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RESEARCH ARTICLE

MITIGATION EFFECT OF PROBIOTIC BACTERIA *LACTOBACILLUS RHAMNOSUS* ON THE GILL TISSUE OF FLUORIDE INTOXICATED FISH *MYSTUS MONTANUS*

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ABSTRACT

The toxic effect of NaF (1/10 of LC_{50} & 1/5 of LC_{50}) was investigated after calculating the LC_{50} value, on gill tissues of the fish *Mystus montanus* after 90 days. Possible ameliorative effect by Probiotic bacterium *Lactobacillus rhamnosus* was investigated after 60 days. Fluoride induced hyperplasia, desquamation of the epithelial cells, complete fusion of secondary gill lamellae and congestion of blood sinuses were the significant histopathological lesions observed in the gill tissues of *Mystus montanus.* Fish fed with Probiotic bacterium *L. rhamnosus* ameliorated the lesions of gill tissue. The recovery was more pronounced in the fish group fed diet supplemented with Probiotic bacterium *L. rhamnosus.* However, slow recovery was observed in depuration group of fish.

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INTRODUCTION

Fluoride, which is included in the list of toxicants, is reported to have adverse effects in animals as well as human beings (Gutowska *et al*., 2011). Fluoride is a persistent bioaccumulator that accumulates in visceral organs of aquatic animals including fish being continuously exposed to the contaminated medium (Bhatnagar *et al*., 2007). It is imperative that histological biomarkers are the indicators of pollutants in the overall health of the entire populations in the ecosystem (Velkova-Jordanoski and Kostoski, 2005). Several metabolic activities are disturbed due to alteration in regulatory enzymes and biomolecules (Kumar *et al.,* 2007), after exposure to fluoride. Prevention of an infection using the probiotic strain to intentionally colonize the site in susceptible host tissues that are normally colonized by a pathogen is called probiotic therapy. The use of probiotics is now prevalent in the aquaculture industry as a means of controlling disease, improving water quality and enhancing the immune system of cultured species (Chae-Woo Ma *et al.,* 2009). The paramount aim of the present study was to analyze the toxic effect of fluoride on the gill tissue of the fish *Mystus montanus* and amelioration of toxic effects by using Probiotic bacterium *L. rhamnosus.*

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MATERIALS AND METHODS

Experimental design for fluoride toxicity analysis

Well-acclimatized, healthy fishes of *M. montanus* with 6.9±0.507cm in length and weighing 3.94±0.292 gram were reared in 35 liter capacity plastic tubs. LC50 value of fluoride was calculated by adopting standard protocol given by Finney 1971. The LC50 value of F of fish *M. montanus* for 96 hour is 178.88 mg/l. The experimental design for the present experiment is shown in Table-1. Feed ingredients and its proximate composition is listed in Table-2. After 60 days, ten numbers of fish from the control group, group I and group II fishes were sacrificed for observations. Gill tissue was dissected out and histopathological analysis was done. The remaining ten fish in the control group and four experimental groups were maintained for further study for 60 days. One group of 17.8 mg F/L and 35.6 mg F/L exposed fish were maintained as depuration group and another one group of 17.8 mg F/L and 35.6 mg F/L exposed fish were maintained as probiotic treatment group. Depuration group were supplied with basic diet and probiotic group were fed by diet supplemented with 10^6cfu/g of *L. rhamnosus*. After 60 days, gill tissue was analyzed to find out the effect of depuration and Probiotic treatment. The tissue was rinsed in physiological saline (0.85% NaCl) and quickly fixed in Bouin's solution. Paraffin sections, 5-µm thick, cut on Rotary microtome at room temperature, were processed by double staining using alcoholic Eosin and haematoxylin. Sections were dehydrated in graded alcohol series and mounted in DPX.

Table 1. Experimental Design

Fish group	no of fish	treatment	duration	feeding %
Control	20	normal water & prepared diet	150 days	5% body mass
Group I	10	water with 17.8mg F/l & prepared diet	90 days	5% body mass
Group II	10	water with 35.6 mg F/l & prepared diet	90 days	5% body mass
Group III	10	Depuration from 1/10th LC_{50} dose of F & prepared diet	90 days + 60 days	5% body mass
Group IV	10	Withdrawal from 1/10th LC_{50} dose of F & probiotic diet	90 days + 60 days	5% body mass
Group V	10	Depuration from 1/5th LC_{50} dose of F & Prepared Feed	90 days +60 days	5% body mass
Group VI	10	Withdrawal from 1/5th LC_{50} dose of F & Probiotic Feed	90 days +60 days	5% body mass

