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Research Article

Evaluation of antifungal agent ketoconazole against *Aphanomyces invadans* infection in *Labeo rohita*

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Abstract

Epizootic ulcerative syndrome (EUS), caused by *Aphanomyces invadans*, is a major threat to the sustainability of freshwater aquaculture. This study presents the first evaluation of ketoconazole (KTZ) as a novel therapeutic strategy against EUS, applying a precision intervention with biomarker-driven assessment of its *in vitro* antifungal activity and *in vivo* efficacy in *Labeo rohita* fingerlings. KTZ exhibited concentration-dependent inhibition of *A. invadans*, with a minimum inhibitory concentration of 24 mg mL^{-1} and inhibition zones of 10–32 mm. *In vivo*, fish fed KTZ at 0, 10, 20, and 30 mg kg^{-1} body weight for 30 days showed significant ($p<0.05$) improvements in erythrocyte and leukocyte counts, haemoglobin, packed-cell volume, respiratory burst, and anti-protease activity, indicating enhanced haematopoiesis and innate immunity. Elevated liver enzyme activities, including glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP), at higher doses suggested mild hepatocellular stress. At the same time, transient reductions followed by increases in blood glucose reflected adaptive metabolic responses. In a 10-day post-infection therapeutic trial, the 20 mg kg^{-1} group achieved the highest relative per cent survival (80%), compared with 60% in the 10 and 30 mg kg^{-1} groups and 0% in the infected control. Overall, dietary KTZ at 20 mg kg^{-1} effectively controls EUS in *L. rohita* through combined antifungal and immunophysiological benefits, offering a practical and promising strategy for sustainable carp aquaculture.

Keywords: *Aphanomyces invadans*, EUS, Immunomodulation, Ketoconazole, *Labeo rohita*

Highlights

- Ketoconazole (KTZ) inhibited *A. invadans* effectively
- Optimal dose of KTZ was 20 mg kg fish biomass^{-1}
- It enhanced innate immunity in *L. rohita*
- It improved survival against EUS infection
- It promoted precision therapeutics in aquaculture

INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors globally, contributing 130.9 million tonnes of aquatic animals in 2022 (Food and Agriculture Organization of the United Nations [FAO], 2024). Among freshwater species, carps such as *Cyprinus carpio*, *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* dominate Indian aquaculture production (FAO, 2024). In north eastern India, rich freshwater resources and indigenous fish diversity make aquaculture a rapidly expanding sector (Mishra et al., 2017). However, disease outbreaks, particularly fungal infections like epizootic ulcerative syndrome (EUS), pose serious challenges to its sustainability and profitability (Debnath et al., 2019; Mohanty et al., 2020). EUS, caused by *Aphanomyces invadans*, an aseptate oomycete of the family Saprolegniaceae, is a destructive transboundary disease affecting a wide range of freshwater fish (Kamilya & Baruah, 2014). The pathogen invades healthy tissues, forming haemorrhagic lesions and necrotic ulcers that often lead to mortality. Environmental stressors such as poor water quality and temperature fluctuations further promote disease outbreaks (Sujon et al., 2026). Recent advances in aquatic animal health highlight the integration of targeted therapeutics with biomarker-based diagnostics to enhance disease management efficiency. Current control strategies largely depend on improved husbandry and broad-spectrum chemicals such as formalin, methylene blue, CIFAX, salt, and lime, which provide only limited efficacy (Treves, 2000). Although biofungicides such as *Bacillus licheniformis* and *Trichoderma harzianum* show promise (Figueroa-Brambila et al., 2025; Kumar et al., 2026), effective antifungal agents specifically targeting *Aphanomyces invadans* remain lacking.

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Azole antifungals remain among the most potent agents against fungal infections. Ketoconazole (KTZ), an imidazole derivative developed by Janssen Pharmaceuticals (Heeres et al., 1979; Drouhet & Dupont, 1980), acts by inhibiting cytochrome P450-dependent lanosterol 14-α-demethylase, thereby disrupting ergosterol biosynthesis in fungal membranes (Monk & Keniya, 2017). Despite its proven efficacy in human and veterinary medicine, its potential application in aquaculture, particularly against *A. invadans,* remains unexplored. The present study evaluates the *in vitro* antifungal efficacy of KTZ against *A. invadans*, investigates the immune, haematological, and biochemical responses of *L. rohita* to varying drug concentrations, and determines its therapeutic effectiveness in infected fingerlings. The work aligns with emerging trends in precision therapeutics in aquaculture, integrating targeted chemotherapeutic strategies with biomarker-based health monitoring to improve fungal disease management and sustainability.

