Mapana – Journal of Sciences
2024, Vol. 23, No. 1, 121-134
ISSN 0975-3303 | https://doi.org/10.12723/mjs.68.6

Effect of *Simarouba glauca L*eaf Aqueous Extract on the Growth, Immunological, Haematological and Biochemical Parameters of *Oreochromis niloticus*

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Abstract

The present study was conducted to evaluate the effect of aqueous leaf extract of *Simarouba glauca* on the growth, haematology, immunological and biochemical parameters of *Oreochromis niloticus*. Concentration ranging from 2500mg /kg to 7500mg/kg were supplemented through diet. Fishes were fed with C1(2500 mg of plant extract/ kg of basal diet),C2 (5000 mg/kg of basal diet) and C3 (2500 mg/kg of basal diet), each with two replicates for 60 days. The results showed that fish fed dietary *Simarouba glauca* gained more weight in C1 (83.14 ±1.11 g) than control ($P< 0.01$). Fish fed with C1 had a higher level of hematological content while C3 shows highest value of WBC (33.79±3.11). A significant increase in glucose level and decrease in protein level is reported at C1. Better results were reported in fish fed with diet 2500mg/kg and 5000mg/kg reveals that at lower specific concentration these can be used as a feed supplement.

Keywords: *Simarouba glauca*, *Oreochromis niloticus*, growth, haematology, biochemistry, immunoglobulin

1. Introduction

The aquaculture industry has expanded quickly over the past 30 years in order to keep- up with the demands of humans and the growing

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world population. Fish and other aquatic products make up more than half of the protein and minerals used in the poorest nations of Africa and South Asia[1]. Tilapia refers to a group of cichlid fish found in brackish and fresh water systems. It is a significant source of both animal protein and income. Currently, much of the world's tilapia is produced outside of the fish natural environment because tilapia is resilient to environmental stress and may live and breed in a broad range of environments [2]. Intense aquacultural practices resulted in an increase in disease outbreaks, leading to partial or entire loss of fish [3].

In the aquaculture industry, infectious diseases are major hindrance. Infections in fishes are commonly treated using antibiotics used in veterinary medicine[4]. Although antibiotics are frequently successful in treating illness, their use is discouraged since it leads to the deposition of drug residues in the environment or in piscine tissue and the rise of bacteria that are resistant to antibiotics[5]. Medicinal plants have emerged as a viable and useful disease control alternative in light of the adverse effects of veterinary pharmaceuticals used in aquaculture on the environment, human health, and animal welfare. Aquaculture uses medicinal plants as feed additives and chemotherapeutics because they contain a variety of minerals and chemicals [6]. Because of the higher content of phytochemical compounds and nutrients [7] medicinal herbs have been shown to promote growth, hunger stimulation, immunological stimulation, antibacterial, and anti-stress effects in fish [8]. The existence of various active components, including alkaloids, steroids, phenolics, tannins, terpenoids, saponins, glycosides, and flavonoids is associated to the action mechanism of such herbs and their metabolites [9]. Accessibility and low cost are also driving elements for their widespread usage in aquaculture to improve growth and protection. Herbal extracts can typically be supplied to fish in aquaculture in three different ways: orally, intravenously, or submerged. The objectives of administration, the fish size and breed, the sort of extracts being utilized, and the farming system all play a significant role in the choice of administration method [10]. Injection and immersion methods might also be successful [11].

Simarouba glauca has a great history of usage in herbal medicine across many nations. Even though many studies reveals that the plant

Simarouba glauca have anti cancerous, antimicrobial effect, the effect of the same in aquaculture sector is not yet studied. So, aim of the current study is to evaluate the effects of dietary supplementations of aqueous extract of *Simarouba glauca* on the growth performance, immunological, haematological and biochemical indices and of Nile tilapia (*Oreochromis niloticus*) after 60 days.

2. Materials and Method:

2.1 Collection of Plant Material:

Simarouba glauca fresh, disease-free leaves were collected on and around the Kerala University campus. Healthy leaves were powdered and plant extract were prepared by serial extraction method.

