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CADMIUM TOXICITY ON ESTUARINE EDIBLE CRAB SCYLLA SERRATA WITH REFERENCE TO OVARIAN MATURATION

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ABSTRACT

Cadmium is considered as a potent toxic metal to a varied variety of creatures; the main impacts are in the development and replica process. Dissolved cadmium salts released in drainage water from mines may represent a significant threat to aquatic wildlife. Pollutant stress leads to significant modification in biochemical and physiological functions in reproductively energetic aquatic animals and further interrupt reproductive processes. In the edible crab Scylla serrata, the protein, carbohydrate, lipid, marker enzymes and phosphatase have been estimated in the ovary, hepatopancreas, muscle and haemolymph during several periods of the ovarian maturation. The results revealed that, there were declined levels of protein, carbohydrates, lipid and phosphatase in the cadmium reared crabs when compared to the control. The marker enzymes were augmented in the haemolymph. The observed results of this experiment were tested statistically.

Keywords: Edible crab, Scylla serrata, cadmium, aquatic animals, Heavy metals

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I. INTRODUCTION

Heavy metals are an imperative group of pollutants in the ecosystem and it has got great consideration due to their capability to pervade cell membrane. In aquatic food chains, these metals can be accumulated and biomagnified [1]. Due to the increased human activities, the levels of toxic trace (elements) metals such as mercury, copper, chromium, silver, lead, cadmium, etc., are increased in the ecosystem. The environmental hazard in the aquatic ecosystem is because of great toxicity or persistence characteristic features of trace contaminants [2]. Polyaromatic hydrocarbons are a persistent hydrophobic chemical enters and accumulates in aquatic organism through different routes. Major entry pathways are through the outer skin surface, via ingesting of polluted food and through the water with gills. Haemolymph engaged in transporting the nutrient reserves and involved xenobiotics process and metabolites in various tissues [3]. Cadmium is one of the foremost pollutants among heavy metals and found it to occur in combination with other elements as such Sulphur naturally (cadmium sulphide). Attempts for both pre-and post-industrial times to qualify and the relative importance of anthropogenic. Cadmium contribution from anthropogenic sources (e.g. Non-ferrous metal industry) has augmented significantly over the past century and it currently governs the cadmium biogeochemical cycle [4]. The geochemical mobility of cadmium has been augmented by the process of acid rain and the subsequent acidification of soils and surface waters [5]. Molluscs and numerous microorganisms readily accumulate the cadmium than other organisms. Through the outlet of mines and industries dissolved cadmium salt mix with drainage water and this may represent a significant threat to aquatic wildlife. Due to several favorable opinions, such as tasty, rich in nutritious worth, bigger in size, high unit value and great demand in import and export market, the mud crabs of the genus *Scylla* are highly considered comparatively to other edible crabs. *Scylla serrata* have a diversity of morphological, behavioral and physiological conversion in their growth and development stages [6], *Scylla serrata* life cycle have different aquatic habitats and environmental conditions [7].

In crustaceans, female reproduction is greatly associated with vitellogenesis mechanism which is considered as an important physiological, energy-demanding process. It is characterized by vitellogenin synthesis, and subsequent processing and accumulation within the developing ovary is a vitellin. Normally and recurrently environmental pollutants mainly harm the reproductive tissues [8]. Yolks are rich in lipid where the pollutants are gathered and these lipids rich yolk are militarized in organ development during larval progress. A disturbed reproductive mechanism is defined in reproductively dynamic marine animals when they are exposed to toxic polluted undergo uncharacteristic biochemical and physiological functions. Biochemical constituents are used for assessing the influence of toxins in aquatic organism reproductive physiology [9]. The Destructiveness of polyaromatic hydrocarbons is their straight attachment to macromolecules hydrophobic sites triggering instabilities in their normal functioning [10]. Because of high sensitivity, minimum variable, more conserved among species and measuring anxiety indices are easier to analyze biochemical elements and enzyme to estimate toxicity in marine animals. These biochemical and enzymes modifications are the primitive and measurable criteria and treated as a vital market for quantifying toxicant exposure and their effect on the organism. The present experiment was commenced to analyses the effect of cadmium on edible crab, *Scylla serrata* regarding ovarian maturation [11].

II. MATERIALS AND METHODS

A. Experimental Animal

Scylla belongs to the family Portunidae also known as mud crabs, an economically important crab species found in the bays. Regarding the biology of crustaceans several research works have been carried out [12]. However, little information is available on the effect of pollutants on decapod crustaceans. Hence, it is proposed to inspect the effect of cadmium on the edible estuarine crab, *Scylla serrata* regarding the maturation of ovarian.

B. Collection of the Animals

Female *Scylla serrata* were collected from the Pulicat Lake near Chennai, Tamil Nadu, India. They were acclimatized to the laboratory condition (28°-2°C) in large glass aquaria for a week. The volume of water was adjusted so that the individuals were just submerged. Flesh of fresh prawns was fed to *Scylla serrata*. Every day the water was changed and the crabs were adapted to the laboratory environments.

C. Toxicant

Cadmium is a non-essential metal [13] and a non-degradable pollutant. It is a common impurity in zinc, and it is most often isolated during the manufacture of zinc. It is highly toxic at relatively low concentrations, readily accumulates in tissues and can adversely affect the organisms. It has a long biological half-life [14]. It is found in small quantities in natural waters and is common in the effluents of mining, electroplating and paint industries.

D. Experimental Design

After acclimatization to the laboratory condition, crabs were separated into two groups. The Group I crabs were reared in cadmium free-seawater and treated as control. Crabs fitting to group II were exposed to cadmium concentration at 20mg/L. Up to 96h the treatments were continued. The ovarian stages were divided based on the principles explained by Vijayavel *et al* after exposure to cadmium [15].

Stage I - Immature, prepubertal and reproductively inactive ovary; white and threadlike in appearance.

Stage II - Reproductively active, showing peripheral undulations for the formation of ovarioles and white in colour.

Stage III - Ovariole formation and oogonial proliferation completed. The ovary is white in colour, opaque and thicker than the previous stage.

Stage IV - Beginning of vitellogenesis and the ovary acquires colouration from pale to deep yellow.

Stage V - Bright orange – coloured ovary with vitellogenesis at its peak lipid yolk deposition completed.

E. Collection of Tissues and Haemolymph

Ovary, muscle and hepatopancreas were separated from the exposed and control crabs. The haemolymph was collected after prechilling the animals for 5 min [16]. This procedure prevents alterations and clumping of haemocytes and melanization. From the cut end of propodus or dactylus of an appendage the haemolymph was collected and decanted directly into prechilled centrifuge tube. The whole haemolymph including the haemocytes was used for biochemical analysis.

F. Biochemical Analysis

1. Estimation of Protein

Protein reacts with Folin complex. Alkaline copper reacts with the protein and phosphomolybdate reduction by tyrosine and tryptophan present in the protein the color is formed [17].

2. Estimation of Total Carbohydrates

Sulphuric acid in anthrone reagent hydrolysis disaccharide and oligosaccharide into monosaccharide and dehydrates all monosaccharide into furfural derivatives. These two components react with number of phenolic compounds and one such is anthrone, which produces a complex colored product. The intensity of which is proportionate to the number of saccharides present in the sample [18].

