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Research Article

INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF WHOLE FLOWER EXTRACT OF *BUTEA MONOSPERMA* BY EX-VIVO AND IN-VITRO TECHNIQUES AND THEIR CORRELATION

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ABSTRACT

Bark, leaves, roots and flowers of *Butea monosperma* (Kesuda/Palash) are being investigated for antibacterial, anti-viral, anti-inflammatory activity. From the study of all the activities already done, the authors found out that anti-rash or anti-allergic activity is not investigated. Traditionally flowers of *Butea monosperma* (Kesuda/Palash) are used to treat rash on baby skin. These references lead us to take up this project. Dried flowers of *Butea monosperma* (Kesuda/Palash) were extracted using 80% ethanol using continuous soxhlet extraction apparatus. The extract was evaporated to dryness using rota evaporator followed by drying in the desiccator. This dry extract was treated as whole drug & tested for in–vitro and ex-vivo anti-inflammatory activity using albumin denaturation technique and Carrageenan induced rat paw edema method with Diclofenac sodium as standard. Successful in-vitro results were confirmed by animal study where anti-inflammatory activity was proved on rats. The dry extract was formulated into soothing Antihistaminic gel formulation. The activity of this formulation on animal was finally investigated which showed the promising results.

Keywords: *Butea monosperma,* ex-vivo, In-vitro anti-inflammatory activity

INTRODUCTION

Butea monosperma

Butea monosperma belongs to family fabeacae. It is a medium sized tree with 20-40 feet height and found in mountain region of India, Burma and few asian countries[1]. In literature, the *Butea monosperma* is known for several medicinal properties. The flowers are widely used internally in treatment of hepatic disorders, viral hepatitis, and diarrhoea [2]. The flowers have anticonvulsant, anti-hepatotoxic, anti-implantation, hypoglycaemic, astringent, diuretic aphrodisiac properties [3-4]. The flowers are good source of flavanoids[5-6]. The contents of flowers are butein, butrin, isobutrin, plastron, coreipsin and isocoreipsin. The roots are useful in treatment of night blindness, filariasis, piles, ulcers and tumors[7]. The stem and bark posses antifungal activity. The compounds isolated from stem and barks are stigmasterol, stigmasterol-βD-glucopyranoside, nonacosanoic acid, 3 α-hydxoxyeuph-25-ene and 2, 14-dihydroxy-11, 12-dimethyl-8-oxo-octadec-11- encyclohexane[8]. The literature survey revealed that less work is done on flowers of *Butea monosperma.* The present work is a study about *in* vitro and ex-vivo Anti inflammatory activity of ethanolic extract of flowers of *Butea monosperma*. Inflammation is tissue reaction to infection or irritation to foreign substances and is an integral part of host defence mechanisms.

Inflammation

Inflammation was recognized as a simple allergic reaction for many decades[9]. There are four cardinal signs of inflammatory conditions and they are redness, heat, swelling, and pain.

Fig. 1: Flowers of *Butea monosperma*

There are several tissue factors or mechanisms that are known to implicate in the inflammatory reaction such as histamine, bradikinin and prostaglandins[10]. Inflammation is homeostatic phenomenon. The present work was done to screen anti inflammatory activity of plant to throw more light in this direction.

MATERIALS AND METHODS

Collection of plant materials[11]

The plant material of *Butea monosperma* flowers used for investigation was collected from Ayurvedic shop and was identified and authenticated by Botanical survey of India Pune. The voucher specimen of the plant has been deposited in the herbarium collection of the department.

Preparation of plant extract[11-12]

The flowers of *Butea monosperma* were shade-dried and powdered to a coarse powder and passed through a 40-mesh sieve, this powder was subjected to continuous hot extraction in Soxhlet apparatus with petroleum ether (60% v/v) The marc left after petroleum ether extraction was dried and extracted with ethanol (80% v/v) in Soxhlet apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent had been removed to give an extract sample with a yield of 8.5% w/w. Ethanolic extract of *Butea monosperma* was used for further studies.

