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Evaluation of Anti-Arthritis Activity of *Butea monosperma* Bark Extract on Albino Wistar Rats

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Mudit Pal, Sunil Kumar Shah*, Narendra Patel, C.K Tyagi

Department of Pharmacology, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, Madhya Pradesh, India.

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ABSTRACT

The Anti- Arthritis potential of the Methanolic extract of the bark of *Butea monosperma* has been evaluated using several experimental models in Wistar albino rats. Methanolic extract was then tested for the presence of various phytochemical constituents such as carbohydrates, flavonoids, steroids, tannins, glycosides, alkaloids, proteins and amino acids. The antioxidant assay was done by performing a DPPH assay and reducing power assay. Antioxidant activity was calculated in terms of % inhibition. The results obtained indicated a dose-dependent antioxidant activity, the higher the % inhibition the better the activity. To get an idea of dose dependency, the results were calculated with respect to concentrations ranging from 20 µg/ml to 100 µg/ml, results obtained ranged from 36.84211% to 69.62719%. The IC_{50} was found to be 43.52%. Reducing power assay was calculated there was the significant antioxidant activity of the extract was found when compared to the ascorbic acid. Acute oral toxicity was calculated by administrating three doses 5 mg/kg, 300 mg/kg and 2000 mg/kg by dividing animals into groups of three. No mortality was observed. Formalin-induced arthritis was checked and the extract was given to check the reduction in symptoms. Indomethacin was taken as control, extract was given two doses in two different groups 200 mg/kg and 400 mg/kg. The study was performed for 10days. % inhibition was noted and both the extracts had effect against arthritis but the extract given in dose 400 mg/kg gave better results on the day when compared with the 200 mg/kg extract dose.

1. INTRODUCTION:

Arthritis is a joint disorder featuring inflammation. Joint is an area of the body where two different bones meet. Joint functions to move the body parts connected by its bones. Arthritis literally means inflammation of one or more joints[1]. Arthritis usually begins in the small joints of the hands and the feet, spreading later to the larger joints. The inflamed joint lining or synovium extends and then erodes the articular cartilage and bone, causing joint deformity and progressive physical disability[2]. Due to the side effects and the high cost of conventionally used anti-inflammatory drugs, arthritic patients are increasingly using complementary alternative medicine (CAM) modalities of treatment. CAM, a system of healing that originated in India, involves using individually prescribed combinations of herbs, found that classic, individualized Ayurvedic approaches, methotrexate (a conventional medication frequently used to treat RA) or a combination of both are equally effective in reducing symptoms of RA[3-4].

Natural herbal remedies can effectively inhibit the inflammatory process safely and offer an alternative to synthetic an anti-inflammatory drug. Studies show that plant drugs were used by traditional practitioners to treat the inflammatory conditions. Nevertheless, on the basis of the results obtained from animal models of RA as well as the delineation of multiple immunological and molecular targets of the indicated herbal products, it is found that herbs like *Vitex leucoxylon, Calotropis procera, Clerodendron serratum, Curcuma longa, Azadirachta indica, Coriandrum sativum, Phyllanthus niruri, Hibiscus vitifolia, Ocimum sanctum* and herbal products inclusive of tea polyphenols, Celastrol, Triptolide, Curcumin and Bowellic acids as promising candidates for further preclinical and clinical trials in RA[5-6]. Agents derived from plants such as flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, can modulate the expression of pro-inflammatory signals and thus act against arthritis[7].

2. MATERIALS AND METHODS

Plant Material The bark of *Butea monosperma* was collected during July 2017 from PBRI Institute Bhopal, district, Madhya Pradesh, India. The authentication of the plant was done by the botanist. A herbarium of plants was submitted to the specimen library of Safia college of arts and science, peer gate Bhopal and authentify by Dr. Zia-Ul-Hassan, Professor, and head of the Department of Botany, Safia college of arts and science, peer gate Bhopal. The

herbarium specimen of *Butea monosperma Roxb* has been deposited at the college for further reference.

