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Research Article

EVALUATION OF CARRAGEENAN INDUCED ANTI-INFLAMMATORY ACTIVITY OF STEM EXTRACTS OF *CUSCUTA REFLEXA* (ROXB) IN RATS

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ABSTRACT

Alcoholic (AESCR) and aqueous (AQESCR) extracts of stem of *Cuscuta reflexa* were evaluated for their anti-inflammatory activity in carrageenan induced paw edema model in rats, and compared to the activity of standard drug, Ibuprofen. These extracts were given orally in a concentration of 100, 200 and 400 mg/kg bd.wt. before carrageenan injection. Both the extracts with medium and higher doses i.e. 200 mg/kg and 400 mg/kg have reduced oedema volume by 47.27%, 72.72% and 57.72%, 80.00% respectively at 5th h as compared to standard drug Ibuprofen 96.36%. Thus the present study revealed that the selected extracts of *C. reflexa* exhibited a significant anti-inflammatory activity in carrageenan induced paw oedema model in rats.

Keywords: *C reflexa*, stem extracts, carrageenan, ibuprofen, anti-inflammatory activity.

INTRODUCTION

Inflammation, a local response of living mammalian tissues to injury, is a body defense reaction to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, the present investigation was carried out to evaluate the anti-inflammatory potential of C. reflexa in experimental animal models.

C. reflexa, commonly known as Amarbel is a leafless, delicate yellow coloured total stem parasite, belonging to the plant family Convolvulaceae. The tiny white flowers appear in bunches and the fruit are pea shaped and seeds are black in colour[2]. It is found throughout India. The plant is acrid; bitter; astringent , aphrodisiac, alternative, tonic and useful in diseases of the eye and of the heart, also used in biliousness and in “kapha;[3]

The herb has a bitter sharp taste; used as expectorant, carminative, tonic, anthelmintic, diuretic, blood purifier and lessens inflammation. It is also useful in jaundice, pain in the muscles and joints, headache, paralysis and in lumbago.

It was reported that decoction prepared with stem is useful in constipation, flatulence, liver complaints and bilious affections. The seeds have a bitter bad taste; sedative, emmenagogue, diuretic; useful in diseases of the liver and the spleen, chronic fevers, griping. hiccough; used to purify the blood and cleanse the bowels; the infusion is given in opthalmia, the decoction in biliousness as a purgative (Unani). The plant is purgative, it is

used externally against itch and internally to protract fevers. The stems are specially useful in bilious disorders [2,3,4].

On the basis of these common uses of this plant in traditional folk medicine, it is planned to evaluate the anti-inflammatory effect of AESCR and AQESCR in carrageenan induced inflammatory model in rats.

MATERIALS AND METHODS

Plant material

Stems of *C. reflexa* collected in the month of May were identified by a botanist Prof. V. Hemanth Kumar, V.L. College of Pharmacy, Raichur and dried in shade at room temperature then subjected to size reduction to a fine powder with the help of mixer grinder.

Chemicals

Ibuprofen was obtained as a gift sample from Cipla, Mumbai, India. Carrageenan was purchased from HiMedia Laboretories, Mumbai, India.

Animals:

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25g were procured from National Centre for Laboratory Animal sciences, C/0 Sri.Venkateswara Enterprises, Bengaluru for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry condition. i.e.

Room temperature - 26 ± 2^0 C
Relative humidity - 45-55%
Light/ dark cycle - 12:12 h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to the guidelines of CPCSEA, No. 425 with registration number 557/02/c/CPCSEA and Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur (Karnataka) and all the procedures were followed as per rules and regulations.

Preparation of extracts[5]

Preparation of alcoholic extract

The stem powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C. The extract was finally air dried thoroughly to remove all traces of the solvent.

Preparation of aqueous extract

About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform used as a preservative for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and the extract was concentrated on a water bath maintained at 50°C .

