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Original Article

Preliminary evaluation of antinociceptive and anti-inflammatory activities of petroleum ether extracts of *Butea monosperma* (L.) leaves in laboratory animals

Vivek D. Rathod*[1], Jitendra O. Bhangale[2], Pradnya J. Bhangale[2]

[1]Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431 004 Maharashtra, India
[2]Smt. N. M. Padalia Pharmacy College, Navapura, Ahmedabad, 382 210 Gujarat, India

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ABSTRACT

The petroleum ether extract of the leaves of *Butea monosperma* was investigated for its antinociceptive and anti-inflammatory activity in respective animal models. In the present study leaves were extracted successively to obtain extract. The phytochemical analysis of extract contains alkaloids, saponins, flavonoids and glycosides. This extract was screened at a dose 100, 200 and 400 mg/kg orally for its antinociceptive activity using writhing, tail immersion and hot plate test while for anti-inflammatory activity, carrageenan, serotonin and histamine induced rat paw edema model used. Oral administration of 200 and 400 mg/kg of extract exhibited significant ($p<0.001$) antinociceptive activity in writhing, tail immersion and hot plate test. In rat paw edema model by carrageenan, serotonin and histamine, the extract was found to be reduce significantly ($p<0.001$) the formation of edema at a dose of 200 and 400 mg/kg at 1, 2, 3, 4 and 6 h. *Butea monosperma* possesses evident antinociceptive and anti-inflammatory activities. The results signify the traditional uses of *butea monosperma* for inflammation and pain.

Keywords: *Butea monosperma*, Anti-inflammatory, Antinociceptive activity

INTRODUCTION

Inflammation is primary physiological protective and defense mechanism that helps body to protect from microbial pathogen infection, chemical irritation and wounding. The production of inflammatory mediators is triggered by microbial products or by host proteins that are activated by damaged tissues. Currently inflammatory diseases are treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) that exert their effects by inhibiting the metabolism of arachidonic acid by both cycloxygenase and lipoxygenase enzyme pathways. Use of NSAIDs is associated with several adverse effects. Because of this effect, development of new compound is still necessary as this may overcome the toxicity associated with NSAIDs. Several plants have shown potential for anti-inflammatory activity. Thus, the search for natural products from plant origin having protective properties and possessing minimal side effects. *Butea monosperma* (Lam.) is commonly known as Flame of forest, belongs to the family Fabaceae [1]. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, Bastard Teak, Bengal Kino, Nourouc and is common throughout India, Burma and Ceylon except in very acrid parts. They comprise one of the largest families of flowering plants, numbering 630 genera and 18,000 species widely distributed throughout India [2]. *Butea monosperma* is extensibly used in Ayurveda, Unani and Homeopathic medicine. Commonly *Butea monosperma* is used as tonic, astringent, aphrodisiac and diuretics [3]. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumors [4]. It is also reported to possess antifertility, aphrodisiac and analgesic activities [2]. Flowers are useful in diarrhoea, astringent, diuretic, depurative and tonic [5]. The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. It has also Anthelmintic activity [6], Anticonceptive activity [7], Anticonvulsive activity [8], Antidiabetic activity [9], Anti-diarrhoeal activity [10], Antiesterogenic and antifertility activity [11-12], Antistress activity [13], Radical scavenging activities [14]. The *B. monosperma* is the reservoir for many potentially active chemical compounds which acts as drugs for anti-arthritic

Corresponding Author Address: Vivek D. Rathod, Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, 431 004, Maharashtra, India

activity. So this gives strong evidence for the use of plants in different medicines. Since no detail scientific data is available regarding the antinociceptive and anti-inflammatory activities of *B. monosperma* leaves, therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of *B. monosperma* leaves as antinociceptive and anti-inflammatory activity in laboratory animals.

MATERIAL AND METHODS

Collection of plant material: Fresh leaves of *Butea monosperma* were collected from local area of Jalgoan district, Maharashtra, India in the months of July-October. This plant was identified and authenticated by Dr. A. Benniamin, Scientist C and HOD, Botanical Survey of India, Pune. Voucher specimens No. (BSI/WRC/Tech./2014/32(JOB-3)) have been kept in Botanical Survey of India, Pune, Maharashtra, India.

Animals: Adult male Wistar albino rats, weighing between 180 - 220 g and albino mice (25-30 g) were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pelleted rats chow and water *ad libitum* as a standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with CPCSEA.

Preparation of leaf extract: The leaves were collected and dried in shade and ground. Coarsely powdered leaves were used for the study. Coarsely powdered leaves material (1000 g) was subjected to successive extraction with petroleum ether (60 – 80ºC) in a soxhlet extractor at a temperature of 45-50ºC to 45 cycles per batch for 2 batches. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish. The yield was 5.6 g/100 g.

