Haematopoietic Activity of *Asteracantha longifolia* on Cyclophosphamide-induced Bone Marrow Suppression

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Chloroform extract of the leaves of *Asteracantha longifolia* at different doses (250 and 500 mg/kg body weight) was examined on anaemic albino rat for certain haematological parameters like erythrocyte count, leukocyte count, and haemoglobin count. Chloroform extract was found to significantly ($^{b,b1,b2,b3}P<0.05$) improve erythrocyte and haemoglobin count on the 22nd day (d) after 7 d of continuous cyclophosphamide (3 mg/kg body weight) treatment. Treatment with chloroform extract along with cyclophosphamide was found to significantly ($P<0.001$) increase the bone marrow cellularity (11.5×10^6) as compared to cyclophosphamide alone treated group (6.2×10^6). Administration of extract increased the number of α-esterase positive cells (1062/4000) in the bone marrow of cyclophosphamide-treated animals, compared to the cyclophosphamide alone treated group (650/4000). The major activity of *A. longifolia* may be stimulation of stem cell proliferation. The observations that *A. longifolia* could reduce the cyclophosphamide-induced toxicity may indicate its usefulness in cancer therapy.

From ancient time, medicinal plants classified as *Rasayana* in Ayurveda are believed to be useful in strengthening the haematopoietic and immune system of an individual[1]. Anaemia is a common form of nutritional disorder, the principal cause of which is iron deficiency. It is prevalent in both industrialized and developing countries. Infants, children, women of childbearing age, pregnant women, and the elderly are considered to be particularly vulnerable to iron deficiency because their intake or absorption is poor. There is need to evaluate the potential of Ayurvedic drugs to counteract toxicity of modern therapy. *A. longifolia* Nees (Fam: Acanthaceae) is a wild herb commonly found in moist places on the banks of tanks, ditches, paddy fields, etc., throughout India and Sri Lanka. It is a source of the Ayurvedic drug *Kokilaaksha* and the Unani drug *Talimakhana.* The seeds are acrid, bitter, aphrodisiac, tonic, sedative, and useful in diseases of the blood. However, there is no information available about its effect on the blood profile as well as on the stem cell proliferation. The present investigation was designated to explore the haematological and stem cell proliferation profile on the experimental rats exposed to the extracts of *A. longifolia*[2-3].

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MATERIALS AND METHODS

Plant material and extraction:
The fresh leaves of *A. longifolia* were collected in the month of November to December from the Patharia village in Sagar and authenticated at the Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar. The powdered leaves (600 g) were successively extracted in a Soxhlet apparatus for 18 h with solvents. The extract was weighed, and percentage w/w was found to be 2.07 for chloroform extract. One gram of chloroform extract was mixed in 10 ml of groundnut oil.

Animal study was performed at the Division of Pharmacology, Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar, with due permission from institutional animal ethical committee (Registration No. 397/01/ab/CPCSEA). Cyclophosphamide was purchased from German Remedies Ltd., Mumbai. All other chemicals used were of analytical reagent grade. Swiss rats (90-110 g) and mice (25-30 g) of either sex, maintained in standard conditions for temperature, relative humidity, light/day cycles and fed with normal diet and

water *ad libitum*, were used.

Acute toxicity study:
Toxicological study revealed that Swiss rats tolerated considerably high dose of chloroform extract (1 g/kg body weight i.p.) without any toxic manifestations.

Studies on haematopoietic activity:
Test animals were divided into five groups – six Swiss rats in each group. Group I kept as control (without drug treatment), Group II was cyclophosphamide control (3 mg/kg body weight i.p.), Group III included animals treated with chloroform extract (500 mg/kg body weight i.p.), Group IV was given cyclophosphamide and chloroform extracts (3 mg/kg and 250 mg/kg body weight i.p., respectively), Group V was given cyclophosphamide and chloroform extracts (3 mg/kg and 500 mg/kg body weight i.p., respectively). Haematological parameters were evaluated in anaemic animal model. Anaemia was produced by cyclophosphamide (3 mg/kg body weight) given i.p. for 7 d[4-6]. On day 7, blood samples were collected from the orbital plexus vein of rat eye in vials containing heparin as anticoagulant and evaluated for blood parameters. Significant lowering of erythrocyte count, haemoglobin count, leukocyte count, haematocrit value, etc., was observed by haematology cell counter (Erma, Japan). After the 7th day, Groups III was treated with chloroform extract at a dose of 500 mg/kg body weight i.p., group IV and V were treated with chloroform extract at doses of 250 mg/kg body weight i.p. and 500 mg/kg body weight i.p., respectively for the next 15 d, and blood was collected on the 22nd day and evaluated for haematological parameters[7-10].