Formulation of topical preparation[13]

Herbal gel was prepared using Sodium carboxy methyl cellulose as a gelling agent in 1% w/w concentration with deionized water as a vehicle under mechanical stirrer. Flower extract of *Butea monosperma* (5% w/w) was added to the gel (1% Sodium Carboxy methyl cellulose) and stirred for sufficient time for homogeneous mixing of extracts in gel base. Prepared gel was filled in collapsible tubes and stored at cool and dry place.

Ex Vivo antiinflammatory activity[12]

Animals

Male Wistar albino rats of either sex, weighing 150–200 g were subjected to carrageenan rat paw edema induced inflammation tests (n=5, in each group). All the animals were housed in standard environmental conditions and fed with standard rodent diet with water *ad libitum*. Four groups (Negative control, Positive control, Test and Standard) of five animals in each group were used for experiment. The animals had free access to food and water and

maintained under 12:12 hr light and dark cycle. All experiments were carried out during day time from 09.00 to 17.00 hr. The institutional animal ethical committee approved the protocol and care of animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of animal welfare, Govt of India.

Carrageenan induced rat paw edema

Animals (Albino Wister Rats) were fasted for 24 hrs before the experiment with water *ad libitum*. The rats were divided into six groups containing five rats in each group. Acute inflammation was induced[11] by o.1 ml of 1.0% of carrageenan in normal saline (0.9%w/v NaCl).This solution was injected to the sub planter region of left hind paw of rats. Rats of the control groups received the plain gel base and 0.2 g 1% diclofenac gel applied in the same manner was used as a standard. The 5% gel containing extract was applied to the paws of the test group of five rats, 1 hr before carrageenan injection. Paw volume was measured immediately after carrageenan injection and at 1, 2, 3 and 4 hrs intervals by using a Digital plethysmometer (PLM-01 plus ORCHID™Scientific).

Different groups treated were as follows:

Group 1: carrageenan (1.0% 1ml) + vehicle

Group 2: carrageenan (1.0% 1ml) + Diclofenac (1%)

Group 3: carrageenan (1.0% 1ml) + ethanolic extract (5%)

Group 4: carrageenan (1.0% 1ml)

The paw volume was measured at half, 1, 2, 3, 4 hrs after carrageenan injection using plethysmometer. The anti inflammatory activity was evaluated based on the ratio of the changes in paw diameter in treated and untreated groups as per the formula given below:

Anti inflammatory activity (%) = [1- (V_t / V_c)] x 100

V_t and V_c are edema volumes in drug treated and control group respectively

In vitro- Anti-inflammatory screening[14]

The *Butea monosperma* flower extract was screened for anti-inflammatory activity using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification[15]. The standard drug and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5%. Test solution (1 ml, 100 µg/ml) was mixed with 1 ml of 1% albumin solution in phosphate buffer saline and incubated at 27 ± 1° C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1° C in a water bath for 10 min[16]. After cooling, the turbidity was measured at 660 nm with UV visible spectrophotometer. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was calculated. Diclofenac sodium was used as standard drug. The percentage of inhibition was calculated using the following formula.

% Inhibition of denaturation= [(Vt / Vc) - 1] × 100

Where, V_t = mean absorbance of test compound,

V_c = mean absorbance of control.

RESULTS

It is inferred from the Table 1 that *Butea monosperma* flower extract (2a-e), has shown significant in vitro anti-inflammatory activity determined using albumin denaturation method. The diclofenac sodium was used as standard drug. The percentage of inhibition was calculated using the above formula.

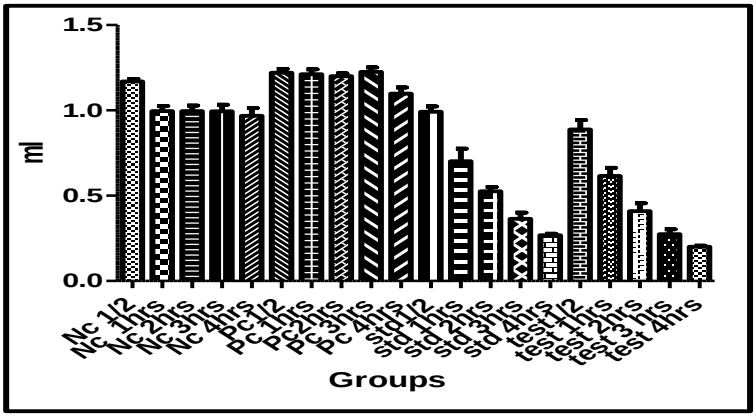
Table 1: Screening of in-vitro anti-inflammatory activity