2.1 Preparation of Plant Extracts:

The bark was dried in the shed and coarsely powdered. The powder was extracted with methanol in a Soxhlet apparatus for 72h[8]. The ethanolic extract was evaporated in vacuo giving the residue (24%). The ethanolic extract obtained was suspended in distilled water in small amounts and was extracted successively and exhaustively with petroleum ether (60-80°C), benzene, chloroform, and acetone in the order of increasing polarity. The extract and fractions were concentrated in a rotary evaporator at reduced pressure[9-11].

2.2 Preliminary Phytochemical Analysis:

The methanolic extract and the fractions isolated from it were screened for the presence of various phytoconstituents. Adult Wistar strain rats (150 to 200 gm) were used for all the experiments in the present study[12-13]. The animals were maintained under standard husbandry conditions in the animal house of the institute (temperature 25 ± 2°C) in a natural light-dark cycle and fed with standard rodent diet and water ad libitum. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/c/09/CPCSEA).

2.3 Acute oral toxicity (OECD 423):

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step[14-15]. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses[16-17]. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight[18-20].

2.4 Formalin-induced Paw Edema:

Experimental arthritis was induced in rats according to the method proposed by Newbould (1963) with some modifications[21]. The left footpad of each rat was injected (Intra palmetry) with 0.1 ml of Formalin (2%). Rats in drug test groups were treated with Methanolic extracts of *Butea monosperma Roxb* (200, 400 mg: kg, p.o.) 24 h before the injection of 2 % formalin and then with daily treatment until 10 days after Formalin challenge. The animals in the control group received saline only[22]. Another group of rats was administered with indomethacin (10 mg/kg, p.o.) as a standard reference. The edema and inhibition rate was measured with the same method as described above.

$$\text{Inhibition rate (I) \%: } E_c\text{-}E_t/E_c$$

Where E_c = rate of the control group

E_t = rate of the treatment group

2.5 Biostatistical Interpretation:

All data are presented in Mean ±SD. data were analyzed by one way ANOVA followed by Bonferroni $P<0.05$ was considered as the level of significance (n=4).

3. RESULT AND DISCUSSION

3.1 Phytochemical analysis:

The dried bark of the plant *Butea monosperma* was taken for experimentation. They were exposed to extraction in methanol. The methanolic extract was then tested for the presence of various phytochemical constituents such as carbohydrates, flavonoids, steroids, tannins, glycosides, alkaloids, proteins and amino acids.

Table 1: Phytochemical analysis

Test	Petroleum ether extract	Methanolic Extract
Carbohydrates	+ ve	+ve
Protein and amino acid	- ve	+ve
Glycosides	- ve	+ve
Alkaloids	+ ve	+ve
Saponins	- ve	+ve
Flavonoids	+ ve	+ve
Triterpenoids and steroids	+ ve	+ve
Tannin and phenolic compounds	+ve	+ve
Percentage Yield	2.80	12.23

3.2 Quantitative Phytochemical Assay:

3.2.1 Total Phenolic Content:

Table 2: Total Phenolic content of Extracts

S. No.	Petroleum ether extract	Methanolic Extract
1	0.097	0.123
2	0.096	0.127
3	0.091	0.128
4	0.098	0.127
5	0.109	0.128
TPC	17.60 mg/gm equivalent to the Gallic acid	31.50 mg/gm equivalent to the Gallic acid

