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Research Article

HAEMATOLOGICAL EVALUATION OF *ECLIPTA ALBA* ROOT EXTRACT IN CATFISH, *CLARIAS BATRACHUS* (LINNAEUS, 1758)

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ABSTRACT
A 28 day study was undertaken to evaluate the effect of aqueous and ethanolic extracts of root of *Eclipa alba* in Asian catfish, *Clarias batrachus* on haematological variables. The fishes of mixed sexes with a mean weight of 70-80 g were selected as experimental model. After acclimation of one week in laboratory condition, fishes were randomly selected into three group of 20 fishes (n = 20) each. Group A served as control and received vehicle only where as group B and C served as test received 10 ppm and 20 ppm of aqueous or ethanolic extract of *Eclipta alba* root respectively up to 28 days. Blood samples were collected on day 7, 14, 21 and 28 for hematological analysis. Result of test groups were compared statistically with control. RBC, Hb, PCV and WBC counts increased significantly. Increase in MCV and MCH was noticed whereas no clear-cut trend was observed in MCHC.
Keywords: *Eclipta alba,* Root, Extracts, *Clarias batrachus*

INTRODUCTION

Botanicals are used as herbal remedies from the ancient time, for the prevention and cure of various diseases and ailments[1]. Their applications are found to be good in improvement of the health of organisms. Some plants are blood booster and used as hematinics. Use of medicinal plants, as herbal remedy for the maintenance of good health may be helpful in production of healthy fishes and improving the pisciculture[2]. One of the important medicinal herb is *Eclipta alba* (family-Asteraceae), commonly called as Bhringraja[3]. All parts (leaf, stem and root) of this herb exhibited medicinal properties.[4] reported several secondary metabolites such as coumestans, alkaloids, thiophanes, flavonoids, polyacetylene, triterpens and their glycosides in this herb. Several of these can boost the immune system, protect the body from free radicals and kill pathogenic germs. The root of this plant is used as an emetic, antiseptic to ulcer and wound and purgative[5]. In aquaculture the economic development is affected by diseases, malnutrition, pollution etc. Various disease preventive measures are required for profitable aquaculture. Plants and their products are safer, biodegradable and eco-friendly[2]. They are easily available and extensively used in aquaculture. The Indian catfish *Clarias batrachus*, commonly known as Magur[6] is native to South East Asia. It is highly nutritious live fish and recommended to the anemic and malnourished individuals and growing children. Hematological parameters are used for the explanation of healthy state of fish and serve as a sensitive index for controlling fish diseases and improving fish cultivation[7]. The proposed study was planned to investigate the effect of root extract (aqueous /alcoholic) on hematological variables of *C. batrachus.*

MATERIAL AND METHODS

Plant material

Eclipta alba roots were obtained from paddy field nearby Raipur city, India. The roots were cleaned and made free from sand and other foreign particles. The shed dried roots were powdered in an electric blender. 15 g of the root powder was taken for extraction using a Soxhlet apparatus at 60°C by the method[8] 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 with aqueous extract was 13.18 % whereas with alcoholic extract ant 11.00 %. The extracts were stored at 4°C as stock solution. These extracts were further used as per the requirement.

Fish and Experimental design

Disease free experimental fish, *Clarias batrachus* (average weight 70-80 g) were procured from local market and disinfected with 1 % potassium permagnet[9]. After 7 days of acclimation in laboratory conditions fishes of mixed sexes were divided into three groups of 20 fishes. Group I was treated as control (received no treatment) and Group II and III administrated with 10 and 20 ppm of root extract (aqueous/ alcoholic). Experimental fishes were fed with goat liver *ad libitum* every alternate day and water was changed before treatment. Blood sample was collected on day 7, 14, 21 and 28 for blood analysis.

Hematological analysis

Red blood cell (RBC) count and White blood cell (WBC) count were estimated by visual means using the new improved Neubauer counting chamber according to[10]. Hemoglobin was estimated by Sahlis method, PCV was determined by Wintrobe method[11]. The derived hematological indices of mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) were calculated using the standard formula[10].

$$\text{MCHC (\%)} = \frac{\text{MCH}}{\text{MCV}} \times 100$$

$$\text{MCH (picograms)} = \frac{\text{Hb (g \%)} \times 10}{\text{RBC} \times 10^6 \mu l}$$

$$\text{MCV (femtoliter)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC} \times 10^6 \mu l}$$

Statistical analysis of the Data

Experimental data obtained were statistically analyzed by means of analysis of variance (Two Way ANOVA). Differences amongst means were determined using Duncan's Multiple-Range Test (DMRT).

