Liver Morphological Deformities in *Anabas* sp. Resulting from Long-Term Exposure to Thiamethoxam-Contaminated Water

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ABSTRACT

The present study evaluated the histopathological effects of thiamethoxam on the liver tissues of *Anabas* sp. following 21 days of exposure to increasing concentrations. Fish were subjected to control, low (0.12 $\mu g \cdot L^{-1}$), medium (0.56 $\mu g \cdot L^{-1}$), and high (1.15 $\mu g \cdot L^{-1}$) doses to assess dose-dependent alterations. The control group exhibited normal hepatic architecture with well-defined hepatocytes and intact sinusoidal spaces. In contrast, the low-dose group showed mild disorganization, early vacuolation, and slight cytoplasmic granulation. Medium-dose exposure produced more pronounced degeneration, with disrupted hepatic cords, expanded vacuolated regions, and notable cytoplasmic deterioration. The high-dose group displayed severe structural damage characterized by extensive homogenization, loss of cellular boundaries, and focal necrotic areas, indicating advanced hepatotoxicity. The progressive nature of these lesions across increasing concentrations highlighted the accumulation of oxidative and metabolic stress induced by thiamethoxam. Overall, the findings demonstrated that chronic exposure, even at environmentally relevant levels, can significantly impair liver structure and function in *Anabas* sp. These results underscore the ecological risks posed by neonicotinoid contamination in freshwater habitats and emphasize the need for stricter monitoring and regulation of pesticide use.

Keywords: Thiamethoxam; *Anabas* sp.; Liver Histopathology; Neonicotinoids; Hepatotoxicity; Aquatic Toxicology.

1. Introduction

The contamination of freshwater environments by agricultural pesticides had been recognized as a major threat to aquatic organisms globally. Neonicotinoid insecticides, particularly thiamethoxam, had been widely applied in crop protection due to their systemic action and high insecticidal efficacy; however, their persistence and mobility had allowed them to enter aquatic systems through runoff and leaching (Morrissey et al., 2015). Once introduced into freshwater habitats, thiamethoxam had been shown to exert sub-lethal yet biologically significant effects on non-target organisms, including fish, by inducing physiological, biochemical, and cellular disturbances (Sánchez-Bayo & Goka, 2014).

The liver, being the central metabolic and detoxification organ in fish, had been identified as a sensitive indicator of xenobiotic exposure. Histopathological alterations such as vacuolar degeneration, hepatocyte hypertrophy, sinusoidal dilation, and necrosis had been frequently documented in fish subjected to chronic pesticide contamination, highlighting the organ's vulnerability to long-term toxic stress (Velisek et al., 2021). International studies had demonstrated that continuous exposure to neonicotinoids resulted in hepatic tissue remodeling and impaired metabolic function, while similar observations had been reported in Indian freshwater fish exposed to commonly used agricultural pesticides (Kole et al., 2013; Ali et al., 2020). These findings underscored the ecological significance of assessing hepatic health in fish inhabiting pesticide-contaminated waters. *Anabas* sp., a hardy and widely distributed freshwater species, frequently inhabited agricultural landscapes in India, its exposure risk to pesticide residues had been considerable. Despite this, limited attention had been given to the chronic hepatic effects of thiamethoxam on this species. Therefore, the present study had been undertaken to characterize the morphological

deformities in the liver of *Anabas* sp. resulting from prolonged exposure to thiamethoxam-contaminated water, thereby contributing to a broader understanding of neonicotinoid-induced toxicity in freshwater ecosystems.

2. Materials and Methods

2.1. Experimental Fish and Acclimatization

Healthy *Anabas* sp. individuals had been collected from local markets and transported to the laboratory in oxygenated containers. Fish of uniform size had been selected after screening for external abnormalities. A 7-day quarantine period had been followed by a 14-day acclimatization under controlled conditions (temperature 26–28 °C, pH 7.0–7.8, dissolved oxygen ≥ 5 mg L^{-1}, 12:12 h photoperiod). Fish had been fed a commercial diet twice daily until 24 h before exposure.

