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ANTINOCICEPTIVE ACTIVITIES OF THE ETHANOLIC EXTRACTS OF *OCIMUM KILIMANDSCHARICUM* BAKER EX GÜRKE AND *OCIMUM KENYENSE* AYOB. EX A.J. PATON LEAVES

Peter W. Mwangi[1a], Stanley Wambugu[2], David K. Kariuki[3], Paul M. Mbugua[1], Titus I. Kanui[2]

[1]Department of Medical Physiology, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya.
[2]Department of Veterinary Anatomy and Physiology, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya.
[3]Department of Chemistry, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya.

ABSTRACT

Ocimum kilimandscharicum and *Ocimum kenyense* are two closely related species endemic to Kenya. They find wide application in a diverse array of medicinal applications, including pain relief. The present investigation was carried out to study their antinociceptive activity using the radiant tail-flick test in mice. At 100, 200, 400 and 800 (mg/kg Bwt) dosages, the ethanolic leaf extracts of both *O. kilimandscharicum* and *kenyense* exhibited statistically significant antinociceptive activities ($p < 0.01$), in a dose dependent manner. The experimental results obtained in this study therefore validate the traditional uses of these plant species as analgesics. Further, this study provides a springboard into future phytochemical and pharmacological studies of these plant species.

KEY WORDS: *Ocimum kilimandscharica*, *O. kenyense*, Pain, Antinociception, Medicinal plants.

INTRODUCTION

The International association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Bonica, 1979). Currently, most of the conventional pain therapies are unsatisfactory in terms of efficacy, tolerability and toxicity (Scholz and Woolf, 2002). The recent banning/withdrawal of rofecoxib a selective Cyclooxygenase 2 enzyme (COX-2) inhibitor due to its cardiotoxic effects (McGettigan and Henry, 2006) is a salutary reminder of the toxicity concerns surrounding current pain therapies. This makes the development and discovery of more efficacious drugs a priority in view of the disabling effects of chronic pain conditions with the attendant socio-economic costs and consequences (Apkarian *et al*., 2009).

There is increasing recognition that natural products may provide a viable source for new drug molecules especially in view of the failure of the more popular combinatorial conventional synthetic chemistry approaches (Prasad *et al*., 2005). The failures in the combinatorial chemistry approaches has been especially marked in the area of CNS therapeutics with the result that many multinational pharmaceutical companies have drastically scaled down or even closed many CNS drug discovery programs (Agid *et al*., 2007).

The genus Ocimum which belongs to the Lamiaceae family consists of sixty four recognized species (Paton *et al*., 1994) many of which are of economic as well as medicinal importance. *Ocimum basilicum* L. is a culinary

Corresponding Author

Peter Waweru Mwangi
Email:- waweruk2001@gmail.com

herb of worldwide renown while *Ocimum sanctum* L. is a key herb in Ayurvedic medicine (Gupta *et al*., 2007). *Ocimum kilimands charicum Baker ex Gurke* and *Ocimum kenyense Ayobangira ex A.J. Paton* are two closely related species that are endemic to Kenya. They find wide application in traditional medicine among ethnic communities in Kenya as analgesics and antipyretics (Njoroge and Bussman, 2005; Kanui, 2006).

This study was undertaken to evaluate the antinociceptive activity of ethanolic extracts prepared from the leaves of these two plant species.

MATERIALS AND METHODS

Extract preparation

The leaves of the two plant species were collected at the Lenana area of Nairobi Kenya. Identities of the collected plant materials were verified by the staff of the University Herbarium, Department of Botany, University of Nairobi, where voucher specimens were deposited.

Fifty grams of the each plant material were placed in a soxhlet evaporator and extracted at 40 degrees centigrade for three hours using absolute ethanol as the at a ratio of 1:10 w/v to ensure complete extraction. The resulting extract was then evaporated to dryness in a rotary evaporator (Ugo Basile, Italy) at 40˚C and 376 Pascals pressure. The extracts were then weighed and placed in airtight amber colored sample bottles.