RESULTS

In the present study, effect of aqueous and alcoholic extracts of root of *E. alba* on hematological changes of *C. batrachus,* at different durations was assessed. The mean values of hematological parameters are disclosed in Table 1 aqueous, Table 2 alcoholic and Figure 1 (A, B and C). Result of ANOVA showed the statistically significant treatment effect ($p < 0.01$) on RBC, Hb, PCV and WBC by application of both the extracts whereas sampling time was significant at 5 % of confidence limit with the two extracts on WBC only. Increment in the mean values of RBC, Hb and PCV was noticed but significant increase was observed immediately in WBCs with both the extracts. Figure 1 (A, B and C) shows the effect of root extract on red cell indices at different time points. Significant variation in the values of MCH and MCHC was noticed at different sampling points with aqueous and alcoholic extracts. The test of significance shows the significant effect ($p < 0.05$) of treatment on MCV and MCH with aqueous extract whereas the duration effect was significant at 5 % of confidence limit with aqueous extract on MCV only. During different sampling points, no significant change was observed on MCV after exposure of aqueous and alcoholic extracts. Significant variation in MCH and MCHC was noticed after application of both the extract.

. **Table 1: Effect of aqueous extract of root of *Eclipta alba* on hematology of *C. batrachus***

Variable	Treatment	Duration (days)				ANOVA
		7	14	21	28	
RBC (10^6/mm^3)	Control	2.35 ± 0.04 aB	2.33 ± 0.03 aB	2.34 ± 0.03 aB	2.36 ± 0.04 aB	T**,D^{ns} T x D^{ns} – 0.12$^{\$}$
	10 ppm	2.48 ± 0.05 aA	2.51 ± 0.04 a A	2.54 ± 0.04 aA	2.56 ± 0.03 aA	
	20 ppm	2.53 ± 0.06 aA	2.56 ± 0.05 aA	2.57 ± 0.05 aA	2.59 ± 0.02 aA	
Hb (g%)	Control	7.65 ± 0.27 aB	7.55 ± 0.17 aB	8.15 ± 0.17 aB	8.00 ± 0.21 aB	T** D^{ns}, T x D^{ns} – 0.70$^{\$}$
	10 ppm	8.60 ± 0.21 aA	8.75 ± 0.22 aA	9.15 ± 0.27 aA	8.75 ± 0.43 aA	
	20 ppm	8.80± 0.14 aA	8.85 ± 0.17 aA	9.25 ± 0.22 aA	8.90 ± 0.31 aA	
PCV (%)	Control	21.30 ± 0.68AB	21.80 ± 0.84 abB	23.40 ± 0.87abB	23.80 ± 0.87 aA	T**D^{ns} T x D^{ns} – 2.00$^{\$}$
	10 ppm	24.50 ± 0.65 aA	24.73 ± 0.62 aA	26.43 ± 1.13 aA	26.20 ± 0.98 aA	
	20 ppm	25.80 ± 0.45 aA	25.28 ± 0.38 aA	26.95 ± 0.83 aA	26.10 ± 0.63 aA	
WBC (10^4/mm^3)	Control	2.60 ± 0.04 aB	2.57 ± 0.03 aB	2.62 ± 0.03 aB	2.62 ± 0.04 aB	T**D*, T x D^{ns} – 0.26$^{\$}$
	10 ppm	2.79 ± 0.05 bA	3.07 ± 0.04 aA	3.15 ± 0.04 aA	3.05 ± 0.03 abA	
	20 ppm	2.85 ± 0.06 bA	3.12 ± 0.05 aA	3.20 ± 0.05 aA	3.19 ± 0.02 aA	

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 alcoholic extract of root of *Eclipta alba* on hematology of *C. batrachus*

