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Assessment of Analgesic Activity of the Extract of *Sesbania* genus Plants in Animal Models

Dinesh Eknath Deokar

Shri Jagdishprasad Jhabarmal Tibrewala University, Rajasthan, India
deokar.dinesh[at]gmail.com

Abstract: ***The objective of this study was to evaluate the analgesic activities of the hydro-alcoholic extract ofthe leaves of Sesbania grandiflora and Sesbania bispinosa individually and in 1:1 combination, and an analgesic effect of different fractions in animal models. The analgesic effect was examined by Eddy's hot plate model. Application of different doses of Sesbania genus plants and their combinations had significant analgesic effects on Eddy's hot plate edema model. The findings showed that all the Sesbania genus plants and their combinations at given doses of 250 and 500mg/Kg demonstrated analgesic activity. Plant extracts with 1:1 Combination has shown better analgesic activity than individual plant extract and are comparable with Pentazocine. This study demonstrated the analgesic effects of Sesbania genus extracts in animal models and supports traditional use of this plant in the treatment of pain management more studies are required to identify the active components.***

Keywords: Eddy's Hot plate model, *Sesbania grandiflora*, *Sesbania bispinosa*, Analgesic activity.

1. Introduction

Pain is common nonspecific manifestations of many diseases. "Pain" is part of the defense response against organ dysfunction or potentially harmful stimuli. The pain is usually short-lived and only the unpleasant sensation does not go away or lasts until the wound or underlying condition has healed. However, some painful conditions (such as arthritis, peripheral neuropathy, cancer, and idiopathic pain) can last for several years. Pain that lasts for long time is called Chronic pain while acute pain is the pain which relieved quickly. Stimulation of peripheral nerve fibers causes nasal pain.

It only reacts to stimuli near or above harmful intensities (nociceptors) and can be divided into thermal, mechanical and chemical stimuli according to the type of disturbing stimulus. Ascending pathways of pain include spinal deformities of the thalamic tract, lateral punctures, thalamus in the center of the brain, and finally the somatosensory cortex to determine the position, intensity, and depth of the brain.

Although many opiates are used in this condition, these drugs can have some side effects, such as: Gastrointestinal diseases, kidney damage, respiratory diseases, depression and possible addiction. In recent years, people have become more interested in finding new analgesic drugs with fewer side effectsfromnaturalsourcesandmedicinalplants.

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 pain 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 is found in Africa, and the reminder in Australia, and Asia.

Sesbaniagrandiflora 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 is an annual shrub which can grow to seven metres in height but usually only reaches one to two metres. 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 analgesic activity of methanolic extract of *Sesbania grandiflora individually*.

In the present study, analgesic effects were evaluated for individual plants and 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa* species and its fractions in rat using Eddy's hot plate model.

2. Experimental

Plant Material

The leaves of *Sesbania bispinosa* and *Sesbania grandiflora* were selected from Junnar taluka region and identified by Dr. Savita S. Rahangdale, Ph.D. the voucher specimen number 212 and 23719 respectivelyand deposited in the Department of Botany, B J 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 (250 g and 500 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

Phytochemicalinvestigationsofthe*Sesbania* genus plants (individual and 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa*) were carried out using standard methods and tests. Preliminary Phyto-chemical analysis of extract was performed for analysis of tannins, alkaloids, Steroids, saponins, carbohydrates, terpenes and coumarins and flavonoids according to Khandelwal, 2006. The extract was solubilized in normal saline (0.9% w/v sodium chloride) for use in in-vivo experimental animals. Results of analysis are given in Table 1.

Animals

48 adult swiss albino mice(25-35g) were housed in animal unit under standard laboratory conditions (temperature 23±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.

Analgesic Activity

Eddy's hot plate method:

Place the animals on a hotplate maintained at a constant temperature (55 ˚C) and finally react the animals, e.g. Feet licking or skipping reaction [1]. Randomly select the test animals of each sex and divide them into eight groups such as Group I, Group II, Group III, Group IV, Group V, Group VI, Group VII and Group VIII. Each group consisted of six rats as control, positive control, and test specimens. Each group received particular treatment as per experimental planning. The animals were positioned on Eddy's hot plate kept at a temperature of 55 ± 0.5˚C. A cut off period was observed to avoid damage to the paws. The reaction time in control and in treated animals was recorded at 0, 15, 30, 45 and 60 mins. After the treatment. Data is represented in table 2 and Table 3.

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 250mg/kg and 500 mg/Kg p.o. and Pentazocine 30mg/kgp.o. as positive control group (Standard) and Normal saline 10mL/kgp.o. as negative control group (Blank).

