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COMPARATIVE EFFECT OF *ECLIPTA ALBA* ON HEMATOLOGICAL PARAMETERS OF ASIAN CATFISH, *C. BATRACHUS*

PREETI MISHRA[a1] AND SEEMA GUPTA[b]

[ab]Department of Zoology, Govt. N. P.G. College of Science, Raipur, Chhattisgarh, India

ABSTRACT

The present study was planned to evaluate the comparative effect of root, stem and leaf of *Eclipta alba* on hematology of *Clarias batrachus*. Fishes of mixed sexes (70-80 g weight) were treated with the dose of 10 and 20 ppm of extracts (aqueous /alcoholic) of leaf, stem and root for 28 days. Blood samples were collected on day 7, 14, 21 and 28 after treatment for analysis of haematological parameters. The effect of aqueous extract of all plant parts had significant role in enhancing the RBC number, hemoglobin %, PCV and WBC counts in *C. batrachus*. The application of 10 and 20 ppm extracts of leaf, stem and root did not show any significant differences amongst themselves. With aqueous extract, treatments, plant part and their interaction showed significant effect ($p<0.01$) except RBC in interaction effect. The results further indicate that aqueous extract of stem performed significantly better in enhancing the RBC level followed by root and leaf extract. Stem and root extracts performed significantly better over leaf extract for hemoglobin level. Leaf and stem extracts gave significantly better result as compared to root extract, in changing the pack cell volume level. Stem and root extracts had statistically at par on WBC count which were significantly superior over leaf extract. With alcoholic extract of all plant parts, the results of ANOVA showed significant effect of treatments ($p<0.01$) on RBC count, Hb, PCV and WBC. The plant parts revealed significant effect at 1% of probablity on Hb and PCV whereas at 5% for WBC. Interaction between treatment and plant part showed significant effect at $p<0.01$ with Hb and PCV. Significant effect on PCV level over control was noticed by exposure of alcoholic extract of all plant parts however, only stem alcoholic extract could performed significant role in haemoglobin level. The results indicate that alcoholic stem extract performed significantly better followed by root and leaf in enhancing the PCV value. Significantly higher WBC values were recorded in treated group over control with all extracts of leaf, stem and root. Root and leaf extracts of alcoholic solution significantly elevated the WBC count over stem extract.

KEYWORDS: *Clarias batrachus, Eclipta alba*, *Plant parts, Extracts, Hematology*

India is the largest producer of medicinal herbs and is called as botanical garden of the world (Seth and Sharma, 2004). Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. India is known for its traditional medicinal systems—Ayurveda, Siddha, and Unani. The literal meaning of Ayurveda is "science of life," and is also called the "science of longevity" because it offers a complete system to live a long healthy life. The plants have ability to synthesize a variety of chemical compounds. These phytochemicals show beneficial effects and can be used effectively treat diseases.One important medicinal herb is Bhringraja (*Eclipta alba*, Family Asteraceae) can be found growing wildly and considered as a weed by farmers. The herb is with diverse medicinal values and use commonly for treatment of gastrointestinal disorders, respiratory tract disorders, fever, hair loss and graying of hair, liver disorders, skin disorders, spleen enlargement, cuts, wounds and also for modulation for blood parameters (Jahan *et al*., 2014). Positive effect of leaf, stem and root has been reported in *C. batrachus* and *C. gariepinus* (Mishra, 2013).

Clarias batrachus is a species of fresh water air breathing catfish, hardy species, one of the most widespread catfish genera in the world. It is medicinally important fish food in its native lands and introduced to several other countries for farming purposes. This species is found in lowland streams, swamp and rice fields. It is threatened (Hossain *et al*., 2005; Ahmad *et al*., 2012) and becoming critically endangered. In view of this, present study may be worthwhile helpful to enhance the culture of *C. batrachus* and to make it more competitive to fish farmers.

MATERIALS AND METHODS

Collection of Plant Material

Eclipta alba, mostly grown in paddy fields during the rainy and summer season in Chhattisgarh state. This herb plant was identified in the Department of Medicinal and Aromatic Plants, Indira Gandhi

[1]Corresponding author

Agricultural University, Raipur (C. G.). The mature plants (including roots) were collected from adjoining village area of Raipur city and brought to the laboratory, washed thoroughly with running tap water then with deionized water to keep the plant material free from any soil particles and chemicals like fertilizer, weedicide, pesticide etc. Different parts of the plant (leaf, stem and root) were removed separately and shade dried at room temperature for more than 15 days. The air dried plant parts were powdered in an electric blender and passed through 200 mesh sieve. The dried powder form of leaf, stem and root were packed in plastic bags and stored at room temperature till further use.

