IN VITRO NEUTRALIZATION OF THE SCORPION, *BUTHUS TAMULUS* VENOM TOXICITY

Y. VENKATESWARLU, B. JANAKIRAM
AND G. RAJARAMI REDDY*

Department of Zoology,
S. V. University P. G. Centre, Kavali - 524 202 (A. P.)

(Received on April 3, 1987)

Summary : Scorpion *(Buthus tamulus)* venom was subjected to neutralization by treating the venom with various chemicals such as hydrochloric acid, sodium hydroxide, thiourea, formaldehyde, zinc sulphate, acetic acid and trichloroacetic acid. The venom was also subjected to heat treatment. The levels of total protein, free amino acids and protease activity in neutralized venom decreased significantly. The decrease in venom protein and free amino acids was in proportion to the duration of the heat treatment and the concentration of chemicals used except zinc sulphate, sodium hydroxide and thiourea. Protease activity of neutralized venom samples also showed a decrease except with zinc sulphate which enhanced the enzyme activity. Intramuscular injection of formaldehyde, trichloroacetic acid and heat treated venoms into albino rats produced low mortality while thiourea and zinc sulphate were not effective in reducing the mortality. Hydrochloric acid and acetic acid treated venoms reduced the mortality by 50% with a decrease in the symptoms of envenomation. The changes were attributed to the denaturing of venom protein by chemical and heat treatments.

Key words : albino rats *Buthus tamulus* protein free amino acid protease

INTRODUCTION

Neutralization of venom toxicities by various compounds has recently become important. Toxicity was destroyed when the toxins from the venom of *Androctonus australis* and *Buthus occitanus* were subjected to chymotryptic digestion (1). Thes tability of neurotoxin of *A. australis* towards denaturing agen s such as temperature and variations in pH has been reported (2). The venom of *Centruroides sculpturatus* is moderately stable to heat treatment (3). The lethal toxins of venom of *Leiurus quinquestriatus* are dialysable (4). Keeping in view that the toxic fractions of scorpion venom is proteinaceous (5), several protein denaturing agents

*Corresponding Author

were employed in the present study to neutralize the toxicity of *Buthus tamulus* venom, and the changes in the levels of venom proteins, free amino acids and protease activity were studied.

MATERIAL AND METHODS

The lyophilized scorpion *(Buthus tamulus)* venom was purchased from Haffkine Institute, Bombay (India). Venom solutions (0.1%) were prepared in physiological saline. The chemical treatments include hydrochloric acid (0.1 N, 1 N, 5 N), sodium hydroxide (0.1 N, 1 N, 5 N), thiourea (0.1%, 1%, 5%), formaldehyde (0.1%, 1%, 5%), zinc sulphate (0.1%, 1%, 5%), acetic acid (1%, 10%, 50%) and trichloroacetic acid (0.1%, 1%, 10%). These solutions were prepared with distilled water.

One *ml* of venom solution was mixed with 0.5 *ml* of test solution and allowed for 30 min. For heat treatment of venom solution, 1 *ml* of venom was heated in a boiling water bath at 100°C for 10 min, 20 min, and 30 min. The solution was then cooled and made upto 1 *ml* with distilled water. All the venom samples were centrifuged for 5 min and the supernatants were used for estimating the proteins, protease activity and free amino acids, and for lethality studies.

Venom was injected intramuscularly into the thigh muscle of albino rats (Wistar Strain) weighing 70±2 *gm*. Protein content of venom was taken as criterion for the amount of venom injected. LD_{50} (50% lethal dose) for 24 hr period calculated following the method of Reed and Muench (8) as given by Carpenter (9) was found to be 3.8 μg^{g-1}. Mortality studies were made by injecting different chemically treated venom samples and heat treated venom. All the venom samples contained LD_{50} venom protein prior to treatments. Control animals received either the saline or the respective chemical solutions with no venom. Protein content (6), protease activity and free amino acid (FAA) content (7) were estimated in the venom samples. The data was analysed for statistical significance following the method of Singh and Bhasin (10).

RESULTS

The levels of total protein, free amino acids and protease activity of venom samples treated with different chemicals decreased significantly (Figs. 1-3). The decrease observed in the protein content of venom treated with HCl, thiourea, formaldehyde, acetic acid and TCA was in proportion to the concentration of chemical used. Maximum decrease was observed with 5 N HCl (60.17%), 5% thiourea (49.14%), 5% formaldehyde (50%), 50% acetic acid (73.28%) and 10% TCA (55.17%). The decrease in venom protein was in proportion to the duration of heat treatment exhibiting maximum decrease (79.83%) with 30 min. treatment.

