



Protective Role of Folic Acid against Tartrazine-induced Hepatotoxicity in Nile Tilapia: Bridging the Biochemical Complexity and Clinical Reliance

Supriyo Acharya^{1,2}, Muthu Sampath², Malabika Bhattacharjee¹

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ABSTRACT

Background: Tartrazine, a synthetic azo dye commonly used as a food colorant, has been implicated in various adverse health effects, including hepatotoxicity. It has been associated with multiple adverse health outcomes, including renal dysfunction. Contamination of tartrazine occurs in various aquatic systems to certain extent by direct exposure from manufacturing bodies or additionally through hotel kitchen run-off water, leftover food and drinks. This study aimed to evaluate the hepatotoxic effects of tartrazine on *Oreochromis niloticus* (Nile tilapia) and to assess the potential protective role of Folic acid as a dietary intervention which showed promising anti-inflammatory action in many studies.

Methods: This study investigates the hepatotoxic effects of tartrazine on *Oreochromis niloticus* (Nile tilapia) and explores the potential mitigation of these effects through dietary intervention of folic acid. *O. niloticus* specimens were exposed to tartrazine through water medium over a specified period. Hepatotoxicity was assessed through biochemical parameters including liver enzyme activities and histological analysis.

Result: Histological examination confirmed hepatocellular degeneration and necrosis. Results revealed a significant increase in liver enzyme activities, indicating hepatocellular damage suggestive of oxidative stress, in tartrazine-exposed fish. To mitigate tartrazine-induced hepatotoxicity, a dietary compound, in this case, Folic Acid, was administered to another group of fish concurrently exposed to tartrazine. Biochemical analysis showed that fish receiving the dietary intervention exhibited reduced liver enzyme activities compared to the tartrazine-only group. Histological examination of liver tissues from the intervention group revealed amelioration of hepatocellular damage, with reduced signs of degeneration and necrosis compared to the tartrazine-only group. These findings suggest that the dietary compound effectively mitigated tartrazine-induced hepatotoxicity in *O. niloticus* by attenuating oxidative stress and enhancing antioxidant defenses.

Key words: Folic acid, Hepatotoxicity, Liver enzymes, Liver histopathology, *Oreochromis niloticus*, Tartrazine.

INTRODUCTION

Tartrazine (E102) is a synthetic azo dye widely used as a food colorant due to its bright lemon-yellow appearance. Chemically, it is known as trisodium-5-hydroxy-1-(4-sulfonatophenyl)-4-(E)-(4-sulfonatophenyl)diazenyl)-1H-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$), with a molecular weight of 534.3 kDa. It is commonly present in soft drinks, sports beverages, flavored chips, ice creams, jams, jellies and chewing gums (Mittal *et al.*, 2007). Tartrazine is also identified by several regulatory and commercial names, including FDandC Yellow No. 5, E102, ISN No. 112, CAS No. 1934-21-0 and CI 19140. Despite its extensive application, concerns regarding its safety persist due to accumulating evidence of adverse effects on neurological development, vascular integrity and organ function across various experimental models (Khayyat *et al.*, 2017; Haridevamuthu *et al.*, 2023; Thanh *et al.*, 2024; Visternicu *et al.*, 2025).

The toxicity of tartrazine is primarily associated with its reductive metabolic biotransformation, which generates metabolites such as sulfanilic acid and aminopyrazolone, particularly following its release into aquatic environments (Suma, 2019). These metabolites can induce the

¹Molecular Cell Biology Lab, Post Graduate Department of Zoology, Vivekananda College, Kolkata-700 063, West Bengal, India.

²Department of Bioengineering and Biotechnology, Birla Institute of Technology, MESRA, Kolkata-700 063, West Bengal, India.

Corresponding Author: Supriyo Acharya, Molecular Cell Biology Lab, Post Graduate Department of Zoology, Vivekananda College, Kolkata-700 063, West Bengal, India.

