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Consequences of *Mesobuthus tamulus gangeticus* (Pocock, 1900) envenomation in albino mice

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Abstract: The present study aimed to investigate the effects of *Mesobuthus tamulus gangeticus* Pocock (Buthidae) venom on albino mice (NIH strain). Whole venom was obtained by electrical stimulation and its toxicity was determined in albino mice by subcutaneous envenomation. The venom LD_{50} was 2.5 mg kg^{-1} of mouse body weight. Toxic effects on different biochemical and enzymatic parameters in blood serum and other tissues of albino mice were determined after experimental envenomation with sublethal doses of *M. tamulus gangeticus* venom. Increased levels of glucose, uric acid and cholesterol, as well as decreased serum total proteins, were observed at 2 and 4 hours after the envenomation. In the liver and muscles, glycogen content dropped after venom injection. Moreover, *M. tamulus gangeticus* venom elevated the enzymatic activity of acid phosphatase (ACP), lactic dehydrogenase (LDH) and alanine aminotransferase (ALT) in the serum of albino mice. In conclusion, *M. tamulus gangeticus* can be considered a lethal scorpion species.

Key words: *Mesobuthus tamulus gangeticus*, scorpion venom, hyperglycemia, serum enzymes.

INTRODUCTION

Scorpion sting is a serious health problem of poor communities in tropical and subtropical countries. Of the 1,500 scorpion species distributed all over the world, 50 are lethal to humans (1). The family Buthidae includes the most toxic genera such as *Androctonus*, *Mesobuthus*, *Centruroides*, *Leiurus* and *Tityus*. In India, 99 species belonging to all six scorpion genera have been reported, of which 45 are members of the family Buthidae, the largest scorpion family, including most toxic scorpion species (2-6). The clinical symptoms of scorpion sting vary according to the scorpion species and age, the venom composition and the victim's physiological response to the venom. Scorpion sting causes a wide range of conditions from severe local skin reactions to neurological, respiratory and cardiovascular collapse. Scorpion venom is a rich source of various polypeptides which act mainly by affecting specific functions of target channels (7). Buthoid venom causes severe consequences for a wide variety of vertebrate and invertebrate organisms and its toxicity has been attributed to the presence of a large variety of basic polypeptides having three to four disulfide bridges (8).

Scorpion venoms cause initial transient hypertension followed by hypotension (6). Symptoms like severe pain, inflammation, hypersalivation, dysphagia and restlessness appear within a few minutes after the sting and reach maximum severity within five hours, persisting for 24 to 72 hours. The victim's death usually occurs by anaphylaxis, bronchoconstriction, pharyngeal secretion, diaphragmatic paralysis and respiratory

failure. Most scorpion sting symptoms are due to release of catecholamines from the adrenal glands and release of acetylcholine from postganglionic parasympathetic neurons. Adrenergic symptoms occur with low venom doses, whereas cholinergic symptoms occur with high venom doses, and this duality is the result of the difference in the sensitivity to these neurotransmitters among different organ systems (9). The venom of some red scorpions of the Buthoid family causes acute myocarditis, transient hypertension followed by hypotension, intravascular coagulation, acute pancreatitis, pulmonary edema, increased levels of catecholamines, angiotensin II, glucagon and cortisol, as well as decreased thyroxine and insulin secretion (3, 6, 10-15). Due to their heterogeneous nature, scorpion venoms show variable reactions in the victim. However, the closer the phylogenic relationship among scorpions, the more similar the symptoms and the immunological properties of their venoms. Furthermore, the various venom constituents may act directly or indirectly and individually or synergistically to exert their effects.

In the present investigation, the effect of venom from the red scorpion *Mesobuthus tamulus gangeticus* Pocock on biochemical and enzymatic parameters in blood serum and different tissues of albino mice after experimental envenomation was studied.

MATERIALS AND METHODS

Isolation of *M. tamulus gangeticus* Venom

Living scorpions *M. tamulus gangeticus* 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 x *g*, 4°C, for five minutes. The supernatant was collected, lyophilized and stored at –4°C until use. The venom protein content was determined by the method of Lowry *et al.* (16).

