Original Research Article

Root bark of *Cordia millenii* essential oil: anti-inflammatory and anti-nociceptive activities

ABSTRACT

Aims: Considering the lack of scientific studies focused on the pharmacological activity of *Cordia millenii* essential oil, this work was designed to evaluate the anti-inflammatory and anti-nociceptive activities of essential oil from the root bark.

Study design: The design of the study include collection of root bark of *Cordia millenii,* hydrodistillation of essential oil from the plant and evaluation of its anti-inflammatory and anti-nociceptive potentials.

Place and Duration of Study: Department of Chemistry, Lagos State University, Nigeria between May 2017 and April 2018.

Methodology: The root bark of *C. millenii* were collected from Ayetoro, Ilesha (7°37′0N 4°43′0E), Osun State, Nigeria in June 2017. Essential oils were obtained from the air-dry sample by hydrodistillation procedure in an all glass Clevenger-apparatus. The anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema. The anti-nociceptive action was established from the hot-plate analysis. Statistical analysis was performed using GraphPad Prism (version 7.02).

Results: The anti-inflammatory activity of the essential oil was statistically significant ($p < 0.001$) at 1st and 3rd h for the 100 mg/kg p.o., at 1st-3rd h for the 200/kg mg p.o. and at 3rd and 4th h for the 400 mg/kg p.o. In addition, the 100 mg/kg p.o. showed significant activity ($p < 0.01$) at 2nd h. Also, the anti-inflammatory activity was significant ($p < 0.05$) for 100 mg/kg p.o. (4th h), 200 mg/kg p.o. (4th h) and 400 mg/kg p.o (1st h). The essential oil of *C. millenii* displayed high activity ($p <0.001$) for all doses in the hot plate anti-nociceptive assay which was time and dose independent.

Conclusion: Results demonstrate that the essential oil of *C. millenii* was effective in the treatment of inflammatory conditions, thereby supporting the traditional use of this herb.

Keywords: Cordia millenii, essential oil, anti-inflammatory activity, anti-nociceptive activity

1. INTRODUCTION

Cordia millenii (Bak.) is a medicinal plant belonging to Boraginaceae family. It is widely distributed in tropical Africa. The plant can grows to a height of 60 to 100 ft, bole cylindrical, but rarely straight, 30 to 40 ft. in length; trunks about 3 ft in diameter above buttresses [1]. The plant has been used in ethnomedicine for the treatment of fever, cough, stomachache, mild tonic, astringent, toothache and inflammation related disorders. Extracts from *C. millenii* have shown the antifertility [1], antimicrobial [2] and antioxidants [2] effects. In addition, the extracts have prevented lipopolysaccharide-induced neuroinflammation [3]. The phytochemical compounds previously isolated from the plant include cordiachromes A–F [4]. Previously, the main constituents of essential oil from the leaf of *C. millenii* [5] were identified as limonene (19.9%), diallyl disulfide (18.4%), β-caryophyllene (16.6%), linalool (13.4%) and nonanal (10.6%). In addition, the leaf essential oil did not possess any significant anti-nociceptive property [5]. However, the essential oil only displayed anti-inflammatory activity

at the 1st h ($P < .01$) for the 200-mg p.o. Till moment, no information is available on biological activity of essential oils from other parts of *C. millenii*.

Generally, the inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischemia, antigen-antibody interaction, and thermal or physical injury. Inflammation is usually associated with pain as a secondary process resulting from the release of analgesic mediators: nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immunosuppressant drugs, which have been used usually in the relief of inflammatory diseases by people around the world for a long time [6]. However, these drugs were often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcers [6]. Recently, many natural medicines derived from medicinal plants were considered as effective and safer for the treatment of various diseases including inflammation and pain [7].

This paper describes the observed anti-inflammatory actions of *C. millenii* essential oil. Recently, the chemical constituents, anti-inflammatory and anti-nociceptive activities of essential oils from Nigerian plants were reported [5,8-10].

2. MATERIALS AND METHOD

2.1 Drug and chemicals

Carrageenan drug (Batch Number: SLBR0530V) of analytical grade was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Acetyl salicylate injection (RX, Nigeria Ltd; Batch Number: MT2056) and Diclofenac Injection (FITZKING LINK LIMITED, Nigeria Ltd; Batch Number: 180606) were purchased from Lagos State University Pharmacy.

