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Dietary Quercetin Mitigates Aflatoxin B_1-Induced Toxicity in *Labeo rohita*: Evidence from Physiological and Tissue Micro-Architecture Responses

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Abstract

This study evaluated the protective effect of dietary quercetin (QUE) against aflatoxin B_1 (AFB_1) toxicity in *Labeo rohita* fingerlings during a 90-day feeding trial. Fish were assigned to different dietary treatments, including a control (CON), AFB_1-contaminated diets, and AFB_1 diets supplemented with QUE (200 mg kg^{-1}). Growth performance, biochemical parameters, antioxidant enzyme activities, and tissue micro-architecture of the liver, and kidney were assessed. Results revealed that QUE supplementation significantly improved growth, enhanced biochemical and antioxidant responses, and reduced tissue damage, particularly at lower AFB_1 exposure. However, higher AFB_1 levels caused noticeable physiological disturbances and histopathological alterations. QUE was effective in alleviating oxidative stress and preserving tissue integrity in exposed fish. Overall, the study demonstrates that dietary QUE can mitigate AFB_1-induced toxicity and improve fish health, suggesting its potential as a natural feed additive for enhancing resilience against mycotoxin contamination in aquaculture systems.

Keywords: Flavonoid supplementation, mycotoxin detoxification, oxidative stress modulation, physiology

Introduction

Mycotoxin contamination in aquaculture feeds has become a major concern affecting fish health, feed safety, and overall productivity. These toxic secondary metabolites, produced by various fungi, commonly contaminate agricultural commodities and feed ingredients, with nearly 25% of global crops affected annually (Eskola et al., 2020). Among them, aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, and zearalenone are the most significant in animal production systems (Pitt, Taniwaki, & Cole, 2013). Common plant-based feed ingredients such as corn, soybean meal, wheat, DDGS, canola, and cottonseed are particularly prone to fungal contamination. The growing reliance on these ingredients in aquafeeds has increased the risk of mycotoxin exposure in fish,

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highlighting the need for effective mitigation strategies to ensure sustainable aquaculture practices. Among mycotoxins, AFB_1 is one of the most toxic and carcinogenic, primarily produced by *Aspergillus flavus* and *A. parasiticus* (Bhatt & Pandey, 2022). Its contamination can occur both during crop growth and storage, especially under warm and humid conditions (Peng et al., 2022). The presence of AFB_1 in aquafeeds poses a serious risk to fish health and food safety, as it negatively affects growth, immune function, and metabolic activities (Gonçalves et al., 2018). Numerous studies across various fish species have demonstrated its effects in species such as tilapia (*Oreochromis aureus* × *O. niloticus*) (Deng et al., 2010; Hussain, Mateen, & Gatlin, 2017), rohu (*L. rohita*) (Bhatt, Holeyappa et al., 2024; Bhatt, Pandey, Holeyappa, Bansal, & Khairnar, 2024; Bhatt, Pandey, Holeyappa, Hundal, & Khairnar, 2024), and common carp (*Cyprinus carpio*) (Tasa, Imani, Moghanlou, Nazdar, & Moradi-Ozarlou, 2020; Nasirin et al., 2023). AFB_1 exposure has been shown to impair growth performance, disrupt hepatic function, and alter haematological and physiological parameters, highlighting its significant impact on aquaculture systems.

In recent years, natural bioactive compounds, particularly polyphenols, have gained considerable attention as dietary supplements to improve fish health and mitigate toxic stress in aquaculture. These compounds are known to enhance growth performance, antioxidant capacity, immune responses, and disease resistance in fish (Ahmadifar et al., 2021; Nasirin et al., 2023; Terzi, Tahiluddin, & Kadak, 2023). Among them, QUE, a flavonoid widely present in fruits and vegetables such as onions, apples, and cabbage, has attracted significant interest due to its strong antioxidant, anti-inflammatory, and protective properties (Dong et al., 2020). QUE acts as an efficient scavenger of reactive oxygen and nitrogen species, thereby protecting biological systems from oxidative damage (Boots, Haenen, & Bast, 2008), and has been shown to regulate antioxidant defence mechanisms (Pan et al., 2023). Studies in fish such as zebrafish, tilapia, and silver catfish (*Rhamdia quelen*) have demonstrated that QUE supplementation alleviates oxidative stress, inflammation, and lipid peroxidation while enhancing antioxidant status (Pês et al., 2018; Deng et al., 2019; Zhang, Jiang, Wang, & Qi, 2021). Additionally, it may improve gut health, nutrient absorption, and growth performance (Shabbir et al., 2021; Xin et al., 2024). However, complete prevention of AFB_1 contamination in aquafeeds remains challenging, and its adverse effects can only be partially controlled, highlighting the need for effective dietary interventions.

