Pharmacogn. Commn. 2019;9(4):139-142
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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Original Article

Hematopoietic Effect of *Amaranthus cruentus* Extract on Cyclophosphamide Induced Toxicity in Rats

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ABSTRACT

Introduction: *Amaranthus cruentus* (Amaranthaceae) is one of the popularly grown leafy vegetables in the Indian subcontinent. The leaves of the plant are rich in polyphenols, tannins, flavonoids, steroids, terpenoids, saponins and betalains. The plant is also rich in protein, calcium, iron, as well as vitamins A, E, C and folic acid. The present work was undertaken to evaluate the anti-anemic effects of *Amaranthus cruentus*. **Methods:** An ethanol extract of *Amaranthus cruentus* was prepared and the of folic acid, ascorbic acid, iron, polyphenol and flavonoid contents were estimated. Acute oral toxicity of the extract was determined according to OECD guideline-423 at doses of 200 mg/kg and 400 mg/kg. Cyclophosphamide (0.3 mg/kg, i.p.) was used to induce anemia in rats. After anemia induction, animals were treated with standard preparation (Ferritop Syrup) and the extract. **Results:** *Amaranthus cruentus* extract significantly aided to restore the levels of RBCs, WBCs, hemoglobin and increased hematocrit levels. **Conclusion:** Thus, it was concluded that *Amaranthus cruentus* is a rich source of phytochemicals that demonstrate hematopoietic effects.

Key words: *Amaranthus cruentus*, Hematopoietic, Cyclophosphamide, Hematocrit, Anemia.

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DOI: 10.5530/pc.2019.4.29.

INTRODUCTION

Anemia is a type of a 'nutritional disorder' characterized by deficiency of red blood corpuscles (RBCs) in the blood. Deficiency of iron, whether due to poor intake or poor uptake, is the main cause of this disease. Anemia affects all age groups viz. infants, child, adolescent, adult, pregnant woman and the elderly.[1] Apart from drugs, dietary management of anemia can play a leading role in controlling the disease. Intake of iron and vitamin B complex rich food and vegetable may also be advocated.

Amaranthus cruentus L. (Family: Amaranthaceae) is a widely grown leafy vegetable in Indian sub-continent. Amaranth is rich source of proteins, fibers, iron, calcium, vitamins A, E, C and folic acid.[2,3] Amaranth is also a rich source of polyphenols, tannins, flavonoids, steroids, terpenoids, saponins and betalains.[4] The herb also finds special place in traditional African medicine for the treatment of fever, hemorrhage, anemia and kidney complaints.[5] Being a rich source of iron and folic acid,[5] the present work was proposed to evaluate anti-anemic potential of *A. cruentus*.

MATERIALS AND METHODS

Plant collection and identification

The leaves of *A. cruentus* were freshly purchased from a local market of Jabalpur (23.1815° N, 79.9864° E). The plant material was identified by Dr. A. B. Tiwari, Sr. Scientist, Department of Plant Physiology, Jawaharlal Nehru Agricultural University, Jabalpur, India. The plant materials was thoroughly washed and dried at room temperature.

Chemicals

Petroleum ether and phenyl hydrazine were obtained from CDH India. All chemicals used were of analytical grade.

Drug

Ferritop-Z (Ind-Swift Ltd, India) was used as standard.

Extraction

A mass of 150 gm of dried leaves (powder) of *A. cruentus* was extracted with petroleum ether in a Soxhlet apparatus. Ethanolic extract was obtained by extracting the same plant material in ethanol in Soxhlet apparatus. The extraction was continued until the solvent became clear. The ethanolic extract was filtered and concentrated at 40°C. The remainder of the solvent was removed in air to dryness. The extracts obtained was stored at 4°C

Phytochemical Screening

Phytochemical screening of the extract was done as per standard methods.[6]

Phytoanalytical studies

Estimation of folic acid

Folic acid content in the extract was determined by method reported by Ruengsitagoon and Hattanat.[7]

Estimation of iron

Iron content was determined spectrophotometrically by the potassium thiocynate method.[8]

Estimation of Vitamin C

Ascorbic acid was determined using standard methods.[9]

Estimation of total phenolic compounds

Total phenolic content was determined by reported method.[10]

Estimation of total flavonoids content

Total flavonoids were calculated as per method reported by Orhan *et al.*[11]

Animals

Healthy adult albino rats between 10 and 12 months of age and weighing about (100-150g) were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; 25±3°C; 35-60% relative humidity) located at Shri Ram

Institute of Technology-Pharmacy animal house facility. The experimental procedure was carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guideline and was approved by Institutional Animal Ethics Committee (Protocol Approval No: CPCSEA/ Dec/20144/03; dated 22/12/2014).

Acute Oral Toxicity Studies

Ethanolic extract of *A. cruentus* was studied for acute oral toxicity as per revised OECD guidelines No. 423.[12] The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence 200 and 400 mg/kg doses of extract were used for the study.

