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ORIGINAL ARTICLE

Behavioral effects of *citrus paradisi* in rats

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Abstract Anxiety and depression adversely affect behavior though these conditions may be handled through environmental and dietary changes. Diet rich in flavonoids and vitamins may support reducing anxiety and depression. Depression is most common but serious illness making life miserable; however the usage of dietary and herbal complements to treat anxiety and depression had been grown by the time. The purpose of this investigation was to characterize the behavioral properties of *Citrus paradisi* in rats at diverse doses i.e. 0.1, 0.3 and 0.5 ml/kg. Anxiolytic and antidepressant actions were particularly measured twice in 15 days through elevated plus maze, open field and forced swimming tests. *C. paradisi*, revealed increase in the locomotor activity and the exploratory skills of the animals, as assessed in the open-field. Indeed *C. paradisi* had a strong anxiolytic effect in elevated plus-maze, as assessed by an augmented number of entries and the proportion of time spent in the open arms. Moreover there was decline in duration of immobility and rise in duration of climbing during forced swimming test. At the tested doses these results suggest that *C. paradisi* have the potential to exert a range of CNS-mediated biological activities and thus encourage more investigations in this field.

Keywords *Citrus Paradisi* · Open field test · Forced swimming test · Elevated plus maze

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Introduction

Referring to the World Health report around 450 million persons experience a mental or behavioral disorder (WHO 2001), which extent to 12.3 % of the global dilemma of the disease, that will increase to 15 % by 2020 (Reynolds 2003), however a small number are only treated properly (WHO 2001). Medicinal plants are under continuous investigation throughout the world in various animal models to pursuit new remedy for the treatment of neurological disorders (Zhang 2004). Anxiety is considered as a condition of extreme fear categorized by sympathetic hyperactivity, motor tension, apprehension and caution (Sadock 2003), which may obstruct with psychomotor function, memory and intelligence (Pine et al. 1999). Depression is the most widespread mental disorder with heterogeneous symptomatic, psychological and biological symptoms (Arieti and Bemporad 1980; Thase and Howland 1995). The increasing complexity of day to day life in contemporary culture normally causes unpredictable degree of anxiety and depression. Disorders affecting mood have been found to be related with chronic pain among medical patients in both developed and developing countries (Gureje et al. 1998; Evans et al. 2005; Gupta et al. 2010a, b, c).

These considerations lead to the exploration for new anxiolytic and antidepressant agents that have a quick onset of action with least side effects and a wider margin of safety. It has diverted the attention of scientists to explore plants, usually employed in conventional and alternative system of medication for sleep and associated disorders (Spinella 2001). Many plants are being used as complementary and alternative medicines for treatment of anxiety. Citrus fragrances have been mainly renowned with mood enhancing properties by aroma therapists. Volatile oils separated from grapefruit (*C. paradisi*), lemon (*C. limon*), bergamot (*C. bergamia*), lime (*C. aurantifolia*), mandarin (*C. nobilis*) and orange

(*C. aurantium*) are frequently employed in the management of anxiety (Komiya et al. 2006; Palazzolo et al. 2013). Analysis of literature reveals that *C. paradisi* has been broadly used in herbal medicine and aromatherapy (Gupta et al. 2010a, b, c; Yang et al. 2010). However no substantial work has been done to explore the anxiolytic and anti-depressant effects of this plant. Thus present investigation was carried out to evaluate the anxiolytic and anti-depressant effects of various doses of *C. paradise* juice.

C. paradisi, also known as Grapefruit has been an important herb in the genus Citrus (Rutaceae). It is native to island of Barbados. Today it is the second most important citrus fruit worldwide. It has been used as popular medicine in many countries for antibacterial, antifungal, anti-inflammatory, antimicrobial, antioxidant, antiviral and astringent properties. It is also stated to be employed for cancer prevention, cellular regeneration, detoxification, lupus nephritis, rheumatoid arthritis, weight loss and hypocholesterolemic effects (Gupta et al. 2011).

The Chemical components found in fruit include naringin, neohesperidin, hesperidin, poncirin, limonene, tangeretin, auraptene, nobiletin, umbelliferone, β-sitosterol, stigmasterol, α-pinene and sabinene. Earlier grapefruits were used as brunch food but current in-vivo and in-vitro studies specify its antidiabetic, anti-inflammatory, antioxidant, anticancer, antiatherogenic, antidepressant, hepatoprotective and antibacterial properties (More et al. 2013).

