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## Histopathological study in stomach and intestine of *Anabas testudineus* (Bloch)

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### Introduction

The current examination was to explore the histopathological adjustments in the stomach and digestive tract of Indian freshwater teleost, *Anabas testudineus* (Bloch) both under lab and field conditions. Extraordinary sort of confine was arranged and introduced in the lake for the field explore. Obsessive adjustments in the concerned fish organs specifically stomach and digestive tract were surveyed through light microscopy, examining and transmission electron microscopy. Cytopathological modifications saw under light and electron microscopy uncovered that the level of reactions were distinctive in various fish tissues just as under conditions, here specifically impacts in stomach were more noticeable in lab condition. The general reactions enlisted in the fish tissues under lab condition were more articulated than field condition.

In the farming fields, the utilization of herbicides to shield the yields from the assault of nuisances and undesirable plants has been considered as an indispensable aspect of the cutting edge rural practices around the world. Be that as it may, its aimless utilization may imperil the amphibian biological systems and fish cultivates near the farming fields, as they at last reach to these sea-going bodies as spillover and made destructive impacts the characteristic occupant live in water particularly non-target oceanic creatures, for example, sea-going creepy crawlies, molluscs and fish. It is utilized for controlling the wide leaf weeds and sedges, for example, *Cyperus iria* (Linnaeus, 1753), *Cyperus deiformis* (Linnaeus, 1836), *Frimbristylis* sp., *Eclipta alba* (Linn), *Ludwigia parviflora* (Roxb, 1820), *Cyanotis axillaris* (Don, 1826), *Monochoria vaginalis* (Presl, 1827), *Marsilea quadrifoliata* (Linn), and so on., both in the earthbound and oceanic framework. It is a particular, both pre-developing and post-rising herbicide and annihilate the undesirable plants both through contact and precise pathway. It was applied in the field at a low use rate i.e., 8 g for each section of land and didn't show any volatilization property; hence don't influence the neighboring yields. Sentinel creatures assume a significant function in the appraisal of natural quality and all the while gives a delicate just as dependable way to deal with assess the defilement level brought about by xenobiotic substances in oceanic bodies. Fish, among them considered as a phenomenal exploratory aliquot for poisonousness examines in light of the fact that they are the best perceived creatures in the sea-going condition, held at the head of the trophic level lastly, they are legitimately presented to these xenobiotic substances straightforwardly by means of surface run-off or by implication through evolved way of life. Herefore, the utilization of fish for better understanding the contamination incited ecological conditions in the amphibian condition have increased significantly more significance worldwide in most recent couple of decades and assists with observing the wellbeing status of the whole oceanic condition [1-5]. In the current investigation, *Anabas testudineus* (*Anabantidae*) was chosen as exploratory model for poisonousness study. A portion of the qualities of this fish species make them as incredible trial model, for example, wide dispersion in sea-going condition, non-intrusive property, wide accessibility consistently, financial significance and simplicity acclimatization etc.

Various examinations exhibited the histopathological adjustments including ultra-basic perceptions (checking electron microscopy and transmission electron microscopy) which is considered as an efficient and widely utilized techniques to assess the wellbeing status of the living beings presented to an unpredictable blend of ecological contaminants both in the lab and field conditions [6-8]. One of the most significant favorable position of utilizing histopathological biomarkers in checking the natural quality is that it permits just the

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assessment in the particular objective organ poisonousness, specifically, stomach and digestive tract. Furthermore, histopathological biomarkers additionally assume a significant part in surveying the general wellbeing status of the whole populace in the oceanic biological system. Besides, the changes saw in these objective organs are simpler and dependable to distinguish explicitly than the utilitarian ones, and at last fill in as notice sign of decay in creature wellbeing [9-11]. Hence biomarkers, in most recent couple of decades, have opened up another vista in surveying the oceanic biological system toxicology as the fish wholesome channel are constantly being uncovered during the assimilation of ingested food stuff sullied with xenobiotic substances legitimately through essential maker life forms. In most recent couple of years, various investigations are accessible on biochemical, physiological and metabolic changes of this herbicide in various fish species [12].

As to obsessive modifications through histological and ultrastructural perceptions of this herbicide on different organs in various fish species are insufficient as significant headway in science has been made lately. Herefore, considering this scant data of this agrochemical, the destinations of the current examination was to portray and think about the histological and ultrastructural adjustments actuated, with specific accentuation on stomach and digestive system of *Anabas testudineus*.

