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Research Article

Preliminary Anti-Inflammatory Screening of *Ammania baccifera*

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ABSTRACT
Ammania baccifera Linn (Lythraceae)is popular traditional medicinal plant in rural India. The study was intended to evaluate the anti inflammatory potential Alcoholic and aqueous extracts of *ammania baccifera* Linn (Lythraceae) against acute inflammation which was induced by inflammogens like carageenan, histamine and 5HT. The extracts were administered in a dose of 250 and 500 mg/kg to the animals oraly. Both the extracts shown dose dependent decrease in the paw edema in tested animals. In carageenan induced inflammation the extracts significantly ($p<0.05$) inhibit the paw edema and rectifies the deranged biochemical parameters. The extracts are also able to supress the inflammation induced by mediators like histamine and 5HT. Among the extracts alcoholic extracts show more profound effect than the aqueous effect. This gives rational for the use of this plant in the traditional medicines.

Keywords: *Hybanthus Enneaspermus*, Carrageenan induced edema, histamine, 5HT, alcoholic extract, aqueous extract

INTRODUCTION

Inflammation is an important physiological reaction, which occurs in response to a wide variety of injurious agents (bacterial infection or physical trauma) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair (Nathan, 2002)[1]. It requires the participation of various cell types expressing and reacting to diverse mediators along a very precise sequence (Gouwy et al., 2005)[2]. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di and Willoughby, 1971)[3] whereas prostaglandins are detectable in the late phase of inflammation (Salvemini et al., 1996)[4]. Eventhough NSAIDs, steroidal anti-inflammatory drugs are being used for different inflammatory disorders the potential side effects give a limitation for their use. Now it is a growing concern allover for the development of new safe, potent, less toxic anti-inflammatory drug [5,6]. Hence, there is a need to explore for more naturally available alternatives and supplements, so that their therapeutic values can be assessed and expanded [7]. *Ammania baccifera* Linn (Lythraceae) is a glabrous, erect branching herb, found as weed in rice-fields and marshy localities throughout India. It is commonly known as Kurandika in Sanskrit, Blistering ammaniain English, Dadamari, Kuranta in hindi, Kalluruvi in tamil, agnivendrapaku in telugu. The leaves are acrid and used in the treatment of rheumatic pain, as laxative, rubifacient and external remedy for ring worm [8]. This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial, seminal weakness, fever, flatulence and CNS depressant activities [9,10]. Current study aimed to find out the possible role of this plant extracts against carrageenan and autacoids induced inflammation and to give a scientific rational for their use.

MATERIALS AND METHODS

Collection of plant material: The plant *ammania baccifera* Linn. was collected from rural belt of dhenkanal dist in the month of October . It was confirmed by Dr. Sitaram Panda, Research Scientist, Regional Plant Resource Centre, Bhubaneswar and a voucher specimen copy was deposited in herbarium of University department of pharmaceutical sciences.

Extraction: The freshly collected plant parts were dried under shade and powdered. The powdered material was sieved in sieve no 20. The coarsely powdered material was extracted with ethanol and water successively by soxhlet apparatus for 72 hours. The extract obtained was concentrated to a small volume under vacuum (50^{o}c) and then dried in desiccators to get a constant weight. The dried extracts were then subjected to phytochemical and pharmacological analysis.

Phytochemical analysis: The extracts are subjected to phytochemical analysis for constituent identification using standard protocol [11,22].

Animals: Adult Swiss albino mice 20-25gm and rats 150-200gms were used for the study. The animals were kept in the animal house of university department of pharmaceutical sciences, Utkal University, Bhubaneswar. They were housed in polypropylene cages and fed with standard pellet and water ad libitum animals were exposed to alternate cycle of 12hr dark and light. All the experiments in this study were approved by the institutional animal ethical committee with CPCSEA

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Table: 1. Effect of Ammnania baccifera extracts and diclofenac on carrageenan induced paw edema in rats

Groups	Drugs	dose	1st hour	2nd hour	3rd hour	5th hour	6th hour
1	Normal saline	5mg/kg (oral)	2.84±0.12	3.26±0.18	3.96±0.27	3.46±0.24	2.92±0.34
2	Standard (diclofenac)	10mg/kg	2.08±0.16 (26.76)	1.66±0.23* (49.07)	1.74±0.16* (56.06)	1.22±0.21* (64.73)	0.96±0.12* (67.12)
3	Ethanolic extract	250mg/kg	2.58±0.22 (9.15)	2.46±0.26 (24.53)	2.62±0.22* (33.83)	2.26±0.16* (37.57)	1.81±0.22 (38.08)
4	Ethanolic extract	500 mg/kg	2.42±0.16 (14.78)	2.34±0.32 (28.22)	2.48±0.18* (37.37)	1.98±0.15* (42.77)	1.54±0.21 (47.26)
5	Aqueous extract	250mg/kg	2.46±0.24 (13.38)	2.38±0.32 (26.99)	2.74±0.24* (30.80)	2.28±0.26* (34.10)	1.96±0.24 (32.87)
6	Aqueous extract	500 mg/kg	2.38±0.18 (16.19)	2.24±0.28* (31.28)	2.52±0.25* (36.36)	2.08±0.26 (39.88)	1.73±0.14 (40.75)

All values represent the inAvg±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: 2. Effect of Ammnania baccifera extracts on various biochemical changes in carrageenan-induced paw edema in rats.