MATERIALS AND METHODS

Experimental design and site: Three experiments were conducted at the Department of Aquatic Health and Environment, College of Fisheries, CAU (I), Lembucherra, Tripura, India, to evaluate (i) *in vitro* antifungal activity of KTZ against *Aphanomyces invadans*, (ii) immuno-biochemical responses of *L. rohita* to dietary KTZ, and (iii) its therapeutic efficacy against experimentally induced *A. invadans* infection. Experiments were designed following a Completely Randomized Design (CRD) in triplicate.

Fish collection and maintenance: Healthy *L. rohita* fingerlings (15–17 g) were procured from Battala market, Agartala, and acclimatized for two weeks in 500 L Fiber-Reinforced Plastic (FRP) tanks with continuous aeration. Fish were fed pelleted feed twice daily, with 30% daily water exchange. Temperature (29±2°C) and water quality were maintained following American Public Health Association [APHA] (2005). Before the feeding trial, fish were fasted for 24 h to enhance feed intake. The increased appetite following fasting facilitated rapid and complete consumption of the medicated feed, ensuring accurate and effective administration of the therapeutic dose. A total of 280 fish were used across all experiments. Water quality parameters remained stable and optimal throughout the experiment, i.e., temperature (28.4–30.9°C), pH (6.7–7.3), dissolved oxygen (4.9–5.7 mgL^{-1}), total alkalinity (100–137 mgL^{-1}), and total ammonia-N (0.01–0.05 $mg\ L^{-1}$).

***In vitro* antifungal activity:** *A. invadans* COF_AHE_F(01) was cultured on Potato Dextrose Agar and sporulated in Glucose Peptone Yeast (GPY) broth (Hatai & Hoshiai, 1992). Zoospore suspensions were prepared in autoclaved pond water, and viable counts were determined on GPY agar. KTZ (Hi Media, India; Mol. Wt. =531.43) was dissolved in dimethyl sulfoxide (DMSO) (Ekanem et al., 2003) to prepare a 50 mg mL^{-1} stock solution. The antifungal activity was assessed using the agar well diffusion assay (Xue et al., 2013) with KTZ concentrations of 0.375–48 mg mL^{-1}. DMSO served as the negative control, and fluconazole as the positive control. Plates were incubated at 18±2°C for 24 h, and inhibition zones were measured. The minimum inhibitory concentration (MIC) of KTZ against the fungal isolate was determined using the broth microdilution method (Sewell et al., 1994). Serial dilutions of the stock solution were prepared to obtain final concentrations of 48.0, 24.0, 12.0, 6.0, 3.0, 1.5, 0.75, and 0.375 mg mL^{-1}. Wells containing fungal spores (3×10^5 spores mL^{-1}) without the test compound served as the positive control, while wells containing only nutrient broth served as the negative control. The initial optical density (OD_o) was measured at 405 nm using a MULTISKAN GO spectrophotometer (Thermo Scientific). After incubation at 20°C for 24 h, the final optical density (OD_o) was recorded. Fungal growth inhibition was calculated using the change in optical density ($\triangle OD = OD_{24} – OD_o$), and the percentage inhibition was determined as: Per cent inhibition= (1–OD of test solution /OD of control) ×100. The MIC was recorded as the lowest concentration showing complete inhibition of fungal growth.