2.2. Experimental Design and Exposure

A completely randomized design had been adopted with four groups:

1. Control (0 mg L^{-1} thiamethoxam)
2. Low dose (1%)
3. Medium dose (5%)
4. High dose (10%) (1% = 0.12 µg·L^{-1}, 5% = 0.56 µg·L^{-1}, 10% = 1.15 µg·L^{-1})

Each treatment had included three replicate tanks, containing three fish per tank (n = 9 per group). Sub-lethal concentrations had been selected based on LC_{50} fractions from range-finding tests. The experiment had been conducted for 21 days using a static-renewal system, with test solutions replaced every 24–48 h. Water quality parameters had been monitored regularly, and concentrations had been prepared using analytical-grade thiamethoxam.

2.3. Sampling and Tissue Collection

Fish had been sampled at Day 0, Day 7, Day 14, and Day 21. Euthanasia had been performed using buffered MS-222. Livers had been dissected through a ventral incision, rinsed in 0.9% saline, trimmed to ≤ 5 mm thickness, and fixed immediately in 10% neutral buffered formalin for 24–48 h.

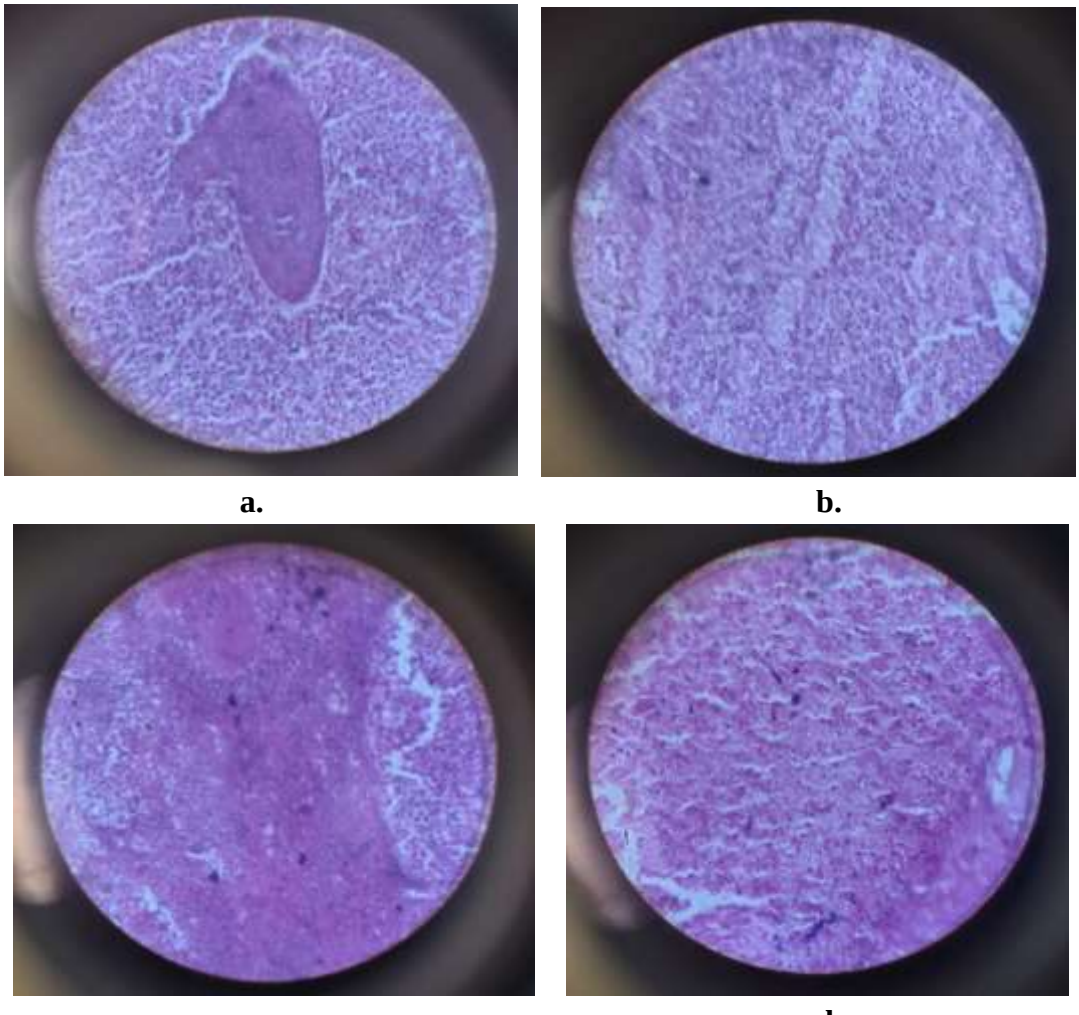
2.4. Histological Processing and Staining