Variable	Treatment	Duration (days)				ANOVA
		7	14	21	28	
RBC (10^6/mm^3)	Control	2.39 ± 0.02 aA	2.37 ± 0.01 aB	2.38 ± 0.02 aB	2.41 ± 0.03 aB	T** D^{ns} T x D^{ns} – 0.26$^{\$}$
	10 ppm	2.33 ± 0.06 bA	2.65 ± 0.09 aA	2.72 ± 0.15 aA	2.70 ± 0.17 aA	
	20 ppm	2.58 ± 0.08 aA	2.71 ± 2.16 aA	2.75 ± 0.04 aA	2.72 ± 0.07 aA	
Hb (g%)	Control	7.90 ± 0.12 aB	7.95 ± 0.02 aB	8.35 ± 0.22 aB	8.25 ± 0.17 aB	T**D^{ns} T x D^{ns} – 1.00$^{\$}$
	10 ppm	8.90 ± 0.44 aA	9.05 ± 0.27 aA	9.45 ± 0.53 aA	9.25 ± 0.72 aA	
	20 ppm	9.30 ± 0.51 aA	9.30 ± 0.53 aA	9.60 ± 0.35 aA	9.15 ± 0.34 aA	
PCV (%)	Control	23.20 ± 0.96 aB	23.18 ± 0.47 aB	24.93 ± 0.68 aB	24.08 ± 0.61 aA	T**D^{ns}, T x D^{ns} – 3.31$^{\$}$
	10 ppm	26.28 ± 1.12 aA	27.10 ± 0.78 aA	27.40 ± 1.62 aAB	26.90 ± 2.29 aA	
	20 ppm	27.10 ± 1.69 aA	27.60 ± 1.69 aA	28.10 ± 1.42 aA	27.10 ± 0.99 aA	
WBC (10^4/mm^3)	Control	2.65 ± 0.03 aA	2.60 ± 0.04 aB	2.66 ± 0.04 aB	2.63 ± 0.05 aB	T**D* T x D^{ns} – 0.27$^{\$}$
	10 ppm	2.86 ± 0.16 bA	3.14 ± 0.08 aA	3.21 ± 0.09 aA	3.20 ± 0.04 aA	
	20 ppm	2.91 ± 0.19 bA	3.18 ± 0.04 abA	3.24 ± 0.05 aA	3.25 ± 0.03 aA	