Experimental animals

Male adult Swiss albino mice aged 5-6 weeks and weighing between 18 and 25g were used. The animals were procured from Kenya Agricultural Research Institute (KARI) Muguga, Kenya. They were housed in standard animal cages within the departmental animal house. Care was taken to maintain ambient temperatures of 23-25°C and the relative humidity in the animal house being at between 45 and 55%. A 12/12 hour light-dark cycle was maintained within the animal house. The animals were fed with food pellets obtained from Unga Feeds (K) Ltd. Water and feed were provided ad libitum. The mice were acclimatized in the animal house for at least one month prior to the actual start of the experimentation. In the test room, they were habituated for an hour prior to the start of testing. Care was taken to ensure that the ambient conditions in the test room were as similar to those in the animal house as possible.

Experimental protocol

a) Tail Flick Test

The analgesic activities of the extracts were screened using the radiant heat variant of theTail Flick Test in the manner described by D'Amour and Smith (1941), with slight modifications. Briefly the mice were randomized to receive either extract or solvent mixture which acted as the negative control. Before injection, the extract was suspended in a solvent mixture composed of Dimethyl sulfoxide (DMSO) and normal saline in a ratio of 1:9 respectively. The extract was administered at four dosage levels of 100, 200, 400 and 800 (mg/kg) body weight. Paracetamol (400 mg/kg) was used as a positive control. There were 6 experimental groups for each extract (4 experimental, 1 positive control and 1 negative control). Each experimental group had five animals.

The extract was injected intraperitoneally and the Tail Flick Test performed 1 hour later to ensure maximal absorption. The beam intensity of the analgesiometer (IITC Model no. W84) was set at 80 and cut-off point response duration was set at twenty seconds to avoid tissue damage. The observer was blind to the treatment group. The tail flick latencies were determined using a stopwatch and recorded in seconds and rounded off to one decimal place.

b) Sensorimotor Activity Testing

The pull-up test (Deacon and Gardner, 1984) was performed in order to verify that any antinociceptive activity shown by the extracts was independent of any confounding muscle relaxant and sensorimotor retardation effects. Briefly the procedure was as follows; the mice were held in a fully extended inverted position one hour after administration of extract/control. The end point of the experiment was when the mouse in attempting to gain an upright position touched the hand or fingers of the experimenter with both forepaws simultaneously. The latency to the end point was recorded using a stop watch. The cut-off point of the experiment was set at fifteen seconds.

Statistical Analysis

The results were analyzed using the Kruksal-Wallis non-parametric test using GraphPad Prism ™ suite of statistical software. Post-hoc analysis was performed using the Wilcoxon-Mann/Whitney U test. The significance level was set at $P< 0.05$.

RESULTS

The ethanol extract of *Ocimum kenyense* leaves possessed significant antinociceptive activity in the tail flick test ($p < 0.001$) (Fig. 1). The extract had significant antinociceptive activity compared to control at all the dosage levels tested in this experiment. The maximal antinociceptive activity was observed at the 800 mg/kg Bwt dose level (11.2 ± 0.4 vs. 4.1 ± 0.2 sec) for the control.

The ethanol extract of *Ocimum kilimandscharicum* leaves possessed significant antinociceptive activity in the tail flick test ($p < 0.001$). This extract had significant antinociceptive activity compared to control at all the dosage levels tested in this experiment. The maximal antinociceptive activity was observed at the 800 mg/kg Bwt dose level (11.8 ± 0.5 vs. 4.1 ± 0.2 sec) for the control.

The ED50 values for the *Ocimum kilimandscharicum* and *Ocimum kenyense* leaves were estimated Bioassay ™ Excel- add-in (Onofri, 2001) to be 125 and 262 (mg/kg) respectively. Preliminary phytochemical analysis of the extracts indicated the presence of steroids, alkaloids and flavonoids.

