The Journal of Phytopharmacology 2021; 10(6):510-513
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(6): 510-513
November- December
Received: 24-10-2021
Accepted: 12-12-2021

doi: 10.31254/phyto.2021.10614

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Phytochemical evaluation and Pharmacological screening of *Cuscuta reflexa* roxburg on anti-arthritic activity

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ABSTRACT

Cuscuta reflexa Roxb, a rootless, leafless, twining annual parasite with a wide variety of species, is an extensive climber found in temperate and tropical regions. It is widely used in Ayurvedic (traditional medicine native to the Indian subcontinent) medicine to relieve and treat many diseases. There are numerous countries in which it is widely used for treating urination disorders, bilious disorders, diabetic disorders, and inflammatory diseases, including Afghanistan, Malaysia, India, and China. The aim of this study is to demonstrate the anti-inflammatory and antiarthritic properties of the methanolic extract of *Cuscuta reflexa* Roxburg. A variety of phytoconstituents are found in it, such as alkaloids, tannins, coumarins, phenolic compounds, flavonoids, and saponins. These phytoconstituents are screened by various conformation tests. The anti-inflammatory properties were evaluated in vivo in rats using CFA-Complete Fruend's adjuvant induced polyarthritis model. This study shows that methanolic extract at a concentration of 400mg/kg inhibits arthritic activity. This result was compared with the standard drug, prednisolone 5mg/kg. The anti-arthritic activity of *Cuscuta reflexa* Roxberg was evaluated by considering paw volume, paw thickness and body weight. Visual criteria were used to monitor the morphological features of arthritis such as redness, swelling, erythema. The scores were recorded during these specific days of the project- 1, 4, 10, 14, 17 and 21. The test compound at 200mg/kg doesn't show any anti-arthritic property, but at the dose of 400mg/kg it proved its significant action to reduce the inflammation and pain induced by complete Freund's Adjuvant (CFA). Based on the systemic analysis, the extract maintained normal joint parameters and greatly restored the normal architecture of the joints in animals. Thus, *Cuscuta reflexa* Roxburg would be an interesting source for antiarthritic activity.

Keywords: *Cuscuta reflexa* R0poxburgh, Anti Arthritic activity, Anti Inflammatory activity, Fruend's Adjuvant, Prednisolone.

INTRODUCTION

Globally, almost 80% of the population believes that traditional medicines are better for their health needs due to cultural acceptance, fewer side effects, and better compatibility with the human body, according to the World Health Organization (WHO).

Cuscuta reflexa Roxburgh (Convolvulaceae) is commonly known as Giant Dodder [2]. This type of plant species is usually observed in the Indian Subcontinent and the Greater Himalayas [3]. Plants of this species are twined and leafless and grow over their hosts. In a short amount of time, it can cover the host plant with hundreds of branches and drain its life. White, bell-shaped flowers with yellow filaments are compact, compact, and compact. Fruits and seeds originated from the flower. This species is taken in preparation methods of traditional and cultural medicines for the cure of headache, labour pain, bone fracture, fever, rheumatism [3], anti-microbial [4], antitumor [5], anti-inflammatory [6].

Arthritis is a chronic autoimmune disease that affects primarily the joints [7]. The most common symptoms are warm, swollen, and painful joints. In many cases, stiffness and pain worsen following rest. On either side of the body, the same joints are typically involved, commonly the wrists and hands. In addition to affecting the skin, eyes, lungs, heart, nerves, and blood, the disease may also affect various parts of the body severely. Reduced red blood cell count, inflammation of the lungs, and inflammation of the heart may result from this. There may also be fever and low energy [7]. Symptoms usually emerge over weeks to months [8].

There is no known cause of rheumatoid arthritis, but scientists believe that genetics and environment play a role. The body's immune system attacks the joints as the primary mechanism [7]. Joint capsules become inflamed and broaden due to this process. It also affects the underlying bone and cartilage. An individual's symptoms and signs are the most important factors in diagnosing their condition [8]. Radiology and laboratory testing may help determine a diagnosis or rule out other diseases with

homogeneous symptoms. There are other diseases that are similar to lupus erythematosus, psoriatic arthritis, and fibromyalgia. Considering the anti-inflammatory activity, we have screened for anti-arthritic activity.

MATERIALS AND METHODS

Experimental animals

The study was conducted on male wistar rats of 150-200g. They were kept in polypropylene cages and maintained at 27*2 C, relative to 65*10%, under 12h light/dark cycles. 48 hours before the experiment began, the animals were given an opportunity to acclimatize to laboratory conditions. The animals were supplied with standard rodent pellet diet and water (libidum) in all sets of experiments, and the food was withdrawn 18-24 hours before the experiments were conducted after obtaining permission from the Institutional Animal Ethical Committee (IACE).

