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Original Research Article

Evaluation of haematological properties of normal Albino rats exposed to ethanolic extract of *Hydrilla verticillata* (L.F.) Royle collected from unpolluted and polluted water sources

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A B S T R A C T

Keywords

Hydrilla verticillata, Ethanol extract, Haematological parameters, Plant samples, Platelets

Present study was undertaken to investigate the haematological properties of whole plant ethanol extract of *Hydrilla verticillata* collected from polluted and unpolluted areas. The results showed that as days increased, (21st days), the RBC counts increased by ethanol extracts of *H. verticillata* from unpolluted than the sample from polluted water. The RBC counts in induced and standard condition are lower than the plant samples from unpolluted water sample. A significant decrease in the level of WBC was observed in induced and standard conditions. Regarding the platelets counts, increased condition was reported in ethanol extract of *H. verticillata* from unpolluted plant samples.

Introduction

Analysis of blood parameters is relevant to risk evaluation of alterations of the haematological system in humans (Olson *et al*., 2000). Haematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Hoeney, 1985). It is an established fact that chronic diseases affect the blood cells adversely. Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions, which have generally been considered to be linked with many chronic health problems such as diabetes, cancers, inflammation, aging and atherosclerosis. All organisms possess antioxidant defense and repair systems that have evolved to protect them against free radicals. However these systems are insufficient to protect them completely against oxidative damage.

Several reports indicate that the aquatic plant *Hydrilla verticillata* pharmacologically

important alkaloids and glycosides are conferred its medicinal ability. The present study is aimed to investigate the possible effects of whole plant ethanolic extracts of *H. verticillata* grown in unpolluted and polluted water source on some haematological properties of albino rats.

Materials and Methods

Plant materials: *H. verticillata* samples were collected from the unpolluted and polluted water sources of Asaripallam, Nagercoil, Kanyakumari district, Tamilnadu and were authenticated by the Taxonomist of Botanical Survey of India (BSI), Coimbatore.

Water analysis: Both unpolluted and polluted water samples were collected separated from a depth of one ft below the surface and kept in one liter prewashed polythene containers separately. Half part of the water samples were analyzed for the physico-chemical parameters within 24 hours of collection and the other half part kept in refrigerator at -4°C with 1 ml conc. HNO_3 per 500 ml in order to avoid precipitation and used to analyze the heavy metals (Table 1) (APHA, 1995).

Preparation of whole plant extract: Whole plant extract of *H. verticillata* collected from unpolluted and polluted water dried separately were dried under shade and were ground to obtain the smooth powder. Ethanol extracts of the powered material were prepared separately by soaking 20 gm of the material for 72 hrs and after every 24 hrs the mixture were stirred with a sterile glass rod. After the completion of 72 hrs time period the extracts were filtered with Whatmann filter paper no.1 in order to obtain the filtrate. The filtrate was kept in water bath to obtain the crude extracts (Alade and Irobi, 1993) and is used for haematological assay.

Assay of haematological parameters in rats: Wistar albino rats (180–200 gm) were divided into seven groups of each with six animals (n=6). Group-1 served as Induced - Carrageenan and Group-2 as Standard - Indomethacin. Group-3 and 4 served were administered with whole plant ethanolic extract of *H. verticillata* collected from unpolluted water source at 200 (low) and 400 (high) mg/kg/day p.o. doses respectively. Similarly group 5 and group 6 were administered with whole plant ethanolic extract of *H. verticillata* collected from polluted water source at 200 (low) and 400 (high) mg/kg/day p.o. doses, respectively. Blood samples were collected from all animals through retro orbital sinus from 6^{th} to 21^{st} days of the treatment for blood samples collection. Rats were sacrificed using chloroform anesthesia. Blood samples were collected by cardiac puncture into EDTA capped bottles with the aid of a 2 ml syringe. The blood samples parameters were then used for evaluating various haematological parameters such red blood cells (RBC), White blood cells (WBC), platelets were estimated using an automated haematological analyser, SYSMEX-KX21 (SYSMEX Corporation, Japan) by conventional procedure as described in Baker *et al.* (1998).

