

Assessment of analgesic activity of *Pterocarpus marsupium* leaf extracts in Swiss albino mice

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ABSTRACT

Pterocarpus marsupium Roxb. (Leguminosae), commonly known as Indian kino or Malabar kino in English, is a large deciduous tree widely distributed in different regions of India. It is traditionally used in India for several medicinal purposes. The present study assessed three different successive solvent extracts from *P. marsupium* leaf for their analgesic potential by acetic acid induced writhing assay in Swiss albino mice. All the test extracts exhibited significant analgesic activity. The methanol extract was found to be the most potent followed by the ethyl acetate and petroleum ether extracts respectively. The present preliminary study demonstrated marked analgesic activity of *P. marsupium* leaf in Swiss albino mice.

Key words: Analgesic, *Pterocarpus marsupium*, writhing, polyphenols, leaf.

Introduction

Traditional or herbal medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. The major merits of traditional medicine seem to be their perceived efficacy, low incidence of serious adverse effects and comparatively low cost.

Pterocarpus marsupium Roxb. (Leguminosae), commonly known as *Bijayasara* or *Asana* in Bengali, *Bijasal* in Hindi, Indian kino or Malabar kino in English, is a large deciduous tree widely distributed in the central, Western and Southern regions of India. It is an important medicinal plant of Indian traditional system of medicines and has been used in India for several medicinal purposes. It is a good source of tannins and hence used as powerful astringent and also in treatment of diarrhea and dysentery, passive haemorrhage, toothache and in diabetes. [1] It is also used as anti-inflammatory, anthelmintic, analgesic and

in treatment of indigestion, diabetic anaemia, elephantiasis, erysipelas, urethrorrhea and ophthalmic complications. The role of *P. marsupium* as anti-diabetic has been very well established. [2] Its main chemical constituent is a glucosidal tannin namely kinotannic acid. Several other chemical constituents like pterostilbene, (-)-epicatechin, pterosupin, marsupsin, etc. have been identified and isolated. It also shows promising results in treatment of cataract and hypertriglyceridaemia. This plant also finds its use as cardiogenic and hepatoprotective agent. Studies have also been reported to demonstrate its ability as a specific COX-2 inhibitor. [3] Several pharmacological studies have been performed on this plant by the previous researchers. However, the analgesic assessment of successive petroleum ether, ethyl acetate and methanol extracts from *P. marsupium* leaf are still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *P. marsupium*.

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Materials and Methods

Plant material

The mature leaves of *Pterocarpus marsupium* Roxb. (Family: Leguminosae), were collected during November 2012 from Kalyani, Nadia, West Bengal,

India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/80/2012/Tech.II/574) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

Preparation of plant extracts

The dried powdered material was first extracted with petroleum ether (60-80°C), the percentage extractive value was 7.36 % w/w. The marc thus obtained was further extracted successively with ethyl acetate and methanol for 72 h. The solvent was distilled off under reduced pressure resulting in semisolid mass that was vacuum dried to yield the dry extracts and the percentage extractive values were accordingly 1.89 % w/w and 7.52 % w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts. [4]

Drugs and chemicals

Acetyl salicylic acid (aspirin) and glacial acetic acid were from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

Experimental animals

Adult male albino mice of Swiss strain weighing 20 ± 2 g were procured from registered breeders (Reeta Ghosh & Co., Kolkata, India) and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ with dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee.

Analgesic evaluation: acetic acid-induced writhing test

Swiss albino mice were divided into five groups ($n = 6$). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes. Group II received aspirin (100 mg/kg b.w. p.o.) which served as reference. Groups III, IV and V received the petroleum ether, ethyl acetate and methanol extracts at the doses of 120 mg/kg b.w., p.o. respectively. Thirty minutes after aspirin and extracts administration, group II to V received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes [5, 6]. The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:-

$$\left(\frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \right) \times 100 \%$$

Statistical analysis

The data are represented as mean \pm standard error of mean (SEM). Degree of significance was assessed by Student's 't' test.

Results and Discussion

Preliminary phytochemical studies revealed the presence of triterpenoids and steroids in the petroleum ether extract; triterpenoids, steroids and polyphenolic compounds in the ethyl acetate extract; and triterpenoids, steroids, glycosides, polyphenolic compounds and carbohydrates in the methanol extract of *P. marsupium* leaf.

The analgesic activity of *P. marsupium* leaf extracts was evaluated by acetic acid induced writhing method in mice to assess peripheral (non-narcotic) type of analgesic activity. [7] Acetic acid induced writhing is chemically induced nociception by intraperitoneal injection of dilute acetic acid solution to mice. The chemical agents can produce nociceptive reactions in mice. Intra-peritoneal injection of phenyl para quinone, bradykinin or dilute acetic acid (1-3% v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning

of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain. [7, 8]

Acetic acid induced writhing test is known as a visceral pain model nociception. Several mediators like kinins, acetylcholine, substance P, calcitonin-gene-related peptide and prostaglandins (PG) take part in visceral pain model nociception and transmission of the nociception from the viscera. In this test both central and peripheral analgesics are detected. Analgesics of narcotic (central) e. g. morphine, pentazocin, pethidine etc and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin etc can inhibit the writhing response in mice. [7-10] The results of the present study are in agreement with those of other previous researchers. [11-13]

Present results indicated that the methanol extract was the most potent followed by the ethyl acetate and petroleum ether extracts respectively (Table 1). Preliminary phytochemical studies revealed the presence of polyphenols in the ethyl acetate and

methanol extracts. *P. marsupium* is rich in polyphenols content which is the basis of its different pharmacological properties (see introduction section). Polyphenols are well known natural products possessing several important pharmacological activities. [14] The better analgesic effects of ethyl acetate and methanol extracts may be due to the presence of polyphenols in them.

Based on the results obtained from the present preliminary investigation, it can be inferred that all the test extracts had effective peripheral analgesic actions. The present preliminary study confirms marked analgesic activity of *Pterocarpus marsupium* leaf in Swiss albino mice. Further studies are presently necessary to confirm the identity of the bioactive principles responsible for these actions.

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Table 1: Analgesic effect of *P. marsupium* leaf extracts on acetic-acid induced writhing in mice.

Treatment	Dose	Number of writhes	% Protection
Acetic acid (1% v/v)	10 ml/kg	52.83 ±1.400	-
Acetic acid + Aspirin	100 mg/kg	17.26 ±1.606*	67.32
Acetic acid + Pet. ether extract	120 mg/kg	25.27 ±1.361*	52.17
Acetic acid + Ethyl acetate extract	120 mg/kg	21.18±1.371*	59.90
Acetic acid + Methanol extract	120 mg/kg	18.59±1.491*	64.81

Values are mean ± SEM (n = 6). *p < 0.001 when compared to control.

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