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Effects of Almix Herbicide on histopathological and ultrastructural alterations in freshwater fish, *Heteropneustes fossilis*: A Comparative Study

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ABSTRACT

Present study concerned with exposure of herbicide, almix in stomach and intestine of Heteropneustes fossilis at a dose of 8 g/acre and 66.67 mg/l for 30 days under field and laboratory conditions respectively on histopathological and ultrastructural levels. Stomach showed vacuolation in gastric glands, thinning of mucosal folds and disappearance of the brush border under laboratory condition, but secretion of mucus from damaged columnar epithelial cells (CEC) were prominent in field. In stomach, scanning electron microscopy (SEM) showed fragmentation of CEC and damage in microridges, while transmission electron microscopy (TEM) displayed deformed mitochondria, vacuolation and damage in tubular network under laboratory condition but in field condition alterations in shape of CEC and mucin droplets under SEM, and cellular vacuolation under TEM study were prominent. In intestine, disruption and vacuolation of connective tissue of lamina propria and severe damage in CEC were seen under laboratory condition but in field condition liver showed almost normal appearance. Under SEM observation intestinal epithelium showed shrinkage in mucosal folds, empty mucus pit and secondary cellular growth in laboratory condition but debris of fragmented secondary mucosal folds were prominent under field condition. On the other hand, TEM study showed dilated mitochondria and swelled endoplasmic reticulum under laboratory condition but intestinal epithelial cells remain unaltered under field condition. Therefore, present study revealed that the responses were more profound in laboratory condition than field condition. Finally, these responses could be considered as biomarker of herbicide toxicity in aquatic ecosystem for monitoring the state of the entire ecosystem.

Keywords: Almix, Histopathological, Ultrastructural, *Heteropneustes fossilis*

INTRODUCTION

Almix® 20 WP is one of the most widely used herbicide in agriculture particularly in rice paddy field to control broad leaf weeds and sedges both through contact and systematic pathway such as *Cyperus iria, Cyperus defformis, Frimbristylis* sp., *Eclipta alba, Ludwigia parviflora, Cyanotis axillaris, Monochoria vaginalis, Marsilea quadrifoliata etc.*, as well as in aquatic bodies. It is a fourth generation herbicide belonging to the sulfonylurea group of herbicide. It is a selective, pre-emergent and post-emergent herbicide and acts both through contact and systematic pathway. It is composed of 10.1% metsulfuron methyl ($C_{14}H_{15}N_5O_6S$) [methyl 2-(4-methoxy-6-methyl-1,

3, 5-triazin-2-yl-carbamoyl-sulfamoyl) benzoate], 10.1% chlorimuron ethyl ($C_{15}H_{15}ClN_4O_6S$) [ethyl 2-(4-chloro-6-methoxy-pyrimidin-2yl-carbamoyl-sulfamoyl) benzoate] and 79.80% adjuvants [1]. Almix did not show any volatilization property, therefore do not harm adjacent crops such as mustards, cotton, vegetables, fruit crops, castor *etc.*, unless they are sprayed onto them [1]. Due to wide spread use in the rice fields, almix ultimately reach to the nearby aquatic bodies which are in close proximity with agricultural fields as agricultural run-off or directly due to careless application in agricultural sectors. It is highly soluble in water and is stable at normal temperature, although bioaccumulation property of almix herbicide has not yet been reported by any other authors. Its high water solubility, extensive usage in the agricultural fields and continuous exposure to non-target organisms under aquatic environment might pose potential hazards to these aquatic organisms as well as health of the entire population.

In modern agricultural practices, the acknowledgment of new technology in crop production and protection has increased use of herbicides. But, indiscriminate use of herbicides, careless handling, accidental spillage, or discharge of untreated effluents into natural waterways can contaminate fish population and other animals or plants inducing a kind of effects including secondary effects sometimes due to indirect contamination [2,3]. The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding in recent times, and for aquatic systems, fish have become indicators for the evaluation of the effects of these noxious compounds. The use of fish as bio-indicators is being more and more used in last few decades since fishes are very sensitive to changes and play a significant role in assessing potential risk arising due to contamination in aquatic environment. In aquatic toxicological study, laboratory experiments are performed to estimate the potential hazard of chemicals and to establish ‘‘safe’’ levels of pollutants [4]. *Heteropneustes fossilis* as an ecotoxicological aliquot has several characteristics of choice, such as wide distribution in freshwater environment, ease availability throughout the year, easy acclimatization to laboratory conditions and commercial importance.

