

## MODULATORY EFFECT OF ECLIPTA ALBA ON BIOCHEMICAL PARAMETERS OF CATFISH, CLARIAS BATRACHUS

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### ABSTRACT

The present study was designed to observe the effect of leaf extract (aqueous and ethanolic) of *E. alba* on biochemical parameters in Asian cat fish, *Clarias batrachus*. Fishes of average weight of 70-80g were selected for the experimentation. Three sets of healthy fishes (n=20) were kept in concret tanks. Set I act as control received vehicle only, set II and III were as test groups, exposed to low and high concentration (10 and 20 ppm) of aqueous/ ethanolic extract of leaf of *E. alba* up to 28 days. Blood serum was collected on day 7, 14, 21 and 28. After serum analysis, the obtained data were subjected to statistical analysis such as two ways ANOVA and DMRT etc. The statistical

analysis revealed significant increase in total protein and globulin level and insignificant increase in albumin in test groups. Whereas the Albumine/Globuline (A/G) ratio were declined in experimental fishes as compare to control.

**KEY WORDS:** *Clarias batrachus*, *Eclipta alba*, extracts, biochemical parameters.

### INTRODUCTION

Biochemical parameters of blood especially proteins can be used to detect the fish health [1] and play crucial role in immune response. The basic function of immune system is to protect individuals against infectious agents and pathogens. Proteins are the most abundant compounds in the serum and are used in forming defensive molecules that helps the body to fight against infections. There are two groups of serum proteins, albumins and globulins. Albumins are very strong predictor of health and important for tissue growth and healing. Globulins play an important role as defensive proteins and include gamma globulins and a

variety of enzymes and carrier/transport proteins. Many medicinal plants classified as Rasayanas in Ayurveda are believed to be useful in strengthening the immune system of an individual [2].

Herbs and herbal products have a major role in aquaculture [3] and have long been used in India for promotion of health, prevention and treatment of diseases. One of the most important methods for controlling diseases in aquaculture is strengthening the defense mechanism of fish with the help of immunostimulants [4]. Recently, plants have received more attention towards their immune stimulating functions in fish culture [5, 6, 7]. [8] reviewed the reasearch currently being carried out on the herbs and herbal extract that have shown immunostimulating activity in fishes.

*Eclipta alba* (L) family - Asteraceae, commonly known as false daisy and Bhringaraja [9], used as traditional medicine in many countries. *Eclipta alba* is important plant of Ayurvedic matria media [10]. Several secondary metabolites such as coumestans, alkaloids, thiophanes, flavonoids, polyacetylene, triterpens and their glycosides etc are present in this herb [11] which carries out a number of protective functions in human body. They can improve the immune system also protect the body from free radicals and kill pathogenic germs.

The proposed study was planned to investigate the changes in biochemical parameters in *C. batrachus* after the exposure of leaf extract of *E. alba*. Such study may be successful for planning and production of fishes as healthier and wealthier management.

## MATERIAL AND METHODS

### Preperation of extract

The mature plants were collected from adjoining villege area of Raipur city. After washing with running tap water and deionized water leaves were removed separately and shade dried at room temperature for more than 15 days. The air dried leaves were powdered in an electric blender. 15grams of the leaf powder was taken for extraction using a Soxhlet apparatus at 60°C by the method of [12] with water/ethanol as a solvent. The extracts were concentrated in a water-bath individually until semi solid phase was formed. The paste was weighed and actual yield for aqueous and alcoholic extracted substance was estimated. The yield obtained was 35.23% (aqueous) and 15.75% (ethanol). The extracts were stored at 4°C as stock solution. These extracts were further used as per the requirement.

### Experimental model

**Fish** - *Clarias batrachus* with an average weight of 70-80 were collected from local fish market. After disinfection with 0.1% KMnO<sub>4</sub> [13], they were acclimatized for seven days in laboratory conditions.

