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Biochemical and Histopathological Changes in Certain Tissues of *Oreochromis mossambicus* (Trewaves) Under Ambient Urea Stress

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Modern agricultural practices result in indiscriminate use of various agrochemicals, which usually enter into the aquatic environment. Srinivasan et al. (1980) have reported the pollution of River Cauvery in South India from industrial effluent, agricultural run-off and urban wastes. The use of agrochemicals in the field has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem.

These agrochemicals adversely affect the non-target organisms, especially plankton and fish. Protein, carbohydrate and lipids in gill, muscle, liver, intestine and kidneys of *Oreochromis mossambicus* decreased with increased cadmium (Cd) intake (Sheela et al. 1995). *Oreochromis mossambicus* exposed to sub-lethal concentrations of the organochlorine insecticide endosulfan, showed decreased levels of protein, carbohydrate and lipid in their liver (Gansan et al. 1989).

Many authors have found urea in different ambient water bodies probably due to run-off from fields. Urea is highly soluble in water, penetrates into the tissues of fishes and induced alterations in the skin and gastric lining of the fish *Channa punctatus* (Srivastava and Srivastava 1979).

An attempt has been made in the present study to determine the urea-induced alterations in certain tissues of the fresh water fish *Oreochromis mossambicus* (Trewaves).

MATERIALS AND METHODS

The fish *O. mossambicus* used in the present study is edible, commercially valuable and distributed all over India. Live fish were obtained and stocked in glass aquaria after dipping in a 1% salt solution to prevent any parasitic attack. The standard length and weight of the fish ranged between 9-9.5 cm and 20-23 g, respectively. They were acclimatized to laboratory conditions for ten d. The fishes were fed on boiled egg white *ad libitum* for 2 hrs/d. Water was changed once a day.

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In the present study, a sublethal concentration of 80 ppm urea was used, which is less than 1/10th of the LC_{50} value for 96 hrs (Sriwastava and Srivastava 1985). The fish were exposed to urea for 20 d at 28°C ± 1°C. Simultaneously, controls were maintained. The uneaten food and faeces were collected every day with pipette. After 20 d, the fish were collected individually and blood samples were collected in a micropipette pre-rinsed with cold water by cutting the caudal region. After the blood was collected, the fish were sacrificed and the tissues were removed for biochemical (liver and muscle) and histological studies (liver and gill).

The protein content of the liver and muscles was estimated using the method of Gornall et al. (1949). Total free sugars (Roe 1955) and cholesterol (Zarrow et al. 1964) were estimated in blood, liver and muscles. The Bouin-fixed tissues were processed by adopting the procedure of Gurr (1959), and sections of 6µ thickness were obtained. The sections were stained using hematoxylin and eosin and permanent slides were prepared. The slides were observed using a "NIKON photomicrographic unit" and photographs were taken.

RESULTS AND DISCUSSION

It is well established that exposure to toxicants produces many biochemical changes in fish, which precede cellular systemic dysfunction. Srivastava & Srivastava (1979) showed that urea induced changes in the skin and gastric lining of the freshwater fish *Channa punctatus*.

When the fish *O. mossambicus* was exposed to a sublethal concentration of 80 ppm of urea, it revealed a slight increase in the operculum movement for the first hour which then became normal. The sub-lethal concentration of urea induced striking alterations in the total free sugars and cholesterol of blood, and total free sugars, proteins and cholesterol of liver and muscle of experimental *O. mossambicus*.

After 20 d of exposure to urea, the total free sugars in the blood of treated fish increased significantly when compared to the control ($P<0.001$). The average level of total free sugars in the blood was found to be 153.17 ± 12.33 mg/100 mL in the controls and 255.45 ± 37.30 mg/100 mL in treated fish (Fig. 1). The significant increase in the blood total free sugars may be attributed to the greater energy demand posed by the urea stress. A stress-induced increase in blood sugar has been reported by earlier workers like Sriwastava and Srivastava (1985).

The blood cholesterol of experimental fish increased significantly ($P<0.001$) from 11.88 ± 2.22 to 125.47 ± 9.58 mg/100 mL (Fig.1). The liver & muscle cholesterol levels showed significant increase. The liver mean cholesterol level increased ($P<0.001$) from 3.00 ± 1.61 mg/g dry wt. in the controls (C) to 17.05 ± 5.04 mg/g dry wt. in treated fish (Fig.2). The muscle mean cholesterol

