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SOME CHANGES IN THE BIOCHEMICAL COMPOSITION OF THE CENTRAL NERVOUS SYSTEM OF *SCHIZODACTYLUS MONSTROSUS* D. (ORTHOPTERA) AFTER APPLICATION OF PYRETHRUM

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Pyrethrum a deadly neurotoxicant, selectively acts on nerve ganglia. In this study changes were examined in the enzymes like acetylcholinesterase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase and in the macromolecules such as proteins, carbohydrates and total free amino acids in brain and ventral nerve code with ganglia in the case of both control and pyrethrum treated *Schizodactylus monstrosus* Drury. All the organic constituents under toxic stress showed uneven activities. The significance of such changes had some relation with the phenomena of metabolic compensation and interdependence among enzymes studied, to meet the toxic stress.

INTRODUCTION

It is known that the toxic substances used for the purposes of insect control are selected on the basis of their differential toxicity in respect of their mode and mechanism of action on the target cells, tissues and organs. The neurotoxic substances pyrethrum is being preferred now for its first acting insecticidal properties (quick knockdown), favourable persistence and nontoxic effects on humans and pets.

The literature available deals with cholinergic transmission in relation with the neuroenzyme acetylcholinesterase (AChE) and the effects of neurotoxic substances like pyrethrum on the central nervous system (CNS) of different insect species (Yamamoto, 1970; Burt & Goodchild, 1974; O'Brien, 1978; Casida, 1980).

But there exists scanty evidence which can distinctly show the effects of pyrethrum on the concentration and activity of different enzymes like Glutamic Oxalacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and macromolecules like protein, carbohydrate, total free amino acid (TFAA) etc of the CNS of insects. However, a few attempts have been made to reflect the activity of these enzymes by applying some other neurotoxic substances in CNS of different animals. Agarwal *et al.* (1978) observed increasd in the activities of GOT and GPT in the brain of rhesus monkey after application of DDT. Similar effects after injection of cobra venom in the brain of rabbits has also been reported by Alkhayat & Abdel-Aziz (1979). It is yet to be settled whether pyrethrum initiates or inhibits the synthesis/utilization of various macromolecules and enzymes in the CNS of insects. The present study has been aimed at evaluating the biochemical status of the CNS *in vivo* of an orthopteran insect, *Schizodactylus monstrosus* D. after pyrethrum application

MATERIAL AND METHODS

Newly emerged adult males and females of *Schizodactylus monstrosus* were reared by the method of Banerjee *et al.* (1984) for experimental purpose. The pyrethrum of petroleum ether suspension (2% w/w) was used and a dose of 0.8 μg/g body weight of 4×10^{-4}M concentration was injected by means of a microlitre syringe. The control insects received equivalent quantum of petroleum ether in the same manner as the pyrethrum treated ones.

Both the control and the treated insects were sacrificed after, 6, 12 and 18 posttreatment hours and dissected under chilled insect's Ringer solution. The brain and ventral nerve cord along with three thoracic and three abdominal ganglia were placed on ice-bath at 4°±1°C for enzyme assay. The tissues were dried to constant weight (14–29 hr at 60–70°C) and weighed in a electrically operated monopan chemical balance.

The following biochemical components were measured by different standard methods : (1) Total protein by Folin-phenol method, using bovine serum albumin (BSA) as standard (Lowry *et al.*, 1951), (2) Total carbohydrate by anthrone method (Roe, 1955), (3) TFAA by Ninhydrin method (Rosen, 1957), (4) AChE was determined by Sigma colorimetric determination (Technical bulletin 420), (5) GOT and GPT activity were determined by following the method of Reitman & Frankel (1957).

The appropriate statistical analy is of the results obtained included SE, SEM, and CD and this statistical method are analysed by Panse & Sukhatme (1978).

RESULTS

In control insects the differences in the activities of AChE, GOT and GPT in male and female as well as in different tissues were clearly evident (Figs. 1 & 2). AChE showed higher activity in male than in female (Fig. 1) The reverse was the result in case of transaminase activity. GPT activity was much higher than GOT activity in both tissues irrespective of sex (Fig. 2) which agreed with the findings of Kibby & Neville (1957). The protein and TFAA contents in

