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Original Research Article

Evaluation of effect of *Calotropis gigantea* root bark in some animal models of *Wister albino* rats

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ABSTRACT

Background: *Calotropis gigantea* is a well documented traditional medicinal plant useful for many disease conditions. The present study deals with the effect of alcoholic extract of roots bark of *Calotropis gigantean* in some animal models.

Methods: Acute anti-inflammatory activity of root extract was assessed on carrageenin induced paw edema and chronic antiinflammatory activity on cotton pellet granuloma formation in rats and compared with standard diclofenac sodium. Antiasthmatic activity was assessed in histamine and acetylcholine induced bronchoconstriction in guinea pigs.

Results: 100 and 200 mg/kg p.o. root extract of CG showed significant anti-inflammatory and bronchodilator activity. Antiinflammatory activity was higher than Diclofenac Sodium 50mg/kg. The combination of low dose diclofenac sodium 25mg/kg potentiated the effect. Inhibitory effect on synthesis and release of various inflammatory mediators might contribute to both of these actions. Steroid like phytoconstituents might be responsible for these effects. Further research is needed to understand the exact mechanism behind these effects.

Conclusions: In the present study *Calotropis gigantea* root bark exhibited anti-inflammatory activity in animal models.

Keywords: Antiasthmatic, Bronchoconstriction, *Calotropis gigantea*, Anti-inflammatory

INTRODUCTION

From ancient time's plants remain valuable source medicinal compounds for pharmaceutical industry. Many clinically applicable novel pharmacological drugs are derived from plants. Plants synthesize chemicals to combat diseases and predators. *Calotropis gigantea* (CG) (species-Arka, family-*Apocynaceae asclepiadaceae*) is a roadside perennial shrub with lavender flowers and *Calotropis provera* is white flowered plant. Both have nearly similar effects. It is known world over as crown flower or giant milk weed. It is well documented medicinal plant well known to many cultures. It displayed number of pharmacological activities including

analgesic, anti-inflammatory, hepatoprotective activity, antifungal, antibacterial activity, anticoagulant, aphrodisiac, free radical scavenging activity, activity in neurological diseases like epilepsy, infertility etc.^{1,2} The aerial and underground parts of the plant had been traditionally used by ayurveda physicians for many health ailments including DM, bronchial asthma, rheumatoid arthritis, tumors, ulcers, leprosy, leucoderma, wound healing, piles, migraine, snake poisoning, liver and spleen diseases.³⁻⁸ The plant is known by various names in India, called Arka in Sanskrit, Madar in Hindi, Rui in Marathi. Its flowers are used to treat asthma, cough, inflammation.^{9,10} latex (milk) for ring worm infection, skin eruption, arthritis, wound healing, piles, scorpion

bites.¹¹ Root bark for antispasmodic, laxative, anthelmintic effects.^{12,13} Leaves are used for arthralgia and swelling locally in diarrhoea dysentery, gaseous dissention, candidiasis, bacterial infections.^{14,15} It is also used as analgesic in toothache, earache, sprains, stiff joint.¹⁴ The phytochemicals detected in the plant and mentioned in literature consists of cardiac glycosides, flavonoids, terpenoids, alkaloids, saponins, steroids, tannins, calotropin, calotropiol, cyanidin, Pregnanes, procesterol etc.^{8,17,18} The plant is toxic hence minute doses are used internally, most traditional uses are local. Accidental entry of Milky latex sap into eyes is known to cause keratoconjunctivitis and reversible vision loss.¹⁹ Scientific studies to validate the above effects are limited. Hence present investigation was planned to study antiasthmatic and anti-inflammatory activity of *Calotropis gigantea* root bark in animals.

METHODS

The plant roots were collected, dried in shed, and the bark grounded to powder. Dried powdered drug was then extracted with 90% ethanol. The solvent was removed by evaporation and dried extract was obtained. Albino male Wistar rats weighing (150-200 gm) were used. Animals were maintained under standard laboratory conditions (12 hours light: temperature 30±2°C) with access to food and water ad libitum. Experiments were carried out according to CPCSEA guidelines and after IAEC approval. No toxicity reported up to 1000mg/kg of AECG. Hence 100 and 200 mg/kg doses were selected for present study. Chemicals utilized were of analytical grade. Tab diclofenac sodium 50mg (Medico remedies limited) was procured locally. AECG root bark was available from regional pharmacy lab.

