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Neuroendocrine regulation and pesticidal impact on freshwater crab, *Barytelphusa guerini* (H. Milne Edwards)

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Abstract: *The extensive use of pesticides to control agricultural pests poses a serious threat to many non-target organisms of the aquatic environment such as the freshwater crab, B. guerini. The deleterious influence of the pesticide causes physiological, biochemical, histological and such other disorders in the animal exposed. In the present study impact of an organophosphate pesticide, monocrotophos and neuroendocrine regulation on the biochemical contents of hepatopancreas of B. guerini has been studied. Experimental studies revealed that glycogen and protein content decreased in normal crabs when exposed to sub lethal concentrations of monocrotophos, while lipid content was increased. In the ablated and pesticide exposed crabs glycogen, protein and lipid contents decreased. In case of ablated and exposed crabs when injected with eyestalk extract, glycogen and protein contents declined, whereas lipid content hiked. It was observed that glycogen, protein and lipid contents in eyestalk extract injected crabs were similar to those of normal exposed crabs. This indicates the vital role of eyestalk in the regulation of biochemical contents. Histological studies of the hepatopancreas indicate structural changes such as large number of vacuolated cells and phagocytes when exposed to the pesticide.*

Key words: *Barytelphusa guerini, Neuroendocrine, Monocrotophos, Hepatopancreas, Glycogen, Protein, Lipid*
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Introduction

Crustacean hepatopancreas, originally considered only as digestive gland, is now being increasingly suspected to function also as a centre of intermediary metabolism and as an important storage depot for fat bodies similar to vertebrate liver and adipose tissue (Bunt, 1968; Smith *et al.,* 1975; Bhide *et al.,* 2006). It is a well known storage site for carbohydrates, proteins and lipids in addition to some minerals. In crustaceans, eyestalk hormones are attributed with control of a number of physiological processes namely, somatic changes, blood glucose level, osmoregulation, moulting, reproduction and oxygen consumption (Fingerman, 1970). It has been shown that carbohydrates, proteins and lipids in crustaceans are regulated by neurohormonal factors released from eyestalk (Gilbert and O'Connes, 1970).

The application of pesticides has become an essential part of present day agricultural practices. The benefits due to use of pesticides are numerous, but at the same time they cause considerable harm to the ecosystem. Pesticides contaminate through agricultural run off to the streams, lakes and ponds during rainy season and adversely affect the non-target aquatic flora and fauna. The pesticide derivatives are known to alter the physico-chemical properties of water, these in turn interface and interact with various physiological activities of organisms. Biochemical constituents like glycogen, protein and lipid are considered as sensitive indicators of metabolic activities. Pesticides have been known to influence carbohydrate, protein and lipid contents in crustaceans, *viz., M. malcolmsonii* exposed to carbaryl (Saravana *et al.,* 2002); *B.cunicularis* exposed to endosulfan (Shanmugam and Venkateshwarulu, 2000).

The present work is undertaken to find out the histological and biochemical changes in hepatopancreas of *B. guerini* caused by monocrotophos toxicity and neuroendocrine control.

