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RESEARCH ARTICLE

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Growth performance, physiological response, and tissue microarchitecture of the carp *Labeo rohita* challenged with AFB1 are improved by supplementing with turmeric

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Abstract

Aim of study: To examine the impact of dietary supplementation with turmeric (TM) (*Curcuma longa*) on growth, haematological, biochemical parameters, and histoarchitecture in rohu (*Labeo rohita*) challenged with aflatoxin B1 (AFB1).

Area of study: Ludhiana, Punjab, India.

Material and methods: A completely randomized design involved the utilization of 225 fingerlings distributed across five treatments with three replicates each. Diets were allocated as follows: T1 denoted the negative control diet, T2 comprised AF100, while T3, T4, and T5 were formulated with AF25TM, AF50TM, and AF100TM, respectively.

Main results: The results indicated a negative correlation between AFB1 dosage in feed and fish growth, with higher doses resulting in decreased growth. Significant changes were observed in haematological parameters, including reductions in total erythrocyte count, total leukocyte count, haemoglobin, and packed cell volume, alongside alterations in biochemical parameters, such as decreases in total protein, albumin, and globulin levels, and an increase in glucose levels and albumin/globulin ratio. Additionally, elevated levels of aspartate aminotransferase, alanine aminotransferase, catalase and superoxide dismutase were noted in T2 and T5 compared to other treatments. AFB1 exposure also led to damage in the microarchitecture of the brain, kidney, and liver tissues, although inclusion of TM at 25 ppb AFB1 showed signs of recovery.

Research highlights: The changes observed were dose-dependent, and supplementation of TM showed increased resistance against AFB1 and the greatest improvement in T3. Therefore, a diet containing 5 g TM kg^{-1} would lower AFB1 contamination of 25 ppb compared to 50 and 100 ppb. In conclusion, supplementing TM in fish feeds can help regulating the AFB1, which in turn can improve sustenance-based output.

Additional key words: aflatoxin B1; aquaculture; blood metabolic profile; *Curcuma longa*; rohu; tissue microarchitecture; toxicity.

Abbreviations used: AFB1 (aflatoxin B1); ALT (alanine aminotransferase); AST (aspartate aminotransferase); CAT (catalase); FCR (feed conversion ratio); Hb (haemoglobin); K (condition factor); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration); MCV (mean corpuscular volume); NBWG (net body weight gain), PCV (packed cell volume); SGR (specific growth rate); SOD (superoxide dismutase); TEC (total erythrocyte count); TLC (total leucocyte count); TM (turmeric meal).

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Introduction

Carps contribute more than 85% to total fish production from inland aquaculture in India, and approximately 35% of India's principal carp production is accounted for by the popular fish species rohu, *Labeo rohita* (FAO, 2018). However, a number of illnesses are causing the aquaculture sector to decline. In addition, secondary metabolites are frequently the cause of fungal infections, or mycoses, which typically occur in hatcheries and culture systems, and have a detrimental effect on fish output and financial loss.

Amongst several fungal metabolites, aflatoxin B1 (AFB1) is the most toxic, exhibiting harmful hepatotoxic, carcinogenic, teratogenic and mutagenic compounds generated mainly by two fungal species, *Aspergillus flavus* and *Aspergillus parasiticus* which eventually have an impact on the cattle and aquaculture sectors and cause financial losses (Bhatt & Pandey, 2022; Bhatt, 2023). AFB1 and related mycotoxins are of great concern in many tropical countries because they contaminate grains and seeds used as feed ingredients, due to their ubiquitous nature and favourable climate (Gonçalves et al*.,* 2018). Therefore, control of AFB1 contamination is necessary, and feasible by including medicinal plants, herbs, essential oils and spices as they act as detoxifying agents for the presence of flavonoids, phenolics, phytoalexins and thiosulfonates compounds.

Since ancient times, ayurvedic medicine has employed turmeric (*Curcuma longa* L.), a widely utilized medicinal plant (Bhatt et al., 2023a). Numerous studies have shown that turmeric has a wide range of biological properties and therapeutic effects as antioxidant, immunostimulant, anti-bacterial, anti-inflammation, anti-stress agent and for gut health promotion in fish (e.g., Fagnon et al., 2020). Turmeric contains 1.5-5% essential oils and 3-4% curcuminoids. Its main bioactive ingredient is curcumin, which is well known for its antioxidant action. Other active ingredients in turmeric powder include curlone, curcuminoids, aromatic turmerones (32.5%), alpha (15.6%), and beta (17.1%) turmerones (Ferreira et al., 1992). Mooraki et al. (2019) found that turmeric elevates the white blood cell count in the carps (*Cirrhinus mrigala)*. Additionally, prawns (*Macrobrachium rosenbergii*) and fish that have been exposed to *Pseudomonas aeruginosa* and *Vibrio alginolyticus* have demonstrated improvement when given turmeric supplements in their diets (Sivagurunathan et al., 2011; Alambra et al., 2012).

Besides, physiological responses are utilized as suitable indicators for understanding the health status and toxic effects of xenobiotics in various animals including fish. Hitherto, studies are not yet undertaken to counteract the AFB1 using herbs and/or their extracts in fish. Therefore, turmeric meal (TM) efficacy is standardised in the proposed study for reducing AFB1 contamination in turn improve fish immunity and thereby production, which would ultimately benefit stakeholders such as farmers, entrepreneurs and the feed industry.

Thus, the current study set out to investigate the effects of a turmeric diet supplemented with AFB1 on the rohu's growth, survival, physiological response, and tissue cell microarchitectures.

Material and methods

Rohu fish (*Labeo rohita*) were obtained from the College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab, India. A total of 225 fingerlings, initially measuring 12.40 ± 0.06 cm in body length and weighing 15.20 ± 0.33 g were randomly distributed into 15 (5 treatments × 3 replicates in each treatment) fiber-reinforced plastic (FRP) experimental tanks, each measuring 1.5 × 1.0 × 0.75 m, with a stocking density of 15 fingerlings per replicate. Five test diets (treatments) were prepared as given in Table 1, where T1 was negative control, fed with basal diet composed of rice bran (40%), mustard oil cake (30%), soybean meal (26%), carboxy methyl cellulose (2%) and vitamin-mineral mixture (2%). T2 = AF100 (positive control), the fish were fed on basal diet, however supplemented with 100 ppb AFB1. Aflatoxin B1 (AFB1: $C_{17}H_{12}O_6$, MW: 312.27, purity: 98%) was purchased from the Sigma Aldrich Co., USA), and handled with care using appropriate protective gear. The AFB1 was dissolved in 70% methanol to prepare different concentrations for treatments including T3 (25 ppb AFB1 + 5 g TM kg^{-1} feed), T4 (50 ppb AFB1 + 5 g TM kg^{-1} feed) and T5 (100 ppb AFB1 + 5 g TM kg^{-1} feed). The turmeric rhizomes were obtained from the College of Horticulture and Forestry at Punjab Agricultural University, Ludhiana, (Punjab), India. They were carefully dried and then processed with a grinder to a fine powder meal.

Pellets were formulated with varying ingredient levels according to the formulations outlined in Table 1. Precisely weighed ingredients were thoroughly blended in a grinder and pressure-cooked in an autoclave at 115°C pounds per square inch (15 psi) of pressure for 15 minutes. Following cooking, the dough was cooled to room temperature, and AFB1 dilutions at specific concentrations were uniformly sprayed onto the cooked feed ingredients using a hand sprayer. Subsequently, the ingredients were left to undergo methanol evaporation in the shade. Once dried, all ingredients were meticulously mixed with the prescribed amounts of turmeric (5 g kg^{-1}), vitamin-mineral premix, and binder, ensuring homogeneous blending, and hand-kneaded to achieve the desired consistency, adding sufficient water to form dough. The dough was then extruded through a mechanical pelletizer. The resultant pellets were dried in a hot air oven at 40°C overnight until the moisture content reached 10%. Each diet was individually packed in polythene bags, labelled, and stored in a refrigerator at 4 °C until further use as described earlier (Kaur et al., 2020).