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

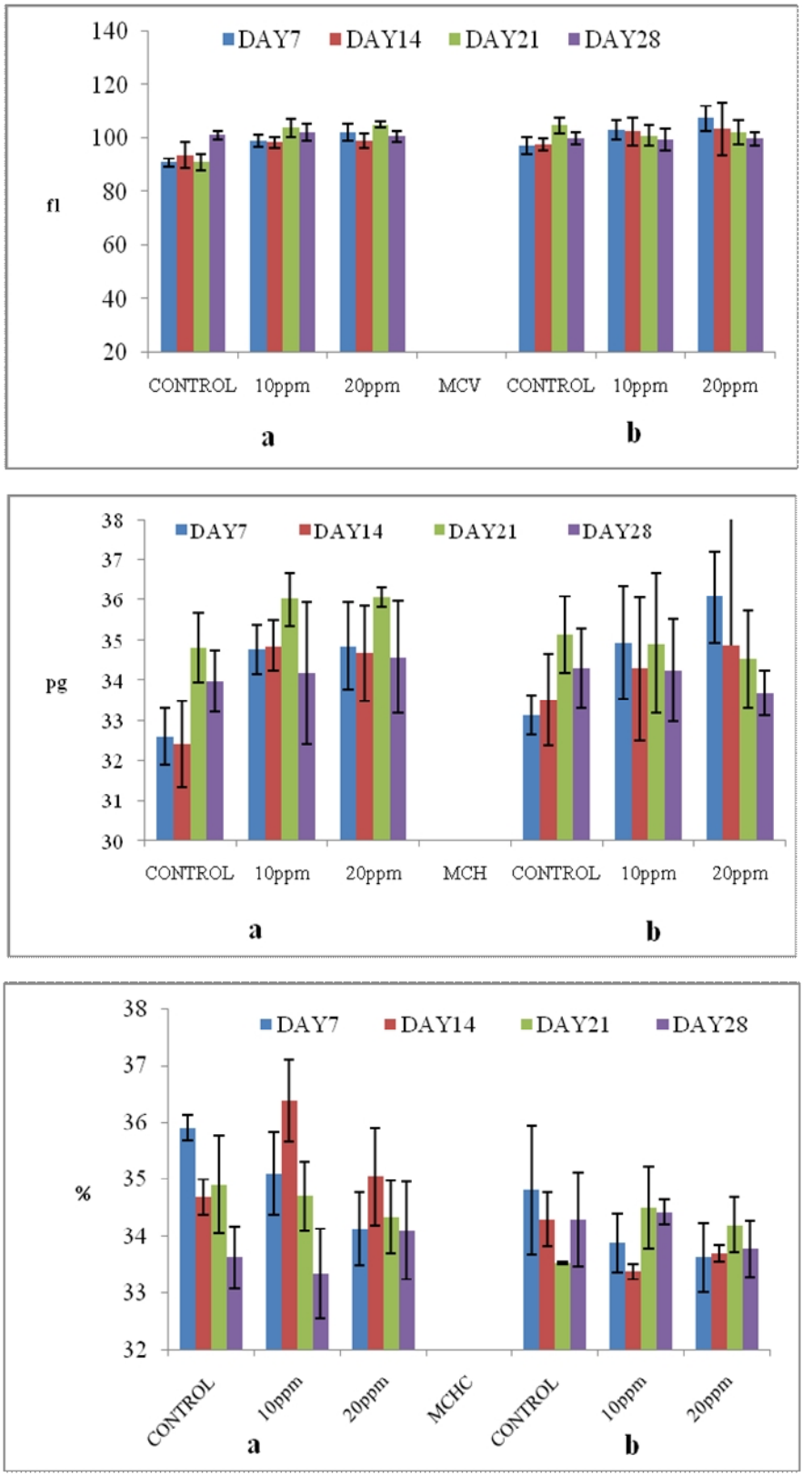

Figure 1: Effect of aqueous (a) and alcoholic (b) root extract of *E. alba* on Red cell indices of *C. batrachus*

DISCUSSION

The haematological parameters such as RBC, Hb, PCV and WBC counts in the present investigation were significantly higher in treated group over control. The study of[12] is in favor of present study, who reported enhanced values of RBC, haemoglobin and PCV after the administration of aqueous extract of leaf of *E. alba* on Swiss albino mice. Identical results were reported in *Labeo rohita* fingerlings fed *Mangifera indica* kernel[13], in *C. gariepinus,* treated with different parts of *Garcinia mangostana*[14]*,* with *Coriandrum sativum*[15] and *Plumbago rosea*[16] and with *Cynodon dactylon* in *Catla catla*[17,18], in *Cyprinus carpio* treated with *Epilobium hirsutum* mixed diet[19] and in *Oreochromis mossambicus* treated with *Andrographis paniculata*[20]. The investigations in Rainbow trout fed with *Allium sativum*[21] and in *Pangasianodon hypophthalmus* with aqueous extract of *Garcinia* gummi gutta[22] were in agreement with the present findings. Increased level of RBC, Hb and PCV by the exposure of root extract may be due to enhanced erythropoiesis[20,23]. Elevated value of RBC is associated with the iron content that enables to stimulate erythropoiesis and ultimately increased level of hemoglobin due to high absorption of iron[24]. Presence of iron in all parts of *E. alba* was investigated[4]. Antioxidants presence in the plant extract may trigger erythropoiesis[20]. *Eclipta alba* has antioxidant properties[25]. Flavonoids have been reported for antioxidant activity which may maintain the heam iron in its ferrous state and this could enhance erythropoiesis[26]. The saponin in the extract hydrolyzes and produces steroid or triterpene and the stimulatory effect of steroid on bone marrow results in increased erythropoiesis[27]. Presence of saponin tannin, flavonoids and phenolic compounds and cardio glycosides in aqueous and alcoholic extract of root was reported[28]. Increased production of WBC in mice treated with methanol extract of *E. alba* was reported[29]. Increased WBC may be due to the constituents like vitamin A, B and C, calcium iron, potassium and protein, traces of carotenoids, saponins and phenolic compounds[30]. The polyphenols, alkaloids, glycosides and reducing sugar might be responsible for increased WBC[31]. In present study decrease in MCV and increase in MCH and MCHC was observed after the exposure of aqueous and alcoholic extract. Increase in MCHC may be attributed to improvement of fish health[32]. In gamma irradiated mice pretreated with aqueous extract of *E. alba* regained MCV and MCH values after 15 days was reported[12]. They concluded that aqueous extract of *E. alba* maintains the MCV and MCH level in mice and further believed that aqueous extract modulates the hematological alterations due to its antioxidant activity. The findings of present study were agreement with[33] who studied in *Oreochromis niloticus* fed with different doses of *Allium sativum* and chlomphenicol mixed diet. They concluded that the decrease or increase in blood indices may be attributed to a defense reaction which occurs by stimulation of erythropoiesis.

CONCLUSION

The root extracts of *Eclipta alba* used in this study, had a positive effect on hematological parameters and may be helpful to increase the health status of *Clarias batrachus*.

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