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Research Article

Evaluation of crude saponins extract from leaves of *Sesbania sesban* (L.) Merr. for topical anti-inflammatory activity

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ABSTRACT

Sesbania sesban is a short-lived shrub up to 8 m tall, which belongs to the family Leguminosae. Leaves are found to be rich in saponin content. The leaves of *Sesbania sesban* has traditionally been used as purgative, demulcent, maturant, anthelmintic and for all pains and inflammation. This study was intended to evaluate the topical anti-inflammatory activity of the crude saponins extract by carrageenan induced rat paw edema method by preparing the gel formulation. The activity was carried on Wistar albino rats, receiving two strengths of crude saponin gel at a concentration of 1% w/w and 2%w/w respectively and Diclofenac sodium gel (1%w/w) was used as reference drug. The crude saponins extract in 2% w/w gel formulation showed significant anti-inflammatory activity in comparison to control group and the results were comparable to the activity shown by reference drug.

Keywords: *Sesbania sesban;* topical anti-inflammatory activity; carrageenan induced paw edema.

INTRODUCTION

Sesbania sesban, commonly known as 'Egyptian sesban' is one of the six species of genus sesbania which is commonly found to be grown in tropical region of India. The plant is widely grown for its nitrogen fixing ability and as wind shades. The plant has got good medicinal importance (Wealth of India, 2003). According to ethno medicinal claims the poultice of leaves of *S. sesban* promotes suppuration of boils and abscesses and absorption of inflammatory rheumatic swellings. Juice of fresh leaves is credited with anthelmintic properties (Kirtikar et al., 1996). *S.sesban* leaves are found to have clinical application in Vicharchika, a skin disease like Eczema (Kendra et al., 2000).

MATERIALS AND METHODS

Plant Material

Plant *Sesbania sesban L*. was collected from the Nasik district of Maharashtra. The plant was authenticated from the Botany Department of S.S.V.P.S College of Science, Dhule by Dr. Khirsagar.

Preparation of extract

All the solvents and reagents used during the study were of AR grade.

Powdered leaves of *Sesbania sesban* was extracted by successive extraction in Soxhlet apparatus using petroleum ether and methanol as a solvent. The solvent was removed under vacuum by rotary evaporation, producing dry extracts. The methanol extract was further fractionated with butanol: water (1:1) proportion to get butanol extract which was precipitated in solvent ether to get crude saponins (SAP) (Rajpal et al., 2006).

Phytochemical analysis

The preliminary phytochemical analysis was performed for crude saponin extracts to check the presence of secondary metabolite groups (Khandelwal 2005).

Thin layer chromatography (TLC)

TLC for crude saponin extract was performed to further confirm the presence of saponins. Various solvents such as chloroform, ethyl acetate, acetone, methanol, glacial acetic acid and water were used for optimizing mobile phase and aluminium plates precoated with silica gel $60F_{254}$ was used as stationary phase. The best separation was found in the mobile phase of Chloroform: Glacial acetic acid: Methanol: Water in the proportion 3: 1.5: 0.6:0.2. The anisaldehyde reagent was used as derivatising agent (Wagner et al., 2009).

Formulation of Gel

Gel formulations containing 1% w/w and 2 % w/w of the crude saponins were prepared by using carbopol 934 (0.25 gm) as gelling agent, triethylamine (0.1 ml) and water (q.s).

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Animals

Albino Wistar rats, weighing 150–200 g were used. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. All animal procedures were followed in accordance with the approved protocol for use of experimental animals set by the standing committee on animal care. Four groups of five animals were used for the experiment.

Table 1: Groups of animals receiving test drug, reference drug and placebo

Sr. No	Group	Animals
1	Control	5
2	Diclofenac sodium gel (1%)	5
3	SAP gel (1%)	5
4	SAP gel (2%)	5

Carrageenan-induced rat paw edema

Four groups each having five rats whose portion of

Table 2: Mean increase in Paw volume with mean±SEM values

Time Interval	Control	Standard	SAP 1%	SAP 2%
30	1.168± 0.039	0.898± 0.020**	0.996±0.023	0.962± 0.016*
60	1.292± 0.032	0.968± 0.022***	1.102±0.030	1.044±0.015**
90	1.378 ± 0.026	0.932± 0.033***	1.15±0.024*	1.076±0.013***
150	1.428 ± 0.024	0.926±0.028***	1.128±0.030**	1.032±0.018***
210	1.438 ±0.034	0.904±0.033***	1.088±0.027***	0.994 ±0.025***
270	1.428 ± 0.031	0.852± 0.018***	1.028± 0.027***	0.908±0.030***

Values are expressed as mean±S.E.M, n=5. Data were analyzed by one way ANOVA followed by Dunnett's Multiple comparison test. ***P<0.0001 and **P<0.001 was considered as significant. SAP 1%: Gel containing 1% w/w of crude saponin, SAP 2%: Gel containing 2% w/w of crude saponin.

