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Quantification of Antinociceptive and Anti-inflammatory Potentials of Different *Ocimum gratissimum* Linn. Leaf Extracts in Male Whistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis and managed the analyses. Author OSJ managed the literature searches. Author JSA wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at quantifying the antinociceptive and anti-inflammatory potency and efficacy of aqueous (AQ) and ethanolic leaf extracts of *O. gratissimum* (ET) in Whistar rats.
Methodology: Inflammation (paw edema) was induced in rats (n=5 per group) with fresh egg albumin (0.1 ml); groups for anti-inflammatory experiment were pretreated (0.5 h), while those for anti-nociceptive experiment were post-treated (2.5 h) with AQ (200, 400 or 800 mg/kg, p.o.), ET (200, 400 or 800 mg/kg, p.o.), piroxicam (20 mg/kg, i.p.), or normal saline (1 mL/kg, i.p.). Paw sizes

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of anti-inflammatory group rats were measured at 0 and 2 h after injection of phlogistic agent using plethysmometer, whereas mechanical nociceptive thresholds were measured in anti-nociceptive group rats before and 2 h after test agent treatments using analgesymeter.
Results: There was neither change in inflammatory reaction (after egg albumin injection at time 0 or 2 h) nor threshold in control rats before or after normal saline treatment. AQ inhibited (P = .05) paw edema at 400 mg/kg (26%) and 800 mg/kg (64%); whereas all three doses of ET caused inhibitions (35, 39 and 32%, respectively) compared to the egg albumin induced values. AQ and ET treatments increased (P = .05) mechanical nociceptive thresholds, but maximum effects were observed at 400 mg/kg for both extracts (144 and 270%, respectively) compared to values obtained before extract treatment. Piroxicam produced significant inhibitions in inflammation (20%) and nociception (100%).
Conclusion: ET is more potent, but AQ is about twice as efficacious as ET in anti-inflammatory activity. Also, ET is more potent and has a higher analgesic efficacy than AQ.

Keywords: Anti-inflammatory; antinociceptive; efficacy; O. gratissimum extract; potency.

1. INTRODUCTION

Ocimum gratissimum Linn (Family- Lamiaceae) is an herbaceous perennial shrub, widely distributed in the tropics of Africa and Asia. It belongs to the genus *Ocimum*, which consists of over 30 other species including, *O. americanum, O. basilicum* and *O. micranthum*. The plant is identified by different local names: African Clove, Shrubby Basil (in English) and Basilic Sauvage, Gabonaise (in French). In Nigeria, because of its wide pungent application, the plant is generally called 'Scent Leaf'.

O. gratissimum is extensively used throughout West Africa by traditional medicine practitioners in the treatment of several conditions. The decoctions of the leaves of *O. gratissimum* is used in the treatment of mental illness, convulsion, stomach ache and rhinitis, while the oil from the leaves is used to treat bacterial and fungal infections [1]. Also, the plant is used in the treatment of high fever, diarrhea and ulcer [1,2]. In the southern parts of Nigeria, *O. gratissimum* leaf is widely used in the dressing of neonatal umbilical cord and wounds as it is believed to keep the baby's umbilical cord and wound surfaces sterile [3]. It is formulated in creams against different dermatological diseases, and it is very valuable as a seasoning agent because of its aromatic flavor [4]. Previous research works have also shown that the plant possesses potent pharmacological properties including, antioxidant [5-7]. antimicrobial [8-10], anti-diabetic [11,12], insecticidal [13,14] and anti-ulcer activities [13,15].

The phytochemical constituents of *O. gratissimum* have been severally evaluated to show the presence of alkaloids, tannins, phytates, flavonoids, oligosaccharides [16], tolerable cyanogenic content, non-cyclic sesquiterpenes, phenols [17,18]. *O. gratissimum* leaf is rich in aromatic oil, consisting mainly of thymol and eugenol and other components like xanthones, terpenes and lactones [17-19]. The above phytochemical constituents are believed to be responsible for the many medicinal properties of the plant.

Previous studies have demonstrated anti-inflammatory and antinociceptive properties of the plant [20-22], authenticating the folkloric analgesic use of the plant. This study is aimed at quantifying and comparing the antinociceptive and anti-inflammatory potency and efficacy between aqueous and ethanolic extracts of *O. gratissimum* leaf in Whistar albino rats.