Therefore, developing effective nutritional strategies to mitigate AFB_1-induced toxicity in aquaculture is essential, as information on the protective role of QUE in Indian major carps remains limited. *L. rohita*, a key species in South Asian aquaculture, is highly susceptible to mycotoxin-contaminated feeds. This study evaluated the effects of dietary AFB_1 exposure and the protective role of QUE on growth, biochemical responses, antioxidant status, and tissue integrity to support improved fish health and sustainable aquaculture.

Materials and Methods

AFB_1 and QUE (≥ 95% purity), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Five experimental diets (CON $AFB_1$100, $AFB_1$25+QUE, $AFB_1$50+QUE, and $AFB_1$100+QUE) were formulated to be isonitrogenous and isocaloric along with a negative CON (basal feed+$AFB_1$0), and a positive control ($AFB_1$100). The dietary AFB_1 concentrations (25, 50, and 100 ppb) were selected based on previous studies reporting toxicological effects of AFB_1 in fish at comparable exposure levels (Ahmed et al., 2020; Hoseyni et al., 2024). These concentrations represent low to moderate contamination levels that may occur in aquafeeds under practical farming conditions. The quercetin supplementation level (200 mg kg^{-1} diet) was selected based on previous studies demonstrating its beneficial effects on growth performance, antioxidant status, and immune responses in fish (Xu et al., 2019; Armobin et al., 2023). All basal feed ingredients were finely ground using a grinder and passed through a 60-mesh sieve to obtain uniform particle size. QUE was carefully weighed and thoroughly mixed with the other feed ingredients to ensure uniform distribution before pellet formation. 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 (psi) for 15 minutes. Following cooking, the dough was cooled to room temperature, and AFB_1 dilutions was dissolved in 70% methanol to create a stock solution, which was then serially diluted (25, 50, and 100 ppb). These solutions were then uniformly sprayed onto the

Table 1. Details of treatments with respect to feed formulation and their inclusion level

	Details of diet groups				
Diet groups	Dosage				
$AFB_1$0 (CON)	Basal diet* without AFB_1 kg^{-1} feed (CON)				
$AFB_1$100 (+ve control)	Basal diet with 100ppb AFB_1 kg^{-1} feed				
$AFB_1$25+QUE	Basal diet with 25ppb AFB_1 and 200 mg QUE kg^{-1} feed				
$AFB_1$50+QUE	Basal diet with 50ppb AFB_1 + 200 mg QUE kg^{-1} feed				
$AFB_1$100+QUE	Basal diet with 100ppb AFB_1 + 200 mg QUE kg^{-1} feed				
	Ingredient formulations and compositions of experimental diets				
Ingredients	CON	$AFB_1$100	$AFB_1$25+QUE	$AFB_1$50+QUE	$AFB_1$100+QUE
Soyabean (%)	40	40	40	40	40
Rice bran (%)	30	30	30	30	30
Mustard 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
QUE (mg)	-	-	200	200	200
AFB_1 (ppb)	-	100	25	50	100

QUE: Quercetin; AFB_1: Aflatoxin B_1

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, 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 high-density polythene bags, labelled, and stored in a refrigerator at 4 °C until further use, consistent with the methodology previously described (Bhatt, Holeyappa et al., 2024). Proximate composition analysis of diets (moisture, crude protein, crude fat, crude fiber, and ash) was performed using standard AOAC (2012) methods, and results are presented in Table 2.

Healthy fish fingerlings were sourced from Fish Farm, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (India) and then acclimated for 20 days in fibre-reinforced plastic (FRP) experimental tanks under a controlled temperature range of 24-28 °C. During this period, they were fed a standard basal diet and a constant temperature (24-28 °C) was maintained before the experiment. A total of 180 fish (mean initial length: 12.40 ± 0.06 cm; mean initial weight: 17.31 ± 0.03 g) were allocated into 15 FRP (1.5x1.0x0.75 m) tanks (12 fish/tank), with triplicate groups assigned to each dietary treatment and maintaining a stocking density of 12 fish per tank with continuous aeration. The fish were fed twice daily at 2-3% of their body weight (9-10 AM and 4-5 PM) for 90 days. Before each feeding, leftover feed and fecal matter were siphoned out to maintain water quality. Water in the tanks was partially exchanged (33%) with pre-aerated water every week to remove accumulated waste. The experimental diets and pellets were prepared following the methods described in previous studies (Bhatt, Holeyappa et al., 2024; Bhatt, Pandey, et al., 2024). The water quality parameters were maintained as follows: dissolved oxygen >6.0 mg L^{-1}, total ammonia nitrogen <0.2 mg L^{-1}, temperature 28-30 °C, and pH 7.4-7.8.

