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ANTI-INFLAMMATORY AND ANTIARTHRITIC POTENTIAL OF *AMMANIA BACCIFERA* LINN

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ABSTRACT:

The anti arthritic potential of *Ammania baccifera* Linn (Lythraceae) extracts were evaluated by taking chronic inflammatory models like cotton pallet induced granuloma and complete Freund's Adjuvant (CFA)induced arthritis in albino rats. Arthritis was induced by injecting 0.1ml of complete Freund's adjuvant below the plantar aponeurosis of the right hind paw. Treatment with the extracts and standard started on the day of induction of inflamogens and continue up to 28 days. In this study both the alcoholic and aqueous extracts significantly ($p<0.01$) decrease the paw edema on 28th day. The extracts also significantly rectified the deranged hematological parameters. In cotton pallet granuloma inflammation was induced by implanting the sterilized pre weighed cotton pallet subcutaneous in the ventral region of the groin. Treatment continued up to 8 day. The decrease in weight of the cotton pallet as compared to the control was considered as anti-inflammatory effect of the extracts. In both the inflammatory models alcoholic extracts show more potency then the aqueous extracts in terms of percentage of inhibition of inflammation.

KEYWORDS: Anti-inflammatory, *Ammania baccifera* Linn, complete Freund's adjuvant, cotton pallet granuloma, Hematological parameter

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction and commonly lead to significant disability[1]. It affects about 1% of the population of world in a female and male ratio of 2.5:1 [2]. It caused by no of pro-inflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines.[3,4] The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions [5,6]. Eventhough various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, 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 antiarthritic drug [7, 8]. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded [9]

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 [10]. This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial, seminal weakness, fever, flatulence and CNS depressant activities [11, 12]. Current study aimed to find out the possible role of this plant extracts against freund's adjuvant and cotton pallet induced arthritis and give a scientific rational for their use.

EXPERIMENTAL:

Plant material : It has collected freshly dried under shade to prevent loss of volatile components and powdered. The powdered materials were sieved in no #20. The coarse powdered material successively extracted with ethanol and water using a soxhlet apparatus for 72 hrs. The extracts were evaporated under vacuum using rotavapour. The percentage yield was calculated.

Phytochemical analysis: The extracts are subjected to phytochemical analysis for constituent identification using standard protocol [13].

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 pallet 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 registration number IAEC/999/UDPS, Utkal University.

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 upto 48 hours for seeing any motility and LD 50 values were calculated by the method of Ghosh (1994)[14].

Antiarthritic screening:

Cotton pallet granuloma test:

The method used by Penn et al [15] was followed to screen the effect of the drug in exudative and proliferative phases of inflammatory by forming granuloma pouch. The granuloma was produced by implanting the sterilized pre weighed cotton pallet subcutaneous in the ventral region of the groin under light phenobarbitone (30mg/kg.s.c.) anesthesia. After 7 days of consecutive treatment with extracts and standard drug the cotton pellets are taken out on 8^{th} day and dried at 60^0 till constant weight. The weight of granuloma estimated. The difference in weight of granuloma from control group to that of treated group indicates the anti-inflammatory activity.

Adjuvant arthritis:

The rats were divided into 6 groups the 1^{st} group was taken as control, which receive only the vehicle (5% normal saline), the 2^{nd} group was administered with standard drug indomethacin, 3^{rd} and 4^{th} group receive alcoholic extracts 250mg/kg and 500mg/kg respectively, 5^{th} and 6^{th} group receive aqueous extracts 250mg/kg and 500mg/kg respectively. On 0 day arthritis was induced by injecting 0.1ml of complete freund's adjuvant below the plantar aponeurosis of the right hind paw of the rat [16] the treatment with standard and the test drug were continue up to 28days. The difference in the paw edema from the control group to that of treated group is taken as the anti arthritic potential of the extracts.

The rats were anaesthetized under light ether anesthesia and blood was collected by cardiac puncture for biochemical and haematological estimationRBC and WBC count were estimated according to the method of Chesbrough and Mc Arther in an improved neubauer chamber[17].ESR was estimated by the method of westergren [18].

