Research Article
ISSN: 0974-6943

S. Gopalakrishnan et al. / Journal of Pharmacy Research 2010, 3(7),1547-1549

Available online through
www.jpronline.info

Pharmacological evaluation of *Ammania baccifera L*inn.

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Received on: 15-04-2010; Revised on: 12-05-2010; Accepted on:13-06-2010

ABSTRACT

This study was intended to evaluate the anti-inflammatory and anti-arthritic activities of different extracts *Ammania baccifera* Linn. In this study both acute and chronic inflammation models were used to evaluate the anti-inflammatory and anti-arthritic activities of the *Ammania baccifera.* In acute model carrageenan was used to induce inflammation in rat hind paw and in chronic model , Freund's adjuvant induced arthritis model was performed. Indomethacin (10 mg/kg bw) was used as a standard drug. The ethanol extract of *A. baccifera* exhibited significant dose dependent activity in acute inflammation and the doses of 100 mg/kg bw and 200 mg/kg bw produced 38.27% and 43.39% inhibition respectively after 3 h as compared with that of the standard drug which showed 48.52% inhibition. In Freund's adjuvant induced arthritis model, the doses of 100 mg/kg bw and 200 mg/kg bw of the ethanol extract produced(38.83%) and (44.08%) inhibition respectively after 19 days when compared with that of the standard drug (55.47%). In conclusion the ethanol extracts of both doses of *A. baccifera* exhibit considerable anti-inflammatory and anti-arthritic activities.

Key words: *Ammania baccifera,* anti-inflammatory, anti-arthritic, Carrageenan-induced oedema, Freund's adjuvant induced arthritis.

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity of the working force throughout the world. This has been called the 'King of Human Miseries'[1]. Many compounds were introduced as a result of laboratory search for drugs with anti-inflammatory activity (AIA) and anti- arthritic activity (AAA); though many of them produced a dramatic symptomatic improvement in rheumatic processes, did not arrest the progress of the diseases and all of them shared the common side effect i.e., gastro-intestinal irritations, ulceration and bleeding [2]. Thus the presently available anti-inflammatory drugs such as steroids, non-steroids, immuno suppressive agents, immuno regulatents and gold compounds provide only symptomatic relief and are not free from side effects. The target should be to discover newer drugs from plant kingdom which may provide therapeutic cure and would be free from undesirable effects as well as economical, which would be accepted by the developing nations like India[3].

Ammania baccifera Linn. (Fam : *Lythraceae*) is commonly known as "Neerumelneruppu" in Tamil, "Agnigarava" in Sanskrit and "Dadmari" in Hindi respectively. .*A. baccifera* is an erect glabrous reddish herb, growing upto 70 cm or more. It grows as weed in all over India in rice fields and marshy localities [4]. The plant is considered bitter, acrid and used in the treatment of rheumatic pains, intermittent fevers and hepatic eruptions [5]. The leaves of the plant are used to raise the blister in rheumatism when applied to the skin for half an hour or little longer[6]. The fresh bruised leaves are used as rubifacient and external remedy for ringworm and other skin diseases[7]. In the Konkan the plant juice is given with water to animals when in heat to extinguish sexual appetite. The plant contains vitamin C and tannins[8]. Roots contain betulinic acid and lupeol; leaves contain lawsone, ellagic acid, quercetin, hentriacontane, dotriacontanol and ß-sitosterol glucoside and fruits contain triacontan-1, 30-diol [9].

Inflammation is an important causative agent of human morbidity and mortality, such as Systemic Inflammatory Response Syndrome, Multiple Organ Dysfunction Syndrome and Multiple Organ Failure. All the steroidal and non-steroidal anti-inflammatory drugs currently available are probably poly component in that they are able to modulate more than one mediator or cellular event concerned with the inflammatory response[10]. In the screening of new anti- inflammatory compounds,carageenan-induced oedema in the rat hind paw [11] and Freund's adjuvant induced arthritis [12] are widely employed. The perusal of literature reveals that there is no work related to anti-inflammatory and anti-arthritic activities of this whole plant. Hence the objective of the present study was to investigate the anti-inflammatory and anti-arthritic activities of the ethanol extract of the whole plant powder of *A. baccifera* using different experimental animal models.

