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Evaluation of anti-inflammatory activity of the plant extract *Smithia Sensitiva*

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ABSTRACT

To evaluate the anti-inflammatory property of the whole plant of Smithia sensitiva. Smithia sensitiva belonging to the family Fabaceae is a plant used as an anti inflammatory and anti oxidant drug by tribal peoples in kerala. Smithia sensitiva is a low growing annual herb 30-90cm long and it is distributed widely in Hilly areas. The whole plant is traditionally used as Refrigerant, Galactogogue and as lotion in headaches. The present study aimed at the evaluation of the anti-inflammatory activity of the methanolic extract of Smithia sensitiva (MESS) by both in vitro and in vivo method. In vitro method was estimated by bovine serum albumin denaturation (BSA) method and in vivo method was estimated by Cotton pellet-induced granuloma method. Both the methods showed significant anti-inflammatory property of the methanolic extract. The MESS at a concentration of 400µg/ml showed potent activity on comparing with the standard drug.

Keywords: *Smithia sensitiva*, BSA, Cotton pellet-induced granuloma, Anti- inflammatory, MESS.

INTRODUCTION

Inflammation is the response of living tissues to injury and is caused by a variety of stimuli including physical damage, ultraviolet (UV)-irradiation, microbial invasion and immune reactions. Inflammation involves an increase of blood supply to the affected region by means of vasodilation [1]. The classical NSAIDs are effective for the treatment of pain and inflammation, however, their chronic use particularly in patients with arthritis or other chronic inflammatory diseases is associated with adverse effects such as gastrointestinal perforation, ulceration, bleeding (PUB), and renal toxicity mainly due to the blockade of COX-1[2]. In Ayurveda and traditional medicines, there are several records of treating people suffering from pain and inflammation with phytochemicals. In recent times, focus on plant research has increased and non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the most widely used classes of drugs [3]. The family Fabaceae consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phytochemical constituents. *Smithia sensitiva* belonging to the family Fabaceae is a plant used as an anti inflammatory and anti oxidant drug by tribal peoples in kerala. The effect is due to the property of any of the constituents present in the plant [4]. *Smithia sensitiva* is a low growing annual herb 30-90cm long and it is distributed widely in Hilly areas. The whole plant is traditionally used as Refrigerant, Galactogogue and as lotion in headaches [5]. Our aim is to extract the dried plant with methanol and study the anti-inflammatory activity by *in vitro* and *in vivo* method.

Recently, we have reported the significant anti–inflammatory activity of methanolic extract of *C.siamensis* and *Smithia sensitiva* using Carrageenan induced paw oedema method. *Smithia sensitiva* was also reported the cox1 and cox2 inhibitory activity. Based on the information from previous literature and considering the anti–inflammatory

activity *in vivo*, the present study was undertaken to evaluate the anti-inflammatory activity of *Smithia sensitiva* by *in vitro* and *in vivo* method.

MATERIALS AND METHODS

2.1 Collection and identification of plant

The plant *S.sensitiva* was collected from Wayanad district and taxonomically identified and authenticated by Dr. Jayasmitha S J, Parassinikkadavu Ayurveda Medical College, Kannur. The voucher specimen has been preserved in our department for future reference. The plant materials were dried under shade, sliced into small pieces, pulverized using a mechanical grinder and passed through 40 mesh sieve and stored in an airtight container for further use [6].

2.2 Preparation of the Extract

The powdered whole plant of *S.sensitiva* was extracted with n-hexane, chloroform, ethyl acetate, methanol and water successively at room temperature [7]. After exhaustive extraction, the solvent was collected and filtered. The solvent was concentrated under reduced pressure at 50-55°C. The concentrated n-hexane, chloroform, ethyl acetate, methanol and water extracts were kept in desiccators for further use. The methanolic extract of *Smithia sensitiva* (MESS) found to have most phytoconstituents during the preliminary phytochemical study.

2.3 Chemicals and instruments.

All chemicals used in the study were of analytical grade. Reference standard drug diclofenac sodium and indomethacin was obtained as gift sample from MRL Labs Chennai.Shimadzu-A-1800 UV-Visible spectrophotometer was used for the *in vitro* study. All other chemicals were purchased from S.D.fine chemicals Ltd (Mumbai, India).

