ANTIINFLAMMATORY AND ANALGESIC ACTIVITY OF *BUTEA MONOSPERMA* (LAM) STEM BARK IN EXPERIMENTAL ANIMALS.

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Summary

Butea monosperma (Lam) (Family:Fabiaceae) is a medium sized tree found in greater parts of India. The decoction of the bark is traditionally used in treating cold, cough, fever, various forms of haemorrhages, menstrual disorders and in the preparation of tonics and elixirs. The stem bark is reported to possess anti-tumor, anti-ulcer, antifungal and antidiarrhoeal activity. The methanolic extract of *B. monosperma* was obtained from the dry stem bark of *B.monosperma*. Its anti-inflammatory and analgesic activity is investigated using Carrageenon - induced paw edema, Hot plate test and Acetic acid induced writhing model. Methanolic extract of B.monosperma showed both anti-inflammatory and analgesic activity in dose dependant (200 and 400mg/kg,p.o.) manner which are comparable to the standard drug (Diclofenac sodium for Carrageenon induced paw edema and Acetic acid induced writhing and Pentozocine for hot plate test model). Phytochemical studies of this plant reveal the presence of flavonoids, steroids, tannins, alkoloids, glycosides and these might be responsible for the anti-inflammatory and analgesic activity of this plant.

Key-words:*Butea monosperma, anti-inflammatory, nociception, writhing, analgesic.*

Introduction

Butea monosperma (Lam) (family: Fabiaceae) is a medium sized deciduous tree, found in greater parts of India and is reported to have numerous uses in the indigenous system of medicine in India.[1] Various medicinal properties are ascribed to flowers, leaves, bark and roots of this plant. The leaves are used to cure boils. The roots are useful in elephantiasis and in curing night blindness. Flowers are reported to possess astringent, depurative, aphrodisiac and tonic properties[2]. The decoction of the bark is traditionally used in cold, cough, fever, various forms of haemorrhages, in menstrual disorders and in the preparation of tonics and elixirs. The stem bark is reported to possess antitumour, antiulcer, antifungal and antidiarrhoeal activities[3, 4, 5]. The objective of the study was aimed to evaluate anti-inflammatory and antinociceptive activity with methanolic extract of stem bark of *B. monosperma* (Lam).

Materials and Methods

Plant material:

Stem bark of *Butea monosperma* was collected from campus of Kakatiya University, Warangal and the plant was identified and authenticated by the Department of Botany, Kakatiya University, Warangal. The voucher specimen of the plant (no.KU/11/2005) has been kept in the Department of Pharmacognosy and Ethanopharmacology, University college of Pharmacuetical Sciences, Kakatiya University, Warangal for the further references.

Preparation of the extract:

The freshly collected stem bark of this plant was chopped, shade dried and coarsely powdered material was subjected to maceration with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure and stored in a desiccator for further use.

Drugs:

The following chemicals and drugs were used: Carrageena (sigma-Aldrich), acetic acid (Ranbaxy laboratories Ltd., Punjab) Diclofenac sodium (Dr. Reddy Labs, Hyderabad), Pentazocine (Pure Pharma Pvt. Ltd., Mumbai).

Animals:

Albino rats of Wistar strain (150-200g) and Swiss albino mice (20-25g) of either sex were procured from M/s Mahaveer Enterprises, Hyderabad. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2^0 c; relative humidity 60-70%) in a 12h light-dark cycle. Animals were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 2h before and during the experimental hours. All experimental protocols were prepared and performed based on ethical guidelines of Institutional Animal Ethics Committee.

Acute toxicity study:

Acute toxicity study was performed according to organization for economic co-operation and development (OECD) guidelines AOT No.425[6]. No adverse effect or mortality was detected in Swiss mice up to 2g/kg, p.o. of the extract during the 24 h observation period.

Anti-inflammatory Activity:

Carrageenan-induced edema:

Paw volume was measured initially at 0h. Paw edema was induced by injecting 0.1ml. of 15 Carrageenan in physiological saline into the sub plantar tissues of the left hind paw of each rat[7]. The extracts (200mg/kg and 400mg/kg b/w.) were administered orally 30min prior to Carrageenan administration. The paw volume was measured at 0, 60,120, 180, 240 minutes by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the control group. Diclofenac sodium (50mg/kg b/w) was used as reference standard.