3. Estimation of Total Lipids

Homogenate was discharged into a separating funnel and the biphasic layer was obtained by mixing with 0.95% saline solution. After 24 h of interval, the lower chloroform phase containing the lipid was poured into a pre-weighed test tube. Repetitively the methanolic phase was washed with chloroform and collected. This crude extract was evaporated to constant weight. Through subtracting the initial weight from the final weight of the test tube, the total lipid content was measured. In the case of ovary, hepatopancreas, muscles, the results were delivered as mg/100 mg wet tissue and mg/ml for haemolymph.

4. Assay of Acid and Alkaline Phosphatase

The liberated p-Nitro phenol, due to the enzyme activity with p-Nitro phenyl phosphate was estimated spectrophotometrically using the method [19] as described [20].

5. Statistical Analysis

The data were displayed as mean ± Standard Deviation. Statistically the results were investigated by a student T-test using SPSS software student's version.

III. RESULTS

A. Biochemical fluctuations

Crustaceans such as prawns, crabs and lobster are one of the sources of nutritious food. Therefore, efforts are being made all over the world to investigate and exploit both marine and freshwater bodies for food production. The crustaceans are chosen for consumption based on their nutritional values. The nutritional value of crustacean is expressed in terms of its major organic constituents like protein, carbohydrates and minerals, amongst which protein is a very important component. With a view to understand the extent of such fluctuations in invertebrates, especially in crustaceans with reference to ovarian maturation the proteins, carbohydrates, lipid, phosphatases were estimated in response to cadmium stress.

B. Protein

Habitually animal tissues are composed of biological composition called proteins. In the body, proteins are in the form of amino acids and other metabolites celebrated as building blocks of the body. It is well known the hepatopancreas and haemolymph proteins are utilized during the ovarian maturation of crustaceans [21]. Considering the extent of studies on the biochemical fluctuations during the ovarian stages in *Homaeus americanus*, [22] *callinectus sapidus* [23], Paraplphusa hydrodromus [24] and *Scylla serrata* [25] information on their impact on environmental pollutant is meager as compared to studies available in fishes. Therefore, it is of interest to study the effect of cadmium on protein content of *Scylla serrata* in relation of ovarian maturation.

1. Ovary

The effect of cadmium on the ovarian protein content of *Scylla serrata* during the ovarian maturation is presented in table (1) and figure (1a). The ovarian protein content of the crab reared in cadmium free medium was 2.456, 2.021, 1.934, 4.036 and 2.877mg/100mg wet tissue at stage I, II, III, IV and V correspondingly was decreased to 2.262,1.844,0.886,3.224 and 1.179mg/ 100mg wet tissue in crabs reared in cadmium medium. The reduction in the ovarian

protein content was statistically more significant ($p<0.01$) at stage I and II and highly significantly ($p<0.001$) at stage III, IV and V.

2. Hepatopancreas

Influence of cadmium on the hepatopancreatic protein content of *Scylla serrata* during the ovarian maturation is presented in fig. (1b). The hepatopancreatic protein content of *Scylla serrata* when reared in cadmium free medium was 2.694, 2.009, 2.068, 4.019 and 3.116 mg/100 mg wet tissue at stage I, II, III, IV and V correspondingly. It is evident from the present investigation revealed that the protein content of control crabs between stage IV and V indicates intense vitellogenic activity. When the crab reared in cadmium medium the protein content of hepatopancreas decreased to 2.484, 1.780, 1.906, 3.762 and 3.002 mg/100 mg wet tissue at stage I to V respectively. The reduction of the hepatopancreatic protein content was statistically more significant ($p<0.01$) at stage I and II while highly significant ($p<0.001$) at stage III, IV and V respectively.

3. Muscle

The effect of cadmium on the muscle protein content of *Scylla serrata* during the ovarian maturation is presented in fig. (1c). The muscle protein content of *Scylla serrata* when reared in cadmium free medium was 1.836, 1.997, 1.999, 1.919 and 1.056mg/100mg wet tissue at stage I, II, III, IV and V respectively was decreased to 1.736, 1.892, 1.894, 1.712 and 1.375 mg/100mg wet tissue in crabs reared in 20mg/L cadmium concentration. From the fig. It reveals that the reduction of the muscle protein content at the stage I and III was more significant ($p<0.01$) while highly significant ($p<0.001$) at stage II, IV and V correspondingly.

4. Haemolymph

The effect of cadmium on the haemolymph protein content of *Scylla serrata* during the ovarian maturation is presented in fig. (1d). The haemolymph protein content of *Scylla serrata* reared in cadmium free medium (control) was 2.944, 1.976, 1.956, 4.000 and 3.848 mg/ml at stage I to V respectively. When *Scylla serrata* reared in cadmium, the haemolymph protein content was reduced to 2.513, 1.903, 1.874, 3.924 and 3.548 mg/ml at stage I to V respectively. The decrease in haemolymph protein content was significant ($p<0.05$) at stage V while more significant ($p<0.01$) at stage I, II and IV and highly significant ($p<0.001$) at stage III correspondingly.

A. Carbohydrates

Carbohydrate metabolism, provides fuel and get oxidized to supply energy for further metabolic events. Metabolic intermediates are used for numerous biosynthetic reactions. Administration of the pollutant was shown to alter the carbohydrate metabolism [26]. A review of literature, however, reveals that effect of cadmium on carbohydrate metabolism of *Scylla serrata* during the ovarian maturation.

1. Ovary

Influence of cadmium on the ovarian carbohydrate content of *scyll serrata* during the ovarian maturation is presented in the fig. 2a. The ovarian carbohydrate of *Scylla serrata* reared in cadmium-free medium was 0.845, 0.851, 0.885, 0.745 and 0.399 mg/100 mg wet tissue at stage I to V respectively was decreased to 0.527, 0.472, 0.538, 0.572 and 0.253 mg/100 mg wet tissue in crab reared in 20mg/L concentration of cadmium medium. The reduction in the ovarian carbohydrate content was statistically more significant ($p<0.01$) at stage I and highly significant ($p<0.001$) at stage II and III.

2. Hepatopancreas

The effect of cadmium on the hepatopancreatic carbohydrate content of *Scylla serrata* during the

ovarian maturation is presented in the fig. 2b. The hepatopancreatic carbohydrate content of *Scylla serrata* when reared in cadmium-free medium was 0.778, 0.980, 0.526, 0.785 and 0.397 mg/100 mg wet tissue at stage I to IV correspondingly. The carbohydrate level was reduced during stage V, due to utilization of carbohydrate for developing oocytes during the ovarian maturation. *Scylla serrata* exposed to cadmium medium the carbohydrate level was decreased to 0.553, 0.578, 0.321, 0.754 and 0.365 mg/100 mg wet tissue at stage I to V. From the fig. 2b, it is revealed that the reduction of the hepatopancreatic carbohydrate level at stage II and IV was significant ($p<0.05$) while more significant ($p<0.01$) at stage I, II, V correspondingly.

3. Muscle

Influence of cadmium on muscle carbohydrate content of *Scylla serrata* during the ovarian maturation is presented in the fig. 2c. Carbohydrate content of the muscle of crab reared in the cadmium-free medium was 0.696, 0.678, 0.334, 0.796 and 0.079 mg/100mg wet tissue at stage I, II, III, IV and V correspondingly, was decreased to 0.509, 0.388, 0.294, 0.715 and 0.065 mg/ 100 mg wet tissue in crabs reared at a 20mg/L concentration of cadmium medium. The decrease in carbohydrate level in muscle of control crab may be due to heavy intake of water. The present investigation also confirms the previous worker's view because when the *Scylla serrata* reared in cadmium medium, the muscle carbohydrate content was statistically decreased.