The toxicity of almix herbicide to *H. fossilis* has been reported recently [5–10]. Low concentrations of almix, such as those used in rice fields, might cause changes in metabolic and enzymatic parameters of catfish, *H. fossilis* [5–10] along with other two fish species namely *Anabas testudineus* and *Oreochromis niloticus* [5–10] such as reduction of protein level and glutathione-S-transferase (GST) in different tissues and enhancement of acetylcholinesterase (AChE), lipid peroxidation (LPO) and catalase (CAT) in different tissues of *A. testudineus* and *O. niloticus* [5–10]. The metabolic and physiological activities of the organism alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Therefore, to know the extent of severity of tissue damage it is very much essential to go the insight the histological analysis, although the severity of damage depends on the toxic potentiality of the particular toxic compound as they act as biological markers to assess the toxic condition [11–14]. The major advantages of using histopathological biomarkers in environmental monitoring are that it allows examination in the specific target organs including stomach and intestine, which are responsible for vital functions, such as digestion of food stuff, and absorption of these food materials in the fish [15,16]. A large number of studies were reported by several authors to understand the biochemical physiological and metabolic alterations caused by chronic exposure of different pesticides and/or herbicides on animals and fishes [17–20], but relatively a very few studies regarding the histopathology and ultrastructural effects of almix herbicide on different fish tissues and other aquatic invertebrates are scanty and still need to be studied to establish the model to compare with mammalian tissues [21]. Nevertheless, field studies using histopathology and ultramicroscopic observations of fish as biomarker of aquatic contamination by almix herbicide have not yet been reported. Therefore, the present study was designed to investigate the histopathological and ultrastructural responses of almix herbicide in stomach and intestine of the air-breathing freshwater teleostean fish, *Heteropneustes fossilis* under both laboratory and field conditions.

MATERIALS AND METHODS

Chemicals

Commercial formulation of the almix herbicide (Almix® 20 WP, DuPont India Pvt. Ltd., Gurgaon, Haryana, India) was used in both the experiments. Delafield’s haematoxylin stain, eosin yellow, xylene, DPX, amyl acetate, acetone, glutaraldehyde solution, sodium hydroxide, tricaine methanesulphonate, uranyl acetate (EM grade), ethanol, disodium hydrogen phosphate, dihydrogen sodium phosphate, lead citrate (EM grade), epoxy resin (EM grade), paraformaldehyde (EM grade) and araldite CY212 (EM grade) of analytical grade were purchased from Merck Specialities Private Limited. Osmium tetraoxide was purchased from Spectrochem Pvt. Ltd., Mumbai, India.

Fish

Freshwater teleostean carnivorous fish *Heteropneustes fossilis* (Bloch) of both sexes with an average weight of 37.91±5.43 g and total length of 18.58±0.959 cm respectively were procured from local market and were acclimatized under congenial laboratory conditions for 15 days separately in aquaria of 250 L capacity. Fish were kept in continuously aerated water with a static system and experiment was conducted with a natural photoperiod (12-h light/12-h dark) and at an ambient water temperature. During the acclimatization period, the average value of

laboratory water parameters were measured: temperature, 18.61±0.808^{o}C; pH, 7.23±0.082; electrical conductivity, 413.67±0.90 µS/cm; total dissolved solids, 295.11±1.16 mg/l; dissolved oxygen, 6.46±0.215 mg/l; total alkalinity, 260.00±16.90 mg/l as $CaCO_3$; total hardness, 177.33±5.50 mg/l as $CaCO_3$; sodium, 19.20±0.36 mg/l; potassium, 2.45±0.22 mg/l; orthophosphate, 0.02±0.002 mg/l; ammoniacal-nitrogen, 2.31±0.43 mg/l and nitrate-nitrogen, 0.30±0.058 mg/l. After acclimatization, fish were divided into two groups: one set of fish was transferred to field ponds situated at Crop Research Farm premises of the University of Burdwan and other was transferred to laboratory aquarium. The fish were fed once a day with commercial fish pellets (32% crude protein, Tokyu) during both acclimation and exposure periods. Therefore, the study was carried out under two different experimental conditions: field pond and laboratory for duration of 30 days.