Preparation of Plant Extracts

Extract of different plant parts were prepared by Soxhlet apparatus separately with aqueous and alcoholic solvents.

Experimental Design

Clarias batrachus of average weight, 70-80 g was procured from local fish farm. Fishes were disinfected with 0.1% potassium permagnet (Joshi *et al*., 2002) and acclimatized for seven days in laboratory conditions in circular cement tank (1 x 1.5 feet). Fishes of mixed sexes were divided in to three groups (n = 20) and kept in cement tank containing 10 liter of water separately. Group I was treated as control, group II and III was exposed to 10 and 20 ppm dose of aqueous/alcoholic extracts of leaf, stem and root respectively every alternate day till 28 days. The fishes were fed with goat liver every alternate day and water was changed after 24 hours of feeding. Blood samples were collected on day 7, 14, 21 and 28 for different analysis

Blood Parameter Analyses

Blood was collected by cutting caudal peduncle in EDTA coated tubes. The total erythrocyte and leukocyte were counted in an improved Neubaeur haemocytometer using Hayem's diluting fluid and Turk's fluid respectively. Hemoglobin (Hb) concentration was measured by Sahlis method. Pack cell volume (PCV) was determined by Wintrobe method (Blaxhall and Diasley, 1973).

Data Analyses

Experimental data and those of control were statistically analyzed Two Way ANOVA. 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 and alcoholic extracts of leaf, stem and root of *E. alba* on RBC, Hb, PCV haemoglobin in *C. batrachus* at different sampling points are disclosed in Table 1 and 2. Table 1 displays the effect of aqueous extract of leaf, stem and root of *E. alba* on RBC count, Hemoglobin, PCV and WBC of *Clarias batrachus* in relation to treatments. The test of significance as evidenced by ANOVA, revealed that treatments (T), plant part (P) had significant effect ($p<0.001$) and their interaction (TxP) had also significant effect ($p<0.001$) except RBC. The effect of aqueous extract of all plant parts had significant role in enhancing the RBC number, hemoglobin level, PCV and WBC level in *C. batrachus*. The application of 10 and 20 ppm extract of leaf, stem and root did not show any significant differences amongst themselves. The results (Table 1) further indicated that aqueous extract of stem performed significantly better in enhancing the RBC level followed by root and leaf extract. Stem and root extracts performed significantly better over leaf extract for hemoglobin level. The results indicate that leaf and stem extracts gave significantly better result as compared to root extract in changing the pack cell volume level. Stem and root extracts had statistically at par effect on WBC count which were significantly superior over leaf extract.

Table 2 represents the effect of alcoholic extracts of leaf, stem and root of *E. alba* on red blood cell counts, hemoglobin, PCV and WBC of *C. batrachus* in relation to different treatment (T). The results of ANOVA showed significant effect of treatments ($p<0.01$) on RBC count, Hb, PCV and WBC.

The plant parts revealed significant effect at 1% of probability on Hb and PCV whereas at 5% for WBC. Interaction between treatment and plant part showed significant effect at $p<0.01$ with Hb and PCV. Significantly higher RBC values were recorded

between control and 10 ppm doses with all extracts of leaf, stem and root and further increase in dose (20 ppm) did not respond in RBC counts significantly. The RBC value enhanced under stem extract treated group as compared to leaf and root. Alcoholic extracts of stem exhibited significant role in influencing the haemoglobin level over control. The exposure of alcoholic extracts of all plant parts exhibited significant role in influencing the PCV level over control. The results indicate that alcoholic stem extract performed significantly better followed by root and leaf in enhancing the PCV level.

Significantly higher WBC values were recorded in treated group over control with all extracts of leaf, stem and root. Root and leaf extracts of alcoholic solution elevated the WBC count significantly over stem extract.

DISCUSSION

In the present study, effect of varied concentrations of aqueous and alcoholic extracts of leaf, stem and root of *Eclipta alba* on hematological changes of *Clarias batrachus* at different time points have been assessed.

In *C. batrachus,* aqueous extractants of the leaf and stem had delayed effect whereas no duration effect was found with root extract. Similarly, the RBC values remarkably changed after the application of alcoholic extract of three plant parts but immediate increase was noticed with leaf and root extracts exposure. Among the three plant parts, aqueous and alcoholic extracts of stem were more effective in enhancing the RBC level followed by root and leaf extracts.

