Original Research Paper

Volume - 12 | Issue - 07 | July - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

PHARMACOLOGY

EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES OF ALCOHOLIC EXTRACT OF CUSCUTA REFLEXA ROXB. STEM ON EXPERIMENTAL ANIMALS

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ABSTRACT **Aim of the study:** To evaluate the anti-Inflammatory activity of alcoholic extract of Cuscuta reflexa stem on experimental animals **Materials and Methods:** Stems of Cuscuta reflexa were collected, air dried and powdered. Methanolic extract was obtained by percolating the dried powder with 99.8% methanol. Alcoholic extracts of Cuscuta reflexa stem (100mg/kg) was used as test drug. Anti-inflammatory activity was on acute inflammation was evaluated by carrageenan induced rat paw oedema method and chronic inflammation was studied by Freund's complete adjuvant-induced arthritis method. For each of the studies albino rats of either sex weighing 100-200gms were divided into 3 groups with 5 animals in each group. Group A received normal saline 10 ml/kg. Test groups B, received alcoholic extracts of Cuscuta reflexa stem (100mg/kg), Standard group C received Aspirin (100mg/kg). Acute oral toxicity test of the extract was performed as per OECD 425 (OECD Guidelines, 2001). **Results:** The results were analysed by ANOVA followed by Dunnett's multiple comparison test. Alcoholic extracts of Cuscuta reflexa stem (100mg/kg) showed significant ($p<0.01$) anti-inflammatory activity when compared to the control in carrageenan induced acute inflammation. AECR was also effective in chronic arthritis model. **Conclusion:** The present study indicates that alcoholic extracts of Cuscuta reflexa stem (100mg/kg) showed significant ($p<0.01$) anti-inflammatory activity against acute inflammation as well as chronic inflammation.

KEYWORDS : Alcoholic Extract, Anti-inflammatory, Cuscuta reflexa, carrageenan, rat paw oedema, Aspirin.

INTRODUCTION

Inflammation by involving innate immune components with multiple effectors like leucocytes, mast cells, macrophages and locally produced cytokines tries to protect, repair, and remodel tissues. It is a complex process. It has systemic response and local response. Though these inflammatory responses are usually beneficial, but often drug therapy is needed to suppress and prevent tissue damage, chronicity caused as a result of inflammation induced functional impairment.[1]

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.[2]

Cuscuta reflexa Roxb. is a leafless yellow twining, parasitic annual belonging to convolvulaceae family. Commonly known as amarlati, akashilata, distributed throughout India.[3]

The stems bear parasitic roots known as haustoria which penetrate the host tissue. After establishing contact with the vascular bundles of the host, the roots absorb water and readymade foods.[4]

Whole plant, stem, seeds are used for various medicinal purposes. The herb is used as expectorant, carminative, tonic anthelmintic, purgative, diaphoretic, diuretic, purifies blood and cleanses the body, lessens inflammations, useful in jaundice, pains in the muscles and the joints, heat of the brain, headache, paralysis, diseases of the spleen, vomiting, and lumbago. The seeds are used in the diseases of the liver and the spleen, quartan fever, griping hiccough, purify blood and cleanse the bowels.[3]

Various studies on *Cuscuta reflexa* showed that the plant has anticonvulsant, antimicrobial, antioxidant, anti-steroidal, anti-inflammatory, antitumour, diuretic, anti-HIV, hair growth promoting, hypotensive, hypoglycaemic activity.[5]

Stem of *Cuscuta reflexa* contains flavonoids, phenolic compounds,[6] reflexin [5- hydroxy-7-methoxy-6-(2,3-epoxy-3-methyl butyl)-flavanone, apigenin 7-O- glucoside, kaempferol,myricetin have been isolated from stems of *Cuscuta reflexa*.[7] The study aimed to evaluate anti-inflammatory activity of alcoholic extract of *Cuscuta reflexa* stem on inflammation on albino rats.

MATERIALS AND METHODS

Plant Material

The study was carried out in the department of pharmacology at Assam Medical College. Stems of *Cuscuta reflexa* were collected within Dibrugarh district of Assam. A taxonomist of Dibrugarh University identified and confirmed the stem samples.

