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Protective action of Spirulina on blood chemistry and lipid profile of stinging catfish: *Heteropneustes fossilis*, induced by mercuric chloride

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Abstract: *The effect of mercuric chloride on blood, glycogen, and lipid contents of ovaries of carp, Heteropneustes fossilis has been studied. The fish were exposed to various concentrations of Mercuric chloride (0.01, 0.1, 0.5 mg/ml) respectively for 30 days. For recovery of health, the spirulina was given in feed at 5mg/ml dose for 30 days. The toxic group treated with mercury chloride showed a decrease in glycogen and lipid content in ovaries. But after feeding spirulina, the level of haematological and biochemical contents maintained and reached almost normal ranges. Therefore, from the study, it can be concluded that the significant alterations are due to the toxic effects of heavy metals at biochemical and hematological levels in ovaries.*

Key Words: *Heteropneustes fossilis, Mercuric chloride, Biochemical and Hematological.*

1. INTRODUCTION:

Toxicity is the primary subject of research in today scenario. The laboratories all over the globe played a leading role in developing technologies for rapid toxicity testing and analysis. For evaluation of adverse effects of toxic chemicals and pesticides in fishes specific testing methods and the proper system is required. Literature was available regarding the noxious status of the environment, but no particular role and mechanism studied for safety prospects. Therefore, in the present study we designed the study of biochemical, toxicological, and haematological parameters in fish to know the organ-specific damage at various concentrations of mercury chloride. In addition to this treatment phase of mercury in fish, the recovery of all changes was also studied using spirulina as a food additive.

Changes in biochemical markers in organisms are mostly due to exposure of chemicals and it has direct correlation with the changes in growth (Graney and Giesy, 1986) reproduction (Jarvenian et al., 1983), neurotransmission (MacLee and Knowles, 1988), energy metabolism (Bhaktavatsalam, 1988) and immune system (Gopal et al., 1992). Tort and Torres (1988) observed that changes in blood parameters are often quick in response to environmental or physiological alteration; furthermore, they are easily measurable and provide an integrated measure of the physiological status of the organism. Blood chemistry offers the potential for several biomarkers of toxicant stress, and it may be possible, in some cases to use biochemistry to extrapolate back from the fish to some features of habitat quality (Lockhart and Metner, 1984). Among various kinds of pollution, heavy metal pollution seems to be the most persistent one (Margarat et al., 2001). It is one of the challenging problems to the environmental biologists, as varieties of heavy metals have potentially harmful effects on the biological organisms (Jagadeesan and Kavitha, 2006). It is easily mixed with surface water and groundwater, and ultimately, they affect the aquatic environment.

The heavy metals are slow poisoning and slowly degradable substance and produce a toxic effect to prolonged period. They are also considered as a significant source of pollution toxicity to the living organisms. Recently, Mercuric chloride exposure increased the ROS levels and decreased the antioxidant potential of gilthead seabream (Sparus aurata) serum while increased the SOD, CAT and GR activities in the liver (Guardiola et al., 2016). Besides, significant alterations in the expression of the antioxidant enzyme genes sod, cat, gst, gpx, and gr have been observed in the freshwater fish Yamú (Brycon amazonicus) after Hg exposure leading to oxidation of lipids and proteins (Monteiro et al., 2010). Several scientists observed the toxic effect of heavy metal on fishes as well as the therapeutic effect of some organisms. High concentrations of metals can accumulate in the tissues of most aquatic organisms because their feeding and metabolic processes concentrate the metals in specific body parts. Some studies of this process on fish have indicated that cadmium may have toxic effects that result in alterations of the physiological processes in the blood and tissue of fish (Drastichoa J et al., 2004).

Several studies have shown that Hg produces an imbalance between the reactive oxygen species (ROS) production and its clearance by the antioxidant system in the known oxidative stress response. Thus, in fish, it has been described the production of ROS after Hg exposure in vivo (Capello T et al., 2016). The cell death mechanism has been

demonstrated in fish exposed to Hg in vitro fish systems including cell lines and primary cultures of head-kidney leucocytes (Morcillo et al., 2017; Morcillo et al., 2015). Thus, it is important to monitor the concentrations of heavy metals in aquatic environments. In this work, we studied about "remedial effects of spirulina on liver and gonad of heteropneustes fossilis after exposing to mercuric chloride."

