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Research Article

EAT AN ORANGE TO KEEP ANXIETY AT LONG RANGE

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ABSTRACT

Orange fruit *(citrus sinensis)* is a favorite fruit of all sections of the society, particularly among patients suffering from different ailments for its nutritional and medicinal properties. This study was undertaken to test the efficacy of orange juice in the management of anxiety. A total of 72 mice divided in 12 groups were employed in this study. Orange juice (2.5%, 5%, 7.5%, 10% v/v) was administered orally to mice with the help of an oral feeding needle. Elevated plus maze and light - dark model served as behavioral models in this study. Peripheral cholesterol, reduced glutathione and thiobarbituric acid levels were estimated before and after administration of orange juice. *Citrus sinensis* significantly increased the time spent by mice in open arm in elevated plus maze test. Furthermore, there was enhancement in the time spent by mice in lit compartment of the light - dark model. Orange juice significantly ($P<0.01$) increased the GSH levels of brain. On the other hand, orange juice significantly ($P<0.01$) decreased the TBARS and cholesterol levels. All these findings, when combined together reflect the anti-anxiety potential of orange juice.

KEY WORDS: *Citrus sinensis*, Anti-anxiety, Elevated plus maze, Light and dark model

INTRODUCTION

From times immemorial, whole Orange plant including ripe and unripe fruits, fruit-juice, orange peels, leaves and flowers are used as traditional medicines. The major medicinal properties of orange fruit include anti-bacterial, anti-fungal, anti- diabetic, cardio-protective, anti-cancer, anti-arthritic, anti-inflammatory, anti-oxidant, anti-tubercular, anti-asthmatic and anti-hypertensive properties[1]. Phytochemically, the orange plant contains limonene, citral, neohesperidin, naringin, rutin, rhamnose, eriocitrin, vitamin-C, B1, B2, B3, nobiletin, tangetin, citbrasin and hesperidin. Anxiety disorders are senile neurological disorders, which are widely prevalent in modern fast-paced life[1]. More than 20% of the adult population suffers from anxiety at some time during their life[2]. Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat[3]. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyper-activity, apprehension and vigilance syndromes[4]. It may interfere with intelligence, psychomotor function and memory[5]. The development of anxiety-related disorders involve complex defense mechanisms of the body involving the limbic system and the hypothalamic-pituitary adrenal axis; their interactions play a significant role in the manifestation of the disease pathology[6, 7]. Benzodiazepines (BZDs) form the main stay for the treatment of anxiety[8] along with barbiturates and tricyclic antidepressants (TCA's).[9]

Objective: In the light of above, the present study was undertaken to explore the anti-anxiety potential of *Citrus sinensis* using elevated plus maze and light - dark model.

MATERIALS AND METHODS

Plant material

Fresh orange fruits were purchased for this study from the local market of Hisar and were authenticated from Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (Ref. NISCAIR/RHMD/Consult/-2011-12/1895/195). Orange juice was administered in different concentrations (2.5%, 5%, 7.5% and 10% v/v p.o.) to mice daily for duration of 10 days with the help of an oral feeding needle.

Animals

Adult (6 months old) mice of either sex, weighing around 20-25g were procured from the Disease Free Small Animal House of Lala Lajpat Rai University of Veterinary Sciences, Hisar. A total of 72 Swiss mice divided into 12 groups were employed in this study. Each group comprised of a minimum of 6 animals. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and the care of animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (Registration number 0436).

Drug protocol

Normal saline (vehicle, p.o), Alprazolam (0.5mg/kg) and *Citrus sinenis* (2.5%, 5%, 7.5% and 10% v/v p.o.) were administered for 10 successive days to mice. Biochemical studies were carried out on 10th day after drugs/vehicle/CS administration.

Experimental Design

Elevated plus maze (EPM)

The Elevated Plus-Maze test has been widely used for measuring anxiolytic and anxiogenic activities in mice. The procedure was performed as described by Lister; 1987.[10]

Light and Dark model

Light and dark model is commonly employed for evaluation of anxiolytic activity. The experimental procedure was performed as described by Bourin et al; 2003.[11]

Collection of Blood and Brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 10th day, 90 min after the administration of last dose of CS or standard drugs. Immediately after the decapitation, the trunk blood was collected. Then, whole brain was carefully removed from the skull. The collected blood was centrifuged at 3000 rpm for 15 min so as to separate serum. The serum was used for estimation of cholesterol levels. For preparation of homogenate, the whole brain was weighed and transferred to glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the

resultant cloudy supernatant liquid was used for estimation of reduced glutathione and thiobarbituric acid reactive substance (TBARS) levels present in the brain.

Biochemical Estimations

Estimation of Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, was estimated spectrophotometrically. The procedure was performed according to Okhawa et al; 1979 [12].

