



Evaluation of *Butea monosperma* as an immunomodulatory agent

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ABSTRACT

The leaves of *Butea monosperma* (Lamk) Taub (Fabaceae) have been traditionally used as tonic. Ethanolic extract of *Butea monosperma* contains flavonoids, glucosides, butin, butrin, isobutrin, palastrin. The present study was undertaken to evaluate the immunomodulatory activity of *Butea monosperma* leaves. Ethanolic extract of *Butea monosperma* leaves was administered at dose of 100, 200 and 400 mg/kg to healthy mice. The immunomodulatory activity was carried out by testing non-specific immune responses using carbon clearance test (CCT), Neutrophil adhesion test (NAT) and cecal ligation and Puncture induced abdominal sepsis models. *Butea monosperma* ethanolic extract (BMEE) caused increase in the Phagocytic index in Carbon clearance test as well as increased neutrophil index in NAT. Increased percentage survival of animals was observed in Cecal ligation and Puncture induced abdominal sepsis model. The results of the present study indicate that the BMEE stimulates the immune system non-specifically.

Key words: *Butea monosperma*, phagocytic index, neutrophil adhesion, sepsis

INTRODUCTION

Some plants are believed to promote positive health and maintain resistance against infection by establishing body equilibrium. It is tempting to speculate that the restorative and rejuvenating power of these herbs may be due to their action on the immune system. Immunomodulation is related to activation of immune cells such as macrophages, complement, natural killer cells, granulocytes and also to the production of various effector molecules produced by activated cells. These effects against variety of pathogenic microorganisms offer an alternative to routine chemotherapy^[1]. Traditional Indian systems of medicine like Siddha and Ayurveda have suggested means to increase the body's natural resistance to diseases. A number of Indian medicinal plants have been claimed to possess Immunomodulatory activity. Leaves of *Butea monosperma* (Lamk) Taub (Family: Fabaceae) are tonic, astringent, appetizer, anthelmintic, aphrodisiac and also lesson inflammation. Roots are useful in filariasis, nightblindness, helmenthiasis, piles, ulcers and tumors^[2]. Flowers are tonic, astringent, aphrodisiac and diuretic^[3]. Methanolic extract of seeds of *Butea monosperma* has potent anthelmintic activity^[4]. Anticonvulsant activity

was observed on administration of petroleum ether extract of flowers^[5]. Ethanolic extract of *Butea monosperma* had potent antidiabetic activity^[6]. The antifungal activity was shown by ethyl acetate and petroleum ether extracts of stem^[7]. Alcoholic extract of bark showed wound healing activity^[8]. The bark extract fraction showed potent antiasthmatic activity^[9]. The selected plant contains variety of constituents like flavonoids, alkaloids, monospermosides, d-lactone^[10, 11, 12].

The aim of the mentioned research was to study immunomodulatory activity of ethanolic extract of the leaves of *B. monosperma* (Lamk) Taub in animal models.

MATERIALS AND METHODS

Animals: Swiss albino mice of either sex (National Toxicology Centre, Pune, India) weighing between 20 to 25 g were used. Animals were housed under standard temperature condition and proper light/dark cycle was maintained. Animals were given pellet diet and water. The Institutional Animal Ethics Committee approved the study protocol.

Plant material: Fresh leaves of *Butea monosperma* were collected from local gardens of Pimpri in Maharashtra state, India. The plant was authenticated at Botanical Survey of India, Pune by Mr. P. S. N. Rao, Jount Director BSI (Voucher specimen no. BSI/WC/Tech./2006/603).

Preparation of Extract: Leaves of *Butea monosperma* were dried in shade at room temperature. The dried, coarsely powdered leaves were extracted with 95% ethanol by soxhlet extraction method. The yield was 12.8%w/w . The *Butea monosperma* ethanolic extract (BMEE) was then concentrated and air dried. It was further used to study immunomodulatory activity.

