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Impact of Tartrazine on Kidney Function in Tilapia and the Mitigating Effect of Folic Acid: A Biochemical and Histopathological Evaluation

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ABSTRACT

Background: Tartrazine, a synthetic azo dye widely used as a food colorant with considerable amount of demand in market. It has been associated with multiple adverse health outcomes, including renal dysfunction. Substantial amount of contamination of tartrazine takes place 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 nephrotoxic 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 role in many studies.

Methods: Fish were exposed to tartrazine *via* the aquatic medium for a defined duration. Renal toxicity was evaluated by measuring serum urea and creatinine levels, alongside histological examination of renal tissues. Tartrazine exposure led to a significant elevation in serum urea and creatinine, accompanied by marked histopathological changes, including tubular degeneration and necrosis, indicating oxidative stress-mediated renal damage. To counteract these effects, folic acid was incorporated into the diet of a separate group of tartrazine-exposed fish.

Result: Biochemical assessments demonstrated a notable reduction in serum urea and creatinine levels in the folic acid-treated group. Histological analysis further confirmed a considerable improvement in renal architecture, with diminished signs of tubular degeneration and necrosis. These findings suggest that dietary folic acid effectively mitigates tartrazine-induced nephrotoxicity in *O. niloticus*, likely through its antioxidant and cytoprotective properties.

Key words: Folic acid, Histopathology, *Oreochromis niloticus,* Oxidative stress, Renal toxicity tartrazine.

INTRODUCTION

Tartrazine is a synthetic azo dye belonging to the monoazo and pyrazolone families, primarily valued for its intense lemon-yellow to orange hue. Its chemical designation is trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(E)-(4-sulfonatophenyl)diazenyl]-1H-pyrazole-3-carboxylate, with the molecular formula $C_{16}H_9N_4NA_3O_9S_2$ (Fig 1) and a molecular weight of 534.36 g·mol^{-1}. The dye exhibits high water solubility and is characterized by a strong absorption maximum (λmax) at approximately 425 nm in aqueous solutions, properties that underpin its widespread application as a food and beverage colorant. Tartrazine is registered under multiple commercial identifiers, including INS No. 102, FD and C Yellow No. 5, E102, CAS No. 1934-21-0 and CI 19140 (Smith *et al.,* 2010).

Tartrazine is extensively used in a wide variety of consumables, including ice creams, desserts, sweetened rice preparations (such as biriyani and pulao), soft drinks (*e.g.*, Mountain Dew), popcorn, chewing gum, fruit cordials, fermented beverages, cotton candy, puddings, laddu-modaks, jelly, pickles, marmalade, instant soups, Maggie masala, custards and falooda mixes, among others, owing to its desirable yellow to greenish-brown hue (Sharma and Chattopadhyay, 2012). Despite its widespread use, growing concerns have emerged regarding the environmental and biological toxicity of tartrazine. Several studies have reported that tartrazine induces oxidative stress, histopathological damage and organ-specific toxicity in both terrestrial and aquatic models, highlighting its potential health risks (Al-Shaheen *et al.,* 2021; Ma *et al.,* 2021). The persistence of tartrazine in aquatic ecosystems is particularly alarming, as urban and peri-urban areas often witness the discharge of improperly discarded food waste and kitchen effluents from restaurants and hotels directly into local water bodies. Such contaminated runoffs, enriched with synthetic dyes

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like tartrazine, pose significant ecological threats to aquatic fauna (Shahid *et al.,* 2024).

Among vital organs, the kidneys play a crucial role in the removal of waste products and toxins, as well as in the maintenance of water, electrolyte and acid-base balance (Shivashakthi *et al.,* 2019). Renal toxicity is therefore a sensitive and reliable endpoint in ecotoxicological assessments. In this context, the Nile tilapia, *Oreochromis niloticus*, has been selected as the experimental model due to its ecological significance, economic value and extensive use as a bioindicator in biomonitoring studies. Alterations in the activities of biotransformation enzymes in this species have previously been employed to reflect the impact of diverse environmental contaminants (Seena *et al.,* 2024), thereby justifying its suitability for the present study.

The primary objectives of the current investigation are: (i) to evaluate the renal toxicity of tartrazine in *O. niloticus* through histopathological analysis of kidney tissues, (ii) to assess serum urea and creatinine levels as reliable biochemical indicators of kidney function (Rao, 2006) and (iii) to explore a mitigation strategy through dietary supplementation of folic acid. Folic acid, a synthetic form of folate (Vitamin B9), plays an essential role in DNA and RNA synthesis, red blood cell generation and cellular repair. Recent evidence suggests that folic acid exhibits promising therapeutic potential in counteracting oxidative stress and tissue damage induced by environmental pollutants in aquatic organisms (Huang *et al.,* 2019; Al-Shaheen *et al.,* 2023; Zhou *et al.,* 2024). Therefore, its dietary administration in tartrazine-exposed fish is assessed here as a novel approach to alle*via*te renal damage and restore physiological balance.

MATERIALS AND METHODS

Reagents used for histological slide preparation

The following reagents were used for the histological processing of kidney tissues:

· **Fixative:** Neutral buffered formalin (10%).

· **Embedding medium:** Paraffin wax (melting point: 50-60°C).

· **Adhesive for slides:** Mayer's albumen.