4. Haemolymph

The effect of cadmium on the haemolymph carbohydrate content of *Scylla serrata* during the ovarian maturation is presented in fig. 2d. The haemolymph carbohydrate content of *Scylla serrata* when reared in cadmium-free medium was 0.766, 0.529, 0.421, 0.744 and 0.651 mg/100 ml of haemolymph at stages I, II, III, IV and V respectively. The carbohydrate content of the haemolymph of the crab when exposed to cadmium chloride medium decreased to 0.445, 0.214, 0.350, 0.709 and 0.625 mg/ml at stages I to V correspondingly. The decrease in haemolymph carbohydrate content was statistically significant ($p<0.05$) at stage III; more significant ($p<0.001$) at stage I and II and highly significant ($p<0.001$) at stage IV and V correspondingly.

A. Lipids

Next to carbohydrates, lipids are the best energy producers of the body. Several workers have studied the impact of pollutants on lipid content [27]. A review of the literature, however, reveals that the effect of cadmium on lipid content in crab has not been studied. Hence, it is of interest to study the influence of cadmium chloride on lipid content of *Scylla serrata* during the ovarian maturation.

1. Ovary

Influence of cadmium on the ovarian lipid content of *Scylla serrata* during the ovarian maturation is presented in fig. 3a. The variation in the ovarian lipid content of *Scylla serrata* when reared in control and cadmium chloride was 1.9, 3.1, 4.8, 5.5 and 6.8 mg/100 mg wet tissue, reduced to 1, 1.2, 2.2, 2.6 and 2.9mg/100 mg wet tissue at stages I to V correspondingly. The decrease of ovarian lipid content was statistically highly significant ($p<0.001$) at stage I, II, III, IV and V correspondingly.

2. Hepatopancreas

The effect of cadmium on the hepatopancreatic lipid content of *Scylla serrata* during the ovarian maturation is presented in the fig. 3b. The hepatopancreatic lipid content of *Scylla serrata* reared in cadmium-free medium was 4.1, 5.1, 5.9, 7.7 and 7.2 mg/100mg wet tissue at stage I to V respectively, and it was decreased to 0.63,

2.2, 2.1, 3 and 2.4 mg/100 mg wet tissue at stage I to V when reared in 2o mg/L concentration of cadmium medium. The reduction of hepatopancreatic lipid content was statistically highly significant ($p<0.001$) at stage I, II, III, IV and V correspondingly.

3. Muscle

The effect of cadmium on the muscle lipid content of *Scylla serrata* during the ovarian maturation is presented in the fig. 3d. The Muscle lipid content of *Scylla serrata* when reared in cadmium free medium was 4.3, 5.6, 5.2, 5.1 and 4.3 mg/100 mg wet tissue at stage I to V respectively. The lipid content of the muscle of the *Scylla serrata* when exposed to cadmium medium decreased to 1.2, 3.1, 2.4, 2.2 and 2.3 mg/100mg wet tissue at stage I to V respectively. The decrease of muscle lipid content was statistically highly significant at stage I, II, III, IV and V correspondingly.

4. Haemolymph

The effect of cadmium on the haemolymph lipid content of *Scylla serrata* during the ovarian maturation is presented in fig. 3d. The haemolymph lipid content of *Scylla serrata* reared in cadmium-free medium was 3.1, 4.3, 4.7, 5.3 and 5.0 mg/ml of haemolymph at stage I to V correspondingly and it decreased to 0.70, 1.8, 2.2, 2.5 and 2.0 mg/ml of haemolymph at stage I to V when reared in 20mg/L concentration of cadmium medium. It is revealed that the decrease in the haemolymph lipid content at stage I, II, III, IV and V was statistically highly significant ($p<0.001$).

A. Acid phosphatase

Acid phosphatase is regarded as a well-known lysosomal enzyme which hydrolyses the phosphorus ester in acidic medium. It is reasonable that the enzyme is hydrolytic in its action and performances as one of the numerous acids hydrolyses in the autolytic process of the cell after its death. Administration of pollutants was shown to Alanine transaminase the acid phosphatase [28]. It has been revealed that, cadmium effect on activity of acid phosphatase in crab has not been studied except the work of [29]. Hence, it is about attention to analyze the impact of cadmium on acid phosphatase activity of *Scylla serrata* during the ovarian maturation.

1. Ovary

Influence of cadmium on the ovarian acid phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in the fig. 4a. The ovarian acid phosphatase activity of *Scylla serrata* reared in cadmium-free medium was 2.540, 1.265, 0.679, 0.520 and 0.153 mg p-nitrophenol/mg protein/h at stage I to V respectively and it was increased to 3.210, 1.846, 0.954, 0.681 and 0.241 mg p-nitrophenol/mg protein/h at stage I to V respectively, when reared in 20mg/L concentration of cadmium medium. The increase in the ovarian acid phosphatase activity was statistically significant ($p<0.05$) at stage IV while more significant ($p<0.01$) at stage I, II and V while highly significant ($p<0.001$) at stage III.

2. Hepatopancreas

The effect of cadmium on the hepatopancreatic acid phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in fig. 4b. The hepatopancreatic acid phosphatase activity of *Scylla serrata* when reared in chemical free medium was 1.655, 0.982, 0.543, 0.296 and 0.183 mg p-nitrophenol/mg protein/h at stage I to V respectively and it increased to 1.689, 1.777, 0.859, 0.491 and 0.306 mg p-nitrophenol/mg protein/h at stages I to V, when exposed to 20 mg/L concentration of cadmium medium. The increase in the hepatopancreatic acid phosphatase activity was statistically significant ($p<0.05$) at stage II and III; while more significant ($p<0.01$) at stage I and highly

significant ($p<0.001$) at stage IV and V correspondingly.

3. Muscle

The effect of cadmium on muscle acid phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in fig. 4c. The muscle acid phosphatase activity of *Scylla serrata* when reared in cadmium-free medium was 2.162, 0.362, 1.126, 1.012 and 0.258 mg p-nitrophenol/mg protein/h at stage I to V respectively, and it was increased to 2.997, 0.656, 1.692, 1.284 and 0.497 mg p-nitrophenol/mg protein/h at stages I to V correspondingly when exposed to the cadmium medium. The increase in the muscle acid phosphatase activity was statistically significant ($p<0.05$) at stage III, while more significant ($p<0.01$) at stage I and highly significant ($p<0.001$) at stage II, IV and V correspondingly.

4. Haemolymph

The effect of cadmium on the haemolymph acid phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in the fig. 4d. The haemolymph acid phosphatase activity of crab reared in cadmium-free medium was 1.543, 0.294, 0.259, 0.154 and 0.081 mg p-nitrophenol/mg protein/h at stage I, II, III, IV and V correspondingly. When *Scylla serrata* reared in cadmium the haemolymph and phosphatase activity was increased to 1.950, 0.892, 0.503, 0.408 and 0.188 mg p-nitrophenol/mg protein/h at stage I to V. The increase of haemolymph acid phosphatase activity was more significant ($p<0.01$) at stage IV and V while highly significant ($p<0.001$) at stage I, II and III correspondingly.