Statistical analysis:

The statistical significance was assessed by using one-way analysis of variance (ANOVA) and followed by dunnet's comparisons test. All the data are presented as mean ± SEM and $p<0.05$ was considered as significant.

RESULTS:

From the extraction the percentage of yield was found to be 15.2% for ethanol and 7.8% for water extract.

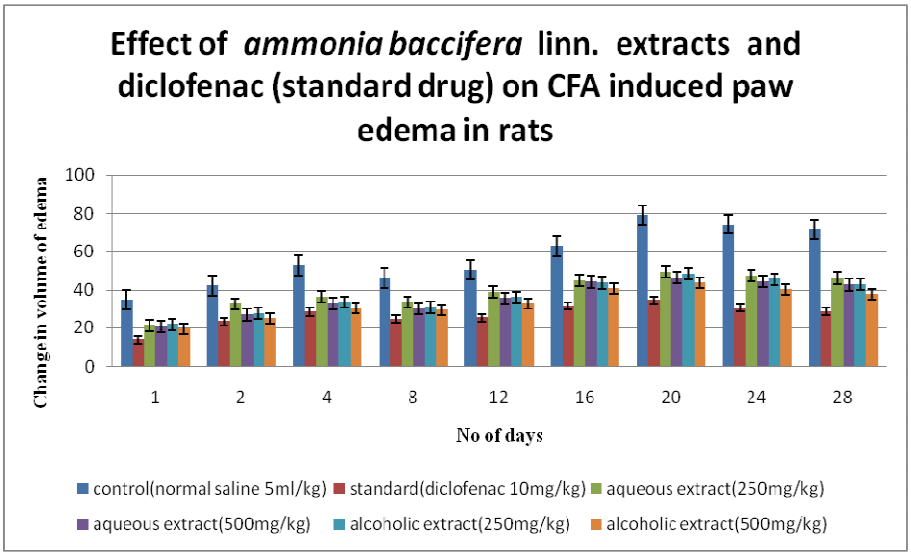
Preliminary phytochemical screening shows the presence of sterols, glycosides, alkaloids, triterpenoids in alcoholic extract and saponins, glycosides, carbohydrates in aqueous extract. The acute toxicity studies for the alcohol and aqueous extracts has showed that extracts are safe up to 5000mg/kg body wt. therefore $1/10^{th}$ of the acute toxicity dose is taken as test dose.

Table: 1

Effect of *Ammania baccifera* linn. extracts and diclofenac (standard drug) on CFA induced paw edema in rats

Groups	Volume of paw edema (edema rate%)								
	1day	**2 day**	**4 day**	**8 day**	**12 day**	**16 day**	**20 day**	**24 day**	**28 day**
Control (Normal saline)	34.6±4.8	42.4 ±6.2	52.8±4.6	46.3±8.2	50.4±5.6	62.6±5.8	79.2± 9.8	74.3±8.4	71.4± 8.6
Standard (diclofenac 10mg/kg)	13.8±2.6* (60.11%)	23.2±3.2* (45.28%)	28.3±2.3* (46.4%)	24.5±7.8* (47.08%)	25.2±3.6* (50%)	31.3±8.2* (50%)	34.6±8.6* (56.31%)	30.4±6.3* (59.08%)	28.5±4.8* (60.08%)
Aqueous extracts (250mg/kg)	21.2±3.6* (38.72%)	32.6±4.6** (23.11%)	36.4±3.8* (31.06%)	33.7±8.4** (27.21%)	38.8±8.4** (23.01%)	44.8±7.4** (28.43%)	49.4±6.4* (37.62%)	47.4±8.2* (36.20%)	46.2±7.4* (39.29%)
Aqueous extracts (500mg/kg)	20.6±4.5* (41.04%)	26.8±2.4* (36.79%)	32.8±4.8* (37.68%)	30.2±3.5* (34.77%)	35.6±5.4** (29.36%)	44.2±6.3* (29.39%)	46.5±3.8* (41.28%)	44.4±4.8* (40.24%)	42.7±6.2* (40.19%)
Alcoholic extracts (250mg/kg)	21.7±3.4* (37.28%)	27.6±4.3* (34.90%)	33.6±3.6* (36.36%)	30.8±6.4* (33.47%)	36.2±7.6** (28.17%)	43.7±7.4* (30.19%)	48.4±6.8* (38.88%)	45.6±6.4* (38.62%)	42.8±7.4* (40.05%)
Alcoholic extracts (500mg/kg)	19.2±5.4* (44.50%)	24.7±3.4* (41.74%)	30.4±4.6* (42.42%)	29.2±5.8* (36.93%)	32.8±4.6 * (34.92%)	40.7±6.6* (34.98%)	43.8±7.3* (37.12%)	40.4±4.8* (45.62%)	37.8±6.5* (47.05%)

