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PHYTOCHEMICAL SCREENING AND IN-VIVO ANTI-INFLAMMATORY POTENTIAL OF *SESBANIA GRANDIFLORA*

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ABSTRACT
The aim of recent study was to estimate anti-inflammatory potential of methanolic extract of leaves of *Sesbania grandiflora* using animal model. The anti-inflammatory study of methanolic extract (SGME) was performed by using 200 mg/kg and 400mg/kg dose in carrageenan induced and formalin induced rat paw edema model. The phytochemical screening of extract also carried out as supportive study. Acute toxicity study was carried out to determine the dose and safety of extracts by using OECD guidelines. The indomethacin was used as standard drug for evaluation of anti-inflammatory potential. The phytochemical screening illustrated the presence of secondary metabolites such as alkaloids, glycosides, tannins, flavonoids, saponins. The result depicted that SGME showed remarkable anti-inflammatory potential in both doses in formalin induced rat paw edema and demonstrated maximum inhibition (400mg/kg) of paw edema after 24 hr ($^{**}P< 0.01$). The oral administration of SGME at the doses 200 & 400 mg/kg demonstrated maximum inhibition at 24 hr in carrageenan induced paw edema. The SGME illustrated inhibition of paw edema in 1-2 hours of carrageenan and formalin induction. Therefore, we conclude that the anti-inflammatory potential of SGME may be due to inhibition of histamine, serotonins and kinins.

KEYWORDS: *Sesbania grandiflora*, Anti-inflammatory, Carrageenan, Formalin.

INTRODUCTION
Inflammation is the first line of complex protective mechanism to infection, cell damage, injury or trauma by immune system.[1] The two distinct phases of inflammatory responses are the acute transient phase intended by changes in the blood vessels diameter and increased capillary permeability followed by a slightly delayed sub acute phase highly characterized by the infiltration of leukocytes and phagocytic cells and a chronic proliferative phase; in which tissue degeneration and fibrosis occur.[2] The inflammatory symptoms occurs due to release of chemical mediators like Prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin and more recently platelet-activating factor (PAF) and interleukin-1 from tissues and migrating cells.[3] Inflammation is linked to a wide range of progressive diseases, including sepsis, auto-immune disorders, neurological diseases, metabolic disorder and cardiovascular disease[4, 5] which impose severe social and financial burdens including poor quality of life, high health-care costs and substantial loss of productivity.

Sesbania grandiflora is popularly known as Sesban or Hadga and is a short-lived, quick-growing, soft-wooded tree, 6-9 m in height and is an ornamental plant belonging to family Fabaceae.[6, 7] It is native to Malaysia and grown in many parts of India such as Punjab, Delhi, Assam and Bengal and grows in hot and humid environment of the world.[8] The bark of *Sesbania grandiflora* possesses astringent, cooling, bitter, tonic, and anthelmentic and antipyretic properties.[9] The fruits are believed to be laxative and stimulant.[10]

The bark and leaves are reported to cure diarrhea, dysentery, snake bite, malaria, smallpox, eruptic fever, scabies, ulcer, and stomach disorders in children; in high doses it causes vomiting and mild diarrhea.[11, 12] Leaves used as tonic, diuretic, laxative, antipyretic, chewed to disinfect mouth and throat. Flower used in headache, dimness of vision.[13] Traditionally the plant has been used for the treatment of head ache, in fever, as a tonic, in catarrh and as an astringent.[14]

In present, either narcotics e.g. opioids or nor-narcotis e.g. salicylates and corticosteroids are the drug of choice

for the management of pain and inflammatory conditions. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop. On the divergent to this many medicines of plant origin had been used since long time with minimum adverse effects.[15] The healing power of plants is explored from an ancient time. Thus the present study oriented towards the assessment of anti-inflammatory potential of *Sesbania grandiflora* leaves.

MATERIAL AND METHODS

Collection of plant material

The leaves of *Sesbania grandiflora* were collected from different areas of Nanded District, Maharashtra during December to January and were authenticated from Dr. Mulani sir, School of Life Sciences, S.R.T.M. University, Nanded (Voucher specimen no. 2/10/08/2015). The selected parts of plant were air dried in shade at temperature of 21-25 ^{o}C for 15 to 30 days. After this the leaves are chopped and ground. Finally the extraction was carried by following procedure.