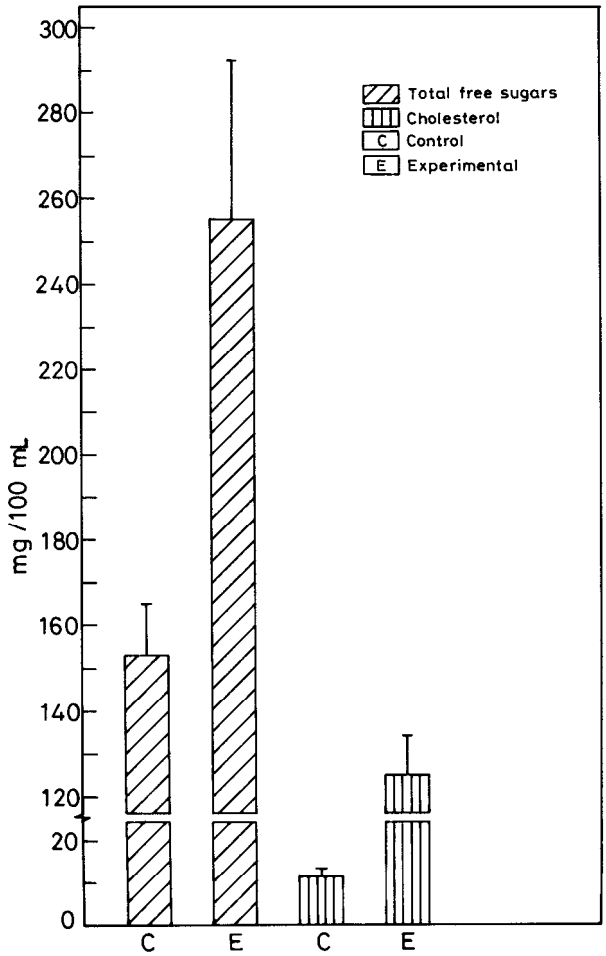

Figure 1. Changes in the levels of total free sugars and cholesterol in blood of *Oreohromis mossambicus* after exposure to urea for 20 days

level also increased significantly ($P<0.001$) from 1.54 ± 0.65 mg/g dry wt. (C) to 8.29 ± 1.476 mg/g dry wt. in treated fish (Fig.3). Hilmy et al. (1983) reported enhanced serum cholesterol in two euryhaline freshwater fishes which were exposed to sub-lethal concentrations of DDT and Endrin. The authors suggested that the insecticides either enhanced cholesterol production by the liver or inhibited its excretion to the bile duct after a certain period of exposure. This may be the reason for the significant increase in serum and tissue cholesterol of urea-treated fish.

As a result of urea stress, the level of total free sugars in the liver and muscle decreased significantly in treated fish when compared to the controls. The mean levels of total free sugars in the liver from 75 ± 9.13 mg/g dry wt. (C)

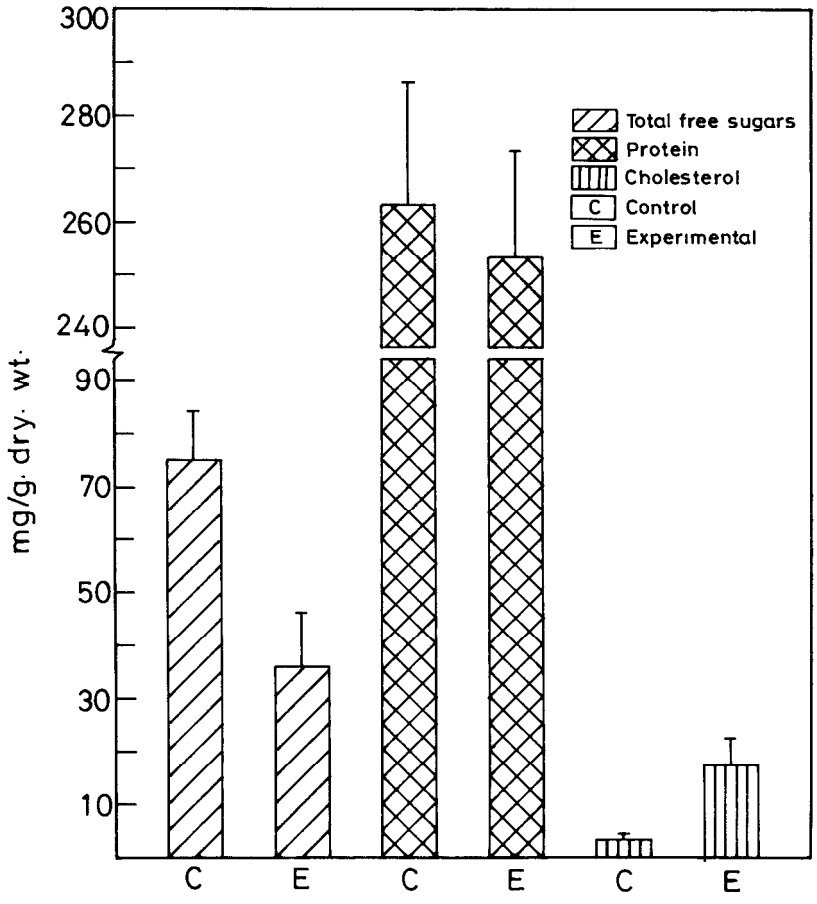

Figure 2. Changes in the levels of total free sugars, protein and cholesterol in liver of *Oreohromis mossambicus* after exposure to urea for 20 days

