ANTI-ARTHRITIC PROPERTY OF THE PLANT *RUBIA CORDIFOLIA* LIN.

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Summary

Objective: To study the anti arthritic effect of ethanolic extract of the plant *rubia cordifolia*.
Methods: Arthritis is induced in albino rats by injecting Freund's Complete Adjuvant and Bovine type II Collagen. Effectiveness of the plant extract is evaluated by comparing with that of a standard non-steroidal anti inflammatory drug Aspirin. Treatment is assessed by using various blood parameters and also by taking the change in paw volume.
Results: Ethanolic extract of rubia cordifolia showed significant anti arthritic activity which was statistically similar to aspirin. The results suggest that the ethanolic extract of *rubia cordifolia* exhibits significant anti-arthritic potential.

Key words: Ethanolic extract, Arthritis, Freund's Complete Adjuvant, Bovine type II Collagen, Aspirin.

Rubia cordifolia (Family: Rubiaceae) is a climbing perennial herb with long reddish, fibrous roots. It is widely distributed in the hilly areas of India and is also found in China and Japan. Root of the plant has been suggested in Indian and Chinese traditional medicines to treat haematemesis, haematuria, inflammations, ulcers and skin diseases.[1] However, there are no clear references on the evaluation of the anti-arthritic properties of this plant in the current literature. So this study was undertaken to appraise the anti-arthritic activity of the ethanolic extract of the plant *rubia cordifolia*.

Materials and Methods

Plant material

Rubia cordifolia roots were collected from Udupi (Karnataka district, India) in the month of July 2006. Two kg of dried roots were blended to fine powder and extracted with ethanol (95%) using soxhlet method. The extract was concentrated by distillation under reduced pressure and evaporated to dryness. The total yield of the dried powder was 52.5 g.

Test animals

Male albino rats (Wistar strain) of 180-200 g were used for the studies. They were kept in cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 $\pm$ 5ºC). The rats were provided with commercial rat diet and water *ad libitum* and were divided into groups of six. The ethical guidelines for the investigation of the animals used in experiments were followed in all the tests.

Acute toxicity test

A group of 6 rats was given graded doses of 0.25, 0.5, 1 and 2 g of plant extract. Rats were continuously observed for their mortality and behavioral responses for 48 hrs and there after once daily until the 14th day. Selection of dose is done by taking 1/10 of the lethal dose. Ld50 obtained from this experiment was 3000mg/kg rat and the dose of the *rubia* extract selected for our experiment was 300mg/kg rat. The Aspirin was used as a standard drug to compare the effectiveness of *rubia.* The dose of Aspirin for the experiment was taken as 360mg/kg rat by using human-rat conversion factor.

Induction of arthritis

Arthritis was induced by injecting Freund's Complete Adjuvant (FCA) and Bovine type II Collagen. In the first method, 0.5 ml of FCA containing 10mg of dry heat killed Mycobacterium butyricum/ml of sterile paraffin oil (Difco Laboratories, Detroit, MI) was injected in to the plantar surface of left hind foot of the animal.[2,3] In the second method, 0.1ml of collagen emulsified with Incomplete Freund's adjuvant (IFA) was injected to the left hind foot.[4] The hind paw swelling in each animal was examined using a plethysmograph.

Experimental design

Animals were divided into four groups of six rats each as follows. Group I – Normal rats; Group II – Arthritic rats; Group III - Arthritic rats administered with the drug aspirin; Group IV - Arthritic rats administered with the extract of *rubia cordifolia.* Administration of *rubia cordifolia* and aspirin started on the 20th day of the induction of arthritis and continued for 20 days. During the 20 days of treatment the paw volume and the weight of the animals were recorded at regular intervals. At the end of the 20th day, animals were killed by cervical dislocation. The serum separated from the blood was collected for further biochemical assays.

Biochemical assays

Hemoglobin content was estimated by the method of Drabkin and Austin. Red blood cell and White blood cell counts were estimated according to the method of Chesbrough and Mc Arthur in an improved Neubauer chamber. Estimation of erythrocyte sedimentation rate was followed by the method of Westergren. For the estimation of copper level, the Colorimetric Bathocuproin disulphonate method of Zak and Landers was used. C-reactive protein level was estimated using the ELISA kit obtained from Alpha Diagnostics Intl., USA.

Statistical analysis

The results were analyzed using one way Analysis Of Variance followed by Bonferroni test. The values are expressed as mean $\pm$ standard deviation.

Results

From the acute toxicity test, the Ld50 obtained with the *rubia cordifolia* extract was 3000mg/kg and the $1/10^{th}$ of the Ld50 was taken as the safe dosage for the present experiment

Paw volume

Paw volume obtained its maximum value on 20^{th} day in both the arthritic models and maintained almost the same volume for the next 20 days in the arthritic group (group II). There was a significant diminution in paw volume in *rubia* treated group (group IV) and in aspirin treated group (group III) when compared to group II. The results are depicted in figure 1.