Field experimental design

Fish of field set were grouped as follows: three control groups, each comprises of ten (10) fish in three cages, and similarly three exposure groups with 10 fish species in separate cages for 30 days. The desired dose of 8 g/acre corresponds to concentration recommended for use in rice culture was dissolved in water and applied once. It was sprayed on first day of the experiment on the surface of each cage of almix-treated plots. During experimentation almix-treated and control groups were subjected to same environmental conditions. For field experiment a special type of cage was prepared and installed separately at pond of Burdwan University Crop Research Farm, the University of Burdwan. The cages were prepared for the culture of the experimental fish species as per Chattopadhyay *et al.* [22] with some modifications. All the cages were square in shape having an area of 2.5 m x 1.22 m and a total height of 1.83 m (submerged height was 0.83 m). The cages were framed by light strong bamboo. The four-sided wall, floor of the cage and top of the cage cover was fabricated with nylon net and was embraced by two PVC nets: the inner and outer bearing mesh sizes of 1.0 x 1.0 mm^2 and 3.0 x 3.0 mm^2 respectively. During the experimentation period (30 days) in the field, values of average limnological parameters were: temperature, 15.67±0.145^{o}C; pH, 7.89±0.033; electrical conductivity, 390.33±2.19 µS/cm; total dissolved solids, 276.33±1.45 mg/l; dissolved oxygen, 7.47±0.088 mg/l; total alkalinity, 101.33±0.67 mg/l as $CaCO_3$; total hardness, 152.00±2.31 mg/l as $CaCO_3$; sodium, 20.56±0.294 mg/l; potassium, 2.89±0.111 mg/l; orthophosphate, 0.12±0.007 mg/l; ammoniacal-nitrogen, 6.06±0.875 mg/l and nitrate-nitrogen, 0.58±0.016 mg/l.

Laboratory experimental design

Under laboratory condition fish were maintained into two groups of 40 L capacity of six aquaria 3 for control and 3 for almix-treated, containing 10 fish in each aquarium in the Ecotoxicology Lab, Department of Environmental Science, the University of Burdwan. The fish were exposed to sub-lethal dose of almix, *i.e.*, 66.67 mg/l for a period of 30 days [5–10]. Dose was applied for every alternate day. During experimentation almix-treated and control were subjected to same environmental conditions. During experimentation period, the average water parameters were recorded as follows: temperature, 19.67±0.293^{o}C; pH, 7.48±0.052; electrical conductivity, 478.33±9.70 µS/cm; total dissolved solids, 341.44±6.56 mg/l; dissolved oxygen, 5.82±0.394 mg/l; total alkalinity, 317.30±15.60 mg/l as $CaCO_3$; total hardness, 188.89±8.58 mg/l as $CaCO_3$; sodium, 21.36±0.76 mg/l; potassium, 2.80±0.29 mg/l; orthophosphate, 0.02±0.001 mg/l; ammoniacal-nitrogen, 6.63±1.15 mg/l and nitrate-nitrogen, 0.46±0.108 mg/l.

Sampling

During experimentation period the quality of the water was assessed as per APHA [23]. After completion of the experiment *i.e.*, 30 days the fish were collected from both the sets of aquarium and pond conditions of controls and almix-treated and were anesthetized with tricaine methanesulphonate (MS 222) and stomach and intestine were taken immediately after dissection and proceeded in specific ways for histological, scanning and transmission electron microscopic study.

Histopathological analysis

Fish tissues namely stomach and intestine from control and treatment were collected and fixed in aqueous Bouin's fluid solution, dehydrated through graded series of ethanol and finally embedded in paraffin. Paraffin sections were cut at 3-4 micron using Leica RM2125 microtome. These sections were then stained with haematoxylin-eosin (H&E) and observed under Leica DM2000 light microscope.

