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Metacid Induced Histopathological Changes in Stomach of Fingerlings of *Channa punctatus* (Bloch)

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ABSTRACT

The present study reports histopathological and histochemical changes in the stomach of fingerlings (three groups) of the freshwater, air-breathing, snake-headed murrel fish, *Channa punctatus* (Bloch), following exposure to a sub-lethal concentration (8.4ppm) of Metacid 50 (an organophosphate) for 96 hours. Histopathological changes observed in both the cardiac and pyloric regions of the stomach included disruption of the inner epithelial lining, loss of nuclear integrity in cells, glandular deformities, and damage to the serosa, along with disorganized submucosa and muscularis layers. Histochemically, Metacid intoxication led to an increase in mucins in the serosa and a higher concentration of glycogen in the neutral muscularis. The muco-substances present in both control and exposed fingerlings may play a vital role in detoxification by neutralizing the toxicant (Metacid) or protecting the gastric mucosa from chemical injury. These findings highlight the need for regular biochemical monitoring and timely restoration of freshwater bodies to support aquatic life.

INTRODUCTION

It is now an established fact that environmental problems have increased exponentially in recent decades. With rapid industrialization, many water sources have become contaminated due to the discharge of effluents from various industrial units. These industrial discharges often contain toxic and hazardous substances, including heavy metals and other chemical contaminants **(Untoo *et al.*, 2002)**, which pollute aquatic ecosystems and pose significant threats to aquatic organisms including fish.

The influx of toxic substances into the gut microbiome has led to declines in fish populations and promoted bioaccumulation along higher trophic levels. For instance, Endosulfan-induced pathological alterations in the histoarchitecture of the optic nerve, such as thinning of the fibrous sheath, degeneration of nervous tissue, and the presence of scattered blood cells, have been documented in the freshwater catfish *Heteropneustes fossilis* (Bloch) **(Sandhu *et al.*, 2021)**.

The present study aimed to investigate the effects of Metacid on the histopathological and histochemical changes in the stomach of the freshwater, air-breathing, snake-headed Murrel fish, *Channa punctatus*, with the goal of evaluating specific alterations limited to the alimentary canal within an aquatic ecosystem context.

MATERIALS AND METHODS

Live specimens of *Channa punctatus* (Bloch) fingerlings, ranging in size from 3 to 10cm, were procured from local fish dealers and maintained in laboratory conditions. During a minimum acclimation period of seven days, the fish were fed daily with a diet consisting of chopped goat liver and live earthworms.

Metacid 50 (an organophosphorus compound containing 50% w/w Methyl Parathion = O, O-dimethyl O-(p-nitrophenyl) phosphorothioate; Bayers India Ltd., Mumbai) was used as the test chemical. To study the effects of Metacid on developmental stages, fingerlings were categorized into three size-based groups: Group I (2–4cm), Group II (5–6cm), and Group III (7–8cm).

Prior to experimentation, the median tolerance limit (TLm or LC_{50}) of Metacid was determined at 29.0 ± 1.0°C using static acute bioassay methods **(Kumari, 2007; APHA, 2023)**. The LC_0, LC_{50}, and LC_{100} values at 96 hours of exposure were found to be 5.09, 14.04, and 21.0mg/ L, respectively. The safe concentration, as calculated by the method of **Hart *et al.* (1945)**, was 4.12mg/ L. All experimental exposures were conducted at a concentration of 8.4 mg/L.

At the end of the exposure period, the fish were euthanized by decapitation. The alimentary canal was dissected, and samples from the cardiac stomach, pyloric stomach, and pyloric caeca were collected and fixed in a solution of 2% calcium acetate dissolved in 10% formalin (ICAF). After 24 hours, tissues were thoroughly washed in chilled distilled water followed by running tap water for 3–4 hours.

The samples were then dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax. Sections were cut at a thickness of 4–6µm. Some sections were routinely stained with hematoxylin and eosin (H&E), while others were stained with Mallory's triple and PAS stains for histopathological and histochemical analysis.

