

Comparing the anti-arthritic activities of the plants *Justicia gendarussa* Burm F. and *Withania somnifera* Linn

Jaijesh Paval, Srinivasan Keloth Kaitheri¹, Bhagath Kumar Potu², Sreejith Govindan³, Raju Suresh Kumar⁴, Sareesh Naduvil Narayanan⁴, Sudheer Moorkoth¹

Departments of Anatomy, ³Microbiology and ⁴Physiology, Melaka Manipal Medical College, ¹Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Science, ²Department of Anatomy, Kasturba Medical College, Manipal, Karnataka, India

The aim of this study was to compare the anti-arthritic activities of the plants *Justicia gendarussa* and *Withania somnifera*. Arthritis is induced in male albino rats using Freund's complete adjuvant and bovine type II collagen. Leaves of *J. gendarussa* and roots of *W. somnifera* were powdered and extracted with ethanol (95%) using the soxhlet method. The effect of these plant extracts on arthritic rats were assessed by various blood parameters and also by taking the change in paw volume. The plants *J. gendarussa* and *W. somnifera* suppressed the anti-arthritic changes induced in rats and the results were statistically significant.

Key words: Arthritis, collagen, paw volume, plant extract

INTRODUCTION

Withania somnifera (Family: Solanaceae), commonly known as ashwagandha, is a medicinal plant widely found in India and North America.^[1] The root of this plant contains flavonoids and various compounds of the withanolide group. Pharmacological studies on animal models support the antitumour and radio-sensitizing effects of this plant.^[2] The alkaloid fraction of the root extract exhibits hypotensive, bradycardiac and respiratory stimulant activities in dogs. Studies also provide information about the antibacterial, antitumour, anti-arthritic and immunosuppressive properties of this plant.^[3]

Justicia gendarussa (Family: Acanthaceae) is a shade loving, quick growing, ever green plant which is mostly found in moist areas. It is considered to be a native of China and is distributed widely in India, Sri Lanka and Malaya. In the Indian and Chinese traditional medicines, the leaves of the plant is recommended to treat ailments such as fever, hemiplegia, rheumatism, arthritis, headache, ear ache, muscle pain, respiratory disorders and digestive troubles.^[4,5]

Although the anti-arthritic effect of *W. somnifera* is well known, there are no published scientific studies on the anti-arthritic activities of the leaves of *J. gendarussa*. The objective of this study was to

compare the anti-arthritic potential of these two plants using two arthritic rat models.

MATERIALS AND METHODS

Plant Material

Leaves of *J. gendarussa* and the roots of *W. somnifera* were collected from Udupi (Karnataka district, India) in the month of August 2006. Two kilograms of dried leaves of *J. gendarussa* and 2 kg of dried roots of *W. somnifera* were blended to fine powder separately and extracted with ethanol (95%) using the soxhlet method. The extracts were concentrated by distillation under reduced pressure and evaporated to dryness. The total yield of the *J. gendarussa* extract was 65 g and that of the *W. somnifera* extract was 20.97 g.

Test Animals

Male albino rats of Wistar strain weighing around 180-200 g were used for the studies. The animals were housed in cages under standard laboratory conditions (12:12 h light/dark cycle at 25 ± 5°C). The rats were fed 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 six rats was given graded doses of 0.25, 0.5, 1 and 2 g of plant extracts. The rats were continuously observed for their mortality and behavioural responses for 48 h and thereafter once daily until the 14th day. The selection

Address for correspondence: Dr. Jaijesh Paval, Department of Anatomy, Melaka Manipal Medical College, Manipal, Karnataka, India.
E-mail: jaijesh@yahoo.co.in

Received: 21-03-2008; **Accepted:** 31-12-2008; DOI: 10.4103/0973-8258.59732

of a dose is done by taking 1/10 of the lethal dose. Ld₅₀ obtained from *J. gendarussa* extract was 1000 mg/kg rat and that from *W. somnifera* extract also was 1000 mg/kg rat. The experimental dose of both the extracts was selected as 100 mg/kg rat.