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<0.01, **p<0.05 for control untreated Vs treatment (Dunnett's t-test)*



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The edema rate versus time could be divided into two phages. In the first phase edema rate of the injected footpad increased and reached a peak on 4th day. The swelling then subsided until 12th day after which it peaked in 20th day which is the second phase of rise. Both the extracts significantly P<0.01 decrease the paw edema of the test groups at the end of 28th day treatment in adjuvant induced arthritis in a dose dependent manner which is depicted in table 1. Alcoholic extracts have got higher efficacy both in acute and chronic phase of inflammation induced by freund's adjuvant. The alcoholic extract more potently (47.05%) inhibits the paw edema as compare to the aqueous extract 40.19% where as standard diclofenac decrease the edema by 60.08% at the end of 28th day.

Table 2
Effect of aqueous extract of *ammania baccifera* stem on various hematological parameters in FCA induced poly arthritis in rats

Parameters	Control (normal saline 5ml/kg)	Standard (diclofenac 10mg/kg)	Aqueous extract (250mg/kg)	Aqueous extract (500mg/kg)	Alcoholic extract (250mg/kg)	Aqueous extract (500mg/kg)
ESR (mm) Total	7.4±1.4	2.6±0.96*	4.8±1.2*	3.6±0.84*	4.16±0.86*	3.32±0.68*
WBC (cmm)	10860±746	7976±458*	8657±564*	8348±624*	8294±724*	7984±586*
Lymphocytes(%)	62±3.6	38±2.4*	42±1.8	36±2.6*	39±2.6*	34±3.2*
RBC (million/cmm)	3.46±0.52	3.9±0.46	3.74±0.62	3.85±0.72	5.96±0.86	6.12±0.65

Statistically significant from control *p<0.05 for control untreated Vs treatment (Dunnett's t-test)
All values represent the inAvg±S.E.M of 6 rats for each group.

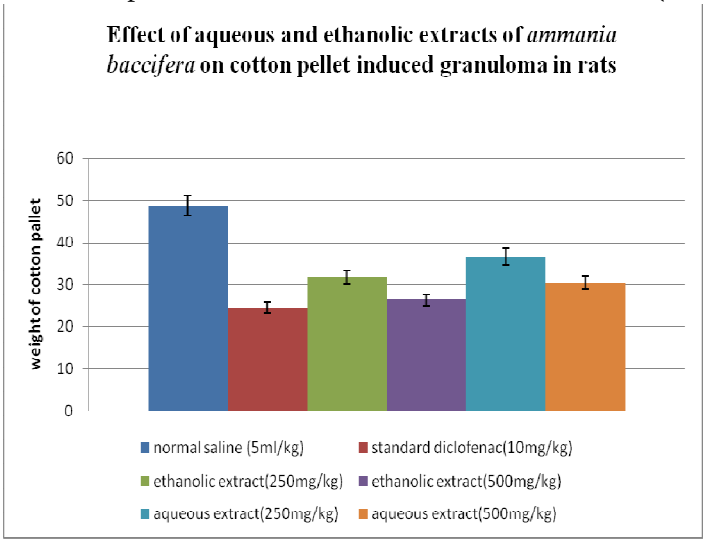
With induction of adjuvant there is marked increase in the ESR and WBC count in control group. However upon treatment with diclofenac and the extracts there is significant (p<0.05) correction in these levels dose dependently. This justifies the significant inhibitory role of these extracts on arthritis.

