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# HEMATINIC ACTIVITY OF ECLIPTA ALBA ON CLARIAS GARIEPINUS (BURCHELL) 1822

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#### **ABSTRACT**

The purpose of present study was to evaluate the effect of aqueous and ethanolic extracts of Eclipta alba stem on hematological indices of Clarias gariepinus. Experimental fishes of average weight 70-80g were randomly divided into three groups (n=20) and kept in cement tanks. The fishes of group I served as control received only vehicle, the remaining 2 groups (II & III) act as experimental groups, administered with the 10 and 20 ppm of aqueous and ethanolic extracts every alternate day for 28 days. Blood samples were collected on day 07, 14, 21 and 28 for hematological analysis. Positive alteration in the values of RBC, Hb, PCV and WBC were recorded with both the extracts at

different sampling points, as compared to control. In red cell indices increasing trend in MCV and MCH while decreasing in MCHC was noticed after the exposure of the different doses of both the extracts. Increase in the level of RBC, PCV and MCV suggest that the stem extract is capable of stimulating erythropoiesis that could be useful as antianemic, for the treatment of anemia. Study suggested that E. alba may be used for the treatment of anemia in farmed fishes.

**KEYWORDS**: Clarias gariepinus, E. alba, hematology.

# **INTRODUCTION**

The most frequent disorder of the blood is anemia which is caused mainly by iron inadequacy. It is very common in both industrialized and developing countries. The iron insufficiency is related to its poor intake or absorption. The goodness and healing properties of herbal plants were explored by people since ancient time. Herbs are the nature's gift for living beings and as medicine they can be helpful in the treatment of anemia. For these reasons, herbs seem like a good alternative. Nutritionists feel that blood nourishing herbs aid

in the absorption of iron as well as providing other benefits to the blood. It may be useful in strengthening the hematopoietic and immune system of an individual.

Eclipta alba (Linn.) Hassk a medicinal herb is often known as false daisy or bhringraja belongs to the Family Asteraceae. It has been used by traditional healers as medicine and used in the treatment of hepatic-malfunction, hair diseases and anemia since ancient time<sup>[1]</sup>. In E. alba, presence of secondary metabolites i.e. coumestans, alkaloids, thiophanes, flavonoids, polyacetylene and their glycosides was reported by<sup>[2]</sup>. [3] and [4] discussed its pharmacological activities.

In culturable fishes blood deficiency is very unexceptional, may be caused by bacterial, fungal, viral and parasitic infections or toxicity, stress or malnutrition. Therefore the current study was designed to investigate the effect of aqueous and ethanolic extracts of stem on hematological parameters of the C. gariepinus which is a culturably important catfish of family clariidae.

### **MATERIAL AND METHODS**

## **Preparation of extract**

The plant Eclipta alba was collected from adjoining village area of Raipur city and washed thoroughly with running tap water then with deionized water. Stem was removed separately and shed dried at room temperature for two weeks then powdered in an electric blender. The extract was prepared by soxhlet apparatus using ethanol and distilled water. This cycle was repeated for 3-4 times. The solvent was concentrated by evaporating with water bath till it changes into semi solid phase. The obtained yield was 34.73% and 15.17% with aqueous and ethanolic extractants respectively from 15g stem powder.

### **Experimental Design**

The experimental fish C. gariepinus of average weight, 70-80 g were acquired from local fish market and treated with dip treatment of 0.1% potassium permagnet<sup>[5]</sup> to remove dermal infections. After acclimation of one week in laboratory conditions, fishes of mixed sexes were randomly divided in to three groups (n = 20) in concret tanks with 10 litter of water separately. Group I was kept as control received no treatment, group II and III were exposed to 10 and 20 ppm dose of aqueous or ethanolic extracts of stem every alternate day up to 28 days. The fishes were fed with goat liver ad libitum every alternate day and water was changed after 24 hours of feeding. Blood samples were collected by cutting caudal peduncle in EDTA coated tubes at fixed time on day 7, 14, 21 and 28 for hematological evaluation.



Fig.1: Eclipta alba



Fig.2: Experimental tanks



Fig.3: C. gariepinus



Fig. 4: Soxhlet apperatus

# Hematological analysis

The total RBC and WBC were counted by Neubaeur's improved haemocytometer using Hayem's and Turks fluid respectively. Hb concentration was measured by Sahlis method and PCV was determined by Wintrobe method<sup>[6]</sup>. The red cell indices viz., Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated following<sup>[7]</sup>.