Over a period of 90 days, the experimental fish were manually fed *ad libitum* with the prepared pellets supplemented with TM and AFB1 as specified above. Proximate analysis of both diets and ingredients (Tables 1 and 2) was conducted as per the AOAC standards (2012).

Water quality

During the experiment, fish were kept in continuously aerated tanks. All the water quality parameters, including

Table 1. Formulation (%) and proximate analysis (% of dry matter) of the experimental diets

Ingredients	T1	T2	T3	T4	T5
	Control diet	AF100	AF25TM	AF50TM	AF100TM
Rice bran (%)	40	40	40	40	40
Mustard oil cake (%)	30	30	30	30	30
Soybean meal (%)	26	26	26	26	26
Vitamin-mineral mixture (%)	2.0	2.0	2.0	2.0	2.0
Carboxy methyl cellulose (%)	2.0	2.0	2.0	2.0	2.0
Turmeric (g)	-	-	5	5	5
Aflatoxin B1 (ppb)	-	100	25	50	100
Proximate analysis of experimental diets (% of dry matter)					
Crude protein	24.48	24.37	24.32	24.43	24.35
Ether extract	3.22	3.26	3.28	3.23	3.25
Crude fat	19.82	19.89	19.91	19.96	19.93
Ash	9.26	9.25	9.24	9.18	9.24
NFE[1]	43.22	43.23	43.25	43.21	45.23

[1] NFE: nitrogen-free extract.

Table 2. Proximate analysis of feed ingredients

Ingredients[1]	Proximate composition (% of dry matter) [2]				
	CP	EE	CF	Ash	NFE
Rice bran	12.38	1.28	15.64	11.51	59.19
Mustard meal	38.91	1.93	11.54	8.16	39.46
Soybean meal	42.9	1.13	4.01	7.2	44.76
TM	14.95	2.88	0.04	11.85	70.28

[1] TM: turmeric meal. [2] CP: crude protein. EE: ether extract. CF: crude fat. NFE: nitrogen-free extract.

temperature (28.70-27.12ºC), pH (7.75-7.73), dissolved oxygen (8.35-7.40 ppm), total alkalinity (156.26-174.64 ppm), total hardness (216.37-251.34 ppm), and NH_3-N (0.055-0.056 ppm) were within the acceptable range as recommended (Boyd, 1998) for fish growth.

Sampling and analysis

Fish feeding was ceased one day prior sampling. At intervals of 0, 30, 60, and 90 days into the experiment, fish from each experimental pool were anesthetized in 2-phenoxyethanol (1 mL L^{-1}), using 0.45 × 13 mm syringes (Hindustan Syringes & Medical Devices Ltd.). After the fish were weighed and measured for length, they were returned in their designated pools.

Nine fish per treatment or 45 fish were used for blood analysis per sampling session when three rohu fish were randomly selected for blood collection from each FRP pool. Using 1.5-mL syringes washed with a solution containing 25,000 U heparin ammonium salt (Sigma H6279) per 3 mL of 0.9% NaCl, blood samples (100-150 µL) were drawn from the caudal vein.

After centrifugation (4000 g, 3 min, 4°C) to remove the blood serum, the sample was frozen in liquid nitrogen and kept at -80°C until further examination. Subsequently, fish were decapitated and dissected for the brain, liver, and kidney samples and placed in empty sample collection tubes filled with 10% neutral buffered formalin (Sigma-Aldrich, CAS #HT501128). Likewise, flesh samples were obtained for flesh quality analysis and stored at -20°C until analysis.

Growth performance. Monthly records were maintained for fish survival and growth, with measurements taken for

total body length and weight. Growth parameters were calculated using standard methodologies as outlined by Halver (1957).

Blood metabolic profile. The blood samples obtained from anaesthetized fish (n=9) were placed in anticoagulant vials. Haematological parameters, including total leucocyte counts (TLC) and total erythrocyte counts (TEC), were measured using a haemocytometer (Naubauer Counting Chamber). The microcapillary method was used to measure the packed cell volume (PCV) or haematocrit, while Sahli's (1962) approach was used to evaluate hemoglobin (Hb). The blood indices viz., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Mukherjee (1988). The total protein (Cat. #120231), albumin (A) (Cat. #120223), and glucose (Cat. #120235) were quantified in accordance with standard protocols using ERBA Mannheim Kits.

Liver enzyme profile. The manufacturer's instructions (ERBA, Lachema s.r.o) for measuring the liver profile parameters, namely aspartate aminotransferase (AST, Cat. #120204) and alanine aminotransferase (ALT, Cat. #120207), were followed while measuring fish serum. A standard technique was followed to quantiy the enzymes using 50 µL serum in a biochemical analyzer (CHEM-7, ERBA, Mannheim). Antioxidant parameters, including superoxide dismutase (SOD) and catalase (CAT), were calculated according to the standard protocol (Nishikimi et al., 1972).

Tissue microarchitecture analysis. After the termination (90 days) of the experiment, fish tissues such as the brain, kidney and liver were removed and preserved in 10% neutral buffered formalin (NBF, Sigma Aldrich, CAS #HT501128). Then, the tissues were processed. Briefly, tissues were dehydrated using different concentrations of methanol (70, 80, 95 and 100%), followed by chloroform (100%) and then embedded in paraffin. After that, tissues were sectioned (4-6 µm) using a Leica RM2125RT rotary microtome. Sections were mounted onto microscope slides (Super Frost, #G10106, Abdos, India) and dried at ~35 °C overnight. The sections were then examined under a microscope using the previously mentioned Harris hematoxylin and eosin staining technique (Shanthanagouda et al., 2014). Then the slides were mounted using DPX and allowed to dry overnight. The images were captured using a digital camera mounted microscope (Nikon 80i).

Statistical analysis

Statistical analysis of the data was performed using SPSS v16. One-way ANOVA was applied to understand the effect of TM on the growth and blood metabolic profile of fish challenged with AFB1 at $p \leq 0.05$ level of significance, followed by Duncan's multiple comparisons to determine the significant differences among the treatments.

Results

Growth performance

The current study showed percentage (%) average survival of fish as 100.00±0.00, 77.77±2.77, 97.22±2.67, 91.66±0.01 and 80.55±2.77 % in control diet (T1), AF100 (T2), AF25TM (T3), AF50TM (T4) and AF100TM (T5) treatments, respectively. Maximum survival was observed in the negative control diet (100%) followed by AF25TM (97.22%), whereas, in AF100, the lowest survival was observed. The negative control diet group did not experience any mortality during the experiment, and there was a significant ($p \leq 0.05$) increase in growth indices as measured by net body weight gain (NBWG) and specific growth rate (SGR) (Table 3). However, the fish in the AF100 group saw a notable decline in growth indices when compared to the negative control group. Conversely, the average NBWG in treatments T3, T4, and T5 was significantly higher ($p \leq 0.05$) than that of the positive control (T2) and higher than that of T4 and T5. Similarly, the average final body weight (g) and NBWG (g) were maximum in T1 and minimum in T2 and the differences among different treatments were significant ($p \leq 0.05$). Likewise,

Table 3. Growth performances of *L. rohita* in different treatments at the termination of experiment