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 10 mg of dry heat-killed *Mycobacterium butyricum*/ml of sterile paraffin oil (Difco Laboratories, Detroit, MI, USA) was injected into the plantar surface of left hind foot of the animal.^[6,7] In the second method, 0.1 ml of collagen emulsified with incomplete Freund's adjuvant (IFA) was injected to the left hind foot.^[8] 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 root extract of *W. somnifera*; Group IV – arthritic rats administered with the leaf extract of *J. gendarussa*. Administration of *J. gendarussa* and *W. somnifera* was 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, the animals were killed by cervical dislocation. The serum separated from the blood was collected for further biochemical assays.

Phytochemical Screening

Since the components of the ethanolic root extract of *W. somnifera* were well known, only the *J. gendarussa* extract was tested to identify the presence of alkaloids, steroids, flavonoids, etc. using standard procedures.

Biochemical Assays

Hemoglobin content was estimated by the method of Drabkin and Austin.^[9] Red blood cell and white blood cell counts were estimated according to the method of Chesbrough and Mc Arthur in an improved Neubauer chamber.^[10] Estimation of erythrocyte sedimentation rate was followed by the method of Westergren.^[11] For the estimation of copper level, the colorimetric bathocuproine disulfonate method of Zak and Landers was used.^[12] C-reactive protein level was estimated using the ELISA kit obtained from Alpha Diagnostics Int., USA.

Statistical Analysis

The results were analyzed using one-way analysis of variance followed by the Bonferroni test. The values are expressed as mean + standard deviation.

RESULTS

Paw Volume

In arthritic rats, the paw volume reached its maximal size on 20th day in both the arthritic models. Paw maintained its inflamed condition for the next 20 days in Group II rats. A significant reduction in paw volume was observed in *W. somnifera*-treated group (group III) and *J. gendarussa*-treated group (group IV) when compared to group II. The results are illustrated in Figure 1.

Body Weight

Figures 2 and 3 shows the body weight of normal and experimental groups of rats. There is a significant decrease in the body weight of arthritic rats (group II) when compared to normal rats (group I). Administration of *J. gendarussa* and *W. somnifera* extracts improved the body weight significantly when compared to group II in both collagen and FCA-induced arthritic rats.

Hematological Parameters

Tables 1 and 2 shows hematological parameters such as Hb, RBC count, WBC count, ESR, serum copper level and