Email: sa2.zoology@sajaipuriacollege.ac.in

ORCID: 0009-0002-1157-6243

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generation of reactive oxygen species (ROS), leading to oxidative stress and subsequent hepatic and renal damage, as reflected in altered biochemical and structural profiles (Chequer *et al.*, 2011; Chung and Cerniglia, 1992; Himri *et al.*, 2011). Recent studies further demonstrate that tartrazine-induced oxidative stress can trigger

mitochondrial dysfunction, apoptosis and genotoxicity, thereby intensifying tissue injury and systemic toxicity (Haridevamuthu *et al.*, 2023; Khayyat *et al.*, 2017; Visternicu *et al.*, 2025). Given the complexity of its metabolic pathways and organism-specific responses, comprehensive investigations are required to better elucidate its ecological and biological risks (Amchova *et al.*, 2024; Alshehrei, 2025).

This study focuses on *Oreochromis niloticus* (Nile tilapia), a well-established model species in aquatic toxicology due to its ecological relevance and sensitivity to environmental contaminants. The primary objective is to evaluate histopathological alterations in hepatic tissue following exposure to tartrazine-contaminated water. Additionally, liver function will be assessed through key enzymatic biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. Considering emerging evidence on dietary protective agents, the study also investigates the potential mitigating role of folic acid, known for its antioxidant and anti-inflammatory properties, against tartrazine-induced hepatotoxicity (Amchova *et al.*, 2024).

MATERIALS AND METHODS

Toxic heavy element considered for the study

Tartrazine was purchased from Merck (Germany). Its molecular weight is 534.36 and $C_{16}H_9N_4Na_3O_9S_2$ is the formula of it. The chemical structure of Tartrazine is shown in Fig 1.

Mitigating agent considered for the study

Folic acid (Vitamin B9 powder) is used as mitigating supplementation to nullify the toxic impact of tartrazine. It was administrated with tartrazine in an equivalent dose strategy as mentioned in Table 1.

Reagents

Reagents used in histological slide preparation

Neutral buffered formalin fixative; Paraffin (m.p. 50-60°C); Mayer's albumen; Xylene; Graded series of alcohols (50%, 70%, 90%, 100%); Distilled water; Delafield's hematoxylin; 2% alcoholic eosin; DPX Mountant.

Reagents associated with protein profiling

Alkaline Sodium carbonate solution; $CuSO_4$ Na-K Tartarate solution; BSA Stock Solution; Alkaline $CuSO_4$ working solution; Working Folin-Ciocalteu Phenol solution

Reagents used in biochemical assay

GOT [AST-Aspartate aminotransferase] measurement- Kit manufactured by ARKRAY Healthcare private limited; 2,4-DNPH colour reagent; Working pyruvate standard; Sodium hydroxide.

GPT [ALT-Alanine amino transferase] measurement- Kit manufactured by ARKRAY Healthcare private limited; Buffered Alkaline- α -KG substrate; 2,4-DNPH colour reagent; Working oxaloacetate standard; Sodium hydroxide.

ALP [Alkaline phosphatase activity] measurement- Kit manufactured by ARKRAY Healthcare private limited; Chromogen reagent; Phenol standard; Buffered substrate.

SOD [Superoxide dismutase], CAT [Catalase activity], GPx [Glutathione peroxidase] and MDA [Malondialdehyde test] measurement- Kit manufactured by Sigma Aldrich 19184-2-KT-F, phosphate-buffered saline, xanthine oxidase, Nitroblue tetrazolium.

Animals and experimental design

Juvenile *Oreochromis niloticus* (28-30 g) were acclimatized for six days and assigned to control and treatment groups ($n = 10$). Fish were maintained at $\sim 30^\circ C$, fed twice daily (2-2.5% body weight), fasted before sampling and monitored daily during the 96-h exposure. All experimental procedures were carried out in compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and CPCSEA.

The LC_{50} value for Tartrazine had been worked out to be 0.072 g/L following the method developed by Pichhode *et al.* (2022). Based on this value, two exposure concentrations were selected: $2 \times LC_{50}$ (0.144 g/L) and $3 \times LC_{50}$ (0.216 g/L) which were administered in aquaria containing 40 L of water. For folic acid supplementation, equivalent concentrations were co-administered with the respective tartrazine treatments. The experiment was conducted in the Molecular Cell Biology Laboratory of the Postgraduate Department of Zoology, Vivekananda College (affiliated to the University of Calcutta), between February and October 2025.