· **Clearing agent:** Xylene.

· **Dehydrating agents:** Graded series of ethanol (50%, 70%, 90% and 100%).

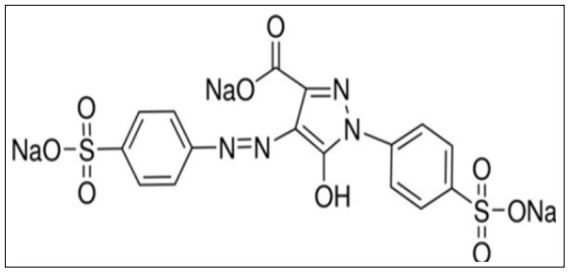


Fig 1: Chemical structure of tartrazine.

· **Staining reagents**

o Delafield's hematoxylin.
o 2% Alcoholic eosin.

· **Mounting medium:** DPX.

· **Washing agent:** Distilled water.

Reagents used for kidney function tests

· **Anticoagulant:** EDTA.

· **Commercial kits:** Urea and creatinine estimation kits (based on enzymatic colorimetric method) provided by Beacon, were used for assessing Urea and Creatinine level.

Reagents used for superoxide dismutase (SOD) and catalase activity (CAT) tests

· **Commercial kits:** Abbkine superoxide dismutase assay kit and elabscience assay kit were used for subsequent determination of SOD and CAT value in the tissue sample.
· Ehanol.

Toxicant used in the study

Tartrazine, a synthetic azo dye, was selected as the test compound in this study due to its significant environmental relevance. To evaluate its toxicological effects, *Oreochromis niloticus* (Nile tilapia) was exposed to tartrazine at concentrations based on its experimentally determined LC_{50} (lethal concentration for 50% mortality), which was found to be 0.072 g/L. To investigate sub-lethal toxicity, two elevated exposure doses were selected: 2X LC_{50} (0.144 g/L) and 3X LC_{50} (0.216 g/L). These concentrations were introduced into separate aquaria, each containing 40 liters of water, to study the dose-dependent physiological and histopathological effects of tartrazine on the test species.

Mitigating agent

To evaluate its protective role against tartrazine-induced toxicity, Folic acid was considered to be administered at doses equivalent to the respective tartrazine exposure levels. Specifically, a dose of 0.144 g/L was used in the 2X tartrazine-exposed group and 0.216 g/L was used in the 3X tartrazine-exposed group. This approach allowed for a comparative assessment of efficacy of Folic acid in mitigating dose-dependent renal damage induced by tartrazine.

Experimental animals and design

The test species selected for the study was juvenile *Oreochromis niloticus* with an average body weight of 28-30 g. Each control and experimental group comprised ten fish. Specimens were procured from a local estuarine fish farm and acclimatized for four days in laboratory aquaria maintained at ambient room temperature (~30°C). To minimize nitrite toxicity and ensure a stable aquatic environment, sodium chloride (NaCl; reagent grade) was added to all experimental tanks at a final concentration of 15 g/L (Dhanasiri *et al.,* 2023). Fish were fed either a high-

quality commercial tilapia pellet (≥30% crude protein, extruded) or an equivalent laboratory-prepared diet. Feeding was performed twice daily, at 09:00 and 17:00, at a ratio of approximately 2-2.5% of body weight, evenly divided between meals. To avoid water fouling, only the amount of feed consumed within 3-5 minutes was provided and uneaten pellets were promptly removed.

Prior to terminal sampling and blood collection, fish were fasted for 24 h to stabilize biochemical parameters. Throughout the experimental period, feeding behavior, mortality and water quality parameters (temperature, dissolved oxygen, pH, ammonia and nitrite) were monitored daily; mortalities were recorded and dead fish were immediately removed. The experimental exposure lasted for 96 h across all treatment groups. All procedures were conducted in compliance with the Institutional Animal Ethics Committee (IAEC), BIT Mesra, Ranchi (Registration No. 326/GO/ReBiBt/D/2001/CPCSEA).

The experimental design consisted of five groups as follows:

1. **Negative control (NC):** No exposure to tartrazine or folic acid.
2. **Tartrazine 2 × LC_{50} (0.144 g/L) (2 × Tz):** Exposure to twice the median lethal concentration of tartrazine.
3. **Tartrazine 3 × LC_{50} (0.216 g/L) (3 × Tz):** Exposure to three times the median lethal concentration of tartrazine.
4. **Tartrazine 2 × LC_{50} + Folic acid 2 × dose (0.144 g/L each) (2 × TzFa):** Co-exposure to tartrazine at 2 × LC_{50} and folic acid at 0.144 g/L.
5. **Tartrazine 3 × LC_{50} + Folic acid 3 × dose (0.216 g/L each) (3 × TzFa):** Co-exposure to tartrazine at 3 × LC_{50} and folic acid at 0.216 g/L.

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.

Rationale for dose selection

The median lethal concentration (LC_{50}) of tartrazine is established at 0.072 g/L. In this study, exposure concentrations equivalent to 2x and 3x the LC_{50} dose were employed to investigate the sub-lethal and organ-specific toxicological impacts of tartrazine, with a particular focus on renal tissue. These elevated concentrations, although exceeding the immediate lethal threshold, were essential to simulate acute exposure scenarios and to elicit measurable histopathological and biochemical responses. This approach enabled a more precise evaluation of nephrotoxicity, aiding in the identification of early biomarkers of renal dysfunction. The results offer valuable insights into the ecological risks associated with tartrazine contamination in aquatic ecosystems and support the formulation of evidence-based regulatory guidelines to manage the use of synthetic food dyes in industrial processes.