Diet preparation and feeding trial: Experimental diets were formulated by incorporating KTZ at 1 g kg^{-1} (Diet T1), 2 g kg^{-1} (Diet T2), and 3 g kg^{-1} (Diet T3) feed, with a control diet (0 g kg^{-1}). Doses were selected based on preliminary toxicity and antifungal screening studies (unpublished). Ingredients were mixed, autoclaved at 120°C for 2 min, and pelleted using a hand pelletizer. After drying (48 h), pellets were sealed and stored. Proximate composition (moisture, ash, protein, lipid, carbohydrate) was analyzed following Association of Official Analytical Chemists [AOAC] (2000). A total of one hundred eighty *L. rohita* fingerlings (16.2±1.9 g) were randomly allocated to 12 tanks (200 L capacity), with 15 fish per tank. Fish were fed twice daily (1% body weight in the morning with the medicated feeds; *ad libitum* with the control diet in the evening) for 30 days. One per cent feeding

rate has been calculated in such a way that the mentioned feeding rate will provide the exact KTZ dosages of 0, 10, 20 and 30 mg kg BW^{-1} to the respective treatments and are denoted as control (C), T1, T2 and T3, respectively (Saha et al., 2017; Singh et al., 2018b). Blood samples were collected on days 7, 14, 21, and 30 for haematological, biochemical, and immunological analyses.

Haematological, biochemical, and immunological analyses: Blood was collected from the caudal vein using 1 mL syringes under clove oil anaesthesia (50 µL L^{-1}). Plasma was separated by centrifugation (3500 rpm, 15 min, 4°C). Haematological parameters such as total erythrocyte count (EC) and total leucocyte count (LC), haemoglobin (cyanmethemoglobin method), and packed cell volume (PCV) were determined following Schaperclaus (1991). Plasma glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP) were assayed using commercial kits (Diatek Healthcare, India) as per Trinder (1969), Reitman and Frankel (1957), and International Federation of Clinical Chemistry and Laboratory Medicine guidelines. Immunological parameters, including nitro blue tetrazolium (NBT) activity and antiprotease activity (APA) levels, were determined following Anderson and Siwicki (1995), and Zuo and Woo (1997), respectively.

Experimental infection and therapeutic efficacy: The therapeutic efficacy of KTZ was evaluated using a completely randomized design comprising five treatment groups, each with two replicates. Experimental L. rohita fingerlings were challenged with A. invadans following the ami-momi method (Hatai & Hoshiai, 1992) and exposed to 3×10^3 zoospores L^{-1} (Firouzbakhsh et al., 2014; Singh et al., 2018a). A total of 100 fish were used, including 80 infected and 20 uninfected fish, randomly distributed into 10 plastic tanks (10 fish per tank). The treatment groups were: C+CF (uninfected fish fed control feed), I+CF (infected fish fed control feed), I+T1F (infected fish fed KTZ at 10 mg kg BW^{-1}), I+T2F (infected fish fed KTZ at 20 mg kg BW^{-1}), and I+T3F (infected fish fed KTZ at 30 mg kg BW^{-1}). Fish were fed once daily at 1% of body weight for 10 days, with the ration adjusted according to daily mortalities. Water quality was maintained by replacing 20% of the tank water daily. Mortality was recorded throughout the experimental period, and therapeutic efficacy was expressed as Relative Percentage Survival (RPS) (Singh et al., 2018a).

RPS = [1– (mortality in treated group/mortality in control)] ×100

Statistical analysis: All data were expressed as mean ± standard error (SE). One-way ANOVA followed by Duncan's multiple range test was used to determine significance at $p<0.05$ using SPSS v16.0 (SPSS Inc., USA).

RESULTS

Three experiments were conducted to evaluate the efficacy of KTZ against *A. invadans* COF_AHE_F (01) and its effects on haematological, biochemical, and immunological parameters in *L. rohita* fingerlings. KTZ exhibited a concentration-dependent antifungal activity in the agar well diffusion assay (Table 1). The highest tested concentration (48.0 mg ml^{-1}) produced the maximum inhibition zone of 32 mm. A progressive decline in inhibition zone and per cent inhibition of *A. invadans* spores was observed with decreasing KTZ concentration. Significant differences ($p<0.05$) were observed among concentrations, confirming the dose-dependent antifungal efficacy of KTZ. The MIC of KTZ against *A. invadans* was 24 mg/ ml^{-1} (Table 1).