Table 3

Effect of aqueous and ethanolic extracts of *ammania baccifera* on cotton pellet induced granuloma in rats

Group	Drug	Before treatment	After treatment
1.	Normal Saline 5ml/kg p.o.	10mg	48.8±3.26
2.	Standard(diclofenac 10mg/kg)	10mg	24.6±2.58 (49.17) *
3.	Ethanolic extract (250mg/kg)	10mg	31.8±2.25 (34.29) *
4.	Ethanolic extract (500mg/kg)	10mg	26.4±1.82 (45.45) *
5.	Aqueous extract(250mg/kg)	10mg	36.7±2.42 (24.17)
6.	Aqueous extract(500mg/kg)	10mg	30.5±2.28 (36.98) *

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)



Cotton pallet granuloma: both the extracts decrease the paw edema in a dose dependent manner. Among the extracts alcoholic extract has shown more pronounced effect 45.45% as compared to aqueous extract 36.98% in reducing the weight of the granuloma. However standard diclofenac is able to decrease the weight of the cotton pellet by 49.17 at the end of 8th day treatment.

DISCUSSION:

Plants continue to serve as possible source for new drug and chemicals. They are extremely useful as a lead structure for synthetic modification and optimization of bioactivity. The secondary metabolites available from the plant are very difficult to synthesize externally which limits the synthetic derivation of various potentially usable photochemical. The folklore treatment of rheumatoid arthritis using plants is well known to the master of traditional medicinal practices. The plant products and their combinations are running well now in the market due to their lower side effects efficacy and less cost. This experimental work just is an attempt to establish the rational use of *Ammania baccifera* extracts as an anti-inflammatory agent against rheumatoid arthritis.

Cotton pellet granuloma method is the indication of chronic inflammation due to proliferation of inflammatory cells like macrophages, neutrophils, fibroblasts etc, which accumulates at the site of implant. With the increase in days the volume of

granuloma increases. So that decrease in the weight of granuloma or weight of the cotton pellet is an indication for suppression of inflammation by the test compounds. However both the extracts significantly $P<0.05$ decrease the weight of cotton pellet. The ethanolic extract has shown to be more pronounced effect as compare to aqueous extract which indicate the inhibition of chemical mediators of inflammation by the ethanolic extract is more than the aqueous extracts.

Rheumatoid arthritis mostly involve in immunological derangements. The adjuvant arthritis model satisfies mostly the allied conditions of arthritis in rat which resembles human. In adjuvant arthritis bacterial peptidoglycan and muramyl dipeptide are responsible for its induction[19]. It can occurs through cell mediated auto immunity by structural mimicry between mycobacteria and peptidoglycan in rats[20].The response to the CFA administration arthritis is biphasic it consists of acute phase and polyarthritic for a chronic phase, correspond to day 0-10 and 10-28 post CFA inoculation respectively. The acute phase response is characterized by unilateral inflammatory edema of the ipsalateral paw peaking around a 4-6 followed by subsequent arthritis and chronic phase response which begin around day 10 characterized by inflammatory edema in contralateral paw. CFA induced arthritis involves highly significant increase paws thickness of rat, significantly decrease in serum cortisol, highly significantly increase in c-reactive proteins [21,22] however both the extracts decrease the paw volume in the present experimental conditions. The high efficacy of alcoholic extracts can be correlated with the presence of alkaloids, triterpenoids fraction in this part.

In the studies there is an increased ESR level which is a common diagnostic feature in patient in chronic arthritis [23]. Increase in the erythrocyte sedimentation rate is an indication of active but obscure disease process which elevate in response to stress, inflammation and cell necrosis [24]. In arthritic condition there is mild to moderate increase in the WBC count which plays a major role in body defense mechanism. WBC count increase is may be due to the release of interleukins, responsible for production of both granulocytes and macrophages colony stimulating factor [25].Treatment with the extracts significantly decrease the ESR and the WBC count indicate the significant recovery from the arthritic progress.

Thus it can be concluded that the aerial parts of *Ammonia baccifera* posses' significant anti-inflammatory and antiarthritic activity in rats. Further studies involving the isolation of the potent chemical constituent of the plant an investigation of the detail biochemical pathway responsible for this anti-inflammatory action may result in the development of a potent anti-inflammatory agent having low toxicity and low cost of preparation.

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