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HISTOPATHOLOGICAL ANALYSIS OF ANABUS TESTUDINEUS (BLOCH)

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ABSTRACT:

The purpose of the present study was to investigate, under laboratory and field conditions, the histopathological changes in the stomach and intestine of Indian freshwater teleost, *Anabas testudineus* (Bloch) after Almix exposure. The field (8 g / acre dose) and laboratory (66.67 mg / l dose) experiments were conducted for a duration of 30 days. For the field experiment, a special form of cage was prepared and placed in the pond. Pathological modifications of the fish organs involved, namely the stomach and intestines via light microscopy, scanning and transmission electron microscopy,

they were evaluated.

KEYWORDS:

Cytopathological; Stomach; Intestine; Anabas testudineus; Almix.

INTRODUCTION:-

The use of herbicides for plants in agricultural fields is seen as central to contemporary agricultural practices worldwide in defending against attacks by pests and unwanted plants. However, the indiscriminate use of water and fish farms near farming fields might endanger aquatic environments as they eventually enter those aquatic bodies as a rush, causing harmful effects on the natural population residing in water, especially on non-target aquatic organisms, including aquatic insects, molluscs and fish.

Almix is one of Indian farmers' most common herbicides in the recent past. It consists of 10.1 percent methyl sulphonyl urea, 10.1 percent ethyl chlorimuron, and 79.80 percent of other adjuvants [1]. It is a sulphonyl urea class herbicide. The scheme of controls of the large leaf weeds is used, as are the *Cyperus arifolius* (Linnaeus, 1753), *Cyperus deformis* (Linnaeus, 1836), *Fimbristylis* (Presl, 1828) and *Marsilea quadrifoliata* (Linn), etc. in terrestrial and aquatic systems, for instance, *Ludwigia parviflora* (Roxb, 1820), *Cyanotis axillaris* (Don, 1826) and the *Monochoria vaginalis* (Presl, 1827). It is a selective herbicide, both previously emerging which post-emergent, and both contact and systemically kills unwanted plants. This has been used in the field at a low rate of usage, i.e. 8 g per acre and has no volatilizing

effect; therefore, the adjacent crops are not affected [1]. Sentinel organisms play an important role in the assessment of environmental quality and simultaneously provides a sensitive as well as reliable approach to evaluate the contamination level caused by xenobiotic substances in aquatic bodies [2]. Fish, among them considered as an excellent experimental aliquot for toxicity studies because they are the best understood organisms in the aquatic environment, held at the top of the trophic level and finally, they are directly exposed to these xenobiotic substances directly via surface run-off or indirectly through food chain [3,4]. - Formerly, fish has gained significant worldwide significance in recent decades and helps track the health status of the entire aquatic ecosystem for better

understanding the pollution-induced environmental conditions in the aquatic ecosystem [4,5]. In this analysis, the experimental toxicity model *Anabas testudineus* was selected as the test model. Some of these fish species are excellent experimental models, including widespread distribution in water, non-invasive properties, wide availability all year round, economic importance and ease of acclimation, etc.

Some studies have demonstrated an effectiveness, widely used tool to assess the health status, both in laboratory and field conditions, of organisms subject to a complex mixture of environmental pollutants like ultrastructural observations (scanning electron microscopy or transmission electron microscopy). One of the key benefits of the use of histopathological biomarkers in environmental quality controls is that only the exam in particular stomach and intestine can be tested in the same target organ toxicity. In the assessment of the overall health status of the entire population in the aquatic environment, histopathological biomarkers also play a key role. In addition, the changes detected in these target organs are simpler and more accurate than functional ones to precisely identify [9] and eventually act as an alert signal of animal health deterioration [10,11].

