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Gonadosomatic and Hepatosomatic Indices of Freshwater Fish *Oreochromis mossambicus* in Response to a Plant Nutrient

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Abstract: Plant nutrients have been used extensively and aggressively by the Indian farmers for the production of the crops. These chemicals pollute freshwater bodies through agricultural runoff. The present study was carried out to determine the toxicity of micronutrient Librel™ on freshwater fish *Oreochromis mossambicus*. The LC_{50} value (5000 mg L^{-1}) was obtained and the fishes were exposed to sublethal concentration (250 mg L^{-1}) for the period of 45 days. Reproductive aspects in addition to condition factor, gonadosomatic index (GSI) and hepatosomatic index (HSI) were analyzed based on weight of the body length, Total body weight and organ weights of the fish. The results obtained indicated adverse effects on the gonads as well as on liver weight. These GSI and HSI alterations were accompanied by histopathological changes in liver, ovary as well as in testis. This study is the first histological evidence of reproduction disturbance related to a micronutrient Librel™ and the high levels of gonad and liver histopathology recorded raise concerns about the long-term health of fish populations.

Key words: *Oreochromis mossambicus* · Micronutrient · Hepatosomatic Index · Gonadosomatic Index · Histopathology and Histomorphology

INTRODUCTION

A sustainable Agriculture is a system of agriculture that is committed to maintain and preserve the agricultural base of soil, water and atmosphere ensuring future generations the capacity to feed themselves with an adequate supply of safe and wholesome food [1]. To satisfy human hungers with the increasing population growth and changing dietary patterns have resulted in more and more land moving from forest or grasslands into agricultural production. Initially the crop productivity could be maintained with nitrogen fertilizer alone as the other major nutrients needed by the plants were provided by the soil. However, due to gradual usage of the nutrients the same is no longer possible by applying N alone. Tiwari [2] in his studies have reported the growing emergence of plant nutrient deficiencies in the farm fields and is of the view that the Indian agriculture is in an era of multiple nutrient deficiencies. Hence to sustain the high yields farmers have to apply the six main nutrients (N, P, K, S and Zn). Most of the soils of Gujarat have been reported to be deficient in Zn and Fe [3]. The supplementation of micronutrients through multi-micronutrients mixture under such situations becomes more important to provide balanced nutrition to the crops [4].

India has come a long way since independence, in respect of production and consumption of fertilizers. From a mere 69.8 thousand tons of fertilizers (1950-1951) to 22.57 million tons (2007-2008), the fertilizer consumption increased from a mere 0.7 Mt in 1951 to 28.3 in the year 2011. This resulted in the food grain production from 640 kg/ha to 1921 kg/ha in Rao [5]. This clearly states that as earth can only supply limited amount of nutrition for every additional ton increase in the food production external application is needed. This increased use of fertilizer with the motive of soil nutrient enrichment has led to eutrophication and deterioration of surface water quality due to transpiration of nutrients applied through fertilizers via leaching and /or runoff. Further eutrophication may lead to excessive growth of phytoplankton and filamentous algae, increase in aquatic plant life, increase in turbidity (cloudiness) of water, increase in rate of sedimentation, development of anoxic

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conditions (low oxygen levels), decrease in species diversity and an increase in the frequency of algae blooms causing a dearth of oxygen and a change in fish species composition [6]. Honeyfield and coworkers [7] have documented the mortality of alligators (*Alligator mississippiensis*) in the lake Griffin, Florida caused due to the production of a neurotoxin by microscopic floating algae, *Cylindrospermopsis.*