Determination of the effect of *A. longifolia* on the bone marrow cellularity and α-esterase activity:
Three groups of Swiss Mice (six mice/group) were used to carry out this study. Group one (albino mice) was treated with cyclophosphamide (25 mg/kg per body weight), group two was treated with cyclophosphamide and *A. longifolia* (20 mg/dose per animal; i.p.), and group three was treated with *A. longifolia* alone. Three albino mice from each group were sacrificed on the 15th and 22th day of treatment, and the bone marrow was collected from the femur into a medium containing 2% FCS. The number of bone marrow cells was counted using a haemacytometer and expressed as total live cells/femur. Bone marrow preparation was smeared on clean glass slides and stained with Harris Haematoxylin to determine the non-specific α-esterase activity according to reported method[11-12].

Statistical analysis:
Statistical analysis was performed with GraphPad Instat software (version 3.00, GraphPad Software, San Diego, California, USA) using one-way ANOVA, followed by Tukey-Kramer multiple comparison test. Difference with $P < 0.05$ was considered statistically significant.

RESULTS

Haematopoietic activity was evaluated by using cyclophosphamide-induced anaemia in animal model. Studies on cyclophosphamide control group II showed significant decrease in blood parameters as compared to control animals group I (without drug treatment). Comparison of group II with III, IV, and V exhibited significant ($^{a,a1,a2,a3}P < 0.05$) (Table 1) increase in haematological parameters after 7 d. Recovery period observations of group II indicated that cyclophosphamide-induced anaemia was not restored to normal count even after discontinuing the use of cyclophosphamide after 7 d. In continuation of this study, the screening of chloroform extract at the doses of 250 mg/kg and 500 mg/kg body weight i.p., respectively. Comparison of group II with III, IV, and V showed significant ($^{b,b1,b2,b3}P < 0.05$) (Table 2) increase in haematological parameters. However, change in values after 15 d showed that the haematological count was approximately restored to normal even after discontinuing of cyclophosphamide treatment. This further indicates that chloroform extracts are effective and

TABLE 1: EFFECT OF *ASTERACANTHA LONGIFOLIA* EXTRACTS IN CYCLOPHOSPHAMIDE-TREATED ALBINO RATS (AFTER 7 DAYS)

Group	I (control)	II (CP control 3 mg/kg)	III (Chloroform extract 500 mg/kg)	IV (CP + chloroform extract 250 mg/kg)	V (CP + chloroform extract 500 mg/kg)
Mean RBCs (10^6/μl)	6.05±0.750	3.45±0.945	6.59±0.210[a]	5.13±0.256[a]	5.2±0.342[a]
Mean Hb (gm/dl)	13.1±0.230	7.0±0.358	13.9±0.857[a1]	8.9±0.857[a1]	10.8±1.72[a1]
Mean WBCs (10^3/μl)	7.4±0.254	1.7±0.349	6.90±0.655[a2]	4.00±1.24[a2]	5.5±0.925[a2]
Mean HCT (%)	46.5±1.28	35.28±2.11	47.12±2.98[a3]	36.80±2.96[a3]	38.50±3.46[a3]

n=6 rats per group, tabular value represents mean±SEM, $^{a,a1,a2,a3}P<0.05$ (Comparison of II with III, IV, and V)

TABLE 2: RECOVERY PERIOD OBSERVATIONS AFTER WITHDRAWAL OF CYCLOPHOSPHAMIDE (ON 22ND DAY)

Group	I	II	III	IV	V
Mean RBCs (10^6 /µl)	6.05±0.750	4.63±0.195	6.80±0.132 ^{b}	5.35±0.100 ^{b}	8.12±0.204 ^{b}
Mean Hb (gm/dl)	13.1±0.230	7.9±0.131	14.2±0.391 ^{b1}	10.9±0.225 ^{b1}	12.1±0.468 ^{b1}
Mean WBCs (10^3/µl)	7.4±0.254	5.9±0.122	7.0±0.162 ^{b2}	5.6±0.214 ^{b2}	6.2±0.184 ^{b2}
Mean HCT (%)	46.5±1.28	35.90±1.37	47.52±1.26 ^{b3}	43.5±1.650 ^{b3}	45.8±1.15 ^{b3}

n=6 albino rats per group, tabular value represents mean±SEM, $^{b,b1,b2,b3}P<0.05$ (Comparison of II with III, IV, and V)

possess good haematopoietic activity of *A. longifolia.*

The effect of administration on the bone morrow cell number on the 15th day in the cyclophosphamide alone treated animals was 5×10^6 cells/femur, while that of cyclophosphamide in the presence of extract was 10×10^6 cells/femur. Bone marrow cellularity in the cyclophosphamide alone treated animals did not reach the normal values even after 19 d (6.2×10^6 cells/femur), whereas extract and cyclophosphamide treated group showed a bone marrow cellularity of 11.5×10^6 cells/femur on the next day. The group that was treated with extract showed normal value (15×10^6 cells/femur) (Table 3). The number of α-esterase positive cells in the bone marrow of cyclophosphamide-treated animals was low (650/4000 cells) as compared to normal animals (1090/4000 cells). A significant rise in the α-esterase positive cells was observed in the bone morrow of the group treated with cyclophosphamide along with chloroform extract of *A. longifolia* (1062/4000 cells) as well as in chloroform extract treated group (1709/4000 cells) (Table 4).

