EVALUATION OF ANTI-RHEUMATOID ACTIVITY OF CUSCUTA REFLEXA IN FREUND’S ADJUVANT INDUCED ARTHRITIC RATS

Damerakonda Kumaraswamy*1, Goverdhan Puchchakayala2, Prashanth Yatla3

Center for Pharmacological Screening and Herbal studies
Department of Pharmacology, Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Warangal – 506001, Telangana, INDIA.
Department of Medicinal Chemistry, Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Warangal – 506001, Telangana, INDIA.

Email: dks.july12@gmail.com

Received on 15-05-2016 Accepted on 18-06-2016

Abstract:

In the present study, ethanolic extract of Cuscuta reflexa (EECR) was assessed for anti-arthritic activity in rats using in-vitro and in-vivo methods. Diclofenac sodium was used as a standard drug. Complete Freund’s adjuvant (CFA) was used as inducing agent. A significant ($P ≤ 0.001$) inhibition of paw edema volume and body weight was observed from 0th, 7th, 11th, 14th, 17th, 21st and 28th day in the treated groups. The biochemical parameters like erythrocyte sedimentation rate (ESR), red blood cell (RBC), Haemoglobin (Hb), Platelets and total White blood cells (WBC) count was observed which are the major markers of arthritis and serum uric acid analysis, Rheumatoid Factor (RF), in-vitro studies were observed. A significant increase in the level of all the markers were found in the Treatment I and Treatment II compared to that of disease. Results revealed that the ethanolic extract of Cuscuta reflexa (EECR) at two different concentrations (200mg/kg and 400mg/kg) possess significant anti-arthritic activity as compared to that of standard. The plant extract showed a significant dose dependent activity.

Key words: Complete Freund’s adjuvant (CFA), Cuscuta Reflexa (EECR), Diclofenac sodium, Rheumatoid arthritis (RA).

Introduction

Rheumatoid arthritis is a chronic autoimmune disease and is characterised by inflammation of lining, synovium, destruction of articular cartilage. It leads to bone erosion and damage of joints [1]. Occurs mainly in middle age, but it often occurs in 20 to 30 years old age peoples also. It effects in females three times more than males [2]. Commonly using for rheumatoid arthritis treatment is Non-Steroidal anti-inflammatory drugs (NSAIDs), Disease modifying anti-
rheumatic drugs (DMARDs), and Corticosteroids. The goal of these drugs has been to relieve pain and to decrease joint inflammation, to prevent joint destruction and these drugs are known to produce various side effects [3].

*Cuscuta reflexa* was reported for its anti-spasmodic, anti-hypertensive, muscle relaxant, anti-viral, anti-convulsant, hair growth, anti-steroidogenic, hypoglycaemic effect, anti-fertility, and bradycardiac effects, *in-vitro* anti-inflammatory, anti-epileptic and anti-tumour activity [4-5]. We investigated the anti-rheumatoid activity experimentally by using *in-vitro* and *in-vivo* methods.

**Materials and methods**

**Collection of plant material**

*Cuscuta reflexa* was collected from Near Tekumatla village, Adilabad District, Andhra Pradesh, India and the plant was authentified by Dr. E. Narasimha Murthy, Department of Botany, Shatavahana University, Karimnagar, Andhra Pradesh and India. Plant was dried under shade and then stored in air tight container.

**Chemical procurement:** Complete Freund’s adjuvant-Sigma Aldrich Laboratories, Hyderabad.

All other chemicals were purchased from local firms (India) and were analytical grade.

**Plant preparation and extraction**

The dried plant was reduced to a coarse powder. Then the powder was subjected to maceration with ethanol as a solvent for 72 hours at a temperature of 50-60 ºC. The extract was concentrated and the solvent was completely removed. The extract was freeze dried and stored in the vacuum desiccators until further use [6].

**Animals**

Male Wister Albino rats (weighing 200-220 grams) were used for this experiment were procured from Sanzyme scientific, Hyderabad, India. The animals were housed in poly acrylic cages (38cmx23cmx10cm) with not more than six animals per cage, at an ambient temperature of 18±2ºc with 12hr. The rats were accessed with standard chow diet and water *ad libitum*. The maintenance and the handling of animals were performed according to Committee for the Purpose of Control and Supervision on Experimental Animals(CPCSEA) guidelines and the Institutional Animal Ethical Committee (IAEC) approved all the experimental procedures (IAEC NO: 1047/ac/07/CPCSEA), Vaagdevi college of Pharmacy, Warangal, Andhra Pradesh, India.