Methods

Carbon Clearance Test: The animals were divided into 4 groups consisting 5 animals each. Group I received Sodium carboxymethylcellulose (1%, 1 ml/kg, p.o.) whereas group II, III and IV received *Butea monosperma* ethanolic extract (BMEE) 100, 200 and 400 mg/kg/day, p.o., respectively, for five days. On day 7, animals were given colloidal carbon by intravenous route. Blood samples were withdrawn from retro orbital plexus at 0 and 30 min of administration of colloidal carbon ink. Samples of 25 μ l were lysed in 3 ml of sodium carbonate solution. The optical density was spectrophotometrically measured at 650 nm.

Neutrophil adhesion test: Animals were divided into four groups comprising of five animals in each group. Blood samples were collected from retro-orbital plexus under mild ether anesthesia on day 7 of the treatment. Vials containing di-sodium EDTA were used to collect blood samples. Samples were analyzed for total leukocyte count (TLC) and differential leukocyte count by fixing blood smears and staining with Field stain I and Leishman's stain. Later blood samples were incubated with nylon fiber 80 mg/ml of sample for 15 minutes at 370°C followed by repetition of TLC and DLC. Neutrophil index was found by multiplying TLC with % Neutrophil count^[14, 15].

Cecal ligation and puncture induced abdominal sepsis: Five groups of animals were made. Animals from group I (Sham-Laparotomy) and group II (Sham- Cecal Ligation and Puncture i.e.CLP) received Sodium carboxymethylcellulose (1%, 1 ml/kg, p.o.) at 18 hr \pm 2 hr and 2 hours before laparotomy and cecal ligation respectively. Group III, IV and V received *Butea monosperma* ethanolic extract (BMEE) as 100, 200 and 400 mg/kg, p.o., respectively. Mice were anesthetised by Ketamine 100 mg/kg, i.p. A small midline incision (1 to 2 cm) was made through abdominal wall; the cecum was located and ligated 1 cm away

from the tip. Atmost care was taken to prevent bowel obstruction. With help of 20- gauge needle a single puncture was made in the cecal wall. To ensure full thickness puncture the cecum was lightly squeezed and a small amount of stool was expressed from the puncture site. The cecum was placed back in the abdominal cavity and the incision was closed with surgiclips. Sham mice underwent anesthesia and midline Laparotomy; the cecum was exteriorized, returned to the abdomen and the wound was closed with surgiclips. Measurement of mortality was carried out for 7 days after CLP^[16].

Statistical Analysis: All values were expressed as mean \pm SEM. The data were statistically analyzed using one way ANOVA followed by Tukey Kramer multiple comparison test and values at p < 0.05 are considered as significant.

RESULTS

Carbon clearance test: Albino mice treated with BMEE (100 - 400 mg/kg, p.o.) showed dose dependent increase (47.3% to 77.7%) in the phagocytic activity when compared with the control group as observed in the carbon clearance test (Table 1).

Neutrophil adhesion test: The neutrophil adhesion was increased significantly by BMEE (400mg/kg) when compared to the control group (Table 2).

Cecal ligation and puncture induced abdominal sepsis: In this animal model, mortality due to CLP-induced abdominal sepsis was observed and results were expressed as percentage survival. All three doses of MBEE showed significant protection against death at 12, 24, 48 and 168 hr as compared to control group (Table 3).

DISCUSSION

Modulations of immune response helps in maintaining a disease free state. Agents activating host defense mechanisms in presence of impaired immune responsiveness can provide supportive therapy to conventional chemotherapy^[17]. Interest is growing worldwide in identifying herbal modulators ever since their possible use in modern medicine^[18]. Phagocytosis is primarily the removal of microorganisms, foreign bodies and also the elimination of dead or injured cells. On injection the circulating carbon particles in the body are removed by intravascular phagocytes in the liver and spleen. Kupffer cells of liver approximately take up 90% of carbon and splenic macrophages take up 10%. The rate of removal of carbon particles by intravascular phagocytes in liver and

spleen from bloodstream is the measure of reticuloendothelial phagocytic activity [19].