2. MATERIALS AND METHODS:

2.1 Fish Heteropneustes fossilis

The live fish Heteropneustes fossilis will be collected from the local fisherman of Indore and acclimated for 7 days in Pinnacle Biomedical Research Institute, Bhopal and kept large in glass aquaria (40 x 30 x 35 cm). Live fishes (irrespective of sex and almost medium size group) were brought to the laboratory. Fishes would acclimatize into dechlorinated water for 30 days and treated with 0.01% of potassium permagnate (KMnO4) solution to avoid dermal infection. They were fed dried pellet feed every day, and water was renewed alternate days, leaving no fecal matter, unconsumed food or dead fish in aquaria. Water in all aquaria replaced by freshwater at every 48hrs.The fishes were examined for any bruise, lesions, or external infections. Heteropneustes fossilis was selected for the recent investigation, as it is available in abundance and could be handled very easily during experimentations.

2.2 Toxicant

In the present study, mercury was used as a toxicant. Stock solution was prepared then the solution of various concentrations of mercury (0.05mg/ml, 0.1 mg/ml and 0.5 mg/ml) was prepared.

2.3 Spirulina

Freshwater spiruilna was used for the experiment. It is collected in a sterile container. Spirulina was isolated from serial dilution method, which was developed in the Microbiology department of Pinnacle Biomedical Research Institute, Bhopal. Microscopic observations were made, and the total count of blue-green algae was enumerated by Lackey's drop count method using an Olympus Microscope under 100x magnification. Spirulina strain was maintained in Zarrouk's agar media slants at 4^{o}C. A loop full of spirulina culture was inoculated in 50 ml of the flask containing 10 ml sterile (Zarrouk's agar media) under sterile condition. pH was adjusted from 8.8 to 9.0. Growth and maintenance of culture was done in an illuminated cabinate at 30 ±2o C under 12/12 hour light-dark cycles. Manual shaking was done a day thrice.

50 ml of Spirulina culture was centrifuged at 4000 rpm, 4oC for 10 min. Then, the pellet was washed twice with 15 mL of a 10 mm EDTA solution. The resulting 2 × 15 mL (separated by centrifugation) was analyzed for the biomass. The culture was harvested for the determination of dry weight. Cells were collected by the filtration Whatsman no. 1 filter paper. After filtration, the filter paper was dried in an oven at 100^{o}C for 16 hrs, kept at dessicator allowed to cool at room temperature and dry weight was calculated. The filtrate or pellets were reserves at -20^{o}C temperature for further studies.

2.4 Exposure Procedure:

The fishes were kept in an aquarium. The various concentrations of mercury solution were introduced separately in each tank and observed for 1-3 hours for any mortality during the exposure time. The healthy stock of fishes was kept in aquaria containing 50 l of freshwater as control. During the exposure in different concentrations of mercuric chloride, the behavioral changes of fish were recorded. Those fishes which did not show any tactile response were considered dead. The dead fishes were removed from the aquaria immediately after death to avoid depletion of oxygen. Water was exchanged in each alternative day, and leftover feed and excreta were siphoned out every day. In every fortnight, sampling was done randomly from the stocked specimen for their growth.

2.5 Toxicity evaluation

The percent organ toxicity of fish in different concentrations of mercury chloride was determined. Fish was divided into 3 groups of 20 fishes each and would be kept in glass aquaria; each containing 50 liters stored dechlorinated tap water. Toxicity status was observed and recorded for all the concentration 0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml for 10, 20 and 30 day. A normal batch of fish was also maintained simultaneously in freshwater medium served as control. All the experiments were performed thrice to confirm the results. To evaluate the influence of mercuric chloride on ovaries, the hematological and biochemical assay was performed. The study was conducted on Heteropneustes fossilis. They were acclimatized to laboratory conditions in glass troughs for fifteen days. They were fed with commercial fish food pellets during acclimation. After 15 days, if fishes were in normal behavioral activity and good health conditions, those species were selected for experiment purpose. The fish were divided into three groups. One group without mercury chloride served as the control, and the other group was exposed to a lethal concentration of mercury chloride for 10, 20 and 30 days During this experiment, the behavioral changes were critically observed.

