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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anti-inflammatory activity of *Cuscuta reflexa* Roxb: A Parasite

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ABSTRACT

Medicinal plants have therapeutic potential and are used worldwide to treat various diseases. Cuscuta reflexa Roxb.; family convolvulaceae has been used for the treatment of astringent to the bowels, aphrodisiac, alterative, tonic, expectorant, carminative, anthelmintic, purgative, diuretic, purifies the blood, sedative, diuretic and emmenagogue. This study was designed to investigate the phytochemical and anti-inflammatory activity of methanolic extract of Cuscuta relexa Roxb. (CRM). The anti-inflammatory activity of methanolic extract of C. relexa Roxb. was evaluated by inflammatory models of carrageenan induced paw edema in rats and acetic acid induced vascular permeability in Mice. The result that preliminary phytochemical analysis showed presence of flavonoids, tannins, phenolic compounds along with primary metabolites. Methanolic extract of C. relexa Roxb significantly decreased carrageenan-induced paw edema and acetic acid-induced capillary permeability. Paw edema is dramatically reduced at 3, 4, and 5 h after carrageenan injection by CRM at 400 mg/kg along with vascular permeability too. In conclusion, the findings suggested that methanolic extract of C. relexa Roxb. produced potential anti-inflammatory activity which support the claim for its traditional use in the treatment of various diseases.

Keywords: *Cuscuta reflexa*, Anti-inflammatory activity, paw edema, vascular permeability

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INTRODUCTION

Globally, medicinal plants have a remarkable effect on health. For many people, knowledge of medicinal plants is their only source of therapeutic information. [1,2]. Using medicinal plants has frequently been the sole way to receive basic healthcare in rural or village communities [3]. India has an enormous variety of medicinal plants, and several plant-derived extracts are utilized in traditional systems of medicine viz. Siddha, Ayurveda and Unani to treat different diseases. Few of them have undergone scientific investigation. Recent years have seen a significant increase in interest in plant-derived natural compounds like flavonoids, terpenes, glycosides, and alkaloids because of their wide range of pharmacological features, which include inflammatory, antipyretic, and analgesic effects. [4]. Convolvulaceae is another name for the morning glory family. Twining or climbing woody or herbaceous plants, which frequently have heart-shaped leaves and funnel-shaped blooms, predominate in this family. The Convolvulaceae is a family of approximately 60 genera and 1,650 species, mainly of herbaceous vines but also including trees, shrubs, and herbs.

Cuscuta reflexa Roxb. is an annual stem parasite that twines over other plants with leafless, thread-like orange, red, or yellow stems. Widespread in India, it is especially widespread in the Bengal lowlands, Ceylon, and grows as high as 2800 meters in some areas of Himachal Pradesh. It is known as akaswel or amarbel because it only develops as a parasitic thread on other plants and lacks a root system underground [5].

The glabrous, pale greenish yellow stems are branching, very long, thick, densely twinning, and occasionally spotted with crimson. Bracts are 1.5 mm long, oval-oblong, and fleshy; flowers are solitary, in umbellate clusters of 2-4, or in short racems; pedicels are short, glabrous, and typically curved. Broadly oval, slightly uneven, 3 mm lobes, calyx divided almost to the base. White corolla with a tube measuring 6-8 by 4 mm and almost cylindrical lobes measuring 2.5-3 mm long, big, and acutely deltoid. The corolla tube's

neck contains stamens. Ovarian ova l, style short, thick, and simple; two separate, ovoid, long stigmas. Capsules: glabrous, 6-8 mm in diameter, somewhat curcumscissile near base.
The aim of the present study is to study anti-inflammatory properties of methanol extract of *C. reflexa* Roxb. stems in comparison to a number of experimental models in rats and mice in order to verify its ethno medicinal uses.
An essential protective mechanism of the body against harmful stimuli including toxins and pathogens is inflammation. Uncontrolled or excessive inflammation, though a protective mechanism, can cause or worsen a variety of illnesses. Reactive oxygen species and free radicals are known to be generated during inflammation, and this can set off a sequence of events that prolong healing. It has also been demonstrated that the inflammatory process generates large levels of reactive oxygen species (ROS), which highlight the pathophysiology of numerous chronic illnesses, such as rheumatoid arthritis, cancer, cardiovascular, and neurological disorders. Thus, anti-inflammatory and antioxidant medicines have an important function in both preventing and curing a wide range of human illnesses. Unfortunately, a number of adverse effects are linked to the anti-inflammatory medications presently in use, such as NSAIDs (non-steroidal anti-inflammatory medicines), which restricts their usage. Therefore, it's necessary to design strong anti-inflammatory medications with fewer adverse effects. Therefore, plants or substances having antioxidant characteristics may assist to alleviate inflammatory illnesses by absorbing reactive oxygen species and free radicals during the inflammatory process [6].

