Original Article

ISSN Print 2231 – 3648
Online 2231 – 3656

ANTI-INFLAMMATORY EFFECT OF ETHYL ACETATE ACTIVE FRACTION OF *BUTEA MONOSPERMA* LEAVES

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Abstract

The present study was undertaken to assess the anti-inflammatory potential of the ethyl acetate active fractions of *Butea monosperma leaves* against Complete Freund's Adjuvant (CFA) induced inflammation in wistar rats. Three different ethyl acetate active fractions of *Butea monosperma leave* (50, 100 & 250 mg/kg) showed a significant dose-dependent protective effect against CFA-induced inflammation. At the dose of 250 mg/kg, the percentage inhibition of inflammation was 30.45, 35.54, 39.88 and 44.1 on day 4th, 8th, 14th and 21st day respectively and oral administration of E.A active fraction of *Butea monosperma* leaves for 21 days significantly increased($P<0.05$) the body weight in all treatment groups. These observations suggest possible therapeutic potential of the ethyl acetate active fractions of *Butea monosperma* leaves in inflammatory disorders like rheumatoid arthritis and other inflammatory diseases.

Keywords: Anti-inflammatory, *Butea monosperma*; ethyl acetate active fractions, Complete Freund's adjuvant.

Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Progressive destruction of the tissue would compromise the survival of the organism. However chronic inflammation can also lead to a host of diseases such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g. gall bladder carcinoma). It is for that reason that inflammation is closely regulated by the body.

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Inflammation can be classified as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Current therapies to relieve pain have some limitations such as, NSAIDS having GI irritation, opioids having dependency etc. Novel therapies for pain treatment are essential to overcome the adverse effects of existing therapies for pain treatment[1]. It is indeed a need of hour to treat the pain of specific pathologic origin such as cancer pain, neuropathic pain etc. The thirsts for new therapies to treat such painful conditions are alarming. Hence global scenario for new drug discovery is to develop new therapies to treat pain.

The study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy, in the search for analgesic drugs[2][3]. Different pharmacological activities of *Butea monosperma* are reported in last few years, although *Butea monosperma* has a reported use as folklore medicine but has not established through any scientific report. Present study is an attempt to prove the pharmacological evaluation for anti inflammatory activity of *Butea monosperma* leaves in rodents. This makes us to evaluate the analgesic activity of the ethyl acetate active fraction of *Butea monosperma* leaves.

Materials and Methods

Collection of plant material

Leaves of *Butea monosperma* were collected, and it was authenticated by Dr. Sri Ram Murthy, Department of Botany, Andhra Loyola College, Vijayawada. A voucher specimen was deposited. The plant materials were shade dried, coarsely powdered and used for extraction.

Procedure for extraction and isolation

Powdered leaves were soaked in ethyl acetate (E.A) for 72hrs and extracted using Soxhlet apparatus with intermittent shaking. The solution was filtered and the filtrate was concentrated under reduced pressure using rotary evaporator. All the filtrates were air dried. Ethyl acetate extract of *Butea monosperma* leaves was subjected to column chromatography over silica gel, Silica gel; 60-120 mesh, 1200 g, 68 column was prepared using hexane, eluent: hexane–ethyl acetate with increasing polarity (90:10–50:50) collecting 42 fractions of 500 ml each from leaves, which were combined to 12 major fractions for leaves on the basis of TLC analysis. One of the obtained fractions was evaluated for anti-inflammatory activity.

Drugs and chemicals

Test compound:

Isolated fraction of E.A extract of *Butea monosperma* leaves

Standard drug:

Prednisolone (10 mg/kg Body weight *P.O*)

Inducing agent:

Complete Freund 's adjuvant (0.1ml)

Animals

Studies were carried out using male wistar rats of 10-11 weeks. The animals were grouped in polyacrylic cages with not more than three animals per cage and maintained under standard laboratory conditions at temperature 25 ± 2°C with dark and light circle (12/12 hrs). The rats were acclimatized to laboratory condition at least for 7 days before the commencement of the experiment. Prior to and after treatment, the animals were fasted for 12hrs and 7hrs respectively. However, water was made available *ad libitum*. The study was approved by institutional animal ethical committee (Registration No. 1221-a/08/CPCSEA). This research was conducted in accordance with the internationally accepted principles for laboratory animals use and care [4].

