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Physio-biochemical Response of *Labeo rohita* (Hamilton, 1822) fed with Antifungal Drug, Fluconazole

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Abstract

An experiment was carried out to delineate the biochemical responses in *Labeo rohita* fingerlings fed with fluconazole (FLZ). There were twenty-four treatments divided into four groups (Control, T1, T2, and T3 fed with 0, 10, 20 and 30 mg FLZ kg^{-1} body weight, respectively) and each group with 6 exposure times (0, 6, 12, 24, 48, and 96 h) in triplicates. Biochemical parameters in plasma like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP), glucose, albumin, albumin globulin ratio (A/G) were measured. Significant increase ($p<0.05$) in the GOT, ALP, and decrease ($p<0.05$) in glucose were observed in the treatment groups as compared to control. However, homeostasis was achieved by rohu within 48 h of administration. Fish fingerlings treated with fluconazole at higher doses exhibited marked changes in physiology.

Keywords: *Labeo rohita*, fluconazole, feed, biochemical, fingerlings

Introduction

Many pharmaceuticals contaminate our environment as micro pollutants (Heberer, 2002). Among various pharmaceuticals, triazole fungicides are important antifungal pesticides, used in large scale for many agricultural crops (Konwick et al., 2006; Chen et al., 2008; Li & Randak, 2009) and in human and animals (Brammer & Tarbit, 1987). However, triazole exposure causes skeletal defects, craniofacial malformations and hydrocephaly in mammalian embryo (Menegola et al., 2005; Farag & Ibrahim, 2007).

Triazoles including fluconazole are long lasting with low bio-degradability in natural conditions (Wang et al., 2011; Bergheim et al., 2014). Fluconazole is a bis-triazole antifungal compound (Saag & Dismukes, 1988), which remains almost unchanged when excreted by human beings or animals (Hess et al., 1986; Watt et al., 2000) and become persistent in soil and water (Bromillow et al., 1999). They are taken up by non-target animals through contaminated food and water and thereafter affect their homeostasis. However, only few studies have been conducted on acute toxicity of fluconazole on fishes (Kim et al., 2009; Brilhante et al., 2011; Hermsen et al., 2011; Zhu et al., 2014) but not with feed containing fluconazole. With this background in formation, the present study was designed to evaluate the effect of orally administered fluconazole in rohu.

Materials and Methods

The experiment was carried out at the wet laboratory of Department of Aquatic Health and Environment, College of Fisheries, Lembucherra, Tripura, India. Apparently healthy *L. rohita* fingerlings (average length 12.27 cm and average weight 17.23 g) were collected from the Battala fish market, Agartala, Tripura. The fish were acclimatized in circular fibre reinforced plastic (FRP) tanks of 500 l under laboratory conditions for 30 days and then used for experiments. Fish were provided with adequate aeration and fed with control diet. Satiation level feeding was done twice a day during acclimatization and 30% of water was exchanged daily.

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There were twenty-four treatments that were divided into four groups (Control, T1, T2, and T3 fed with 0, 10, 20 and 30 mg FLZ kg^{-1} body weight, respectively) and each group with 6 exposure times (0, 6, 12, 24, 48, and 96 h) in triplicates. The doses were decided based on our earlier study where rohu fingerlings did not show any significant mortality at higher dose of 1600 mg kg^{-1} body weight (unpublished data). Seventy two aquarium tanks (2x1x1 cu ft) with 40 l water were used for the experiment and each tank was randomly assigned for each replicate. A Total of 576 of healthy rohu fingerlings were equally distributed in all the aquarium tanks following a completely randomized design (CRD). The experimental trial was conducted for 10 days and the sampling of blood for haematological and immunological parameters was done at 0, 6, 12, 24, 48, and 96 h. Round the clock aeration was provided.

Four iso-nitrogenous and iso-caloric diets were prepared with graded levels [0 (control diet), 100 (diet A), 200 (diet B) and 300 (diet C) mg fluconazole 100 g^{-1} feed] of fluconazole (Himedia Laboratories, Mumbai, India) (Table 1). Each experimental group; control, T1, T2, and T3 received control diet, diet A, diet B, and diet C, respectively at 1% of their body weight which ultimately provided a dose of 0, 10, 20 and 30 mg FLZ kg^{-1} body weight to the respective group. Experimental feeds were given once to evaluate the effect of different doses of FLZ on the fish homeostasis in terms of biochemical parameters. Proximate composition of the experimental diets was analysed following standard method (AOAC, 1995).