Ultrastructural analysis

For scanning electron microscopic study, tissues were fixed in 2.5% glutaraldehyde in phosphate buffer (0.2 M, pH 7.4) for 24 h at 4^{o}C and then post-fixed with 1% osmium tetraoxide in phosphate buffer (0.2 M, pH 7.4) for 2 h at 4^{o}C, dehydrated through graded acetone, subsequently followed by amyl acetate and subjected to critical point drying with liquid carbon dioxide. The tissues were then mounted on metal stubs and sputter-coated with gold with thickness of approximately 20 nm. The tissues were examined with a scanning electron microscope (Hitachi S-530) at University Science Instrumentation Centre of the University of Burdwan, Burdwan, West Bengal, India.

For transmission electron microscopic study, tissues were fixed in Karnovsky fixative (mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer) for 12 h at 4^{o}C and then post-fixed with 1% osmium tetraoxide in phosphate buffer (0.2 M, pH 7.4) for 2 h at 4^{o}C, dehydrated through graded acetone, infiltrated and embedded in epoxy resin, araldite CY212. Ultrathin sections (0.5 - 1 µm) were then cut by using a glass knife on an "Ultracut E Reichart – Jung" with the thickness of 70 nm, collected on naked copper-meshed grids, and contrasted with uranyl acetate and lead citrate. The tissues were examined under TECHNAI G2 high resolution transmission electron microscope at Electron Microscope Facility, Department of Anatomy, AIIMS, New Delhi, India.

Ethical statement

The experiment was carried out in accordance with the guidelines of the University of Burdwan and approved by the Ethical Committee of the University.

RESULTS

Stomach

The most evident pathological alterations in stomach of *Heteropneustes fossilis* due to almix intoxication under laboratory condition were thinning of mucosal folds, disappearance of the brush border, severe degenerative changes and vacuolation in gastric glands (Fig. 1.2) as compared to control one (Fig. 1.1), while secretion of mucus from damaged columnar epithelial cells were more prominent in the field condition (Fig. 1.3).

