Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

ANTI- INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY OF *Pterospermum acerifolium* LEAVES

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Received 30spetember 2011 :, Revised and Accepted: 30 november 2011

ABSTRACT

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) has long been used traditionally for the treatment of painful inflammatory conditions in the Indian folk medicine. *Objective:* In the present study we have evaluated antinociceptive effects and anti-inflammatory effects of unsaponified petroleum ether extract of *Pterospermum acerifolium* leaves (USPEL, 100 and 200 mg/kg orally) and isolated β-sitosterol (10 and 20 mg/kg) from the leaves. USPEL and β-sitosterol are evaluated for its anti-inflammatory activity in carrageenan-induced paw edema model in rats and analgesic activity in acetic acid-induced writhing, hot plate and formalin induced paw licking models in mice. USPEL (200mg/kg) and β-sitosterol (20mg/kg) significantly ($p<0.05$) inhibited the writhing response 71.95%, 60.84% respectively. In hot plate method USPEL and β-sitosterol showed highest increase in reaction time which is comparable to the standard pentazocin. The USPEL significantly ($p<0.05$) and in dose dependent manner decreased the time spent on licking in both the first and second phases in formalin-induced nociception in mice. USPEL and β-sitosterol significantly ($p<0.05$) reduced the carrageenan induced rat paw edema in a dose dependent manner.

These findings demonstrate that other constituents alongwith β-sitosterol may be responsible for analgesic and anti-inflammatory activity of USPEL.

Keywords: *Pterospermum acerifolium*, β-sitosterol, anti-inflammatory, antinociceptive, writhing.

INTRODUCTION

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) commonly known as 'Dinner plate tree' is a large deciduous tree widely distributed in North Canada and in many parts India [1,2]. In traditional system of medicine, the flowers are used as a general tonic, anti-tumor agent, analgesic and for the treatment of diabetes, gastrointestinal disorders, leprosy, blood troubles, bronchitis, cough, cephalic pain, migraine and inflammation. Manna et al., have reported antiulcer and wound healing activity of bark in experimental animals [3, 4]. Muhit et al., have reported antioxidant activity of the bark[5].

The bark contains Kaempferol, kaempferol-3-O-galactoside, luteolin-7-O-glucoside, luteolin-7-O-glucoronide, kaempferide-7-O-beta-D-glucopyranoside, D-galactouronic acid, D-galactose and L-rhamnose. Flowers contain 24-beta ethylcholest-5-in-3-beta-O-alpha-cellobioside, 3,7-dimethyl-7-methyl1:5pentacosanolide, n-hexacosan-1, 26-diol-dilignoserate, β-amyrine, β-sitosterol and a mixture of acids and saturated hydrocarbons. The seeds contain palmitic, stearic, arachidic, behenic, myristic, lignoceric, oleic, linoleic, linolenic acids. Trunk bark and seeds gave the amino acids tyrosin, cystine, glycine, alanine and leucine [6, 7, 8, 9].

The purpose of the study reported here was to isolate the pure constituent responsible for analgesic and anti-inflammatory activity from the leaves of the plant.

MATERIALS AND METHODS

Plant Material and extraction

Leaves of *P. acerifolium* was collected from Nasik district of Maharashtra State (INDIA) in January 2010 and authenticated at Botanical Survey of India, Pune, where a sample (voucher number-RASPTA1) has been deposited. Shade-dried and powdered leaves were extracted with petroleum ether by maceration with frequent stirring. Solvent was evaporated under reduced pressure. The extract was further purified by separating saponified and unsaponified matter through treatment of alcoholic alkali[10]. The unsaponified petroleum ether extract of leaves (USPEL, 15 g) obtained was applied to the column of silica gel 60 (60-120 mesh) packed in benzene slurry and the column was developed with benzene, from which 10 fractions 300-400 ml were collected. Fractions 6-9 were combined on the basis of similar TLC pattern (Si gel Plates, benzene and vanilline-sulphuric acid spray). These fractions were further resolved by preparative TLC on silica gel GF_{254} using benzene as mobile phase, resulting in isolation of β-sitosterol (R_F-0.57) confirmed by melting point and superimposable 1H-NMR and mass spectral analysis. β-sitosterol: white amorphous powder, m.p.- 136–138^{0}C. IR spectrum: KBr (ν_{max} cm−1) 3404.5 (–OH group), 2924.0 (–CH3 stretch), 1635.0 (C=C stretch), 1455.0 (C–H bending). Mass spectra: *m/z* [414] M^+ 399, 396, 381, 329, 303, 301, 275, 273, 272, 271, 255, 253, 231, 229 and 213. ^{1}*H-NMR:* 1×01 (2H, *m*, H-1), 1×37 (2H, *m*, H-2), 3×82 (1H, *m*, H-3), 2×62 (2H, *m*, H-4), 5×32 (1H, *t*, H-6), 1×93 (2H, *m*, H-7), 1×54 (1H, *m*, H-8), 0×94 (1H, *m*, H-9), 1×44 (2H, *m*, H-11), 1×69 (2H, *m*, H-12), 1×10 (1H, *m*, H-14), 1×51 (2H, *m*, H-15), 4×61 (2H, *m*, H-16), 1×74 (1H, *m*, H-17), 0×67 (3H, *s*, H-18), 0×98 (*s*, 3H, H-19), 1×90 (1H, *m*, H-20), 0×92 (3H, *d*, *J* = 2×9 Hz, H-21), 1×62 (2H, *m*, H-22), 1×65 (2H, *m*, H-23), 1×58 (1H, *m*, H-24), 1×56 (1H, *m*, H-25), 0×82 (3H, *d*, *J* = 7 Hz, H-26), 0×80 (3H, *d*, *J* = 7 Hz, H- 27), 1×52 (2H, *m*, H-28), 0×84 (3H, *t*, H-29)[11, 12].

