PRELIMINARY EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF *OCIMUM GRATISSIMUM* LEAVES

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ABSTRACT

The aqueous extract of *Ocimum gratissimum (Linn)* leaves was investigated for its possible anti-nociceptive activity. The extract (100 - 800 $mgkg^{-1}$) in a dose - dependent fashion significantly ($p < 0.005$ and $p < 0.0005$) reduced the number of writhes and stretching induced by 0.6% aqueous solution of acetic acid in Wistar rats. At a higher dose of 800 $mgkg^{-1}$, the extract conferred 76.49% protection to the rats as compared with a low (100 $mgkg^{-1}$) dose which conferred 39.19% protection on the rats. The effect of the higher dose in protecting the rats against the acetic acid induced writhing was closer to that of the reference drug Pethidine hydrochloride at a dose of 20 $mgkg^{-1}$. In the acute toxicity studies, the aqueous extract did not cause any mortality up to a dose at 10g kg^{-1}. However, there were clinical signs of loss of appetite, decreased exploratory behaviour, and abnormal body posture.

INTRODUCTION

Ocimum gratissimum, Linn, of the (family; Labiaceae) is a herbaceous plant commonly found in tropical Asia especially in Pakistan and India, where it is used for aromatic baths, for fumigations, in the treatment of rheumatism and paralysis (Uphof, 1968). In West Africa and in Nigeria, the plant is found both in the Savannah and the coastal areas. In the coastal areas it is used to treat epilepsy (Gbile, 1986; Osifo, 1992) whilst in the Savannah areas decoctions of the leaves is used to treat mental illness (Fanna, 1992). The ethanol extract of the leaves has been reported to have analgesic activity (Adesina, 1982) in mice. It is also used as spice in Nigeria.

In view of its many varied uses and the finding that its ethanol (70%) extract has been found to exhibit analgesic activity, the present study was undertaken to evaluate the effect of the aqueous extract on pain threshold in rats.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh leaves (500g) of the shrub were collected in August 1997, from the University of Maiduguri. The leaves were identified by Plant Taxonomist, Department of Biology, Faculty of Science, University of Maiduguri.

Preparation of Extract

Fresh leaves of *Ocimum gratissimum* were kept in the oven at 80°C for 10 min and then at 60°C for 30 min. They were then sun-dried and ground into coarse powder. Thirty grams of the powdered leaves were boiled with distilled water for 5 min. The mixture was then kept aside off the hot plate, for 30 min to allow it to infuse, it was then filtered through cheese cloth. The filtrate was kept in refrigerator at 4°C until used.

Acute Toxicity Studies

Acute toxicity studies were carried out using Wistar strain of rats weighing between 180 – 240g, obtained from the Department of Pharmacology, College of Medical Sciences, University of Maiduguri. Five groups of six rats in each were used. They were administered (intraperitoneally) with varying logarithmic doses (1000, 2000, 4000, 8000 and 10,000 $mgkg^{-1}$) of the aqueous extract of *Ocimum gratissimum* leaves. The rats were allowed access to food and water *ad libitum* and were observed for a period of 24 hrs for clinical signs of toxicity and death (Loomis, 1978).

Writhing Test

Thirty six healthy albino rats weighing between 180-200g and of mixed sexes were used for the study. They were fed with growers mash (ECWA feeds, Maiduguri) and water *ad libitum* throughout the period of the experiment. The rats were randomly separated into six groups of 6 rats each. Group 1 served as the control while groups 2-5 were given various doses (100, 400 and 800 $mgkg^{-1}$ body weight) of the extract intraperitoneally. These doses did not elicit any serious signs of toxicity or neurological effects. The animals in group six were treated with 20 $mgkg^{-1}$ body weight of Pethidine hydrochloride (Sigma Chemical Company, U.S.A.).