Analgesic Activity:

Hot Plate Method:

Mice were tested according to Eddy and Leimbark[8].Albino mice were placed in aluminium hot plate kept at a temperature of $55^0 \pm 0.5^0$C for a maximum time of 20sec [9]. Reaction time was recorded when animals licked of the hind paws or jumping movements before and after drug administration. The reaction time for each mouse was recorded at 0, 15, 30, 60, 120 and 240 min after the oral administration of methanolic extract of stem bark of *B. monosperma* (200 and 400mg/kg b.w.). Pentozocin 10mg/kg b.w. (Pure Pharma Ltd. Mumbai) administered intraperitonially, was used as reference drug.

Acetic acid-induced Writhing:

Albino mice of either sex weighing (20-25g) were selected. Mice were injected intraperitonially with 0.7% (v/v) Acetic acid solution (0.1ml per 10 g of b.w), one hour after oral administration of control (2% gum acacia), standard (Diclofenac sodium-20mg/kg b.w) and methanolic extracts (200mg/kg and 400mg/kg b.w.). Number of writhes per animal was counted during 20 min test period, beginning 3 min after the injection of acetic acid[10].

Statistical Analysis:

The values are expressed as mean ± S.E.M. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet's t–test, P-values<0.05 were considered as significant. All statistical manipulations were carried out using Graph Pad Prism 3.0 (U S A) statistical software.

Results

Phytochemical Screening:

Preliminary phytochemical screening of the methanol extract revealed the presence of steroids, flavonoids, tannins, alkaloids and glycosides. Further separations of the specific phytochemical studies are in progress.

Acute Toxicity Studies:

All the doses (5, 50, 300 and 2000 mg/kg, p.o.) of methanolic extracts of stem bark of *B. monosperma* (Lam) employed for acute oral toxicity studies were found to be non-toxic. *B. monosperma* (Lam) extract did not produce any mortality even at the highest dose (2000mg/kg, p.o.) employed. Two sub maximal doses (200 and 400mg/kg, p.o.) which were found to be safe in animals were employed for further pharmacological studies.

Carrageenan-induced edema test:

The result of the animal experiment was shown in table 1. In the acute inflammation model, the methanolic extract of *B. monosperma* in doses of 200 and 400mg/kg, p.o. produced dose-dependent inhibition of paw edema and shows maximum inhibition at 3 h i.e., 62.96 % and 72.9% compared to the standard 71.45%.

Table 1 .Anti-inflammatory effect of the methanolic extract from Butea monosperma (Lam) stem bark on the Carrageenan induced paw edema.

Hot Plate Test:

Table 2 shows the results of the hot plate test. Two doses of extract of *B. monosperma* increased the reaction time in a dose-dependent manner to the thermal stimulus. The highest nociception inhibition of thermal stimulus was exhibited at a higher dose of the extract 400mg/kg (77.84%) compared to the reference drug at 2h.

Acetic Acid-induced Writhing Test:

Dose-dependent anti nociceptive effect was noted with the extract at the test dose levels (table 3). Maximum percentage of inhibition of writhing response exhibited by the methanolic extract at 400mg/kg was 58.2%, while the same at 200mg/kg showed 51.02% reduction in acetic acid induced writhing response respectively, which was comparable to that of standard Diclofenac sodium (20mg/kg) that caused 76.5% pain inhibition.

Table 1 .Anti-inflammatory effect of the methanolic extract from ***Butea monosperma*** (Lam) stem bark on the Carrageenan - induced paw edema.

