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Evaluation of Phytochemical Screening and Anti-Inflammatory Activity of Root Extracts of *Solanum Sisymbriifolium* Lam

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ABSTRACT

Nonsteroidal anti-inflammatory (NSAIDs) drugs are generally used as a part of drug therapy in inflammation. However, these drugs have severe side-effects. Therefore, naturally originated compounds with very little side-effects are required to substitute chemical therapeutics and have better safety profile. *Solanum sisymbriifolium* (Family: Solanaceae) has many therapeutic uses mentioned in Ayurveda. Moreover, the plant has diverse pharmacological activity such as antihypertensive, diuretic, analgesic, contraceptive, antisyphilitic, hepatoprotective. Therefore, the present study was designed to investigate the preliminary phytochemical screening and anti-inflammatory activity of methanolic extract of roots of *Solanum sisymbriifolium* (MERS). Carrageenan-induced rat paw edema model was used for assessing the anti-inflammatory activity. The phytochemical analysis of MERS confirmed the presence of alkaloids, carbohydrates, flavanoids, terpenoids, tannins and saponins. Furthermore, MERS at doses of 50,100, 200 and 300 mg/kg showed anti-inflammatory activity in dose-dependent manner.

Keywords: Anti-inflammatory activity, Aspirin, Carrageenan-induced paw edema, root methanolic extract, *Solanum sisymbriifolium,*

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INTRODUCTION

The use of plants to treat ailments is as old as antiquity since 6000 to 4000 years ago in Ayurvedic and Traditional system of medicines in India. [1] Globally, the use of traditional medicine and medicinal herbs in developing countries is most attracting by scientists for the maintenance of good health.[2,3] Synthetic molecules such as nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase (COX)-2 inhibitors increase the incidence of adverse cardiovascular thrombotic effects. So, in order to overcome, main research is focused on the scientific exploration of herbal drugs alleged folkloric use as pain relievers, anti-inflammatory agents having fewer side effects. [4,5]

Solanum sisymbriifolium (Family: Solanaceae) is an annual or perennial erect, rhizomatous herb about 1.5 m in height. The steroidal glycol-alkaloids, solasodine and other closely related glycol-alkaloids were found in the *S. Sisymbriifolium.* [6] Solasodine serves as an important intermediate in the synthesis of steroidal hormones [7] and is a potential alternative to diosgenin as precursor in the synthesis of steroidal hormones.[8] Moreover, the plants are used in the diverse pharmacological activities such as diuretic, analgesic, contraceptive, antisyphilitic and hepatoprotective [9], anti-inflammatory. [10,11,12] Further, the various parts of plant are also used in the treatment of hypertension, diarrhoea and in the regulation of fertility. [13,14,15] In the light of above background, the present study was aimed to evaluate the preliminary phytochemical screening and anti-inflammatory activity of methanolic extract of roots of *S. sisymbriifolium.*

MATERIALS AND METHOD

Drugs and chemicals

Carrageenan was purchased from Sigma-Aldrich Co., Spruce Street, St. Louis, MO, USA and sodium carboxy methyl cellulose (CMC) from Hi-media, Mumbai, India. Pure sample of aspirin was obtained from Cadila Pharmaceutical Ltd, Ahemdabad, Gujarat, India. All other chemicals used in this study were of analytical grade.

Plant materials

The roots of plant *S. sisymbriifolium* were collected from young matured plant in Saurashtra University campus, Rajkot. The root was washed cleanly, cut into small pieces and then air dried under shade. The plant was taxonomically identified and authenticated by Dr. Reddy, Department of Bioscience, S. P. University, V. V. Nagar, Gujarat, India. A voucher specimen (Herbarium/2007-08/03) was retained in the depository of Department of Pharmaceutical Sciences, Saurashtra University, Rajkot for future reference.

The roots of *S. sisymbriifolium* were coarse powdered. The obtained powder (100 g) was sequentially extracted with different solvent by increasing polarity order i.e. petroleum ether (60-80°C), benzene, chloroform, acetone, methanol and water for 48 hr at ≤ 40 °C by soxhlet extraction process. [16] The powdered material was dried each time before extracting with the solvent in oven below 50° C. The resultant extracts were concentrated under reduced pressure at room temperature using rotary flash vacuum evaporator. The final obtained extracts were weighed and calculate its percentage in terms of the air-dried weight of the plant material. Further, this concentrated extract then completely dried in freeze drier to yield solid residue and stored in vacuum sealed air tight containers until further use.

