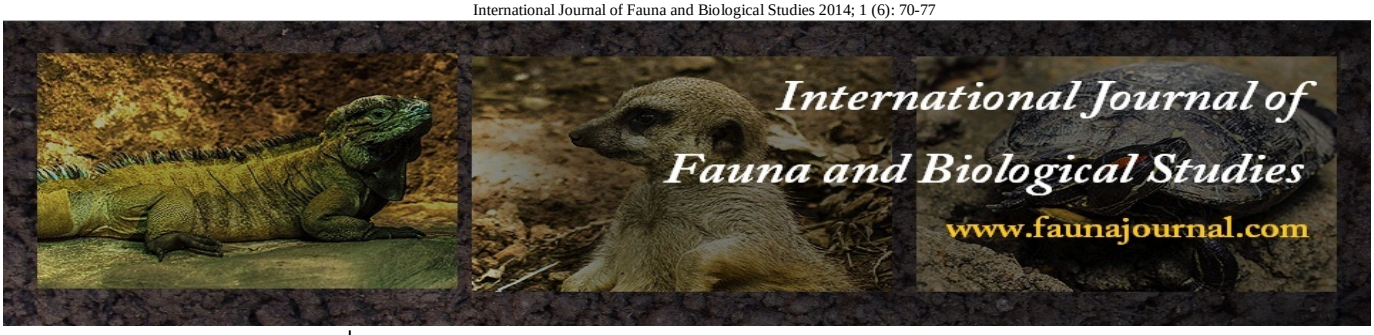

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Role of *Pedalium murex* in the enhancement of growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings

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Abstract
The effect of dietary ethanolic extract of *Pedalium murex* (Bada Gokshru) on growth, metabolism and hemato immunological parameters of an Indian Major Carp, *Labeo rohita* fingerlings were studied. Fishes were fed with an experimental diet containing four graded levels (0.0, 0.06, 0.08 and 0.1 g/100 g diet) of *Pedalium murex.* Net weight gain, specific growth rate (SGR) and food conversion ratio significantly ($p < 0.05$) improved in *Pedalium murex* fed *fish*. Still the highest weight gain and specific growth rate (SGR) were recorded in 0.08 g/100 gm *Pedalium murex* supplemented diet. The increase in digestive enzymes (i.e. protease, amylase and lipase) supported the results of the increase in growth of treatment level 0.08 g/100 gm. Whereas, the enzymes of protein metabolism were found the increased in herbal extract supplemented group as compared to control. Further, the haematological parameters such as packed cell volume (PCV), haemoglobin (Hb) and erythrocytes (RBC) were not significantly different in *Pedalium murex* supplemented diets fed fish. A significant ($P < 0.05$) proliferation of the leukocytes (WBC) and improvement in respiratory burst activity was observed. The results of this study proved the significant role of ethanolic extract of *Pedalium murex* in on growth, metabolism and immunity defence mechanism of *Labeo rohita* fingerlings.

Keywords: *Pedalium murex*, growth, hematology, *Labeo rohita*

1. Introduction
Pedalium murex, "Gokshru" is rich in flavonoids, sapogenin, sterol and soluble proteins. [1] In Indian Ayurvedic medicine system gokshru is used as analgesic and antipyretic activities [2, 3]. It is mainly used as tonic, aphrodisiac and useful in strangury, urinary discharges, vascular calculi, cough, asthma, pain, skin diseases, heart troubles, piles leprosy, stomachache, headache, diarrhea, dysentery, cough intestinal affections etc [4, 5]. Pharmacologically, the plant has been investigated for antiulcerogenic, nephroprotective, hypolepidemic, aphrodisiac, antimicrobial and insecticidal properties [6, 7, 8, 9].
The aqueous extract of the whole plant has been found to possess analgesic and anti-inflammatory properties [11]. Extensive phytochemical investigations have revealed the presence of Pedalitin and Pedalin (major flavanoids) along with Diosmetin, Dinatin, Dinatin-7-glucoronide, Quercetin, Quercimeritin, and Quercetin-7-glucorhamnoside in the plant [12]. Triterpenoids such as α-amyrin acetate, Rubusic acid, ursolic acid, and lupeol acetate are reported [13]. Presence of steroids such as, β-sitosterol [14], Sapogenins [15] and Diosgenin [16] has also been reported. Lipids [17], phenolic acids such as caffeic acid, ferulic acid, protocathechic acid, vanillic acid [15], and amino acids such as aspartic acid, glutamic acid, and histidine are other phytoconstituents of *P. murex* [18].
Herbs have been widely used in veterinary and human medicine as they are not only safe for consumers but also widely available throughout Asia. Wild satavari (*Asparagus racemosus*), which is widely used in India as Ayurvedic medicine for promoting growth in humans, produced a similar result in *Labeo rohita* fry [19] thus, looking at the importance of herbal medicines in aquaculture, the present study was designed to investigate the effect of the ethanolic extract of *Pedalium murex* on growth, metabolism and haemato-immunology of *Labeo rohita* fingerlings.