Compound	Absorbance value	Inhibition of denaturation (in %)
Control	0.0110 + 0.0003	____
2a	0.0209 + 0.0005	90.12
2b	0.0169 + 0.0006	53.63
2c	0.0194 + 0.0002	76.36
2d	0.0138 + 0.0002	25.45
2e	0.0200 + 0.0002	81.81
Diclofenac Na	0.0240 + 0.0004	118.18

Table 2: Effect of topical administration of *Butea monosperma* flower extract gel using carrageenan-induced rat paw edema method

Time interval	30 Min	60 Min	120 Min	180 Min	240 min
Negative Control	1.17±0.014,	0.996±0.029,	0.996±0.032,	0.994±0.039,	0.966±0.046
Positive control	1.20±0.04	1.212±0.028	1.200±0.017	1.22±0.026	1.098±0.036
Standard	0.992±0.03	0.702±0.07	0.526±0.02	0.364±0.03	0.268±0.08
Butea monosperma Flower extract	0.888±0.05	0.616±0.04	0.41±0.045	0.282±0.02	0.200±0.07
% Inhibition of edema (Standard)	15.214	29.519	47.189	63.381	72.22
% Inhibition of edema(*Butea monosperma* Flower extract)	24.11	38.153	58.84	71.63	79.92

Significant P<0.005



Graph 1: Anti-inflammatory activity of 5% *Butea monosperma* flower extract using rat paw edema method result graphical form

The results of anti inflammatory activity after topical application of ethanolic extract are reported in Table 1. Statistical analysis showed that the inhibition of edema preparations containing extract were significantly different from control group at all the concentrations tested. The results showed that the anti inflammatory effect of the formulation containing 5% of the ethanolic extract was similar to the effect of gel containing 1% of Diclofenac sodium

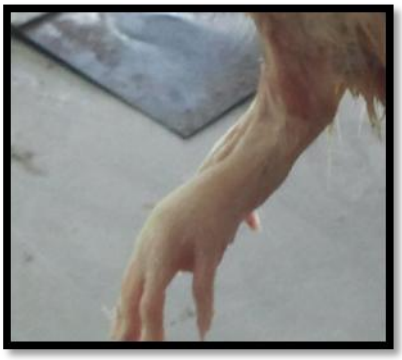


Fig. 2: Carrragenan (1%) induced paw edema (positive control)

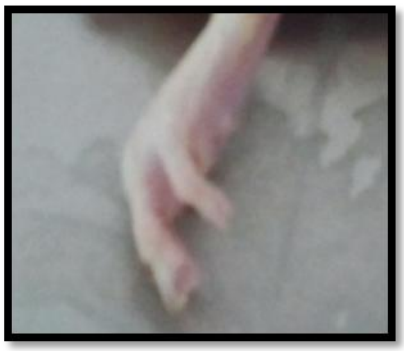


Fig. 3: Carrragenan (1%) induced paw edema (treated group 5% extract)

Statistical Analysis

ANOVA followed Student-Newman-Keuls test was used to determine significant differences between groups and $P < 0.005$ was considered significant.

DISCUSSION

The anti-inflammatory activities after topical administration of Butea monosperma extract gel formulations were studied, using the standard experimental model of acute inflammation. Moreover, the experimental model exhibits a high degree of reproducibility. The results of in vitro anti-inflammatory activity by albumin denaturation method of extract are reported in Table 1 where as results of ex-vivo anti-inflammatory activity after topical application of extract gel are reported in Table 2. Statistical analysis showed that the edema inhibition by preparation containing extract is significantly more from control group The results showed that the anti-inflammatory effect of the formulation containing 5% of the extract gel (1% Na CMC) was equivalent to the effect of standard Diclofenac gel formulation. From these overall results, we can conclude that topical gel preparation containing at least 5 % of extract possesses anti-inflammatory effect which can be useful for the treatment of local inflammation. The data presented in this study demonstrated that the ethanolic dry flower extract of *Butea monosperma* possess significant topical anti-inflammatory properties, supporting their traditional use for the treatment of rash on skin

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