A. Alkaline phosphatase

Brush border enzyme alkaline phosphatase fragmented various phosphorous esters at alkaline pH. Alkaline phosphatases are known to have significant role in various biological mechanisms such as metabolism of carbohydrate, growth and differentiation, synthesis of protein, certain enzymes synthesis, secretory activity and phosphorylated intermediates transportation across the cell membranes [30]. There is paucity of information regarding the influence of pollutants on alkaline phosphatase activity of crab with reference to ovarian maturation. Hence, it is proposed to study the influence of 20ppm of cadmium on alkaline phosphatase activity of *Scylla serrata* during ovarian maturation.

1. Ovary

Influence of cadmium on the ovarian alkaline phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in the fig. 5a. The ovarian alkaline phosphatase activity of *Scylla serrata* when reared in cadmium-free medium was 1.556, 1.226, 0.436, 0.620 and 0.542 mg p-Nitro phenol/mg protein/h increased to 2.192, 1.512, 0.436, 0.620 and 0.542 mg p-Nitro phenol/mg protein/h at stages I to V correspondingly.

2. Hepatopancreas

The effect of cadmium on the hepatopancreatic alkaline phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in the fig. 5b. The hepatopancreatic alkaline phosphatase activity of *Scylla serrata* when reared in cadmium free medium was 1.255, 0.656, 0.329, 0.541 and 0.094 mg p-nitrophenol/mg protein/ h increased to 1.562, 0.864, 0.608, 0.706 and 0.260 mg p-nitrophenol/ mg protein/ h at stages I to V respectively.

3. Muscle

The influence of cadmium on the muscle alkaline phophatase activity of *Scylla serrata* with reference to the ovarian maturation is presented in the fig. 5c. The alkaline phosphatase activity of *Scylla serrata* reared in cadmium free medium was 1.531, 0.782, 0.304, 0.628 and

0.092 mg p-nitrophenol/ mg protein/h at stage I to V respectively, and it was decreased to 2.301, 0.966, 0.786, 1.125, 0.344 mg p-nitrophenol/mg protein/h at stage I to V when reared in 20mg/L concentration of cadmium medium. The increase of muscle alkaline phosphatase activity was statistically highly significant ($p<0.001$) at stage I –V correspondingly.

4. Haemolymph

The effect of cadmium on the haemolymph alkaline phosphatase activity of *Scylla serrata* during the ovarian maturation is presented in the fig. 5d. The haemolymph alkaline phosphatase activity of *Scylla serrata* reared in cadmium free medium was 1.327, 0.545, 0.159, 0.114 and 0.066 mg p-nitrophenol/mg protein/ h increased to 1.944, 0.866, 0.309, 0.312 and 0.133 mg p-nitrophenol/mg protein/h at stages I to V respectively when reared in cadmium medium.

Table 1. Variation in the total protein level of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages.

Ovarian Developmental stages		Ovary	Hepatopancreas	Muscle	Haemolymph
Stage I	Control	2.456±0.465	2.694±0.817	1.836±0.255	2.944±0.893
	Treated	2.262±0.448**	2.484±0.084**	1.736±0.263**	2.513±0.901**
Stage II	Control	2.021±0.002	2.009±0.010	1.997±0.003	1.976±0.004
	Treated	1.844±0.006**	1.780±0.021**	1.892±0.005***	1.903±0.004**
Stage III	Control	1.934±0.687	2.068±0.003	1.999±0.010	1.956±0.004
	Treated	0.886±0.002***	1.906±0.006***	1.894±0.023**	1.874±0.004***
Stage IV	Control	4.036±0.008	4.019±0.004	1.919±0.009	4.000±0.010
	Treated	3.224±0.002***	3.762±0.004***	1.712±0.005***	3.924±0.001**
Stage V	Control	2.877±0.007	3.116±0.003	0.005±0.003	3.848±0.039
	Treated	1.179±0.004***	3.002±0.342***	1.375±0.004***	3.548±0.143*

The results were expressed in mg/100mg wet tissue while for haemolymph the results were expressed mg/ml.
Values were expressed as mean ± SD of 6 observations. Asterisks indicate values that are significantly different from control.
*p<0.5, *p<0.01, *p<0.001

Table 2. Variation in the Carbohydrate level of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages.

Ovarian Developmental stages		Ovary	Hepatopancreas	Muscle	Haemolymph
Stage I	Control	0.845±0.047	0.778±0.022	0.696±0.015	0.766±0.024
	Treated	0.527±0.019**	0.553±0.040**	0.509±0.017***	0.445±0.020**
Stage II	Control	0.851±0.009	0.980±0.010	0.678±0.020	0.529±0.006
	Treated	0.472±0.010***	0.578±0.008*	0.388±0.006***	0.214±0.010**
Stage III	Control	0.885±0.023	0.526±0.178	0.334±0.268	0.421±0.374
	Treated	0.538±0.009***	0.321±0.010**	0294±0.008***	0.350±0.016*
Stage IV	Control	0.745±0.005	0.785±0.008	0.796±0.009	0.744±0.006
	Treated	0.572±0.035*	0.754±0.009*	0.715±0.015**	0.709±0.010***
Stage V	Control	0.399±0.029	0.397±0.039	0.065±0.014	0.651±0.007
	Treated	0.253±0.029**	0.365±0.015**	0.065±0.013***	0.625±0.009***

The results were expressed in mg/100mg wet tissue while for haemolymph the results were expressed mg/ml.
Values were expressed as mean ± SD of 6 observations. Asterisks indicate values that are significantly different from control.
*p<0.5, *p<0.01, *p<0.001

Table 3. Variation in the Lipid content of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages.

Ovarian Developmental stages		Ovary	Hepatopancreas	Muscle	Haemolymph
Stage I	Control	1.90±0.38	4.10±0.95	4.30±0.96	3.10±0.90
	Treated	1.10±0.35***	0.63±0.28***	1.20±0.40***	0.70±0.23***
Stage II	Control	3.10±0.48	5.10±1.10	5.60±0.30	4.30±0.49
	Treated	1.20±0.69***	2.20±0.43***	3.10±0.50***	1.80±0.36***
Stage III	Control	4.80±1.18	5.90±1.20	5.20±0.49	4.70±0.89
	Treated	2.20±0.41***	2.10±0.38***	2.40±0.38***	2.20±0.28***
Stage IV	Control	5.50±0.82	7.70±1.18	5.10±0.58	5.30±0.72
	Treated	2.60±0.43***	3.00±0.33***	2.20±0.36***	2.50±0.37***
Stage V	Control	6.80±1.20	7.20±1.37	4.30±0.51	5.00±0.75
	Treated	2.90±0.34***	2.40±0.21***	2.30±0.24***	2.00±0.34***

The results were expressed in mg/100mg wet tissue while for haemolymph the results were expressed mg/ml.
Values were expressed as mean ± SD of 6 observations. Asterisks indicate values that are significantly different from control.
*p<0.001

Table 4. Variation in the Acid phosphatase activity of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages

Ovarian Developmental stages		Ovary	Hepatopancreas	Muscle	Haemolymph
Stage I	Control	2.540±0.060	1.655±0.022	2.162±0.022	1.543±0.023
	Treated	3.210±0.041**	1.689±0.352**	2.997±0.416**	1.950±0.226***
Stage II	Control	1.265±0.024	0.982±0.010	0.362±0.026	0.294±0.039
	Treated	1.846±0.019**	1.777±0.001*	0.656±0.082***	0.892±0.124***
Stage III	Control	0.679±0.007	0.543±0.023	1.126±0.004	0.259±0.006
	Treated	0.954±0.095***	0.859±0.136*	1.692±0.292*	0.503±0.068***
Stage IV	Control	0.520±0.004	0.296±0.005	1.012±0.004	0.154±0.005
	Treated	0.681±0.020*	0.491±0.035***	1.284±0.110***	0.408±0.007**
Stage V	Control	0.153±0.006	0.183±0.033	0.258±0.004	0.081±0.006
	Treated	0.241±0.015**	0.306±0.033***	0.497±0.069***	0.188±0.020**

The results were expressed in mg p-nitrophenol/mg protein/h.

