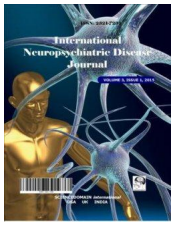

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Anxiolytic Effect of *Citrus paradisi var. Marsh seedless* Using Different Models

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VG, PB and PG designed the experiments and wrote the first draft of the main manuscript. Authors VG, PB and KK preformed all the scientific experiments and typed the manuscript and statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was designed to evaluate the anti-anxiety activity of various extracts of the leaves of *Citrus paradisi* var. *Marsh seedless* using light dark model and hole board methods in Swiss albino mice.
Methodology: Swiss Albino mice were treated with different doses of the extracts (50,100,200 and 400 mg/kg p.o.) and Diazepam (2 mg/kg, p.o) was used as a positive control using Hole Board test and light dark model.
Results: Results showed that methanol extract in doses of 100 mg/kg p.o. and 200 mg/kg p.o. possesses marked anti anxiety activity. This effect was comparable to the effect produced by standard drug, diazepam.
Conclusion: Hence this plant may be developed as a potentially useful anxiolytic agent. Further studies are going on to find out the active constituent responsible for this activity.

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Keywords: Anxiety; Citrus paradise; diazepam; hole board; light dark model.

1. INTRODUCTION

The complexities of modern life frequently lead to mood and anxiety disorders in developed as well as developing countries. Benzodiazepines are the most widely used anxiolytics used by the physicians but it is associated with variety of risks like psychomotor impairment and dependence liabilities. In recent years, anxiolytic drugs have been among the front-runners in terms of the number of prescription written in medical practice. This may be due to the tense life style imposed on man by the competitive atmosphere. Some degree of anxiety is a part of normal life, but treatment is needed when it is disproportionate to the situation and excessive [1-2]. Therefore, natural product scientists are exploring natural resources especially plants to develop newer, safer and cost effective medicines [3-5]. Exploitation of traditionally used plants seem to be viable and rational approach. Fragrances from the genus citrus are well known by the aromatherapists to elevate mood and have been used in the treatment of anxiety and other mood disorders [6].

Citrus fragrances have been particularly attributed with mood enhancing properties by aroma therapists. The volatile oils obtained from genus Citrus (*Citrus paradisi*) have been recommended and used for the treatment of anxiety. A review of literature also reflects that *Citrus paradisi* is widely employed in herbal medicine and aromatherapy and significant work has already been reviewed and carried out by authors on the anxiolytic effects of the plant extracts using elevated plus maze model [6-11]. The present study was designed to evaluate the anti anxiety activity of different leaves extracts of *Citrus paradisi* var. *Marsh seedless* using the various animal behavioural models.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Citrus paradisi* var. *Marsh seedless* were procured and identified from a cultivated source: Punjab Agricultural University Regional Centre at Abohar (Punjab, India) in the month of March-April 2013.

2.2 Preparation of Extracts

Leaves of *Citrus paradisi* var. *Marsh seedless* were dried in shade and powdered. The powdered leaves (100 g) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity i.e. petroleum ether, chloroform and methanol and water. Before each extraction the powdered material was dried in hot air-oven below 50ºC. 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 (CPMSP), chloroform (CPMSC), methanol (CPMSM) and water (CPMSW) was 3.43%, 4.54%, 3.29%, 3.41% w/w, respectively. All the extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents.

2.3 Phytochemical Screening

The extract was subjected to preliminary phytochemical screening [12-13].

2.4 Drugs and Chemicals

Diazepam was used as standard anxiolytic drug from Ranbaxy, Pvt. Ltd. All other chemicals used were procured from SD Fine Chemicals and were of analytical grade. Simple syrup IP and carboxy methyl cellulose (2%), was used as vehicle. Different leaves extracts of petroleum ether (CPMSP), chloroform (CPMSC), methanol (CPMSM) and water (CPMSW) of *Citrus paradisi* var. *Marsh seedless* at different doses viz.50, 100, 200 and 400 mg/kg respectively.