Analysis of literature also reveals that *C. paradisi* is widely used in herbal medicine and aromatherapy, while there are also reports for the anxiolytic effects of the plant extracts (Komiya et al. 2006; Lather et al. 2012).

Consequently it seems relevant to endorse the effect of *C. paradisi* juice for its anxiolytic effects, open field test and elevated plus maze are considered effective experimental methods for the measurement of anxiety (Herskin and Jensen 2002; Mansouri et al. 2014). Forced swimming test is a commonly employed technique to assess depressive state in animals, since some aspects of human depression matches with the behavioral immobility of rats during forced swimming (Porsolt et al. 1977, 1987; Wilner 1984; Petit-Demouliere et al. 2005; Carbajal et al. 2009).

Hence present study investigates the behavioral effect of *C. paradisi* at three different doses in experimental rat models, to develop an economic herbal based drug that may serve as substitute for existing anti-anxiety drugs.

Materials and methods

Animals

Adult male Wister rats with mean body weight 220 ± 10 g were used for the Study. Animals were kept in precise condition of temperature 23 ± 2 °C and humidity 50–60 %. Rats were maintained throughout the experiment on a 12/12 h light and dark cycle with incessant access to rat chow and tap water. Five rats were housed in each plastic cage measuring 81 × 46 × 41 cm. The use of animals in this study was in agreement with the National Institute of Health (NIH) guide for the care and usage of Laboratory Animals (National Research Council 1996) and approved by the Board of Advance Studies and Research University of Karachi.

Citrus paradisi juice

The *C. paradisi* (Grapefruit) was bought from local market, recognized by center of plant conservation, University of Karachi. The coupon specimen no C.P 09-10 was submitted to department of Pharmacognosy, University of Karachi. The fruits were unpeeled and squeezed to yield fresh juice, which was then filtered and given through feeding tube in three doses i.e. 0.1 0.3 and 0.5 ml/kg.

Drug treatment

All rats were divided into six groups each comprising of ten animals. One group served as control and received vehicle; three groups were treated with *C. paradisi* at 0.1, 0.3 and 0.5 ml/kg and two groups received standard drugs diazepam and imipramine in the dose of and 3 mg/kg and 25 mg/kg respectively according to body weight (Carbajal et al. 2009 and Gupta et al. 2010a, b, c). *C. paradisi* juices, vehicle, diazepam and imipramine were administered daily between 8.0 to 10.0 am orally for 15 days.

Open field test (OFT)

Open field test is used to assess movement and anxiety associated behavior (Prut 2003). The open field was made up of Plexi glass, 75 cm long, 75 cm wide and 40 cm high in the shape of quadratic box. Black lines were drawn on floor, which divides the floor into twenty-five (15 × 15 cm) squares. A central square (30 × 30 cm) was drawn around the middle centre squares to count number of central entries of animal. Cross section length of squares was 21 cm. The open field was located in test room, illuminated by 60 W light bulbs. All tests were recorded by camera (Samsung Handycam) from the top view of the field. OFT for all animal groups was performed twice on 8th day and 15th day, just 1 h after administration of drugs.

Procedure

Animals were positioned into the center of the open field maze by holding their tail. Time of exploration of field by the animal was 30 min, after which rats were returned in their home cages

and arena was cleaned with 70 % ethanol solution. Variables measured were, (Alves et al. 2005) distance traveled by the animal in the field by line crossing all four paws, (Arieti and Bemporad 1980) total number of entries in central square (30 × 30 cm), (Buddenberg et al. 2009) number of rearing's i.e. frequency with which animal stood or rising up on hind legs with the forelegs along the wall of maze or in the air, (Budzynska et al. 2013) Duration of rearing's i.e. time spent by animal in rising up or standing position.

Elevated plus-maze (EPM)

EPM was employed to measure the anxiety related behavior (Hogg 1996) of *C. paradisi*. Animals used in open field test underwent testing in EPM just 30 min after OFT. The elevated plus-maze comprised of two open and two closed arms opposite to each other, arranged around (10× 10 cm) central platform. Open arms were 50 cm long and 10 cm broad. While closed arms were 50 cm long, 10 cm broad and 38.5 cm tall with open roof.

Procedure

Animals behavior in all test was recorded by means of a video camera (Samsung Handycam) mounted 100 cm above the maze to have the top view. Animals were positioned into the central platform, facing one of the open arms. During the test rats are permitted to move freely in the maze and explore environment for 5 min, variables measured were, (Alves et al. 2005) time expended in open arms, (Arieti and Bemporad 1980) time expended in close arms, (Buddenberg et al. 2009) number of entries, when animal entered with all four paws into the open or closed arms.