2. MATERIALS AND METHODS

2.1 Animals

Eighty male Whistar albino rats (weighing 250 to 280 g) were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were maintained with standard rat chow in a well-ventilated room at room temperature and a 12 h light/dark cycle. They were allowed access to tap water *ad libitum*. All animal experiments were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Animal experimental procedures were approved by the Ethics Committee on Animal Experiments of the College of Health Sciences, University of Port Harcourt, Nigeria.

2.2 Extraction of Plant Materials

Fresh leaves of *O. gratissimum* L. were collected from a garden within the premises of the University of Port Harcourt, Nigeria. The plant was identified and authenticated by a senior botanist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The leaves of *O. gratissimum* were air-dried and ground into powder. The powdered leaves were separately macerated in 10 liters of distilled water and 70% ethanol for 8 hours and filtered through cheese cloth, glass wool and Whatman No. 1 filter paper. The filtrates were evaporated to dryness by heating in a water bath at a temperature of 40℃ to obtain brownish residues. Their percentage yields were calculated (16.1 and 18.9%, respectively) and the extracts were stored in air-tight containers and preserved in a refrigerator until use.

2.3 Experimental Design

A total of eighty rats were used, forty each for anti-inflammatory and antinocicepive experiments. The rats for each of the experiments were divided into eight groups (n = 5 /group) and administered normal saline (Juhel Nigeria Ltd., Nigeria), piroxicam (Pfizer Inc., USA) and different dose levels of aqueous extract of *O. gratissimum* (AqOG) and ethanolic extract of *O. gratissimum* (EtOG).

2.3.1 Anti-inflammatory study

Fresh egg albumin (0.1 mL) was injected into the subplanter surface of the left hind paw of all the rats to induce acute inflammation [23,24]. Thirty minutes before induction of inflammation, the animals were treated with AqOG (200, 400 and 800 mg/kg, p.o.), EtOG (200, 400 and 800 mg/kg, p.o.), piroxicam (20 mg/kg, i.p.), or normal saline (10 mL/kg, i.p.). Edema in animal paws were measured plethysmographically at 0 and 2 h after injection of phlogistic agent using plethysmometer (Ugo Basile 7140, Italy) by volume displacement as described by Anseloni et al. [25] The percent inhibition of edema was calculated using the formula: % edema inhibition = $[(V_b - V_a)/V_t] \times 100$. V_b and V_a are edema volume after 0 and 2 h of phlogistic agent treatment, respectively.

2.3.2 Antinociceptive study (Egg albumin-induced mechanical hyperalgesia)

Inflammation was induced with fresh egg albumin in left hind paw of experimental rats as described above under anti-inflammatory study. Mechanical nociceptive threshold was determined in the rat paw before and after administration of experimental agents using an analgesymeter (Ugo Basile 372150, Italy) based on the Randall-Selitto test as described by Anseloni et al. [25]. Briefly, incremental pressure was applied gradually to the left hind paw until the rat withdrew the paw. The pressure (grams) required to elicit paw withdrawal was recorded as paw withdrawal threshold (PWT). Baseline measurement was taken before animals were injected phlogistic agent and PWTs were determined again 2 h after egg albumin to establish that mechanical hyperalgesia had developed. AqOG (200, 400 and 800 mg/kg, p.o.), EtOG (200, 400 and 800 mg/kg, p.o.), piroxicam (20 mg/kg, i.p.), or normal saline (10 mL/kg, i.p.) were administered 2.5 h post egg albumin treatment, and PWTs were obtained again 2 h after treatment of test agents. The percent inhibition of nociception was calculated using the formula: % nociception inhibition = $[(PWT_a - PWT_b)/PWT_b] \times 100$. PWT_a and PWT_b are paw withdrawal thresholds after and before extract or piroxicam treatments, respectively.

2.4 Statistical Analysis

The results are presented as mean ± SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnette's multiple range Post hoc test for pair wise comparisons. Data were analyzed using GraphPad Prism 5 software and values were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Anti-inflammatory Effect of *Ocimum gratissimum* Leaf Extracts

There was no change in paw edema in the control rats, as the paw volumes obtained in normal saline treated rats after egg albumin injection were not significantly different from each other (Figs. 1A and 1B). Aqueous extract of *O. gratissimum* (AqOG) produced no significant effect on egg albumin induced paw edema at 200 mg/kg, but decreased ($P = .05$) inflammation at 400 mg/kg (26%) and 800 mg/kg (64%), when compared with egg albumin induced treatment (Figs. 1A and 3). Ethanolic extract of *O. gratissimum* (EtOG) caused significant reduction of paw edema ($P = .05$) at all the doses, corresponding to 35, 39 and 32%

reductions, respectively, compared with egg albumin induced values (Figs. 1B and 3). Paw edema in piroxicam treated rats was also decreased significantly, *P* = .05 (Figs. 1A and 1B), which was equivalent to 20% reduction in inflammation (Fig. 3).