2.5 Test Animals

The experimental animals [Swiss albino mice (20-30 gm) of either sex] were procured from the Animal House, Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur (CPCSEA no. ATRC/05/13). The animals were given standard laboratory feed and water *ad libitum*. The experiments were performed between 6.00 am to 11.00 am. The experiments were conducted in a semi-sound proof laboratory. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee, Akal College of Pharmacy and Technical Education,

Mastuana Sahib, Sangrur, Punjab, India (CPCSEA no. ATRC/05/13).

2.6 Acute Toxicity Study

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, neurological abnormality and mortality for 14 days.

2.7 Anti Anxiety Activity

2.7.1 Hole–board model

The apparatus consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The extracts in above mentioned doses and vehicle were administered for 5 days p.o. once daily and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the number of line crossing and number of head dipping were calculated [14].

2.7.2 Light dark test

The apparatus consisted of two 20 cm × 10 cm × 14 cm plastic boxes: one was made dark and the other transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min after the mouse stepped into the dark box [15].

2.8 Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's 't'-test, $p<0.05$ were considered as significance.

3. RESULTS

3.1 Phytochemical Screening

The preliminary phytochemical analysis of *C. paradisi var Marsh seedless* extracts is given in the Table 1. Phytochemical tests revealed the presence of saponins, steroids, flavanoid, alkaloids and glycosides.

3.2 Acute Toxicity Study

Acute oral toxicity studies suggested the non-toxicity of extracts. There were no neurological, behavioural abnormalities and mortality even at the dose of 2000 mg/kg by any of the extracts showing safety profile of the plant.

3.3 Hole-Board Model

The number of line crossing and head dipping was significantly increased in case of Diazepam treated animals as compared to the control animals. All doses of CPMSM showed an increase in the number of line crossing and head dipping significantly and the results were very near to the effects of standard drug, Diazepam (Tables 2a & b).

3.4 Light-Dark Model

The time spent in lit box was significantly increased in case of administration of 100 and 200 mg/kg dose of CPMSM and the result was comparable to the standard drug, Diazepam (Table 3).

Table 1. Photochemical screening of *C. paradisi var Marsh seedless* extracts

Phytoconstituents	CPMSP	CPMSC	CPMSM	CPMSW
Carbohydrates	-	-	+	+
Proteins & amino acids	-	-	+	+
Fats	+	+	-	-
Glycosides	-	-	+	-
Tannins	-	-	+	+
Alkaloids	-	-	+	+
Flavonoids	-	+	+	+
Steroids	-	+	+	-
Saponins	-	-	+	+

Table 2a. Effect of *C. paradisi var Marsh seedless* extracts in hole-board model

Groups	Treatment	No. of head dipping					
		Extracts				Controls	
		CPMSP	CPMSC	CPMSM	CPMSW	Negative	Positive
I	Vehicle					13.64±3.22	
II	Diazepam 2 mg/kg p.o.						45.25±2.21*
III	50 mg/kg	12.21±0.64	20.43±0.26	38.34±2.14*	23.71±1.76		
IV	100 mg/kg	14.32±0.28	22.54±0.32	44.18±1.15*	34.11±0.56*		
V	200 mg/kg	17.72±1.11	33.10±1.55*	45.76±1.25*	37.54±0.44*		
VI	400 mg/kg	16.22±1.20	34.26±0.72*	40.26±0.25*	35.91±1.01*		

*Values are Mean±SEM (n=6); *p<0.05; one way ANOVA followed by Dunnett's 't' test*

Table 2b. Effect of *C. paradisi var Marsh seedless* extracts in hole-board model

Groups	Treatment	No. of line crossing					
		Extracts				Controls	
		CPMSP	CPMSC	CPMSM	CPMSW	Negative	Positive
I	Vehicle					111.35±1.22	
II	Diazepam 2 mg/kg p.o.						198.43±1.55*
III	50 mg/kg	109.22±0.95	122.84±0.53	149.74±0.22	134.53±1.42		
IV	100 mg/kg	112.14±0.88	132.54±0.97	189.96±2.53*	156.73±0.54		
V	200 mg/kg	117.63±2.64	135.65±0.33	190.09±0.22*	158.31±0.44*		
VI	400 mg/kg	120.13±0.44	132.53±2.12	172.13±0.42*	154.27±1.19*		