Histological assessment

For histopathological examination, the kidneys of freshly sacrificed *Oreochromis niloticus* specimens were dissected out immediately in normal saline, weighed and processed. After that, they were weighed and cleaned using a cooled saline solution at a concentration of 0.9%. The tissues were chopped and homogenized (10% w/v) in a Potter-Elvehjem-type homogenizer with 1.15% KCl in ice-cold sodium phosphate buffer (0.01 M, pH 7.4). The homogenates were centrifuged at 10,000 Xg. for 20 minutes at 4°C. (Al-Saeedi *et al.,* 2025).

Tissue samples were fixed in 10% neutral buffered formalin and subsequently dehydrated through a graded ethanol series, followed by embedding in paraffin wax according to standard histological procedures (Parvin *et al.,* 2019). Paraffin-embedded tissues were sectioned at 6 μm thickness using a rotary microtome and stained with Hematoxylin and Eosin (H and E) for microscopic examination. All experimental and control groups were maintained under similar conditions for comparative analysis. The various enzyme activity, free radicals and biochemical parameters were analyzed using the resulting supernatants.

Collection of blood samples

Fish were gently caught individually in a small hand net. After the preliminary investigation of the length and weight, the fish were then placed belly upwards on dissection tray and blood samples obtained from the caudal vein circulation with the aid of a heparinized 2 cm^3 disposable plastic syringes and a 21 gauge disposable hypodermic needle (Parvin *et al.,* 2019). The use of plastic syringe is usually recommended because contact with glass results in decreased coagulation time. The site chosen for puncture (about 3-4 cm from the cloaca) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted perpendicularly to the vertebral column of the fish and gently aspirated during penetration followed by pushing gently down until blood started to enter as the needle punctured a caudal blood vessel (Abdul *et al.,* 2021). Blood was taken under gentle aspiration until about 1cm^3 has been obtained, then the needle was withdrawn and the blood gently transferred into lithium heparin anticoagulant tube and allowed to clot at room temperature for 30-40 minutes (Abdul *et al.,* 2021).

Centrifuging of blood sample

The blood in the anticoagulant tubes were collected and then transferred into clean dry centrifuge tubes and centrifuged at 4000 rpm for 10 minutes, followed by serum separation (Agbozu *et al.,* 2007).

Separation of serum from blood

The serum was separated from the blood after centrifuging for 10 minutes by using a pasteur pipette and transferred into a anticoagulant free test-tube and stored in a refrigerator until analysis (Chatterjee *et al.,* 2004).

Kidney function assessment

For the biochemical assessment of renal function, blood samples (1 mL/kg wet body weight) were collected from the caudal vein of each fish using sterile plastic syringes. Half of the collected volume was transferred into tubes containing EDTA for hematological analysis (Blaxhall *et al.,* 2006), while the remaining blood was placed in plain gel tubes without anticoagulant for serum separation. Samples were transported on ice to maintain integrity. Blood samples were centrifuged at 3000 rpm for 10 min and the separated serum was carefully aspirated with micropipettes into labeled Eppendorf tubes and stored at -40°C (Chng *et al.,* 2003) until further use. Biochemical estimations were carried out using a Cobas CIII automated biochemical analyzer (Roche Diagnostics, Germany; 2014).

Creatinine estimation

Sample volume

Serum creatinine was estimated using the Jaffe spectrophotometric laboratory method as described by Pratt (1996). Prior to analysis, the working reagent, standard and serum samples were equilibrated to room temperature. For each assay, 1 mL of working reagent was dispensed into labeled tubes for the standard (S) and test (T). Subsequently, 100 µL of standard solution and 100 µL of serum sample were added to the respective tubes. Following gentle mixing, absorbance was recorded at 500 nm after 30 s and 90 s, using distilled water as blank. Serum creatinine concentrations were expressed in mg/dL and further validated at 340 nm in accordance with the manufacturer's protocol (Centromic GmbH, Germany).

Urea estimation

Serum urea was quantified using the Nesslerization method following the protocols of Pratt (1996) and Aitken *et al.* (2003). A commercial urea assay kit (Centromic GmbH, Germany) was employed for the analysis. Three tubes were designated as blank (B), standard (S) and test (T). To each tube, 1 mL of working reagent was added, followed by 10 µL of distilled water. The tubes were incubated at room temperature for 10 min and absorbance was measured at 340 nm against the blank. Serum urea concentrations were expressed in mg/dL.

SOD and CAT estimation

To investigate toxicity-induced stress in the exposed and treated groups, two key antioxidant biomarkers were selected to evaluate the renoprotective efficacy of folic acid. Kidney tissues from all five experimental groups were excised and processed for biochemical assays. The tissues were perfused with phosphate-buffered saline (PBS) containing 0.16 mg/mL heparin, weighed, minced and homogenized in ice-cold isotonic buffer (10 mM Tris-HCl, 50 mM sucrose and 2 mM EDTA, pH 7.4) at a ratio of 10 mL buffer per gram of tissue using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 5000 rpm for 15 min at 4°C and the resulting supernatant was collected as the crude nuclear fraction for antioxidant enzyme analysis (Seena *et al.,* 2024).

