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Original Article

Musanga cecropioides leaf extract exhibits anti-inflammatory and anti-nociceptive activities in animal models

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ABSTRACT

Extract obtained from the leaves of *Musanga cecropioides* R. Br. ex Tedlie, Urticaceae, a tree growing in Africa, is used traditionally in the treatment of edema and rheumatism. The anti-inflammatory and anti-nociceptive properties of ethanol extract were studied using the carrageenan, histamine, serotonin and xylene-induced edema tests as well as the formalin, mouse writhing and tail clip tests. Significant dose dependent inhibition was observed in the carrageenan model with peak inhibition at 150 mg/kg (71.43%, 90 min, $p < 0.001$). In the histamine and serotonin models, the extract caused significant inhibition of 83.33% ($p < 0.05$) and 45% ($p < 0.01$) at 120 min respectively. For the xylene model, the extract showed maximum inhibition (59.25%) at 200 mg/kg. Also, *M. cecropioides* produced significant anti-nociceptive activity in the mouse writhing (55.12%, $p < 0.01$), formalin (81.88%, $p < 0.01$) and tail clip (11.78%, $p < 0.001$) tests at 200 mg/kg respectively. The results obtained in this study demonstrated that the ethanolic leaf extract of *M. cecropioides* possesses anti-inflammatory effect possibly mediated via histaminergic and serotonergic inhibition and anti-nociceptive effect mediated via peripheral mechanism with mild central involvement.

Introduction

Musanga cecropioides R. Br. ex Tedlie, Urticaceae, is a deciduous or evergreen, dioecious medium-sized tree of up to 30 m tall with an umbrella-shaped crown. Traditionally, the leaves are used to prepare a vaginal douche for painful menstruation and also used in the treatment of gonorrhea and cough (Burkill, 1985; Ayinde et al., 2003). The bark decoction is taken to treat arterial hypertension, constipation, pain during childbirth, cough, diabetes and schizophrenia. The stem sap is used to treat dysmenorrhoea and as galactagogue, while the root sap is used to treat stomach spasms, diarrhea, gonorrhea, pulmonary complaints, trypanosomiasis, skin diseases, otitis, rheumatism, edema, epilepsy, and to ease childbirth. The root bark is eaten with kolanut to cure cough, and the bark is tied on wounds where it is said to effect a cure (Adejuwon, 2001). The sheath-like stipules are applied as emmenagogue and oxytocic, and to treat stomach complaints, hiccough and wounds (Burkill, 1985).

Pharmacological activities such as the uterotonic, antidiabetic, hypotensive and hypoglycemic properties of the leaf and stem bark as well as the antimicrobial activity of the root sap of *M. cecropioides* have been reported (Kamanyi et al., 1992, 1996; Dongmo et al., 1996; Ayinde et al., 2003, 2006; Adeneye et al., 2006, 2007; Senjobi et al., 2012; Uwah et al., 2013). There is, however, no record of the anti-inflammatory or analgesic activity in literature; hence, the aim of this work is to investigate the anti-inflammatory and analgesic properties of the plant with a view to validating its ethno-medicinal use.

Materials and methods

Plant collection and extraction

The leaves of *Musanga cecropioides* R. Br. ex Tedlie, Urticaceae, leaves were collected at Abatadu, Oke-ode, a village about 3 km to Ikere, Osun State, Nigeria (7°30′ N 4°30′ E), in February, 2013. The plant specimen was identified and authenticated at the Herbarium of the Department of Botany and Microbiology, University of Lagos, Akoka, Lagos, Nigeria where a voucher specimen (LUH 5637) was deposited. The leaves were pulverized and macerated with absolute

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ethanol at room temperature. The extract was filtered and concentrated using the rotary evaporator (Buchi, Switzerland). The yield was 4.58% (w/w).

Animals

Wistar rats (100–200 g) and Swiss albino mice (18–30 g) of either sex used in this study were purchased from the Animal House of the National Agency for Food and Drug Administration and Control (NAFDAC), Lagos. The animals were maintained under standard laboratory conditions (12 h light/dark cycle at 22 ± 2 °C) and fed standard rodent pellets (Livestock Feed PLC, Lagos, Nigeria) and water ad libitum. The protocol was approved by the Experimentation Ethics Committee of the College of Medicine, University of Lagos (CM/COM/08).

Acute toxicity

Acute oral toxicity assay was performed on three groups of six mice each fasted for 12 h prior to the experiment. The plant extract was administered at doses of 1, 2 and 3 g/kg (*p.o.*). The animals were observed for immediate signs of toxicity and mortality for 24 h and further observed for seven days for signs of delayed toxicity.