At the end of the 90-day feeding trial, the experimental fish were starved for 24 h to clear the gut contents prior to sampling. Subsequently, the fish were anesthetized using 2-phenoxyethanol at a

Table 2. Proximate composition of different feed ingredients and experimental diet (% of dry weight)

Ingredients/ diets	[1] 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
QUE	9.53	0.97	0.07	2.3	87.13
(Experimental diets)					
CON	25.47	3.32	19.82	9.40	41.99
$AFB_1$100	23.38	3.12	19.12	9.12	45.26
$AFB_1$25+QUE	24.26	3.28	19.91	9.24	43.31
$AFB_1$50+QUE	24.41	3.25	19.89	9.21	43.24
$AFB_1$100+QUE	23.38	3.13	19.13	9.16	45.2

[1] CP: Crude protein; EE: Ether extract; CF: Crude fiber; NFE: Nitrogen-free extract

concentration of 0.4 mL L^{-1}. Each fish was then individually weighed and measured to evaluate growth performance parameters before being returned to their respective tanks. For hematological analysis, blood samples were collected from three randomly selected fish per tank through the caudal vein using 26G × 1/2" (0.45 × 13 mm) disposable syringes (Hindustan Syringes & Medical Devices Ltd.). The collected blood was transferred into EDTA-coated tubes using heparinized syringes to prevent clotting and was used for the determination of hematological parameters. For serum biochemical analysis, blood samples were collected from three fish per tank without anticoagulant. The samples were kept at 4 °C for 24 h to allow clot formation and were subsequently centrifuged at 3000 rpm for 10 min at 4 °C. The obtained serum (supernatant) was carefully separated, aliquoted, and stored at -80 °C until further biochemical analysis. For histological examination, approximately 1 cm tissue segments of liver and kidney, were collected from four randomly selected fish. The tissues were immediately fixed in 10% neutral buffered formalin (NBF) and stored at room temperature until further histological processing and analysis.

Growth performance indices were calculated using standard formulas (Halver, 1957):

Specific growth rate (SGR; % weight gain day^{-1}) = log (ln) final body weight (g) – ln initial body weight (g)/t (time interval in days) x 100

Feed conversion ratio (FCR) = feed intake (g)/ weight gain (g)

Weight gain (%) = [Final body weight (g) – Initial body weight]/Initial body weight (g) x 100

For proximate composition analysis, fish samples were subjected to autoclave treatment (120 °C for 20 min), followed by homogenisation, oven drying, and grinding into fine powder prior to chemical analysis. The proximate composition, including moisture, crude protein, crude lipid, crude fibre, and ash content, was determined following standard procedures recommended by the Association of Official Analytical Chemists (AOAC, 2012).

Serum biochemical parameters, including alanine aminotransferase (ALT; Cat. #120207), aspartate aminotransferase (AST; Cat. #120204), total protein (TP; Cat. #120231), glucose (GLU; Cat. #120235), and albumin (Cat. #120223), were determined using commercially available ERBA® Mannheim diagnostic kits. The analyses were performed using a CHEM-7 biochemical analyser, following the manufacturer's instructions. For each biochemical parameter, 50 µL of serum was used for analysis according to the standard assay procedure provided with the diagnostic kits. Both standards and serum samples were analysed in triplicate to ensure analytical accuracy and reproducibility of the results. The activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), were determined following established stan-

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

Parameters [1]	Treatments				
	CON	$AFB_1$100	$AFB_1$25+QUE	$AFB_1$50+QUE	$AFB_1$100+QUE
Initial body weight (g)	17.36+0.13	17.31+0.08	17.34+0.10	17.31+0.15	17.32+0.19
Final body weight (g)	30.59^{a}+0.11	26.25^{d}+0.06	30.31^{a}+0.05	29.15^{b}+0.14	28.38^{c}+0.12
NBWG (g)	13.26^{a}±0.73	8.94^{c}±0.12	12.97^{ab}±0.12	11.84^{b}±0.05	11.06^{bc}±0.04
SGR	0.89^{a}±0.02	0.59^{d}±0.01	0.81^{b}±0.04	0.69^{c}±0.03	0.64^{c}±0.07
FCR	1.19^{c}+0.01	1.26^{a}+0.01	1.20^{b}+0.02	1.21^{b} +0.01	1.25^{a}+0.05
K-value	1.23^{a}+0.01	0.64^{d}+0.01	1.22^{a}+0.02	1.04^{b}+0.01	0.92^{c}+0.03
Survival rate (%)	100.00^{a}+0.00	79.76^{e}+2.77	98.21^{b}+2.77	94.59^{c}+0.45	91.54^{d}+2.77

[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 ($p \leq 0.05$)

dard protocols (Nicholls, 1962; Nishikimi, Rao, & Yagi, 1972). Prior to analysis, tissue samples were subjected to appropriate homogenisation and preparation procedures. All steps related to sample preparation, tissue homogenisation, reagent preparation, and enzyme assay procedures were performed strictly according to the manufacturer's guidelines supplied with the assay kits to ensure accuracy, reliability, and consistency of the analytical results.