BMEE treated groups exhibited significantly high phagocytic index in the carbon clearance test, which is indicative of stimulation of the reticuloendothelial system. Phagocytosis caused by macrophages is important against small parasites and its effectiveness is enhanced by opsonisation of the parasite with the antibodies and complement activation leading to faster clearance of parasites from blood [20]. Function of neutrophils is to patrol body via bloodstream in quest of invading microbes. In this study, BMEE significantly evoked increase in the adhesion of neutrophils to the nylon fibers. It correlates with the process of margination of cells blood vessels [21]. The neutrophil adhesion was significantly increased by BMEE 400 mg/kg when compared to control, indicating possible immunostimulant effect.

Sepsis is a common complication of traumatic injury, developing in nearly one-half of all trauma patients. The early events of sepsis may trigger long term consequences such as immunosuppression and can lead to late mortality in one-fourth of survivors of severe sepsis [22]. The Cecal ligation and puncture induced abdominal sepsis model is associated with the presence of the pathogens such as *Escherichia coli*, *Proteus*

mirabilis, *Enterococcus cloacae*, *Alcaligenes faecalis* and it closely resembles the pathophysiology of human sepsis [23]. In cecal ligation and Puncture Induced Abdominal sepsis model, the survival rate of the animals after administration of BMEE was increased which indicates that this drug may be used to cure the multiple infections which occur as a result of immunosuppression due to sepsis.

CONCLUSION

Present investigation suggests that ethanolic extract of *Butea monosperma* leaves stimulates the non-specific immune response. It could be effective in treating infections caused by smaller parasites by exhibiting its phagocytic action as well as; could be used to take care of multiple infections caused as a result of immunosuppression. Further studies to elucidate the exact immunostimulatory mechanisms are in progress.

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Table 1: Effect of graded dose of orally administered (p.o.) *Butea monosperma* ethanolic extract (BMEE) on Phagocytic Index:

Gr. No.	Drug Treatment (mg/ kg, p.o.)	Phagocytic Index	% increase in Phagocytic Index
I	Control (1% CMC)	3.76±0.12	100 ± SEM
II	BMEE 100	5.54±0.16*	147.3± SEM
III	BMEE 200	6.66±0.66***	177.1± SEM
IV	BMEE 400	6.68±0.36***	177.7± SEM

Values are expressed as mean ± SEM (n=5); *p<0.05, ***p<0.001 vs control; Drug treated groups were compared with control group (I).

Table 2: Effect of *Butea monosperma* ethanolic extract (BMEE) on neutrophil activation by Neutrophil Adhesion Test:

Sr. No.	Groups (n=5)	% Neutrophil Adhesion (Mean ± S.E.M.)
I	Control	13.86 ± 1.32
II	BMEE 100	18.82 ± 2.07
III	BMEE 200	25.31 ± 7.17
IV	BMEE 400	39.76 ± 7.42*

Values are expressed as Mean ± S.E.M.; * = p<0.05. ; Control : 1% Sodium CMC, 10 ml/kg, p.o. Drug treated groups were compared with control group (I).

Table 3: Effect of graded doses of orally administered (p.o.) *Butea monosperma* ethanolic extract (BMEE) on survival rate after Cecal Ligation and Puncture induced abdominal sepsis:

Gr. No.	Drug Treatment (mg/kg, p.o.)	% Survival (After 12h)	% Survival (After 24h)	% Survival (After 48h)	% Survival (After 168h)
I	Sham Laparotomy (LAP) (1% CMC)	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.3 ± 6.7
II	Control-CLP (1% CMC)	60.0 ± 1.5	53.3 ± 6.7	6.7 ± 6.7	0 ± 0.0
III	BMEE 100 + CLP	73.3 ± 6.7	60.0 ± 11.5	26.7 ± 6.7	0 ± 0.0
IV	BMEE 200 + CLP	100.0 ± 0.0***	86.9 ± 6.7**	46.7 ± 6.7**	26.3 ± 6.7*
V	BMEE 400 + CLP	100.0 ± 0.0***	93.3 ± 6.7**	53.3 ± 11.5**	26.3 ± 6.7*

Values are expressed as mean ± SEM (n=15); *p<0.05, **p<0.01, ***p<0.001 vs control; Drug treated-CLP groups were compared with control-CLP group.

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