The water used for the experiment was analysed for various environmental parameters following APHA (2005). pH, temperature, dissolved oxygen (DO), total alkalinity, and total ammonia-N of the water were found to be in the range of 6.6-7.4, 28.5-30.0°C, 6.2-7.6 mg l^{-1}, 110-125, and 0.01-0.03 mg l^{-1}, respectively, during the experiments, which were in the optimum range required for rohu (Kumar et al., 2012). At different sampling hours, plasma was collected and stored as described by Das et al. (2015). For the determination of glucose in plasma, glucose diagnostic kit (Crest Biosystems, India) was used which is based on Trinder (1969) GOD/POD method. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity was determined using the GOT diagnostic kit (Crest Biosystems, India) as described by Reitman & Frankel (1957). Alkaline phosphatase activity in

Table 1. Composition of experimental diets

Ingredients (in g) /100 g^{-1} of feed	Control diet	Treatment diets		
		Diet T1	Diet T2	Diet T3
Casein (vitamin free)	32.00	32.00	32.00	32.00
Gelatin	8.00	8.00	8.00	8.00
Dextrin	230.00	30.00	30.00	30.00
Cellulose powder	12.97	12.87	12.77	12.67
Carboxymethylcellulose	2.00	2.00	2.00	2.00
Sunflower oil	4.50	4.50	4.50	4.50
Cod liver oil	4.50	4.50	4.50	4.50
Vitamin Mineral premix*	6.00	6.00	6.00	6.00
Betaine hydrochloride	0.02	0.02	0.02	0.02
Butylated hydroxyl toluene	0.01	0.01	0.01	0.01
Fluconazole	0.00	0.10	0.20	0.30
Total	100	100	100	100

*Composition of vitamin-mineral premix (PREEMIX PLUS) (quantity/2.5 kg): Vitamin A, 5500000 IU; Vitamin D_3, 1100000 IU; Vitamin B_2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B_6, 1000 mg; Vitamin B_{12}, 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 ppm.

plasma was assayed using alkaline phosphatase diagnostic kit (Crest Biosystems, India) as described by Kind & King (1954). For albumin and globulin ratio (A/G), albumin was estimated by the bromocresol green binding method of Doumas et al. (1971) using albumin kit (Qualigens Diagnostics). Globulin was calculated by subtracting albumin values from total plasma protein (Schaperclaus, 1991). Total plasma protein was measured using a diagnostic kit (Crest Biosystems, India) based on Biuret method (Raghuramulu et al., 2003). Albumin and globulin ratio (A/G) was calculated by dividing albumin values by globulin values as describe by Schaperclaus (1991). Comparisons of mean values were determined by one way ANOVA and Tukey test using SPSS-15.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Biochemical properties of plasma are used as biomarkers for determining fish health condition (Vutukuru, 2003). Higher plasma glucose levels indicate stress response in fish (Nemcsok & Boross, 1982; Sheridan, 1986; Hontela et al., 1993) and it depends on the nutritional status of the fish (McLeay, 1977). However, the increase in blood glucose is usually correlated with the mobilization of glycogen. From the study, it was found that the plasma glucose content of T1, T2 and T3 were significantly ($p<0.05$) lower than the control at 6 h of administration. However, they attained homeostasis by 24 h of administration (Fig. 1).

The total plasma protein is also an indicator of health and metabolic response to stress. Furthermore, their level and the ratio of albumin and globulin depend on the quality and quantity of dietary protein consumed by the fish (Onifade & Tewe, 1993) and the presence of toxicants (Naganna, 1989). During stress, fish may also direct proteins to meet energy demand for maintenance of homeostasis (Martinez et al., 2004). However, in the present investigation, albumin contents of blood plasma were significantly ($p<0.05$) lower than the control at 12 h and onwards of sampling. Albumin content of the entire experimental group as compared to control kept fluctuating throughout the experiment and no defined pattern was observed (Fig. 1). Similarly, A/G ratio fluctuated significantly ($p<0.05$) as compared to the control from 48 h onwards of administration. This indicates that the fluconazole affected the A/G ratio in the blood of *L. rohita*. Furthermore, in terms of albumin content and A/G

