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Effect of Fruit Rind Extract of *Garcinia gummi-gutta* on Haematology and Plasma Biochemistry of Catfish *Pangasianodon hypophthalmus*

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ABSTRACT

A 45 day study was undertaken to evaluate the effect of dietary aqueous extract of *Garcinia gummi-gutta* in catfish, *Pangasianodon hypophthalmus* on growth performance, basic haematological and biochemical parameters. Fishes were randomly distributed in to glass aquaria at ten fish/tank in triplicate. Five diets (30% crude protein) containing varying levels of aqueous extracts of *G. gummi-gutta* at 0, 500, 1000, 2000 and 3000 mg kg^{-1} were prepared and fed to the fishes twice daily at 3% of their body weight. The results for each test group were compared statistically with those for the control. Analysis of the results showed a significant increase in erythrocytes, leucocytes, thrombocytes, haemoglobin, packed cell volume, mean corpuscular haemoglobin concentration, protein and high density lipid and significant decrease in weight gain, specific growth rate, glucose, total cholesterol, triglycerides and low density lipid. The increase in cellular immunological indicators such as RBC, WBC and thrombocytes in the experimental fish may be due to the increase in the levels of immunity which in turn could be due to the action of the extract present in the diets. The decrease in total cholesterol, triglycerides and LDL could be due to the action of the hydroxy citric acid present in the dried fruit rind of *G. gummi-gutta* which has a known hypolipidaemic property. This decline in LDL cholesterol is by reducing the endogenous synthesis of cholesterol and triglyceride due to the decreased production of acetyl COA. The critical examination of the parameters of the various treatment groups shows that fish treated with an extract of 2000 mg kg^{-1} of feed exhibited more optimum features in most of the biochemical, haematological and growth factors. The results prove that the inclusion of *G. gummi-gutta* extract at a rate of 2000 mg kg^{-1} feed will enhance growth, elicit immunity and modify lipid profile to a significant level.

Key words: Haematological parameters, hydroxyl citric acid, immunostimulant, hypolipidaemic, *Garcinia gummi-gutta, Pangasianodon hypophthalmus*

INTRODUCTION

The recent expansion of intensive aquaculture practices has led to a growing interest in understanding fish diseases, so that they can be treated and/or prevented. Disease outbreaks are particularly prevalent in rapidly developing aquaculture industries, affecting the economic development of this sector. There is a need to look for ecofriendly disease prevention measures to

promote suitable culture. There are studies on the inclusion of plant derived materials or whole plants such as Sheeno and Sahu (2006) and Chowdhury *et al.* (2008) as nutrient sources. The most effective method may be the development of natural disease resistance in fish, with immunostimulants which can increase the immunocompetency and disease resistance of fish. Immunostimulants increase resistance to disease by enhancing the non-specific immune system and their use has been given considerable attention in aquaculture. The use of natural immunostimulants in fish culture for the prevention of diseases is a promising new development (Anderson, 1992; Sakai, 1999). Natural immunostimulants are biocompatible, biodegradable and safe for the environment and human health (Ortuno *et al.,* 2002). Immunostimulants do not eradicate pathogens in most cases but can increase non-specific immunity and may lead to reduction in mortality caused by opportunistic pathogens or stressors. Immunostimulants, used in vaccines to amplify the specific immune response or administered as feed additives to modulate non-specific immunity, have been demonstrated to play an important role in protection against diseases in fish and enhancing fish growth. Several immunostimulants facilitate the function of phagocytic cells, increase the cell's bactericidal activities and stimulate lysosomes and the antibody responses of fish (Sakai, 1999). The recent studies on this aspect include those of Biradar *et al.* (2007), El-Barbary and Mehrim (2009) and Soltani *et al.* (2010). They attach to specific receptors on the cell surface of the phagocytes and lymphocytes, activating these cells to produce enzymes capable of destroying pathogens (Raa, 1996). Different factors affect lipid content in Siluriforms as in other fish: body weight, season, feeding, dietary protein and dietary lipid. Large differences in lipid content between strains (Erickson, 1992) and hybrids (Smitherman *et al.,* 1983) are reported. Recently much concern has arisen over the quality of fish flesh especially in lipid content of the cultured fish and the control of lipid content in cultured fish could be an important aim for quality studies in future to ensure a minimum amount of lipid (development of aroma and pigmentation) and to avoid fatty fish. Any organic compound in fish feed which acts both as immunostimulant and obese inhibitor will be a novel idea in the fish culture since healthy low fat fishes will have a high consumer acceptance in any market.

