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Phytochemistry and Ethanopharmacological Studies on *Rubia cordifolia* Linn. (Rubiaceae)

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Abstract

Ethanolic extracts of the leaves of *Rubia cordifolia* L. (Rubiaceae) (ERC) were investigated for their phytocompounds, in vitro and in vivo immunopharmacological properties. Immunomodulatory effects of this plant extract were proved in Cyclophosphamide (CP) -induced immunosuppressed animal models. Albino rats were treated with the aqueous extract of ERC and Cyclophosphamide. The Total count (TC) and Differential count (DC) of microphages and blood clotting assay were affected due to the suppressive effect of Cyclophosphamide. Administration of plant extract to CP- exposed animals resulted in enhanced immune responses.

Keywords: mmucopharmacology, Rubia cordifolia L., cyclophosphamide and blood clotting assay.

The family Rubiaceae comprises about 450 genera and 6500 species and includes trees, shrubs and infrequently herbs. *Rubia cordifolia* L. is a perennial, herbaceous climbing plant, with very long roots, cylindrical, flexuous, with a thin red bark. Stems often have a long, rough, grooved, woody base. Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots. The traditional therapeutic use of the plant has been for skin disorders and for anticancer activity. Furthermore, the anthraquinones of the Rubiaceae family exhibit some interesting in vivo biological activities, such as antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic and mutagenic functions. Apart from its medicinal value, this plant has also

been used as natural food colourants and as natural hair dyes. The interest in the isolation of natural dyes and colouring matters is increasing due to their applications in food, drugs and other human consumptions (Chopra et. al., 1992).

Rubia cordifolia L. was extensively used in traditional medicinal systems of India, China, Tibet, Nepal, and Sri Lanka for the treatment of various immune-related diseases (Jain and DeFillips, 1991; Varier, 1995). *Rubia cordifolia* L. was predominant in the Western Ghats of South India. Based on its use in Ayurvedic medicine, the plant was selected in our search for immuno bioactive compounds. To evaluate this activity, a series of test were performed.

Methodology

Collection of plant materials

Healthy plant leaves were collected from South-Western Ghats of Tirunelveli range, Tamil Nadu, India. They were collected in early morning and were washed in tap water. They were shade dried for 10 days and powdered mechanically. The collected plant materials were botanically authenticated by the Botany Department, V.H.N.S.N.College, Virudhunagar.

Preparation of plant extract

Plant extracts were prepared by the method of Tiwari et al. (2004), with slight modification of the method used to purify the ethanolic extract. Briefly, the extract was filtered through Whatman filter paper no. 1 (WHATMAN, ENGLAND) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. To obtain a concentrated crude ethanolic extract, the crude extracts were evaporated at 45oC. The quantity was determined by weighing.

Phytochemical screening

Standard phytochemical screening tests were carried out for various constituents of the ERC according to the methods of Trease and Evans (1983). Observations were carried out for colour change, precipitation or formation of an emulsion. The presence or absence of saponins tannins, alkaloids, glycosides, flavonoids, essential oils were observed.

Experimental animals

Swiss albino rats weighing 100 – 125 g of either sex were used to study the immunopharmacological activity. Rats were kept in 12 h light/12 h dark cycles under standard conditions of temperature (28oC) and relative humidity (RH: 60%) with free access to food and water. All protocols performed in this study were conducted in accordance with internationally accepted

principles for use and care of laboratory animals.

Experimental design

In each experiment, the animals were randomly divided into four groups and each group consisted of six animals. The animals in Group I served as a test control. Animals in Groups II & III treated with ERC 100 mg/ kg, b.w. orally for 21 days, on 14th day group III received a single dose of CP 30 mg/kg b.w. (i.p.) for induced immunosuppressant, and group IV received above the same concentration of CP without ERC treatment.

Hematological changes

Group II and III animals, respectively, received ERC orally at a dose of 100 mg/kg for 21 days. On the 21st day, blood samples were collected from the orbital plexuses of individual animals and the total WBC and RBC counts were determined with a haemocytometer (ROHAM, India).