Histamine and acetyl choline aerosol induced bronchospasm in Guinea pigs

Experimental asthma was induced in guinea pigs by exposing them to histamine and acetyl choline aerosol using histamine chamber. Guinea pigs of either sex (350-450 g) were selected and randomly divided into 4 groups each of 6 guinea pigs. Group I and III were exposed to histamine di-hydrochloride (0.1%) aerosol while Group II and IV were exposed to acetyl choline bromide (0.5%) aerosol. Exposed guinea pigs showed progressive dyspnoea. The end point, preconvulsive dyspnoea (PCD) was noted from time to aerosol exposure to onset of dyspnoea leading to appearance of convulsion as described by Sheth et al. As soon as PCD commenced the animals were removed from chamber and placed in fresh air. This time of PCD was taken as day 0 value. GII and III were treated with alcoholic extract of *Calotropis gigantea* (AECG) root bark 100 mg/kg p.o. while GI and IV were treated with alcoholic extract of *Calotropis gigantea* (AECG) root bark 200 mg/kg p.o. once a day for 7 days. On 7th day one hour after the dose, time of PCD onset was recorded. The % increase in time of PCD was calculated using formula as below.

$$\% \text{ Increase in time of PCD} = \left(\frac{T_1}{T_2} \right) \times 100,$$

Where T₁ = time for PCD onset on day 0 and T₂ = time for PCD onset on day 7.

Anti-inflammatory activity

Carrageenin induced inflammation test: acute inflammation can be produced using phlogistic agents (irritants) like carrageenin, egg albumin, brewer's yeast etc. This study used 1% carrageenin to cause acute inflammation. Rats were divided into four groups consisting of 6 rats in each group. Rats were fasted for 24 hour before test. Water was provided ad libitum. The Rats received following treatments. Control group received (distilled water 3 ml, p.o.), reference group received (diclofenac sodium 50 mg/kg, p.o.), test groups received (alcoholic extract-AECG) 200 mg/kg p.o.) and (alcoholic extract (AECG) 100 mg/kg p.o.+diclofenac sodium 25 mg/kg p.o.). After 1 hour freshly prepared carrageenin suspension 0.1ml of (1% w/v) (Sigma chemicals USA) in normal saline was injected in sub plantar region of right hind paw. Paw was marked with ink at level of lateral malleolus and immersed in mercury up to the mark. Change in Paw volume was measured at 1, 3, 5 hours after carrageenin injection using plethysmometer. Percent inhibition of inflammation was calculated using formula as below;

$$\% \text{ Inhibition} = (1 - V_t / V_c) \times 100$$

Where V_c was inflammation (paw edema) of control group at a given time while V_t was inflammation (paw edema) after drug treatment (alcoholic root bark extract of *Calotropis gigantea* and reference diclofenac sodium) at a same time.¹⁶ Treatment groups were compared with control.

Cotton pellet granuloma: rats were divided into 4 groups, 6 rats in each group. In anaesthetized rats sterile cotton pellets (10±1 mg) were implanted in axilla. Control group received (distilled water 3 ml p.o.), reference group received (diclofenac sodium 50 mg/kg p.o.), test groups received (alcoholic extract, AECG) 200 mg/kg p.o. and alcoholic extract (AECG) 100 mg/kg p.o.+diclofenac sodium 25 mg/kg p.o. for 7 days after cotton pellet implantation. On 8th day under anaesthesia cotton pellets were removed and dried at 60°C. Increase in dry weight of pellet indicates granuloma formation. Percent inhibition of inflammation was calculated using formula;

$$PI = (\text{Control} - \text{Test} / \text{Control}) \times 100$$

Statistical analysis

All values were expressed as mean±SEM. Results were analyzed for statistical significance (p) by using one way ANOVA (SPSS Version 15.0) followed by Bonferroni test, p<0.05 was considered statistically significant.

RESULTS

Effect of alcoholic extract of root bark of *Calotropis gigantea* (AECG) on histamine and acetylcholine aerosol induced bronchospasm in guinea pigs was tested. Results are depicted in (Table 3 and Figure 3).

Table 1: Effect of EECG (p.o.) on histamine and acetylcholine aerosol induced bronchospasm in guinea pigs.

Group	Dose p.o. mg/kg	Preconvulsive dyspnoea prolongation time (PCD) in seconds		% increase in time to PCD
		Before treatment	After treatment	
Histamine aerosol 0.1% w/v				
AECG	100	115 ±1.62	387±4.08	70
AECG	200	119.8±0.9	423±8.87	72
Acetylcholine aerosol 0.1% w/v				
AECG	100	39.8±0.17	350±0.22	61
AECG	200	134±0.33	430±0.33	71

Values are m±SEM, (n=6), p<0.01, AECG: ethnolic extract of *Calotropis gigentia*.