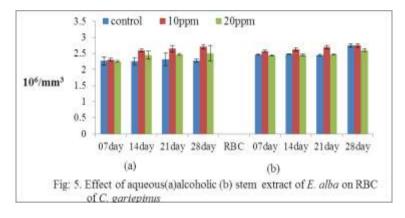
# Statistical analysis of the data

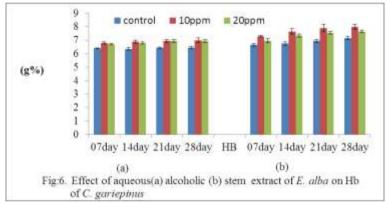
Experimental data obtained were statistically analyzed by two way analysis of variance<sup>[8]</sup>. Differences amongst means were determined using Duncan's Multiple-Range Test<sup>[9]</sup>.

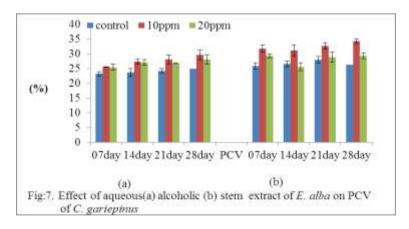
### **RESULTS**

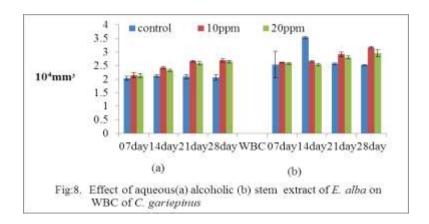
The effect of aqueous and ethanolic extracts of stem of E. alba on hematological variables (RBC, Hb, PCV and WBC) of C. gariepinus at different sampling time points from 7 to 28 days are shown in Fig. 5-8 (aqueous and ethanolic).

ANOVA shows significant treatment effect (p<0.01) on RBC, Hb, PCV and WBC after the application of aqueous and ethanolic extracts. The duration was significant at p< 0.01 for all variables exposed to extract with alcoholic solution except PCV. With aqueous extract the duration effect was significant at p<0.01 for WBC and p<0.05 for RBC and Hb only. The interaction between T x D was significant (p<0.01) for only WBC in ethanol treated group.



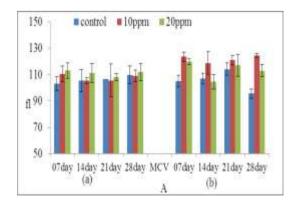


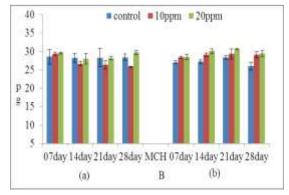




In treated with 10 and 20 ppm dose of both the extracts, increment in RBC count was noticed (Fig.5). The RBC values ranged from 2.26±0.11 to 2.71±0.07 with aqueous and 2.45±0.02 to 2.75±0.06 with alcoholic extract during all the sampling points. In alcoholic extract treated group the low dose had pronounced effect. As compared to control, Hb level was higher in test groups (Fig.6). Hb level was ranged from 6.35±0.10 to 7.00±0.18 (aqueous) and 6.65±0.10 to 8.00 ±0.18 (alcoholic). The level of PCV rose from 23.13±0.70 to 29.50±1.70 (aqueous) and 25.80±1.16 to 34.20±0.80 (alcoholic) during each sampling points. The low dose of alcoholic extract significantly alters the PCV level (Fig.7). The WBC count ranged from 2.03±0.07 to 2.69±0.05 with aqueous and 2.51±0.02 to 3.17±0.03 with alcoholic extract. Significant difference between 10 and 20 pm was observed on 28<sup>th</sup> day sampling with alcoholic extract only (Fig. 8).

The effect of aqueous and ethanolic extracts of stem of E. alba on red cell indices of C. gariepinus at different sampling time points from 7 to 28 days is shown graphically in Fig.9 (A,B and C). The effect of treatment was found to be significant at p<0.01 level with alcoholic extract on MCV, MCH whereas at 5% confidence limit on MCHC. After the exposure of the two doses of both the extract, increase in MCV and MCH and decrease in MCHC was observed.





