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Anti-inflammatory and analgesic activity of different fractions of *Boswellia serrata*

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Abstract

The study was designed to investigate the anti-inflammatory and analgesic effect of different fractions of *Boswellia serrata.* The effect of different fractions of *Boswellia serrata* were studied using carrageenan induced paw edema, acetic acid induced writhing response, formalin induced pain, hot plate and tail flick method for studying anti-inflammatory and analgesic activity, respectively. The different fractions of *B. serrata*, essential oil (10 ml/kg), gum (100 mg/kg, resin (100 mg/kg) oleo-resin (100 mg/kg) and oleo-gum-resin (100 mg/kg) significantly reduces carrageenan induced inflammation in rats and shows analgesic activity, as determined by acetic acid induced writhing response, formalin induced pain, hot plate and tail flick method. The different fractions of *B. serrata* showed prompt anti-inflammatory and analgesic activity due to the inhibition of 5-lipoxygenase enzyme.

Keywords: Analgesic; *Boswellia serrata*; Inflammation; 5- lipoxygenase; Burseraceae.

Introduction

Boswellia serrata (family Burseraceae) is an oleo-gum-resin found in dry hilly parts of India. It is a large branching medium size tree known as 'Dhup', Indian frankincense or Indian Olibanum [1]. *Boswellia serrata* (*B. serrata*) has been used for a variety of therapeutic purposes such as cancer, inflammation, arthritis, asthma, psoriasis, colitis and hyperlipidemia [2-8]. The essential oil of *B. serrata* is a mixture of mono, di and sesquiterpenes whereas gum portion consists of pentose and hexose sugar with oxidizing and digestive enzymes [9].

Chemically resin is pentacyclic triterpenoid in nature in which boswellic acids (β-boswellic acid, acetyl-β-boswellic acid, keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid) is the main moiety [10]. BA_s and its derivatives are novel, specific, non-redox inhibitor of 5-lipoxygenase (5-LOX), an enzyme in neutrophils responsible for the conversion of arachidonic acid to 5- HETE and leukotrienes which causes vasoconstriction, bronchoconstriction, increase vascular permeability and chemotaxis [11].

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Materials and methods

Materials

Carrageenan, acetic acid, gum acacia, formalin, PVP were purchased from CDH, India. All chemicals used were of analytical reagent grade.

Plant material

The oleo-gum-rein was collected from the local market and was authenticated in Botany Department of Dr. H.S. Gour University, Sagar (M.P.) India.

Extraction and Isolation of gum essential oil and resin

About 100 g shade dried samples were extracted with petroleum ether (60-80^{0}C) in a soxhlet apparatus to get oleo-resin (70.6g). The marc, which contains gum was dried and extracted with hot water to get aqueous extract (29.0 gm). The petroleum ether extract (oleo-resin) was freed from the solvent and hydrodistilled using clavenger apparatus to isolate essential oil (11.0 ml). After the complete removal of essential oil the water layer from the flask was decanted off. The resin (59.6 g) was dried and weighed [12].

Animals

Albino rats (100-150 g) of either sex maintained in standard conditions for temperature, relative humidity light/day cycle and feed with food and water ad libitum.

Preparation of suspension of different fractions:

The different fraction of *B. serrata* suspended in 2% gum acacia for oral administration while essential oil was given in the form of emulsion.

Anti-inflammatory activity

Carrageenan induced paw edema in rats

Pedal inflammation in rats (100-150g) was described by Winter *et al*. (1962). Oedema was induced by subcutaneous administration of 0.1ml of 1% aqueous solution of carrageenan into right hind paws [13]. The test drug (oil, resin, oleo-resin and oleo-gum-resin) is suspended in 1% solution of PVP and diluted with saline. The control group received the vehicle (10 ml/kg body wt.). A test drug suspension (100mg/kg or 10ml/kg) was administered orally for 7 consecutive days prior to the infection of carrageenan paw volume were measured upto 5h after the carrageenan administration at an interval of 60 min and paw volume was measured with plethysmometer. Indomethacin (Guillen *et al*., 1997) and Ibuprofen (Choi *et al*., 2003) were used as standard drug [14, 15].

Analgesic activity

Acetic acid induced writhing response

Whittle (1999) performed acetic acid induced writhing response (abdominal constriction) in rats [16]. Vehicle, indomethacin (10 mg/kg) and test solution (100 mg/kg) were administered orally 30 min before the experiment and 0.1 ml per 10 g of 0.7% acetic acid saline was then injected i.p. 10 min after the injection. The number of writhing during the following 20 min period was counted. The per cent inhibition (% analgesic activity) was calculated by

$$\% \text{ inhibition} = \frac{N - N^t}{N} \times 100$$

Where, N = Average number of stretching of control per group

N^t = Average number of stretching of test per group.