Each group was maintained under identical environmental conditions and all concentrations were selected based on preliminary LC_{50} studies to evaluate dose-dependent toxicological effects and the potential protective role of folic acid.

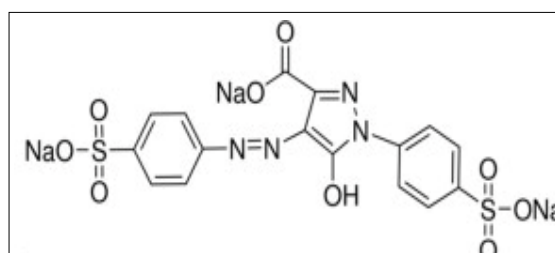


Fig 1: Chemical structure of tartrazine.

Table 1: Animal Groups that were maintained throughout the study.

Experimental animal groups with concentration of treated chemicals	Abbreviations
Control	NC
2xTartrazine [0.144 g/L]	2xTt
Control folic Acid [0.144 g/L]	CFa1
2xTartrazine [0.144 g/L] + FA [0.144 g/L]	2xTtFa
3xTartrazine [0.216 g/L]	3xTt
Control folic acid [0.216 g/L]	CFa2
3xTartrazine [0.216 g/L] + FA [0.216 g/L]	3xTtFa

Histological assessment

Livers from dead fish were immediately excised, processed and analyzed. Tissues were homogenized (10%) in 0.25 M sucrose, centrifuged and the supernatants stored for enzymatic assays (Deepika *et al.*, 2023). Portions were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned and stained with haematoxylin-eosin for histological evaluation (Parvin *et al.*, 2019; Kundu, 2018).

Total liver protein estimation

Protein content was estimated according to the method of Lowry *et al.* (1951) using BSA as standard (Reitman and Frankel, 1957).

Estimation of GPT [Alanine amino transferase] activity

GPT activity from liver tissues from control and experimental sets were measured by using Alanine and α -ketoglutarate as substrates and monitoring the concentration of a brown coloured pyruvate hydrazone formed on addition of 2, 4 dinitrophenyl-hydrazine (Reitman and Frankel, 1957). The absorbance was taken in at 505 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119) and the activity was expressed as nM/min/mg protein (Kundu 2018).

Measurement of GOT [Aspartate aminotransferase] activity

GOT activity from liver tissues from control and experimental sets were measured by using Aspartate and α -ketoglutarate as substrates and monitoring the concentration of a brown coloured pyruvate hydrazone formed on addition of 2, 4 dinitrophenyl- hydrazine (Reitman and Frankel, 1957). The absorbance was taken at 505 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119) and the activity was expressed as nM/min/mg protein.

Measurement of alkaline phosphatase (AP) activity

AP activity (μ moles/min/mg protein) of liver were detected according to the method of Kind and King (1954) by measuring the intensity of the colour formed when AP hydrolyses di Sodium Phenylphosphate substrate to form phenol that further reacts with 4-Aminoantipyrine in the presence of Potassium Ferricyanide. The reading was taken at 510 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119) (Kundu, 2018).

Antioxidants level assay

The activities of antioxidant enzymes including the catalase enzyme (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed in liver tissue of the treated group specimen using commercial assay kits according to the manufacturer's instructions. The lipid peroxidation end product, malondialdehyde (MDA) was measured as thiobarbituric acid reactive substance (Ayaz *et al.*, 2016).