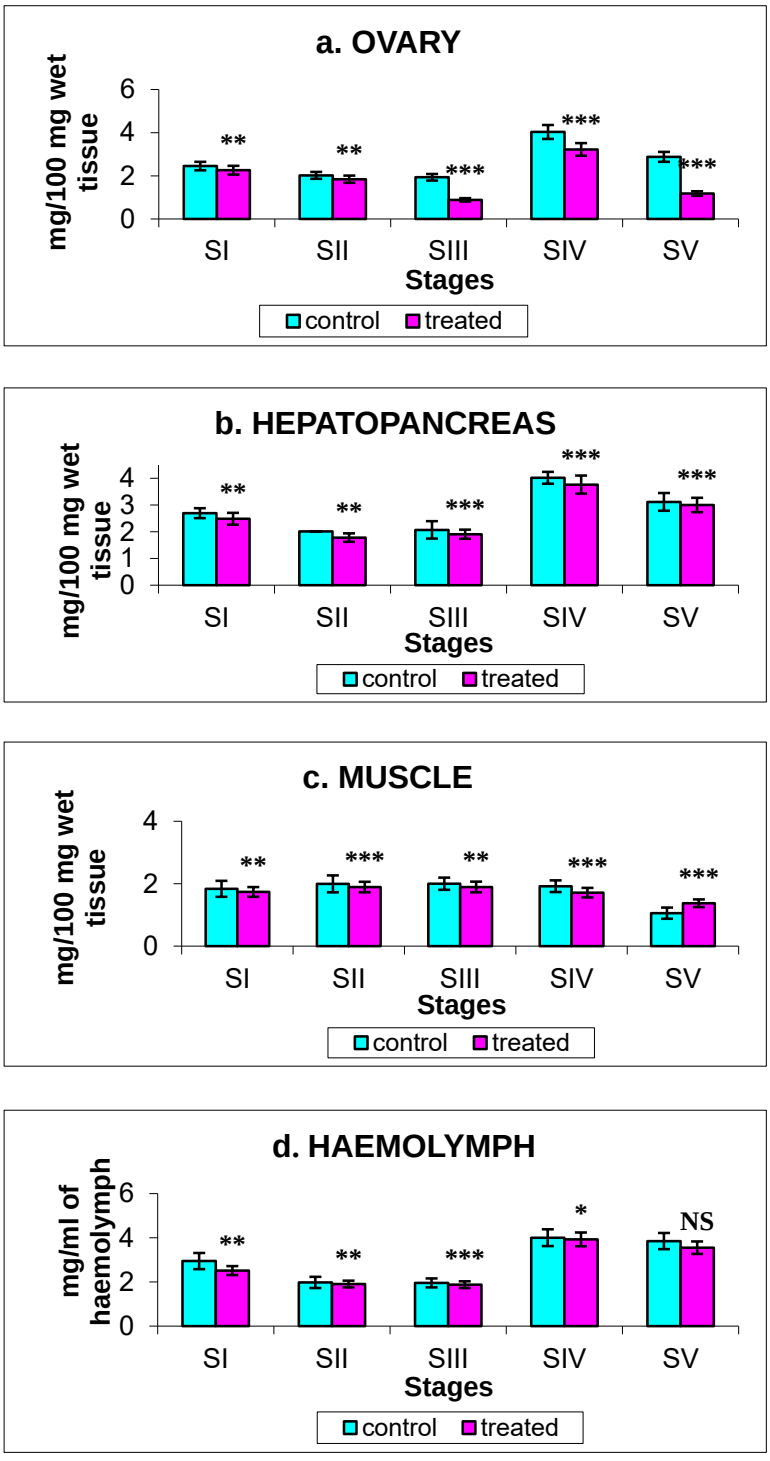
Values were expressed as mean ± SD of 6 observations. Asterisks indicate values that are significantly different from control. *p<0.5, *p<0.01, *p<0.001

Table 5. Variation in the Alkaline phosphatase activity of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages. The results were expressed in mg p-nitrophenol/mg protein/h.

Ovarian Developmental stages		Ovary	Hepatopancreas	Muscle	Haemolymph
Stage I	Control	1.556±0.004	1.255±0.005	1.531±0.004	1.327±0.004
	Treated	2.192±0.240**	1.562±0.222***	2.301±0.328***	1.944±0.274**
Stage II	Control	1.226±0.004	0.656±0.004	0.782±0.004	0.545±0.003
	Treated	1.512±0.248***	0.864±0.095***	0.966±0.105***	0.866±0.078***
Stage III	Control	0.436±0.021	0.329±0.007	0.304±0.003	0.159±0.003
	Treated	0.724±0.072**	0.608±0.117***	0.786±0.151***	0.309±0.051***
Stage IV	Control	0.620±0.017	0.541±0.004	0.628±0.005	0.114±0.003
	Treated	0.764±0.019***	0.706±0.061***	1.125±0.081***	0.312±0.011***
Stage V	Control	0.542±0.004	0.094±0.004	0.092±0.023	0.066±0.003
	Treated	0.621±0.021***	0.260±0.015***	0.344±0.092***	0.133±0.014***