Experimental induction of inflammation

On the 0th day, the basal paw volume of left hind paw of each animal was measured using mercury plethysmometer. On the 1st day all the animals except normal group were once anaesthetized. They were injected at the ankle joint of left hind paw i.e. through intra planetary route with 0.1 ml of Complete Freud's Adjuvant (Sigma Aldrich, USA) containing 0.1 mg of heat killed *Mycobacterium tuberculosis* cells in liquid paraffin and were allowed to recover to serve as control. Dosing with standard drug Prednisolone, the extracts were introduced on the same day i.e. on 1st day and continued for 21 days. Normal and disease control group rats received normal saline throughout study while the experimental groups of animals received respective treatment once daily by oral route. The DMSO 0.5%w/w was used as vehicle for suspending the E.A fractions. Paw volume was measured on 4th, 8th, 14th and 21st day of study period. The body weight of the animals was measured by digital balance to access the course of the disease at the initial day before induction and at the end of 21st day [5].

Statistical Analysis

The experimental results were statistically analyzed with analysis of variance (one-way ANOVA) followed by Dunnett's multiple comparison test. All results were expressed as mean ± standard error of the mean (S.E.M). P values< 0.05 were considered as significant.

Results

Body weight

The mean changes in body weights of all the animals were observed normal except in the disease control. Body weight was significantly decreased in the disease control group when compared with normal group. Oral administration of E.A active fraction of *Butea monosperma* leaves for 21 days significantly increased ($P<0.05$) the body weight in all treatment groups. (Ref: Table 2 and Figure 1)

Paw volume

The mean changes and percentage inhibition in Paw volumes of all the animals were shown in table 3 & 4. Paw volumes were significantly increased in the disease control group when compared with normal group. Oral administration of E.A active fractions of *Butea monosperma* leaves for 21 days significantly decreased ($P<0.05$) the Paw volumes in all treatment groups in a dose dependent manner when compared with the disease control group. (Ref: Table 3, 4 and Figure 2)

Paw thickness

The mean changes and percentage inhibition in Paw thickness of all the animals were shown in table 5 & 6. Paw thickness were significantly increased in the disease control group when compared with normal group. Oral administration of E.A active fractions of *Butea monosperma* leaves for 21 days significantly decreased ($P<0.05$) the Paw volumes in all treatment groups in a dose dependent manner when compared with the disease control group. (Ref: Table 5, 6 and Figure 3)

Animal Model

Table 01: Treatment schedule

S. No	Group	Treatment
1	Normal Control	Normal saline
2	Disease control	Normal saline
3	Test control-1(low dose)	Active fraction of *B.M* leaf (50 mg/kg B.wt *p.o*)
4	Test control-2(medium dose)	Active fraction of *B.M* leaf (100 mg/kg B.wt *p.o*)
5	Test control-3(high dose)	Active fraction of *B.M* leaf (250 mg/kg B.wt *p.o*)
6	Standard control	Prednisolone (10 mg/kg B.wt *p.o*)

Table 02: Changes in body weight in Adjuvant-induced arthritis in rats.

Treatment groups	Mean body weight(gm)		
	Before induction 0th day(gm)	On 21st day (gm)	Mean changes in body weight (gm)
Group 1	200.67 ± 5.75	226.83 ± 4.49	26.17 ± 6.97
Group 2	203.83 ± 7.14	204.00 ± 7.95	0.17 ± 1.94
Group 3	198.83 ± 6.71	218.00 ± 12.08	19.17 ± 8.89
Group 4	200.17 ± 11.69	218.83 ± 6.11	18.67 ± 11.79
Group 5	202.33 ± 13.25	225.17 ± 11.70	22.83 ± 5.91
Group 6	206.50 ± 10.11	232.33 ± 10.97	25.83 ± 9.02

Table 03: Mean changes in paw volume using plethysmometer in Adjuvant-induced arthritis in rats.

Treatment groups	Mean changes in paw volume ±SD			
	4th day	8th day	14th day	21st day
Group 1	1.29 ± 0.03	1.30 ± 0.04	1.31 ± 0.04	1.31 ± 0.04
Group 2	3.39 ± 0.13	3.49 ± 0.11	3.57 ± 0.13	3.67 ± 0.10
Group 3	3.24 ± 0.05*	3.20 ± 0.06**	3.16 ± 0.06**	3.16 ± 0.03**
Group 4	3.04 ± 0.08**	3.00 ± 0.07**	2.96 ± 0.07**	2.90 ± 0.05**
Group 5	2.75 ± 0.09**	2.71 ± 0.09**	2.67 ± 0.08**	2.63 ± 0.09**
Group 6	1.97 ± 0.02**	2.01 ± 0.04**	2.05 ± 0.04**	2.09 ± 0.05**

Table 04: Percentage inhibition of paw volume in Adjuvant-induced inflammation in rats.