**Acute toxicity studies**

Acute oral toxicity studies are performed as per OECD 423 guidelines (acute toxic classic method). Male Wister Albino rats were selected randomly and divided into two groups the animals fasted over night and EECR (ethanolic
extract of Cuscuta reflexa) at the dose of 2000mg/kg b.wt administered orally to one of the group. Another group received normal saline. The animals were observed continuously for 24 hrs. Any behavioural changes and mortality were observed.

**Induction of arthritis**

Arthritis was induced by single intra-dermal injection of 0.1 ml of Complete Freund’s Adjuvant (CFA) containing 1 mg/ml mycobacterium tuberculosis H37Ra suspension in sterile paraffin oil into a foot pad of the left hind paw of male rats with the help of glass syringe and 26 G needles. The rats were anesthetized with ether inhalation prior to and during adjuvant injection [7].

**Experimental Design**

Animals were divided into five groups of six animals each as follows:

- **Group-I** - Normal rats given vehicle alone 0.1% Carboxy methyl cellulose (CMC) (p.o).
- **Group-II** - Arthritic untreated rats (Induced with CFA 0.1ml single intra-dermal injection).
- **Group-III** - Arthritic rats treated with 10mg/kg (p.o) standard Diclofenac sodium.
- **Group-IV** - Arthritic rats treated with 200mg/kg (p.o) of ethanolic extract.
- **Group-V** - Arthritic rats treated with 400mg/kg (p.o) of ethanolic extract.

**NOTE:** Standard and test extracts were dissolved in 0.1% CMC.

2.8. **Measurement of body weight and paw volume.**

Body weight was measured at every four days up to 28th day after immunization. Both the injected and contralateral hind paw volume was measured by means of a plethysmometer. Immediately before arthritis induction and 7th, 11th, 14th, 17th, 21st, 28th days thereafter.

The following formula was used to calculate this increase:

\[
\text{Increase} = \left( \frac{\text{Volume on the test day} - \text{Volume before adjuvant injection}}{\text{Volume before adjuvant injection}} \right) \times 100
\]

The value thus obtained was corrected for 100 g body weight [8].

**Measurement of hematological parameter**

On the 28th day after arthritis induction, rats were anaesthetized with ether and blood samples were collected into Ethylenediamine tetra acetic acid (EDTA) coated tubes from retro orbital junction. The number of leukocytes from each rat was determined using a counting chamber (HORIBA, ABX MICRO ESP 60) and differential analysis of every sample was performed on staining blood smears using Jenner's stain.
A total of 100 WBC were counted to determine the percentage of neutrophils. ESR was determined using the Wintrobe method [9] and RF, Hb, RBC were also determined.

**Measurement of spleen weight**

The rats were sacrificed with ether on the 28th day, the spleen removed and the wet weight of the spleen was recorded and corrected for 100 g body weight.

**Uric acid analysis:** The blood sample was collected from the rat retro orbital and uric acid parameter was analyzed in auto analyzer (Turbochem100, CPC).

**Radiological analysis of bone destruction**

After scarification on 28th day, knee joints were removed and certified radiologist from Vijetha Scans and Diagnostics, Warangal, and Andhra Pradesh. Who was unaware of the different drug treatments was scored the condition of tibiotarsal joints and graded as follows: periosteal reaction, 0–3 (none, slight, moderate, marked); erosions, 0-3 (none, few, many small, many large); joint space narrowing, 0–3 (none, minimal, moderate, marked); joint space destruction, 0–3 (none, minimal, extensive, ankylosis) [10].

**Histological processing and assessment of arthritis damage**

After sacrifice on 28th day, knee joints were removed and fixed for 4 days in 5% formaldehyde. After decalcification in 10% formic acid, processed for paraffin embedding tissue sections (7 μm thick) were stained with haematoxilin and eosin or safranin O. Certified pathologist from VBR Diagnostics, Hanamkonda, and Andra Pradesh. Who was unaware of the different drug treatments, scored the condition of tibiotarsal joint, Histopathological changes were scored as follows: inflammatory cells in the synovial tissues scored, 0–3; destruction of articular cartilage, 0–3 (ranging from the appearance of dead chondrocytes to complete loss of the articular cartilage); bone erosion, 0–3 (ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head); Cartilage and bone destruction by pannus formation, 0–3 (none, mild, moderate, 3, severe); and vascularity , 0–3 (almost no, few, some, many)[11-13].

