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Effect of Asian Black Scorpion *Heterometrus Fastigiousus* Couzijn Envenomation on Certain Enzymatic and Hematological Parameters

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Abstract

Accidental scorpion sting is a serious health problem in poor communities in tropical and subtropical areas throughout the world. Toxic actions of several scorpion species have been studied, but, mode of action of asian black scorpion *Heterometrus fastigiousus* Couzijn (Family: Scorpionidae) have not yet been explored. In the present study, effect of *H. fastigiousus* venom on alkaline phosphatase (ALP), acid phosphatase (ACP), lactic dehydrogenase (LDH) and glutamate-pyruvate transaminase (GPT) enzyme activity; and red blood cells (RBCs) count, white blood cells (WBCs) count, blood hemoglobin, mean corpuscular hemoglobin (MCH), packed cell volume (PCV) and plasma hemoglobin in experimentally envenomed albino mice was studied. Venom was obtained by electrical stimulation and its toxicity was determined in albino mice by subcutaneous envenomation. Effect of sub-lethal doses of *H. fastigiousus* venom on ALP, ACP, LDH and GPT enzyme activity; and RBCs count, WBCs count, blood hemoglobin, MCH, PCV and plasma hemoglobin in experimentally envenomed albino mice was studied. The LD_{50} was 18.6 mgkg-1 body weight mice. *H. fastigiousus* venom caused significant increase in ALP, ACP, LDH and GPT activity in liver tissue of albino mice. This venom reduced RBC count and increased WBC count, blood hemoglobin, MCH, PCV and plasma hemoglobin. Findings of this study will help to understand the mechanism of Asian black scorpion, *H. fastigiousus* venom toxicity

Keywords : *Heterometrus Fastigiousus*; Scorpion Venom; Hemolysis; Envenomation

Introduction

Accidental scorpion sting is a serious health issue of poor communities in tropical and subtropical areas throughout the world. Out of 1500 scorpion species distributed throughout the world, only 50 scorpion species have been proved lethal to human [1]. The symptoms of scorpion envenomation depend on species, age, venom composition and the victim's physiological response. Scorpion sting may induce local skin reactions, neurological, respiratory and cardiovascular disorders. Scorpion venom is a cocktail of various polypeptides with diverse pharmacological and physiological activities and exerts its effects by targeting ion channels [2]. Asian black scorpions belonging to genus *Heterometrus* (Family: Scorpionidae) are the largest scorpions living in Southeast Asian regions and are responsible for most of the accidental stings after their equivalents of family Buthidae. The toxic effect of these scorpion venoms and mechanism by which envenomation exerts its effects has not been clearly known. However, several scientific groups have reported pharmacological effects of some asian black scorpions. *H. scaber* venom causes prolong and sharp burning sensation around the site of sting [3]. *Palmaneus gravimanus* envenomation results in localized irritation, edema and itching [4]. H. fulvipes venom induces hemotoxic effects and inhibits acetylcholineesterase activity [5]. *H. bengalensis* venom produces irreversible nerve blockage [6]. *H. longimanus* and *H. spinifer* venoms produce contractile responses in rat anococcygeus muscle [7]. Black scorpion venoms contain high concentration of acetylcholine and nor-adrenaline, and cause reversible contraction of chick biventer cervicis muscle by cholineregic and adrenergic action [8]. *P. gravimanus* envenomation increases glucose, creatinine, blood urea nitrogen, alanine aminotransferase, creatine phosphokinase and lactic dehydrogenase and decreases total protein, uric acid, cholesterol, calcium and phosphate in serum of albino mice [9]. In the present investigation, the effect of black scorpion *H. fastigiousus* venom was studied for its effects on enzymatic and hematological parameters in albino mice after experimental envenomation.

Materials and Methods

Isolation of *H. fastigiousus* Venom

Living scorpions *H. fastigiousus* were purchased from Eastern Scientific Emporium, Gorakhpur, UP, India. Venom was obtained by electric stimulation of telson, dissolved in phosphate buffer (50 mM, pH 7.2) and centrifuged (MP01, Tarson Co., India) at

3,000×g and 4°C for five minutes. The supernatant was collected, lyophilized and stored at - 4 °C until use. The venom protein content was estimated by Lowry et al. method [10].

Toxicity Determination

H. fastigiousus venom was injected in mice weighing 25±5g subcutaneously and LD_{50} was determined by Kankonkar et al. method [11]. Median lethal dose (LD_{50}) was determined by injecting 0.1 ml of different dilutions of venom proteins subcutaneously. For each dose, four albino mice were used and mortality in experimental animals was recorded after 24 h of treatment. The LD_{50} represented dose at which half of the tested animals were died.

