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## **ORIGINAL ARTICLE**

# The effect of aqueous leaf extract of *Phyllanthus emblica* on Biochemical parameters of catfish, *Clarias batrachus*

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## ABSTRACT

The objective of the current study was to estimate the effect of leaf extract (aqueous) of Phyllanthus emblica on biochemical parameters, i.e., total serum protein, albumin, and globulin, of Clarias batrachus. Fish of average weight (80–120 g) were randomly divided into three groups (n = 7) control and treated with 2% and 5% of leaf extract (aqueous) for up to 30 days. After treatment, serum was collected 7, 15, and 30 days later. A two-way ANOVA was used for statistical analysis, which revealed that significant differences in the values of total serum protein and albumin were obtained due to differences in dose concentration (p<0.05). The differences in globulin values obtained due to both dose concentration and duration were non-significant. The results suggested that the aqueous leaf extract of Phyllanthus emblica may help stimulate the immune potential of Clarias batrachus fish and may be useful in aquaculture when used as a natural immunostimulant.

KEY WORDS: Clarias batrachus, Phyllanthus emblica, aqueous leaf extract, biochemical parameters.

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## INTRODUCTION

Fish is an important source of animal protein in our diet. It contains all important vitamins, minerals and trace elements in appropriate amounts. Enhancing fish yield was essential to supplement the protein food needs of this country. India produced 108 million tons of fish in 2016, the third highest in the world after China and Indonesia. According to FAO, India's share in global production of fish is 7%, second to China (2018). Fishes are not only an important delicacy for human being, but they are broadly used for different biological experiments [3].

*Clarias batrachus* has been found in many continents, successfully adapted itself and found all over Asia and Africa. *Clarias batrachus* is considered as a medicinal fish and rich in nutritional value, found in different parts of India, mostly in Tripura and West Bengal. In some Indian states like Bengal and Tripura intensive culture of *Clarias batrachus* have better prospects towards income resources, generation of employment and ensuring dietary enhancement in the normal diet among the people [4].

The importance of medicinal plants and herbs to people's health and the health of society has always been proven. Numerous scientific studies on conventional herbal treatments for various diseases have been conducted recently, which has sparked an increase in the use of alternative drugs and therapeutic modalities [5].

*Phyllanthus emblica* L. is commonly known as Indian gooseberry. *Emblica officinalis* is a synonym of *Phyllanthus emblica*. It (amla) belongs to the family Euphorbiaceae as a strong rejuvenating aromatic plant and has been extensively used from a medicinal perspective and as an edible plant. All plant parts, including the leaves, flowers, seeds, bark, roots, and fruits, are utilised for therapeutic purposes in Ayurveda. It is reported as a good dietary source of vitamin C, amino acids, and minerals [6]. It consists of a number of secondary metabolites like alkaloids, glyceroids, tannins, glyceroids, flavonoids, saponins,[7].

Natural immunostimulants are good replacements for synthetic drugs in aquaculture, they are biocompatible, biodegradable and safe [8] [9]. Therefore, this investigation was designed to study the effect of the two doses of 20 g/kg and 50 g/kg (2% and 5%) of aqueous leaf extracts of *Phyllanthus emblica* incorporated in fish diets on the biochemical parameters of *Clarias batrachus* at the end of seven, fifteen, and thirty days. This may be helpful in increasing the production of disease-free, healthy fish.

### MATERIAL AND METHODS

*Phyllanthus emblica* leaves were procured from nearby areas of Jabalpur. The leaves were shade-dried, and an aqueous extract was prepared following the procedure by [10]. The extract was then incorporated into the fish feed. Fish diet was prepared using an adequate amount of ingredients, viz., rice bran powder, soya bean powder, fish meal (dried prawn), wheat flour, CMC (carboxymethylcellulose) binder, vitamin mixture, and cod liver oil. The autoclaved dough was then pressed through a fine poking machine, shade dried, and stored in an airtight container at 20 °C [11] [12] [13].

#### Experimental design -

Fish with an average body weight of 80–120 g of mixed sexes were purchased from local fishermen in Jabalpur. They were rinsed with 0.1% potassium permanganate and acclimatised for fifteen days under laboratory conditions, and an adequate oxygen supply was ensured [14]. The fish were divided into three groups of seven each, in triplicate, in the aquarium. Group I was the control, and Group II and Group III were experimental groups in which fish were fed with two doses of (2% and 5%) leaf extract (aqueous) *Phyllanthus emblica* incorporated into the fish diet. The fish of the control and experimental groups were fed twice at a rate of 2% body weight daily for 30 days, and the water was changed every alternate day [15] [16], with some modifications.

## **Biochemical parameter determination-**

At seven, fifteen, and thirty days, blood samples were taken from the caudal vein of experimental fish that had been given anaesthesia. Blood samples were taken in eppendorf tubes, left to clot for 15 minutes at room temperature, and then centrifuged at 3000 rpm for 5 minutes to extract the serum. After being collected, the serum was stored at -20°C until further evaluation. Studies on total protein, albumin, and globulin were done on blood plasma. All these measurements were calculated in triplicate. Total serum protein was assessed by the Lowry method [17] using BSA (bovine serum albumin) as a standard. Albumin was evaluated by the bromocresol green dye method [18]. By deducting the albumin value from the total protein value, the globulin fraction was calculated.