Statistical analysis

All data were statistically analyzed using SPSS software (version XX, IBM Corp., Armonk, NY, USA). One-way analysis

of variance (ANOVA) followed by appropriate post-hoc tests was employed to determine significant differences among groups, with results expressed as mean $\pm$ standard error (SE). A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Histological assessment of liver tissue

In Tilapia, exposure to Tartrazine at elevated doses Fig 2, such as double and triple doses (2xTt and 3xTt) Fig 2C and 2D with respect to Normal Fig 2A and 2B, has been shown to induce hepatocyte degeneration, necrosis and inflammation, indicating significant liver damage. These histological changes collectively impair liver function and compromise the overall health of Tilapia, making them more susceptible to various diseases and physiological

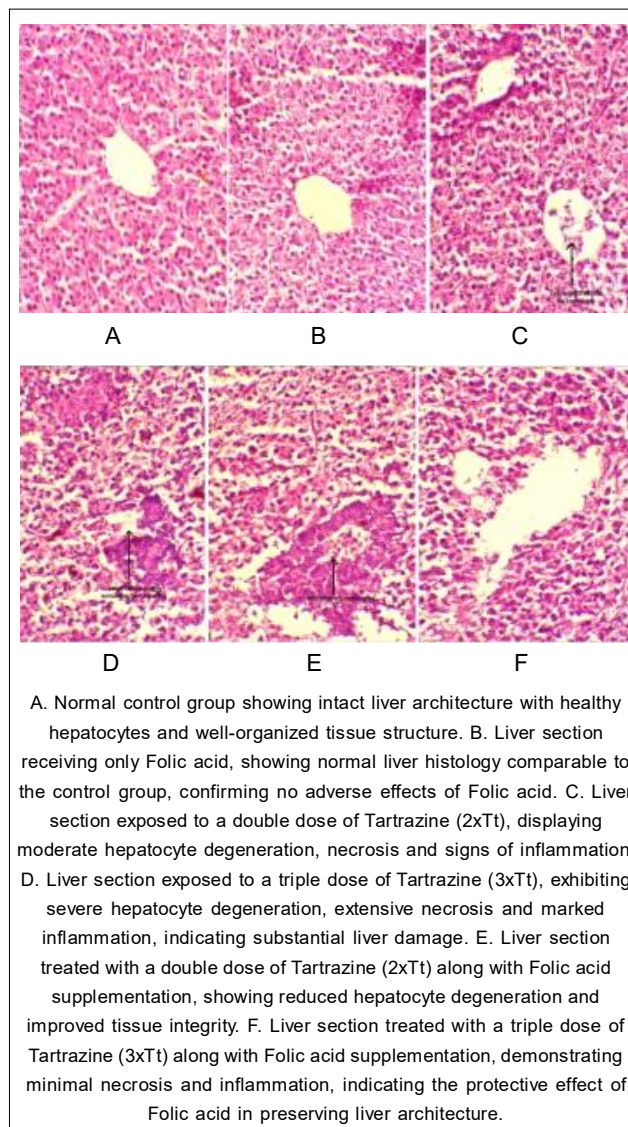


Fig 2: Histological sections of Tilapia liver under different treatment conditions.

stressors. However, the concurrent supplementation of Folic acid alongside Tartrazine exposure appears to counteract these detrimental effects on Tilapia liver tissue *viz* Fig 2E and 2F. In Tilapia exposed to Tartrazine and supplemented with Folic acid, a notable reduction in hepatocyte degeneration, necrosis and inflammation are observed, indicating the preservation of liver tissue architecture.

Liver function assessment

The liver is a very important organ which breaks down chemicals and as a result, liver cells are often among those that are damaged by toxic chemicals, hence estimating certain liver parameters assume prime importance.

Hepatosomatic index

The hepatosomatic index (HSI) is a metric used to assess the relative size and condition of the liver in relation to the total body weight of an organism, typically fish. It is calculated by dividing the liver weight by the total body weight and multiplying by 100. Changes in the HSI value suggests alterations in liver size or structure, indicating potential liver damage or dysfunction as seen in Table 2.

In our observation, the Normal Control (NC) group has an HSI value of 5.075, providing a baseline for comparison. When Tartrazine is administered at 2xTt, the HSI decreases to 2.14, suggesting a significant impact on liver health. This reduction might indicate potential hepatotoxic effects of Tartrazine at this concentration. Similarly, a further decrease in HSI is observed at 3xTt with a value of 1.888, indicating a dose-dependent effect on liver health. Conversely, the addition of Folic acid alongside Tartrazine (2xTtFa and 3xTtFa) shows a noticeable increase in HSI values. At 2xTtFa, the HSI rises to 3.333 and at 3xTtFa, it rises to 1.98. More efficient recovery is seen in 2xTtFa dose [3.33] compared to 3xTtFa [1.88]. These results suggest a potential protective effect of Folic acid at a lower dose against the hepatotoxicity induced by Tartrazine.

