# IMPACT OF SCORPION *HETEROMETRUS FULVIPES* VENOM ON CHOLINESTERASE RHYTHMICITY IN THE TROPICAL MOUSE *MUS BOODUGA*

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**Summary ;** Rhythmicities of acetycholine (ACh) and acetylcholinesterase (AChE) were studied in the mouse, *Mus booduga* (Gray), following intramuscular injection of scorpion, *Heterometrus fulvipes* (C. Koch) venom. Envenomation inhibited the activity levels of ACh and AChE in all the tissues selected for experimentation. Control animals exhibited diel rhythmicity in ACh and AChE while envenomated animals showed fluctuation in the Phase ($\Delta\phi$), amplitude (A), Acrophase ($\phi$) and the extreme activity hours (X and $X^1$).

**Key words :** envenomation rhythmicity phase shift amplitude acrophase

## INTRODUCTION

The effects of *H. fulvipes* venom were studied in cockroaches (2), (9) lizards (6) and mice (9) on general metabolism and neurotransmission. Scorpion venom inhibits neurotrasmission at presynaptic sites of both cholinergic and adrenergic nerves (12, 24). So far no attempts were made to find out the effect of scorpion venom on the Physiological rhythmicities in the animals except a few parallel works such as X-rays (22), denervation (17, 18) and drugs (16). Since the rhythmic patterns reflect the general awareness and activity of organisms in relation to time, the effect of envenomation on rhythmic patterns of ACh and AChE in the field mouse, *Mus booduga* was attempted in the present investigation.

## MATERIAL AND METHOD

Venom was collected from adult healthy scorpions following the method of Babu *et al.* (2) and was diluted with physiological saline (0.9% NaCl) before using for experimentation.

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Laboratory acclimatized male mice weighing $10 \pm 2$ *gms* were selected for experimentation since reproductive cycles in femles were known to affect the rhythmicity (7). 50% lethal dose ($LD_{50/2}$ days) was determined by the method of Dragstedt – Behren as given by (5). Sublethal dose of venom (9.4 *ugms* – i.e. 1/3 of $LD_{50}$) was administered intramuscularly to the mice. This venom administration was done at 08 h on the day preceded by two days before the experimentation. Since venom fractions are mostly proteins (25) the amount of venom injected is expressed as protein content in *µgms*. Envenomated animals were designated as experimental animals and saline injected animals as controls.

To study the circadian rhythmicity of ACh and AChE, six timings were selected arbitrarily for convenience from 24 h natural solar day viz., 08, 12, 16 h (light hour half cycle i.e. day time) 20, 00, 04 h (dark hour half cycle i.e. night time).

The tissues selected for the study were the envenomated leg muscle (EM), Contralateral leg muscle (CM), brain and liver and the studies were made two days after envenomation (i.e. on 3rd of post envenomation) so that the animals may be relieved of the envenomation shock (10).

ACh content was estimated by the method given by Augustinsson (1), the AChE activity was assayed following the method of Metcalf (11).

Statistical analysis of the data was done by linear regression analysis as per (3).

## RESULTS

Scorpion venom inhibited AChE and ACh levels significantly and the rate of percent inhibition was in the order of EM>brain>liver>CM for ACh and EM>CM>liver >brain for AChE (Tables I, II). In both controls and experimental animals the mean activity levels (MELs) of ACh content were higher during light hour half cycle than the dark hour half cycle, while a reverse trend was observed in the MELs of AChE (Figs. 1 and 2, Tables I and II).

In control animals all the tissues exhibited diel rhythmicity with crests alternating with troughs in both ACh and AChE levels. The ACh rhythm was 180° out of phase with the rhythm of AChE (Figs. 1 and 2). The computed rhythm characteristics viz., amplitude (A), acrophase ($\phi$), crest (X) and trough ($X^1$) activity hours in all the experimental tissues of control animals were in good synchrony with the observed values (Figs. 1 and 2, Tables I and II).