## **RESULTS AND DISCUSSION**

Results of the current study are presented in Table 1. Table 1 presents aqueous leaf extract of the two doses of 20 g/kg and 50 g/kg (2% and 5%) of *Phyllanthus emblica* incorporated into the fish diet of *C. batrachus*. The experimental data were recorded, and a two-way ANOVA was used to statistically assess them at p<0.05





## Total serum protein (g/dl) estimation in response to Phyllanthus emblica leaf extract treatments -

The total serum protein showed a rise in all experimental groups fed with leaf extract of *Phyllanthus emblica* at various concentrations. In this treatment, the average value in controls was  $(3.37\pm0.41)$ , the highest level of protein was seen in 2% feeding at 15 days  $(4.04\pm0.47)$  and the value obtained at 5% feeding after 30 days was  $4.18\pm0.18$ . The findings obtained are shown Figure 1.

## Albumin estimation in response to Phyllanthus emblica leaf treatment. -

In the present study, the mean albumin (g/dl) was  $1.59\pm0.07$  in controls. In Group II fish, the highest value obtained was  $2.07\pm0.20$  at a 2% dose after 15 days of treatment. In Group III fish, the best value obtained was  $2.05\pm0.05$  at a 5% dose after 30 days of treatment. The results obtained are shown in Figure 2.



Fig. 2 : Effect of *Phyllanthus emblica* leaf on Albumin (g/dl) of *Clarias baratchus* after 7, 15 and 30 days

## Globulin (g/dl) estimation in response to *Phyllanthus emblica* leaf treatment.

In the present investigation, the mean globulin (g/dl) was  $1.82\pm0.04$  in controls. The maximum value obtained was  $2.17\pm0.14$  in Group II fish at 2% feeding after 15 days of treatment. In Group III fish, the highest value obtained was  $2.13\pm0.14$  at 5% feeding after 30 days of treatment. The results obtained are shown in and Figure 3.



Fig. 3: Effect of leaf extract *Phyllanthus emblica* on Globulin (g/dl) of *Clarias baratchus* after 7, 15 and 30 days

The researchers studied the immunomodulatory activity of aqueous leaf extract of *Ocimum basilicum* in *Clarias batrachus*. They obtained an increase of the total protein from 2.25 in control to 3.05 at 15 days after feeding *Ocimum basilicum* crude extracts, slightly higher values were found after 30 days [19]. The results obtained indicated that albumin drops from 1.32 to 0.95 g/dl after 15 days and from 1.44 to 0.86 after 30 days due to the effect of *Ocimum basilicum* extract feeding with the corresponding increase in globulin (1.42-2.37 g/dl) after 15 days and (1.52-2.60g/dl) after 30 days at 5% feeding [19].

In the current investigation the fishes treated with *Phyllanthus* emblica leaf extract, the value of total protein arose from 3.37 to 3.92 g/dl at 5% feeding after 30 days. The starting value in the present investigation was higher than both the above instances.

The base value for albumin in present investigation was  $1.59\pm0.07$  which increased to  $2.13\pm0.14$  after 30 days at 5% feeding of leaf extract. Similar results with a slight increase in albumin, was reported. They found an increase from  $2.24\pm0.03$  in control to  $2.26\pm0.08$  in fishes fed with 20 ppm of *Eclipta alba* extracts after 28 days [20].

In the work done by some workers on aqueous leaf extract of *Ocimum kilimandscharicum Guerke* in *Clarias batrachus* the albumin decreases from 1.32 to 0.75 g/dl (at 5%) with a corresponding increase in globulin 1.42 to 2.30 g/dl after 15 days [21]. Globulin value was reported to be 3.14g/dl which is a base value for 120-150 gm fish [22]. These values are somewhat greater than the values obtained in the present investigation.

They worked on effect of *Eclipta alba* on *Clarias batrachus* found slightly higher values of globulin, the values increases from 2.92 to 3.02 g/dl after 28 days of feeding. The results are quite similar taking into consideration that their control values were higher. The differences are probably due to the environmental variations [20].

In the present investigation the value of globulin increased 1.8 to a maximum of 2.0 g/dl after 30 days of feeding of *Phyllanthus emblica* leaf extract. The increase of albumin in *Phyllanthus emblica* extract amount up to 2.13 (because the total protein was also greater) and it reached 2.07±0.14 after 15 days of 2% feeding.

## CONCLUSION

The present study revealed enhanced biochemical parameters in *C. batrachus* when treated with the aqueous extract of the leaf of *P. emblica*. The use of the extract of *P. emblica* may help to enhance the various aspects of immune capacity & can be used as an effective immunomodulators when added to fish feed at the recommended dose and time periods. This will contribute much to improve the yield of *Clarias batrachus* in culture.

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## **CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

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