Fixed liver tissues had been dehydrated in ascending ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5–7 µm thickness had been prepared using a rotary microtome and stained with Hematoxylin and Eosin (H&E). Additional stains had been applied when required for specific lesion identification.

3. Results and Discussion

The liver section of the control *Anabas sp.* examined after the 21-day experimental period exhibited normal hepatic architecture, indicating that experimental conditions and handling did not induce stress-related lesions. The hepatocytes appeared polygonal with well-defined cellular boundaries and uniform cytoplasmic staining, consistent with healthy liver tissue. The hepatic cords were arranged in a regular, radially oriented pattern around the central vein, which remained structurally intact without signs of congestion, dilation, or hemorrhage.

Sinusoidal spaces were clearly visible and evenly distributed, showing no evidence of occlusion, dilation, or inflammatory infiltration. No necrotic foci, vacuolation, fatty degeneration, or cellular hypertrophy were observed, confirming the absence of degenerative or toxic changes. The overall staining quality suggested well-preserved tissue integrity, with nuclei maintaining normal shape and distribution. The lack of pathological alterations validated the suitability of the control group as a baseline for comparing pesticide-induced hepatic deformities in treated groups. In summary, the control liver tissue exhibited normal histomorphology, indicating that the fish were healthy and unaffected by environmental or procedural stressors during the 21-day period, thereby serving as an appropriate reference for evaluating thiamethoxam-induced hepatic changes in exposed groups.



a. **b.**

c. **d.**

Fig. 1. Histological Tissue Section of Liver of Anabas sp. for 21 Days Exposure in Thiamethoxam-Contaminated Water for a. Control b. Low dose c. Medium Dose d. High Dose

The low-dose exposed liver section shows early and mild hepatic alterations compared to the control tissue. The normal cord-like arrangement of hepatocytes appears less distinct, with mild disorganization of hepatic parenchyma. Hepatocytes exhibit slight cytoplasmic vacuolation, suggesting early-stage fatty changes or metabolic stress. The sinusoidal spaces appear narrowed and irregular, indicating beginning sinusoidal congestion or reduced perfusion. No clear necrotic areas are observed, but the tissue shows signs of hepatocellular swelling, reflecting early toxic injury. Overall, the changes are mild and indicate initial hepatic responses to low-dose thiamethoxam exposure, without severe degenerative lesions.

The liver section exposed to 21 days of medium-dose thiamethoxam has appeared diffusely disorganized, with the normal hepatic cord pattern largely obscured. The tissue has shown a homogenous, pale-to-dark purple staining pattern suggestive of hepatocellular vacuolation and cytoplasmic degeneration. Patchy areas of increased granularity and lighter zones have indicated possible focal necrosis or proteinaceous accumulation. Overall, the sample has exhibited diffuse structural deterioration consistent with pesticide-induced hepatotoxic stress.

The liver section exposed to the high dose of thiamethoxam for 21 days has appeared markedly deteriorated, with the normal hepatic architecture severely disrupted. Hepatocytes have shown extensive cytoplasmic degeneration and loss of clear cellular boundaries, producing a diffuse, granular, and homogenized tissue texture. Areas of pale, irregular patches have indicated pronounced vacuolation and possible coagulative necrosis, while darker regions have suggested condensed, damaged cellular material. Overall, the tissue has exhibited advanced hepatotoxic injury consistent with sustained high-dose pesticide exposure.