Parameters[1]	T1	T2	T3	T4	T5
	Control diet	AF100	AF25TM	AF50TM	AF100TM
Initial body weight (g)	15.24+0.15	15.31+0.17	15.34+0.21	15.31+0.27	15.20+0.19
Final body weight (g)	28.22^{a}+0.34	24.25^{e}+0.18	27.31^{b}+0.26	26.25^{c}+0.44	25.38^{d}+0.33
NBWG (g)	13.10^{a}+0.02	8.94^{e}+0.03	11.97^{b}+0.01	10.94^{c}+0.01	10.18^{d}+0.02
SGR	0.69^{a}±0.02	0.51^{e}±0.03	0.64^{b}±0.01	0.59^{c}±0.05	0.56^{d}±0.02
FCR	1.07^{e}+0.02	1.23^{a}+0.03	1.16^{d}+0.05	1.19^{c} +0.01	1.21^{b}+0.03
K-value	1.10^{a}+0.02	0.65^{e}+0.04	1.09^{b}+0.03	1.02^{c}+0.06	0.94^{d}+0.05
Survival rate (%)	100.00^{a}±2.12	77.77^{e}±4.68	97.22^{b}±6.35	91.66^{c}±5.26	80.55^{d}±5.05

[1] NBWG: net body weight gain. SGR: specific growth rate. FCR: feed conversion ratio. K: condition factor. Values are mean ± S.E. ($p \leq 0.05$). $^{a, b,...e}$: values with same superscript in a row do not differ significantly ($p \leq 0.05$)

SGR, condition factor and feed conversion ratio (FCR) demonstrated greater improvements in both the control diet and T3 compared to T2, T4 and T5.

Blood metabolic profile

Haematological parameters

The blood parameters including TEC, TLC, PCV, and Hb levels, revealed significant reductions ($p \leq 0.05$) in fish exposed to higher concentrations of AFB1, particularly in group T2 and T5, compared to T1, T3, and T4 groups (Table 4). The results indicated that in *L. rohita* fingerlings, the parameters were significantly enhanced ($p \leq 0.05$) in treatments where AFB1 was supplemented with turmeric, particularly AF25TM (T3), which had the lowest AFB1 concentration.

Biochemical parameters and liver enzyme profile

Table 5 presents the biochemical parameters results for each treatment, indicating significant variations in total protein, albumin, globulin, and albumin/globulin ratio (A/G ratio). The lowest levels were observed in T2 and T5 compared to the negative control (T1), whereas the highest blood glucose level was observed in T2. Similarly, decreased total protein, albumin, and globulin levels were noted in T2, followed by T5, in comparison to T1, T3, and T4. Additionally, the A/G ratio was highest in the positive control (AF100), while the minimum values were observed in AF50TM and AF25TM (Table 5).

The outcomes of the liver enzyme profile, as depicted in Table 5, indicated a notable rise ($p \leq 0.05$) in the plasma AST, ALT, CAT, and SOD due to dietary AFB1, particularly evident in the AF100 and AF100TM groups. On the other hand, there was little reaction in the negative control diet group, which does not take TM supplements. Notably, a

Table 4. Response of haematological parameters of *L. rohita* in different treatments at the termination of experiment

Parameters[1]	T1	T2	T3	T4	T5
	Control diet	AF100	AF25TM	AF50TM	AF100TM
TEC ($\times 10^6$ mm^{-3})	$1.94^{a} \pm 0.01$	$1.64^{e} \pm 0.02$	$1.78^{b} \pm 0.03$	$1.76^{c} \pm 0.02$	$1.71^{d} \pm 0.01$
TLC ($\times 10^3$ mm^{-3})	$2.55^{a} \pm 0.02$	$1.65^{c} \pm 0.01$	$1.74^{b} \pm 0.03$	$1.71^{b} \pm 0.05$	$1.65^{c} \pm 0.04$
Hb (g %)	$5.37^{a} \pm 0.02$	$4.20^{d} \pm 0.03$	$5.02^{b} \pm 0.06$	$4.46^{c} \pm 0.01$	$4.23^{d} \pm 0.06$
PCV (%)	$20.16^{a} \pm 0.04$	$18.13^{e} \pm 0.04$	$19.77^{b} \pm 0.03$	$19.59^{c} \pm 0.02$	$19.38^{d} \pm 0.01$
MCV (fl)	$103.65^{c} \pm 0.24$	$110.53^{b} \pm 0.24$	$111.15^{a} \pm 0.14$	$111.15^{a} \pm 0.12$	$112.74^{a} \pm 0.13$
MCH (pg)	$27.60^{b} \pm 0.11$	$25.62^{c} \pm 0.09$	$28.24^{a} \pm 0.03$	$25.28^{c} \pm 0.02$	$24.60^{d} \pm 0.04$
MCHC (g dL^{-1})	$26.62^{a} \pm 0.08$	$23.18^{c} \pm 0.03$	$25.40^{b} \pm 0.01$	$22.75^{d} \pm 0.01$	$21.81^{e} \pm 0.02$

[1] TEC: total erythrocytes count. TLC: total leucocytes count. Hb: haemoglobin. PCV: packed cell volume. MCV: mean corpuscular volume. MCH: mean corpuscular haemoglobin. MCHC: mean corpuscular haemoglobin concentration. Values with same superscripts in a row do not differ significantly ($p \leq 0.05$)

Table 5. Blood metabolic profile of *L. rohita* in different treatments at the termination of the experiment

Biochemical parameters[1]	T1	T2	T3	T4	T5
	Control diet	AF100	AF25TM	AF50TM	AF100TM
Blood glucose (mg dL^{-1})	$68.60^{e} \pm 0.11$	$97.99^{a} \pm 0.15$	$71.49^{d} \pm 0.11$	$73.45^{c} \pm 0.10$	$88.27^{b} \pm 0.23$
Total protein (g dL^{-1})	$3.95^{a} \pm 0.02$	$1.13^{e} \pm 0.01$	$2.66^{b} \pm 0.01$	$2.46^{c} \pm 0.06$	$1.25^{d} \pm 0.02$
Albumin (g dL^{-1})	$1.48^{a} \pm 0.07$	$0.52^{d} \pm 0.05$	$0.74^{b} \pm 0.01$	$0.63^{c} \pm 0.04$	$0.59^{d} \pm 0.03$
Globulin (g dL^{-1})	$2.47^{a} \pm 0.01$	$0.61^{e} \pm 0.03$	$1.92^{b} \pm 0.01$	$1.83^{c} \pm 0.02$	$0.65^{d} \pm 0.04$
A/G ratio	$0.60^{c} \pm 0.02$	$0.85^{b} \pm 0.01$	$0.34^{d} \pm 0.02$	$0.39^{d} \pm 0.03$	$0.91^{a} \pm 0.06$
Liver enzyme profile					
ALT/SGPT (IU L^{-1})	$17.22^{e} \pm 0.03$	$45.20^{a} \pm 0.01$	$21.46^{d} \pm 0.13$	$27.38^{c} \pm 0.04$	$42.18^{b} \pm 0.01$
AST/SGOT (IU L^{-1})	$97.50^{e} \pm 0.22$	$171.61^{a} \pm 0.17$	$115.95^{d} \pm 0.25$	$127.21^{c} \pm 0.17$	$161.94^{b} \pm 0.12$
Catalase	$2.14^{d} \pm 0.01$	$5.15^{a} \pm 0.04$	$3.12^{c} \pm 0.02$	$3.25^{c} \pm 0.02$	$4.97^{b} \pm 0.05$
SOD (U mg^{-1} Hb)	$0.45^{d} \pm 0.02$	$0.84^{a} \pm 0.01$	$0.56^{c} \pm 0.04$	$0.58^{c} \pm 0.06$	$0.78^{b} \pm 0.03$

[1] A/G ratio: albumin/globulin ratio. ALT: Alanine aminotransferase. SGPT: Serum glutamic pyruvic transaminase. AST: Aspartate aminotransferase. SGOT: Serum glutamic-oxaloacetic transaminase. SOD: Superoxide dismutase. Values with same superscripts in a row do not differ significantly ($p \leq 0.05$)

significant improvement was observed in the AF25TM group, characterized by a low AFB1 dose (25 ppb) and TM inclusion.

