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Research Article

Anti-nociceptive activity of methanolic extract of *ocimum gratissimum* (*labiate*) on experimental animals

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ABSTRACT

The methanolic leaves extract of *Ocimum gratissimum* was investigated for anti-nociceptive effects in mice. The models used to study the effect on nociception are the acetic acid induced abdominal constriction test, hot- plate method in mice. The extract demonstrated a significant (P< 0.05) anti-nociceptive activity at all the doses (50,100 and 200 mg/kg body weight i.p.) tested group and standard group (piroxicam 20mg/kg) compared to control normal saline. The activity resides more at the highest dose 200mg/kg body weight i.p. that was found to have the highest percentage (92.5 %) of inhibition of the abdominal constriction induced by acetic acid in mice. Also the extract was found to have a significant (p <0.05) inhibitory effect on hot-plate method at all the three doses. The extract caused a significant (p<0.05), dose dependent inhibition of acetic acid-induced writhing and hot-plate method. The intraperitoneal LD50 value of the extract was 1285.5mg/kg body weight in mice. Preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins and flavonoids. The results suggest the extract contained pharmacologically active principles, and supports the local application of the plant in painful conditions. Further studies may reveal the exact mechanisms of action responsible for the analgesic activities of *O. gratissimum* leaves extract.

Keywords: *Ocimum gratissimum; Labiatae;* Anti-nociceptive; Piroxicam; Acetic acid-induced writhing; hot-plate.

INTRODUCTION

Ocimum gratissimum is commonly known as fever leaf in general but is has different native names in different part of the country. In Yoruba language, it is known as Ewfirin ajase, Nchu-nwu in Ibo, Bunsuru daji in Hausa, Ireru in Ebira, Ebaubokho in Benin, ufuo-yibo in Urhobo and ntion in Efik (Sofowora, L.A, 1993). There are about 60 or more species of *Ocimum* and numerous varieties, belonging to the Family *Labiatae.* This different types of species are represented by the five most important representatives of the more that 60 *Ocimum* species and these include (i) *Ocimum gratissimum,* (ii) *Ocimum basilicum,* (iii) *Ocimum americanum,* (iv) *Ocimum sanctum* and (v) *Ocimum americanum* (Mandal, J, 2000; Martin, A.P 1999).

Ocimum gratissimum Linn. (Labiaceae) is a herbaceous plant commonly found in tropical Asia especially India. It is used in the treatment of epilepsy in the coastal area of Nigeria (Osito, N.G. 1992), High fever (Oliver, B. 1980), and Diarrhoea (Oliver, B. 1980; (Sofowora, L.A 1993). The plant is also used to treat typhoid fever and diabetes (Adjanahoun, E et al., 1991; Igoli, J.O et al., 2002; Tor-Anyin, T.A et al., 2003). Today, basil is used mainly as a culinary herb. It medicinal value is not as widely appreciated in Western World. In France it is used in perfumes and cosmetics (Ross,Ivan A. 2003).

This research was aimed at investigating the possible anti-nociceptiactivities of methanolic leaves extract of the plant in order to support or refute the claims by traditional herbalists.

MATERIAL AND METHODS

Preparation of Extract

The fresh leaves of *Ocimum gratissimum* was chopped, cleaned and air dried for minimum one week. After that the size was reduced with a mortar and pestle into a fine powder. 100 g of the powder was extracted with 90% methanol (2.5 litres) using Soxhlet apparatus process for 50-55 h. The liquid extract was then concentrated on a water bath to give a brownish solid extract with a mean yield of 10% w/w.

Methanolic extract obtained was washed with hexane to obtain purified methanolic extract. Methanolic fraction was successively extracted with ethanol (90 % w/w) in Soxhlet apparatus for 50-55 h (Trease, G.E 1989).

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Table 1: Effect of methanolic extract of *O. gratissimum* on writhing assay

S.No	Treatment	Mean wriths
1	Vehicle (Placebo/control)	22.5
2	Piroxicam (Standard)	5.65*
3	Ocimum gratissimum 50ml/kg	7.54*
4	Ocimum gratissimum 100ml/kg	7.15*
5	Ocimum gratissimum 200ml/kg	2.64*

*P < 0.05 compared to vehicle (control)

Table 2: Effect of methanolic extract of *O.gratissimum* on Hot-plate assay

S.No	Treatment	Reaction Latency (30 mins)	Reaction Latency (60 mins)	Reaction Latency (120 mins)
1	Vehicle (Placebo/control)	8.2	11.4	9.5
2	Piroxicam (Standard)	20.4	49.8*	47.4*
3	Ocimum gratissimum 50ml/kg	27.6*	33.8*	37.5*
4	Ocimum gratissimum 100ml/kg	23.3*	36.4*	37.2*
5	Ocimum gratissimum 200ml/kg	44.2*	44.4*	46.8*

*P < 0.05 compared to vehicle (control)

Acute Toxicity Study

This was conducted by using the method described by Lorke. In the initial phase, mice were divided into 3 groups of three and treated with the methanolic leaves extract of the plant at doses of 10, 100 and 1000mg extract/ kg body weight Intra-peritoneal (i.p.) and were then observed for 24 hrs for signs of toxicity including death .In the final phase, mice were divided into 4 groups of one mouse each and treated with the ethanol extract at doses of 600, 1000, 1600 and 2900 mg / kg body weight i.p. The median lethal dose (LD50) was calculated from the second phase (Lorke, D 1983).