**In-vitro methods**

**Protein denaturation method**

Test solution of 0.5ml consisted of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test solution (50,100,250,500, and 1000 µg/ml). Test control solution of 0.5ml consisted of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05ml of distilled water. Standard solution of 0.5ml consisted of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05ml of Diclofenac sodium (50,100,250,500, and 1000 µg/ml).
Product control of 0.5ml consisted of 0.45ml of distilled water and 0.05ml of test solution (50, 100, 250, 500, and 1000µg/ml). All of above solutions were adjusted to pH 6.3 using small amount of 1N HCl. The samples were incubated at 37°C degree centigrade for 20min and heated at 57°C for 3min. After cooling, add 2.5ml of phosphate buffer to the above solution. The absorbance of the above solutions was measured using UV-Visible spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula:

\[
\text{Percentage inhibiton} = 100 - \frac{[(\text{OD of test solution} - \text{OD of product control})]}{\text{(OD of test control)}} \times 100
\]

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium (250µg/ml) treated samples [14].

**Membrane stabilization method**

The principle concerned in the following method is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. Blood was collected from (2ml) from healthy volunteers and was mixed with equal volume of sterilized Alsever’s solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, 0.42% sodium chloride, in distilled water) and centrifuged at 3000rpm for 5min.

The packed cells were washed with isosaline solution and 10%v/v suspension was prepared with normal saline and kept at 4°C disturbed until use. Procedure is following as: Test solution of 4.5ml contained 2ml hypotonic saline (0.25% w/v), 1ml of phosphate buffer (pH 7.4), 1ml of test extract (50, 100, 250, 500, 1000 µg/ml) and 0.5ml of 10% HRBC suspension. Product control of 4.5ml contained 2ml of hypotonic saline(0.25% w/v), 1ml of phosphate buffer (pH-7.4) and 1ml of test extract (50, 100, 250, 500, 1000 µg/ml) in normal saline and 0.5ml of isotonic saline. Test control of 4.5ml contained 2ml of hypotonic saline (0.25% w/v), 1ml of phosphate buffer (pH-7.4) and 1ml of isotonic saline and 0.5% of 10% HRBC suspension. Standard solution of 4.5ml contained 2ml of hypotonic saline(0.25% w/v), 1ml of phosphate buffer (pH-7.4) and 1ml of Diclofenac sodium (50, 100, 250, 500, 1000µg/ml) in normal saline 0.5ml of 10% of HRBC suspension.

The above solutions were incubated at 56°C for 30 min. The tubes were then cooled in running tap water for 30min. After that they were centrifuged and the supernatant liquid was separated and the absorbance of supernatant solution was measured at 560nm by UV-spectrophotometer [15].

The percentage of membrane stability was calculated as follows:

\[
\text{Percentage stabilization} = 100 - \frac{[(\text{OD of test solution} - \text{OD of product control})]}{\text{(OD of test control)}} \times 100
\]
Statistical Analysis: The result was expressed as Mean ± S.D statistical difference between two means was determined by one-way ANOVA followed by Dunnett’s multiple comparisons test by using Graph pad prism V.5 software. Only those mean values showing statistical difference ***P<0.001, **P<0.01 and *P<0.05 were considered as statistically significant.

Results

Acute toxicity study: No adverse effects and no mortality of animals during the period of acute toxicity studies were observed up to the dose of 2 mg/kg administered through oral route. Since EECR was found to be non toxic, the doses of 200mg/kg b.wt and 400mg/kg b.wt was selected for studies in experimental animals.

Bodyweight

Animals in which arthritis had been induced gained less weight after induction, which was significantly lower than negative controls on 14\textsuperscript{th}, 17\textsuperscript{th}, 21\textsuperscript{th}, 28\textsuperscript{th} day (P <0.01). In Cuscuta reflexa treated arthritic rat’s weight was not decrease as such to disease control animals in dose-dependent manner (Table 1).

Spleen weight

In an experiment, the mean spleen weight of the adjuvant control rats was increased (Table 2) it was suggested spleenomegaly was apparent. Both extracts significantly reduced spleen weight of the adjuvant treated rats.

