

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Original article

doi: 10.12980/jclm.4.2016j5-153

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Analgesic, anti-inflammatory and antipyretic activities of methanolic leaf extract of *Maerua crassifolia*

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ARTICLE INFO

Article history:

Received 19 Aug 2015

Received in revised form 6 Sep 2015

Accepted 8 Oct 2015

Available online 1 Mar 2016

Keywords:

Maerua crassifolia

Leaf

Analgesia

Inflammation

Pyrexia

Rodents

ABSTRACT

Objective: To investigate the the analgesic, anti-inflammatory and antipyretic activities of the methanolic leaf extract of *Maerua crassifolia* in mice and rats.

Methods: Acetic acid-induced writhing and tail immersion methods were used to assess analgesic activity, while xylene and carrageenan-induced paw oedema methods were used to evaluate the anti-inflammatory effect of the leaf extract. Yeast and amphetamine-induced pyrexia were used to investigate the antipyretic activity. The phytochemical analysis and oral acute toxicity of the methanolic leaf extract of *Maerua crassifolia* were also evaluated.

Results: The leaf extract (100, 200, and 400 mg/kg) showed a dose dependent and significant ($P < 0.05$) inhibition of pain in acetic acid-induced writhing and tail immersion tests. The extract also produced significant ($P < 0.05$) anti-inflammatory activity in both paradigms. A significant ($P < 0.05$) reduction in hyperpyrexia was also observed with the leaf extract. The phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids, resins, saponins and cardiac glycosides. The oral median lethal dose of the leaf extract was estimated to be greater than 5000 mg/kg in rats.

Conclusions: The findings confirmed its ethnomedical use in the treatment of pains and feverish conditions.

1. Introduction

Many plants synthesize substances that are useful to the maintenance of human health. Before the availability of synthetic drugs, man was completely dependent on natural medicinal plants for curing diseases[1]. Natural medicines produced from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. Many herbal remedies have been used traditionally for the treatment of diseases in Nigeria[2]. The World Health Organization encourages the inclusion of herbal

medicine in health care because of the great potentials they possess. Natural plant products such as herbs, fruits and vegetables became more popular in recent years due to public awareness and increasing interest among consumers and scientific community[3]. Epidemiological evidence has revealed that constituents in natural products show many biological and pharmacological activities, including anti-inflammatory and antiviral effects[4,5].

Maerua crassifolia (*M. crassifolia*) which belongs to the family Capparaceae is mainly found in the Saharan Africa, especially Nigeria and Niger Republic. The leaf of this plant has been long used for the treatment of malaria[6], toothache and intestinal diseases[7,8], antibacterial and antioxidant[9]. However, the plant has not been experimentally tested for its analgesic, anti-inflammatory and antipyretic activities. Hence, an effort was made to investigate the same with the leaf extract in experimentally induced pains and hyperpyrexia in rodents.

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Materials and methods

2.1. Collection of plant material

Fresh leaves of *M. crassifolia* were collected in the month of March, 2009 from Sokoto (North West), Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria where a voucher specimen (6406) was deposited in the herbarium for reference. The international plant number index is Fl. Aegypt.-Arab. P. Cxiii. 1775 (1 Oct. 1775).

2.2. Plant extraction

The leaves were washed, reduced to smaller pieces and dried at room temperature for 7 days and pulverized to a coarse powder. The powder (500 g) was macerated in methanol for 24 h. The resultant filtrate was dried on a water bath at reduced temperature to obtain 13.28% w/w of methanol extract. The extract was stored in an airtight container and later used for the study.

2.3. Phytochemical analysis

The phytochemical screening of methanol extract of *M. crassifolia* leaf was carried out to determine using standard procedures[10,11].