Tissue microarchitecture

Brain. Fish fed on the negative control (T1) diet displayed no discernible alterations in brain tissue. Across systematically arranged layers from the ependyma to the outer surface, including Stratum periventricular (SPV), Stratum album central (SAC), Stratum griseum central (SGC), Stratum fibrosum et griseum superficial (SFGS), Stratum opticum (SO), and the Stratum marginale (SM), integrity was maintained with no detachment of granular cells or lesions observed in any of the stratified zones in the tectum (Fig. 1A). However, in AF100 group, outer layers such as SO and SM exhibited cell detachments, varied focal necrosis, cell degenerations, and vacuolization in SFGS, SAC, and SPV particularly evident in AF100 (Fig. 1B). On the other hand, different tectum layers were seen strikingly compactly arranged in the AF25TM treatment (Fig. 1C), while in AF100TM treatment (Fig. 1D) less distinction in the separation of layers (LDSL) were observed, accompanied by the presence of pyknotic nuclei (PN).

Kidney. In the negative control group (T1), the kidney exhibited a normal structure, characterized by distal and proximal convoluted tubules and glomeruli (Fig. 2A). However, in the positive control group (AF100), significant histological alterations were observed, including clumping of glomeruli and mononuclear cell infiltration in inter-tubular tissues (Fig. 2B). Conversely, in the AF25TM group (Fig. 2C), dilatation and congestion of blood vessels, multi-clumping of the glomerulus, and deposition of melanomacrophages were observed. Furthermore, the kidney micro-architecture in the AF100TM group (Fig. 2D) showed vacuolization and degenerated glomeruli.

Liver. In the current study, the negative control group, which did not receive AFB1, exhibited normal oval to hexagonal hepatocytes with centrally located nuclei, en-

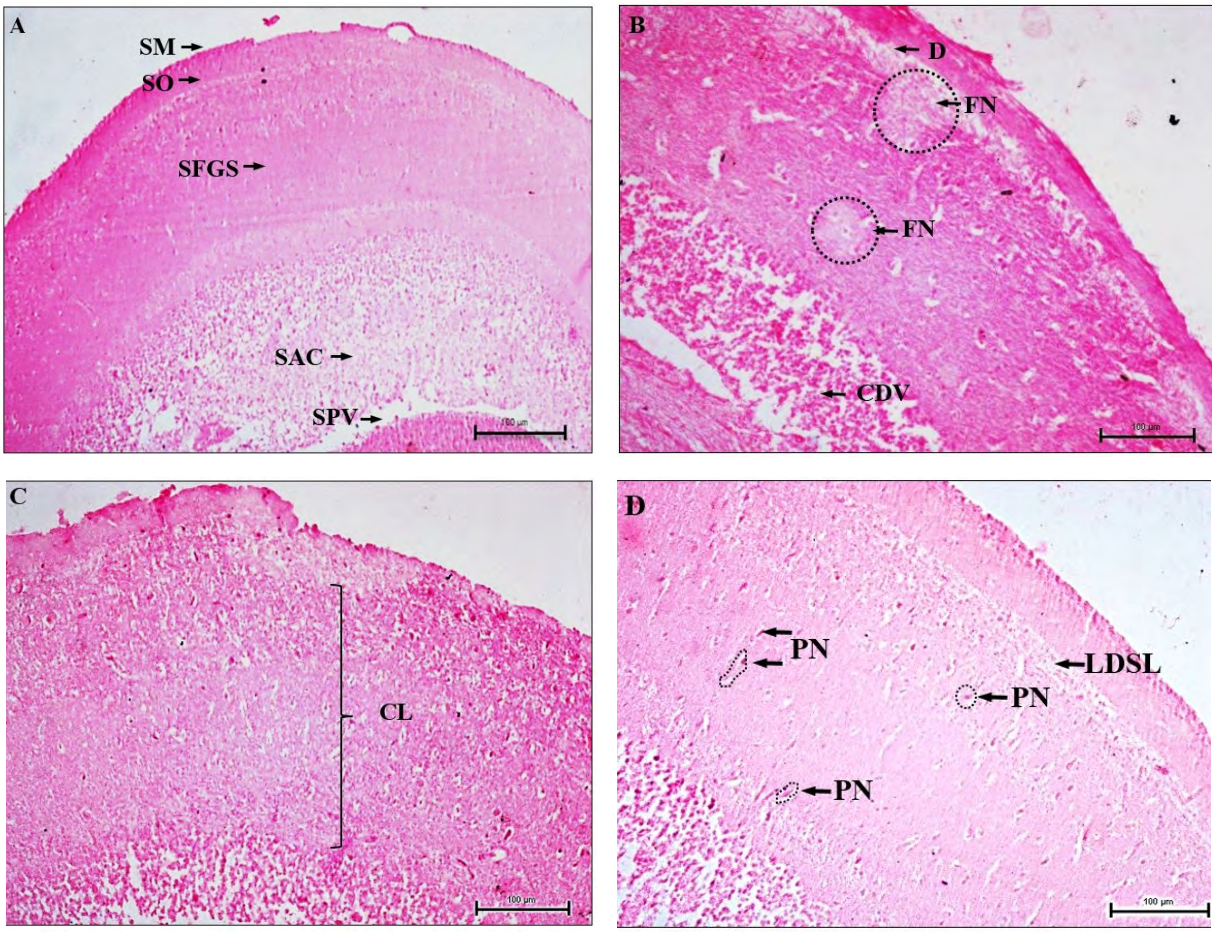

Figure 1. Representative pictures of the brain of *L. rohita.* (A) Negative control diet: layers of tectum- Stratum marginale (SM), S. opticum (SO), S. fibrosum et. grisium superficial (SFGS), S. album central (SAC), S. periventricular (SPV). (B) AF100: detachment (D) of SM and SO layers, focal necrosis (FN), cell degeneration and vacuolization (CDV). (C) AF25TM: compact layers of tectum (CL). (D) AF100TM: less distinction in the separation of layers (LDSL), and pyknotic nuclei (PN). Scale bar = 100 µm, H & E staining.

