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Original Article

Studies on the anti-inflammatory and anti-nociceptive properties of *Blepharis maderaspatensis* leaves

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A B S T R A C T

Blepharis maderaspatensis (L.) B. Heyne ex Roth, Acanthaceae, is a procumbent or scrambling perennial herb used traditionally for treatment of snakebites, wounds, edema and gout. The anti-inflammatory and anti-nociceptive properties of the ethanol extract of the whole plant of *B. maderaspatensis* was investigated using carrageenan-induced paw edema in rats, xylene-induced edema in mice, mouse writhing and tail clip tests respectively. The effect of the extract on inflammatory mediators, serotonin and histamine, using the most active dose (75 mg/kg) was also carried out. The results showed that the extract of *B. maderaspatensis* in carrageenan-induced test caused a significant inhibition (84.5%, 90 min) of paw edema at a dose of 75 mg/kg while the xylene-induced test caused a significant inhibition (62.65%) at 50 mg/kg. The histamine-induced test showed significant inhibition (90.9%, 90 min) while serotonin-induced test showed moderate inhibition (54.10%, 180 min). In the mouse writhing and tail clip tests, the extract produced a significant inhibition of 66.21% and 15.81% at 75 mg/kg, respectively. These results collectively demonstrate that the ethanol extract of *B. maderaspatensis* possesses anti-inflammatory and anti-nociceptive properties, and this supports the ethnopharmacological use of the plant in the treatment of inflammation.

Introduction

Inflammation is a fundamental defensive reaction of the body to an invasion of pathogens or injury. It involves a complex array of enzyme activation; mediators release, extravasation, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). The classic signs of inflammation such as pain, redness, swelling and loss of function are produced by inflammatory agents such as nitric oxide, prostaglandins, bradykinin, serotonin, leukotrienes and histamine (Banasik, 2000; Chandrasoma and Taylor, 2005). Uncontrolled and persistent inflammation contributes to the progression of many chronic diseases such as multiple sclerosis, rheumatoid arthritis, atherosclerosis, psoriasis and inflammatory bowel disease (Talwar et al., 2011). Developments of new drugs with little or no side effects are still needed as some of the antiinflammatory drugs cause undesired and harmful effects. Nowadays, public interest in phytopharmaceuticals to inhibit chronic diseases is gathering momentum.

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Blepharis maderaspatensis (L.) B. Heyne ex Roth (BM), a member of the family Acanthaceae, is a procumbent or scrambling perennial or rarely annual herb with stems up to 2.5 m long. It can be found in a wide variety of grassland, shrub and woodland habitats as well as forest-merging in the savannah zone, from Senegal to South Nigeria (Burkill, 1985). Traditionally it is used in treatment of swellings, edema and gout. A paste of the leaves is mixed with black gram powder, crushed onion and white egg yolk and the mixture is applied topically over fractured bones in Nigeria (Burkill, 2004). The whole plant is burnt to ash and mixed with oil for rubbing onto swollen legs after it has been washed in warm water in Tanganyika (Burkill, 1985).

Although the cytotoxic activity against colon cancer cells, wound healing and antioxidant activities of the plant have been reported (Baskar et al., 2012; Rajasekaran et al., 2012), the anti-inflammatory and anti-nociceptive activities have not been scientifically confirmed. Hence, the present study aimed to evaluate the anti-inflammatory and anti-nociceptive activities of the ethanol extract of *Blepharis maderaspatensis*.

Materials and methods

Plant material and extraction

Fresh samples of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth, Acanthaceae (whole plant), were collected from Odofin village in Ikire town, Osun State, Nigeria, and authenticated at the Herbarium of the Department of Botany and Microbiology where a voucher specimen was deposited (LUH 4592). The plant was dried in a hot air oven at 40 °C and grounded in a mechanical grinder. The powdered material (500 g) was macerated with absolute ethanol for 72 h at room temperature. The extract was filtered and evaporated to dryness in a water bath at 40 °C. The percentage yield was 6.52% (w/w).