Preliminary phytochemical studies: Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the petroleum ether extract of leaves of *Butea monosperma* has been carried out [15].

Acute oral toxicity of the extract: Adult Albino mice (25-30 g) were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Group I received only vehicle (distilled water). Group II, III, IV and V animals received with different doses of petroleum ether extract of leaves of *Butea monosperma* (PEBM) i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality [16-17].

ANTINOCICEPTIVE ACTIVITY

Writhing test: Male Swiss albino mice (25-30 g) were divided into five groups containing six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Indomethacine (10 mg/kg, p.o.), Group III: PEBM (100 mg/kg, p.o.), Group IV: PEBM (200 mg/kg, p.o.), Group V: PEBM (400 mg/kg, p.o.) [18] . All the drug treatments were given 1 hour before i.p. injection of 0.6 % (v/v) acetic acid, at a dose of 10 ml/kg [19]. Writhing is a syndrome characterized by a wave of contraction of the abdominal musculature followed by a wave of contraction of hind limbs. The hind limbs contractions that occurred over a period of 10 min were counted. A reduction in time of writhing initiation and number of writhing as compared to the vehicle treated group was considered as evidence for the analgesia.

Tail immersion test: Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Aspirin (100 mg/kg, p.o.), Group III: PEBM (100 mg/kg, p.o.), Group IV: PEBM (200 mg/kg, p.o.), Group V: PEBM (400 mg/kg, p.o.). The lower 5 cm portion of the tail was immersed in a beaker containing water and temperature maintained at 55 ± 0.5ºC [20]. The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion set at 10s. The reaction time was measured 1 h before and 0.5, 1, 2, 3, 4 and 6 h after oral administration of drugs [21-22].

Hot Plate Method: Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Pentazocine (10 mg/kg, i.p.), Group III: PEBM (100 mg/kg, p.o.), Group IV: PEBM (200 mg/kg, p.o.), Group V: PEBM (400 mg/kg, p.o.). Mice were placed on a hotplate maintained at a temperature of 55 ± 1ºC for a maximum time of 15 s. The time between placement of animal on the hot

plate and occurrence of licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before distraction (basal) and 30, 60 and 90 min. after treatment. The cut off time for hotplate latencies was set at 15 s [22-23].

ANTI -INFLAMMATORY ACTIVITY

Carrageenan induced rat paw Oedema: The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, p.o.), Group III: PEBM (100 mg/kg, p.o.), Group IV: PEBM (200 mg/kg, p.o.), Group V: PEBM (400 mg/kg, p.o.). After selection of animals, 0.1 ml of 1% carrageenan solution was injected into the left hind paw. The pretreatment time was 1 h before carrageenan injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured [24-25].

Serotonin and Histamine induced rat paw Oedema: The animal were treated in a manner similar to that of carrageenan induced rat paw edema protocol, different only in the administration of the inflammatory stimulus which was induced by sub-planter injection of serotonin (0.05 ml of 1 %) and histamine (0.05 ml of 1 %), respectively. The paw volume was measured as mentioned earlier [26-27].

Statistical analysis: All the values were expressed as mean ± SEM. Statistical evaluation of the data was done by two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at P <0.001 using Graph-Pad Prism 5, San Diego, CA software.

RESULTS AND DISCUSSION

Inflammation is a complex process which involves enzyme activation, mediator release, cell migration, extravasation of fluid, tissue breakdown, as well as tissue proliferation. Several experimental protocol of inflammation and pain are used for evaluating the potency of drugs. The present study establishes the antinociceptive and anti-inflammatory activity of the petroleum ether extract of the leaves of *Butea monosperma* using different animal models.

*Butea monosperma (*L.) is the reservoir for many potentially active chemical compounds which acts as drugs against various diseases and disorders. The petroleum ether extract of *Butea monosperma* (L.) showed the presence of alkaloids, saponins flavonoids, and glycosides. The extract was found to be safe at all doses used and found no mortality up to the dose of 4000 mg/kg when administered orally. In view of this, we have taken 400 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

Many studies have revealed that inflammation and pain are associated with disease of various clinical conditions like arthritis, vascular and cancer disease. Moreover, many medicinal plants have been found to alleviate inflammation and pain in vitro and in vivo system. *Butea monosperma* is a medicinal plant commonly used traditionally for pain relief and other medicinal use.

Antinociceptive activity was tested using writhing, hot plate and tail immersion test. The extract exhibited antinociceptive activity. In the acetic acid induced writhing test, local peritoneal receptors are postulated to be partly involved in the abdominal writhing response and the mechanism of the reaction to this nociceptive stimulus seems to be revealed to the prostanoid system [28]. The abdominal constriction produced by acetic acid is a very sensitive procedure for peripheral analgesic agents, and has also been associated with prostanoid [29-30] as well as lipoxygenase products [31].

