Journal of Ethnopharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Ethnopharmacological communication

Anti-nociceptive activity of the crude extract of *Myrianthus arboreus* P. Beauv (Cecropiaceae) in mice

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ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form
12 March 2015
Accepted 4 May 2015

Chemical compounds studied in this article:
Morphine (PubChem CID: 5464110)
Acetylsalicylic acid (PubChem CID: 2244)
Naloxone (PubChem CID: 5464092)
Formalin (PubChem CID: 712)
Acetic acid (PubChem CID: 176)

Keywords:
Myrianthus arboreus
Anti-nociception
Tail-flick
Writhing reactions
Tail immersion

ABSTRACT

Ethnopharmacological relevance: Myrianthus arboreus P. Beauv (Cecropiaceae) is a shrub or a tree plant widely distributed in Tropical Africa. In the South Eastern part of Nigeria, the leaves are used in traditional medicine as an analgesic for muscular pains, and also as an enema to relieve pain in the back and loins. Although no scientific study has been performed to validate its traditional use in pain management, this study therefore aims at investigating the anti-nociceptive activity of *M. arboreus* leaves extract in mice.

Materials and methods: Anti-nociceptive activity of *M. arboreus* was investigated using acetic acid induced writhing, formalin induced paw licks, hot plate, and tail flick tests. Acute toxicity was determined using a slightly modified Lorkes method.

Results: The extract of *M. arboreus* produced a significant dose-dependent [$F(4, 20)=13.48$ $p<0.001$] inhibition of abdominal writhings induced by acetic acid. In the formalin paw licking test, it produced a significant dose-dependent inhibition of neurogenic and inflammatory pain [$F(4, 17.5)=60.13$ $p<0.001$]. It also produced a significant dose dependent [$F(4, 20)=30.5$ $p<0.001$; $F(4, 20)=0.321$ $p<0.0001$] prolongation of the latency and reaction time in the hot plate and tail immersion tests. Peak effect was observed at the highest dose (40 mg/kg). LD_{50} of the plant was found to be 894 mg/kg.

Conclusion: M. arboreus possesses potent antinociceptive activity mediated centrally and peripherally, an effect which may justify its traditional use in the management of pain.

1. Introduction

Pain is defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Merskey et al., 1979). It could be purely psychogenic, though this is somewhat rare, but more often than not it is an organic physio-emotional experience occurring either as a result of the primary activation of visceral or somatic nociceptors by disease or trauma or from potentially damaging stimuli, or as a result of actual damage to the peripheral or central nervous system (neurogenic or neuropathic pain) (Baldry, 1993). Nociception is the detection of pain-producing stimuli by primary sensory neurons. Most bodily pains have inflammation as the underlying cause and current therapeutic interventions are directed toward the inhibition of the formation of inflammatory mediators (Dray, 1995). The 'classic' analgesic drugs, notably opiates and non-steroidal anti-inflammatory drugs (NSAIDs) have been used for centuries in the management of pain (Rang et al., 1999). However, most of these pain relievers are known to produce undesirable side effects, which tend to limit their clinical usefulness (Sycha et al., 2005). As alternatives to these drugs, a number of plants and plant-derived compounds that are well tolerated are being sought as new medicines for pain.

Myrianthus arboreus P. Beauv (Cecropiaceae) is a common tree found in the forest region of West and Tropical Africa. It is a dioecious shrub or tree which grows up to 14–20 m length. Several pentacyclic triterpenoids have been isolated from the wood and the roots of this tree. Euscaphic acid, myrianthic acid, tormentic acid, ursolic acid and a derivative of ursenoic acid have been isolated from its stems (Ojinnaka, 1985). Myrianthinic acid was isolated from the bark. The wood also contains myrianthiphyllin, a lignan cinnamate (Ngounou et al., 1990). Bark extract of *M. arboreus* showed antiplasmodial, antimycobacterial and antitrypanosomal effects *in vitro* (Tshibangu et al., 2002), which supports some of its uses in traditional medicine (for instance in the treatment of malaria). The leaves also have been shown to have good antibacterial activity (Agwa et al., 2011). The sweet pulp around the seed is edible and the young leaves are eaten as vegetables. The leaves, fruit, bark, and sap are parts of the plant used for traditional medicine (Okafor, 2004). The decoctions of the leaves, roots, and bark are used in Nigeria and in other parts of West, Central and East Africa for the treatment of several

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http://dx.doi.org/10.1016/j.jep.2015.05.005
0378-8741/

sicknesses and diseases including fever, pains, diarrhoea, dysentery, and heart troubles. The leaf or leaf-petiole is beaten into a plaster for application to boils, sap from the young leaves or the terminal buds is applied topically to toothache, applied to the chest to treat bronchitis, or applied to the throat for laryngitis or sore throat. A recent study showed that the leaves of the plant contains flavonoids which are known to target prostaglandins which are involved in the late phase of acute inflammation, tannins which are reported to play roles in anti-nociceptive activities, and alkaloids which are well known for their effects to inhibit pain perception (Otitoju et al., 2014). It is in this regard that this study aimed at evaluating the anti-nociceptive activity of the crude extract of *M. arboreus* in mice.

2. Materials and methods

2.1. Plant material

The leaves of *M. arboreus* (local name: obishere/ibishere, or eweade) were collected in February 2012 from Gambari Forest Reserve, Ibadan, Oyo state. The botanical identification and authentication of the plant were performed by Mr. Oshiyemi O.A., a Plant Taxonomist at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen of the plant was deposited at the herbarium of the Institute (FHI 109662).