Forced swimming test (FST)

FST was employed to measure depression like behavior. Test was performed twice following administration of *C. paradisi* and vehicle to respective groups on 15th day 1 h just after the EPM, marked as day-1 (pre-test phase) and 16th day, 24 h after 1st FST, marked as day-2 (test-phase).

Procedure

FST was performed in Plexiglas cylinder 46 cm high having 20 cm diameters. The cylinder was filled with water at 25 °C to 20 cm from bottom. The forced swim test apparatus was situated in a sound isolated and dimly illuminated room. On the day-1, rats were positioned independently into the water for 15- min period. After the completion of pre-test phase, animals were detached from the water and then positioned under a light heating lamp in a plastic cage for about 15–20 min to make them dry. Same experimental condition of FST was applied to animals after 24 h i.e. placed into the water for 15-min. Water was replaced after each animal testing. All tests were documented by camera (Samsung Handycam) from the front view of the whole cylinder.

Variables measured were: duration of immovability i.e. time at which animal showed lack of motion, except only those movement required to keep his head above water, duration of swimming i.e. time in which animal showed vigorous movements with forepaws, duration of climbing i.e. time at which animal showed vigorous movements both with fore and hind paws against the walls of cylinder (Buddenberg et al. 2009).

Statistical analysis

Data was entered and analyzed by Superior Performance Statistical Software (SPSS) version 17. Values were shown as mean ± SEM with 95 % confidence interval. ANOVA followed by post hoc was done to compare values with control. Values of $p \leq 0.05$ were taken statistically significant and $p \leq 0.005$ as highly significant.

Results

Table 1 reveals the results of *C. paradisi* and diazepam on behavior of rats in open field. There was significant increase in no. of rearing's, no. of central entries and distance travelled at 0.3 and 0.5 ml/kg on 15th day as compare to control, while no considerable difference was observed in duration of rearing's at these doses, while there was no significant change in any parameter on 8th day. However animals received diazepam showed significant increase in number of rearing's, central entries and distance travelled on both 8th and 15th day as compare to control.

Table 2 shows results of *C. paradisi* and diazepam on behavior of rats in elevated plus maze. There was significant increase in no. of entries in open arm at 0.3 and 0.5 ml/kg both on 8th and 15th day as compare to control, however there was no significant change in time spent in open arm, close arm and no. of entries in close arm at any dose of *C. paradisi* both on 8th and 15th day as compare to control. Animals received diazepam exhibited significant increase in number of entries in open arm and time spent in open arm, while there was significant decrease in number of entries in close arm.

Table 3 shows results of *C. paradisi* and imipramine on behavior of rats in forced swim test. There was significant decline in duration of immobility and considerable rise in duration of climbing at 0.5 ml/kg but no considerable difference in the duration of swimming was observed during first and second exposure to forced swimming test in comparison to control group.

Table 1 Effect of *C. paradisi* and diazepam on behavior of rats in open field test

Group/Dose ml/kg/day	Days	Parameters			
		No. of rearing's	Duration of rearing's (s)	No. of centre entries	Distance travelled (cm)
Control	8	50.1 ± 5.40	200.1 ± 47.25	4.9 ± 0.87	2137.8 ± 151.89
	15	51.5 ± 5.59	207.0 ± 48.45	5.6 ± 0.96	2329.9 ± 187.50
C. paradisi 0.1	8	54.5 ± 5.09	210.5 ± 47.42	5.2 ± 0.84	2646.9 ± 317.48
	15	53.4 ± 5.04	203.4 ± 46.75	4.8 ± 0.84	2191.0 ± 147.84
C. paradisi 0.3	8	53.5 ± 5.04	211.0 ± 47.55	5.7 ± 0.98	2647.9 ± 317.50
	15	63.7 ± 2.19*	213.9 ± 47.75	7.7 ± 0.64*	3231.0 ± 334.95*
C. paradisi 0.5	8	55.0 ± 5.11	206.5 ± 47.48	6.5 ± 1.06	2595.4 ± 127.06
	15	64.1 ± 2.33*	220.5 ± 50.05	8.1 ± 0.54*	3225.4 ± 350.30*
Diazepam	8	62.7 ± 2.96*	235.8 ± 48.66	7.5 ± 0.46*	2812.5 ± 256.24*
	15	63.2 ± 2.93*	237.50 ± 48.71	8.0 ± 0.38*	2851.3 ± 223.59*

$n = 10$

Mean ± S.E.M

*$P \leq 0.05$ significant difference as compared to control

There was highly significant decrease in duration of immobility and significant rise in duration of climbing by imipramine on first and second exposure to forced swimming as compare to control. However no significant changes in duration of immobility, duration of climbing and duration of swimming were observed at 0.1 and 0.3 ml/kg on both exposures in comparison to control.