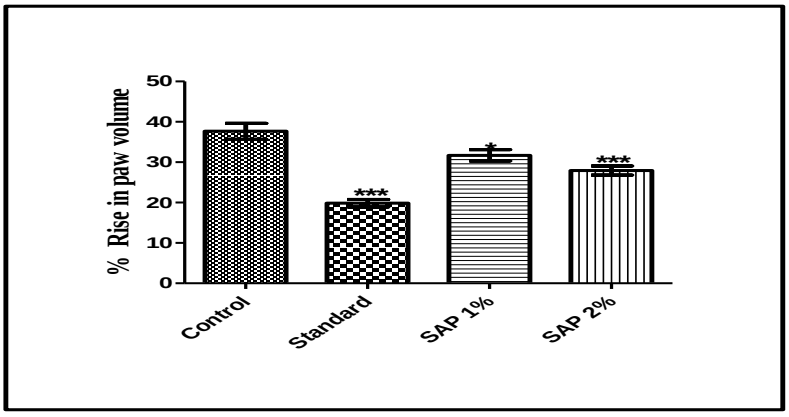

Figure 1: Anti-inflammatory effect of topical administration of crude saponin extract of Sesbania sesban (SAP 1% and SAP 2%) on the first phase (90 min) of carrageenan-induced paw edema in rat. Values represent the mean±S.E.M. ***p < 0.001 vs. control value.

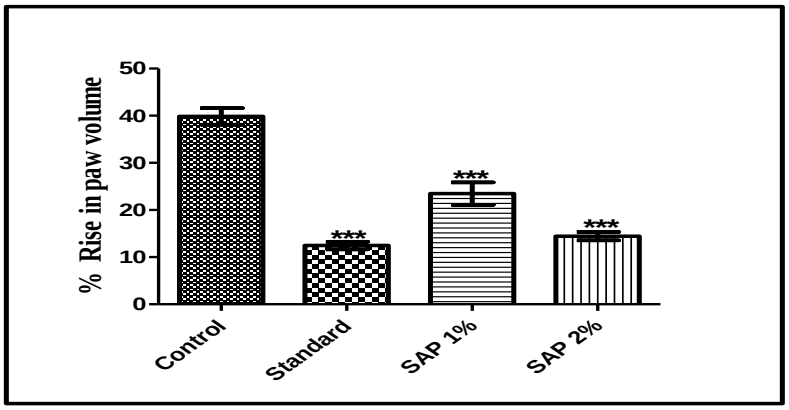

Figure 2: Anti-inflammatory effect of topical administration of crude extract of Sesbania sesban (SAP 1% and SAP 2%) on the late phase (270 min) of carrageenan-induced paw edema in rat. Values represent the mean±S.E.M. ***p < 0.001 vs. control value

back (2.5cm^2) was depilated and shaved 24 h earlier, were applied with crude saponin extract (SAP 1% and 2% gel), Diclofenac sodium gel (1% w/w) and placebo (control) respectively to the right hind paw and shaved portion. Four hour later, 0.1 ml of 1% carrageenan was injected to the right hind paw. A cardboard collor was placed on their necks to prevent ingestion of the preparation. Paw diameter was measured with the help of plethysmometer (Ugo Basile, Italy) at 0 min, 30 min, 60 min, 90 min, 150 min, 210 min and 270 min post-carrageenan injection (Winter et al., 1962).

Statistical analysis

Data are reported as the mean ±S.E.M. and were analysed statistically by means of Two-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. P value ($P<0.0001$) was considered as statistically significant

RESULTS

Phytochemical investigation and TLC:

Preliminary phytochemical investigation confirmed the presence of triterpenoidal and steroidal saponin in crude saponin extract. Other than saponin, the extract also gave positive test for flavanoids and phenolic compounds. The TLC profile depicted total 11 different peaks after spraying it with anisaldehyde sulphric acid reagent, at Rf values 0.029, 0.043, 0.25, 0.5, 0.61, 0.65, 0.78, 0.82, 0.87, 0.92, 0.98 with brown, light brown, blackish green, yellowish brown ,violet, blue, yellowish brown, reddish violet, blue, black and green colour respectively which confirmed the presence of both triterpenoid and steroidal saponin.

Anti inflammatory activity

The effect of crude saponin gel formulation (1% and 2%) with respect to positive and negative control is depicted in the table 1. It was found that the crude saponin gel at a concentration of 2% has shown significant decrease in the paw volume when compared to control group. The percent decrease in paw volume shown by saponin gel 2% was comparable to the standard drug.

DISCUSSION

The Phytochemical investigation of crude saponin extract revealed presence of various constituents like triterpenoidal and steroidal saponins, tannins and flavonoids which had been reported to have anti-inflammatory activity (Gepdiremen et al., 2004, Wang et al., 2008, Sparg et al., 2004).

The development of carrageenan induced inflammation is a biphasic event, the first phase occurs within an hour of injection is attributed to the release of histamine, 5-HT and kinins, while second phase which can be measured around 3-4 h time is related to release of prostaglandins . Diclofenac sodium is used as standard reference drug as it is reported to inhibit inflammation by its effect upon plasma exudation associated with carrageenan mediated inflammation (Olufunmilayo et al., 2008).

Inhibition of carrageenan oedema by the crude saponin extract was recorded at the early, middle and late stages, from 30 min onward. Three phases have been postulated for carrageenan induced edema namely, histamine and 5-hydroxytryptamine (5- HT) release in the first (early) phase, kinins release in the second phase and prostaglandins release in the third (late) phase. From the results, it is clear that the crude saponin extract have been able to control the increase in paw edema in the early phase; however it has exhibited significant activity at the 3rd hour and further time point, which support the fact that it is related to the inhibition of prostaglandins release which inhibits the second phase of inflammation. Hence, it can be said that the present anti-inflammatory activity of crude saponin extract might be due to its action on the early and later phase of inflammation.

CONCLUSION

Based on these results, we can say that the crude saponin extract of leaves of *Sesbania sesban* shows promising anti inflammatory activity and can be further evaluated to be used as anti inflammatory drug.

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