Assay of blood clotting time

A modified method of Lee and White, as reported by Wintrobe (1967) was used for the assay of blood clotting time. Glass test tubes containing 0.1–1 ml each of ERC and makeup with PBS, it was equilibrated in a water bath at 370C. Blood was collected from healthy adult volunteers by venipuncture into sterile plastic disposable syringes. The blood donors had been screened for the study by a medical practitioner to ensure that they had not taken any medications for at least 1 week before the blood was collected. Starting a stopwatch, 1ml of this blood was immediately transferred into each of the equilibrated test tubes by carefully allowing the blood to run down the side of the tube. At intervals of 30 seconds, the tubes, still in the water bath, were gently tilted to an angle of 450C to check for blood clot formation. This was continued until the tubes could be inverted without blood flowing; the stopwatch was immediately stopped and the blood clotting time was recorded.

Statistical analysis

The results are presented as mean ± standard deviation (S.D.). Statistical significance between the groups was analyzed by the Student’s t-test and $P<0.01$ was considered to be statistically significant.

Results

Phytochemical screening

Phytochemical studies showed positive for alkaloids, cardiac glycosides, tannins, flavonoids and phenols, and negative for saponins, volatile oils, anthraquinones and cyanogenic glycosides, and very

trace for steroids.

Hematological changes

Total white blood cells and red blood cells in the control and plant extracts treated rats for 21 days are given in table 1. In the control rat the mean total WBC count was 13.08 ± 4.62 x 10 3/ µl. In *R. cordifolia* L. treated rats showed 13.98 ± 2.68 x 103/µl respectively. When the rats were given immune suppressive drugs cyclophosphamide treated rats total WBC count was much reduced (4.8 ± 1.3 x 10 3/ µl). If plant extract were given along with immune suppressant drugs the decrement in total WBC was less when compared to the treatment in which immunosuppressant cyclophosphamide alone was given. The results indicate that the active compounds in the extract of the plants had enhanced haematopoiesis.

Assay of blood clotting time

The blood coagulation time of 4 adult volunteers varied between 4 and 8 min, with a mean of 6.83 ±1.95 min. In the presence of 1ml of ERC, the mean of clotting time was significantly ($P < 0.01$) reduced to 2.83±1.01 min with a range of 2–4 min. The use of 1ml PBS instead of ERC, as a control, gave a mean clotting time of 7.29 ±1.98 min with a range of 5–9 min. In the tubes containing anticoagulant, the blood sample did not clot, as expected; this was a control used to ensure the integrity of the blood samples.

Discussion

The preliminary chemical constituents of selected plant *Rubia cordifolia* L. was analysed and the results showed the bioactive properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, cardiac glycosides, tannins, flavonoids and phenols. Alkaloids are the lead molecules of therapeutic importance from the selected plants. These are heterocyclic Indole compounds which have proved to be have pharmacological properties such as Antifungal activity (Ali et al., 2001), antiprotozoal, antimicrobial and antimalarial activities (Frederich et al., 2002).Literature review on the phytochemical constituents of these plants revealed that alkaloids are the major components of Rubia cordifolia L. The presence of alkaloids in *Rubia cordifolia* L. (Toshihiro et al., 1992) had been reported.

In the present study, the immuno-pharmacological activity of Rubia cordifolia L., an important plant in indigenous medicinal practice, was explored. Administration of *Rubia cordifolia* L. increased total counts of WBC and RBC cell count was significantly high in immunocomparmised animals. It

indicated that the extract had stimulated the haemopoetic system.

Blood clotting process is very complex, involving many factors found in the plasma and tissues. It involves both the intrinsic and extrinsic pathways (Brown, 1988; Jandl, 1996). Inhibitors (anticoagulants) and activators (procoagulant) of blood coagulation may affect any of the factors. The blood clotting test is used for distinguishing between the effects of ERC on the extrinsic and intrinsic pathways. Bio-active substances from the selected plant *Rubia cordifolia* L., which affect the clotting factors, are thought to act on the extrinsic pathway factors: factors V, VII, X, prothrombin and fibrinogen. The results reported here the effect of ERC show little or no anti-clotting effect when examined by this test.