2. Material and Method

2.1. Experimental Design

The present experiment was conducted in eighteen cemented tanks of 3 m^3capacityfor 90 days. Healthy rohu, *Labeo rohita* fingerlings were obtained from a fish seed production unit of MUPAT, Udaipur (India). Prior to the start of the experiment, the fish were fed with commercial feed for 7 days to make the fish acclimatized to experimental environments. The healthy fingerlings of uniform size (14.18±0.00 g) were randomly distributed in six experimental groups each with three replicates following a complete randomized design. Each cemented tank (3x3x1 m) was filled with 5 m^3 water and stocked with 10 fingerlings. Fishes were fed daily at 3.0% of their body weight for 90 days period.

2.2. Preparation of Extract

The seeds of *Pedalium murex* were procured locally and authentication was done by the expert of the Botany Department (College of Science, MLS University, Udaipur, India). Thus the seeds procured were washed using tap water and then rewashed with distilled water to remove the dust. The seed was dried under shade for 7 days and seed coat was removed before grinding them to fine powder. Later, it was transferred into 5 lit. glass jar and ethanol was added as solvent until the fine particles of the seed were completely soaked. The container was gently shaken for 72 h at every 1 h interval (until the color of solvent became colorless) and the filtrate was vacuum concentrated to remove the moisture content [20]. The percentage yield of an extract from seed was around 8.5%. The dried extract powder was packed in sealed polythene bags and placed in deep freezer till further use.

2.3. Formulation of Experimental Diet

A basal diet having 35% crude protein was formulated using different ingredients (Table 1). The ingredients were powdered, thoroughly mixed and moistened with water to form dough. Thus, the dough prepared was placed in autoclave (121 ºC at 15l bs/cm^2 pressure) for 15 minutes. After cooling, graded levels of ethanolic extracts of *Pedalium murex* seeds were added to the basal diet at 0 (T1), 0.06(T2), 0.08(T3) and 0.1 g (T4)/100 g of basal diet. The feedstuffs were thoroughly mixed using a die of 8 mm diameter. The diets were air-dried at ambient temperature for 72 h, broken into small pellet sizes, packed in air-tight containers, labelled and stored.

Table 1: Ingredients (g/100 gm dry matter) of the experimental diets for experiment.

Ingredients	T1	T2	T3	T4
Fishmeal	10	10	10	10
GNOC	44.96	44.96	44.96	44.96
Rice bran	21.52	21.42	21.22	20.82
Wheat flour	22.52	22.52	22.52	22.52
Mineral mixture(Agrimin)	1.0	1.0	1.0	1.0
Ethanolic extract of *Pedalium murex*	0.00	0.06	0.08	0.10
Moisture	8.25±0.016[a]	8.31±0.023[b]	8.56±0.029d	8.45±0.006c
protein	34.91±0.023[a]	34.88±0.012a	35.04±0.017b	35.15±0.058b
ether extract	12.33±0.017[b]	12.24±0.023a	12.49±0.012c	12.63±0.029d
carbohydrate	30.8±0.1[a]	30.93±0.018a	30.64±0.024a	30.4±0.231a
Ash	13.71±0.006[d]	13.64±0.012d	13.27±0.017a	13.37±0.029b
Energy(Kcal/gm)	431.93±0.005[b]	431.445±0.006b	433.495±0.009d	434.435±0.003f

Mineral Mixture Agrimin forte-Nutritional value per kg.-Vit. A-7,00,000I.U., Vit.D3-70,000I.U., Vit.E-250 mg, Nicotinamide-1000 mg, Cobalt-150 mg, Copper-1200 mg, Iodine- 325 mg, Iron -1500 mg, Magnesium-6000 mg, Maganese-1500 mg, Potassium-100 mg, Selenium-10 mg, Sodium-5.9 mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5% and Phosphorus-12.75%. (supplied by Virbac Animal Health India Pvt. Ltd., Mumbai).