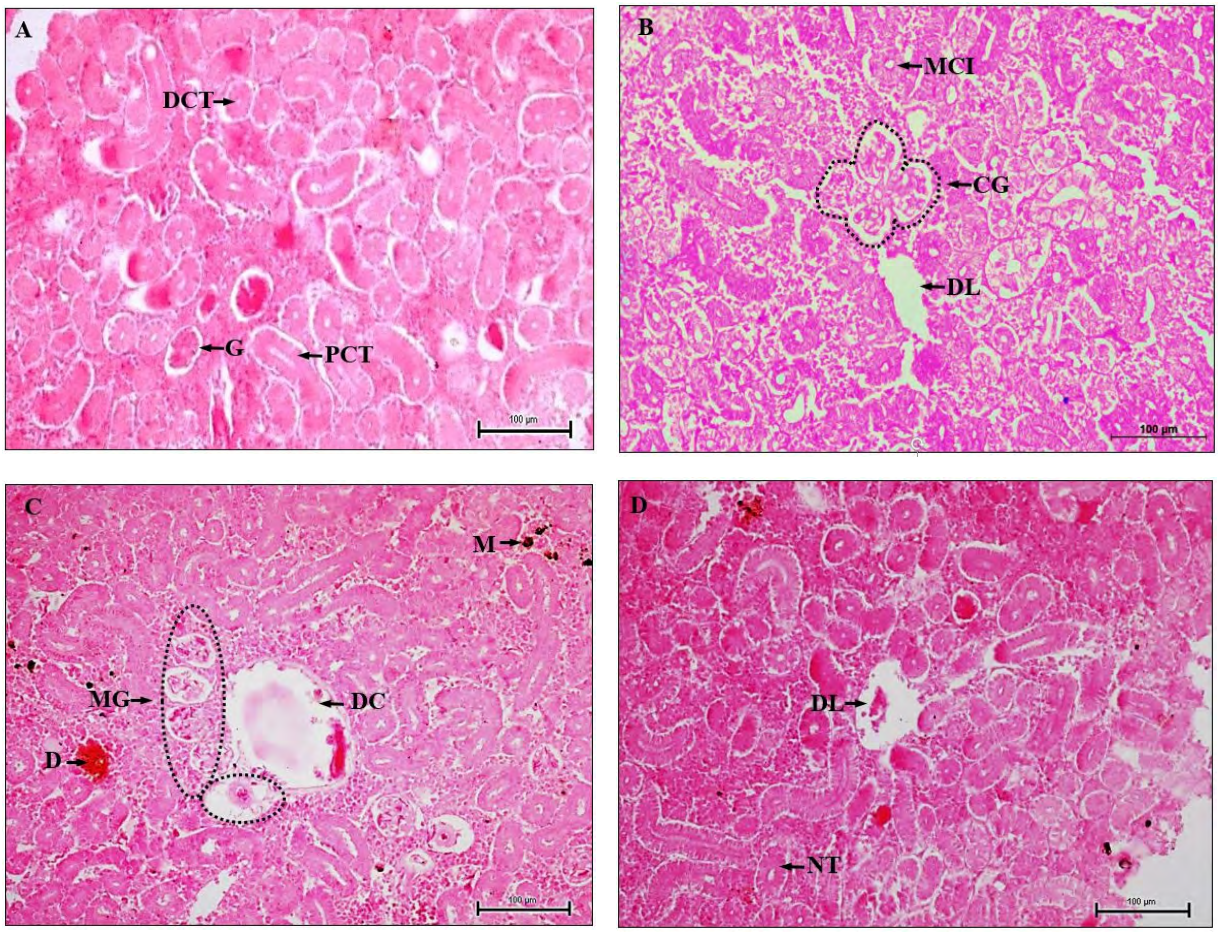

Figure 2. Representative pictures of the kidney of *L. rohita.* (A) Control diet: distal convoluted tubule (DCT), proximal convoluted tubule (PCT), glomerulus (G). (B) AF100: clumping of glomerulus (CG), mononuclear cell infiltration in inter tubular tissue (MCI), dilatation (DL). (C) AF25TM: dilatation and congestion of blood vessels (DC), multiclumping of the glomerulus (MG), deposition (D), melanomacrophages (M). (D) AF100TM: normal tubules (NT) and dilatation (DL). Scale bar= 100 µm, H & E staining.

dothelial cells, and a uniform cytoplasm (Fig. 3A). However, in AF100 (positive control), notable alterations, including vacuolization of hepatocytes, blood cell congestion in the central vein, and depositions were observed (Fig. 3B). In contrast, in AF25TM (Fig. 3C), reduced dilatation of the central vein, vacuolization, and binucleated hepatocytes were recorded. Meanwhile, in AF100TM (Fig. 3D), an obliterated central vein and possible deposition of TM particles were observed.

Discussion

Mycotoxins, produced by significant fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species, have been associated with severe toxic effects on vertebrates. Among these, AFB1 was the first mycotoxin investigated in aquaculture. Aflatoxins, the fungal metabolites produced by *A. flavus* and *A. parasiticus*, exhibit a range of harmful effects including teratogenic, neurotoxic, hepatotoxic, mutagenic, and carcinogenic effects in fish, along with suppression of the immune system in animals (Bhatt & Pandey, 2022). Despite this, only a limited number of studies have explored the potential efficacy of herbs or herbal extracts in mitigating the adverse effects of aflatoxins in aquatic animals.

Growth indices

The high dietary AFB1 levels significantly affected various growth performance and health status parameters in fish. Over a 90-days period of exposure to 100 ppb AFB1 reduced growth indices, including NBWG, SGR, and survival rate

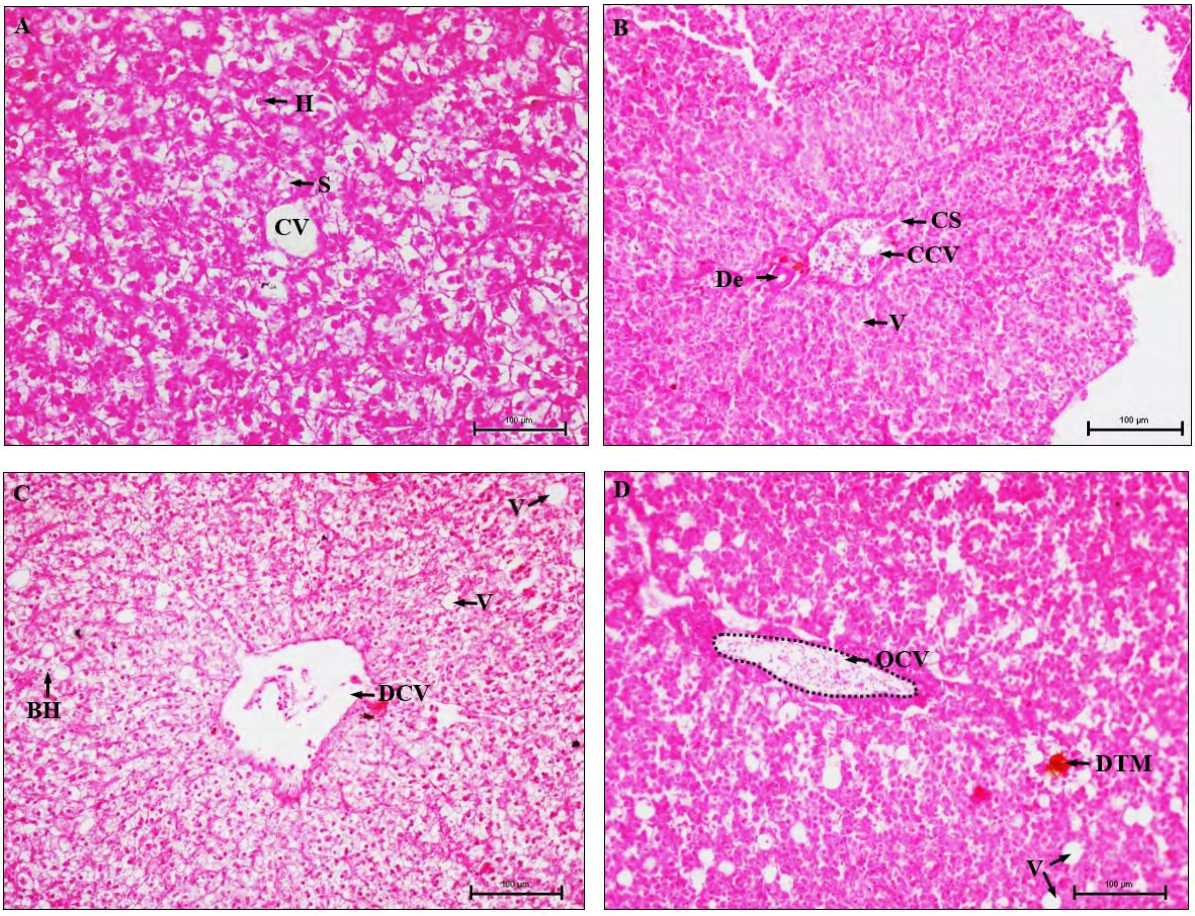

Figure 3. Representative pictures of the liver of *L. rohita*: (A) Control diet: hepatocytes (H), sinusoids (S), central vein (CV). (B) AF100: congestion in central vein (CCV), congestion in sinusoids (CS), deposition (De), vacuolization of hepatocytes (V). (C) AF25TM: dilation of central vein (DCV), vacuolization (V), binucleated hepatocytes (BH). (D) AF100TM: obliterated central vein (OCV), deposition of turmeric particles (DTM), vacuolization (V). Scale bar= 100 µm, H & E staining.

compared to diets with 0 and 25 ppb AFB1. These findings indicate that the ingestion of low to moderate doses of AFB1 leads to significant growth depression, reduced weight gain, and decreased feed efficacy in a dose-dependent manner. The amount of toxin and duration of exposure are related to AFB1's detrimental effect on growth parameters. Similar outcomes have been observed in broiler chicks (Manafi et al., 2014), and in juvenile Nile tilapia (Tuan et al., 2002).