Librel, is an EDTA chelated Micronutrient mixture used extensively in Gujarat. A chelate is a complex in which an organic coating is given by EDTA around inorganic elements which make them stable. It contains Zn, Mn, Fe, Cu and B in trace amounts. However, this stability enhances the solubility and makes them favorable to enter into the aquatic environment. Additionally, metals are non-biodegradable. Various studies have proved the deleterious effects of such xenobiotics/stressors entering into the aquatic environment. El Nemeki and Badawy [8] & El Nemeki and Badawy [9] established that the concentrations of different heavy metals exceeded the maximum permissible concentrations in both *Mugil cephalus* and *Ctenopharyngodon idella* tissues. Barakat [10] in his study has reported that the dissolved, metals and organic contaminants or their metabolites in the fish and their eggs affected the spawning behavior and duration. Other than that several publications that revealed the existence of pesticide residues in various aquatic ecosystems were presented by several investigators [11-16]. According to Marcovecchio and Moreno [17], the advantage of studying the fish is that its results reflect the bioavailability of pollutants in the system and thus helps in assessing the true degree of pollution in the environment. Tilapia (Cichlidae) are native of Africa, but have been introduced to other countries. They are among the most studied groups of fish in African waters and most prominent in the freshwater aquaculture at many regions of the world due to their economic importance [18, 19]. The reproductive health is studied so as to know the health of the fish population in the aquatic ecosystem. To attend the producers' demand tilapia reproduction plays an important role in the process of the production of fish system as it greatly depends on quantity and quality of fingerlings to attend the producers demand. The inspection of liver is pertinent as it plays an important role in the metabolism and excretion of xenobiotic compounds [20]. Due to their toxicity and accumulation metals have got a great ecological concern. According to Mason [21] fish are capable to accumulate significant concentration of metals in water that are below the limit of detection in routine water samples. Further they can either increase or decrease hepatic enzyme activities leading to histopathological changes, depending on the metal type and concentration, fish species, length of exposure and other factors [22]. For an accurate and effective assessment of the effects of xenobiotic compounds in field and experimental studies the proper monitorization of histological changes in fish liver is a highly sensitive and accurate way. Hence, the present study aims to investigate the effect of Librel on the reproductive and metabolic health of freshwater fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Oreochromis mossambicus, commonly known as Tilapia, was brought from the local pond of Baroda and acclimatized to the laboratory conditions in a well aerated dechlorinated tap water for the period of 10 days. Fishes having an average weight (25±2 g) and size (12±3 cm) were selected for the study. Commercial fish food was supplied to the fishes during whole experimental period. Test animals were categorized into treated and control groups (20 animals in each group). After establishing the LC_{50} values (5000 mg/l) acclimatized fishes were exposed to a sublethal dose of 250 mg/l ($LC_{50}/20$) for the period of 15, 30 and 45 days and were considered as treated group. Control group was kept in dechlorinated water without any treatment. 30% water was changed after every 24 hours and physicochemical properties of water were measured twice in a week.

After the period of exposure fishes were removed and washed with freshwater. Control as well as treated groups were killed by decapitation and weighed; blood was allowed to drain and dissected to take out organs. The prime goal of the study was to know the reproductive and the metabolic health of the fish for the xenobiotic i.e. an EDTA chelated micronutrient mixture (LIBREL™). Hence the total organ weight (liver and gonads) was taken for the Hepatosomatic (HSI) and Gonadosomatic (GSI) indices. The indices were calculated according to the following formula:

$$HSI = (\text{Liver weight (g)} / \text{Fish weight (g)}) \times 100,$$

$$GSI = (\text{Gonad weight (g)} / \text{Fish weight (g)}) \times 100.$$

Further the tissues were fixed in 10% formalin for 24 hours, hydrated, embedded in paraffin wax, sectioned at 7-8 µm, stained with hematoxylin and eosin, observed microscopically to know the histological alterations.

Means ± standard deviation (SD) were calculated for both group. Statistical analysis (Two Way ANOVA and Post tests after Two Way ANOVA) was done using the software Graphpad Prism 5, at a 5% significance level. The results of the experimental groups were compared with the control group to quantify the effect of the xenobiotic on the test animal.