Table 1. *In-vitro* antifungal activity of ketoconazole (KTZ)

KTZ concentration (mg ml^{-1})	Inhibition zone (mm)*	Percent inhibition**
48.0	32	103 ± 0.72^{a}
24.0	28	100 ± 0.81^{b}
12.0	23	90.98 ± 1.04^{c}
6.0	22	46.66 ± 0.92^{d}
3.0	21	28.83 ± 1.53^{e}
1.5	19	19.98 ± 0.88^{f}
0.75	16	15.56 ± 0.68^{g}
0.375	10	3.33 ± 0.5^{h}

*Agar well diffusion assay, **: Broth microdilution assay. Data are presented as mean±SE (n= 3). Different superscripts indicate statistically significant differences ($p<0.05$) among the concentration groups.

The proximate composition of experimental diets remained largely consistent across treatments, with moisture content ranging from 9.71±0.09% to 9.92±0.14%, crude protein from 34.41±0.17% to 35.23±0.43%, total lipid from 2.04±0.01% to 2.08±0.01%, ash from 5.63±0.01% to 5.87±0.04%, and carbohydrate from 49.74±0.34% to 50.29±0.71% (Table 2).

KTZ-medicated feed significantly influenced haematological parameters (Table 3). Total erythrocyte count (EC) increased in treated groups, peaking at $3.23\pm0.01 \times 10^6$ mm^{-3} in T3 on day 14, and remained

Table 2. Composition of control and treatment diets (500 g) used in the experiment

Ingredients (in g)	Control	Diet T1	Diet T2	Diet T3
Casein	80	80	80	80
Gelatin	45	45	45	45
Dextrin	150	150	150	150
Fish meal	80	80	80	80
Carboxymethyl cellulose (CMC)	10	10	10	10
Veggetable oil	20	20	20	20
Cod liver oil	20	20	20	20
Vit-min mixture*	30	30	30	30
Butylated hydroxytoluene (BHT)	1	1	1	1
Cellulose	64	63.5	63	62.5
Ketoconazole (KTZ)	0	0.5	1	1.5
Total	500	500	500	500

*Composition of vitamin-mineral premix (quantity 2.5 g kg^{-1}): Vitamin A, 5,500,000 IU; Vitamin D3, 1,100,000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; L-lysine, 10 g; DL- Methionine, 10 g; Selenium, 50 mg L^{-1}

higher than the control throughout the trial. Total leukocyte count (LC) also showed a significant elevation in treated groups compared to the control, with the highest count observed in T2 and T3 on day 14 (142.06±0.56 × 10^3 mm^{-3}). Haemoglobin content increased from 4.13±0.04 g dL^{-1} in the control on day 7 to 4.89±0.02 g dL^{-1} in T3 on day 14 and stabilized across treatments thereafter. PCV showed a similar trend, with maximum values recorded in T3 (36.03±0.01%) on day 14 and a slight elevation maintained through day 30. Biochemical parameters were also positively affected by KTZ treatment. Blood glucose initially decreased in treated groups, with the lowest value in T2 (61.28±0.22 mg dL^{-1}) on day 7, and subsequently increased, peaking in T3 (78.79±0.03 mg dL^{-1}) on day 21. Liver enzyme activities reflected a dose-dependent response to medicated feed. GPT levels were significantly higher in T2 and T3 from day 14 onwards, peaking at 50.02±0.23 U L^{-1} in T3 on day 21. GOT activity increased gradually in T2 and T3, with T3 recording the highest value (61.11±0.05 UL^{-1}) on day 30. ALP activity followed a similar pattern, reaching a maximum of 56.65±0.40 IU L^{-1} in T3 on day 21 (Table 3).

Immunological responses were significantly enhanced in treated groups. NBT reduction assay revealed increased respiratory burst activity in T2 and T3, with the highest value in T2 (0.57±0.02 OD) on day 14, followed by a gradual normalization by day 30. APA peaked in T2, reaching 90.52±0.01 g dL^{-1} on day 30, while T3 consistently showed lower values (Table 3). Curing efficacy was assessed by RPS. Infected control fish fed a non-medicated diet showed 0% survival, confirming the virulence of the pathogen, whereas uninfected control fish exhibited 100% survival. Among the treated groups, T2-fed fingerlings

Table 3. Haematological, biochemical, and immunological parameters of *Labeo rohita* fingerlings fed with different doses of KTZ-based medicated feed on various sampling days