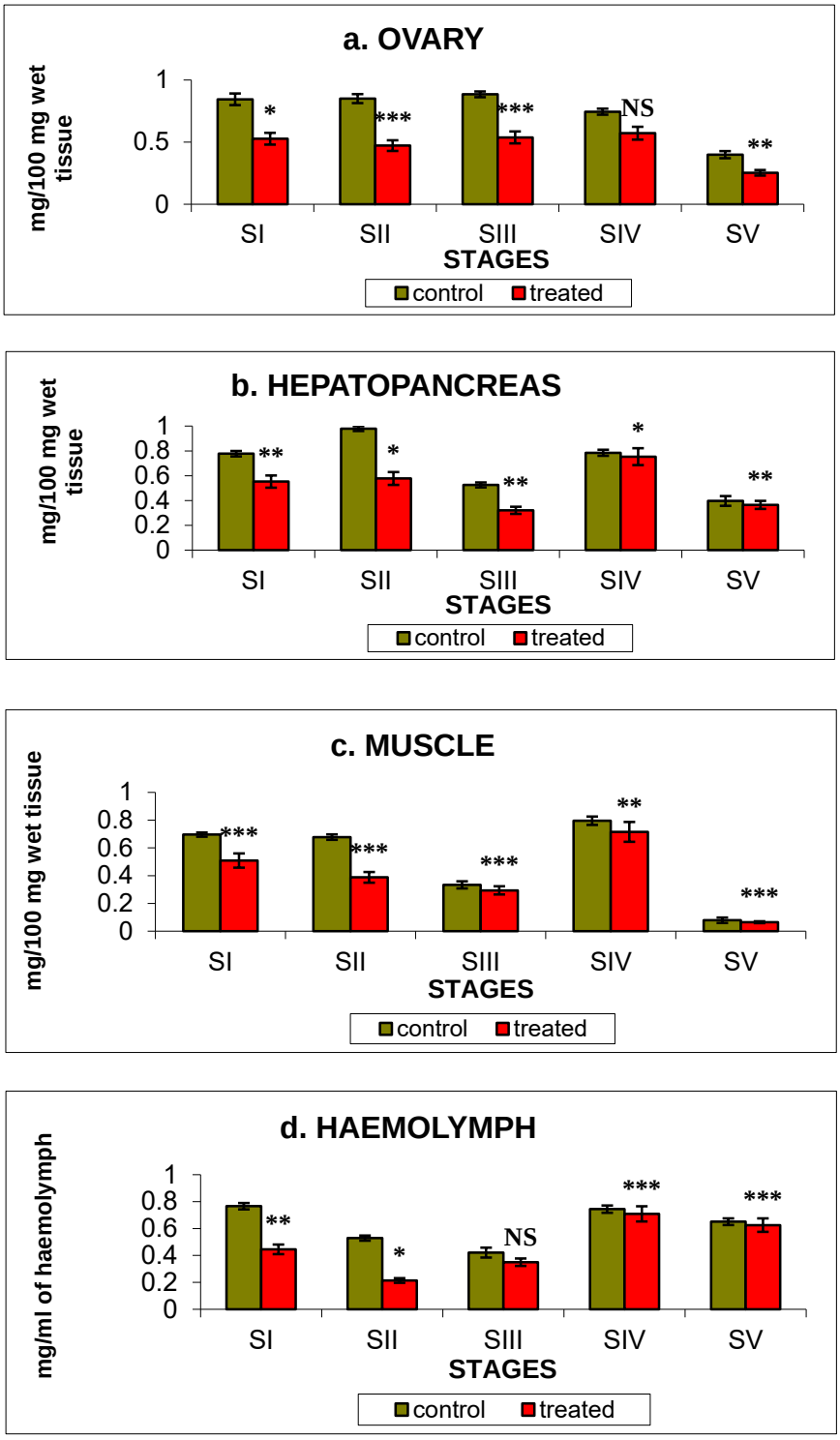
Values were expressed as mean ± SD of 6 observations. Asterisks indicate values that are significantly different from control. *p<0.5, *p<0.01, *p<0.001

Figure. 1 Variation in the protein level of ovary, hepatopancreas, muscle and haemolymph in the control and cadmium exposed Scylla serrata with special focus to ovarian stages.



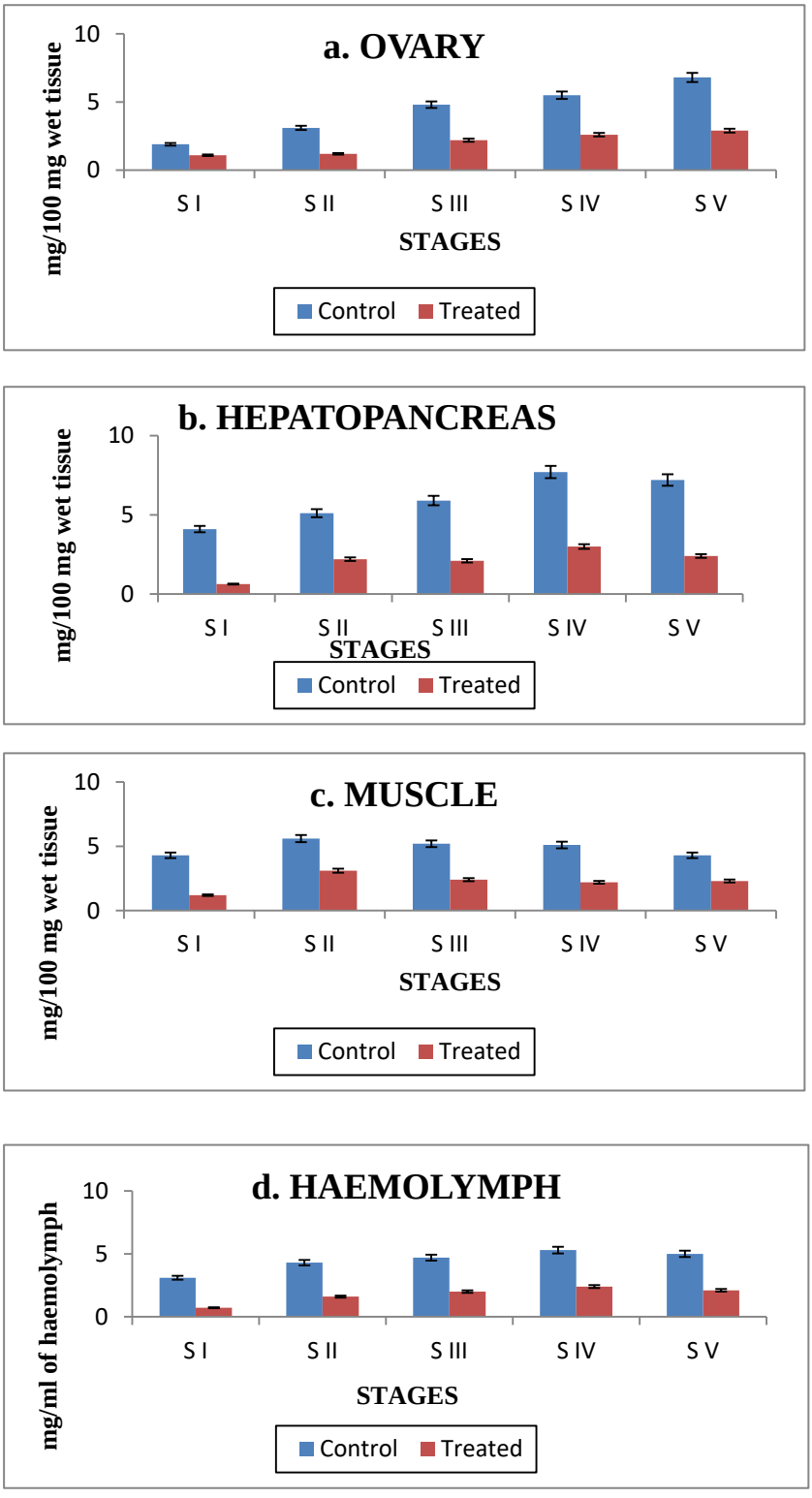
The results were expressed in mg protein/100 mg wet tissue while for haemolymph the results were expressed in mg/ml.

Figure 2 Level of cadmium on carbohydrate level of ovary, hepatopancreas, muscle and haemolymph in different ovarian stages of Scylla serrata.