Animals

All the animals (male Wistar rats (100-200 g) and Swiss albino mice (20-30 g) used in this study were obtained from the National Agency for Food and Drugs Administration and Control, Yaba. The animals were kept in hygienic and well ventilated compartments and maintained under standard laboratory conditions (12 h light/dark cycle at 22 ± 2 °C) as approved by the Experimentation and Ethics Committee of the College of Medicine, University of Lagos (CM/COM/08). All animals were acclimatized for one week, fed rodent diet (Livestock Feeds PLC, Ibadan, Oyo State, Nigeria) and had free access to drinking water. They fasted for at least 12 h prior to experimentation.

Acute toxicity test

The method of Lorke (1983) was used with minor modifications for this study. A single oral dose of the extract (5 g/kg) was administered to a group of six healthy male mice the control group only received the vehicle (10 ml/kg, *p.o.*). The animals were observed for general behavioural changes, physiological function and mortality at 1, 4 and 24 h after treatment. The mice were further observed for up to seven days for any signs of delayed toxicity and mortality.

Anti-edematogenic models

Carrageenan-induced rat paw edema

In this assay, the rats were divided into seven groups (n = 6 each), which were orally treated with distilled water (control) or indomethacin (reference drug, 10 mg/kg), or the extract (12.5, 25, 50, 75 and 100 mg/kg). After 1 h after oral administration, 0.1 ml of 1% suspension of carrageenan in normal saline was injected into the sub-plantar tissue of the right hind paw to induce edema (Winter et al., 1962). The paw edema was measured at 30 min interval for 3 h after carrageenan injection. The linear paw circumference was measured using the cotton thread method of Bamgbose and Naomesi (1981). The difference between the initial and subsequent values gave the actual oedema value, which was compared to control. The inhibition of inflammation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \left(Vc - \frac{Vt}{Vc} \right)$$

where Vc represents mean edema in control and Vt mean edema in group treated with standard/extract.

Histamine and serotonin-induced rat paw oedema

Three groups of six rats each were treated with the extract (75 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*), and distilled water (10 ml/kg, *p.o.*). Paw edema was induced after 1 h by sub-plantar administration of 0.1 ml histamine or serotonin (10^{-3} mg/ml) on the right hand paw (Amann et al., 1995). The linear paw circumference was measured at 0 min and thereafter every 30 min for 3 h.

$$\% \text{ inhibition} = 100 \left(Vc - \frac{Vt}{Vc} \right)$$

where Vc represents mean edema in control and Vt mean edema in group treated with standard/extract.

Xylene-induced ear edema

The animals were divided into five groups of six mice each. Edema was induced by applying 0.03 ml of xylene to the inner surface of the right ear 30 min after oral administration of distilled water (10 ml/kg), extract (12.5-100 mg/kg) and dexamethasone (1 mg/kg) respectively. The left ear was considered as control. Fifteen minutes after the application of xylene, the mice were killed under ether anesthesia and both ears were cut off and weighed (Nunez Guillen et al., 1997). The mean of the difference between the right and left ears was calculated.

$$\% \text{ inhibition} = 100 \left(Vc - \frac{Vt}{Vc} \right)$$

where Vc represents difference in weight of ear in control and Vt difference in weight of ear in group treated with standard/extract.

Anti-nociceptive activity

Mouse writhing test

Mice divided into seven groups of six animals each were pre-treated with distilled water (10 ml/kg, *p.o.*); extract (12.5-100 mg/kg, *p.o.*) and acetylsalicylic acid (10 mg/kg, *p.o.*). After 1 h, the mice were injected with acetic acid (0.6% v/v in saline, 10 ml/kg, *i.p.*). The number of writhing and stretching movements was counted for a period of 30 min (Koster et al., 1959). The percentage of anti-nociceptive activity was calculated as:

$$\% \text{ inhibition} = 100 \left(Vc - \frac{Vt}{Vc} \right)$$

where Vc represents mean number of writhing in control and Vt mean number of writhing in group treated with standard/extract.