Acetic acid induced abdominal constriction

Swiss mice (20-25gm) were divided into 5 groups of 6 mice each. The first group was given 10 ml/kg of Normal saline i.p. and served as control or placebo, groups 2 received Piroxicam 20mg/kg as a positive control or standard, 3, 4 and 5 received 50, 100 and 200 mg of extract per kg of body weight i.p. respectively. Thirty mins later, mice in all the groups were treated with Acetic acid (1%v/v, 10ml/kg body weight i.p.). Five minutes after Acetic acid injection, mice were placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 mins (Koster, R 1959).

Hot-plate assay :

Swiss mice (20-25gm) were divided into 5 groups of 6 mice each. The first group was given 10 ml/kg of Normal saline i.p. and served as control or placebo, groups 2 received Piroxicam 20mg/kg as a positive control or standard, 3, 4 and 5 received 50, 100 and 200 mg of extract per kg of body weight i.p. respectively. A 500 ml of glass beaker was placed on hot-plate. The temperature of the hot-plate was then regulated to 50 ± 20 C in order to obtain the animal's response to electric heat-induced nociceptive pain stimulus (licking of the fore-paws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time taken for each mouse to jump out of the beaker (i.e. reaction time) recorded in seconds. Readings were taken at intervals of 30, 60 and 90 mins after extract administration. Cut off time in the absence of response was 60 s to avoid tissue damage to the mice paws (Sharma, K.K 1982).

Statistical analysis

Results were expressed as mean ± Standard Error of Mean (SEM). The data was statistically analyzed using the one-way ANOVA to determine whether results in a particular group were significantly different from those in the corresponding control groups. Results were statistically significant when P values are less than 0.05 (P <0.05) as described by Duncan (Duncan, R. C).

RESULT

The freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids and steroids. The sign of toxicity was first noticed after 4-6 hrs of extract administration. There was decreased locomotor activity and decreased sensitivity to touch and jerking. Also there was decreased feed intake, and prostration after 10 hrs of extract administration. The median lethal dose (LD50) in mice was calculated to be 1285.5 mg/kg body weight

The extract demonstrated a significant (P< 0.05) anti-nociceptive activity at all the doses (50,100 and 200 mg/kg body weight i.p.) tested compared to control normal saline. The activity resides more at the highest dose 200mg/kg body weight i.p. that was found to have the highest percentage (92.5 %) of inhibition of the abdominal constriction induced by acetic acid in

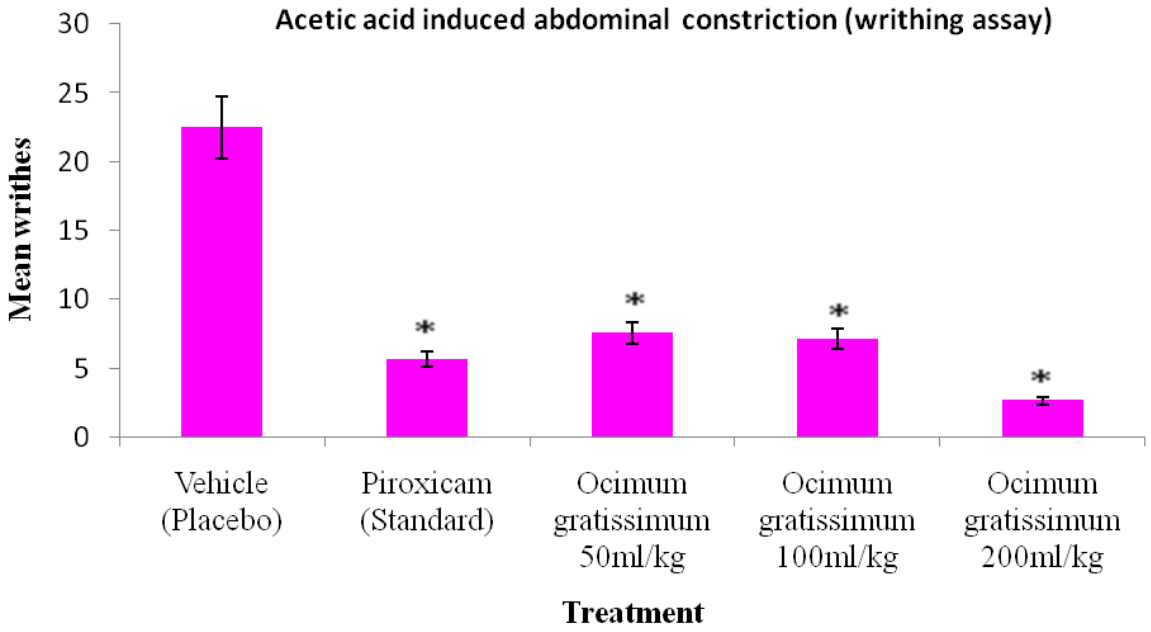

Figure 1: Effect of methanolic extract of *O.gratissimum* on writhing assay, *P < 0.05 compared to vehicle