The decrement in protein content of venom did not alter significantly with increase in concentration of NaOH and $ZnSO_4$, however maximum decrease was noticed with 5 N NaOH (43.1%) and 5% $ZnSO_4$ (39.31%) (Fig. 1).

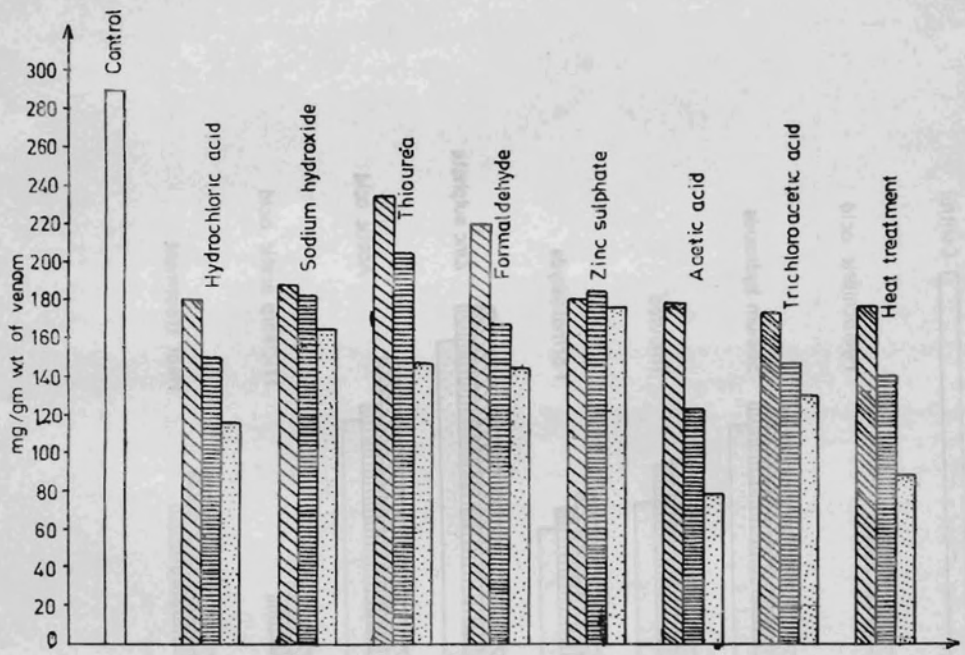

Fig. 1 : Changes in the level of proteins in chemically treated samples of *Buthus tamulus* venom.

- Control
- Low concentration/10 min. heat
- Medium concentration/20 min. heat
- High concentration/30 min. heat

The Free amino acid (FAA) content of venom showed a decrease in all the chemically treated and heat inactivated venom samples (Fig. 2). The decrease in FAA content was concentration dependent with HCl, formaldehyde, acetic acid, TCA and heat treatment,

exhibiting maximum decrease with 5 N HCl (96.12%), 5% formaldehyde (51.93%), 50% acetic acid (27.88%), 10% TCA (82.52%) and 30 min heat (95.26%). NaOH, $ZnSO_4$ and thiourea resulted in a decrease of 31.32%, 11.66%, 47.04% respectively at high concentrations while low and medium concentrations did not exhibit significant decrease (Fig. 2).

