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(Research article)

IN VIVO ANALGESIC ACTIVITY OF PETROLEUM ETHER EXTRACT OF BOSWELLIA OVALIFOLIOLATA LINN. LEAVES

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ABSTRACT

Boswellia ovalifoliolata Linn. (Family: Burseraceae) Leaves were extracted in petroleum ether (60°-80°C) to evaluate for centrally acting analgesic potential using tail immersion and peripheral pharmacological actions using acetic acid induced writhing test in mice. *Boswellia ovalifoliolata* Linn. extracts were found to have been significant ($p < 0.05$) analgesic activity at the oral dose of 200 & 400 mg/kg b. wt., in the tested models. In tail immersion test *Boswellia ovalifoliolata* Linn. extracts showed increased tail withdrawal time whereas in acetic acid induced writhing test *Boswellia ovalifoliolata* Linn. extracts showed reduced number of writhes at two dose levels, which are significant ($p < 0.05$) compared to control. The results obtained support the use of leaves of *Boswellia ovalifoliolata* Linn. extracts in painful conditions acting both centrally and peripherally.

Keywords: *Boswellia ovalifoliolata* Linn. Writhing Test, Tail Immersion Test.

INTRODUCTION

Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs [1].

Boswellia ovalifoliolata Linn. (Family: Burseraceae) Leaves are imparipinnate. Alternate or crowded at the ends of branches; leaflets sessile, ovate-oblong, unequal at base, glabrous beneath, veins reddish. The leaves are generally alternate, spiral, and odd-pinnately compound with opposite, frequently long-

petiolate, entire to serrate, pinnately veined leaflets whose symmetry is distinctive in some genera. Some members are known to have trifoliolate or unifoliolate leaves. The leaf and leaflet stalks and axis may be brown and scurfy, while the leaf base is swollen and may be concave adaxially. The family members tend to be without stipules. The flowers may have 4-5 faintly connate but imbricate sepals with an equal number of distinct, imbricate petals. Also, the stamens, that may contain nectar discs, have distinct glabrous filaments that come in 1-2 whorls and in numbers equal lying or twice the number of petals; the tricarpellate pollen is contained within two locules of the anthers that open longitudinally along slits. The gynoecium contains 3-5 connate carpels, one style, and one stigma that is head-like to be lobed. Flowers in axillary panicles. Calyx 5-toothed. Petals 5, imbricate. Its leaves are effective against pain and inflammation [2]. The present study was undertaken to evaluate the analgesic activity of *Boswellia ovalifoliolata* Linn. in mice using two experimental pain models.

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MATERIALS AND METHODS

Plant material

The leaves of *Boswellia ovalifoliolata* Linn. was collected from Tirumala hills, Tirupati, Andhra Pradesh, India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected leaves were dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extract is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extracts was around 15.5%w/w.

Animals Used

Albino mice (25-30 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 °C with free access to feed (Sai durga feeds and foods, Bangalore) and water. Moreover, the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

The acute toxicity of Petroleum Ether extracts of *Boswellia ovalifoliolata* Linn. leaves was determined as per the OECD guideline no.423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg 2000 mg/kg doses. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose was selected for further study [3].

Analgesic Activity

Writhing test

Abdominal constriction induced by intraperitoneal injection of acetic acid was carried out by method of

Koster et al., [4]. *Boswellia ovalifoliolata* Linn. extract was tested at 200 and 400 mg/kg, p.o and Indomethacin (10 mg/kg, p.o) a reference analgesic and anti-inflammatory drug, the writhing inhibition produced by the plant extract was determined by comparing the reference drug. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 minutes, 10 minutes after the application of acetic acid.

Tail Immersion test

The basal reaction time to radiant heat by placing the tip of the tail in a beaker of water maintained at 55°C was carried out. The *Boswellia ovalifoliolata* Linn. extract was tested at 200 and 400 mg/kg, p.o and compared to the reference compound Indomethacin 10 mg/kg. p.o. Tail withdrawal is taken as the end point, a cutoff point of 15 sec is observed to prevent the damage to the tail [4]. The percent increase in reaction time at each time an interval was calculated.