FIG-1

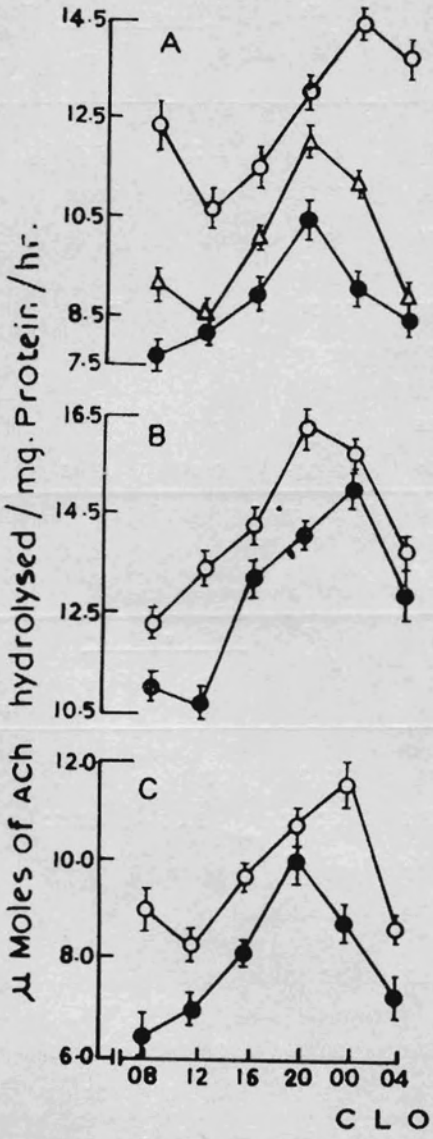


FIG.2

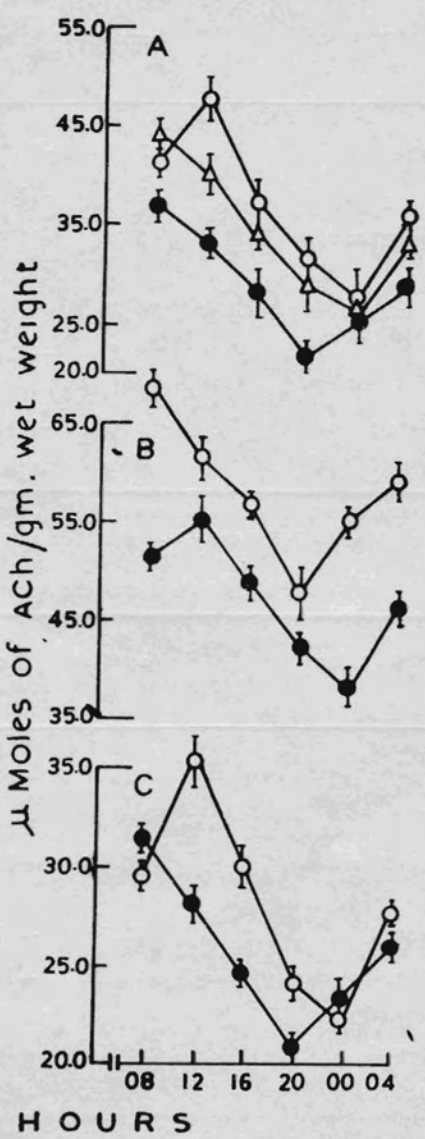


Fig. 1 : Cyclical variations in AChE activity (Fig. 1) and ACh content (Fig. 2) in muscle (A), brain (B) and liver (C) tissues of *Mus booduga*.

and 2 : 0—0 = Control ; ●—● = Experimental ; Δ—Δ = Contralateral muscle
Each value in figures is an average of six individual observations.

Envenomation induced phase shifts in both AChE and ACh rhythms of the tissues. The crest values of AChE in EM, CM and liver exhibited an advance phase shift (+Δϕ)

TABLE I : Rhythm characteristics of AChE in control and envenomated mice

| *Name of the tissue* | *A* μmoles | *ϕ* | *X* (h) | *X1* (h) | *MEL (μmoles)* | | *% change over control (μmoles)* |
|---|---|---|---|---|---|---|---|
| | | | | | *A* | *B* | |
| *Muscle* | | | | | | | |
| E. Muscle | 2.36 | –172.82° | 20.48 | 8.48 | 8.28 | 9.39 | 30.34 |
| Control | 3.62 | –106.59° | 24.84 | 12.84 | 11.56 | 13.81 | — |
| Contralateral muscle | 3.30 | –166.00° | 20.94 | 8.94 | 9.33 | 10.73 | 20.96 |
| *Brain* | | | | | | | |
| Experimental | 4.35 | –146.94° | 22.20 | 10.20 | 11.65 | 14.05 | 11.38 |
| Control | 3.98 | –172.99° | 20.47 | 8.47 | 13.69 | 15.30 | — |
| *Liver* | | | | | | | |
| Experimental | 3.12 | –171.70° | 20.55 | 8.55 | 7.25 | 8.71 | 18.08 |
| Control | 1.54 | –118.81° | 0.08 | 12 08 | 9.05 | 10 42 | — |

A : amplitude; ϕ: acrophase; X: computed creast; $X^1$ : Computed trough
MEL : Mean AChE level (A - light hour half cycle; B - dark hour half cycle).