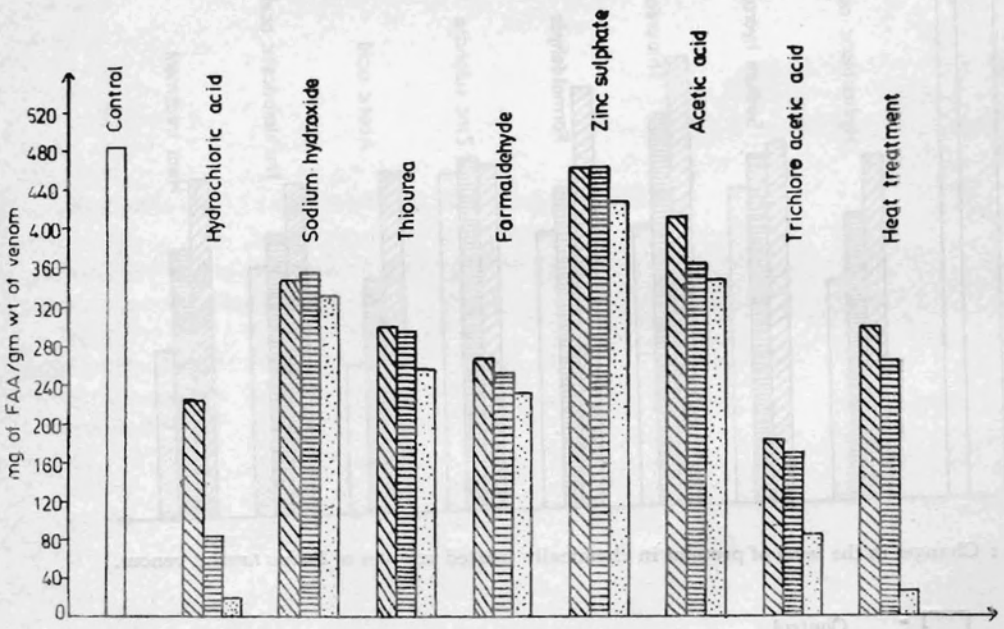

Fig. 2 : Changes in the level of free amino acids (FAA) in chemically treated and heat treated samples of *Buthus tamulus* venom.

- Control
- Low concentration/10 min. heat
- Medium concentration/20 min. heat
- High concentration 30 min. heat

Protease activity also showed a decrease with all the treatments except $ZnSO_4$ which produced a slightly enhanced activity (Fig. 3). Unlike the protein and FAA content, protease activity showed maximum change with low concentrations of chemicals except $ZnSO_4$, formaldehyde and heat. Maximum decrease in activity was observed with 0.1 N HCl (85.71%),

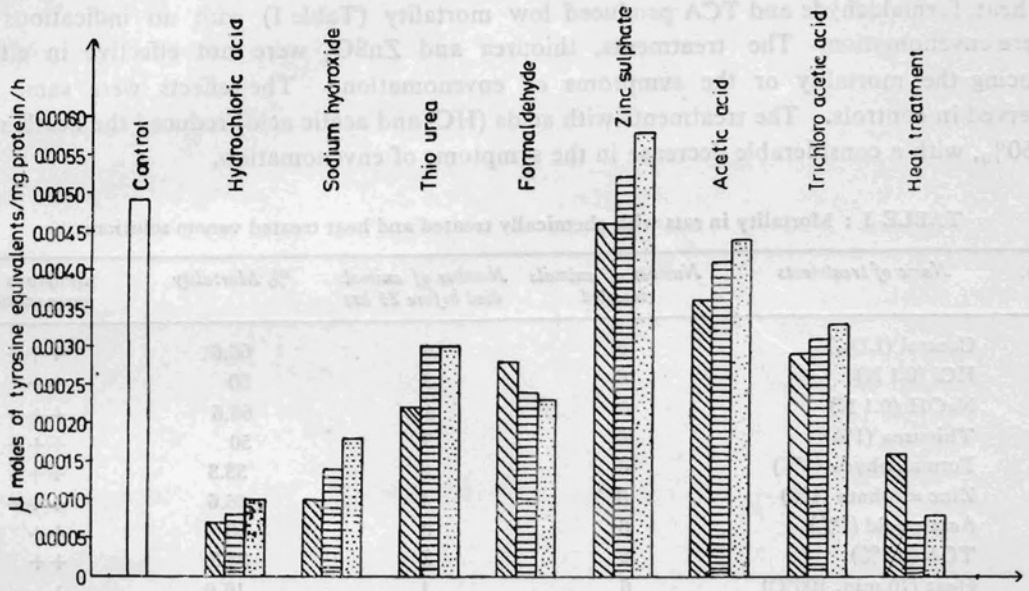

Fig. 3 : Changes in the level of protease activity in chemically treated and heat treated samples of *Buthus tamulus* venom.

- Control
- Low concentration/10 min. heat
- Medium concentration/20 min. heat
- High concentration 30 min. heat

0.1 N NaOH (79.59%), 0.1% thiourea (55.1%), 1% acetic acid (26.53%), 0.1% TCA (40.82%) and 20 min heat (85.71%) while 5% $ZnSO_4$ resulted in a maximum increase (18.37%) in enzyme activity (Fig. 3). The changes observed in the levels of proteins, FAA and protease activity of venom samples subjected to various treatments were found to be significant ($P<0.05$-0.001) except the decrease in the FAA and increase in the protease activity of the venom samples treated with $ZnSO_4$.