to 36.67 ± 10.75 mg/g dry wt. in treated fish (P<0.001). The same has been in muscle (P<0.01) from 27.78 ± 9.30 mg/g dry wt. (C) to 16.67 ± 6.09 mg/g dry wt. in treated fish (Fig.3). The reason for this decrease may be attributed to urea stress. The same was reported earlier by workers Sriwastava and Srivastava (1985).

The mean protein level in the liver decreased (P>0.1from 263.33 ± 23.38 mg/g dry wt. (C) to 253.33 ±20.66 mg/g dry wt. in treated fish (Fig.2). Sublethal concentration of the organochlorine insecticide endosulfan decreased the levels of proteins, carbohydrate and lipid in the liver of *O. mossambicus* (Gansan et al. 1989).

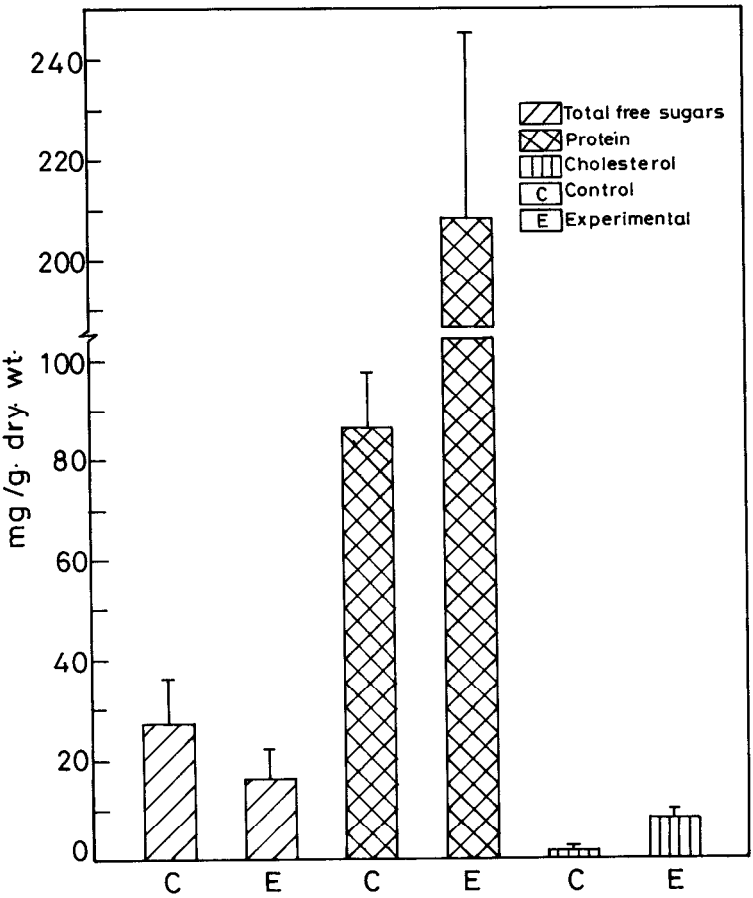

Figure 3. Changes in the levels of total free sugars, protein and cholesterol in muscle of *Oreohromis mossambicus* after exposure to urea for 20 days

However, in the case of muscle, protein level increased significantly ($P<0.001$) from 86.67 ± 11.15 mg/g dry wt. (C) to 208.89 ± 37.40 mg/g dry wt. in treated fish (Fig.3). Sivaprasad Rao and Ramana Rao (1979) have reported stress-induced protein synthesis in the muscle of *Tilapia mossambica,* using labelled aminoacids.

Histopathological examination of the tissues showed that the liver of the normal fish comprises a continuous mass of hepatic cells with cord-like formation. The cells are large in size, hexagonal in shape with more or less centrally placed nucleus and homogenous cytoplasm. Twenty days of exposure to urea resulted in widespread vacuolation, degeneration of nucleus, histolysis, hemocytic infiltration and necrosis of the hepatic cells (Plate I). Radhaiah and Rao (1992) have reported that when *T. mossambica* was exposed to sublethal concentration of fenvalerate for 10 and 20 d, histopathological lesions such as