2.4. Estimation of Growth Performance

The fish growth and nutrient utilization data were collected. Gross energy was calculated according to [21] with multiplier factors of carbohydrate, 4.1 kcal/g, protein, 5.4 kcal/g and lipids, 9.5 kcal/g. The following formulae were used to calculate, specific growth rate (SGR), food conversion ratio (FCR) and feed efficiency ratio (FER):

$$SGR = \frac{(\text{Log e Final Wt.} - \text{Log e Initial Wt.})}{\text{Culture Period}} \text{ x } 100$$

$$FCR = \frac{\text{Total feed given}}{Totalweightgain}$$

$$FER = \frac{\text{Total wet weight gain}}{Totalfeedgiven}$$

2.5. Proximate composition analysis

The proximate analysis of experimental feed and fish were done following the method of [22]. Feed composition was estimated before the initiation of experiments, while that of the experimental fish, the proximate composition was performed initially and at the termination of the experiments.

2.5. Haematological Parameters

At the end of 90 days feeding trial, fish were fasted for 24 hours immediately prior to blood sampling and five fish per tank were randomly chosen and anesthetized with tricaine methane sulfate (20 mg/L). The blood was extracted from the caudal vein and poured in Eppendorf tubes. One capillary of 50microliter was filled and placed in blood analyzer (Exigo Vet.) and result for RBC,MCV, HCT, HGB, PLT,WBC, MCHC, HCT, Lymphocytes and granulocyte were obtained.

2.5.1. Blood glucose
Five hundred micro litre (µl) of blood sample was deproteinized by mixing with 4.75 ml of zinc sulphate followed by addition of 4.75 ml of barium hydroxide [23]. The solution was mixed vigorously and filtered using a filter paper and the filtrate was collected in a dry test tube and 1 ml of alkaline copper sulphate was added to it. The test tubes were placed in a boiling water bath for 20 min. The test tubes were then cooled to room temperature and 1 ml arsenomolybdate reagent was added. The absorbance was recorded at 540 nm against blank.

2.5.2. Nitroblue tetrazolium (NBT) assay
The NBT assay was done following [24] method modified by [25]. Fifty micro litre of blood was placed into the wells of "U" bottom microtitre plates and incubated at 37 ^{0}C for 1 hour to facilitate adhesion of cells. The supernatant was removed and the loaded wells were washed three times in PBS. After washing, 50 µl of 0.2% NBT was added and was incubated for further 1 hr. The cells were then fixed with 100% methanol for 2-3 min and again washed thrice with 30% methanol. The plates were then air dried. Sixty microliters 2N potassium hydroxide and 70 microliters dimethyl sulfoxide were added into each well to dissolve the formazon blue precipitate formed. The OD of the turquoise blue coloured solution was then read in ELISA reader at 620 nm.

2.6. Serum Protein
For separation of serum, blood samples were withdrawn from the caudal vein and transferred to Eppendorf tubes without anticoagulant. The blood samples were centrifuged at 3000 x g for 15 minutes and the supernatant serum was collected and stored at -20 ^{0}C until used. Serum protein was estimated by biuret method [26].

2.6.1. Albumin- globulin ratio
Albumin was estimated by bromocresol green binding method [27]. Whereas, the globulin was obtained by subtracting albumin values from total plasma protein. Albumin- globulin ratio was calculated following formula:

$$A/Gratio = \frac{Albumin(g\%)}{Globuline(g\%)}$$

2.6.2. Alanine &Aspartate amino transferase (sAST)
Serum Alanine & Aspartate amino transferase were measured by the auto-analyser MERCK Selectra Junior, Merck, Germany by using commercially available kit from Qualigens Diagnostics following standard protocols.

2.7. Functional immune response assays (Serum lysozyme)
The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg ml-1 lyophilized *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. Fifty µl of serum was added to 2 ml of the bacterial suspension and the reduction in the absorbance at 540 nm was determined after 0.5 and 4.5 minutes incubation at 22 °C. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min-1.

