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ACUTE AND CHRONIC ANTI-INFLAMMATORY EVALUATION OF *CRATEVA RELIGIOSA* IN RATS

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Abstract:

Crateva religiosa is popular traditional medicinal plant in rural belt of India for inflammatory conditions and kidney stones. The study was intended to evaluate the anti inflammatory potential of *Crateva religiosa* and give a scientific basis for this. Acute and chronic inflammation was induced by inflammogens like carrageenan, histamine, 5HT and formalin. The reduction in the volume displacement of the paw as compared to the control was considered as the anti-inflammatory effect of the extracts. The alcoholic and aqueous extracts of the plant were administered in a dose of 250 and 500 mg/kg to the animals orally one hour before the induction of inflammogens. Both the extracts showed dose dependent decrease in the paw edema in tested animals. In carrageenan induced inflammation the extracts show significant activity ($p < 0.001$) at 6 hours. The extracts also significantly suppress the inflammation induced by mediators like histamine and 5HT. In chronic inflammation induced by formalin the extracts show significant ($p < 0.05$) activity in the second phase i.e. after 6th day in a 10 day study. Among the extracts alcoholic extracts show more profound effect than the aqueous effect which can be correlated to the presence of flavonoids and triterpenoids in it. The current study thus supports the traditional utilization of this plant against the inflammatory disorders.

Keywords: Alcoholic extract, Aqueous extract, Carrageenan induced edema, Cotton pellet induced granuloma, *Crateva religiosa*.

Introduction:

Inflammation is a pathophysiological response to injury leads to the accumulation of various mediators like Prostaglandins, Histamines, 5-HT, Leukotrienes etc at the site of injury. Though it is a defense mechanism of the body different events and complex mechanisms involve in it are responsible to maintain and aggravate many type of inflammatory disorders including Rheumatoid arthritis (RA) [1]. Inflammatory diseases are currently treated with steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) [2]. Unfortunately, both of these widely prescribed drug classes have significant negative side effects, reducing their use in certain segments of the population [3, 4]. Hence, there is a need to develop new drugs with novel modes of action that do not produce considerable side effects. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [5]. Natural product-based anti-inflammatory agents with a transcriptional mode of action, good efficacy, and lower risk of side effects offer promising treatment and prevention of inflammation-related conditions.

Crateva religiosa hook & frost belonging to family Capparidaceae (cappaceae) is a tree usually found in the vicinity of temples of central and eastern India [6,7] It is popularly known as pasugandha in Sanskrit ,three legs capper in English, varuna in hindi Ethanomedically the plant used as diuretic, laxative, lethinotriptic, antirheumatic, tonic antiperiodic etc. In folklore the bark is specially used in urinary disorders including kidney and bladder stone , calculi effection, anti emetic etc [8,9]. The present study is an attempt to give a scientific proof for the use of this plant against inflammatory conditions taking rat as animal model.

Materials and methods

Plant material

The plant was collected from rural belt of Bhubaneswar, Orissa. The plant was identified and authenticated in Regional Research Laboratory, Bhubaneswar, Orissa, India. The voucher specimen bearing no.9995 was deposited at the herbarium of Regional Research Laboratory Bhubaneswar for further reference. The bark was collected in bulk and washed with tap water to remove the soil and dirt particles and then shad dried. The dried plant materials were

milled into coarse powder by a mechanical grinder and sieved in sieve 20. The coarse powder was extracted successively using soxhlet apparatus with Ethanol (95%) and water for 72 hour [10,11]. The extracts were concentrated by distilling off the solvent under reduced pressure and kept inside desiccators. The % of yield was calculated separately for each extract with respect to the air dried weight.

Experimental animals

Adult male albino mice 20-25gm and rats 150-200gms were used for the study. Animals were kept in the animal house of university department of pharmaceutical sciences, Bhubhaneswar, maintained under standard husbandry condition with free access to food and water *ad libitum*. The animals were acclimatized for 7 days to the laboratory conditions before doing experiments. All the experiments were carried out according to the IAEC and CPCSEA guidelines.