Fish from the CON and experimental groups were fixed in 10% NBF for 24 hours and washed under running tap water. The tissues were dehydrated in graded alcohol (70% to absolute), cleared in xylene, and embedded in paraffin wax using molds. Thin sections (4 μm) were prepared using a rotary microtome (Leica RM2125 RT, Germany) and mounted onto Super Frost slides (#G10106, Abdos, India). After drying overnight at ~33 °C, the sections were stained with hematoxylin and eosin, mounted with DPX (Product Code 23140, Molychem, Mumbai), and images were captured using a Leica DM3000 LED light microscope.

All experimental data were expressed as mean ± standard error (SE). Statistical analysis was performed using appropriate statistical software. Differences among treatment groups were evaluated using one-way analysis of variance (ANOVA), followed by suitable post hoc tests to determine significant differences between means. A significance level of $p < 0.05$ was considered statistically significant.

Results and Discussion

During the experiment, no mortality was observed in the CON group. Growth parameters, including net body weight gain (NBWG) and SGR, were highest in the control group; however, the differences were not statistically significant ($p > 0.05$) compared to the other treatment groups. (Table 3). However, growth indicators declined notably in the $AFB_1$100 group exposed to 50 ppb AFB_1. Interestingly, dietary supplementation with 25 ppb AFB_1 and 200 mg QUE per kg of diet ($AFB_1$25) significantly enhanced growth indices in *L. rohita* fingerlings, with NBWG and SGR increasing by 12.97% and 0.81%, respectively, compared to other AFB_1-exposed treatments ($AFB_1$50 and $AFB_1$100). Among the AFB1-exposed groups, the $AFB_1$25 treatment showed the highest NBWG; however, the difference was not statistically significant ($p > 0.05$), highlighting the potential protective effects of QUE.

In addition to growth impairment, prolonged dietary exposure to AFB_1 adversely affected survival, with the $AFB_1$100 group exhibiting the lowest survival rate among the treatments. These findings indicate that higher dietary AFB_1 concentrations negatively influence both growth and survival in *L. rohita* fingerlings. The observed reduction in growth may be attributed to impaired protein metabolism and disruptions in glucose and lipid utilisation, as reported by Zaki and Fawzy (2012) and Zychowski et al. (2013). Conversely, dietary quercetin supplementation improved growth performance and survival, suggesting a protective role against AFB_1-induced toxicity. This beneficial effect may be

Table 4. Biochemical Parameters and Anti-oxidant Enzymes of *L. rohita* in different treatments at the termination of experiment

Biochemical Parameters[2]	Treatments				
	CON	$AFB_1$100	$AFB_1$25+QUE	$AFB_1$50+QUE	$AFB_1$100+QUE
Blood glucose (mg dL^{-1})	67.4^{d}±0.05	98.15^{a}±0.18	72.85^{c}±0.19	74.46^{c}±0.05	82.20^{b}±0.12
Total protein (g dL^{-1})	3.75^{a}±0.01	2.24^{d}±0.03	3.71^{a}±0.02	3.24^{b}±0.04	2.97^{c}±0.06
Albumin (g dL^{-1})	1.47^{a}±0.04	0.54^{d}±0.02	1.25^{b}±0.05	1.04^{b}±0.02	0.76^{c}±0.05
Globulin (g dL^{-1})	2.27^{b}±0.03	1.71^{d}±0.01	2.46^{a}±0.01	2.19^{c}±0.02	2.20^{b}±0.01
A/G Ratio	0.65^{a}±0.04	0.31^{d}±0.03	0.51^{b}±0.04	0.47^{c}±0.01	0.34^{d}±0.002
ALT/SGPT (IU L^{-1})	21.46^{d}±0.03	26.75^{a}±0.01	23.16^{c}±0.04	24.26^{b}±0.06	26.14^{a}±0.05
AST/SGOT (IU L^{-1})	97.28^{e}±0.02	140.35^{a}±0.01	105.49^{d}±0.03	115.51^{c}±0.02	131.29^{b}±0.05
Anti-oxidant Enzymes					
CAT (U mg Hb^{-1})	2.14^{d}±0.03	3.00^{a}±0.03	2.32^{c}±0.01	2.68^{b}±0.02	2.92^{b}±0.01
SOD (U mg Hb^{-1})	0.35^{c}±0.02	0.60^{a}±0.04	0.38^{c}±0.05	0.56^{b}±0.03	0.58^{b}±0.04

[2] ALT: Alanine aminotransferase, SGPT: Serum glutamic pyruvic transaminase, AST: Aspartate aminotransferase, SGOT: Serum glutamic-oxaloacetic transaminase, SOD: Superoxide dismutase. CAT: Catalase. Values with same superscripts in a row do not differ significantly ($p \leq 0.05$), using one-way ANOVA; Post hoc: Duncan's multiple comparison

associated with the antioxidant and anti-inflammatory properties of quercetin, as previously reported by Han and Huang (2006).