Rats in the control group were injected with 10 $mgkg^{-1}$ aqueous solution of 0.6% acetic acid intraperitoneally. The number of stretching movements was counted for 10 min starting 5 min after acetic acid injection. The effect of aqueous extract of *Ocimum gratissimum* on the stretching movement was determined by administering rats with various doses (100 - 800 $mgkg^{-1}$) of the extract 30 min before acetic acid injection. Stretching movements was counted as was done in the control groups. The effect of Pethidine hydrochloride (20 $mgkg^{-1}$ body weight) on the writhing induced by acetic acid was studied in the sixth group of rats. Using the number of writhes/stretches, the percentage protection was calculated using the following formula:

$$\text{Percentage Protection} = \frac{\text{Mean writhings of the Control group - Mean writhings of the treated group}}{\text{Mean writhings of the control group}} \times 100$$

as reported by Hernandez-Perez *et al.* (1995).

RESULTS

Acute Toxicity Studies

Fifteen minutes after the extract administration there was decreased locomotor activity. The rats were sedated and grouped together at one corner of their cage. There was body sag. Thirty minutes after extract administration, there was no movement with the animals being sedated but did not sleep. There was loss of appetite. Three hours after extract administration, some rats had facial oedema and pruritis especially at the flanks of the rats that received high doses (8 and 10g kg^{-1}). There were some neurological effects like, lack of exploratory behaviour and abnormal body posture. There was no mortality upto 24 hrs after administration of the extract. The rats exhibited complete loss of pain perception, since they did not react to pressure applied to the tail or the hindlimbs.

Effect of Extract on Writhing

The intraperitoneal administration of the decoction of *Ocimum gratissimum* in the dosage range (100 - 800 $mgkg^{-1}$) caused an increase in pain threshold of all the rats (Table 1) when compared with the control. The effect was dose dependent. At doses of 100 $mgkg^{-1}$ and 800 $mgkg^{-1}$ the number of writhes were 15.01 ± 1.86 and 5.80 ± 0.78 respectively when compared with the control (24.67 ± 1.99). These decreases were significant ($p < 0.005$) and conferred 39.19 and 76.49%, protection on the rats, respectively. The group that received Pethidine hydrochloride (20 $mgkg^{-1}$) intraperitoneally did not respond to a challenge with 0.6% acetic acid (10$mlkg^{-1}$) indicating 100% protection on the rats.

Table 1: The Effect of Aqueous Extract of *Ocimum gratissimum* leaves on stretches (Writhes) Induced by Acetic Acid (0.6%).

Treatment	Extract Dose ($Mgkg^{-1}$)	Number of Writhes (/Min)	Percentage Protection
Control	-	24.67 ± 1.99	0
Extract + A.A.	100	15.0 ± 1.86*	39.19
Extract + A.A.	200	12.67 ± 1.70*	48.64
Extract + A.A.	400	6.67 ± 1.20**	72.96
Extract + A.A.	800	5.80 ± 0.78**	76.49
Pethidine HCl + A.A.	20	0	100

A.A. = Acetic Acid, N = 6 (Number of rats in a group) * $P < 0.005$ ** $P < 0.0005$ significantly different from controls (Students t - test)

DISCUSSION

The results of the present investigation have shown that the decoction of the *Ocimum gratissimum* leaves possesses potential antinociceptive activity in rats. The extract at a dose of 800 $mgkg^{-1}$ body weight, conferred 76.49% protection on the rats. This was found to be similar to the effect of 20 $mgkg^{-1}$ of Pethidine hydrochloride in the extent to which the writhings or

stretching induced by acetic acid were suppressed or abolished. Acetic acid is believed to trigger the production of noxious substances within the peritoneum which cause the writhing response (Bartolini, *et al.* 1987). The similarity in the effect of the extract and Pethidine against the noxious stimulus indicates that they depressed the production of the irritants and thereby reduced the number of writhes in the animals.

The results has provided an evidence that the decoction of *Ocimum gratissimum* possess potential antinociceptive effect. This finding also supports the report of Adesina (1982) showing that the alcoholic extract of *Ocimum gratissimum* leaves possess analgesic activity. The extract was found to be safe since it did not cause any death even at a very high dose of 10g kg^{-1} body weight. Further work to isolate the active chemical principle(s) and to elucidate the mechanism of analgesic action of the plant extract is needed to be determined.

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