2.4. Animals

Adult Albino rats (180–220 g) and Albino mice (18–22 g) of both sexes were purchased from animal house of University of Nigeria, Nsukka, Enugu State, Nigeria. They were kept to acclimatize in laboratory condition for 14 days before commencement of experiment. Animals were grouped and housed in cages with not more than six per cage and exposed to standard pellets and water. All experiments were carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals[12].

2.5. Acute toxicity test

The acute toxicity of *M. crassifolia* leaf extract was tested to determine its safety adopting the Guidelines for the Testing of Chemicals[13]. The studies were done in two phases. Nine rats, randomized and divided into three were used in the first phase. The rats were orally administered with 100, 600 and 1 000 mg/kg of the leaf extract, respectively. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. This was followed by the second phase in which 2000, 3000 and 5000 mg/kg of the extract was administered to the next three groups of three rats per cage. The signs of toxicity and mortality were observed for 24 h, 48

h and 72 h, respectively.

2.6. Pharmacological tests

2.6.1. Acetic acid-induced writhing test

Analgesic activity of methanol leaf extract of *M. crassifolia* against acetic acid-induced writhing was carried out following the procedure of Nwafor and Okwuasaba[14], Akuodor *et al.*[15]. The adult Albino mice used for this study were randomized into 5 groups of 6 mice per cage. Group 1 which served as control received distilled water (20 mL/kg), while Groups 2–4 received 100, 200 and 400 mg/kg of the leaf extract. Group 5 received 150 mg/kg of acetylsalicylic acid. All administered via cannula. Thirty minutes after drug administration, each mouse was injected 0.7% acetic acid intraperitoneally to create pain sensation. Each mouse was later placed in a transparent observation box. The number of abdominal constrictions for each mouse was counted for 30 min, commencing 5 min after acetic acid injection.

2.6.2. Tail immersion test

This was based on the method described by Jansen and Jagenau[16]. The Albino mice selected for this study were randomized into 5 groups of 6 per cage. Group 1 (negative control) received distilled water (20 mL/kg), while Groups 2–4 received 100, 200 and 400 mg/kg of the leaf extract of *M. crassifolia*, respectively. Group 5 received 10 mg/kg of morphine subcutaneously. Thirty minutes after drug administration, each mouse was restrained in a horizontal cylinder leaving the tail hanging freely. The 4 cm portion of the tail was marked and this part of the tail was immersed in a water bath thermo-statically maintained at $(50 \pm 1) ^\circ\text{C}$ and the time taken for the animal to remove its tail out of the water was recorded. The initial reading was taken immediately before administration of test and standard drugs. The tail flick latency was evaluated at 30, 60, 90 and 120 min. The initial reading was taken immediately before administration of test samples. All the drugs were given by oral route.

2.6.3. Xylene-induced ear oedema in mice

The mice used for this study were randomly divided into 5 groups of 6 in each cage. Group 1 which served as control (negative) received 20 mL/kg distilled water, while Groups 2–4 received 100, 200 and 400 mg/kg of the leaf extract of *M. crassifolia* (oral), respectively. Group 5 received 4 mg/kg of dexamethasone (oral). One hour after drug administration, oedema was induced in each mouse by applying a drop of xylene at the inner surface of the right ear. Three hours afterwards, the animals were sacrificed under light ether anaesthesia and both ears were cut off to approximately equal size and weight. The mean differences between the right and left ear were determined for each group and recorded as an indication of inflammation[17].

2.6.4. Carrageenan-induced inflammation in rats

Adult Albino rats (180–220 g) of both sexes used for this study were divided into 5 groups of 6 per cage. Group 1 which served as control (negative) received 20 mL/kg of distilled water, and Groups 2–4 received 100, 200 and 400 mg/kg of the leaf extract, respectively. Group 5 was administered 150 mg/kg of aspirin. After one hour, inflammation of the hind paw was induced in rat by injection of 0.1 mL of freshly prepared carrageenan suspension (1%) in distilled water into the subplantar tissue of the right hind paw to all the groups[18-20]. The paw volumes were measured at 0.5, 1, 2, 3, 4 and 5 h, respectively by using plethysmometer. The average swelling of paws in the groups of extract treated was compared with control group and the standard. The extract and standard drug were given orally.