Acute toxicity studies

Oral acute toxicity studies were carried out with Albino mice weighing 20-25gm. The extracts were administered as per the staircase method [12,13]. The mice were fed with alcoholic and aqueous extracts of *Crateva religiosa* separately suspended in 5% w/v normal saline at dose 500, 1000, 1500, 2000, 2500 and 5000 mg/kg bodyweight. The animals were observed continuously for 2 hours for the gross behavioral changes and then intermittently once in every 2 hours and finally at the end of 24 and 72 hours to note for any signs of toxicity including death.

Anti-inflammatory studies:

Carrageenan, histamine, serotonin and formalin were used as inflamogens for evaluating the anti inflammatory potential of the extracts. For each model rats were divided in 6 groups (n=6). Normal saline (5%w/v), diclofenac (10mg/kg) and extracts at a dose of 250 and 500mg/kg were administered orally one hour before the induction of inflamogens. The paw thickness was measured by volume displaced in plethysmograph. The reduction in the volume displacement of the paw as compare to the control was considered as the anti-inflammatory effect of the extracts.

$$\% \text{ edema inhibition} = [1 - (V_t / V_c)] \times 100$$

V_t and V_c are edema volume in the drug treated and control groups respectively.

Carrageenan induced paw edema:

In this method, acute inflammation was produced by injection of 0.1ml 1% carrageenan (sigma USA) in sub plantar region of the rat left hind paw to produce edema [14]. The paw volume as measured before and at 1st, 2nd, 3rd, 5th and 6th hours after administration of carrageenan.

Histamine and serotonin induced paw edema in rats:

One hour after dosing, the rats are challenged by a subcutaneous injection of 0.1ml of 1% solution of histamine or serotonin into the sub-plantar side of the left hind paw. The paw volume is measured again at 3 hours after challenge [15, 16] the increase in paw volume is calculated as percentage compared with the basal volume.

Formaldehyde induced inflammation:

Inflammation was induced by sub-planter injection of 0.1ml of 2%v/v formaldehyde was administered to the right hind paw on the first and 3rd day of experiment. The rat paw volume was measured daily for 10 days [17]. On the 3rd day of treatment, the paw volume was measured before the injection of formaldehyde [18, 19].

Statistical Analysis

Results were expressed as mean \pm S.D. and statistical analysis was performed using ANOVA, to determine significant differences between groups, followed by student's t- test. $P < 0.05$ implied significance.

Result and discussions:

Due to the increase frequency of NSAID and their common side effects the use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists worldwide [20]. *Crateva religiosa* is used as an anti inflammatory agent in folklore. To give a scientific validation to this plant an attempt was made to study the anti inflammatory activity.

The percentage of yield was found to be 10.3 and 9.6 (%w/w) for Ethanol and Aqueous extracts respectively. The preliminary phytochemical analysis reveals the presence of saponins, triterpinoids, flavonoids, alkaloids and sugars in the extracts.

From the acute toxic study the extracts were found to be safe up to 5000mg/kg body weight so 1/10th of this dose i.e. 500mg/kg and a sub maximal dose 250 mg/kg were taken as the test doses for the anti-inflammatory screening.

Sub planter injection of carrageenan in rats shows time dependent increase in paw thickness. Carrageenan induced rat hind paw edema has been widely used for the discovery and evaluation of many anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience [14]. Development of edema in the paw of the rat after injection of Carrageenan is a biphasic event [21]. The first phase is due to the release of histamine and serotonin and the second phase is due to the release of prostaglandins kinin like substances, protease and lysosomes [22]. It has been reported that second phase of edema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-edematous effect of natural products [23]. Both the extracts dose dependently decrease the paw edema at the end of 6 hour study which can be seen in table 1. However the extracts at a concentration of 500mg/kg significantly decrease the paw edema induced by Carrageenan throughout the two phases which can be seen from the table. The maximum percentage of decrease was found at 3rd hour for the extracts and standard drug. Standard diclofenac decrease the edema significantly from 1st hour where as alcoholic extracts at dose of 500 mg/kg decrease it from 2nd hour and other treatment groups shows significant activity from 3rd hour. Based on these reports, it can be inferred that the inhibitory effect of the extract of *Crateva religiosa* (Capparaceae) on carrageenan-induced inflammation in rats may be due to the inhibition of release or antagonism to the mediator induces inflammation.