Estimation of total liver protein content

The total liver protein content is a crucial parameter that reflects the metabolic activity and health status of the liver in fish. The Normal Control (NC) group recorded a total liver protein content of 25.05 mg g⁻¹ wet weight, serving as the physiological baseline. Exposure to tartrazine at a

double dose (2xTt) caused a significant reduction to 17.707 mg g⁻¹ wet weight, indicating marked impairment of hepatic metabolism and protein synthesis. This effect was further aggravated at the triple dose (3xTt), where protein content declined to 14.711 mg g⁻¹ wet weight, clearly demonstrating dose-dependent hepatotoxicity. Notably, folic acid co-administration (2xTtFa and 3xTtFa) partially restored liver protein levels to 23.774 and 19.293 mg g⁻¹ wet weight, respectively. This recovery highlights the protective role of folic acid in mitigating tartrazine-induced metabolic disruption, likely through enhancement of protein synthesis and preservation of hepatic cellular integrity.

Liver enzymes assay

Estimation of GPT [ALT]

GPT activity from liver tissues from control and experimental sets were measured by using Alanine and α -ketoglutarate as substrates and monitoring the concentration of a brown coloured pyruvate hydrazone formed on addition of 2, 4 dinitrophenyl-hydrazine (Reitman and Frankel, 1957). The absorbance was taken in at 505 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119) and the activity was expressed as IU/min/mg protein. Monitoring ALT levels helps in diagnosing liver conditions, evaluating disease progression and assessing the effectiveness of treatments. Early detection of elevated ALT levels allows for timely intervention and management of liver diseases, potentially preventing further liver damage and improving overall patient outcomes.

As observed in Fig 3, in Control (C) fishes the liver GPT activity is 36.99 IU/min/mg protein. In case of 2xTt the activity is sharply increased to 94.57 nM/min/mg protein. For 3xTt the GPT activity is strikingly decreased to 2.15 IU/min/mg protein. The values of GPT in the groups treated with two different doses of folic acid (FA) are 32.61 IU/min/mg protein and 32.64 IU/min/mg protein respectively, that are insignificant compared to the control group. Interestingly, GPT activity approached the control values in case of both 2xTtFa (28.86 IU/min/mg protein) and 3xTtFa (39.77 IU/min/mg protein). The sharp fall in GPT activity in 3xTt exposed fish is correlated to damage of the cellular integrity of the hepatocytes including disintegration of the central vein, leading to change in membrane permeability

Table 2: Weight of liver tissue and body weight in different experimental groups along with the reflection of HSI index and corresponding total liver protein.

Test groups	Liver weight [g]	Body weight [g]	HSI	Liver total protein [mg g ⁻¹ wet wt]
NC	3.045	60	5.075	25.05
2xTt	1.177	55	2.14	17.707
CFa1	2.993	61	4.907	24.318
2xTtFa	2	60	3.333	23.774
3xTt	1.001	53	1.888	14.711
CFa2	2.993	61	4.907	25.48
3xTtFa	1.092	55	1.98	19.293

and thus leakage of the enzymes into blood. The subsequent recovery in GPT activity upon treatment with folic acid is reflected as revival of the cellular integrity and the regenerative capability of folic acid.

Estimation of GOT [AST]

GOT activity from liver tissues from control and experimental sets were measured by using Aspartate and α -ketoglutarate as substrates and monitoring the concentration of a brown coloured pyruvate hydrazone formed on addition of 2, 4 dinitrophenyl-hydrazine (Reitman and Frankel, 1957). The absorbance was taken at 505 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119) and the activity was expressed as IU/min/mg protein. Measuring liver aspartate aminotransferase (AST) levels is significant in assessing liver health and function. AST is an enzyme present in various tissues, including the liver, heart and muscles. Measuring AST helps in diagnosing liver conditions such as viral hepatitis, cirrhosis and drug-induced liver injury.