2.6.5. Yeast-induced hyperpyrexia

Adult Albino rats (180–220 g) of both sexes were employed for this study. The rats were randomized into 5 groups of 6 in each cage. The basal temperature of each rat was taken and subcutaneously injected with 20 mL/kg of 15% aqueous suspension of Brewer's yeast suspended in methylcellulose to induce pyrexia. Twenty-four hours after yeast injection, the rectal temperature was again recorded and rats showing a minimum increase of less than 0.6 °C were discarded. Group 1 which served as control (negative) received 20 mL/kg of distilled water, whereas the leaf extract of *M. crassifolia* was administered at a dose of 100, 200 and 400 mg/kg to rats in Groups 2, 3 and 4, respectively. Group 5 received 20 mg/kg of paracetamol. The rectal temperature of each rat was again taken at 1 h interval for 5 h[21,22]. All drugs were administered by oral route.

2.6.6. Amphetamine-induced hyperlexia

The method described by Akuodor *et al.*[23], and Mbagwu *et al.*[24] was adopted with slight modification. Adult Albino rats used in this experiment were randomized into 5 groups of 6 rats each. The anal temperature of each mouse was taken before administration of amphetamine (10 mg/kg, *i.p.*) to induce pyrexia. Thirty minutes after dose administration, Group 1 (negative control) received 20 mL/kg of distilled water, while Groups 2–4 received 100, 200 and 400 mg/kg *p.o.* of the leaf extract of *M. crassifolia*, respectively. Group 5 was treated with the standard drug, paracetamol (20 mg/kg *p.o.*). Both the extract and the standard drug were orally administered. Thereafter, the rectal temperature reading of each rat was however recorded at 1 h interval for 5 h.

2.6.7. Statistical analysis

Data were expressed as mean ± SEM. The significant difference between mean was determined using One-way ANOVA to analyse results between groups. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Phytochemical screening

Phytochemical screening of the methanol leaf extract of *M. crassifolia* revealed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids, resins, saponins and cardiac glycosides, while anthraquinones and phlorotannins were not detected.

3.2. Acute toxicity test

There was no lethality observed at any of the doses of methanolic leaf extract of *M. crassifolia* used in the study. All animals were alive, healthy and active during the observation period. The oral acute toxicity test of the leaf extract was estimated to be greater than 5000 mg/kg in rats. Thus, the experimental doses used (100, 200 and 400 mg/kg *p.o.*) were within safe margin.

3.3. Acetic acid-induced writhing in mice

The methanolic leaf extract of *M. crassifolia* (100, 200 and 400 mg/kg) dose-dependently reduced abdominal constrictions in mice. The reduction was significant ($P < 0.05$) when compared with control (Table 1). The effect of the extract was comparable to that of the standard drug, aspirin (150 mg/kg).

Table 1

Effect of methanolic leaf extract of *M. crassifolia* on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Writhing	% Inhibition
Control	0.2 mL/kg	18.65 ± 1.40	-
<i>M. crassifolia</i>	100	7.29 ± 0.30	61*
	200	5.60 ± 0.33	70*
	400	3.71 ± 0.42	80*
Aspirin	150	3.10 ± 0.21	83*

Results were expressed as mean ± SEM, $n = 6$. *: Significant at $P < 0.05$ when compared to control.

3.4. Tail immersion

The mean latency of nociceptive response to thermal stimuli in the tail immersion test was summarized in Table 2. *M. crassifolia* leaf extract significantly ($P < 0.05$) exerted protective activity from heat-induced pain in mice. However, the standard drug, morphine produced a greater protection than the extract.