	7 day	14 day	21 day	30 day
Treatments		Total erythrocyte count (EC, 10^6 mm^{-3})		
Control	2.70±0.04^a	2.83±0.01^a	2.89±0.04^a	2.93±0.02^a
T1	2.87±0.16^b	2.99±0.01^b	3.01±0.02^b	3.06±0.03^b
T2	3.16±0.04^c	3.19±0.01^c	3.06±0.04^b	3.04±0.02^b
T3	3.18±0.02^c	3.23±0.01^d	2.99±0.02^b	3.01±0.02^b
		Total leukocyte count (LC, 10^3 mm^{-3})		
Control	126.25±3.06^a	130.36±0.51^a	134.90±1.15^a	136.07±0.56^a
T1	131.31±3.03^a	136.91±0.12^b	137.05±0.58^{ba}	138.97±0.03^b
T2	134.83±2.89^a	142.06±0.56^c	141.25±0.51^c	140.65±0.53^c
T3	132.36±2.37^a	140.53±1.12^c	138.41±0.40^b	139.38±0.61^{cb}
		Haemoglobin content (Hb, g dL^{-1})		
Control	4.13±0.04^a	4.24±0.03^a	4.43±0.03^a	4.56±0.04^a
T1	4.29±0.04^b	4.46±0.02^b	4.71±0.06^b	4.82±0.02^b
T2	4.63±0.03^c	4.84±0.02^c	4.80±0.07^b	4.76±0.02^b
T3	4.71±0.02^c	4.89±0.02^c	4.67±0.07^b	4.71±0.03^b

Cont. Table 3.

Table3., Cont. ...

	7 day	14 day	21 day	30 day
		Packed cell volume (PCV, %)		
Control	35.10±0.08[a]	35.29±0.09[a]	35.58±0.02[a]	35.72±0.01[a]
T1	35.48±0.20[b]	35.61±0.08[b]	35.78±0.02[bc]	35.87±0.58[a]
T2	35.84±0.02[b]	36.01±0.02[c]	35.89±0.03[c]	35.82±0.60[a]
T3	35.88±0.09[b]	36.03±0.01[c]	35.69±0.07[b]	35.85±0.05[a]
		Glucose (mg dl^{-1})		
Control	70.50±0.16[a]	74.01±0.09[a]	73.47±0.09[a]	75.17±0.03[a]
T1	63.16±0.33[b]	71.32±0.20[b]	75.34±0.08[b]	76.67±0.02[b]
T2	61.28±0.22[c]	68.44±0.04[c]	75.85±0.08[c]	75.62±0.07[c]
T3	64.80±0.21[d]	78.65±0.04[d]	78.79±0.03[d]	77.68±0.02[d]
GPT		Glutamate pyruvate transaminase (GPT, U L^{-1})		
Control	41.20±1.66[a]	42.60±0.10[a]	44.77±0.09[a]	41.55±0.41[a]
T1	40.12±1.34[a]	39.37±0.98[b]	40.41±0.69[b]	41.20±0.05[a]
T2	40.24±0.93[a]	44.69±0.13[c]	46.52±0.25[c]	42.95±0.05[b]
T3	40.68±0.46[a]	46.46±0.25[d]	50.02±0.23[d]	44.78±0.40[c]
		Glutamate oxaloacetate transaminase (GOT, U L^{-1})		
Control	43.82±0.36[a]	45.65±0.27[a]	49.14±0.23[a]	47.14±0.09[a]
T1	39.39±0.13[b]	42.07±0.27[b]	43.90±0.05[b]	49.23±0.35[b]
T2	40.27±0.40[b]	43.29±0.05[c]	47.75±0.40[c]	47.22±0.23[a]
T3	40.96±0.35[b]	50.77±0.30[d]	56.13±0.05[d]	61.11±0.05[c]
		Alkaline phosphatase (ALP, IU L^{-1})		
Control	41.44±0.88[a]	45.67±0.46[a]	46.31±0.78[a]	43.56±0.40[a]
T1	36.66±0.28[a]	43.09±0.72[b]	45.76±0.21[a]	43.42±0.63[a]
T2	37.07±0.58[a]	40.20±0.64[c]	48.93±0.62[b]	46.31±0.14[b]
T3	38.36±0.60[a]	53.84±1.10[d]	56.65±0.40[c]	54.17±0.62[c]
		Nitrobluetetrazolium (NBT, OD at 540 nm)		
Control	0.13±0.01[a]	0.20±0.03[a]	0.27±0.01[a]	0.30±0.06[a]
T1	0.24±0.03[b]	0.38±0.02[b]	0.42±0.01[b]	0.45±0.05[b]
T2	0.44±0.03[c]	0.57±0.02[c]	0.56±0.02[c]	0.48±0.03[c]
T3	0.36±0.01[d]	0.49±0.02[d]	0.46±0.02[d]	0.43±0.02[d]
		Anti-protease activity (APA, g dL^{-1})		
Control	85.69±0.02[a]	87.04±0.05[a]	88.01±0.01[a]	87.31±0.01[b]
T1	84.28±0.03[b]	86.53±0.03[b]	87.40±0.02[b]	88.23±0.01[b]
T2	84.47±0.04[c]	88.84±0.03[c]	89.80±0.02[c]	90.52±0.01[c]
T3	84.16±0.03[d]	86.01±0.02[d]	86.42±0.02[d]	86.81±0.01[d]