Superoxide dismutase (SOD) activity in the kidney extracts was determined following the method of Marklund and Marklund (1974), which is based on the inhibition of pyrogallol auto-oxidation by superoxide radicals. Enzyme activity was expressed as units per mg protein. Catalase (CAT) activity was measured by monitoring the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm and results were expressed as µmol of H_2O_2 decomposed per mg protein per minute (Aebi, 1984).

All assays were performed at room temperature, with reagents equilibrated before use. The total reaction volume was adjusted to 1.5 mL, with appropriate amounts of deionized water added to balance reagent volumes. Reagents were briefly vortexed before use to ensure homogeneity. For SOD determination using the xanthine-xanthine oxidase-NBT method, the reaction mixture contained deionized water, 25 × reaction buffer, xanthine solution and nitroblue tetrazolium (NBT), to which the tissue lysate was added. The reaction was initiated by adding xanthine oxidase (XOD) and the absorbance was recorded at 550 nm every 30 seconds for 5 minutes using a visible spectrophotometer. The initial reading was taken at 30 seconds, with the final measurement at 5 minutes and 30 seconds, enabling calculation of enzyme activity.

Histological assessment

Kidneys from moribund or freshly dead fish were immediately excised in normal saline and weighed. Portions of the kidney tissue were fixed in 10% neutral buffered formalin for histological examination. The tissues were dehydrated through graded alcohol series, embedded in paraffin and sectioned at a thickness of approximately 6 µm. Sections were stained with hematoxylin and eosin following standard histological protocols (Parvin *et al.,* 2019). Histological analyses were carried out for all experimental groups as described above.

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 ± standard error (SE). A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Histological assessment of kidney tissue

The interstitial spaces between nephrons are occupied by blood vessels, lymphatics and a variety of renal cell types, including interstitial cells and macrophages, which are essential for maintaining the renal microenvironment and supporting overall kidney function (Karaca and Durna, 2019). In *Oreochromis niloticus*, the kidney demonstrates distinct adaptations for osmoregulation, enabling the

species to survive in aquatic habitats with fluctuating salinity. The histological organization of the kidney thus highlights specialized structural and functional features that facilitate filtration, reabsorption, secretion and osmoregulation-processes fundamental for maintaining homeostasis in aquatic environments.

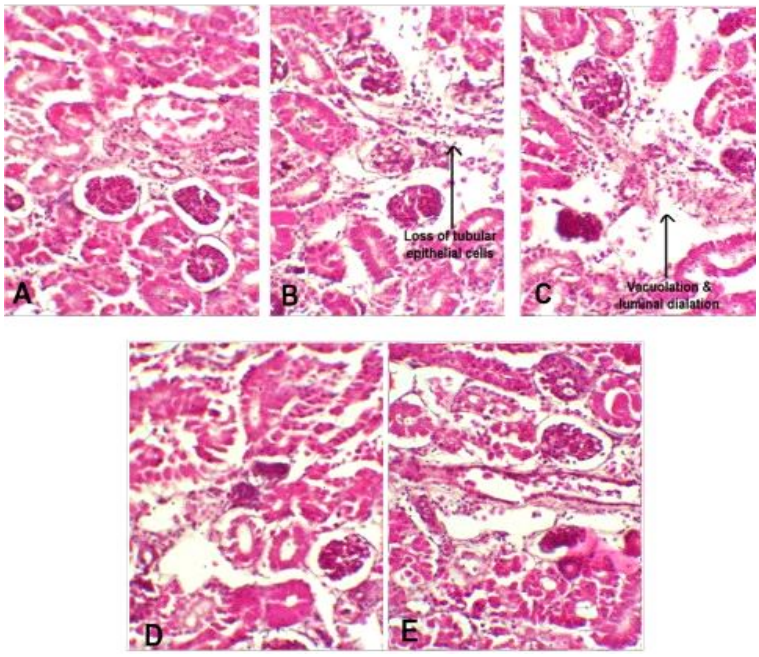
In the control group (Fig 2A), the kidney exhibits a typical histoarchitecture characterized by well-defined glomeruli, intact tubular structures and negligible interstitial inflammation. The glomeruli appear round and compact within Bowman's capsule, with a distinct separation between the glomerular tuft and Bowman's space. Proximal and distal convoluted tubules show normal epithelial lining and uniform luminal contents, indicative of preserved renal function.

In contrast, kidneys of *Oreochromis* exposed to sublethal concentrations of tartrazine (Fig 2B and 2C) display marked histopathological alterations consistent with renal injury and functional impairment (Landrigan *et al.,* 2018). Glomerular changes include congestion, dilation of capillary loops and thickening of Bowman's capsule, suggesting increased glomerular permeability and compromised filtration (Thakur *et al.,* 2022). Tubular alterations are evident as degeneration of tubular epithelium, vacuolation and luminal dilatation. Furthermore, interstitial regions exhibit pronounced inflammatory responses, including infiltration of lymphocytes and macrophages, reflecting tartrazine-induced renal inflammation (Toghan *et al*., 2022; Lohiya *et al.*, 2018).