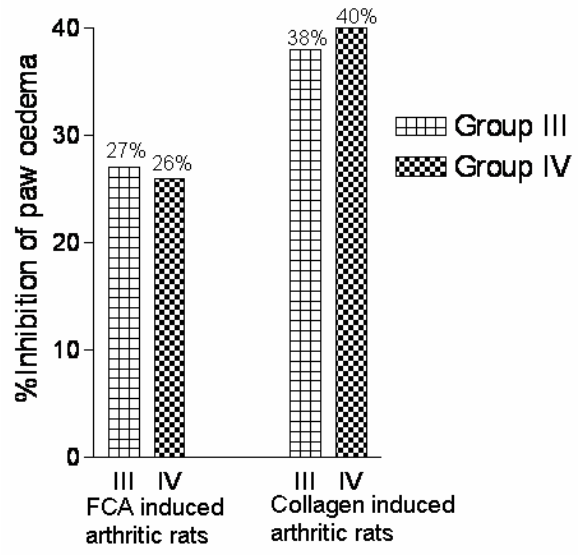

Figure I – showing the %inhibition of paw edema in FCA induced and Collagen induced arthritic rats treated with aspirin (Group III) and *rubia* extract (Group IV)

Body weight

Figure 2 and 3 demonstrates the body weight of normal and experimental group of rats. A significant decrease in the body weight was exhibited by the arthritic rats (group II) when compared to normal rats (group I). Administration of *rubia* extract (group IV) improved the body weight significantly when compared to group II in both collagen and FCA induced arthritic rats. There was no significant improvement in the weight of aspirin group (group III) when compared to group II.

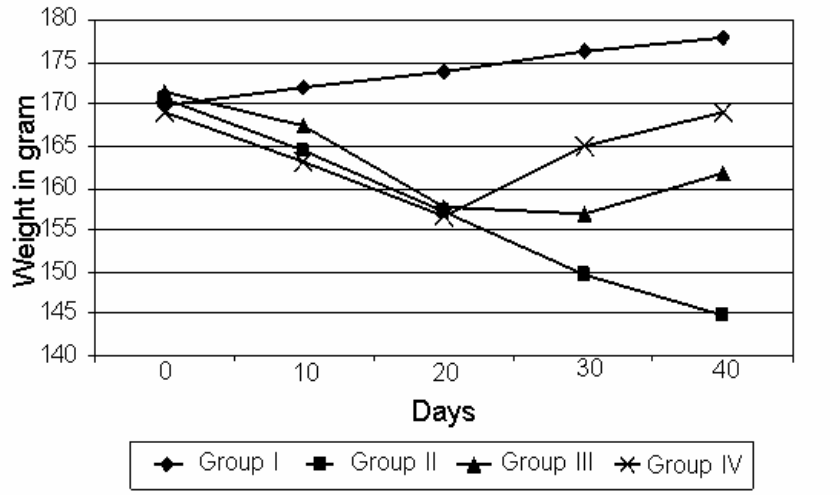

Figure II – Effect of aspirin and *rubia* extract on body weight changes in FCA induced arthritic rats.

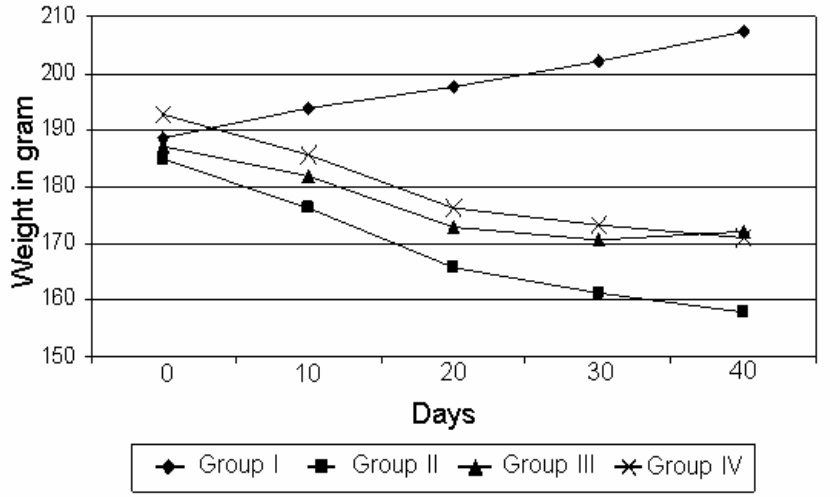

Figure III – Effect of aspirin and *rubia* extract on body weight changes in collagen induced arthritic rats

Haematological parameters

Table 1 and 2 shows haematological parameters such as Hb, RBC count, WBC count, ESR, serum copper level and C-reactive protein level of normal and experimental group of rats. A significant decrease in levels of RBC and Haemoglobin were observed in arthritic rats (group II) when compared to normal rats (group I). There was a significant improvement in the levels of Hb and RBC in *rubia* treated rats (group IV). The increased levels of WBC, ESR, serum C-reactive protein and serum Copper were significantly suppressed in the extract administered arthritic group (group IV).