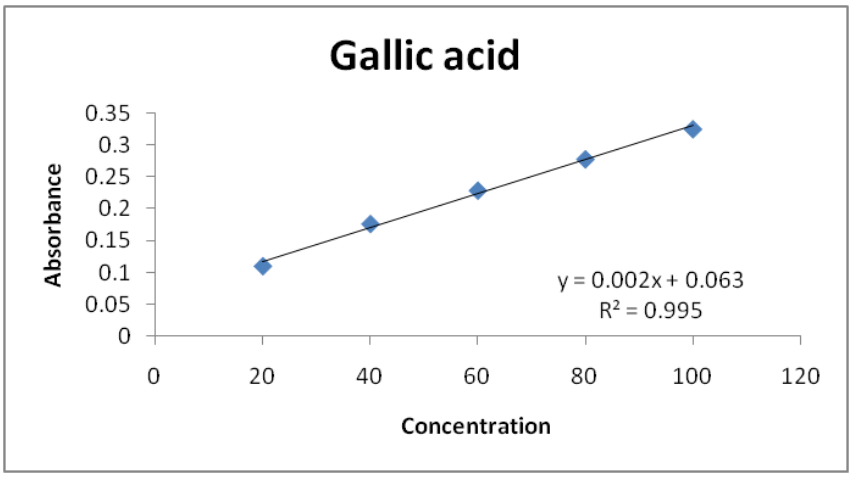

Figure 1: Standard curve of Gallic acid

3.2.2 Total Flavonoid content of Extracts:

Table 3: Total Flavonoid content of Extracts

Sr. No.	Petroleum ether Extract	Methanolic extract
1	0.112	0.152
2	0.11	0.149
3	0.098	0.148
4	0.112	0.145
5	0.112	0.143
TFC	16.00 mg/gm equivalent to Rutin	55.00 mg/gm equivalent to Rutin

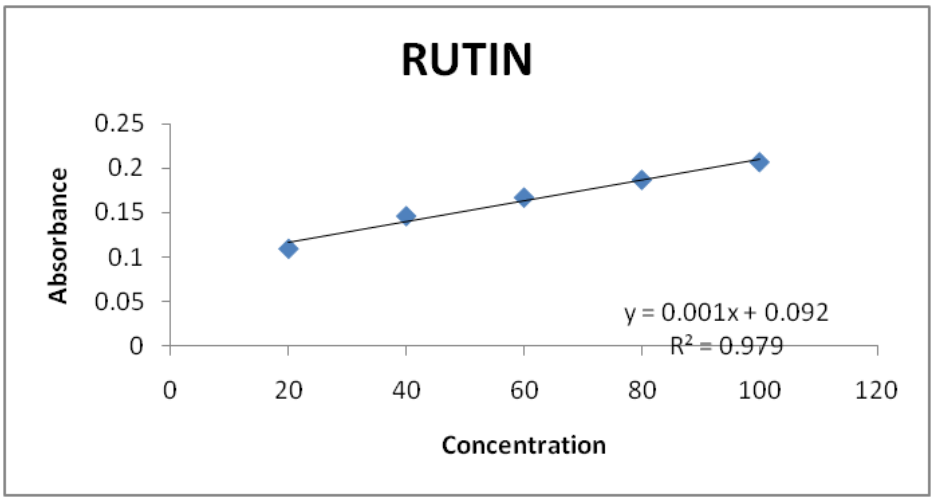

Figure 2: Standard curve of Rutin.

3.3 Antioxidant assay:

3.3.1. DPPH assay of Ascorbic acid:

Table 4: DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) assay of Ascorbic acid

Sr. No.	Concentration	% Inhibition of Ascorbic acid	IC_{50} Value
1	20 µg/ml	52.74123	11.54
2	40 µg/ml	56.35965	
3	60 µg/ml	61.51316	
4	80 µg/ml	68.9693	
5	100 µg/ml	71.71053	

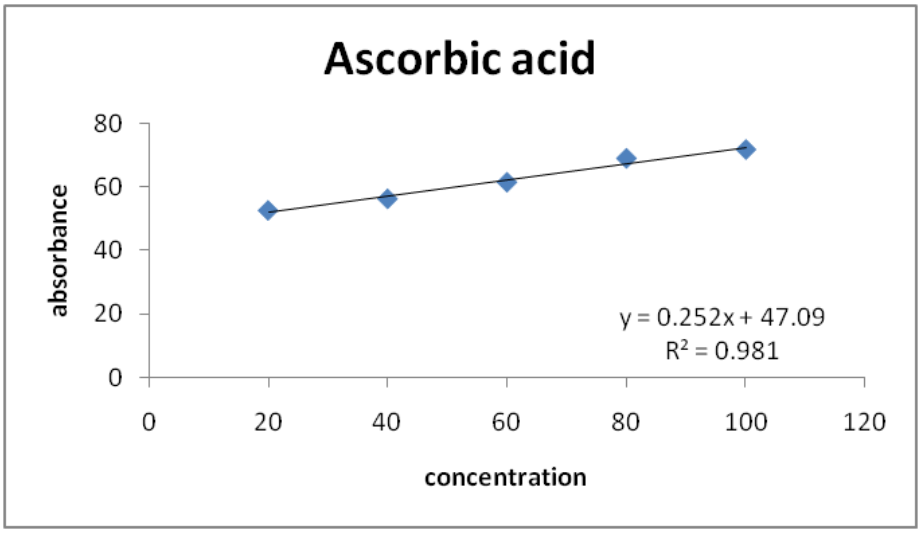

Figure 3: Standard curve of Ascorbic acid.