Conversely, co-supplementation with folic acid alongside tartrazine exposure (Fig 2D and 2E) demonstrates a dose-dependent attenuation of these pathological changes. At lower doses, folic acid partially mitigates glomerular and tubular abnormalities, with observable structural improvements. At higher supplementation levels, the protective effect is more evident, with near-restoration of renal architecture and only minimal histopathological alterations. These ameliorative outcomes are likely attributable to the antioxidant potential of folic acid and its role in enhancing cellular repair and regeneration.

Analysis of serum urea and creatinine level

The estimation of serum urea and creatinine is a crucial diagnostic tool for assessing renal function, particularly in the context of exposure to eco-toxicants such as Tartrazine, a widely used synthetic azo dye (Ibrahim and Mahmoud, 2005). These biochemical markers serve as sensitive indicators of kidney health, with elevated levels typically



(A) Control group (NC) showing normal kidney structure on longitudinal section, with intact renal tubules, including both proximal and distal convoluted tubules (400×). (B) Kidney from fish exposed to 0.144 g/L tartrazine (2xTz) displaying loss of tubular epithelial space and disruption of tubular morphology. (C) Kidney from fish exposed to 0.216 g/L tartrazine (3xTz) showing marked vacuolation and luminal dilation (arrow). (D) Kidney from fish treated with 0.144 g/L tartrazine supplemented with folic acid (2xTz+FA) exhibiting reduced intertubular vacuolation, indicating partial protection. (E) Kidney from fish treated with 0.216 g/L tartrazine and folic acid (3xTz+FA) revealing improved epithelial cell population and better preservation of tubular organization compared with tartrazine-only groups.

Fig 2: Histopathological changes observed in the kidney of *Oreochromis niloticus* following exposure to tartrazine and folic acid.

reflecting impaired glomerular filtration and possible nephrotoxicity (Salem *et al.,* 2021). The present study demonstrates that tartrazine exposure leads to significant, dose-dependent renal toxicity in fish, as evidenced by elevated levels of serum creatinine and urea-key indicators of impaired kidney function.

Serum creatinine and urea levels exhibited (Table 1) marked changes upon exposure to four respective doses of tartrazine and folic acid as illustrated in Fig 3. In the negative control (NC; no exposure to tartrazine or folic acid) group, creatinine (0.30 mg/dL) and urea (8.44 mg/dL) were within normal physiological ranges. Treatment with tartrazine resulted in a significant elevation of both markers.

Table 1: Serum urea and creatinine level under different doses of tartrazine and folic acid.

Doses	Creatinine level (mg/dL)	Urea level (mg/dL)
NC	0.30	8.44
2×Tz	1.92	10.55
3×Tz	2.03*	11.12 *
2×TzFa	0.32	8.21
3×TzFa	0.34	8.40

(Asterisks show the highest level of creatinine and urea in serum).

In fish exposed to tartrazine at 2 × LC_{50} (0.144 g/L; 2 × Tz), creatinine rose to 1.92 mg/dL and urea to 10.55 mg/dL, while in the group exposed to tartrazine at 3 × LC_{50} (0.216 g/L; 3 × Tz), creatinine further increased to 2.03 mg/dL and urea to 11.12 mg/dL. One-way ANOVA indicated that these increases were statistically significant compared with the control ($p<0.05$) and Tukey's post hoc test confirmed pronounced differences, particularly at the higher tartrazine dose (3 × Tz). Interestingly, co-treatment with folic acid effectively counteracted these changes. In the group co-exposed to tartrazine 2 × LC_{50} and folic acid 2 × dose (0.144 g/L each; 2 × TzFa), creatinine and urea levels were maintained at 0.32 mg/dL and 8.21 mg/dL, respectively. Similarly, in the group co-exposed to tartrazine 2 × LC_{50} and folic acid 3xdose (0.216 g/L each; 3 × TzFa), creatinine (0.34 mg/dL) and urea (8.40 mg/dL) remained comparable to the control. These values showed no statistically significant difference from NC ($p>0.05$), strongly suggesting that folic acid supplementation provides protective efficacy against tartrazine-induced renal toxicity.

Analysis of SOD and CAT Level

The activities of the antioxidant enzymes SOD and CAT in kidney tissue lysates were assessed across the five experimental groups, as illustrated in Fig 4 and 5.

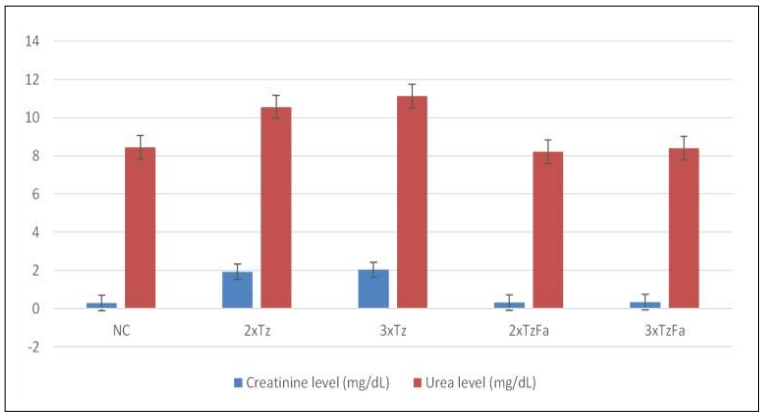


Fig 3: Effect of Tartrazine on renal function and the protective role of Folic acid in *O. niloticus*.