2.2 Fish and Experimental Design:

The experimental fish *Oreochromis niloticus* were collected from local fish farm (Alans's aquarium, Balaramapuram, Thiruvananthapura m, Kerala, India). All the fishes were acclimatized in water tank for 15 days. They were evenly and randomly distributed to seven glass aquariums (total of 6 fishes per replicate). There were four experimental groups- control, C1, C2, and C3, each with two replicates were used in a randomized manner in the study. The *Simarouba glauca* aqueous extract was added to the feed at 2000 mg kg^{-1} (C1) 5000mg kg^{-1} (C2), 7500 mgkg^{-1} (C3) and control diet contained no supplementation of plant extract. Fish were given daily diets containing three doses of *Simarouba glauca* aqueous leaf extract at 9.30 am and 4 pm at a rate of 3% of their body mass per day for 60 days.

Fig 1.1 *S. glauca* leaf extracts

Figure 1.2 Experimental diet

2.3 Blood Sampling:

After 60 days of experimental study, fishes were randomly selected from each tank. They were starved for a period of 24 hours and the fishes were anesthetized with clove oil and blood samples were collected from caudal vein using syringe rinsed with EDTA (10%) for haematological and total immunoglobulin tests. For collecting serum, blood samples were collected without EDTA coated syringe and allowed to clot at room temperature.

2.4 Growth Parameters:

Six fish were randomly chosen from each tank at the completion of the feeding trial and their weights were recorded. Following formulas were used to determine WG, SGR, PER and FCR.

Weight Gain (WG)(g) = final body weight– initial body weight

Feed Conversion Ratio (FCR) = feed consumed (g)/ weight gain(g)

Protein Efficiency Ratio PER = Weight gain(g)/ Protein ingested(g)

Specific Growth Ratio SGR = 100 ×ln (final weight-initial weight)/ days of experiment.

2.5 Haematological Parameters:

Sahil's haemoglobinometer was used to estimate hemoglobin using the acid-haematin method. Packed cell volume (haematocrit) was measured as per the Micro haematocrit technique [12].

Total erythrocyte count ($\times 10^6/\mu l$), total WBC ($\times 10^3/\mu l$) count and total thrombocyte count were evaluated using Neubauer's haemocytometer. MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) with the following formulae [13].

MCV (nm^3) = Hct (%) ×10/ RBC ($\times 10^{6}/\mu l$)

MCH (pg/cell) = Hb (g/dl) × 10/ RBC ($\times 10^6/\mu l$)

MCHC (g/dl) = Hb (g/dl) ×100/Hct (%)

2.6 Biochemical Parameters:

Biochemical tests were carried out in blood serum. All parameters were determined using analytic test kits (Agappe diagnostics Ltd, Kerala) and measured with a UV-Visible double beam spectrophotometer.

2.7 Total Plasma Immunoglobulin Test:

Plasma is separated from the blood and total protein of blood plasma were estimated by Biuret method using total protein kit. 50 µl of polyethylene glycol were added to 50 µl of plasma. The mixture was centrifuged at 7500 rpm for 15 minutes after being incubated for two hours at room temperature with continuous shaking. The protein content of the supernatant was evaluated using a UV-Visible double beam spectrophotometer and this value is deducted from the total protein level, and the result was equivalent to total immunoglobulin in the blood plasma [14].

2.8 Statistical Analysis

The statistical significance difference in the mean and standard deviation was analysed by One Way Analysis of Variance (ANOVA) followed by Tukey's test to compare the differences among individual means using R software (Version 4.2.1). Differences were considered significant at levels < 0.05.