Mahmoud et al. (2014) proposed that the augmented growth response with TM supplementation may stem from enhanced feed consumption and utilization, suggesting increased nutrient digestibility and antioxidant activity of turmeric, thereby promoting protein synthesis through enzymatic systems. Consistent with earlier studies, Ashry et al. (2021) observed significant enhancements in growth rate, SGR, and FCR in Gilthead seabream diet supplemented with TM. Similarly, curcumin has demonstrated improvements in growth performance in *L. rohita* (Kaur et al., 2020).

Haematological parameters

The study revealed significant increase ($p \leq 0.05$) in haematological parameters (TEC, TLC, Hb, hematocrit value, and MCV) in groups receiving higher doses of AFB1 compared to those receiving lower doses. Similar findings were reported in *O. niloticus* (Tuan et al., 2002) and *L. rohita* (Bhatt et al., 2023b). In contrast, diets with low doses of AFB1 and supplemented with TM (5 g kg^{-1}) exhibited elevated Hb, PCV, TEC, and TLC counts, suggesting potential immune-modulatory effects of TM (Kumar et al., 2013). In line with our findings, green terror (*Andinoacara rivulatus*) fed TM diets showed an increase in TLC, as observed by Mooraki et al. (2019). Similarly, the elevation in Hb content may be attributed to increased enzyme synthesis or red blood cell size. The decrease in TEC, TLC, Hb, and hematocrit values suggests anemia, possibly due to inhibition of enzyme activities in heme biosynthesis or increased erythrocyte disruption in hematopoietic organs

(Jenkins et al., 2003). However, the decline in TLC count might be attributed to epinephrine release during stress, leading to spleen contraction and reduced leukocyte count, thereby compromising the immune system and rendering fish susceptible to infection (Mooraki et al., 2019). Blood indices such as MCV, MCH, and MCHC play a crucial role in assessing erythrocyte relevance and oxygen-carrying capacity, as well as diagnosing anaemic conditions in fish and other animals (Houston, 1997).

Biochemical parameters

In the present study, a high blood glucose level was observed probably due to increased liver glycogenolysis and a decrease in the secretion of insulin as an effect of AFB1, which in turn reduces the process of glycogenesis, resulting in increased blood glucose level. Similar results were obtained by El-Boshy et al. (2008) who reported increased blood glucose levels in AFB1-challenged *O. niloticus*. The results indicated that the blood glucose level closely correlates to stress level in fish and represents the state of respiratory and nutritional disturbance.

Furthermore, there was a gradual decline in total protein, albumin and globulin in AFB1-exposed fish, indicating decreased protein synthesis as reported in *Onchorhynchus mykiss* (Ruby et al., 2014), which could be due to the damaging effects of AFB1 on hepatocytes. The current study´s results are consistent with earlier research on *O. niloticus* (Shehata et al., 2009), showing that fish fed diets containing plant extracts had higher humoral immunity. This suggests that TM supplementation may fortify the immune system and offer long-term resistance (Sahu et al., 2008), exhibiting stronger antioxidant activity. Addition of 0.5% rosemary to AFB1-contaminated food significantly increases total protein and globulin levels, activating the immune system and improving fish health (Naiel et al., 2019).

Response of liver enzyme

The liver, being the primary site for AFB1 metabolism, is highly susceptible to damage. Dietary AFB1 significantly increased plasma AST and ALT activity ($p \leq 0.05$), indicating potential liver dysfunction or swelling, leading to enzyme release into the bloodstream (Akrami et al., 2015). However, in the current study, fish exposed to 25 ppb AFB1 + 5 g kg^{-1} TM supplementation (T3) showed significantly reduced serum AST and ALT activity, indicating ameliorative efficacy. Similar findings were also observed by El-Barbary & Mehrim (2009) in *O. niloticus*. Moreover, increased SOD and CAT activities in T2 and T5 suggest dietary AFB1-induced oxidative stress, prompting antioxidant enzyme activation as defence mechanisms against AFB1 effects. Furthermore, by converting superoxide anion free radicals, SOD reduces the damage to cellular DNA. Though the fish were challenged with AFB1, supplementation of TM enhanced health by increasing the protein concentration which might be due to the antioxidant and therapeutic properties of TM. This suggests that the application of TM in aquaculture may feed the aquatic species´ general well-being.

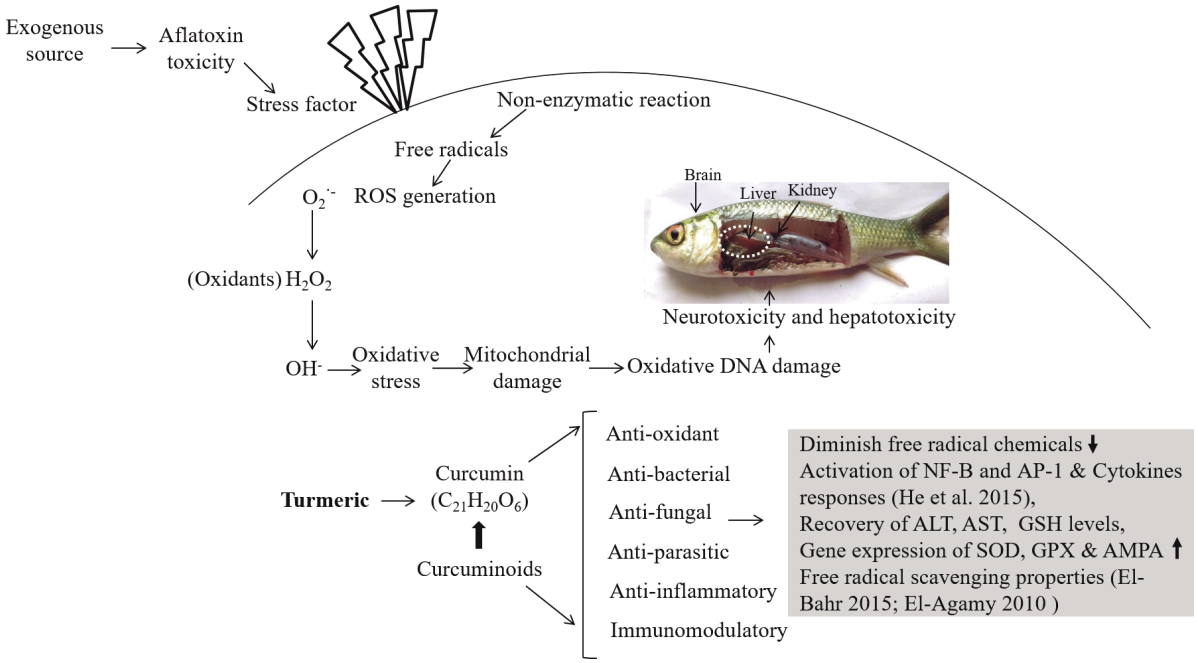

Figure 4. Aflatoxin-induced toxicity and ameliorative action of turmeric

Tissue micro-architecture

Histopathology has been widely used as a suitable biomarker in assessing the health of fish exposed to xenobiotics and or stress factors since they reflect the actual health status of the animals. Amongst several xenobiotics, exposures to aflatoxin have caused serious damage to fish, and the current study has investigated the histopathological responses in key organs and efficiency of TM in amelioration of AFB1 in the most widely cultured tropical fish, rohu.