Haffner's tail clip test

In this model, a initial sensitivity test was carried out by applying a metal artery clip to the base of the tail to induce pain, animals that failed to attempt to dislodge the clip in 10 s were discarded. The responsive mice were then divided into seven groups of six animals each. The pre-treatment reaction time of all mice was determined and treatment was then administered using distilled water (10 ml/kg, *p.o.*); extract (12.5-100 mg/kg, *p.o.*) and the standard drug, morphine (10 mg/kg, *s.c.*). The reaction time of each animal was determined 60 min post-treatment for oral administration and 30 min post-treatment for subcutaneous administration (Adeyemi et al., 2004; Bianchi and Franceschini, 1954). A post-treatment cut-off time of about 60 s was used.

$$\% \text{ Inhibition} = \frac{(\text{Post - treatment latency}) - (\text{Pre treatment latency})}{(\text{Cut - off time - Pre treatment latency})} \times 100$$

Quantitative analysis

Determination of total phenolic content

The concentration of total phenolics in the ethanol extract of *Blepharis maderaspatensis* was determined by the Folin-Ciocalteu colorimetric method (Slinkard and Singleton, 1977). Briefly, 500 µl of the solution (containing 1 mg/ml) extract in methanol was added to 2.5 ml Folin-Ciocalteu reagent (diluted with water 1:1) and 2 ml (75 g/l) sodium carbonate. After 30 min of incubation at 40 °C, the resulting absorbance was measured at 760 nm using a spectrophotometer (T80 spectrometer, PG Instrument Ltd.). A calibration curve, using gallic acid in a concentration range of 0.01-0.05 mg/ml was prepared. Measurements were carried out in triplicate. The total phenolic content was expressed as milligram gallic acid equivalent (GAE) per gram of sample.

Determination of total flavonoid content

The total flavonoid content of the extract was determined using the method of (Ordonez et al., 2006). Quercetin was used as standard. Solution of 2% AlCl3 in ethanol (1 ml) was added to 1 ml of the extract. The absorbance was measured at 420 nm after 1 h of incubation at room temperature. Measurements were carried out in triplicate. Total flavonoid content was expressed as milligram quercetin equivalent (QE) per gram of sample.

Statistical analysis

Data were presented at mean ± SEM. The data were analysed using GraphPad Prism for windows version 5 (GraphPad Software, San Diego, CA, USA). Values were considered significant when $p \leq 0.05$.

Results

Acute toxicity test

Oral administration of BM extract at 5 g/kg did not produce any mortality. The extract did not produce significant changes in behavior during the time of observation.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

The subplantar injection of carrageenan produced edema development which increased progressively with time in the control group. Peak edema development was observed at 180 min (2.53 ± 0.15). The administration of BM (12.5, 25, 50 and 75 mg/kg, *p.o.*) showed significant ($p < 0.05$) inhibition from 30 min after carrageenan injection with peak effect (84.5%) produced at the dose of 75 mg/kg at 90 min. Oral administration of indomethacin (10 mg/kg) also significantly ($p < 0.01$) reduced the edema with 86.7% inhibition at 180 min (Table 1). However pro-inflammatory activity was observed for the plant extract at 100 mg/kg.

Histamine and serotonin-induced rat paw edema

A significant ($p < 0.001$) reduction in the edema of mice treated with the extract was observed in the histamine-induced paw edema model (Fig. 1). The maximum inhibition was observed at 90 min (90.9%).

In the serotonin-induced paw edema model, the extract had low effect on the inhibition of edema. The maximum inhibition was observed at 180 min (54.1%) (Fig. 1).

Xylene-induced ear edema

The effect of *B. maderaspatensis* was dose-dependent with peak effect (62.65% inhibition) produced at the dose of 50 mg/kg (Table 2). This effect was greater than but not significantly ($p < 0.05$) different from that produced by dexamethasone (50.2% inhibition). Pro-inflammatory activity was also observed for the extract at 100 mg/kg.

Anti-nociceptive activity

Mouse writhing test

In this model, the oral administration of *B. maderaspatensis* produced a reduction in the number of writhes at all the five doses tested, but the maximum inhibition was observed at 75 mg/kg (20.50 ± 3.56, 66.21%) (Fig. 2). Aspirin produced a greater inhibition (15.50 ± 2.89, 74.45%), however it was significantly different ($p < 0.05$) from that of the extract (75 mg/kg) within the same period

Table 1
Effect of extract of BM on carrageenan induced rat paw oedema.