### Experimental Design

Experimental fishes of mixed sexes were divided into three groups. Each group was kept in concrete tank containing 10 liter of water separately, at a density of 20 fish / tank in replicate. Group I was as control, received vehicle only, group II & III were exposed to 10 and 20 ppm dose of aqueous / ethanolic extracts of leaf every alternate days up to 28 days. Fishes were fed with goat liver every alternate day and water was replenished before the treatment.

### Sample collection

Blood samples were collected during morning hours at every four successive seven days. Blood (without anticoagulant) collected from fish was kept overnight at 4°C for serum separation. The serum was separated by spinning down at 3000 rpm for 15-20 min in centrifuge. The supernatant was collected and stored in sterile vials at -20°C for further analysis.

### Biochemical Investigation

Total protein content was determined by the method of [14] using Bovine Serum Albumin (BSA) as standard and globulin was estimated by biuret method. Albumin was determined by subtracting the globulin value from total protein. Serum A/G ratio was determined by the method of Reinhold *et al.* (1950) as described by [15].

### Statistical analysis

The data obtained were analysed using two-way analysis of variance (ANOVA) to test the level of significance. Differences amongst means were determined using Duncan's Multiple-Range Test (DMRT). Standard deviation (SD) was calculated. Significance level was set at P = 0.05 confidence limit.

## RESULTS

The effect of aqueous/ethanolic extracts of leaf of *E. alba* on total serum protein (g/dl), albumin, globulin and A/G ratio at different sampling points of seven days interval are presented in Table1 & 2.

ANOVA revealed statistically significant effect of treatment on total protein, globulin and A/G ratio at 1% of confidence limit with both the extracts whereas different sampling points was at 5% level of probability only for total protein. Significant increase was not observed with albumin. The results also indicated that mean values on total serum protein and A/G ratio between 10 and 20 ppm extracts were statistically at par. Decreasing trends in A/G ratio were noticed from control to treated group with all extracts at all sampling points.

The globulin level in treated with aqueous extract was more than alcoholic extract treated group. The A/G ratio was higher with ethanolic than aqueous extract.

**Table.1. Effect of leaf extract (aqueous) of *E. alba* on biochemical parameters of *C. batchus*.**

Day	Treatment			ANOVA
	Control	10ppm	20ppm	
	TP (g/dl)			
07	4.93 ± 0.06 a B	5.09 ± 0.05 b A	5.16 ± 0.04 a A	T** D <sup>ns</sup> T x D <sup>ns</sup> -0.17 <sup>\$</sup>
14	4.95 ± 0.06 a B	5.19 ± 0.03 abA	5.23 ± 0.07 a A	
21	4.91 ± 0.02 a B	5.27 ± 0.04 a A	5.30 ± 0.04 a A	
28	4.96 ± 0.02 a B	5.29 ± 0.06 a A	5.28 ± 0.08 a A	
	Albumin (g/dl)			
07	2.18 ± 0.05 a A	2.21 ± 0.04 a A	2.24 ± 0.03 a A	T <sup>ns</sup> D <sup>ns</sup> T x D <sup>ns</sup> -0.13 <sup>\$</sup>
14	2.22 ± 0.07 a A	2.27 ± 0.02 a A	2.23 ± 0.03a A	
21	2.18 ± 0.04 a A	2.26 ± 0.02 a A	2.26 ± 0.01a A	
28	2.20 ± 0.02 a A	2.27 ± 0.02 a A	2.26 ± 0.08 a A	
	Globulin (g/dl)			
07	2.74 ± 0.01 a B	2.88 ± 0.01 b A	2.92 ± 0.01 b A	T** D** T x D <sup>ns</sup> -0.08 <sup>\$</sup>
14	2.73 ± 0.01 a C	2.92 ± 0.01 b B	3.00 ± 0.02ab A	
21	2.73 ± 0.02 a B	3.01 ± 0.02 a A	3.05 ± 0.04 a A	
28	2.76 ± 0.01 a B	3.03 ± 0.04 a A	3.02 ± 0.01 a A	
	A/G ratio			
07	0.79 ± 0.01 a A	0.76 ± 0.01 a A	0.77 ± 0.01 a A	T** D <sup>ns</sup> T x D <sup>ns</sup> -0.05 <sup>\$</sup>
14	0.81 ± 0.02 a A	0.78 ± 0.01 a A	0.75 ± 0.07 a B	
21	0.80 ± 0.02 a A	0.75 ± 0.01 a B	0.74 ± 0.01 a B	
28	0.80 ± 0.01 a A	0.75 ± 0.01 a B	0.75 ± 0.02 a B	