RESULTS AND DISCUSSION

The shape of the stomach in fishes varies depending on the available space within the body cavity. In *Channa punctatus*, the stomach is differentiated into three regions: a broad anterior part near the heart, known as the cardiac stomach; a narrower middle section called the pyloric stomach; and the terminal portion, referred to as the pyloric caeca. Histologically, the stomach is composed of four distinct layers: an outer serosa, followed by the muscularis, submucosa, and the innermost mucosal layer, which is lined with epithelial cells **(Dar *et al.*, 2022)**.

The cardiac stomach shares a similar four-layer structure with the oesophagus, except that its mucosal layer is further differentiated into a surface epithelial layer and a layer containing gastric glands. Upon exposure to Metacid, several histopathological changes were observed in this region, including disruption of the epithelial lining, nuclear degradation in epithelial cells, glandular deformities, rupture of the serosa, and disorganization of the submucosa and muscularis.

Histochemically, Metacid intoxication led to thickening of the submucosa with reduced levels of neutral mucins, decreased glycogen in the muscularis, and moderate amounts of mucins in the gastric gland cells. Histological examination revealed blunt rugae in the gastric mucosa. Notably, the cardiac glands lacked mucous neck cells, contained some endocrine cells, and displayed uniform epithelial cells—distinct features in the fingerlings of *C. punctatus*.

The toxic effects of Metacid induced severe dose- and time-dependent histopathological changes in the cardiac stomach, such as complete destruction of surface mucosal cells, necrosis in the muscularis, submucosal thickening, and rugae shortening.

Histochemical studies identified different cell types within the mucosa, including columnar epithelial cells secreting neutral mucosubstances, and three types of goblet cells: type I (neutral mucins), type II (sulfomucins), and type III (sialomucins). During Metacid exposure, all mucin-secreting cell types showed reduced activity compared to controls, especially in the mucosal layer. However, acid mucosubstance secretion increased with higher concentrations and longer exposure times. Serosal mucins remained unchanged.

Glycogen content (as detected histochemically) was depleted in response to Metacid exposure, with greater depletion observed at higher concentrations. The mucosubstances present in both control and exposed fingerlings may play a protective role by neutralizing the toxicant or safeguarding the gastric mucosa from chemical injury.

Similar histopathological effects, such as fused microvilli, damaged outer microvillar membranes, and hemorrhaging in the submucosal region, have also been reported in *Channa punctatus* following acute exposure to Endosulfan **(Haloi *et al.*, 2013)**.

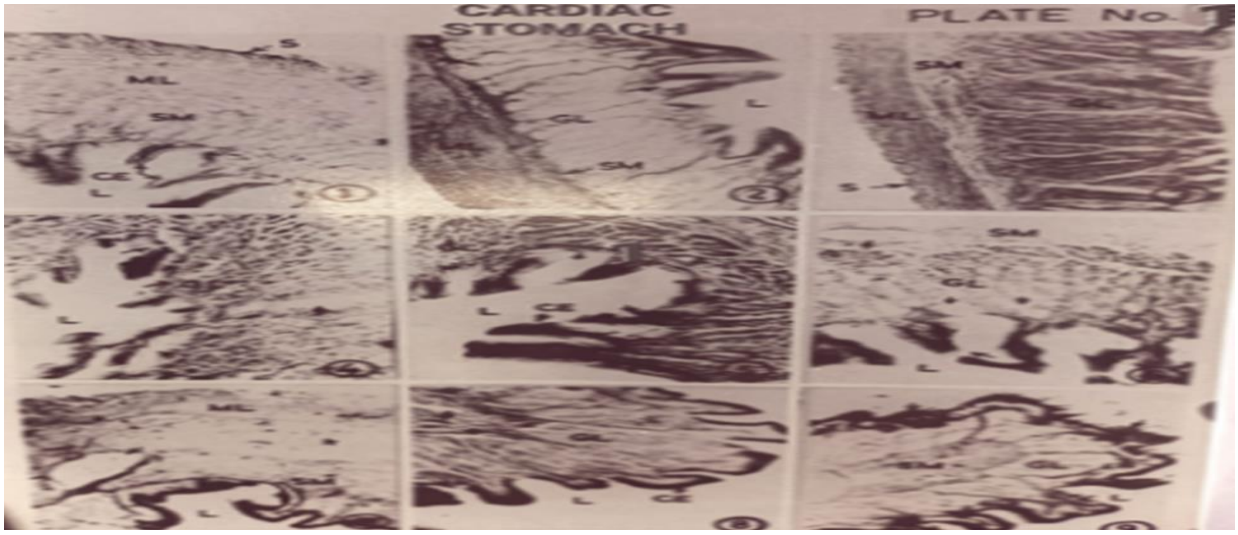

Plate I. Histopathological and histochemical alterations in the cardiac stomach of fingerlings of *Channa punctatus* following Metacid intoxication (8.4 ppm for 96 Hours)