Preparation of Extract

The powdered leaves 800 g was subjected for extraction process by maceration with 80% of methanol at room temperature for 7 days. Then the extract was filtered and concentrated to dryness by using rotary evaporator. The yield was found to be approximately 5.12 % w/w.

Animals

The Wistar albino rats of both sexes weighing 150-200g were used for the study of anti-inflammatory potential. Animals were maintained under controlled room temperature (22 ± 2∘C) and humidity (55 ±5∘C) with a 12:12 hours' light: dark cycle. The animals were acclimatized to laboratory hygienic conditions for 7 days before commencing the experiment. The animals were fed with standard laboratory food diet and pure drinking water *ad libitum*. The ethical clearance was granted by Institutional Animal Ethics Committee (R-3-XIII/2/06/2014).

Phytochemical screening

The qualitative analysis for confirming different groups of phytoconstituents in SGME was carried out based on standard protocols.[16-18]

Acute toxicity study

The acute oral toxicity study was studied on Wistar albino rats of either sex (150-200 g weight). The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the rats up to 4 g/ kg, p.o., during the 24 h observation period. Based on the results obtained from this study, the dose for anti-inflammatory activity was fixed to be 200 mg/ kg b.w. and 400 mg/kg for dose dependent study.[19]

Anti-inflammatory model

The animals were divided into four groups (n = 6). Group I served as Control received the vehicle only (1% CMC, 10 ml/kg p.o.). Group II served as Standard, received Indomethacin Sodium at dose of 200 mg/kg b.w. Group III and IV served as test, received methanolic extract at doses of 200 and 400 mg/kg b.w. p.o. respectively.

Carrageenan induced rat paw edema

Carrageenan model is widely used animal model to test anti-inflammatory potential. The test was used to determine the anti-inflammatory activity of the extract by the method of Winter et al.[20] The animals pretreated with extract or indomethacin sodium one hour before were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after carrageenan application at 0, 1, 2, 3 and 24 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

Formalin induced rat paw edema

Initial paw volume of individual rats was noted and vehicle/extract/standard was administered accordingly. One hour after the administration of vehicle or extracts or standard drug, all the rats were injected with 0.05 ml of formalin (2.5%) in normal saline in the sub-plantar region of the right hind paw and the left hind paw served as reference. Immediately thereafter the paw oedema volumes were measured plethysmographically at fixed time intervals.[21]

Statistical analysis

All values were expressed as mean ± standard error mean (SEM or SD). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett test. P values < 0.05 were considered to be statistically significant when compared to control.

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical investigation of methanolic leaf extracts showed the presence of flavonoids, alkaloids, glycosides, phenols and saponins as depicted in Table 1. Preliminary phytochemical screening showed presence of flavonoids in methanolic extract of *S. grandiflora*. The flavonoids have been shown to possess different biological properties related with anti-inflammatory, antioxidant and anti-nociceptive by targeting reactive oxygen species and prostaglandins which involve in the late phase of acute inflammation and pain.[22-24]

Table 1: Phytochemical screening of *S. grandiflora* methanolic leaf extract.

Sr. No.	Phytochemical Tests	SGME of Leaves
1	**Alkaloids**	
	1. Dragendorff's test	+
	2. Mayer's test	+
	3. Wagner's test	+
	4. Hager's test	+
2	**Glycosides**	
	1.Raymond's test	+
	2. Legal's test	+
	3. Bromine water test	-
	4. Kellar Kiliani test	+
3	**Flavonoids**	
	1. Lead acetate test	+
	2. Shinoda's test	+
	3. Alkaline reagent test	+
	4. Zn-HCl Reduction test	+
4	**Tannins**	
	1. FeCl3 test	-
	2. Lead acetate test	-
	3. Alkaline reagent test	-
5	**Phenols**	+
6	**Resins**	-
7	**Saponins**	+