Trt	Dose mg/kg	Increase in paw circumference (cm)						
		T_0	T_{30}	T_{60}	T_{90}	T_{120}	T_{150}	T_{180}
BM	12.5	1.93 ± 0.03	2.18 ± 0.03 (53.7)	2.22 ± 0.01 (42.3)	2.25 ± 0.03 (44.8)	2.28 ± 0.01[b] (59.1)	2.28 ± 0.03[c] (23.9)	2.32 ± 0.01 (48.0)
BM	25	1.95 ± 0.02	2.15 ± 0.04 (63.0)	2.22 ± 0.01 (48.1)	2.25 ± 0.04 (48.2)	2.28 ± 0.05 (50.0)	2.3 ± 0.05 (23.9)	2.32 ± 0.03 (50.7)
BM	50	1.92 ± 0.04	2.05 ± 0.02a (75.9)	2.03 ± 0.05b (78.9)	2.13 ± 0.05[a] (69.0)	2.21 ± 0.05 (56.1)	2.25 ± 0.06 (28.3)	2.25 ± 0.08[a](56.0)
BM	75	1.97 ± 0.05	2.08 ± 0.07 (81.4)	2.07 ± 0.03[a] (82.7)	2.07 ± 0.03[a] (84.5)	2.15 ± 0.04[a] (74.2)	2.22 ± 0.05 (47.8)	2.32 ± 0.04[b](66.7)
BM	100	1.74 ± 0.02	2.33 ± 0.04 (-9.3)	2.13 ± 0.03 (25.0)	2.33 ± 0.06 (-1.7)	2.4 ± 0.05 (0.0)	2.29 ± 0.09 (-19.6)	2.3 ± 0.05 (25.33)
Indn	10	1.92 ± 0.05	2.14 ± 0.08 (59.3)	2.18 ± 0.06 (50.0)	2.18 ± 0.06 (55.2)	2.13 ± 0.05[b] (68.2)	2.05 ± 0.03 (71.7)	2.02 ± 0.01[c] (86.7)
D.W	10ml/kg	1.78 ± 0.03	2.32 ± 0.05	2.30 ± 0.07	2.36 ± 0.1	2.44 ± 0.1	2.24 ± 0.1	2.53 ± 0.15

Figures in parenthesis represent percentage inhibition of edema development.
Trt, Treatments; Indn, Indomethacin; D.W, Distilled water; BM, *Blepharis maderaspatensis*. Values are expressed as mean ± SEM, n =6.
[a]$p < 0.05$.
[b]$p < 0.01$.
[c]$p < 0.0001$ compared to the control.

Haffner's tail clip test

The effect of BM on the tail clip test is shown in Table 3. The animals of the control group elicited reactions towards clip removal with pre-treatment latency being 4.43 ± 1.13 s and post-treatment latency being 5.79 ± 2.74 s. BM showed a significant ($p < 0.05$) dose-dependent increase in reaction latency. Maximum inhibition was produced at 75 mg/kg (15.81%). This effect was however less than that elicited by morphine (75.67%) at 10 mg/kg.