Figs. (1–9) illustrate transverse sections (T.S.) of the cardiac stomach from three size groups of fingerlings, showing both histopathological and histochemical changes:

1. T.S. of Group I fingerlings, stained with H&E, showing lumen (L), surface mucous cells (CE), submucosa (SM), muscularis (ML), and serosa (S). Magnification: ×300.
2. T.S. of Group II fingerlings, stained with H&E, showing lumen (L), gastric glands (GL), submucosa (SM), and muscularis (ML). Magnification: ×300.
3. T.S. of Group III fingerlings, stained with H&E, showing gastric glands (GL), submucosa (SM), muscularis (ML), and serosa (S). Magnification: ×300.
4. T.S. of Group I fingerlings, stained with PAS, showing lumen (L), surface mucous cells (CE), and gastric glands (GL). Magnification: ×300.
5. T.S. of Group II fingerlings, stained with PAS, showing lumen (L), surface mucous cells (CE), and gastric glands (GL). Magnification: ×300.
6. T.S. of Group III fingerlings, stained with PAS, showing lumen (L), surface mucous cells (CE), and gastric glands (GL). Magnification: ×300.
7. T.S. of Group I fingerlings, stained with PAS, showing lumen (L), submucosa (SM), and muscle layer (ML). Magnification: ×300.
8. T.S. of Group II fingerlings, stained with H&E, showing lumen (L), surface mucous cells (CE), and gastric glands (GL). Magnification: ×300.
9. T.S. of Group III fingerlings, stained with PAS, showing lumen (L), gastric glands (GL), and submucosa (SM). Magnification: ×300.

Histologically, the pyloric stomach of *Channa punctatus* exhibited the same four distinct layers as observed in the cardiac stomach: the outer serosa, muscularis, submucosa, and the innermost mucosal epithelium.

Histopathologically, Metacid intoxication led to marked inflammation and acute proliferation of the mucosal epithelium. Degenerative loss of epithelial cells was evident, accompanied by thickening of the connective tissue and rupture of the serosal layer.

Histochemically, Metacid exposure resulted in increased mucin content in the serosa, an enhancement of neutral mucins in the submucosa, and a higher concentration of glycogen in the muscularis.

Histological examination of the pyloric stomach across all three fingerling groups revealed structurally similar features, consistently showing the typical four-layer organization. However, distinct histopathological changes—comparable to those observed in the cardiac stomach—were evident under toxicant exposure.

Sharma et al. (2018) reported similar tissue damage in the liver, gills, gonads, and kidneys of *Channa punctatus* correlated with the accumulation of 4-Nonylphenol. These findings align

with the present observations at both lower and higher concentrations of Metacid, indicating a shared pathological response pattern.

The observed changes in the pyloric stomach—such as degenerative epithelial loss in localized areas, thickening and vacuolation of the submucosa, a mesh-like appearance in the muscularis, and ruptured serosa—are clearly dose- and time-dependent. These alterations can be attributed to the direct toxic effects of Metacid on the gastrointestinal tract of the fish.

