Research Article **JHMR (2016), Vol. 1, Issue 01**

Journal of Herbal Medicine Research
(ISSN: 2474-106X)

Anti-anxiety Activity of Citrus paradisi var. duncan Extracts in Swiss Albino Mice - A Preclinical Study

Vikas Gupta[1*], Parveen Bansal [1], Junaid Niazi [2], Kamlesh Kohli [3], Pankaj Ghaiye[4]

[1]University Centre of Excellence in Research, BFUHS, Faridkot, India
[2]Department of Pharmaceutical Chemistry, Bahra Institute of Pharmacy, Patiala, India
[3]Department of Pharmacology, Guru Gobind Singh Medical College, BFUHS, Faridkot, India
[4]Department of Pharmaceutics, ACPTE, Sangrur, India

ABSTRACT

Background: Fragrances from aromatic oils of Citrus have been particularly attributed with mood enhancing properties by aroma therapists. Volatile oils are available in very small amounts hence authors intend to establish antianxiety activity of leaf extracts that can be made available for commercial purposes. Objectives: The present study was designed to authenticate the already established anti-anxiety activity (by using elevated plus maze model) of various extracts of the leaves of Citrus paradisi var. Duncan by authors using different animal models. Methods: Swiss Albino mice were treated with different doses of the leaf extracts (50, 100, 200 and 400 mg / kg p.o.) and Diazepam (2mg/ kg, p.o) was used as a positive control. Antianxiety activity was determined using Y maze model, light dark model and hole board methods. Results: Results of study show that methanol extract in higher doses (100, 200, 400 mg/kg) possesses marked anti anxiety activity and was comparable to the effect produced by diazepam. Conclusion: The plant can be developed as a commercial source of anxiolytic agent. Further studies are in-process to isolate the active constituent responsible for this activity and mechanism of action.

Keywords:
Anxiety, Grapefruit, Hole board test, Light dark model, Y maze

***Correspondence to Author:**
Vikas Gupta
University Centre of Excellence in Research, BFUHS, Faridkot, India.
E-mail: vikas_4308@rediffmail.com

How to cite this article:
Vikas Gupta, Parveen Bansal, Junaid Niazi, Kamlesh Kohli and Pankaj Ghaiye. Anti-anxiety Activity of Citrus paradisi var. duncan Extracts in Swiss Albino Mice-A Preclinical Study. Journal of Herbal Medicine Research, 2016,1(1): 0001-0006.

Received 31 May 2016; accepted 10 June 2016; published 13 June 2016.

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

INTRODUCTION

Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes. Interest in alternative medicine and plant-derived medications that affect the "mind" is growing day by day. Benzodiazepines are the major class of compounds used in the treatment of anxiety, despite the unwanted side effects like sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiating and tolerance.[1] Plants have long been used to treat central nervous system (CNS) disorders and various herbal remedies have been used as anxiolytic drugs in different parts of the world.[1] Therefore, natural product scientists are exploring natural resources especially plants to develop newer, safer and cost effective medicines.[2] Fragrances from the genus citrus are well recomended by the aromatherapists to elevate mood and have been used in the treatment of anxiety and other mood disorders. The volatile oils obtained from genus Citrus (Citrus paradisi) have been used for the treatment of anxiety. A significant work has already been reviewed and carried out by authors on the anxiolytic effects of the plant extracts using elevated plus maze model. [3-7] The present study is designed to authenticate the anti anxiety activity of Citrus paradisi var. duncan using other behavioural models.

MATERIALS AND METHODS

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

Preparation of extracts and Phytochemical Screening: 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 i.e. petroleum ether, 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 (CPDP), chloroform (CPDC), methanol (CPDM) and water (CPDW) 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 phyto-constituents.

Phytochemical Screening: The extract was subjected to preliminary phytochemical screening as per standard methods.

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 (CPDP), chloroform (CPDC), methanol (CPDM) and water (CPDW) of Citrus paradisi var. duncan at different doses viz.50, 100, 200 and 400 mg/kg respectively.

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.

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 behavioural, neurological abnormality and mortality for 14 days.

Anti anxiety Activity

Y–Maze Model: Mice were treated with the extracts of Citrus paradisi var duncan (50, 100, 200 and 400 mg / kg p.o.) and vehicle for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment and kept individually in one arm of the apparatus. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. The total numbers of visits to different arm were measured for a period of 10 minutes. [8]

Hole–Board Model: The apparatus used for experiment consisted of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distrib

-uted 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. The number of line crossing and number of head dipping were calculated for a period of 10 minutes. [9]

Light dark test: The apparatus used for this experiment 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 30cm 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. [10]

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 values less than 0.05 were considered as significance.

RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of C. paradisi var duncan extracts is given in the table 1.

Acute toxicity Study

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

Y-Maze Model

A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. CPDM showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam (Table 2). 100, 200 and 400 mg/kg dose of CPDM showed ceiling effect.

Hole-Board Model

The number of line crossing and head dipping was increased significantly in case of Diazepam treated animals as compared to the control animals. All doses of CPDM showed an increase in the number of line crossing and head dipping significantly and the result near to the standard drug, Diazepam (Table 3a and 3b).