Plant Extract

Stems of *Cuscuta reflexa* were air dried at room temperature. Dried stems of *Cuscuta reflexa* were powdered in electrical grinder. The dried powder then be mixed with 99.8% methanol and allowed to stand for few minutes in a tightly covered container. The entire solution was transferred to Soxhlet apparatus and sufficient quantity of stem extract was obtained.[8]

Animals

Healthy albino rats of the species *Rattus norvegicus* of either sex weighing 100 - 200 gm, a total of 30 animals were used for the study with 5 animals in each group.

All the animals used in the study were taken care of under ethical consideration with approval from the institutional ethics committee (Registration no.-634/02/a/CPCSEA), Assam Medical College.

Toxicity studies: Alcoholic extract of *Cuscuta reflexa* stem was subjected to acute oral toxicity (OECD Guidelines, 2001). Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000 mg/kg.[9]

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Experimental Design: Fifteen albino rats of either sex weighing 100-200 gm were taken for each study. The animals were divided randomly into 3 groups of five animals per group. The rats were maintained on a standardized diet and water ad libitum. For experimental purpose, the animals were kept fasting overnight, but allowed free access to water. Drugs in the study were administered orally or intraperitoneally.

Groups	Treatment
Group A (Control)	Normal saline 10ml/kg/p.o
Group B (Test)	Alcoholic extract of Cuscuta reflexa stem(AECR) 100mg/kg/p.o
Group C (Standard)	Aspirin 100mg/kg/p.o

(1) Acute inflammation: Carrageenan induced rat paw oedema

1 hour after administration of normal saline, AECR and aspirin orally,

acute inflammation was produced in all animals by injecting 0.1 ml of freshly prepared 1% carrageenan in normal saline in the left hind paw. The paw volume was measured plethysmometrically just before carrageenan injection i.e., 0 hr and then 1st, 2nd, 3rd and 4th hrs after carrageenan injection.[10]

(2) Evaluation of anti-inflammatory activity on chronic inflammation

The chronic anti-inflammatory activity of AECR was tested by Freund's adjuvant-induced arthritis method in rats. On Day 1, the animals were injected into the sub-plantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant. Dosing with the test compounds or the standard to the respective groups was started on the same day and continued for 12 days. The paw volumes of both sides were measured plethysmographically and the body weights were recorded on the first day of injection. On Day 5 the volume of the injected paw was measured again. The severity of the disease was followed by measurement of the non-injected paw volume (secondary lesions). From Day 13 to 21, the animals were not administered with the test compound or the standard.[11]

The non-injected paw volume and the body weight were measured again on 21st day and the polyarthritis severity score was graded on 0 to 4: 0= No swelling; 1=Phalanx joint involvement; 2= Phalanx joint and all digits involvement; 3=involvement of the entire region down to the ankle; 4=involvement of entire paw, including ankle.[12][13]

Evaluation:[11]

a) *For primary lesions*: The percentage inhibition of paw volume of the injected left paw over vehicle control was measured at day 5.

b) *For secondary lesions*: The percentage inhibition of paw volume of the non-injected right paw over control was measured at day 21.
c) *An arthritic index* was calculated as the sum of the scores as indicated above for each animal. The average of the treated animals was compared with the control group.

Statistical Analysis

The data obtained were subjected to statistical analysis using one way ANOVA followed by Dunnet's multiple comparison test. A probability level of $p<0.01$ obtained and considered to be significant.

Results

The present study showed that both alcoholic extract of *Cuscuta reflexa* stem and standard drug aspirin possessed significant antiinflammatory activity against acute inflammation induced by carrageenan. AECR showed significant anti-inflammatory activity at the selected dose at 1st hour, 2nd hour 3rd hour and 4th hour and maximum percentage of inhibition of paw oedema was observed at 4th hour after carrageenan injection. Standard drug aspirin also showed maximum percentage of inhibition of paw oedema at 4th after carrageenan injection.