3. Results and Discussion

3.1 Biochemical Parameters:

Fish blood serum biochemical changes are commonly used as reference values to understand the effects of feed ingredients, fish health and fish metabolism [15]. Table 1.1 shows the results of biochemical parameters after feeding trials**.** There are significant variations in the biochemical parameters of all treatment groups under different dietary inclusion levels of *Simarouba glauca*. Serum glucose level showed the maximum value in C3 (107.81±8.64 mg/dl) and least value was observed in control (74.05±4.86mg/dl). Total protein in serum increased in all the three treatment groups compared with control. Maximum value was reported in C1 (5.90±0.60 mg/dl). The protein level is increased significantly ($p< 0.01$) in C1 and C2. The value of serum albumin ranged between 1.02 to 3.98 mg/dl. According to other studies, blood glucose levels rise together with stress levels in

aquaculture applications [16]. The current study shows significantly different serum glucose levels ($p < 0.01$). That is the glucose values of C3 significantly increased when compared with the control group and other treatment groups (C2 and C3) ($P< 0.01$) which can be considered as an indicator of stress. Serum protein tests helps in the diagnosis of disorders including kidney and liver ailments. While an increase in total serum protein of an organism may indicate increased feed utilization and vice versa [17]. According to the current investigation, fish supplemented with *S. glauca* showed significantly higher blood total protein levels when the inclusion levels were increased from 2500 mg/kg to 5000 mg/kg ($P<0.05$). This suggests that *S. glauca* extracts can increase the efficiency of protein utilization in diet. According to reports, some herbal extracts can raise fish serum total protein level, which is a sign of the activated immune system [14]. WBC, which is the primary source of protein production, may have increased, contributing to this rise [18]. However, at a dosage of 7500 mg/kg, both the glucose levels significantly increase and this concentration reported a significant decrease in serum protein level. This might be due to increased protein catabolism, a process in which both blood and structural protein are transformed into energy, may be caused by the body's increased energy requirement to combat stress. This lowers serum protein levels [19]. Nutrition, enzyme activity, and hepatic function can all have an impact on blood cholesterol levels. In the current study, cholesterol was found to be significantly lower in higher concentrations(7500 mg/kg). fish fed diets with *S. glauca* aqueous leaf extracts. Similar results occur in rodents after dietary supplementation of plant leaf extracts [20].

Table 1.1 Biochemical parameters of *O. niloticus* fed with *S. glauca*

Parameters	Control (Mean ± SD)	Treatment group		
		C1(Mean ± SD)	C2(Mean ± SD)	C3(Mean ± SD)
Protein (g/dl)	4.03±0.31	$5.90±0.60^{a**}$	$5.71±0.39^{a**}$	$4..09±0.50^{b**c**}$
Albumin (g/dl)	1.41±0.50	$3.73±0.40^{a**}$	$3.17±0.73^{a*}$	$2.05±0.13^{b*}$
Glucose (mg/dl)	74.05±4.86	62.00±3.07	$81.92±6.43^{c*}$	$107.81±8.64^{a**b**c**}$

Cholesterol (mg/dl)	193.66±7.09	180.66±15.82	157±5.56[a]*	129±8.71[a]**[b]**

3.2 Growth Parameters:

The growth parameters of *Oreochromis niloticus* fed with diets containing three various concentrations of *S. glauca* aqueous extract are shown in table 1.2. Highest weight-gain has been observed in C1 (83.14 ±1.11 g). A significant decrease in the weight gain is reported in C1, C2 and C3 when compared to control ($P< 0.01$). FCR levels in all treatment groups demonstrate a significant decrease ($P<0.01$. SGR of fish supplemented with *S. glauca* leaf extract also shows an increase in all treatment groups compared with fish fed with control diet and the value ranged between 2.27 to 2.64 %. The PER increases dosage dependently in all treatment groups. C1 and C2 increases significantly at the level of 1% compared to control. And C3 value of PER shows a significant decrease at the level of 5% when compared with control and C1. Similarly, no significant difference ($P>0.05$) was reported in the SR (Survival Rate). The findings imply that adding *S. glauca* leaf extract to the diets of Nile tilapia, *Oreochromis niloticus,* at doses of 2500 mg/kg and 7500 mg/kg may enhance weight increase and feed utilisation more effectively than control. However, a lower concentration of leaf extracts as feed supplement at 2500mg/kg and 5000mg/kg is preferable to any amount above this. The causes for the declining performance with increasing the concentration of leaf extract concentration could be attributed to metabolic, and digestive issues, rising feed bitterness with a subsequent low palatability, as had been explained by prior researchers [21]. According to reports, most medicinal herbs include bioactive chemicals including saponins and tannins that may impart a bitter flavour that may serve as a feed deterrent [22]. Using lower doses of medicinal herbs, similar improvements in growth and feed utilisation have been noted in other experiments [23]. The use of medicinal plants in fish has numerous benefits, but there are also drawbacks due to the active ingredients and overdose. And at the right dosage, they do not cause any physiological issues [24].