AECG significantly and dose dependently increased time to PCD following histamine and acetylcholine aerosol. 200/kg dose of root extract more significantly increased the time taken for onset of PCD than 100mg/kg dose. After treatment of 200/kg dose PCD latency was 423±8.87 after histamine aerosol and 430±0.33 after acetylcholine aerosol compared to pretreatment PCD of

119.8±0.9 after histamine aerosol and 134±0.33 after acetyl choline aerosol.

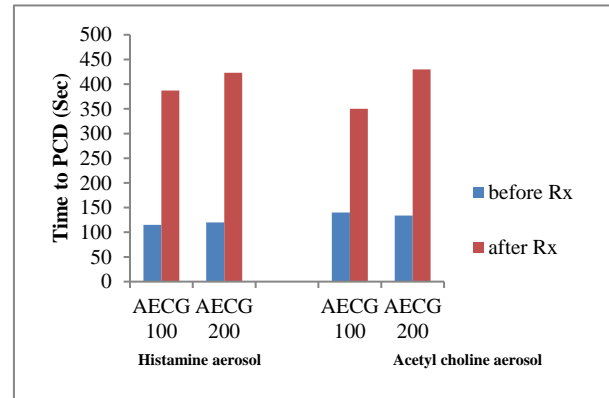


Figure 1: Effect of AECG (p.o.) on histamine induced bronchoconstriction, each bar represents mean±SEM, (n=6).

The root extract possess significant bronchodilator activity and may have potential in the treatment of asthma. This effect might be due to the some phytoconstituents in the extract blocking some mediators. Anti-inflammatory potential was explored using carrageenin model. Carrageenin edema is categorized into two phases based on time and release of mediators. The first hour is initial phase attributed to histamine and serotonin release. The second phase commence 3-5 hours after is due to prostaglandin, bradykinin and lisosomal release contributing in edema development. Results are shown in (Table 2 and Figure 2).

Table 2: Effect of AECG (p.o.) on carrageenin induced paw edema in rats.

Group	Dose p.o. mg/kg	Mean increase in paw volume (ml) in rats.			% inhibition at 1, 3, 5 hr
		1 hr	3 hr	5 hr	
Control	-	0.39±0.005	0.65±0.003	0.68±0.003	-
Diclofenac	50	0.22±0.002	0.26±0.003	0.23±0.003	44, 60, 66
AECG	200	0.22±0.005	0.17±0.002	0.18±0.003	62, 74, 81
AECG+ diclofenac	200+25	0.16±0.003	0.15±0.003	0.13±0.002	59, 66, 74

Values are m±SEM, (n=6), p< 0.01, AECG: ethnolic extract of *Calotropis gigentia*.

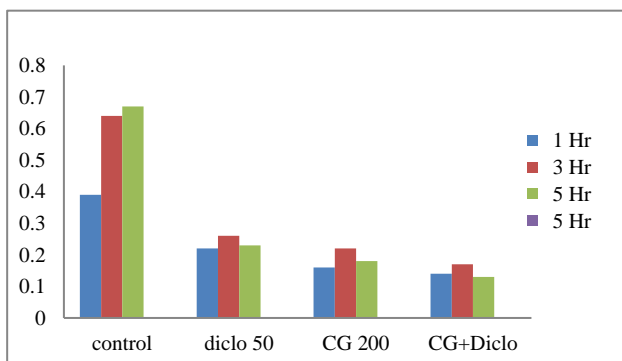


Figure 2: Effect of AECG (p.o.) on paw oedema in rats, each bar represents mean±SEM, (n=6).

The present study had found statistically significant (p<0.01) inhibition of both phases of inflammation after oral administration of alcoholic extract of *Calotropis gigantea* (200 mg/kg). In 1 hour there was 44%, 62%, 59% inhibition by diclofenac 50 mg/kg, AECG 200 mg/kg, AECG100+diclofenac 25 mg/kg respectively. At 5 hr inhibition was 66%, 81%, and 74% respectively with treatments. Thus AECG and combination had greater AI activity than diclofenac alone. The mechanism of anti-inflammatory effect could be due to inhibition of synthesis and release of mediators like histamine, serotonin, prostaglandin and bradykinin. The effect on these mediators might be due to some phytochemicals in the plant. AECG Significantly reduced granuloma

formation in rats. Results are shown in (Table 3 and Figure 3). The results suggest effect on reduction of chronic inflammation also. The % decrease in dry weight was 51%, 47%, 57 % after diclofenac 50 mg/kg, AECG 200 mg/kg, and combination of AECG 100 mg/kg+diclofenac 25 mg/kg suggesting significant anti-inflammatory effects. Reduction was more significant with AECG 200 mg/kg than diclofenac 50 mg/kg or AECG 100 mg/kg+diclofenac 25 mg/kg.