The study of hematological status is one of the important ways for diagnosis of the root cause of diseases. Alternation in blood parameters may be due to changes in cellular integrity, membrane permeability and metabolism or even due to exposure to toxic chemicals (Brosche *et al*., 1990). These hematological studies give an indication that the plant extract contains phytochemicals that may stimulate or inhibit the formation or secretion of erythroprotein in the stem cells of the animals. Erythroprotein is a glycoprotein hormone which stimulates stem cells in the bone

marrow to produce red blood cells (Ohlsson and Aher, 2009). Since haemoglobin, RBC etc. are related to the total population of red blood cells in the blood (Adebayo *et al*., 2005) it could thus imply that though the extract may stimulate the production of red blood cells and hemoglobin, it could have an inhibitory effect on haemoglobin incorporation into red blood cells and a consequent reduction in oxygen exchange.

The present study showed that the extracts of *H. verticillata* whole plant have both positive and negative effect on the haematological parameters. The results (Table 2) indicate that the extract of *H. verticillata* caused an increase in the RBC and platelet counts while it reduces the WBC counts. This finding suggests that the extract of the plant contains agents that influence the production of RBC, leucocytes and platelets. The presence of such agents had been reported for *Viscum album* and other commonly prescribed medicinal plants (Bendich, 1993; Mamary, 2002). The significant increase in RBC and platelets counts implies that there was a change in the oxygen caring capacity of the blood and the transferring respiratory gases (Degruchy, 1976). The higher values of RBC and other associated parameters are suggestive of polycythemia (Nwibani *et al*., 2008). White blood cell differentials are the indicators of the ability of an organism to eliminate infection. The increase in RBC and platelets counts observed in this study suggested that *H. verticillata* extracts may be pressured for their clinical relevance in the management of anemia and immunity dependent disorders.

Polluted plant samples of *H. verticillata* showed a decline in the hematological profile as compared the plant sample from unpolluted water source. Exposure to a pollutant can impact on the growth of an organism by direct effect or indirectly by channeling the energy budget of the organism as it attempts to detoxify the contaminant (Spurgeon and Hopkins, 1996). The haematological parameters significantly decreased in polluted plant samples was due to the heavy metals which disturbed the formation of haemoglobin due to which their percentage level is decreased in the blood. Similar results were observed by Christopher *et al*. (1977); Kostic *et al*. (1993); Christensen *et al*. (1998) and Ognjanovic *et al*. (2000). They reported that chronic treatment with cadmium induced oxidative damage in erythrocytes of rats causing destruction of cell membranes as well as alternation of the oxidative enzyme system, energy metabolism and the appearance of anemia. In the results of present study, RBC, WBC and platelets counts were significantly decreased due to the cytotoxic effect of polluted source plant samples extract on the erythropoietin tissue, bone marrow etc (Fig.1). Such a disturbance in bone marrow leads to alteration of cell cycle and reduction in erythropoiesis (Nunes *et al*., 2001; Sharma and Dubey, 2007).

Oxidative stress can disrupt normal physiological pathways and cause erythrocyte destruction as observed by Kanter *et al*. (2009) who studied the cadmium toxicity. Some antioxidants can exert a protective role against heavy metals induced destruction of RBCs. There are reports in literature that tocopherol treated groups protect cells from oxidation and neutralizes unstable free radicals which can cause damage (Olcott, 1937). Thus it could be concluded that heavy metals are highly toxic compounds which cause decrease in haematological parameters while tocopherol is a good antioxidant that can overcome the heavy metals toxicity to a certain extent.

In rats, the effect of *H. verticillata* whole plant ethanol extracts on haematological activity was increased with the increase of RBC counts and duration and it was higher in samples of unpolluted water sources than the polluted sources. A significant decrease in the level of WBC was observed in standard conditions. The ethanol extract of *H. verticillata* from unpolluted water sources increased the platelets counts than the plant samples from the polluted water sources.