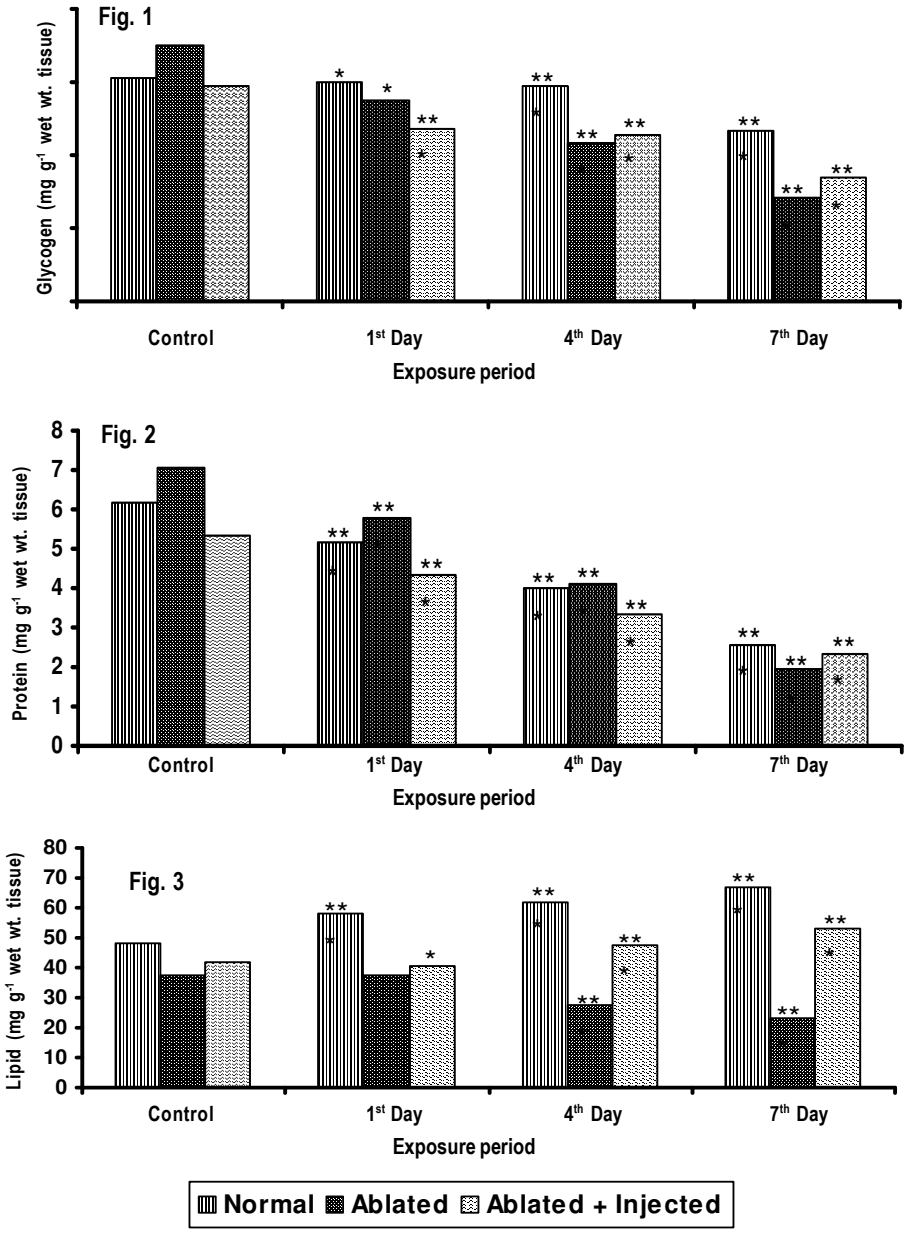
Materials and Methods

The freshwater crab, *Barytelphusa guerini* with intact appendages were collected from paddy fields. Adult male and female crabs with an average weight of 35-40 g were used in the experiment. The crabs were acclimated to laboratory conditions for a week by maintaining them in plastic troughs. Feeding was stopped a day prior to and during experimentation.

Commercial grade pesticide, monocrotophos 36% EC (manufactured by Ciba Geigy) was used for the study. Stock solution was prepared and further dilutions were made as per requirement. Lethal toxicity tests were performed according to standard procedures in vogue. Sub-lethal concentrations were worked out as 1.10 ppm for normal crabs, 0.86 ppm for ablated, 1.02 ppm for eyestalk ablated + injected crab and these were exposed for duration of 1day,4 day and 7day. Exposed crabs were sacrificed at fixed time and hepatopancreas was dissected carefully for biochemical estimations following various methods *viz.*, glycogen (Carrol *et al*., 1956), total protein (Lowry *et al.,* 1951) and total lipids (Barnes and Blackstock, 1973). Observations were made and statistical analysis was carried out and significance is represented in graphs. Hepatopancreas of the exposed crabs were fixed in Bouin's fluid further processed for dehydration, embedding, sectioning and staining with counter staining (haematoxylin and eosin) technique were followed for histological studies.