Butea monosperma extract at a dose 200 and 400 mg/kg significantly ($p<0.001$) reduced writhings and stretchings induced by acetic acid (nociceptive stimuli) (Figure 1A). The first phase of tail immersion and hotplate test results showed central protective effect of extract. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (k) and delta receptors [32-33]. The reaction time of animal showed a significant increase ($p<0.001$) with increasing latency (time). Oral administration of PEBM (200 and 400 mg/kg) showed significant ($p<0.001$) increased pain latencies at 1, 2, 3, 4 and 6 h as compared to vehicle treated animal in tail immersion test (Figure 1B). Treatment with PEBM (100 mg/kg, p.o.) did not show significant activity. The latency response was found to be significantly ($p<0.001$) increased with the pretreatment of PEBM (200 and 400 mg/kg) at 20, 60 and 90 min in hot plate test (Figure 1C).

Using the carrageenan induced paw edema, which is the most widely used primary model for the screening of new anti-inflammatory agents. Carrageenan induced oedema is a multimediated phenomenon that liberates diversity of mediators

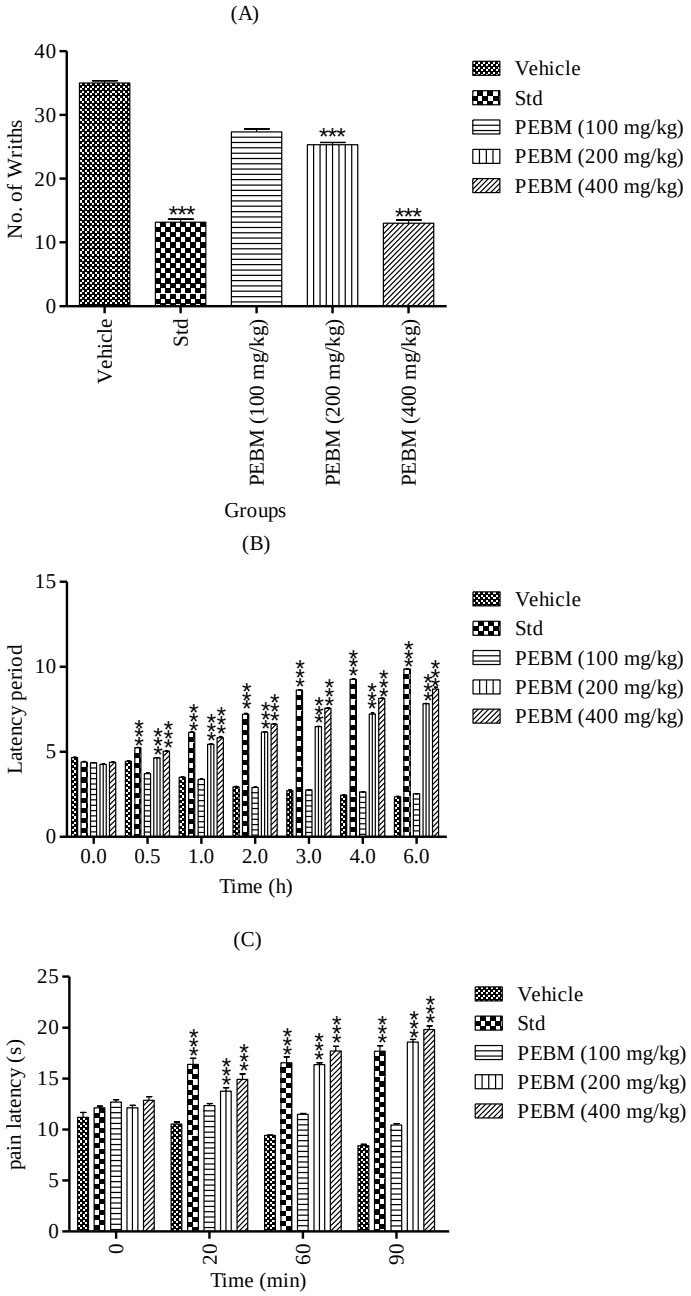
like histamine, 5-HT, kinins and prostaglandins at various time intervals. It is believed to be biphasic the first phase (60 min) involves the release of serotonin and histamine while the second phase (over 60 min) is mediated by prostaglandins, the cyclooxygenase products, and the continuing between the two phase is provided by kinins [34-35]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [36]. Oral administration of PEBM (200 and 400 mg/kg) showed significant ($p<0.001$) reduction in paw volume at 1, 2, 3, 4 and 6 h compared to vehicle treated animals in carrageenan induced rat paw edema (Figure 2A).