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The significance of differences between the group was assessed using one way, and the multiple way analyses of variance (ANOVA). The test followed by Dunnett's test, p values less than 0.05 were considered as significance.

RESULTS

ANALGESIC ACTIVITY

Writhing test

In case of the acetic acid writhing test, at doses of 200 and 400 mg/kg the PEBO inhibited the writhing responses and the number of writhes were significantly lower than the control group and the maximum inhibition is seen at 400 mg i.e., 67.63% Indomethacin has produced as protective effect and exhibited 76.52% of inhibition at a dose of 10 mg/kg (Table 1).

Tail immersion test

The PEBO at the doses of 200 and 400 mg/kg produced the significant delay in response of tail withdrawal compared to control, and it was higher at 400 mg/kg and the delay in response was higher by Indomethacin at a dose of 10 mg/kg (Table-2).

Table 1: Analgesic effect of petroleum ether extract of *Boswellia ovalifoliolata* Linn. in acetic-acid induced writhing test

Group	Design of treatment	Number of writhings	% inhibition
I	Control (Normal saline, 10 ml/kg)	56 ± 1.14	---
II	PEBO (200mg/kg bw, p.o)	35.32 ± 1.22**	36.93
III	PEBO (400 mg/kg bw, p.o)	18.13 ± 1.17**	67.63
IV	Indomethacin (10 mg/kg bw, p.o)	13.15 ± 1.35**	76.52

n=6, values are expressed as mean ± SEM, *P < 0.05, **P < 0.01 when compared with control

Table 2 : Analgesic effect of petroleum ether extract of *Boswellia ovalifoliolata* Linn. in tail immersion method.

Group	Design of treatment	Tail withdrawal in sec					
		0 min	15 mins	30 mins	60 mins	120 mins	180 min
I	Control (Normal saline, 10 ml/kg, p.o)	2.36 ± 0.31	3012 ± 0.16	2.52 ± 0.13	2.83 ± 0.30	3.16 ± 0.21	2.83 ± 0.30
II	PEBO (200mg/kg bw, p.o)	3.14 ± 0.32	4.25 ± 0.30	4.16 ± 0.28*	4.83 ± 0.30**	5.16 ± 0.13*	5.25 ± 0.30*
III	PEBO (400 mg/kg bw, p.o)	3.17 ± 0.20	4.16 ± 0.12	5.32 ± 0.13**	5.16 ± 0.31**	6.13 ± 0.23**	7.14 ± 0.16**
IV	Indomethacin (10 mg/kg bw, p.o)	3.47 ± 0.2	5.17 ± 0.14*	6.32 ± 0.24**	6.5 ± 0.34**	7.43 ± 0.14**	9.17 ± 0.60**

n=6, values are expressed as mean ± SEM, *P < 0.05, **P < 0.01 when compared with control.

DISCUSSION AND CONCLUSION

In the present study, the potential analgesic effect of the petroleum ether extracts of *Boswellia valifoliolata* Linn. was investigated. The results indicate that the oral administration of PEBO exhibit significant and dose dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli at the doses of 200 and 400 mg/kg and indicate that PEBO possess both peripheral (writhe reduction) and central (prolongation of tail withdrawal) effects.

The intraperitoneal administration of acetic acid that irritate serous membranes provokes a stereotypical behavior in mice and rats, which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination [5]. The quantification of prostaglandins by Radio immuno

assay in the peritoneal exudates of rats obtained after the intraperitoneal injection of acetic acid demonstrated high levels of prostaglandins PGE_{2α} and PGF_{2α} during 30 min after stimulus [6].

On the other hand, the lack of influence of extracts of *Boswellia ovalifoliolata* Linn. on the reaction time of mice submitted to the tail immersion is consistent with the interpretation that its analgesic property does not have a central origin, having an analgesic effect in the acetic acid writhing test that is mostly mediated via a peripheral mechanism by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonising the action of pain mediators at the target sites [7]. Finally, the results of the present study confirm that *Boswellia ovalifoliolata* Linn. has analgesic properties.

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