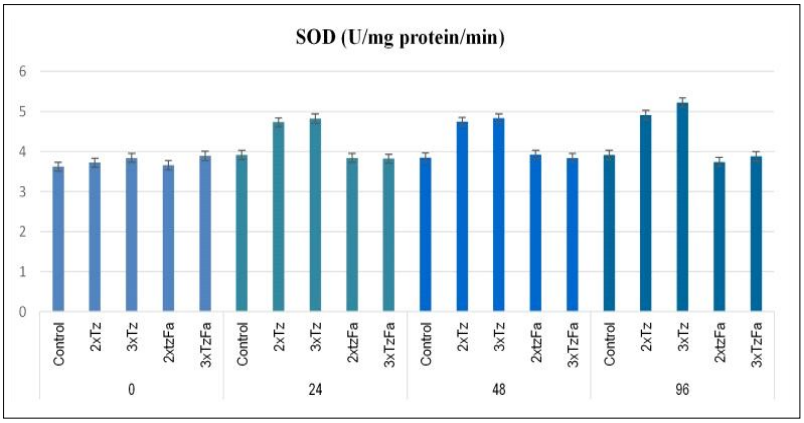


Fig 4: Impact of tartrazine toxicity and folic acid supplementation on SOD activity in the kidney of *O. niloticus*.

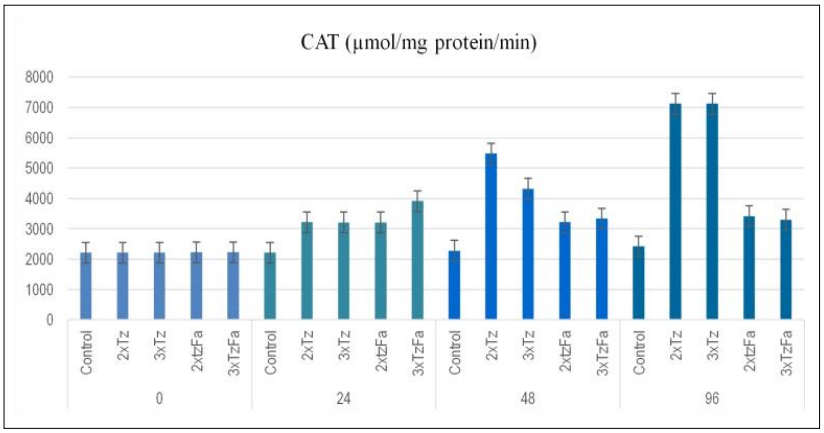


Fig 5: Impact of tartrazine toxicity and folic acid supplementation on CAT activity in the kidney of *O. niloticus*.

The analysis of SOD activity across treatment groups and time points revealed significant differences in response to tartrazine exposure and folic acid supplementation. At baseline (0 h), no notable variation was observed among groups, indicating comparable starting conditions. However, with increasing exposure duration (24-96 h), tartrazine treatment (2 × Tz and 3 × Tz) caused a marked, dose- and time-dependent elevation in SOD activity compared to the control ($p<0.01$), reflecting oxidative stress and compensatory upregulation of the antioxidant system. In contrast, the folic acid co-treated groups (2 × TzFa and 3 × TzFa) maintained SOD levels close to control values, showing significant reduction compared to tartrazine-only groups ($p<0.01$) but non-significant differences relative to control, suggesting a protective effect. Overall, two-way ANOVA confirmed significant effects of both treatment and time, with post-hoc analysis establishing that folic acid effectively mitigates tartrazine-induced oxidative alterations in SOD activity.

The catalase (CAT) activity data reveal significant treatment- and time-dependent changes in response to tartrazine and folic acid. At 0 h, CAT activity was similar across all groups (~2210-2225 µmol/mg protein/min), showing no baseline difference. From 24 h onward, tartrazine exposure (2 × Tz and 3 × Tz) led to a marked elevation in CAT activity compared to control, with values rising progressively and peaking at 7118.88 and 7119.88 µmol/mg protein/min, respectively, at 96 h ($p<0.01$). This indicates that tartrazine induces oxidative stress, stimulating an adaptive increase in CAT activity. Interestingly, folic acid co-treatment moderated this response: at 24 h, 2 × TzFa and 3 × TzFa groups showed CAT activities higher than control but lower than tartrazine-only groups, while by 48 h and 96 h, folic acid groups (3218.55-3418.22 µmol/mg protein/min) were significantly reduced compared to tartrazine-only groups ($p<0.01$) yet remained above control, suggesting partial but consistent protection. Two-way ANOVA would confirm significant main effects of both treatment and time, with post-hoc analysis highlighting that tartrazine significantly upregulates CAT activity while folic acid supplementation mitigates, but does not fully normalize, this oxidative response.