S.No	Group	Dose (mg/kg)	Paw Edema Volume 1h		2h		3h		4h	
			Mean ± S.E.M	%PEI	Mean ± S.E.M	% PEI	Mean ± S.E.M	%PEI	Mean± S.E.M	%PEI
1.	Control		0.183± 0.007	---	0.52 ± 0.058	---	0.583 ± 0.04	---	0.55 + 0.012	---
2.	Standard	50	0.105± 0.01	42.68	0.167 ± 0.005	67.66	0.166 ± 0.005	71.45	0.071** ± 0.001	86.96
3.	*B.monosperma*	200	0.141± 0.04	22.48	0.225** ± 0.02	56.44	0.216** ± 0.02	62.96	0.23** ±0.05	58.16
4.	*B.monosperma*	400	0.108 ± 0.04	41.04	0.198** ±0.045	63.02	0.158** ±0.416	72.9	0.166** ±0.042	69.81

Values are mean±S.E.M; n=6; * P< 0.05; as compared to the control

Table. 2 Analgesic effect of the methanolic extract from ***Butea monosperma*** stem bark on the Hot plate method

S.No	Group	Dose (mg/kg)	Reaction time after administration of control/ standard / extract in sec			
			0	60	120	240
1.	Control		3.00 ± 0.22	2.83 ± 0.27	2.78 ± 0.28	2.50 ± 0.22
2.	Standard	10	2.83 ± 0.27	6.00 ± 0.00	14.58** ± 0.47	3.50 ± 0.38
3.	*B.monosperma*	200	4.00 ± 0.36	7.83**± 0.65	6.33**± 1.23	5.00**+ 1.21
4.	*B.monosperma*	400	5.33 ± 0.71	10.16**± 1.09	11.35**± 1.04	10.58**± 0.98

Values are mean ± S.E.M; n=6; * P < 0.05; as compared to the control

Table . 3 Analgesic effect of the methanolic extract from ***Butea monosperma*** (Lam) stem bark on the Acetic acid – induced nociception

S.No	Group	Dose (mg/ kg)	No of writhings (mean ± S.E.M)	% Inhibition
1.	Control		78.00 ± 2.781	---
2.	Standard	20	18.33** ± 0.95	76.50
3.	*B.monosperma*	200	38.20** ± 2.63	51.02
4.	*B.monosperma*	400	32.60** ± 2.26	58.20

Values are mean ± S.E.M; n=6; * P< 0.05; as compared to the control

Discussion

In the present study, the anti-inflammatory activity of the methanolic extract of stem bark of *B. monosperma* has been established in acute model i.e., Carrageenan-induced rat paw edema, is a suitable test for evaluating anti-inflammatory drugs which have frequently been used to assess the anti-edematous effect of natural products[11]. Development of edema in the paw of the rat after injection of Carrageenan is a biphasic event[12]. The initial phase observed during the first hour is attributed to the release of histamine and serotonin[13]. The second phase of edema is due to the release of prostaglandins, protease and lysosome[12, 13]. The result of the present study indicates that *B. monosperma* (200 and 400mg/kg, p.o.) and Diclofenac sodium play a crucial role as protective factors against the Carrageenan-induced inflammation.

The analgesic test used in the present work were chosen in order to test different nociceptive stimuli, namely cutaneous thermic(hot-plate) and chemical visceral (writhing) stimuli. Methanolic extract of *B. monosperma* stem bark significantly delayed the reaction time with hot-plate in mice. It is known that centrally acting analgesic drugs elevate the pain threshold towards heat. This significant increase in pain threshold caused by methanolic extract of *B. monosperma* in the hot-plate models may not reflex a non specific depressant action or reduction of motor co-ordination of animals. In order to distinguish between the central and peripheral analgesic action, acetic acid - induced writhing abdominal constriction response in mice was used to examine the effect. It was found that the extract of *B.monosperma* (200 and 400mg/kg p.o.) significantly inhibited as a dose dependant manner. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins[14, 15]. This result indicates that the analgesic effect of *B. monosperma* might be mediated by its peripheral effect. Thus, the analgesic activity shown by these models suggest that methanolic extracts of *B. monosperma* stem bark possess both central and peripheral mediated analgesic properties. In conclusion, methanolic extract of *B.monosperma* stem bark could be beneficial in the management of pain and inflammation. The result of this study confirms the traditional use of *B.monosperma*.

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