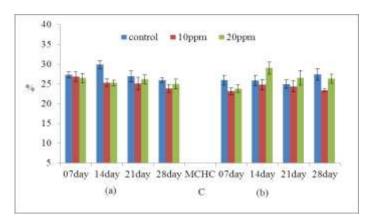


Fig. 9. Effect of aqueous (a) and alcoholic (b) extract of E. alba on MCV (A), MCH (B) and MCHC (C) of C.gariepinus

# **DISCUSSION**

The present study disclosed the supporting effect of the aqueous and ethanolic extract of stem of Eclipta alba on hemopoietic system of the C. gariepinus. This was confirmed by incremented values of RBC, Hb, PCV and WBC and border line changes in red cell indices after the application of stem extract to the experimental fishes.<sup>[10]</sup> reported recovery of hematological indices in endosulfan treated mice after the treatment of aqueous leaf extract of E.alba. Incremented values 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<sup>[11,12]</sup>, Cynodon dactylon<sup>[13,14]</sup>, in Cyprinus carpio treated with Nelumbo nucifera<sup>[15]</sup>, Zataria multiflora and Eucalyptus globolus essential oil<sup>[16]</sup> and Epibolium hirsutum<sup>[17]</sup>. Identical observations were also noticed in Oreochromis mossambicus treated with Andrographis paniculta<sup>[18]</sup> and C. dactylon<sup>[19]</sup>. The investigation of<sup>[20]</sup> in Oreochromis mykiss fed with Allium sativum and ginger<sup>[21]</sup>, <sup>[22]</sup> in Pangasianodon hypophthalmus with aqueous extract of Garcinia gummi gutta also validated the elevated hematological values. Similar finding was reported in C. batrachus exposed to the ethanolic extract of C. dactylon<sup>[23]</sup>. Allied results were noticed in C. gariepinus treated with Garcinia mangostana<sup>[24]</sup>. In C. gariepinus significantly higher level of RBC, Hb, PCV and WBC was noticed after feeding of 0.5% garlic supplemented diet<sup>[25]</sup>.

Rise in the level of RBC, Hb and PCV on the exposure of two extracts of stem of E. alba might be resulting enhanced erythropoiesis due to stimulation of erythropoietic system<sup>[18&26]</sup>. Erythropoiesis may be triggered by phyto-constituents present in the stem extracts. Saponin in the extract hydrolyses and steroid or triterpene produced that stimulate bone marrow and resulted as increased erythropoiesis<sup>[27&28]</sup>. Antioxidants present in the extracts also triggers

erythropoiesis<sup>[18]</sup>. The antioxidant activity may maintain the heme iron in its ferrous state and could enhance erythropoiesis<sup>[29]</sup>. Presence of alkaloids, saponin, tannin, terpanoid and phenol in both the extract was reported by<sup>[30]</sup>. investigated the presence of iron in all part of E.alba. It is correlated with iron content and because of high absorption of iron the hemoglobin level increased<sup>[31]</sup>.

The Red cell indices are important for the diagnosis of anemia<sup>[32]</sup>, provide information about the hemoglobin content and size of erythrocytes. Increasing trend in MCV and MCH and decreasing trend in MCHC was observed in current study. Higher values were noticed in MCV after the exposure of alcoholic extract. No difference in MCH was found between aqueous and alcoholic extract. Decline in MCHC level was induced by both the two extracts. <sup>[33]</sup> reported that gamma irradiated mice pretreated with aqueous extract of E. alba recovered MCV and MCH values after 15 days. Finally they concluded that in mice, aqueous extract of E. alba conserves the MCV and MCH level. The change in blood indices may be allocated to the defense reaction which occurs by stimulation of erythropoiesis<sup>[34]</sup>. Alteration in red cell indices was also reported in O. massambicus fed with C. dactylon mixed diet<sup>[19]</sup>.

WBCs are important blood cell of the immune system with a significant role in defending the body against infections, diseases and foreign materials. In WBC noteworthy increase was noticed immediately after the treatment of both the extracts which is an outcome of viable stimulation of non specific immune system<sup>[35]</sup>.<sup>[36]</sup> reported similar result in mice treated with methanol extract of E. alba. Increased leucocytes in fishes were documented by the use of botanicals viz., in C. gariepinus with different parts of Garcinia mangostana<sup>[24]</sup>, in Catla catla treated with ethanolic extract of Cynodon dactylon mixed diet <sup>[13]</sup>, with Coriandrum sativum and Plumbago rosea<sup>[35]</sup>.<sup>[36]</sup> and in Oreochromis mossambicus fed with different doses of methanol extract of Andrographis paniculta mixed diet<sup>[18]</sup>. In C. batrachus enhanced level of WBC was noted after the application of two doses of ethanolic extract of C. dactylon<sup>[23]</sup>. Some constituents from stem extract have stimulatory effect on bone marrow for leucocytosis<sup>[37]</sup>.

# **CONCLUSION**

Present study reveals that the extracts (aqueous and ethanolic) of stem of Eclipta alba possess hemopoietic potential and may be useful as agent to improove the hematological parameters.