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MATERIALS AND METHODS

Plant material

The plants were collected based on ethnopharmacologic information The plant specimens for the present study were harvested in the month of November from the wet land area around Five falls, Courtallam Hills , Western Ghats, Tirunelveli District, Tamil Nadu and identified by Dr.V.Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Palayamkottai. A voucher specimen (MSU– 036) was preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu, India.

Preparation of extracts

The fresh and mature plants were cleaned with distilled water to remove extraneous matter, shade-dried until to get constant weight and then powdered. The dried powder of the whole plant (500 g) was successively extracted using petroleum ether (40°-60°C), benzene, chloroform, ethanol and water. The last trace of solvent is removed by reduced pressure distillation and then vacuum dried. All the vacuum dried extracts in 100 mg/kg bw dose (Table 1) was used for preliminary anti-inflammatory study as a suspension in normal saline (0.5% w/v). The ethanol extract of the plant was chosen for detailed anti-inflammatory and anti-arthritic studies since it showed the maximum inhibition.

Animals

Colony in breed strains of male Wistar albino rats weighing about 150-170 g from inbred stock were used throughout the experiments. They were

given commercial diet (Hindustan Lever Ltd., Bangalore) and tap water *ad libitum*. For experiment, rats were randomly selected into groups comprising of 6 rats. All the animal experiments were carried out in the Department of Pharmacology, Periyar College of Pharmacy, Trichirappalli, Tamil Nadu, India and were approved by the ethical clearance committee of the Institute (IAEC- 265/ CPCSEA). The animals were given a week time to get acclimatized with the laboratory conditions. The animals were made to fast overnight before the experiment.

Drugs

Dose selection of the test drug, the ethanolic extract of whole plant of *A baccifera* was based on the acute toxicity test carried out over a varying dosage ranging from 50 to 800 mg/kg bw orally and the LD_{50} was estimated as 400 mg/kg bw[13]. According to the results of acute toxicity test, the doses of 100 mg/kg bw and 200 mg/kg bw were chosen for the experiments. The test drug in the form of suspension in normal saline was fed orally in a volume of 10 ml/kg bw. The standard drug, indomethacin (Ciba Geigy, India) was used as a 2% suspension in normal saline in a dose of 10 mg/kg bw.

Preliminary pharmacological screening

Preliminary pharmacological screening was performed using Carrageenan induced paw oedema method. The rats were divided into seven groups of 6 rats each. The group I received normal saline 0.5 mg/kg bw and served as control. The group II received the standard drug, indomethacin 10 mg/kg bw. The groups III, IV, V, VI and VII received the doses of 100 mg/kg bw of the petroleum ether extract, benzene extract, chloroform extract, ethanol extract and water extract of *A.baccifera* respectively. Oedema was induced by injecting 0.1 ml of carrageenan (1% w/v; Sigma, USA) in normal saline into the subplantar region of the left hind paw after 1 h of drug administration. The paw volume was measured with the help of mercury displacement plethysmometer (Model 7140, UGO Basile, Italy) at 3 h after administration of drugs.

Anti-inflammatory activity

Anti-inflammatory activity of the ethanolic extracts was performed using Carrageenan induced paw oedema method. The rats were divided into four groups of 6 rats each. The group I received normal saline and served as control. The groups II and III received the doses of 100 mg/kg bw and 200 mg/kg bw of the ethanol extract of *A. baccifera* respectively. The Group IV received the standard drug, indomethacin 10 mg/kg bw. Oedema was induced by injecting 0.1 ml of carrageenan (1% w/v; Sigma, USA) in normal saline into the subplantar region of the left hind paw after 1 h of drug administration. The paw volume was measured with the help of mercury displacement plethysmometer (Model 7140, UGO Basile, Italy) first at zero hour and then at 1, 2, 3 & 4 h after administration of drugs. The dosage details are presented in Table 2. The percentage inhibition of oedema compared with that of the control was taken as anti-inflammatory activity. The percentage inhibition of oedema was calculated by the formula: Percentage Inhibition of oedema = (A – B)/ A × 100 where, A represents the paw volume of the control group at 3 h and B represents the paw volume of the test drug treated group at 3 h.