Preliminary phytochemical screening

Phytochemical screening of *S. sisymbriifolium* root extracts was performed for the qualitative detection of various phytoconstituents such as alkaloids, glycosides, carbohydrates, phenolic and tannins, phytosterols, fixed oils, amino acids, flavonoids, saponins, gums and mucilage using reported methods.[17]

Animals

Wistar albino rats of either sex (150-200 g) were obtained from the central animal house of Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. All animals were housed in polypropylene cages (3 in each cage) at an ambient temperature (25 ± 1 °C), relative humidity (55 ± 5%), and were maintained under a 12 hr light/dark cycle (light on 07.30-19.30 hr). Animals had free access to standard pellet diet (Amrut, Pranav Agro Industries Ltd, Vadodara, Gujarat, India) and water given *ad libitum.* The care and the use of these animals were in accordance with the guidelines of the committee for purpose of control and supervision of experimental on animal (CPCSEA).

Experimental design

For the present study, rats were divided into seven groups of six animals each and allowed to acclimatize for one week prior to the experiments. Paw edema was induced [18] by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. The group - I received 1% sodium CMC solution. In the group - II, 1% w/v carrageenan, suspended in 1% CMC was given. The reference standard group - III was treated one hr before with a suspension of aspirin in 1% sodium CMC at a dose of 150 mg/kg body weight, p.o. The groups IV-VII received methanolic extract of roots of *S. sisymbriifolium* (MERS) in 1% sodium CMC solution before one hr.

- Group I Normal control (Vehicle only, 1% CMC)

- Group II Disease control (1% w/v carrageenan, suspended in 1% CMC)
- Group III Standard (Aspirin, 150 mg/kg, suspended in 1% CMC)
- Group IV MERS (50 mg/kg, suspended in 1% CMC)
- Group V MERS (100 mg/kg, suspended in 1% CMC)
- Group VI MERS (200 mg/kg, suspended in 1% CMC)
- Group VII MERS (300 mg/kg, suspended in 1% CMC)

The paw edema volume was measured before and upto four hrs after the injection of carrageenan by the volume displacement method using mercury column plethysmometer. The edema volume was determined and expressed as percentage swelling, compared with the initial hind paw volume of each rat.

Percentage inhibition in edema volume was calculated by using the following formula,

Percentage inhibition = $(V_0 – V_t) / V_0$ X 100

Where, V_0 = Volume of the paw of control at time 't'.

V_t = Volume of the paw of drug treated at time 't'

Data analysis

All the data are expressed as mean ± Standard Error of Mean (SEM). Results were analyzed using one-way ANOVA followed by multiple comparisons Dunnett's test using Graph Pad Prism (Version 5). A *p*-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of plant materials

The phytochemical screening of the different extracts of *S. Sisymbriifolium* showed the presence of alkaloids, carbohydrates, flavanoids, terpenoids, tannins and saponins (Table 1), Major phytoconstituents were present in the MERS. Therefore, MERS was selected for evaluation of anti-inflammatory activity.

Table 1: Phytochemical profile of *S. sisymbriifolium root* extracts

Phytochemicals	PERS	BERS	CERS	AERS	MERS	WERS
Alkaloids	-	-	+	+	+	-
Glycoside	-	-	-	-	-	-
Saponin	-	-	-	+	+	+
Phytosterol	-	-	-	+	+	+
Phenol & Tannin	-	-	+	+	+	+
Protein	-	-	-	+	+	+
Fixed Oil	-	-	-	-	-	-
Carbohydrate	-	-	-	-	+	+
Gum & Mucilage	-	-	-	-	-	-

Flavanoids	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	-

Abbreviations: PERS: Petroleum ether, BERS: Benzene, CERS: Chloroform, AERS: Acetone, MERS: Methanol, WERS: Water extract , (+) present/detected, and (-) not detected.