Experimental Protocol for Hematological and Enzymatic Assays

Three sets of albino mice weighing 25±5g were used to study the effect of scorpion venom. Animals of the first set consisting of 12 albino mice were injected with 40% of 24-h LD_{50} and those of second set also consisting of 12 albino mice were injected with 80% of 24-h LD_{50} of scorpion venom subcutaneously. Mice of both sets were sacrificed 8 hours after envenomation for hematological and enzymatic analysis. The third set consisted of six mice receiving only phosphate buffer (50 mM, pH 7.2) were used as control. At the end of experimental period, mice were anesthetized using vapours of ether. Blood was collected by cardiac puncture in tube containing anticoagulant ehylenediaminetetraacetic acid (EDTA) and used for hematological analysis. The liver was taken out by dissecting the animal for enzymatic analysis.

Hematological analysis

Determination of RBC count, blood hemoglobin, MCH, WBC count, PCV and plasma hemoglobin was done according to Dacie and Lewis method [12].

Enzymatic Analysis

Determination of alkaline phosphatase (ALP) and acid phosphatase (ACP) activity

ALP and ACP enzyme activity in liver tissue was determined by Bergmayer's method [13]. A 50 mg of liver tissue was homogenized in 1ml 0.9% sodium chloride solution and centrifuged at 5,000 g for 15 min at 0^0C. The supernatant was used as enzyme source. For ALP enzyme activity determination, in 0.1 ml enzyme source, 1 ml alkaline buffer substrate was added, mixed and incubated for 30 min at 37^0C. A 5 ml aliquot of 0.02 M NaOH was then added to the incubation mixture. For ACP enzyme activity determination, in 0.2 ml enzyme source, 1 ml acid buffer substrate was added, mixed and incubated for 30 min at 37 C. Now, 4 ml NaOH (0.1M) was added to the incubation mixtures. The intensity of yellow colour developed was measured at 420 nm. A standard curve was drawn with different known concentration of p-nitro phenol. Enzyme activity was expressed as µ mol p-nitro phenol formed 30 min^{-1} mg^{-1} protein.

Determination of lactic dehydrogenase (LDH) activity

LDH activity in liver tissue was determined by Annon's (1984) method [14]. A 50 mg of tissue was homogenized in 1 ml phosphate buffer (0.1M, pH 7.5) in ice bath and centrifuged at 10,000 g for 30 min at 4^0C. The supernatant was used as enzyme source. In 0.05 ml enzyme source, 0.5 ml pyruvate substrate was added and incubated at 37^0C for 45 min. Now 0.5 ml of 2, 4-dinitrophenylhydrazine solution was added to mixture. After 20 min, 5 ml NaOH (0.4M) was added and left for 30 min at room temperature. The absorbance of reaction mixture was measured at 540 nm. Enzyme activity was expressed as µ moles of pyruvate reduced 45 min^{-1} mg^{-1} protein.

Determination of glutamate-pyruvate transaminase (GTP) activity

GPT activity in liver tissue was determined by Reitman and Frankel's (1957) method [15]. A 50 mg of tissue was homogenized in 1 ml chilled sucrose (0.25M) in ice bath and centrifuged at 3,000 g for 15 min at -4^0C. Supernatant was used as enzyme source. To 0.1 ml enzyme source, 0.5 ml GPT substrate and 0.5 ml 2, 4- dinitrophenylhydrazine solution was added and the mixture was incubated for 15 min at room temperature. Now, 5 ml NaOH (0.4M) was added, mixed and incubated at room temperature for 20 min. The absorbance of mixture was measured at 505 nm. The enzyme activity has been expressed in units of GPT activity h^{-1} mg^{-1} protein.

Statistical Analysis

Results were expressed as mean±SE of six replicates. Student's t-test was used to detect significant changes [16].

Results

Toxicity Determination

The median lethal dose (LD_{50}) of *H. fastigiousus* venom was 18.6 mg kg-1 body weight.

Effect of *H. fastigiousus* venom on Hematological Activity

RBC count was decreased to 79.35 and 62.38% of the control after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom respectively (Table 1). Blood hemoglobin level was increased to 122.12 and 140.71% of the control after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom respectively (Table 1). MCH level was increased to 129.63 and 225.52% of the control after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom respectively (Table 1). Increase in WBC count was 117.19 and 144.31% of control after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom (Table 1). PCV was increased to 114.38 and 138.91% of the control after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom respectively (Table 1). Hemoglobin level in plasma was increased to 0.7 and 1.6 g100 ml^{-1} plasma after 8 hours of treatment with 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom respectively (Table 1). The

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variations in RBCs count, WBCs count, blood hemoglobin, MCH, PCV and plasma hemoglobin were dose-dependent ($p<0.05$, Student t-test).