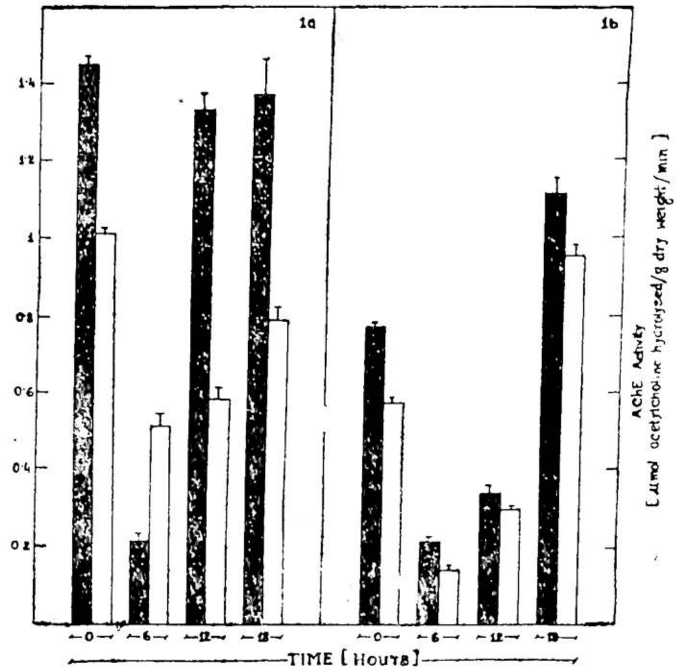

Fig. 1. Showing the changes in the activity of AChE in brain (1 a) and ventral nerve cord with ganglia (1 b) after pyrethrum treatment. Enzyme activity in zero hours indicates control. Activity expressed in terms of micromoles of acetylcholine hydrolyzed/g dry weight/min. Black bar represents brain and ventral cord in male. Hollow bar represents brain and ventral nerve cord in female.

brain of male were higher than in female but just reverse result was found in case of carbohydrate level.

After pyrethrum application symptoms like knockdow, tremor, convulsion abdominal bloat was found. After 6 hr post-treatment the AChE activity significantly decreased as compared to the control, but the activity increased gradually with time in both tissues (Fig. 1). But the GOT and GPT activity significantly increased with the passage of time in both the tissues after pyrethrum application. After 6, 12 and 18 hr post-treatment the total protein, content significantly depleted, whereas the TFAA content significantly boosted up (Table I). But only in male after 18 hr post-treatment the protein content showed marked increase which corroborated the earlier observation of Feroz & Ahmad (1974). In case of carbohydrate level after pyrethrum treatment the

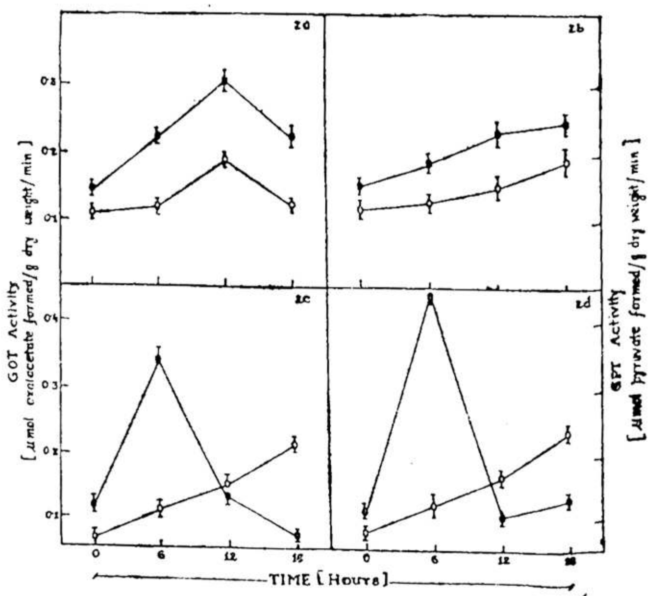

Fig. 2. Showing the charges in the activity of GOT in brain (2 a & 2 b) and ventral nerve cord with ganglia (2 c & 2 d) after pyrethrum treatment. Hollow points represents GOT in brain and ventral nerve cord of male and female Solid points represent GPT in brain and ventral nerve cord of male and female.

result showed steep fall with time in both sexes and also in both tissues.

DISCUSSION

Although this study produced evidence against the exclusive importance of AChE inhibition in pyrethrum poisoning, a relationship was observed between AChE inhibition level at the time to knockdown and reaction of pyrethrum. The abnormalities like tremor, convulsions, paralysis induced by pyrethrum, assumed a physiochemical effect, presumably due to the selective action on nerve ganglia and the destruction of their cells

After pyrethrum treatment, in both tissues the AChE activity decreases

Table 1. Showing the changes in the concentration of total proteins, total free amino acids and total carbohydrates in the brain and ventral nerve cord with ganglia of adult *Schizodactylus monstrosus* D. after pyrethrum treatment. Concentration expressed in terms of mg/g dry weight of the tissue.