As observed in Fig.4, in Control (C) fishes the liver GOT activity is 0.69 IU/min/mg protein. In case of 2xTt group

the activity is significantly decreased to 0.04 IU /min/mg protein but is comparatively increased to 0.52 IU /min/mg protein in 3xTt group. The GOT activities in the groups treated with two different doses of folic acid are 0.63 IU/min/mg protein and 0.59 IU/min/mg protein respectively. Interestingly, GOT regained the control values in case of 2xTtFa (0.63 IU/min/mg protein). But in 3xTtFa group the activity declined to 0.29 IU/min/mg protein compared to both 3xTt and control groups. The decrease in GOT activity in 2xTt is correlated to damage of the cellular integrity of the hepatocytes leading to change in membrane permeability accompanied by leakage of the enzymes into blood. However, the increase in GOT activity in 3xTt compared to 2xTt may be due to increase in enzyme synthesis to overcome the acute stress effect of the higher dose of tartrazine. The subsequent recovery in GOT activity to the control level upon treatment with folic acid along with 2xTt can be correlated with the revival of the histological profile.

Estimation of AP

ALP activity (KA/min/mg protein) of liver were detected according to the method of Kind and King (1954) by

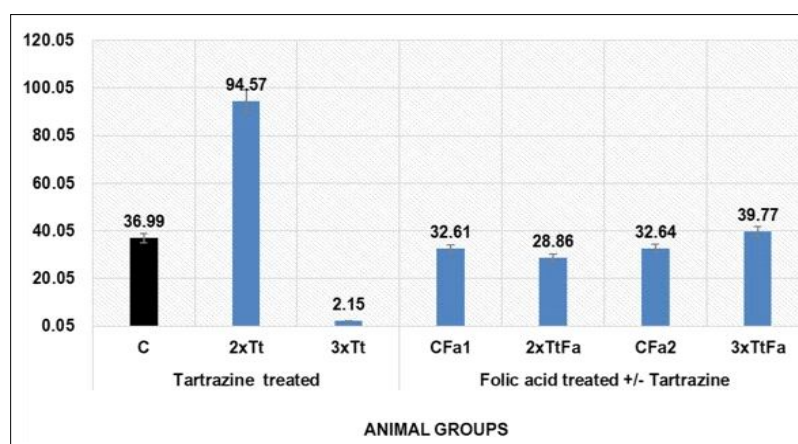


Fig 3: Activity of GPT/AST (IU/min/mg protein) in different experimental group.

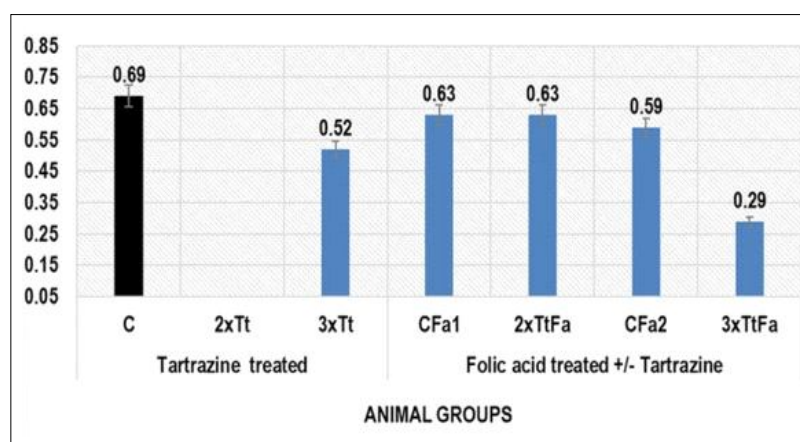


Fig 4: Activity of GOT/AST (IU/min/mg protein) in the different experimental group.

measuring the intensity of the colour formed when AP hydrolyses di Sodium Phenylphosphate substrate to form phenol that further reacts with 4-Aminoantipyrine in the presence of Potassium Ferricyanide. The reading was taken at 510 nm in a spectrophotometer (Systronix UV-Vis Spectrophotometer-119).