### **AKNOWLEDGEMENT**

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#### REFERENCES

- 1. Sharma A, Deo A D, Tandel Riteshkumar S, Chanu T I, Das A, Effect of Withania somnifera (L. Dunal) root as a feed additive to immunological parameters and disease resistance to Aeromonas hydrophila in Labeo rohita (Hamilton) fingerlings. Fish and Shellfish Immunology, 2010; 29: 508-512.
- 2. Muruganantham S, Anbalagan G and Ramamurthy, N., FT-IR and SEM-EDS comparative analysis of medicinal plants, Eclipta alba Hassk and Eclipta prostrata Linn. Romanian J. Biophys., 2009; 19: 285-294.
- 3. Mithun N M, Shashidhara S and Vivek kumar R, Eclipta alba (L.) A review: on its phytochemical and pharmacological profile. Pharmacologyonline, 2011: 1: 345-357.
- 4. Jahan R, Al-Nahain A, Majumder S and Rahmatullah M, Ethnopharmacological Significance of Eclipta alba (L.) Hassk. (Asteraceae), International Scholarly Research Notices, 2014; Article ID 385969.
- 5. Joshi P K, Harish D and Bose M, Effect of lindane and malathione exposure to certain blood parameters in a fresh water teleost fish Clarias batrachus. Pollution Resources, 2002; 21: 55 57.
- 6. Blaxhall P C and Diasley K W, Routine haematological methods for use with fish blood. J.Fish Biol., 1973; 5: 771-781.
- 7. Dacei J W, Lewis S M, Practical Haematology (9<sup>th</sup> edition). Churchill Livington, London, 2001.
- 8. Bruning JL, Kintz BL, "Computational Handbook of Statistics". Scott, Foresman and Glenview, Illinois, 1977.
- 9. Duncan D B, Multiple range-multiple F-tests. Biometrics, 1955; 11: 1-6.
- 10. Singh T, Singh A, Nivedita, Singh S K, and Singh J K, Efficacy of Eclipta alba (L/) against sub lethal dose of endosulphan induced biochemical and hematological alterations in Swiss albino mice. Int. J. Pharm. Sci. Rew. Res., 2014; 27: 216-221.
- 11. Innocent B X, Fatima M S A, Dhanalakshmi, Studies on immunostimulant activity of Coriandrum sativum and resistance to Aeromonas hydrophila in Catla catla, Journal of Applied Pharmaceutical Science, 2011; 1: 132-135.

- 12. Innocent B X, Fatima M S A, Siva rajani S, Immune response of Catla catla fed with oral immunostimulant Plumbago rosea and post challenged with Aeromonas hydrophila, International Journal of Applied Pharmaceutical Technology, 2011; 2: 447-454.
- 13. Kaleeswaran B, Ilavenil S, Ravikumar S, Changes in biochemical, histological and specific immune parameters in Catla catla (Ham.) by Cynodon dactylon (L.), Journal of King Saud University (Science), 2012; 24: 139-152.
- 14. Kaleeswaran B, Ilavenil S, Ravikumar S, Enhancement of specific immune response in Catla catla (Ham.) against Aeromonas hydrophila by the extract of Cynodon dactylon (L.), Hitek J Bio. Sci. and Bioengg, 2012; 1: 1-15.
- 15. Vinodhini R, Detoxifying effect of Nelumbo nucifera and Aegle marmelos on haematological parameters of common carp (Cyprinus carpio L.). Interdiscip. Toxicol., 2010; 3: 127-131.
- 16. Sheikhzadeh N, Soltani M, Ebrahimzadeh-Mousavi H A, Shahbazian N, and Norouzi M, Effects of Zataria multiflora and Eucalyptus globolus essential oils on haematological parameters and respiratory burst activity in Cyprinus carpio. Iranian Journal of Fisheries Sciences, 2011; 10: 316-323.
- 17. Pakravan S, Hajimoradloo A, and Ghorbani R, Effect of dietary willow herb, Epilobium hirsutum on growth performance, body composition, hematological parameters and Aeromonas challenged on common carp, Cyprinus carpio. Aquaculture research, 2012; 43: 861-869.
- 18. Prasad G, and Mukthiraj S, Effect of methanolic extract of Andrographis paniculata (Nees) on growth and hematology of Oreochromis mossambicus (Peters). World Journal of Fish and marine Sciences, 2011; 3: 473-479.
- 19. Aruldoss K, Kannan R, Chandira A, Effect of Cynodon dactylon on the heamatological parameters in the blood of Oreochromis mossambicus. International Journal of Morden Research and Reviews, 2014; 2(5): 171-177.
- 20. Farahi A, Kasiri M, Sudagar M, Irael M S, Shahkolaei M D, Effect of garlic (Allium sativum) on growth factors, some hematological parameters and body compositions in rainbow trout (Onchorhynchus mykiss). Aquaculture, Aquarium, Conservation and Legislation International Journal of the Bioflux Society, 2010; 3: 317-323.
- 21. Haghighi M, and Rohani M S, The effect of powdered ginger (Zingiber officinale) on the haematological and immunological parameters of rainbow trout Oncorhynchus mykiss. Journal of Medicinal Plant and Herbal Therapy Research, 2013; 1: 8-12.