These two extracts were stored in airtight containers in a refrigerator below 4°C and were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies

The acute toxicity of AESCR and AQESCR was determined by using albino mice of either sex (16-20 g), maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts and observed for the mortality upto 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. A maximum dose of 2000 mg/kg bd. wt was tested for its mortality. From the LD_{50} doses of each extracts 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively[6].

Carrageenan induced paw oedema model[7,8]

Albino rats of either sex weighing 125 – 150 g were selected and were maintained on standard pellet diet and free access to water. The animals were divided into 8 groups each containing 6 animals. The groups were treated as follows:

Group A - Toxicant control (Carrageenan 1% w/v,0.1 ml s.c)
Group B - Standard (Ibuprofen 40 mg/kg, p.o)
Group C - AESCR (low dose 100 mg/kg, p.o)
Group D - AESCR (medium dose 200 mg/kg, p.o)
Group E - AESCR (high dose 400 mg/kg, p.o)
Group F - AQESCR (low dose 100 mg/kg, p.o)
Group G - AQESCR (medium dose 200 mg/kg, p.o)
Group H - AQESCR (high dose 400 mg/kg, p.o)

Initial paw volume of individual rats (right paw) was noted at '0' h. One hour after the administration of vehicle, extracts, standard

drug, all the rats were injected with 0.1 ml of histamine (0.1%) in normal saline in the sub-plantar region of the right hind paw and the left hind paw was served as reference. Immediately thereafter the oedema volume of the injected paws were measured plethysmographically at fixed time intervals i.e., 3rd h and 5th h.

The difference between paw volumes of the treated animals was measured and the mean oedema volume was calculated. Percentage reduction in oedema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{V_0 - V_t}{V_0} \times 100$$

Where

V_o = Paw volume of the control at time't'.

V_t = Paw Volume of the drug treated at time't'.

Stastical analysis

All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). P< 0.05*, 0.01** and 0.001*** were considered statistically significant.

RESULT

The AESCR and AQESCR with three dose levels tested i.e. 100, 200 and 400 mg/kg had exhibited a significant reduction in paw oedema volume in carrageenan induced paw oedema (acute) model in rats. Results are tabulated in (Table 1). Ibuprofen (40 mg/kg) is used as standard reference and it has reduced paw oedema volume by 93.61% at 5th h. AESCR and AQESCR with medium and higher doses i.e. 200 mg/kg and 400 mg/kg have reduced oedema volume by 38.29%, 53.19% and 48.93%, 61.70% respectively at 5th hr. (Figure 1).

DISCUSSION

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltration of leukocytes and the third one by granuloma formation[9]. Histamine is one of the important inflammatory mediators and it is potent vasodilators substance and increases the vascular permeability[10]. Inflammatory diseases affecting majority of the peoples, are very common and are known to be as oldest diseases as that of mankind. No substantial progress has been achieved till today for their permanent cure. Anti-inflammatory activity was investigated in acute models of inflammation in rats as the inflammation was induced by sub-plantar injection of carrageenan. It was reported that carrageenan administration causes release of various mediators like histamine, serotonin (initial phase), kinins (middle phase) and PG (final phase) that play an important role in the development of inflammation[11]. AESCR and AQESCR have inhibited the initial, middle and final phases suggesting that the extracts can block the mediators like histamine, kinins and PGs. In histamine induced paw edema model AESCR and AQESCR exhibited significant inhibitory action against histamine induced paw edema and this indicates that these extracts exhibited their anti-inflammatory action by means of inhibiting the synthesis, release or action of various inflammatory mediators like kinins and histamine.[12] The study showed that the AESCR and AQESCR effectively suppressed the oedema produced by the carrageenan, which is indicate that the extracts exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin's might be involved in the inflammation. Phytoconstituents reported for the anti-inflammatory activity are glycosides, flavonoids, sterols and saponins present in both the extracts hence these can be accounted for its anti-inflammatory activity[13]. The AQESCR was found to possess relatively better anti-inflammatory activity than AESCR.