The analysis of antioxidant enzyme activities (SOD and CAT) demonstrated consistent patterns of tartrazine-induced oxidative stress and folic acid-mediated protection. At baseline (0 h), both enzymes showed comparable values across groups, confirming the absence of pre-treatment differences. With prolonged exposure (24-96 h), tartrazine treatment (2 × Tz and 3 × Tz) significantly elevated SOD and CAT activities compared to the control ($p<0.01$), with the highest responses observed at 96 h (SOD: 4.91-5.22 U/mg protein/min; CAT: ~7119 µmol/mg protein/min). This indicates a dose- and time-dependent oxidative stress response characterized by compensatory upregulation of enzymatic defenses. In contrast, folic acid co-treated groups (2 × TzFa and 3 × TzFa) maintained enzyme levels close to or slightly above control values, showing significant reductions compared to tartrazine-only groups ($p<0.01$) but no significant differences from control. These findings suggest that while tartrazine exposure induces oxidative imbalance, folic acid supplementation effectively counteracts the stress, stabilizing antioxidant enzyme activities and thereby exerting a protective role against tartrazine-mediated toxicity.

Future scopes

The present study provides strong evidence of tartrazine-induced nephrotoxicity and the protective efficacy of folic acid in *Oreochromis niloticus*, yet several avenues remain open for future exploration. Molecular-level studies, including gene expression profiling of antioxidant, apoptotic and inflammatory markers, are needed to unravel the mechanistic basis of toxicity and protection. Long-term and low-dose exposure experiments should be undertaken to simulate environmental conditions more realistically and establish safe thresholds. Comparative analyses with other natural antioxidants may identify more effective or synergistic protective strategies, while investigations into additional organs such as liver, gills and brain could provide a comprehensive view of systemic toxicity. Furthermore, proteomic and metabolomic approaches could reveal novel

biomarkers of oxidative damage, whereas ecological and food safety studies may highlight risks of bioaccumulation and human exposure through fish consumption. Finally, applied research on folate-enriched diets in aquaculture could pave the way for practical interventions to mitigate dye-induced toxicity in aquatic organisms.

Limitations of the study

This study was limited to short-term exposure (96 h), a single organ (kidney) and selected biochemical markers (serum urea, creatinine, SOD and CAT), which may not reflect the full systemic or chronic effects of tartrazine. Molecular analyses and comparisons with other antioxidants were not included, restricting mechanistic depth and broader applicability. Moreover, findings based on *O. niloticus* may not be directly extrapolated to other species or ecological contexts.

CONCLUSION

The present investigation demonstrates clearly that Tartrazine, severely damages the kidneys of *Oreochromis niloticus*. Histopathology showed significant kidney injury: glomerular congestion, Bowman's capsule thickening, and tubular degeneration/vacuolation, indicative of impaired function. Biochemical markers like elevated serum urea and creatinine confirmed this nephrotoxicity. Additionally, the significant rise in superoxide dismutase (SOD) and catalase (CAT) activities pointed to excessive reactive oxygen species and an overstressed antioxidant defense. Crucially, higher doses of folic acid co-treatment demonstrated a nephroprotective effect. It restored renal architecture, normalized serum urea/creatinine and maintained SOD/CAT levels near control values. This suggests folic acid protects by reducing oxidative stress and supporting cellular repair.

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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. Both authors contributed equally to this work.

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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).

Disclaimer

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abdul, Lateef, E.N., Hussein, M.A., Mustafa, S.R., Taen, M.M., Ali, S.A. and Saheab, N. (2021). Toxicity of anthracene on the function of the liver and kidney of the common carp (*Cyprinus carpio*). *Egyptian Journal of Aquatic Biology and Fisheries*. **25(3):** 831-840.

Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*. **105:** 121-126.

Agbozu, I.E., Ekweozor, I.K.E. and Opuene, K. (2007). Survey of heavy metals in the catfish *Synodontis clarias*. *International Journal of Environmental Science and Technology*. **4(1):** 93-97.

Aitken, M.M., Hall, E., Allen, W.M., Scott, L. and Davot, J.L. (2003). Liver-related biochemical changes in the serum of dogs being treated phenobarbitone. *Veterinary Record*. **153:** 13-16.

Al-Saeedi, R.F., Hussain, A.S., Raghad, K.M., Noor, A.J., Zina, F.H., Ahmed, F.H. and Hany, M.E.W. (2025). Nephrotoxicity of iron oxide nanoparticles in male mice. *Agricultural Science Digest*. **41(2):** 340-344. doi: 10.18805/ag.DF-751.

Al-Shaheen, A., Al-Mayah, A. and Ali, R. (2021). Subchronic exposure to tartrazine induces biochemical and histological alterations in rats. *Toxicology Reports*. **8:** 1021-1028.

Al-Shaheen, A., Mohammed, R. and Jasim, S. (2023). Protective effects of folic acid against oxidative stress in aquatic organisms exposed to pollutants. *Fish Physiology and Biochemistry*. **49:** 713-725.

Blaxhall, D., Daisley, G., Alptekin, Ö., Tükel, S. and Canli, M. (2006). Response of catalase activity to Ag^{+}, Cd^{2+}, Cr^{6+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish *Oreochromis niloticus*. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology*. **143(2):** 218-224.

Chatterjee, N., Pal, A.K., Manush, S.M., Das, T. and Mukherjee, S.C. (2004). Thermal tolerance and oxygen consumption of *Labeo rohita* and *Cyprinus carpio* early fingerlings acclimated to three different temperatures. *Journal of Thermal Biology*. **29(6):** 285-290.