Parameter	*Group I*	*Group II*	*Group III*	*Group IV*
Hb (g/dL)	12.25 ± 0.21	9.00 ± 0.18*	8.67± 0.25	10.33± 0.21*
RBC (x10^6/mm^3)	4.48 ± 0.01	3.76± 0.02*	3.78 ±0.01	4.49 ±0.02*
WBC (x10^3/mm^3)	7.34 ± 0.14	17.43 ± 0.16*	12.78 ± 0.27*	9.36 ± 0.19*
ESR	3.33 ± 0.33	10.67 ± 0.42*	10.50 ± 0.43	7.53 ± 0.26*
CRP (µg/ml)	172.9 ± 3.47	425.3 ± 9.62*	254.2 ± 3.95*	373.0 ± 3.13*
Copper (µg/ml)	103.2 ± 2.46	186.1 ± 3.25*	138.1 ± 5.00*	133.3± 4.72*

*P<0.001

Table I - Effect of aspirin and *rubia* extract on haematological parameters in normal and FCA induced arthritic rats

Parameter	*Group I*	*Group II*	*Group III*	*Group IV*
Hb (g/dL)	12.67 ± 0.21	8.92 ± 0.15*	9.17 ± 0.28	11.00 ± 0.18*
RBC (x10^6/mm^3)	4.78 ± 0.06	3.52 ± 0.09*	3.41 ± 0.15	4.16 ±0.13*
WBC (x10^3/mm^3)	6.38 ± 0.16	4.23 ± 0.25*	8.24 + 0.22*	9.25 ± 0.22*
ESR	4.17 ± 0.31	11.83 ±0.31*	11.67 ± 0.67	5.88 ±0.29*
CRP (µg/ml)	76.87 ±2.43	411.7 ±10.59*	288.3 ±5.97*	372.9 ±3.14*
Copper (µg/ml)	118.5 ± 7.27	187.8 ± 4.68*	142.0 ± 4.63*	150.4 ± 5.59*

*P<0.001

Table II - Effect of aspirin and *rubia* extract on haematological parameters in normal and collagen induced arthritic rats.

Discussion

Freund's complete adjuvant (FCA) induced arthritis and collagen induced arthritis are the two models which are extensively used to study the pathogenesis of rheumatoid arthritis for testing therapeutics.[3] Collagen-induced arthritis (CIA) is an experimental model sharing several clinical and pathological features with rheumatoid arthritis (RA). The importance of T cells in the pathogenesis of CIA and RA has been established and numerous studies have been performed to determine the cytokines and susceptibility factors involved in arthritis development.[5, 6]

Paw swelling is one of the major factors in assessing the degree of inflammation and therapeutic efficacy of the drugs.[7] Here the *rubia* treated rats showed 26% paw edema inhibition in FCA induced arthritic model and 40% inhibition of paw edema in collagen induced arthritic model. Aspirin treated rats showed 27% of paw edema inhibition in FCA induced arthritic model and 38% inhibition in collagen induced arthritic model.

In this present study, the arthritic rats (group II) exhibited a reduced RBC count, reduced Hb level and an increased ESR. All these indicate the anaemic condition which is a common diagnostic feature in patients with chronic arthritis.[8,9] The treatment with the *rubia* extract improved the RBC count, Hb level and the ESR to a near normal level indicating the significant recovery from the anaemic condition.

White blood cells are a major component of the body's immune system. Indications for a WBC count include infectious and inflammatory diseases.[10] WBC count was increased in arthritic rats. The migration of leukocytes is significantly suppressed in *rubia* treated rats (group IV) as seen from the significant decrease in the WBC count

C-reactive protein is a member of the class of acute phase reactants and is used mainly as a marker of inflammation. Measuring and charting C-reactive protein values can prove useful in determining disease progress or the effectiveness of treatments as its levels rise dramatically during inflammatory processes.[11] The level of CRP is significantly reduced in plant treated groups as well as in aspirin treated groups

Increased level of copper ion indicates the inflammatory condition.[12] Serum copper concentration was measured in normal and arthritic rats. The arthritic rats exhibited a significant elevation of copper level and this was suppressed in *rubia* and aspirin treated rats.

In brief, this study clearly shows the anti arthritic properties of the plant *rubia cordifolia*. This plant is reported to contain anthraquinones such as rubiadin, munjistin and purpurin.[13] Various research works in the current literature indicate the anti arthritic properties of anthraquinones[14] and the presence of this agent may be one of the reasons behind the anti arthritic potential exhibited by this plant. More works need to be done in the field of separation of the active components from the ethanolic extract of the plant rubia, that may involved in determining the effectiveness of this plant towards the treatment of arthritis.

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