TABLE 3: EFFECT OF *A. LONGIFOLIA* ON BONE MARROW CELLULARITY

Treatment	Bone marrow cellularity (cells/femur)	
	15th day	19th day
Normal	$14.4\times10^6\pm1.2$	
CP	$5\times10^6\pm1$	$6.2\times10^6\pm1$
CP + *A. longifolia* (chloroform extract)	$10\times10^6\pm1.2$*	$11.5\times10^6\pm1.5$*
A. longifolia (Chloroform extract)	$13.9\times10^6\pm1.3$*	$15\times10^6\pm1.6$*

All the animals were treated with ten doses (3 mg/kg body wt.) of CP. Treated animals were given ten doses of (500 mg/kg body wt.) Chloroform Extract of *A. longifolia* *$P<0.05$

TABLE 4: EFFECT OF *A. LONGIFOLIA* ON α-ESTERASE ACTIVITY

Treatment	Number of α-esterase positive cells/4000	
	15th day	19th day
Normal	1090±30	
CP	774±25	650±50
CP + *A. longifolia* (chloroform extract)	959±30*	1062±33*
A. longifolia (Chloroform extract)	1437±5*	1709±6.7*

All the animals were treated with ten doses (3 mg/kg body wt.) of CP. Treated animals were given ten doses of (500 mg/kg body wt.) Chloroform Extract of *A. longifolia**$P<0.05$

DISCUSSION

From the above results, we conclude that chloroform extract of the leaves of *A. longifolia* may be used for haematopoietic activity in Ayurvedic system of medicine. The reliable criteria for judging the haematopoietic activity of a plant is to observe haematological indices in anaemic animal model. Cyclophosphamide belongs to the nitrogen mustard subclass of alkylating agents under cytotoxic drugs. In rats, cyclophosphamide has bone marrow suppressive effect and induces aplastic anaemia. Cyclophosphamide treatment (3 mg/kg body weight i.p.) resulted in significant lowering of erythrocyte count, leukocyte count, haemoglobin content, and haematocrit value %. Recovery study also indicates the potency of this plant as haematopoietic activity enhancer. All the haematological indices were restored to almost normal counts after continuous administration of the extract. A significant increase in the number of bone marrow cells and α-esterase cells observed in *A. longifolia* treated group compared to cyclophosphamide alone treated animals indicate the ability of *A. longifolia* to enhance the proliferation of stem cells. These investigations validate the use of the leaves of this plant for haematopoietic activity in Indian system of medicine.

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REFERENCES

1. Charak Samhita (Trans.). Shree Gulab Kunverba Ayurvedic Society, Jamnagar, India, 1949, 1.
2. Chopra, R.N., In; Suppliment to Glossary of Indian Medicinal Plants. Publication and Information Directorate, New Delhi. 1986, 29.
3. Kirtikar, K.R. and Basu, B.D, Eds., In; Indian Medicinal Plants, Periodical Export Books Agency, New Delhi, 1987, 1253

4. Ziauddin, M, Phansalkar, N., Patki, P., Diwanay, S. and Patwardhan, B., **J. Ethnopharmacol**., 1996, 50,69.
5. Mazumsdar, U.K., Gupta, M., Maiti, S. and Mukherjee, D., **Indian J. Exp. Biol.**, 1997, 35, 473.
6. Mazumdar, U.K., Gupta, M. and Maiti, S., **Indian J. Exp. Biol.**, 1996, 34, 1201.
7. Agrawal, R., Diwanay, S., Patki, P. and Patwardhan.B., **J. Ethnopharmacol.**, 1999, 67,27.
8. Owoyele, B.V., Alabi, O.T., Adebayo, J.O., Abioye, A.I.R. and Jimoh, S.A., **Fitoterapia**, 2004, 75, 322.
9. Pedrazzi, A.H.P., Rodrigues, E.R., Zanardo-Filho, A. and Franco, J.J., **Fitoterapia.** 1997, 38, 26.
10. Hardman, J.G. and Limbird, L.F., In; The Pharmacological Basis of Therapeutics, McGraw Hill International Edition, 1992,1410.
11. Bancroft, J.D. and Cook, H.F., In; Manual of Histologic Technique, Churchill-Livingtone, London, 1984,171
12. Davis, L. and Kuttan, G., **J. Ethnopharmacol.,** 1998, 62, 209.

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