- Control group: In this group, fishes are fed with prepared pellet feed
- Treatment group: In this group, fishes get mercuric chloride in different concentration (0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml) was added in water including normal diet.
- Recovery group: In this group, fishes are fed with Spirulina powder mixed with pellet feed with different concentration of mercuric chloride (0.05 mg/ml, 0.1 mg/ml, 0.5 mg/ml).

On 10, 20 and 30-day fishes of control, experimental and recovery groups were dissected. Liver and gonad were separated and preserved for the biochemical and haematological studies.

2.6 Haematological Study (Blaxhall and Daisley, 1973)

Blood samples were collected on 10, 20, and 30 days. During sampling, care was taken to minimize stress in the netted fish and fish remaining in the tank. All fishes were netted within 20 seconds. Blood samples were collected from the caudal tail vessels with 21 or 23 gauge needles and 1 or 3 cc syringe before ventilator response was noticeably depressed. To prevent repeated sampling, fish were not returned to the same tanks after blood collection.

2.7 Biochemical Study (Shelke et al., 2013)

Blood was collected as described for biochemical analysis as firstly blood of healthy (non-intoxicated) fish were collected directly in dry and sterilized centrifuge tube s by cutting the caudal peduncle of living fish with a sharp, sterilized dry knife. Before cutting the caudal peduncle, the fish was blotted with blotting paper to avoid haemolysis. The blood was allowed to clot for 10 minutes and centrifuged at 3000 rpm for 20 minutes. The serum was carefully taken out with the help of pipette and stored at 40^0C. All the tests were performed within 72 hours.

3. RESULTS:

Healthy control group fishes did not show any signs of toxicity from 10th day to 30th day. They were sensitive to light and moved to the bottom of the tank. The behavioral changes were observed in the treatment group fishes when exposed to Mercury chloride. They showed erratic swimming, imbalance posture, opercular movement, and spreading of the excess of mucus all over the surface of the body. The fish eventually died with their mouth and opercular wide opened. In lower concentration of mercuric chloride treatment, the behavior of the fish was slowly disrupted during the 10 day. The hyperactivity, excitement, hyperventilation, etc., increased exposure to the higher concentrations of mercuric chloride. In recovery group decrease in such activities was found when treated with spirulina. They behave normally. Further, the fish at 30th days of exposure exhibited normal balanced swimming and active feeding. The haemetalogical analysis of control, treatment, and recovery group of Heteropneustes fossilis were shown in Table: 1 and Table: 2.

Parameters	Healthy Control	0.05mg/ml			0.1 mg/ml			0.5 mg/ml		
		10 DAYS	20 DAYS	30 DAYS	10 DAYS	20 DAYS	30 DAYS	10 DAYS	20 DAYS	30 DAYS
RBC (x 10^6/µL)	4.44 ±0.274	2.97±0.012	2.64±0.006	1.94±0.061	2.53 ± 0.006	2.10 ± 0.006	1.33 ± 0.010	2.86 ± 0.010	2.07 ± 0.006	1.72 ± 0.010
WBC (x 10^3/µL)	2.51±0.053	5.61±0.010	5.97±0.006	6.02 ±0.006	5.85 ± 0.010	6.24 ± 0.006	6.60 ± 0.010	5.91 ± 0.010	6.55 ± 0.0060	6.97 ± 0.010
Hb (g/dL)	11.44±0.101	8.88±0.010	8.12±0.010	7.67±0.015	8.76 ± 0.010	7.13 ± 0.010	6.46 ± 0.10	8.29 ± 0.010	6.92 ± 0.015	4.31 ± 0.010
Haemocrit (fL)	29.42 ±0.010	36.11±0.010	38.20±0.010	41.75±0.010	35.41 ± 0.015	36.75 ± 0.010	37.84 ± 0.010	35.32 ± 0.006	36.21 ± 0.006	37.62 ± 0.010
MCH (pg)	25.53±0.015	28.94±0.012	35.54±0.036	45.95±0.010	34.48 ± 0.015	33.90 ± 0.006	48.27 ± 0.010	29.95 ± 0.070	37.92 ±0.032	38.76 ± 0.020
MCHC (g/dL)	37.32±0.006	31.36±0.010	29.33±0.012	28.88±0.010	31.02 ± 0.01	26.16 ± 0.010	24.47 ± 0.010	29.90± 0.006	26.53 ± 0.015	18.03 ±0.010
LYMPHOCYTES (x 10^3/µL)	30.21±0.006	36.23± 0.006	44.20 ± 0.006	52.84 ± 0.006	36.81 ± 0.010	44.33 ± 0.012	54.85 ± 0.010	39.02 ± 0.006	45.61 ± 0.010	56.49 ± 0.010
GRANULOCYTES (x 10^3/µL)	3.62±0.006	5.11 ± 0.010	5.36 ± 0.012	5.337 ± 0.015	5.26 ± 0.006	5.61 ± 0.010	5.73 ± 0.006	5.31 ± 0.006	5.72 ± 0.006	6.18 ± 0.006