Treatment groups	% inhibition of paw volume			
	4th day	8th day	14th day	21st day
Group 1	-	-	-	-
Group 2	-	-	-	-
Group 3	7.15	13.06	17.95	21.55
Group 4	16.61	22.48	27.15	32.44
Group 5	30.45	35.54	39.88	44.10
Group 6	67.33	67.35	67.03	66.93

Table 05: Mean changes in paw thickness using Vernier calipers in Adjuvant-induced inflammation in rats.

Treatment groups	Mean changes in paw thickness ±SD			
	4th day	8th day	14th day	21st day
Group 1	4.24 ± 0.04	4.25 ± 0.04	4.26 ± 0.04	4.26 ± 0.04
Group 2	10.34 ± 0.18	10.45 ± 0.18	10.60 ± 0.12	10.73 ± 0.11
Group 3	9.99 ± 0.26**	9.93 ± 0.26**	9.87 ± 0.26**	9.81 ± 0.27**
Group 4	9.37 ± 0.19**	9.19 ± 0.22**	9.00 ± 0.25**	8.90 ± 0.26**
Group 5	8.70 ± 0.23**	8.53 ± 0.21**	8.33 ± 0.23**	8.17 ± 0.22**
Group 6	6.83 ± 0.12**	6.64 ± 0.13**	6.44 ± 0.18**	6.29 ± 0.17**

Table 06: Percentage inhibition of paw thickness in Adjuvant-induced inflammation in rats.

Treatment groups	% inhibition of paw thickness			
	4th day	8th day	14th day	21st day
Group 1	-	-	-	-
Group 2	-	-	-	-
Group 3	6.07	8.67	11.79	14.48
Group 4	17.35	21.58	26.42	29.51
Group 5	28.17	32.25	37.01	40.72
Group 6	60.66	64.52	68.49	71.56

Group 1: Normal (Normal saline)

Group 2: Disease Control (Complete Friend's adjuvant 0.1ml)

Group 3: Ethyl acetate active fraction of *Butea monosperma* leaf extract (50 mg/kg P.O)

Group 4: Ethyl acetate active fraction of *Butea monosperma* leaf extract (100 mg/kg P.O)

Group 5: Ethyl acetate active fraction of *Butea monosperma* leaf extract (250 mg/kg P.O)

Group 6: Prednisolone (10 mg/kg P.O)

Values are expressed in Mean ± S.D

Significance of therapeutic level is indicated by *

Where * indicates **P <0.05,** ** indicates **P < 0.01** and *** indicates **P<0.001**

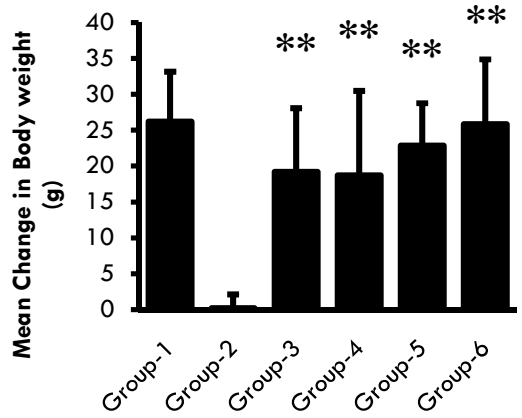

Figure 01: Effect of *Butea monosperma* on body weight in Adjuvant induced inflammation in rats

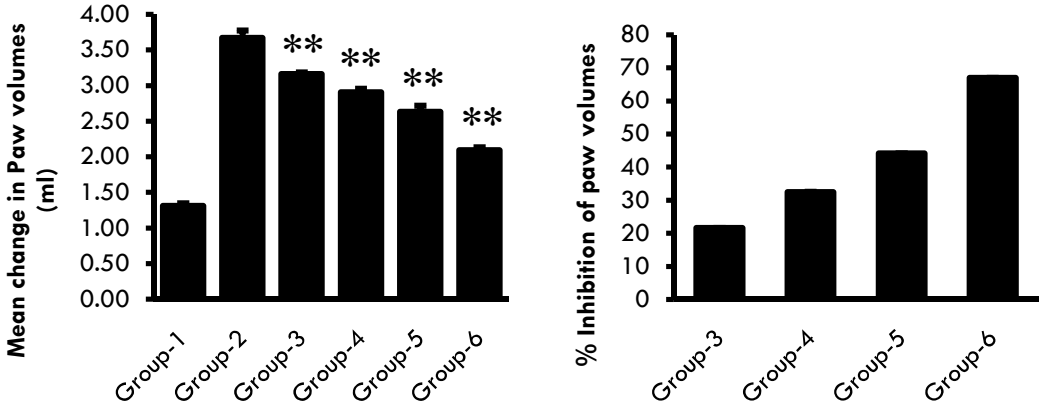

Figure 02: Effect of *Butea monosperma* on Mean changes and Percentage inhibition of Paw Volume in Adjuvant induced inflammation in rats