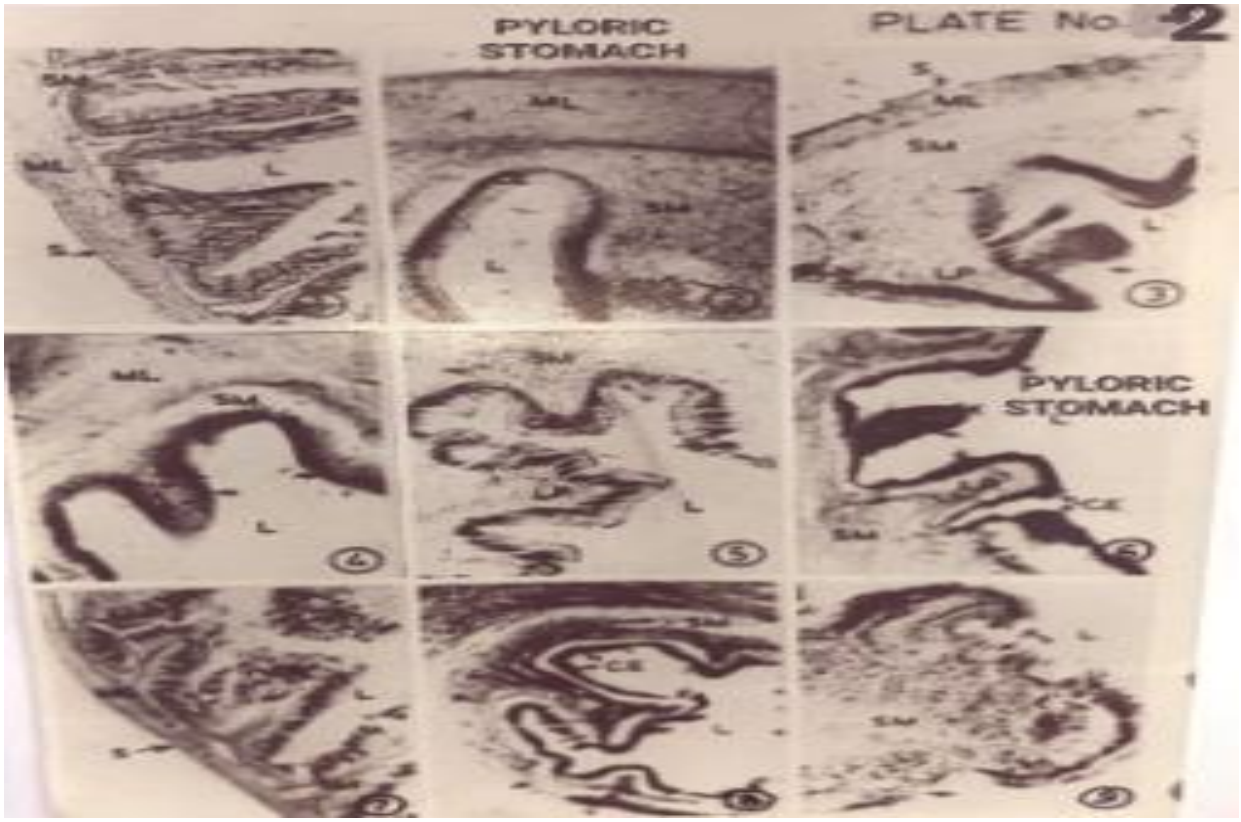

Plate II. Histopathological and histochemical alterations in the pyloric stomach of fingerlings of *Channa punctatus* following Metacid intoxication (8.4 ppm for 96 Hours)

Figs. (1–9) illustrate transverse sections (T.S.) of the pyloric stomach from three size groups of fingerlings, showing histopathological and histochemical changes:

1. T.S. of Group III fingerlings, stained with H&E, showing lumen (L), submucosa (SM), muscular layer (ML), and serosa (S). Magnification: ×300.
2. T.S. of Group II fingerlings, stained with H&E, showing lumen (L), surface mucous cells (CE), submucosa (SM), and muscle layer (ML). Magnification: ×300.
3. T.S. of Group I fingerlings, stained with H&E, showing lumen (L), surface mucous cells (CE), lamina propria (CP), submucosa (SM), muscle layer (ML), and serosa (S). Magnification: ×300.
4. T.S. of Group III fingerlings, stained with PAS, showing lumen (L), submucosa (SM), and muscle layer (ML). Magnification: ×300.