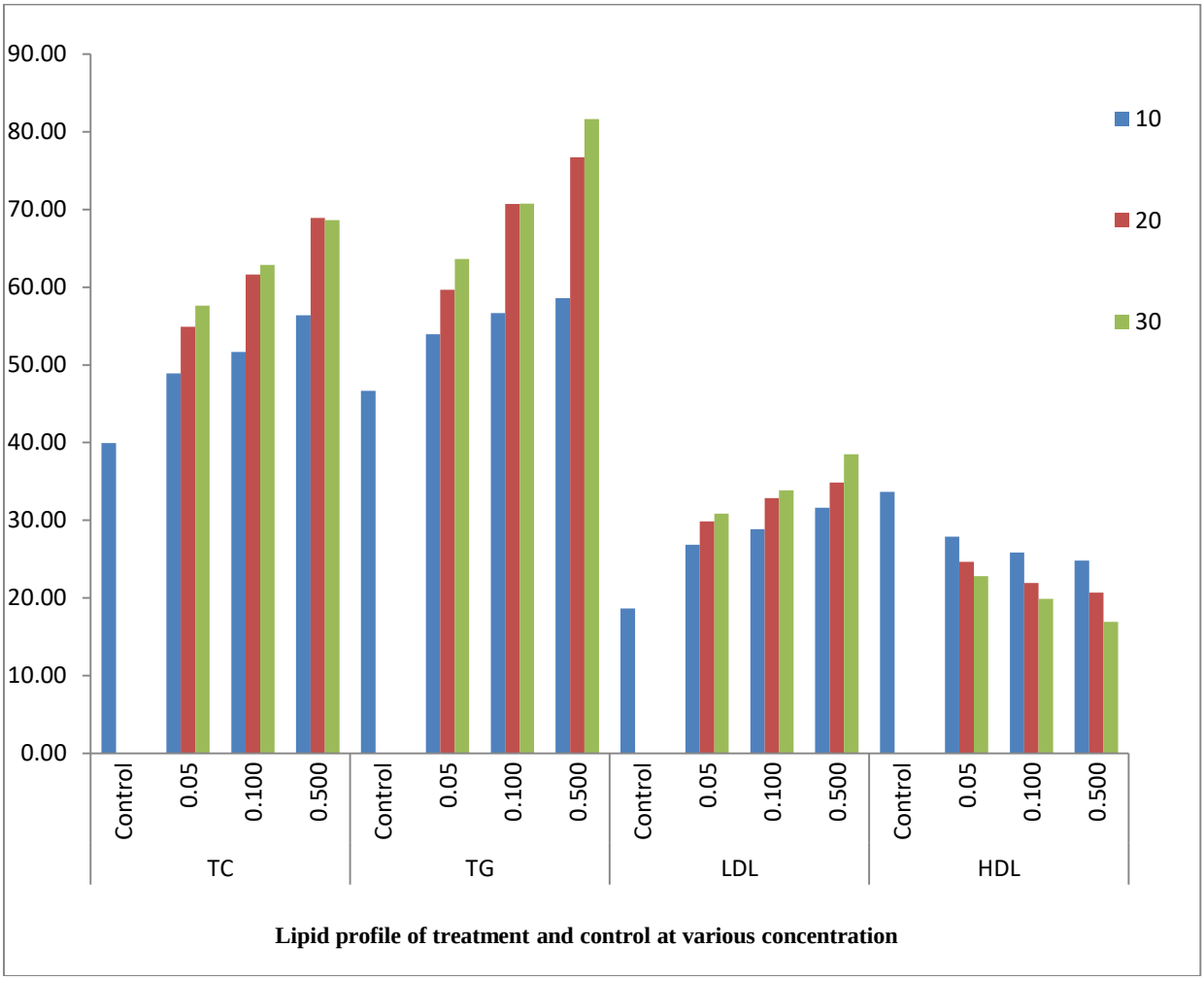
Table 1: Haematologigal profile of healthy control and treatment group with various concentration and time duration