2.8. Aspartate amino transferase (AST)
The AST activity was assayed in different tissue homogenates as described by [28]. The substrate comprised of 0.2 M D, L-aspartic acid and 2 mM α-ketoglutarate in 0.05M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5 ml of substrate was added. The reaction was started by adding 0.1 ml of tissue homogenate. The assay mixture was incubated at 37 ^{0}C for 60 minutes. The reaction was terminated by adding 0.5 ml of 1mM 2, 4 dinitrophenyl hydrazine (DNPH). In the control tubes. The enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5 ml of 0.4 ml NaOH solution was added, the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank.

2.9. Digestive enzyme assays
Three important digestive enzymes like intestinal amylase, protease and lipase were assayed using standard protocols. Protease activity was determined by the casein digestion method [29]. Amylase activity was estimated using dinitro-salicylic-acid (DNS) method. While, the lipase activity (EC 3.1.1.3) was assayed by the method [30].

2.10. Alkaline& Acid Phosphatase (ALP)
The ALP (E.C. 3.1.3.1) activity was determined by [31]. The procedure adopted for the estimation of ALP activity was same as that for ACP activity estimation except that the buffer which comprised of 0.2 M Sodium Acetate and Acetic Acid, having pH 5.0. The ACP activity was expressed as nanomoles P-nitrophenol released / mg protein / minute at 37 °C.

2.11. Statistical Analysis
The data collected for fish growth parameters, blood analysis etc. were statistically analyzed using SPSS (SPSS Inc., Chicago, IL, USA, version 16.0) programme for windows.). As such, standard error of mean, ANOVA, and Duncan's Multiple Range Test were calculated to know the significance of experimental results.

3. Result and Discussion
3.1. Growth parameters
The growth parameters such as weight gain, FCR, FER and SGR are given in Fig. 1 and 2. The weight gain (%), FCR, FER and SGR of the treatment groups fed with ethanolic extract of *Pedalium murex* were significantly different ($p<0.05$) as compared to control. Significantly higher values of percent weight gain (139.55±2.95), FER (0.36±0.01) and SGR (1.37±0.01) were observed in T-3, while the lowest values were in T-1 (control). The lowest (2.81±0.04 and highest (3.365±0.01) values of FCR were in T-3 and T-1 respectively. The effects of herbs have been studied on many aquatic animals. Improvement in the growth of fish has been reported by feeding herbs supplemented diet [32, 33]. In the present study, the growth and nutrient utilization by fish got decreased with the increasing level of *Pedalium murex* extract inclusion level in fish diets. This could probably be a result of persistent consumption of the *Pedalium murex* extract which could retard the animal growth rate. Siddhuraju P, Ojha ML [34, 35, 36] found similar results in common carp fed with *Mucuna pruriens*. They observed no significant difference in fish growth as compared to control and concluded that the sensitivity of common carp, to the antinutritional factors contained in the mucuna seed meal produced low growth performance at higher inclusion levels. Further, the studies of [37] have shown that the use of processed mucuna seeds significantly improved the

growth performance and feed utilization in tilapia compared with raw mucuna seed. Supplementation of *Gynostemma pentaphyllum*, a traditional Chinese herbal medicine, to grass carp feed resulted in increased weight gain, feed conversion efficiency and specific growth rate [38].

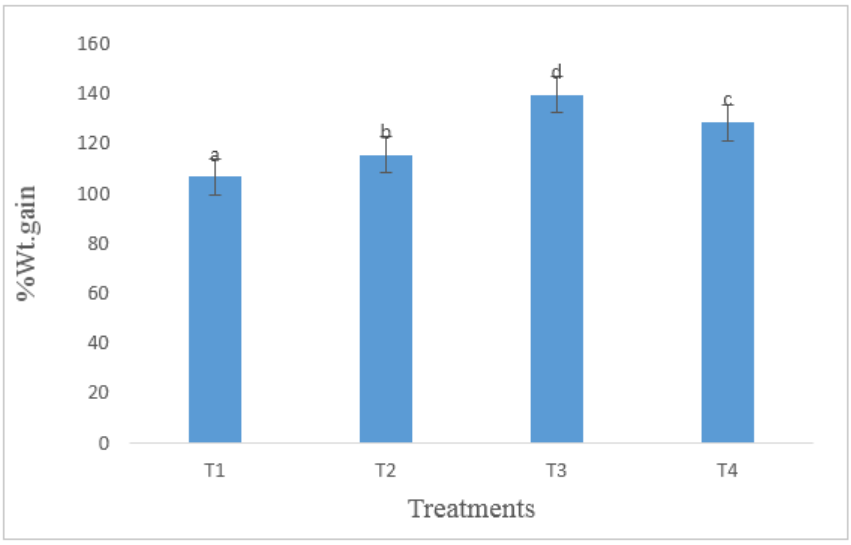

Fig 1: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on % Wt. gain of *Labeo rohita* fingerlings

