Effect of Mercuric Chloride on the Biochemical Constituents of Some Tissues of a Freshwater Crab, Barytelphusa cunicularis [Westwood, 1836]

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Abstract: The crab has immense tolerance for wide ranging changes in the environmental factors by physiological adjustments. The present study was undertaken to assess the effect of sublethal [1/5 of 24 h LC50 (0.208 ppm)] concentration of mercuric chloride on protein, lipid and glycogen in the freshwater crab, Barytelphusa cunicularis, which is an important food source in this parts of Marathwada region. The crabs were exposed for 7, 10 and 15 days. Protein, lipid and glycogen contents in ovary, hepatopancreas, thoracic muscles, gills and spermathecae of treated crabs showed significant (P<0.05) reduction as compared to untreated controls. Significance of these changes is discussed.

Key words: Barytelphusa cunicularis • HgCl2 • Protein • Lipid • Glycogen

INTRODUCTION

Crustaceans comprises 15% of the total fishery catch, among them crabs form highly nutritious and are excellent means of obtaining protein, lipids, minerals and vitamins hence are economically important group of crustaceans [1, 2].

Due to rapid industrialization, water pollution has become an alarming hazard for the edible aquatic shellfish fauna, particularly to the bivalves and crabs. Worldwide the heavy metals and are extensively used in textile processing, paper printing, pharmaceutical; food and agrochemicals and discharging their effluents into nearly water bodies [3].

In recent years, it has been evident that anthropogenic activities are adding trace metals to the aquatic environments. It is of prime importance to understanding biochemical changes in organisms under the stress of pollutants [4-6]. Biochemical constitutes like glycogen, protein and lipid are considered as sensitive indicators of pollution effects in crabs [7-10].

The objective of the present work is to find out variations in the biochemical components like protein, glycogen and lipid in different tissues of the crab, Barytelphusa cunicularis exposed to sublethal concentration of mercuric chloride.

MATERIALS AND METHODS

The freshwater female crab, Barytelphusa cunicularis were collected from the freshwater ponds on the outskirts of Aurangabad. They were brought to laboratory in live condition and kept in the plastic trough (18" diameter) with sufficient dechlorinated tap water for a period of 10 days for acclimation under 11 D: 13 N illumination. During acclimation they were fed ad libitum with pieces of earthworm and bivalve meat. Healthy intermoult (Stage C3: Diwan) crabs having equal size (carapace width 30 to 35 mm) and weight (25 to 30 g) were used. Food and aeration were not provided during experimentation. Stock solution of mercuric chloride [HgCl2] was prepared by dissolving appropriate amount of salt in distilled water.
The crabs were exposed to sublethal concentration [1/5 of 24 h LC$_{50}$ (0.208 ppm)] of mercuric chloride. The biochemical changes were analyzed after exposing crabs for 7, 10 and 15 days along with control crabs which were maintained in dechlorinated tap water. The ovary, hepatopancreas, gills and thoracic muscles were dissected out from both the control and experimental crabs to assess changes if any in protein, glycogen and lipid using following procedures.

The total protein was estimated by the Biuret method [11]. 100 mg of tissue was homogenized in 5 ml of cold distilled water. 5 ml of 30% TCA was immediately added to precipitate the protein. The protein content was calculated with the help of standard graph and expressed as mg/100 mg wet tissue.

The total Lipid was estimated by the method [12] using vanillin reagent. 100 mg tissue was homogenized by adding 10 ml of chloroform: methanol (2:1) mixture. The homogenate was filtered and 1 ml of this filtrate was kept at room temperature in laboratory at 37°C for 2 days. Lipid content was calculated with the help of standard graph and expressed as mg/100 mg wet tissue.

The total glycogen was estimated, employing the method [13] using anthrone reagent. 50 mg tissue was homogenized in 30% KOH and the mixture was kept in boiling water bath for 3 to 5 minutes to dissolve the tissue and then cooled. To this 2 ml of 96% ethyl alcohol was added and the mixture was kept overnight in refrigerator at 12 °C. Next day this mixture was centrifuged at 3000 rpm for 15 minutes to settle down glycogen cake. Glycogen content in tissue was calculated with the help of standard graph and expressed as mg/100 mg wet tissue.

The obtained data are statistically analyzed by using student ‘t’ test [14].

**RESULTS AND DISCUSSION**

The freshwater crabs were exposed to sublethal concentration of mercuric chloride 1/5 (0.208) for 7, 10 and 15 days. The results are presented in the Figs.1-3, clearly reveal a marked biochemical alteration in the ovary, hepatopancreas, muscles, gills and spermathecae of *Barytelphusa cunicularis*.