Toxicity Determination

The toxicity of *M. tamulus gangeticus* venom was determined in male albino mice (NIH strain) weighing 25 ± 5 g by the method of Chaubey and Upadhyay (17). LD_{50} was determined by subcutaneously injecting 0.1 mL of different scorpion venom dilutions (1.5, 2.0, 2.5, 3.0 mg/kg body weight). For each dose group, four mice were used.

Experimental Protocol for Biochemical Assays

Three sets of albino mice weighing 25 ± 5 mg were used to study the effect of scorpion venom. The first set consisted of 12 albino mice injected with 40% of 24-hour LD_{50} (1.0 mg/kg body weight) and the second set also consisted of twelve albino mice injected with 80% of 24-hour LD_{50} (2.0 mg/kg body weight) of scorpion 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 2 and 4 hours after envenomation, respectively, for biochemical analysis. The third set consisted of six mice serving as control that received only phosphate buffer (50 mM, pH 7.2). The estimation of biomolecules and the determination of enzymatic activities were done separately, using the same experimental protocols already described.

Determination of Serum Biochemical and Enzymatic Parameters

The effects of *M. tamulus gangeticus* venom on serum glucose, uric acid, cholesterol, total protein, acid phosphatase, lactic dehydrogenase and alanine aminotransferase were determined in albino mice. At the end of each experimental period, mice were anesthetized using vapors of ether. Blood was then collected by cardiac puncture and allowed to clot; clear serum was isolated for further analysis.

Glucose determination

Serum glucose level was determined according to the method of Mendel *et al.* (18) and expressed as milligram/100 mL of serum.

Uric acid determination

Serum uric acid level was determined by the method of Folin (19) and expressed as milligram/100 mL of serum.

Cholesterol determination

Serum cholesterol level was determined by the method of Abell *et al.* (20) and expressed as milligram/100 mL of serum.

Total protein determination

Total serum protein level was determined by the method of Lowry *et al.* (16) and expressed as milligram/100 mL of serum.

Determination of acid phosphatase (ACP) activity

ACP activity in blood serum was determined by the method of Bergmayer (21) and expressed as micromoles of formed p-nitrophenol/30 minutes x milligram of protein.

Determination of lactic dehydrogenase (LDH) activity

LDH activity in blood serum was determined by the method of Annon (22) and expressed as micromoles of reduced pyruvate/45 minutes x milligram of protein.

Determination of alanine aminotransferase (ALT) activity

ALT activity in blood serum was determined by the method of Reitman and Frankel (23) and expressed as units of ALT activity/hour x milligram of protein.

Determination of glycogen content

Glycogen content in liver and muscle tissues was determined in albino mice after *M. tamulus gangeticus* envenomation. Thus, anesthetized mice previously envenomated according to the experimental protocol were sacrificed for the collection of liver and muscle tissues, which were kept at –20°C until analysis. The glycogen level in liver and muscle tissues was determined by the method of Dubois *et al.* (24) and expressed as gram/100 g of tissue.

Statistical Analysis

Results were expressed as mean ± SE of six replicates. Student's t-test was used to verify significant differences relative to controls and between sublethal 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 *M. tamulus gangeticus* venom was 2.5 mg/kg of body weight of albino mice.

Effect of *M. tamulus gangeticus* Venom on Serum Metabolite Level

Glucose level was 147.94%, compared to control, after two hours of treatment with 80% of 24-hour LD_{50} of scorpion venom. This level increased to 174.74% after four hours of the same treatment (Table 1). Uric acid level was 131.44%, compared to the control group, and increased to 149.82% after four hours of treatment with 80% of 24-hour LD_{50} of scorpion venom (Table 1). After two hours of treatment with 80% of 24-hour LD_{50} of scorpion venom, serum cholesterol level was 130.62%, compared to control. This level peaked (157.42%) after four hours of treatment with 80% of 24-hour LD_{50} of scorpion venom (Table 1). A maximum decrease in total protein level (61.20%) was found after four hours of treatment with 80% of 24-hour 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 *M. tamulus gangeticus* Venom on Glycogen Level

Glycogen level in the liver tissue of albino mice decreased to 73.13% and 51.6% after 2 and 4 hours of treatment with 80% of 24-hour LD_{50} of scorpion venom, respectively (Table 2). Similarly, glycogen level in the muscle tissue of albino mice decreased to 74.23% and 53.56% after 2 and 4 hours of treatment with 80% of 24-hour LD_{50} of scorpion venom, respectively (Table 2). The decrease in glycogen level in the liver and muscle of albino mice after treatment with scorpion venom was time- and dose-dependent (Table 2, $p < 0.05$, Student's t-test, F-test).

