ANXIOLYTIC EFFECT OF *OCIMUM GRATISSIMUM* ON THE ELEVATED PLUS MAZE MODEL OF ANXIETY IN MICE

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Summary

In traditional system of medicine *Ocimum* species has been recommended for relief of anxiety and other central nervous system disorders. In the present study the petroleum ether, chloroform, methanol and aqueous extracts of *Ocimum gratissimum* were evaluated for anxiolytic effect using elevated plus maze (EPM) model in mice. The various extracts have produced statistically significant ($p<0.05$) and considerable anxiolytic activity. The aqueous extract at the dose of 100 mg/kg has produced maximum anxiolytic activity. These findings justify the traditional use of the plant in the treatment of anxiety and CNS disorders.

Key words: *Ocimum gratissimum*, Anxiolytic activity, Elevated plus maze, Diazepam

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Introduction

Anxiety disorders in a modern society have a relatively high prevalence and common considerable financial resources. Currently the most widely prescribed medication for anxiety disorder is Benzodiazepines (BDZs). However the clinical uses of BDZs are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drug and dependence liability. Therefore the development of new medication possessing anxiolytic effect without the complication of BDZs would be of great importance in the treatment of anxiety related disorders. Medicinal plants are good source to find new remedies for these disorders. Several *Ocimum* species belonging to family Lamiaceae are used to treat central nervous system (CNS) disorders in various parts of world. Its depressive [1] and insect control [2] activity has been reported. Leaves from *Ocimum* species release a pleasing odour when squashed between the fingers and could be used as a culinary condiment [3]. Published data from ethnopharmacological sources indicate the use of *Ocimum basilicum* as a sedative in Spain [4].

O. gratissimum have also been reported as sedative [5], cytotoxic [6], antidiarrhoeal [7], blood coagulating agent [8]. It is also useful in the treatment of mental illness [9], malaria [10].

Materials and Methods

Plant material

Plants of *O. gratissimum* were collected from the cultivated source from the nursery of National Institute of Pharmaceutical Education and Research, Mohali (Punjab, India in April 2005). The material was authenticated by Dr. H.B Singh, Head of Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (Consult no. 912/96) and a voucher specimen was deposited at Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India for further references.

Preparation of extract

Aerial parts of *O. gratissimum* were dried in shade, powdered and passed through sieve (#60). The dried aerial parts were successively extracted with petroleum ether, chloroform and methanol. Finally the aqueous extract was prepared by decoction. The different extracts were dried using rotary vacuum evaporator and preserved in vacuum dessicator. The yield of the petroleum ether (OGPE), chloroform (OGCH), methanol (OGM) and aqueous extract (OGA) were 2.5, 5.3, 12.2 and 6.8% respectively.

Animals

Swiss albino mice of either sex (20 – 25 gm) were procured from College of Veterinary Science, Izatnagar, Bareilly, India and used for the experiment. The animals were allowed to standard diet and water *ad libitum*. The experiment was approved from Institutional Animal Ethical Committee (IAEC) and experiments were carried out in accordance with the ethical committee guidelines laid down by the local committee regarding the care and use of animals for experimental procedures.

Drugs

Different extracts and diazepam were suspended in the vehicle i.e., 1% (v/v) Tween 80 in normal saline. Drugs and extracts were freshly prepared on the day of the experiment.

Pharmacological evaluation

Animals were divided into different groups of six mice each. One group served as control and received 1% (v/v) Tween 80 in normal saline, another group was treated with diazepam (2 g/kg) and the rest groups were treated with different extracts in different doses as shown in Table 1.

Anti-anxiety activity

Anti-anxiety activity was studied by elevated plus-maze test. The plus maze apparatus consisting of two open arms (16x5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof with the plus maze elevated (25cm) from the floor was used to observe anxiolytic behaviour in animals [11]. The animals were fasted 18 hrs prior to the

experiment and extracts were administered orally using a tuberculine syringe fitted with oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45 minutes after the administration of the dose. Each mouse was placed at the center of the EPM with its head facing the open arms and during the 5 minutes of experiment the behaviour of the mouse was recorded for preference of the mouse for its first entry into the open or closed arms, the no of entries into the open arm and average time spent by the mouse in the open arm (total duration in arms/no of entries). During the entire experiment, the animals were allowed to socialize and every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals [12, 13].

Statistical analysis

Results are expressed as mean ± S.E.M. and statistical difference were analyzed using student's t- test and results were considered significant when $p<0.05$.