In recent decades, therefore, biomarkers have uncovered a new viewpoint for the evaluation of toxicological aquatic environments as the fish food canal is constantly exposed to xenobiotic material contaminated foods by primary organisms as digestion is carried out. A number of studies have been available in recent years regarding biochemical, physiological and metabolic changes in dienerent species in this herbicide [12-20]. The pathological modifications of the herbicide on various organs of Diabetes supplementing fish species by histological and ultrastructural observations are scarce [19,20], because significant advances have been made in science in recent years. The present study was intended to describe, compare the histological and ultrastructural alterations induced by the Almix, with special focus on the stomach and intestines of the *Anabas testudineus* in light of this scarce knowledge of this agrochemical type.

MATERIALS AND METHODS

Fish

Anabas testudineus (Bloch, 1792) were bought from the local fish farm and acquired for fifteen days, with an average weight of $23,58 \pm 2,05$ grammes and a total length of $11,15 \pm 0,548$ cm, respectively. Duree was held with a static system and at natural photoperiod 12 h light/12 h dark in continuously airy water (250 L capacity). During the acclimation time, the average value of water parameters was the following: CaCO₃ gross hardness, $177,33 \pm 5,50$ mg / l as CaCO₃; sodium, $19,20 \pm 0,36$ mg / l; potassium, $2,45 \pm 0,22$ mg / l; orthophosphate, $0,02 \pm 0,002$ mg / l; ammoniacal-nitrogen, $2,31 \pm 0,43$ mg / l and $0,43 \pm 0,43$ g / l; temperature $18,61 \pm 0,81$ mC; pH, $7,23 \pm 0,082$; electrical conductivity, $413,67 \pm 0,90$ mS / cm; total solids dissolved, 295. Fish were divided into two components for their acclimatisation, one group of fish was moved into crop research field ponds at University of Burdwan's CRSMF sites, and the remaining fish were transported to the laboratory aquarium. Fish were divided into two parts. During acclimation and exploration once a day fish were fed commercial pellets of fish (32% raw protein, Tokyu). The experiments were carried out in compliance with the animal experiments policy of Burdwan University and accepted by the University's Ethical Committee.

Experimental Set-Up

Again, the area was divided as follows: three cages and three cages that have been handled, each cage containing 10 fish. The prescribed dose (750 gsm) was dissolved in water for rice cultivation. On the first day of the experiment it was implemented [21,22]. During the exposure time the concentration of glyphosate was calculated using the Jan et al. [23] method and 1.20 mg / l was reported. A special cage kind has been made with some improvements and put in the middle of the pools on the basis of Chattopadhyay et al. [24] for fish rearing. The cages had a rectangular shape of 2.5 m to 1.22 m and the cage's height was 1.83 m. The cage was submerged at 0.83 m in height. Strong bamboo was formed into cages. Two nylon nets (PVC netting) were made from the four-sided wall, floor cage and cage cover: 1.0 to 1 mm² of internal mesh and 3.0 to 3.2 to 3,0 mm², respectively, external mesh sizes. During the field studies the following parameters for waters measured in APHA [26] were shown: temperature, $24,03 \pm$

0.203 ° C; pH, 6.56 ± 0.087; electric conductivity, 347 ± 1.15 µS / cm; total solid dissolvables, 247 ± 1.45 mg / l; dissolving oxygen, 7.00 ± 0.157 mg / l; total alkalinity, 221 ± 3.53 mg / l as CaCO₃; total hardness, 140 ± 2.31 mg / l as CaCO₃; Sodium, 63.4 ± 2.67 mg

Similar equipment, three control aquariums containing 10 fish and three for care, were maintained in the laboratory. The experiment was conducted on a sublethal dose of 17.20 mg / l [27,28]. A dose was administered on each alternative day. During the study era, measured water levels of glyphosate were 16.88 mg / l. Water parameters displayed during exposure time: Temperature: 26.6 ± 0.120 ° C, pH: 7.93 ± 0.075; Electric Conductance: 426 ± 5.93 µS / cm, Total dissolved solids (CAO): 303 Complete Alkalinity: 210 ± 10.5 mg / l, CaCO₃: Farce hardness; 163 ± 3,04 mg / l, DIS, dissolved oxygen, 5.06 ± 0.43 mg / l ± Complete alkalinity: l as CaCO₃.

