

ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITIES OF DIFFERENT EXTRACTS FROM *CITRUS PARADISI* VAR. *DUNCAN*

Vikas Gupta^{*1}, Parveen Bansal¹, Pawan Kumar², Richa Shri³

¹National Institute of Ayurvedic Pharmaceutical Research, Patiala, Punjab, India.

²Department of Pharmaceutical Sciences, GPCG, Patiala, Punjab, India.

³Senior Lecturer, Punjabi University, Patiala, Punjab, India.

Mr. Vikas Gupta, National Institute of Ayurvedic Pharmaceutical Research, Patiala, Punjab, India. E-mail - vikas_4308@rediffmail.com

Abstract: The present study was designed to evaluate the anti-anxiety activity and anti-depressant activity of various extracts viz petroleum ether, chloroform, methanol and water, of the leaves of *Citrus paradisi* var. *duncan* using elevated plus maze (EPM) model and forced swimming test (FST) respectively in swiss albino mice. Albino mice were treated with different doses of the extracts (i.e.100, 200 and 400 mg/kg orally) and behavior was observed on the EPM and FST. Results showed that methanol extract at the dose of 100 mg/kg of the leaves of *Citrus paradisi* var. *duncan* markedly increased the average time spent in the open arms in EPM and methanol extract at the dose of 400 mg/kg showed a significant decrease in the time spent immobile by mice in FST. These results provide support for the potential anxiolytic and antidepressant activity of *Citrus paradisi* var. *duncan*.

Keywords: Anti-anxiety, anti-depressant, *citrus paradisi* var. *duncan*, elevated plus maze, forced swimming test.

INTRODUCTION

According to the World Health report [1], approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [2]. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [3]. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes [4]. Anxiety may interfere with intelligence, psychomotor function and memory [5]. The benzodiazepines are considered the drug of choice in the treatment of anxiety. Unfortunately, there are several side effects [6-7]. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous [8-10]. The complexity of daily life in modern society frequently leads to varying degree of anxiety and depression. Mood, depression and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries [11-12]. These considerations implicate the search for new anxiolytic and antidepressant agents that have a fast onset of action present with less side effects and a wider safety margin. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases [13]. Various plants are being used in complementary and alternative medicines for management of anxiety. *Citrus* fragrances have been particularly attributed with mood enhancing properties by aroma therapists. Volatile oils isolated from grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), bergamot (*Citrus bergamia*), lime (*Citrus aurantifolia*), mandarin (*Citrus nobilis*) and orange (*Citrus aurantium*) are often used in the treatment of anxiety [14-15]. A review of literature revealed that *Citrus paradisi* is highly reputed plant, and has been widely employed in herbal medicine and aromatherapy [15] but no significant work has been carried out on the anxiolytic effects and anti-depressant activity of the plant extracts. So, the present study was designed to evaluate the anti anxiety activity and anti depressant of different extracts of *Citrus paradisi* var. *duncan*.

MATERIALS AND METHODS

Plant material

The leaves of *Citrus paradisi* var. *duncan* were procured and identified from a cultivated source: Punjab Agricultural University Regional Centre at Abohar, Punjab, India in the month of March-April 2007.

Preparation of extracts

Leaves of *Citrus paradisi* var. *duncan* were dried in shade and powdered. The powdered leaves (100g) were subjected to successive soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60 – 80 °C), chloroform and methanol

and water. Before each extraction the powdered material was dried in hot air-oven below 50 °C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material. The yield of the extract petroleum ether (60-80 °C), chloroform and methanol and water was 1.97%, 3.61%, 4.12%, 4.11% w/w respectively.

Test animals

The experimental animals [swiss albino mice (20-25 gm) of either sex] were procured from the Animal House, Punjabi University, Patiala. The animals were given standard laboratory feed and water *ad libitum*. The experiments were performed between 8.00 am to 1.00 pm. The experiments were conducted in a sound proof laboratory. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee.

Anti-anxiety activity

Elevated plus maze model

The elevated plus-maze model is well established animal model for testing anxiolytic drugs [16]. The elevated plus-maze apparatus consist of two open arms (16 x 5 cm for mice and 50 x 10 cm for rats), two closed arms (16 x 5 x 12 cm for mice and 50 x 10 x 40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor [17]. The animals were placed individually in the centre of the maze, head facing towards open arms and the stop watch was started and following parameters were noted for 5 min. a) First preference of mice to open and closed arm. b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm) c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries).

Treatments

Animals were divided into five (I-V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, diazepam (2mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were given petroleum ether (60-80°C), chloroform and methanol and water extracts of leaves of *Citrus paradisi* var. *duncan* at different doses viz.100, 200 and 400mg/kg respectively.

All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test.