Results

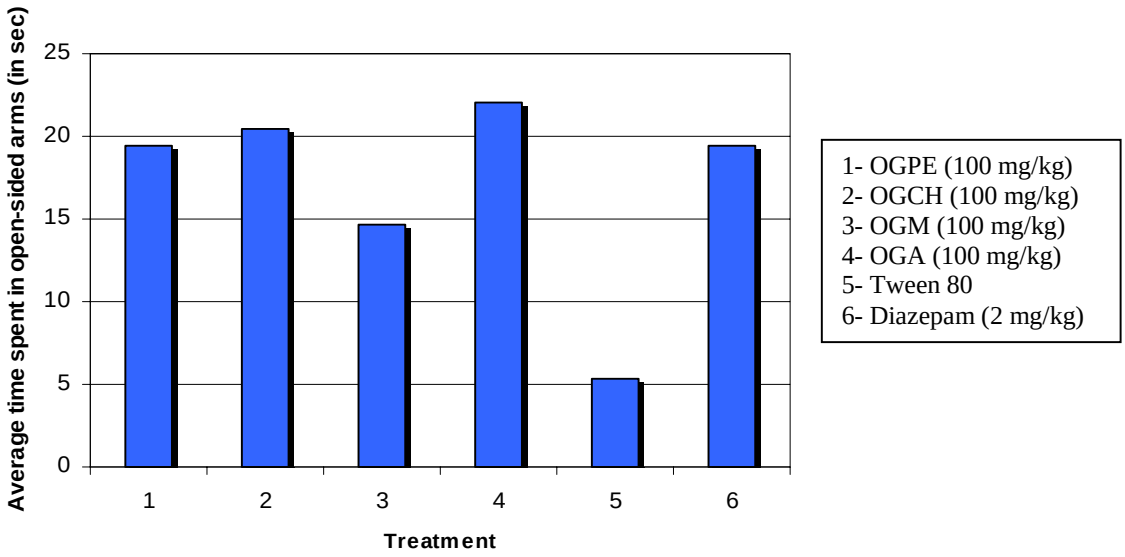
Petroleum ether, chloroform, methanol and aqueous extracts of *O. gratissimum* significantly ($p<0.05$) increased the time spent by the mice in the open-sided arms starting at a dose of 50 mg/kg (Table 1). All the extracts produced maximum activity at the dose of 100 mg/kg and aqueous extract was the most potent one (Figure 1). However, in all the extracts the effect started decreasing by increasing the dose to 200, 400 and 800 mg/kg. The anxiolytic activity of all the extracts was comparable with the standard drug Diazepam.

Table 1: Effect of different extracts of *O. gratissimum* on anxiolytic response in the elevated plus-maze test in mice

Group	Dose (mg/kg) p.o.	Average time spent in open-sided arms (in sec)			
		OGPE	OGCH	OGM	OGA
Tween 80	-	5.3 ± 0.3			
Diazepam	2	19.4 ± 0.3*****			
Extract	50	15.2 ± 0.5*****	16.7 ± 0.8*****	13.3 ± 0.4*****	18.2 ± 0.5*****
	100	18.2 ± 0.9*****	20.4 ± 1.2*****	14.7 ± 0.5*****	22.0 ± 0.9*****
	200	17.5 ± 0.4*****	17.1 ± 1.1*****	14.6 ± 0.7 *****	17.8 ± 0.7*****
	400	14.0 ± 0.5*****	8.4 ± 1.0*****	12.4 ± 1.2*****	14.0 ± 1.0*****
	800	6.8 ± 0.6*****	6.3 ± 0.5*****	10.1 ± 0.7*****	7.3 ± 0.9*****

Values are expressed as mean ± S.E.M.; n = 6. * $p<0.05$ vs. control. p.o.: per oral.
OGPE, petroleum ether extract; OGCH, choloroform extract; OGM, methanolic extract; OGA, aqueous extract.

Figure 1: Effect of different extracts of *O. gratissimum* on anxiolytic response in the elevated plus-maze test in mice



OGPE, petroleum ether extract; OGCH, choloroform extract; OGM, methanolic extract; OGA, aqueous extract.

Discussion

The elevated plus maze is currently one of the most widely used models of animal anxiety [14, 15]. All the extracts of *O. gratissimum* increased the time spent in open-sided arms of the plus-maze by the mice in the dose range of 50 to 100 mg/kg. Maximum activity by all the extracts were produced at 100 mg/kg and the response was reverted when the doses were increased to 200, 400 and 800 mg/kg. This suggests that all the extracts possess sedative effect.

Aqueous extract of the aerial parts of *O. gratissimum* was found to possess maximum anxiolytic activity. Plants containing tannins, sterols, flavonoids etc. are reported to have anxiolytic activity [16-18] and preliminary phytochemical screening revealed the presence of sterols, tannins, flavonoids and carbohydrates in the aerial parts of *O. gratissimum*. Therefore, the anxiolytic activity of *O. gratissimum* may be due to the presence of tannins, sterols, flavonoids etc. However, further investigations are required to isolate the phytoconstituents responsible for anxiolytic activity and to find their mechanism of action.

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