Results and Discussion

The results of changes in glycogen, protein and lipid levels of hepatopancreas of the freshwater crab, *B. guerini* exposed to sub-lethal concentrations of the pesticide monocrotophos are presented in Fig. 1-3. The biochemical contents are presented as mean values ± SE. The glycogen content in pesticide exposed normal crabs decreased from 6.231 ± 0.010 to 4.683 ± 0.130 mg g^{-1} wet wt of tissue on day 7 showing a significant decline ($p < 0.001$). Enhancement of glycogen level of hepatopancreas was evident in eyestalk ablated crabs from 6.231 ± 0.010 to 6.980 ± 0.019 mg g^{-1} wet wt of tissue, was highly significant ($p < 0.001$), glycogen level of hepatopancreas in the ablated exposed crabs exhibited declining trend from 6.980 ± 0.019 to 2.833 ± 0.046 mg g^{-1} wet wt. of tissue on day 7 compared to normal group and found to be highly significant ($p < 0.001$), eyestalk extract injection of the ablated crabs also brought similar trend in glycogen content by a decrease of 5.10%. Eyestalk extract injected crabs on exposure to pesticide also exhibited a decrease in glycogen content from 5.191 ± 0.048 to 3.366 ± 0.03 mg g^{-1} wet wt of tissue on day 7 of the experiment (Fig.1).



Figs. 1,2,3: Biochemical changes of hepatopancreas of *B. guerini* exposed to sub-lethal concentrations of monocrotophos . Values are significant at $p < 0.05$*, $p < 0.001$**

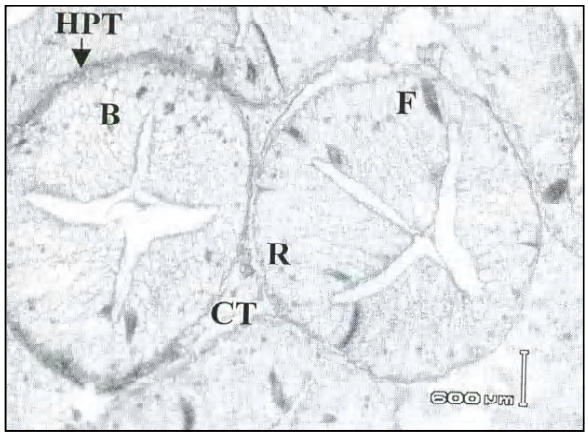


Fig. 4: Normal - Compact hepatic tubles (HPT) with B-cells, F-cells, R-cells, connective tissue (CT)

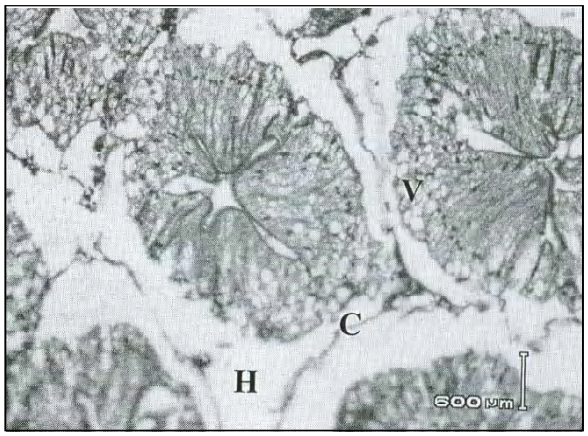


Fig. 7: Normal exposed - Narrowed lumen (L), disturbed Connective (DCT), large number of vacuoles (V), cuticle separated (C)

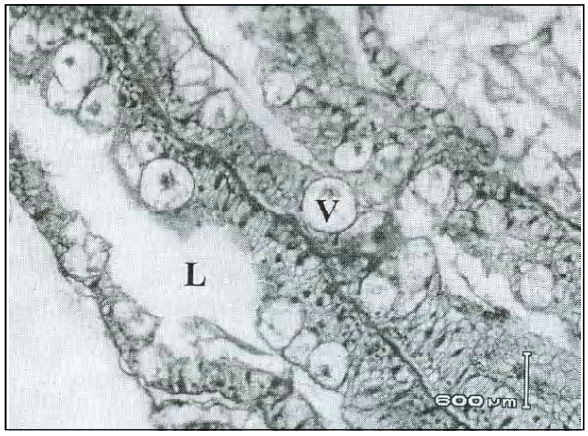


Fig. 5: Eyestalk ablated - Increased lumen (L), with vacuoles (V)