Table 1: Effect of 40 and 80% of 24 h LD_{50} of scorpion *H. fastigiousus venom* on RBCs count, WBCs count, blood Hb, MCH, PCV and plasma Hb in albino mice after 8 hours of envenomation.

Parameters	Control	40% of 24-h LD_{50}	80% of 24-h LD_{50}
RBCs	6.54±0.12	5.19±0.17	4.08±0.23
	(100)	(79.35)	(62.38)
WBCs	4.13±0.008	4.84±0.06	5.96±0.09
	(100)	(117.19)	(144.31)
Blood Hb*	11.30±0.14	13.80±0.16	15.90±0.24
	(100)	(122.12)	(140.71)
MCH	17.28±0.05	26.58±0.17	38.97±0.25
	(100)	(129.63)	(225.52)
PCV	42.40±1.14	48.50±1.13	58.90±1.17
	(100)	(114.38)	(138.91)
Plasma Hb*	0.00±0.00	0.7±0.16	1.60±0.18

Results were expressed as mean±SE

Values in parentheses indicate per cent change with respect to control taken as 100%.

*Values have been represented as gm 100 ml^{-1}

Effect of *H. fastigiousus* Venom on Liver Enzyme Activity:

Table 1: Effect of 40% and 80% of 24-h LD_{50} of *H. fastigiousus venom* on ALP, ACP, LDH and GPT activity in liver tissue of albino mice after 8 hours of envenomation

Enzymes	Control	40% of 24-h LD_{50}	80% of 24-h LD_{50}
ALP*	1.72±0.09	2.38±0.13	2.98±0.17
	(100)	(138.37)	(173.25)
ACP*	3.81±0.16	5.67±0.17	6.74±0.23
	(100)	(144.81)	(173.71)
LDH**	508.66±5.13	674.32±6.73	799.31±8.79
	(100)	(132.56)	(157.15)
GPT***	84.74±2.84	141.79±2.53	193.67±2.88
	(100)	(167.25)	(228.54)

Results were expressed as mean±SE

Values in parentheses indicate per cent change with respect to control taken as 100%.

*ALP and ACP enzyme activity: μ moles of p-nitro phenol formed 30 min^{-1} mg^{-1} protein

**LDH: micromoles of reduced pyruvate 45 min^{-1} mg^{-1} of protein

***GPT: units of GPT activity h^{-1} mg^{-1} protein

The increase in ALP activity was 138.37 and 173.25% of the control after 8 hours of treatment with 40 and 80% of 24-h LD_{50} of *H. fastigiousus* venom respectively (Table 2). ACP activity was increased to 144.81 and 173.71% of the control after 8 hours of treatment with 40 and 80% of 24-h LD_{50} of *H. fastigiousus* venom respectively (Table 1). The increase in LDH activity was 132.56 and 157.15% of the control after 8 hours of treatment with 40 and 80% of 24-h LD_{50} of scorpion *H. fastigiousus* venom respectively (Table 2). GPT activity was increased to 162.75 and 228.54% of the control after 8 hours of treatment with 40 and 80% of 24-h LD_{50} of scorpion *H. fastigiousus* venom respectively (Table 2). The variations in the activity of these enzymes were dose-dependent ($p < 0.05$, Student's t-test). (Figure 1-2).

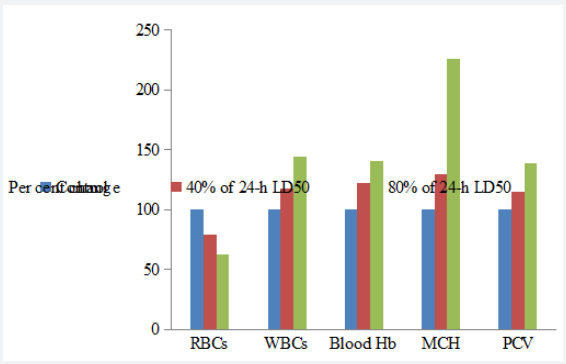

Figure 1: Effect of 40 and 80% of 24 h LD_{50} of scorpion *H. fastigiousus* venom on RBCs count, WBCs count, blood Hb, MCH and PCV in albino mice after 8 hours of envenomation.