3.5. Xylene-induced ear oedema

Anti-inflammatory effect of leaf extract of *M. crassifolia* against xylene-induced ear oedema in mice was shown in Table 3. The extract at various doses administered, significantly ($P < 0.05$) reduced the xylene-induced ear oedema in mice. The effect was comparable to the standard drug, aspirin (150 mg/kg).

Table 2Effect of methanolic leaf extract of *M. crassifolia* on thermal stimuli [(50 ± 1) °C hot water] response in the tail immersion test in mice.

Treatment	Dose (mg/kg)	Pre-treatment			Post-treatment			
		0 min	30 min	60 min	90 min	120 min	100 min	
Control	0.2 mL/kg	7.20 ± 1.30	7.90 ± 0.42	8.30 ± 1.35	10.22 ± 1.20	12.40 ± 1.30	13.33 ± 1.22	
<i>M. crassifolia</i>	100	7.35 ± 0.56	11.10 ± 0.20	13.38 ± 0.17	14.09 ± 0.62	15.64 ± 0.55*	14.67 ± 0.08	
	200	7.63 ± 0.43	13.19 ± 0.30	14.50 ± 0.43	16.40 ± 0.28	18.28 ± 0.22*	15.83 ± 1.05	
	400	7.92 ± 0.55	15.35 ± 0.77	16.60 ± 0.62	18.37 ± 0.52	20.17 ± 0.20*	17.00 ± 0.62	
Morphine	10	7.73 ± 1.64	17.70 ± 1.33	20.45 ± 0.71	22.00 ± 0.14	23.66 ± 0.33*	23.50 ± 1.09	

Results were expressed as mean ± SEM, n = 6. *: Significant at P < 0.05 when compared to control.

Table 3Effect of methanolic leaf extract of *M. crassifolia* on carrageenan-induced paw oedema in rats.

Treatment	Dose (mg/kg)	Paw oedema volume (mL) versus time (h)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	0.2 mL/kg	1.23 ± 0.05	1.66 ± 0.04	1.74 ± 0.04	1.85 ± 0.03	1.88 ± 0.03	1.98 ± 0.03	2.11 ± 0.05
<i>M. crassifolia</i>	100	1.25 ± 0.04	1.69 ± 0.03	1.60 ± 0.03	1.49 ± 0.04	1.41 ± 0.04	1.34 ± 0.04	1.29 ± 0.04*
	200	1.18 ± 0.05	1.62 ± 0.05	1.54 ± 0.04	1.41 ± 0.02	1.36 ± 0.03	1.30 ± 0.03	1.24 ± 0.04*
	400	1.20 ± 0.03	1.60 ± 0.05	1.54 ± 0.04	1.46 ± 0.02	1.33 ± 0.03	1.28 ± 0.03	1.21 ± 0.03*
Aspirin	150	1.22 ± 0.04	1.70 ± 0.04	1.62 ± 0.04	1.54 ± 0.03	1.39 ± 0.04	1.27 ± 0.03	1.18 ± 0.03*

Results were expressed as mean ± SEM, n = 6. *: Significant at P < 0.05 when compared to control.

3.6. Carrageenan-induced oedema

The result of the effect of methanolic leaf extract of *M. crassifolia* on carrageenan-induced paw oedema was shown in Table 4. The extract at various doses administered significantly (P < 0.05) exhibited dose-dependent anti-inflammatory activity in rat when compared to control. The effect of the extract was comparable to that of the standard drug, dexamethasone (4 mg/kg).

Table 4Effect of methanolic leaf extract of *M. crassifolia* on xylene-induced ear oedema in mice.

Treatment	Dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% Inhibition
Control	0.2 mL/kg	0.088 ± 0.02	0.046 ± 0.01	0.042 ± 0.01	-
<i>M. crassifolia</i>	100	0.065 ± 0.01	0.046 ± 0.01	0.019 ± 0.01	55*
	200	0.052 ± 0.00	0.041 ± 0.00	0.011 ± 0.00	74*
	400	0.044 ± 0.00	0.036 ± 0.00	0.008 ± 0.00	81*
Dexamethasone	4	0.045 ± 0.00	0.037 ± 0.00	0.008 ± 0.00	83*

Results were expressed as mean ± SEM, n = 6. *: Significant at P < 0.05 when compared to control.

