

## Antinociceptive activity of *Sesbania sesban* (Linn) wood extracts, a preliminary study

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The wood of the plant *Sesbania sesban*, is reported to have antinociceptive activity. To validate its folk use in the treatment of pain, wood was extracted successively with petroleum ether, chloroform, ethyl acetate, ethanol, and water to produce respective extracts. The extracts (50 and 100 mg/kg, ip) were screened for antinociceptive activity using hot plate test and acetic acid-induced writhing test in mice. Petroleum ether, chloroform, and ethyl acetate extracts showed significant and dose-dependent activity in both the tests. In order to find out the involvement of opioid receptors, effect of naloxone (1 mg/kg, sc) on the action of extracts was checked in hot plate test. Petroleum ether, chloroform, and ethyl acetate extracts showed significant and dose dependant antinociceptive activity. The antinociceptive action of the extracts was blocked by naloxone, suggesting involvement of opioid receptors in the action.

**Keywords:** Hot plate test, Naloxone, *Sesbania sesban*, Writhing test

*Sesbania sesban* (Linn) Merrill [Syn *S. aegyptiaca* (Poir) Pers.] (Papilionaceae) is a small soft wooded tree found wild and cultivated in almost all parts of India, especially in Southern India. It is commonly called as 'Jayanti' in Marathi, 'Egyptian pea' in English and 'Bichu' in Hindi. Traditionally the plant is used in the treatment of inflammatory rheumatic conditions, diarrhea, in excessive menstrual flow, to reduce enlargement of spleen and in skin diseases<sup>1,2</sup>. Aerial flowering top and leaves contains a derivative of oleanolic acid and a saponin 3-*O* -[ $\alpha$ -1-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -d-glucuronopyranosyl]-oleanolic acid<sup>3</sup>. Plant contains rare kaempferol trisaccharide, which acts as anti-tumor promoter. The unsaponifiable matter from fixed oil from the seed yields the saponin, stigmasta-5,24(28)-diene-3 $\alpha$ -*O*- $\alpha$ -D-galactopyranoside<sup>3</sup>. Traditionally, wood powder is used as analgesic hence present preliminary work has been undertaken to prove it scientifically.

### Materials and Methods

**Plant material**—The wood of *S. sesban* was collected from Ahmednagar district (74° E longitude) during August 2010 and authenticated by Mr. S C Mujumadar (Botanical Survey of India, Pune). A voucher specimen was deposited for future reference (Voucher no- GUR-6).

**Preparation of the extracts**—Shade dried and coarsely powered wood (5 kg) of *S. sesban* was subjected to successive solvent extraction in Soxhlet extractor using solvents in the following order; petroleum ether (60°-80°C), chloroform, ethyl acetate, and absolute ethanol. Marc left was refluxed with water. All the extracts were vacuum dried to obtain petroleum ether extract (PEE; 1.25%), chloroform extract (CLE; 2.32%), ethyl acetate extract (EAE; 1.56%), ethanol extract (ETE; 5.50%), and aqueous extract (AQE; 4.59%), respectively.

**Animals**—Male Swiss albino mice weighing 25-30 g procured from National Toxicological Center, Pune, were housed for at least one week in the laboratory animal room prior to testing. Feed with standard rodent diet and water were given *ad libitum*.

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The Local Institutional Animal Ethical Committee approved all the experimental procedures.

**Drugs and chemicals**—The following drugs were used: pentazocine lactate (Ranbaxy, India), paracetamol (Heilenlab, India), and naloxone hydrochloride injection (Samarth Life Sciences Pvt. Ltd., India). Acetic acid and dimethylsulfoxide (DMSO) (PCL, India), petroleum ether, chloroform, ethyl acetate, ethanol (SD Fine, India), and saline water (Claris life sciences Pvt. Ltd., India) were used. All the extracts were suspended into minimum amount of DMSO and the volume was adjusted with saline water. All drug solutions and extract dilutions were prepared immediately before starting the experiment.

**Pharmacological screening for analgesic activity**—**Hot plate test:** Central nociceptive activity was evaluated using hot plate method<sup>4</sup>. Mice were divided into 18 groups of 6 animals each. The first group served as control and received only vehicle (5% DMSO into saline water), second group was administered standard drug pentazocine (10 mg/kg, ip). The animals of groups 3-12 were treated with PEE, CLE, EAE, ETE, and AQE (50 and 100 mg/kg, ip, each), respectively. Doses were selected on the basis of toxicity study. The animals of groups 13-18 were treated with PEE, CLE, EAE, ETE, AQE (100 mg/kg, ip, each), and pentazocine (10 mg/kg, ip) 15 min after naloxone (1 mg/kg, sc) administration. The mice were placed individually on the hot plate maintained at  $55 \pm 0.2^\circ\text{C}$  and latency of nociceptive response such as licking, flicking of the hind limbs or jumping was noted. The readings were taken at 0, 30, 60, 90, 120, and 150 min after treatment. The experiment was terminated 20 sec after their placement on the hot plate to avoid damage to the paws.