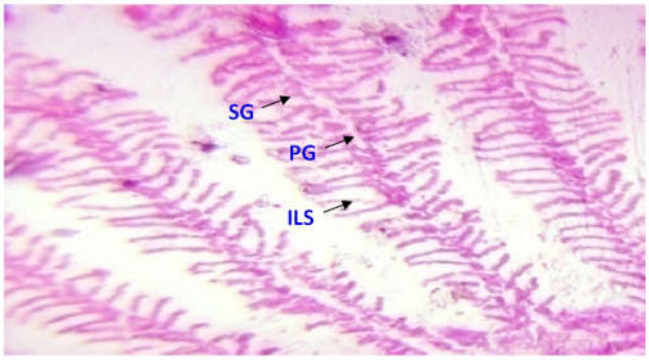
Table 2. Feed ingredients and its proximate composition

S. No	Ingredients	Purpose of inclusion	Inclusion level (%)	Protein (%)	Lipid (%)	Carbo hydrate (%)
1	Fish meal	animal protein	16	62	5.8	3.7
2	Groundnut oil cake	plant protein	16	45.6	40.9	8.7
3	Sesame oil cake	plant protein	16	54.3	24.8	8.2
4	Soya flour	plant protein	16	71.6	10.7	9.8
5	Rice bran	carbohydrate	18	13.5	1.8	75.5
6	Tapioca flour	binder	18	5.8	12.5	76.3
7	Vitamins & minerals mix	vitamins & minerals	0.5	-	-	-
8	PrePro KID	*Lactobacilus rhamnosus*	10^6cfu/g	-	-	-

RESULTS

Gills of control fish

Plate-1shows normal histological structures of primary gill lamellae with haemal system and secondary gill lamellae and normal squamous epithelial cells and pillar cells.



PGL: Primary Gill Lamella SGL: Secondary Gill Lamella ILS: Inter Lamellar Space

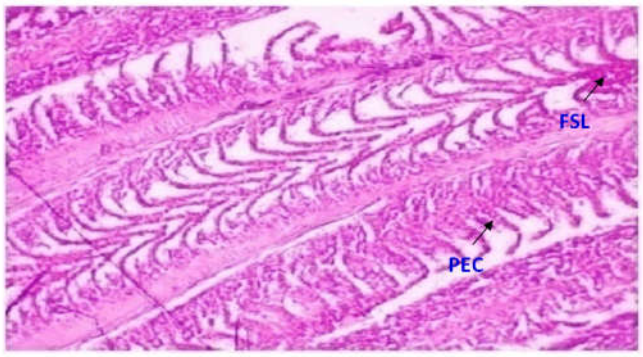
Plate 1. Histophotograph of Gill tissue of control fish (Haematoxylin Eosin, X 250) showing normal aspect of the gill with filament and distinct separate secondary gill lamellae. These are parallel and perpendicular to the filament

Gills of Group I fish

Group I fish showed bulging of tips of primary gill filaments. The secondary gill filament lost their original shape and curling of secondary gill filaments was also observed. There was no interlamellar space between the secondary gill lamellae. There is a tendency of fusion of disorganized secondary gill filaments (Plate-2).

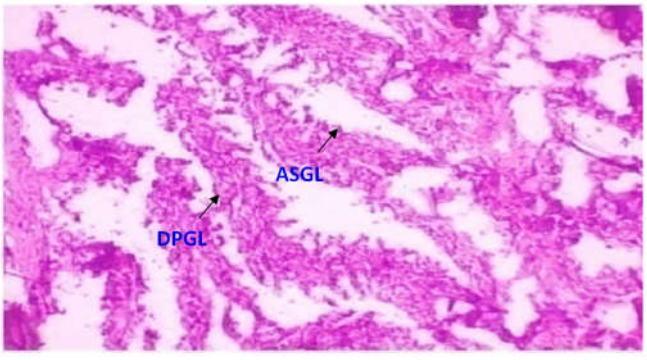
Gills of Group II fish

The gills of group II fish were shortened. Clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary gill lamellae were well marked. Besides these changes, vacuolization and disintegration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also identified (Plate 3).