Pharmacological studies

In vivo anti-inflammatory activity

Carrageenan-induced paw edema. Increase in the rat hind paw linear circumference induced by plantar injection of the phlogistic agent was used as the measure of acute inflammation (Henriques et al., 1987). The rats ($n = 6$) were administered the plant extract (50, 100, 150 and 200 mg/kg, *p.o.*) while the control rats received indomethacin (10 mg/kg *p.o.*) and distilled water (10 ml/kg, *p.o.*) respectively. One hour after treatment, 0.1 ml of carrageenan (1%, w/v in water) was administered into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured immediately before injection of the phlogistic agents and at 30 min interval for 3 h using the cotton thread method (Bamgbose and Naomesi, 1981).

Anti-inflammatory activity is determined by analyzing the reduction in edema size and calculating % inhibition of edema. A mean reduction in edema when compared with control and an increase % inhibition in the treated groups is an indication of anti-inflammatory activity.

Serotonin and histamine induced rat paw edema. In order to elucidate the mechanism of action of *M. cecropioides*, selected anti-inflammatory mediators (histamine and serotonin) were used. The dose which gave maximum inhibition in the carrageenan assay for was used for the tests.

Adult rats (100–200 g) fasted overnight were divided into three groups of six animals each. Distilled water, 10 ml/kg was administered to group one (control), group two received 10 mg/kg indomethacin and group three, 150 mg/kg *M. cecropioides*. All treatments were done orally. One hour post treatment, edema was induced by injection of 0.1 ml serotonin or histamine (10^{-3} mg/ml) into the sub-plantar tissue of the right hind paw. The linear circumferences of the paws were measured using cotton thread method. Measurements were made at 0 min and thereafter at an interval of 30 min for 3 h. The mean of the paw size were computed and percentage inhibitions were calculated (Agbaje and Fageyinbo, 2011).

Xylene-induced ear edema. Adult mice (18–30 g) fasted overnight were divided into six groups of six animals each and were treated as follows: *M. cecropioides* (50, 100, 150 and 200 mg/kg, *p.o.*), dexamethasone (1.0 mg/kg *p.o.*) and distilled water (10 ml/kg *p.o.*). Ear edema was induced by applying 0.03 ml of xylene to the inner surface of the right ear. 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 removed and weighed. Increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections (Nùñez Guillén et al., 1997).

Anti-nociceptive activity

Mouse writhing test. Mice used for this experiment were divided into five groups of six animals each. Group I was given distilled water (10 ml/kg, *p.o.*); Group II received the standard drug acetylsalicylic acid (100 mg/kg, *p.o.*) while the remaining Groups III, IV and V were given the plant extract (50 mg/kg, 100 mg/kg and 200 mg/kg, *p.o.*) respectively. Sixty minutes after treatment, acetic acid (0.6% v/v in saline, 10 ml/kg *i.p.*) was administered. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was counted for 30 min (Singh and Majumdar, 1995; Mbagwu and Anene, 2007).

Formalin test. In this study, the animals ($n = 6$) were arranged into five groups which received distilled water (10 ml/kg), extract (50, 100 and 200 mg/kg) and morphine (10 mg/kg *s.c.*) respectively. For the induction of pain, formalin (20 μl of 1% solution) was injected into sub-plantar tissue of the right hind paw of each mouse 60 min after administration for the oral route and 30 min for the subcutaneous route. The nociceptive response was considered as the time spent in licking and biting of the injected paw. The responses of the mice were observed for 5 min (first phase) and 15–30 min (second phase) post formalin injection (Shibata et al., 1989; Vianna et al., 1998).

Haffner's tail clip test. Mice used in this experiment were prescreened by placing a metal artery clip 1 in. from the base of the tail and animals which did not respond to the clip placement by turning or biting at the clip within 10 s were discarded. Eligible mice were divided into five groups of five animals each. The pretreatment reaction time of all mice to clip was determined after which the animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with the extract at 50 mg/kg, 100 mg/kg and 200 mg/kg respectively, Group 5: Morphine 10 mg/kg *s.c.* The presence or absence of anti-nociceptive activity was determined 60 min after drug administration for oral administration and 30 min for subcutaneous administration (Adeyemi et al., 2004). A post-treatment cut-off time of 30 s was used (Adeyemi et al., 2004; Agbaje and Adeneye, 2008).

Quantitative analysis

Determination of total phenol content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). Gallic acid was used as a standard with a concentration range of 0.01–0.05 mg/ml prepared in methanol (Folin and Ciocalteu, 1927). The extract (0.5 ml) (0.1 mg/ml) together with the gallic acid was mixed with 2.5 ml Folin–Ciocalteu reagent (previously diluted with distilled water 1:10, v/v) and 2 ml (75 g/l) of sodium carbonate. The mixtures were vortexed for 15 s and allowed to stand for 30 min at room temperature before the absorbance was measured at 765 nm using a spectrophotometer. All determinations were performed in triplicates. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per gram of sample.

Determination of total flavonoid content

Total flavonoid was estimated using the method of Miliauskas et al. (2004). A 2% $AlCl_3$ in ethanol (2 ml) was added to 2 ml of