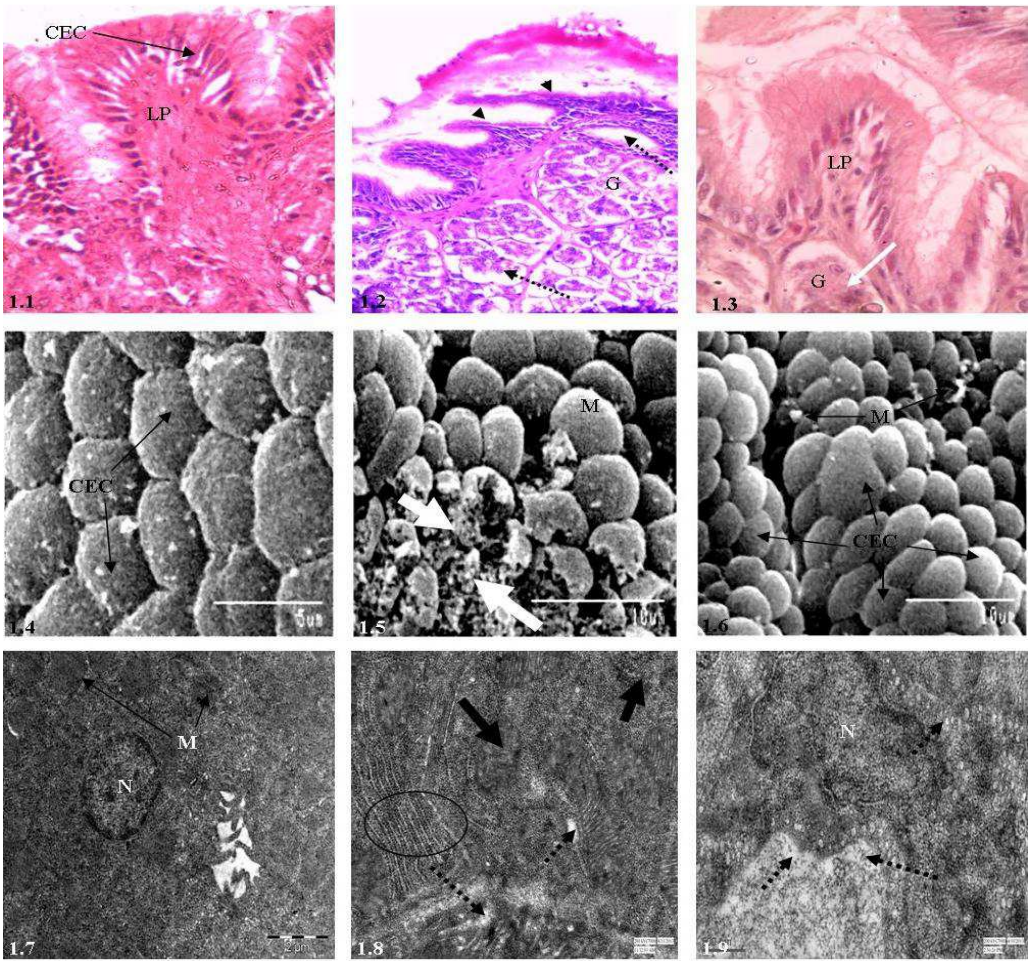

Fig. 1. Histopathological photomicrographs of stomach of *H. fossilis* under control condition (C), almix treated laboratory condition (AL), almix treated field condition (AF). 1.1 Showing regular arrangement of columnar epithelial cell (CEC), lamina propria (LP) and gastric gland (GG) with prominent nucleus under light microscopy (Cx1000). 1.2 Mucosal folds showing degeneration (white arrow), vacuolation (broken arrow) and thinning (arrow head) (ALx400). 1.3 Showing damage in gastric gland (white arrow) light microscopy (AFx1000). 1.4 Scanning electron microscopy showing normal arrangement of mucosal folds (MF) supported by oval or rounded CEC with stubby microvilli (MV) (Cx8000). 1.5 Showing fragmentation of MF in CEC (bold arrow) and severe mucus secretion (M) on CEC under SEM (ALx5000). 1.6 Showing almost normal structure of mucosal folds and CEC under SEM (AFx4000). 1.7 Normal appearance of gastric glands and nucleus (N) with prominent mitochondria (M) and rough endoplasmic reticulum (RER) (Cx2500). 1.8 Showing deformed mitochondria (bold arrow), vacuolation (broken arrow) and appearance of vast amount of endoplasmic reticulum (oval) under TEM study (ALx7000). 1.9 Showing vacuolation (broken arrow) under transmission electron microscopy (AFx7000)

Under scanning electron microscopic study stomach of test fish species showed severe degenerative changes of columnar epithelial cells such as fragmentation, profound mucus secretion over the epithelial surface and damage in microridges (Fig. 1.5), but under field condition only the shape of the epithelial cells was changed and mucin droplets were seen over the epithelial cells (Fig. 1.6).

On the other hand, transmission electron microscopy showed deformed mitochondria, appearance of vast amount of endoplasmic reticulum, vacuolation and damage in tubular network under laboratory condition (Fig. 1.8) compared to control condition (Fig. 1.7), but only severe vacuolation was noticed in stomach of *H. fossilis* and the damages were comparatively less in field condition (Fig. 1.9).

Intestine

The most conspicuous histopathological alterations after almix exposure in intestine of *H. fossilis* seen under light microscopy were disruption and vacuolation of connective tissue of lamina propria and columnar epithelial cells; severe damage in columnar epithelial cells and complete disappearance of brush border (Fig. 2.2), while under the field condition, cellular structure of stomach of *H. fossilis* showed almost normal appearance but secretion of mucus from epithelial region was noticed in some places (Fig. 2.3).