India is a large repository of medicinal plants but studies on the effects of commonly available medicinal plant on the immunostimulation and growth promotion of cultured fish are fragmentary. *Garcinia gummi-gutta,* a common medicinal plant, has been used historically to treat respiratory infections such as sore throat and cough (Oluyemi *et al.,* 2007). The phytochemical constituents include biflavonoid, xanthone and benzophenones (Iwu, 1993) and the principle acid in the fruit and rind is hydroxy citric acid (-HCA 16-26%) (Antony *et al.,* 1998; Jayaprakasha and Sakariah, 1998). This acid has been found to suppress fatty acid synthesis, lipogenesis, food intake and to promote glycogenesis, while inducing weight loss (Jena *et al.,* 2002). The sun-dried rind of the fruit is astringent, antiseptic and purgative. The efficacy of *G. gummi-gutta* on the lowering of fatty acid composition in mammals has already been reported but its effects on immunostimulation and fatty acid modification in fish have not yet been reported and hence, the present experiment has been conducted to understand the efficiency of *G. gummi-gutta* fruit rind extract on the modification of different forms of lipids and eliciting the immunostimulatory effects in a pangasid catfish *Pangasianodon hypophthalmus*.

MATERIALS AND METHODS

Experimental design: The sun-dried rind of *G. gummi-gutta* was obtained from a local market in Kerala and authenticated by the Department of Botany, University of Kerala. The aqueous

Table 1: Ingredients and proximate compositions (g kg^{-1} dry matter) of the experimental diets

		Experiment distte				
Ingredients	% level	D1 (Control)	D2	D3	D4	D5
Fish meal	25	250	250	250	250	250
Ground nut oil cake	20	200	200	200	200	200
Soya bean flour	25	250	250	250	250	250
Tapioca flour	9	90	90	90	90	90
Wheat flour	15	150	150	150	150	150
Vitamin/Mineral mixture [1]	5	50	50	50	50	50
Fish oil	1	10	10	10	10	10
Aqueous extract of *G. gummigutta* (mg kg^{-1})		0	500	1000	2000	3000

[1]Vitamin mineral mixture: Vitamin A: 700000 IU, Vitamin D_3 : 70000 IU, Vitamin E: 250 mg, Nicotinamide: 1000 mg, Cobalt: 150 mg, copper: 1200 mg, Iodine: 325 mg, Iron: 1500 mg, Magnesium: 6000 mg, Manganese: 1500 mg, Pottasium: 100 mg, Selenium: 10 mg, sodium: 5.9 mg, Sulphur: 0.72%, Zinc: 9600 mg, Calsium: 25.5%, Phosphurus: 12.75%. The basal diets contains 33.62% crude protein, 8.94% crude lipid, 33.75% nitrogen free extract, 6.58% crude fiber and 9.06% ash

extraction of sun dried rind of the *G. gummi-gutta* was done in a soxhlet apparatus for 72 h. The extract was concentrated using a rotary vacuum evaporator and after complete evaporation of the solvent, residues of the extract were bottled and refrigerated. This extract was used in different concentrations for the preparation of experimental diets. A basal diet (D1) containing 30% crude protein which has been proved to be the optimum protein content for the fast growth of this cat fish (Jantrarotal *et al.,* 1992) has been prepared. Graded levels of aqueous extract of *G. gummi-gutta* were added to the basal diet at 500, 1000, 2000 and 3000 mg kg^{-1} and designated as treatments D2, D3, D4 and D5, respectively. The feed stuffs were thoroughly mixed and hot water was added at specified intervals to gelatinize starch, subsequently this was cooked in a pressure cooker. After cooking, vitamin mineral mixture, fish oil and plant extract were added. Then the diets were pelletized using a hand pelletiser and sun dried to a moisture level of below 10%. These pellets are broken into small pieces and stored in airtight containers and labeled. The proximate composition of the experimental diets is given in Table 1. The experiment has been conducted at the wet lab facility of the department of Zoology, University of Kerala, Kerala, India from July to September 2010.