The histamine is a basic amine related with inflammatory and allergic process causing both vasodilation and increase of vascular permeability [37]. Oral administration of PEBM (200 and 400 mg/kg) showed significant ($p<0.001$) reduction in paw volume at 1, 2, 3, 4 and 6 h compared to vehicle treated animals in serotonin (Figure 2B) and histamine (Figure 2C) induced rat paw edema.
The phytochemical analysis of this extract revealed that it contains alkaloids, saponins, flavonoids and glycosides. On these, flavonoids and saponins are well known for their ability pain perception. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [38].

The results of this study showed that *Butea monosperma* can be effective in acute inflammatory disorders. Considering that there are only a few preliminary data reported in the literature regarding the anti-inflammatory properties of *Butea monosperma*, and that it has been largely used in folk medicine to treat inflammatory disorders mainly rheumatism.

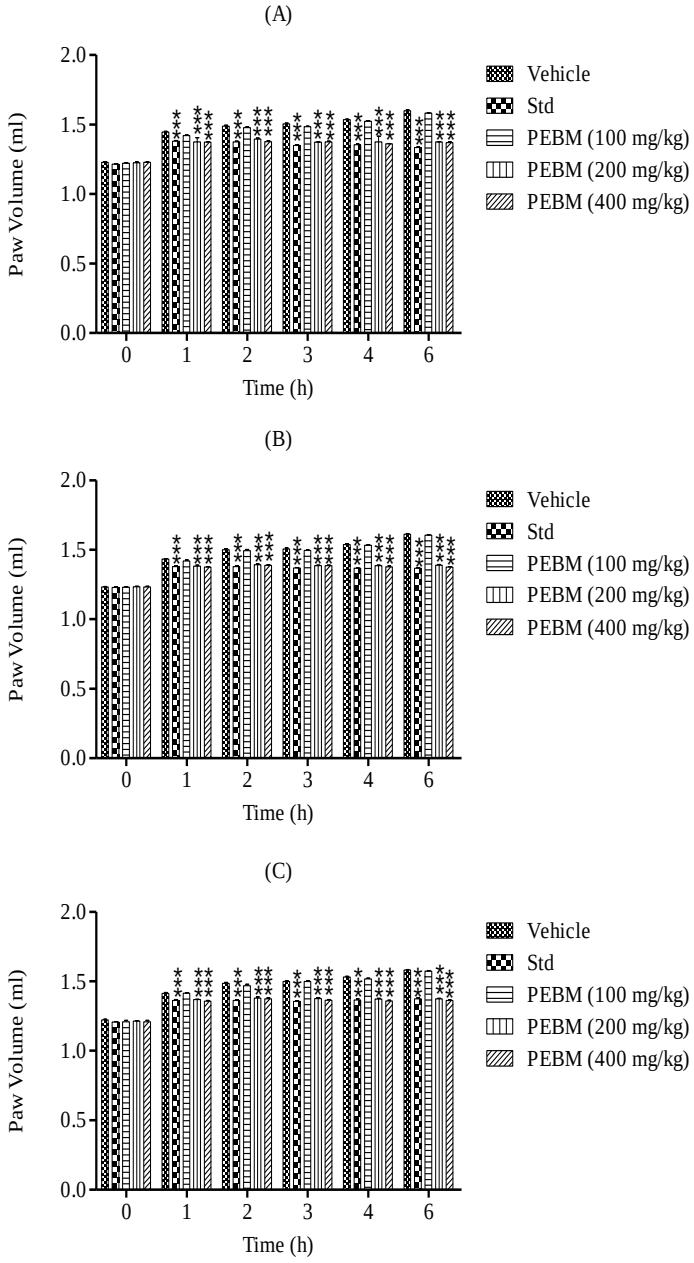
Phytochemical screening of AqCD has shown the presence of alkaloids, flavonoids and glycosides. The potent activity may be attributed to the presence of these phytoconstituents. The ability of the extract to cause oedema inhibition produced by these inflammatory mediators suggests that it contains phytochemically active constituent(s) with antiarthritic properties [(34)]. Amongst them, flavonoids may play a major role as they are proved as anti-inflammatory agents due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation
So we can conclude that the present study has shown that the petroleum ether extracts of *Butea monosperma* leaves possess antinociceptive and anti-inflammatory activities. The potent activity may be attributed to the presence of these phytoconstituents. The ability of the extract to cause oedema inhibition produced by these inflammatory mediators suggests that it contains phytochemically active constituent(s) with antinociceptive and anti-inflammatory properties. Amongst them, flavonoids may play a major role as they are proved as anti inflammatory agents due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation such as bradykinin and prostaglandins. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

Figure 1 : Effect of Petroleum ether extract of *Butea monosperma* on (A) Writhing test, (B) Tail Immesion test, (C) Hot Plate test



Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***p<0.01 compared to vehicle treated animals.

Figure 2 : Effect of Petroleum ether extract of *Butea monosperma* on (A) Carrageenan, (B) Serotonin and (C) histamine induced rat paw oedema



Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***p<0.01 compared to vehicle treated animals.

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