MATERIAL AND METHODS

Herbal preparation and extraction

Stems of *Cuscuta reflexa* Roxb., family Convolvulaceae, were collected from western ghat, Satara, Maharashtra. The plant sample was authenticated from Joint Director, Botanical Survey of India, Western Regional Centre, Pune, India (No.: BSI/WRC/IDEN.CER./2020/H3/90) and the departmental library kept the voucher specimen (TVC-01) for further references. The coarsely ground, shade-dried stems were first defatted for 4 hours at 60–80°C using petroleum ether, and then they were extracted for 10–12 hours using methanol in a soxhlet apparatus. A dry residue yield of 34% w/v was obtained by filtering the extract through Whatman filter paper (No. 1) and then concentrating it to dryness in a rotary vacuum at a lower pressure.

Drugs and chemicals

The following chemicals were used: Thermosil Fine Chem Industries (Pune, India) provided the carrageenan, methanol, and indomethacin. We bought acetic acid, Evans blue, and Na-CMC from Shivaji Scientific Supplier (Pune, India).

Animal Preparation

Animals used in present study, consisting Swiss albino mice (18 – 22 g; GBL/250/2023) and adult wistar albino rats (180–220 g; GBL/251/2023) were purchased from Global Bioresearch Solution Pvt. Ltd., Pune. (Maharashtra, India). They were kept in typical lab cages in a 12-hour light-dark cycle room with moderate humidity (50 ± 5%) and constant temperature (22 ± 1 ◦ C). Throughout the trial, food and water were available to all animals without restriction. CPCSEA committee of JSPM's Rajarshi Shahu College of Pharmacy and Research, Pune, India – 411033 was approved the experiment protocol (Ethics approval: IAEC/2023/03).

Anti-inflammatory activity

1. Carrageenan induced paw edema in rats:

Four groups of rats were prepared: Group 1: Control (Inflammation control, 0.5 % CMC-Na); Group : 2 Indomethacin treated (10 mg/kg, dispersed in 0.5 % CMC-Na); Groups 3 and 4 : received an oral CRM treatment at 200 and 400 mg/kg, respectively, 30 minutes before to the carrageenan injection. 100 µL of 1% w/v carrageenan was sub-plantarly injected into the right hind paw of each rat. Rat paw volume was measured using a varnier caliper at 0, 1, 2, 3, 4 and 5 h. The edema thickness was calculated using the millimeter difference between the contralateral and inoculated footpads at the same evaluation time [7,8].

Acetic acid induced Vascular Permeability in mice:

This model represents, mice of both sex consisting of four groups (n = 6 per group). Group 1: Control group, Group 2: indomethacin (10 mg/kg, positive group), Group 3 and 4: CRM (200 and 400 mg/kg) were administered via oral gavage. Mice were given two injections: One i.p. injection of 0.6% (volume by volume) acetic acid and one intravenous injection of 2 percent solution of Evans blue made in saline solution. Mice were cervical dislocated and killed 20 minutes after the acetic acid was given. A 10 ml saline solution was used to rinse the peritoneal cavity. After collecting the washing solutions in a collecting tube, they were centrifuged for five min at 1000 × g for 5 min. The capillary permeability of the exudates was indicated by

the absorbance of Evans blue at 590 nm, which was determined in the supernatant using spectrometry. An oral dose of CRM, and Indomethacin was given one hour before the acetic acid injection [9].

Statistical Analysis

The data will have presented as the means ± standard error of the mean (SEM) (n = 6). Significant differences between group (means) will be analyzed by one-way ANOVA followed by Dunnett's t-test for multiple comparisons test. Data were defined as the statistical significance when *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Figures and statistical analysis were generated using Graph Pad Prism 10

RESULTS AND DISCUSSION:

The existence of triterpenoids, steroids, flavonoids, tannins, phytosterols, and carbohydrates was confirmed by preliminary phytochemical research results.