Discussion

Current study provides an indication for valuable effect of *C. paradisi* in anxiety and depression since have decreased the symptoms related with these disorders.

OFT and EPM models are used in rodents to evaluate the anxiolytic effect. In OFT 0.3 and 0.5 ml/kg dose of *C. paradisi* showed significant increase in number of rearing's and number of centre entries on 15th day as compare to control, which was comparable to that of diazepam treated group. While there was significant increase in distance travelled on 15th day at both doses 0.3 and 0.5 ml/kg of *C. paradisi* that was comparable to diazepam treated group, these significant changes in animal behavior indicates an exploratory and anxiolytic effect of *c. paradisi* at 0.3 and 0.5 ml/kg. Increase in distance travelled by diazepam treated animals could be justified since diazepam has been found to produce a dose dependent biphasic effect (Soderpalm et al. 1991).

Table 2 Effect of *C. paradisi* and diazepam on behavior of rats in elevated plus-maze

Group/Dose ml/kg/day	Days	Parameters			
		No. of entries in open arm	Time spend in open arm(s)	No. of entries in close arm	Time spend in close arm(s)
Control	8	3.6 ± 0.71	125 ± 23.0	6.5 ± 0.76	175 ± 23.0
	15	4.2 ± 0.74	140 ± 24.8	5.6 ± 0.85	160 ± 24.8
C. paradisi 0.1	8	5.2 ± 0.78	138 ± 25.3	5.2 ± 1.02	162 ± 22.6
	15	5.1 ± 0.82	122 ± 21.2	5.5 ± 1.12	178 ± 21.9
C. paradisi 0.3	8	6.8 ± 1.09*	123 ± 21.2	6.4 ± 1.14	177 ± 25.0
	15	7.1 ± 1.01*	111 ± 22.0	6.3 ± 1.08	189 ± 22.0
C. paradisi 0.5	8	6.1 ± 1.04*	132 ± 22.8	6.2 ± 1.10	168 ± 22.8
	15	7.2 ± 0.91*	139 ± 20.6	6.9 ± 0.92	161 ± 22.6
Diazepam	8	6.8 ± 0.67*	158 ± 18.0	3.1 ± 0.54*	142 ± 17.9*
	15	7.0 ± 0.84*	208 ± 22.0*	2.3 ± 0.61*	92 ± 22.0*

$n = 10$

Mean ± S.E.M

*$P \leq 0.05$ significant difference as compared to control

Table 3 Effect of *C. paradisi* and imipramine on behavior of rats in forced swimming test

Group/Dose ml/kg/day	Days	Parameters		
		Immobility Duration(s)	Climbing Duration(s)	Swimming Duration(s)
Control	15	151.5 ± 13.39	67.5 ± 8.84	681.0 ± 12.56
	16	160.5 ± 20.61	66.4 ± 6.43	673.1 ± 18.20
C. paradisi 0.1	15	148.3 ± 12.69	90.7 ± 12.75	661.0 ± 18.91
	16	156.3 ± 23.35	88.7 ± 8.57	655.0 ± 28.30
C. paradisi 0.3	15	126.2 ± 26.75	83.3 ± 10.63	690.5 ± 25.92
	16	130.2 ± 28.92	86.3 ± 8.46	683.5 ± 27.10
C. paradisi 0.5	15	100.1 ± 9.90*	101.5 ± 3.48*	698.4 ± 10.55
	16	111.1 ± 13.42*	94.6 ± 3.87*	694.3 ± 17.90
Imipramine	15	89.2 ± 6.87**	92.3 ± 7.93*	718.5 ± 14.02
	16	87.8 ± 8.92**	99.3 ± 8.76*	712.9 ± 29.01

$n = 10$

Mean ± S.E.M

*$P \leq 0.05$ significant difference as compared to control

**$P \leq 0.005$ highly significant difference as compared to control

There is no way to confidently differentiate a drug effect as either stimulant or anxiolytic due to a similar phenotype, and there could be certainly an overlap in terms of underlying mechanisms. Hence increase in distance traveled is an indication of increase in motor activity as well as stimulation of central nervous system (Kennett et al. 1987; Czech Donald 2002) and number of rearing's is an index of locomotor activity (Alves et al. 2005).