Table 1.2 Growth parameters of *O. niloticus* fed with *S. glauca*

Parameters	Control (Mean ± SD)	Treatment group		
		C1 (Mean ± SD)	C2 (Mean ± SD)	C3 (Mean ± SD)
Initial weight (g)	23.94±0.72	24.02±0.45	22.14±2.11	24.42±0.72
Final weight (g)	95.55±1.43	107.16±0.77	103.37±4.68	101.60±2.48
Weight gain (WG)(g)	71.86±9.90	83.14±1.11^{a**}	81.23±2.83^{a**}	77.18±1.93^{a*b*}
Feed Conversion Ratio (FCR)	1.15±0.01	1.00±0.01^{a**}	1.02±0.03^{a**}	1.07±0.02^{a*b*}
Specific Growth Rate (%)	2.30±0.04	2.49±0.04^{a*}	2.57±0.09^{a**}	2.37±0.02^{c*}
Protein Efficiency Ratio (%)	2.19±0.02	2.53±0.03^{a**}	2.47±0.08^{a**}	2.35±0.05^{a*b*}
Survival Rate (%)	91.66±11.78	74.99±11.78	91.83±11.55	74.99±11.78

^{a} indicates significant difference with control and ^{b}and ^{c} indicates the significant difference with the treatments c1 to c2. ** and * indicates significance at 1% and 5% respectively.

3.3 Haematological Parameters:

Table 1.3 shows the haematological parameters of fish fed with *S. glauca* aqueousleaf extract. In thisstudy, fish fed with *S. glauca* extract diets had significantly higher levels of WBCs, RBCs, PCV, Hb, and Hct than fish fed the control diets. But the values were decreased in higher concentration (7500mg/kg). This indicates that *S. glauca* aqueous leaf extract incorporated in Nile tilapia diets has the ability to improve its health at lower concentrations. C1 shows a significant increase in the RBC count than the control ($P<0.05$) and other higher concentrations. The current increase in haematological parameters could be related to the active elements in *Simarouba glauca* playing stimulatory effects on the level of haematopoiesis in the body. Even though the overall haematology counts of experimental groups are higher than that of control a constant decrease in the counts can be seen within the

concentrations especially in C3. It might be due to certain stress caused by the increasing level of phytochemicals in the plant extract. After identifying and estimating harmful ingredients, *S. glauca* had to be detoxified from alkaloids (1.01g/100g), phenolics (0.95g/100g), phytic acid (0.73g/100g) and saponin (0.95g/100g) [25] before feed formulations. In the present study, we used the leaf extract without any detoxification from higher concentrations of phytochemicals. It might be the reason for the lowering of haematological parameters in C3 than the other experimental diet and drastic increase in the WBC content. The results of the current investigation showed that administration of plant extract significantly increased red cell swelling (decreased MCHC) but did not significantly alter MCH. According this study, plant extracts at levels of 2500 mg/kg and 7500 mg/kg significantly increased MCV compared to the fish fed with control diet. Even though the number of erythrocytes has decreased in higher concentrations, an increase in MCV may indicate that the erythrocytes size has increased. The MCV represents a normal or aberrant cell division during erythropoiesis and provides an indication as to the condition or size of the RBCs. In this study the number of RBCs and value of MCV is changing in a proportional manner which indicates that the supplemented feed has no adverse effect on this haematological index.