Bioactive substances present in natural goods provide promise for the creation of innovative pharmaceutical treatments [10]. The initial phytochemical screening may help identify plant bioactive components, which may result in the creation of new medications. [11]. Primary source of a plant's antioxidant activity is its phenolic components, including tannins, phenolic acids, and flavonoids. It has been demonstrated that these plant-derived compounds have anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic qualities [12]. Recently, there has been a lot of focus on searching for novel anti-inflammatory compounds to treat inflammation as quickly as possible [13].

Paw-edema caused by Carrageenan induction is a well-known model which determined to assess acute anti-inflammatory drugs. Cell recruitment at the injury site results in inflammation through the vascular endothelium, which expresses adhesion molecules and promotes inflammatory cell migration into injured tissue. Rats that receive it, reveal a biphasic pattern of inflammation that may be divided into early and late phase. Bradykinins, serotonin, and histamine are among the pre-synthesised inflammatory mediators that are released during the about hour-long early phase. After the first hour, the late phase starts when early phase mediators trigger actions that cause neutrophil infiltration and further prostaglandin production by cyclooxygenases (COX) [14].

Pretreatment of CRM at 400 mg/kg results in dramatically reduction of paw volume (***=p<0.001) at 3, 4 and 5 hour's time interval while Indomethacin (10 mg/kg,p.o) also significantly reduced (****=p<0.0001) paw volume (Fig. 1). The mice receiving CRM treatment did not develop stomach ulcers or hemorrhage after receiving inflammatory medication.

The cyclooxygenase and lipoxygenase pathways, which result in prostaglandins and leukotrienes, begin with phospholipids. Prostaglandins, specifically PGE 2 and LTB 4, induce vasodilation, which is a prevalent indication of inflammation [15]. Two important inflammatory mediators can be blocked by CRM, which lowers the volume of provocative paw volume.

A basic and early model of acute inflammation for assessing possible anti-inflammatory agents is mice's vascular permeability caused by acetic acid. It was thought that acetic acid caused mice's muscular constriction and nociception by indirectly encouraging the peritoneal cavity's production of prostaglandins and related mediators [16].

Histamine, kinin, fibrinolysin, phospholipase A2, and PLA2 are also examples of inflammatory mediators that may contribute to edema. These mediators induce edema by promoting vasodilation and increasing vascular permeability (17).

Acetic acid (10ml/kg) was injected intraperitoneally. CRM extract (200, 400 mg/kg,p.o) dose dependently greatly decreased (**=p<0.01) the acetic acid-induced plasma exudation in mice. Indomethacin (10 mg/kg,p.o) also clearly, greatly inhibited (***=p<0.001) the exudation (Fig. 2).

In this paradigm, the increased acetic acid-induced vascular permeability and inflammation were both mitigated by the CRM extract. These findings indicate that CRM extract may have an anti-inflammatory effect through interfering with the functioning of inflammatory mediators. A small number of phytochemicals have been shown in animal models to have anti-inflammatory and antioxidant properties, including polyphenols, flavonoids, and triterpenes. Phytochemicals such flavonoids, glycosides, saponins, reducing sugars, polyphenols, and flavonoids are present in extract CRM.

Because of this, the anti-inflammatory properties of CRM may be associated with the availability of various phytochemicals, particularly flavonoids and polyphenols. It has been demonstrated that flavonoids have potent anti-inflammatory properties, making them useful for the treatment of chronic inflammatory illnesses [18,19].

Table 1: Effect of CRM extracts on Carrageenan induced paw edema in rat.

Sr. No.	Group	Parameter (Paw edema at mm)					
		0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
1.	Control	1.16 ± 0.05	1.42 ± 0.02	1.51 ± 0.01	1.61 ± 0.01	1.59 ± 0.01	1.51 ± 0.06
2.	Indomethacin	1.13 ± 0.21	1.18**** ± 0.16	1.31**** ± 0.05	1.34**** ± 0.03	1.23**** ± 0.03	1.25**** ± 0.06
3.	CRM 200 mg/kg	1.08 ± 0.07	1.31* ± 0.03	1.47* ± 0.01	1.57** ± 0.01	1.55** ± 0.01	1.41*** ± 0.01
4.	CRM 400 mg/kg	1.20 ± 0.03	1.31 *± 0.04	1.46 **± 0.01	1.56***± 0.02	1.54*** ± 0.02	1.39**** ± 0.03