Biochemical measures showed a substantial difference between the AFB_1 and QUE treated groups. The highest blood GLU levels were seen in $AFB_1$100, followed by the lowest in $AFB_1$0. Similarly, $AFB_1$100 and $AFB_1$100+QUE had lower TP, albumin, and globulin levels than $AFB_1$0, $AFB_1$25, and $AFB_1$50. The enzymatic activity of AST, ALT, CAT, and SOD in fish subjected to varied amounts of AFB_1 feeding treatments correlated negatively with rising AFB_1 inclusion levels. The treatment that received (combined dose) AFB_1 and QUE exhibited lower antioxidant levels as observed in the $AFB_1$25 group. As AFB_1 concentrations increased, enzymatic activity also increased, with the $AFB_1$100 group exhibiting the highest levels, followed by the $AFB_1$100+QUE group. The differences among the treatment groups were statistically significant (Table 4).

Significant changes in biochemical and antioxidant parameters were observed in AFB_1 and QUE-treated groups, including reduced TP, globulin, and A/G ratio, along with increased GLU, ALT, AST, SOD, and CAT levels. These alterations indicate a stress response involving metabolite mobilisation (Koolhaas et al., 2011). Elevated GLU may result from enhanced glycogenolysis and reduced insulin activity, as reported by El-Boshy, El-Ashram, and El-Ghany (2008) in *O. niloticus*, while similar responses have been linked to immune activation by Akrami, Gharaei, Mansour, and Galeshi (2015). Evaluation of liver enzymes is essential for assessing toxicological effects (Sudová, Piaèková, Kroupová, Pijáèek, & Svobodová, 2008), while dietary bioactive compounds can further enhance physiological performance (Hossain, Small, Kumar, & Hardy, 2024).

The dietary intake of AFB_1 resulted in a non-significant increase in AST and ALT activities ($p > 0.05$), indicating potential liver dysfunction or swelling, leading to enzyme leakage into the bloodstream (Fawole et al., 2020). These enzymes serve as biomarkers for cellular injury, including degeneration and necrosis (Akrami et al., 2015). However, co-administration of AFB_1 and QUE mitigated the elevated enzyme levels. Similarly, Akrami et al. (2015) reported reduced ALT and AST activity in juvenile beluga (*Huso huso*) fed 1% onion, attributed to onion's antioxidant properties, which protect cell membranes from lipid peroxidation and enzyme diffusion. SOD and CAT activity increased with higher AFB_1 levels but decreased with QUE, consistent with QUE's role in regulating endogenous antioxidants and suppressing apoptosis (Moloi, Ziqubu, Mazibuko-Mbeje, Mabaso, & Ndlovu, 2024).