Sample

Acclimatization and experimental water quality was tested in accordance with APHA[29]. At the end (thirty days) of the experiment fish were collected using a hand grid and anaesthetized with tricaine methane sulfonate (@ 100 mg / l) under both conditions. Fish were dissected after anesthetization and desired organs were immediately taken and fixed in the various fixative agents prescribed for the electron microscopic histology, scanning and transfer analysis, namely the stomach and intestine.

Histopathological analysis

Fish were anaesthetized with tricainemethanesulphonate (MS 222) at the end of the exposure time and, eventually, the stomach and intestines were dissected and fixed for further analysis in the respective fixatives. Tissues were fixed overnight in the aqueous Bouin 's fluid solution for histological analysis and dehydrated by graded ethanol series (70 percent, 90 percent and 100 percent) and ultimately embedded in paraffin. For paraffin sectioning at 3-4 µ, Leica microtome RM2125 was used. Parts were eventually stained with

Ultra structural analysis

In the case of the SEM sample, a 2,5% glutardaldehyde solution was fixed for overnight at 4 ° C, followed by a 1 % osmium tetroxide solution for 2 h post-fixing at 4 ° C. The tissues were then dehydrated by graded acetone, followed by amyl acetate, and tissue was eventually dried with a crucial dryer point with liquid carbon dioxide. Tissue was then mounted on stubs of metal and gold-coated sputter (20 nm thickness). Finally, the scanning of electron microscopes (Hitachi S-530) mounted at University Science Instrumentation Center Burdwan, Burdwan, West Bengal, India is examined for the purpose of gold-coated tissues. For the TEM analysis the Karnovsky fixative was fixed to the tissues at 4 ° C overnight, and the 1 % osmium tetroxide was fixed for a 2-hour post-fixation period at 4 ° C. After fixation, tissue has dehydrated, preceded by infiltration, by graded sequence of acetones and eventually by epoxy resin (araldite CY212). The ultrathine was then cut using a glass knife (thickness 70 nm) and parts were collected and dried on the naked copper grids. Finally parts of the TECHNAI g2 high resolution electron transmission microscope mounted at Electron Microscope Facilities, Department of Anatomy, AIIMS, New Delhi, India were stained with uranyl acetate and lead citrate.

RESULTS

Stomach

As normal, the stomach consists of four layers, i.e., mucosa, submucosa, muscular muscles and serosa. Single layer of compactly organised epithelial cell columnae (CEC) with centrally located nuclei is lined with –e gastric mucosa. The basal part of gastric mucosa is present at e tubular gastric glands. In the gastric gland, the central nucleus gastric cells such as the central lumen are present. Easy, tubular and rounded or elongated gastric glands are developed. The sub mucosa of the loosening of connective tissue is well vascularized with a dense layer (Figure 1.1). Degenerative changes to columnary Epithelial cells, fatty deposition in the basal zone, disappearance of brush line, thinning of the top plate

and damages to stomach glands and muco folds of stomach A were the most important changes observed in light microscopy in laboratory conditions. Although no such significant changes have been observed under field conditions (figure 1.2) (Figure 1.3).

In addition, SEM study reported damages observed under light microscopy such as significant degeneration in CCE, such as fractured CEC, extreme mucosal secretion over epithel surface, and damage in micro-crest structures (Fig. 1.4 and 1.5). The microscopic observed transmission electron revealed nucleus and mitochondrial deformations (figure 1.7), endoplasmic rough reticular damages, as well as vacuolations in stomach A. The field conditions were only deformed mitochondria and vacuolations (Figure 1.8), but damage was lower than laboratory conditions (Figure 1.9).