3.2 Antinociceptive Effect of *Ocimum gratissimum* Leaf Extracts on Mechanical Hyperalgesia

There was no change in nociceptive threshold in the control rats when the thresholds obtained before and after normal saline treatment were compared (Figs. 2A and 2B), thus producing zero inhibition (Fig. 4). AqOG treatment significantly increased (*P* = .05) nociceptive threshold, as higher weights were required to cause paw withdrawal than the hyperalgesic states, i.e, before extract treatment (Fig. 2A). The maximum of this effect was observed at 400 mg/kg wherein 80.00±0.0 and 195.00±15.0 g weights were needed to elicit paw withdrawal before and after administration of extract, respectively (Fig. 2A). This was equivalent to about 144% elevation of pain threshold (i.e., decrease in nociception), when compared with the pre extract treatment values (Fig. 4). The ethanol extract also significantly increased (*P* = .05) nociceptive threshold in treated rats, which was equally maximum at 400 mg/kg (Fig. 2B). The nociceptive threshold that was obtained at this dose corresponded to about 270% elevation of pain threshold when compared with the pre extract treatment threshold (Fig. 4). Nociceptive threshold in piroxicam treated rats was increased by 100% (Figs. 2A, 2B and 4).

O. gratissimum L. has been shown to possess several pharmacological properties, including analgesic and anti-inflammatory activities. The present study evaluated the analgesic and anti-inflammatory potency and efficacy of the aqueous and ethanolic extracts of the plant using egg albumin mediated mechanical hyperalgesia and inflammatory tests.

Egg albumin (phlogistic agent) acts as irritant foreign body and induces acute phase inflammatory response. It damages proteins and evokes the release of inflammatory mediators like histamine and serotonin, leading to vascular permeability and expression of adhesion molecules with subsequent extravasation of fluids, plasma proteins and neutrophils to the site of inflammation to cause edema [26]. Time course studies, using this model, have shown that the pathological effects of egg albumin occurs maximum between 1 and 3 h [24,27,28], so potency and efficacy evaluations of the test agents were measured after 2 h of treatment. The analgesymeter and plethysmometer were used which are very sensitive models that can detect anti-nociceptive and anti-inflammatory effects of substances, respectively at doses that may not be detectable by other models.

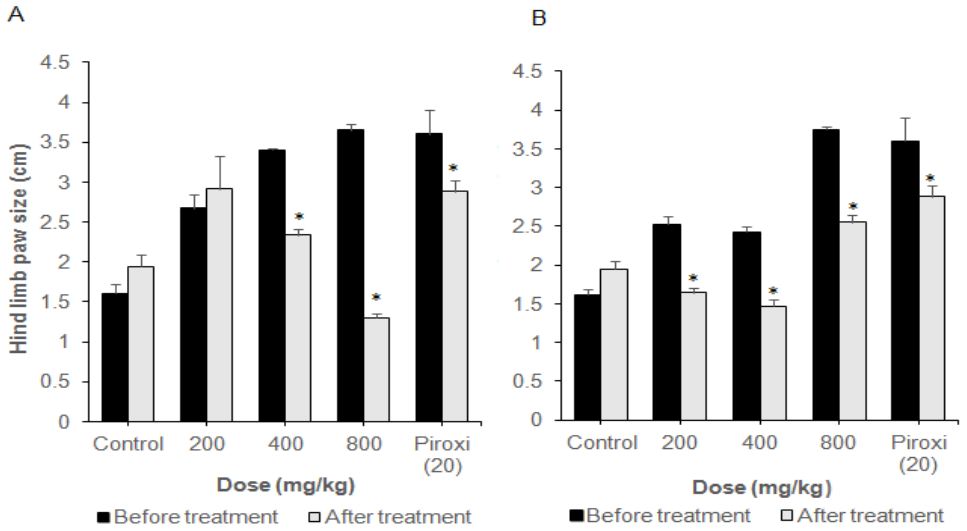

Fig. 1. Hind limb paw sizes obtained after 0 and 2 h of egg albumin injection **in male Whistar albino rats pretreated with (A) Aqueous extract of *Ocimum gratissimum* leaf and piroxicam (piroxi), and (B) Ethanolic extract of *Ocimum gratissimum* leaf and piroxicam**