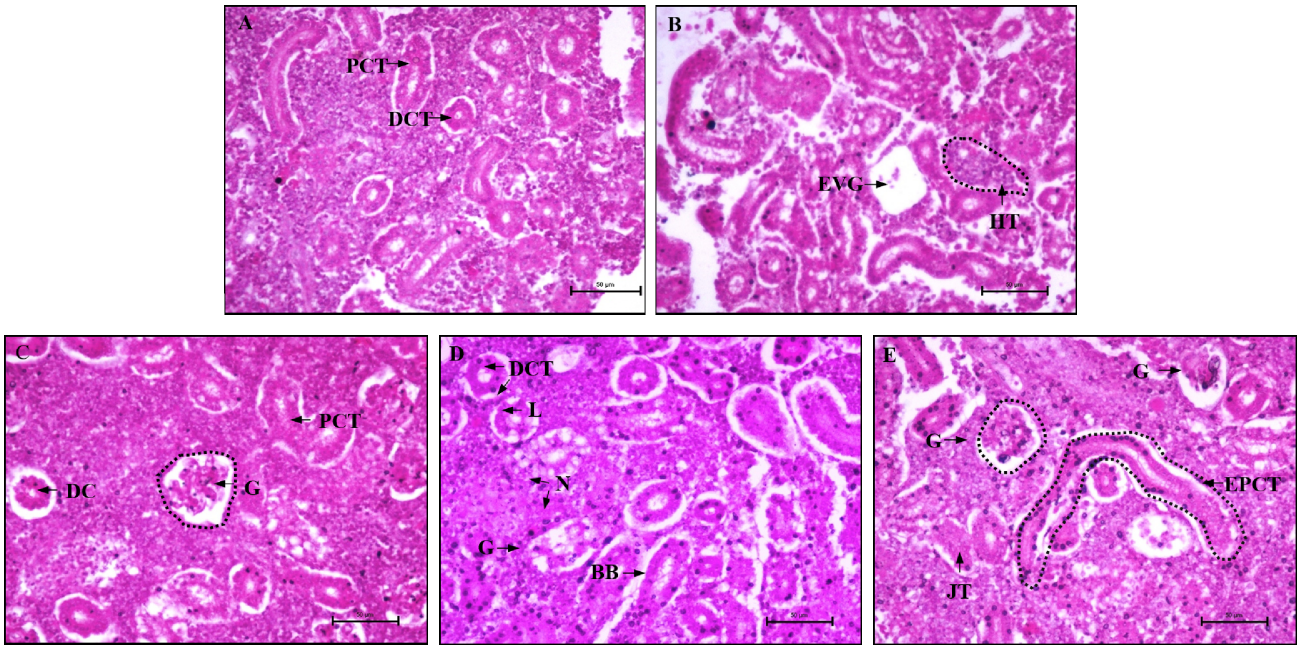


Fig. 1. Photomicrograph of H & E stained kidney sections of *L. rohita* showing A: (CON: 0 ppb AFB_1) Distal convoluted tubule (DCT), Proximal convoluted tubule (PCT), B: (Positive control, 100 ppb AFB_1) Evacuation of glomerulii (EVG), Haematopoetic tissue (HT); C: (25 ppb AFB_1+200 mg QUE) Distal convoluted tubule (DCT), Proximal convoluted tubule (PCT), Glomerulus (G); D: (50 ppb AFB_1+200 mg QUE) Distal convoluted tubule (DCT), Lumen (L), Nucleus (N), Glomerulus (G), Brush border (BB); E: (100 ppb AFB_1+200 mg QUE) Glomerulus (G), Enlarged proximal convoluted tubule (EPCT), (D) Scale bar = 50μm, H & E staining

SOD catalyzes O^{2-} into O_2 and H_2O_2, whereas CAT and glutathione peroxidase (GPx) disintegrate H_2O_2 into O_2 and H_2O, effectively eliminating excessive free radicals and preventing oxidative stress (Engwa, EnNwekegwa, & Nkeh-Chungag, 2022; Jerald, Ravindran, & Babu, 2024) (Fig. 3).

In this study, a comprehensive examination of photomicrographs depicting the tissue microarchitecture of kidney structures was conducted across different experimental groups. The CON group ($AFB_1$0) had regular characteristics, including normal glomerulus, proximal convoluted tubule, and distal convoluted tubule architecture. Conversely, significant deviations were observed in the AFB_1-exposed group ($AFB_1$100), including the evacuation of glomeruli and the presence of hematopoietic tissue. Group $AFB_1$25 displayed degenerative changes primarily in the proximal and distal tubules, while the glomerulus maintained its typical structure with no significant alterations noted. Notably, group $AFB_1$50 showcased nearly normal proximal tubules with centrally placed lumens, a typical glomerular morphology and a well-defined brush border of epithelial cells. Conversely, group $AFB_1$100 presented enlarged proximal tubules and glomerulus, accompanied by a slight joining of tubules (Fig. 1).

The kidneys of teleost fish are vital hematopoietic and osmoregulatory organs and are highly susceptible to pollutants (Shahid et al., 2022; Smorodinskaya et al., 2023). In this study, high AFB_1 exposure caused significant alterations in kidney microarchitecture, indicating physiological stress and metabolic disturbances at cellular and tissue levels (Liu et al., 2024). Similar studies have linked flavonoid-rich compounds to nephroprotective effects through their antioxidant and free radical scavenging properties (Adeneye & Benebo, 2008). QUE, due to its polyphenolic nature, may exert similar protective effects by reducing oxidative stress and restoring tissue integrity in AFB_1-exposed fish.