Table 1.3 Haematological parameters of *O. niloticus* fed with *S. glauca*

Parameters	Control (Mean ± SD)	Treatment group		
		C1 (Mean ± SD)	C2 (Mean ± SD)	C3 (Mean ± SD)
RBC (×10 ⁶/µl)	1.08±0.05	1.48±0.05^{a*}	1.32±0.21	1.10±0.01^{b*}
WBC (×10³/µl)	21.13±1.16	23.66±1.36	28.44±1.03^{a**}	33.79±3.11^{a**b**c*}
Thrmb (10³/µl)	11.02±1.50	19.81±2.42^{a**}	17.22±0.92^{a**}	13.93±1.45^{b*}
Hb (g/dl)	4.1±0.2	6.56±0.32^{a**}	6.46±0.58^{a**}	5.56±0.20^{a**b*}
Hct (%)	18.73±1.10	36±3.5^{a**}	25.33±2.51^{b**}	25.42±3.00^{b**}
MCV (nm³)	172.44±7.62	242.88±14.69^{a*}	193.62±20.70	230.55±29.54^{a*}
MCH (pg/cell)	37.77±2.18	18.72±0.30^{a*}	49.78±9.01	50.53±4.22
MCHC (g/dl)	22.23±0.23	18.72±0.30^{a*}	25.80±1.58^{a*b**}	22.02±1.68^{b*c*}

[a] indicates significant difference with control and [b]and [c] indicates the significant difference with the treatments c1 to c2. ** and * indicates significance at 1% and 5% respectively

3.4 Total Immunoglobulin*:*

Table 1.4 shows the data of fish total immunoglobulin after the feed supplementation. The best overall immunoglobulin was obtained in fish fed with diet C1. And the immunoglobulin value was marked significantly increased ($P< 0.01$) compared to control in all experimental groups. The value of this parameter ranged between 19.23 to 37.25 mg/ml. The maximum value of immunoglobulin was reported in C1 (34.58±2.51 mg/ml) and the minimum value was reported in fish fed with control diet (21.23±1.73 mg/ml). Effector B cells are the primary generator of immunoglobulins in the blood and serve as pathogen or toxin neutralizing agents in the fishimmune system. In the present study, *S. glauca* leaf extract incorporation in the diet resulted in the elevation of plasma immunoglobulin contents in groups receiving 2500mg/kg to 7500mg/kg of diet compared to fish fed with control diet. The most effective herbal bioactive components, including saponins, may be poisonous to fish and other cold-blooded animals at specific quantities[26]. Therefore, giving fish higher amounts of herbal extracts may cause their immune systems to be suppressed. Regular ingestion may result in immune system overstimulation, which interferes with the fish natural metabolic processes, and long- term overdosing may limit their efficiency [9]. The current investigation suggests that dietary inclusion level of 7500 mg/kg of *S. glauca* may be related to the appearance of immune system suppression or overstimulation.

Table 1.4 Total plasma immunoglobulin of *O. niloticus* fed with *S. glauca*

Parameters	**Immunoglobulin (mg/ml)**
Control (Mean ± SD)	21.23±1.73
C1(Mean ± SD)	34.58±2.51[a]**
C2(Mean ± SD)	33.73±2.79[a]**
C3(Mean ± SD)	29.58±0.58[a]**

Conclusion

The present study was conducted to evaluate the effect of aqueous leaf extract of *Simarouba glauca* on the growth, haematology,

immunological and biochemical parameters of *Oreochromis niloticus.* Different concentration ranging from 2500 mg /kg to 7500 mg/kg were supplemented through diet. And better results were reported in fish fed with diet 2500mg/kg and 5000mg/kg. Even while the bioactive substances are found in most therapeutic plants, when present in high concentrations, this could have an adverse impact on growth. But at lower specific concentration these herbal extracts can be used as a good feed supplement to teleost. It's necessary to quantify the bioactive compounds in the plant extract for a real estimation of their effectiveness. And further detoxification and purification of leaf extract from higher concentrations of phytochemicals should be practiced in order to understand the proper effect. This information is essential for expanding the utilisation of *Simarouba glauca* as a feed and its applicability to other pharmaceutical fields as well. Further research of this kind is therefore deemed necessary.

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