In *C. batrachus,* the hemoglobin level enhanced by stem extract with duration effect and other extracts (leaf and root) showed identical results. Aqueous extracts of stem performed significantly superior over root and leaf extracts. The alcoholic extract of all plant parts also elevated the hemoglobin level. The comparative effect of aqueous extracts of leaf, stem and root on pack cell volume indicated that stem and leaf extracts gave significantly better result as compared to root extract in changing the level of pack cell volume. The result of pooled effect showed higher number of WBC produced by root followed by leaf and stem extracts.

The results of present study are in agreement with the study of Singh *et al*. (2011) who reported enhanced values of RBC, haemoglobin and PCV after the administration of aqueous extract of leaf of *E. alba* on Swiss albino mice. Singh *et al.* (2014) reported recovery of hematological incides in endosulfan treated mice after the treatment of aqueous leaf extract of *E.alba*.

The enhancement of haematological parameters such as RBC, hemoglobin percentage and pack cell volume was reported after the treatment with *Garcinia cola* seed (Dada and Ikuerowo, 2009) and different parts of *Garcinia mangostana* such as leaf, shoot and rind (Soosean *et al*., 2010), in *C. gariepinus*.

Increased level of RBC, hemoglobin and PCV were reported by various medicinal plants in different fish species *viz*., in *Catla catla* treated with *Coriandrum sativum* and *Plumbago rosea* (Innocent *et al.* 2011a & b), *Cynodon dactylon* (Kaleeswaran *et al*., 2012 a & b), in *Cyprinus carpio* treated with *Nelumbo nucifera* (Vinodhini, 2010), *Zataria multiflora* and *Eucalyptus globolus* essential oil (Sheikhzadeh *et al*., 2011), and *Epibolium hirsutum* (Pakrawan *et al*., 2012). Similar results were also reported in *Oreochromis mossambicus* treated with *Andrographis paniculta* (Prasad and Mukthiraj, 2011) and *C. dactylon* (Aruldoss *et al*. 2014). The findings of Farahi *et al.* (2010) in *Oreochromiis mykiss* fed with *Allium sativum* and ginger (Haghighi and Rohani, 2013), Prasad and Priyanka (2011*)* in *Pangasianodon hypophthalmus* with aqueous extract of *Garcinia* gummi gutta also validated the elevated hematological values. Similar observations were reported in *C. batrachus* exposed to the ethanolic extract of *C. dactylon* (Jurry *et al*., 2014). Allied results were noticed in *C. gariepinus* significantly higher level of RBC, Hb, PCV and WBC was noticed after feeding of 0.5% garlic supplemented diet (Nwabueze, 2012).

Increased level of RBC, hemoglobin and PCV levels by the exposure of different extracts of *E. alba* may be due to enhanced erythropoiesis (Uboh *et al*., 2010; Prasad and Mukthiraj, 2011; Koffuor *et al.,* 2012). The elevation in the blood indices could be related to the chemical composition of the different part of the *E. alba.* According to the Singh *et al.* (2010) and Kulkarni and Khatwani (2011), the chemical composition of this plant includes protein,

carbohydrates, tannins, steroids, glycosides, alkaloids, alkenes, ethers, organic halogen compounds, polyacethylenes, triterpenes, flavanoids (apegenin and luteolin) and traces of nicotine, phytosterol essential oils and coumestan. Most of these constituents have haematological inducing factors that influence the production of blood from the bone marrow (Ganong, 1997). These chemicals can stimulate the formation and secretion of erythropoietin in the stem cells of animal which is the humoral regulator of RBC production (Ohlsson and Aher, 2006, Oyedemi *et al*., 2011, Ozougwu, 2011 and Mbaka and Owolabi, 2011).

Prasad and Mukthiraj (2011) reported that the presence of antioxidants in the plant extract may trigger erythropoiesis. This seems in agreement with the present study as the extract of *Eclipta alba* has antioxidant properties (Karthikumar *et al*., 2007; Kim *et al.,* 2008; Bhaskar Rao *et al*., 2009; Majumdar *et al.,* 2010 and Uddin *et al.,* 2010). The flavonoids, tannins, phenols and flavonols have been reported to possess strong antioxidant capacity (Akah *et al*., 2007). Uboh *et al*. (2010) and Shatoor (2011) suggested that flavonoids have been reported for antioxidant activity. The antioxidant activity of flavonoids may maintain the heme iron in its ferrous state and this could enhance erythropoiesis (Shatoor, 2011). Mbaka and Owolabi (2011) suggested that saponin in the extract hydrolyze and produce steroid or triterpene and the stimulatory effect of steroid on bone marrow results in increased erythropoiesis. Ikpeme *et al*. (2011) suggested that different plant part contain similar bio-reactive but in different proportion and hence vary in their action. It is quite worth information generated that hematology of the fish under study exposed to varied concentration of aqueous/alcoholic extract of root, stem and leaf of *E. alba* in different sampling points showed positive modulation.