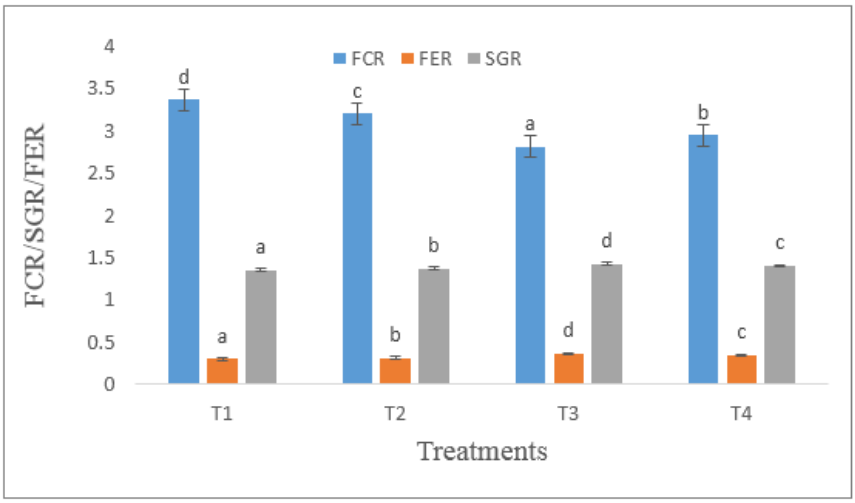

Fig 2: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on Food Conversion Ratio, Food Efficiency Ratio and Specific Growth Rate of *Labeo rohita* fingerlings

3.2. Digestive Enzyme analysis

The digestive enzymes like protease, amylase, lipase, alkaline phosphatase and acid phosphatase activities in intestinal tissue of different experimental groups are given in Table 2. A significant effect on amylase activity due to *P. murex* supplementation diet fed fish was observed. As such, the highest amylase activity was observed in T-3 (25.49±0.31), while the lowest was in T-1(23.49±0.27). The value of amylase activity in T-1 (23.49±0.27), T-2 (24.13±0.58) and T-4 (23.83±0.35) were not significantly (P>0.05) different. The protease activity was highest in treatment T-3 (27.49±0.32) and lowest in T-1(21.16±0.65). The lipase activity was highest in treatment T-4 (0.606±0.004) and lowest in Treatment T-1(0.517±0.003). The enzyme activities of alkaline and acid phosphatase were significantly different. The highest value of alkaline phosphatase was in treatment T-3 (10.137±0.024) and lowest was in treatment T-1(9.948±0.017). The acid phosphatase activity of treatment T-3(4.028±0.011) was highest and in T-1(3.839±0.009) it was lowest. The level of digestive enzymes in fish may be influenced by type of feeding [39], biochemical composition of food and onset of sexual maturity [40]. It is also known that age and stage of development significantly influence the digestive enzyme activities in different fish species [41].

Amylase is one of the major carbohydrases which hydrolyzes glycosidic bonds between sugar residues in large carbohydrate molecules. Amylase specifically breakdowns starch into glucose molecules. Low amylase activity in the carnivorous (with stomach) and high activity in omnivorous fishes (without stomach) is the general assumption [42]. Proteases are digestive enzymes which hydrolyzes the peptide bonds between the adjacent amino acids in the proteins. Protease activities in intestine were higher than the hepatic protease activity, which was supported by the result of [43]. Kumar *et al*., 2006 [43]

reported functional efficacy of digestive proteases of catla (*Catla catla)*, rohu (*Labeo rohita)*, and silver carp (*Hypophthalmichthys molitrix)* total protease activity was higher in rohu followed by silver carp, and catla. Lipases hydrolyzes the ester bonds among the fatty acids and glycerol in lipids. Alkaline phosphatase activity was reported to be an indicator of the intensity of nutrient absorption in enterocytes of fish [44]. Abalaka *et al* [45] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed significant increases in the activity of alkaline phosphatase changed with increasing concentrations of both extracts