2.2. Extraction

The dried leaves were pulverized using a mechanical grinder and 200 g of the material was macerated in 2 L of 50% ethanol for 48 h. The extract obtained was concentrated to dryness with a rotary evaporator (BUCHI rotavapor R-205). The yield was 9.87%. The green coloured solid extract obtained was always reconstituted in 5% Tween 80 to appropriate concentrations on each day before administration to experimental animals.

2.3. Laboratory animals

Swiss albino mice (20–25 g) were used in this study. The animals were obtained from central animal house, College of Medicine, University of Ibadan, Nigeria, and were housed in plastic cages at room temperature with a 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet and water ad libitum. The animals were acclimatized for at least 1 week before being used for experiments. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4. Drugs and chemicals

The drugs and chemicals are acetic acid (Analar, England), formalin (BDH, England), acetylsalicylic acid (Sigma), naloxone, and morphine (Sigma-Aldrich, U.S.A).

2.5. Acute toxicity test

The acute toxicity and lethality (LD_{50}) in mice ($n=24$) was estimated using a modified method of Lorke (1983). In the first instance, experimental animals were administered 10, 100, or 1000 mg/kg of MA i.p ($n=5$) and were observed for number of deaths in 24 h. After the initial toxicity test and absence of death in the experimental animals, another set of animals were given 500, 800, 2000, and 3000 mg/kg ($n=3$) of the extract and monitored for 24 h. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

2.6. Analgesic assays

2.6.1. Acetic acid-induced writhing test

Acetic acid-induced writhing in mice was carried out according to the method described by Koster et al. (1959). The animals were divided into five groups of 5 animals per group ($n=5$). Animals in group 1 were administered 5% Tween 80 (10 ml/kg i.p), while animals in groups 2–4 were administered 10, 20 and 40 mg/kg ethanol extract of *M. aboreus* leaves respectively. Thirty minutes after treatment, each mouse was administered 10 ml/kg of 0.6% acetic acid (i.p.) before being assessed for 30 min inside the Plexiglas's cage (25 cm × 25 cm × 30 cm). The number of writhing displayed by each mouse was counted and recorded. Acetylsalicylic acid (150 mg/kg, i.p.) which served as the reference drug was administered to group 5.

2.6.2. Formalin-induced paw licking test

Formalin-induced tonic pain was carried out in a similar manner to the method previously described by Janssen et al. (1963). Mice were pretreated with *M. arboreus* (10–40 mg/kg), morphine (5 mg/kg) or vehicle (5% Tween 80, 10 ml/kg) i.p. Thirty minutes later, each mouse received an intra-plantar injection of 50 μl of 1% formalin in the sub-planter space of the right-hind paw and the duration of paw licking was determined at 0–5 min (1st Phase) and 20–30 min (2nd Phase) after formalin administration. The 1st phase is regarded as the neurogenic mechanism and the 2nd phase is the inflammatory mechanism (Oyemitan et al., 2008). In another set of experiment, 2 groups of mice consisting of 5 mice each were selected and pretreated with naloxone (2 mg/kg, i.p.) 15 min prior to administration of the extract (40 mg/kg) and morphine (5 mg/kg i.p.). Thirty minutes later, they were administered 1% formalin and assessed as earlier described above.

2.6.3. Hot plate test

Mice used in this experiment were initially screened by placing the animals in turn on a hot plate set at 55 ± 2 °C and those which failed to lick their hind paw or jump (nociceptive responses) within 5 s were discarded. Eligible animals were divided into five groups of six animals each. Group 1 received 5% Tween 80 (10 ml/kg, i.p.); Groups 2–4 received *M. arboreus* (10, 20 and 40 mg/kg, i.p.) respectively, while Group 5 received morphine (5 mg/kg, i.p.). Thirty minutes after treatment, the animals were placed individually on the hot plate and the reaction time was again recorded. A post-treatment cut-off time of 10 s was used (Gupta et al., 2005). In order to assess possible involvement of opioid receptors, another 2 groups containing 5 mice each were randomly selected, a dose of *M. arboreus* (40 mg/kg) and morphine (5 mg/kg) was interacted with naloxone (1 mg/kg, i.p.). Naloxone was administered 15 min prior to administration of *M. arboreus* (40 mg/kg) or morphine (5 mg/kg, i.p.). Thirty minutes later, the mice were tested as described above.

2.6.4. Tail immersion test

The hot water-induced tail withdrawal reflex as a model of nociception was carried out according to the method of Janssen et al. (1963). Mice were administered *M. arboreus* (10–40 mg/kg i.p), morphine (5 mg/kg i.p) or vehicle (5% Tween 80). Thirty minutes later, the tail of each animal (up to 5 cm) was dipped in hot water maintained at 55.0 ± 0.5 °C. The time (in seconds) it took each animal to withdraw its tail clearly out of the water was taken as the reaction time to pain. The cut-off time of 15 s was used to avoid tissue damage. In another sets of experiment, 2 groups consisting of 5 mice each were randomly selected. Group 1 was pretreated with naloxone (2 mg/kg, i.p) 15 min prior to administration of 40 mg/kg *M. arboreus* extract while group 2 was pretreated with naloxone (2 mg/kg, i.p.) 15 min prior to morphine

Please cite this article as: Olonode, E.T., et al., Anti-nociceptive activity of the crude extract of *Myrianthus arboreus* P. Beauv (Cecropiaceae) in mice. Journal of Ethnopharmacology (2015), http://dx.doi.org/10.1016/j.jep.2015.05.005