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Pharmacological Changes after Black Scorpion *Heterometrus fastigiousus* Couzijn Envenomation in Mice

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Inspite of the extensive researches regarding the pharmacological consequences of scorpion envenomation, toxic effect of Asian black scorpion, *Heterometrus fastigiousus* (*H. fastigiousus*) Couzijn (Family: *Scorpionidae*) venom and mechanism by which envenomation exerts its effects in the victim have not yet been clearly known. The present study was aimed to investigate the effects of *H. fastigiousus* venom in albino mice in order to understand the mechanism of toxicity. Venom was obtained by electrical stimulation and its toxicity was determined in albino mice by subcutaneous envenomation. Effects on different biomolecules and enzymes in blood serum of albino mice were determined after experimental envenomation with sub-lethal doses of *H. fastigiousus* venom. Student's t-test and F-test was used for analysis of data. The LD_{50} of *H. fastigiousus* venom was 18.6 mg kg^{-1} b.wt. Increased levels of serum glucose, pyruvic acid, lactic acid and creatinine were observed after *H. fastigiousus* envenomation. Elevated levels of circulating lactic dehydrogenase and creatine kinase were observed. Decrease in glycogen content was observed in the liver and gastrocnemius muscle tissue after experimental envenomation with sub-lethal doses of *H. fastigiousus* venom. This study helps to understand the mechanism of toxicity of Asian black scorpion, *H. fastigiousus* venom. This will help the pharmacologists to design drugs for the treatment of accidental *H. fastigiousus* envenomation.

Key words: *Heterometrus fastigiousus*, scorpion venom, hyperglycemia, serum enzymes, envenomation

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INTRODUCTION

Scorpion sting is a major health problem for poor communities in tropical and subtropical countries. About 1750 scorpion species reported throughout the world, only 25 scorpion species have been reported for their lethality in human[1,2]. The signs and symptoms of scorpion sting depend on scorpion species, its venom composition, age of the scorpion and the victim's physiological response[3]. Scorpion sting may induce severe local skin reactions, neurological, respiratory and cardiovascular collapse. *Buthidae* is the largest scorpion family and includes most toxic scorpion species[4-7]. Being rich source of various polypeptides, scorpion venoms have diverse pharmacological and physiological activities. These venoms exert their effects by targeting ion channels[8].

Scorpions belonging to genus *Heterometrus* (Family: *Scorpionidae*) are the largest scorpions living in Southeast Asian regions and are responsible for the most of the accidental stings after their equivalents of family *Buthidae*. The effect of this scorpion venom and mechanism by which envenomation exerts its effects has not been clearly known. However, different scientific groups have reported effects of some black scorpion species venoms[9-15]. Prolong and sharp burning sensation occurs around the site of sting by *H. scaber*[9]. *Palamneus gravimanus* envenomation results in localized irritation, edema and itching[10]. The *H. fulvipes* venom induces hemotoxicity and inhibits acetylcholine esterase activity[11]. The antigenic protein (Hb) of *H. bengalensis* venom produces irreversible nerve blockage[12]. *H. longimanus* and *H. spinifer* venom produce contractile responses in rat anococcygeus muscle[13]. Black scorpion venoms contain high concentration of acetylcholine and noradrenaline and cause reversible contraction of chick biventer cervicis muscle by cholineregic and adrenergic actions[14]. An increase in glucose, creatinine, blood urea nitrogen, alanine aminotransferase, creatine phosphokinase and lactic dehydrogenase and decrease in total protein, uric acid, cholesterol, calcium and phosphate in serum of albino mice was observed in experimental *Palamneus gravimanus* envenomation[15]. The present investigation, the effect of black scorpion *H. fastigiousus* venom on biochemical and enzymatic parameters in serum of albino mice after experimental envenomation have been studied to understand the mode of action of envenomation.