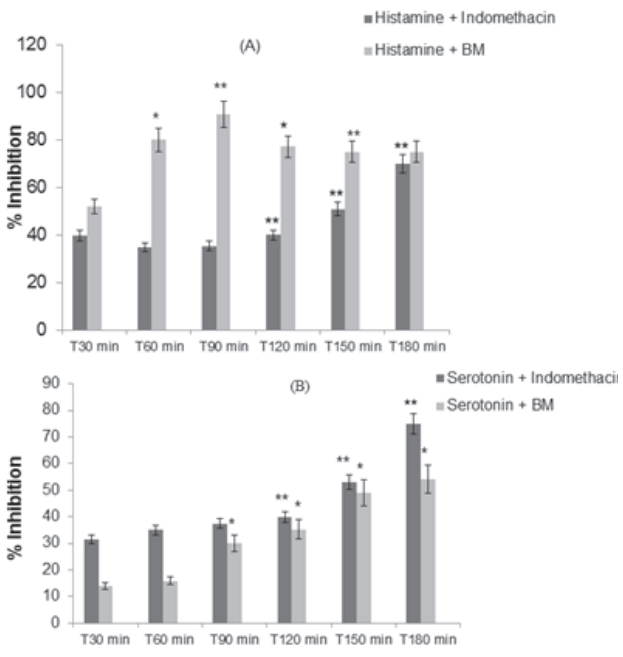

Fig. 1 - The effects of *Blepharis maderaspatensis* and indomethacin on rat's hind paw edema induced by (a) histamine and (b) serotonin. Data represented as mean ± S.E.M (n=6). *$p < 0.01$. **$p < 0.0001$ (Two-way ANOVA followed by Bonferroni's test).

Quantitaive analysis

The total phenolic and flavonoid content of the extract were 88.73 ± 0.26 and 221.02 ± 0.004 respectively.

Table 2
Effect of extract of BM on xylene induced edema in mice.

Treatment	Dose (mg/kg)	Ear swelling (g)	Inhibition (%)
BM	12.5	0.025 ± 0.004	12.84
BM	25	0.002 ± 0.004[a]	21.40
BM	50	0.004 ± 0.004[b]	62.65
BM	75	0.009 ± 0.002[a]	30.74
BM	100	0.018 ± 0.002[a]	-37.98
Dexamethasone	10	0.007 ± 0.003[a]	50.20
Distilled water	10 ml/kg	0.013 ± 0.006	_

BM, *Blepharis maderaspatensis*. Values are expressed as mean ± SEM, n = 6.
[a]$p < 0.05$.
[b]$p < 0.01$ compared to the control..

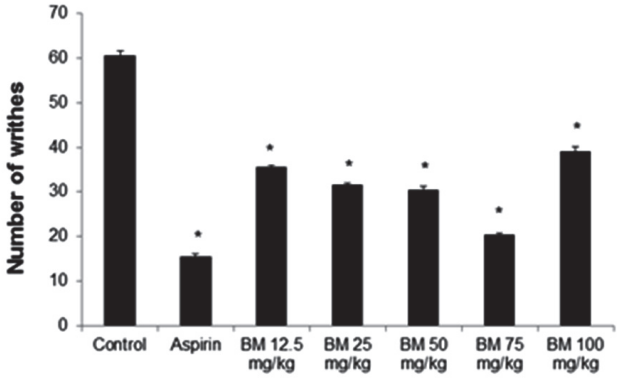

Fig. 2 -Effect of *Blepharis maderaspatensis* on mouse writhing test in mice. Values are expressed as mean ± SEM (n=6). *$p < 0.05$ compared to the control.

Table 3
Effect of BMon Haffner's tail clip reflex in mice.

Treatment	Dose mg/kg	Pre-treatment(s)	Post-treatment(s)	% Inhibition
BM	12.5	3.35 ± 0.69	5.39 ± 1.14[a]	3.60
BM	25	3.49 ± 0.54	6.65 ± 1.43[a]	5.59
BM	50	2.89 ± 1.18	10.22 ± 3.14[a]	12.83
BM	75	3.20 ± 0.70[a]	12.18 ± 2.50[a]	15.81
BM	100	2.86 ± 0.55[a]	4.81 ± 0.53[a]	3.41
Morphine	10	5.96 ± 0.96	46.85 ± 7.56[a]	75.67
Distilled water	10 ml/kg	4.43 ± 1.13	5.79 ± 2.74	2.45

BM, *Blepharis maderaspatensis*. Values are expressed as mean ± SEM (n = 6).

Discussion

Modern medicine has gained immensely from the research of natural products as a number of the therapeutic drugs currently in use have been derived from pharmacologically active agents obtained from plants (Shu, 1998). However, in spite of this progress, the potential of higher plants as sources for new drugs is still largely unexplored.There is also an urgent need for effective anti-inflammatory and anti-nociceptive agents in medicine.

The result from the acute toxicity test carried out in this work indicated that the ethanol extract of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth, Acanthaceae, has low oral toxicity as the 5 g/kg dose did not cause death in the animals even after seven days of administration.