5. T.S. of Group II fingerlings, stained with Alcian Blue (AB) pH 1.0, showing lumen (L), surface mucous cells (CE), and submucosa (SM). Magnification: ×300.
6. T.S. of Group I fingerlings, stained with AB pH 2.5 + PAS, showing lumen (L), surface mucous cells (CE), lamina propria (CP), and submucosa (SM). Magnification: ×300.
7. T.S. of Group III fingerlings, stained with PAS, showing lumen (L), surface mucous cells (CE), muscle layer (ML), and serosa (S). Magnification: ×300.
8. T.S. of Group II fingerlings, stained with PAS, showing lumen (L), surface mucous cells (CE), submucosa (SM), and muscle layer (ML). Magnification: ×300.
9. T.S. of Group I fingerlings, stained with PAS, showing lumen (L) and submucosa (SM). Magnification: ×300.

Histologically, the pyloric caeca of *Channa punctatus* exhibited the same four typical layers as observed in the pyloric stomach: the outer serosa, muscularis, submucosa, and the innermost mucosal layer.

Following Metacid intoxication, several notable histopathological alterations were observed. The mucosal folds (villi) were severely damaged, often completely destroyed, forming debris of dead cells in the lumen. Many villi were shortened and exhibited sloughing of epithelial cells. The submucosa was either entirely destroyed or significantly reduced, while the muscularis appeared markedly diminished. The serosa persisted as a thin layer surrounding the tissue. In some villi, thickening and bifurcation at the tips were observed, along with an increased number of goblet cells.

Similar findings have been reported by **Adhana and Garg (2023)**, who observed necrosis, vacuolar degeneration, and congestion in the liver, along with hyperplasia in the gills and kidneys of exposed fish.

Histochemically, after Metacid exposure, the villi showed reduced concentrations of neutral mucins, while acid mucosubstances—particularly those associated with goblet cells—were markedly increased. Submucosal neutral mucins were present at moderate levels. A slight increase in glycogen was noted in the muscularis, and neutral mucins in the serosa were enhanced.

Anjum and Kumari (2023) also reported organ dysfunction in *C. punctatus* exposed to ammonium sulphate, with desquamation of epithelial cells in the villi and necrosis in the gills, liver, intestine, and kidney.

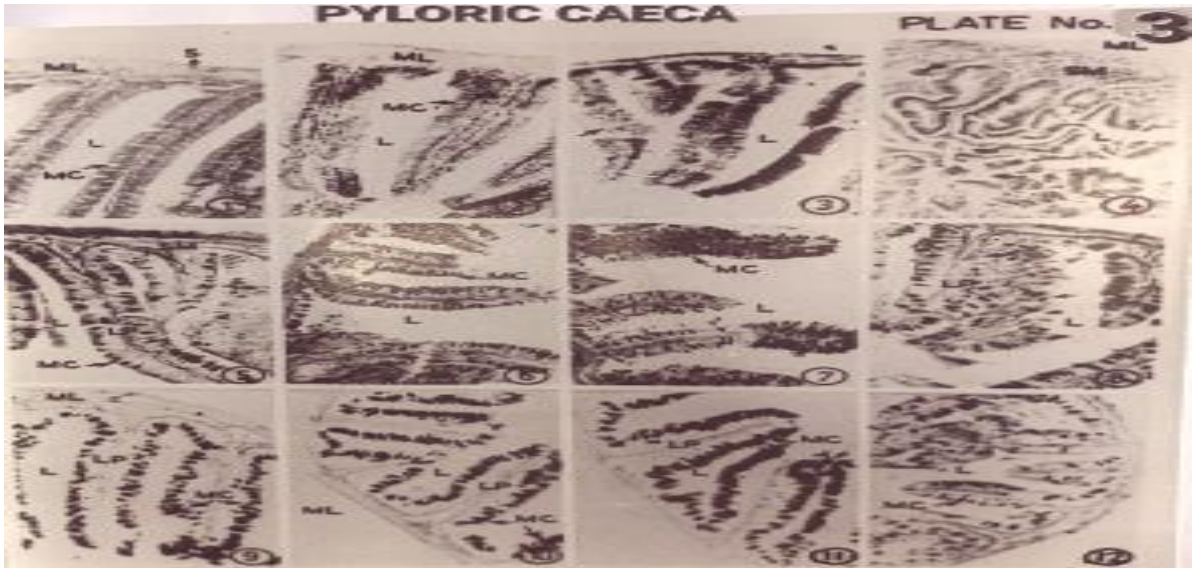

Plate III. Histopathological and histochemical alterations in the pyloric caeca of fingerlings of *channa punctatus* following Metacid intoxication (8.4 ppm for 96 Hours)