MATERIALS AND METHODS

Isolation of *H. fastigiousus* venom: Living *H. fastigiousus* scorpions were purchased from Eastern Scientific Emporium, Gorakhpur, UP, India. Their venom was obtained by electric stimulation of the telson, dissolved in phosphate buffer (50 mM, pH 7.2) and centrifuged (MP01, Tarson Co., India) at 3,000 rpm and 4°C for 5 min. The supernatant was collected, lyophilized and stored at -4°C until use. The protein content in venom was estimated by Lowry *et al*.[16] method. This study was done during January-April, 2006.

Toxicity determination: The toxicity of *H. fastigiousus* venom was determined in male albino mice weighing 25±5 g[5]. LD_{50} was determined by injecting 0.1 mL of scorpion venom (10, 15, 20, 25 mg kg^{-1} b.wt.) subcutaneously. For each dose, four mice were used.

Experimental protocol for biochemical assays: Three sets of albino mice weighing 25±5 g were used to study the effect of *H. fastigiousus* venom. The animals in first set consisted of 12 albino mice injected with 40% of 24 h LD_{50} and those in the second set, also consisted of 12 albino mice injected with 80% of 24 h LD_{50} of *H. fastigiousus* venom subcutaneously. Mice of the first two sets were divided into two groups: Group I and Group II including six animals each. Group I and Group II mice from the first and second sets were used at 4 and 8 h of envenomation, respectively, for biochemical analysis. Mice from the third set consisting of six mice served as control received only phosphate buffer (50 mM, pH 7.2).

Determination of serum biochemical and enzymatic parameters: At the end of each experimental period, mice were anesthetized using vapours of ether. Blood was collected by cardiac puncture, allowed to clot and clear serum was obtained for further analysis.

Determination of glucose: Serum pyruvic acid level was determined according to Mendel *et al*.[17] method and expressed as mg/100 mL of serum.

Determination of pyruvic acid: Serum lactic acid level was determined according to Friedeman and Haugen method and expressed as mg/100 mL of serum[18].

Determination of lactic acid: Serum glucose level was determined according to Shang *et al*.[19] method and expressed as mg/100 mL of serum.

Determination of creatinine: Serum creatinine was determined according to Bonsnes and Taussky method and expressed as mg/100 mL of serum[20].

Determination of creatine kinase (CK) activity: The CK activity in serum was measured using the method of Duncan as modified by Duncan *et al*.[21] and Saha[22]. CK activity was expressed as Mmol h^{-1} mL^{-1}.

Determination of lactic dehydrogenase (LDH) activity: LDH activity in serum was measured by Annon method and expressed as micromoles of reduced pyruvate 45 min^{-1} mg^{-1} of protein[23].

Determination of glycogen content: Glycogen content in liver and muscle tissues was determined in by DuBois *et al*.[24] method and expressed as g/100 g tissue.

Statistical analysis: Results were expressed as Mean±SE of six replicates. Student's t-test was used to verify significant differences ($p<0.05$) relative to controls and between sub-lethal doses and exposure periods[25]. F-test was performed to verify the regression coefficient equality[26].

RESULTS

Toxicity determination: The median lethal dose (LD_{50}) of *H. fastigiousus* venom was 18.6 mg kg^{-1} b.wt., of albino mice.

Effect of *H. fastigiousus* venom on serum biochemical level: Glucose level was increased to 152.04% of the control, after 4 h of treatment with 80% of 24 h LD_{50} of scorpion venom. This level increased to 206.23% of control after 8 h of the same treatment (Table 1). Pyruvic acid level was increased to 174.07% of the control after 4 h of treatment with 80% of 24 h LD_{50} of scorpion venom and increased to maximum (237.04%) after 8 h of treatment with 80% of 24 h LD_{50} of scorpion venom (Table 1). Serum lactic acid level was 191.45% of the control after 4 h of treatment with 80% of 24 h LD_{50} of scorpion venom. This level was increased to 228.35% of the control after 8 h of treatment with 80% of 24 h LD_{50} of scorpion venom (Table 1). A maximum increase in creatinine level (175%) was found after 8 h of treatment with 80% of 24 h LD_{50} of scorpion venom (Table 1). All these changes in different biochemical parameters were time and dose-dependent (Table 1, $p<0.05$, Student's t-test, F-test).