Some of the chemical constituents isolated from *Rubia cordifolia* L. include alkaloids, cardiac glycosides, tannins, flavonoids and phenols (Gupta et al., 1995 and Ranjit et al., 2008). Many of these constituents isolated from *Rubia cordifolia* L. plant sources possess immunomodulatory activity (Ranjit singh et al., 2004). Most of the studies concerning the evaluation of ethanopharmacological activities of the plants have been undertaken utilizing the crude extracts.

Table 1. Effect of ethanolic extract of R.cordifolia on peripheral blood count.

Group	**treatment**	**Dose Mg/kg**	**WBC X 10^3/µl**	**RBC X 10^6/µl**
I	Control (sterile water)	-	13.08±4.62	10.73±1.34
II	*R. cardifolia* L. extract	100	13.98±2.65* (6.3)	11.23±1.04* (4.7)
III	*R. cordifolia* L. extract (100 Mg/kg) and cyclophosphamide (30 mg/kg)	130	5.97±2.1 (-50.01)	9.73±0.04 (-9.3)
IV	Cyclophosphamide	30	4.8±1.3 (-63.9)	9.93±0.84 (-7.5)

P<0.05 when compared with control.

References

Ali.Y., Munir. O ., Vahit B., 2001, Antioxidant activity of leaves *Cydonia vulgaris*. J Agric Food Chem. 29; 52(26):7884-90.

Brown, B.A., 1988. Haematology: Principles and Procedures, 5th ed. Lea and Febiger, Philadelphia, pp. 195–215.

Chopra RN. Chopra IC, Handa KL, Kapur ID. 1992. Indigenous Drugs of India, UN Dhar and Sons Pvt. Ltd., Calcutta.31 – 37.

Frederich M, Jacquier MJ, Thepenier P, De Mol P, Tits M, Philippe G, Delaude C, Angenot L and Zaches Hanrot M,2002. Antiplasmodial Activity of Alkaloids from Various *Strychnos* Species J. Nat. Prod., 65, 1381.

Gupta, A.K., Chaugule, M.A., Pakdalkar, R.K., 1995. NMR structural characterization of gallactomannan from *Cassia occidentalis*. Indian Journal of Chemistry 34, 169–170.

Jain, S.K., DeFillips, R.A., 1991. Medicinal Plants of India, vol. 2. Reference Publications, Algonac, MI, p. 558.

Jandl, J.H., 1996. Blood: Textbook of Haematology, 2nd ed. Little, Brown & Co., Boston, pp. 1213–1275.

Ranjith M.S., Ranjith singh A.J.A. and Gokulshanker, S., 2008. Enhanced phagocytosis and antibody production by *Tinospora cardifolia* – A new dimension in immunomodulation. African Jr. of. Biotechnology, 7 (2), 081 – 085.

Ranjith singh A.J.A., Dhasarathan, P., Sujatha N. and Jeypal. C., 2004, Antibacterial activities of *Cassytha* capillaries, Asian Jr. of microbial. Biotech. Env. Sc, 6 (4), 609 – 612.

Tiwari U, Rastogi B, Thakur S, Jain S, Jain NK, Saraf DK (2004). Studies on the Immunomodulatory effects of *Cleome viscose*. Ind .J. Pharm. Sci. 66: 171-176.

Toshihiro, A., Gebreyesus, T., Hiroshi, H., Toshitake, T., 1992. Cooccurrence of C-24 epimeric 24-ethylsterols possessing and lacking a _25 bond in Kalanchoe petitiana. Phytochemistry 31, 163–166.

Trease, G.E., Evans, M.S., 1983. Textbook of Pharmacognosy, 12. Bailliere Tindall, London, pp. 343–383.

Varier, P.S., 1995. Indian Medicinal Plants, A Compendium of 500 Species, vol. 4. Orient Longman,

Hyderabad, India, pp. 269–272.

Wintrobe, M.W., 1967. Clinical Haematology, 6th ed. Lea and Febiger, Philadelphia, pp. 365–367.