## Materials and Methods

### Fish

Indian Freshwater teleost, *Anabas testudineus* (Bloch, 1792) with a normal load of  $23.58 \pm 2.05$  g and all out length of  $11.15 \pm 0.548$  cm, separately were bought from the nearby fish ranch and were adjusted for 15 days. During acclimatization, fish were kept in constantly circulated air through water (250 L limit) with a static framework, and at common photoperiod of 12 h light/12 h dull. Normal estimation of water boundaries during the acclimatization time frame were as per the following: temperature,  $18.61 \pm 0.81^\circ\text{C}$ ; pH,  $7.23 \pm 0.082$ ; electrical conductivity,  $413.67 \pm 0.90$   $\mu\text{S}/\text{cm}$ ; all out broke up solids,  $295.11 \pm 1.16$  mg/l; disintegrated oxygen,  $6.46 \pm 0.22$  mg/l; all out alkalinity,  $260.00 \pm 16.90$  mg/l as  $\text{CaCO}_3$ ; complete hardness,  $177.33 \pm 5.50$  mg/l as  $\text{CaCO}_3$ ; 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 nitrate-nitrogen,  $0.30 \pm 0.06$  mg/l. after fruition of acclimatization, fish were isolated into two sections: one gathering of fish was moved to handle lakes arranged at Crop Research and Seed Multiplication Farm (CRSMF) premises of the University of Burdwan, and remaining fishes were brought to the lab aquarium. Fish were taken care of business fish pellets (32% rough protein, Tokyu) when daily during acclimatization and experimentation. He tests were directed in agreement to the rules of the University of Burdwan for creature try and were approved by the Ethical Committee of this University

### Histopathological analysis

Stomach and digestive system aier dismemberment were fixed in fluid Bouin's answer for the time being. Hen got dried out through reviewed arrangement of ethanol (70%, 90% and 100%) lastly inserted in paraffin for setting up the paraffin block. Tissue segments were cut at 3-4  $\mu$  utilizing

Leica RM2125 microtome and recolored with haematoxylin-eosin (H&E). At last recolored areas were inspected under Leica DM2000 light magnifying lens and photos were taken by Leica Image Organizer software to look at the obsessive adjustments.

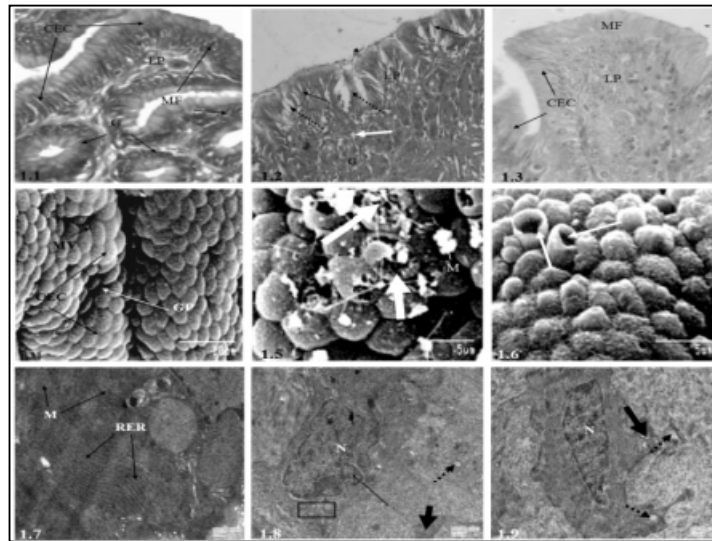
For transmission electron tiny (TEM) study, stomach and digestive system ( $2 \times 2$  mm in size) were fixed in Karnovsky fixative arranged in 0.1 M phosphate support for 12 h at  $4^\circ\text{C}$  and afterward post-fixed with 1% osmium tetra oxide arranged in phosphate cradle (0.2 M and pH 7.4) for 2 h at  $4^\circ\text{C}$ . Her obsession tissues were washed with phosphate cradle and afterward dried out through evaluated arrangement of  $\text{CH}_3\text{I}_2\text{CO}$ , invaded lastly installed in epoxy pitch (araldite CY212). Ultrathin areas of the individual tissues were then cut by utilizing a glass blade on "Ultracut E Reichart-Jung" machine (thickness 70 nm). Segments were then gathered on stripped copper-fit networks, and recolored with uranyl acetic acid derivation and lead citrate. At last, tissues were inspected under TECHNAI G2 high goal transmission electron magnifying instrument at Electron Microscope Facility, Department of Anatomy, AIIMS, New Delhi, India and photos were caught by Image Organizer software.