Paw volume

The volume of ipsilateral paw as well as contralateral paw in the adjuvant induced arthritis (AIA) rats increased progressively. The differences in the volume of ipsilateral paw and contralateral paw between the AIA and drug-treated rats were statistically significant in dose-dependent manner. The 6 h acute inflammation was not affected by either drug administered. Especially significant effects of Cuscuta reflexa were observed at days 11 to 28 after immunization (Table 3).

Uric acid analysis

Here the uric acid analysis was done by the TURBO CHEM 100 CPC (Table 4). The treatment II should shows significant effect than treatment I.

Radiological analysis of bone destruction

Bone destruction, which is a common feature of adjuvantarthritis, was examined by radiological analysis. Gross destruction of the joints of the untreated paws was observed in5 out of 6 rats in the adjuvant control group. Adjuvant-treated rats had developed definite joint space narrowing of the in tertarsal joints, diffuse soft tissue swelling that
Damerakonda Kumaraswamy* et al. International Journal Of Pharmacy & Technology included the Digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudo widening of all joint spaces. In contrast, in rats given ethanolic extract of *Cuscuta reflexa* attenuate these abnormalities predominantly localized to the proximal areas of the paws (Table 5). Ethanolic extract at 200 mg/kg dose alone failed to produce any significant improvement. Ethanolic extract at 400 mg/kg dose alone should produce slightly good significant improvement than before dose.

**Histological analysis of bone destruction**

Histological changes like infiltration of a few neutrophils into mildly edematous synovium, destructive lesions in articular cartilage, vascularity formation into the joint space, more extensive shown in adjuvant-treated animals (Fig. below). Ethanolic extracts (400 mg·kg⁻¹) of *Cuscuta reflex* produced knee joints protective effect compared to control in dose-dependent manner (Table 6). Ethanolic extract at 200 mg/kg dose alone failed to produce any significant improvement.

**Figure 1: Radiological analysis of bone destruction.**

![Normal](image1) ![Disease](image2)  
Normal  Disease  
Diclofenac sodium (10mg/kg).  
![EECR (200mg/kg)](image3) ![EECR (400mg/kg)](image4)
Figure 2: Histological analysis of bone destruction.

A-Disease
B-Diclofenac sodium (10mg/kg)
C-EECR (200mg/kg)
D-EECR (400mg/kg)

Hematological parameter

Total WBC count increases with significantly increased in arthritic animals. ESR and RF were also significantly increased, while Hb, RBC were decreased in AIA animals. Results shown in below (Table 7) suggest that total WBC count, ESR and RF are significantly decreased, while hemoglobin, RBC was slightly increased in treated animals in dose-dependent manner as compared to disease control ($P < 0.01$).
In-vitro anti-arthritic activity

Inhibition of Protein Denaturation

The percentage inhibition of protein denaturation of different extracts and standard were shown in (Table 8). *Cuscuta reflexa* extract exhibited maximum percentage inhibition of protein denaturation of 89.76% at 1000µg/ml and this effect was compared with the standard anti-inflammatory drug Diclofenac which showed the maximum inhibition 93.54% at the same concentration.

Membrane stabilization method

In this in-vitro method it was observed that *Cuscuta reflexa* showed significant activity compared to standard Diclofenac sodium. Percentage stabilization was found to be 92.85% and 94.84% at concentration of 1000µg/ml of *Cuscuta reflexa* and Diclofenac sodium respectively. The activity was found to be concentration dependent, with increase in concentration the activity also increased. These results may be attributed to the presence of flavonoid content and good anti-oxidant properties (Table 9).

Discussion

Most of the investigators have reported that inhibition of adjuvant-induced arthritis in rats is one of the most suitable test procedures to screen anti-arthritic agents since it closely resembles human arthritis. Freund’s adjuvant induced arthritis is thought to occur through cell-mediated autoimmunity structural mimicry between mycobacteria and cartilage Proteoglycan in rats. It activates macrophages and lymphocytes by adjuvant inoculation or their products like monokines, cytokines, and chemokines may be involved in abnormal lipid and protein metabolism. The CFA administered rats showed soft tissue swelling around the ankle joints during the Development of arthritis which was considered as edema of the particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities [16].