2.2 Animals

Wistar rats (150-200 g) of both sexes were accommodated in the Biochemistry Department animal facility of Lagos state University, Ojo-Lagos. The animals were kept in metal steel cages, where they had unrestricted supply to water and standard pellet food. They were acclimatized for two weeks before commencement of experiment. The animals were assigned at random to a group of 5 consisting of 6 animals per group:

Group 1- Control group (Saline solution); Group 2- Diclofenac treated group 100 mg/kg (Standard Group); Group 3- 100 mg/kg of essential oil of *C. millenii*; Group 4- 200 mg/kg of essential oil; and Group 5- 400 mg/kg of *C. millenii*.

The rationale for selecting the studied doses was that animals of similar weight were grouped together to obtained average weight. The weight recorded was similar across the groups of animals. The dose was therefore determined from the weight of animals in the assigned group. The essential oil of *C. millenii* was dissolved in a saline vehicle and administered to the animal in the order of 100, 200 and 400 mg/kg.

2.3 Plant sample

The root barks of *C. millenii* were collected from Ayetoro, Ilesha (7°37′0N 4°43′0E), Osun State in June 2017. Botanical identification was achieved by Mr. Dotanus E. of Herbarium, Department of Botany, University of Ibadan, Nigeria. A voucher specimen (UIH-22607) was deposited at the herbarium. Samples were air-dried under laboratory shade (27°C) for two weeks.

2.3.1 Hydrodistillation of essential oil

In this experiment, 260 g of air-dried and pulverized roots of *C. millenii* was used. The pulverized sample was carefully introduced into a 5 L flask and distilled water was added until it covered the sample completely. Essential oils were obtained by hydrodistillation which was carried out in distillation unit designed according to the specification as described previously [5, 8-10]. The distillation time was 3 h and conducted at normal pressure. The volatile oils which distilled over water were collected by running through the tap in the receiver arm of the apparatus into clean and weighed sample bottles. The oils after drying were kept under refrigeration (4^{o}C) until the moment of analyses.

2.4 Anti-inflammatory and anti-nociceptive tests

2.4.1 Toxicity test

The essential oil was tested for acute toxicity study. Twenty-five Wistar rats (both sexes, 150-200 g each) divided into 5 animals in each groups were used for the toxicity study. Wistar rats were administered 500, 1000, 1500 and 2000 mg/kg of *C. millenii* per oral route. One group received normal saline that served as a negative control. The animals were observed for 12 h continuously for changes in their behavior. Mortality for the next 14 days was also noted.

2.4.2 Carrageenan-induced paw edema in rats

Carrageenan induced rat paw edema experiment was carried out according to a modification form of an established procedure as described previously [5,8-10]. Thirty Wistar rats (both sexes, 150-200 g each) divided into 6 animals in each groups were used for study. The animals were induced by subcutaneous injection of 0.1 mL of 1% freshly prepared carrageenan in saline in the right hind paw. In addition, 1mL of all other solutions was administered for all doses. Paw volume of the injected rats was measured every hour for four hours using a plethysmometer (Ugo Basile, Italy). All treatments were administered orally using the canula syringe.

2.4.3 Hot plate anti-nociceptive test

The experiment was carried out according to the method described previously [5,8-10]. Twenty-five (25) mature Wistar rats of both sexes were randomly divided into 5 groups of equal rats. The animals were fasted for 12 h with provision of clean water *ad libitum*. Doses were administered as follows: Group 1- 10 mL/kg of saline solution (control); Group 2- 10 mg/kg (sodium salicylate, ASA, standard control; Group 3- 100 mg/kg of *C. millenii* oil p.o.; Group 4- 200 mg/kg of *C. millenii* oil p.o.; Group 5- 400 mg/kg of *C. millenii* oil p.o.

Each mouse was placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55 ^{0}C within the restraining glass cylinder. Animal response to the heat varies and such changes includes kicking of hind foot and jumping about, licking of foot, raising the foot, holding the foot tightly to its body or shaking of the foot. The reaction time was recorded 30, 60, 90 and 120 min after the administration of the treatments. The maximum reaction time was fixed at 30 s to prevent any injury to the tissues of the paws. If the reading exceeds 30 s, it would be considered as maximum analgesia.

2.4.4 Statistical analysis

Repeated Measures Two way ANOVA Analysis using Bonferotti multiple comparisons post hoc test was performed using GraphPad Prism (version 7.02), San Diego CA, USA, www.graphPad.com) to compare activity between the control groups and rat treated with the test compounds and values were considered significant at $P < .05$ and above. Results were expressed as mean ± SEM [5,8-10].

3. RESULTS AND DISCUSSION

3.1. Yield of the essential oil

The yield of the essential oil was 0.11% (v/w), calculated on a dry weight basis. The essential oil was colourless and odourless. The yield of the oil was higher than the 0.026% observed for the leaf sample [5].