Anti-depressant activity

Forced swimming test

The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity [18]. The swimming test includes two exposures to a water tank, spaced 1 day apart. For these experiments, the tank sizes were 22 cm in diameter and 40 cm in

Table 1. It shows anti-anxiety activity of various extracts of leaves of *Citrus paradisi* var. *duncan*

Group	Treatment	Average time spent in open arms (in sec)					
		Extracts			Control		
		Petroleum ether	Chloroform	Methanol	Aqueous	Negative	Positive
I	Vehicle	-	-	-	-	7.734±0.613	-
II	Diazepam	-	-	-	-	-	22.102±0.628*
III	100mg/kg	13.576±0.628*	16.864±1.619*	21.264±0.519*	11.391±0.691*	-	-
IV	200mg/kg	11.994±0.388*	12.984±1.456*	19.086±0.268*	12.354±0.257*	-	-
V	400mg/kg	11.926±0.989*	10.146±1.151	12.162±0.831*	11.906±0.544*	-	-

Values are Mean ± SEM (n=6); one way ANOVA and Tukey's multiple range test. *p<0.05 compared to control

Table 2. It shows anti-depression activity of various extracts of leaves of *Citrus paradisi* var. *duncan*

Group	Treatment	Duration of immobility(in sec)					
		Extracts			Control		
		Petroleum ether	Chloroform	Methanol	Aqueous	Negative	Positive
I	Vehicle	-	-	-	-	220.73 ± 1.941	-
II	Diazepam	-	-	-	-	-	108.361±1.514*
III	100mg/kg	201.133±0.928	166.824±2.639*	143.958±2.017*	180.410±1.844	-	-
IV	200mg/kg	190.633±1.788	162.181±3.516*	134.186±1.268*	160.754±1.256*	-	-
V	400mg/kg	166.213±1.961*	160.914±2.251*	114.907±3.102*	149.414±3.587*	-	-

Values are Mean ± SEM (n=6); one way ANOVA and Tukey's multiple range test. *p<0.05 compared to control

height. The tank had a rounded lid and contained 20-cm-high fresh water at 25 °C. During the first exposure, mice not yet treated were placed in the tank and left there for 15 min. During the second exposure (test session), 30 min after the treatment, mice were placed in the tank and left there for 5 min during which their immobility time was observed. A mouse was considered immobile when it remained floating in the water, without struggling, making only very slight movements necessary to keep its head above the water.

Treatments

Animals were divided into five (I-V) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, imipramine (20mg/kg, orally), suspended in the vehicle. Group III-V were treated as test groups and were given petroleum ether (60-80°C), chloroform and methanol and water extracts of leaves of *Citrus paradisi* var. *duncan* at different doses viz.100, 200 and 400mg/kg respectively.

All the test solutions, standard drug and control were administered orally 30 minutes prior to experiment.

Statistical analysis

The anxiolytic activities of the extracts, diazepam and control were analyzed by one-way analysis of variance (ANOVA). The test groups were compared with standard/control by Tukey's Multiple Range Test. Difference were considered significant at p<0.05.

RESULTS

The results obtained from the EPM model, indicates that methanol extract showed significant (p<0.05) anti anxiety activity as compared to diazepam. The average time spent in open arms increased from 7.734 ± 0.613 (sec) in control to 21.264 ± 0.519 (sec) in methanol extract at a dose of 100mg/kg. The petroleum ether extract, chloroform extract and aqueous extract appeared to be devoid of anti anxiety activity since their p values are insignificant. Results obtained are presented in Table 1.

The results obtained from the FST, indicates that methanol extract showed significant (p<0.05) anti depression activity as compared to imipramine. Methanol extract at the dose of 400mg/kg showed a significant decrease in the time spent immobile by mice from 220.73 ± 1.941(sec) in control to 114.907 ± 3.102(sec.). The petroleum ether extract, chloroform extract and aqueous extract appeared to be devoid of antidepressant activity since their P values are insignificant. Results obtained are presented in Table 2.

DISCUSSION

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms [19]. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically [20]. The widespread use of this

simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rodents (rats and mice), following initial escape-oriented movements, develop an immobile posture when placed inside an inescapable cylinder with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior, meaning the loss of the animal's ability to cope with stressful stimuli [21]. The methanol extract of *Citrus paradisi* var. *duncan* (100mg/kg), markedly increased the percentage of average time spent by the animals in the open arms. The anxiolytic effect of the plant extract was more prominent at 100mg/kg and doses higher or lower than this did not show a consistent anxiolytic effects. The lack of significant anxiolytic effects at doses higher than 100mg/kg could be due to strong sedative properties of the plant extracts. Lower doses (less than 100mg/kg) of the plant extract did not show any significant anxiolytic effects. The methanol extract of *Citrus paradisi* var. *duncan* (400mg/kg), markedly showed a significant decrease in the time spent immobile by mice. The anxiolytic and antidepressant effects of methanolic extract of *Citrus paradisi* var. *duncan* may be related to their flavonoid content. However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic and anti-depressant effect of methanol extract at dose 100 mg/kg and 400mg/kg orally respectively and to explain mechanism.

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