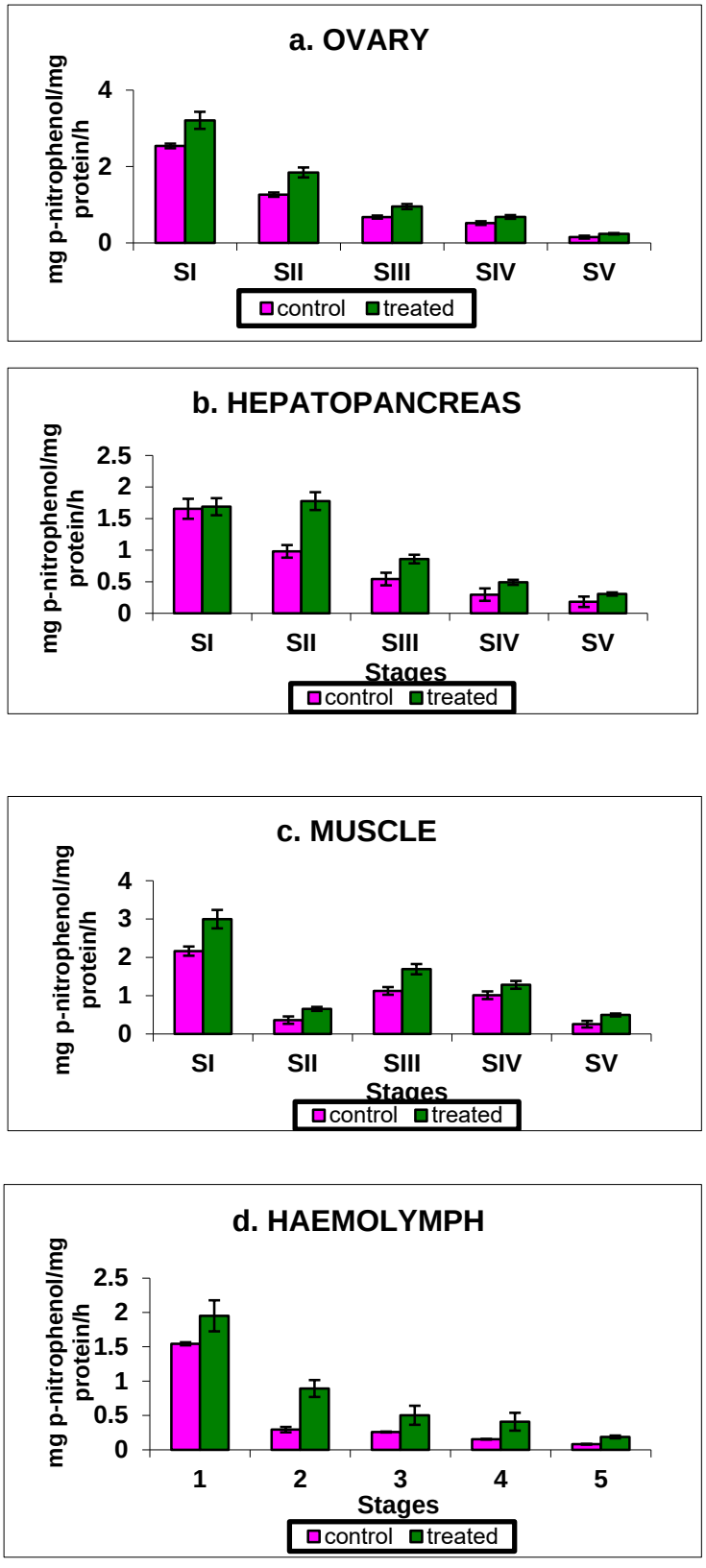
The results were expressed as mg/glucose/100mg wet tissue in the case of ovary, hepatopancreas, muscle and mg/glucose/ml for haemolymph

Figure 3 Variation in the total lipid content of ovary, hepatopancreas, muscle and haemolymph in the edible estuarine crab Scylla serrata with reference to different stages of ovarian maturation.



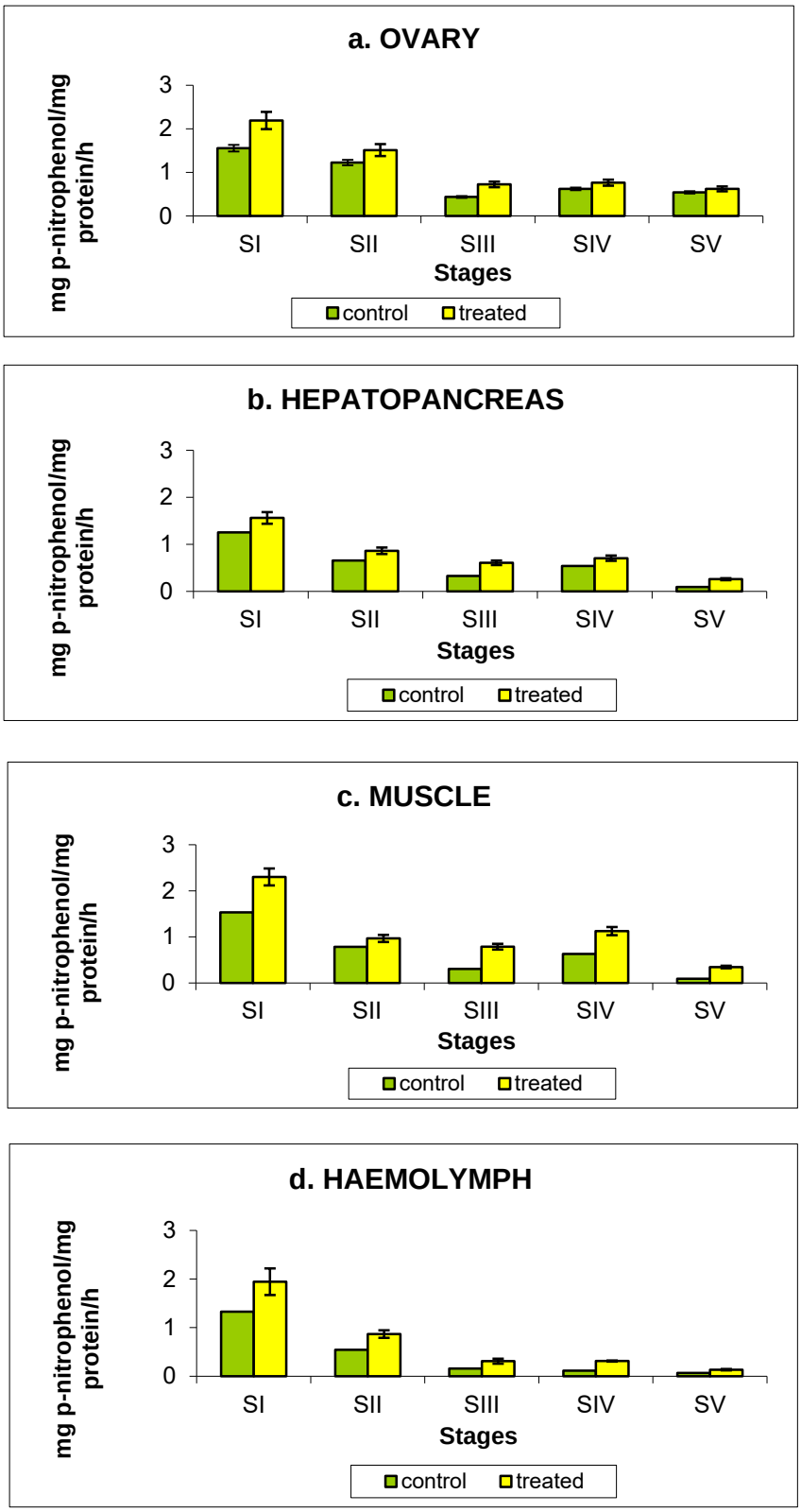
The results were expressed as mg/100 mg wet tissue in the case of ovary, hepatopancreas, muscles and mg/ml for haemolymph.