Animals

Albino rats (100-120 g) and Swiss mice (20–30 g) of either sex were used. Animals were randomly assigned to groups and maintained in plastic boxes at controlled room temperature (25–28 ◦C) with free access to food and water, under a 12:12 h light/dark cycle. All the experimental procedures were carried out during the light period of the day (11:00 a.m. to 04:00 p.m.).

Acute Toxicity Study

Acute oral toxicity was performed in mice by following Organization for Economic Cooperation and Development (OECD) guidelines 425. In the acute toxicity study, USPEL did not produce any mortality even at the highest tested dose 2000 mg/kg, p.o. during the 24 hour period. There was no change in the gross behaviour also. The two doses (100 and 200 mg/kg, i.p.) of USPEL were selected for further pharmacological studies.

Acetic acid-induced writhing test in mice

This test was performed as described by Collier et al. [13]. Mice were divided in groups of 6 each. Acetic acid (0.6%, v/v) was administered i.p., at a volume of 0.1 mL and the number of writhes, a response consisting of contraction of the abdominal wall, pelvic rotation followed by hind limb extension, was counted during 30 min beginning from the acetic acid injection. USPEL (100 and 200 mg/kg, body wt., i.p.) or β-sitosterol (10 and 20 mg/kg, body wt., i.p.) or the reference drug ibuprofen (40 mg/kg p.o.) were administered

30 min before the acetic acid injection. Antinociceptive activity was expressed as percent inhibition of the writhing.

Hot-plate test in mice

This test was performed according to the method of Woolfe and Macdonald[14]. Mice were treated with USPEL (100, 200 mg/kg, i.p.), or β-sitosterol (10 and 20 mg/kg, body wt., i.p.) or with pentazocine (10 mg/kg, i.p.) and were placed individually on a metallic plate warmed to 55±0.5 ◦C (n = 6/group). The time elapsed until the appearance of paw licking was recorded as an index of nociception. Measurements were performed at time 0, 30, 60, 90, 120 and 180 min after the administration. In order to avoid damage to the animal's paws the cut-off time was limited to 20 s.

Formalin test

Male Wistar rats were pretreated with USPEL (100, 200 mg/kg, i.p.), or β-sitosterol (10 and 20 mg/kg, body wt., i.p.) or ibuprofen (40 mg/kg i.p.)), p.o., 60 min before subcutaneous injection of 50 µl of 1% formalin into the dorsal surface of the right hind paw was applied. The procedure was similar to that described previously by Hunskaar and Hole[15] . Animals were observed in the chambers.Animals were observed from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase) and the time that they spent licking the injected paw was recorded and considered as indicative of nociception.

Anti-inflammatory activity

The anti-inflammatory effect of was assessed in acute inflammation method already described by Winter et al.[16]. Initially normal paw volume of each rat was noted. acute inflammation was produced by sub plantar injection of 0.1 ml of 1% carrageenan suspension into the region of the right hind paw of each rat one hour after oral administration of USPEL (100, 200 mg/kg body weight), or β-sitosterol (10 and 20 mg/kg, body wt.) or ibuprofen (40 mg/kg), control group received 2.5 ml/kg of saline. The paw volume, up to tibiotarsal articulation, was measured using a plethysmometer. The measures were determined at 0 h (V0: before edematogenic agent injection) and 1, 2, 3 and 4 h intervals later (VT). The difference between VT (1, 2, 3 and 4 h) and V0 was taken as the edema value. The percentages of inhibition were calculated according to the following formula:

% inhibition = ((VT −V0) control − (VT −V0) treated group)×100/(VT −V0)control.