RESULTS AND DISCUSSIONS

Periodic variations due to addition of plant nutrient on the GSI and HSI of the males and females are shown in Tables 1 and 2 (Mean ± SD) respectively. The HSI values showed significant difference in both males and females as compared to the control group. The values for males for 15 days and 30 days were (0.7768±0.267) and (0.7531±0.142) respectively compared to control (2.1367±0.149), $p<0.05$. And in case of females they were (0.8722±0.230) and (0.9935±0.087) 15 days and 30 days respectively as compared to control group (1.9046±0.100), $p<0.05$. GSI values showed significant difference in both cases males and females. In males as compared to the control group (2.0698±1.566) significant difference was seen in the group treated for 30 days (0.1455±0.0605) while in case of females both the groups treated for 30 days (1.6774±0.113) and 45 days (0.7091±0.265) showed the significant difference when compared with control group (3.4354±0.722), $p< 0.05$.

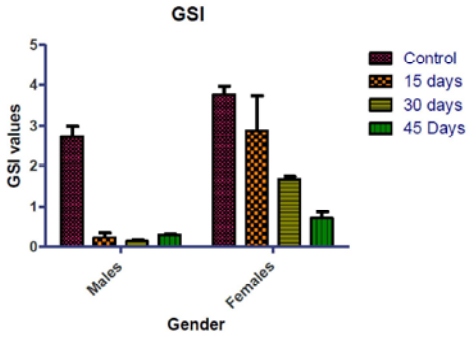

Fig. 1: Showing the time dependent changes in GSI values on both the sexes of *Oreochromis mossambicus*

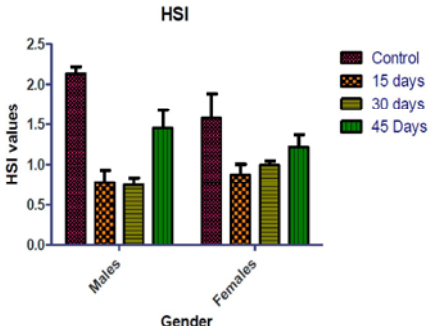

Fig. 2: Showing the time dependent changes in HSI values on both the sexes of *Oreochromis mossambicus*

The Two way ANOVA result confirms the same (Figs. 1, 2 and Table 3). The Interaction P value in case of GSI and HSI was found to be 0.0246 and in 0.1265 respectively. This suggests that the interaction was significant in case of GSI, but not in case of HSI.

Table 1: Effect of addition Plant nutrient on gonadosomatic index (GSI) and hepatosomatic index (HSI) of *Oreochromis mossambicus* males after treatments

		HSI			GSI		
Treatments	No. of fishes	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
Control	5	2.0133	2.3025	2.1367±0.149	0.2682	3.1065	2.0698±1.566
15 Days	5	0.5751	1.0803	0.7768±0.267***	0.1009	0.4576	0.2332±0.195
30 Days	5	0.6325	0.9107	0.7531±0.142***	0.0859	0.2069	0.1455±0.0605*
45 Days	5	0.9950	1.7173	1.4505±0.396*	0.2504	0.3404	0.29691±0.045

Table 2: Effect of addition Plant nutrient on gonadosomatic index (GSI) and hepatosomatic index (HSI) of *Oreochromis mossambicus* females after treatments

		HSI			GSI		
Treatments	No. of fishes	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
Control	5	0.9516	1.9726	1.9046±0.100	0.6987	4.1420	3.4354±0.722
15 Days	5	0.6582	1.1166	0.8722±0.230***	1.1708	3.7335	2.8780±1.478
30 Days	5	0.9158	1.0883	0.9935±0.087***	1.5841	1.8034	1.6774±0.113*
45 Days	5	1.0589	1.5283	1.2161±0.270**	0.4152	0.9324	0.7091±0.265**

Table 3: Two Way ANOVA analysis showing the P values and significance depending on time and gender

	Interaction		Column (Time)		Row (Gender)	
Factors	GSI	GSI	GSI	GSI	GSI	GSI
p-values	0.0246	0.1265	<0.0001	<0.0001	<0.0001	0.3156
Significance	*	ns	****	****	****	ns

Table 4: Bonferroni multiple comparison tests showing the P values and significance of GSI depending on time and gender