Intestine

Intestine also has four prominent histological layers as estomach, histologically speaking. Number of intestinal villi is slim and narrow. The intestinal mucosa consists of simple, long absorbing epithelial columnary cells, each of them centrally and fundamentally located. There are mucous cells distributed around the bowel mucosa. The loose connective tissue fibres of submucosa are projected into the lamina propria mucosal folds. Narrow, long vascular and mucous cells are scattered. The epithel cell of the columns is prominent and the nucleus is central and deeply stained (Figure 2.1). Strict damage in CEC, distortion of the lamina propria connective tissue, detachment of the lamina propria epithelial layer and extreme mucus secretion were the most visible changes in the intestine in laboratory condition (Figure 2.2). Under field conditions the intestine displayed almost normal appearances, but mucus secretions were conspicuous (Figure 2.3)

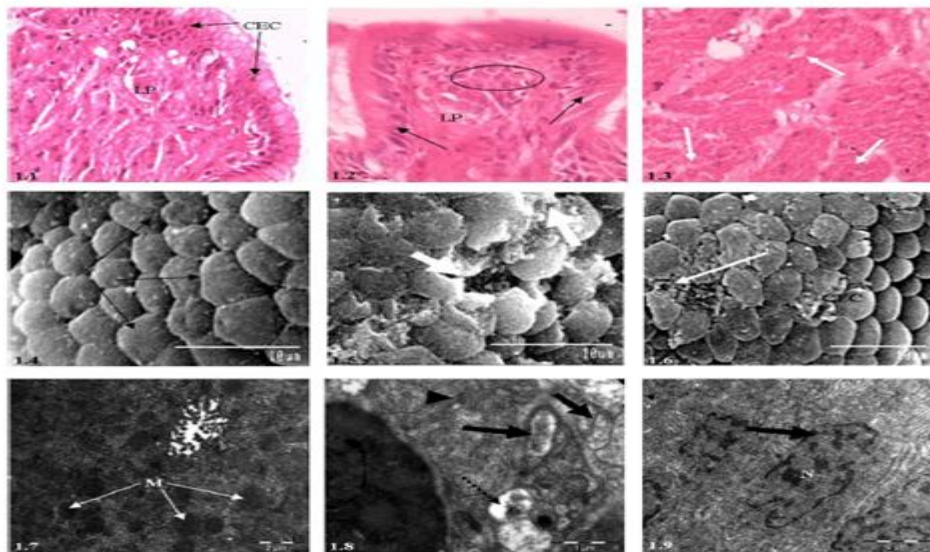


Figure 1: Stomach photomicrograms in A. Control (C), laboratory condition (AL) and field condition (AF) testudineus are seen.

Extensive mucus secretion in the epithelial surface and laboratory necrosis was shown by ultrastructural lesions (Figures 2.4-2.9). During the examination of the SEM, during mucosal folds and CEC, less damage was observed compared to laboratory condition but between the primary mucosal folds, fragmentary, secondary mucosal folds (Figure 1.6). The TEM research also showed fatty deposition and vacuolation, damage to the glycocalyxes, dilated mitochondria and damage to the tubular network as a result of laboratory conditions (Figure 1.8) and prominent field-specific mitochondrial deformation and vacuolations (Figure 1.9).

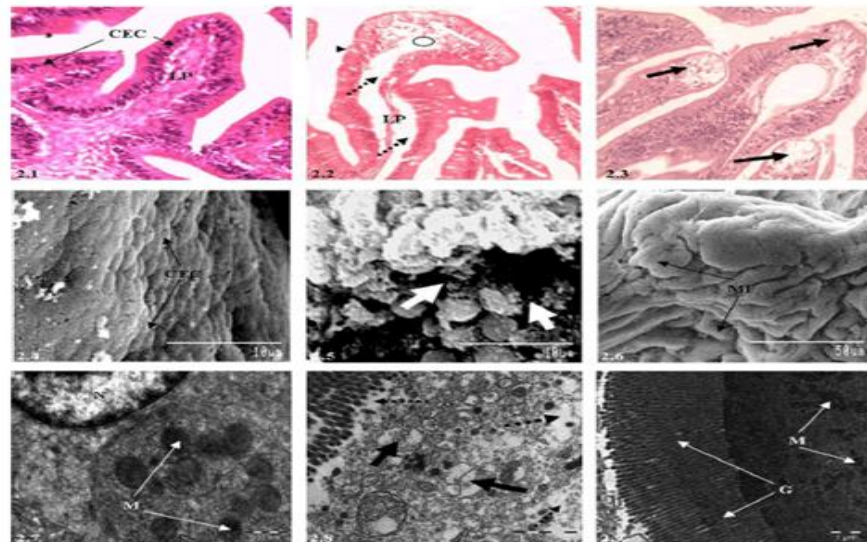


Figure 2: Photomicrographs of intestine in A. Control conditions (C), laboratory (AL), field (AF) conditions shown testudineus.