*Post treatments: significant from pretreatments, * P < 0.05*

Mean ± S.E.M = Mean values ± Standard error of means of five experiments

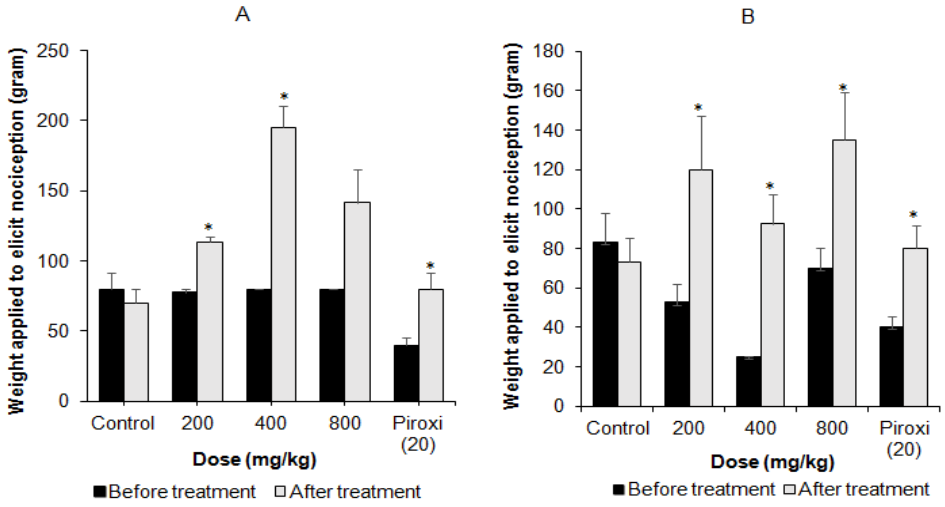

Fig. 2. Mechanical nociceptive pain thresholds obtained before and 2 h post treatment of (A) Aqueous extract of *Ocimum gratissimum* leaf and piroxicam (piroxi), and (B) Ethanolic extract of *Ocimum gratissimum* leaf and piroxicam in male Whistar albino rats that were previously administered egg albumin

*Post treatments: significant from pretreatments, * P < 0.05*
Mean ± S.E.M = Mean values ± Standard error of means of five experiments

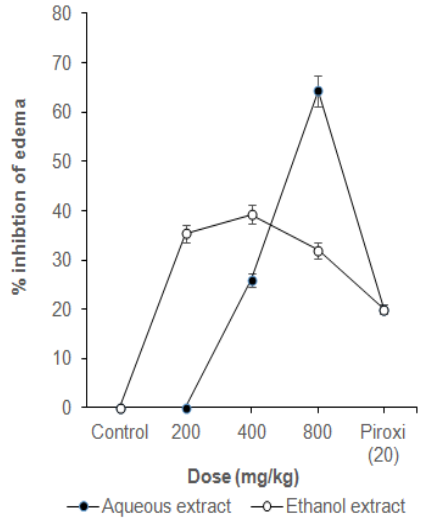

Fig. 3. Percentage inhibition of egg albumin induced paw edema by aqueous and ethanol extracts of *Ocimum gratissimum* leaf and piroxicam (piroxi) in male Whistar albino rats

The results show that aqueous and ethanol leaf extracts of the plant exhibit antinociceptive and anti-inflammatory activities comparable to piroxicam, a potent nonsteroidal anti-inflammatory drug (NSAID). The antinociceptive study basically measured the ability of the test agents to reduce or inhibit hyperalgesia induced by egg albumin in the rats. The results revealed that antinociceptive activity of the plant (aqueous and ethanol extracts) is dose-dependent and maximum at 400 mg/kg. Above this dose, the antinociceptive effect reduced, with the indication that the extracts may fail to produce analgesia at much higher doses. This may reflect the dual redox activities of the plant reported in an earlier study, where it was shown that the crude aqueous leaf extract exhibits antioxidant property at a particular dose, but at higher doses antagonizes the same property [29]. Further, the results indicated that the ethanolic extract is more potent and has a higher (about twice) analgesic efficacy (270%) compared to the aqueous extract (144%). Both extracts produced higher levels of antinociceptive effects than piroxicam, which produced 100% reduction in inflammation. However, piroxicam would be more efficacious than the aqueous extract at the other doses.