For the present study, *P. hypophthalmus* was collected from a commercial aquarium in Thiruvananthapuram district, Kerala. The experiment was conducted in glass aquaria with water from an open well which was aerated and kept for three days for stabilization. After measuring the individual weights, fish with body weight ranging from 17.50 to 30.50 g were randomly distributed into glass aquaria, ten fish per tank in triplicate treatments. These fish were acclimatized for five days in the respective aquaria and fed with basal diets. All the tanks were aerated throughout the period of experiments. The water quality in glass aquaria was maintained by removal of faecal matter and the water was partially (a quarter) replaced every day with the same quantity of matured well water of the same source. The water quality parameters like temperature and pH were monitored throughout the study using mercury-in-glass thermometer and digital pH meter respectively. Animals were fed daily at a rate of 3% of the body weight and the daily ration is divided into two equal parts and given in morning and evening. The dead fish were removed whenever observed. On every occasion when it was found that the feed acceptance rate was higher, the quantity of the feed was increased accordingly. The final weights of the fish were taken after 45 days of rearing.

Blood sample collection: At the end of the experiment, feeding was suspended for 24 h before blood samples were collected. From randomly picked fish, after anaesthetizing with MS-222, blood was collected from the caudal vein with a 1 mL plastic syringe with heparin. Individual fish were sampled only once to avoid the influence on the assays due to multiple bleeding and handling stress on fish. Samples were divided into two parts, one part is used for the haematological parameters and the other part was centrifuged at 5000 rpm for 10 min and the plasma separated and kept at -20°C until biochemical analyses were carried out.

Determination of haematological parameters: Haematological analyses were carried out by standard methods suggested by Blaxhall and Daisley (1973). Haemoglobin estimation was done by acid-haematin method using Sahli's haemoglobinometer and the value is expressed in g%. The haematocrit was determined by microhaematocrit tube method (Bull *et al.*, 2000) and packed cell volume (pcv) has been calculated using the following formula:

$$PCV = \frac{\text{Height of RBC column after centrifugation}}{\text{Total height of the blood column}} \times 100$$

Total erythrocyte count (10^6 mm^{-3}), total leucocyte count (10^4 mm^{-3}) and total thrombocytes (10^4 mm^{-3}) were determined by using a Neubauers haemocytometer after examining at 40 and 10x magnification using a research microscope. Hendricks solution (Hendricks, 1952) is used as the diluting fluid for counting of RBC, WBC and thrombocytes. These data were used to calculate the Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Haemoglobin Concentration (MCHC) suggested by (Dacie and Lewis, 1984).

Determination of blood plasma biochemical parameters: Plasma separated by centrifugation at 5000 rpm for 10 min was used for estimation of total plasma protein, plasma glucose, triglycerides, total cholesterol, HDL and LDL. Total plasma protein was determined by the Lowry's method (Lowry *et al.*, 1951) using Bovine Serum Albumin (BSA) as standard in a double beam UV-VIS spectrophotometer (Labtronics LT-2900) at 660 nm. Plasma glucose concentration was estimated by glucose oxidase/peroxidase method (GOD/POD method) using commercially available glucose estimation kit (Span Diagnostics Ltd, India). The total cholesterol concentration (cholesterol oxidase/peroxidase method), HDL cholesterol concentration (precipitation method) and triglyceride concentration (GPO-PAP method) were estimated using commercially available kits (Agappe Diagnostics Ltd, India). All the parameters were estimated using a double beam UV-VIS spectrophotometer (Labtronics LT-2900) at 505 nm. From these values, the LDL cholesterol concentration was calculated using the formula:

$$\text{LDL-Cholesterol concentration in mg } dL^{-1} = \text{Total cholesterol concentration} - (\text{HDL cholesterol} + \text{Triglycerides}/5)$$

Statistical analysis: Experimental data are presented as Mean±SD and were analysed with one way Analysis of Variance (ANOVA) followed by Tukey's test using the SAS programme (SAS, 1989) to compare the means between individual treatments in SPSS at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