Table-1: Effect of *Crateva religiosa* extracts and diclofenac on carrageenan induced paw edema in rats

Groups	Drugs	Dose	1 st hour	2 nd hour	3 rd hour	5 th hour	6 th hour
1	Normal saline	5mg/kg (oral)	2.12±0.14	2.68±0.13	3.38±0.26	2.62±0.22	1.96±0.32
2	Standard (diclofenac)	10mg/kg	1.22±0.14* (42.45%)	1.48±0.21* (44.77%)	1.46±0.14* (56.8%)	0.92±0.16* (64.88%)	0.64±0.24* (67.34%)
3	Alcoholic extract	250mg/kg	1.76±0.22 (16.98)	1.66±0.24 (38.05%)	1.82±0.21* (46.15%)	1.64±0.14* (37.40%)	1.28±0.16 (34.69%)
4	Alcoholic extract	500 mg/kg	1.58±0.26 (25.47)	1.32±0.22 (50.74)	1.56±0.16* (53.84)	1.24±0.22* (52.67)	0.84±0.08 (57.14)
5	Aqueous extract	250mg/kg	1.88±0.14 (11.32%)	1.9±0.22 (29.10%)	2.12±0.13* (37.27%)	1.76±0.22* (32.82%)	1.42±0.18 (27.55%)
6	Aqueous extract	500 mg/kg	1.64±0.14 (22.64)	1.62±0.24* (37.85)	1.84 ±0.21* (45.56)	1.46±0.12* (44.27)	1.12±0.24* (42.85)

All values represent the in Avg \pm S.E.M of 6 rats for each group.

Each value in parenthesis indicates the percentage inhibition rate

Statistically significant from control * $p < 0.05$ for control untreated Vs treatment (Dunnett's t-test).

Histamine and serotonin are one of the important inflammation mediators and it is potent vasodilators substance and increases the vascular permeability [24]. The treated animals found to decrease the edema induced by histamine and serotonin significantly and dose dependently at the end 3 hours which can be seen from the table. At the interval of 3 hours ethanolic and aqueous extracts 500 mg/kg body weight decrease the paw volume by 43.06% and 32.11% respectively where as standard diclofenac decrease the edema by 55.1% in histamine induced inflammation. In serotonin induced inflammation the percentage of decrease in edema for the aqueous and alcoholic extracts (500mg/kg) and standard drug was found to be 37.80, 42.04 and 53.35. Among the extracts ethanolic extract proves to be more effective than the aqueous extracts in terms of percentage of inhibition in autacoids induced inflammation. It can suggested from the study that the effectiveness for suppression of edema is due to the ability of extracts to either inhibiting the synthesis, release or action of autacoids like histamine and Serotonin involved in the inflammation.

It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis [25]. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The inflammatory effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response [26]. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Extract of *crateva religiosa* shows significant ($p < 0.05$) inhibitory effect on the formalin-induced arthritis in a dose dependent manner. After repeated induction of formalin on 3rd day after measuring the paw volume the organisms develop a chronic swelling. Standard diclofenac decrease the paw edema significantly from the first day of experiment. On the initial days extracts does not show significant effect which indicates its ineffectiveness in suppressing the immediate phase reactions induced by formalin. At a dose of 500mg/kg the alcoholic and aqueous extracts shows significant inhibitory activity from the 4th

and 8th day. The extracts are found to be capable of inhibiting the late phase of the inflammation which was seen from the table. These findings justify the usefulness of ethanolic extract of *crateva religiosa* in the treatment of inflammation associated diseases like arthritis. The effect of the extract is may be due to the presence of different phytochemicals present in this.

Table-2: Effect of *Crateva religiosa* extracts histamine induced paw edema in rats.

Group	Drug	Dose	Paw volume	Percentage of inhibition
1.	Normal Saline p.o.	5ml/kg	5.48±0.52	
2.	Standard(diclofenac)	10mg/kg	2.46±0.28	55.1*
3.	Ethanolic extract	250mg/kg	3.63±0.42	33.75*
4.	Ethanolic extract	500mg/kg	3.12±0.34	43.06*
5.	Aqueous extract	250mg/kg	4.16±0.36	24.08
6.	Aqueous extract	500mg/kg	3.72±0.44	32.11*

All values represent the in Avg ± S.E.M of 6 rats for each group.

