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Phytochemical Evaluation and Pharmacological Screening of Cuscuta Reflexa Roxburg on Anti Arthritic Activity

Gowtham Reddy Cheruku[1], Pavani Anumula[2], Divya Jyothi G.S.V[3], Tejaswini S.S Oruganti[4], Akhil Babu Gangi[5]

Department of Pharmacology, Shri Vishnu College of Pharmacy, Bhimavaram, 534202.

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ABSTRACT:

CuscutareflexaRoxb. Is an extensive climber, rootless, leafless twining annual parasite found in temperate and tropical regions of the world with huge species diversity. It is widely used in Ayurvedic (traditional medicine native to the Indian subcontinent) medicine to relieve and treat many pathologies. It is widespread in Afghanistan, Malaysia, India, China and in many more countries where it is used to treat urination disorders, bilious disorders, diabetic disorders and inflammatory diseases. The main purpose of this study is to experimentally demonstrate the anti-inflammatory properties and antiarthritic properties of the methanolic extract of CuscutareflexaRoxburg. It contains different types of phytoconstituents such as, Alkaloids, tannins, coumarins, phenolic compounds, flavonoids and saponins. These phytoconstituentsare 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 CuscutaReflexaRoxbergwas evaluated by considering paw volume, paw thickness and body weight. The morphological features of the arthritis like redness, swelling, erythema was monitored by set visual criteria The scores were recorded on these particular 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). Systemic analysis shows that the extract maintained the studied parameters very close to normal and greatly restored the normal architecture of the joint in animals. CuscutaReflexaRoxburg would therefore be a very promising source for antiarthritic activity.

Key words: CuscutaReflexaRoxburgh, Anti Arthritic activity, Anti Inflammatory activity, Fruend's Adjuvant, Prednisolone.

I. INTRODUCTION:

According to the World Health Organization (WHO), almost 80% of the world's population believes in traditional medicines for their health needs due to better cultural acceptability, fewer side effects and better compatibility with the human body[1].

CuscutaReflexaRoxburgh (Convolvulaceae) is commonly known as Giant Dodder[2]. This type of plant speciesis usually observed in the Indian Subcontinent and the Greater Himalayas[3]. This parasitic plant species is a leafless twined sprawling and grows over a host plant. This species is capable of producing a huge number ofbranches which can cover the host plant within a very short period of time and suck life out of the host plant. Flowers are compact, bell shaped and white in colour with yellow filaments. Fruits and seeds originated from the flower. This species is used in preparation methods of traditional medicines for the treatment of headache, labour pain, bone fracture, fever, rheumatism[3], anti microbial[4], anti tumour[5], anti inflammatory[6].

Arthritis is a long-term autoimmune disorder that majorlyaffects joints[7]. It mostly results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Frequently, the wrist and hands are involved, with the same joints typically involved on either side of the body. The disease may also cause severe affects to the other parts of the body, including skin, eyes, lungs, heart, nerves and blood. This may result in decrease of red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be

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present[7]. Often, symptoms appear gradually over weeks to months[8].

While the cause of rheumatoid arthritis is not clear, it is believed to involve a merger of genetic and environmental factors. The primary mechanism involves the body's immune system attacking the joints[7]. This results in severe inflammation and thickening of the joint capsule. It also attacks the underlying bone and cartilage.The diagnosis is made majorlyon the basis of a person's signs and symptoms[8]. X-rays and laboratory testing may support a diagnosis or exclude other diseases with homogeneous symptoms. Other diseases that may present similarly include systemic lupus erythematosus, psoriatic arthritis, and fibromyalgia among others. Considering the anti inflammatory activity, we have screened for anti arthritic activity.

II. MATERIALS AND METHODS:

Experimental animals:

The study was conducted on male wistar rats of 150-200g. They were housed in polypropylene cages and maintained at 27±2˚C,relative to 65±10% under 12h light/dark cycles.The animals were allowed to acclimatize to laboratory conditions 48h before the start of the experiment.group f 6 rats were used in all sets of experimental animals were provided with standard rodent pellet diet and water (libidum) and the food was withdrawn 18-24h 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 crudeextract:

Cuscutareflexa leaves were air dried and coarsely powdered leaves of the plant were used for the extraction process. The coarse powder of the plant was successively extracted by soaking process with the solvents in increasing order of polarity starting with petroleum ether,chloroform,ethylacetate,and methanol. Powdered sample was initially soaked in methanol in a conical flask and allowed to stand for 15 days with occasional shaking. After 15 days, the solvent along with components were collected and was filtered using whatman # 1 filter paper. By using a rotary evaporator, the extracts were concentrated under reduced pressure and then dried in open air. The dried methanolic extract was suspended in saline water (vehicle) and used for anti-arthritic activity.