Effect of *H. fastigiousus* venom on serum enzyme activity: The increase in circulating CK and LDH activity was 199.1 and 153.03% respectively after 4 h of treatment with 40% of 24 h LD_{50} of scorpion venom (Table 2). The CK and LDH activity further increased to 232.75 and 183.95%, respectively after 8 h of treatment with 80% of 24 h LD_{50} of scorpion venom (Table 2). The increase in the activity of these circulating enzymes was time and dose-dependent (Table 2, $p<0.05$, Student's t-test, F-test).

Effect of *H. fastigiousus* venom on glycogen level: Glycogen level in the liver and gastrocnemius tissues of albino mice was decreased to 65.85 and 60.49%, respectively after 4 h of treatment with 40% of 24 h LD_{50} of scorpion venom, respectively (Table 3). This level in liver and gastrocnemius tissues was decreased to 49.59 and 38.27% of the control after 8 h of treatment with 80% of 24 h LD_{50} of scorpion venom, respectively (Table 3). The decrease in glycogen level in the liver and gastrocnemius muscle of albino mice was time and dose-dependent (Table 3, $p<0.05$, Student's t-test, F-test).

DISCUSSION

In the present study, venom from Asian black scorpion *H. fastigiousus* was isolated by electric stimulation and its

Table 1: Effect of 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom on glucose, pyruvic acid, lactic acid and creatinine levels in the serum of albino mice

		After 4 h		After 8 h	
Parameters	Control	40% of 24 h LD_{50}	80% of 24 h LD_{50}	40% of 24 h LD_{50}	80% of 24 h LD_{50}
Glucose*	68.23±2.87 (100)	86.64±3.14 (126.98)	103.75±3.75 (152.04)	119.38±3.88 (174.96)	140.71±4.87 (206.23)
Pyruvic acid*	0.27±0.003 (100)	0.35±0.004 (129.63)	0.47±0.007 (174.07)	0.051±0.006 (188.89)	0.64±0.008 (237.04)
Lactic acid*	30.54±1.27 (100)	45.13±2.37 (147.77)	58.47±2.58 (191.45)	56.20±2.67 (184.02)	69.74±3.66 (228.35)
Creatinine*	0.64±0.09 (100)	0.80±0.08 (125.0)	0.97±0.07 (151.56)	0.91±0.09 (142.18)	1.12±0.11 (175.0)

*mg/100 mL serum, Values in parentheses indicate percent change with respect to control taken as 100%, Results were expressed as mean±SE

Table 2: Effect of 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom on creatine kinase (CK) and lactic dehydrogenase levels in the serum of albino mice

		After 4 h		After 8 h	
Parameters	Control	40% of 24 h LD_{50}	80% of 24 h LD_{50}	40% of 24 h LD_{50}	80% of 24 h LD_{50}
Creatine kinase (CK)*	22.26±1.33 (100)	34.4±1.34 (154.53)	41.32±1.54 (199.1)	30.64±1.47 (173.58)	51.81±1.12 (232.75)
Lactic dehydrogenase (LDH)**	105.46±3.87 (100)	136.42±4.16 (129.35)	161.39±4.97 (153.03)	158.64±5.33 (150.42)	193.99±5.21 (183.95)

*Mmol h^{-1} mL^{-1}, **µmol of pyruvate reduced 45 min^{-1} mg^{-1} protein, Values in parentheses indicate percent change with respect to control taken as 100%, Results were expressed as mean±SE

Table 3: Effect of 40 and 80% of 24 h LD_{50} of *H. fastigiousus* venom on glycogen levels in liver and gastrocnemius muscle of albino mice

		After 4 h		After 8 h	
Tissues	Control	40% of 24 h LD_{50}	80% of 24 h LD_{50}	40% of 24 h LD_{50}	80% of 24 h LD_{50}
Liver	2.46±0.04 (100)	2.03±0.08 (82.52)	1.62±0.12 (65.85)	1.73±0.13 (70.32)	1.22±0.11 (49.59)
Gastrocnemius muscle	0.81±0.005 (100)	0.66±0.003 (81.48)	0.49±0.004 (60.49)	0.47±0.002 (58.02)	0.31±0.002 (38.27)

