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Evaluation of protective effects of *Cuscuta reflexa* Roxb. against FCA induced arthritis in rats

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Graphical Abstract



Abstract:

Introduction: *Cuscuta reflexa* (Convolvulaceae), is an Indian traditional medicinal plant with claims of antibacterial, antiproliferative, anti-inflammatory properties. Present study was designed to investigate the protective effect of extracts of *Cuscuta reflexa* Roxb. against adjuvant induced arthritis.

Materials and methods: Arthritis was induced by Complete Freund's adjuvant. Arthritic rats were treated with methanol and aqueous extract of *Cuscuta reflexa* Roxb. Diclofenac sodium was used as standard treatment. Protection against arthritis was investigated using various parameters like paw thickness, changes in whole body weight, weight of spleen and thymus, pain in inflammation by using thermal stimulus, radiological and histological analysis of the joint and serum levels of biochemical markers and inflammatory mediators (TNF- α and IL-6). Methanol extract of *Cuscuta reflexa* Roxb. (MECR) was fractionalized and its fraction was subjected to IR and LC/MS analysis.

Results: Treatment of arthritic rats with methanol and aqueous extracts of *Cuscuta reflexa* Roxb. significantly protected the animals from the arthritic changes as compared to the arthritic control group. Elevated levels of inflammatory cytokines, lipid peroxidation and other biochemical and pathological changes associated with arthritis were normalized by the treatment of MECR and AECR. Results suggest significant antiinflammatory and antiarthritic effects.

Keywords: Dodder; Freund's Complete Adjuvant; Arthritis; Cytokines; quercetin



Ethical Use of Animals

Rats of the strain Wistar Albino in the weight range of 180-200 g were used for the Evaluation of protective effects of *Cuscuta reflexa Roxb.* against FCA induced arthritis in rats. All of the animals were procured from National Toxicological Centre, Pune. The experimental protocols were approved by Institutional Animal Ethics Committee (CPCSEA Reg. no. 1329/ ac/ 10/ CPCSEA).

Collection of Plant material

Stems of *Cuscuta reflexa* growing on the plants of *Ziziphus* were collected from local region of Igatpuri, District Nasik. Plant material was identified with the help of local community and was authenticated by Dr. J. Jayanthi from Botanical Survey of India, Pune (Ref no. BSI/WC/Tech/374).





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flora mellone a1

Introduction

Cuscuta reflexa Roxb. (Dodder), (Convolvulaceae), is also referred as Amarwel because of its immortal nature. This parasitic twinner has no roots but grows on host plant like hairy stems which eventually covers host plant (Nadkarni, 1986).

Chronic inflammation of synovial joints is one of the important characteristic of Rheumatoid arthritis (RA) which is an autoimmune inflammatory disease resulting in severe damage to the joints as well as muscles surrounding joints leading to a painful condition as difficulty in movements of joints (Robins, 2007; Gullaiya et al., 2013).

Cuscuta reflexa Roxb. is a traditional herbal medicine used in the form of decoction for various conditions like liver disease, cough, itching (Udavant et al., 2012a). Traditional healers have also been using the herb for various conditions associated with inflammation (Pal et al., 2006; Patel et al., 2012). Dodder has also been found to possess antiinflammatory activity (Suresh et al., 2011; Udavant et al., 2012b). Focus was given to find out the usefulness of extracts of *Cuscuta reflexa* Roxb. in arthritis induced in animals by FCA



Results and discussion

Effect of extracts of *Cuscuta reflexa* Roxb. on paw thickness changes in FCA induced arthritis

Important clinical signs of arthritis like swelling, hyperemia and weight loss started appearing in five to six days after injection of the adjuvant. Freund's complete adjuvant induced arthritic rats presented with significant elevation of paw thickness as compared with normal rats and treatment of arthritic rats with MECR and ECR significantly inhibited paw edema as compared to arthritic rats (Table 1).

Treatment group	Paw thickness in mm (mean \pm SEM)			Inhibition (%)
	0 th day	28 th day	Edema	
Normal	4.48 \pm 0.13	4.53 \pm 0.09	0.05 \pm 0.07**	—
Arthritic control	4.69 \pm 0.14	6.78 \pm 0.13	2.09 \pm 0.04	—
DCS 10	4.49 \pm 0.11	5.21 \pm 0.11	0.72 \pm 0.03**	65.47 \pm 1.40**
MECR300	4.65 \pm 0.15	5.44 \pm 0.13	0.78 \pm 0.02**	62.44 \pm 1.16**
AECR300	4.56 \pm 0.15	5.59 \pm 0.16	1.03 \pm 0.02**	50.39 \pm 1.01**

Table : Effect of extracts of *Cuscuta reflexa* on FCA induced arthritis.