DISCUSSION

This research records for the first time the toxicity of the commercial agrochemical, Almix sulfonylurea by means of scanning and transmission microscopical electrons in *A* with regard to histological and ultrastructural observations. Comparative testudineuses in field and laboratory conditions, while Senapati has documented histopathological alterations in oesophagus, mouth pharynx, stomach and intestine *A* under laboratory conditions. Testudineus and Samanta in various types of fish, like *A*, regarding certain biochemical parameters. Testudineus but only laboratory [13-37]. Stomach is one of the key food canal organ of fish and is essential for the digestion of ingested food for fish species' development and growth. Histopathological light-microscopic studies have demonstrated substantial variations between two conditions in gill epithelium. In the present research, vacuolated basal zone, degenerative changes in columnar epithelial cells, brush border disappearance,

Intestine plays an essential role in the digestion and absorption of foods, and is a sensitive toxicity body to determine xenobiotic substances in fish species because they are exposed directly to a complex mixture in toxic substances through the ingestion of food contamination or indirectly through blood an Intestine is the next most essential component of a fish canal. A number of historical studies have been reported with respect to the histopathological effects of di-tenerent agents on the intestines of fish, but the histology and ultrastructure linked to almix exposure have been relatively poor [39-42]. [39-43]. In both *Cyprinus carpio* and *Cirrhinus mrigala* exposed to atrazine and fenvalerate, Walsh and Velmurugan have seen degeneration of the tip of villi, loss of structural integrity in mucosal folds, hypertrophy, vacuolation and necrosis [42,43]. Our results in the current research, such as damage to CEC, tissue distortion in the lamina propria, epithelial separation from the lamina propria and excessive mucus secretion can also be likened to these pathological changes. Also in C was recorded the same type of pathological changes found in this study. Mandal and Sharma exposure to batrachus and *C. mrigala* a daisy pesticide [44,45]. Vacuolations, villial and serosal layer injuries, necrosis, bloody capillar congestion, as well as extreme secretion of mucous tissue in *Tilapia mossambica* were observed in the case of Ravanaiah and Narasimha Murthy [46]. Ghosh also suffered damage to brush borders and blood vessels, suggesting a decrease in absorption from the intestinal lumen of various macromolecules [47]. The microscopic scanning of electron revealed a serious mucus secretion and laboratory necrosis. – These results are also in line with Senapati findings that documented damages in CEC and mucosal folds and degeneration in the *A*-intestinal microvilli structure. Laboratory testudineus following

exposure to Almix [19]. Ghosh in the intestines of *Notopterus notopterus* a dieteric arsenic exposure and Bose in *A* have also identified similar observations. Cadmium lead *testudineus* and sensitivity to cadmium. Extreme mucus secretion under the current study revealed the stress on fish and attempt to compensate for this stress. Under SEM analysis in field environment, debris of the broken secondary mucosal folds between the primary mucosal folds was seen. Transmission electron micrographic observation depicted severe vacuolations, damage in glycocalyx structure, dilated mitochondria and damage in the tubular network under both conditions, indicating fish were in stress and approached to protect the imposed stress. Comparatively less pathological responses in field condition might be due to dilution capability and self-regulating mechanism of the natural environment. Herefore, the alterations impaired the intestinal transportation process as well as absorption of food materials.

CONCLUSION

This study shows that exposure to Almix caused significant pathological changes in *A*'s stomach and intestines. Laboratory disease *testudineus*. In contrast to field research, pathological lesions showed stronger laboratory responses.

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