Each value in parenthesis indicates the percentage inhibition rate.

Statistically significant from control *p<0.05 for control untreated Vs treatment.

Table: 2.11 Effect of *crateva religiosa* extracts and diclofenac (standard drug) on formalin induced paw edema in rats.

Groups	Dose	Paw edema (mm)									
		1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day
Control (Normal saline)	5 ml/kg	8.6 ±3.6	6.2 ±2.5	2.6 ±2.6	2.4 ± 4.1	7.3 ± 3.2	5.4 ±2.9	3.8 ±3.2	2.3 ±2.6	1.2 ± 3.2	
Standard (diclofenac)	10 mg/kg	8.4 ± 1.7* (20.98%)	4.8 ± 2.2* (24.67%)	2.6 ± 2.1* (23.47)	8.7 ± 2.6* (26.14)	5.6 ±1.9* (24.73)	1.8 ± 2.2* (29.95)	0.2 ± 2.1* (31.05)	7.6 ±2.4* (34.75)	4.6 ± 2.3* (40.29)	
Ethanolic extract	250 mg/kg	4.2 ± 2.4 (9.05)	2.8 ± 3.2 (7.35)	9.2 ± 2.6 (7.98)	3.8 ± 3.8 (16.41)	9.2 ± 2.5 (17.12)	7.6 ± 2.4 (17.18)	4.2 ± 1.8* (21.9)	2.8 ± 2.5* (22.27)	0.8 ± 2.7* (25.24)	
Ethanolic extract	500 mg/kg	2.1 ± 2.7 (13.37)	9.8 ±2.4 (13.8)	6.3 ±3.1 (14.78)	0.6 ± 4.2* (22.51)	7.8 ±2.8* (20.08)	5.9 ±1.7* (20.92)	3.7 ±2.1* (23.05)	0.2 ±2.8* (28.6)	7.5 ±1.6* (33.25)	
Aqueous extract	250 mg/kg	3.6±2.5 (10.28)	1.4±2.2 (10.38)	9.2±2.4 (7.98)	3.4±2.8 (17.17)	1.7±2.4 (11.83)	9.6±2.8 (12.77)	7.2±3.3 (15.06)	4.8±2.4* (17.73)	1.4±2.8* (23.78)	
Aqueous extract	500 mg/kg	2.8±2.3 (11.93)	9.7±2.6 (14.06)	8.6±2.2 (9.38)	2.4±3.6 (19.08)	8.2±2.6 (19.23)	7.5±2.4 (17.40)	4.3±2.7* (21.68)	2.5±2.2* (23.16)	0.2±2.4* (26.69)	

All values represent the in Avg. ± S.E.M of 6 rats for each group.

Each value in parenthesis indicates the percentage inhibition rate.

Statistically significant from control *p<0.05 for control untreated Vs treatment (Dunnett's t-test).

Table-3: Effect of *crateva religiosa* extracts serotonin induced paw edema in rats.

Group	Drug	Dose	Paw volume	Percentage of inhibition
1.	Normal Saline p.o.	5ml/kg	5.66±0.42	
2.	Standard(diclofenac)	10mg/kg	2.64±0.28	53.35*
3.	Ethanolic extract	250mg/kg	3.94±0.32	30.15
4.	Ethanolic extract	500mg/kg	3.28±0.36	42.04*
5.	Aqueous extract	250mg/kg	4.12±0.26	26.14
6.	Aqueous extract	500mg/kg	3.52±0.28	37.80*

All values represent the in Avg ± S.E.M of 6 rats for each group.

Each value in parenthesis indicates the percentage inhibition rate.

Statistically significant from control *p<0.05 for control untreated Vs treatment.

Conclusion:

Thus it can be concluded from that the aqueous and alcoholic extracts of the stem bark of *Crateva religiosa* possess significant anti-inflammatory activity in rats. This may be due to the presence of saponins, flavonoids and triterpenoids in the extracts as reported earlier [27, 28]. Further studies involving purification of chemical constituents and investigation of detail mechanism of anti-inflammatory activity may results in development of potent anti-inflammatory agent with low toxicity and better therapeutic index.

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