	15 days		30 days		45 days	
GSI	Males	Females	Males	Females	Males	Females
P-values	<0.001	>0.05	<0.001	=0.01	=0.001	<0.0001
Significance	***	ns	***	**	***	****

Table 5: Bonferroni multiple comparison tests showing the P values and significance of HSI depending on time and gender

	15 days		30 days		45 days	
GSI	Males	Females	Males	Females	Males	Females
P-values	>0.05	<0.001	>0.05	<0.001	>0.05	>0.05
Significance	ns	***	ns	***	ns	ns

Thus HSI of both male and female fishes was affected in the similar way on the contrary in case of GSI the effect of the treatment was not consistent in both cases. The Column Factor (Time) P value in both cases GSI (Fig. 1) and HSI (Fig. 2) were found to be <0.0001 which clearly states that the control and treated means are significantly different. This rejects the null hypothesis that the treatment has no effect on the fishes. The P value for Row factor (gender) in HSI was not significant (0.3516) while in case of GSI it was found to be significant (<0.0001). This shows that females were seen to be more sensitive than males when it comes to GSI Index. The reduction of GSI of both sexes of blue tilapia subjected to phenol may possibly reflect the reduction of the gonad mass. Similar findings were reported by Barse *et al*. [23] and by Mir *et al.* [24] when subjected to 4-tert-butylphenol and Dichorvos respectively on carp (*Cyprinus carpio*).

Bonferroni multiple comparison tests (Tables 4 and 5) were done to find the exposure dependent effect of the treatment on the fish. It states that at the 15th day there was a significant change in the GSI values of males ($p<0.001$) but there was no significant change for the females ($p>0.05$). At the 30th day there was a significant change in the values in both sexes males ($p<0.001$) and females ($p=0.01$) but females were affected more. On the contrary at the 45th day females showed significant effect ($p<0.0001$) than males ($p=0.001$) though both were affected. Thus we can say that males were seen to be affected earlier but they tried to recover back as time passed but females though they got affected late they could not recover back at the 45th day.

The biological parameters are sometimes indicative of toxicant effects [25]. In the present study, the expression of GSI was time dependent and gender specific. In females it was more prominent compared to males and was evident by the histological alterations. Our results are in agreement with Hanson *et al*. [26] who have also documented that female GSI decreased significantly than males as compared to control in 3 fishes *O. niloticus*, *C. gariepinus* and *C. nigrodigitatus*. The histological alteration makes the picture clearer. (Fig. 3) Normal histology of the ovary of *O. mossambicus* reveals that it is surrounded by an ovarian wall that is differentiated into an outer thin peritoneum, a thicker tunica albuginea made up of connective tissues, muscle fibers and blood capillaries. The innermost layer is the germinal epithelium which joins with the tunica albuginea at several places and projects into the central lumen, the ovocoel, in the form of finger like projections called ovigerous lamella. Fish exposed to the plant nutrient showed progressive thinning and degeneration of ovarian wall which is apparent at the 30th and 45th day. Oocytic stages were not intact. Degeneration of germinal epithelial cells of oocytes caused vacuolation. At the 30th and 45th day more vacuolated follicular epithelium and degerative cytoplasm were seen. The cytoplasm showed vacuolization at the periphery of oocyte which gradually extended towards the centre. Thus the histopathological alterations were seen to get adversed with time.

Analogous histopathological findings were reported by Hossain *et al*. [27] in the ovaries of *Anabas testudineus* and *C. punctatus* due to the exposure of pesticide, dimecron 100SCW, Giri *et al*. [28] by insecticide