The CON group's liver cell microarchitecture during the experimental period showed typical hepatocyte morphology, characterized by uniform cytoplasm, centrally located nuclei, and a discernible central vein (Fig. 2A). In contrast, the liver tissue of the

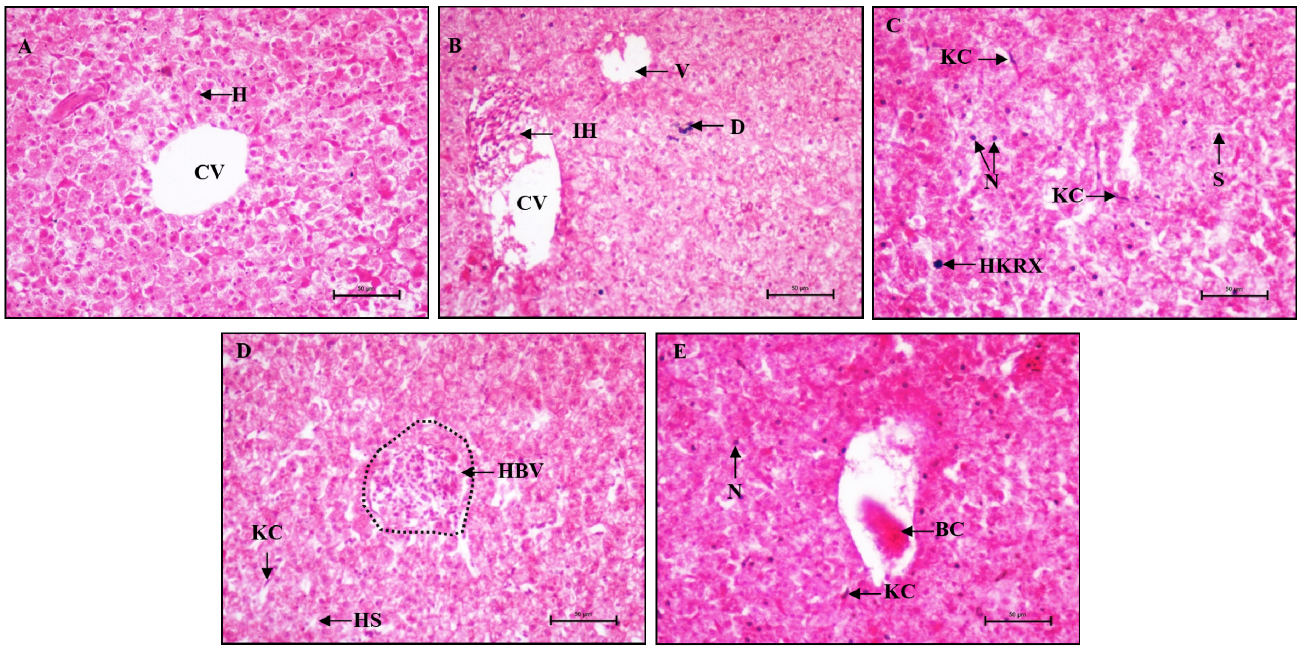


Fig. 2. Photomicrograph of H & E stained liver sections of *L. rohita* showing A: (CON: 0 ppb AFB_1) Hepatocytes (H), Central vein (CV), B: (Positive control, 100 ppb AFB_1) Invasion of hepatocytes in central vein (IH), Deposition (D) Vacuolization (V), C: (25 ppb AFB_1+200 mg QUE) Kupffer cells (KC), Nucleus (N), Sinusoids (S), Hepatocytes shows Karyorrhexis (HKRX) D: (50 ppb AFB_1+200 mg QUE) Kupffer cells (KC), Hepatic sinusoids (HS), Hemolysis of blood vessels (HBV); E: (100 ppb AFB_1+200 mg QUE) Kupffer cells (KC), Blood congestion (BC) Scale bar= 50μm, H & E staining

$AFB_1$100 group exhibited several notable alterations, notably including hepatocyte congestion and infiltration into the central vein, vacuolisation, intravascular hemolysis, and dilation of hepatic sinusoids, forming a distinctive tree-like pattern (Fig. 2B). Conversely, observations of the $AFB_1$25 group showed an existence of Kupffer cells, sinusoidal structures, and karyorrhexis (aggregation of chromatin material within hepatocytes) (Fig. 2C). Furthermore, the liver tissue of the $AFB_1$50 group displayed features such as hepatic sinusoids, Kupffer cells within macrophages, and complete occlusion of the central vein lumen with blood (hemolysis) (Fig. 2D). Notably, the $AFB_1$100 group demonstrated the presence of Kupffer cells and congestion within the central vein (Fig. 2E).

Evaluation of liver microarchitecture is a reliable indicator of health impairment in fish (Mokhtar, 2021). In the present study, higher AFB_1 concentrations caused hepatic injury and structural alterations, including dilated sinusoids and congestion of major veins. This disruption in blood flow from the hepatic portal vein and artery to the central vein can lead to cellular degeneration and necrosis (Hinton, 2021). These findings are consistent with Azzam and Gababl (1997) and Tessari, Oliveira, Cardoso, Ledoux, and Rottinghaus (2006) who reported similar effects at comparable AFB_1 levels. QUE, known for its strong antioxidant and free radical scavenging properties (Wu et al., 2021), showed a protective effect when co-administered with AFB_1 by enhancing antioxidant enzyme activity and reducing tissue damage (Venkataraman, Krishnamoorthy, Selvakumar, & Arunakaran, 2009). The study highlights the impact of AFB_1 on organ integrity and provides valuable insights into the mitigating role of QUE in *L. rohita*, contributing to future research on AFB_1 toxicity.

