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Effect of *Phyllanthus niruri* on the biochemical parameters of freshwater fish, *Labeo rohita*

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Abstract:

Nowadays, herbal extracts of the medicinal plants are important in the aquaculture sector for the enhancement of growth and reproductive factors of freshwater fishes. In India, *Labeo rohita* is one of the most commonly cultured freshwater fish for human consumption. *Phyllanthus niruri* (Family: Euphorbiaceae) has various pharmacological properties due to their phytocompounds which are used to treat various ailments. After acclimatization, the L. rohita adult fishes (n=10) were treated with leaf extracts of *P. niruri* (5, 10, 20, 40 and 80mg/gm) based diet for 14 days. Fish gill, muscle and liver tissues - total glucose, total protein, total cholesterol, transaminases (AST, ALT) and glutamate dehydrogenase enzymes were statistically analysed. Our study concluded that *P. niruri* leaf extract based diet to the freshwater fish *L. rohita* showed an improved biochemical parameter than control fishes.

Keywords: *Phyllanthus niruri*, Labeo rohita, biochemical, phytoextract, transaminase

1.Introduction

Herbal extracts of the medicinal plants are important in the aquaculture sector nowadays (Kumar *et al.*, 2010). The use of various herbs has been demonstrated in numerous studies for the prevention or treatment of fish disease (Tan *et al.*, 2017; Panase *et al.*, 2018a); in addition, selected medicinal plant extract have been found to speed up fish development (Pratheepa and Sukumaran, 2014; Panase and Tipdacho, 2018).

Phyllanthus niruri (Family: Euphorbiaceae) typically grows as a winter weed in the drier regions. In the tropical and subtropical regions, the *Phyllanthus* genus encompasses more than 600 species of shrubs, trees, and annual or biennial herbs which grows up to 60 cm. It has many phytochemicals and pharmacological characteristics (Calixto *et al.*, 1998).

From several portions of P. niruri, the bioactive chemicals alkaloids, flavonoid, lignans, terpenoid, tannins, polyphenol, saponins and coumarin have been found. Numerous clinical trials have demonstrated the medicinal benefits of this herb's extracts. Numerous illnesses, including vaginitis, influenza, dysentery, tumours, jaundice, diuretics, dyspepsia, kidney stones, antihepatitis-B, antihepatotoxic, antihyperglycemic, diabetes, as well as antimicrobial qualities, can be treated with the help of this plant (Naik and Juvekar 2003).

With increasing concentrations of the phytoextract, farm fish's total protein, albumin, and globulin levels increased noticeably (Goda, 2008). This suggested that some of the extract's bioactive chemicals were stimulating the fishes' immune responses (Wiegertjes *et al.*, 1996). This was hypothesised since a larger innate immune response has been linked to higher levels of total protein, albumin, and globulin (the source of antibodies or Ig) in fish (Misra *et al.*, 2006). Since monosaccharides (glucose) plays a significant part in animal bioenergetics, which is translated to ATP generation, glucose is widely known to be a critical byproduct of cellular respiration (Lucas, 1996).

The Krebs cycle receives α-ketoglutarate from the mitochondrial enzyme glutamate dehydrogenase (GDH), which catalyses the deamination of glutamate by oxidation (Reddy and Venugopal, 1990). According to Gould *et al.* (1976), the AST, ALT, and GDH activities are sensitive indicators of stress. The primary goal of the study is to raise the biochemical indices of *Labeo rohita*, a freshwater fish, after it has been exposed to *Phyllanthus niruri* leaf extract for a period of 14 days.

2. Materials and Methods

2.1. Phytoextraction

The *Phyllanthus niruri* fresh plants were bought at a local botanical garden. Plant leaves cleaned with water for the removel of dirt before being immersed 20 mins in a Clorox mixture (10%), as per the procedures outlined by Panase *et al.*(2018a). They are dried by air in sterile circumstances. The leaves were weighed and combined in 1000 mL conical flasks with a 50% ethanol solution at a weight-to-volume ratio of 1:2.