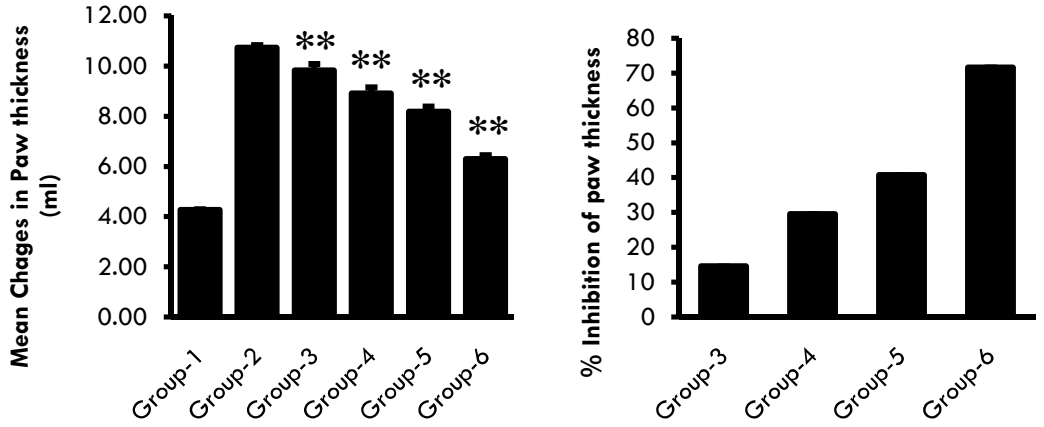

Figure 03: Effect of *Butea monosperma* on Mean changes and Percentage inhibition of Paw Thickness in Adjuvant induced inflammation in rats

Discussion

The results of the present study indicate that the E.A active fraction of *Butea monosperma* leaves exhibits anti-inflammatory effects in rats with Freund's Adjuvant-induced inflammation. The model of adjuvant induced inflammation in rats has been extensively used in the study of inflammatory processes[4]. Freund Adjuvant is an antigen solution emulsified in mineral oil that is used as an immune-potentiator. The Complete Freund's Adjuvant (CFA) is composed of inactivated and dried mycobacterium and is effective in stimulating cell mediated immunity and may lead to the potentiation of the production of certain immunoglobulins. Shortly after the administration of CFA into hind paw; pronounced swelling appears in the hind paw which persists for weeks (primary reaction). After few days, the contra lateral paw as well as front paw also becomes swollen and inflammatory nodules will be observed (delayed systemic response)[5]. In the present study, we observed that E.A fraction of *Butea monosperma* leaves could significantly inhibit the progression of the inflammation in treated animals. The inflammation continued to grow until day 21 after CFA injection[6]. In the animals treated with the E.A fraction of *Butea monosperma* leaves, the percentage of the inflammatory response was clearly reduced. Administration of 50, 100, 250 mg/kg of the E.A fraction of *Butea monosperma* leaves leads to a significant ($p<0.01$) decrease in the percentage of inflammation in dose dependent manner. The increased body weight during the treatment with Prednisolone (10 mg/kg B.wt *p.o*) and the E.A fraction of *Butea*

monosperma leaves as observed in this work may be due to the restoration of the absorption capacity of the intestine. The presence of phytoconstituents like flavonoids and polyphenols has been previously found to be responsible for anti-inflammatory activities in plants [7][8]. These constituents may be responsible for the anti-inflammatory activities observed in this study since they are present in the ethyl acetate extract of *Butea monosperma* leaves.

Conclusion

It was proved that the three different doses of *Butea monosperma* leaves (50, 100, 250 mg/kg B.wt *P*.O) showed significant dose dependent anti-inflammatory activity on CFA induced inflammation in wistar rats by normalizing the paw volume, paw thickness and body weight. The results were comparable with the reference drug Prednisolone (10 mg/kg B.wt *P*.O). This protective action may be attributed towards the presence of flavonoids and polyphenols. We would like to conclude that it is worthwhile to use *Butea monosperma* as drug and further studies should be initiated to establish exact mechanism of action and elaborative phytochemical investigations to find out the active constituents responsible for anti-inflammatory activity. These reports may serve as a foot step in the research of potent anti-inflammatory drug.

Acknowledgement

It is my privilege to express my gratitude to my highly learned supervisor of research, Rev.Fr.Dr.S.Emmaneul, Dept. of Botany, Loyola Academy, Secunderabad, for his unparalleled guidance, sustained interest, analytical suggestions, judicious advices and encouragement during the course of research and in the preparation of the thesis.

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