Statistical analysis

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

3. Results

Preliminary phytochemical study of the hydroalcoholic extract of individual plant and 1:1 combination of *Sesbania bispinosa* and *Sesbania grandiflora* showed the presence of saponins, terpenes, carbohydrates, alkaloids, tannins, steroids, anthraquinones and flavonoids.

Eddy's hot plate method:

The findings of hot plate test showed that *Sesbania* genus plants show analgesic activity. 1:1 combination of *Sesbania grandiflora* and *Sesbania bispinosa* plant extract possesses significant analgesic effect in comparison to other genus plants and control group. It is comparable with standard drug Pentazocine (30mg/kg). However, standard drug Pentazocine (30mg/kg) showed highly significant ($p<0.05$) analgesic activity.

4. Discussion and Conclusion

Several natural products have been used to treat pain, *Sesbania bispinosa* and *Sesbania grandiflora* are plants used for treatment of pain conditions for many years. In this study, analgesic 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 Eddy's hot plate edema model.

Table 1: Results of Preliminary Phytochemical Analysis

Extract	Steroids	Alkaloids	Tannins	Carbohydrate	Flavonoids	Saponins	Terpenes
Sesbania grandiflora(SG)	+	+	+	+	+	+	+
Sesbania bispinosa (SB)	+	+	+	+	+	+	+
1:1 mixture of *Sesbania grandiflora* & *Sesbania bispinosa* (SG & SB)	+	+	+	+	+	+	+

(+)Hydroalcoholic extract of Present. (+) Hydroalcoholic extract of Absent

Table 2: Evaluation of analgesic effect by Eddy's hot plate method

Group	Treatment	Dose mg/kg	Reaction Time in Sec.				
			Basal Reaction	After 15 mins	After 30 mins	After 45 mins	After 60 mins
I	Control	Normal Saline	2.50 (0.1103)	2.80 (0.1106)	2.90 (0.0901)	3.10 (0.0902)	2.60 (0.1205)
II	*Sesbania bispinosa* (SB)	250	2.37 (0.1224)	3.87 (0.0989)	5.1 (0.0749)	6.55 (0.0651)	7.78 (0.0576)
III	*Sesbania bispinosa* (SB)	500	2.65 (o.1104)	4.08 (0.0708)	5.47 (0.0806)	6.88 (0.0503)	8.11 (0.0405)
IV	*Sesbania grandiflora* (SG)	250	2.05 (0.1410)	3.72 (0.0955)	4.97 (0.0749)	6.31 (0.0651)	7.67 (0.0594)
V	*Sesbania grandiflora* (SG)	500	2.22 (0.1530)	3.85 (0.0962)	5.13 (0.0727)	6.47 (0.0643)	7.87 (0.0581)
VI	1:1 mixture of SB & SG	250	2.45 (0.1647)	4.03 (0.1081)	5.30 (0.0729)	6.75 (0.0641)	8.08 (0.0578)
VII	1:1 mixture of SB & SG	500	2.72 (0.1198)	4.22 (0.0836)	5.63 (0.0874)	7.05 (0.0566)	8.21 (0.0500)
VIII	Pentazocine	30	2.93 (0.1053)	4.42 (0.0969)	5.78 (0.0783)	7.28 (0.0539)	8.49 (0.0505)

Values are expressed as Mean ±Coefficient of variations; n=6, $p<0.05$

Table 3: Tukey's honest significant difference (HSD) test

Group	Treatment	Dose mg/kg	q value				
			Basal Reaction	After 15 mins	After 30 mins	After 45 mins	After 60 mins
II	*Sesbania bispinosa* (SB)	250	1.3802524	7.3958091	13.6050204	21.1899832	30.8108716
III	*Sesbania bispinosa* (SB)	500	3.6489402	8.2173671	15.3082285	22.7254893	32.0401342
IV	*Sesbania grandiflora* (SG)	250	1.1602475	6.4191548	12.7998674	19.7056607	29.1565867
V	*Sesbania grandiflora* (SG)	500	3.4007682	7.0386464	13.7805024	20.6883846	31.3461956
VI	1:1 mixture of SB & SG	250	1.7870479	8.0734966	14.8437172	22.4081514	32.5853716
VII	1:1 mixture of SB & SG	500	3.7867491	8.6214999	15.8966095	23.2373246	33.3387906
VIII	Pentazocine	30	3.8200042	9.2532505	16.8772445	24.2405219	34.4490923