Data are presented as mean±SE (n= 3). Mean values with different superscripts (a, b, c and d) within a column for a parameter are significantly different ($p<0.05$).

demonstrated significantly higher RPS (80±10%) compared to T1 and T3 (60±10%) (Table 4).

DISCUSSION

The present investigation was conducted to evaluate the antifungal potential of KTZ against *A. invadans*, emphasizing its *in vitro* efficacy, its effects on the pathophysiological responses of *L. rohita* fingerlings, and its therapeutic suitability for managing fungal infections. *In vitro* assays clearly demonstrated the strong antifungal activity of KTZ against *A. invadans*. Inhibition zones increased in a dose-dependent manner, ranging from 10 mm at 0.375 mg ml^{-1} to 32 mm at 48 mg ml^{-1}, with a MIC of 24 mg ml^{-1} producing complete growth inhibition. This result aligns with previous reports that established azole-based antifungals as potent inhibitors of *Aphanomyces* spp. (Hatai & Egusa, 1979; Xue et al., 2013). Comparable antifungal activities were observed with other azoles, such as fluconazole and miconazole nitrate, which effectively protected *L. rohita* against *Saprolegnia parasitica* (Saha et al., 2016; Singh et al., 2018b). Collectively, these findings reinforce the view that KTZ serves as a potent antifungal chemotherapeutant suitable for sustainable aquaculture applications.

Feeding KTZ-incorporated diets for 30 days significantly altered haematological indices of *L. rohita*. The increase in erythrocyte count across all treatment groups indicates stimulation of erythropoiesis or enhanced red blood cell stability, consistent with reports describing haematopoietic enhancement by azoles and phyto-based immunostimulants

Table 4. Relative percentage survival (%) of *Labeo rohita* fingerlings fed with multiple doses of KTZ-based medicated feed

Experimental groups	Relative percentage survival
Infected fish + Control feed:(I+CF)	0±0[a]
Uninfected fish + Control feed:(C+CF)	100±0[b]
Infected fish + T1 feed (KTZ): (I+T1F)	60±10[c]
Infected fish + T2 feed (KTZ): (I+T2F)	80±10[d]
Infected fish+ T3 feed (KTZ):(I+T3F)	60±10[c]

All data are presented as mean±SE. Mean values with different superscripts (a, b, c and d) are significantly different ($p<0.05$).