Statistical analysis

The statistical analyses were performed by one-way ANOVA, followed by Dunnett's test. The statistical analyses were carried out using Graph Pad Prism version 5.0. The results were expressed as the mean ± S.E.M. to show variation in groups.

RESULTS

The effect of unsaponified petroleum ether extract and β-sitosterol were evaluated for central as well as peripheral analgesic, along with anti-inflamatory activity.

Acetic acid-induced writhing in mice

Mice treated with acetic acid exhibited writhing behaviour which was significantly ($p<0.05$) reduced by USPEL and β-sitosterol as well as ibuprofen. At the dose of 200mg/kg body wt. USPEL inhibited the writhing response 71.95% almost to the same degree of ibuprofen 72.49%. The observations are given in Table 1.

Table 1: Effect of β-sitosterol on acetic acid induced writhing test in mice .

Treatment (dose: mg/kg)	No. of writhings	% inhibition
Vehicle	63±2.503	-
Ibuprofen (40)	17.33±1.116	72.49
USPEL(100)	28.67±1.174	54.49
USPEL(200)	17.67±1.022	71.95
β-sitosterol (10)	31.33±1.406	50.27
β-sitosterol (20)	24.67±0.8819	60.84

n = 6, the data is significant at P < 0.05 compared to the vehicle treated group.

Hot plate test in mice

Pretreatment with pentazocine or USPEL or β-sitosterol did not produce any significant changes of paw licking time in early phase of pain. However in the late phase of pain, a dose dependent and significant ($p<0.05$) increase in licking time was observed. The maximum activity was observed with and β-sitosterol (20 mg/kg) at the 90 min time interval which is comparable to the standard pentazocin. The maximum analgesia induced by USPEL (200mg/kg body wt.) was at 60 min time interval and persist upto 120 min. The observations are given in Table 2.

Table 2: Effect of β-sitosterol on latency to paw licking in mice placed on hot plate.

Treatment (dose: mg/kg)	latency to paw licking (s) (Mean ± SEM) at					
	0 min	30 min	60 min	90min	120 min	180 min
Vehicle	5.85 ± 0.371	6.66 ± 0..3413	7.87±0.314	7.915±0.477	5.7± 0.1442	6.83±0.5702
USPEL(100)	6.424± 0.196	8.29± 0.339*	11.76±0.3812*	15.32±0.366*	16.53± 0.5678*	8.17±0.6221
USPEL(200)	5.6± 0.352	11.21±.4203*	15.11±0.3024*	18.12±0.424*	20. 0*	13.82±0.4971*
β-sitosterol (10)	6.09± 0.2995	7.96± 0.27	11.31±0.376*	14.11±0.299*	14.26±0.5588*	8.98±0.4107
β-sitosterol (20)	5.77± .3352	10.06±0.466*	11.12±0.587*	16.23±0.386*	15.55± 0.2694*	9.36±0.7708*
Pentazocine (10)	6.19± 0.4265	11.16±0.274*	16.83±0.3632*	17.69±0.212*	17.67± 0.409*	11.02±0.88*

n = 6, the data is significant at P < 0.05 compared to the vehicle treated group.

Table 3: Effect of β-sitosterol on formalin-induced pain in rats

Treatment	Licking time (s)	% Inhibition	Licking time (s)	% Inhibition
	First phase		Second phase	
Control	72.67±4.0393		73.5±1.31	-
USPEL(100)	36.83±4.191	49.32	31.17±1.138	57.59
USPEL(200)	24.17±1.352	66.74	21.17±0.7923	71.2
β-sitosterol(10)	37±2.556	49.08	35.17±1.493	52.15
β-sitosterol(20)	33.33±2.418	54.14	30.67±1.358	58.27
Ibuprofen(40)	19.67±1.687	73.93	16.17±1.195	78

n = 6, the data is significant at P < 0.05 compared to the vehicle treated group.

Table 4 : Effect of β-sitosterol on carrageenan-induced paw edema in rats.

