Anti-inflammatory and Anti-arthritic Activities of *Merremia tridentata* (L.) Hall. f.

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Abstract: The various extracts of *Merremia tridentata* were investigated for its anti-inflammatory and anti-arthritic activities in male albino rats. The anti-inflammatory activity was carried out using carrageenan-induced rat paw oedema model and the anti-arthritic activity was carried out using complete Freund's adjuvant induced arthritis model. Indomethacin (10 mg/kg bw) was used as a standard drug. The ethanol extract of *M. tridentata* exhibited significant dose dependent activity in acute inflammation and the doses of 100 mg/kg bw and 200 mg/kg bw produced 38.3% and 42.8% inhibition respectively after 3 h as compared with that of the standard drug which showed 48.5% inhibition. In arthritis model, the doses of 100 mg/kg bw and 200 mg/kg bw of the ethanol extract produced 49.0% and 51.7% inhibition respectively after 19 days when compared with that of the standard drug (55.5%). Both doses of the ethanol extract of *M. tridentata* exhibit significant anti-inflammatory and anti-arthritic activities.

Keywords: *Merremia tridentata*, Anti-inflammatory, Carrageenan-induced oedema, Freund's adjuvant induced arthritis.

Introduction

Merremia tridentata (L.) Hall. f. (Fam. *Convolvulaceae*) commonly known as "Mudiarkunthal" or "Savulikodi" or "Thrippan Pullu" in Tamil and "Prasarini" in Sanskrit is reported to possess a number of medicinal values¹. It is a thick climbing herb with woody rootstock spreading on the walls and on the grounds. It grows in all plain districts of Tamilnadu, Western peninsula and Bengal². The plant is considered bitter, astringent, tonic and used in the treatment of rheumatism, piles, swellings and urinary disorders¹. The decoction of the root is also used in toothache. diosmetin, luteolin, diosmetin-7-O- β -D-glucoside and luteolin-7-O- β -D-glucoside have been isolated from *M. tridentata*³. Ergosine alkaloids have been isolated from the seeds of *M. tridentata*⁴. Pyrolidine alkaloids like hygrins and nicotine

have been isolated from the root and the aerial parts of *M.tridentata*⁴. 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 response⁵. In the screening of new anti-inflammatory compounds, carrageenan-induced oedema in the rat hind paw⁶ and Freund's adjuvant induced arthritis⁷ are widely employed. In the present work, the anti-inflammatory and anti-arthritic activities of the ethanol extract of the whole plant have been performed using these models.

Experimental

Preparation of the extracts

The plant was collected in the month of August 2007 from St. Xavier's College campus, Palayamkottai, Tirunelveli District, Tamilnadu, India and identified by Dr.V.Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU- 034) was preserved in the Herbarium of the Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli-627 012, Tamilnadu, India. The fresh 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) were 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.

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

S. No.	Drug	Dose mg/kg	Increase in paw volume after three hours.	% Inhibition in paw volume
1	Control	0.5, mL/kg	0.34 ± 0.01	-
2	Standard Indomethacin	10	0.12 ± 0.01	48.52 ± 2.01
3	Petroleum ether (40°-60°C) extract	100	0.32± 0.01	15.6± 3.14
4	Benzene extract	100	0.28 ± 0.01	17.7 ± 3.06
5	Chloroform extract	100	0.24 ± 0.01	29.4 ± 1.97
6	Ethanol extract	100	0.16 ± 0.01	38.3* ± 1.97
7	Water extract	100	0.22 ± 0.01	35.3* ± 2.04

* $P < 0.001$ as compared to control (ANOVA followed by Dunnett's *t* test)

Each value is the mean ± SEM of 6 rats weighing 150-170 g.

Animals

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, Tamilnadu, India and were approved by the Institutional Animal Ethical Committee (IAEC – 265/CPCSEA). The animals were given one week time to get acclimatized with laboratory conditions. The animals were made to fast over night before the experiment.

Drugs

Dose selection of the test drug, the ethanolic extract of *M. tridentata* was based on the acute toxicity test carried out over a dosage range of *M. tridentata* varying from 50, 100, 200, 400, 600 and 800 mg/kg bw orally and the LD₅₀ was estimated⁸ as 400 mg/kg bw. According to the results of acute toxicity test, the doses of 100 mg/kg bw and 200 mg/kg bw were chosen for the experiment. 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.

Carrageenan-induced oedema in rats

The rats were divided into four groups of 6 rats each. Group I received normal saline and served as control. Group II and III received the doses of 100 mg/kg bw and 200 mg/kg bw of the ethanol extract of *M. tridentata* respectively. 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 sub plantar region of the left hind paw after 1 h of drug administration⁶. The paw volume was measured with the help of mercury replacement 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:

$$\text{Percentage Inhibition of oedema} = (A - B) / A \times 100$$

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.