The body weight of control AIA rats was significantly decreased compared with that of no immunized normal rats. The data suggest that oral *Cuscuta reflexa* prevents inflammatory body weight loss in AIA rats. Thus, give *Cuscuta reflexa* protective action.

The spleen provided a readily available source of cells known to be involved in arthritis [17]. As stated, there is increased cellularity in the spleen of arthritic rats. *Cuscuta reflexa* inhibit splenomegaly, which can enhance inhibitory effect of drug by suppression of spleenic lymphocytes, which might then result in reduced immunological activation, and subsequent inhibition of the infiltration of circulating lymphocytes into the synovium.
The present study revealed that the paw volume increases with ankle stiffness in adjuvant-challenged animals. *Cuscuta reflexa* administration delayed the onset and suppressed severity of clinical arthritis, as demonstrated by decreased both the paw volume.

The decrease in plasma uric acid in arthritic animals might be due to its continuous utilization by the system during free radical quenching reaction. It has been reported that uric acid serves as antioxidant *in vivo*, scavenging singlet oxygen, peroxyl and hydroxyl radicals and hypochlorous acid [18]. However, it is degraded on continuous exposure to OH and HOCl. The concentration of uric acid oxidation products has reported to be increased in serum and synovial fluid (SF) from patients with RA [19]. This supports our present result. Thus, the increase in uric acid in drugs treated rats might exert protection to the joint cartilage against the deleterious effects of ROS/RNS [20]. Alterations observed in the above parameters during arthritic conditions were normalized to a greater extent in *Cuscuta reflexa* treated animals.

In synovial tissue, erosion of subchondral and cortical bone is common, leading to the characteristic erosions seen on radiography. Osteoclasts can be seen in the areas of bone destruction during AIA. Here, we report that *Cuscuta reflexa* treatment in established AIA markedly reduced bone erosions, examined by radio graphical and Histopathological analysis. Here *Cuscuta reflexa* should give good response on high dose.

As observed from the present study a similar decrease in Hb and increase in the WBC count and ESR levels in AIA rats were reported by Agarwal and Rangari [21]. The decrease in Hb and RBC levels in arthritic rats reflects the presence of anemia in these rats. Anemia is the most common extracellular manifestation in RA [22] and a moderate hypochromic; Normocytic anemia due to reduction in the RBC count with a modest reduction in the MCHC is a common feature of RA. The most important cause might be the decreased level of plasma iron due to sequestering of iron in the reticuloendothelial system and synovial tissue that lead to failure of bone marrow to respond to anemia [23]. The decrease in plasma iron in turn was induced by IL-1 in association with the acute phase response [24-25]. Hence, it is provocative to speculate that the sequestration of less deformable erythrocytes by endothelial cells in the spleen plays a causative role in the shortened halflife of erythrocytes and subsequently anemia resulting in adjuvant arthritis.

The increase in total WBC count in AIA rats falls in line with the reports of [26-27]. The increase in both WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism [28]. This is evident by the infiltration of inflammatory mononuclear cells in the joints of AIA rats [29]. ESR is an indirect measurement of acute phase response for determining the disease activity in RA [30]. Although CRP is a better marker...
for inflammation and though ESR is influenced by several factors such as the plasma concentration of fibrinogen, Immunoglobulins, RF and Hb, the increased level of ESR in arthritic rats adds information reflecting the chronicity and severity of the disease better than CRP [31]. Hence, a combination of the tests might be worthwhile. The above-mentioned changes were brought back to near normal levels upon *Cuscuta reflexa* treatments, which emphasizes the beneficial effect of the drugs on AIA.

Anti-arthritic effect of ethanolic extract of *Cuscuta reflexa* was studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of ethanolic extract of *Cuscuta reflexa* on inhibition of protein denaturation is shown in table 8. Extract of *Cuscuta reflexa* at different concentrations provided significant protection against denaturation of proteins.

Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that *Cuscuta reflexa* could be used as potent anti-arthritic agent [32].

Anti-arthritic effect of ethanolic extract of *Cuscuta reflexa* was studied significantly by using *in-vitro* inhibition of membrane stabilization model. The effect of ethanolic extract of *Cuscuta reflexa* on inhibition of membrane stabilization is shown in table 9. Extract of *Cuscuta reflexa* at different concentrations provided significant protection against Stabilization of the HRBC. Membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti-inflammatory action of *Cuscuta reflexa*. Membrane stabilization action was studied to establish the mechanism of anti-arthritic effect of *Cuscuta reflexa*.