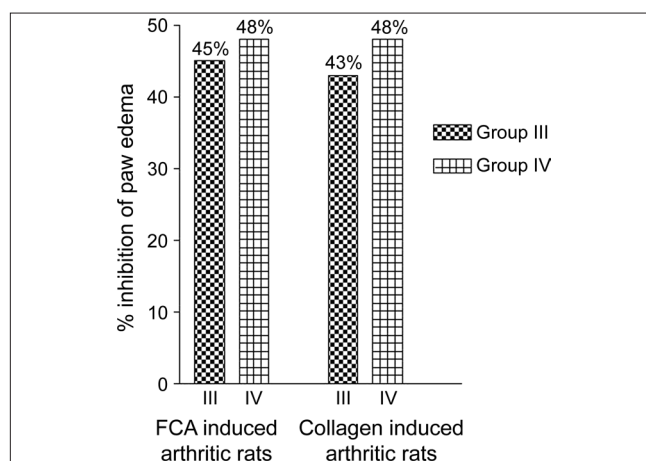


Figure 1: Percentage inhibition of paw oedema

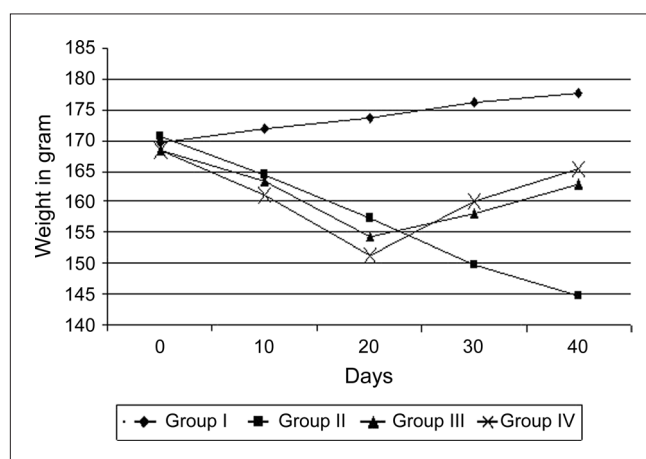


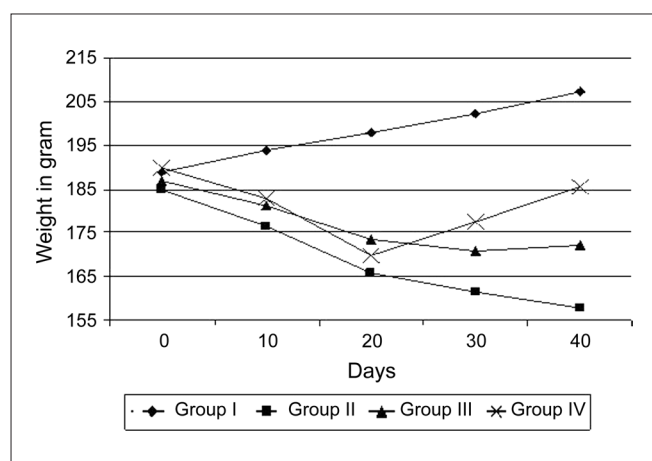
Figure 2: Change in the weight of normal and Freund adjuvant-induced arthritic rats

Table 1: Change in the hematological parameters of normal and Freund adjuvant-induced arthritic rats

Parameter	Group I	Group II	Group III	Group IV
Hb (g/dL)	12.25 ± 0.21	10.92 ± 0.20*	8.67 ± 0.25*	11.75 ± 0.21*
RBC ($\times 10^6/\text{mm}^3$)	4.48 ± 0.01	3.76 ± 0.02*	4.15 ± 0.01*	4.14 ± 0.17*
WBC ($\times 10^3/\text{mm}^3$)	7.34 ± 0.01	17.43 ± 0.16*	9.16 ± 0.24*	8.0 ± 0.18*
ESR	3.33 ± 0.33	10.67 ± 0.42*	6.30 ± 0.28	5.17 ± 0.31*
CRP ($\mu\text{g}/\text{ml}$)	172.9 ± 3.47	425.7 ± 9.62*	372.7 ± 3.64*	285.0 ± 3.14*
Copper ($\mu\text{g}/\text{ml}$)	103.2 ± 7.27	186.1 ± 3.25*	140.1 ± 3.66*	124.3 ± 6.26*

* $P < 0.001$ **Table 2: Change in the hematological parameters of normal and collagen induced arthritic rats**

Parameter	Group I	Group II	Group III	Group IV
Hb (g/dL)	12.67 ± 0.21	8.92 ± 0.15*	10.58 ± 0.33*	11.67 ± 0.28*
RBC ($\times 10^6/\text{mm}^3$)	4.78 ± 0.06	3.52 ± 0.09*	4.20 ± 0.06*	4.40 ± 0.12*
WBC ($\times 10^3/\text{mm}^3$)	6.38 ± 0.16	14.23 ± 0.25*	9.35 ± 0.20*	7.48 ± 0.13*
ESR	4.17 ± 0.31	11.83 ± 0.31*	5.05 ± 0.15	5.83 ± 0.40*
CRP ($\mu\text{g}/\text{ml}$)	76.87 ± 2.43	411.7 ± 10.59*	199.3.2 ± 5.79*	111.4 ± 0.69*
Copper ($\mu\text{g}/\text{ml}$)	118.5 ± 7.27	187.8 ± 4.68*	127.80 ± 4.58*	127.3 ± 4.41*