**Acetic acid induced writhing test:** Peripheral nociceptive activity was evaluated using acetic acid-induced writhing test<sup>5</sup>. Mice were divided into 12 groups of 6 animals each. First group served as control and was treated with vehicle (5% DMSO into saline water) only. Second group was administered standard drug paracetamol (50 mg/kg, ip). The animals of 3-12 groups were treated with PEE, CLE, EAE, ETE, and AQE (50 and 100 mg/kg, ip, each), respectively 30 min before intra-peritoneal injection of 0.6% solution of acetic acid (10 ml/kg). After acetic acid injection the individual mice was observed for onset of writhing and number of writhing responses for 30 min.

**Statistical analysis**—All data were expressed as mean  $\pm$  SE. The statistical analysis of all the observations was carried out using one-way ANOVA followed by the multiple comparison test of Tukey-Kramer, where necessary.  $P < 0.05$  was considered as significant compared with the control group.

## Results and Discussion

**Hot plate test:** In the hot plate test, petroleum ether, chloroform and ethyl acetate extracts (50 and 100 mg/kg, ip) showed significant increase in reaction time compared to control (Fig. 1). Ethanol and aqueous extracts (50 and 100 mg/kg, ip) did not show significant results (data not shown). To study, involvement of opioid receptors, the extracts were given after opioid antagonist, naloxone (1 mg/kg, sc). In the mice pretreated with naloxone, the extracts exhibiting antinociceptive activity did not show any significant increase in reaction time (Fig. 2).

The hot plate test is the specific central antinociceptive test. Petroleum ether, chloroform and

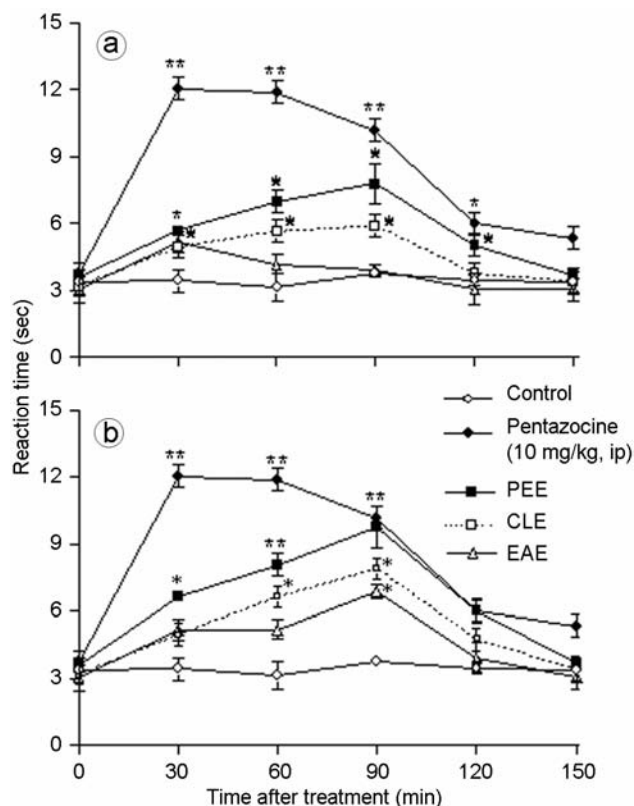


Fig. 1—Effect of 50 (a) and 100 (b) mg/kg, ip doses of various extracts of *S. sesban* wood on thermal stimulus-induced pain (Hot plate test). [Values are mean  $\pm$  SE from 6 animals each.  $P$  values: \*\* $<0.0001$ ; \* $<0.01$ , only significant data are presented, PEE = petroleum ether extract, CLE = chloroform extract, EAE = ethyl acetate extract]

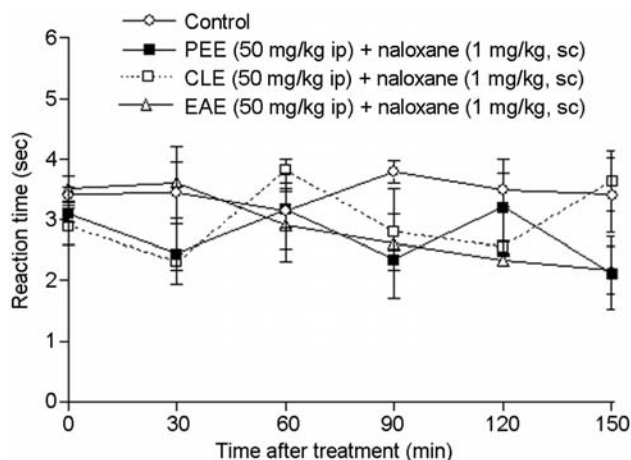


Fig. 2—Effect of naloxone on the antinociceptive activity of various extracts of *S. sesban* leaves on thermic stimulus-induced pain (Hot plate test). [Values are mean  $\pm$  SE from 6 animals each. PEE = petroleum ether extract, CLE = chloroform extract, EAE = ethyl acetate extract]

ethyl acetate extracts showed significant results in this test while their action was blocked by opioid antagonist, naloxone suggesting that there may be involvement of opioid receptors. The opioid agents exert their analgesic action via supraspinal ( $\mu_1$ ,  $\kappa_3$ ,  $\delta_1$ ,  $\sigma_2$ ) and spinal ( $\mu_2$ ,  $\kappa_1$ ,  $\delta_2$ ) receptors<sup>6</sup>. Therefore it is possible that the extracts exert their effect through central opioid receptor or promoted release of endogenous opioid peptides.