Treatment groups	Mean Body wt Before immunization	Mean Body wt On 28 th day	Body wt changes
Normal Control	190.83±2.82 ^{**}	229.76±3.78 ^{**}	38.92±2.93 ^{**}
Arthritic Control	190.01±1.89	197.32±1.57	7.30±1.04
DCS	189.58±2.23 ^{**}	220.31±3.41 ^{**}	30.73±1.40 ^{**}
ME300	193.95±2.30 ^{**}	232.75±4.42 ^{**}	38.81±2.93 ^{**}
AE300	189.02±2.48 [*]	223.66±3.78 ^{**}	34.65±1.56 [*]

Table : Effects of extracts of *Cuscuta reflexa* on body wt changes of FCA induced arthritis in rats.

DCS- Diclofenac sodium, PECE- Pet ether extract of *Cuscuta reflexa*, MECE- Methanolic extract of *Cuscuta reflexa* and AECE- Aqueous extract of *Cuscuta reflexa*



Weight changes on Spleen and Thymus

Treatment groups	Mean wt of spleen	Mean wt of thymus
Normal Control	0.88±0.01**	0.13±0.006**
Arthritic Control	1.17±0.05	0.21±0.006
DCS	0.94±0.03**	0.14±0.007**
ME300	0.95±0.01**	0.145±0.009**
AE300	0.96±0.02**	0.153±0.007**

Table : Effects of extracts of *Cuscuta reflexa* on weight changes of spleen and thymus of of FCA induced arthritis in rats.

DCS- Diclofenac sodium, PEER- Pet ether extract of *Cuscuta reflexa*, MEER- Methanolic extract of *Cuscuta reflexa* and AEER- Aqueous extract of *Cuscuta reflexa*