Estimation of brain reduced Glutathione level

Glutathione was measured spectrophotometrically by using Ellman's method [13]

Estimation of serum total cholesterol level

CHOD – PAP method was used for the estimation of serum cholesterol [14].

Statistical Analysis

All the results were expressed as mean ± standard error (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's *t*-test. *p*-values < 0.05 were considered as statistically significant.

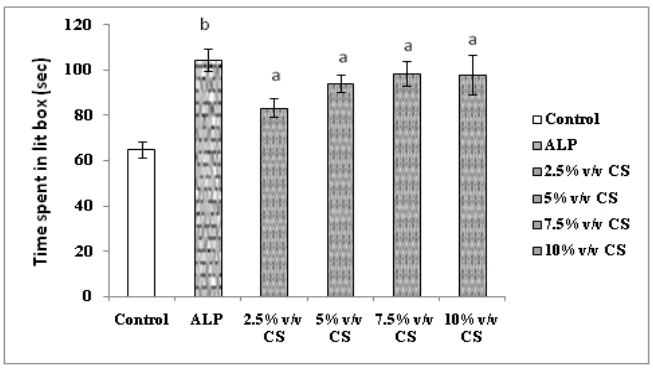

Figure 1: Effect of Citrus sinensis on time spent in lit box by mice.
ALP = Alprazolam (0.5 mg/kg, i.p.), CS= *Citrus sinensis*
a denotes p<0.01 as compared to control group,
b denotes p<0.001 as compared to control group.

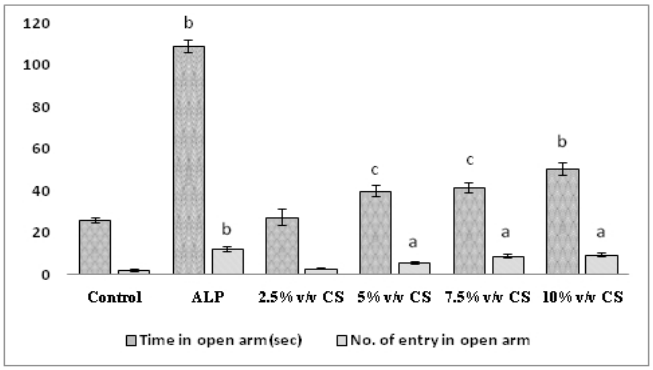

Figure 2: Effect of Citrus sinensis on time spent in open arm and number of entries in open arm of mice using Elevated plus maze.
ALP = Alprazolam (0.5 mg/kg, i.p.), CS= *Citrus sinensis*
c denotes p<0.05 as compared to control group,
a denotes p<0.01 as compared to control group,
b denotes p<0.001 as compared to control group.

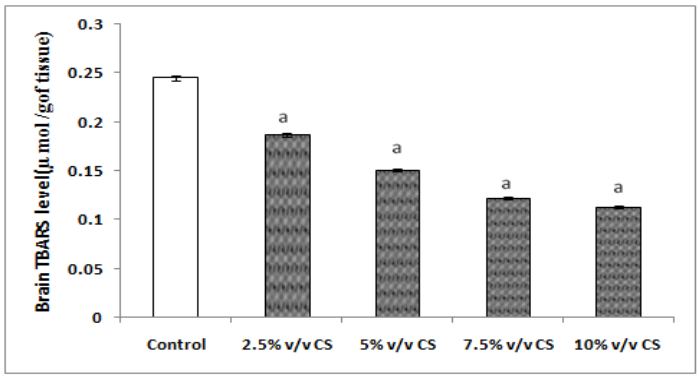

Figure 3: Effect of *Citrus sinensis* on Brain TBARS level.
Values are expressed in mean ± S.E.M (n=6).
a denotes p<0.01 as compared to control group.

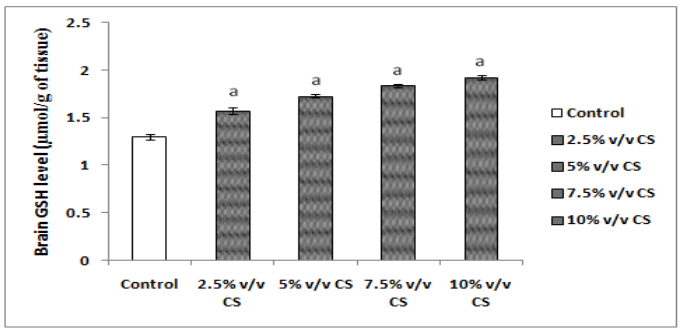

Figure 4: Influence of *Citrus sinensis* on Brain GSH level.
Values are expressed in mean ± S.E.M (n=6).