Therefore, our *in-vitro* studies on *Cuscuta reflexa* extracts demonstrate the significant anti-arthritic activity.

**Table1:** *Effect of Cuscuta reflexa on Body weight in Control and Experimental Animals.*

<table>
<thead>
<tr>
<th>TIME(n&lt;sup&gt;th&lt;/sup&gt; day)</th>
<th>Control</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>207.50±8.80</td>
<td>203.33±14.02</td>
<td>201.66±6.83</td>
<td>200.00±11.40</td>
<td>205.83±6.64</td>
</tr>
<tr>
<td>7</td>
<td>215.00±6.32</td>
<td>189.16±10.20</td>
<td>195.83±3.76</td>
<td>193.33±12.11</td>
<td>200.83±7.35</td>
</tr>
<tr>
<td>11</td>
<td>216.66±5.16</td>
<td>183.33±6.83</td>
<td>193.33±2.58</td>
<td>190.33±9.17</td>
<td>205.00±5.47</td>
</tr>
<tr>
<td>14</td>
<td>218.33±4.08</td>
<td>180.00±8.94</td>
<td>198.33±2.58*</td>
<td>193.33±8.16*</td>
<td>208.33±4.08*</td>
</tr>
<tr>
<td>17</td>
<td>219.16±3.76</td>
<td>175.83±5.84</td>
<td>205.00±4.47**</td>
<td>199.16±9.70**</td>
<td>211.66±2.58**</td>
</tr>
<tr>
<td>21</td>
<td>220.8±</td>
<td>168.33±2.58</td>
<td>209.16±2.04**</td>
<td>201.66±9.30**</td>
<td>212.50±2.73**</td>
</tr>
</tbody>
</table>
All values expressed as mean ± SD, n=6, *P<0.05, **P<0.01, ***P<0.001 as compared to disease control group comparisons are done by one way ANOVA using Dunnett’s test.

Table 2: Effect of *Cuscuta reflexa* on spleen weight in control and experimental animals.

<table>
<thead>
<tr>
<th>Spleen weight (gm)</th>
<th>Normal</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.51</td>
<td>0.65</td>
<td>0.50***</td>
<td>0.46**</td>
<td>0.49***</td>
</tr>
</tbody>
</table>

Table 3: Effect of *Cuscuta reflexa* on paw volume in control and experimental animals.

<table>
<thead>
<tr>
<th>TIME (n&lt;sup&gt;th&lt;/sup&gt; day)</th>
<th>Normal</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.53±0.051</td>
<td>0.55±0.054</td>
<td>0.53±0.051</td>
<td>0.53±0.051</td>
<td>0.55±0.054</td>
</tr>
<tr>
<td>7</td>
<td>0.55±0.054</td>
<td>0.93±0.051</td>
<td>0.91±0.075</td>
<td>0.91±0.075</td>
<td>0.96±0.081</td>
</tr>
<tr>
<td>11</td>
<td>0.55±0.054</td>
<td>1.11±0.075</td>
<td>1.08±0.075</td>
<td>1.10±0.063</td>
<td>1.16±0.081</td>
</tr>
<tr>
<td>14</td>
<td>0.56±0.051</td>
<td>1.25±0.083</td>
<td>1.18±0.075**</td>
<td>1.20±0.063**</td>
<td>1.28±0.075**</td>
</tr>
<tr>
<td>17</td>
<td>0.56±0.051</td>
<td>1.35±0.083</td>
<td>1.06±0.081***</td>
<td>1.10±0.063**</td>
<td>1.18±0.075**</td>
</tr>
<tr>
<td>21</td>
<td>0.58±0.040</td>
<td>1.40±0.063</td>
<td>0.93±0.051***</td>
<td>1.00±0.063***</td>
<td>0.98±0.075**</td>
</tr>
<tr>
<td>28</td>
<td>0.61±0.040</td>
<td>1.45±0.054</td>
<td>0.68±0.075***</td>
<td>0.81±0.040***</td>
<td>0.71±0.075**</td>
</tr>
</tbody>
</table>
Table 4: Effect of \textit{Cuscuta reflexa} on serum uric acid parameter in control and experimental animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (mg/dl)</td>
<td>54.128±0.1</td>
<td>51.193±0.1</td>
<td>53.483±0.23</td>
<td>52.019±0.091</td>
<td>52.986±0.104</td>
</tr>
<tr>
<td>80</td>
<td>70</td>
<td>1**</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

All values expressed as mean ± SD, n=6. **P<0.01, as compared to disease control group comparisons are done by one way ANOVA using Dennett’s test.