The protein content in the control ovary was found to be 77.17 mg %. A significant decrease of 33.7, 31.4 and 23.3 mg % was observed. In the hepatopancreas of control crabs protein content was observed to be 42.35 mg %, whereas in the experimental, a significant decrease in protein content was of 24.0, 21.0 and 18.2 mg % respectively. The protein content in the control gill was found to 56.6 mg %. Decline in the protein content in gill exposed to HgCl$_2$ were from 31.2, 29.6 and 24.0 mg % was observed. In muscle of experimental crabs significant decrease (P<0.05) in protein content was found to be 28.9, 24.9 and 17.5 mg % as compare to control value which was observed to be 45.65 mg %. In the spermathecae of control crabs protein content was observed to be 54.7 mg %, whereas in the experimental, a significant decrease in protein content was of 33.8, 34.4 and 24.6 mg % respectively after 5, 10 and 15 days respectively Fig. 1.
Maximum decrease in protein content was observed in ovary as compared to hepatopancreas, gill, muscle and spermathecae.

Protein is the main energy source in crustaceans [15, 16]. The tissue specific time dependent loss in protein in the tissues of freshwater field crab, Barytelphusa guerini after heavy metal intoxication [17]. In the present investigation, reduction in total protein content was noted in the tissues of the test crabs exposed to mercury. This was possibly due to the direct effect of the mercury on protein metabolic demands.

The lipid content in the control ovary was found to be 63.2 mg %. In the experimental crab lipid content was of 20.6, 25.3 and 35.1 mg %. In the hepatopancreas of control crab lipid content was observed to be 71.7 mg % whereas in the experimental crab, a significant decrease of 16.2, 28.3 and 37.5 mg %. In the muscle of control crabs lipid content was observed to be 23.4 mg % whereas in the experimental crabs, a significant decrease of 6.8, 8.59 and 10.6 mg % was noticed after exposure respectively. The lipid content in the control gill was found to be 16.51 mg %. A significant decrease of 4.5, 5.4 and 10.4 mg %. The lipid content in the control spermatheca was found to be 39.5 mg %. A significant decrease of 11.6, 11.9 and 20.9 mg % after 7, 10 and 15 days respectively Fig. 2.

Maximum decrease in lipid content was observed in hepatopancreas and muscle as compared to ovary, spermathecae and gills.

Lipid, an important dietary constituent, serves as reserve energy when food supply is scanty. In stressful environmental conditions, after glycogen, lipid is used as energy source [18]. In stress condition induced by pesticides, the lipid content depleted to meet the energy demands. In the present study, stress imposed by sublethal doses of the mercury salt to Barytelphusa cunicularis resulted in decrease in lipid content in the ovary, hepatopancreas, gills muscles and spermathecae tissues, indicating high energy demands.

The glycogen content in the control ovary was found to be 27.1 mg %. A significant decrease of 8.2, 11.9 and 15.2 mg %. In the hepatopancreas of control crab, glycogen content was observed to be 34.3 mg %, whereas in the experimental crabs a significant decrease in glycogen content in the hepatopancreas was found to be 10.2, 14.9 and 19.0 mg %. In the muscle of control crab, glycogen content was observed to be 19.1 mg %, whereas in the experimental, a significant decrease in glycogen content was of 8.2, 9.5 and 11.3 mg %. The glycogen content in the gills of control crab was found to 19.8 mg %, a significant decrease of 7.4, 9.7 and 10.6 %. The glycogen content in the spermathecae of control crab was found to 19.8 mg %, a significant decrease of 8.5, 10.8 and 12.9 % was observed after exposure for 7, 10 and 15 days respectively Fig. 3.

Maximum decrease in glycogen content was observed in hepatopancreas and gill as compared to ovary, spermathecae and muscle.

Depletion in glycogen content in the crab, Barytelphusa cunicularis exposed to sevimol [19]. The depletion of glycogen may be due to its rapid utilization to meet the energy demands under the impact of heavy metal pollution stress.

In recent years substantial information on effect of toxicants such as aldrin and monocrotophos in crab, Paratelphusa masomiana [20]; heavy metals in crab, Barytelphusa guerini [21]; hildan in crab, Barytelphusa guerini [22]; cadmium in the crab, Barytelphusa cunicularis [23] and carbaryl in the prawn, Macrobachium malcomsonii [24] resulting into the stress and affecting the metabolic pathways and depletion in protein, glycogen and lipid contents of different tissues of aquatic animals has been dealt in detail.

Crustaceans including prawn, shrimps and crabs have become the major source of animal protein to the low income earners due to its low price and availability [25]. However, the chemical composition and functional properties of crabs have not been reported in detail which has generated considerable interest to study biochemical composition in Barytelphusa cunicularis which are consumed by the local communities in Aurangabad region.

The results of present study on freshwater crab, Barytelphusa cunicularis (Westwood, 1836) suggest that decrease in levels of protein; glycogen and lipid in different tissues after exposure to HgCl2 may be due to increased glycogenolysis, glycolysis, proteolysis and lipolysis under stress to meet increase energy demands for survival.

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