The water temperature during the period of experimental study ranged from 28 to 31°C and the pH ranged from 6.9 to 7.1. The values are found to be in the optimum range for the rearing and growth of *P. hypophthalmus*. The growth performance of catfish *P. hypophthalmus* fed with diets containing different concentrations of *G. gummi-gutta* extracts are summarized in Table 2. The weight gain decreased in all experimental groups compared with the control in a statistically significant manner but this trend is not dose-dependent. The specific growth rate of fish fed with various concentrations of plant extract shows a decrease in all experimental groups compared with the control and this decrease is statistically significant but not dose-dependent. The D3 shows significant decrease at 5% level ($p<0.05$) compared with control and 1% level ($p<0.01$) with D2. In D4 it is significantly ($p<0.01$) decreased when compared with control but in D5 significant decrease ($p<0.01$) occurs compared with both control and D2. The survival rate has no significant difference between the experimental groups and control except in D4 which shows significant increase at 5% level ($p<0.05$) compared with D3.

The results of haematological parameters and indices of *P. hypophthalmus* are given in Table 3. The RBC count was increased in group D2, D4 and D5 and decreased in D3 compared with the control (D1) but the significant increase at 1% level ($p<0.01$) occurs only in group D5 compared with control and all other experimental groups. The WBC count is dose-dependently increased in all the experimental groups compared with the control. Statistically significant increase occurs in D3, D4 and D5 compared with control and other experimental groups. D3 shows 1% level ($p<0.01$) significance with control and 5% level ($p<0.05$) with D2 and all other significant increase is at 1% level ($p<0.01$). The highest mean value for WBC is 6.10±0.12 and is reported in D5. The thrombocyte count is increased in all the experimental groups compared with the control and the significant increase at 5% level ($p<0.05$) are shown by both D3 and D4 and the highly significant increase at 1% level ($p<0.01$) occurs in D5 compared with the control. The values of haemoglobin (Hb) showed an increasing trend in D3, D4 and D5 when compared to control fish and the significant increase ($p<0.01$) is shown only by D5 with reference to control and other experimental groups. The Packed Cell Volume (PCV) increases in all the experimental groups compared with the control and significant increase ($p<0.01$) is shown only by D5 compared with the control and other experimental groups. The MCV and MCH values are more or less similar in the experimental groups except in D5 in which significant decrease has been observed. The MCHC has been maintained at a steady state in the experimental groups when compared to the control except in D5 where the value showed an increase.

The plasma biochemical parameters of *P. hypophthalmus* are shown in Table 4. The plasma glucose level shows a dose-dependent decrease in all experimental groups compared with the control and this decrease is also statistically significant. The level of glucose in D2 is significantly decreased at 5% level ($p<0.05$) when compared to control. In D3, D4 and D5, the level of glucose is significantly decreased at 1% level when compared to control and other experimental groups. Protein level is significantly increased in group D2 and D4 and almost similar values are reported in group D3 and D5 when compared to control. The concentration of total cholesterol level in plasma is decreased in all experimental groups in a dose-dependent manner compared with the control and the decrease is highly significant ($p<0.01$) in all cases. The values for this parameter showed significant decrease between the treatments also. The value for this parameter ranged from 122.68 to 191.42 with the maximum (186.54±2.01) in the control and the minimum in D5 (28.78±1.29). Similar to the trend shown by total cholesterol, the plasma triglyceride level also

Table 2: The growth performance of cat fish, *P. hypophthalmus* fed with different diets

	Experiment disets									
	D1 (Control)		D2		D3		D4		D5	
Parameters	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Initial weight (g)	17.50-30.50	22.15±0.85	20.00-30.00	23.97±0.93	20.00-28.50	23.90±0.32	20.00-28.50	24.30±0.94	20.00-27.00	22.02±0.09
Final weight (g)	22.00-40.00	29.70±0.54	24.00-33.00	28.04±0.63	23.00- 31.00	26.71±0.46	23.00-32.00	27.83±0.53	23.00-27.00	25.04±0.24
Weight gain (g)	4.00-13.00	7.55±0.32	1.50-8.50	4.06±0.46^{a**}	1.50-6.00	2.80±0.29^{a**b**}	2.00-5.00	3.53±0.17^{a**}	1.50-5.50	3.02±0.28^{a**b**}
Specific growth rate (%)	8.89-28.89	16.78±0.12	4.44-18.89	9.03±1.05^{a**}	2.22-13.33	6.23±0.64^{a**b**}	4.44- 11.11	7.85±0.39^{a**}	3.30- 12.22	6.71±0.63^{a**b**}
Survival rate (%)	80.00- 82.00	81.00±6.84	70.00-80.00	76.66±5.77	70.00- 78.00	74.00±5.44	80.00-90.00	83.33±5.77^{c*}	70.00-80.00	76.66±5.77

[a]Indicates significant difference with control. [b,cd]Indicates the significant difference with the treatments D2 to D4. ** and * indicates significance at 1 and 5% level, respectively.