TABLE II : Rhythm characteristics of ACh in control and envenomated mice.

| *Name of the tissues* | *A (μmoles)* | *Φ* | *X (h)* | *$X^1$ (h)* | *MEL (μmoles)* | | *% change over control (μmoles)* |
|---|---|---|---|---|---|---|---|
| | | | | | *A* | *B* | |
| *Muscle* | | | | | | | |
| E. Muscle | 13.47 | +20.37° | 9.36 | 21.36 | 32.98 | 25.17 | 21.69 |
| Control | 16.86 | +43.85° | 10.92 | 22.92 | 42.00 | 32.22 | — |
| Contraateral muscle | 16.55 | +30.32° | 10.02 | 22.02 | 39.52 | 29.68 | 6.76 |
| *Brain* | | | | | | | |
| Experimental | 15.76 | +47.37° | 12.49 | 24.49 | 51.92 | 42.27 | 18.84 |
| Control | 16.62 | +7.87° | 8.52 | 20.52 | 62.03 | 53.91 | — |
| *Liver* | | | | | | | |
| Experimental | 9.36 | +11.74° | 8.78 | 20.78 | 28.28 | 23.62 | 8.16 |
| Control | 11.35 | +52.05° | 11.47 | 23.47 | 31.68 | 24.83 | — |

A : amplitude; ϕ : acrophase; X: computed crest; $X^1$ : computed trough;
MEL : Mean ACh level (A – light hour half cycle; B – dark hour half cycle).

by 4 h. In liver both crest and trough values had established a 4 h $+\Delta\phi$. But in brain both values showed a delay phase shift $-\Delta\phi$ by 4 h (Figs. 1. a, b, c). The phases of Ach rhythmicity also witnessed the same changes i.e. a 4 h $+\Delta\phi$ in both crest and trough values in EM and in crest of CM. In brain both values exhibited a 4 h $-\Delta\phi$, while in liver these values documented 4h $+\Delta\phi$ (Fig. 2. a, b, c).

On envenomation the computed rhythm characteristics were also varied in accordance with the fluctuations of the observed values. The changes in A values of both ACh and AChE rhythms in all the tissues were significant (Tables I and II). The $\phi$ values of AChE rhythm were lengthened by about 66° in EM, 60° in CM, and 53° in liver over their respective controls. But in liver the $\varnothing$ value was shortened by 26° (Table I). In ACh rhythm the $\phi$ value was shortened in EM, CM and liver by about 23°, 7°, 41°, respectively but in brain it was lengthened by about 40° over their respective controls (Table II). The fluctuations in the X and $X^1$ values of AChE rhythm were advanced by about 4 h in EM, CM and liver and 2 h in brain in their respective phases (Table I). These Values in Ach rhythm were shortened in EM, CM and liver but lenthened in brain in their phases (Table II). All these variations in the computed values are in good agreement with the observed values (Tables I and II and Figs. 1 and 2).

## DISUCSSION

AChE activity and ACh content were decreased in the tissues of mice due to envenomation implying an inhibitory effect of scropion venom on neuronal activities. Similar observations were made in Cockroaches (2, 21) guinea pigs (20) and mice (9). Scorpion venom damages the neuromuscular junctions (19) which may result in decreased activity of cholinergic system as observed in the present study.

The inverse relationship observed in AChE and ACh was also reported in Cockroaches (22, 23) and snails (14) suggesting a probable lack of efficiency in replenishment mechanism for ACh and hence the synthetic and releasing phases for ACh might be different during different periods of 24 h diel cycle.

The computed crests (X), troughs ($X^1$), amplitude (A) and the acrophase ($\phi$) values of AChE and ACh rhythm in different tissues of control animals were closely synchronized with the visual chronograms indicating the existence of the diel rhythmicity in cholinesterase system of *Mus booduga*. This inturn corresponds to the nocturnal motoric activity of the animal (13, 15).

Similar synchrony of rhythm characteristics with the visual chronograms of metabolic rhythms was also observed in the tissues of envenomated animals (10). Phase shifts of both +ve and –ve as observed in the AChE and ACh rhythms of mice during post envenomation were also observed in different physiological activities of animals under various stresses (16, 18, 22, 23). Various patterns of phase shifting in the experimental tissues, in response to envenomation, may be due to the fact that different components appear to shift at different rates towards new equilibrium state. Since the mice are nocturnal the $+\Delta\phi$s in EM, CM and liver reflects the general awareness of the animal to shift it's cholinesterase metabolic activity more towards "eveningness", while the $-\Delta\phi$s in brain towards "morningness". These shifts in the phases of the said rhythms may be due to derangement of cholinesterase metabolism in response to envenomation.

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