3.3.2. DPPH assay of Petroleum Ether Extract:

Table 5: DPPH assay of Petroleum Ether Extract

S. No.	Concentration	Percentage Inhibition of Petroleum ether extract	IC_{50} Value
1	20 µg/ml	36.84211	43.52
2	40 µg/ml	52.63158	
3	60 µg/ml	57.56579	
4	80 µg/ml	66.00877	
5	100 µg/ml	69.62719	

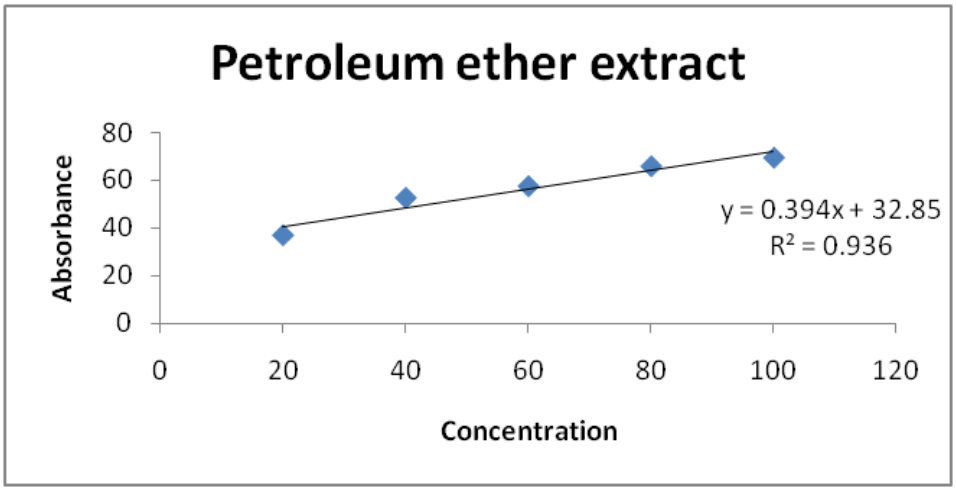

Figure 4: DPPH assay of Petroleum Ether Extract.

3.3.3. DPPH assay of Methanolic Extract:

Table 6: DPPH assay of Methanolic Extract

S. No.	Concentration	% Inhibition of Petroleum ether extract	IC_{50} Value
1	20 µg/ml	43.85965	36.15
2	40 µg/ml	48.13596	
3	60 µg/ml	64.14474	
4	80 µg/ml	68.42105	
5	100 µg/ml	76.42544	

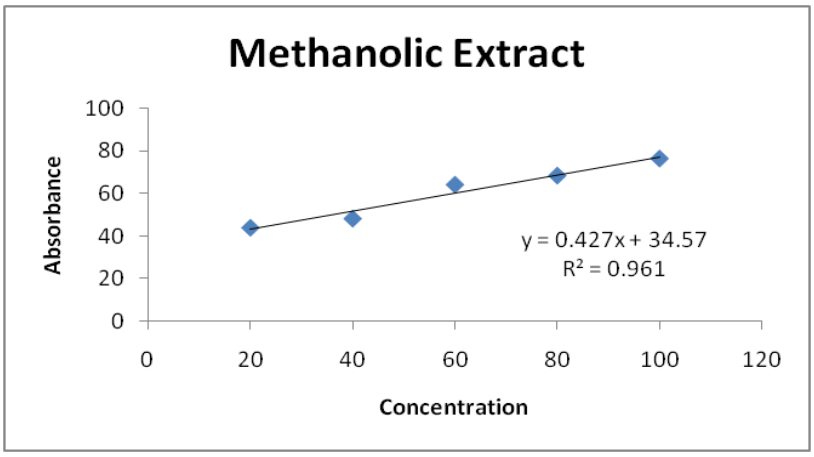

Figure 5: DPPH assay of Methanolic Extract.