It is well known that benzodiazepines act as anxiolytics at low doses and encourage sedation and muscle relaxant effects at higher doses (Novas et al. 1988). Hence in present study *C. paradisi* has shown this effect in open field (Table 1). This hyperactivity revealed as increased distance travelled may be due to its anxiolytic effect which was almost identical to the effect diazepam used as a positive control for anxiolytic-like effects.

Fear of height prompts anxiety in the animals when positioned on the elevated plus maze. The subsequent indicator of anxiety and fear in the animals is revealed by reduction in the motor activity and inclination to remain at safer places. Anxiolytic agents are estimated to raise the motor activity, which is evaluated by the time spent in open arms by the animals (Gupta et al. 2010a, b, c).

In EPM the *C. paradisi* showed significant increase in number of entries to open arm at 0.3 and 0.5 ml/kg both on 8th and 15th day. This increase in number of entries to open arm were quite similar to standard drug diazepam, while comparison of animals regarding time spent in open arm vs time spent in close arm does not reveals any significant change as compare to control animals. Hence a general stimulatory and antianxiety behavior can be speculated by total or open arm entries (Pellow and Sandra 1986; Budzynska et al. 2013; Mansouri et al. 2014).

The marked increase in number of entries in animals treated with *C. paradisi* juice both at 0.3 and 0.5 ml/kg dose, shows the anti-anxiety effect of the citrus juice. Anxiolytic effects of *C. paradisi* may be related to their flavonoid content, since flavonoids exert anti-anxiety activity through gamma-Amino butyric Acid (GABA) receptors.

In the Central Nervous System (CNS) numerous flavones attach to the benzodiazepine site on the $GABA_A$ receptor causing sedation, anxiolytic or anticonvulsant effects. Flavonoids with anti-anxiety effects have been described in many plant species used in traditional medicine e.g. *Passiflora coerulea* (Kumar and Sharma 2005). This action has been recognized due to the affinity of flavonoids for benzodiazepine receptors (Wolfman et al. 1994; Griebel et al. 1999). However, more studies are being carried out to ascertain the phytoconstituent responsible for the detected anxiolytic effect of citrus juice.

FST is the most extensively employed pre-clinical tool to assess antidepressant activity. The prevalent use of this simple model is largely due to its capacity to perceive wide range of antidepressant agents. The test is centered on the observation that rodents, after initial escape-oriented movements, cultivate an immobile posture when located inside an inevitable cylinder with water. The immobility is believed to replicate either a failure of diligence in escape-directed behavior (i.e. despair behavior) or the growth of a passive behavior i.e. loss of the animal's capability to manage with traumatic stimuli (Gupta et al. 2010a, b, c).

The *C. paradisi* juice has significantly reduced immobility duration and increased duration of climbing at 0.5 ml/kg, indicating an antidepressant effect; flavonoids of numerous groups are inhibitors of monoamine oxidase A or B, thus anti-depressants effect of *C. paradisi* juice may be due to its high flavonoids contents, though further studies are desirable to recognize the mechanism of phytoconstituent responsible for apparent anti-depressant activity of *C. paradisi.*

Since some aspects of human depression matches with the behavioral immobility of rats during forced swimming. Immobility is observed when animal is likely to lose hope for survival in forced swimming and become depressive (Porsolt et al. 1977, 1987; Wilner 1984; Petit-Demouliere et al. 2005; Carbajal et al. 2009). Present study also characterized the effect of the *C. paradisi* on rat in forced swimming test (FST) following 15 day treatment.

Reduction in duration of immobility represents antidepressant effect (Taiwo Adefunmilayo et al. 2012). Result of the present study reveals that 0.5 ml/kg treated animals shows significant reduction in duration of immobility, which is comparable to that of imipramine. 0.5 ml/kg treated group also showed significant increase in duration of climbing, as a compensatory mechanism of reduction in immobility.

The beneficial effects of flavonoids are now unanimously accepted on human health and it is suggested that *C. paradisi* juice may play important role in all the observed effects. Thus on the basis of present data it may be concluded that the *C. paradisi* have maximum CNS stimulant, anxiolytic and antidepressant effects in rats at 0.5 ml/kg, however there is need for further investigation on more doses. Hence *C. paradisi* might serve as an appealing alternative therapeutic target, particularly for the high occurrence of treatment-resistant depression as well as anxiety.

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