Alkaline phosphatase (ALP) activity serves as an important biomarker of liver function and metabolic status. As shown in Fig 5, control (C) fish exhibited an ALP activity of 0.44 KA/min/mg protein. In the 2xTt group, ALP activity increased markedly to 1.06 KA/min/mg protein, indicating enhanced metabolic stress and altered energy regulation. In contrast, a drastic decline to 0.02 KA/min/mg protein in the 3xTt group reflects severe metabolic impairment and hepatic failure at higher tartrazine exposure. In folic acid-treated groups, ALP activities (0.50 and 0.52 KA/min/mg protein) were not significantly different from control, suggesting metabolic stability. Partial restoration was evident in 2xTtFa (0.78 KA/min/mg protein) and 3xTtFa (0.65 KA/min/mg protein), demonstrating the hepatoprotective and metabolic modulatory role of folic acid. The elevated ALP in 2xTt may result from a metabolic shift toward phosphatase-dependent energy pathways and increased enzyme turnover under stress condition, whereas the sharp decline in 3xTt possibly indicates metabolic collapse under excessive toxic load. Slightly higher ALP in folic acid-only

groups may reflect minor assay interactions rather than physiological stress.

Individual impact of tartrazine shock and folic acid supplementation on antioxidant level status

Treatment of tartrazine doses (2xTt and 3xTt) caused significant ($p < 0.05$) decreases in the hepatic tissue SOD, CAT and GPx activities while causing significant ($p < 0.001$) increases in the hepatic tissue MDA level. Surprisingly, administration of folic acid supplementation (2xTtFa and 3xTtFa) in the groups under tartrazine exposure significantly ($p < 0.05$) attenuated decreases in the hepatic tissue activities of SOD, CAT and GPx while significantly attenuated increases in the hepatic tissue MDA levels (Table 3).

Histological examination revealed clear dose-dependent hepatic damage in tartrazine-exposed fish, with pronounced alterations in the 2xTt and 3xTt groups. Pathological features such as hepatocyte degeneration, necrosis and inflammatory infiltration indicate impaired detoxification and tissue dysfunction, consistent with xenobiotic-induced liver injury (Donmez and Cengizier, 2020; Chequer *et al.*, 2011). Although inflammation represents a protective response (Vishwakarma *et al.*, 2021), the progressive severity from 2xTt to 3xTt suggests enhanced oxidative stress and cellular injury at higher tartrazine doses. Similar dose-dependent hepatic alterations following azo dye exposure have been reported

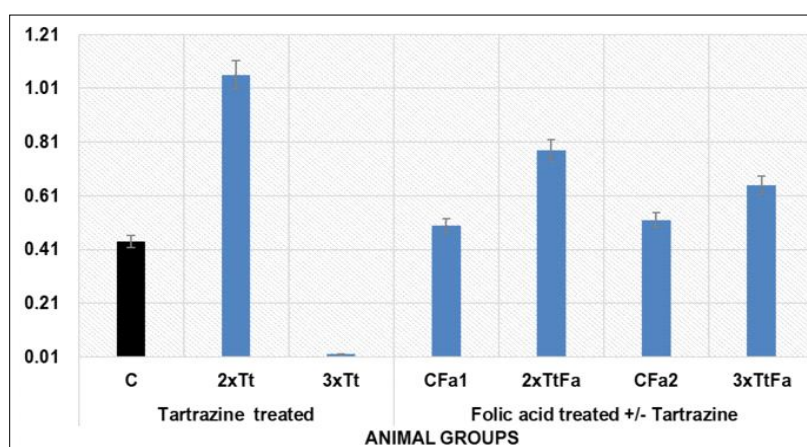


Fig 5: Activity (KA/min/mg protein) of ALP in the different experimental groups.

Table 3: Mitigatory impact of aqueous folic acid supplementation on liver tissue SOD, CAT, GPx activities and MDA level in experimental fish groups pre-exposed to tartrazine.