Values are expressed as Mean ± SE. Means in a column followed by different lower case letters and mean in a row followed by different capital letters are significantly different at 5% level by DMRT. \*p<0.05; \*\* p<0.01;\*\*\*p<0.001, ns= not significant, \$ = LSD at 5% level

**Table.2. Effect of leaf extract (ethanolic) of *E. alba* on biochemical parameters *C. batrachus*.**

Day	Treatment			ANOVA
	Control	10ppm	20ppm	
	<b>TP (g/dl)</b>			
<b>07</b>	4.95 ± 0.05 a B	5.24 ± 0.05 b A	5.31 ± 0.05 a A	<b>T** D* T x D<sup>ns</sup> -0.16<sup>\$</sup></b>
<b>14</b>	4.99 ± 0.04 a B	5.32 ± 0.03 ab A	5.39 ± 0.07 a A	
<b>21</b>	5.00 ± 0.02 a B	5.39 ± 0.02 ab A	5.46 ± 0.04 a A	
<b>28</b>	4.98 ± 0.04 a B	5.45 ± 0.06 a A	5.48 ± 0.07 a A	
	<b>Albumin (g/dl)</b>			
<b>07</b>	2.27 ± 0.05 a A	2.29 ± 0.03 a A	2.32 ± 0.03 a A	<b>T<sup>ns</sup> D<sup>ns</sup> T x D<sup>ns</sup> -0.13<sup>\$</sup></b>
<b>14</b>	2.28 ± 0.06 a A	2.34 ± 0.03 a A	2.34 ± 0.03 a A	
<b>21</b>	2.26 ± 0.03 a A	2.33 ± 0.02 a A	2.35 ± 0.01 a A	
<b>28</b>	2.29 ± 0.02 a A	2.35 ± 0.01 a A	2.33 ± 0.07 a A	
	<b>Globulin (g/dl)</b>			
<b>07</b>	2.68 ± 0.03 a B	2.96 ± 0.02 b A	2.99 ± 0.02 b A	<b>T** D** T x D<sup>ns</sup> -0.11<sup>\$</sup></b>
<b>14</b>	2.71 ± 0.08 a C	2.99 ± 0.02 ab B	3.05 ± 0.02 ab A	
<b>21</b>	2.74 ± 0.01 a B	3.06 ± 0.01 abA	3.12 ± 0.04 a A	
<b>28</b>	2.69 ± 0.02 a B	3.10 ± 0.05 a A	3.15 ± 0.01 a A	
	<b>A/G ratio</b>			
<b>07</b>	0.84 ± 0.02 a A	0.77 ± 0.01 a B	0.78 ± 0.01 a B	<b>T** D<sup>ns</sup> T x D<sup>ns</sup> -0.06<sup>\$</sup></b>
<b>14</b>	0.85 ± 0.04 a A	0.78 ± 0.01 a B	0.77 ± 0.01 a B	
<b>21</b>	0.83 ± 0.01 a A	0.76 ± 0.01 a B	0.75 ± 0.01 a B	
<b>28</b>	0.85 ± 0.01 a A	0.76 ± 0.01 a B	0.74 ± 0.02 a B	

Values are expressed as Mean ± SE. Means in a column followed by different lower case letters and mean in a row followed by different capital letters are significantly different at 5% level by DMRT. \*p<0.05; \*\* p<0.01;\*\*\*p<0.001, ns= not significant, \$ = LSD at 5% level

## DISCUSSION

In the present study, effect of different concentrations of aqueous and ethanolic extracts of leaf of *Eclipta alba* on biochemical changes of *Clarias batrachus* at different time points have been assessed.