Group	Drug	Dose	SGOT (U/ml)	SGPT (U/ml)	Alkaline Phosphate (U/ml)
1.	Normal Saline p.o.	5ml/kg	106.7±6.2	83.83±2. 92	86.67±2.3
2.	Ethanolic extract	250mg/kg	92.32±4.7*	63.17±3.5*	72.17±3.2*
3.	Ethanolic extract	500mg/kg	78.83±3.6*	56.50±2.8*	69.50±3.2*
4.	Aqueous extract	250mg/kg	97.32±4.7	68.17±1.9	78. 7±3.2
5.	Aqueous extract	500mg/kg	82.8±3.12*	54.42±2.2*	68.2±2.8*

Values are mean ± SEM (n = 6).
Experimental groups were compared with control p < 0.001.
*Statistically significant from control *p<0.05 for control untreated Vs treatment (Dunnett's t-test)*

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Acute toxicity studies: Adult Swiss albino mice 20-25gm were taken for acute toxicity tests. The mice were divided in to control and test groups containing 6 animals each. The control group receive vehicle (5%of normal saline) and the test group receive graded doses of extracts. The animals were observed carefully up to 4hours then occasionally up to 48 hours for seeing any motility and LD 50 values were calculated by the method of Ghosh (1994)[12].

Carrageenan induced paw edema: The acute phase inflammation induced by carrageenan was measured by the volume displaced in the plethysmograph by the animal left hind paw. In this method the animals were divided into 6 groups containing 6 animals each. 5% normal saline solution, standard drug ibuprofen (10mg/kg) and the extracts (250mg/kg) were administered orally by gastric intubations method 1 hour before the injection of 0.1ml 1% carrageenan (sigma USA) in sub plantar region of the rat left hind paw to produce edema [13, 14] .The paw volume as measured at 1st, 2nd, 3rd, 5th and 6th hours after administration of drug the reduction in the volume displacement of the hind paw as compare to the control was considered as the anti-inflammatory effect of the given extract. Lysosomal enzyme content in carrageenan induced paw edema was estimated at 6 h as it is in maximum level at this time. The rats were anaesthetized under light ether anaesthesia and blood samples were collected by retro-orbital plexus route for biochemical estimation. Serum was separated and SGOT, SGPT, ALP were determined by the colorimetric method (Reitmen and Frankel, 1957)[15] using standard kits.

Histamine and serotonin Induced Paw Edema in Rats: Animals were divided into 6 groups (n=6) starved overnight with water ad-libitum prior to the day of experiment. The control group receives vehicle orally, while other groups receives standard drug and test drug (in different conc.) respectively. Left paw is marked with ink at the level of lateral malleolus; basal paw volume is measured plethysmographically by volume displacement method using Plethysmometer by immersing the paw till the level of lateral malleolus. The animals are given drug treatment. 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[16]. The paw volume is measured again at 3 hours

Table: 3. Effect of Ammania baccifera extracts histamine induced paw edema in rats

Group	Drug	Dose	Paw volume	Percentage of inhibition
1.	Normal Saline p.o.	5ml/kg	5.64±0.56	
2.	Standard(diclofenac)	10mg/kg	2.56±0.24	54.6*
3.	Ethanolic extract	250mg/kg	4.52±0.36	19.85
4.	Ethanolic extract	500mg/kg	3.16±0.4	43.97*
5.	Aqueous extract	250mg/kg	4.82±0.46	14.53
6.	Aqueous extract	500mg/kg	3.76±0.42	38.65*

Values are mean ± SEM (n = 6).
Experimental groups were compared with control p < 0.001.
*Statistically significant from control *p<0.05 for control untreated Vs treatment (Dunnett's t-test)*

Table: 4. Effect of ammania baccifera extracts serotonin induced paw edema in rats

Group	Drug	Dose	Paw volume	Percentage of inhibition
1.	Normal Saline p.o.	5ml/kg	5.82±0.36	
2.	Standard(diclofenac)	10mg/kg	2.72±0.32	53.26
3.	Ethanolic extract	250mg/kg	3.55±0.36	39.0
4.	Ethanolic extract	500mg/kg	3.08±0.42	47.07
5.	Aqueous extract	250mg/kg	3.98±0.36	31.61
6.	Aqueous extract	500mg/kg	3.66±0.44	37.11

Values are mean ± SEM (n = 6).
Experimental groups were compared with control p < 0.001.
*Statistically significant from control *p<0.05 for control untreated Vs treatment (Dunnett's t-test)*

after challenge. The increase in paw volume is calculated as percentage compared with the basal volume. The percent inhibition of the inflammation is calculated using the formula and compared with control group.