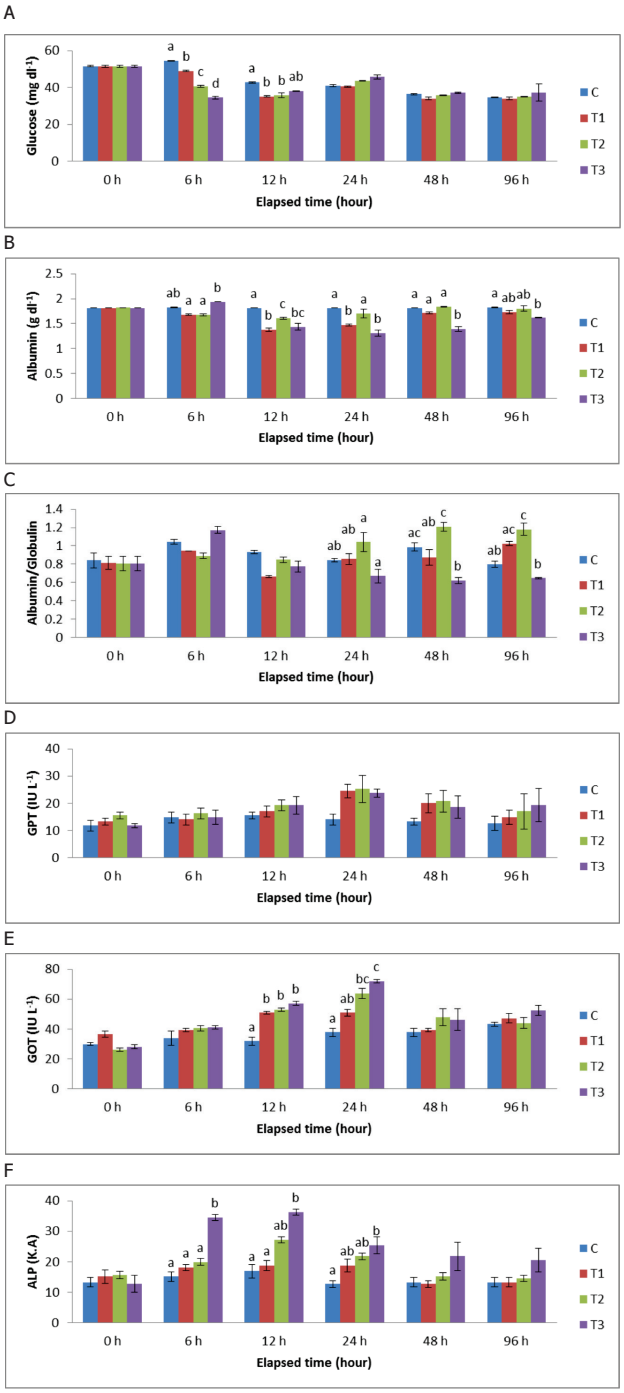

Fig. 1. Glucose (A), Albumin (B), A/G (C), GPT (D), GOT (E), and ALP (F) of *L. rohita* fingerlings of C, T1, T2, and T3 group fed with fluconazole containing feed at the rate of 0, 10, 20 and 30 mg FLZ kg^{-1} body weight, respectively and sampled at 0, 6, 12, 24, 48, and 96 h (n=3). Data (mean± SE) at the same sampling hour with different letters (a, b, and c) signiûcantly differ ($p<0.05$) among different experimental groups.

ratio, the homeostasis was not achieved till 96 h of administration as compared to the other biochemical parameters. This might be due to food deprivation, as no feed was given during the experiment and food directly influences the protein level (Onifade & Tewe, 1993).

Liver is one of the main target organs for the accumulation of any toxicant and the elevated levels of GPT, and GOT in plasma are indicative of liver injury (Kumar et al., 2013). In the present study, there was no significant ($p>0.05$) difference in GPT activity among the experimental groups. However, the treatment groups T1, T2 and T3 showed non-significant difference ($p>0.05$) from 12 to 48 h of administration (Fig. 1). GOT activity in T1, T2 and T3 groups were significantly ($p<0.05$) higher than the control at 12 h of administration. However, at 24 h of administration only T3 was significantly higher than the control. At 48 h and then onwards, there was no significant difference among the experimental groups (Fig. 1) and this suggests that fluconazole might cause some adverse effect on fish but the recovery was quick.

Alkaline phosphatase activity can be used as a biomarker for growth, illness, spawning, stress, hepatic damage, kidney dysfunction and bone disease (Goldemberg et al., 1987; Matusiewicz & Dabrowski, 1996, Suvetha et al., 2015; Ram & Sathyanesan, 1987; Barse et al., 2006). In the present study, ALP level increased significantly ($p<0.05$) in T3 groups from 6-24 h as compared to the other groups indicates that there is an adverse effect on the fish.

It is thus evident from the present study that fluconazole has some adverse effects on the fish health at higher dose of administration; however, the affect get neutralized quickly within 48 h in terms of majority of the biochemical parameters. However, more detailed studies need to be undertaken for the better understanding of its mechanism of action during long term feeding trial.

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