Table 5Effect of methanolic leaf extract of *M. crassifolia* on yeast-induced pyrexia in rat.

Treatment	Dose (mg/kg)	Rectal temperature (°C)						
		0 h	24 h	1 h	2 h	3 h	4 h	5 h
Control	0.2 mL/kg	35.55 ± 0.17	36.67 ± 0.15	36.79 ± 0.14	36.90 ± 0.13	37.45 ± 0.15	37.62 ± 0.14	37.88 ± 0.16
<i>M. crassifolia</i>	100	36.15 ± 0.12	37.44 ± 0.08	37.00 ± 0.09	36.67 ± 0.10	36.55 ± 0.11	36.27 ± 0.10	36.17 ± 0.11*
<i>M. crassifolia</i>	200	35.66 ± 0.10	36.90 ± 0.10	36.45 ± 0.11	36.35 ± 0.08	36.22 ± 0.07	35.85 ± 0.05	35.61 ± 0.07*
<i>M. crassifolia</i>	400	35.68 ± 0.11	36.50 ± 0.07	36.44 ± 0.07	36.21 ± 0.05	36.00 ± 0.10	35.69 ± 0.11	35.56 ± 0.60*
Paracetamol	20	35.70 ± 0.10	36.88 ± 0.06	36.66 ± 0.10	36.40 ± 0.08	36.20 ± 0.06	35.70 ± 0.50	35.50 ± 0.11*

Results are expressed as mean ± SEM, n = 6. *: Significant at P < 0.05 when compared to control.

Table 6Effect of methanolic leaf extract of *M. crassifolia* on amphetamine-induced pyrexia in rat.

Treatment	Dose (mg/kg)	Rectal temperature (°C)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	0.2 mL/kg	35.70 ± 0.04	36.66 ± 0.03	36.70 ± 0.04	36.79 ± 0.03	36.85 ± 0.02	36.95 ± 0.02	37.40 ± 0.02
<i>M. crassifolia</i>	100	35.60 ± 0.12	36.55 ± 0.11	36.42 ± 0.10	36.20 ± 0.07	36.11 ± 0.08	35.99 ± 0.08	35.64 ± 0.09*
<i>M. crassifolia</i>	200	35.52 ± 0.06	36.85 ± 0.08	36.55 ± 0.06	36.55 ± 0.09	36.20 ± 0.04	35.84 ± 0.06	35.54 ± 0.05*
<i>M. crassifolia</i>	400	35.54 ± 0.03	36.76 ± 0.05	36.49 ± 0.06	36.30 ± 0.05	36.15 ± 0.03	35.79 ± 0.03	35.52 ± 0.04*
Paracetamol	20	35.53 ± 0.05	36.77 ± 0.04	36.45 ± 0.05	36.20 ± 0.06	36.10 ± 0.03	35.75 ± 0.04	35.50 ± 0.05*

Results were expressed as mean ± SEM, n = 6. *: Significant at P < 0.05 when compared to control.

3.7. Yeast-induced pyrexia

The extract at various doses administered, significantly (P < 0.05) decreased the temperature of rats in a dose-dependent manner compared to the control (Table 5). The reduction was comparable to the standard drug, paracetamol (20 mg/kg).

3.8. Amphetamine-induced pyrexia

The extract at various doses used showed significant (P < 0.05) and dose-dependent reduction of pyrexia when compared to control (Table 6). The effect of the extract was comparable to the standard drug, paracetamol (20 mg/kg).