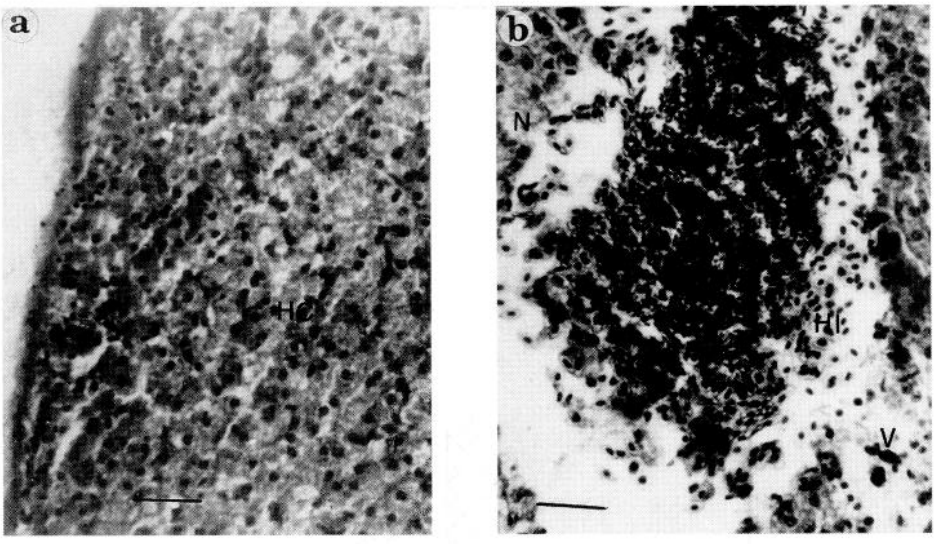

Plate I Cross section through liver tissue of *Oreochrornis mossambicus.* **a.** Normal liver showing hepatic cells (HC) **b.** After exposure to 80 ppm urea for 20 d, showing vacuoles (V), necrosis (N) and haemocytic infiltration (HI). Scale bar = 20 µm

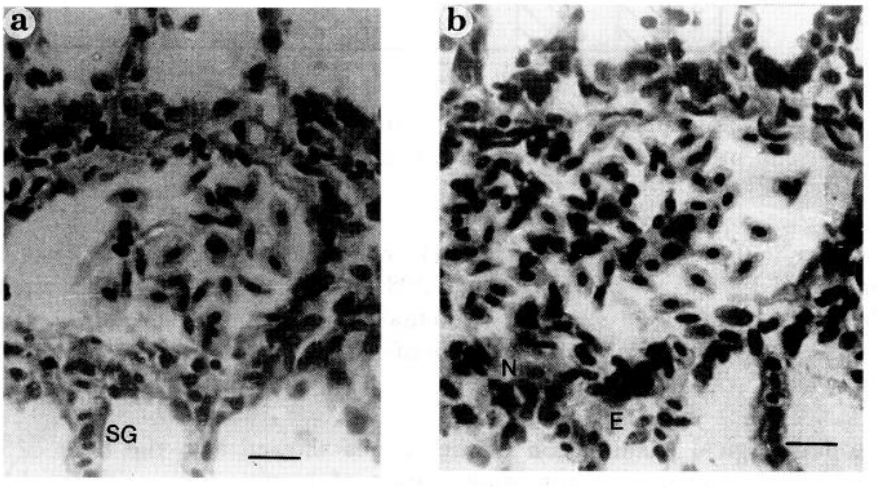

Plate II Longitudinal section through gill tissue of *Oreochromis mossambicus.* **a.** Normal gill showing secondary gill lamellae (SG) **b.** After exposure to 80 ppm urea for 20 d, showing degenerated epithelium (E) and necrosis (N). Scale bar = 20 µm

vacuolated hepatocytes, cell necrosis, movement of nuclei to the cell periphery, pycnotic nuclei and cytoplasmic degeneration were noted in the liver tissue. Ram and Singh (1988) also showed that, *C. punctatus* was exposed to a safe dose (4.5 ppm) of the carbamate pesticide carbofuran for six months. The liver exhibitedvarious histopathological changes including cytoplasmolysis, nuclear pyknosis and necrosis leading to disintegration of hepatocytes, degeneration of proliferated hepatocytes and the rupturing of blood sinus causing invasive infiltration of leucocytes.

As a result of urea exposure, the mucous cells of the gill epithelium degenerated. The fusion of secondary lamellae and also inflammatory alterations of lamellar epithelium were observed. Furthermore, gills exhibited hypertrophy of epithelium, some necrosis of epithelial cells and separation of epithelium due to edema. The number of blood cells were observed to be more in treated fish when compared to control fish (Plate II). Roy and Munshi (1991) demonstrated changes in the gills of *Cirrhinus mrigala* exposed to malathion. A sublethal dose of malathion caused inflammatory alterations of lamellar epithelium and hyperplasia on short-term exposure and proliferative loss of epithelium following long-term exposure.

Thus urea, at the sublethal level, affects the levels of tissue metabolites and brings about pathological changes in liver and gills.

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