Chemicals and drugs

Complete Freund's Adjuvant (CFA), prednisolone [9] (tablets), methanol, saline water and all other chemicals used in this study were analytical grade and procured from approved chemical suppliers.

Preparation of crude extract

For the extraction process, leaves of *Cuscuta reflexa* were air dried and coarsely powdered. The coarse powder of the plant was extracted using the following solvents in order of increasing polarity: petroleum ether, chloroform, ethyl acetate, and methanol. Initial methanol bath of powdered sample was conducted over 15 days in a conical flask with occasional shaking. We collected the solvents along with components after 15 days and filtered them using whatman # 1 filter paper. Under reduced pressure, the extracts were concentrated using a rotary evaporator, and they were then dried in the open air. In order to treat arthritis, dried methanolic extracts were dissolved in saline (vehicle) water.

Freund's adjuvant induced arthritis

In this study, albino male rats (wistar stain) were divided into four groups, including diseased or control, standard, and drug-treated (two groups of methanolic extract low and high dose treated group tested for 1-200 mg/kg, test for 2-400 mg/kg). Group1 served as the control group and received 1% saline water (1 ml/1kg body weight), group II was the standard group and received diclofenac sodium 10mg/kg suspended in saline water, group III was the first test group receiving methanolic extract at dose of 200 mg/kg orally and the last 4th group was given methanolic extract at dose of 400 mg/kg orally. Male albino rats were given 0.1 ml of Freund's complete adjuvant (FCA) in the plantar region of the left hind foot. On the day of adjuvant injection, both hind paw volumes were measured using plethysmometer and the body weight was recorded. On the day of Freund's adjuvant injection, concentrates of plant aerial parts were dosed at 200 mg/kg and 400 mg/kg, respectively, and prednisolone was administered at 10 mg/kg orally for 14 days. Following Freund's adjuvant injection, paw volumes were measured at different times up to 21 days after the injection. An inflammatory response was measured and recorded using mercury plethysmometer on the 1st, 7th, 14th and 21st days following the adjuvant injection. On the 1st, 7th, 14th and 21st days following the adjuvant injection, rats were weighed using digital weighing balance.

Arthritic score

Monitoring morphological features of arthritis, such as redness, swelling, and erythema, was done visually: normal paw = 0, mild swelling and erythema of digits = 1, swelling and erythema of the digits = 2, severe swelling and erythema = 3, gross deformity and inability to use the limb = 4 on respective days. As a result, both hind paws could be scored at the maximum of 8.

Paw volume

In order to measure the volume of the left hind paw of the animals on day 0 and day 21, we used a plethysmometer (UGO Basile, Italy). As a result, the final paw volume differed from the initial paw volume. Using a Vernier caliper the left hind paw joints were measured after arthritis was induced on the above–mentioned testing days [10].

The percentage inhibition of paw volume was calculated by using below formula

$$\% \text{ inhibition of paw volume} = 1 - (Vt/Vc) \times 100$$

Where,
Vc = Paw oedema volume of control group (untreated)
Vt = Paw oedema volume of treated group

Paw thickness

The hind paw thickness of all rats were measured by using vernier callipers at days 0,1,7,14 and 21. The following formula was used to calculate the inhibition percentage in paw thickness,

$$\% \text{ inhibition of paw thickness} = 1 - (Tt/Tc) \times 100$$

Where,
Tc = Paw thickness of control group (untreated)
Tt = Paw thickness of treated group

Body weight

Body weight of all rats was noted every day from day 0 to day 21

The percentage change in body weights was calculated for day 1, 7, 14 and 21

$$\% \text{ change in body weight} = 1 - (Wo/Wt) \times 100$$

Where,
Wo = Body weight of rats at day 0
Wt = Body weight of rats at time t

RESULTS

Evaluation of antiarthritic activity

Table 1: Effect of CRE on Paw volume

Group	Day 0	Day 7	Day 14	Day 3	Day 21
Disease control	0.28	0.4	0.5	0.5	0.6
standard	0.32	0.4	0.6	0.6	0.5
Test 1	0.28	0.8	0.6	0.5	0.4
Test 2	0.32	0.6	1.1	0.6	0.6

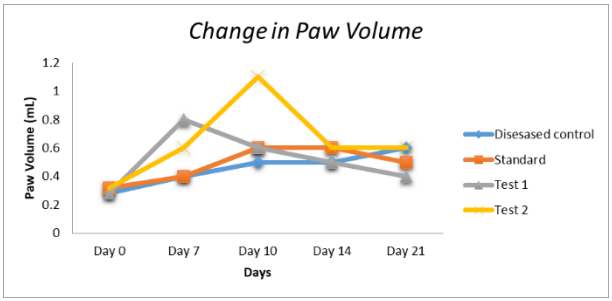

Figure 1: Percentage inhibition on paw volume

Paw volume should be decreased in case of test compounds compared to diseased simultaneously 400mg of test shows more potent action as like standard.