The mixture were put in a muslin cloth for filtration, after being shaken for 24 hours at room temperature in an automated shaker. Following completion of this process, all the phytocompounds were combined in a 5000ml flask and maintained at 4°C for seven days in a refrigerator. By using rotary evaporator, solvents were evaporated at 65°C in accordance with the procedure employed by Harikrishnan *et al.*(2009), and the dry weight was then calculated using a freeze-drying methodology. Until use, the powder form was maintained at 20°C.

2.2. Experimental animal

Labeo rohita fingerlings were bought from a local fish farm. All of the fish were brought to the lab and were given 4 weeks to acclimatise in cement ponds (2x3x0.5m) with 300 L of water and a natural photoperiod. Every week, a system of continual air circulation and water was maintained. Throughout the acclimatisation period, parameters such as the dissolved oxygen, pH and temperature were maintained as 7.70±1.3 mg/L, 7.40±1.21 and 27.4±2.09°C respectively. Additionally, the fish feed was prepared based on rice and wheat bran (for control) and phytoextract based feed (for treatment) and fed to the fishes twice daily, during the acclimatisation period 40% crude protein were fed.

2.3. Extract Treatments

After acclimatization, the adult fishes (n=10) were selected for further study. One control and five treatment groups were maintained. The leaf extract based feed was provided to the treated groups and their concentrations are 5, 10, 20, 40 and 80mg/gm. Fishes were feed with phytobased diet for 14 days. Water parameters were maintained according to the APHA. During the experimental period, the remaining food and fecal matter were siphoned properly. After 14 days, the fishes were randomly selected from each group and dissected. Gills, liver and muscle tissues were collected and used for biochemical analysis.

2.4. Tissue biochemical studies

Gills, liver and muscle tissues were homogenized and centrifuged in a cooling centrifuge at 2500rpm for 15 min (Athif *et al.*, 2020). Total glucose, total protein and total cholesterol of the fish tissues were analysed by glucose oxidase method, Lowry method and Zak method respectively (Trevor, 2008; Lowry 1951; Zak *et al.*, 1954). Aspartate aminotransferase and alanine aminotransferase were studied by Reitman and Frankell (1957) method. Glutamate dehydrogenase was assayed by the method of Lee and Hardy (1965). Triplicate analysis was carried for each parameter. Data were analysed using a ANOVA and test with a 95% confidence interval ($P< 0.05$). For statistical analyses, SPSS 17.0 software version for Windows (SPSS Inc.) was used. All data were presented as mean ± SD.

3. Results

After the chronic exposure (14days) of *Phyllanthus niruri* leaf extract of five different concentrations such as 5, 10, 20, 40 and 80mg/gm based diet to adult freshwater *Labeo rohita* showed an enhanced biochemical parameter in the treated fishes than compared to the control fishes.

Tissues with a high concentration of mitochondria were crucial in maintaining the osmo-ionic balance of the system, which allowed more enzymes and biomolecules to be involved in the stress-induced system. To fulfil the energy requirement caused by nanoparticle exposure, liver tissues were heavily involved in detoxifying the body by creating several types of enzymes that improved glucose, lipid, and protein metabolic activities. Because gluconeogenesis used the cholesterol deposited in the hepatocytes, there was less total cholesterol in the tissues. Animals begin using the protein in their numerous organs throughout metabolism by synthesising transaminases, which create the building blocks for gluconeogenesis.

3.1. Total glucose level

In gill tissues, the control fish total glucose levels were observed as 52.09±2.13mg/dL whereas the P. niruri leaf extract treated fishes showed an improved total glucose level (F=0.87, P<0.05) as 51.36±1.46, 52.87±1.35, 52.93±1.84, 54.47±1.18 and 55.64±1.29mg/dL for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 1). In muscle and liver tissues, the total glucose levels were observed as 67.38±1.27, 70.22±1.28, 72.93±1.41, 74.17±1.10, 79.28±1.49mg/dL (F = 0.83, P<0.05) and 72.28±1.38, 72.29±1.83, 79.28±1.28, 81.47±1.18 and 85.48±1.19mg/dL (F = 0.77, P<0.05) respectively (Table 2 &3).