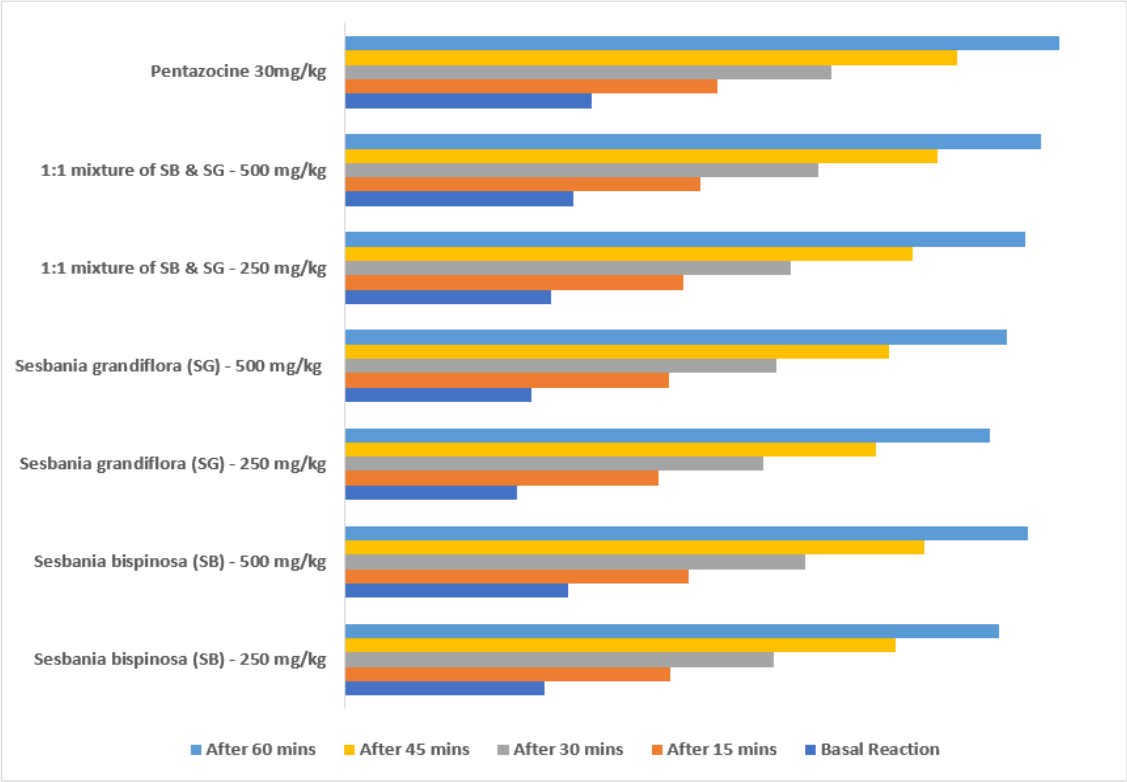

Figure 1: Effect of *Sesbania* genus plants on latency time of mice exposed to hot plate test

It is well established that chemical mediators are responsible for the inflammatory pain. NSAIDs are known to reduce pain by reducing inhibition by synthesis and release of prostaglandins. As a criterion for this study, Pentazocine reduced pain by inhibiting the formation of painful substances in the surrounding tissues, where prostaglandins and bradykinin are considered important. Hot plate tests were used to study central analgesics effect.

In the hot plate method, stimulation is given in the high intensity phase. Pain caused by thermal stimulation of the hotplate is typical of center-mediated activity. Opoids exert an analgesic effect through receptors in the spinal cord ((μ_1 , κ_3 , σ_2 , γ_1) and spinal cord (μ2, κ1, γ2). EMO shows anti-analgesic action by increasing the incubation period of discomfort in hot plate testing. This effect can be achieved by activating the periaqueductal gray matte to release endogenous peptides (endorphins or enkephalin).

This endogenous peptide descends to the spinal cord and acts as an inhibitor of pain impulse transmission in the dorsal horn sink. The potential mechanism of EMO leaves may be due to their effect on central opioid receptors or to stimulate the release of endogenous opioid peptides.

The observed analgesic activity in the hydroalcoholic extracts of *Sesbania* genus is attributed to the presence of compounds including flavonoids, tannins and saponins. This connection already exists. Phytochemical screening was performed during the research process. Flavonoids play a role in analgesics by targeting prostaglandins and inhibiting prostaglandin synthesis (especially endoproxidase) their activity. In Central analgesic tests, *Sesbania* genus plants individual and mixture of 1:1 composition of *Sesbania grandiflora* and *Sesbania bispinosa* significantly increased the reaction time implying its central analgesic activity.

From this study, it is established that hydro alcoholic extract of S*esbania* genus plants possesses significant analgesic effect.

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.

5. Conflict of Interest

We have no conflict of interest to declare.

6. Acknowledgements

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