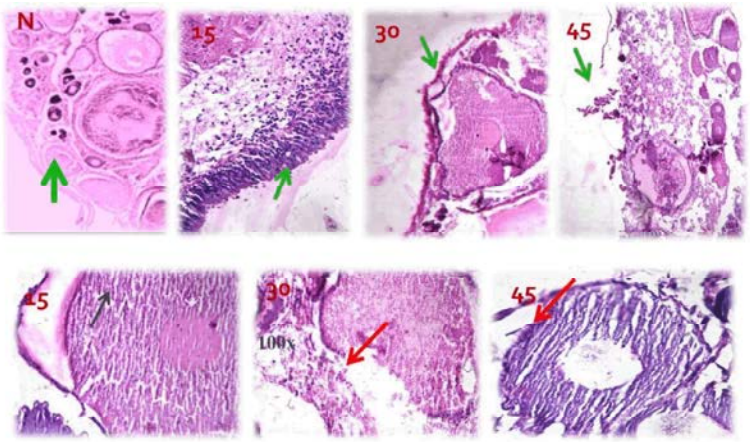

Fig. 3: Histological alterations in Ovary
N: Normal, 15: Treatment for 15 days, 30: Treatment for 30 days, 45: Treatment for 45 days; Thinning and degeneration of Ovarian Wall (↓) at the 30th and 45th day. Oocytic stages are not intact, Degeneration of germinal epithelial cells of oocytes (↓)

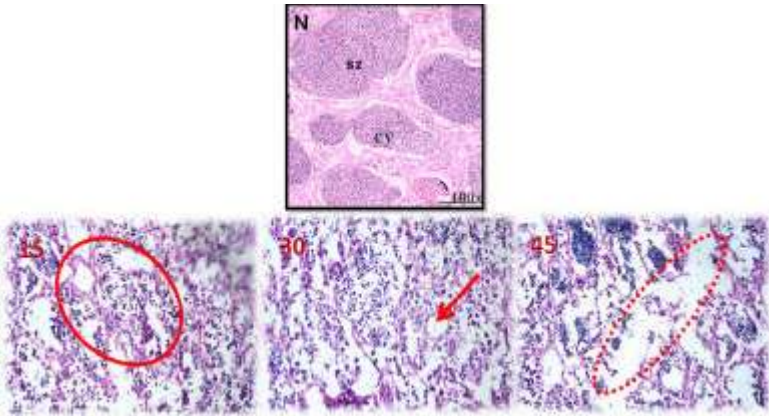

Fig. 4: Histopathological alterations in Testis
N: Normal, 15: Treatment for 15 days, 30: Treatment for 30 days, 45: Treatment for 45 days; Cy-undisturbed cyst; sz-spermatozoa; Disorganization (○) and degeneration (⁘) of tubular cysts associated with Intertubular vacuolization (↓).

basathrin induced on catfish, *H. fossilis*. They reported marked damage in germinal epithelium, atresia of ocyte, stromal hemorrhage, vacuolization of oocytes and general inflammation. Hilderbrand *et al*. [29], Sankar and Mandal [30] have reported similar observations in lead treated rats.

Exposure dependent histological alterations in the testis were seen. Progressively there was an increase in the vacuolization, disorganization and distortion of seminiferous tubules (Fig. 4). At the 45th day of exposure condensation of spermatocytes besides inflammation and inter-tubular vacuolation was very much prominent. According to some investigators [31-34] testicular inflammation has been documented as one of the common responses on the aquatic animals exposed to environmental toxicants. Testis in fish represents the most dynamic organ having a high cell turnover during the reproductive period which makes it vulnerable to a wide variety of chemical toxicants. Scientists such as Katti and Sathyanesan [35], observed exposure time dependent and concentration-mediated changes in testis of *Clarias batrachus* treated with lead [36] in *Xiphophorus maculates* [37] in *Glossogobius giuris* [38] in *Glossogobius giuris* and [39] in *H. fossilis* have reported various cytotoxic effects on testis due to the exposure of the different toxicants. These changes may culminate in a partial or total arrest of spermatogenesis. Thus the present study suggests that the extent of damage in the fish gonads depends on the time of exposure of the toxicant.