2.4 Animals.

Wistar albino rats (150-200g) of either sex were used for experimental study. The animals were housed in colony cages at 25 ± 2°C and relative humidity (50 ± 5%) with 12 h light and dark cycle. They were provided with free access to food and water ad libitum [8]. The animals were cared and used in accordance with the CPCSEA guidelines and experimental protocols approved by institutional animal ethics committee (CPCSEA No.CADD/27/282).

2.5 Acute Toxicity Studies [9]

Acute Toxicity Studies was done according to the OECD guidelines 423(Acute toxicity class method)

2.6 *In vitro* anti-inflammatory activity:

In vitro anti-inflammatory activity of *Smithia sensitiva* was performed by using bovine serum albumin denaturation method. The reaction mixture 3ml contained, 50µl of the test solution (100, 200, 400µg/ml) and diclofenac sodium (100µg/ml) was prepared in methanol, 450µl of 5% w/v BSA was added to all the above test tubes. For control tests, 50µl of distilled water instead of test solution. The test tubes were incubated at 37^0C for 20 minutes and then heated at 57^0C for 3 minutes [10, 11]. After cooling the test tubes, 2.5 ml phosphate buffer saline (PH 6.3) was added to each tube. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm.

$$\% \text{ Protein Denaturation Inhibition} = \frac{\text{Abs of control - Abs of treated}}{\text{Abs of control}} \times 100$$

2.7 *In vivo* anti-Inflammatory Screening:

Cotton pellet-induced granuloma was performed by the method of Winter and Porter (1957)**.** The animals were shaved and anaesthetized. Sterile pre-weighed cotton pellets (50 ± 1 mg) were implanted in the axilla region of each rat through a single needle incision by aseptic method. In this study wistar rats of either sex weighing 150-200g were used and each group contains three animals [12, 13]. All the groups of animals were given the extracts at a dose of 100,200&400 mg/kg, p.o, Standard Indomethacin 10 mg/kg, p.o, throughout the experimental period of

seven days, where the control received 1% Tween 80 (10ml/kg), p.o. The drugs were administered to the respective group of animals for seven consecutive days from the day of cotton pellet implantation. On the eighth day, the animals were anaesthetized again and the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C constant weight. The increment in the dry weight of the pellets was regarded as a measure of granuloma formation.

2.8 Statistical analysis: Results obtained were evaluated by ANOVA by Bonferroni test; values of $p<0.05$ were considered statistically significant.

RESULTS

3.1 Acute Toxicity Studies

MESS when orally administered in the dose range of 5-2000mg/kg to mice did not produce any significant changes in the autonomic or behavioral response during the observation period. The extracts were safe for administration up to the dose of 2000 mg/kg. LD_{50} was found to be more than 2000mg/kg.

3.2 *In vitro* anti-inflammatory activity.

In the BSA denaturation inhibition assay, MESS (100,200&400 µg/ml) displayed significant activity. The methanolic extract of *Smithia sensitiva* at 400µg/ml showed % inhibition of 39.82 ($P<0.01$) by Bovine serum albumin denaturation method. The results were tabulated in (**Table 1**). MESS at a concentration of 400 µg/ml showed maximum activity**.**

Table: 1. Effect of Methanolic extract of *S. sensitiva* on *In vitro* Bovine serum albumin denaturation inhibition

Group	Absorbance at 660 nm	% Inhibition of denaturation
Control	0.4670± 0.00474	----
Diclofenac (100µg/ml)	0.1480± 0.00441**	68.30
MESS (100µg/ml)	0.3433± 0.00682**	26.48
MESS (200µg/ml)	0.3190±0.00584**	31.69
MESS (400µg/ml)	0.2810±0.0042**	39.82

*Values are Mean±SEM, n=3. ** - Significant (p value < 0.01) compared to control*

3.3 *In vivo* anti-Inflammatory Screening:

In cotton pellet induced granuloma, MESS significantly ($p<0.01$) diminished the formation of granuloma when compared to the sham operated rats. MESS at the dose of 400mg/kg displayed maximum activity. The results were tabulated in (**Table** 2).