Anti-anemic studies

Induction of anemia

Anemia was induced by intraperitoneal injection of cyclophosphamide (3 mg/kg, i.p.).[13] Anemia was considered to be induced when the red blood cell (RBC) level, as well as hemoglobin concentration, of the blood reduced by about 30%.

Animals were divided into five groups containing six animals each

Group I : Normal control

Group II : Cyclophosphamide (3 mg/kg, i.p.)

Group III : Cyclophosphamide (3 mg/kg, i.p.) + Standard treatment (Ferritop-Z)

Group IV : Cyclophosphamide (3 mg/kg, i.p.) + Extract (200 mg/kg)

Group V : Cyclophosphamide (3 mg/kg, i.p.) + Extract (400 mg/kg).

Hematological analysis

A volume of 0.5 ml of blood was collected from each rat into a 5 ml EDTA tubes (BS MediChem, Jabalpur, India) via the tail vein using syringe needles. The hematological analyses were carried out within 24 hr of blood collection. The first set of blood samples of the animals in all four groups were collected before the induction of anemia (day 1). The second set of blood samples were collected 3 days after the induction of anemia (day 3). The third set of blood samples was collected 12 days later (day 15).

RBC Count

The number of RBCs were determined by the method reported by Dacie and Lewis.[14]

WBC Count

The number of WBCs were determined by standard methods.[14]

Packed cell volume/ Hematocrit

Packed cell volume was determined by the micro-hematocrit method of Baker and Silverton.[15]

Hemoglobin

Hemoglobin levels were quantified by standard methods.[15]

Statistical Analysis

Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where the negative control group was compared with rest group. Significance was set at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Table 1: Quantitative analysis of ethanolic extract of *A. cruentus*.

Constituents	Value
Folic acid	12.55±1.39 µg/g of extract
Ascorbic acid	5.47±0.86 mg/g of extract
Iron	7.31±2.55 mg/g of extract
Total Phenolics	569.37 ± 2.44 mg GAE/100g of extract
Condensed Tannins	171.23 ± 1.55 mg QE/100g of extract

Results of phytochemical investigation are:

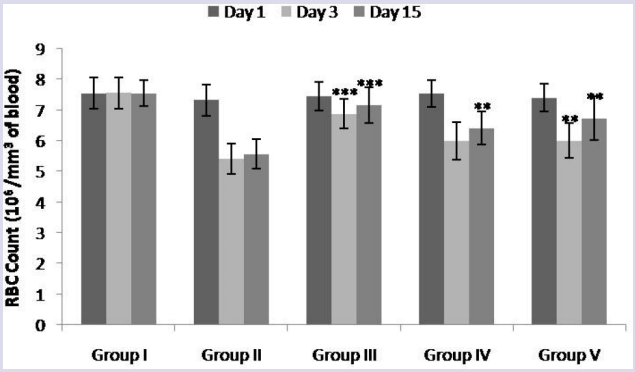

Figure 1: Effect of *A. cruentus* extract on RBC count in cyclophosphamide induced anemic rats.
Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where GroupII was compared with rest group. Significance was set at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

RESULTS

Phytochemical screening

Results of phytochemical screening revealed the presence of glycosides, flavonoids, polyphenols, carbohydrates and tannins in the extract.

Acute oral toxicity studies

The ethanolic extract of *A. cruentus* was studied for acute oral toxicity as per revised OECD guidelines No. 423. The extract was devoid of any toxicity in rats when given in doses up to 2000 mg/kg by oral route. Hence 200 and 400 mg/kg doses of extract were used for the study.

Antianemic studies

Administration of cyclophosphamide in rats caused a noteworthy decrease in the levels of red blood cells (Figure 1). Administration of standard hematinic (Ferritop-Z) caused a significant increase ($7.17 \pm 0.58 \times 10^6$/mm^3 of blood) in the level of RBCs (as observed on day 15). Comparable significant results ($^{**}p<0.01$) were observed after treatment with the extract (Group V $6.74 \pm 0.71 \times 10^6$ /mm^3 in red blood cell content) on day 15.

Following injection of cyclophosphamide, on day 3, there was notable change in levels of white blood cells ($7.19 \pm 0.44 \times 10^3$/mm^3 of blood) in the test animals (Figure 2). *A. cruentus* extracts significantly augmented in the levels of white blood cells ($7.28 \pm 0.45 \times 10^3$/mm^3 of blood in Group IV animals, $^{**}p<0.01$; $7.57 \pm 0.48 \times 10^3$/mm^3 of blood in Group V animals, $^{***}p<0.001$) after 15 days of treatment with extract.