3.2. Total protein level

In gill tissues, the control fish total protein levels were observed as 10.10±0.74gm/dL whereas the P. niruri leaf extract treated fishes showed an improved total protein level (F=0.46, P<0.05) as 10.11±0.65, 10.45±0.58, 10.88±0.63, 10.91±0.61 and 11.99±0.59gm/dL for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 1). In muscle and liver tissues, the total protein levels were observed as 19.47±0.51, 19.48±0.58, 20.38±0.38, 21.97±0.55, 22.92±0.79gm/dL (F = 0.45, P<0.05) and 14.38±0.65, 14.60±0.68, 14.88±0.61, 15.11±0.38 and 15.37±0.48gm/dL (F = 0.22, P<0.05) respectively (Table 2 & 3).

3.3. Total cholesterol level

In gill tissues, the control fish total cholesterol levels were observed as 37.07±1.33mg/dL whereas the P. niruri leaf extract treated fishes showed an improved total cholesterol level (F=0.57, P<0.05) as 36.35±1.04, 37.11±1.11, 37.43±1.20, 37.89±1.24 and 38.59±1.21mg/dL for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 1). In muscle and liver tissues, the total cholesterol levels were observed as 50.48±1.28, 52.89±1.48, 58.28±1.28, 59.38±1.29, 61.38±1.59mg/dL (F = 0.80, P<0.05) and 39.35±1.68, 40.11±1.19, 43.18±1.10, 45.82±1.18 and 48.14±1.38mg/dL (F = 0.55, P<0.05) respectively (Table 2 & 3).

3.4. Transaminases level

Aspartate transaminase levels in control fish gill, muscle and liver tissues were observed as 9.67±0.34, 7.48±0.28 and 11.47±0.28IU/L respectively whereas alanine transaminase levels were observed as 6.99±0.56, 5.38±0.17 and 8.37±0.04IU/L for gill, muscle and liver tissues respectively (Table 1). Treated fishes AST levels observed as 9.01±0.21, 8.30±0.35, 8.35±0.13, 8.32±0.14, 8.21±0.25 (F=0.11, P<0.05) for gill tissues; 7.01±0.19, 6.49±0.28, 6.19±0.15, 6.09±0.11, 6.00±0.17 (F=0.16, P<0.05) for muscle tissues; 11.41±0.26, 11.30±0.35, 11.31±0.30, 11.20±0.53, 11.15±0.11 (F=0.20, P<0.05) for liver tissues for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 2 & 3).

Treated fishes ALT levels observed as 5.67±0.32, 5.57±0.12, 5.31±0.21, 5.21±0.25, 5.11±0.24 (F=0.09, P<0.05) for gill tissues; 5.13±0.16, 4.57±0.17, 4.31±0.14, 4.21±0.24, 4.01±0.47 (F=0.11, P<0.05) for muscle tissues; 8.27±0.05, 8.47±0.05, 8.31±0.04, 8.21±0.05, 8.11±0.06 (F=0.15, P<0.05) for liver tissues for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively.

3.5. GDH

Control fish glutamate dehydrogenase enzyme observed as 7.92±0.47IU/L (gill), 6.22±0.27IU/L (muscle) and 7.92±0.15IU/L (liver) whereas treated fish GDH levels significantly varied as 6.84±0.33, 6.31±0.37, 6.24±0.24, 6.11±0.11 and 5.99±0.32IU/L for gill tissues (F=0.12, P<0.05) for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 1).

In muscle and liver tissues, GDH levels were observed as 6.01±0.26, 5.83±0.21, 5.58±0.23, 5.11±0.29, 5.09±0.38IU/L (F=0.13, P<0.05) and 7.84±0.27, 7.61±0.48, 7.54±0.12, 7.31±0.23, 7.09±0.34IU/L (F=0.13, P<0.05) for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm groups respectively (Table 2 & 3).