The present study was designed to evaluate the protective efficacy of dietary quercetin against AFB_1-induced toxicity in *L. rohita*, the experimental design focused primarily on assessing its effects under toxin-challenged conditions. Inclusion of a quercetin-only group and dose-matched AFB_1-only controls (25 and 50 ppb) would have provided additional insight into the intrinsic effects of quercetin and enabled a more precise assessment of its protective efficacy across different toxin exposure

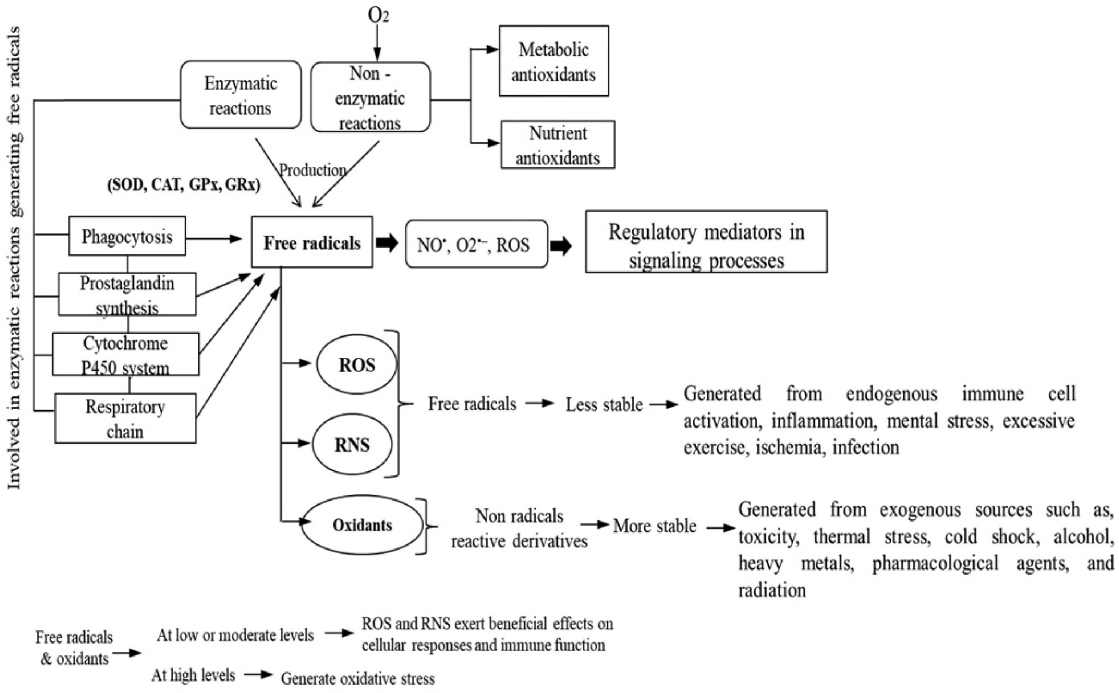


Fig. 3. Schematic representation of enzymatic reactions generating free radicals and antioxidant system during oxidative stress

levels. Nevertheless, the consistent improvements observed in growth performance, physiological and biochemical responses, antioxidant status, and tissue microarchitecture underscore the potential of quercetin as a dietary strategy for mitigating AFB_1-induced stress in fish. Future studies incorporating these additional control groups would further strengthen mechanistic understanding and support its application in aquaculture.

Overall, the present study demonstrated that dietary quercetin supplementation (200 mg kg^{-1} diet) effectively mitigated the adverse effects of aflatoxin B_1 (AFB_1) in *L. rohita*. Quercetin improved growth performance, physiological and biochemical responses, antioxidant status, and tissue microarchitecture in AFB_1-exposed fish, indicating its protective role against mycotoxin-induced stress. The beneficial effects were observed across all AFB_1 exposure levels evaluated, supporting its potential as a natural feed additive in aquaculture. Although the extent of recovery varied among treatments, this variation is likely associated with differences in toxin burden rather than quercetin efficacy. The study was conducted under controlled conditions; therefore, further validation under field conditions is required. Future studies incorporating quercetin-only groups, dose-matched AFB_1 controls, and molecular approaches such as transcriptomics and proteomics are recommended to elucidate the mechanisms of protection.

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