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EFFECT OF METHANOLIC EXTRACT OF THE LEAVES OF CALOTROPIS GIGANTEA R.BR. ON LEUKOCYTE AND NEUTROPHIL MIGRATION

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Abstract

Calotropis gigantea R.Br. is a perennial under-shrub found chiefly in wastelands throughout India. It has been reported as a traditional folkloric medicine for a variety of ailments. Calotropis gigantea belongs to family Asclepiadaceae. The leaves of Calotropis gigantea are traditionally used in the treatment of paralysis, arthralegia, swellings, and intermittent fevers. The aim of the present study is to evaluate the anti-inflammatory activity of the methanolic extract of the leaves of Calotropis gigantea following in-vitro method by evaluating leukocyte and neutrophil infiltration. The study was carried out using the doses of 100, 200 and 300mg/kg p.o. and indomethacin 10mg/kg p.o. as standard. The pharmacological screening of the extract showed significant dose-dependent inhibition in leukocyte and neutrophil count which confirms its anti-inflammatory activity.

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Introduction

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluids and blood cells. Though it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain, or aggravate many diseases[1]. However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use[2]. Hence, the development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

The leaves of Calotropis gigantea have been used in Indian folk medicine to treat various inflammatory conditions. Traditionally Calotropis gigantea is used alone or with other medicinals to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhoea. According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and anthelmintic. The dried root bark is a substitute for ipecacuanha and also used as febrifuge, anthelmintic, depurative, expectorant, and laxative. The powdered root is used in asthama, bronchitis, and dyspepsia. The flowers are bitter, digestive, astringent, stomachic, anthelmintic, and tonic[3-7].

Calotropins D_1 and D_2 have been isolated, crystallized and studied from Calotropis gigantea[8]. The new oxiopregnane-

oligoglycosides named calotropis A and B have been isolated from the root of Calotropis gigantea and their chemical structure have been elucidated by chemical and spectroscopy methods[9]. The cytotoxic principles of *'Akond mul'* (Root of Calotropis gigantea) cardenoloids glycosides, calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside were obtained as the cytotoxic principles[10].

Materials and methods

Collection of Plant Material

The leaves of *Calotropis gigantea* were collected at Bundelkhand University, Jhansi, Uttar Pradesh, India. The leaves were taxonomically identified by an experienced taxonomist at National Botanical Research Institute (NBRI) Uttar Pradesh, India. A voucher specimen has been preserved in their laboratory.

Preparation of plant extraction

The collected leaves of *Calotropis gigantea* were shade dried and reduced to coarse powder using a mechanical grinder. The powdered material of the leaves was exhaustively extracted with methanol under the maceration process. The macerated mixture was filtered and evaporated to yield a green solid extract. The extract was stored in a desiccator and dilutions of the extract were made in 2% gum acacia for pharmacological studies.

Animals

Adult male mice (20-25 g) were used to study the anti-inflammatory activity. The animals (six per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27° C ± 2° C), with access to food and water ad libitum. The experiment was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the Institutional Animal Ethical Committee (IAEC) approved all the procedures. Experimental studies were undertaken according to their rules and regulations [11].

Acute Toxicity Study

Acute oral toxicity study was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No. 425 [12].

Carrageenan-induced Peritonitis

Inflammation was induced by the modified method of Griswold et al. 1987 [13]. Male Swiss albino mice weighing 20-25 g were divided into five groups (n = 6). Group 1 served as control and treated with 2% gum acacia. Group 2 served as standard and was dosed with indomethacin (10 mg/kg, p.o.) and group 3-5 were administered with Calotropis gigantea methanolic extract at the doses of 100, 200, 300 mg/kg, p.o., respectively. The standard drug and extract doses were administered orally 1 hour prior to the induction of peritonitis. After 1 hour, carrageenan (0.25 ml, 0.75% w/v in saline) was injected intraperitonially. Four hours later, the animals were sacrificed by cervical dislocation and 2 ml of Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count was determined in a Neubauer's chamber and the differential cell count was determined [14-15]. The percentage of leukocyte inhibition was calculated using the following formula:

% of leukocyte inhibition (% L. I) = (1 – T/C)X100

Where 'T' represents the leukocyte count of the treated group and 'C' represents the leukocyte count of the treated control group. Inhibition of neutrophil migration was calculated by the following equation:

Inhibition of neutrophil migration = 100 – {(N T / N C) X 1 0 0 }
here NT = neutrophil counts of treated groups and NC = neutrophil counts of control groups.

Statistical analysis

The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by Dunnett's t-test. P value of <0.05 was considered statistically significant.

Results

Test for acute toxicity

In the acute toxicity study, no mortality was observed during the 24 hr period at the doses

tested and the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxia, diarrhea, or increased diuresis and salivation.

Carrageenan-induced pleurisy

The *Calotropis gigantea* methanolic extract inhibited peritoneal leukocyte migration at the rate of 35.2, 52.5 and 64.4% at the dose of 100, 200, and 300 mg/kg, respectively. Whereas the inhibition produced by indomethacin (10 mg/kg) was found to be 67.6%carrageenan-induced peritonitis model as shown in table. The inhibition of neutrophils infiltration of *Calotropis gigantea* methanolic extract was 29.3, 51.4 and 62.7%, respectively, whereas indomethacin shows 63.2%.

Table : Effect of methanolic extract of Calotropis gigantea leaves on leukocytes migration and neutrophils migration in peritoneal exudation in carrageenan-induced mice

Group	Dose (mg/kg)	Leukocytes	% Inhibition of Leukocyte migration	Neutrophils (105/ml)	% Inhibition of Neutrophil migration
Control	-	(105/ml) 4.02±0.06	-	2.32±0.11	-
Indomethacin	10	1.12±0.11**	67.6	0.76±0.02**	63.2
Calotropis gigantea	100	2.32±0.02**	35.2	1.52±0.04**	29.3
Calotropis gigantea	200	1.52±0.07**	52.5	1.11±0.05**	51.4
Calotropis gigantean	300	1.21±0.03**	64.4	0.79±0.01**	62.7

Values are mean ±S.E.M. (n=6),

** Experimental groups were compared with control ($p<0.01$).

DISCUSSION

Carrageenan-induced pleurisy was taken as a prototype of exudative phase of acute inflammation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan-induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin [16-17].

Intraperitonial injection of carrageenan leads to inflammation of the peritoneum resulting from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorphonuclear cells by a variety of processes including adhesion and cell mobility [18-20]. Leukocyte aggregation and neutrophil migration are the fundamental events during inflammation. These cells release due to the defense process of our body to counter inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility.

Conclusion

From the above discussion, the methanolic extract from the leaves of *Calotropis gigantea* exhibited significant anti-inflammatory activity by inhibiting leukocyte and neutrophil infiltration. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities. These results support the traditional use of *Calotropis gigantea* in some inflammatory conditions and the delayed phase is sustained by the leucotrienes and prostaglandins. The present research work will enhance the confidence amongst its users to use its leaves in inflammatory disorders.

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