Light-Dark Model

The time spent in lit box was increased significantly in case 100, 200, 400 mg/kg dose of CPDM and the result was comparable to the standard drug, Diazepam (Table 4).

DISCUSSION AND CONCLUSION

High prevalence of neuropsychiatric disorders, especially anxiety and depression has significantly increased public concern on mental health. Present drug modules for these conditions used demonstrate adverse side effects. So, growing attention is being paid to traditional herbal medicines.

In the anxiety disorder involvement of GABAergic, serotonergic, adrenergic and dopaminergic neurotransmission is well established. [11] Despite the widespread use of Citrus paradisi var duncan, its anxiolytic activity has still not been established. The present study showed that CPDM has anxiolytic properties using Y maze, 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 100mg/kg body weight in four varieties of Citrus paradise. [12] 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 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. [13] This effect has been attributed to the affinity of flavonoids for the central benzodiazepine receptors.

[14] In another study a sedative effect on the central nervous system has been shown for quercetrin and isoquercetin glycosides in mice. Phytochemical tests of CPDM 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 GAB-A_A-BZD complex. In support of this, it has been found that flavonoids bind with high affinity BZD site of the $GABA_A$ receptor. [15]

From the above observations, authors conclude that the methanolic extract of Citrus paradisi var duncan shows significant anxiolytic activity at 100, 200 and 400 mg/kg dose, which is comparable with the reference drug. The activity does not change significantly with variable dose from 100-400 mg/kg thus indicating good results even at a lower dose of 100mg/kg. However, further studies are under process to isolate the active constituent and the exact mechanism responsible for this activity.

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Table-1: Phytochemical screening of C. paradisi var duncan extracts

Phytoconstituents	CPDP	CPDC	CPDM	CPDW
Carbohydrates	-	-	+	+
Proteins & amino acids	-	-	+	+
Fats	+	+	-	+
Glycosides	-	-	+	+
Tannins	-	-	+	+
Alkaloids	-	-	-	+
Flavonoids	-	+	+	+
Steroids	-	+	+	-
Saponins	-	-	+	+

Table 2: Effect of C. paradisi var duncan extracts in Y-maze model

		No. of visits					
Groups	Treatment	Extracts				Controls	
		CPDP	CPDC	CPDM	CPDW	Negative	Positive
I	Vehicle					57.67± 3.65	
II	Diazepam 2 mg/kg p.o.						29.33± 2.06*
III	50 mg/kg	54.18±1.15	50.22±2.66	38.42±1.24*	49.22±1.47		
IV	100 mg/kg	48.89±1.02	44.78±1.47	30.22±1.22*	45.76±2.00		
V	200 mg/kg	43.33±1.07*	39.80±1.35*	29.45±1.24*	43.98±1.58*		
VI	400 mg/kg	41.27±1.89*	35.66±1.89*	31.29±1.45*	40.73±1.43*		

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

Table 3a: Effect of C. paradisi var duncan extracts in Hole-board model

		No. of head dipping					
Groups	Treatment	Extracts				Controls	
		CPDP	CPDC	CPDM	CPDW	Negative	Positive
I	Vehicle					22.34±1.05	
II	Diazepam 2 mg/kg p.o.						42.67± 2.43*
III	50 mg/kg	25.78±1.87	23.89±1.25	40.73±1.50*	31.74±1.04		
IV	100 mg/kg	27.34±1.25	30.21±1.59	43.98±1.36*	38.66±1.02*		
V	200 mg/kg	29.83±1.47	39.80±1.87*	45.76±1.25*	35.89±1.36*		
VI	400 mg/kg	31.77±1.36*	35.66±1.78*	45.22±1.55*	33.08±1.56*		

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

Table 3b: Effect of C. paradisi var duncan extracts in Hole-board model

No. of line crossing							
Groups	Treatment	Extracts				Controls	
		CPDP	CPDC	CPDM	CPDW	Negative	Positive
I	Vehicle					129.33± 2.06	
II	Diazepam 2 mg/kg p.o.						181.09± 3.65*
III	50 mg/kg	138.42±1.09	140.22±1.25	165.22±1.45*	140.18±1.15		
IV	100 mg/kg	130.22±1.22	143.76±1.36*	174.78±1.58*	141.89±1.28		
V	200 mg/kg	129.45±1.36	145.98±1.44*	177.80±1.69*	143.33±1.38*		
VI	400 mg/kg	131.29±1.24	147.73±1.77*	178.66±1.27*	145.27±1.66*		

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

Table 4 Effect of C. paradisi var duncan extracts in light dark model

Time spent in lit-box (sec/5min.)							
Groups	Treatment	Extracts				Controls	
		CPDP	CPDC	CPDM	CPDW	Negative	Positive
I	Vehicle					2.8±1.69	
II	Diazepam 2 mg/kg p.o.						22.3±2.69*
III	50 mg/kg	3.2±0.20	9.0±0.25	11.7±0.39*	9.7±0.99		
IV	100 mg/kg	7.2±0.65	13.9±0.36*	19.9±0.25*	12.8±0.21*		
V	200 mg/kg	4.8±0.58	11.9±0.58	20.5±0.25*	15.6±0.55*		
VI	400 mg/kg	4.0±0.59	12.0±0.78*	20.9±0.45*	16.3±0.31*		

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