Figure 4. Acid phosphatase levels in ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages.



The results were expressed as mg p-nitro phenol/mg protein/hr.

Figure 5. Variation in the alkaline phosphatase activity in ovary, hepatopancreas, muscle and haemolymph in the control and cadmium chloride exposed Scylla serrata in different ovarian stages.



The results were expressed in mg p-nitrophenol/mg protein/h.

IV. DISCUSSION

Biochemical investigations dealing with changes in organic compositions on crustaceans are limited mainly to the vitellogenic oocytes. Only very little is known on the biochemical fluctuations in the extra ovarian synthetic and storage sites such as hepatopancreas and muscle occurring during various stages of ovarian maturation and development [31]. Biologically active molecules such as amino acids, proteins and enzyme systems are severely affected by the activity pollutants. The study of the biochemical constituents of the tissues of an organism is an important feature that provides a key for the physiology of animal [32]. Hence, in the present study, *Scylla serrata* was exposed to 20mg/L cadmium to study the changes in protein, carbohydrates, lipid and alterations in the phosphatases, transaminases, enzymic antioxidants and non-enzymic antioxidants with reference to ovarian maturation. It is a well-known fact the crustaceans, especially prawns and crabs are one of the sources of the nutritious food for human beings. Therefore, efforts are being made all over the world to investigate and exploit both marine and freshwater bodies for prawn and crab production. Hence, it is of interest to study the impact of cadmium on reproductive physiology of estuarine edible crab *Scylla serrata* with reference to ovarian maturation.

The protein is the foremost intake of energy source in addition to being a major form of energy being stored. The protein content of the cell may be considered as an important tool for evaluation of physiology standards. Hence, it is of interest to study the impact of cadmium on protein content in different tissues of *Scylla serrata* with reference to ovarian maturation. Ovary and hepatopancreas protein content of control crabs increased from stage I to V. The pattern of muscle and haemolymph protein content of control animals are high only in the initial stage of vitellogenesis. From the present experiment, it is evident that the protein content of control crabs between IV and V designates an intense vitellogenic activity during this period. In the present analysis, reduction in the protein content in the ovary, hepatopancreas, muscle and haemolymph are observed in cadmium exposed *Scylla serrata*. The diminution in the protein content specifies that tissue protein may involve in formation of free amino acids because of proteolysis and used for energy production in the TCA cycle during stress condition [33]. Decrease in protein content of pollutants exposed crustaceans have been observed by Reddy and Venugopal [34]. The reduction of protein content was due to the reduced synthesis of proteins because toxicant inhibited the bindings of phenylalanyl and lysl t-RNA to ribose. Maximum reduction of protein in hepatopancreas may be due to greater concentrations of enzymes. Since, it is the site of metabolism as suggested by [35]. Decrease of protein in muscle could be due to the increased activity levels of neutral and alkaline proteases, which act preferentially on the structural proteins. In the present investigation, it has been observed that under chemical anxiety protein loss may be credited to the consumption of amino acids in many catabolic reactions. Therefore, from the foregoing investigation, it is inferred that the cadmium affects the ovarian maturation of *Scylla serrata*.

Carbohydrates are the best energy producers of the body of the living organisms. In the tissues of crustaceans, it exists as free sugar. It serves as reserve substance and produce energy during stress conditions of organisms. Carbohydrate is the first degraded organic nutrients in response to stress conditions imposed on animals. In animals, even though the protein is the key basis of energy, there is a speedy shrinking of deposited carbohydrates due to chemical stress,

principally in hepatopancreas and muscle of crustaceans [27]. Therefore, the present investigation was undertaken to estimate the carbohydrate level in *Scylla serrata* with reference to ovarian maturation. Administration of chemical was shown to alter the carbohydrate content [36]. The carbohydrate content of ovary, hepatopancreas, muscle and haemolymph was higher only in the initial stage of vitellogenesis. This high level gradually dropped to trace during vitellogenesis. In the present investigation, decrease in the carbohydrate of ovary, hepatopancreas, muscle and haemolymph was observed in pollutant exposed *Scylla serrata*. This is in accordance with the findings of [37]. The decrease in carbohydrate level might be due to the consumption of energy demand under stress condition during the ovarian maturation as suggested by [26]. After carbohydrates, lipids are considered as superlative energy producers of the body. Several workers have studied the impact of pollutants on lipid content. A review of literature, however, reveals that the effect of cadmium on lipid content in crab about ovarian maturation has not been studied. Hence, it is of interest to study the influence of cadmium on lipid content of *Scylla serrata* during the ovarian maturation.

Lipids forms a major component of yolk is decapod crustaceans. Maximum lipids stored within oocyte is derived from extraneous sources such as the hepatopancreas which are the major stowage compartment in higher crustaceans [24]. The lipid content of control ovary, hepatopancreas, muscle and haemolymph showed a steady elevation from stage I to V. Lipid in the ovary steadily increased from stage II to V with a peak deposition at the end of stage V. In the present study lipid content of ovary, hepatopancreas, muscle and haemolymph of different ovarian stages of *Scylla serrata* decreased after exposure to cadmium. Energy demand under the stress conditions is due to excess utilization of lipids is the explanation for the deterioration of lipid [38]. pH specificity phosphatases are non-specific phosphomonesterase liberate phosphate through hydrolyzing many phosphate esters. In crabs, acid and alkaline phosphatases are scattered in various tissues. Phosphatases may play different roles, depending on the sites of occurrence. Administration of pollutants was shown to alter the acid phosphatase [39]. In the ovary of control crabs, acid phosphatase activity was higher only in initial stages of Vitellogenesis. Acid phosphatase activity was always maintained at a higher level than that of alkaline phosphatases. In the present investigation, a rise in acid phosphatase activity in ovary, hepatopancreas, muscle and haemolymph is observed in cadmium exposed to *Scylla serrata*. After exposure to pollutant the increased ACP activity was observed by [40]. It may be recalled that ACP has been established as a marker for lysosomes [41]. The increased ACP activity in the female *Scylla serrata* may be due to toxic effect of cadmium by which the cellular and lysosomal membrane might have been ruptured [42].

Brush border enzyme alkaline phosphatase splits numerous phosphorous esters at alkaline pH. Alkaline phosphatases laboring in many biological mechanism's likely metabolism of carbohydrate, growth and differentiation, synthesis of protein, enzymes production, secretory activity and transport to phosphorylated intermediates between cell membranes [43]. The alkaline phosphatase activity of ovary, hepatopancreas, muscle and haemolymph of control crabs was higher only in the initial stages of vitellogenesis. After exposure to cadmium the level of alkaline phosphatases activity was elevated.

V. Conclusion

Effect of Cadmium in reproductive physiology of estuarine edible crab *Scylla serata* in relation to ovarian maturation has been investigated. The biochemical estimation was also studied in hepatopancreas, muscle and haemolymph in the corresponding stages of ovarian maturation of *Scylla serrata* after exposure to Cadmium. Cd exhibits biochemical and physiological toxicity for crabs, affecting on the activity of antioxidant enzymes, protein, carbohydrates, lipids and phosphatases. Cd presented noticeable effects on the ovarian maturation of edible crab.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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