Table 1. Effect of *P. niruri* on the gill tissues biochemical parameters of freshwater *Labeo rohita*

Groups	Biochemical parameters					
	Total Glucose (mg/dL)	**Total protein (gm/dL)**	**Total cholesterol (mg/dL)**	**AST (IU/L)**	**ALT (IU/L)**	**GDH (IU/L)**
Control	52.09±2.13	10.10±0.74	37.07±1.33	9.67±0.34	6.99±0.56	7.92±0.47
T1-5mg/gm	51.36±1.46	10.11±0.65	36.35±1.04	9.01±0.21	5.67±0.32	6.84±0.33
T2-10mg/gm	52.87±1.35	10.45±0.58	37.11±1.11	8.30±0.35	5.57±0.12	6.31±0.37
T3-20mg/gm	52.93±1.84	10.88±0.63	37.43±1.20	8.35±0.13	5.31±0.21	6.24±0.24
T4-40mg/gm	54.47±1.18	10.91±0.61	37.89±1.24	8.32±0.14	5.21±0.25	6.11±0.11
T5-80mg/gm	55.64±1.29	11.99±0.59	38.59±1.21	8.21±0.25	5.11±0.24	5.99±0.32
P value (Sig.)	0.87 (P<0.05)	0.46 (P<0.05)	0.57 (P<0.05)	0.11 (P<0.05)	0.09 (P<0.05)	0.12 (P<0.05)

Table 2. Effect of *P. niruri* on the muscle tissues biochemical parameters of freshwater *Labeo rohita*

Groups	Biochemical parameters					
	Total Glucose (mg/dL)	**Total protein (gm/dL)**	**Total cholesterol (mg/dL)**	**AST (IU/L)**	**ALT (IU/L)**	**GDH (IU/L)**
Control	66.09±2.13	19.10±0.49	49.07±1.27	7.48±0.28	5.38±0.17	6.22±0.27
T1-5mg/gm	67.38±1.27	19.47±0.51	50.48±1.28	7.01±0.19	5.13±0.16	6.01±0.26
T2-10mg/gm	70.22±1.28	19.48±0.58	52.89±1.48	6.49±0.28	4.57±0.17	5.83±0.21
T3-20mg/gm	72.93±1.41	20.38±0.38	58.28±1.28	6.19±0.15	4.31±0.14	5.58±0.23

T4-40mg/gm	74.17±1.10	21.97±0.55	59.38±1.29	6.09±0.11	4.21±0.24	5.11±0.29
T5-80mg/gm	79.28±1.49	22.92±0.79	61.38±1.59	6.00±0.17	4.01±0.47	5.09±0.38
P value (Sig.)	0.83 (P<0.05)	0.75 (P<0.05)	0.80 (P<0.05)	0.16 (P<0.05)	0.11 (P<0.05)	0.13 (P<0.05)

Table 3. Effect of *P. niruri* on the liver tissues biochemical parameters of freshwater *Labeo rohita*

Groups	**Biochemical parameters**					
	Total Glucose (mg/dL)	**Total protein (gm/dL)**	**Total cholesterol (mg/dL)**	**AST (IU/L)**	**ALT (IU/L)**	**GDH (IU/L)**
Control	71.09±1.05	14.43±0.28	39.16±1.38	11.47±0.28	8.37±0.04	7.92±0.15
T1-5mg/gm	72.28±1.38	14.38±0.65	39.35±1.68	11.41±0.26	8.27±0.05	7.84±0.27
T2-10mg/gm	72.29±1.83	14.60±0.68	40.11±1.19	11.30±0.35	8.47±0.05	7.61±0.48
T3-20mg/gm	79.28±1.28	14.88±0.61	43.18±1.10	11.31±0.30	8.31±0.04	7.54±0.12
T4-40mg/gm	81.47±1.18	15.11±0.38	45.82±1.18	11.20±0.53	8.21±0.05	7.31±0.23
T5-80mg/gm	85.48±1.19	15.37±0.48	48.14±1.38	11.15±0.11	8.11±0.06	7.09±0.34
P value (Sig.)	0.77 (P<0.05)	0.22 (P<0.05)	0.55 (P<0.05)	0.20 (P<0.05)	0.15 (P<0.05)	0.13 (P<0.05)