The results revealed significant increase in total protein and globulin after application of both (aqueous and ethanolic) extracts in treated groups. Marginal increase in albumin level was noticed in the group treated with aqueous extract. The A /G ratio differed insignificantly in treated groups with all aqueous extracts. Decreasing trend in A/G ratio was observed with

alcoholic extracts. Measurable higher level of total protein, albumin and globulin in the present study suggest the enhancement of non-specific immunity in *C. batrachus* following treatment with *E. alba*.

Increase in serum protein, albumin and globulin levels is thought to be associated with stronger innate immune response [16] and considered as a good indicator of activation of immune system [17]. These are associated with a stronger innate immune response in fishes [18]. Enhanced total protein and decreased A/G ratio is the indicator of health. Increase in globulin level may indicate the potential of plants to stimulate the immune response by increasing defensive molecule [19]. Since albumin is produced in liver, the increased albumin level in the present study may indicate that the extract of *E. alba* promotes good functioning of liver that may prove its traditional use as liver tonic.

[20] suggested that the phyto-constituents of methanolic leaf extract of *Mimosa pudica* plant (flavonoids, glycosides and alkaloids) might be responsible for increase in total protein and albumin. [21] also reported these secondary metabolites in leaf extract of *E. alba*. [22] reported significantly increased total protein, globulin and dose dependent increase in albumin level with aqueous root extract of *Pelargonium reniforme*. Similarly, [23] studied the combined effect of aqueous extract of *E. alba* and *Enicostemma littorale* leaf (1:1 ratio) as hepatoprotective during ethanol induced oxidative stress in liver tissue of albino rats and reported significant increase in total protein level as compared to control. [24] reported increased production of total protein and no significant change in albumin level in rat treated with aqueous extract of *Ocimum sanctum*. [25] evaluated the action of ethanolic extract of *Eclipta alba* leaves and seed of *Piper longum* in combination against  $\text{CCl}_4$  induced hepatic damage in albino rats and reported increase in total protein up to normal value. In Piscine model, [26] reported that dietary intake of aqueous extract of *E. alba* increases the non-specific immune response in *Oreochromis mossambicus*.

Many investigators in fishes with other plants documented similar findings. [5] reported that the diet containing aqueous extract of *Viscum album*, *Utrica dioica* and *Zingiber officinale* increased the total protein level in plasma in rainbow trout (*Oncorhynchus mykiss*). [27] studied the effect of aqueous root extract of *Achyranthes aspera* mixed diet on trypsin activity of *Labeo rohita* and reported high level of protease inhibitors after four weeks on antigen injection and showed elevated values of antigen antibody level, serum globulin in fish

fed with experimental diet. [28, 29] documented increase in total plasma protein and serum protein, respectively in all groups of *Oreochromis niloticus* fed with different doses of *Allium sativum* in the diet.

The higher level of serum protein, albumin, and globulin were documented after feeding with *Magnifera indica* and *Allium sativum* mixed diet [18, 30] in *Labeo rohita*. [31] reported significant increase in total serum protein, albumin and globulin in *Oreochromis niloticus* and *Clarias gariepinus* after treatment with aqueous neem extract. A/G ratio is a measurable humoral component of non-specific defense. Reduction of A/G ratio in fish might be due to increased total globul in level, with significance for the protective mechanisms. [32] documented the modulation of some parameters of innate immune system in tilapia by *Lonicera japonica* and *Ganoderma lucidum*.

A number of investigators on different fishes also documented enhanced level of total proteins, albumin and globulins as in present study by using different plant. Few of them are [33, 34, 29, 35, 36, 37, 38]. [39] reported influence of *Plumbago rosea* diet in *Catla catla* on increased level of serum protein.

## CONCLUSION

The present study reveals enhanced biochemical indices in *Clarias batrachus* when treated with aqueous and ethanolic extracts of leaf of *E. alba*. The effects were more pronounced in *C. batrachus* with aqueous extracts. *Eclipta alba* is documented as an immune stimulant herb besides possessing several other medicinal properties. It would be a matter of further investigation that which of the active phyto-constituent present in *E. alba* is responsible in modulating the health of these fishes.

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