Anti-inflammatory activity

Table 2 depicts the effect of MERS and aspirin in carrageenan-induced experimental rats at different time intervals. The carrageenan-treated rats exhibited significant ($p < 0.001$) increase in paw edema volume when compared with control rats. MERS (50, 100, 200 & 300 mg/kg, p.o.) and aspirin (150 mg/kg, p.o.) treated rats showed anti-inflammatory activity ($p < 0.001$) as compared with carrageenan-induced rats. Further, MERS administered at a dose of 50, 100, 200 & 300 mg/kg p.o prevented carrageenan-induced paw edema with a percentage inhibition of 32.43%, 62.16%, 64.86%, and 72.97% at four hr, respectively, while aspirin at a dose of 150 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition of 81.08% at four hrs.

Table 2: Anti-inflammatory activity of MERS in experimental rats

Group	Initial Paw Volume	1st hrs	2nd hrs	3rd hrs	4th hrs
CON	1.16 ± 0.003	1.16 ± 0.003	1.16 ± 0.003	1.16 ± 0.003	1.16 ± 0.003
DC	1.16 ± 0.005	1.31 ± 0.010[a]	1.43 ± 0.011[a]	1.50 ± 0.006[a]	1.54 ± 0.012[a]
STD	1.15 ± 0.06	1.32 ± 0.010	1.30 ± 0.006[b]	1.27 ± 0.006[b]	1.22 ± 0.006[b]
Test 1	1.14 ± 0.008	1.33 ± 0.007	1.40 ± 0.007	1.43 ± 0.007[b]	1.41 ± 0.007[b]
Test 2	1.16 ± 0.008	1.34 ± 0.004	1.32 ± 0.004[b]	1.31 ± 0.004[b]	1.29 ± 0.004[b]
Test 3	1.14 ± 0.003	1.34± 0.004	1.31 ± 0.004[b]	1.29 ± 0.004[b]	1.27 ± 0.003[b]
Test 4	1.14 ± 0.003	1.32 ± 0.004	1.30 ± 0.004[b]	1.26 ± 0.004[b]	1.24 ± 0.004[b]

Value are expressed as Mean ± SEM (n=6), CON, normal control; DC, disease control; STD, disease animals treated with aspirin (150 mg/kg, p.o.); Test 1, disease animals treated with MERS (50 mg/kg, p.o.); Test 2, disease animals treated with MERS (100 mg/kg, p.o.); Test 3, disease animals treated with MERS (200 mg/kg, p.o.); Test 4, disease animals treated with MERS (300 mg/kg, p.o.). Significantly different from normal control ($p^a < 0.001$); significantly different from disease control ($p^b < 0.001$); One way ANOVA followed by Dunnett's Multiple Comparison Test.

Carrageenan-induced acute inflammation is one of the most suitable and widely accepted animal model for the screening of anti-inflammatory agents. The duration of development of edema in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. [19] The first initiation phase of inflammation occurs within an hour of carrageenan insult. [20] This

model is believed to be sensitive to COX inhibitors in experimental rats and has been used to evaluate the effect of NSAIDS, which primarily acts by inhibiting the COX involved in prostaglandin synthesis. [21] It plays a vital role in the development of the second phase of inflammatory reaction, which is measured after three hrs. [22] The results of the present study showed the effect percentage inhibition of paw edema, 32.43%, 62.16%, 64.86 and 72.97% at doses of 50, 100, 200 and 300 mg/kg, respectively, at four hrs by administration of MERS.

After the three hrs exposure of carrageen, the suppression of edema volume correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents. [22] Therefore, results of these studies together with those of earlier ones, suggest that MERS has an ability to inhibit the COX pathway which is involved in carrageenan-induced paw edema. The present study indicates that there could be herbs that need to be explored as potential treatments for inflammation. Moreover, detailed work has been carried out on isolation and characterization of lead molecules from MERS responsible for its anti-inflammatory property.

Table 3: Effect of MERS on carrageenan-induced paw edema in experimental rats

Sr. No.	Treatment Group (n=6)	Dose (mg/kg)	% Inhibition of edema at 4 hr.
1	Control	-	-
2	Standard (Aspirin)	150	81.08
3	Test-1	50	32.43
4	Test-2	100	62.16
5	Test-3	200	64.86
6	Test-4	300	72.97

CONCLUSION

This research work shown that the MERS possessed a significant antioedematogenic effect on paw oedema induced by carrageenan. Hence, its anti-inflammatory action was confirmed. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation, the results of the present study are an indication that *S. sisymbriifolium* can be effective in inflammatory disorders.

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