- 22. Prasad G, and Priyanka G L, Effect of fruit rind extract of Garcinia gummi- gatta on hematology and plasma biochemistry of catfish, Pangasianodon hypophthalmus, Asian Journal of Biochemistry, 2011; 6: 240-251.
- 23. Jurry R, Gupta S, Mishra P, Investigation of hemopoietic effect of Cynodon dactylon (L.) Pers. On Indian walking catfish, Clarias batrachus (Linnaeus, 1758).J. Exp., India, 2014; 17(2): 519-524.
- 24. Soosean C, Marimuthu K, Sudhakaran S, and Xavier R, Effect of mangosteen (Garcinia mangostana L.) extracts as a feed additive on growth and hematological parameters of African catfish (Clarias gariepinus) fingerlings, European Review for Medical and Pharmacological Sciences, 2010; 14: 605-611.
- 25. Nwabueze A A, The effect of garlic (Allium sativum) on growth and haematological parameters of Clarias gariepinus (Burchell, 1822). Sustainable Agriculture Research, 2012; 1: 222-228.
- 26. Koffuor G A, Sam G H, Dadzeasah P E, Owiafe E O, Gyapong A A, Erythropoietic effect of ethanolic root bark extract of Carissa edulis in phenylhydrazine-induced anemic sprague-dawley rats. Research Journal of Pharmacology, 2011; 6: 20-24.
- 27. Sodipo O A, Abdulrahman F I, Sandabe U K, and Akinniyi J A, Effects of aqueous fruit extract of Solanum macrocarpum Linn on the hematological parameters of triton- induced hyperlipodermic rats. African Journal of Pharmacy and Pharmacology, 2011; 5: 632-639.
- 28. Mbaka G O, Owolabi M A, Evaluation of haematinic activity and subchronic toxicity of Splenocentrum jollyanum (Menispermaceae) seed oil. European Journal of Medicinal plants, 2011; 1: 140-152.
- 29. Shatoor A S, Acute and Sub-acute toxicity of Crataegus aronia Syn. Azarolus (L.) whole plant aqueous extract in wistar rats. American Journal of Pharmacology and Toxicology, 2011; 6: 37-45.
- 30. Mishra P, Gupta S, Phytochemical screening of Eclipta alba. The Asian Journal of Animal Science, 2013; 8(2): 112-114.
- 31. Johnkennedy N, Adamma E, Austin, A, Chukwunyere N E, Influence of Xylopia aethiopica fruits on some hematological, biochemical profile. American J. Med. Sci., 2011; 4: 191-196.
- 32. Agbor GA, Oben Mcnight DC, Mills RG, Bray, JJ, and Craing PA, Human physiology.4<sup>th</sup> Edition, Churchill Livingstone, 1999; 290-294.

- 33. Singh A, Kumar R, Nivedita J K, and Singh T, Radio-protective effect of Eclipta alba (L.) against radiation induced hematological changes in Swiss albino mice, Journal of Natural Products, 2011; 4: 177-183.
- 34. Shalaby A M, Khattab Y A, and Abdel Rahman A M, Effect of garlic (Allium sativum) and chloramphenicol on growth performance physiological parameters and survival of Nile tilapia (Oreochromis niloticus), J. Venom. Animal Toxins Incl. Trop. Dis., 2006; 12: 172-201
- 35. Mohamad S, Abasali H, Effect of plant extracts supplemented diets on immunity and resistance to Aeromonas hydrophila in common carp (Cyprinus carpio), Agricultural Journal, 2010; 5: 119-127.
- 36. Jayathirtha M G, Mishra S H, Preliminary immunomodulatory activities of methanol extract of Eclipta alba and Centella asiatica. Phytomedicine, 2004; 11: 361-365
- 37. Ufelle S A, Ukaejiofo Neboh E E, Achukwu P U, Nwagha U I, and Ghasi S, The effect of crude methanolic leaf extract of Bryophyllum pinnatum on some haematological parameters in wistar rats. Research Journal ofs Pharmacology, 2011; 5: 14-17.