Table 3: Haematological characteristics of *P. hypophthalmus* fed with different diets

	Experiment disets									
	D1 (Control)		D2		D3		D4		D5	
Parameters	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
RBC (x10^6 mm^{-3})	2.86-3.14	3.04±0.04	2.63-3.46	3.11±0.05	2.60-3.26	2.95±0.04	2.78-3.42	3.06±0.05	4.38-6.20	5.13±0.26^{a**b**c**d**}
WBC (x10^4 mm^{-3})	2.88-3.83	3.45±0.02	3.58-3.95	3.76±0.03	3.64-4.91	4.12 ±0.18^{a**b*}	3.04-5.15	4.62±0.18^{a**b**c**}	4.82-6.84	6.13±0.12^{a**b**c**d**}
Thrombocyte (x10^4 mm^{-3})	10.65-14.90	13.57±0.21	12.70-16.40	14.7±0.24	13.88-15.70	14.77±0.165^{a*}	13.10-19.04	14.86±0.55^{a*}	14.10-19.24	15.86±0.74^{a**}
Hb (g %)	8.00-9.20	8.46±0.08	8.00-9.00	8.42±0.09	8.20-9.20	8.64±0.07	8.00-10.00	8.80±0.09	9.00-14.80	12.94±0.58^{a**b**c**d**}
PCV (%)	30.77-42.65	37.00±0.30	34.64-40.91	37.10±0.33	34.04-40.81	37.01±0.17	30.36-45.09	37.42±0.84	38.80-50.94	42.96±1.35^{a**b**c**d**}
MCV (m^3)	99.23-142.97	121.89±1.14	106.15-134.68	118.34±1.58	76.03-134.13	120.72±4.55	105.78-138.87	122.46±1.97	64.56-102.45	84.57±5.98^{a**b**c**d**}
MCH (pg)	25.50-30.45	27.40±0.06	24.12-30.42	26.84±0.05	25.15-31.72	29.27±0.53	26.32-30.77	28.83±0.21	17.24-32.42	25.41±0.49^{a**b**c**d**}
MCHC (%)	1.92-2.97	2.33±0.02	2.05-2.54	2.28±0.03	2.25-2.47	2.34±0.01	2.15-2.70	2.36±0.04	1.88-3.71	3.03±0.21^{a**b**c**d**}

[a]Indicates significant difference with control. [b,cd]Indicates the significant difference within the treatments D2 to D4. ** and *Indicates significance at 1 and 5% level, respectively

Table 4: Biochemical parameters of *P. hypophthalmus* fed with different diets

	Experiment disets									
	D1 (Control)		D2		D3		D4		D5	
Parameters	Range	Mean± SD	Range	Mean± SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Glucose (mg 100 mL)	90.00-134.29	114.00 3.21	90.32-120.00	104.89±2.19^{a*}	62.69-102.11	87.36±6.38^{a**b**}	68.75- 90.04	80.22±3.23$^{a** b**}$	41.19-53.74	46.59 ±1.23$^{a** b**c**d**}$
Protein(g %)	4.07-5.28	4.60±0.04	4.80-6.07	5.19±0.03^{a**}	4.02-4.87	4.39±0.16^{b**}	4.68-6.81	5.22±0.26$^{a** c**}$	4.04-5.73	4.49±0.10$^{b** d**}$
Total cholesterol (mg dL^{-1})	176.98-191.42	186.45±2.01	165.48-188.09	179.21±1.43^{a**}	136.62-190.20	158.21±2.13$^{a** b**}$	127.84-148.00	137.71± 2.42$^{a** b** c**}$	122.68-136.08	128.78 ±1.29$^{a** b** c**d**}$
Triglycerides (mg dL^{-1})	214.78-248.10	237.62±2.34	178.26-274.78	242.38±2.83	181.20-240.00	203.39±7.10^{a**b**}	158.52-176.00	165.88±1.88$^{a** b** c**}$	128.40-153.92	134.31 ± 2.18$^{a** b** c**d**}$
HDL (mg dL^{-1})	51.54-54.60	52.97±0.74	52.46-58.02	54.98±0.62	52.64-65.56	57.74±0.70	65.09-81.00	70.75±3.72$^{a** b* *c**}$	71.00-84.95	75.91± 1.78$^{a** b**c**d*}$
LDL (mg dL^{-1})	80.94-90.83	86.24±0.41	67.64-84.96	75.67±0.39^{a**}	39.71-82.52	59.79±0.34^{a**b**}	24.66-43.04	33.77±3.10$^{a** b** c**}$	20.00-33.69	26.71±1.91$^{a** b** c**d**}$