Fig 1. Response latency in the Tail flick test for the ethanol extract of *Ocimum kenyense* leaves

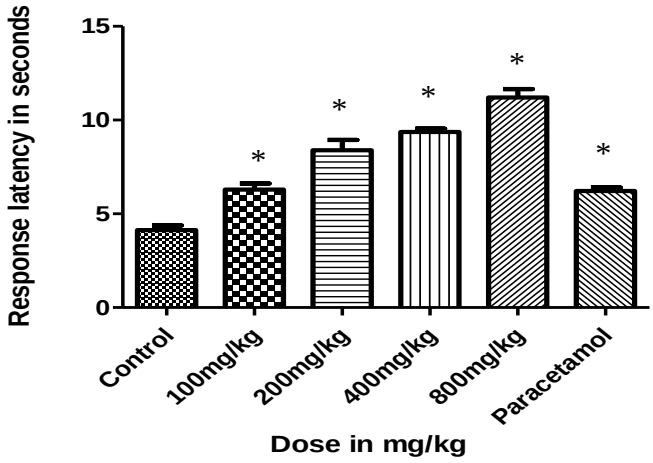
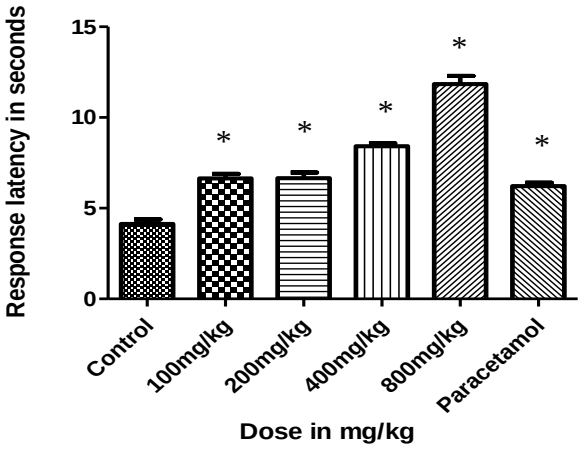


Fig 2. Figure showing the reponse latency in the Tail flick test for the ethanol extracts of *Ocimum kilimandscharicum* leaves



DISCUSSION

The Tail flick test is a popular and convenient method for the evaluation of antinociceptive activity of different pharmacological agents (King *et al*., 1997). This is because the test does not require the use of highly sophisticated equipment (King *et al*., 1997), and results may be obtained rapidly without conditioning effects and without causing undue stress to the experimental animal (Grau, 1987). Although the tail flick response is a spinally integrated reflex, tail flick response latencies have been shown to be sensitive to pharmacological manipulation with analgesics acting at supraspinal levels (Amit and Galina, 1986; Le Bars *et al*., 2001). The foregoing therefore makes the Tail flick test an ideal and robust test for the evaluation of the antinociceptive activity of various pain alleviating drugs.

Both extracts showed significant antinociceptive activity in the tail flick test in mice. The antinociceptive activity was present in all the doses used, in a dose dependent manner. The data therefore supports the traditional/folkloric use of the plants as well as validates their use as analgesics (Kanui, 2006; Njoroge and Bussman, 2007). The results of this study are significant because previous studies on these plants have focused on the isolation of the chemical phytoconstituents of the essential oils contained in these plants (Mwangi *et al*., 1994; Jembere *et al*., 1995; Mwangi *et al*., 2009). Paracetamol (Acetaminophene) was used as a positive control because it is a centrally acting analgesic and not Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Tjølsen A *et al*., 1991; Hole *et al*., 1994; Mallet *et al*., 2008, 2011). Probably the mode of action of the test extracts could be analogous to that of paracetamol.

There were no statistically significant differences between the test and the control groups in the pull-up test at the highest dose of the extract tested (800 mg/kg) (6.23± 0.7s control vs. 6.15± 0.2s). This was an indication that the observed antinociceptive activity was not being confounded by skeletal muscle relaxant activity of the extract (Deacon and Gardner, 1983).

CONCLUSION

The ethanolic extracts of *Ocimum kenyense* and *Ocimum kilimandscharicum* leaves possess significant antinociceptive activity in the animal model of nociception tested. This therefore validates the traditional uses of these closely related herbs in the management of pain related disorders. More studies are needed to evaluate the phytochemical components responsible for the observed antinociceptive activities.

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