FLUORIDE-INDUCED HISTOPATHOLOGICAL CHANGES IN GILL, KIDNEY, AND INTESTINE OF FRESH WATER TELEOST, LABEO ROHITA

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SUMMARY: Thirty four fingerlings, 7 to 10 cm in length, of Labo rohita, a fresh water teleost, were randomly chosen and divided into two groups. To assess damage by fluoride (F) to their soft tissues, one group of 10 fingerlings served as the controls in chlorine-free water, and the other group of 24 fingerlings was exposed to 15 mg/L sodium fluoride (6.8 ppm F ion) in the same kind of water. Members (2 from the control and 6 from the treated) of both groups were sacrificed to assess F damage to the gill, kidney, and intestine after 30, 60, 90, and 120 days of exposure. In the exposed group, in increasing severity with time, the gill tissue developed clubbed lamellae, mucoid metaplasia, and lamellar hyperplasia. The kidney showed renal architecture damage in the form of increased capsular space, shrunken glomeruli, shrunken lumen of renal tubules, and vacuolated cytoplasm. The intestine exhibited flattening and fusion of villi and a cracked clay appearance. None of these changes was observed in the control group. We conclude that chronic exposure to 15 mg NaF/L in water caused soft-tissue damage to teleost fingerlings.

Keywords: Fingerling teleost fish; Gill histopathology; Intestine histopathology; Kidney histopathology; Soft tissue and fluoride; Teleost (Labo rohita).

INTRODUCTION

Sodium fluoride (NaF), which has many uses in commerce and industry, is sufficiently soluble in water to be readily taken up by plants and absorbed by animals. In India, about 62 million people are estimated to be afflicted with various stages of skeletal fluorosis from consuming fluoride-contaminated water.1 Accordingly, a great deal of research has been conducted on skeletal and non-skeletal fluorosis in humans2-4 and animals.5-7 Studies on fluoride (F) toxicity to fish, however, are relatively limited. Although uncontaminated bodies of fresh water generally have low levels of F, the concentration can increase significantly from fertilizer run-off, mining operations, and industrial emissions. In the present investigation histopathological effects of low levels of F on gill, kidney, and intestine of fingerlings of commercial fresh water teleost fish, Labo rohita, were studied after chronic exposure for up to 120 days to 15 mg NaF (= 6.8 mg F ion)/L.

MATERIALS AND METHODS

Healthy teleost fresh water fingerlings (7 to 10 cm in length) belonging to the genus Labo rohita were obtained from local sources in Udaipur, Rajasthan, India. They were kept in the laboratory in a glass aquarium for a period of 15 days prior to beginning the experiment so as to acclimate them to laboratory conditions.

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During this period, and also during the experiment, the fingerlings were fed *ad libitum* on gram flour (powdered Chick Pea: *Cicer arietinum*) and fish food available in the local market.

Thirty-four healthy fingerlings were randomly selected for the experiment and divided into two groups. The first group of 10 fingerlings served as the controls and was kept in chlorine-free water throughout the 120 days of the study. The second group of 24 fingerlings served as the experimental group and was exposed to 15 ppm NaF for the same period of 120 days. Water for both groups was changed at 15-day intervals to clean the aquaria. Care was taken to maintain uniform conditions (water temperature 27 ± 2°C) throughout the experiment.

Six fingerlings in the NaF group and two in the control group were sacrificed by decapitation after 30, 60, 90, and 120 days. (At the end of the experiment the two remaining fingerlings in the control group were set free.) The sacrificed fingerlings were dissected to collect the gill, kidney, and intestine tissues, which were rinsed in physiological saline (0.85% NaCl) and quickly fixed in Bouin’s solution. Paraffin sections, 6-µm thick, cut on Rotary microtome at room temperature, were processed by double staining using alcoholic Eosin and Delafield’s haematoxylin. Sections were dehydrated in graded alcohol series and mounted in DPX.

**RESULTS**

Results of the experiment are documented in Plates 1–3. Plate 1 records the photomicrographs of transverse section of gill tissue of the control fingerling group after 30 days (Figure 1) and the exposed group after chronic exposure to 15 ppm NaF after 30 days (Figure 2), 60 days (Figure 3), 90 days (Figure 4), and 120 days (Figure 5). In the F-exposed group, the distinct histopathological change is lamellar oedema (Figures 2–5). Significant swelling at the tip of secondary gill lamellae and clubbing of lamellae also occurred. Other pathological conditions include mucoid metaplasia and lamellar hyperplasia.

Plate 2 demonstrates the toxic effects on the kidney of fingerlings. In this plate Figure 1 is again that of the control group after 30 days, followed by Figures 2–5 for the F-exposed group after 30, 60, 90, and 120 days, respectively. In this case the kidney tissue shows a thick lining of the Bowman’s capsule, shrunken glomerulus, increased capsular space with swelling, and sloughing off of the epithelium of the capsule cells. The renal tubules exhibit shrunken lumen and vacuolated cytoplasm.