RESULTS

Table-1 Anti-inflammatory activity of AECR against acute inflammationu

Group	Drg dose p.o.	Mean increase in Paw volume (Mean ± S.E.M) (ml) with Percentage Inhibition			
		1st hour	2nd hour	3rd hour	4th hour
Group A (Control)	Normal saline 10 ml/kg	0.26 ± 0.014	0.38 ± 0.02	0.54± 0.014	0.53 ± 0.111
Group B (Test drug)	EEAC 200mg/kg	0.20 ± 0.02[a] (23%)	0.21± 0.010[a] (44.74%)	0.25 ± 0.01[a] (53.70%)	0.22 ± 0.023[a] (58.49%)
Group D (Standard)	Aspirin 100mg/kg	0.14 ± 0.011[a] (46.15%)	0.15± 0.010[a] (60.53%)	0.19 ± 0.011[a] (64.81%)	0.16 ± 0.017[a] (69.81%)

n = 5 rats in each group; ap <0.01 when compared to group A; ANOVA followed by Dunnet□s multiple comparison test

Table- 2 Anti-inflammatory activity of AECR against chronic inflammation

Name of the group	Drug dose p.o.	Mean increase in Paw volume (Mean ± S.E.M) (ml)with Percentage Inhibition	Weight change on 21st day	Arthritis index
		On 5th Day(injected paw)	On 21st Day(Non injected paw		
Group A (Control)	Normal saline 10 ml/kg	1.26±0.019	0.38±0.009	-29.80±0.58	7.80±0.37
Group B (Test Drug)	AECR 100mg/kg	1.10±0.037 12.69%	0.22±0.004 42.11%	-11.00±0.55a	6.20±0.245b
Group D (Standard drug)	Aspirin 100mg/kg	0.74±0.014 41.27%	0.12±0.007 68.42%	-52.40±2.04a	2.60±0.245a
One Way Anova	F Df p	112.1 12,2 <0.01	332.4 12,2 <0.01	268.6 12,2 <0.01	62.591 2,2 <0.01

n = 5 rats in each group; [a]p <0.01, [b]p <0.05 when compared to group A; ANOVA followed by Dunnet's multiple comparison test.

DISCUSSION

Carrageenan induces paw oedema in two phases. By stimulating phospholipase A_2 carrageenan, initiates the early phase of inflammation, and the cytotoxic effects progress the inflammation. In the process there is release of several pro-inflammatory mediators. [14]

In 3 to 5 hours swelling of the rat paw reached a peak, and then for several hours remained about the same degree of oedema. [15]

In the present study AECR at the doses 100 mg/kg, showed significant percentage of inhibition of carrageenan induced paw oedema when compared to the control which was maximum at 4th hour. The anti-inflammatory activity of AECR may be due to the inhibition of release of some mediators of inflammation which are released within initial hours of carrageenan injection. Freund's complete adjuvant was used to induce arthritis for evaluation of chronic anti-inflammatory activity. One of the reasons for the wide utilization of this model is due to the strong correlation between the efficacy of therapeutic agents in this model and in rheumatoid arthritis in humans.[16] AECR at the dose 100 mg/kg showed significant reduction of adjuvant induced paw oedema. *Cuscuta* stem is rich in flavonoids and phenolic compounds.[6] Flavonoids are known to exhibit anti-inflammatory activity.[17] Phenolic compounds present in *Cuscuta* reported to have free radical scavenging activity which results antioxidant property. Antioxidant property increases by increasing the phenolic component.[6] Flavonoids are reported to exhibit anti-inflammatory activities, against both acute and chronic inflammation.[18] AECR has significantly down regulated the arthritis index in adjuvant arthritis of rats. The presence of important phytochemicals like flavonoids and phenolic compounds in the stems may be also responsible for the significant antiinflammatory activity of AECR.

CONCLUSION

All the findings observed in the present study suggest that alcoholic extract of *Cuscuta reflexa* stems possess significant anti-inflammatory activity against both acute and chronic inflammation in experimental animal models.

ACKNOWLEDGEMENT

We are thankful to laboratory staff of Pharmacology Department, Assam Medical College, Dibrugarh, Assam.

Source of Support: Nil
Conflict of Interest: None

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