3.2. Acute toxicity

Test doses of 500, 1000, 1500 and 2000 mg/kg body weight of WIEO showed no adverse effects on the behavioural and physical responses in the tested rats following an observation for 14 days. There was no mortality, flesh or skin peeling, swollen limb or neck, and no weight loss was observed. Therefore, a higher dose of 400 mg/kg given to rats in this study was considered to be safe.

3.3. Anti-inflammatory activity

The evaluation of the anti-inflammatory activity in vivo was conducted using the model of carrageenan-induced paw edema. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [11]. This is a well-defined model of acute inflammation and has been applied in the study of anti-edematous effect of extracts due to the production of different inflammatory mediator in the Wistar rat. This development is time dependent characterised by biphasic release of mediators. The initial phase involves the release of mediators such as histamine, serotonin and bradykin last within the first 1 h, while the latter phase is characterized by infiltration of leukocytes and prostaglandins biosynthesis [12].

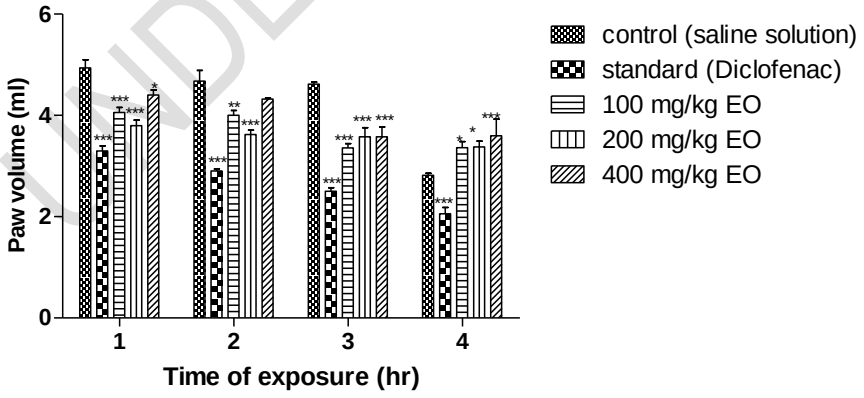

Figure 1: Effect of the essential oils of *C. millenii* roots bark on Carragenan-induced inflammation. Control, standard and *C. millenii* represent 1mL saline solution, 100 mg/kg of diclofenac injection and

1mL of 100, 200 and 400 mg/kg of *C. millenii* leaves essential oil respectively. *$P < .05$, ** $P < .01$, *** $P < .001$ statistically compare to the control.

The anti-inflammatory activity of *C. millenii* root essential oil was statistically ($P < .001$) for the 100 and 200 mg/kg p.o., at 1st and 3rd h, while the 200 mg/kg p.o., also displayed significant activity ($P < .001$) at 2nd h. The 400 mg/kg p.o. displayed anti-inflammatory actions ($P < .001$) at 3rd and 4th h. The anti-inflammatory activity was also statistically ($P < .01$) for the 100 mg/kg p.o at 2nd h, while activity was significant ($P < .05$) for 100 and 200 mg p.o. (4th h) and 400 mg/kg p.o (1st h).

The anti-inflammatory inhibitory activity of *C. millenii* root oil was highly significant. As shown in **Fig. 1**, mediators released in all phases were significantly inhibited. The oil activity at these doses was also equivalent to that of the standard drug used (Ibuprofen). However, at the 4th h, there was significant reduction in the inhibitory activities of the 100 and 200 mg/kg doses. The carrageenan-induced paw edema in rats is believed to be biphasic. The former phase is due to the release of histamine or serotonin (0-1 h post treatment), and the latter phase is characterised by the release of bradykinin, protease, prostaglandin, and lysosome (2nd to 4th h post treatment) [13]. In the present study, oral treatment with *C. millenii* root oil markedly inhibited carrageenan-induced paw oedema in rats in a dose and time dependent manner. This treatment steadily attenuated the paw oedema induced by carrageenan, as well as by numerous inflammatory mediators participating in the carrageenan-induced inflammation such as bradykinin, histamine, substance P and platelet-activating factor [14,15]. This evidence suggests that the anti-inflammatory action of the essential oil of *C. millenii* are related to the inhibition of one or more inflammation mediator pathways involved in the effects of these mediators.

3.4. Anti-nociceptive activity

The anti-nociceptive activity of the essential oils was investigated using the hot plate model. The hot plate test was carried out to ascertain either the peripheral or the central acting effect of the essential oils [16]. The test is widely used to clarify the analgesic and most especially the effect of opioid drugs on the spinal cord. In our study, we found that the essential oil of *C. millenii* showed a very prominent activity at all doses. The ability of the essential oil to inhibit the expressions of the nociceptive neurons was highly significant at all doses ($p<0.001$). The activity of the oil showed a similar activity as the standard drug (ASA), due to statistical significance as shown in **Fig. 2**.