CONCLUSION

The overall assessment between plant parts indicate that leaf extract performed significantly better followed by stem and root extracts. Increasing trend was observed in alcoholic extract treated group whereas, significant delayed effect was noticed with stem and root extracts. The hematology of *C. batrachus* exposed to varied concentration of aqueous/alcoholic extract of root, stem and leaf of *E. alba* in different sampling points showed positive modulation in hematology of fish under study. This might be helpful in improvement of fishes health and also for profitable aquaculture

Table 1: Effect of aqueous extract of leaf, stem and root on hematology of *C.batrachus*

Treatment	Plant parts			ANOVA
	Leaf	stem	Root	
Red Blood Cells (10^6 ×mm^3)				
Control	1.62 ± 0.02 b B	2.35 ± 0.02 b A	2.34 ± 0.01 b A	T**
10ppm	2.00 ± 0.03 a C	2.69 ± 0.07 a A	2.52 ± 0.02 a B	P**
20ppm	1.96 ± 0.03 a C	2.77 ± 0.04 a A	2.56 ± 0.02 a B	T x P* – 0.14
Hemoglobin (**G%**)				
Control	6.83 ± 0.08 b C	7.19 ± 0.05 c B	7.84 ± 0.11 b A	T**
10ppm	7.81 ± 0.14 a B	9.11 ± 0.24 b A	8.81 ± 0.14 a A	P**,
20ppm	7.85 ± 0.15 a C	9.50 ± 0.23 a A	8.95 ± 0.10 a B	T x P** – 0.38
Pack cell Volume (%)				
Control	25.16 ± 0.51 b A	21.72 ± 0.23 b B	22.58 ± 0.51 b B	T **
10ppm	26.83 ± 0.63 a A	27.42 ± 0.80 a A	25.46 ± 0.45 a B	P**,
20ppm	27.23 ± 0.72 a A	28.38 ± 0.66 a A	26.03 ± 0.29 a B	T x P** – 1.51
White Blood Cell (10^4mm^4)				
Control	1.85 ± 0.03 bB	2.60 ± 0.01 bA	2.60 ± 0.02 bA	T **
10ppm	2.71 ± 0.18 aB	3.14 ± 0.08 aA	3.01 ± 0.06 aA	P**
20ppm	2.73 ± 0.11 aA	3.21 ± 0.06 aA	3.09 ± 0.06 aA	T x P** – 0.13

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 leaf, stem and root on hematology of *C. batrachus*

Treatment	Plant parts			ANOVA
	Leaf	Stem	Root	
Red Blood Cells (10^6 ×mm^3)				
Control	2.20 ± 0.06 b B	2.44 ± 0.01 b A	2.39 ± 0.01 b A	T**
10ppm	2.63 ± 0.05 a A	2.78 ± 0.03 a A	2.65 ± 0.05 a A	P^{ns}
20ppm	2.62 ± 0.07 a B	2.83 ± 0.03 a A	2.69 ± 0.05 a AB	T x P^{ns} – 0.16
Hemoglobin (G%)				
Control	7.33 ± 0.15 a A	8.00 ± 0.18 b A	8.11 ± 0.20 b A	T**
10ppm	7.65 ± 0.15 a B	9.84 ± 0.31 a A	9.16 ± 0.47 a A	P**
20ppm	7.68 ± 0.19 a C	10.19 ± 0.33 a A	9.34 ± 0.42 a B	T x P** – 0.80
Pack cell Volume (%)				
Control	23.43 ± 0.80 bA	23.35 ± 0.63 b A	23.84 ± 0.73 b A	T**
10ppm	25.71 ± 1.09 aB	28.72 ± 1.02 a A	26.92 ± 1.41 a B	P**,
20ppm	26.19 ±1.26 aC	29.93 ± 1.00 aA	27.63 ± 1.38 a B	T x P** –1.43
White Blood Cell (10^4mm^4)				
Control	2.37 ± 0.03 b AB	2.24 ± 0.01 b B	2.63 ± 0.02 b A	T**
10ppm	3.03 ± 0.08 a A	2.72 ± 0.06 a B	3.10 ± 0.06 a A	P*
20ppm	3.03 ± 0.08 a AB	2.78 ± 0.69 a B	3.14 ± 0.05 a A	T x P^{ns} – 0.27

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

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