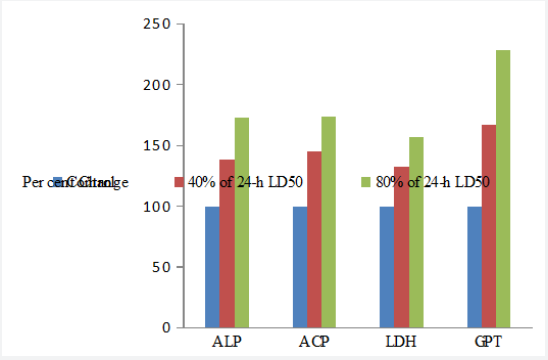

Figure 2: Effect of 40% and 80% of 24-h LD_{50} of *H. fastigiousus venom* on ALP, ACP, LDH and GPT activity in liver tissue of albino mice after 8 hours of envenomation.

Discussion

H. fastigiousus venom reduced red blood cell count and increased white blood cell count, blood hemoglobin, mean corpuscular hemoglobin, packed cell volume and plasma hemoglobin in experimentally envenomed mice. Decreased red blood cell count due to the hemolytic effect of scorpion venom is supported by increased hemoglobin level in plasma [17]. This results in anemia and circulatory hypoxia [12]. Increase in blood hemoglobin after *H. fastigiousus* envenomation may probably be the result of hemo concentration by massive release of catecholamines and angiotensin II [18,19]. Increase in plasma hemoglobin after *H. fastigiousus* envenomation indicates intravascular hemolysis [17]. When the hemolysis rate is high, the plasma extra corpuscular hemoglobin cannot be converted into bilirubin as quickly as it is released. When plasma hemoglobin

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concentration exceeds the hemoglobin binding capacity and kidney tubular capacity, the excess free plasma hemoglobin is filtered and excreted in the urine causing hemoglobinuria. Different scientific groups have given different reasons of intravascular hemolysis. Cobra venom releases an enzyme, phospholipase, which converts lecithin to lysolecithin, a powerful hemolytic and cytotoxic substance [17]. Since lecithin is present in red blood cells, the introduction of the venom into the body stimulates the production of hemolytic substance, lysolecithin. This could be the cause of hemolysis associated with scorpion envenoming [20]. Hemolytic activity of scorpion venom peptides may also be associated with certain structural characteristics formed by the constituent peptides when come in contact with biological membranes [21]. An imbalance is created due to increased secretion of catabolic counter regulatory hormones like catecholamines, epinephrine, norepinephrine, glucagon, cortisol, thyroxine, triiodothyronine and reduction in anabolic hormone, insulin which might have contributed the fragility of red blood cells resulting in hemolysis [22]. Increased mean corpuscular hemoglobin after *H. fastigiousus* envenomation is an indicative of hemolysis [12]. Similarly, increase in white blood cells count was also reported in *Mesobuthus tamulus* envenomation which may probably due to the myocardial infarction [22]. Increased packed cell volume after *H. fastigiousus* envenomation in mice is similar to red scorpion envenomation [22]. The elevation of angiotensin II during scorpion decreases blood volume by shifting the fluid from intravascular to extravascular compartments and consequently increases packed cell volume [19,23].

ALP is a group of membrane bound enzymes which mediate transport of metabolites across the membrane and play an important role in protein and certain enzyme synthesis [24,25]. Increased ALP activity stimulates the pace of protein synthesis in liver which may probably be responsible for elevated serum protein. ACP is a lysosomal enzyme which plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis [26]. Liver ischemia and hypoxia increase the activity of plasma lysosomal enzymes [27]. The increased activity of ACP might probably induce tissue necrosis and increased serum level. Tissue LDH utilizes glucose for energy especially in anaerobic conditions. Increased LDH activity occurs in response to insufficient supply of oxygen suggesting the increased glycolytic activity for obtaining energy in oxygen deficient condition [28]. The increased LDH activity in liver tissue of envenomed mice probably indicates increased glucose utilization under oxygen deficient condition. GPT works as a link between carbohydrate and protein metabolism by catalyzing the conversion of alanine to pyruvate [29]. Increased liver GPT activity is the result of stress caused by scorpion venom as stress is known to increase GPT activity [30]. During stress, energy requirement is too high to recover and glycogen level decreases. To maintain this energy requirement and to make up high decrease in glycogen level, amino acids take an active role and act as precursor of carbohydrate metabolism through transamination reaction [28].

Conclusion

Heterometrus fastigiousus venom increased ALP, ACP, LDH and GPT activity in liver tissue of albino mice. This venom reduced RBC count and increased WBC count, blood hemoglobin, MCH, PCV and plasma hemoglobin. The outcomes of this study help to understand the mechanism of asian black scorpion, *H. fastigiousus* venom toxicity. This will help the pharmacologists to design drugs for the treatment of accidental *H. fastigiousus* envenomation.

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