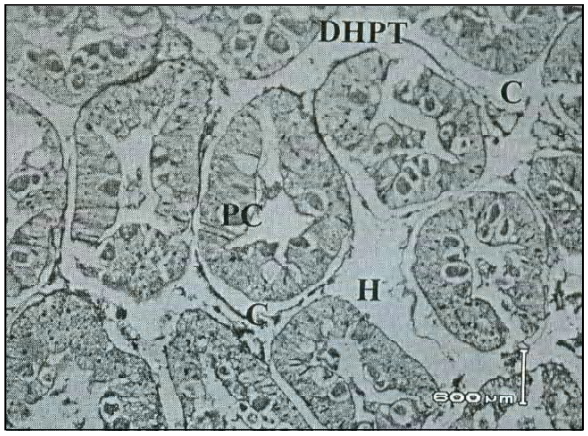


Fig. 8: Ablated exposed - Decreased lumen (L), disturbed connective tissue (DCT), large number of vacuoles (V), separated cuticle (C), hemal space (H), phagocytes (PC), damaged hepatic tubles (HPTD)

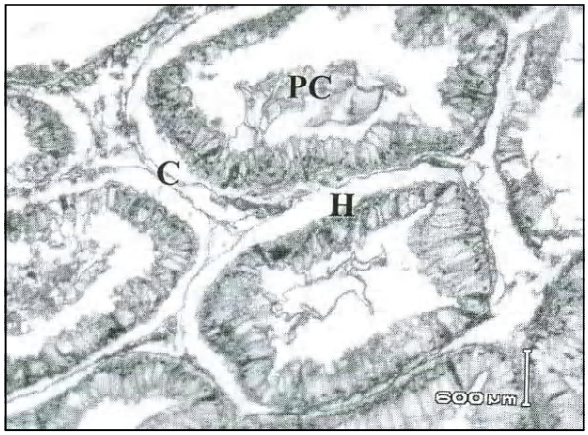


Fig. 6: Eyestalk extracted injected - Increased lumen (L), with phagocyte material (PC), separated cuticle (C), hemal space (H)

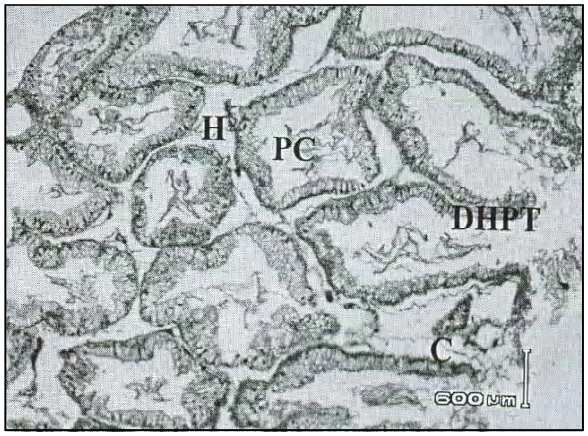


Fig. 9: Ablated injected exposed - Damaged hepatic tubles (HPTD), vacuoles (V), phagocytes (PC), separated cuticle (C), hemal space (H)

Fall in glycogen level of hepatopancreas observed in the present study on exposure to monocrotophos is an indication of the pesticide stress. Instances of glycogen decrease were reported earlier by several authors for heavy metal toxicity, those include Sarojini *et al. (*1990a, b) in *B .gurerini* exposed to cadmium chloride and zinc sulphate. In the present study decrease in glycogen concentration in hepatopancreas indicate an immediate utilization of reserve food under pesticide stress (Venkateshwarlu and Sumita, 1995).