(+): Positive; (-): Negative

Anti-inflammatory activity

Carrageenan induced rat paw edema

Carrageenan acts as an irritant for the inflammation induction. Carrageenan induced edema is a biphasic response which involves the cyclo-oxygenase products of arachidonic acids and production of reactive oxygen species.[25] The methanolic extract of *S. grandiflora* possesses noteworthy anti-inflammatory potential at a dose of 200 mg/kg as well as 400mg/kg (Table 2). The indomethacin showed greater anti-inflammatory potential than the SGME. The both doses of extract have equivalent capacity to inhibit the paw volume after 24 h in carrageenan induced rat paw edema as shown in Figure 1. The first phase mediated through the release of histamine, serotonin and kinins which is up to 1-2 h after carrageenan injection whereas, the second phase related to the release of prostaglandins, oxygen-derived free radicals and production of inducible cyclo-oxygenase which peak at 3 h.[26]

Table 2: Evaluation of methanolic extract of leaves of *S. grandiflora* on carrageenan-induced rat paw edema.

Groups	Paw Volume (ml)				
	0 min	1 h	2 h	3 h	24 h
Control (10ml/kg)	0.72±0.03	1.10±0.03	1.22±0.03	0.96±0.05	0.74±0.03
Standard (200 mg/kg)	0.68±0.03**	0.62±0.03**	0.64±0.02**	0.55±0.02**	0.49±0.01**
SGME 1 (200 mg/kg)	0.66±0.09	0.87±0.02**	0.74±0.01**	0.69±0.01**	0.60±0.07**
SGME 2 (400 mg/kg)	0.70±0.02	0.73±0.01**	0.71±0.09**	0.67±0.09**	0.60±0.01**

Values are expressed as mean ± SEM. (n=6), ANOVA followed by Dunnett test.
*p < 0.05, when compared with control group,
**p < 0.01, when compared with control group.

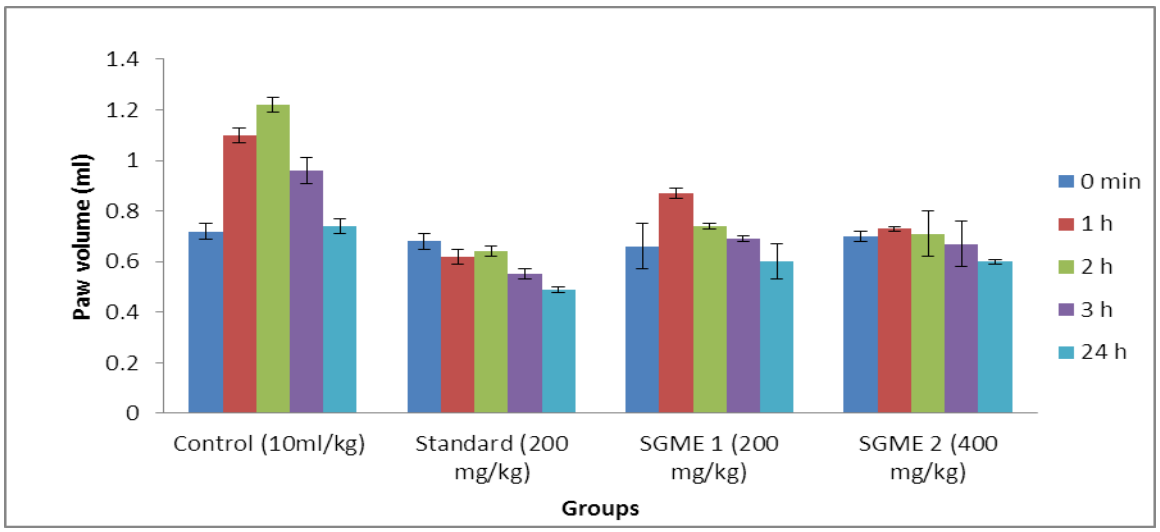

Figure 1: Effect of methanolic extract of *S. grandiflora* on carrageenan induced rat paw edema. The paw volume of extract showed time dependent anti-inflammatory potential as revealed by indomethacin. Values expressed as mean ± SEM (n=6). The ANOVA followed by Dunnett test, *P < 0.05, **P < 0.01 vs. control group.