Treatment with the standard hematinic preparation caused an incremental increase in the levels of hemoglobin (10.43 ± 0.61 g/dl; $^{***}p<0.001$; Figure 3). The *A. cruentus* extract (400 mg/ kg) also promoted increase

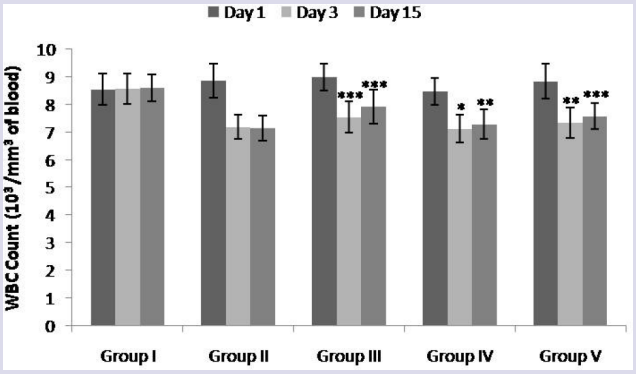

Figure 2: Effect of *A. cruentus* extract on WBC count in cyclophosphamide induced anemic rats.
Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where GroupII was compared with rest group. Significance was set at *p<0.05, **p<0.01, ***p<0.001.

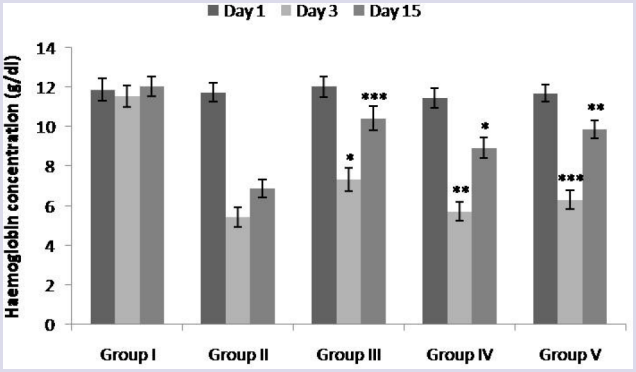

Figure 3: Effect of *A. cruentus* extract on haemoglobin in cyclophosphamide induced anemic rats.
Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where Group II was compared with rest group. Significance was set at *p<0.05, **p<0.01, ***p<0.001.

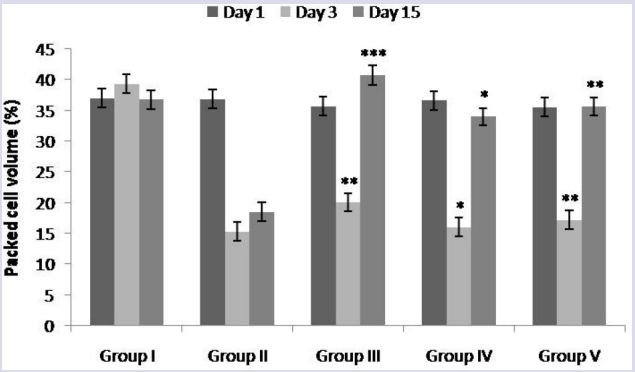

Figure 4: Effect of *A. cruentus* extract on packed cell volume in cyclophosphamide induced anemic rats.
Data are given as mean ± standard error of mean. Data were analyzed with ANOVA where Group II was compared with rest group. Significance was set at *p<0.05, **p<0.01, ***p<0.001.

in the levels of hemoglobin content (9.87 ± 0.47 g/dl; ***p<0.001) on day 15. Extract treatment caused significant restitution of hematocrit (34.02 ± 1.39 % for Group IV, *p<0.05; 35.67 ± 1.51 % for Group V, **p<0.01) as observed on day 15 (Figure 4).

DISCUSSION

Cyclophosphamide is an 'alkylating agent'. It is extensively used in combination with other drugs in 'cancer chemotherapy'. Alone, it is used as immunosuppressant to treat conditions such as lupus nephritis and rheumatoid arthritis. It causes bone marrow toxicity, which adversely influences erythropoiesis,[16] leading to anemia. Anemia is also a recurrent impediment cancer patients receiving cytotoxic drugs like cyclophosphamide.

In the present study, an *A. cruentus* extract was administered to rats receiving cyclophosphamide. Animals treated with cyclophosphamide showed less RBCs as compared to normal control rats. The *A. cruentus* extract, in dose dependent manner, not only promoted and augmented RBCs counts, but also promoted synthesis of WBCs and hemoglobin. The protective effect of *A. cruentus* extract might be due to antioxidant, antihemolytic and cytoprotective effects of flavonoids,[17] especially quercetin. Binding of flavonoid to the membrane of RBCs prevents lipid peroxidation and prevents the chances of 'hypotonic lysis'. Tryptophan residue could be the main site for these antioxidant and antihemolytic effects.[18] Amaranth constituents including flavonoids, polyphenols, betalains, ascorbic acid and iron may be responsible for antianemic effect. Thus, *A. cruentus* is rich source of phytochemicals which are responsible for anti-anemic effect. Further investigations are necessary to reveal the mechanisms involved in the induction of hemoglobin synthesis and RBCs formation.

CONCLUSION

In nutsheel, it can be regarded that *Amaranthus cruentus* is the richest source of hematopoietic phytoconstituents. More studies are necessary to ascertain the above effect.

ACKNOWLEDGEMENT

The authors are thankful to Dr. AB Tiwari, Sr. Scientist, Department of Plant Physiology, Jawaharlal Nehru Agricultural University, Jabalpur, India for identifying the plant material.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

RBC: Red Blood Corpuscles; **OECD:** Organization for Economic Cooperation and Development.

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