Parameters	0.05mg/ml			0.1 mg/ml			0.5 mg/ml		
	10 DAYS	20 DAYS	30 DAYS	10 DAYS	20 DAYS	30 DAYS	10 DAYS	20 DAYS	30 DAYS
RBC (x 10^6/µL)	2.95 ±0.006	2.64±0.000	1.98±0.006	2.54±0.010	2.10±0.010	1.34±0.006	2.85±0.010	2.06±0.006	1.72±0.006
WBC (x 10^3/µL)	5.50±0.100	5.98±0.010	6.02±0.006	5.86±0.006	6.23±0.010	6.60±0.010	5.93±0.010	6.54±0.010	6.98±0.010

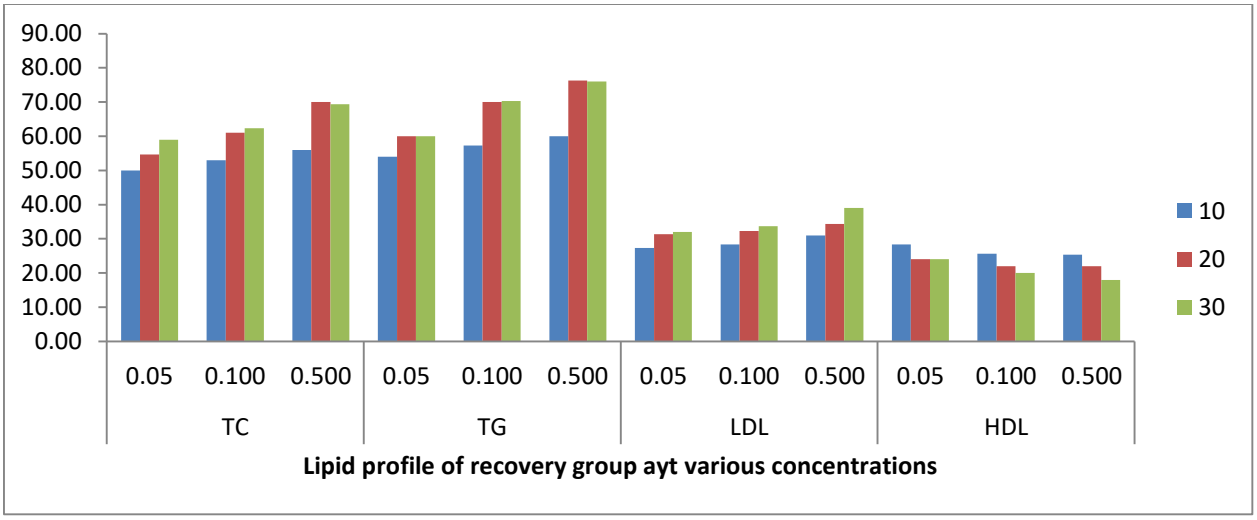
Hb (g/dL)	8.87±0.010	8.11±0.012	7.68±0.010	8.76±0.006	7.11±0.010	6.48±0.006	8.28±0.010	6.94±0.006	4.32±0.010
Haemocrit (fL)	36.11±0.010	38.20±0.010	41.76±0.015	35.41±0.010	36.76±0.006	37.86±0.010	35.31±0.010	36.11±0.010	37.61±0.010
MCH (pg)	28.94±0.010	35.58±0.010	45.95±0.006	34.48±0.010	33.90±0.006	48.28±0.006	30.01±0.010	37.93±0.010	38.78±0.010
MCHC (g/dL)	31.36±0.006	29.31±0.010	28.88±0.006	31.02±0.006	26.18±0.010	24.49±0.010	29.90±0.006	26.52±0.010	18.04±0.006
LYMPHOCYTES (x 10^3/µL)	36.23±0.006	44.20±0.006	52.83±0.006	36.83±0.006	44.34±0.006	52.86±0.010	39.03±0.006	45.61±0.010	56.51±0.010
GRANULOCYTES (x 10^3/µL)	5.11±0.012	5.36±0.010	5.34±0.010	5.28±0.010	5.62±0.010	5.73±0.010	5.32±0.010	5.73±0.010	6.18±0.010