Effect of *M. tamulus gangeticus* Venom on Serum Enzyme Activity

The increase in serum ACP activity was 123.48 and 139.47% after 2 and 4 hours of treatment with 80% of 24-hour LD_{50} of scorpion venom, respectively (Table 3). The increase in circulating LDH and ALT activity was 133.84% and 145.56%, respectively, after two hours of treatment with 80% of 24-hour LD_{50} of scorpion venom (Table 3). LDH and ALT activity further increased to 147.83% and 175.35%, respectively, after four hours of treatment with 80% of 24-hour LD_{50} of scorpion venom (Table 3). The increase in the activity of these circulating enzymes was time- and dose-dependent (Table 3, $p < 0.05$, Student's t-test, F-test).

Table 1. Effect of 40% and 80% of 24-hour LD_{50} of *M. tamulus gangeticus* venom on glucose, uric acid, cholesterol and total protein levels in the blood serum of albino mice

		After 2 hours		After 4 hours	
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[1]	68.85 ± 0.93 (100)	87.02*# ± 0.96 (126.40)	101.86*# ± 0.84 (147.94)	110.04*# ± 0.97 (159.83)	121.68*# ± 0.89 (174.74)
Uric acid[1]	2.62 ± 0.07 (100)	2.98*# ± 0.09 (113.81)	3.44*# ± 0.08 (131.44)	3.40*# ± 0.07 (129.62)	3.92*# ± 0.09 (149.82)
Cholesterol[1]	159.00 ± 3.68 (100)	185.20* # ± 2.81 (116.48)	207.66*# ± 2.42 (130.62)	224.59*# ± 2.74 (141.25)	250.29*# ± 2.97 (157.42)
Total protein[2]	3.29 ± 0.08 (100)	2.90*# ± 0.07 (88.14)	2.45*# ± 0.05 (74.33)	2.41*# ± 0.07 (73.08)	2.01*# ± 0.06 (61.20)

[1]Values represent milligram/100 mL of blood serum; [2]values represent gram/100 mL of blood serum; values in parentheses indicate percent change with respect to control considered 100%; *significant ($p < 0.05$, Student's t-test); #significant ($p < 0.05$, F-test).

Table 2. Effect of 40% and 80% of 24-hour LD_{50} of *M. tamulus gangeticus* venom on glycogen level in the liver and muscle tissue of albino mice

		After 2 hours		After 4 hours	
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 ± 0.07 (100)	2.12*# ± 0.08 (86.42)	1.80*# ± 0.06 (73.13)	1.64*# ± 0.04 (66.79)	1.27*# ± 0.08 (51.60)
Muscle	0.93 ± 0.004 (100)	0.81*# ± 0.001 (87.74)	0.69*# ± 0.003 (74.23)	0.63*# ± 0.002 (67.81)	0.50*# ± 0.001 (53.56)

Values represent gram of glycogen/100 g of tissue; values in parentheses indicate percent change with respect to control considered 100%; *significant ($p < 0.05$, Student's t-test); #significant ($p < 0.05$, F-test).