The total protein content of hepatopancreas exposed to sub-lethal concentrations of monocrotophos was found to decrease from 6.161 ± 0.032 to 2.583 ± 0.015 mg g^{-1} wet wt of tissue and was significant ($p< 0.001$). Increase in protein content was observed on eyestalk ablation (14.76%) when compared to normal crabs; further, ablated crabs on exposure to sub-lethal concentration exhibited decrease in the protein content on day 7, ablated crabs on eyestalk extract injection showed decreased protein level of 7.071 ± 0.70 to 5.308 ± 0.087 mg g^{-1} wet wt of tissue in comparison to earlier values with a trend towards normal level. Further, on exposure to pesticide again, the protein content decreased from 5.308 ± 0.098 to 2.356 ± 0.054 mg g^{-1} wet wt of tissue similar to that of normal exposed crab (Fig. 2).

The total protein content of hepatopancreas decreased significantly on exposure to monocrotophos; ablation of eyestalks showed increase of total protein content in control and gradual decrease at the end of day 7 exposure could be attributed to the presence of eyestalk factors in the blood initially and thereafter. Similar observations were reported in other crustaceans to various pesticides such as those of Kumar *et al* . (1992) in fresh water crab, *P. masonianii;* Radhakrishnaiah *et al.* (1995) in *O. senex senex* exposed to fenvalerate, Yeragi *et al.* (2000) in marine crab, *Uca marinis* to malathion treatment and Shanmugam and Venkateshwarlu (2000) in *B. cunicularis* to endosulphon exposure. The present observations, however, indicate a significant influence of the length of period of exposure to pesticide toxicity and also eyestalk factors.

Lipid content of hepatopancreas increased from 47.866 ± 0.341 to 67.066 ± 0.409 mg g^{-1} wet wt of tissue in normal crabs on exposure; crabs on eyestalk ablation showed decrease in the lipid content (21.14%) from that of normal ones; ablated crabs on further exposure also showed decrease in lipid content from 37.750 ± 0.520 to 23.200 ± 0.247 mg g^{-1} wet wt of tissue and was found to be significant ($p<0.001$). In contrast the ablated crabs on eyestalk extract injection exhibited an increase in lipid levels from 37.750 ± 0.520 to 42.033 ± 0.307 mg g^{-1} wet wt of tissue (rise by 12.19%) and found to be significant ($p< 0.001$).

The lipids provide energy for almost all enderogonic processes and are of utmost importance in maintaining the structural and physiological integrity of cellular and subcellular structure. Lipids are important energy resources in crustaceans and are required during reproductive cycles. In the present study, lipid content increased in normal crabs on exposure to monocrotophos. This observation falls in line with earlier reports (Surendranath *et al*., 1991; Sarojini *et al.,* 1990a; Khan *et al.,* 2001). The increase in lipid content on exposure to pesticide suggests that it may be due to inhibition of lipase activity and other enzymes of lipid metabolism.

Glycogen content increases on eyestalk ablation as a result there is a fall in the respiratory quotient. Carbohydrate may be the main metabolic substrate of crustaceans and it is noticeable that glycogen content decreases in ablated crabs on further exposure. The results of the present study are supported by the reports of Venkataramanaiah and Ramamurthi (1980), who observed that eyestalk ablation leads to increase in free sugars in both hepatopancreas and muscle of *O. senex senex* while eyestalk extract injection into ablated animals results in decrease of the glycogen content and further trying to reach control levels. In the presence of eyestalk hormone, the tissue glycogen is broken down to liberate free sugar and these results in elevation of different carbohydrate fractions of blood and the tissue. Eyestalk ablation results in removal of this factor as a result of which sugars are mobilised from the blood and the tissues to the storage sites where they are converted into glycogen and stored.

Eyestalk ablation caused marked changes in the total protein, which increased in ablated crabs and on exposure to sub-lethal concentrations of pesticide, it declined significantly as observed in *B. guerini*, in the present study which are in consonance with the reports of Sunita *et al.* (1989). The total protein content decreased in muscles and gills of normal and eyestalk ablated crabs and again levels were restored after eyestalk extract was injected in *P. jacquimontii* (Parate *et al*., 2003). The crab*, B. guerini* on eyestalk ablation and on injection with central nervous (brain,thoracic ganglia) extracts did not majorly affect the protein level (Gangothri *et al.,* 1989). Further evidence is from the observation that eyestalk removal in *Orconectes virilis* in premoult stages results in greater incorporation of amino acids and lower protein content in the tissue (Mcwninnie and Mohrhers, 1970). Protein synthesis can be disturbed by a variety of mechanisms either by affecting the nucleic acid metabolism or structure or in the protein forming system itself. Toxic agents acting directly on ribosomes, RNA, enzymes or co-enzymes may also have a drastic influence on protein synthesis. The present study is supported by Venkatachari (1985), showing that eyestalk ablation leads to decrease in the enzyme activity in the hepatopancreas of the *B. guerini*, varies in relation to the physiological state of the animal and is under the neuroendocrine control of an eyestalk principle.