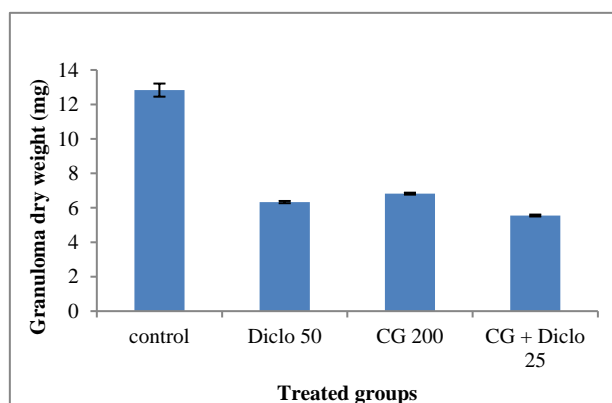


Figure 3: Effect of EECG (p.o.) on granuloma formation in rats, each bar represents mean±SEM, (n=6).

DISCUSSION

The alcoholic extract of *Calotropis gigantea* (AECG) possess antiasthmatic activity. Histamine and acetyl choline are spasmogens that cause immediate bronchoconstriction as measured by appearance of PCD in guinea pigs. In asthma increased airway hyper reactivity is due to mediators like histamine and acetylcholine. Bronchodilator effect of root bark extract of CG was evaluated by observing effect on time of PCD. Time of PCD occurrence was significantly increased suggesting bronchodilator activity of the extract. Bronchial asthma is a chronic inflammatory disease characterized by both bronchoconstrictions and air way inflammation which leads to bronchial hyper-responsiveness to various stimuli.^{20,21} The Paw edema is a standard experimental model of acute inflammation. Carrageenin induced paw edema model exhibit high degree of reproducibility. Carrageenin is a flogistic (irritant) agent. The edema development by Carrageenin is biphasic.²² The initial phase is due to release of histamine, and 5HT during first hour. The second phase is attributed to release of prostaglandin, bradykinin, lysosomes etc. The second phase is sensitive to most of the clinically effective anti-inflammatory agents. The second phase of edema was significantly inhibited by the root bark extract of CG. Thus the extract of CG has inhibitory effect on early inflammatory process. These observations were similar to other researchers.²³⁻²⁷ The Cotton pellet granuloma is a mode l for chronic inflammation. The granuloma assess transudation and

proliferation of granulomatus tissue in chronic inflammation. The wet weight of pellet correlates with transudate and dry weight with formation of granuloma tissue. The AECG 200mg/kg or diclofenac The 50 mg/kg or combination in low dose of both exhibit statistically significant reduction in granuloma formation in rats compared to control. Diclofenac sodium reduce inflammation by inhibiting prostaglandin synthesis and release. Root bark extract of *Calotropis gigantea* might be interfering arachidonic acid pathway similar to diclofenac sodium . Thus root bark extract of *Calotropis gigantea* could be effective in chronic inflammatory ailments. The activity might be due to phytochemicals in the plant like sterol, triterpinoid, saponins.²¹ Further studies to determine exact phyto-constituents responsible are needed. These findings support traditional claims and provide scientific basis for anti-inflammatory effect of the extract.²⁸⁻³⁰

CONCLUSION

The results of this study showed that acoholic extract of root bark of *Calotropis gigantea* 100mg/kg and 200 mg/kg p.o. possess bronchodilator effect justifying the claim of its traditional use in the treatment of asthma. This activity showed by the root bark extract might be because of the steroid like phytochemical in the plant. However further research is needed to characterize the active principle responsible for it. The inhibitory action on leucocytes and neutrophil infiltration by inhibiting mediator synthesis and release might be the reason. Present investigation also suggest anti-inflammatory activity probably through same mechanisms of decreased synthesis and release of mediators, again supporting the traditional use in inflammatory conditions. Further investigations are needed on bioactive phytoconstituents contributing to this mechanism of action. The presently used anti-inflammatory and antiashamatic drugs have shortcomings hence there is a need for newer compounds with improved profile of action.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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