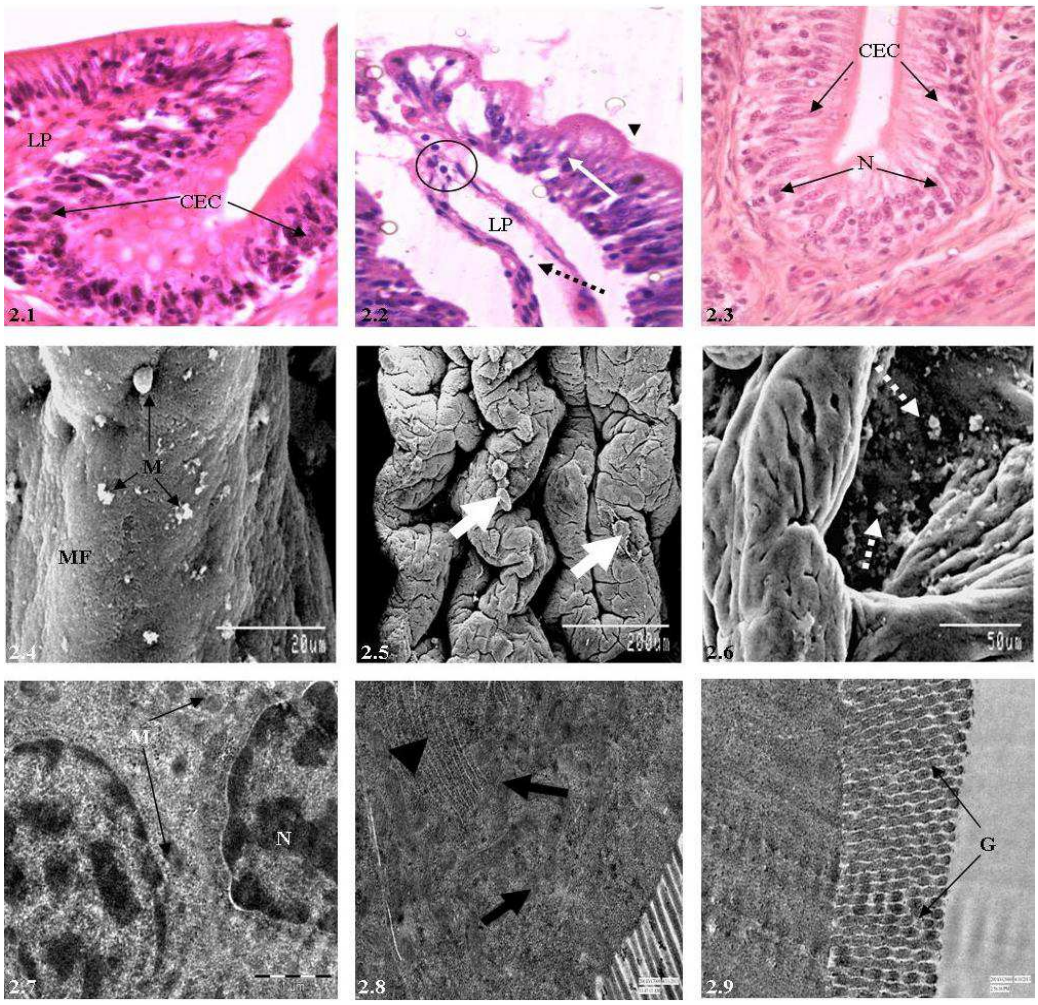

Fig. 2. Histopathological photomicrographs of intestine of *H. fossilis* under control condition (C), almix treated laboratory condition (AL), almix treated field condition (AF). 2.1 Showing normal appearance of lamina propria (LP), columnar epithelial cells (CEC) with prominent nucleus under light microscopy (Cx1000). 2.2 Showing disruption of lamina propria (oval), vacuolation in lamina propria (broken arrow), disappearance of brush border (arrow head) and damage in CEC (white arrow) under light microscopy (ALx1000). 2.3 Light microscopy showing almost normal structure of CEC with prominent nucleus (AFx1000). 2.4 Showing normal mucosal folds packed with oval or rounded CEC under SEM observation (Cx200). 2.5 Showing degenerative changes in CEC (bold arrow) (ALx200). 2.6 SEM observation showing mucus droplet in between MF (broken arrow) (AFx600). 2.7 Showing normal appearance of CEC with prominent nucleus (N) under transmission electron microscopy (Cx6300). 2.8 RER showing swelling (arrow head) and dilated mitochondria (bold arrow) under TEM (ALx7000). 2.9 Intestine showing normal appearance of epithelial cells and glycocalyx (G) under transmission electron microscopy (AFx5000)

Ultrastructural alterations as viewed under SEM study showed severe damage in mucosal folds with shrinkage, empty mucus pit and secondary cellular growth over epithelial cell surface after almix exposure (Fig. 2.5). In the field condition, mucosal folds and columnar epithelial cells were not seriously affected but debris of the fragmented secondary mucosal folds was noted in the concavities in between the primary mucosal folds in *H. fossilis* (Fig. 2.6).

On the other hand, transmission electron microscopic study of intestine showed appearance of large amount of dilated mitochondria and swelled endoplasmic reticulum but glycocalyx remain unalterated after almix intoxication (Fig. 2.8), but in the field condition, intestinal epithelial cells showed almost normal structure along with normal glycocalyx (Fig. 2.9).