The animals injected with different low concentration chemical solutions alone (with no venom) showed no mortality with no signs of damage to the organism while the animals injected with lethal dose of venom showed restlessness, irregular respiration, licking at venom injected region, abdominal twitches, paralysis of injected leg, hemorrhages and necrosis. Sublethal envenomation ($\frac{1}{3}$ of lethal dose) resulted in reduced feeding, temporary paralysis and mild hemorrhages from which the animals recovered in 2-3 days. Of all the treatments, the heat, formaldehyde and TCA produced low mortality (Table I) with no indications of severe envenomation. The treatments, thiourea and $ZnSO_4$ were not effective in either reducing the mortality or the symptoms of envenomation. The effects were same as observed in controls. The treatments with acids (HCl and acetic acid) reduced the death rate by 50%, with a considerable decrease in the symptoms of envenomation.

TABLE I : Mortality in rats with chemically treated and heat treated venom solutions.

S. No.	*Name of treatments*	*Number of animals injected*	*Number of animals died before 24 hrs*	*% Mortality*	*Symptoms of envenomation*
1.	Control (LD_{50})	6	4	66.6	+++
2.	HCl (0.1 N)	6	3	50	++
3.	NaOH (0.1 N)	6	4	66.6	+++
4.	Thiourea (1%)	6	3	50	+++
5.	Formaldehyde (1%)	6	2	33.3	++
6.	Zinc sulphate (1%)	6	4	66.6	+++
7.	Acetic acid (1%)	6	3	50	++
8.	TCA (0.1%)	6	2	33.3	++
9.	Heat (10 min. 100°C)	6	1	16.6	+

+++ : Symptoms of lethal envenomation
++ : Symptoms of sublethal envenomation
+ : Reduced symptoms of envenomation

DISCUSSION

The decreased levels of protein and free amino acid contents and protease activity with acid treatments like HCl, acetic acid and TCA might be due to the denaturing effect of acids

on venom protein and also due to inactivation of enzyme protease. McIntosh and Watt (11) made similar observations in the venom of *C. sculpturatus*. They found partial inactivation of venom with 2.5% HCl and considerable inactivation of venom with nitrous acid. The changes observed in the toxic components of *B. tamulus* venom with acid treatments might be due to extreme acidic pH of venom, and denaturing of venom protein.

As observed in the present study, formaldehyde inactivation of venom was achieved by McIntosh and Watt (11). Formaldehyde was also used for active immunization as well as for the antivenin production (12). The decreased levels of toxic components observed with NaOH might be due to high alkaline pH of venom, while the changes with thiourea are because of damage to sulphydral groups. McIntosh and Watt (11) reported that sulphydral groups (—SH) are necessary for toxicity and the reagents which reduce disulphide bonds reduce lethality, as does O-methylisourea. It was also reported that the compound dihydrothioacetic acid containing sulphydral groups are effective in neutralizing snake venom (13).

Zinc is present in venom hemorrhagic toxins and enhances proteolytic activity. Proteolytic activities of venom disappeared upon removal of zinc (14). Goucher and Flowers (15) reported that there is a direct relationship between hemorrhagic activity and venom protease activity and found that EDTA (chelating agent) reduces both hemorrhage and venom protease activity. The elevation of protease activity in the present study might be due to the stimulating effect of zinc on venom enzyme activity.

The toxicity of venom can be retained indefinitely at 4°C in the lyophilized state. It was found that the toxic principle is slowly inactivated by heat (3) while the toxic effects of heated venoms were attributed to the activity of venom phospholipase A_2, which is relatively heat-stable. The mortality rate and the envenomation symptoms observed with low concentration treatments of LD_{50} venom, suggest that the heat, formaldehyde and TCA are more effective in neutralizing the scorpion venom.

In general, high concentrations of treatments were more effective in bringing out changes in the toxic components of venom but they were considered as rather harsh treatments. Hence, low concentration treatments were suggested for neutralizing *Buthus tamulus* venom. Whether or not the chemically detoxified venom has antigenic activity needs further study for clarification.

ACKNOWEDLGEMENTS

The authors (Y. V. and B. J.) thank the U. G. C. and C. S. I. R., for Senior Research Fellowships.

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