FSL: Fused Secondary. Lamella PEC: Proliferated Epithelial Cells

Plate 2. Gill tissue of group I fish (Haematoxylin Eosin, X 250), showing marked distortion of the secondary gill lamellae, sloughing of the epithelium, fusion of the lamellae and strands of secreted mucus showing fusion between two secondary lamellae due to proliferation of epithelium. The spaces between two adjacent filaments were partially full of epithelial cells



DPGL: Disrupted Primary Gill Lamella ASGL: Atrophy of Secondary Gill Lamella

Plate 3. Histomorphology of Gill tissue of group II fish (HE, x250), shows blunting and congestion. Completely disrupted secondary gill structure and marked hyperplasia of the branchial arch were shown in the figure

Probiotic effect on gill of *M. montanus*

Group IV showed slightly bulged primary gill lamellae tip. Some of the secondary gill lamellae showed clubs at its end. Interlamellar space was clearly seen (Plate-4).

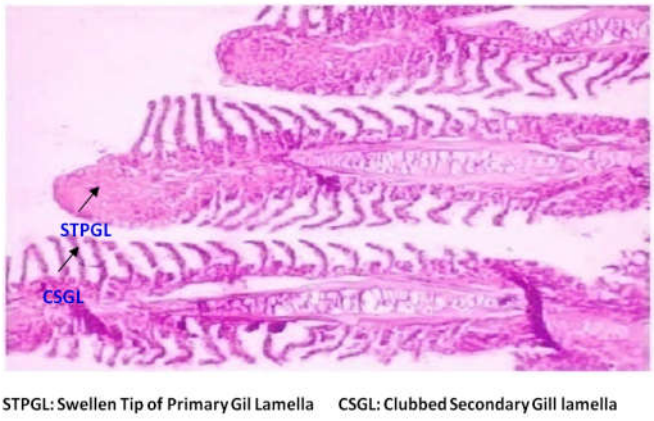


Plate 4. Histomorphology of Gill tissue of group IV fish (HE, x250), showing almost normal primary gill lamellae, except slight bulge at the tip and hyperplasia in some of the secondary gill lamellae

Group VI showed normal primary gill lamellae. Secondary gill lamellae showed clubbed tip with normal gill filaments. No inflammatory cells were noticed in gill filaments. The recovery of gill tissues were rapid than the depuration group of fish (Plate-5).

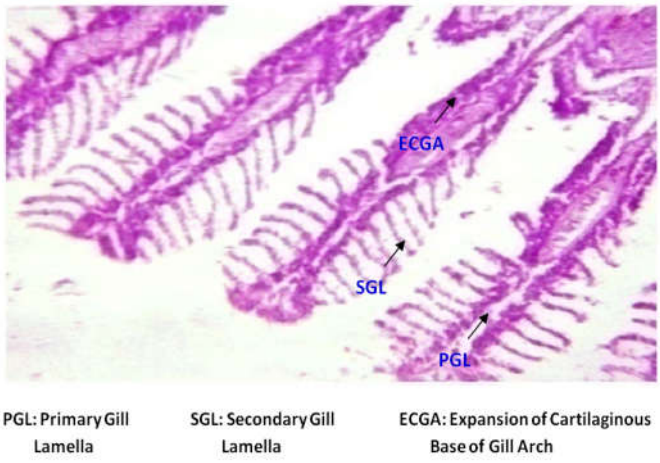


Plate 5. Histomorphology of Gill tissue of group VI fish (HE, x250), showing expansion of the cartilaginous base of gill arches and normal secondary gill lamellae

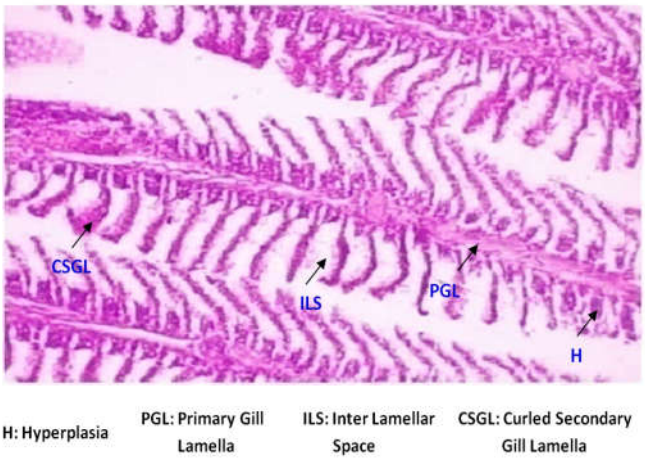


Plate 6. Histomorphology of Gill tissue of group III fish (HE, x250), showing almost normal primary gill lamellae, except slight bulge at the tip and some curled secondary gill lamellae