[a] Indicates significant difference with control and [b,c,d] Indicates the significant difference with the treatments D2 to D4. **and* Indicates significance at 1 and 5% level, respectively.

showed a decreasing trend in all experimental groups in a dose-dependent manner except in D2 when compared to control and this decrease is statistically significant too. The plasma HDL cholesterol level shows a dose-dependent increase in all experimental groups compared with the control. Statistically significant increase is reported in the case of D4 and D5 when compared with the control and all experimental groups. The plasma LDL cholesterol level is decreased in a dose-dependent manner in all the experimental groups compared with the control and this downward trend is highly significant ($p<0.01$).

In aquaculture, there are many reports that herbal extracts can be used as immunostimulants to boost the non-specific immune system of cultured fish species (Sakai, 1999; Shao *et al.,* 2004; Tan and Vanitha, 2004; Rao *et al.,* 2006; Sahu *et al.,* 2007; Ardo *et al.,* 2008). Furthermore, the use of such plant products as immunostimulants in aquaculture systems may also have environmental value because of their biodegradability.

In present study, the increment in the weight of the experimental fish over the control was less than that in the latter. This slow growth in terms of weight gain and Specific Growth Rate (SGR) of fish in all groups fed on *G. gummi-gutta* extract could be due to the effect of HCA present in the extract. It may be noted from the results that in D2 group, where minimum quantity of plant extract has been incorporated, the growth is higher when compared with experimental groups. This proportionate decrease in weight gain with increased concentration of plant extract in the diets clearly shows that higher concentration of HCA will induce weight loss at a faster rate. Similar results are reported by others in mammals (Jena *et al.,* 2002; Oluyemi *et al.,* 2007). The survival rate in the experimental and control groups has not varied significantly which is clear evidence for the non-toxic effects of the plant extract in lower vertebrates, as in the case of higher vertebrates (Bray and Greenway, 1976).

The extract-supplemented diets induced significant increase in all blood parameters like RBC, WBC, thrombocytes, haemoglobin content and the packed cell volume (PCV). The aqueous extract of *G. gummi-gutta* displayed the ability to trigger erythropoesis and these results are in agreement with those obtained by in rats and this mechanism seems to preserve the average life span of individual RBCs (Oluyemi *et al.,* 2007). The increase in RBC in the present study may be partly attributable to the iron composition of *G. gummi-gutta*, since iron is a known erytropoietic agent (Okwu, 2005). Another possible mechanism for erythropoiesis is the decrease in the rate of oxidant-induced haemolysis due to the presence of the antioxidants (Maduinyi, 1983). The proliferation of WBC in the treated groups could be due to leucopoiesis, particularly lymphopoieis as a response to enhanced immunity and this is in agreement with that of the observations made by Dada and Ikuerowo (2009) in *Clarias gariepinus* brood which was stock fed with a diet containing the same compound. Piscine thrombocytes represent a link between innate and adaptive immunity (Passantino *et al.,* 2005) and it is already agreed that fish thromboytes are blood phagocytes that form protective barriers (Kolman *et al.,* 2003; Tavarese-Dias and Moraes 2004; Prasad and Charles, 2010). In the present study, the thrombocyte count is increased in all experiments and the same is significant in D3, D4 and D5. This enhanced concentration of thrombocytes may be due to their participatory role in immune functions as observed by Kollner *et al.* (2004) and the antibiotic properties of the *G. gummi-gutta* have already been reported by Iwu (1993) and Iwu *et al.* (1999). The blood indices are particularly important for the diagnosis of anemia in most animals (Coles, 1986). The MCV and MCH values are more or less similar in the experimental groups except for D5 in which significant decrease has been observed. MCHC has been maintained at a steady state in the experimental groups when compared to the control except in D5 where the value showed an increase.