CONCLUSION

The pharmacological studies on different parts of the plant C. reflexa revealed a significant antibacterial[5], anti-steroidogenic, psychopharmacological activity.

The preliminary phytochemical analysis of the AESCR and AQESCR revealed the presence of carbohydrates, sterols, flavonoids, glycosides, fixed oils fats, saponins and alkaloids.

From the studies it can be concluded that AESCR and AQESCR (200, 400 mg/kg) have shown significant anti-inflammatory activity against carrageenan induced paw edema model in rats (acute model). The AQESCR was found to be more potent than AESCR which is confirmed by its higher percentage reduction in paw oedema volume than the other in carrageenan induced paw oedema (acute) model in rats.

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Table 1: Anti-inflammatory effect of stem extracts of *C. reflexa* carrageenan induced (acute) paw oedema model in rats

Group %ROV	Treatment	Dose mg/kg	at 3rd h Paw oedema (ml)	%ROV	at 5th h Paw oedema (ml)
1 -	Control	10 mL	0.49±0.009	-	0.47±0.004
2 93.61	Standard (Ibuprofen)	40	0.08±0.005**	83.67	0.03±0.003**
3 25.33	AESCR	100	0.04±0.006**	18.36	0.35±0.0011**
4 38.29	AESCR	200	0.34±0.006**	30.61	0.29±0.004**
5 53.19	AESCR	400	0.28±0.008**	42.85	0.22±0.007**
6 34.04	AQESCR	100	0.36±0.006**	26.53	0.31±0.003**
7 48.93	AQESCR	200	0.29±0.006**	40.81	0.24±0.006**
8 61.70	AQESCR	400	0.23±0.006**	53.06	0.17±0.006**

n = 6, Significant at P < 0.05*, 0.01**, and 0.001***, ns = not significant
AESCR – Alcoholic extract of stem of *C. reflexa*, AQESCR – Aqueous extract of stem of *C. reflexa*
% ROV - % reduction of oedema volume

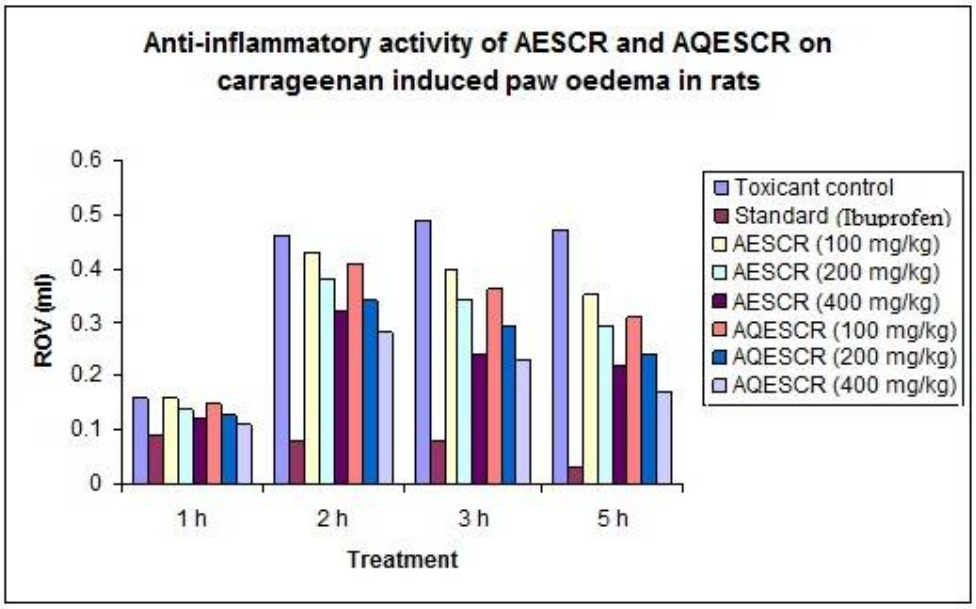

Fig. 1:

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