DISCUSSION

Present study is an attempt to report the toxicity of the sulfonylurea-based commercial herbicide, almix with regard to histopathological and ultrastructural observations through scanning and transmission electron microscopic study in freshwater teleostean fish, *H. fossilis* both under field and laboratory study, although Senapati *et al.* [21,24] reported some histopathological alterations in oesophagus, buccopharynx, stomach and intestine of *A. testudineus* after almix exposure in the laboratory condition and Samanta *et al.* [5–10] in their study reported on responsive changes in several biochemical parameters such as acetylcholinesterase, lipid peroxidation, catalase, glutathione-S-transferase, alkaline phosphatise, alanine and aspartate aminotransferase in different fish species including *H. fossilis* under laboratory condition.

Fishes are considered as sentinel organism for ecotoxicological studies and are continuously exposed to wide variety of environmental contaminants and play a significant role in evaluating the potential risk in aquatic ecosystem [25]. Therefore, use of fish as indicator species against xenobiotic compounds is of great importance [26,27]. Simultaneously, cellular biomarkers revealed through histopathological and ultrastructural study of pollutant-induced organisms represent the intermediate levels of tissue organization between lower-level biochemical effects and higher-level population effects [28,29]. This ultimately provides a better evaluation of organism health than a single biochemical response [30] and is widely used as efficient biomarker of water quality, cellular state and mode of action of the xenobiotic contaminants under microscopic study as well as reflecting the overall health of the entire population in the ecosystem [31,32].

The present study showed that almix exposition caused pathological alterations in the alimentary canal particularly in stomach and intestine of *H. fossilis* both under the laboratory and field conditions and the toxicosis was more pronounced in case of laboratory condition. Stomach is considered as the first organ of the fish alimentary canal and more vulnerable to the changes in aquatic environment because maximum digestion of food-borne contaminants takes place in presence of HCl secreted by cardiac stomach. Detailed description of each histopathological signs observed through light and electron microscopy in stomach of *H. fossilis* in the present study helps to evaluate the degree of damage and the potential consequences for the fish. Severe degenerative changes and vacuolation in gastric glands, thinning of mucosal folds and disappearance of the brush border were the most evident alterations caused due to almix intoxication. Haque *et al.* [33] in their study also reported severe degenerative changes and vacuolation in gastric glands of *Channa puntatus* after sodium fluoride exposition. Similar results were also reported by several other authors in stomach of different fish species after exposure of different contaminants including glyphosate [24, 34–36]. Thinning of mucosal folds and disappearance of the brush border as observed in the present investigation were also similar with the findings of Ghosh [37]. Disappearance of brush border from the gastric epithelium impairs the anchoring of food materials as well as in the absorption of digestible food materials. Severe secretion of mucus was more prominent in the field condition which indicated the stress condition and occurrence of damage in the columnar epithelial cells when fish tried to compensate this stress by releasing mucus secretion. This may be due to formation of organochloride acids in stomach having corrosive property resulted from induction of secretion of HCl from cardiac stomach that triggers the production of this acid. So, digestibility of food materials and their absorption by the intestinal region impaired. Scanning electron microscopic study showed fragmentation of columnar epithelial cells, profound mucus secretion over the epithelial surface and damage in microridges under laboratory condition. These results can be correlated with the findings observed under light microscopy as strands of secreted mucus cell over the columnar epithelial cells. Damage in columnar epithelial cells as observed in the present study was also reported by Senapati *et al.* [21] and this may be due to herbicidal stress imposed on gastric epithelium which ultimately reduces the protection ability of gastric epithelium from xenobiotic exposure. Along with this, excess secretion of mucus indicates that almix exposure induces the activity of the aforesaid cells. Similar results were also reported by Ghosh [37] and Bose [38] in their study in different fish species after heavy metal exposure. Haque *et al.* [33] in their study also reported similar findings in stomach of *C. puntatus* exposed to sodium fluoride as observed under present study. In the field condition, the shape of the epithelial cells changed and the presence of mucin droplets over the epithelial cells were seen and this may be as protection as well as defensive