*Values are Mean±SEM (n=6); *p<0.05; one way ANOVA followed by Dunnett's 't' test*

Table 3. Effect of *C. paradisi var Marsh seedless* extracts in light dark model

Groups	Treatment	Time spent in lit-box (sec/5 min.)					
		Extracts				Controls	
		CPMSP	CPMSC	CPMSM	CPMSW	Negative	Positive
I	Vehicle					10.60±3.01	
II	Diazepam 2 mg/kg p.o.						29.42.3±1.54*
III	50 mg/kg	4.24±1.53	11.32±1.42	21.43±2.12*	10.43±0.32		
IV	100 mg/kg	11.56±0.54	15.34±0.33	27.95±1.32*	18.38±0.88		
V	200 mg/kg	12.43±0.12	20.94±0.99*	27.42±1.31*	18.62±0.35		
VI	400 mg/kg	12.12±1.09	12.81±1.21	24.43±0.42*	17.34±0.53		

*Values are Mean±SEM (n=6); *p<0.05; one way ANOVA followed by Dunnett's 't' test*

4. DISCUSSION

Public concern on mental health has significantly increased given the high prevalence of neuropsychiatric disorders, especially anxiety and depression. Most of the drugs for these conditions used nowadays have adverse side effects, so the need for newer, better-tolerated and more efficacious treatments is gaining high. So, growing attention is being paid to traditional herbal medicines [16]. In the anxiety disorder, involvement of GABAergic, serotonergic, adrenergic and dopaminergic neurotransmission is well established [17-19]. Despite the widespread use of *Citrus paradisi var Marsh seedless,* its anxiolytic activity has still not been established. The present study showed that CPMSM has anxiolytic properties using hole board and light dark model. The data obtained in present study is in line with the results of studies already conducted by authors using elevated plus maze model at dose profile of 100 mg/kg body weight in four varieties of *Citrus paradisi* [20]. The anxiolytic effects of methanolic extract of *Citrus paradisi* may be related to their flavonoid content.

The extracts from the plant shows the presence of flavonoids and the flavonoids exert anti-anxiety activity through GABA receptors. In the CNS several flavones bind to the benzodiazepine site on the GABA, a receptor resulting in sedation, anxiolytic or anti-convulsive effects. Flavonoids of several classes are inhibitors of monoamine oxidase A or B, thereby working as anti-depressants or to improve the conditions of Parkinson's patients. Flavonoids with anxiolytic activity have been described in many plant species used in folk medicine such as Passiflora coerulea [21]. This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors [22-24]. In another study a sedative effect on the central nervous system has been shown for quercetrin and isoquercetin glycosides in mice [25-27]. Phytochemical tests of CPMSM revealed the presence of saponin, steroids, flavonoids and glycosides. The possible mechanism of anxiolytic action of CPMSM could be due to the binding of any of these phytochemicals to the $GABA_A$-BZD complex. In support of this, it has been found that flavonoids bind with high affinity BZD site of the $GABA_A$ receptor [28].

5. CONCLUSION

From the above observations, authors concluded that the methanolic extract of *Citrus paradisi var Marsh seedless* shows significant anxiolytic activity at 100 and 200 mg/kg dose, which is comparable with the reference drug. However, further studies are under process to isolate the active constituent and the exact mechanism responsible for this activity.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agarwal MN, Itankar PR, Patil AT, Vyas JC, Kelkar AJ. Anxiolytic activity of annona squamosa leaf extracts in mice. Ind J Pharm Educ Res. 2009;43(1):99-103.
2. Korwar PG, Beknal AK. Anti-anxiety activity of *Punica granatum* fruit juice in rats. International Journal of Pharma-ceutical Invention. 2012;2(5):22-30.
3. Behnke K, Jehnsen G, Graubaum H, Gruenwald J. *Hypericum perforatum* versus fluoxetine in the treatment of mild to moderate depression. Adv Ther. 2002; 19:43-52.
4. Longo L, Johnson B. Addiction: Part I. Benzodiazepines-side effects, abuse risk and alternatives. Am Fam Physician. 2000; 61:2121-8.
5. Sandhya S, Vinod KR, Kumar S. Herbs used for brain disorders. Hygeia J D Med. 2010;2:38-45.
6. Gupta V, Bansal P, Niazi J, Kaur G. Anti-anxiety activity of *Citrus paradisi var.* starruby extracts. International Journal of Pharm Tech Research. 2011;2:1655-7.
7. Komiya M, Takeuchi T, Harada E. Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. Behav Brain Res. 2006;172:240-9.
8. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *Citrus paradisi var. foster*. Journal Pharmacy Research. 2009; 2:1864-6.
9. Gupta V, Bansal P, Kumar P, Shri R. Anxiolytic and antidepressant activities of different extracts from *citrus paradisi var. duncan*. Asian Journal of Pharmaceutical and Clinical Research. 2010;3:98-100.