Anti-arthritic activity

Anti-arthritic activity of the ethanolic extracts was performed using Freund's adjuvant induced arthritis method. The rats were divided into five groups of 6 animals each. Adjuvant arthritis was induced by subcutaneous injection of complete Freund's adjuvant (CFA - 0.1ml of 0.5% w/v suspension of heat killed *Mycobacterium tuberculosis* cells in liquid paraffin) into the plantar tissue of the right hind paw of each rat. Rats in this group were known as inflamed control group I. The groups II and III received the doses of the test drugs 100 mg/kg bw and 200 mg/kg bw respectively. The group IV received the standard drug (10 mg/kg bw). The group V served as the non-inflamed control group consisted of rats injected with 0.1 ml of liquid paraffin. The test groups consisted of complete Freund's adjuvant (CFA)-injected rats challenged with doses of the test drug administered orally 2 h before induction of arthritis. The drug administrations were continued daily at the same time of the day for 19 more days. Development of adjuvant induced oedema in the paws of both the injected and non-injected paws of each rat were monitored daily as the percentage increase in paw volume. The percentage inhibition of paw volume compared with that of the inflamed control was taken as anti-arthritic activity. The dosage details of the arthritis experiment are presented in Table 3.

Data analysis

The data were expressed as mean ± SEM. Statistical analysis was performed one-way ANOVA followed by Dunnett's multiple comparison test using sigma stat software (Version 2.0, Jandel Scientific Inc. USA). The 'P' values less than 0.001 were considered as significant.

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RESULTS

Effect of Preliminary pharmacological screening

The results of preliminary pharmacological screening revealed that the ethanolic extract of *A. baccifera* exhibited a maximum inhibition of carrageenan induced paw oedema (38.23 ± 1.97) compared to that of the petroleum ether extract (05.88± 2.07), benzene extract (23.52± 1.67), chloroform extract (29.41 ± 2.11), and water extracts (32.35 ± 1.84) as shown in Table 1.

Table 1. Preliminary pharmacological screening of *A. baccifera* extracts on carrageenan induced rat hind paw oedema

Sl. No.	Drug	Dose (mg/kg)	Increase in paw volume after three hours.	% Inhibition in paw volume
1.	Control (Group I)	0.5 (ml/kg)	0.34 ± 0.01	
2.	Standard (GroupII) Indomethacin	10	0.18 ± 0.01	47.05 ± 2.01
3.	Petroleum ether (40°- 60°C) extract (GroupIII)	100	0.31± 0.01	05..82± 2.07
4.	Benzene extract (GroupIV)	100	0.26 ± 0.01	23.52± 1.67
5	Chloroform extract(GroupV)	100	0.24 ± 0.01	29.41 ± 2.11
6.	Ethanol extract (GroupVI)	100	0.21 ± 0.01	38.23 * ± 1.97
7.	Water extract (GroupVII)	100	0.23 ± 0.01	32.35 ± 1.84

** P < 0.001 as compared to Control (ANOVA followed by Dunnett's t-test). Each value is the mean ± SEM of 6 rats weighing 150-170 g.*

Effect of the carrageenan induced paw oedema

The ethanolic extract of *A. baccifera* produced a dose dependent inhibition of carrageenan induced paw oedema. The inhibition started at 1h, which continued till 3 h in comparison to that of the control. Oral administration of 100 mg/kg bw and 200 mg/kg bw doses of the ethanol extract produced 38.27% and 43.39% inhibition respectively after 3 h as compared with that of the standard drug which showed 48.52% inhibition (Table 2).

Freund's adjuvant induced arthritis

The anti-arthritic effects of the ethanol extract of *A. baccifera* started on day 3, which continued till day 19 in comparison to the control (Table 3). In the case of standard drug maximum inhibition was observed on day 5 itself, whereas in the case of 100 mg/kg bw and 200 mg/kg bw doses of the test drug maximum inhibition was noticed on day 7. In all the three cases the inhibition started decreasing after day 7 and again reached the maximum on day 19 (Table 3).

