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Research Article

Anti-inflammatory Activity of *Calotropis gigantea* Linn. Leaves Extract on In-vitro Models.

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ABSTRACT

The leaves of *Calotropis gigantea* are traditionally used to cure cancer[1], intermittent fever, paralyzed part of body, painful joints, swelling, heals wounds. Also used as an antispasmodic, antiasthmatic, externally used for piles, boils, ulcers, scabies, eczema, leprosy[2]**.** It is having promising anti-inflammatory activity[3]. However, its anti – inflammatory activity is not scientifically documented. Hence, the present study was under taken to evaluate anti–inflammatory activity of *Calotropis gigantea* leaves extract using in – vitro method. *Calotropis gigantea* leaves were subjected to successive extraction[4-5] with petroleum ether (60-80^0), chloroform, ethyl acetate, n-butanol and ethanol and Distilled water and the extract was screened for in vitro anti – inflammatory activity by using inhibition of albumin denaturation technique[6] which was studied according to Muzushima and Kabayashi[7] with slight modification. Ibuprofen (100mg/kg) was used as standard reference drug. The % inhibition of denaturation produced by ethanolic extract of *Calotropis gigantea* leaves was comparable with that produced by Ibuprofen (85.71%) which indicates that *Calotropis gigantea* leaves extract possess significant anti – inflammatory activity.

Keywords: Calotropis gigantean, anti-inflammatory

INTRODUCTION

Calotropis gigantean (Asclepiadaeceae) whole plant found allover in India up to an altitude of 900m is including the Andaman. Also found in dry waste places commonly known as mudar in English. Roots are externally whitish grey in colour. Transverse section of mature root shows the cork zone. Composed of 30-50 or rows of polyhedral to nearly cubical thin walled cells .Very small

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sized cubical crystals are found in the inner row. Phellogen is distinct .Cortex is comparatively narrow of few rows of cubical, rectangular or oblong-thin walled cells, most of which are filled with numerous starch grains. Phloem is abroad zone consisting of number of broad radial bands of thin walled cell traversed by very narrow strips of medullary rays. Laticiferous cells and crystal of calcium oxalate are present. Cambium is distinct. Leaves are freshly, obviate, oblong, apex acute, rarely rounded, base cordite, 6-20 Cm long and 3-8Cm wide, glaucous green, smooth above, cottony below. Petioles 0.3-2 Cm long. The stem is woody with yellowish white bark, young stem and branches covered with soft, loosely apprised, whitish, waxy or sometime powdery pubescence. While fruits are single or paired, turgid, recovered, 7 – 10 Cm long**.** Seeds are numerous, broadly ovate, flattened brown in colour 2.5 -3.2 Cm long, including the white tuft of silky hair and pointed end. Flowers with lilac, pale rose or purple, rarely light greenish – yellow or white, inodorous, with spreading reflexed corolla lobes, borne in axillary pedunculate corymbs. Flower almost through out the year but most commonly from November to March in central India.

MATERIAL AND METHODS

Plant Material: About 2 Kg of leaves of *Calotropis gigantea* were collected from local area of Chopda and were positively identified with the authentic sources.

Drying & size reduction: The freshly collected leaves of *Calotropis gigantea* were shade dried under normal environmental conditions and then subjected for size reduction to coarse powder.

Preparation of extract: In the present study, the fresh leaves of *Calotropis gigantea* were collected and shade dried. The dried material was reduced to coarse powder in a mechanical grinder and pass through sieve No. 40 to obtain about 1 kg powder of desired particle size. About 1 kg of powdered material was subjected to successive extraction with petroleum ether ($60\text{-}80^0$), chloroform, ethyl acetate, n-butanol and ethanol and Distilled water. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction, the solvent was distilled off and the extract was concentrated at low temperature. The percentage yield of petroleum ether ($60\text{-}80^0$), chloroform, ethyl acetate, n-butanol and ethanol, distilled water extract was recorded as under. The crude extract obtained was subjected to preliminary phytochemical investigation[7] of various phytoconstituents.

Preparation of Test sample

For in – vitro testing, the extract was prepared by dissolving in dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4) to make final 2.5% concentration.

Drugs and Chemicals

Ibuprofen was used as reference standard while no drug was added as control.

Table No. 1: Results of Percentage Yield of Extracts of Whole Plant

Sr. No.	Extract	Weight of Residue	%age Yield
1	Petroleum-ether	35 gm	5%
2	Chloroform	10 gm	1.5%
3	Ethyl acetate	8 gm	1.27%
4	n-Butanol	7 gm	1.12%
5	Ethanol	15 gm	2.45%
6	Distilled Water	66 gm	11 %

TABLE 2: In-Vitro Anti – inflammatory activity of *Calotropis gigantea*

Compds	Dose (mg / kg)	Absorbance value (Mean ± SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098 ± 0.007	----
Standard (Ibuprofen)	100mg/kg	0.182 ± 0.002	85.71
Petroleum ether extract	200mg/kg	0.151 ± 0.001	54.08
Chloroform extract	200mg/kg	0.141 ± 0.003	43.87
Ethyl acetate extract	200mg/kg	0.124 ± 0.006	26.53
n-Butanol	200mg/kg	0.167 ± 0.009	70.40
Ethanol	200mg/kg	0.175 ± 0.008	78.57
Distilled Water	200mg/kg	0.160 ± 0.003	63.26

Study of anti – inflammatory activity (In – vitro models)

Calotropis gigantea leaves extract was screened for anti – inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification at the doses of 200 mg/kg. The standard drug and test compounds were dissolved in minimum quantity of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). Final

concentration of DMF in all solutions was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1ml of 1mM albumin solution in phosphate buffer and incubated at 27 ^{o}c $\pm$ 1 ^{o}c in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV – Visible Spectrophotometer SL – 159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken.

Statistical Analysis

The percentage inhibition of denaturation was calculated by using following formula.

$$\% \text{ of Inhibition} = 100 \text{ X } [Vt / Vc - 1]$$

Where,

Vt = Mean absorbance of test sample

Vc = Mean absorbance of control

RESULT

The results of anti – inflammatory effect of *Calotropis gigantea* are showed in Table – 2. Significant activity was shown by *Calotropis gigantea* doses as compared to control. But the 200 mg / kg dose of ethanolic extract *Calotropis gigantea* showed maximum anti – inflammatory effect and was comparable to that produced by 100mg / kg of Ibuprofen.

DISCUSSION

The ethanolic extract *Calotropis gigantea* showed significant anti – inflammatory activity, suggesting that it predominantly inhibits the release of inflammatory mediators. However, animal study and other studies are necessary to identify and isolate the active constituents responsible for its anti –inflammatory activity and also there is a need to elucidate its mechanism/s of anti – inflammatory action.

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