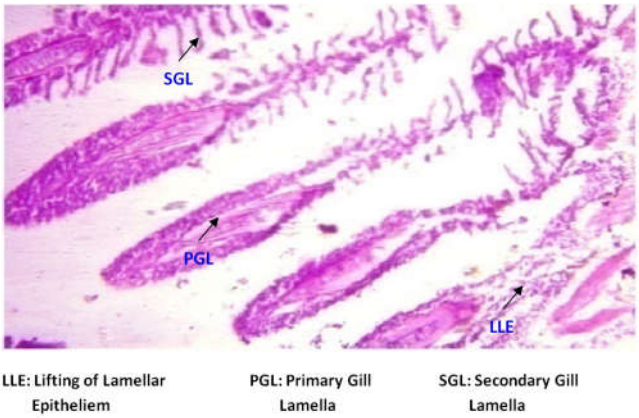


Plate 7. Histomorphology of Gill tissue of group V fish (HE, x250), showing hyperplasia of filaments which was expressed in the expansion of their end sections. Hyperplasia of secondary gill lamellae and expansion of the cartilaginous base of gill arches showing lifting of lamellar epithelium and edema

Depuration effect on gill tissue of *M.montanus*

In group III, fish primary gill lamellae had bulged tips and some of the secondary gill lamellae showed curled filaments and hyperplasia. Inter lamellar space was clearly visible. There was no fusion of secondary gill lamella (Plate-6). Gill tissues of group V showed primary gill lamellae with bulged tips and curled secondary gill lamellae, aggregation of inflammatory cells in gill filaments. Dilation and congestion in blood vessels of gill filaments were observed. However the gill lesions were less severe as compared to Group II fish exposed to $1/5^{th}$ LC50 dose of fluoride (Plate-7).

DISCUSSION

The present research work revealed that adverse effects of fluoride in gill was severe in the group II fish compared to group I fish ($1/10^{th}$ LC_{50} dose of fluoride). In group II fish, the distinctly observed histopathological changes were lamellar atrophy and distorted primary gill lamella. Research findings of Tripathy *et al.,* (2009), suggested that fluoride interferes with cellular activities in fishes, even at a genetic level, inducing chromosomal aberrations. Gills of *C.batrachus* exposed to 35mgF ion/L showed swelling in primary lamellar epithelium, shortening and fusion of secondary lamellae, hyperplasia and hypertrophy in chloride cells (Kumar *et al*., 2010). In this condition fish fail to get adequate oxygen for total metabolic activities, and they therefore visit the surface more frequently (Kumar *et al.,* 2010). From the study it could be implied that when compared to group I fish ($1/10^{th}$ LC_{50} concentration), group II fish ($1/5^{th}$ LC_{50} concentration) was found to be more damaged histopathology in the gill tissue. The study results revealed that recovery of adverse effects of fluoride was very slow in the depuration group of fish compared to the fish fed with Probiotic bacteria. However, all the altered pathological changes were almost recovered near to the control fish gill in the group IV fish and group VI fish indicated that they required further probiotic treatment to get complete recovery. Depuration group of fish III and V showed slow reversal of fluoride toxic changes in the gill tissue. A limited number of studies advocate the management of gastrointestinal tract bacteria can reduce accumulation and increase elimination of some environmental contaminants and they bring about its effect through changes in enzymatic

reactions (Kimura *et al*., 2004 and Gratz *et al.,* 2007). Tian *et al*., (2012) and Zhai *et al*., (2013) reported that binding of heavy metals on the Lactic acid bacteria could be a promising solution for heavy metal removal from water, liquid food and from the body of the humans and the animals. Besides cadmium binding capacity of Lactic acid bacteria they are also known to have antioxidative properties, which maybe another important characteristic for cadmium toxicity protection **(**Watterlot *et al*., 2010). James *et al*., (2008) proved that dietary supplementation of spirulina reduced copper uptake in tissues and elimination of more copper through feces thereby improvement of growth, blood parameters and phospatases activities in carp (*Cirrhinus mrigala*). The above literature evidences support the role of Probiotic bacteria *L. rhamnosus* in ameliorating the toxicity of fluoride in the fish and alleviate the damages of gill tissues.

Conclusion

This research work concluded that toxic effect of fluoride could be slowly reversed by depuration and completely ameliorated by feed supplemented with probiotic bacteria *L. rhamnosus*. Hence the probiotic bacteria *L. rhamnosus* could be used as a therapeutic regimen to ameliorate fluoride toxicity in fish.

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