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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anti-Inflammatory Activity of The Extract of *Sesbania* Genus Plants in Animal Models

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ABSTRACT

The objective of this study was to evaluate the anti-inflammatory activities of the hydro-alcoholic extract of the leaves of Sesbania grandiflora and Sesbania bispinosa individually and in 1:1 combination, and anti-inflammatory effects of different fractions in animal models. The anti-inflammatory effect was examined by carrageenan induced paw edema test. Application of different doses of Sesbania genus plants and their combinations had significant anti-inflammatory effects on Carrageenan induced paw edema animal model. The findings showed that all the Sesbania genus plants and their combinations at given doses of 400 and 600mg/Kg demonstrated anti-inflammatory activity. Plant extracts with 1:1 Combination has shown better anti-inflammatory activity than individual plant extract and are comparable with Diclofenac. This study demonstrated the anti-inflammatory effects of Sesbania genus extracts in animal models and supports traditional use of this plant in the treatment of inflammation. More studies are required to identify the active components.

Keywords: *Carrageenin induced paw edema, Sesbania grandiflora, Sesbania bispinosa, Anti-inflammatory*

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INTRODUCTION

Inflammation is common nonspecific manifestations of many diseases. Although many opiates have been used classically in these conditions, but some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory, depression, and possible dependence. In recent years, there has been an increasing interest to find new anti-inflammatory drugs with possibly fewer side effects from natural sources and medicinal plants. *Sesbania grandiflora and Sesbania bispinosa* (known as Agati and Dhaincha respectively) is a plant belonging to family *Fabeceae*, which has been used traditionally for treatment of inflammatory conditions. Also, it is used for treatment of anti-inflammatory conditions as well. *Sesbania* is a genus of flowering plants in the pea family, Fabaceae and the only genus found in the tribe *Sesbanieae*. River hemp is the common name for the plants in this genus. Some 60 species are currently accepted, with about 39 still unsolved. The largest number of species are found in Africa, and the reminder in Australia, and Asia.

Sesbania grandiflora is a fast-growing tree. The leaves are regular and rounded and the flowers white, red or pink. The fruits look like flat, long, thin green beans. The tree thrives under full exposure to sunshine and is extremely frost sensitive. While *Sesbania bispinosa* is an annual shrub which can grow to seven metres in height but usually only reaches one to two meters. It sends out fibrous, pithy stems with long leaves and bears purple-spotted yellow flowers. It produces pods which contain light brown beans. Pharmacological evaluations have shown anti-inflammatory activity of methanolic extract of *Sesbania bispinosa* and *Sesbania grandiflora individually*. In a preliminary study, acute and chronic Anti-inflammatory activity of the methanol extract of *Sesbania bispinosa and Sesbania grandiflora* has been reported.

In the present study, acute anti-inflammatory effects were evaluated for individual plants and 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa* species and its fractions in rat using Carrageenan induced paw edema model.

MATERIAL AND METHODS

Experimental

Plant Material

The leaves of *Sesbania bispinosa* and *Sesbania grandiflora* were selected from Junnar taluka region and identified by Dr. Savita S. Rahangdale, the voucher specimen number 212 and 23719 respectively and deposited in the Department of Botany, BJ college, Ale, Junnar (Pune), Maharashtra.

Preparation of extract and fractions

For preparation of the hydro-alcoholic extract, dried and grinded leaves of individual plant and 1:1 mixture of *Sesbania bispinosa* and *Sesbania grandiflora* were macerated in ethanol 70% for three times (each time 24h). The extract was then filtered and concentrated with vacuum evaporator.

To yield different fractions (400 g and 600 g), dried hydroalcoholic extract was suspended in water and partitioned by hydro alcohol. Each fraction was evaporated to obtain hydro-alcohol fraction which was used for bioassay.

Phytochemical Screening

Phytochemical investigations of the *Sesbania* genus plants (individual and 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa*) were carried out using standard methods and tests.

The test for tannins was carried out by subjecting 1g of extract in 2 mL of distilled water, filtrate and ferric chloride reagents were added to the filtrate. The extract was subjected to frothing test for the identification of saponins and to Fehling's test for glycosides. Alkaloids were detected in the alkaloid fraction obtained by a classical acid:base extraction procedure for alkaloids and analyzed by TLC in chloroform : methanol : ammonia solution 25% 8: 2: 0.5 as solvent system, spots were detected after spraying with Dragendorff's reagent. The presence of flavonoids was determined using 1% aluminum chloride solution to the extract and yellow coloration. Another test for flavonoids dilute ammonia (5mL) was added to the extract and then concentrated sulphuric acid (1mL) was added. Steroids were detected by adding 1mL of acetic anhydride to 0.25gm ethanolic extract of each sample with 1mL H2SO4. The color changed from violet to blue or green indicating the presence of steroids.

The test for anthraquinones was performed with 0.5g of extract boiled with 10mL sulphuric acid and filtrate. Then filtrate was shaken with 5m $CHCl_3$ and $CHCl_3$ layer was removed to another tube and 1mL of ammonia was added and colour change was observed.

Detection of terpenoids (triterpenoids) was carried out by adding 2 mL of $CHCl_3$ to 0.5 gm of extract and then adding carefully concentrated H_2SO_4 (3mL) to form a layer and reddish to brown color in interface.

Animals

48 adult male wistar rats (150-200g) were housed in animal unit under standard laboratory conditions (temperature23±2˚C) with 12h dark and 12h light cycle. The animals had free access to standard dry pellet diet and tap water and libitum. Pregnant animals and those that had delivered once or used previously for any other experimental purposes were excluded from the study.