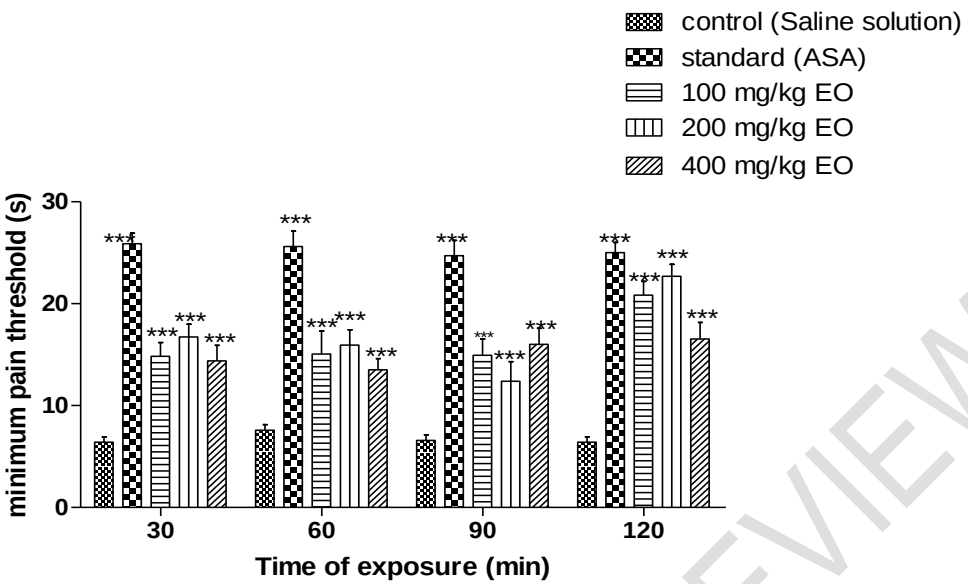

Figure 2 : Effect of the essential oils of *C. millenii* roots bark on hot plate-induced anti-nociceptive. Control, standard and *C. millenii* represent 1mL saline solution, 100 mg/kg of aspirin injection and 1mL of 100, 200 and 400 mg/kg of *C. millenii* leaves essential oil respectively. **P* < .05, ** *P* < .01, *** *P* < .001 statistically compared to the control.

Essential oil has been reported as a good source of anti-inflammatory agent due to their quick penetration after dermal, oral, or pulmonary administration. Their metabolism and elimination occurs in the kidney in the form of phase-II conjugates [17]. Recent information indicated that essential oils and their composition have significantly ameliorated inflammation related ailments. The anti-inflammatory activity of essential oil of *C. millenii* root competes favourably with data from essential oils from other plants studied under the same experimental model. The root barks oil of *C. millenii* posse's considerable anti-inflamamtory activity when compared with the leaf oil [5]. The essential oil of *Phyllanthus muellerianus* [8] and *Waltherica indica* [10] displayed anti-nociceptive effect (*P*< .001) and suppression of inflammatory mediators (*P* < .001) at a rate independent of reaction time and dose. The anti-nociceptive property of the essential oil of *Bounganvillea glabra* [9] was statistically significant (*P*< .001), while for the 1st and 2nd h, at doses of 100 and 200 mg/kg, the anti-inflammatory activity was statistically very significant (*P*< .001). The essential oil of *Melissa officinalis* showed pronounced reduction and inhibition of edema induced by carrageenan at 6 h at 200 and 400 mg/kg [18]. The essential oils of *Senecio flammeus* [19] and *Pycnocycla bashagardiana* [20] significantly reduced inflammation mediators (*P*< .05) 4 h after of carrageenan injection.The anti-inflammatory activity of essential oil of *Cinnamomum longepaniculatum* [21] and *Artemisia aucheri* [22] occurred both in early and late phase and peaked at 4 h after carrageenan injection.

4. CONCLUSION

For the first time, the anti-inflammatory and anti-nociceptive activities of essential oil from the root barks of *C. millenii* were evaluated against carrageenan-induced paw edema and hot plate test, respectively, in rats. Results in this study demonstrated that the essential oils of *C. millenii* were statistically significant and effective in the treatment of both pains and inflammatory conditions, thereby supporting the traditional use of this herb.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interest exists. The plant, Wistar rats, carrageenan drug, acetyl salicylate and diclofenac injection used for this research are commonly and predominantly use products in our area of research and country, Nigeria. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

All experimental procedures were approved under the Lagos State University Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2017/LASU/BCH).

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