Biochemical Components	Sex	Brain				C. D. value at 5% level	Ventral Nerve Cord with Ganglia				C. D. value at 5% level
			Post-treatment hours					Post treatment hours			
		Control	6	12	18		Control	6	12	18	
Total Protein	Male	45.510 (0.150)	39.863 (0.292)	35.293 (0.221)	50 080 (0.135)	0.425	31.630 (0.170)	30.461 (0.197)	22.957 (0.288)	44.274 (0.115)	0.408
	Female	35.470 (0.320)	33.651 (0 225)	32.177 (0.195)	27.475 (0 622)	0.831	25.366 (0.165)	25 104 (0.120)	23.096 (0.661)	21 518 (0.406)	0.760
Total Free Amino Acids	Male	0.358 (0.001)	6.701 (0.203)	8.243 (0.172)	9 427 (0 072)	0.303	2.676 (0.007)	3.226 (0.068)	5.871 (0.130)	14.484 (0.266)	0 309
	Female	2.143 (0.010)	4.326 (0.129)	10.012 (0 310)	11.424 (0.271)	0 447	2.057 (0.123)	4.991 (0.327)	7.705 (0.114)	8.642 (0.126)	0.399
Total Carbohydrates	Male	11.240 (0.400)	9.040 (0.192)	7.464 (0.345)	7.355 (0.446)	0.516	12.680 (0.440)	9 099 (0.473)	8.924 (0.048)	6.059 (0.570)	0.975
	Female	11.780 (0.240)	7.982 (0.232)	4.841 (0.190)	4.133 (0.430)	0.517	5.196 (0.159)	2.980 (0.241)	2.812 (0.256)	2.081 (0.365)	0.449

Results within the table are the mean value ± standard error (within the parentheses) whereas n = 10 more rapidly. This finding corroborates that of Bandyopadhyay (1982). This was probably due to the rapid penetration of pyrethrum into the more excitable nervous tissue (Briges *et al.*, 1974; Narahashi, 1976). After 12 and 18 hr post-treatment the activity of AChE gradually increased thereby exhibiting a tendency to recover the AChE activity. According to Weiss (1961) the length of time required for the recovery of AChE activity depends on the extent of initial inhibition and the chemical formulation of the insecticides. So the recovery period of AChE activity during pyrethrum treatment may be due to the rapid catabolism of pyrethrum in the body of insects.

The differential activity of GOT and GPT indicated that these two enzymes were both sex and tissue specific. The higher GOT and GPT activities in the CNS might be related to the synthesis and maintenance of amino acid pool required for protein synthesis (Pant & Jaiswal, 1981; Banerjee & Choudhuri, 1985). After pyrethrum treatment the increase of GOT and GPT activities might be due to imposition of stress condition which caused on elevation in the activities of transaminases (Knox & Greenguard, 1965; Alkhayat & Abdel-Aziz, 1979). The increase in GOT and GPT activities after treatment is suggestive of more feeding of amino acids inorder to overcome the impeding energy demands under toxic stress, (Banerjee & Choudhuri, 1985).

The metabolism of some macromolecules like protein, TFAA, carbohydrate in the CNS of insects previously had been studied by some workers (Bock, 1978; Strang *et al.*, 1979; Choudhuri *et al.*, 1981; Jabbar & Strang, 1984). Since carbohydrate are the major source of energy for the nervous tissue their concentrations were important during the above mentioned physiological events. During energy demands under toxic stress, the carbohydrate within the neurons, were consumed and thus revealed the lower level of metabolic activity (Banerjee & Choudhuri, 1985). The significant reduction of total protein in both sexes after toxic stress is due to degradation of protein in the cell (Rath & Misra, 1980; Banerjee & Choudhuri, 1985) for yielding excess energy to counter the toxic stress. The increase level of TFAA after pyrethrum treatment is due to proteolysis. The increased TFAA might have been partly routed to gluconeogensis through the transamination and transdeamination reactions to supply necessary keto acids to act as precursors for the sustained carbohydrate metabolism required to meet the energy need when exposed to pyrethrum stress (Natarajan, 1983; Banerjee and Choudhuri, 1985).

From earlier cytochemical to recent biochemical results it will be evident that the neurons are the sites for high protein metabolism that increases with increasing functional demands. This protein play a critical role in regulating the synaptic functions. Since the synapse is the obvious site for a regulatory type of function the quantitative changes of protein content may mediate changes in the synaptic function. With repidly decreased carbohydrate level after toxic stress, it may be suggested that the neurons responsible for action potentials are rapidly depleted of their energy reserves and cease to fire. Ultimately all the metabolic activity is expected to cease resulting paralysis in the insect.

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