% Inhibition = Vc - Vt / x 100

Vt and Vc edema volume in the drug treated and control groups respectively

Statistical analysis: All the experimental results are represented as Avg. ± S.E.M. The data were analyzed statistically by one way ANOVA. P<0.05 was consider as significant value.

RESULT AND DISCUSSION

From the extraction the yield was found to be 13.2%for alcoholic and 7.8 % for aqueous extracts. Preliminary phytochemical screening revel the presence of alkaloids, flavonoids, glycosides, phenols, carbohydrates and tannins in the extracts

From the acute toxicity study it was found that both the extracts are safe up to 5000mg/kg so one tenth of this dose i.e. 500mg/kg and a sub-maximal dose i.e. 250mg/kg was consider as the evaluation doses .

The carrageenan-induced rat paw edema model is widely used to investigate mechanisms of inflammatory processes and also to screen potential anti-inflammatory agents. However, the precise mechanism by which carrageenan produces inflammation in the rat paw is unclear. Di Rosa et al. reported that there are three distinct phases involved in carrageenan-induced inflammation: an initial phase mediated by histamine and hydroxytryptamine; an intermediate phase involving the activity of kinins; and a third phase in which the mediators are most likely prostaglandins. More recently, evidence has been presented for the involvement of nitric oxide as a key mediator in carrageenan-induced rat paw inflammation[4]. The effect of the extracts are shown in table-1. Both the aqueous and ethanolic extracts of *Ammania baccifera* at (250 and 500 mg/kg, p.o.) significantly ($P < 0.05$) reduced the mean paw edema volume at 3 h after carrageenan injection. The aqueous extracts of *Ammania baccifera* at dose 250mg/kg and 500mg/kg inhibit the paw swelling by 32.87% and 40.75% at the end of 6th hours. At the end of 3 hours the percentage of inhibition for standard and the aqueous extract at 250 and 500 mg/kg was found to be 56.06%, 30.80% and 36.36%. The extracts exhibited anti-inflammatory activity in a dose-dependent manner with the percent inhibition of paw edema of 33.83% and 37.37% respectively at the end of 3 hours and 38.08% and 47.26% respectively at the end of 6th hour as compared with the control group for alcoholic extracts. However, the standard drug, diclofenac (10 mg/kg, p.o.) showed highly significant ($P < 0.001$) anti-inflammatory activity with the percent inhibition of 67.12. The dose dependent inhibitory action of the extracts also indicates the effectiveness of the phytochemicals presents in this for either decreasing the release or antagonizing the expression of inflammatory mediators. In the light of that and Table *Ammania baccifera* extracts seems effective in blocking all stages of the acute inflammation. This effect of the extracts is may be due to the presence of the active phytochemicals in it. There is evidence that lysosomal enzymes like SGPT, SGOT and ALP deranged in acute inflammation[17]. Many anti inflammatory drugs exert their effect by either stabilizing the membrane or inhibiting the release of mediators which are responsible

for inflammation[18]. The extract treated rats significantly and dose dependently decreases the increased lysosomal enzyme level which is depicted in table-2.

Histamine and serotonin are the one the important inflammation mediators and it is potent vasodilators substance and increase the vascular permeability [19, 20]. The treated animals found to decrease the edema rate significantly and dose dependently which can be seen from the table-3. Ethanolic extracts at a dose of 500mh/kg body weight decrease the edema by 43.97% where as the aqueous extracts decrease by 38.65%. Standard diclofenac decrease the effect by 54.6%. At a dose of 250mg/kg bodyweight the alcoholic and aqueous extracts decrease the paw volume by 19.85%and 14.53% respectively. Table-4 show the effect of *ammania baccifera* extracts on serotonin induced inflammation. Serotonin is one of the important inflammations which increase the vascular permeability and involve in inflammation process [16] resulting in acute inflammation. The treated animals found to decrease the edema rate significantly and dose dependently which can be seen from the table-4. Ethanolic extracts at a dose of 500mg/kg body weight decrease the edema by 47.07% where as the aqueous extracts decrease by 37.11%. Standard diclofenac decrease the effect by 53.26%. There is a significant increase in response with the increase in dose. At a dose of 250mg/kg bodyweight the alcoholic and aqueous extracts decrease the paw volume by 39.0% and 31.61% respectively. Among the extracts ethanolic extract proves to be more effective than the aqueous extracts in terms of percentage of inhibition. The study showed that the extracts effectively suppressed the edema produced by the autacoids like histamine and serotonin which is indicates that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of these mediators which are acute mediator of inflammation. So, it can be suggested that its anti-inflammatory activity is possible backed by its effect against histamine and serotonin.