Table 2: Effect of CRE on Paw thickness

Group	Day 0	Day 7	Day 14	Day 3	Day 21
Disease control	0.416	0.7	0.6	0.5	0.6
standard	0.414	0.6	0.6	0.5	0.4
Test 1	0.414	0.7	0.7	0.6	0.4
Test 2	0.412	0.7	0.7	0.6	0.6

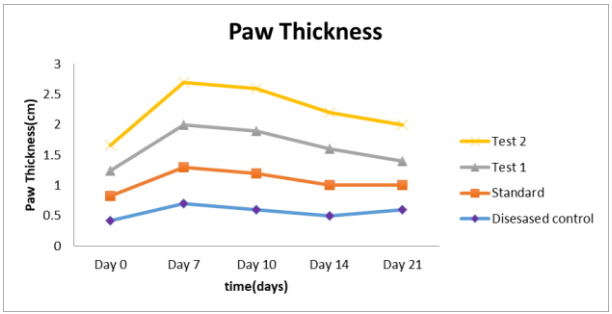

Figure 2: Percentage inhibition of paw thickness

Test 2 (400mg/kg) has more potent action than test 1(200mg/kg).

Table 3: Effect of CRE on body weight

Group	Day 0	Day 7	Day 14	Day 3	Day 21
Disease control	267.5	274	275	272	277
standard	330	312	311	310	299
Test 1	300	323	339	310	299
Test 2	281.5	277	284	269	262

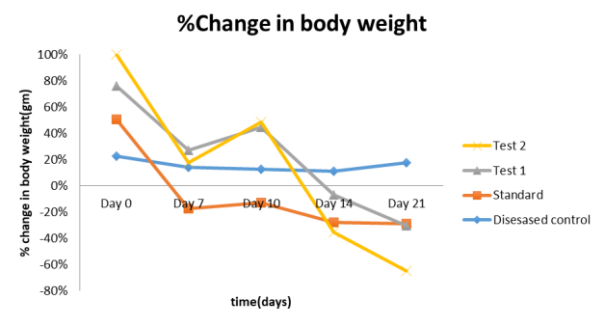

Figure 3: Percentage change in body weight

Body Weight should be decreased in case of test 2 (400mg/kg).

DISCUSSION

Rheumatoid arthritis is an autoimmune disease, and the immunologically mediated Freund's adjuvant induced arthritic model of chronic inflammation is the best experimental model for studying the disease Complete Freund's adjuvant-induced arthritis is one of the models of chronic polyarthritis with characteristics similar to rheumatoid arthritis.

Swelling of the paws is a simple, sensitive, and fast procedure for assessing the severity of inflammation and evaluating the therapeutic effects of drugs.

As a result of the adjuvant-induced arthritis model, rats developed long-term swelling of multiple joints with influence of inflammatory cells, erosion of cartilage, and bone destruction and remodeling similar to human diseases like rheumatoid arthritis. Ultimately, these inflammatory changes result in the complete destruction of joint integrity and function. In addition, rats fed CFA displayed soft tissue swelling around the ankle joints during the nourishment of arthritis, which was considered edema of those tissues.

Cuscuta reflexa Roxburg has anti-inflammatory and anti-arthritic activities. When the albino rats are treated with test 2 (400mg/kg) there is a significant effect compared with test 1 (200mg/kg) and the paw volume (table 1, Figure 1) was decreased parallelly with the standard (prednisolone). Due to decrease in paw volume, paw thickness (table 2, figure 2) and body weight (table 3, figure 3) are also decreased simultaneously. Based on the above results, we conclude that *Cuscuta reflexa* roxburg has anti-inflammatory and anti-arthritic activity.

CONCLUSION

In the given dose of 400 mg/kg EEPL significantly reduced the swelling and erythema of injected paws. It also reduced the arthritic score of rats. It slightly reduced the blood levels of ESR, CRP and TC levels of rats when compared to arthritic control (untreated) rats. Progressive weight loss was prevented by CRE and CRE treated rats started increasing in weight from 2nd week. Thus the present study concluded that CRE has a therapeutic effect on inflammatory arthritis created by CFA-induced arthritis.

Acknowledgement

The study was funded by Shri Vishnu College of Pharmacy, Bhimavaram. Standard drug Prednisolone for anti-arthritic activity was given by Cipla pharmaceuticals, Hyderabad.

Conflict of Interest

None declared.

Financial Support

None declared.

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HOW TO CITE THIS ARTICLE
Cheruku GR, Anumula P, Jyothi GSVD, Oruganti TSS, Gangi AB. Phytochemical evaluation and Pharmacological screening of *Cuscuta reflexa* roxburg on anti-arthritic activity. J Phytopharmacol 2021; 10(5):510-513. doi: 10.31254/phyto.2021.10614