Freund's adjuvant induced arthritis:

The male albino rats (wistar stain)were segregated into four groups, i.e. diseased or control, standard, drug treated (two groups of methanolic extract low and high dose treated group test 1-200mg/kg, test 2-400mg/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 administered with 0.1 ml of Freund's complete adjuvant (FCA) into the plantar region of the left hind paw. Both the hind paws were measured for paw volumes using a plethysmometer and body weight was weighed and recorded on the day of adjuvant injection. The methanolic extract of the aerial parts of the plant (200 and 400 mg/kg) and prednisolone (10 mg/kg) doses were administered orally for 14 days from the day of Freund's adjuvant injection. The changes in the paw volume were measured on various days up to 21 days following Freund's adjuvant injection. The variations in the inflammatory reaction was measured and recorded by using mercury plethysmometer on 1st, 7th, 14th and 21st day from the day of adjuvant injection. The rats were weighed using digital weighing balance on 1st, 7th, 14th and 21st day from the day of adjuvant injection.

Arthritic score:

The morphological feature of the arthritis like redness, swelling and erythema was monitored by set visual criteria as follows: 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. Thus, the maximum possible score for both hind paws was 8.

Pawvolume:

The left hind paw volumes of all animals were measured just before FCA injection on day 0 and thereafter at different time intervals till day 21 using a plethysmometer (UGO Basile, Italy),The change in paw volume was measured as the difference between the final and initial paw volumes.Thejointdiameters of the left hind

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paw were measured using a Vernier caliper on the above-mentionedtestingdaysafterinductionofarthritis[10].

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

% inhibition of paw volume = 1 - (Vt/Vc) × 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 measure by using vernier callipers at days 0,1,7,14 and 21.The percentage inhibition in paw thickness was calculated by using below formula,

% inhibition of paw thickness = 1 - (Tt/Tc) × 100

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

Tt = Paw thickness of treated group

Body weight:

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

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

% change in body weight = 1- (Wo/Wt) × 100

Where, Wo = Body weight of rats at day 0

Wt = Body weight of rats at time t

Results:

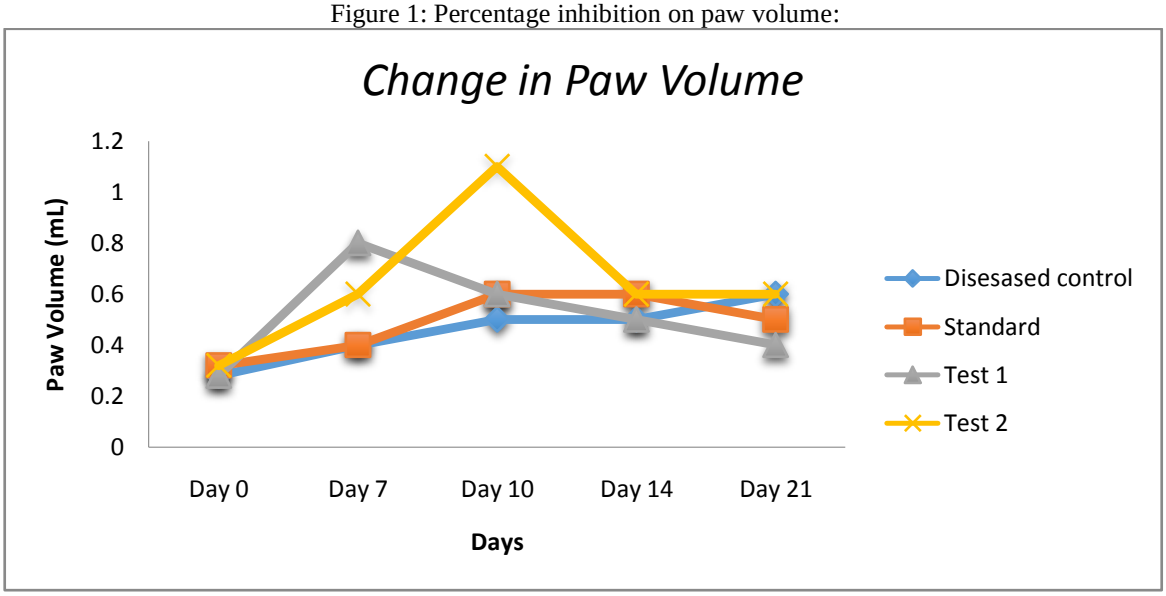
Evaluation of antiarthritic activity:

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

Table 1

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.