Chng, V., Yang, M., Calow, P. and Forbes, V.E. (2003). How do physiological responses to stress translate into ecological and evolutionary processes? *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology*. **120(1):** 11-16.

Dhanasiri, A.K.S., Torres, A.J., Chikwati, A.M., Forberg, T., Kotner, T.M., Krogdahl, A. (2023). Effects of dietary supplementa -tion with prebiotics and Pediococcus acidilactici on gut health, transcriptome, microbiota and metabolome in Atlantic salmon (*Salmo salar* L.) after seawater transfer. *Animal Microbiome*. **10(2):** 10-12.

Huang, X., Wang, J. and Chen, Y. (2019). Protective role of folic acid against oxidative stress in fish models. *Aquatic Toxicology*. **212:** 34-42.

Ibrahim, S.A. and Mahmoud, S.A. (2005). Effect of heavy metal accumulation on enzyme activity and histology in liver of some Nile fish in Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*. **9(1):** 203-219.

Karaca, A. and Durna, Z. (2019). Patient satisfaction with the quality of nursing care. *Nursing Open*. **6(2):** 535-545.

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R. and Baldé, A.B. (2018). The Lancet Commission on pollution and health. *Lancet*. **391(10119):** 462-512.

Lohiya, A., Kumar, V. and Punia, J.S. (2018). Sub-acute oxidant and histopathological effects of imidacloprid on kidney of adult female Wistar rats. *Indian Journal of Animal Research*. **52(9):** 1324-1330. doi: 10.18805/ijar.B-3243.

Ma, Y., Li, H. and Zhang, W. (2021). Impacts of azo dye tartrazine on oxidative stress, inflammatory response and intestinal microbiota in crucian carp. *Aquatic Toxicology*. **238:** 105924.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for SOD. *European Journal of Biochemistry*. **47(3):** 469-474.

Parvin, I., Das, S., Sen, P. and Kundu, S. (2019). A pilot study to evaluate the physiological and behavioural effects of a widely consumed pharmaceutical on *Oreochromis niloticus*. *Research Journal of Chemistry and Environment*. **23(6):** 128-133.

Pratt, P.W. (1996). Laboratory Procedures for Veterinary Technicians. 3rd Edn. Mosby, Philadelphia. pp. 296-400.

Rao, V.J. (2006). Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology*. **143(4):** 492-498.

Roche Diagnostics, Germany and Downs, C.T. (2014). Modelling large spotted genet (*Genetta tigrina*) and slender mongoose (*Galerella sanguinea*) occupancy in a heterogeneous landscape of South Africa. *Mammalian Biology*. **79(5):** 331-337.

Salem, H.S., Hagras, A.E., El-Baghdady, H.A.M. and El-Naggar, A.M. (2021). Biomarkers of exposure and effect in Nile tilapia (*Oreochromis niloticus*) environmentally exposed to multiple stressors in Egypt. *Bulletin of Environmental Contamination and Toxicology*. **107(5):** 889-894.

Seena, P., Narayanasamy, K., Baskaran, K. and Devi, N.K. (2024). Assessment of antioxidant enzymatic activities in liver, gill and brain of Oreochromis mossambicus on ethoxyquin exposure. *International Journal of Pharmacological Science Review*. **84(3):** 1-9.

Shahid, M., Bhat, S.A. and Qureshi, S. (2024). Environmental threats of azo dyes: Ecotoxicological perspectives and management. *Journal of Hazardous Materials Advances*. **7:** 100215.

Singh, Y.P. and Singh, R. (2004). Interaction effect of sulphur and phosphorus on growth and nutrient content of blackgram (*Phaseolus mungo* L.*). Journal of the Indian Society of Soil Science*. **236(52):** 266-269.

Sharma, R. and Chattopadhyay, P. (2012). Studies on the use of synthetic food colours in food products. *Indian Journal of Public Health*. **56(1):** 53-56.

Shivashakthi, R., Sateesh, T., Padmanath, K., Chandrasekhar, M., Sriram, P. and Pandiyan, V. (2019). Urinary cystatin C as biomarker for identification of kidney disease in dogs. *Indian Journal of Animal Research*. **53(2):** 196-199. doi: 10.18805/ijar.B-3481.

Smith, M., Brown, C. and Lee, H. (2010). Characterization of synthetic food dyes and environmental impact. *Food Additives and Contaminants*. **27(5):** 531-539.

Thakur, N., Shukla, S.K., Ahmad, A.H., Jadon, N.S., Singh, J.L. and Chethan, G.E. (2022). Ameliorative, antioxidant and immunomodulatory potential of vitamin D on aminoglycoside induced acute kidney injury in Wistar rats. *Indian Journal of Animal Research*. **56(6):** 655-661. doi: 10.18805/IJAR. B-4471.

Toghan, A., Khairy, M., Mohamed, M.M. and Amer, A.A. (2022). Synthesis of defect-impressive boron graphene as a remarkable electrocatalyst for methanol oxidation reaction. *Journal of Materials Research and Technology*. **16:** 362-382.

Zhou, L., Wang, R. and Chen, J. (2024). Folic acid supplementation mitigates oxidative damage and restores biochemical homeostasis in pollutant-exposed fish. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology*. **266:** 109594.