Finally, Plate 3 shows transverse sections of the intestine of fingerlings, with those of the control group after 30 days in Figure 1, and the F-exposed groups in Figures 2–5 as above. In the exposed group a degenerative effect is evident in the mucosal lining and villi of the intestine. The villi tend to become flattened, and there is sloughing off of the mucosal lining. Hypertrophy of epithelial cells, swelling or oedema of lamina propria, and fusion of villi due to excessive hypertrophies, ultimately leading to rupture of villi at tip, are also evident. Flattening of microvilli and a cracked clay appearance of the tissue are likewise apparent.
Because the histology of the controls remained essentially unchanged throughout the study, only their photomicrographs at the end of 30 days are recorded.

**PLATE 1.** Representative photomicrographs showing transverse sections passing through the gill lamellae of teleost, *Labeo rohita* (40x).

**Figure 1.** Control group after 30 days; **Figure 2.** 15 ppm NaF after 30 days; **Figure 3.** 15 ppm NaF after 60 days; **Figure 4.** 15 ppm NaF after 90 days; **Figure 5.** 15 ppm NaF after 120 days.

Abbreviations: CL Clubbed lamella; EC Epithelial cell; ENC Endothelial cell; ILS Interlamellar space; LF Lamellar fusion; MM Mucoid metaplasia; PC Pillar cell; PGL Primary gill lamella; RBC Red blood cells; SC Salt cell; SGL Secondary gill lamellae; STSL Swollen tip of secondary lamellae.
PLATE 2. Representative photomicrographs showing sections passing through the kidney of teleost, *Labeo rohita* (40x).

**Figure 1.** Control group after 30 days; **Figure 2.** 15 ppm NaF after 30 days; **Figure 3.** 15 ppm NaF after 60 days; **Figure 4.** 15 ppm NaF after 90 days; **Figure 5.** 15 ppm NaF after 120 days.

Abbreviations: BC Bowman's capsule; DCT Distal convoluted tubule; G Glomerulus; ICS Increased capsular space; L Lumen of Tubule; PCT-I Proximal convoluted tubule-I; PCT-II Proximal convoluted tubule-II; SG Shrunken Glomerulus; SL Shrunken lumen; VC Vacuolated Cytoplasm.
PLATE 3. Representative photomicrographs showing transverse sections passing through the intestine of teleost, *Labeo rohita* (20x).

**Figure 1.** Control group after 30 days; **Figure 2.** 15 ppm NaF after 30 days; **Figure 3.** 15 ppm NaF after 60 days; **Figure 4.** 15 ppm NaF after 90 days; **Figure 5.** 15 ppm NaF after 120 days

Abbreviations: CCA Cracked clay appearance; CE Columnar epithelium; CML Circular muscle layer; FLV Flattened villi; FV Fusion of villi; HEC Hypertrophied epithelial cell; LML Longitudinal muscle layer; LP Lamina propria; S Serosa; SLP Swelling in lamina propria; SM Submucosa.
DISCUSSION

F-induced pathological changes are now well established in skeletal as well as non-skeletal tissues. In the present study it was observed that F exposure caused lamellar telangiectasis (clubbed appearance) along with oedema and mucoid metaplasia. The clubbed appearance of lamellae is due to lamellar hyperplasia in which cells are derived from primary lamellae and migrate to the distal end. This results in accumulation of cells at the leading edge of secondary lamella, which is colloquially called ‘clubbing’ of lamellae.\(^8\) Also, the pillar cell architecture was probably altered by increase in chloride cell number and their subsequent bulging to the surface. Mucoid metaplasia is also very distinctly observed as the entire inter lamellar space seems to be filled up by such cells (Plate 1: Figure 5). Similar observations have also been reported due to exposure to pesticides\(^9\) and mercury.\(^10\) Massive lamellar hyperplasia has also led to fusion of many lamellar capillaries thereby causing secondary lamellar fusion (Plate 1: Figure 5).

The pathological changes observed in kidney corroborate results of earlier studies of F toxicity in sheep\(^11\) and mice kidney.\(^12\) Massive doses of F induce tubular necrosis, especially in the convoluted portion, and inflammation of glomerulus leading to impaired kidney functions like polyuria, polydipsia, and increased build-up of non-protein nitrogen.\(^13\) Studies have also indicated that NaF increases intracellular accumulation of calcium\(^14\) and inhibits tubular reabsorption—perhaps due to inhibition of the active chloride pump located in the medullary portion of the ascending limb of the Henle loop.\(^15\) In the present study the severely shrunk lumen of tubules are also suggestive of hindered tubular reabsorption. In addition, clinical findings indicate that F toxicity may lead to osteosclerosis and end-stage renal failure.\(^16\)

F-induced pathogenicity in intestine may be due to the fact that fluoride ion in the presence of HCl secreted in the stomach forms hydrofluoric acid, which has highly corrosive properties. This acid destroys mucous secreting cells of intestinal lining causing the observed abnormalities. Pathological gastrointestinal effects of F including damage to the mucosal lining, loss of microvilli, cracked clay appearance of duodenal mucosa and desquamated epithelial cells of gastric mucosa have also been observed by earlier workers.\(^17\)-\(^19\) Hence F causes irritation and destruction of the mucous membrane of the intestine, thereby hampering absorption.

In this study, therefore, 15 ppm NaF in water (= 6.8 ppm F ion) caused severe pathological changes in the gill, kidney, and intestine of *Labeo rohita*, thereby disturbing normal physiology.

REFERENCES