Acetic acid-induced writhing test: All the extracts (50 and 100 mg/kg, ip) produced significant inhibition of writhing reaction induced by acetic acid compared to control group (Fig. 3). Inhibition of writhings by these extracts was in the order as ethyl acetate > petroleum ether > chloroform > ethanol > aqueous extract. Similarly these extracts delayed onset of writhing response significantly compared to control except ethanol and aqueous extracts (Fig. 4).

Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors<sup>7</sup> or irritation of the visceral surface, which lead to liberation of histamine, bradykinins, prostaglandins, and serotonin<sup>8</sup>. Thus, antinociceptive activity of opioid partial agonist and non-steroidal anti-inflammatory agents can be determined by writhing test<sup>9</sup>. The mechanism of analgesic effect of extracts of *S. sesban* wood could probably due to blockage of effect or release of endogenous substances that excite pain nerve endings.

The petroleum ether, chloroform, and ethyl acetate extracts of *S. sesban* wood showed potent antinociceptive activity in both the tests. Prostaglandins and

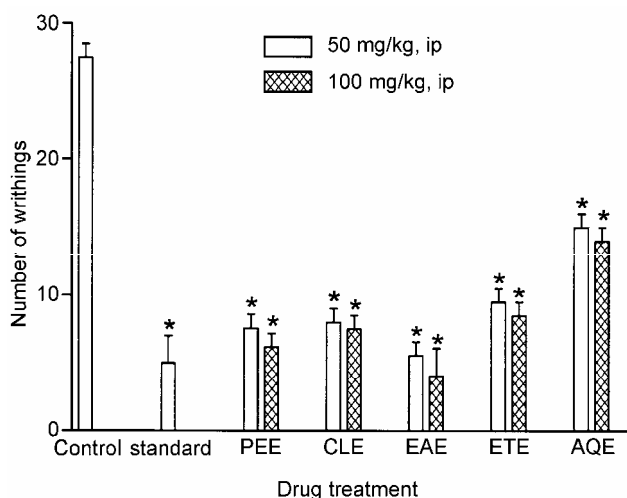


Fig. 3—Effect of various extracts of *S. sesban* wood on acetic acid-induced writhing. [Values are mean  $\pm$  SE from 6 animals each. \* $P$  < 0.05, PEE = petroleum ether extract, CLE = chloroform extract, EAE = ethyl acetate extract, ETE = ethanol extract, AQE = aqueous extract]

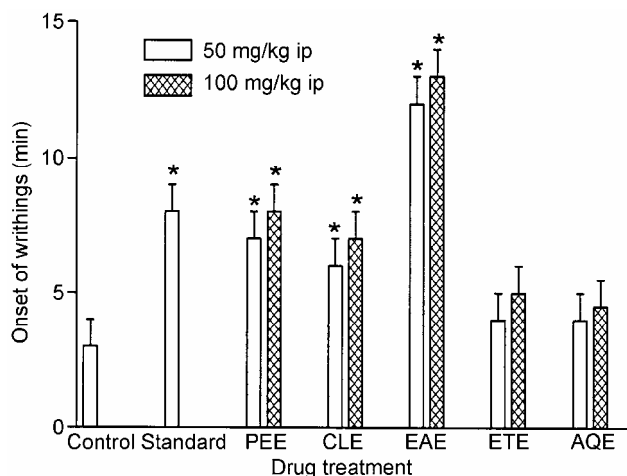


Fig. 4—Effect of various extracts of *S. sesban* wood on onset of acetic acid-induced writhing. [Values are mean  $\pm$  SE from 6 animals each. \* $P$  < 0.001, PEE = petroleum ether extract, CLE = chloroform extract, EAE = ethyl acetate extract, ETE = ethanol extract, AQE = aqueous extract]

bradykinins were suggested to play an important role in nociception<sup>10,11</sup>. Preliminary phytochemical tests showed presence of sterols and triterpenes in petroleum ether, and chloroform extracts and flavonoids in ethyl acetate extract. Flavonoids and sterols are reported to inhibit prostaglandin synthesis<sup>12,13</sup> and a number of flavonoids have been reported to produce analgesic activity<sup>14</sup>. Some sterols and triterpenes are responsible for anti-inflammatory and analgesic activity.<sup>15</sup>

To conclude, the petroleum ether, chloroform, and ethyl acetate extracts of *S. sesban* wood showed potent antinociceptive activity.

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