3.3.4. Reducing Power assay of Ascorbic acid and extracts:

Table 7: Reducing Power assay of Ascorbic acid Pet. ether Extract and Methanolic extracts

S. No.	Ascorbic acid	Pet. ether Extract	Methanolic extract
1	0.987	0.113	0.234
2	1.032	0.127	0.245
3	1.145	0.132	0.267
4	1.159	0.148	0.281
5	1.196	0.167	0.303

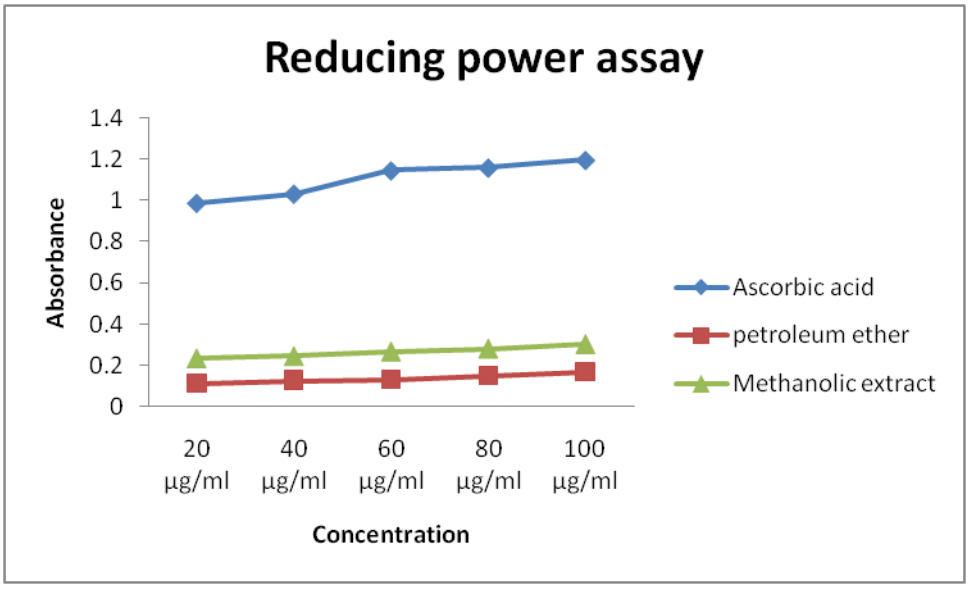

Figure 6: Reducing power assay.

3.4 Acute Oral Toxicity:

Table 8: Acute oral toxicity of Extract

S. No.	Groups	Observations/ Mortality
1	5 mg/kg Bodyweight	0/3
2	300 mg/kg Bodyweight	0/3
3	2000 mg/kg Bodyweight	0/3

3.4. Formalin-induced Arthritis activity (Inhibition rate):

Table 8: Formalin-induced Arthritis activity (Inhibition rate):

Sr. No.	Groups	1st day	3rd Day	10th Day
1.	Control	0.85±0.182	2.22±0.755	2.37±0.646
2.	Indomethacin (10 mg/kg bwt)	0.51±0.393 (25 %)	0.69±0.328 (68.91%)	0.53±0.367 (67.52%)
3.	Extract (200 mg/kg bwt)	0.90±0.503 (5.88%)	1.25±0.890 (43.69%)	1.07±0.517 (54.85%)
4.	Extract (400 mg/kg bwt)	0.83±0.638 (2.35%)	0.99±0.537 (55.40%)	0.76±0.898 (67.93%)