Table 2: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on Amylase, Protease, Lipase, Alkaline phosphatase and Acid Phosphatase in intestine of *Labeo rohita* fingerlings

Treatment	Amylase	Protease	Lipase	ALP	ACP
T1	23.49^{a}±0.27	21.16^{a}±0.65	0.517^{a}±0.003	9.948^{a}±0.017	3.839^{a}±0.009
T2	24.13^{a}±0.58	24.13^{b}±0.58	0.529^{a}±0.006	10.057^{b}±0.011	3.924^{c}±0.004
T3	25.49^{b}±0.31	27.49^{c}±0.32	0.581^{b}±0.006	10.137^{c}±0.024	4.028^{d}±0.011
T4	23.83^{a}±0.35	22.16^{a}±0.02	0.609^{c}±0.004	10.063^{b}±0.015	3.869^{b}±0.007

Amylase activity in micromole maltose released /min/gm protein ; Protease activity in micromole tyrosine released/min/gm protein; Lipase activity in units/mg protein; Alkaline phosphatase activity in nano moles p-nitrophenol released /min/mg protein at 37 ˚C; Acid phosphatase activity in nano moles p-nitrophenol released /min/mg protein at 37 ˚C

3.3. Enzymes of Protein Metabolism

The activities of aspartate amino transferase (AST/GOT) and alanine amino transferase (ALT/GPT) in liver and muscle of *L. rohita* fingerlings are presented in Table 3. The ethanolic extract of *P. murex* showed a significant effect on these metabolic enzymes. The aspartate amino transferase activity in liver and muscles are significantly (P<0.05) different and in liver it was highest in treatment T-3 (9.440±0.287) and lowest in treatment T-1(7.089±0.350). In muscles aspartate amino transferase activity was highest in treatment T-3 (11.879±0.368) and lowest in treatment T-1 (9.719±0.436). The alanine amino transferase activity was significantly (P<0.05) and in liver it was highest in treatment T-3 (3.883±0.006) and lowest in treatment T-1 (3.436±0.012). The alanine amino transferase activity in muscles was highest in treatment T-3 (2.478±0.040) and lowest in treatment T-1 (2.059±0.029). Ananta *et al* [46] studied the effect of ethanolic extract of *Pedalium murex* fruit to ethylene glycol intoxicated rats and *Pedalium murex* reverted the levels of the liver and renal tissues from the damage and maintained the ACP, AST, ALP and ALT in renal and hepatic tissues. Abalaka *et al* [45] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed significant increases in aspartate amino transferase and alanine amino transferase activities.

Table 3: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in *Labeo rohita* fingerlings

Treatment	AST		ALT	
	Liver	**Muscle**	**Liver**	**Muscle**
T1	7.089^{a}±0.350	9.719^{a}±0.436	3.436^{a}±0.012	2.059^{b}±0.029
T2	8.154^{ab}±0.456	10.227^{a}±0.698	3.546^{b}±0.023	2.246^{a}±0.012
T3	9.440^{b}±0.287	11.879^{b}±0.368	3.883^{d}±0.006	
T4	9.305^{b}±0.548	10.606^{ab}±0.469	3.642^{c}±0.011	2.336^{c}±0.031

Aspartate amino transferase activity in nano moles of oxaloacetate formed/min/mg protein; Alanine amino transferase activity in nano moles sodium pyruvate released/min/mg protein