DISCUSSION

Carrageenan-induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic.

Table 2. Anti-inflammatory activity of ethanol extract of *Ammania baccifera* against carrageenan induced paw oedema in albino rats

Treatment	% increase in paw volume ± S.E.M (n=6) Post insult time of assay in hours					% Inhibition in
	0 h	1 h	2 h	3 h	4 h	paw volume (% of AIA)
Control (0.5 ml/kg)	39.91± 1.53	69.32±3.12	97.83±8.13	108.59±9.09	109.81±8.33	-
Extract (100mg/kg)	32.82± 1.95	52.13±4.96	69.92±6.1	67.03*±6.1	61.84*±5.12	38.27
Extract (200mg/kg)	31.53±2.57	46.92±4.3	65.27±5.3	61.47*±4.7	57.62*±4.9	43.39
Indomethacin(10mg/kg)	27.9±0.92	33.8± 1.83	38.8±2.32	55.9*±3.21	58.82*±3.92	48.52

**P < 0.001 as compared to Control (ANOVA followed by Dunnett's t-test). Each value is the mean ± SEM of 6 rats weighing 150-170 g.*

Table 3. Anti-arthritic activity of ethanol extract of *Ammania baccifera* against Freund's adjuvant induced arthritis

Treatment	% Increase in paw volume Post insult time of assay in days									
	1	3	5	7	9	11	13	15	17	19
Control (Inflamed)	101.8±4.18	182.3± 5.12	200.9±7.56	206.8±4.2	192.8±7.3	180.2±7.8	201.3±6.54	219.4±8.19	239.6±9.7	243.9±6.81
Control (Non-Inflamed)	12.5±0.71	24.5±1.6	29.8±1.72	42.8±3.67	26.2±1.52*	25.6±1.34*	27.8±1.69*	23.5±1.38*	19.8±1.20*	18.2±0.89*
A. baccifera extract (100 mg/kg)	91.5±3.2 (10.12)	136.4±6.9 (25.71)	112.5±9.4 (44.00)	114.2±7.4 (44.78)	122.5±4.9* (36.46)	130.6±6.8* (27.53)	134.7±9.6* (33.09)	147.5±10.7* (32.77)	151.4±11.8* (36.81)	149.2)±11.5* (38.83)
A. baccifera extract (200 mg/kg)	89.3±5.4 (12.28)	132.6±9.5 (27.78)	108.7±8.6 (45.89)	107.9±7.5 (47.82)	119.6±5.9* (37.97)	126.9±6.3* (29.58)	129.4±7.1* (35.72)	139.5±10.2* (36.42)	144.7±12.5* (39.61)	136.4±10.6* (44.08)
Indomethacin (10 mg/kg)	69.8±4.32 (31.43)	90.2±5.15 (50.87)	73.7±4.61 (63.32)	81.1±4.82 (60.78)	90.2±6.45* (53.22)	96.7±4.98* (46.34)	97.4±6.05* (51.62)	104.9±6.27* (52.19)	107.1±8.24* (55.30)	108.6±5.72* (55.47)

** P < 0.001 as compared to Control (ANOVA followed by Dunnett's t-test).Each value is the mean ± SEM of 6 rats weighing 150-170 g.The number in the parentheses indicates the percentage inhibition of the inflammation.*