*Values represent glycogen g/100 g tissue, Values in parentheses indicate percent change with respect to control taken as 100%, Results were expressed as Mean±SE

median lethal dose (LD_{50}) was 18.6 mg kg^{-1} body weight of albino mice. The effect of sub-lethal doses of this scorpion venom on certain biochemical and enzymatic parameters was studied. Serum glucose level increased after *H. fastigiousus* envenomation resulting in hyperglycemia. This could be due to reduced insulin secretion, excessive release of catecholamines, decreased thyroid hormone levels and increased cortisol and glucagon levels[4,27-29]. Reduced insulin and increased glucagon secretion causes a sustained fall in glucose clearance and promotes glycogenolysis and gluconeogenesis, thereby increasing blood glucose levels[30]. Increased free fatty acid level after *Mesobuthus tumulus* envenomation inhibits glycogen synthatase and pyruvate dehydrogenase activity and decreases glucose transport resulting in elevated serum pyruvic acid[31]. Increased secretion of glucagon, cortisol and catecholamines along with insulin resistance or reduced insulin level stimulate glycogenolysis in the muscles, thus, promoting lactate formation[32]. Thus, during scorpion envenomation, lactate is produced but not utilized, leading to lactate acidosis[32]. When oxygen level is low, carbohydrates break down to produce energy and makes lactic acid. Lactic acid level becomes higher during strenuous condition, heart failure or when liver is seriously damaged. Serum creatinine is an important indicator of renal health[33]. It is a byproduct of muscle metabolism and is excreted unchanged by the kidney through glomerular filtration. Increased serum creatinine level indicates kidney filtration impairment. Similar results were reported in albino mice when experimented *Palamneus gravimanus*[15].

Creatine kinase (CK) is an enzyme found predominant in the skeletal and cardiac muscle. It plays an important role in providing energy for contraction in skeletal and cardiac muscles[34]. The CK phosphorylates creatine in muscle cells to high-energy molecule, phosphocreatine. Phosphocreatine is used as immediate source of energy in the muscle cells[35]. During the muscle degeneration, muscle cells break and release their contents into the circulation. Since most of the CK exists in muscle, a rise in the circulating level of CK indicates muscle damage[36]. Elevated activity of CK and LDH in the blood serum after *H. fastigiousus* venom administration, indicates myocardial and skeletal muscle damage. The main causes of altered permeability of myocardial and skeletal muscle are circulatory hypoxia, metabolic disorders and inflammation[37]. Similarly, Murthy *et al*.[38] reported an increase in circulating LDH levels during scorpion venom administration. The venom of almost all lethal scorpion species exerts similar pathological abnormalities in experimental animals[15,39].

Liver glycogen is largely related to storage and export of hexose units for the maintenance of blood glucose, whereas muscle glycogen acts as a readily available source of hexose units for glycolysis within the muscle itself[40]. Increased glucagon, corticosteroid and catecholamine levels during scorpion envenomation function synergistically and stimulate hepatic glucose production[38,40]. Under stress conditions carbohydrate reserves are depleted to meet the energy demand[41]. These changes provide ample stimulus for glycogenolysis in the liver and muscles, which indicates rapid utilization of glycogen in response to stress caused by envenomation. Since glycogen depletion is more prevalent under hypoxia condition, a situation similar to hypoxia may occur in the tissues of envenomed mice[42].

CONCLUSION

The findings of this study helps in understanding the mode of black scorpion, *H. fastigiousus* venom lethality. This will help the pharmacologists to design drugs for the treatment of accidental *H. fastigiousus* envenomation.

SIGNIFICANCE STATEMENTS

This study established the lethal nature of Asian black scorpion, *H. fastigiousus*. The outcome of this study helps in understanding the mode of action of *H. fastigiousus* venom and the pharmacologists in drug designing for the treatment.

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