Formalin induced pain in rats

Pain was induced by injecting 0.05 ml of 2.5% formalin (40% formaldehyde) in distilled water in subplantar region of right hind paw. Rats (six per group) were given extract (100 mg/kg), indomethacin (10 mg/kg) and distill water (10 ml/kg) 30 min prior to injecting formalin. These rats were individually placed in transparent Plexiglas cage observation chamber. The amount of time spent licking and biting the injected paw was indicative of pain and was recovered in 0-5 min (first phase) and 15-30 min (second phase) [17].

$$\% \text{ inhibition} = \frac{N - N^t}{N} \times 100$$

Where, N = Average number of licking and biting in control per group
N^t = Average number of licking and biting in test per group.

Tail flick method
This method was described by Asongalem *et al*. (2004). Albino Rats (six per group) were used. This involve immersing extreme 3 cm of rats tail in water bath containing water at a temperature of 55±0.5^{o}C within a few minute, the rats reacted by withdrawing the tail. The reaction time was recorded with a stop watch. Each animal served as its control at 0 and 10 min interval. The average of the two values was the initial reaction time. The test groups were given extract (100 mg/kg), ibuprofen (400 mg/kg) and distilled water (100 ml/kg). The reaction time for the test group was taken at interval 0.5-6 hr after a latency period of 30 min followed by the administration of the extract and drugs [18, 19].

Hot plate method
The device consists of a water bath in which a metallic cylinder was placed. The temperature of the cylinder was set at 55±0.5^{o}C [20]. Each rat (six per group) acted as its control before the treatment; the reaction time of each rat (licking of the fore paw or jumping response) was done at 0 and 10 min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of the extract (100 mg/kg p.o.), indomethacin (10 mg/kg) and distill water (10 ml/kg) was measured at 0.5, 1-5 and 6 hr after a latency period of 30 min.

Statistical analysis
All values are expressed as mean ±S.E.M. Statistical significance was determined by using student's t-test values with $p<0.05$ were considered significant.

Results

Anti-inflammatory activity

Carrageenan induced paw edema in rats
The essential oil (10 ml/kg), gum, resin, oleo-resin and oleo-gum-resin significantly (as compared to control) and dose dependently reduced carrageenan induced paw edema in rats. The standard drug Ibuprofen and Indomethacin shows better inhibitory activity than different fractions of *B. serrata* as shown in Table 1. The lower the paw volume the better the activity. The inhibitory activity of different fractions is very close to ibuprofen.

Table 1. Anti-inflammatory action of *B. serrata* in carrageenan induced paw edema.

Group	Dose (mg/kg, p.o.)	Swelling volume (ml)				
		1(h)	2(h)	3(h)	4(h)	5(h)
Control	10	1.65 ±0.02	1.69±0.01	1.80±0.19	1.42±0.007	1.15±0.012
Essential oil	10	1.01±0.05*	1.19±0.04	1.36±0.03*	1.12±0.05*	1.10±0.04
Gum	100	1.11±0.11*	1.20±0.14	1.34±0.07*	1.15±0.04*	1.13±0.06
Resin	100	1.11±0.03*	1.13±0.31*	1.19±0.03*	1.10±0.09*	0.97±0.05
Oleo-resin	100	1.13±0.04*	1.14±0.007*	0.98±0.06*	0.90±0.04*	0.85±0.05*
Oleo-gum-resin	100	0.80±0.03*	0.89±0.06*	0.93±0.03*	0.97±0.02*	1.15±0.03
Indomethacin	10	0.92±0.04*	0.61±0.10*	0.83±0.03*	0.91±0.03*	0.86±0.03*
Ibuprofen	100	0.96±0.01*	0.98±0.01*	0.93±0.03*	0.90±0.02*	0.70±0.003*

All values are expressed as mean ±S.E.M. p*<0.05 considered significant (n=6).

Analgesic activity

Acetic acid induced writhing response

The different fractions of *B. serrata* reduce acetic acid induced writhing. The oleo-gum-resin fraction shows maximum inhibition (60.54) as compare to oil (20.70) and gum fraction (54.88). The results were shown in Table 2. The % inhibition is calculated by the following formula:

$$\% \text{ inhibition} = \frac{N - N^t}{N} \times 100$$

Where, N = Average number of writhing of control per group

N^t = Average number of writhing of test per group.