Table.1 Analysis of physico-chemical properties and heavy metal analysis of water samples collected from unpolluted and polluted water sources of *Hydrilla verticillata*

Parameters analysed	**Water sample from UPWS of *H. verticillata***	**Water sample from PWS of *H. verticillata***	**WHO permissible limit**
1. pH	7.20	8.60	**6.5-8.5**
2. Dissolved Oxygen	2.40	9.20	**4.00**
3. BOD	0.80	13.50	**3.00**
4. COD	0.12	2.30	**6.00**
5. Total alkalinity	1.53	2.03	**200.00**
6. Total hardness	62.00	66.00	**300.00**
7. Phosphate	0.24	0.10	**500**
8. Ammonia	1.21	3.10	**5.00**
9. Calcium	5.61	16.83	**75.00**
10. Nitrate	1.72	4.30	**45.00**
11. Potassium	0.62	0.21	**30.00**
12. Chloride	23.83	26.10	**250.00**
13. Sulphate	22.96	15.10	**200.00**
14. Magnesium	0.89	1.26	**30.00**
15. Nitrite	0.62	1.09	**40.00**
Heavy metals analysed			
1. Arsenic (As)	84.20	419.40	**100.00**
2. Cadmium (Cd)	0.01	8.11	**0.20**
3. Chromium (Cr)	21.40	72.10	**100.00**
4. Copper (Cu)	1.51	52.10	**5.00**
5. Ferrous (Fe)	99.21	812.10	**200.00**
6. Lead (Pb)	13.00	204.21	**25.00**
7. Manganese (Mn)	3.00	26.20	**5.00**
8. Nickel (Ni)	12.00	81.40	**25.00**
9. Silver(Ag)	NP	9.21	**0.20**
10. Zinc (Zn)	9.20	169.20	**30.00**

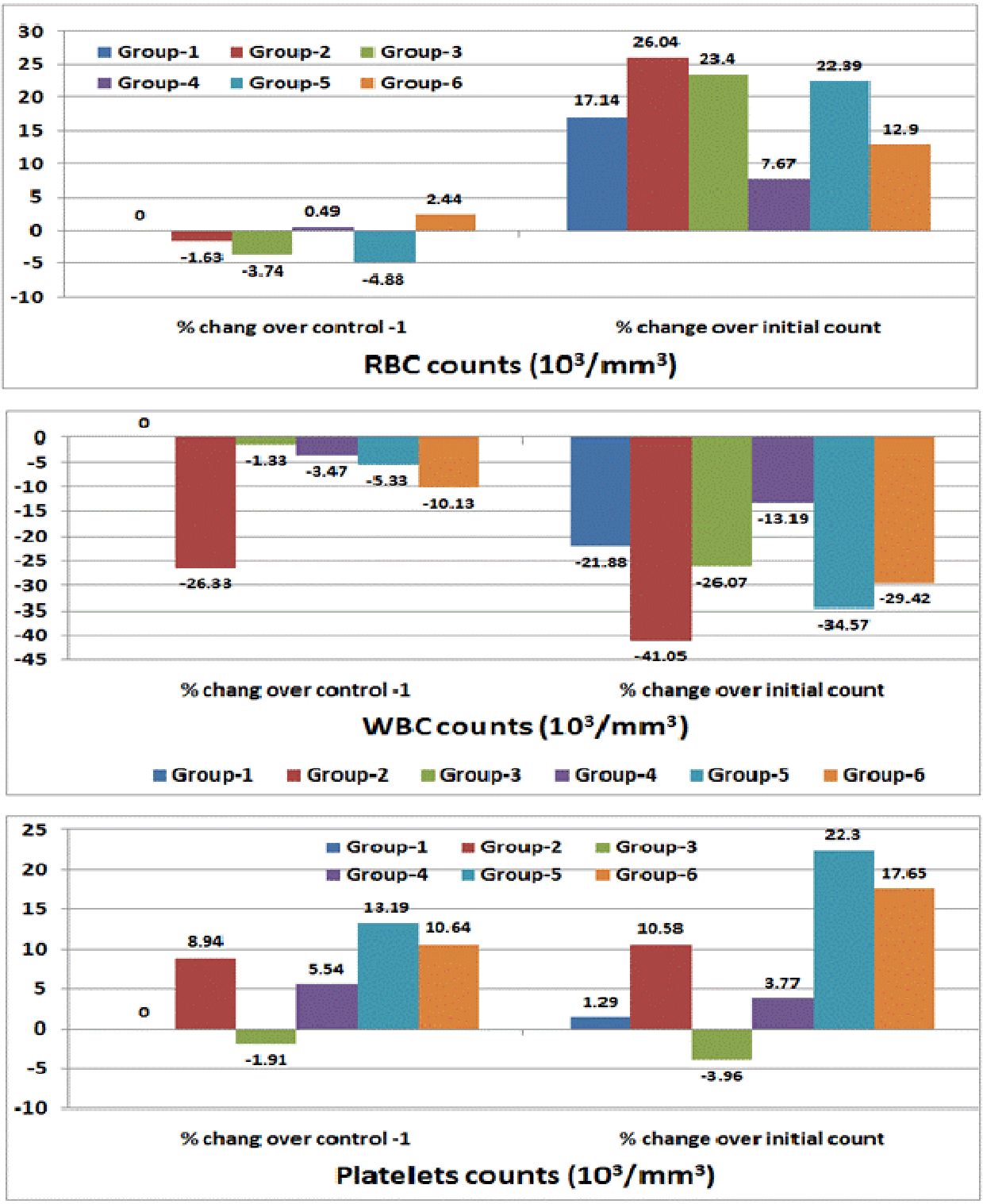
UPWS-Unpolluted Water Source; PWS-Polluted Water Source; NP- Not Present; All the parameters are expressed in mg/l except pH