Table 3. Effect of 40% and 80% of 24-h LD_{50} of *M. tamulus gangeticus* venom on acid phosphatase (ACP), lactic dehydrogenase (LDH) and alanine aminotransferase (ALT) levels in the blood serum of albino mice

		After 2 hours		After 4 hours	
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}
ACP	0.694 ± 0.004 (100)	0.766*# ± 0.005 (110.42)	0.857*# ± 0.006 (123.48)	0.886*# ± 0.007 (127.64)	0.968*# ± 0.004 (139.47)
LDH	105.64 ± 3.04 (100)	132.22*# ± (116.64)	141.39*# ± 1.81 (133.84)	140.12*# ±1.42 (132.64)	156.17*# ± 2.01 (147.83)
ALT	15.98±0.74 (100)	19.73*# ± 0.46 (123.44)	23.26*# ± 0.54 (145.56)	24.96*# ±0.57 (156.18)	28.02*# ± 0.64 (175.35)

ACP: micromoles of formed p-nitrophenol/30 minutes x milligram of protein; LDH: micromoles of reduced pyruvate/45 minutes x milligram of protein; ALT: units of alanine aminotransferase activity/hour x milligram of protein; *significant ($p < 0.05$, Student's t-test); #significant ($p < 0.05$, F-test).

DISCUSSION

In the present study, venom from the scorpion *M. tamulus gangeticus* was isolated by electric stimulation and its median lethal dose (LD_{50}) was 2.5 mg/kg body weight of albino mice. The effect of sublethal doses of this scorpion venom on certain biochemical and enzymatic parameters was studied. Serum glucose level increased after *M. tamulus gangeticus* 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 observed during earlier studies with other scorpion species (5, 14, 15, 27). Reduced insulin and increased glucagon secretion causes a sustained fall in glucose clearance and promotes glycogenolysis and gluconeogenesis, thereby increasing blood glucose levels (28). Serum uric acid level is highly dependent on renal excretion, as well as on endogenous production (29). Plasma volume expansion increases the renal excretion of uric acid by proximal tubular reabsorption and vice versa (30). Increased secretion of glucagon, cortisol and catecholamines along with insulin resistance or reduced insulin level stimulate glycogenolysis in the muscles promote lactate formation (31). Thus, during scorpion envenomation, lactate is produced but not utilized, leading to lactate acidosis which in turn inhibits the secretory mechanism of uric acid, increasing serum uric acid level (31).

Hypercholesterolemia may occur with hypothyroidism, diabetes and acute pancreatitis. The concentration of serum cholesterol is thyroid-dependent, with thyroid hormone enhancing both the rate of cholesterol synthesis and the rate of catabolism. In hypothyroidism, cholesterol utilization is lower than cholesterol synthesis. Hypercholesterolemia is also due to decreased insulin secretion. The net result is an increase in cholesterol level. Radha Krishna Murthy and Medh (32) reported increased serum cholesterol levels during *M. tamulus* envenomation, which supports the present results. The liver produces almost all serum proteins except immunoglobulin, and albumin is the major constituent of serum proteins. The decrease in total serum protein level probably indicates decreased synthetic activity of the liver under pathological conditions. Similar results have also been reported with *Leiurus quinquestriatus* and *Palamneus gravimanus* envenomation (33, 34).

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 (35). Increased glucagon, corticosteroid and catecholamine levels during scorpion envenomation function synergistically and stimulate hepatic glucose production (31, 36). It has also been reported that under stress conditions carbohydrate reserves are depleted to meet the energy demand (37). 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 (37).

Acid phosphatase (ACP) is a lysosomal enzyme that plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis (38). Liver ischemia and hypoxia increase the activity of lysosomal enzymes, which may be the cause of tissue necrosis in cardiac and liver tissues under hypoxia condition, resulting in an increased serum level of these enzymes (39).

Elevated activity of lactic dehydrogenase (LDH) and alanine aminotransferase (ALT) in the blood serum was observed, indicating myocardial and liver damage after *M. tamulus gangeticus* venom administration. The main causes of altered permeability of myocardial and liver cells are circulatory hypoxia, metabolic disorders and inflammation (40). Gajalakshmi (41) reported a significant rise in serum GPT levels during *Buthus tamulus* envenomation. Similarly, Radha Krishna Murthy *et al.* (42) reported an increase in circulating LDH and ALT levels during scorpion venom administration. The venom of almost all lethal scorpion species exerts similar pathological abnormalities in experimental animals (33, 43).

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CONFLICTS OF INTEREST

There is no conflict.

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