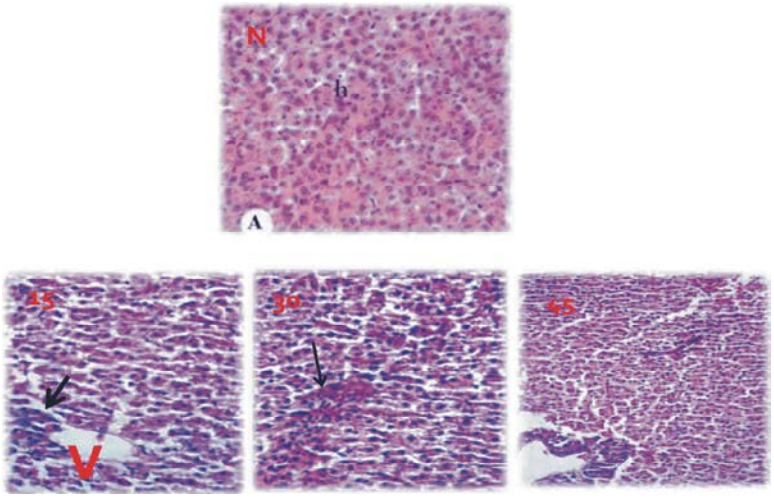

Fig. 5: Histopathological alterations in Liver
N: Normal, 15: Treatment for 15 days, 30: Treatment for 30 days, 45: Treatment for 45 days; H: hepatocytes; Cell outline of hepatocytes became indistinguishable. Dilation and hemorrhage of blood vessels in hepatic sinusoids (⬇) resulting into its blockage. Mild vacualation (V).

Liver is the metabolic organ. It is a target for the metabolism in the fish body, the liver index (HSI) is a useful biomarker to detect the hazardous effects of the environmental stressors [40]. In case of HSI (Fig. 3) the females were not affected in all three cases 15th, 30th as well as 45th day as the P values was found to be >0.05, but in case of males significant effect was seen at the 15th as well as 30th day ($p<0.001$) (Table 3). Akerman *et al*. [41] also found in the decrease in HSI values after 9 weeks in rainbow trout, *Oncorhynchus mykiss* injected with paraquat. On the contrary Figueiredo-Fernandes *et al*. [42] found an increase of HSI in male and female tilapia, *O. niloticus*, exposed to paraquat. It is generally reported that the histopatholoical biomarkers are useful as indicators of the general health of the fish and are considered as a mirror that reflects of the exposure to a variety of anthropogenic pollutants [43]. Liver histology of control and exposed fish is briefly illustrated in Fig. 5. In the control group, the liver exhibited a normal architecture and there were no pathological abnormalities, with hepatocytes presenting a homogenous cytoplasm and a large central or subcentral spherical nucleus, whereas in the tissues of exposed fish cell outline of hepatocytes was indistinguishable and it is evident that this effect increased with the increase in the exposure time. The effect on the cell outline resulted in the mild vacuolation in the cytoplasm which were much more prominent at the 15th day. Hemorrhage of blood vessels in hepatic sinusoids was seen which eventually resulted to its blockage and affecting the metabolic activity. Researchers [44-46] in their work have also identified the same liver damage due to the toxicity of heavy metals.

The toxicant enters the liver through blood circulation, where it gets transformed and excreted through bile secretion or gets transferred to the kidney for filtration and transformation. So liver happens to the most affected tissue as it tries to detoxify and in doing so it gets affected the most.

The study suggests that in situ long-term exposure could be responsible for integrated biological effects related to essential physiological functions, like metabolism and development or reproduction. The liver of *O. aureus* juveniles subjected to phenol showed marked histopathological signs which were found to be directly proportional with the concentration of phenol [48]. The high incidence of histopathological score reflects the degenerative effect of phenol on fish liver. This degenerative effect was reported for carp (*Cyprinus carpio*) subjected to 4-tert-butylphenol. Because the liver is the major detoxification organ [48], altered liver morphology and enzymatic activity in fish is common following exposure to toxicants.

From the present work one can conclude that the plant nutrients are capable of causing the metabolic and reproductive damage in fish. This was apparent in sequential changes that were observed in histomorphological structures of liver as well gonads suggesting a reduction in the reproductive fecundity of

the test organism. However the effects whether reversible or irreversible can be deduced only after the conduction of the recovery studies.

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