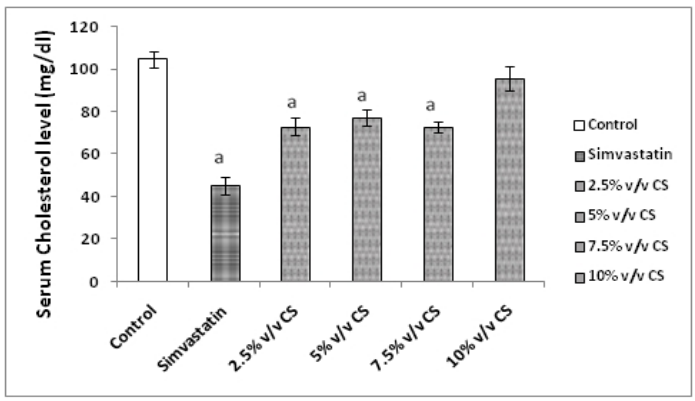

Figure 5: Effect of orange juice (*Citrus sinensis)* on total Cholesterol level.
Values are expressed as mean ± SEM (n = 6)
Simvastatin (5 mg/kg) was used as a standard drug.
a denotes p<0.01 as compared to control group.

RESULTS

Anxiolytic effect of orange (*Citrus sinensis)* juice using light-dark model

Citrus sinensis (CS) juice at the concentration of 5%, 7.5% and 10% v/v increased the time spent in lit box significantly ($p< 0.01$), in Light - Dark model, while at 2.5% v/v dose, no significant ($p> 0.05$) effect was observed.

Anxiolytic effect of orange (*Citrus sinensis)* juice using Elevated plus maze

CS (p.o.) at the concentration of 5% and 7.5% v/v enhanced the time spent in open arm significantly ($p <0.05$) in Elevated plus maze test, while 10% v/v concentration of CS showed remarkable ($p< 0.001$) effect (Figure 2). The effect of CS was found to be comparable to that of Alprazolam (an established anxiolytic agent).

Effect of *Citrus sinensis* on brain TBARS level

TBARS is an important marker of lipid peroxidation. The administration of CS for 10 days in mice produced a significant ($p<0.01$) fall in TBARS levels at 2.5%, 5%, 7.5% and 10% v/v concentrations. (Figure 3)

Influence of orange juice (*Citrus sinensis)* on brain GSH level.

GSH levels give an indication of free radical scavenging property. Orange juice, when administered to mice for 10 successive days significantly ($p< 0.01$) increased brain GSH (µ mol/g of tissue) levels, thereby reflecting increased scavenging of free radicals in the presence of orange juice (Figure 4).

Effect of *Citrus sinensis* on total cholesterol level

CS at different concentrations (2.5%, 5%, 7.5% v/v p.o.) significantly ($p<0.01$) decreased serum cholesterol levels (Figure 5).

DISCUSSION

Anxiety is mental disorder resulting from alterations in the levels of certain neuro-chemicals such as GABA, Serotonin, and Dopamine etc. The common targets for its treatment are GABA and Serotonin. Though several medicines are available, most of them are associated with some or the other limitation. Benzodiazepines are the most commonly prescribed medicines, which act through GABA-ergic system. However, their consumption is associated with problems of sedation and dependence, while serotonin agonists like Aspirones evoke adverse effects like dizziness, paresthesia and sedation. In our previous study, we reported that orange juice can be looked upon as a powerful memory enhancer[15]. Therefore, in the present study, we were interested to explore the potential of *Citrus sinensis* juice in the management of anxiety disorder. Administration of orange (*Citrus sinensis)* juice to mice for 10 successive days showed anti-anxiety potential, as observed by increased time spent by mice in open arm in Elevated plus maze test and enhanced time spent in lit box using Light - Dark model. Orange juice may be useful in anxiety related disorders due to the presence of anti-anxiety constituents such as linalool and flavonoids. Linalool produces anti-anxiety activity by interacting with $GABA_A$ receptor complex. Whereas, limonene and polymethoxylated flavones (active constituents of orange) lower the cholesterol levels of mice (administered with orange juice), which would be may be an additional favorable effect. Furthermore, orange juice contains free radical scavenging compounds like vitamin C, B1, B2 by virtue of which orange juice produces a profound anti-oxidant effect. The results obtained with orange juice in the present study were consistent and favorable. These results uniformly suggest that orange fruit may be looked upon as a promising anti-anxiety agent. Therefore, it is worthwhile to investigate the usefulness of orange juice in the management of human anxiety disorder.

CONCLUSION

In the present study, we observed that orange (*Citrus sinensis)* juice (i) lowered serum cholesterol levels (ii) reduced brain TBARS levels (iii) enhanced GSH levels and (4) reduced anxiety of mice in two test models. These findings reflect the anxiolytic potential of orange juice.

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