Table 2: Hematological profile of recovery group with various concentration and time duration

The present study aimed to determine the composition of cholesterol in the toxic group and recovered group. Dietary lipids play important roles as a source of energy and essential fatty acids necessary for fish growth and development. Changes in the total lipid content (TC, TG, LDL, HDL) in ovaries of Heteropneustes fossilis exposed to mercuric chloride at the various concentration for up to 30 days and in recovery group when treated with spirulina (5mg/kg) are shown in Graph 1 and 2.



Graph 1: Lipid profile of ovaries in the control group and at various time intervals after exposure of mercury at various concentrations for Heteropneustes fossilis in still water condition.



Graph 2: Lipid profile of ovaries in the recovery group after exposure of mercury at various concentrations for Heteropneustes fossilis in still water condition.

The glycogen level of control, treatment, and recovery group of fishes of Heteropneustes fossilis are shown (Table. 3). From the results, the glycogen content in the muscle of the control fishes showed the highest activity (31.8±0.424)whereas the lower value was observed on 30 days at 0.5mg/ml concentration of mercury, i.e. (11.36 ± 0.057) in the treatment group. But on the 30th day at 0.05 concentration, the level of glycogen increase and maintained.

Days	Control	Treatment			Recovery		
		0.05	0.1	0.5	0.05	0.1	0.5
10	31.8±0.424	19.06±0.152	17.46±0.450	16.16± 0.351	20.36±0.321	18.63±0.577	17.20±0.200
20		22.66± 0.577	20.06±0.513	16.90±0.100	22.66 ± 0.577	20.06± 0.513	16.90± 0.100
30		16.23±0.404	12.90±0.100	11.36± 0.57	25.70±0.700	22.40±0.100	18.60±0.305

Table: 3 Glycogen level in ovaries in control, treatment and recovery groups

4. DISCUSSION:

Metabolic activity of an organism reflects the overall utilization of biochemical energy to counteract the stress generated by toxic metal. In every individual, glycogen and lipid play an essential role in metabolism. Due to heavy metal toxicity, the prime sources of energy is affected severely and retard various processes in exposed individuals. (Umminger, 1970) postulated that glycogen is used as the principal and immediate energy precursor in fish Fundulus heteroclitus under stress conditions. (Sastri & Subhadra, 1982) studied the chronic toxic effects of cadmium (0.26mg/L) on the carbohydrate metabolism of a teleost fish, Heteropneustes fossilis after 15, 30 and 60 days of exposure, and observed depletion in liver and muscle glycogen content. Lipids act as the reversed depot of energy from where the energy is supplied as and when required. (Katti & Sathyanesan,1983) reported decreased cholesterol and lipid levels in brain, testis, and ovary of Clarias batrachus exposed to 5 ppm of lead nitrate for 150 days. (Katti & Sathyanesan, 1984) Also reported a decrease in the lipid levels of Clarius batrachus when exposed to cadmium. The results of the present study reveal a significant effect of mercury chloride on blood profile, lipid profile, and glycogen content.

5. CONCLUSION:

The current investigation, therefore, concluded that the depletion in the metabolic rate indicates the fact that the whole metabolic pool of the fish gets altered under the toxic stress. Further, the change in the biochemical profile indicates their rapid utilization to provide excess energy to the cellular biochemical process to cope with the stressful condition. The serum cholesterol level was significantly decreased in recovery group (treated with mercuric chloride and spirulina) as compared to treatment toxicant group(treated with mercuric chloride) on 10th, 20th and 30th day of study protocol in all the three targeted organs (liver, testis, and ovary) There is a significant improvement observed in spirulina treated group when compared to normal. Thus, significant changes observed after mercury intoxication on blood profile and metabolic activity of ovaries of Heteropneustes fossilis. The variation in the concentration indicates

that the cell functioning has been hampered initially, but the affected body has tried to balance and recover for the disturbance.

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