Parameters	Fish groups				
	Normal control	Experimental groups			
		Tartrazine exposed groups		Folic acid supplemented groups	
		2xTt	3xTt	2xTtFa	3xTtFa
SOD (U/mgprotein)	4.35	3.14	2.11	3.91	4.06
CAT (U/mg protein)	2.84	1.61	1.32	2.21	2.12
GPx (mU/mg protein)	35.28	25.23	25.98	33.24	33.45
MDA (nmol/g tissue)	30.44	52.27	52.34	32.11	31.12

in fish and mammalian models (Himri *et al.*, 2011; Wusu *et al.*, 2023). In contrast, folic acid-supplemented groups (2xTtFa and 3xTtFa) exhibited relatively preserved hepatic architecture, reflecting attenuation of tartrazine-induced damage, likely through improved cellular integrity, DNA repair and antioxidant defense (Ibrahim *et al.*, 2019).

The hepatosomatic index (HSI) further corroborated histopathological findings. Marked reductions in HSI in tartrazine-treated groups (2xTt: 2.14; 3xTt: 1.888) indicate liver atrophy and impaired functional capacity, with greater decline at higher exposure levels. Comparable reductions in HSI have been associated with chemically induced hepatic stress and metabolic exhaustion in fish (Schlenk *et al.*, 2008). Folic acid supplementation improved HSI values in 2xTtFa (3.333) and 3xTtFa (1.98), suggesting partial restoration of liver mass and functional resilience against tartrazine.

Total liver protein content also declined significantly in tartrazine-exposed groups (2xTt: 17.707 mg/g; 3xTt: 14.711 mg/g), indicating disrupted protein synthesis and metabolic impairment due to oxidative damage out of chemical insult by tartrazine. Such reductions are characteristic of toxicant-induced metabolic dysfunction (Begum, 2004; Adeyemi *et al.*, 2015). Partial recovery in folic acid-treated groups (2xTtFa: 23.774 mg/g; 3xTtFa: 19.293 mg/g) reflects improved metabolic efficiency and cellular synthetic capacity.

Key hepatic enzymes showed pronounced alterations. GPT activity increased sharply in 2xTt (94.57 IU/min/mg protein), indicating membrane damage and enzyme leakage, but declined drastically in 3xTt (2.15 IU/min/mg protein), suggesting severe hepatocyte destruction. Folic acid normalized GPT activity in 2xTtFa (28.86 IU/min/mg) and 3xTtFa (39.77 IU/min/mg). GOT activity decreased in 2xTt (0.04 IU/min/mg) and increased in 3xTt (0.52 IU/min/mg), reflecting leakage followed by compensatory synthesis under severe stress (Singh *et al.*, 2016). Folic acid restored GOT near control levels in 2xTtFa (0.63 IU/min/mg) and partially in 3xTtFa (0.29 IU/min/mg). ALP activity increased in 2xTt (1.06 KA/min/mg) but declined sharply in 3xTt (0.02 KA/min/mg), while folic acid restored activity in 2xTtFa (0.78 KA/min/mg) and 3xTtFa (0.65 KA/min/mg).

Finally, tartrazine reduced antioxidant enzymes (CAT, SOD, GPx) and elevated MDA, whereas folic acid dose-dependently restored antioxidant status and reduced lipid peroxidation. Collectively, these findings demonstrate that tartrazine induces dose-dependent hepatotoxicity in *O. niloticus* via oxidative stress, while folic acid effectively mitigates structural, metabolic and enzymatic damage.

CONCLUSION

In conclusion, the present findings highlight the protective efficacy of folic acid in mitigating tartrazine-induced hepatotoxicity in *Oreochromis niloticus*. Folic acid supplementation effectively preserved liver histoarchitecture and restored key enzymatic activities, particularly ALP, under chemical stress. These results provide valuable insights

into tartrazine-induced hepatic toxicity and support the potential application of folic acid in environmental risk assessment, regulatory decision-making and sustainable aquatic health management.

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Declarations

Authors' contributions

The research and manuscript preparation were carried out by the corresponding author, *Supriyo Acharya*, under the supervision of *Dr. Malabika Bhattacharjee*. *Dr. Muthu K. Sampath* contributed by providing information regarding reagents.

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Ethical approval

All experimental procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC), BIT Mesra, Ranchi (Registration No. 326/GO/ReBiBt/D/2001/CPCSEA).

Conflict of interest

The authors declare no conflict of interest.

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