Formalin induced rat paw edema

The Table 3 represents significant decrease in paw volume by methanolic extract of *S. grandiflora* in acute model induced by formalin in rats. The both doses of SGME demonstrate correspondent potential to that of indomethacin after 24 h (Fig 1). Both the doses of extract inhibit the paw volume in initial 2 hr of formalin induction. The formalin-induced paw edema assay defines distinctive biphasic nociceptive response termed neurogenic and inflammatory phases.[27] The ability of SGME to have effect on both phases' shows that it contains active anti-inflammatory principle acting both centrally and peripherally.

Table 3: Evaluation of methanolic extract of leaves of *S. grandiflora* on formalin- induced rat hind paw edema

Groups	Paw Volume (ml)				
	0 min	1 h	2 h	3 h	24 h
Control	0.78±0.05	1.18±0.04	1.09±0.05	1.04±0.04	0.86±0.04
Standard (200 mg/kg)	0.76±0.08**	0.72±0.02*	0.74±0.04**	0.72±0.01**	0.67±0.02**
SGME 1 (200 mg/kg)	0.72±0.03	0.78±0.03	0.71±0.02**	0.68±0.02**	0.65±0.02**
SGME 2 (400 mg/kg)	0.76±0.02	0.76±0.025**	0.68±0.02**	0.65±0.01**	0.60±0.02**

Values are expressed as mean ± SEM. (n=6), ANOVA followed by Dunnett test.
*p < 0.05, when compared with control group,
**p < 0.01, when compared with control group.

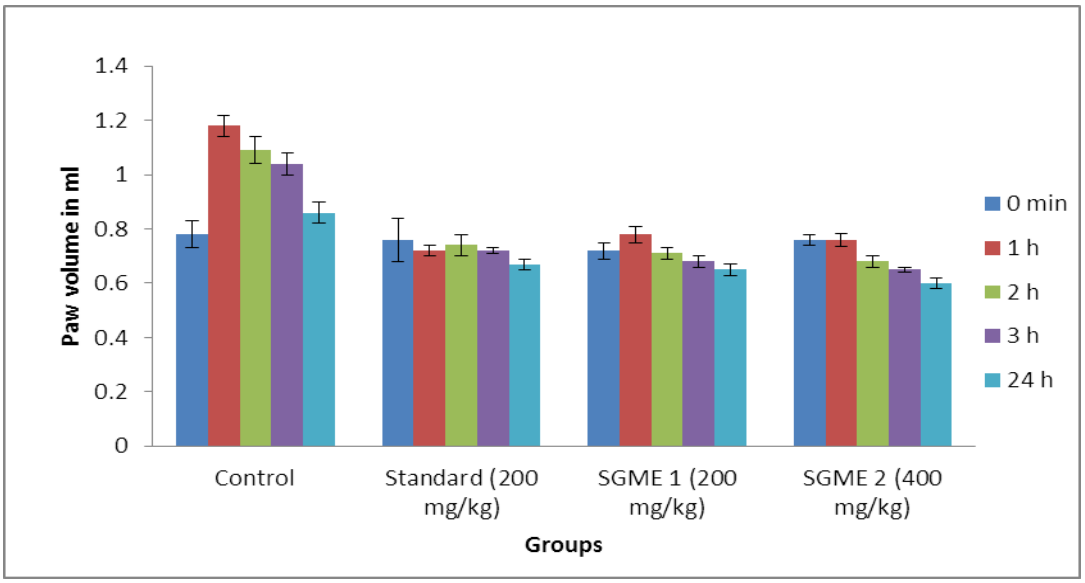

Figure 1: Effect of methanolic extract of *S. grandiflora* on formalin induced rat paw edema. Values expressed as mean ± SEM (n=6). The ANOVA followed by Dunnett test, *P < 0.05, **P < 0.01 vs. control group. SGME1 and SGME2 possesses equivalent potential to that of indomethacin taken as standard.

CONCLUSION

It can be concluded from over findings that the methanolic extract of leaves of *S. grandiflora* may possess significant anti-inflammatory potential due to the presence of flavonoids and other polyphenolic components. The presence of these secondary metabolites seems to support use of this plant in traditional medicine. The accurate mechanism, active constituents involved and site of action are still to be determined, but we just say that the anti-inflammatory potential of SGME may be due to inhibition of the chemical mediator's histamine, serotonin and kinins. The future scope of this study will be to isolation of active constituent for the anti-inflammatory potential and the specific mechanism of action.

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