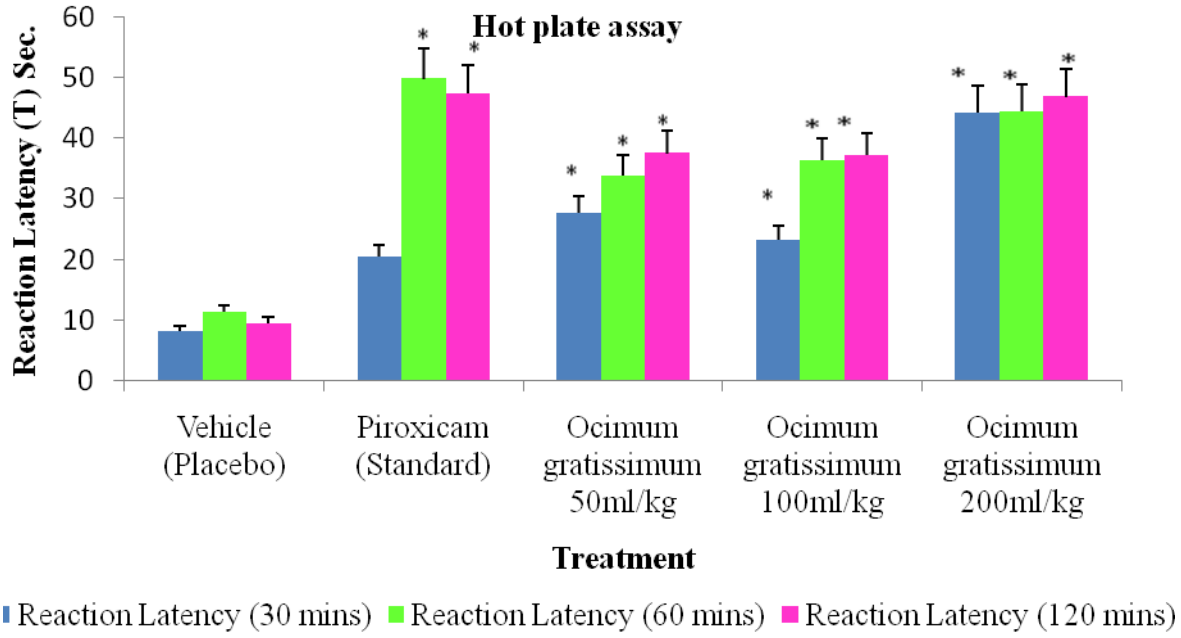

Figure 2: Effect of methanolic extract of *O.gratissimum* on Hot-plate assay, *P < 0.05 compared to vehicle

mice (Table 1). Also the extract was found to have a significant (p <0.05) inhibitory effect on hot-plate method at all the three doses as shown in Table 2.

DISCUSSION

The extract produce significant (* P<0.05) analgesic (anti-nociceptive) activity at all the doses for tested group and standard group compared to control group of animal.

Acute pain as writhing assay is chemical assay that may produce some inflammation and used as model of visceral pain. Possible mediators involved in the acetic acid induced inflammatory pain are not well known. It has been reported that bradykinin, neurokinins and prostanoids involves in the sensory C-fibres activation after Intra-peritoneal injection of acetic acid. The activity demonstrated by the extract might be due to the presence of flavonoids and phenolics that were present in the extract (Bentley, G.A 1983; Besra SE, 1996; Derardt, R et al., 1980).

The Hot-plate assay is somatic pain model that doesn't produce inflammation. The repetitive afferent input enhances the response of the animal to a given noxious stimulus (radiant heat on Hot-plate). Activation of prostanoid receptors increases the opening of voltage sensitive Ca^{2+} channels and enhances primary afferent peptide release. It may be possible that extract exert its therapeutic action by antagonizing prostanoid receptor or inhibiting the synthesis of prostaglandins (Derardt, R et al., 1980; Ahmadiani, A et al., 1998).

CONCLUSION

In conclusion, these preliminary investigations and data obtained from this study demonstrated that effect of herbal plant *Ocimum gratissimum* aqueous leaves extract have good analgesic or anti-nocciceptive activity.

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