Treatment	Paw edema volume after							
	1hr		2hr		3hr		4hr	
	Mean±S.E.M.	% inhibition	Mean±S.E.M.	% inhibition	Mean±S.E.M.	% inhibition	Mean±S.E.M.	% inhibition
Control	0.47±0.08371		0.91±0.07314		1.26±0.06181		0.87±0.1019	
USPEL(100)	0.26±0.02892*	44.68	0.43±0.04868*	53.26	0.55±0.04822*	56.35	0.47±0.04377*	45.98
USPEL(200)	0.23±0.03765*	51.06	0.35±0.04271*	61.96	0.42±0.05914*	66.67	0.38±0.0522*	56.32
β-sitosterol(10)	0.33±0.0313	29.79	0.54±0.03274	41.3	0.63±0.04571*	50	0.52±0.04833*	40.23
β-sitosterol(20)	0.27±0.05125*	42.55	0.40±0.04622*	56.52	0.52 ±0.0572*	58.73	0.43±0.04629*	50.57
Ibuprofen(40)	0.21±0.02512*	55.32	0.35±0.04792*	61.96	0.39±0.04771*	69.05	0.34±0.05188*	60.92

n = 6, the data is significant at P < 0.05 compared to the vehicle treated group

Formalin test

The USPEL and β-sitosterol significantly ($p<0.05$) and in dose dependent manner decreased the time spent on licking in both the first and second phases in formalin-induced nociception in mice (Table 3).

Anti-inflammatory activity

USPEL significantly reduced the carrageenan induced rat paw edema in a dose dependent manner (Table 4). The different doses of the USPEL (100, 200 mg/kg) and β-sitosterol (10, 20 mg/kg) significantly ($p<0.05$) inhibited the inflammation to the extent of 56.35%, 66.67%, 50%, 58.73% at 3 h and 45.98%, 56.32%, 40.23%, 50.57 at 4 h respectively, while the reference drug, ibuprofen (40 mg/kg) reduced the inflammation by 69.05% at 3 h and 60.92% at 4 h.

DISCUSSION

The results indicate that the USPEL and pure β-sitosterol possessed both peripheral (reduction in writhing) and central (delay in reaction time to thermal pain) analgesic effects. The acetic acid induced writhing in mouse and rat is an animal model that measures the peripheral antinociception. Inhibition of writhing by the USPEL indicated that it act peripherally as well. Hirose et al. and Berkenkopf and Weichman have reported production of prostacyclin in mice following intraperitoneal injection of acetic acid[17, 18]. This suggests that the USPEL might reduce production of prostacyclin.

Hot plate analgesiometer has been used to assess central antinociceptive effect [19, 20]. According to Pini et al. [21] the hot-plate test specifically reflexes the involvement of central anti-nociceptive mechanism of extracts/compounds as only the centrally acting drugs were capable of affecting this test [22](Hosseinzadeh and Younessi, 2002). Centrally acting agents were known to activate the release of endogenous peptide by periaqueductal gray matter (PAG), which are carry to the spinal cord to inhibit the pain muscle transmission within the dorsal horn [23]. Inhibition of pain perception by the USPEL indicates its central action. β-sitosterol produced an analgesic effect against thermal induced pain stimuli in mice at various time points post-treatment. The effect observed was dose dependent and statistically significant. Effect of USPEL was initiated first and appears for long duration when compared with different doses of β-sitosterol in hot plate test. This might be due to the other constituents present in the USPEL.

The formalin test consists of two different phases: the first phase measures direct chemical stimulation of nociceptors, whereas the second phase is dependent on peripheral inflammation and changes in central processing. Previous studies demonstrated that bradykinin participate in the first phase, whereas histamine, serotonin, PGs, NO and bradykinin were involved in the second phase of the formalin test [24]. The formalin-induced nociceptive test (paw licking) possesses algesic activity in both phases, reflecting different types of pain. The earlier phase reflects a direct effect of formalin on nociceptors (neurogenic pain) where as the later phase reflects tissue injury or inflammation mediated pain [25]. The experimental results showed that USPEL produced a significant inhibitory effect during first phase and second phase of the formalin test (Table 3). All experimental results from three animal models indicated that the USPEL might produce the analgesic effect centrally as well as peripherally by neurogenic as well as inflammatory mechanisms.

Carrageenan has been widely used as an inflammagen capable to induce experimental inflammation used for the screening of compounds possessing anti-inflammatory activity. It induces an inflammatory reaction in two different phases. The initial phase has been attributed to the release of histamine, serotonin and bradykinin on vascular permeability [26] and the later phase has been due to over production of prostaglandin in tissues [27]. The USPEL and β- sitosterol produced a marked inhibition of carrageenan-induced rat paw inflammation by inhibiting the mediators of acute inflammation indicating its anti-inflammatory activity.

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