3.4. Haemato-immunological Parameters

3.4.1. Blood count

The haematological responses (i.e TEC count, TLC count, haemoglobin, haematocrit, MCV, MCH and MCHC) of experimental fish are presented in Table 4. Significant (P<0.05) difference was observed with respect to the effect of supplementation of ethanolic extract of *P. murex* on these responses. The highest value of total erythrocyte count (TEC) was in treatment T-3 (1.28±0.01) and lowest in treatment T-1(1.15±0.01). The total leucocyte count (TLC) was highest in treatment T-1(232.76±0.60) and lowest in treatment T-3 (230.25±0.10). The haemoglobin content of treatment T-3 (8.88±0.04) was highest and treatment T-1(7.54±0.03) was lowest. Haematocrit (Hct) value in treatment T-1 (24.69±0.04) was lowest and treatment T-3 (27.72±0.05) was highest. The mean cell volume was highest in treatment T-3 (216.37±0.86) and lowest in treatment T-2(211.37±0.80). The mean corpuscular haemoglobin concentration (MCHC) was highest in treatment T-3 (32.01±0.23) and lowest in treatment T-1 (30.53±0.12), but the value of MCHC in treatment T-1(30.53±0.12),T-2(31.10±0.15) and T-4(30.56) were not significant (P>0.05) different. The haemoglobin concentration got decreased with increasing concentration of the plant extracts similar to those reported in *C. gariepinus* to cassava effluents and tobacco (*Nicotina tobaccum*) leaf extracts [47, 48] and aqueous leaf extracts of *Lepidagathis alopecuroides* [49] as compared to control. The RBC values were significantly higher in experimental diet fed fish. Sahu *et al.* [50] have also

reported higher RBC counts in *Labeo rohita* fingerlings fed with *Mangifera indica.* They explained this increase as an indication of enhanced cellular immunity. Chukwudi *et al* [51] observed that WBC counts in rats administered with *Mucuna pruriens* increased significantly in comparison to control. This increase in WBC total count likely had been triggered off by the metabolic assault from alkaloid and/or phenol content in *Mucuna pruriens* [52]. The blood parameters such as MCV, MCH and MCHC are particularly important for the diagnosis of anemia in most of the animals. The MCV values decreased with increasing level of *Pedalium murex* extract inclusion levels in diet. Similar results were observed [32] for Tilapia fed with *A. paniculata* supplemented diet.

Table 4: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on Haematological parameters in *Labeo rohita* fingerlings

Treatment	TEC[1]	TLC[2]	Hb[3]	Hct[4]	MCV[5]	MCH[6]	MCHC[7]
T1	1.15^{a}±0.01	232.76^{c}±0.60	7.54^{a}±0.03	24.69^{a}±0.04	214.23^{ab}±0.55	65.41^{a}±0.12	30.53^{a}±0.12
T2	1.23^{c}±0.02	231.36^{b}±0.11	8.08^{c}±0.04	25.98^{b}±0.01	211.37^{a}±0.80	65.75^{a}±0.35	31.10^{a}±0.15
T3	1.28^{d}±0.01	230.25^{a}±0.10	8.88^{d}±0.04	27.72^{c}±0.05	216.37^{b}±0.86	69.31^{b}±0.51	32.03^{b}±0.23
T4	1.18^{b}±0.03	232.81^{c}±0.05	7.70^{b}±0.03	25.21^{a}±0.36	212.87^{ab}±2.00	65.05^{a}±0.38	30.56^{a}±0.45

[1]TEC=Total Erythrocyte count (10^6 cells/mm^3); [2]TLC=Total lymphocyte count (10^3cells/mm ^{3}); [3]Hb=Haemoglobin (g dl^{-1}); [4]Hct=Haematocrit (%); [5]MCV=Mean cell volume (fl); [6]MCH=Mean corpuscular haemoglobin (pg); [7]MCHC=Mean corpuscular Haemoglobin concentration (gm/dl)

3.4.2. Blood Glucose and Respiratory burst activity (NBT)

Blood glucose levels and the respiratory burst activity (NBT) of various experimental groups are shown in Table 5. There was a significant effect ($p<0.05$) of *P. murex* supplementation on the blood glucose level and the respiratory burst activities of experimental fish. The highest value of blood glucose was in T-1(52.247±0.308) and lowest in T-3(37.903±0.276). The respiratory burst activity was found highest in T-3 (0.173±0.01) and lowest in T-1 (0.130±0.007). Blood glucose concentration in blood serum got reduced significantly in fish fed on diets containing different sources of *Allium sativum.* This condition was attributed to improve the antioxidant system in cells of the pancreas to produce insulin [53]. These results got conformed to those of [54] who found that feeding mice with garlic induced significant decrease of serum glucose levels. Lower levels of plasma glucose in fish have also been reported in the assessment of biochemical effects of *Allium sativum* [55]. Bhaskar *et al* [56] reported that aqueous extract of the seeds of *Mucuna pruriens* significantly reduced the blood glucose levels after an oral glucose load and oral administration of seed extract. Herbal medicine extracts can also enhance phagocytosis in various fish species [57]. Their phagocytic activity is a primitive defence mechanism [58] and an important characteristic of the non-specific immune system.