## Results & Discussion

Stomach histologically, stomach is comprised of as common of four layers viz., mucosa, submucosa, solid muscularis, and serosa. He gastric mucosa is fixed with a solitary layer of minimally masterminded columnar epithelial cells (CEC) with midway positioned cores. He cylindrical gastric organs are available at basal segment of gastric mucosa. In gastric organ, the gastric cells with midway positioned core are available, for example, encompassing the focal lumen. Gastric organs are straightforward, cylindrical alongside either adjusted or prolonged fit as a fiddle. Sub mucosa is very much vascularised with thick layer of free connective tissue (Figure 1.1). Most striking changes saw under light microscopy in the research facility condition were degenerative changes in columnar epithelial cells, greasy affidavit in the basal area, brush outskirt vanishing, top plate diminishing, harm in gastric organs and mucosal folds in stomach of *A. testudineus* (Figure 1.2), while under field condition no such conspicuous changes were watched (Figure 1.3). SEM concentrate additionally affirmed the harms saw under light microscopy, for example, extreme degeneration in CEC, for example, divided CEC, serious bodily fluid discharge over epithelial surface and harm in the microridge structures (Figures 1.4 and 1.5), while under field condition harms were relatively not as much as research facility condition (Figure 1.6). Transmission electron minuscule perception demonstrated distortion in core and mitochondria (Figure 1.7), harm in unpleasant endoplasmic reticulum, and vacuolations in stomach of *A. testudineus* (Figure 1.8), yet just distorted mitochondria and vacuolations were seen under field condition and harms were not as much as research center condition (Figure 1.9). Free connective tissue strands of submucosa extended into the mucosal folds shaping the lamina propria. He lamina propria is limited, long vascular and mucous cells are scattered. Columnar epithelial cell are noticeable and core are halfway positioned and profound recolored (Figure 2.1). He most obvious changes in digestive system under research facility condition were serious harm in CEC, contortion in connective tissues of lamina propria, separation of epithelial

layer from lamina propria and extreme bodily fluid discharge (Figure 2.2), while under field condition digestive system demonstrated practically typical appearance yet in

certain spots bodily fluid emission was conspicuous (Figure 2.3).

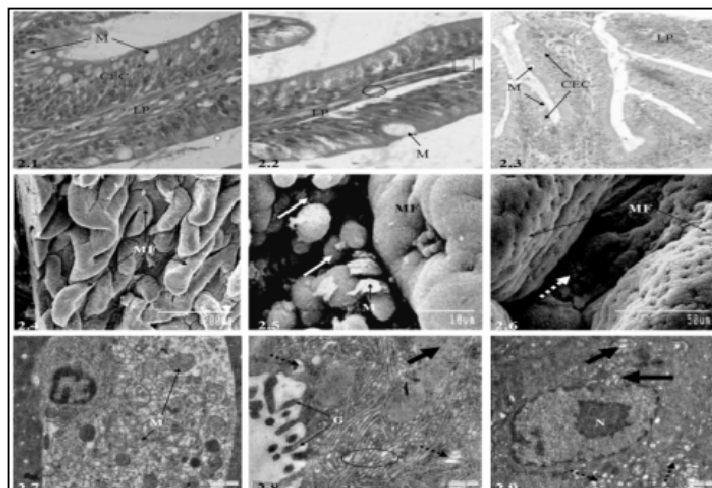


**Fig. 1:** Photomicrographs of stomach in *A. testudineus* showing control condition (C), laboratory condition (AL) and field condition (AF).

