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Evaluation of Anti-inflammatory and Analgesic activities Ethanol Extracts of *Crateva magna* (Lour) DC. (Capparaceae)

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Abstract: **The ethanolic extracts of *Crateva magna* leaves and barks were screened for its anti-inflammatory and analgesic effects in experimental animals. The extracts showed significant inhibition of carrageenan induced paw edema at two different doses used in the study. The activity of the extracts was comparable to that of Indomethacin, the standard anti-inflammatory drug. The extracts also showed significant analgesic activity in rats against tail immersion and hot plate methods. The present study suggests that the ethanolic extract of *Crateva magna* possesses analgesic and anti-inflammatory activities. This result may prove the fact that the plant may be used as analgesic and anti-inflammatory along with its adaptogenic properties.**

Keywords: **Analgesic, Anti-inflammatory, Wistar rats, Traditional medicine.**

1. INTRODUCTION

The biological reaction of vascular tissues as a result of harmful stimuli including pathogens, irritants and damaged cells is inflammation. The inflammation is characterized by classical signs edema, erythrema, pain, heat and above all loss of function. The drugs which are used these days for the management of pain and inflammatory conditions are either steroidal lie corticosteroids or non steroidal lie aspirin. All these drugs have more or less side and toxic results like renal failure, allergic effects, hearing loss or they may increase the risk of haemorrhage by affecting platelet role. [1] Furthermore the rural population of the country is more organized to traditional ways of behaviour because of its easy accessibility and cheaper cost. On the contrary, many plant origin drugs are being used since long time without any adverse effects. The utilization of medicinal plants as herbal remedies to avoid and cure numerous ailments fluctuates from society to society. [2]

A well known plant in herbal world for its extensive range of application in medicinal reasons is the plant *Crateva magna* which belongs to the family Capparaceae. The leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined. [3] It is used as an anti spasmodic, hypotensive, anti-inflammatory, hypoglycemic, anti protozoal, Anthelmintic, analgesic purposes. In folk medicine, its stem pith is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation by the ethnic peoples of Kandhamal district of Orissa known as Eastern Ghats of India. [4, 5, 6]. The major constituent is the Triterpines, which has been shown to have these various activities. Other constituents are the alkaloids, minor flavonoides, sterols, Triterpines and the isothiocyanate glucosides. Based on the above data, in the present study acute and anti-inflammatory and analgesic activity were evaluated in the crude ethanolic extract of *Crateva magna* using animal models.

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2. MATERIALS AND METHODS

Collection of plants

The fresh plant parts (bark & leaves) of *Crateva magna* were collected from Vellamadam, Nagercoil District, Tamil Nadu, India. The gathered samples were cut into small pieces and shade dried until the fracture is identical and even. The dried plant material was crushed or grinded by using a blender and separated to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of plant extracts

The aerial parts of the plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a soxhlet apparatus using ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for anti-inflammatory and analgesic activities.

Acute toxicity study

Acute oral toxicity was performed by following OECD- 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study. [7] The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was viewed in two out of three animals, then the dose managed was allocated as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 upto 2000 mg/kg body weight.

Anti-inflammatory activity

For screening *in vivo* anti-inflammatory activity for each of the extracts, 6 Groups of five animals each were used. Group I served as control. Group II, Group III, Group IV and Group V were treated orally with ethanol extracts of *C. magna* leaf & bark at the doses of 200 mg/kg and 400 mg/kg body weight respectively. Group VI served as Positive control (Indomethacin). The inflammation was enumerated by measuring the volume transferred by the paw, using a plethysmometer at time 0, 60,120 & 180 min following carrageenan injection. The disparity between the left and the right paw volumes (indicating the degree of inflammation) was decided and the percent inhibition of edema was computed in evaluation to the control animals. [8]. The percentage of inhibition of paw edema is calculated by

$$\% \text{ inhibition of paw edema} = \frac{C - T}{C} \times 100$$

where,

C = increase in paw volume of control group

T = increase in paw volume after administration of extracts

Evaluation of analgesic activity

Eddy's hot plate method

The Wistar albino rats were divided into six groups of 5 animals each. Group I served as control. Group II served as standard and were injected Diclofenac (9 mg/kg) intraperitoneal. Group III, Group IV Group V and Group VI were treated orally with ethanol extracts of *C.magna* leaf & bark at the doses of 200 mg/kg and 400 mg/kg body weight respectively. The rats were individually placed on the hot plate maintained at 55^0C, one hour after their respective treatment. The response time was noted as the time at which rats reacted to the pain stimulus either by paw flicking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds. [9]

Tail Immersion method

The Wistar albino rats were divided into five groups of 6 rats each. Group I served as control. Group II served as standard and were injected Diclofenac (9 mg/kg) intraperitoneal. Group III, Group IV, Group V and Group VI were treated orally

with ethanol extracts of *C. magna* leaf & bark at the doses of 200 mg/kg and 400 mg/kg body weight respectively. After one hour, the lower 5cm portion of the tail was immersed in a beaker of freshly filled hot water maintained at 55^0C ± 1.0^0C. The time taken to withdraw the tail was noted as reaction time. A cut of time of 10 seconds was maintained to prevent tissue damage. The time required for flicking of the tail was recorded to assess response to noxious stimulus. [10]

Statistics analysis

For the statistical tests p values of less than 0.001, 0.01 and 0.05 were taken as significance. Analysis of Variance (ANOVA) followed by Dunnett t-test was used for Anti-inflammatory and analgesic activity analysis. The Data were analysed using the statistical analysis system SPSS (SPSS Software for windows release 10.0; SPSS Inc., Chicago IL, USA).