Anti-inflammatory Activity

Carrageenan-induced hind paw edema in rats:

The acute hind paw edema was produced by injecting 0.1 mL of Carrageenin (prepared as 1% suspension in 1% CMC) locally into the planter aponeurosis of the right hind paw of the rats. Initially 48 adult wistar rats were divided into eight groups. The animal in each group were treated with *Sesbania bispinosa, Sesbania grandiflora* and 1:1 mixture of *Sesbania bispinosa* and *Sesbania grandiflora* at doses of 400 mg/kg and 600 mg/Kg p.o. and Diclofenac-10mg/kgp.o. as positive control group (Standard) and Normal saline 10mL/kg p.o. as negative control group (Blank).1 h after drug treatment, paw edema was induced by the injection of carrageenan (an edematogenic agent).The paw volume was measured by a Plethysmometer. The measures were determined at 0 hr(Vo: before edematogenic agent injection) and 3hr intervals later (Vt). The difference between Vt and Vo was taken as the edema value. Increase in the paw edema volume was considered as the difference between 0 and 3 Hr. % inhibition of edema volume between treated and control groups was calculated as follows:

% inhibition = (Vt − Vo)control − (Vt − Vo)treated / (Vt − Vo)control X 100

Where, Vo = volume of the paw of control at time 't', Vt = Volume of the paw of the drug treated at time 't';

Statistical analysis

The results are reported as mean ± S.E.M. The statistical analysis was performed using one-way analysis of variance (ANOVA). Group differences were calculated by post hoc analysis using Tukey's test. Forall tests, differences with values of $P<0.05$ were considered significant.

RESULTS

Preliminary phytochemical study of the hydro-alcoholic extract of individual plant and 1:1 combination of *Sesbania bispinosa* and *Sesbania grandiflora s*howed the presence of saponins, terpens, phenols, alkaloids, tannins, and flavonoids.

Carrageenan-induced hind paw edema in rats:

The mean increase in paw edema volume was about 25.23 in the vehicle treated control rat. 1:1 composition of *Sesbania grandiflora* and *Sesbania bispinosa* (400 and 600 mg/kg p.o.) significantly ($p < 0.01$) reduced the mean paw edema volume at 3 Hr after Carrageenin injection. 1:1 composition of *Sesbania grandiflora* and *Sesbania bispinosa* (400 and 600 mg/kg p.o.) exhibited anti-inflammatory activity in a dose dependent manner with the % inhibition of paw edema of 56.25% and 59.40% respectively as compared with the control group. *Sesbania bispinosa* (400 and 600 mg/kg p.o.) shows 50.35% and 54.60% while *Sesbania grandiflora* shows (400 and 600 mg/kg p.o.) 44.25% and 47.12 % lowest with compare to control group. In general, plant extracts with 1:1 combination has shown better anti-inflammatory activity than individual plant extract. However, standard drug, Diclofenac (10mg/kg) showed highly significant ($p<0.001$) anti-inflammatory activity with the percent inhibition of 62.30% (Table 1).

DISCUSSION AND CONCLUSION

Several natural products have been used to treat inflammation, *Sesbania bispinosa* and *Sesbania grandiflora* are plants used for treatment of inflammation conditions for many years. In this study, anti-inflammatory activities of the hydro-alcoholic extract (70%) of the leaves of 1:1 combination of *Sesbania bispinosa* and *Sesbania grandiflora* were assessed in different well accepted animal models, including Carrageenin-induced hind paw edema test.

Table 1: Evaluation of anti-inflammatory effect of *Sesbania* genus extracts (Acute inflammation)

Group	Treatment	Dose mg/kg	Carrageenin-induced paw edema mean volume (mL) & SEM	% Inhibition
I	Control	Normal Saline	25.23±4.2	
II	*Sesbania bispinosa* (SB)	400	12.53±3.1	50.35
III	*Sesbania bispinosa* (SB)	600	11.45±2.5	54.60
IV	*Sesbania grandiflora* (SG)	400	14.07±2.1	44.25
V	*Sesbania grandiflora* (SG)	600	13.34±2.9	47.12
VI	1:1 mixture of SB & SG	400	11.04±3.7**	56.25
VII	1:1 mixture of SB & SG	600	10.24±4.0**	59.40
VIII	Diclofenac Sodium	10	9.51±2.6***	62.30

Values are expressed as Mean ±SEM; n=6, ***p<0.001; **p<0.01 p<0.05

The edema and inflammation induced by carrageenin is showed to be mediated by histamine and 5-HT, which increased vascular permeability is maintained by the release of kinins up to 3 hrs. the mediators appear to be Prostaglandins, the release of which is closely associated with migrations of leucocytes into the inflamed site. The carrageenin induced paw edema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. In 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa* plant extract produced significant inhibitory activity against histamine, 5-HT and PGE_2 induced hind paw edema in rats.

All three plant extracts were compared in two different doses. Carrageenin paw edema model for acute inflammation showed that hydro alcohol extract of *Sesbania bispinosa* and *Sesbania grandiflora* 1:1 composition 400 and 600 mg/kg dose shows more anti-inflammatory activity compared with control and individual plants (i.e. *Sesbania grandiflora* and *Sesbania bispinosa*). Further studies are required to identify the active ingredients of these plant extracts and possibility of synergism or additive effect with combination. The additional benefit with combination needs to be explored in clinical studies.

CONFLICT OF INTEREST

Author have no conflict of interest to declare.

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