Table 1. Liver Histopathology After 21-Day Thiamethoxam Exposure

Dose	Thiamethoxam Concentration	Observed Histological Features (Past-Participle, Concise)
Control	0 $mg \cdot L^{-1}$	Normal hepatic architecture preserved; hepatocytes appeared uniform; sinusoidal spaces maintained; no degeneration observed.
Low Dose	0.12 $\mu g \cdot L^{-1}$ (1%)	Mild disorganization recorded; hepatocytes slightly granulated; early vacuolation noted; tissue integrity largely retained.
Medium Dose	0.56 $\mu g \cdot L^{-1}$ (5%)	Hepatic cords disrupted; cytoplasmic degeneration pronounced; vacuolation widened; overall architecture moderately deteriorated.
High Dose	1.15 $\mu g \cdot L^{-1}$ (10%)	Extensive structural collapse observed; hepatocellular boundaries lost; diffuse granular homogenization detected; necrotic patches prominently developed.

The liver tissues across control, low-, medium-, and high-dose exposures have shown a clear, dose-dependent progression of thiamethoxam-induced damage. The control section has appeared structurally intact, with well-defined hepatic cords and uniform hepatocytes. The low-dose tissue has displayed mild disorganization, slight cytoplasmic granulation, and early vacuolation. The medium-dose section has exhibited pronounced architectural disruption, diffuse cytoplasmic degeneration, and broader areas of vacuolation. The high-dose tissue has presented the most severe alterations, with extensive loss of hepatocellular boundaries, homogenized granular texture, and patches indicative of necrosis. Collectively, the sections have demonstrated progressive hepatotoxic injury corresponding with increasing thiamethoxam concentration.

The progressive histopathological alterations observed in the liver tissues of *Anabas* sp. under increasing thiamethoxam exposure reflected a clear dose-dependent hepatotoxic response. The control group retained normal hepatic architecture, indicating that the baseline physiological condition had been maintained in the absence of pesticide stress, which aligns with typical teleost liver morphology reported in earlier studies (Hinton & Lauren, 1990).

In contrast, the low-dose exposure resulted in mild hepatocellular changes such as early vacuolation and slight cytoplasmic granulation. Such subtle alterations have been previously associated with early-stage pesticide-induced oxidative stress and metabolic disturbance in fish (Banaee et al., 2011). The medium-dose group exhibited more pronounced degeneration, including disrupted hepatic cords and enlarged vacuolated areas, which suggested escalating cellular damage due to compromised detoxification pathways. Similar findings have been documented for neonicotinoid exposure in aquatic organisms, where hepatocytes showed progressive degeneration with increasing concentrations (Velisek et al., 2006). The high-dose treatment produced severe hepatic deterioration, characterized by extensive cytoplasmic homogenization, loss of cellular boundaries, and necrotic patches. These lesions are consistent with advanced hepatotoxicity caused by prolonged xenobiotic stress, particularly from neonicotinoids known to induce oxidative imbalance and impaired liver function (Simon-Delso et al., 2015). The dose-related escalation in tissue damage across treatments thus supported the conclusion that thiamethoxam exerts cumulative, concentration-dependent toxicity on fish liver tissue.

4. Conclusion

The liver histopathology of *Anabas* sp. exposed to thiamethoxam for 21 days has demonstrated a clear, dose-dependent pattern of hepatic injury. While the control fish maintained normal liver architecture, increasing pesticide concentrations progressively intensified cellular disruption—from mild vacuolation at low dose, to marked cytoplasmic degeneration at medium dose, and extensive necrosis and architectural collapse at high dose. These cumulative alterations indicate that thiamethoxam imposes sustained oxidative and metabolic stress on hepatic tissue, ultimately impairing structural integrity and physiological function. Overall, the findings have confirmed that prolonged exposure to thiamethoxam, even at relatively low environmental concentrations, can exert significant hepatotoxic effects on freshwater fish.

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