Table: 2. Effect of Methanolic extract of *S. sensitiva* on cotton pellet induced granuloma

Groups	Weight of granulation (in mg)	% inhibition
Control –1% Tween 80 (5ml/Kg)	34.89 ± 0.561	-------
Indomethacin (10 mg/ kg)	13.05 ± 1.02**	62.59
MESS (100mg/ kg)	23.65 ± 0.864**	32.27
MESS (200 mg/ kg)	20.54±1.04**	41.12
MESS (400 mg/ kg)	16.05 ± 0.954**	53.99

*Values are Mean±SEM, n=3 **- significant (p value < 0.01) compared to control.*

DISCUSSION

In Ayurveda and traditional medicines, there are several records of treating people suffering from pain and inflammation with phytochemicals. Inflammatory responses occur in three distinct phases, an acute, transient phase characterized by local vasodilation and increased capillary permeability. A sub-acute phase, characterized by infiltration of leucocytes and phagocytic cells. A chronic proliferative phase in which tissue degeneration and fibrosis occur. There are several records on plants in Ayurveda and traditional medicines/ethno medicines which focused on relief from pain, swelling, fever, inflammation and rheumatism. The purpose of the present study is to provide scientific support to rationalize the folklore or traditional claim of the selected plants for treating inflammation [14].

The Preliminary phytochemical screening of the extract showed the presence of alkaloids, sugars and carbohydrates, steroids, tannins and flavonoids. The acute toxicity studies of the extracts showed that there was no lethality or any toxic reactions found at any dose selected until the end of the study period. The extract did not produce any characteristic behavioral changes. Denaturation of proteins is the mainstay of inflammation in the pathogenesis of rheumatoid arthritis. Many anti-inflammatory agents exhibit the anti rheumatoid effect by inhibiting thermally induced protein denaturation [15]. The compounds that inhibit the denaturation of proteins *in vitro* may be used as anti-inflammatory agents. BSA denaturation method was selected for the *in vitro* evaluation of anti-inflammatory property. BSA assay seeks to eliminate the use of live specimens as far as possible in the drug development process. When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction associated with chronic inflammatory diseases [16]. Thus agents that stabilize proteins from denaturation may be of therapeutic value in inflammatory diseases. In the present work, MESS inhibited the protein denaturation in concentration dependent manner and it may be due to anti-denaturation effect of flavonoids, triterpenoids and fixed oils which is line with earlier reports. The cotton pellet granuloma is widely used to evaluate the transudative and proliferative components of chronic inflammation and can serve as a sub chronic and chronic inflammatory model for the study of anti-inflammatory substances. The moist weight of the pellets correlates with transude, the dry weight of the pellet correlates with the amount of granulomatous tissue formed. Chronic inflammation occurs by means of the development of proliferate cells which can be either spread or in granuloma form [17]. Granulomas form in response to immune mediation when macrophages and lymphocytes accumulate around inert foreign particles that have not been eliminated, together with epitheloid and giant cells derived from macrophages to form a ball of cell. In chronic inflammatory states, the efficacy of anti-inflammatory agents can be indicated by the inhibition of fibroblasts and infiltration of neutrophils and exudation [18]. Treatment with MESS exhibits significant reduction of granuloma weight which may be due to the inhibitory effect on granulocyte infiltration and the release of inflammatory mediators that promote cell proliferation and angiogenesis which is in collaboration with previous literature.

The Study was conducted based on the ethno medical background of the plant *Smithia sensitiva.* The anti-inflammatory activity of MESS could be related to the presence of flavanoids, triterpenoids, fixed oils and fats. Methanolic extract significantly ($p<0.01$) inhibited the granuloma formation in rats. The *in vivo* findings also confirm that the plant *Smithia sensitiva* has a potential anti-inflammatory activity and these plants can be a source of anti-inflammatory lead molecules of pharmaceutical interest. This is the first report on the anti-inflammatory activity of MESS.

CONCLUSION

The present investigation provided the scientific support for the ethno medicinal use of the plant and the findings also confirm the potential of Indian medicinal plants as a source of anti-inflammatory lead molecules of pharmaceutical interest.

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