CONCLUSION

Thus it can be concluded that the aqueous and alcoholic extracts of *Hybanthus enneaspermus* possess significant anti-inflammatory activity in rats. Among the extracts alcoholic extracts found to have more significant activity than the aqueous extracts. This may be due to the presence of flavonoids and triterpinoids in these extracts as reported earlier [21]. The results also suggest that there is a high probability for therapeutic effectiveness of the plant extracts against some inflammatory conditions as claimed by folklore practitioners in India. This suggestion is reinforced by the demonstrated absence of toxic effects when the juice extract was given via the oral route in rats. 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.

REFERANCES

1. Nathan C. Points of control in inflammation. Nature 2002; 420:846-852.
2. Gouwy M, Struyf S, Proost P, Van Damme J. Synergy in cytokine and chemokine network amplifies the inflammatory response. Cytokine Growth Factor Rev. 2005; 16: 561-580.
3. Di Rosa M, Willoughby DA. Screens for anti-inflammatory drugs. J. Pharm. Pharmacol. 1971;23: 297-303.
4. Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the carrageenaninduced rat paw edema Eur. J. Pharmacol. 1996; 303: 217-224.
5. Kingsley G, Lanchbury J and Panayi G.Immunotherapy in rheumatic disease: an idea whose time has come or gone. *Immunology Today* 1996;17: 9–12.
6. Badger AM and Lee JC. Advances in antiarthritic therapeutics. *Drug Discovery Today*. 1997; 2: 427–435 .
7. Sarang Bani, Anpurna Kaul, Beenish Khana,Vijay Kumar Gupta, Naresh Kumar Satti, Krishan Avtar Suri and Ghulam Nabi Qazi. Anti-arthritic activity of a biopolymeric fraction from *Euphorbia tirucalli*. *J ethpharm*. 2007;110: 92-98 .
8. Kirtikar KR, Basu BD. Indian Medicinal Plants: vol 2, (m/s periodical experts, Delhi- 32). 1972; p 1071.
9. Dhar MN, Dhavan BN, Mehrotra BN, Srimal RC, Tandon JS. Screening of Indian Medicinal Plants for Biological Activities. *Indian J Exp Biol* 1973; 11:43-54.
10. Bharathi K, Srinivasan KK. Evaluation of *Ammania Baccifera* Linn. for antiurolithic activity in albino rats. *Indian J Exp Boil* 1994; 32(5):311-3.
11. Trease GE, Evans WC. Pharmacognosy Baillere Tindal London 12th edn. 1983;387:475-476-
12. Ghosh MN. Fundamentals of Experimental pharmacology, 2nd Edn.,Scientfic book agency ,Kolkatta. 1994;153-158
13. Chatpaliwar VA, Johrapurkar AA, Wanjari MM, Chakraborty RR, Kharkar VT. Anti-inflammatory activity of martynia diandra glox, Indian Drugs 2002; 39: 543- 545.
14. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats, Journal of Pharmacological Experimental Therapeutics 1969; 66:96-103
15. Reitmen S, Frankel S . Colorimetric method for determination of glutamic oxaloacetate and glutamic pyruvic transaminase. Am. J. Clin. Pathol. 1957; 28: 96-100.
16. Ellman G.L., edema in rats and inflammatory mediators release. *Arch Biochem. Biophy*., 1979; 82, 70-77.
17. Anderson, A. J., Bocklehurst, W. E. and Wills, A. L. : Evidence for the role of lysosomes in the formation of prostaglandins during carrageenin induced inflammation in the rat. Pharmacol. Res. Comm. 1971; 3, 13-19.

18. Nair R B, Ravishankar etal Antiinflammatory effect of strbilanthus heyneanus leaves-A biochemical study,*J Res Ay Sid* 1988;9(1-2)46
19. Okhawa H., Ohish N., Yagi K., assey for lipid peroxides in animal tissues by thiobarbituric acids reaction; *Anal.Biochem*., 1979; 95, 351-358
20. Amann R, Schuligoi R, Lanz, I, Donnerer J. Histamine induced edema in the rat paw-effect of capsaicin denervation and a cgrp receptor antagonist, Europian Journal of Pharmacology 1995; 279: 227-31.
21. Darshan S, Doreswamy R. Potential anti-inflammatory plant drug development from traditional medicines, Phytotherapy research 2004; 18(5):343-357.
22. J.Ramamoorthy, Vanathy, R.Meera, S.Venkataraman, P. Devi , N. Chidambaranathan, Phytochemical Investigation and Anti-inflammatory Activity of Salacia reticulate, IJTPR, 2(3); 81-84