Table 5: Effect of dietary supplementation of ethanolic extract of *Pedalium murex* on serum total protein, albumin, globulin, A/G ratio, serum aspartate amino transferase (AST) and serum Alanine amino transferase (ALT) and lysozyme activities in *Labeo rohita* fingerlings

Treatment	T1	T2	T3	T4
Total protein	5.37^{a}±0.28	7.12^{b}±0.08	8.63^{c}±0.14	5.93^{a}±0.11
Albumin	2.44^{a}±0.14	2.48^{a}±0.04	2.49^{a}±0.02	2.82^{b}±0.08
Globulin	2.93^{a}±0.14	4.64^{b}±0.05	6.13^{c}±0.16	3.12^{a}±0.05
A/G ratio	0.82^{c}±0.01	0.54^{b}±0.01	0.41^{a}±0.01	0.91^{d}±0.02
AST	60.57^{c}±0.37	54.16^{b}±0.49	64.75^{d}±0.36	51.04^{a}±0.14
ALT	29.25^{b}±0.34	25.18^{a}±0.22	32.38^{c}±0.30	25.50^{a}±0.21
Lysozyme	4.04^{a}±0.03	6.82^{c}±0.02	8.72^{d}±0.01	5.96^{b}±0.01
Glucose	52.247^{d}±0.308	43.587^{b}±0.383	37.903^{a}±0.276	47.943^{c}±0.277
NBT	0.130^{a}±0.007	0.159^{ab}±0.0.004	0.173^{b}±0.0.010	0.142^{ab}±0.011

Total protein (gm %); Albumin (gm %); Globulin (gm %); Aspartate transaminase activity in nano moles of oxaloacetate formed/min/mg protein; Alanine transaminase activity in nano moles sodium pyruvate released/min/mg protein; Lysozyme (unit ml^{-1}); Glucose (mg/dl); NBT-Respiratory burst activity (OD at 540 nm)

3.4.3. Serum Parameters

Serum parameters, total protein, albumin, globulin, A/G ratio, lysozyme, aspartate amino transferase (AST) and alanine amino transferase activity of the experimental fish are shown in Table 5. The highest total protein value was in T-3 (8.63±0.14) and lowest in T-1(5.37±0.28). The serum albumin content was highest in T-4 (2.82±0.08) and lowest was in T-1 (2.44±0.14). The values of serum albumin content in T-1(2.44±0.14), T-2 (2.48±0.04) and T-3 (2.49±0.02) were not significantly ($P>0.05$) different. The globulin content in T-3(6.13±0.16) was highest and lowest was in T-1(2.93±0.14). The albumin globulin ratio (A/G ratio) was significant ($P<0.05$) in all treatments, it was highest in T-4 (0.91±0.02) and lowest in T-3 (0.41±0.01). Serum aspartate amino transferase (AST/GOT) and alanine amino transferase activity (ALT/GPT) were significantly ($P<0.05$) different in all treatments. The highest aspartate amino transferase activity was noticed in T-3 (64.75±0.36) and lowest in T-4 (51.04±0.14). The alanine amino transferase activity was highest in T-3 (32.38±0.30) and lowest in T-2 (25.18±0.22). The lysozyme activity in T-3(8.72±0.01) was highest and lowest in T-1 (4.04±0.03). Abalaka *et al* [45] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed plasma total

proteins concentrations increased with increasing extracts concentrations in fish exposed to ethanolic extracts. The results of the present study and specially the improvement in the lysozyme level in *Mucuna pruriens* supplemented diet fed fish found support from the results of crucian carp, large yellow croaker [59] and the common carp [60] when the fish were fed with various herbal extracts that included *Eclipta alba, Radix astragali seu* Hedysari and *Radix angelicae sinensis.*

4. Conclusion

Based on these results and discussions, it is concluded that a *Pedalium murex* extract supplemented diet has the significant role in improving the growth of *L. rohita* besides its ability to enhance metabolism and immunity of the fish. The optimum dose (0.08 gm/100 gm diet) in the feed of *L. rohita* need to be further tested under field condition so that the *Pedalium murex may* be recommended for the commercial aquaculture.

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