Formalin induced pain

The different fractions of *B. serrata* reduce pain, induced by formalin, significantly and dose dependently (see Table 3). Between 0-5 min at a dose 100 mg/kg body wt. essential oil, gum, oleo-resin and oleo-gum-resin are move patent than indomethacin while resin is slightly less potent. The % inhibition is calculated by the following formula:

$$\% \text{ inhibition} = \frac{N - N^t}{N} \times 100$$

Where, N = Average number of licking of control per group

N^t = Average number of licking of test per group

The first (0-5 min) and second (15-30 min) phase of formalin test corresponds to neurogenic and inflammatory pains, respectively. The different fractions of *B. serrata* had analgesic effect on both phase. The results were shown in Table 3.

Tail flick method

A significant reduction of painful sensation due to tail immersion in warm water was observed following oral administration of different fractions at a dose 100 (essential oil 10ml/kg). The effect was noticed after a latency period of 1 hr and it was done dependent. The analgesic effect of oleo-gum-resin is more than other fractions and also standard drug. The results were shown in Table 4.

Hot Plate method

To corroborate that the extract had no central analgesic actions, hot plate method were conducted. Significant results were noted at 100 mg/kg by different fractions was not due to central analgesic acting activities of the extract. This meant there was no opioid like receptor mediation involved. The different fractions at a dose 20 mg/kg shows greater effect than indomethacin 10 mg/kg. The results were shown in Table 5.

Discussion

The anti-inflammatory and analgesic activity of different fractions of *B. serrata* was investigated in the present study. The carrageenan test was selected because of its sensitivity in defecting orally active anti-inflammatory agents particularly in the acute phase of inflammation [21, 22]. The intraplantar infection of carrageenan in rats leads to paw edema. Its first phase (0-2.5 h after injection of carrageenan) results from the concomitant release of mediators: histamine, serotonin and kinins on the vascular permeability. The second phase is correlated with leukotrienes. The oral administration of different fractions of *B. serrata* suppresses inflammation during the second phase. The oleo-gum-resin (200 mg/kg) shows maximum inhibitory response as compared to other fractions.

The mechanism for testing analgesic was selected such that both centrally and peripherally mediated effects were investigated. The acetic acid induced abdominal constriction and tail immersion methods elucidated peripheral and central activity, respectively, while the formalin test investigated both. The hot plate method elucidates peripheral mediated effects [23].

The extract (100 and 200 mg/kg), administered orally, significantly inhibit acetic acid induced writhing in rats. There writhing are related to increase in the peritoneal level of prostaglandins and leukotrienes [24]. The result strongly suggests that the mechanism of action of extract may be linked to lipoxygenase and/or

cycloxygenase. In the formalin test there is distinctive biphasic nociceptive response termed neurogenic and inflammatory phases. Drugs that primarily act on central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [25]. The neurogenic and inflammatory phase is due to the release of substance P, histamine, serotonin, bradykinin prostaglandins and leukotrienes respectively. This test is very useful for not only assessing analgesic drugs but also helping in the elucidation of mode of action. The extract (100 and 200 mg/kg) was able to block both phases of formalin in the second phase (77.13 for essential oil). The oleo-gum-resin shows more inhibition (97.18) in the first phase than second phase (56.74).

Tail immersion model of analgesic assessment in best reserved for evaluating compounds for centrally acting analgesic activity. The oleo-gum-resin (100 mg/kg) shows best effect after a latency period of 6 hr which is more than other fractions. To corrobate that the extract had no central analgesic acid, hot plate test [26] were conducted, significant effect noted for 200 mg/kg of different fraction in hot plate test were not due to central acting activities of the fraction. This mean there was no opioid receptors involved. The oleo-gum-resin (200 mg/kg) shows best activity after 5 h than other fractions and also indomethacin.

Conclusion

In the present study anti-inflammatory and analgesic activity of different fractions of *B. serrata* was investigated by means of acetic acid induced writhing, formalin test, tail immersion model of analgesic assessment and hot plate method in rats. The oral administration of different fractions of *B. serrata* showed suppression of inflammation and mechanism of action of extract might be linked to lipoxygenase and/or cycloxygenase. The oleo-gum-resin showed maximum inhibitory response as compared to other fractions. The result strongly suggests that the oleo-gum-resin can be used efficiently as analgesic and anti-inflammatory agent.

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