75 ± 4.64
4	21.75 ± 2.09	25.50 ± 7.53	28.00 ± 6.96	31.75 ± 6.90	29.50 ± 0.50	21.50 ± 7.07	40.00 ± 0.00	42.00 ± 0.00

[a]T0: 30 min before venom injection, T1: 1 hour after venom injection, T2: 6 hours after venom injection, T3: 12 hours after venom injection, T4: 24 hours after venom injection, T5: 48 hours after venom injected, T6: 96 hours after venom injection, T7: 168 hours after venom injected.
[b]Data are shown as mean ± SD.

Table 4. Effect of Different Time Protocols Treatment on Platelet Count (PLT) of Sheep Injected With *Hemiscorpius lepturus* Venom[a,b]

Group	T0	T1	T2	T3	T4	T5	T6	T7
1	1.81 ± 30.05	1.77 ± 25.93	1.98 ± 48.71	1.49 ± 38.51	1.16 ± 10.49	1.25 ± 27.64	2.24 ± 1.11	Sheep died
2	1.70 ± 23.36	2.12 ± 52.01	2.64 ± 57.12	2.27 ± 24.11	2.37 ± 20.94	2.78 ± 46.38	3.10 ± 45.54	2.57 ±.95.01
3	2.51 ± 19.17	3.14 ± 65.74	2.31 ± 34.65	2.03 ± 7.95	1.59 ± 16.53	1.75 ± 17.63	4.10 ± 70.73	3.93 ± 71.35
4	2.46 ± 44.36	2.73 ± 33.45	2.73 ± 49.47	2.31 ± 41.88	2.94 ± 45.78	2.65 ± 51.32	2.44 ± 5.00	5.14 ± 0.00

[a]T0: 30 min before venom injection, T1: 1 hour after venom injection, T2: 6 hours after venom injection, T3: 12 hours after venom injection, T4: 24 hours after venom injection, T5: 48 hours after venom injected, T6: 96 hours after venom injection, T7: 168 hours after venom injected.
[b]Data are Shown as mean ± SD.

is relatively short and appropriate measures need to be taken, depending on the patients' clinical progress, to re-administer antivenom and intervals less than 8 hours (9). However, lack of studies similar to the present study on large animals, makes it difficult to compare these results with other studies. However, in other available researches (in humans and laboratory animals), the effects of *Hemiscorpius lepturus* venom on coagulation parameters were

noticed.

In the only available study on large animals, Rahravani et al. showed that the scorpion venom in sheep is dangerous (even fatal) and hemoglobinuria is seen in all sheep injected by venom. Hemoglobinuria is seen in most victims probably due to the presence of hemitoxin (6). Pipelzadeh et al. reported a rapid drop in hematocrit levels and severe hemolysis in victims admitted to hospital emergency departments (12). A study on hemolysis induced by *Hemiscorpius lepturus* venom in sheep showed that its amount is larger than human, horse and cat (13). In the present study, all twelve sheep in groups 1, 3 and 4 showed hemoglobinuria in 12 to 24 hours after venom injection; however, rapid onset of treatment (group 2) prevents the occurrence of hemoglobinuria.

The results also showed that by affecting coagulation parameters, *Hemiscorpius lepturus* venom can increase PT and the number of platelets. Brazon et al. demonstrated that by controlling the Xa factor, Tityus discrepans scorpion venom can increase PT and PTT time (14). Contrary to this, in a review, Jalali et al. claimed that following the injection of *Hemiscorpius lepturus* scorpion venom in rats, PT and PTT were within the normal limits and no significant difference was found regarding coagulation parameters between the control group and the group receiving poison (15). Swenson and Markland claimed that Tityus discrepans scorpion venom is active in fibrinogenolysis, which can destroy fibrinogen and affect the coagulation system (16). The results of this study showed no significant difference between PTT factor and fibrinogen levels of treatment groups (2, 3 and 4) and control group.

In the present study, the effects of time and interaction between time and group therapy on the number of platelets of sheep were significant. Song et al. showed that active polypeptides in scorpion venom, (SVAP) prevent platelet aggregation (17). Konca et al. after performing a comparative study of platelet function in 76 children admitted to intensive care unit and 55 healthy children during February and November 2013, reported that the number of platelets in the blood of victims did not show significant difference (18).

In summary, the results of this study showed that *Hemiscorpius lepturus* venom in sheep can cause severe changes in some of the coagulation factors. This should be considered as part of the pathogenesis of the scorpion sting. The difference in intensity of changes observed in animals of this study was due to the difference in time of treatment, and perhaps individual differences between sheep in different groups. Significant differences in the emerged changes in therapy groups highlight the need for faster treatment. However, considering confirmed changes in coagulation factors, they must be considered seriously in the treatment protocol of scorpion sting victims (human or animal).

Footnotes

Authors' Contribution: All authors made substantial contributions to the intellectual content of the paper as conception, acquisition of data, analysis and interpretation of data, drafting of the manuscript and revision of the manuscript.

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