In the present study lipid concentration decreased both in ablated and ablated exposed crab, *B. guerini*. A similar observation has been made in *P. hydrodromus* on eyestalk ablation and exposed to endosulfan by Kallapur and Yadwad (1986). Eyestalk ablation induced a decline in the concentration of lipids of hepatopancreas, whereas in the muscle tissue lipid level was found to be increased in *B. cunicularis* (Diwan, 1973). Further, the same author reported that injection of eyestalk extracts into the destalked crab brought about reestablishment of the lipid level within the normal range both in

hepatopancreas and muscle tissue. The decrease in lipid content in hepatopancreas coupled with an increase in lipid content in haemolymph indicates the mobilization of lipid from hepatopancreas to haemolymph as also observed in *O.senex senex* (Chandramouli *et al*., 1985).

Hepatopancreas of control crab showed compact hepatic tubules with absorptive cells, fibrillar cells and secretory cells surrounding the lumen (Fig. 4). Hepatic tubules increased in size with no lumen and number of secretory cells increased with large number of small vacuoles, cuticle separated with large hemal space between hepatic tubles in normal crabs on exposure to sub-lethal concentration of monocrotophos (Fig.7). In ablated crabs the lumen increases with increase in size of hepatic tubules, with some vacuoles in the cells (Fig. 5). Ablated crabs on exposure showed damages to the hepatic portal tubules with increase in number of vacuoles with phagocytic material and increase in secretory cells with tubules loosely arranged in haemocelomic space in between the tubules (Fig.8). Ablated crabs on eyestalk extract injection showed disappearance of vacuoles, release of phagocytic material in the lumen decrease in size of the cells compared to normal and hepatic tubules are loosely arranged without connective tissue (Fig.6). Hepatopancreas in ablated eyestalk extract injected crabs on exposure was observed with vacuoles increase in lumen with phagocyte material, cells and tubules damaged (Fig. 9).

The histological studies of hepatopancreas of carb, *B. guerini* exposed to sub-lethal concentration of monocrotophos showed increase in number of vacuoles with phagocytes in the cytoplasm, tubules were disfigured and the lumen size was enlarged. Victor *et al.* (1990), observed histopathological changes in the hepatopancreas of *Paratelphusa hydrodromous* in response to cythion resulting in reduction of the height of tubular epithelium, enlargement of lumen, vaculation and atrophy. Such observation was also made in gills of *P. hydrodromus* on exposure to nickel. (Kurian and Radhakrishnan, 2002). The histopathological changes indicate that the animals were not able to digest and store food properly. The lack of nutrients results in atrophy of hepatopancreas. Joshi *et al*. (2007) observe obivious histopathological changes such as vacuolization, necrosis and other damages in the liver of *Heteropneustes fossilis* exposed to cypermethrin. Samanta *et al*. (2005) reported that gill and liver tissues of freshwater fishes, *Arius gagora* and *Pama para* from heavy metal contaminated waters of Hoogly river were severely affected which is attributed to persistant sublethal concentrations of heavy metals.

Glycogen and total protein decreased while the total lipid content increased on exposure in normal crabs; on eyestalk ablation the glycogen and total protein content increased and lipid decreased; where as on eyestalk extract injection of ablated animals the level of glycogen, total protein and lipid content was almost restored to normal indicating the presence of regulating factors in the eyestalk. Histological observations reveal variations in respect of lumen, the vacuoles, phagocytic material and extent of damage of tubules under extract injected in comparison with ablated crabs.

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