mechanism by the fish species against the herbicidal exposure. In the present study, at the ultrastructural level transmission electron micrograph showed deformed mitochondria, appearance of vast amount of endoplasmic reticulum, vacuolation and damage in tubular network under laboratory condition. The results of the present findings were also in agreement with the findings of Rebolledo and Vial [39]. On the other hand, Carrassón *et al.* [40] in their study reported abundant smooth endoplasmic reticulum, mitochondria, rough endoplasmic reticulum, tubule-vascular network and heterochromatinic nucleus in stomach of *Dentex dentex*. But present study depicted differences both in the laboratory and field conditions where the cytopathological responses were more pronounced in the laboratory condition than field and the responses disclosed by the stomach could lead to functional abnormality, like impairment in digestion process and absorption potentiality.

In intestine, light microscopic observation showed disruption and vacuolation in connective tissue of lamina propria, and columnar epithelial cells, severe damage in columnar epithelial cells and complete disappearance of brush border under laboratory condition but the histopathological responses were less in the field condition except marked secretion of mucus. These results also resembled the findings of Mandal and Kulshrestha [41] who observed similar lesions in the intestinal villi of *Clarias batracus* after sumithion exposure and with the findings of Cengiz and U¨nlu¨ [42] who reported necrosis and infiltration of lymphocytes in the intestine of *Gambusia affinis* exposed to deltamethrin. Sharma *et al.* [43] in their study also showed similar type of histopathological alterations in the intestine of *Cirrhinus mrigala* due to pesticidal exposure. Although, Ravanaiah and Narasimha Murthy [44] in their study reported on vacuolization, damage of villi and serosa layer, necrosed mucous epithelium, congested blood capillaries and hyperactivity of mucous cells in fish *Tilapia mossambica* after exposure of industrial pollutants. Damage of the mucosal layer and particularly the columnar epithelial cells in the intestine of *Rasbora daniconius* was also reported by Ghanbahadur and Ghanbahadur [36] due to endosulfan toxicity. Here, the alterations such as disruption of connective tissue of lamina propria, and columnar epithelial cells in the intestine of *H. fossilis* might deteriorate the secretion of different digestive enzyme activity into the lumen of the alimentary canal, which ultimately impair the food digestion. In the present study, damage in brush border on the luminal surface of the intestinal villi seen under light microscopy reduces the ability of absorption of various macromolecules from the intestinal lumen to tissue interior [37]. Damage in mucosal folds with shrinkage and secondary cellular growth over epithelial cell surface as observed under SEM study in present investigation was also reported by Haque *et al.* [33]. Empty mucus pit is another most important findings observed in the present study. Similar results were also reported by Ghosh [37] and Bose [38] in their study after metal exposure. Senapati *et al.* [21] in their study also observed damage in mucosal folds, severe mucus secretion and loss of microridge structures on the apical part of the columnar epithelial cells in intestine of *A. testudineus* after almix exposure. Debris of the fragmented secondary mucosal folds as observed in the concavities between the primary mucosal folds in *H. fossilis* under field condition was also reported by Haque *et al.* [33] in their study after sodium fluoride exposure in intestine of *C. punctatus*. Damage in mucosal folds and severe mucus secretion as observed in the present study might hamper the storage and digestive capacity of the concerned fish species and this may be due to the entering of the aforesaid herbicide into the alimentary tract of fish. At the ultrastructural level seen under transmission electron microscopic observation intestine showed appearance of large amount of dilated mitochondria and swelling of endoplasmic reticulum under laboratory condition which indicated impairment of different metabolic enzyme activity. In the field condition, intestinal epithelium showed almost normal appearance and this may be due to self-perpetuating capability of the natural environment imparts to the organisms.

CONCLUSION

In conclusion, the cytopathological responses observed in these tissue structures displayed stronger pathological alterations in laboratory condition than field experiment. Therefore, these histopathological and ultrastrucural alterations in stomach and intestine of *H. fossilis* caused significant disturbance and impairment in the organisations including the processes of digestion and metabolism, and absorption of food materials. This contamination is also capable of destroying the balance of aquatic ecosystem. Finally, the prominent histopathological and ultrastructural evidences could be considered as biomarkers of herbicidal pollution which have also been able to establish some indicators to monitor the entire health status of aquatic ecosystem.

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