Brain. In this study, the alterations observed in the fish brain may be caused by AFB1 toxicity, as the brain is the most sensitive tissue to AFB1 toxicity. The severity of alterations observed was more pronounced in the groups which received the highest AFB1 over an extended period in comparison to the group fed less or no contaminated diet. Similar results were also reported in channel catfish (*Ictalurus punctatus*) (Jantrarotai & Lovell, 1990). Further, the findings advocated that the brain could accumulate AFB1 as per dose and time-dependent manner, and the toxicity could be mainly attributed to free radicals and oxidative stress produced by the aflatoxin (Fig. 4). Typically, it can combine with the thiol group of enzymes working in antioxidant processes and disrupt the normal functioning of the brain (Baldissera et al*.,* 2018) and the intoxicated fish might have suffered from neurotoxicity. The ability of TM to scavenge ROS through interaction with the oxidative cascade to suppress oxidative enzymes and normalize the antioxidant levels, thereby preventing the cell structures of the brain from more damage, has been evidenced (El-Bahr, 2015).

Kidney. In the present study, the acute toxic effects of AFB1 on the tissue micro-architecture of the kidney of *L. rohita* have been elucidated. The tissue microscopic examinations showed that AFB1 has a great significant effect on the kidney structure that received the highest AFB1 in comparison to the groups fed a less toxin-contaminated diet, as this is consistent with what has been found in previous studies, where fish were exposed to contaminants and pollutants (Hadi & Alwan, 2012). In comparison, fish fed with an aflatoxin-free diet showed normal glomeruli, and proximal and distal convoluted tubules. The damages observed in AFB1-administered groups in the present study could be attributed to the ability of AFB1 to induce free radical generation and increase the activity of catalase in kidney cells (Taheri et al., 2017). This is consistent with several other findings, which report a direct correlation between AFB1-induced oxidant generation and resultant DNA damage (Meki et al., 2001). However, TM seemed to lessen the toxicity by changing the way AFB1 is microsomally activated. It also increased detoxification, protected DNA and stopped the development of future DNA adduct, all of which prevented the toxicity caused by AFB1 from happening.

Liver. The liver is one of the most affected organs due to contaminants, and pollutants. Hepatocytes are the primary target of contaminants. This is evidenced in the present study, where significant alterations observed were more pronounced in the groups which received the highest AFB1 in comparison to the groups fed less or no toxin. Diets contaminated with AFB1 showed dose-dependent changes in histopathological damages similar to those found in the liver of *L. rohita* (Bhatt et al., 2023c). However, the severity of tissue damage in fish treated with different levels of AFB1 increased with an increase in dosage and period of exposure. The present study highlights that the abnormal changes observed are similar to the previous findings having different stressors. However, in the current study, TM appeared to reduce the AFB1 toxicity for its therapeutic properties and probably mediated through its antioxidant and anti-inflammatory action (Aggarwal et al*.,* 2007). Similarly, in the liver and kidney of mice given AFB1, TM restored the activities of both enzymatic and non-enzymatic antioxidants and restored the levels of lipid peroxidation (Verma & Mathuria, 2008). Fish that were poisoned with aflatoxin may have been able to recover through similar processes.

Conclusion

In conclusion, the findings of the present study indicate that aflatoxin presence in feed may have adverse effects on the health and tissue microarchitecture of the brain, kidney, and liver. Interestingly, supplementation of turmeric resulted in reduced damage at the tissue microarchitecture level in fish exposed to aflatoxin. In summary, the results suggest that turmeric supplementation elicited different modulatory responses in biochemical and tissue microarchitecture studies, depending on the dosage and fish physiology. Although turmeric supplementation may alleviate the effects of aflatoxin to some degree, complete recovery was not achieved. Further exploration utilizing transcriptomics and/or proteomics is imperative to gain deeper insights into the precise mechanisms of turmeric action.

Ethical approval: The study was conducted as per approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The approval number for this study was PGS/A-1/21/602-3 dated 01/02/2021.

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Conceptualization, Data curation, Investigation, Project administration, Supervision, Validation, Writing – review & editing. **Abhed Pandey:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Neelam Bansal:** Investigation, Supervision, Validation. **Jaspal Singh Hundal:** Investigation, Supervision, Validation. **Sachin Onkar Khairnar:** Investigation, Software.

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References

Aggarwal BB, Surh YJ, Shishodia S (Eds.), 2007. The molecular targets and therapeutic uses of curcumin in health and disease. 595. Springer Science and Business Media, USA. https://doi.org/10.1007/978-0-387-46401-5

Akrami R, Gharaei A, Mansour MR, Galeshi A, 2015. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish Shellfish Immunol 45(2): 828-834. https://doi.org/10.1016/j.fsi.2015.06.005

Alambra JR, Alenton RRR, Gulpeo PCR, Mecenas CL, Miranda AP, Thomas RC, et al., 2012. Immunomodulatory effects of turmeric, *Curcuma longa* (Magnoliophyta, Zingiberaceae) on *Macrobrachium rosenbergii* (Crustacea, Palaemonidae) against *Vibrio alginolyticus* (Proteobacteria, Vibrionaceae). Aquac Aquar Conserv Legis 5(1): 13-17.

AOAC, 2012. Official Methods of Analysis. 19th Edition. Association of Official Analytical Chemists, Vol II. Washington D.C., USA.

Ashry AM, Hassan AM, Habiba MM, El-Zayat A, El-Sharnouby ME, Sewilam H et al., 2021. The impact of dietary curcumin on the growth performance, intestinal antibacterial capacity, and haemato-biochemical parameters of gilthead seabream (*Sparus aurata*). Animals 11(6): 1779. https://doi.org/10.3390/ani11061779

Baldissera MD, Souza CF, Zeppenfeld CC, Descovi SN, Moreira KLS, da Rocha MIUM, et al., 2018. Aflatoxin B1-contaminated diet disrupts the blood-brain barrier and affects fish behavior: Involvement of neurotransmitters in brain synaptosomes. Environ Toxicol Pharmacol 60: 45-51. https://doi.org/10.1016/j.etap.2018.04.003

Bhatt D, 2023. Efficacy of turmeric and quercetin in Labeo rohita challenged with Aflatoxin B1- Potential alternatives for fungal control in aquaculture. Doctoral Dissertation. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Bhatt D, Pandey A, 2022. Aflatoxins and their repercussions in aquaculture: A review. Fish Technol 59: 69-78.

Bhatt D, Pandey A, Holeyappa SA, 2023a. Turmeric- A versatile multi- potential medicinal plant in aquaculture. Environ Eco 41 (1C): 649-655.

Bhatt D, Pandey A, Holeyappa SA, Hundal JS, Khairnar SO, 2023b. Protective effect of quercetin on haematology and flesh quality in *Labeo rohita* against aflatoxin B1. Natl Acad Sci Lett 46: 1-5. https://doi.org/10.1007/s40009-023-01333-9

Bhatt D, Pandey A, Holeyappa SA, Bansal N, Khairnar SO, 2023c. Histomorphological alterations in the liver of *Labeo rohita* exposed to aflatoxin B1 and quercetin enriched diets. Natl Acad Sci Lett 46: 1-8. https://doi.org/10.1007/s40009-023-01369-x

Boyd CE, 1998. Water quality for pond aquaculture. Research and development series No.43. International Centre for Aquaculture and Aquatic Environment, Auburn University, Alabama. pp 37.