3. RESULTS AND DISCUSSION

Oedema symbolizes the early hour phase of inflammation in carageenan induced paw oedema and is the easiest and the most extensively employed acute inflammatory model for learning anti-inflammatory agents. Carrageenan-induced inflammation is helpful in noticing orally lively anti-inflammatory agents. [11,12] The present study demonstrates the potent anti-inflammatory and analgesic activity of ethanolic extract of different parts of *C. magna* (leaves and bark), indicating the possibility of use of *C. magna* as the cheaper, safer and potent anti-inflammatory therapeutic agent. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and derived from Irish Seamoss (*Chondrus crispus*). The delta type galactan elicited an inflammatory response. The development of the edema in paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase of the edema has been attributed to the release of histamin, serotonin and kinin-like substance, and the second accelerated the phase of swelling to release prostaglandin-like substance

Both the crude ethanol extracts of the selected plant at a dose of 400mg/kg showed highly significant anti-inflammatory activity ($P<0.01$) as compared to control group at 60, 120 and 180 min respectively (Table 1). The standard drug Indomethacin at a dose of 100mg/kg body weight inhibited the development of edema significantly from 120 min onwards. It showed maximum percentage reduction in paw edema at 180 min (Table 1). Intraperitoneal injection of carrageenan leads to inflammation of the peritoneum resulting from carrageenan induced release of interleukin-1 from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorpho nuclear cells by a variety of processes including adhesion and cell mobility. [13] Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility. Since, antinociceptive and/or anti-inflammatory activity of many plants has been attributed to their flavonoids, tannins, triterpenes and coumarins. [14] It is therefore, possible that the antinociceptive and anti-inflammatory effects observed with both plant extracts in the present study may be attributed to the components that are present in abundance in the extracts.

Eddy's hot plate method

Rats treated with ethanol extract of *C. magna* plant parts showed significant (***$p<0.001$) and dose dependent analgesic activity in thermal stimulated pain (hot plate test) in rats. The reaction time at a dose of 400mg/kg (higher dose) was found to be 10.34 seconds after 90 minutes of drug treatment, whereas the standard drug diclofenac showed the tail flick latency11.46 seconds (Table 2).

Tail Immersion method

Rats treated with ethanol extract of *C. magna* plant parts showed significant (***$p<0.001$) increase in the tail flick latency compared to control. The tail flick latency at a dose of 400mg/kg (higher dose) was found to be 8.61 seconds after 90 minutes of drug treatment, whereas the standard drug diclofenac showed the tail flick latency 9.89 seconds (Table 2). The activity was also found to be a significant activity.

The hot plate method is considered to be selective for the drugs acting centrally. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity. [15]. It is an established fact any agent that causes a prolongation of the hot plate latency using this test must be acting centrally. [16] Therefore, the crude extracts of the plant must have a central activity. Again, narcotic

analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain. [17,18] These findings conclude that the ethanol extract of *C.magna* plant parts (leaves and bark) may contain bioactive principles with pharmacological potential.

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APPENDIX – A

List of Tables:

Table 1: Effect of CML and CMB extracts on the Percentage inhibition of Carrageenan induced paw oedema

Oedema volume (ml)						% Inhibition after 180 min
Treatment	Dose mg/kg	0 min	60 min	120 min	180 min	
Group-I	Normal saline	29.16±0.98	68.54±2.08	113.54±2.16	138.56±2.96	-
Group-II	200	33.56±1.54	41.36±1.36*	37.84±1.92***	30.56±1.54***	77.94
Group-III	400	30.11±1.56	37.55±1.36**	28.31±1.65***	21.56±1.08***	84.43
Group-IV	200	31.26±1.84	45.18±1.92*	34.92±1.56***	24.59±1.28***	82.25
Group-V	400	30.65±1.24	40.54±1.84**	30.16±1.39***	22.16±1.38***	84.00
Group-VI	100	29.93±1.22	35.16±1.06**	28.31±1.54***	21.65±1.84***	84.37

Each Value is SEM ± 5 individual observations * P < 0.05 ; ** P<0.01; *** P<0.01 Compared paw oedema induced control Vs drug treated rats.

Table 2: Analgesic activity of ethanolic extracts of CML and CMB Extract on the adult albino rats.

Groups	Dose	Response Time in sec (Mean ± SEM)	
		Eddy Hot Plate Method	Heat Conduction Method
Group I	0.9 mg/dl	2.81±0.231	1.926±0.546
Group II	9 mg/kg	11.465±0.546***	9.896±0.214***
Group III	200 mg/kg	9.018±0.316***	6.881±0.168***
Group IV	400 mg/kg	11.113±0.846***	9.112±0.168***
Group V	200 mg/kg	6.221±0.545**	5.326±0.929**
Group VI	400 mg/kg	10.346±0.129***	8.616±0.326***

One way Analysis if Variance. ANOVA: p value found to be 0.001 is considered extremely significant

NS - Not significant