Figs. (1–12) depict transverse sections (T.S.) of the pyloric caeca from three size groups of fingerlings, highlighting both histological and histochemical changes:

1. T.S. of Group III fingerlings, stained with H&E, showing lumen (L), goblet cells (MC), muscle layer (ML), and serosa (S).
2. T.S. of Group II fingerlings, stained with H&E, showing lumen (L), goblet cells (MC), and muscle layer (ML).
3. T.S. of Group I fingerlings, stained with H&E, showing lumen (L) and submucosa (SM).
4. T.S. of Group I fingerlings, stained with H&E, showing lumen (L), submucosa (SM), and muscle layer (ML).
5. T.S. of Group III fingerlings, stained with Alcian Blue (AB) pH 2.5 + PAS, showing lumen (L), goblet cells (MC), lamina propria (LP), submucosa (SM), and muscle layer (ML).
6. T.S. of Group II fingerlings, stained with AB pH 1.0 + PAS, showing lumen (L) and goblet cells (MC).
7. T.S. of Group I fingerlings, stained with AB pH 2.5 + PAS, showing lumen (L) and goblet cells (MC).
8. T.S. of Group I fingerlings, stained with AB pH 1.0 + PAS, showing lumen (L) and lamina propria (LP).
9. T.S. of Group III fingerlings, stained with AB pH 1.0 + PAS, showing lumen (L), goblet cells (MC), lamina propria (LP), and muscle layer (ML).
10. T.S. of Group II fingerlings, stained with PAS, showing lumen (L), goblet cells (MC), and lamina propria (LP).
11. T.S. of Group I fingerlings, stained with AB pH 2.5 + PAS, showing lumen (L), goblet cells (MC), and lamina propria (LP).

12. T.S. of Group I fingerlings, stained with AB pH 2.5 + PAS, showing lumen (L) and goblet cells (MC).

The main structural feature of the pyloric caeca in *Channa punctatus* is the presence of elongated villi containing numerous goblet cells. Among the various organs studied, the pyloric caeca appeared to be the most affected by Metacid intoxication, particularly in fingerlings. The observed histopathological changes were found to be dose- and time-dependent.

An increase in goblet cell number was correlated with higher concentrations of Metacid. The submucosa exhibited signs of vacuolation and necrosis. The villi became shortened, often resembling buds, and at higher concentrations, they were ruptured, with frequent accumulation of dead cellular debris in the lumen.

These findings are consistent with those of **Mohammad *et al*. (2019)**, who reported that exposure to varying concentrations of insecticides led to significant damage in vital organs of fish. Even small amounts of pesticides, such as Furadan, introduced into freshwater ecosystems were shown to disrupt fish physiology and potentially lead to mortality.

These results underscore the urgent need to consider pesticide toxicity as a significant ecological threat, with implications for fish populations and the stability of aquatic ecosystems in the near future.

CONCLUSION

To date, specific studies on the pyloric caeca of *Channa punctatus* fingerlings under Metacid exposure have not been previously reported. Similar to the pyloric stomach, the histopathological changes observed in the pyloric caeca appear to result from the direct toxic effects of Metacid and may also represent an adaptive response to mitigate toxicity. With increasing doses of Metacid, a decrease in hyaluronic acid and glycogen was noted. The mucous substances may serve an antipeptic function, protecting the villi from peptic proteolysis, while glycogen likely provides an essential energy reserve during environmental stress. Drastic morphological alterations were also evident in the cardiac stomach, including complete disruption of the mucosal surface, necrosis of the muscularis, thickening of the submucosa, and shortening of rugae. The presence of muco-substances in both control and intoxicated fingerlings suggests they play a critical role in detoxification, either by neutralizing the toxicant (Metacid) or by providing a protective barrier for the gastric mucosa against chemical injury. Continued use of such harmful substances in aquatic environments contributes to the accumulation of toxins in the gastrointestinal tract and amplifies the flow of contaminants to vital tissues in fish species that remain in polluted water bodies. This underscores the broader ecological consequences of pesticide pollution on aquatic life.

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DECLERATION OF INTEREST

The authors declare no competing interests.

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