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Original article

Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*

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ABSTRACT

This study analyzes the antinociceptive and anti-inflammatory properties of ethanolic leaf extract of *Alafia barteri* Oliv., Apocynaceae, based on its medicinal use in the treatment of toothaches, inflammation and fevers. The antinociceptive effect was assessed in mice using acetic acid-induced writhing, tail clip, tail immersion and formalin assays. Anti-inflammatory activity was evaluated on carrageenan-induced paw oedema in rats, and xylene-induced ear oedema in mice. In acetic acid-induced writhing test, the extract at different doses (50, 100 and 200 mg/kg, *p.o.*) significantly ($p < 0.05$) and dose-dependently reduced pain by 35.04, 56.49 and 84.25%, respectively. The extract also significantly inhibited both the early and late phases of formalin-induced nociception in mice. In the tail immersion test, the extract caused a significant inhibition of pain (34.43% inhibition, after 90 min) at a dose of 200 mg/kg, while the effect of the extract in the tail clip test was only significant at the 100 mg/kg dose. *A. barteri* caused a significant inhibition of paw oedema development in the carrageenan and xylene-induced oedema tests. There was no mortality recorded following treatment with the extract (5 g/kg, *p.o.*). The results support the traditional use of *A. barteri* in the treatment of various diseases associated with pain and inflammation.

Introduction

Non-steroidal anti-inflammatory drugs (NSAID) are used worldwide for the treatment of inflammation, pain and fever, as well as for cardiovascular protection. However, they often produce significant side-effects, which include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities, thus limiting their use (Burke et al., 2006). Drugs of natural origin are an important source for the treatment of many diseases worldwide (Pandima Devi et al., 2003). The research and analysis of plants employed as pain-relievers and anti-inflammatory agents in traditional ethnomedicine is one of the productive and logical strategies in the search for new drugs (Elisabetsky et al., 1995; Vongtau et al., 2004).

Alafia barteri Oliv., Apocynaceae, is a climbing shrub distributed widely in the tropics. It is valued for its efficacy in the traditional

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medicine system in Nigeria and other African countries, as an anti-inflammatory and fever remedy. The infusion of the leaves and twining stem are used for the treatment of inflammation and fever (Burkill, 1985; Iwu, 1993). The decoction of root and leaves of the plant is also taken internally or applied externally to treat rheumatic pain, toothache and eye infections (Odugbemi, 2008). The extracts of the leaves were found to have antibacterial and antifungal activities (Adekunle and Okoli, 2002; Hamid and Aiyelaagbe, 2011). The aqueous leaf extract was reported to display potent antiplasmodial activity (Lasisi et al., 2012). Aside these reports, and to the best of our knowledge, no other pharmacological effects of this plant related to its traditional use for antinociceptive and anti-inflammatory activities have been reported. Therefore, this study aimed to evaluate the antinociceptive and anti-inflammatory properties of the ethanolic extract of *Alafia barteri* leaves.

Materials and methods

Plant material

The leaves of *Alafia barteri* Oliv., Apocynaceae, were collected from Olokemeji forest, Ibadan (7°25' N, 3°31'E), Nigeria, in January, 2012. The plant sample was authenticated by Mr T. K. Odewo of the herbarium unit of the Department of Botany, University of Lagos, and a voucher specimen (LUH 2880) was deposited in the same unit.

Preparation of the plant extract

The leaves were air-dried and coarsely powdered using Christy and Norriis 8' Lab Milling Machine (serial No. 50158). The powdered plant sample (310 g) was extracted with 96% ethanol (1.8 l) for 24 h, at room temperature with constant stirring. This process was repeated twice for complete extraction. The extract was filtered and the filtrate was concentrated at 45°C on a regulated water bath (Julabo TW20GB, USA Inc.). The percentage yield was 2.8% (w/w).

Spectroscopic methods for quantitative determination of phenolic compounds

Total phenol concentration in the ethanolic extract from leaves of *A. barteri* was determined spectrophotometrically, according to Folin-Ciocalteu colorimetric method (Menichini et al., 2009) and expressed as gallic acid equivalents (mg/g) of dried extract (R^2 = 0.959). Total flavonoid content was measured by the aluminium chloride colorimetric assay (Rajasekaran et al., 2012) and expressed as quercetin equivalents (mg/g) of dried extract (R^2 = 0.961). Proanthocyanidin content was determined by the vanillin-HCl assay as described by Sun et al. (1998). Catechin was used as the standard (R^2 = 0.958) for the calibration curve, and the result was expressed as catechin equivalents (mg/g) of dried extract.

Experimental animals

Swiss albino mice (17-25 g) and Wistar rats (150-200 g) were housed in clean plastic cages and maintained under standard laboratory conditions (temperature 24-28°C and 12:12 light/dark cycles). They were fed with commercial rat food (Livestock Feed PLC, Ikeja, Lagos, Nigeria) and water *ad libitum*. The procedures adopted in this study were in accordance with Guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States (NIH, 1985), and approved by the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos, Lagos, Nigeria (CM/COM/08/VOL.XXV).

Acute toxicity

An acute toxicity assay was performed in accordance to OECD test guidelines 423 (limit test) and as reported by Ong et al. (2011). A single oral dose of *A. barteri* extract (5 g/kg) was administered to a group of seven fasting male mice, while the control group received vehicle (1% Tween 80, 10 ml/kg, *p.o.*). The animals were observed continuously for the first 4 h and then periodically up to 24 h for toxic symptoms and mortality. The mice were further observed for seven days for manifestations of delayed toxicity.

Assessment of antinociceptive effect of the extract

Acetic acid-induced writhing test

The antinociceptive effect was evaluated in mice by acetic acid-induced writhing test (Koster et al., 1959; Singh and Majumdar, 1995). Animals were randomly selected and divided into five groups of six animals each. Group I served as the control and received 1% Tween 80 (10 ml/kg, *p.o.*), groups II, III and IV received plant extract (50, 100 and 200 mg/kg, *p.o.*, respectively) while group V, which served as positive control, received acetylsalicylic acid (ASA) (100 mg/kg, *p.o.*). One hour after the administration of the plant extract or standard drug, the animals received acetic acid (0.6%, 10 ml/kg, *i.p.*). The number of writhes (abdominal contractions and stretches) was counted for 30 min after the administration of acetic acid. The results were evaluated by calculating the mean number of writhes per group and antinociceptive activity was expressed as percentage inhibition of abdominal writhes.

Tail immersion test

The tail immersion test was performed according to the method of Ben-Bassat et al. (1959), as reported by Cha et al. (2011) with minor modifications. The mouse was gently handled and two-thirds of its tail was immersed in a beaker containing water at a temperature of 55 ± 0.5°C. Each animal served as its own control. The reaction time, *i.e.* the amount of time it took the animal to withdraw its tail, was recorded with a stopwatch at 0, 30, 60, 90 and 120 min after the administration of the extract (50, 100 and 200 mg/kg, *p.o.*), vehicle (10 ml/kg, *p.o.*) or morphine (10 mg/kg, *s.c*). To avoid tissue injury, the cut-off time was set at 20 s.

Tail clip test

Mice were screened by applying a metal artery clip to the base of the tail to induce pain. The animals that did not attempt to dislodge the clip within 10 s were not used for the experiment. The selected mice were divided into five groups of six mice each. The extract (50, 100 and 200 mg/kg, *p.o.*), morphine (10 mg/kg, *s.c.*) or 1% Tween 80 (vehicle, *p.o.*)