10. Gupta V, Bansal P, Kumar P, Kaur G. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi var. foster*. Research Journal of Pharmacognosy and Phytochemistry. 2010;2:140-3.
11. Gupta V, Ghaiye P, Bansal P, Shri R. Pharmacopoeial standards and pharmacognostical studies of leaves of *Citrus paradisi var. duncan*. Journal of Pharmacy Research. 2011;4:1084-6.
12. Kokate CK. Practical Pharmacognosy. 5th ed. New Delhi: Vallabh Prakasham; 1991.
13. Kumar AS, Amudha P, Kannan CS. Evaluation of anxiolytic activity of hydroalcoholic activity of *Tephrosia purpuria* (l) pers on swiss albino mice. International Journal of Pharmaceutical Sciences and Research. 2011;2(5): 1262-9.
14. Yadav AV, Kawale LA, Nade VS. Effect of *Morus alba* L. (mulberry) leaves on anxiety in mice. Indian Journal of Pharmacol. 2008;40:32-6.
15. Ghosh MN. Fundamental of experimental pharmacology. Calcutta: Scientific Book Agency; 1984.
16. Muchimapura S, Phachonpai W, Tong-Un T, Wannanon P, Wattanathorn J. Evaluation of neuropharmacological activities of *Stephania venosa* herb consumption in Healthy Rats. American Journal of Agricultural and Biological Sci. 2012;7(3):271-277.
17. Clement Y, Chapouthier G. Biological bases of anxiety. Neuroscience and Biobehavioral Reviews. 1998;22(5): 623-33.
18. Graeff FG, Guimares FS, de Andrade TG, Deakin JF. Role of 5-HT in stress, anxiety and depression. Pharmacol. Biochem. Behav. 1996;54:129–41.
19. Griebel G. 5-hydroxytryptamine pathways in anxiety and its treatment. Pharmacol. Ther. 1995;66:103-48.
20. Gupta V, Bansal P, Kohli K, Ghaiye P. Development of economic herbal based drug substitute from *Citrus paradisi* (grape fruit) for exiting anti-anxiety drug modules. Natural Products Chemistry and Research. 2014;S1:001-004. DOI: 10.4172/2329-6836.S1-001.
21. Kumar S, Sharma A. Anti-anxiety activity studies of various extracts of *Turnera aphrodisiaca* Ward. J Herb Pharmacother. 2005;5:13-21.
22. Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol Biochem Behav. 1994;47:1-4.
23. Medina JH, Viola H, Wolfman C, Marder M, Wasowski C, Calvo D, Paladini AC. Neuroactive flavonoids: New ligands for the Benzodiazepine receptors. Phytomedicine. 1998;5:235-43.
24. Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: Comparison with diazepam. Neuropharmacology. 1999;38: 965-77.
25. Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. Flavonoids and the central nervous system: From forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol. 1999;51: 519-26.
26. Picq M, Cheav SL, Prigent AF. Effect of two flavonoid compounds on central nervous system. Analgesic activity. Life Sci. 1991;49:1979-88.
27. Kang TH, Jeong SJ, Kim NY, Higuchi R, Kim YC. Sedative activity of two flavonol glycosides isolated from the flowers of *Albizzia julibrissin* Durazz. J Ethnopharmacol. 2000;71:321-3.
28. Adeyemi OO, Yemitan OK, Taiwo AE. Neurosedative and muscle-relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. Journal of Ethnopharmacology. 2006;106:312–6.

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