Table 2: Effect of CRM on Acetic acid induced vascular permeability in Mice

Sr. No.	Group	Parameter (Concⁿ. of dye)
1.	Control	3.27 ± 0.25
2.	Indomethacin	1.83*** ± 0.04
3.	CRM 200 mg/kg	2.87** ± 0.18
4.	CRM 400 mg/kg	2.73*** ± 0.03

n=6, Values are expressed as Mean±S.E.M.

*= p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001 when compared to control group. Statistically analyzed by One Way ANOVA followed by Dunnett test.

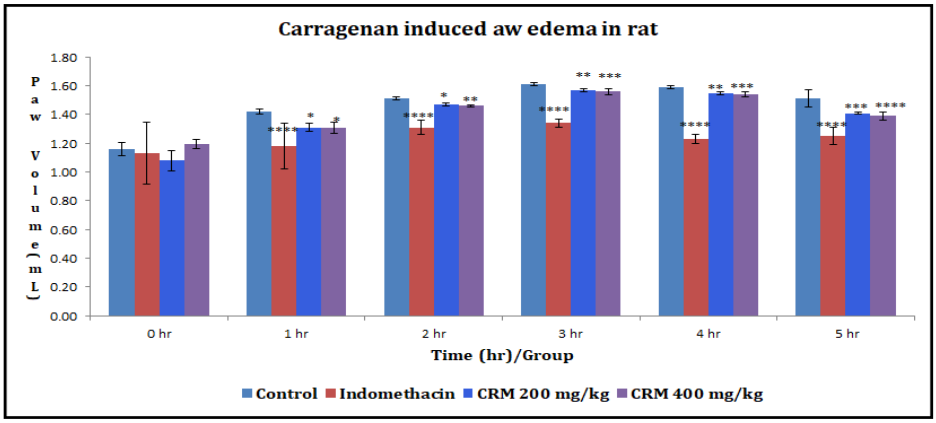

Fig. 1: Effect of CRM extracts on Carrageenan induced paw edema in rat

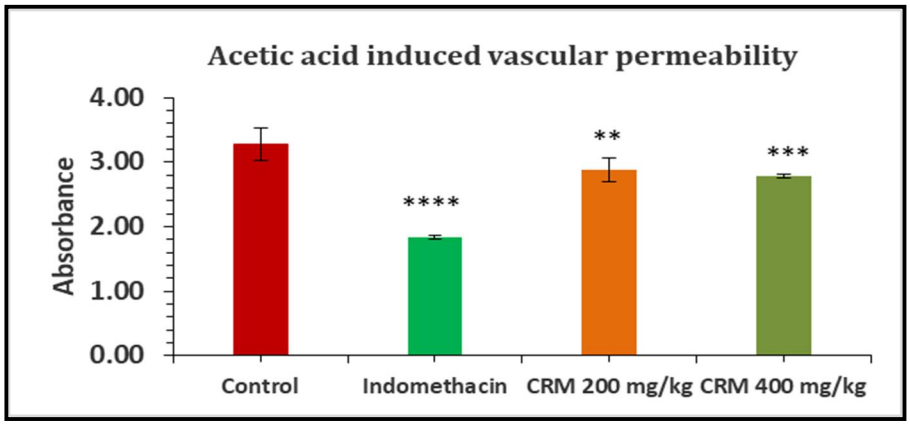

Fig. 2: Effect of CRM *e*xtract on Acetic acid induced vascular permeability in Mice

CONCLUSION

The outcome of this study is the first report on the acute anti-inflammatory effect of *Cuscuta reflexa* Roxb. stem methanolic extract *In Vivo* in paw edema caused by carrageenan and vascular permeability model induced by acetic acid. This provides scientific support for its long-standing applications in the treatment

of inflammatory conditions. The findings demonstrate the high number of secondary metabolites in the CRM. In order to promote healthy living, society will benefit from additional research on the isolation and identification of phytochemical constituents from plant extracts for biological activity, toxicity effect, and pharmacological in-vivo research.

CONFLICT OF INTEREST

No conflicts of interest are disclosed by the authors.

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