4. Discussion

Fish and other animals' health state have both been evaluated using biochemical indices in a variety of contexts. It was determined as insignificant results in the ALT and AST levels across all treatment groups, proving that the extract utilised in this study had no impact on fish liver function. Liu *et al.*, (2007) reported that this is possible due to the high levels of ALT and AST released into the blood plasma, which showed that the fish were under some type of stress indicator which are causing the liver organ to be stressed, impaired and injured.

Plasma Proteins and sugar levels of Labeo rohita fingerlings activated with immunostimulants were increased than those of fish not given an immunostimulant supplement, according to Choudhury *et al.* (2005). Fishes fed with 0.50 and 0.25gkg^{-1}, serum glucose levels were found to have substantially increased, while in the group fed 0.75gkg^{-1}, they had rapidly dropped. Glucose levels are typically chosen as the stress indicator. The liver's breakdown of glycogen allows fish to maintain higher amounts naturally. The monosaccharides are then converted into energy during cellular respiration which are collectively known as glycogenolysis (Vijayan *et al.*, 1997).

In general, the levels of glucose and total protein were at odds with one another through gluconeogenesis proteins can be incidentally converted into glucose and vice versa, both

of which are frequently necessary for the body mechanism. In addition, glucose decreased after being fed the extract at the highest concentration (0.75g/kg) were due to the phenolic compounds present in the phytoextract that lower sugar levels (Lin *et al.*, 2016).

Tissue protein levels were increased marginally and reached their greatest level in the fishes fed with $0.75gkg^{-1}$, glucose levels were greater in the groups fed 0.25 and $0.50gkg^{-1}$ and decreased in the ones fed with $0.75gkg^{-1}$. When A. graveolens extract was taken in higher doses, cholesterol levels rose noticeably. Nutrition, enzyme activity, and hepatic function can all have an impact on blood cholesterol levels. Cholesterol is the predecessor to the five main classes of steroid hormones, which can be affected by the fish reproductive cycle (Berg *et al.*, 2002).

Aspartate amino transferase (AST) and alanine amino transferase (ALT) activity, which is known to change under a variety of pathological and physiological situations, may act as critical linkages between carbohydrate and protein metabolisms, according to Shivakumar's (2005) research. According to Reddy and Venugopal (1990), the mitochondrial glutamate dehydrogenase (GDH) enzyme converts the oxidative deamination of glutamate and supplies α-ketoglutarate to the Krebs cycle. This enzyme performs a number of metabolic tasks with major physiological implications. It is closely related to the tissues' detoxification systems. The extra-hepatic tissues' GDH could be used to direct ammonia generated during proteolysis towards liver detoxification into urea.

Medicinal plants used as dietary supplements which eventually raise fish serum cholesterol. The cholesterol levels in the blood, liver, gonad, and body tissue of Etroplus suratensis that were fed three distinct herbal mixed diets from *Withania somnifera*, *Moringa oleifera* and *Mucuna pruriens* increased (Dhas *et al.*, 2017). Additionally, when compared to the control group, fresh water Catla catla fishes fed with *Cynodon dactylon* ethanolic extracts based diets displayed increased serum cholesterol (Kaleeswaran *et al.*, 2012) in blood.

Conclusion

Phyllanthus niruri leaf extract based diet enriched the biochemical parameters of the adult freshwater fish *Labeo rohita*. These results evidenced that phytocompounds which improved the basic metabolism of the fishes which resulted in the enhanced growth of the cultured fishes.

Conflict of Interest: None

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