Table 2. Anti-inflammatory activity of ethanol extract of *M. tridentata* against carrageenan induced paw oedema.

Treatment	% Increase in paw volume					% Inhibition in paw volume
	Post insult time of assay in hours					
	0	1	2	3	4	
Control,	39.91	69.32	97.83	108.59	109.81	-
0.5 mL/kg	± 1.53	± 3.12	± 8.13	± 9.09	± 8.33	
<i>M.tridentata</i>	27.92	51.24	70.56	67.31*	63.26*	38.27
extract,	± 1.68	± 4.20	± 5.40	± 5.30	± 5.12	
100 mg/kg						
<i>M. tridentata</i>	29.37	47.68	72.17	62.16*	59.92*	42.75
extract,	± 2.57	± 4.30	± 5.30	± 4.70	± 4.90	
200 mg/kg						
Indomethacin,	27.90	33.80	38.80	55.90*	58.82*	48.52
10 mg/kg	± 0.92	± 1.83	± 2.32	± 3.21	± 3.92	

*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.

Freund's adjuvant induced arthritis

Rats were divided into five groups of 6 animals each. Adjuvant arthritis was induced by subcutaneous injection of complete Freund's adjuvant (CFA - 0.1 mL 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 rat⁸. Rats in this group were known as inflamed control group I. Group II and Group III received the doses of the test drugs 100 mg/kg bw and 200 mg/kg bw

respectively. Group IV received the standard drug (10 mg/kg bw). 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 swelling 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 $\pm$ 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 values of 'p' less than 0.001 were considered as significant.

Results and Discussion

The ethanol extract of *M. tridentata* produced a dose dependent inhibition of carrageenan induced paw oedema. The inhibition started at 1 h, which continued till 3 h when compared with the control. Oral administration of 100 mg/kg bw and 200 mg/kg bw of the ethanol extract produced 38.3% and 42.8% inhibition respectively after 3 h as compared with that of the standard drug which showed 48.5% inhibition (Table 2).

The anti-arthritic effect of the ethanol extract of *M. tridentata* started on day 3, which continued till day 19 when compared with that of 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 inhibitions were noticed on day 9. In all the three cases the inhibition started decreasing after day 9 and again reached the maximum on day 19 (Table 3).

Oedema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and the second phase is due to kinin like substances⁹. 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 indomethacin⁶. The results of carrageenan induced rat paw oedema model indicated the dose dependent anti-inflammatory activity i.e. the dose effect of 200 mg/kg bw of the ethanol extract of *M. tridentata* was more active than 100 mg/kg bw which were found to be statistically significant (Table 2). In the present study, 100 mg/kg bw and 200 mg/kg bw of the ethanol extract of *M. tridentata* significantly reduced the complete Freund's adjuvant (CFA) induced chronic inflammation in the knee joint of rats as compared with that of the standard drug. The dose effect of 200 mg/kg bw was maximum (51.7%) than 100 mg/kg bw (49.0%) and almost near value to the standard drug (55.5%) at day 19 (Table 3). In CFA induced poly arthritis, the drug under investigation, significantly reduced the paw volume of the affected rats as compared with that of the control. The cordial signs of the chronic inflammatory reactions like redness, swelling, arthralgia and immobility of affected joints were significantly less in the drug treated animal than those of the control.

Table 3. Anti-arthritic activity of ethanol extract of *M. tridentata* 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	183.6 ± 5.2	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*
<i>M. tridentata</i> extract, 100 mg/kg	78.8 ± 5.6 (22.59)	106.6 ± 7.4 (41.94)	95.4 ± 4.8 (52.51)	89.5 ± 5.3 (56.72)	75.4 ± 4.8* (60.89)	88.9 ± 6.2* (50.67)	104.3 ± 8.2* (48.24)	113.9 ± 9.4* (48.09)	127.4 ± 7.3* (47.77)	122.7 ± 8.1* (48.97)
<i>M. tridentata</i> Extract, 200 mg/kg	78.9 ± 4.2 (22.49)	101.8 ± 8.3 (44.55)	88.9 ± 5.1 (55.75)	83.6 ± 4.9 (59.57)	72.1 ± 3.7* (62.60)	82.5 ± 5.8* (54.22)	99.4 ± 7.2* (50.62)	107.5 ± 9.1* (51.00)	117.6 ± 8.6* (50.96)	115.8 ± 9.5* (51.67)
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 $\pm$ SEM of 6 rats weighing 150-170 g. The number in the parentheses indicates the percentage inhibition of the inflammation.

The present study revealed significant anti-inflammatory and anti-arthritic activities of the ethanol extract of *M. tridentata*. Both activities were dose dependent and the dose of 200 mg/kg bw was more effective than that of 100 mg/kg bw. It may be concluded that 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.

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