

Anti-anxiety Activity of *Citrus paradisi* var. *star ruby* Extracts

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Abstract: *Citrus paradisi* has been used traditionally to reduce stress and anxiety; however no pharmacological work has been done to substantiate these claims. The present study was designed to evaluate the anti-anxiety activity of various extracts viz petroleum ether, chloroform, methanol and water, of the leaves of *Citrus paradisi* var. *star ruby* using elevated plus maze (EPM) model in Swiss albino mice. Albino mice were treated orally with different doses of the extracts (i.e. 100, 200 and 400 mg/kg) and behavior was observed on the EPM. Diazepam (2mg/kg, P.O) was used as a positive control. Results show that methanol extract at the dose of 100mg/kg of the leaves of *Citrus paradisi* var. *star ruby* markedly increased the average time spent in the open arms of the EPM. This effect was comparable to the effect produced by diazepam. Hence this plant may be developed as a potentially useful anti anxiety agent.

Keywords: Anxiety, *Citrus paradisi* var. *star ruby*, Diazepam, Elevated plus maze.

Introduction

The complexity of daily life in modern society frequently leads to varying degree of anxiety. Mood and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries¹⁻². Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability³. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases⁴. 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. The essential oils obtained from genus *Citrus* are recommended for the treatment of anxiety also. 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 anxiety⁵. A review of literature revealed that *Citrus paradisi* is highly reputed plant, and has been widely employed in herbal medicine and aromatherapy but no significant work has been carried out on the anxiolytic effects of the plant extracts⁶. So, the present study was designed to evaluate the anti anxiety activity of different extracts of *Citrus paradisi* var. *star ruby* using the EPM, an exteroceptive behavior animal model.

Materials and Methods

Plant Material

The leaves of *Citrus paradisi* var. *star ruby* 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. *star ruby* 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 2.67%, 3.21%, 2.12%, 1.11% w/w respectively.

Test Animals

The experimental animals [Swiss albino mice (20-25 gm) of either sex] were procured from the Animal House, SD College, Barnala. 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.

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. *star ruby* 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.

Elevated plus maze model

The elevated plus-maze model is well established animal model for testing anxiolytic drugs⁷. 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 floor⁸. 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).

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 methanolic extract showed significant ($p < 0.05$) anti anxiety activity as compared to diazepam. The average time spent in open arms increased from 6.772 ± 0.773 (sec) in control to 23.958 ± 1.087 (sec) in methanolic 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.

Table 1: Anti anxiety activity of various extracts of leaves of *Citrus paradisi* var. *star ruby*

Group	Treatment	Average time spent in open arms(in sec)					
		Extracts				Control	
		Petroleum ether (Mean \pm SEM)	Chloroform (Mean \pm SEM)	Methanol (Mean \pm SEM)	Aqueous (Mean \pm SEM)	Negative (Mean \pm SEM)	Positive (Mean \pm SEM)
I	Vehicle	-	-	-	-	6.772 \pm 0.773	-
II	Diazepam	-	-	-	-	-	24.564 \pm 0.519*
III	100mg/kg	12.576 \pm 0.828*	15.864 \pm 1.629*	23.958 \pm 1.087*	11.910 \pm 0.844*	-	-
IV	200mg/kg	12.664 \pm 0.788*	16.984 \pm 1.556*	22.186 \pm 0.268*	12.754 \pm 0.656*	-	-
V	400mg/kg	13.516 \pm 1.389*	9.946 \pm 0.651	13.162 \pm 0.831*	15.406 \pm 0.587*	-	-

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

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 arms⁹. The methanol extract of *Citrus paradisi* var. *star ruby* (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 anxiolytic effects of methanolic extract of *Citrus paradisi* var. *star ruby* may be related to their flavonoid content. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as *Passiflora coerulea*¹⁰. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors¹¹⁻¹³. Furthermore a sedative effect on the central nervous system has been shown for quercetin and isoquercetin glycosides in mice¹⁴⁻¹⁵. However, further studies are required to identify the phytoconstituent responsible for the observed anxiolytic effect of methanol extract at dose 100 mg/kg and to explain anxiolytic mechanism.

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