El-Bahr SM, 2015. Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. Phytother Res 29(1): 134-140. https://doi.org/10.1002/ptr.5239

El-Barbary MI, Mehrim AI, 2009. Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus*. J Fish Aquat Sci 4(4): 178-190. https://doi.org/10.3923/jfas.2009.178.190

El-Boshy ME, El-Ashram AMM, El-Ghany NAA, 2008. Effect of dietary beta-1, 3 glucan on immunomodulation on diseased *Oreochromis niloticus* experimentally infected with aflatoxin B1. 8th Int Symp on Tilapia in Aquaculture. pp. 1109-1127.

Fagnon MS, Thorin C, Calvez S, 2020. Meta-analysis of dietary supplementation effect of turmeric and curcumin on growth performance in fish. Rev Aquac 12(4): 2268-2283. https://doi.org/10.1111/raq.12433

FAO, 2018. The state of world fisheries & aquaculture. Fisheries and aquaculture development, Rome, Italy.

Ferreira LA, Henriques OB, Andreoni AA, Vital GR, Campos MM, Habermehl GG, et al., 1992. Antivenom and biological effects of ar-turmerone isolated from *Curcuma longa* (Zingiberaceae). Toxicon 30(10): 1211-1218. https://doi.org/10.1016/0041-0101(92)90437-A

Gonçalves RA, Do Cam T, Tri NN, Santos GA, Encarnação P, Hung LT, 2018. Aflatoxin B1 (AFB1) reduces growth performance, physiological response, and disease resistance in Tra catfish (*Pangasius hypophthalmus*). Aquac Int 26(3): 921-936. https://doi.org/10.1007/s10499-018-0259-x

Hadi AA, Alwan SF, 2012. Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum. Int J Pharm Life Sci 3(11): 2071-2081.

Halver JE, 1957. Nutrition of salmonoid fishes. J Nutr 62(2): 225-243. https://doi.org/10.1093/jn/62.2.225

Houston AH, 1997. Review: Are the classical haematological variables acceptable indicators of fish? Trans Am Fish Soc 126: 879-894. https://doi.org/10.1577/1548-8659(1997)126<0879:RATCHV>2.3.CO;2

Jantrarotai W, Lovell RT, 1990. Subchronic toxicity of dietary aflatoxin B1 to channel catfish. J. Aquat Anim Health 2(4): 248-254. https://doi.org/10.1577/1548-8667(1990)002<0248:STODAB>2.3.CO;2

Jenkins F, Smith J, Rajanna B, Shameem U, Umadevi K, Sandhya V, et al., 2003. Effect of sub-lethal concentrations of endosulfan on haematological and serum biochemical parameters in the carp *Cyprinus carpio*. Bull Environ Contam Toxicol 70(5): 0993-0997. https://doi.org/10.1007/s00128-003-0080-7

Kaur A, Holeyappa SA, Bansal N, Kaur VI, Tyagi A, 2020. Ameliorative effect of turmeric supplementation in feed of

Labeo rohita (Linn.) challenged with pathogenic Aeromonas veronii. Aquac Int 28(3): 1169-1182. https://doi.org/10.1007/s10499-020-00518-z

Kumar S, Raman RP, Pandey PK, Mohanty S, Kumar A, Kumar K, 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn.1758) and resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol 34: 564-573. https://doi.org/10.1016/j.fsi.2012.11.038

Mahmoud MMA, El-Lamie MMM, Dessouki AA, Yusuf MS, 2014. Effect of turmeric (*Curcuma longa*) supplementation on growth performance, feed utilization, and resistance of Nile tilapia (*Oreochromis niloticus*) to *Pseudomonas fluorescens* challenge. Global Res J Fish Sci Aquac 1(12): 26-33.

Manafi M, Hedayati M, Yari M, 2014. Aflatoxicosis and herbal detoxification: the effectiveness of thyme essence on performance parameters and antibody titers of commercial broilers fed aflatoxin B1. Res Zool 4(2): 43-50.

Meki ARM, Abdel-Ghaffar SK, El-Gibaly I, 2001. Aflatoxin B1 induces apoptosis in rat liver: protective effect of melatonin. Neuro Endocrinol Lett 22(6): 417-426.

Mooraki N, Batmany Y, Zoriehzahram SJ, Kakoolaki SH, 2019. Evaluating the effect of using turmeric (*Curcuma longa*) on growth performance and haematological parameters of the ornamental fish, Green Terror (*Andinocara rivulatus*). J Surv Fish Sci 5(2): 37-47. https://doi.org/10.18331/SFS2019.5.2.5

Mukherjee, 1988. Medical Laboratory Technology. Tata Mcgraw Hill Publ Co Ltd, 1: 2425-2443. New Delhi.

Naiel MA, Ismael NE, Shehata SA, 2019. Ameliorative effect of diets supplemented with rosemary (*Rosmarinus officinalis*) on aflatoxin B1 toxicity in terms of the performance, liver histopathology, immunity and antioxidant activity of Nile tilapia (*Oreochromis niloticus*). Aquaculture 511: 734264. https://doi.org/10.1016/j.aquaculture.2019.734264

Nishikimi M, Rao NA, Yagi K, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46(2): 849-854. https://doi.org/10.1016/S0006-291X(72)80218-3

Ruby DS, Masood A, Fatmi A, 2014. Effect of aflatoxin contaminated feed on energy reserves of fish *Labeo rohita* (Hamilton). Curr World Environ 9(3): 1037. https://doi.org/10.12944/CWE.9.3.60

Sahli T, 1962. Textbook of Clinical Pathology. Williams and Williams Corporation, Baltimore, USA.

Sahu S, Das BK, Mishra J, Pradhan SK, Samal, Sarangi N, 2008. Effect of dietary *Curcuma longa* on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*. Aquac Res 39: 1720-1730. https://doi.org/10.1111/j.1365-2109.2008.02048.x

Shanthanagouda AH, Guo BS, Ye RR, Chao L, Chiang MWL, Singaram G, et al., 2014. Japanese medaka: A non-mammalian vertebrate model for studying sex and age-related bone metabolism in vivo. PLoS One 9: e88165. https://doi.org/10.1371/journal.pone.0088165

Shehata SA, El-Melegy KM, Ebrahim MS, 2009. Toxicity reduction of aflatoxin B1 by vitamin C in fish. J Arab Aquac Soc 4: 73-86.

Sivagurunathan A, Meera KA, Innocent BX, 2011. Investigation of immunostimulant potential of *Zingiber officinale* & *Curcuma longa* in *Cirrhinus mrigala* exposed to *Pseudomonas aeruginosa*-Haematological assessment. Int J Res Ayurveda Pharm 2(3): 899-904.

Taheri S, Banaee M, Nematdoost Haghi B, Mohiseni M, 2017. Evaluation of nephrotoxic effects of aflatoxins on common carp (*Cyprinus carpio*). Iran J Toxicol 11(2): 51-58. https://doi.org/10.29252/arakmu.11.2.51

Tuan NA, Grizzle JM, Lovell RT, Manning BB, Rottinghaus GE, 2002. Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B1. Aquaculture 212(1-4): 311-319. https://doi.org/10.1016/S0044-8486(02)00021-2

Verma RJ, Mathuria N, 2008. Curcumin ameliorates aflatoxin-induced lipid-peroxidation in liver and kidney of mice. Acta Pol Pharm - Drug Res 65(2): 195-202.