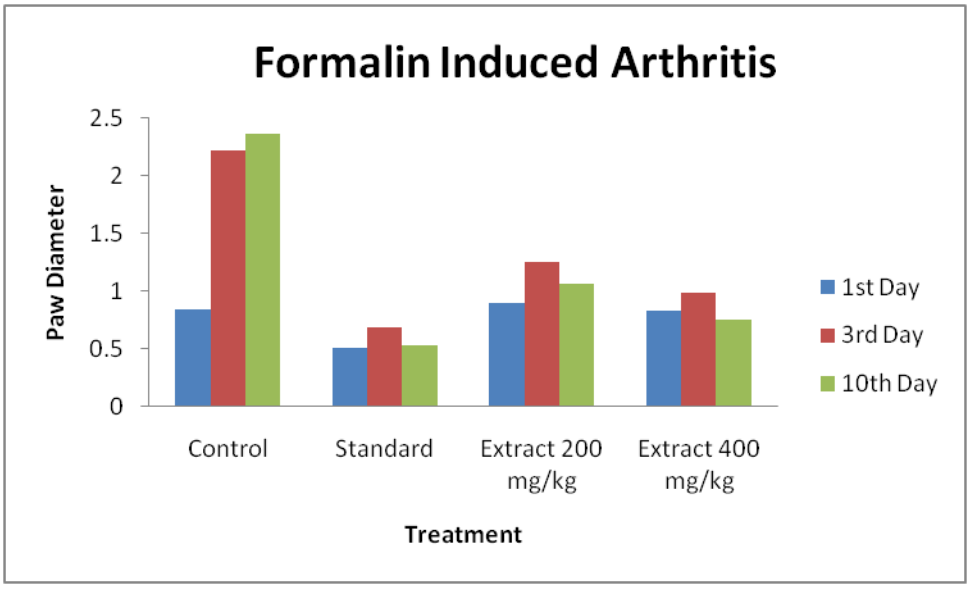

Figure 7: Formalin-induced Arthritis activity.

DISCUSSION:

For the presence of carbohydrates, the tests performed were a Molish test, Fehling's test, and Benedict's test. The extract gave positive results indicating the presence of carbohydrates. Proteins and amino acids were tested for their presence through Biuret test and Ninhydrine test. Both tests were performed and the results were the negative indicating absence of proteins in the sample.

Glycosides were tested through Borntrager test and Keller Killiani test. The extract gave negative results in both the tests indicating unavailability of glycosides in the extract. Alkaloids were tested through Mayer's test, Hager's test and Wagner's test. The extract gave positive results indicating the presence of alkaloids. Saponins were tested with the Froth test and the extract gave negative result indicating the absence of saponin. For flavonoids, the extract was tested for lead acetate and alkaline reagent test and the extract gave positive results in both the cases indicating the presence of flavonoids. The extract was tested for triterpenoids and steroids were and the observations indicated that these were absent in the sample as the extract gave negative results.

Tannin and phenolic were tested through ferric chloride, lead acetate, and gelatine test; the results indicated that these constituents are present in the extract.

The extract was then tested for quantitative estimation by calculating Total Phenolic Compound (TPC) and Total Flavonoid Content (TFC). Gallic acid and rutin were taken as the standard for quantitative estimation. The TPC in the extract was found to be 31.50 mg/g equivalent to gallic acid while the TFC was found to be 55.00 mg/g equivalent to rutin. The antioxidant assay was done by performing a DPPH assay and reducing power assay. Antioxidant activity was calculated in terms of % inhibition. The results obtained indicated a dose-dependent antioxidant activity, the higher the % inhibition the better the activity. To get an idea of dose dependency, the results were calculated with respect to concentrations ranging from 20 µg/ml to 100 µg/ml, results obtained ranged from 36.84211% to 69.62719%. The IC_{50} was found to be 43.52%. Reducing power assay was calculated there was the significant antioxidant activity of the extract was found when compared to the ascorbic acid. Acute oral toxicity was calculated by administrating three doses 5 mg/kg, 300 mg/kg and 2000 mg/kg by dividing animals into groups of three. No mortality was observed. Formalin-induced arthritis was checked and the extract was given to check the reduction in symptoms.

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4. CONCLUSION:

It has been concluded that both extract of *Butea monosperma* here possesses reasonable Anti-arthritic activity indicating that with rigorous research on this plant the therapeutically responsible compound can be isolated and then can be used in the treatment of the disease.

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