4. Discussion

The results of the present study indicated, however, that methanolic

leaf extract of *M. crassifolia* possessed analgesic, anti-inflammatory and antipyretic effects in the experimental animal models used. The acetic acid-induced abdominal constriction and tail immersion methods were used to evaluate for both peripheral and centrally acting analgesic activity. The abdominal constrictions observed in present study were due to irritation of peritoneal cavity caused by acetic acid. This agent (acetic acid) released prostaglandins E_2 and $F_{2\alpha}$ in the peritoneal fluid that excite pain nerve endings[25,26]. This increase in prostaglandin levels then enhanced inflammatory pain by elevating capillary permeability[27]. The analgesic action of the extract can be attributed to the blockade of release of the endogenous mediators of pain, the prostaglandins. It is therefore possible that *M. crassifolia* leaf extract possesses some inhibitory action on the cyclooxygenase pathway which is actually involved in the biosynthesis of prostaglandin. In order to further confirm the analgesic action of the extract, the tail immersion test was carried out. In this method, the leaf extract significantly decreased the pain in thermal stimuli confirming the analgesic activity of the extract. The observed effect further suggests a central mechanism of action for the leaf extract. It has been confirmed that centrally acting analgesic agents prolong the pain threshold of mice towards heat and pressure[28].

In living animal tissues, inflammatory processes mediate the release of several mediators such as prostaglandins, histamine, cytokines and proteinases[29,30]. Xylene caused instant irritation of the mouse ear thereby leading to fluid accumulation and oedema, characteristic of the acute inflammatory response. Suppression of this response may likely be indication of antiphlogistic effect. The leaf extract showed significant inhibition of xylene-induced ear oedema. This suggests the inhibition of phospholipase A_2 which is involved in the pathophysiology of inflammation due to xylene[31]. However, dexamethasone, a steroid anti-inflammatory agent produced significant reduction in the right ear weight of positive control mice, indicating an inhibition of phospholipase A_2 . In the carrageenan-induced paw oedema, *M. crassifolia* leaf extract caused marked inhibition at the early stage of inflammation indicating effect probably on histamine, serotonin and kinins that are involved in the early stage of carrageenan-induced oedema[32]. The extract also reduced later stage of the oedema probably by inhibiting prostaglandin which is known to mediate the second phase of carrageenan-induced inflammation[33].

Antipyretic activity is a characteristic of drugs or compounds which have inhibitory effect on prostaglandin biosynthesis[34,35]. Paracetamol has been reported to inhibit centrally the synthesis of prostaglandin in the brain by inhibiting COX-3[36]. Consequently, elevated plasma prostaglandin level as observed in fever was suppressed. The methanolic leaf extract of *M. crassifolia* demonstrated effective activity as evident in the inhibition of temperature rise in the yeast and amphetamine models of pyrexia. The antipyretic action of the extract may possibly be through inhibition of prostaglandin E production leading to suppression of

elevated plasma level especially since the extract possesses analgesic and anti-inflammatory activities.

The therapeutic benefits of traditional remedies are often attributed to a combination of active constituents. Various flavonoids isolated from medicinal plants have shown remarkable anti-nociceptive and anti-inflammatory effects[37]. However, the analgesic and anti-inflammatory effects observed may be attributed to its flavonoids components, shown to be present during phytochemical analysis. These findings lend pharmacological support to the folkloric use of the plant and also reveal its potential for the development of putative herbal analgesic, anti-inflammatory and antipyretic remedies. The safety of the plant extract when taken orally is justified by the fact that oral administration, 5000 mg/kg, did not produce any mortality and visible toxic signs. Further investigations are ongoing in our laboratory to isolate and characterize the specific active components of the leaf extract responsible for the observed pharmacological actions.

In conclusion, the present study clearly indicated that *M. crassifolia* leaf extract had a remarkable analgesic, anti-inflammatory and antipyretic activities. These findings confirmed its traditional use in the treatment of pains and feverish conditions.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to Dr. (Mrs) Jamilat A. Ibrahim, Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja, Nigeria for her botanical assistance.

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