Piscine plasma proteins have a variety of functions and are especially important for the regulation of water balance in fish (Wedemeyer and Yasutake, 1977). Protein level is significantly increased in group D2 and D4 and almost similar values are reported in group D3 and D5 when compared to the control. These results are in agreement with those reported by Khattab *et al.* (2004) for *O. mossmabicus* treated with plant-derived growth promoter. It has been shown that glucose level increases in infected or stressed animals to ward off the infection or stress (Citarasu *et al.,* 2006). With the increase of plant extract in the diet, a reduction in the quantity of plasma glucose occurs, which could be due to the capability of the selected plant extract to reduce the effect of stressors. Similar observations are made by Sahu *et al.* (2007) who found that glucose levels were reduced in the aquatic animals fed on herbal immunostimulant diets and that this condition could be attributed to improvement of the metabolic and antioxidant systems, especially in pancreatic cells which results in increased production of insulin.

The lipids are the usual economic form of biomolecules used by fish to stock energy and can be stored in many different organs (Guijarro *et al.,* 2003). In the present study, significant decrease in the level of total cholesterol was observed in all the experimental groups fed with diets containing *G. gummi-gutta* extract in a dose-dependent manner. LDL cholesterol decreased significantly ($p<0.01$) in the extract-treated groups in a dose-dependent manner and this could be attributed to increase of HDL cholesterol concentration, since the major function of HDL is the transport of cholesterol from blood to the liver (Brown and Goldstein, 1984). This decrease in LDL values could be due to the action of HCA content of the *G. gummi-gutta*, which is known to be hypolipidaemic. It has been reported that HCA is a competitive inhibitor of ATP-citrate lyase which catalyses the extra-mitochondrial cleavage of citrate to oxaloacetate and acetyl-COA (McCarty, 1995). ATP-citrate lyase inhibition limits the availability of acetyl-COA units required for cholesterol synthesis and triglyceride. The studies made in mammals indicated that HCA suppresses fatty acid synthesis, lipogenesis and induces weight loss (Jena *et al.,* 2002). *G. gummi-gutta* may also reduce cholesterol in fish by interfering with intestinal cholesterol uptake, increasing the conversion of cholesterol into bile acids and increasing the excretion of bile acids. The triglyceride concentration is also decreased significantly ($p<0.01$) in the experimental groups and similar results are reported by Oluyemi *et al.* (2007) in rats.

CONCLUSIONS

The results of the present study indicate the benifical role of *G. gummi-gutta* in augmenting the immunity mediated through cellular and to a certain extent possibly through non-cellular mechanisms, as evident from the enhanced haematological and modified biochemical parameters. Even though the exact modulation of immune response elicited by the selected natural compound in experimental fish is not fully understood the immunostimulant might act directly on the immunopoietic cells. The overall results of the present study proved that the fruit rind extract of *G. gummi-gutta* induced the innate immunity and modified the lipid profile of the fish in all treated groups. The critical examination of the parameters of the various treatment groups shows that fish treated with plant extract of 2000 mg kg^{-1} feed exhibited the more optimum features in most of the biochemical, haematological and growth factors. The results proved that the inclusion of *G. gummi-gutta* extract at a rate of 2000 mg kg^{-1} feed would enhance the growth and immunity, while modifying the lipid profile to a significant level. Hence, it can be stated that the inclusion of *G. gummi-gutta* extract at a rate of 2000 mg kg^{-1} feed in the fish feed would be ideal to elicit immunity and for modifying the lipid profile to desirable levels without compromising growth. It

is postulated that fish such as Sutchi catfish, if given a finisher diet enriched with *G. gummi-gutta* extract towards the end of the culture period which will reduce the incidence of diseases and modify the lipid profile to a desirable level by reducing the concentration of LDL cholesterol.

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