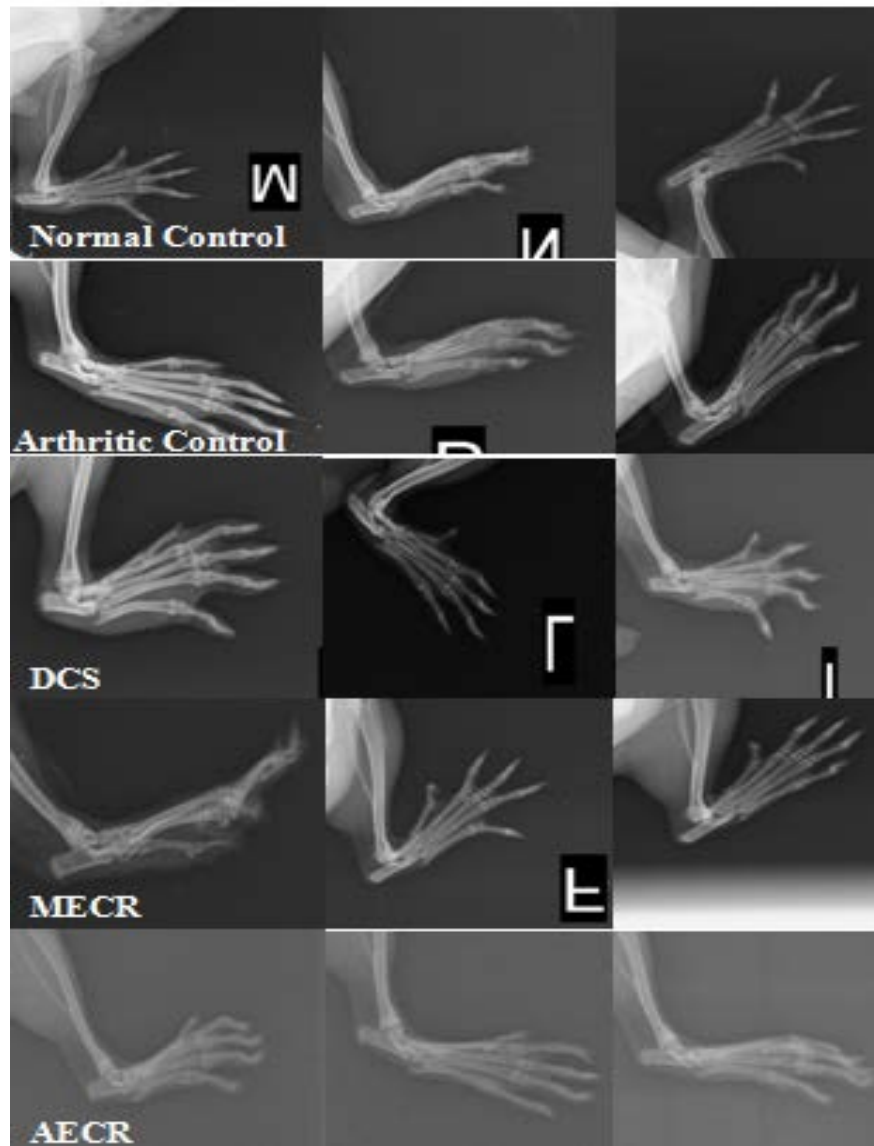


Figure : Effects of extracts of *Cuscuta reflexa* on the joints in FCA induced arthritic rats



HISTOLOGY OF JOINT

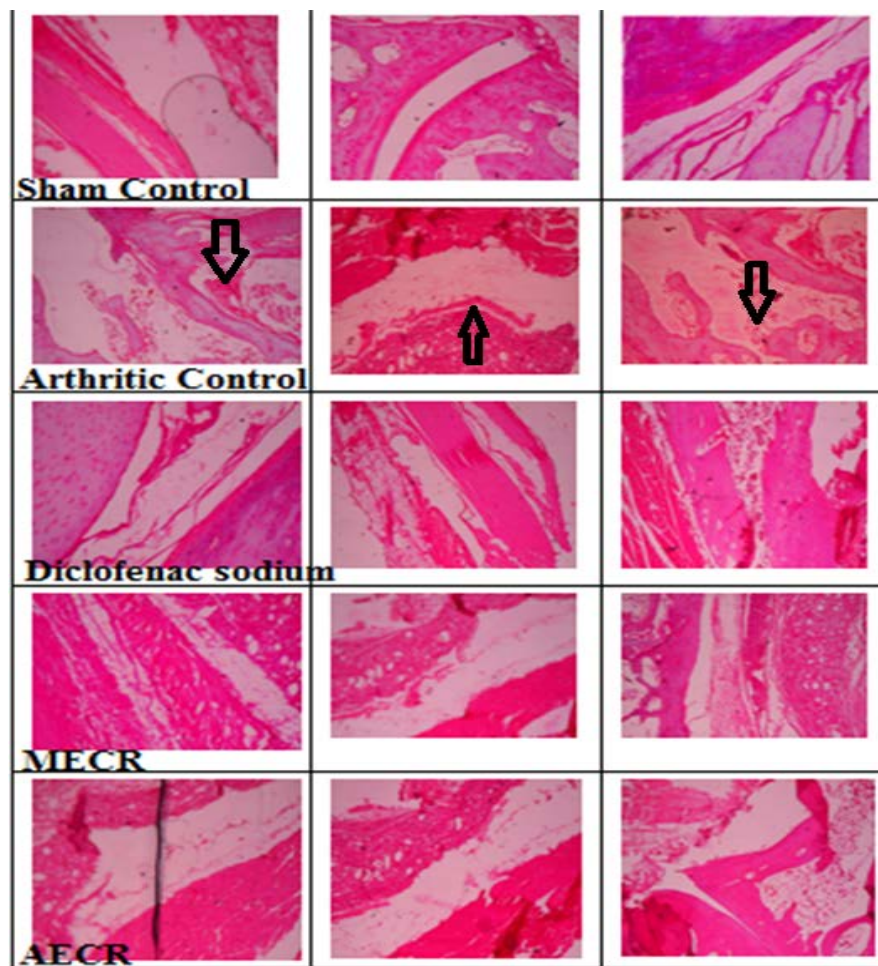


Figure : Effects of extracts of *Cuscuta reflexa* on the joints in FCA induced arthritic rats



Conclusions

Findings of the present investigation suggest that all clinical signs of arthritis have significantly been inhibited or reverted by the extracts of *Cuscuta reflexa Roxb.* and these effects are likely to be exerted by the complex mechanism involving inhibition of action of mediators of inflammation, stabilization of lysosomal membrane, inhibition of prostaglandin synthesis, inhibition of leukocyte infiltration and inhibition of synthesis of proinflammatory cytokines resulting in suppression of cell mediated cytotoxicity suggesting that this herb can be a potential candidate for further investigations



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