(Saglam et al., 2003; Sharma et al., 2014). Unlike toxic antifungal agents such as malachite green, which induce anaemia and haematological depression (Witeska et al., 2013), KTZ exhibited a non-toxic haematological profile. The observed elevations in leukocyte count and haemoglobin concentration indicate enhanced immunophysiological status and oxygen transport efficiency, corroborating the immune-stimulatory potential seen in *L. rohita* exposed to fluconazole and miconazole nitrate (Saha et al., 2016; Singh et al., 2018a, b). PCV values showed a significant rise up to day 21 before stabilising, consistent with adaptive haematological responses reported by Süloğlu et al. (2015). These findings confirm that KTZ supports haematological resilience without inducing cytotoxicity. Plasma glucose, a reliable stress biomarker (El-Sayed et al., 2007), exhibited an initial decline followed by moderate elevation, reflecting early adaptation and mild chronic stress under continued exposure. This pattern corresponds with findings from azole-treated fish reported by Li et al. (2011) and Saha et al. (2018). Liver enzymes GPT and ALP showed dose-dependent changes, i.e., decreasing at lower KTZ doses but rising at higher concentrations, suggesting mild hepatocellular stress during extended or high-dose exposure. Such hepatic responses are consistent with previous observations in fish treated with propiconazole and other synthetic antifungals (Min & Kang, 2008). These results underscore the importance of dose optimisation to ensure therapeutic benefits while maintaining hepatic integrity, as previously emphasised in antifungal feed trials with fluconazole (Saha et al., 2018).

KTZ feeding significantly increased NBT activity across all treated groups, indicating enhanced respiratory burst activity of phagocytes, a key component of innate immunity. This agrees with earlier reports highlighting similar immune activation by azoles, vitamins, and phyto-immunostimulants (Baldauf & Adam, 2000; Jacobs et al., 2003; Saha et al., 2016; Saha et al., 2017). APA activity initially declined on day 7 but subsequently increased, particularly in the medium-dose group, demonstrating adaptive immune modulation, as described by Jeney &Anderson (1993). The observed pattern underscores KTZ's role in fortifying innate immunity and minimizing proteolytic tissue damage during fungal infections, supporting the concept of integrated immuno-therapeutic management in aquaculture (Agbowuro et al., 2018). The therapeutic evaluation revealed that KTZ-medicated feed (20 mg kg $BW^{-1}$for 10 days) yielded the highest RPS, successfully curing *A. invadans* infection in *L. rohita*. EUS, caused by *A. invadans*, is a persistent problem in Indian carps during colder months (Kamilya & Baruah, 2014). Additionally, similar antifungal and immune-ameliorating effects have been reported from natural sources such as *Ocimum basilicum*, *Leucas aspera*, and *Leucas zeylanica* (Kocić-Tanackov et al., 2011; Baba et al., 2016), as well as dietary vitamin C and *Withania somnifera* formulations (Laltlanmawia et al., 2019). Such evidence supports integrating synthetic antifungal chemotherapeutants like KTZ with natural immunomodulators for comprehensive aquatic health management.

The present findings reinforce the emerging paradigm of precision therapeutics in aquaculture, where drug efficacy is evaluated through both pathogen inhibition and host immunophysiological responses. In this context, KTZ demonstrated potent antifungal activity against *A. invadans* in *L. rohita*, while significantly enhancing haematological and immunological defenses. These combined antifungal and immunomodulatory effects highlight its potential as an effective and biologically integrated strategy for EUS management. However, although KTZ was found to be safe and efficacious at optimized doses, its application should be carefully regulated to minimize hepatic stress and ensure ecotoxicological safety in commercial aquaculture systems. Overall, the study establishes KTZ as a promising precision antifungal therapeutic, warranting further validation under field conditions for sustainable fungal disease management.

Conflict of interest: All authors declare that they have no conflicts of interest related to this research.

Author's contribution: PD: Experiment, data collection; HS: Concept, supervision, manuscript writing; KD: Analysis, laboratory support.

Data availability statement: The data that support the findings of this study are available from the corresponding author, H. Saha, upon reasonable request.

Ethical statement: This study was reviewed and approved by the Institutional Animal Ethics Committee of the College of Fisheries, Central Agricultural University, Imphal. All the live fish were handled humanely, and efforts were made to minimize suffering.

Use of artificial intelligence (AI) tools: In the preparation of this manuscript, generative AI tools (such as ChatGPT developed by OpenAI) were used only to assist in language editing, grammar correction, and improving the clarity of expression. The use of AI was limited to linguistic refinement and did not involve any data generation, data interpretation, or content creation that would affect the scientific integrity of the work. The authors take full responsibility for the content, accuracy, and originality of the manuscript.

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