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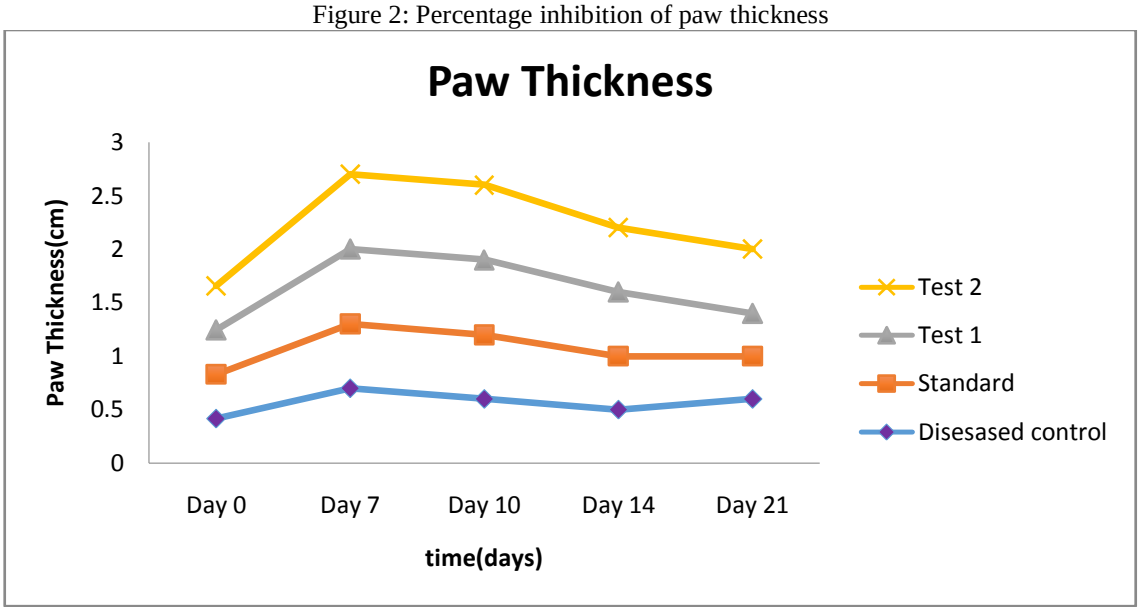
Table 2: Effect of CRE on Paw thickness

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

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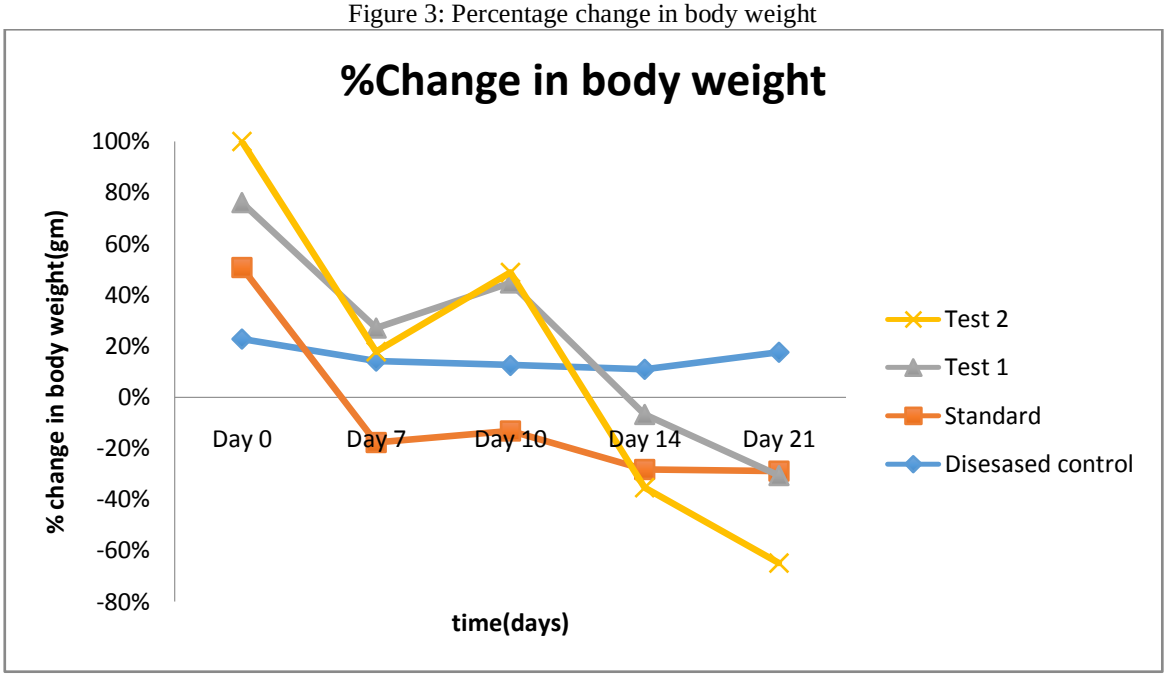
Figure 3: Percentage change in body weight



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

III. DISCUSSION:

Rheumatoid arthritis is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the finest experimental model of rheumatoid arthritis. Complete Freund's adjuvant-induced arthritis is one of the models in chronic polyarthritis with features that resemble rheumatoid arthritis.

The determination of paw swelling is seemingly a simple, sensitive and fast procedure for evaluating the degree of inflammation and assessing thetherapeutic effects of drugs.

In the adjuvant-induced arthritis model, rats developed a long term swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling which have close similarities to human rheumatoid disease. These inflammatory changes finally result in the complete destruction of joint integrity and functions in the affected animal. Also, the CFA administered rats showed soft tissue swelling around the ankle joints during the nourishment of arthritis, which was considered as edema of the particular tissues.

CuscutaReflexaRoxburg 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 cuscutareflexaroxburg has anti inflammatory and anti arthritic activity.

IV. 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

V. ACKNOWLEDGEMENT:

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