Table.2 Effects of ethanol extracts of *Hydrilla verticillata* whole plant samples collected from unpolluted and polluted water sources on haematological activity in rat

Haematological parameters and Treatment Groups of rats.	Blood Counts				
	Initial Blood Count	6th day	12th day	18th day	21st day
A. RBC Counts (10^3/mm^3)					
1. Induced –Carrageenan (control-I)	5.25	5.25	5.92	6.12	6.15
2. Standard –Indomethacin (control-II)	4.80	4.80	6.12	6.03	6.05
3. Ethanol extract of *H. verticillata* from UPWS (200mg/kg body wt.)	5.17	5.15	6.35	6.34	6.38
4. Ethanol extracts of *H. verticillata* from PWS (200 mg/kg body wt.)	5.74	5.74	6.02	6.07	6.18
5. Ethanol extract of *H. verticillata* from UPWS (400mg/kg body wt.)	5.27	5.04	6.02	6.20	6.45
6. Ethanol extracts of *H. verticillata* from PWS (400mg/kg body wt.)	5.58	5.58	6.12	6.24	6.30
B. WBC Counts (10^3/mm^3)					
1. Induced –Carrageenan (control-I)	9.60	9.60	8.05	7.45	7.50
2. Standard –Indomethacin (control-II)	9.50	9.50	5.90	5.72	5.60
3. Ethanol extract of *H. verticillata* from UPWS (200mg/kg body wt.)	10.01	9.86	7.70	7.30	7.40
4. Ethanol extracts of *H. verticillata* from PWS (200mg/kg body wt.)	8.34	8.34	8.12	7.82	7.24
5. Ethanol extract of *H. verticillata* from UPWS (400mg/kg body wt.)	11.02	10.80	7.40	6.98	7.10
6. Ethanol extracts of *H. verticillata* from PWS (400mg/kg body wt.)	9.55	9.55	7.86	6.84	6.74
C. Platelets (10^3/mm^3)					
1. Induced –Carrageenan (control-I)	464	464	450	470	470
2. Standard –Indomethacin (control-II)	463	463	490	504	512
3. Ethanol extract of *H. verticillata* from UPWS (200mg/kg body wt.)	480	452	484	495	461
4. Ethanol extracts of *H. verticillata* from PWS (200 mg/kg body wt.)	478	478	492	490	496
5. Ethanol extract of *H. verticillata* from UPWS (400mg/kg body wt.)	435	428	485	524	532
6. Ethanol extracts of *H. verticillata* from PWS (400 mg/kg body wt.)	442	442	486	532	520
Two way ANOVA					
F-ratio	**RBC**		**WBC**		**Platelets**
1. Between Tr. groups	2.698 NS		2.886**		0.895 NS
2. Between periods	33.534*		25.570*		6.244*

NS-Non significant
*-1 % level of significance
**-5% level of significance
UPWS-Unpolluted Water Source; PWS-Polluted Water Source;
RBC-Red Blood Cells; WBC-White Blood Cells

Fig.1 RBC, WBC and Platelets Counts



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