The first phase (1-2hr) is due to the release of histamine or serotonin, the second phase is due to kinin like substances[14]. The second phase is said to be promoted by prostaglandin like substances. It has been reported that the second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin[11]. The results of carrageenan induced rat paw oedema model indicated the dose dependent anti-inflammatory activity. The 200 mg/kg bw dose of the ethanol extract of *A. baccifera* was more active than 100 mg/kg bw dose which were found to be statistically significant (Table 2).Intraplantar injection of CFA into the right foot paw of rats induced an inflammatory response characterized by paw swelling in both the ipsilateral and the contralateral paw. The response on the injected paw was biphasic. It consists of an acute and a polyarthritic/chronic phase corresponding to day 0 - 10 and day 10 - 28 post CFA inoculation respectively. The acute phase response was characterized by unilateral inflammatory oedema of the ipsilateral paw peaking around days 4 - 6, followed by subsequent polyarthritic/chronic phase response which began around day 10 characterized by inflammatory oedema of the contralateral paw. In the present study, 100 mg/kg bw and 200 mg/kg bw of the ethanol extract of *A. baccifera* considerably reduced the complete Freund's adjuvant (CFA) induced arthritis in the hind paw of rats as compared with that of the standard drug. The effect of 200 mg/kg bw dose was higher (44.08%) than 100 mg/kg bw dose (38.83%) and almost closer to that of the standard drug (55.47%) at day 19 (Table 3). The results of the studies on paw volume showed statistically significant increase in arthritic control, whereas it showed a significant decrease in treated group. The cordial signs of the inflammatory reactions like redness, swelling, arthalgia and immobility of affected joints were significantly less in the drug treated rats than those of the control.

The present study revealed significant anti-inflammatory and anti-arthritic activities of the ethanol extract of *A. baccifera*. Both activities were dose dependent and the dose of 200 mg/kg bw was more effective than that of 100 mg/kg bw. Therefore, it is concluded that the *A. baccifera* extract is capable of inhibiting inflammatory reactions as well as inflammatory pain. The results of the present study support the traditional use of this plant in some inflammation and painful conditions which confirm the presence of active chemical compounds related to these activities. However, further investigations are required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

ACKNOWLEDGEMENT

The authors would like to thank Dr. V. Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Palayamkottai, Tamil Nadu for identification of the plant and Dr. A. Jaswanth, Head, Department of Pharmacology, Periyar College of Pharmacy, Tiruchirappalli, Tamil Nadu for his help in animal experiments. One of the author M. Kamalutheen would like to thank the UGC for financial assistance and thank the correspondent of his college.

REFERENCES

1. Chatterjee GK, Pal SP, Search for anti-inflammatory agents from Indian Medicinal Plants- A review. Indian Drugs, 21, 1984, 413.
2. Rainsford KD, Whitehouse MW, Anti-inflammatory/anti- pyretic salicylic acid esters with low gastric ulcerogenic activity, Agents Action 10,1980, 451-455.
3. Huang KC., The pharmacology of Chinese Herbs. London, CRC Press, 1999, 199.
4. The Wealth of India, Publication and Information Directorate, Vol. VI , CSIR, New Delhi, 1962,354.
5. Chopra RN, Nayar SL, Chopra IC, Glossary of Indian Medicinal Plants. National Institute of Science Communications, CSIR Publications, New Delhi, India, 1986, 330.
6. Nadkarni KM, Indian Materia Medica, Vol. I, Popular Parkashan Pvt. Ltd, Bombay, 1976, 91.
7. Yoganarasimhan SN, Medicinal Plants of India, Tamil Nadu, Vol. II ,Regional Research Institute (Ay.), Bangalore, India, 2000, 40.
8. Atal CK, Srivastava, JB, Wali, BK, Chakravarty RB, Dhawan BN, Rastogi RP, Indian J Exp Biol , 16, 1978,330.
9. Thakkar SM, Deshmukh VK, Saoji AN, Duragkar NJ, J Indian Chemical Soc, 63(6),1986, 619.
10. Whitehouse MW, Introduction and background to the regulation of inflammation and the immune response. In: RA Scherrer and MW Whitehouse (Eds.). Anti-inflammatory agents: Chemistry and Pharmacology, Vol.2. Academic Press, New York: 1964.
11. Winter CA, Risley EA, Nuss CW, Proceedings of Society of Experimental Biology and Medicine 111,1962, 544.
12. Newbould BB, Br.J. Pharmacol., 21, 1963, 127.
13. Miller LC, Tainter ML, Proc Soc Exp Biol Med, 57, 1944, 261.
14. Vinegar R, Schreiber W, Hugo R. Journal of Pharmacology and ExperimentalTherapeutics 166,1969, 96.

Source of support: Nil, Conflict of interest: None Declared