* $P < 0.001$ **Figure 3:** Change in the weight of normal and collagen-induced arthritic rats

C-reactive protein level of normal and experimental groups of rats. A significant decrease in levels of RBC and Hb were observed in arthritic rats (group II) when compared to normal rats (group I). Administration of *J. gendarussa* and *W. somnifera* extracts to arthritic rats enhanced the levels of Hb and RBC to near normal levels. The increased levels of WBC, ESR, serum C-reactive protein and serum copper were significantly suppressed in the extract-administered arthritic groups.

DISCUSSION

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.^[7] There is evidence which indicates that arthritis and certain other connective tissue diseases are caused by hypersensitive mechanisms. Adjuvant-induced arthritis has been widely used as an experimental model of such diseases.^[7] 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 various studies have been performed to determine the cytokines and susceptibility factors involved in arthritis development.^[13,14]

Paw swelling is one of the major factors in assessing the degree of inflammation and therapeutic efficacy of the drugs.^[15] Here, the *J. gendarussa*-treated rats showed 48% paw oedema inhibition in both FCA-induced arthritic model and collagen-induced arthritic models. *W. somnifera*-treated rats showed 45% of paw oedema inhibition in the FCA-induced arthritic model and 43% inhibition in the collagen-induced arthritic model.

In the present study, the arthritic rats (group II) showed a reduced RBC count, reduced Hb level and an increased erythrocyte sedimentation rate. All these indicate the anemic condition and is commonly noted in patients with chronic arthritis.^[16] The two most common reasons for anemia in arthritic patients are gastrointestinal blood loss from arthritis medications and bone marrow changes in patients with inflammatory arthritis, which prevent the release of iron for incorporation into RBCs.^[17,18] The extract-treated groups showed a significant recovery from anemia.

The increase in WBC count in arthritic rats was significantly suppressed in *J. gendarussa*- and *W. somnifera*-treated rats.

C-reactive protein is a member of the class of acute phase reactants as its levels rise dramatically during inflammatory processes.^[19] The level of CRP is significantly reduced in plant-treated groups.

Ceruloplasmin is an enzyme synthesized in the liver containing eight atoms of copper in its structure. Free

copper ions are powerful catalysts of free radical damage. By binding copper, ceruloplasmin prevents free copper ions from catalyzing oxidative damage.^[20] The increased level of copper ion indicates the inflammatory condition.^[21] 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 *J. gendarussa*- and *W. somnifera*-treated rats.

The phytochemical analysis of the *J. gendarussa* extract showed the presence of flavonoids vitexin and apigenin. The plant *J. gendarussa* was reported to yield β -sitosterols, alkaloids, reducing sugars and unidentified sterols.^[22] The compounds such as flavonoids and β -sitosterols are well known for their anti-inflammatory activities and the presence of these compounds in the leaf extract may be the reason behind the anti-arthritic properties shown by this plant.

The chemistry of *W. somnifera* has been widely studied and many chemical components have been isolated.^[23] They include alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII) and withanolides with a glucose at carbon 27 (sitoindoside IX and X).^[24]

There have been various studies to support the anti-arthritic activities of the plant *W. somnifera*. The compound Withaferin A was found to exhibit fairly potent anti-arthritic and anti-inflammatory activities. It was found to suppress the adjuvant-induced arthritic changes in rats without any toxic effect.^[25-27]

CONCLUSION

This study clearly showed that the anti-arthritic property of the plant *J. gendarussa* can be comparable with that of the *W. somnifera*. Further studies are needed in the isolation of other compounds that may be involved in determining the anti-arthritic potential of the plant *J. gendarussa*.

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Source of Support: Nil, **Conflict of Interest:** None declared.

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