Normal and compact arrangement of columnar epithelial cells (CEC) with distinct nucleus under light microscopy and presence of rounded gastric glands (G) separated by lamina propria (LP) (C  $\times$  1000), 1.2: Showing degenerated CEC (arrow), vacuolization (broken arrow), thin top plate (arrow head) and lesions in gastric gland (white arrow) under normal microscopy (AL  $\times$  400), 1.3: Compact CEC with distinct nucleus under light microscopy (AF  $\times$  1000), 1.4: SEM observation showing normal mucosal folds (MF) surrounded by oval or round shaped CEC and stubby microvilli (MV). Note presence of gastric pits (GP) (C  $\times$  3000), 1.5: Showing degeneration in columnar epithelial cells (bold arrow) and mucus secretion (M) under scanning electron microscopy (AL  $\times$  6000), 1.6: Showing damage on tip of CEC (arrow) under SEM (AF  $\times$  6000), 1.7: Showing normal gastric glands with mitochondria (M) and rough endoplasmic reticulum (RER) under TEM observation (C  $\times$  7000), 1.8: Deformed nucleus (arrow) and mitochondria (bold arrow), damage in RER (square) and vacuolization

(broken arrow) under TEM study (AL  $\times$  4000), and 1.9: Presence of deformed mitochondria (bold arrow) and vacuolization (broken arrow) under TEM observation (AF  $\times$  5000).

Ultra basic injuries showed serious bodily fluid discharge over epithelial surface and corruption under research facility condition (Figures 2.4-2.9). While mucosal folds and CEC demonstrated less harm in contrast with lab condition however in the middle of the essential mucosal folds garbage of the divided auxiliary mucosal folds was seen under field condition under SEM study (Figure 1.6). TEM concentrate likewise indicated greasy testimony and vacuolations, harm in the glycocalyx structure, widened mitochondria, and harm in the rounded organization under research facility condition (Figure 1.8), while mitochondrial twisting and vacuolations were unmistakable under field condition (Figure 1.9).



**Fig. 2:** Photomicrographs of intestine in *A. testudineus* showing control condition (C), laboratory condition (AL), field condition (AF).



Normal lamina propria (LP), columnar epithelial cells (CEC) under light microscopy ( $C \times 1000$ ), 2.2: Damage in lamina propria (oval), detachment of epithelium layer from lamina propria (square) under light microscopy ( $AL \times 1000$ ), 2.3: Light microscopy showed almost normal CEC with distinct nucleus ( $AF \times 400$ ), 2.4: Distinct mucosal folds (MF) with oval or round shaped CEC and supported by microvilli (MV) under SEM observation ( $C \times 200$ ), 2.5: Showing deformed MV (arrow) and mucin droplets (M) under SEM observation ( $AL \times 3000$ ), 2.6: Showing normal mucosal folds (MF) and mucin droplets between MF (broken arrow) ( $AF \times 1000$ ), 2.7: TEM observation showing normal appearance of columnar epithelial cells with abundant mitochondria (M) ( $C \times 5000$ ), 2.8: Showing severe vacuolization (broken arrow), dilation in mitochondria (bold arrow), damaged tubular network (oval) and glycocalyx (G) under TEM ( $AL \times 9900$ ), and 2.9: TEM observation showed deformation in mitochondria (bold arrow) and vacuolization (broken arrow) ( $AF \times 4000$ ).

In the Present examination is revealing first time the poisonousness of the sulfonylureabased business agrochemical, Almix concerning histological and ultra-structural perceptions through filtering and transmission electron microscopy in *A. testudineus* under field and lab conditions on near premise, in spite of the fact that Senapati<sup>[11, 12]</sup> announced histopathological adjustments under lab condition in throat, buccopharynx, stomach and digestive tract of *A. testudineus* and Samanta on some biochemical boundaries in different fish species including *A. testudineus* yet just under research facility condition<sup>[12]</sup>. Stomach is one of the prime organs of fish nutritious trench and assumes a significant part in the absorption of ingested food stuff for the development and improvement of fish species. Histopathological concentrate under light microscopy indicated stamped differences in gill epithelium between two conditions. In the current investigation, degenerative changes in columnar epithelial cells, vacuolated basal district, brush fringe vanishing, top plate diminishing, contortion in gastric organs and combination of submucosa with mucosal folds were oftentimes watched neurotic adjustments in stomach of *A. testudineus*. Harm in gastric organs saw under present examination was additionally detailed by Crespo<sup>[18]</sup> and showing lower creation of mucin which eventually decreased the security capacity of the gastric epithelium against the concoction wounds. Contortion in the stomach related organs likewise hampers the stomach related proteins creation which eventually prompts diminished ingestion of food materials by intestinal part. Loss of auxiliary respectability and vacuolation seen under this investigation were additionally detailed by Establier, Sastry and Gupta<sup>[13-14]</sup>. Expanding, mutilation or potentially vacuolation in the mucosal epithelial cells of stomach additionally announced aier incessant introduction of endosulfan and methyl ethyl fluctuating in *Gymnocorymbus ternetzi* by Amminikutty and Rege<sup>[15]</sup>. Vacuolization in sub mucosa, shrinkage of mucosal folds saw under present investigation was additionally detailed by Ghanbahadur in *Rasbora daniconius* aier endosulfan introduction<sup>[16]</sup>. Noticeable ultra-structural changes under lab condition remember extreme degeneration for CEC, for example, discontinuity, serious bodily fluid emission and harm in micro ridge structures saw under checking electron microscopy. Extreme bodily fluid emission, harm in the micro ridge structures and CEC saw under SEM perception