Table 5: Effect of Ethanolic extracts of \textit{Cuscuta reflexa} extract and Diclofenac sodium on X-ray analysis in the adjuvant-treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/b.wt)</td>
<td>-</td>
<td>10</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Radiology score</td>
<td>8</td>
<td>3**</td>
<td>7</td>
<td>5*</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SD, n=6. *P<0.05, **P<0.01, as compared to disease control group comparisons are done by one way ANOVA using Dennett’s test.

Table 6: Effect of Ethanolic extracts of \textit{Cuscuta reflexa} and Diclofenac sodium on Histopathological analysis in the adjuvant-treated rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease</th>
<th>Standard</th>
<th>Treatment I</th>
<th>Treatment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/b.wt)</td>
<td>-</td>
<td>10</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Histology score</td>
<td>8</td>
<td>4**</td>
<td>7</td>
<td>5*</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SD, n=6. *P<0.05, **P<0.01, as compared to disease control group comparisons are done by one way ANOVA using Dennett’s test.

Table 7: Effect of \textit{Cuscuta reflexa} on haematological parameters in control and experimental animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (gm/dl)</th>
<th>RBC ((10^6/mm^3))</th>
<th>WBC ((10^6/mm^3))</th>
<th>Platelets ((10^6/ml))</th>
<th>ESR 30min</th>
<th>ESR 60min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.49±0.03</td>
<td>5.67±0.05</td>
<td>5.38±0.26</td>
<td>43.00±3.76</td>
<td>2.29±0.02</td>
<td>3.91±0.01</td>
</tr>
<tr>
<td>Disease</td>
<td>9.70±0.14</td>
<td>4.35±0.18</td>
<td>10.74±0.17</td>
<td>52.08±7.35</td>
<td>6.61±0.01</td>
<td>8.90±0.05</td>
</tr>
<tr>
<td>Standard</td>
<td>12.76±0.16***</td>
<td>5.37±0.18***</td>
<td>6.09±0.16***</td>
<td>39.73±10.80***</td>
<td>3.08±0.01***</td>
<td>3.73±0.05***</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SD, n=6. ***P<0.001, as compared to disease control group comparisons are done by one way ANOVA using Dennett’s test.
All values expressed as mean ± SD, n=6, **P<0.01, ***P<0.001 as compared to disease control group comparisons are done by one way ANOVA using Dennett’s test.

Table 8: Effect of *Cuscuta reflexa* on Inhibition of Protein Denaturation in control and experimental animals.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>50</th>
<th>100</th>
<th>250</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>% protein denaturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. reflexa</em></td>
<td>63.81</td>
<td>69.29</td>
<td>78.25</td>
<td>86.43</td>
<td>89.76</td>
</tr>
<tr>
<td>% protein denaturation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>65.81</td>
<td>71.54</td>
<td>81.32</td>
<td>87.67</td>
<td>93.54</td>
</tr>
</tbody>
</table>

Table 9: Effect of *Cuscuta reflexa* on Membrane stabilization in control and experimental animals.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>50</th>
<th>100</th>
<th>250</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Membrane stabilization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. reflexa</em></td>
<td>68.74</td>
<td>80.15</td>
<td>85.25</td>
<td>88.76</td>
<td>92.85</td>
</tr>
<tr>
<td>% Membrane stabilization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>69.81</td>
<td>81.21</td>
<td>86.32</td>
<td>89.83</td>
<td>94.84</td>
</tr>
</tbody>
</table>

Conclusion

From the results obtained in the present study, it may be concluded that *Cuscuta reflexa* possess significant anti-arthritic activity. Hence it could be beneficial for further work as active anti-arthritic agent.

Acknowledgement: The authors express sincere thanks to “AICTE, New Delhi for their support and providing necessary facilities to carry out the project work under MODROBS scheme (8024/RIFD/MOD-354(PVT)/policy-III/2011-2012).

References


**Corresponding Author:**

**Damerakonda Kumaraswamy***,

**Email:** dks.july12@gmail.com