The evaluation of the anti-inflammatory activity of *B. maderaspatensis* in this study was carried out using the carrageenan, histamine, serotonin and xylene-induced edema tests. It has been reported that the carrageenan-induced rat paw model is a suitable in vivo model to study anti-inflammatory effects of natural products since it involves several mediators (Woldesellassie et al., 2011). Three phases have been postulated for carrageenan induced edema and these include; the initial phase (between 0 and 1.5 h) which is attributed to the action mediators such as histamine and serotonin; a second phase (1.5-2.5 h) mediated by bradykinin and a third phase (2.5-6 h) mediated by prostaglandins (Di Rosa et al., 1971; Suba et al., 2005). In this investigation, *B. maderaspatensis* showed significant inhibition of rat paw edema in the initial phase. This suggests that the extract (75 mg/kg, *p.o.*) plays an important role as a protective factor against carrageenan-induced acute inflammation and possibly acts by inhibiting the release of/ and the action of histamine and/ or serotonin.

Histamine and serotonin are potent vasodilator substances and are known to increase vascular permeability (Skidmore and Whitehouse, 1967). In order to confirm the results obtained from the carrageenan-induced edema test, the effect of the extract at the most effective concentration (75 mg/kg) was investigated using histamine and serotonin-induced edema models. The results showed that the extract effectively suppressed the edema produced by histamine but had a low effect on the edema produced by serotonin indicating that the extract exhibits its anti-inflammatory action by inhibiting the synthesis, release or action of histamine.

The xylene-induced edema model is useful for the screening of anti-inflammatory agents. It is characterized by fluid accumulation and edema. Suppression of this response is taken as an indication of antiphlogistic effect (Atta and Alkofahi, 1998). The effectiveness of *B. maderaspatensis* (50 mg/kg, *p.o.*) in this model may suggest the inhibition of phospholipase A which is involved in the pathophysiology of inflammation due to xylene (Lin et al., 1992).

In this study, the anti-nociceptive activity of *B. maderaspatensis* was investigated using the mouse writhing and Haffner's tail clip tests. The mouse writhing test is used for the evaluation of peripherally acting drugs and the induction of pain occurs by the release of endogenous substances as well as other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis (Franzotti et al., 2000). The dose-dependent inhibition of writhing of the extract observed in this study, suggests a peripherally mediated anti-nociceptive activity based on the association of the model with stimulation of peripheral receptors (Bentley et al., 1983).

The tail clip test is a confirmatory test to show the centrally acting component of pain mechanism (Richardson et al., 1998). Centrally acting anti-nociceptive drugs are known to elevate the pain threshold of rodents to pressure and heat (Singh and Majumdar, 1995). In this study, *B. maderaspatensis* extract elicited a very low activity (< 20%). This suggests that the extract does not have centrally acting anti-nociceptive properties.

The results obtained from the anti-inflammatory and anti-nociceptive tests suggest that *B. maderaspatensis* is effective against inflammatory and nociceptive pains with a more pronounced effect in the former. It was also observed that in most of the models investigated, the highest inhibtion was at 75 mg/kg after which there was a reduction in activity. This may be due to the filling of the opioid receptors (Bentley et al., 1983).

Previous studies have shown that anti-inflammatory and analgesic effects can be a result of the high polyphenol content of plants especially phenolics and flavonoids (Handa et al., 1992; Orhan et al., 2007). Flavonoids are known to prevent the synthesis of prostaglandins. Biochemical investigations on the mechanism of action of flavonoids have shown that these compounds can inhibit a wide variety of enzymes. The release of arachidonic acid is closely related to the cyclooxygenase and 5-lipoxygenase enzyme systems (Middleton et al., 2000; Williams et al., 1995). The polyphenols present in this plant may be responsible for the observed anti-inflammatory and analgesic activities.

In conclusion, of the present study confirmed that *B. maderaspatensis* possesses significant anti-inflammatory and analgesic activities. The results also support the use of the plant in the treatment of inflammation-related diseases traditionally.

Authorship

MO and TF contributed in collecting plant samples and identification, running the laboratory work and analysis of the data. MSF contributed to biological studies and analysis of the data. AS designed the study, supervised the laboratory work and wrote manuscript. All the authors have read the final manuscript and approved submission.

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