Previously, studies have reported anti-inflammatory activity of *O. gratissimum* using different models. Employing the formalin-induced hind-paw edema, Tanko et al. [20] have demonstrated anti-inflammatory effect of *O. gratissimum* in rats. Chinnasamy et al. [30] have also shown that crude methanolic extract of *O. gratissimum* inhibits the proliferative response on PBMC in mitogenic lymphocyte proliferation

assay, as well as expression of proinflammatory cytokines (tumors necrosis factor, interleukin-1 and interleukin-2) markers. The present study has shown that egg albumin-induced edema is inhibited by both aqueous and ethanolic leaf extracts of the plant. The results further show that the anti-inflammatory effect is higher in the aqueous than ethanolic extract at 800 mg/kg (64% versus 32%), but higher in the ethanolic than aqueous extract at 200 mg/kg (35% versus 0%). Thus, the aqueous extract has a higher anti-inflammatory efficacy than the ethanolic extract, while the ethanolic extract appears to be more potent. The 20% reduction in inflammation that was caused by piroxicam was lower than the effects produced by both extracts except the 200 mg/kg aqueous extract, which failed to produce any significant anti-inflammatory effect. Egg albumin-induced inflammation is used to evaluate the inhibitory activity of drugs against increased vascular permeability and release of inflammatory mediators. Putting this together with the pathophysiological role of egg albumin in pain and inflammation [26], it can be concluded that the antinociceptive and anti-inflammatory effects of the extracts is partly via inhibition of eicosanoids activity, and thus inhibition of peripheral neurotransmission.

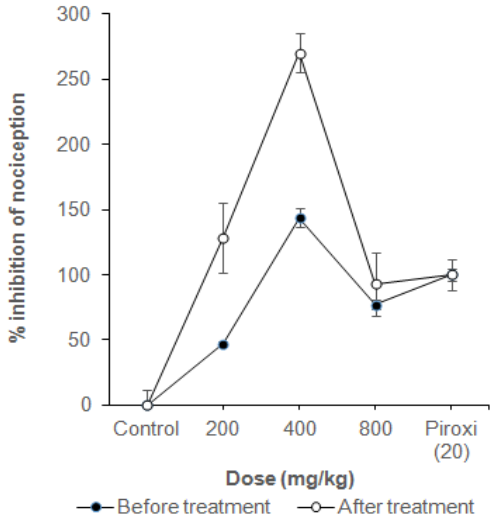

Fig. 4. Percentage inhibition of egg albumin induced mechanical hyperalgesia by aqueous and ethanol extracts of *Ocimum gratissimum* leaf and piroxicam (piroxi) in male Whistar albino rats

These observations can be majorly attributed to the type and amount of phytochemicals in the different extracts. In a previous study, it has been shown that aqueous fractions of *O. gratissimum* leaf have more antimicrobial potentials than its ethanolic fractions [31]. This corroborates the fact that the biological effects of the plant would depend on the phytochemicals and tissue or cell concerned. In this study, the presence of flavonoids, polyphenols, triterpenoids and other chemical constituents in the plant may be responsible for its antinociceptive and anti-inflammatory activities [32]. Phytochemicals are extracted at different degrees in terms of quantity and quality by different vehicles of extraction. In this study, more of these phytochemicals may be extracted in the ethanol than aqueous medium, which may account for the results obtained for the different extracts. A limitation of the study is non-characterization of the extracts, and we recommend further studies to identify and establish the chemical structure(s) of the active components. The findings do not only justify the analgesic and anti-inflammatory application of *O. gratissimum*, but also indicate that effective doses could be achieved at varying concentrations for the different extracts. From the results obtained with piroxicam, it can be considered that 20 and 100% reductions in inflammation and nociception, respectively would be sufficient therapeutically. Apparently, this can be obtained at the lowest dose used in this study (200 mg/kg) for the ethanolic extract, and the mid dose (400 mg/kg) for the aqueous extract.

4. CONCLUSION

The study demonstrates that ethanolic extract of *O. gratissimum* leaf is more potent but less efficacious than the aqueous extract in terms of anti-inflammatory activity; whereas the ethanolic extract has a higher analgesic efficacy and potency than the aqueous extract. A limitation of the study is non-characterization of the biologically active constituents of the extracts, which is an area of future studies.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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