can likewise be confirmed with the discoveries of light infinitesimal perceptions and this may be expected to herbicidal activity which eventually lessens the insurance capacity of gastric epithelium and triggers the movement of the gastric epithelium. His results were additionally concurrence with the discoveries. Haque<sup>[11]</sup> likewise watched harms in columnar epithelial cells which incorporate fracture, significant bodily fluid discharge and loss of microridge structure in stomach of *Channa punctatus* presented to fluoride. At the transmission electron tiny level, gastric epithelium of stomach demonstrated disfigurement in core and mitochondria, harm in unpleasant endoplasmic reticulum, and vacuolation under the two conditions yet the level of changes was similarly less in field condition. Comparative outcome as seen under present examination was additionally detailed by Rebolledo and Vial<sup>[17]</sup>. Then again, Carrassón exhibited plenitude of unpleasant endoplasmic reticulum and mitochondria, harm in tubule-vascular organization, and heterochromatinic core in stomach of dentex<sup>[18]</sup>. Similarly less neurotic modifications under field condition may be because of self-sustaining component as fish are in normal territory and taking practically characteristic food.

Digestive system is the following most significant aspect of the fish nutritious channel aier stomach and assumes a significant part in processing and assimilation of food materials just as considered as a delicate organ for poisonousness appraisal of xenobiotic substances in fish species as they are straightforwardly presented to complex blend of harmful substances by means of ingestion of sullied food studs or in a roundabout way through blood as well as lymph. Various examinations on histopathological effects of different pesticides on fish digestive tract have been accounted for by a few creators yet histological and ultrastructural considers identified with intestinal epithelium due to Almix introduction, are generally inadequate. Walsh and Velmurugan saw degeneration in the tip of villi, loss of auxiliary trustworthiness in mucosal folds, hypertrophy, vacuolation and corruption in *Cyprinus carpio* and *Cirrhinus mrigala* presented to atrazine and fenvalerate, individually. Comparative perceptions were additionally depicted by Ghosh<sup>[12]</sup> in digestive system of *notopterus aier* arsenic introduction and by Bose in *A. testudineus aier* lead and cadmium introduction. Serious discharge of bodily fluid under present examination showed that fish were under pressure and attempting to defeat these worry as compensatory reaction. Flotsam and jetsam of the divided optional mucosal creases in the middle of the essential mucosal folds was seen under SEM concentrate in field condition. Transmission electron micrographic perception portrayed extreme vacuolations, harm in glycocalyx structure, expanded mitochondria and harm in the cylindrical organization under the two conditions, showing fish were in pressure and drawn nearer to ensure the forced pressure. Similarly less obsessive reactions in field condition may be because of weakening ability and automatic instrument of the indigenous habitat. therefore, the modifications hindered the intestinal transportation measure just as ingestion of food materials.

### Conclusion

The current examination uncovered that introduction caused serious obsessive modifications in stomach and digestive system of *A. testudineus* under research facility condition.

Neurotic injuries showed more grounded reactions under research center condition contrasted with field study. At last, these obsessive adjustments to this herbicide introduction could be considered as markers to assess fish wellbeing status under focused on conditions in freshwater biological system, and cautious dealing with and observing ought to be taken before utilization of this herbicide in rural ranches or amphibian bodies for controlling weeds.

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