

Anxiolytic effect of olive leaf tea on mice

Abstract

According to current scenarios, the daily lifestyle of majority of the population is full of stress, worry and anxiety. This life style is also imparting its great role in psychological disorders. To improve the mental conditions, along with keeping a healthy life style, it is better to incorporate natural ingredients in diet. Historically, olive leaves have been used for medicinal purposes as well as being considered a symbol of peace. A study was conducted to evaluate the anxiolytic effect of olive leaf tea on mice. Distilled water 0.2mL was used as control and administered to 6 mice labeled as Group I whereas Olive leaf tea was prepared freshly with dried olive leaves and administered orally at 0.2mL to 6 mice labeled as Group II (treated) for 28 days. To evaluate CNS activity, a number of tests were performed including: Head dip, Cage crossing and Open field test. Upon evaluation of results, marked decrement was seen in number of head dips, cage crossing and also in central and peripheral square crossing. In accordance with the above result, it was concluded that the phenolic compound in olive leaves i.e. Oleuropein possesses some anxiolytic activity, besides its other known various pharmacological activities. Not only can olive leaf tea be prepared freshly at home, it has no known side effects and is economical. Therefore it can bestow a great part in preventing psychological disorder as well as imparting healthy life style.

Keywords: anxiety, cage crossing, head dip, open field, oleuropein, olive leaf.

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Abbreviations: ANOVA, analysis of variance; GABA, gamma amino butyric acid; GAD, generalized anxiety disorder; MAOI: mono amino oxidase inhibitors; SSRI, selective serotonin reuptake inhibitors

Introduction

Anxiety can be defined as the feeling of unease, most often including feelings of apprehension, nervousness, dreadfulness and tension.¹ As long as the subject is in a state of anxiety, additional feelings are also experienced such as panic, fear, insomnia, besides that other physical symptoms are also observed.² Not only mental health but the overall well-being of an individual gets disturbed due to anxiety. The classic symptoms of anxiety include sleep difficulties, inability to rest, breathing problems, heart palpitations, nervous stomach, headaches, neck tension, and worries, being panicked, frightened, etc.³ Bio psychosocial factors interact in situations of fear, trauma or stress which produce clinically significant symptoms of anxiety. Selective mutism, specific phobia, agoraphobia, social phobia, panic disorder, bipolar disorder and generalized anxiety disorder (GAD) are types of anxiety.⁴ Other causes of anxiety are thought to be the dysregulation of neurotransmitters specifically low levels of gamma amino butyric acid (GABA) which is an inhibitory transmitter, and the abnormal neuronal circulation in amygdala that controls fear and sentiments. Both of these mechanisms results in anxiety. Anxiety can be detected on the basis of standardized clinical questionnaires namely Taylor Manifest anxiety scale and Zulf Self-anxiety scale.⁵⁻⁷ Currently the common medications employed to treat anxiety are selective serotonin reuptake inhibitors (SSRI), mono-amino oxidase inhibitors (MAOI) etc.⁸

In ancient times, herbs and other natural products were used to relieve various illnesses which are now largely replaced by allopathic medications. The undesirable side effects and physiological dependence are the major problems associated with allopathic drugs, including those used to alleviate anxiety. This has led to the initiation of many researches to find a safe and effective natural resource to treat various ailments.^{9,10} Olive leaf is the leaf from olive tree (*Olea europaea*) belongs to the family *Oleaceae*. The olive tree is thick and short, mostly ranges from shrubs to 10m height. The leaves are shortly stalked and ovate, leathery, narrow oblong and attenuate, ranges from 4-10cm in length and 1-3cm wide. Olive tree is a monoecious plant and is cultivated in different parts of the world including mostly Mediterranean area in Italy, Spain, Portugal, France and Greece. The dried leaves, olive leaf extract and the oil of the olive fruits are used medicinally. Phenolic compounds, flavonoids, flavones, secoiridoids glycosides are present in all parts of the olive tree. Decoction or tea prepared from dried olive leaves is used to treat multiple infections, gastrointestinal illnesses and many more ailments.¹¹

Oleuropein is the most important and noticeable phenolic compound present in olive plant. It possesses various pharmacological properties including anti-inflammatory, anti-anxiety anti-oxidant, anti-atherogenic, anticancer, antimicrobial, antiviral, skin protectant, anti-aging, neuro-protective, anti-diabetic, hypotensive, antipyretic and many more. Oleuropein is significantly much more concentrated in olive leaf than in other parts of the tree.^{12,13} This current study is designed to evaluate the effect of olive leaf tea on anxiety by developing anxiety models in mice.

Materials and method

Experimental animals

This study has been carried out on albino mice of either sex weighing 20-24g in weight. They were divided into 2 groups each comprising of 6 mice. They were kept in the animal house of Jinnah University for Women. Food and water was provided daily as required, and were kept under the environmental conditions $23\pm 2^{\circ}\text{C}$. The study was approved by the ethical committee of the institute.

Material

Fresh olive leaves were picked from olive trees from Kashmir and were allowed to dry in the dark. The leaves were then bought to and identified by Department of Pharmacognosy, Faculty of Pharmacy Jinnah University for Women, Karachi.

Dosing regime

Group I was taken as the control sample and administered 0.2mL distilled water. Group II was taken as test sample and given 0.2mL of olive tea orally once a day. Stock solution was prepared by dissolving 35mg olive tea leaves in 300 mL of lukewarm, distilled water,¹⁴ the 0.2ml dose was calculated after weight adjustment of the mice. The study was carried out for 28days.

Performance methodology

Head dip test

Head dip or whole board test is a method which is useful for identification of anxiety. It consists of an enclosed wooden rectangular box with a hole of 2.5cm in diameter and found in all walls.¹⁵

Procedure

At the beginning of experiment the mice were familiarized with the wooden box. The temperature was maintained at room temperature. The mice were placed in the center area of the box and allowed to roam freely for 5 minutes. The number of times the mice stuck out its snout from the hole was noted. After performing the experiment, the smell of animal was cleared from the apparatus by cleansing it with 70% alcohol.¹⁶

Open field test

Open field test is used to determine the emotional behavior in rodents. It can also be used to measure exploratory activity, locomotor activity with idea of anxiety related behavior in rodents.¹⁷ The apparatus consists of a large square chamber, measuring 42cm in

height. The floor of the periphery is marked into 25 squares of equal length and width within a diameter of 15cm. A central square of equal size is also present in middle arena.

Procedure

The mice were held gently by the tail as they were placed into the center of the arena in the open field one at a time. The mice moved in the central and peripheral areas. The number of times that it moved was recorded for 5 minutes. The apparatus was cleaned with 70% alcohol to remove the odor of subject.¹⁸

Cage crossing

Cage crossing is an exploratory and behavioral test for rodents. Anxiety-like behavior is also determined by this method. An increased number shows anxiety-like behavior whereas a reduced number shows anxiolytic behavior. The apparatus consists of a square box that is closed on all ends and is transparent in color. The top consists of holes. The cage should be kept empty to avoid distractions for the rodents.

Procedure

One mouse at a time is placed in the apparatus and is observed for 5 minutes. After each reading, clean the apparatus with 70% alcohol to remove or clear off the smell of rodents,¹⁸ for the analysis of results SPSS tool version 19 was used. First the means of all the tests were taken and then the means were compared with the control value. One-way ANOVA was applied followed by post hoc Tukey's test. The result was highly significant if p was ≤ 0.001 .

Results and discussion

Historically, olive leaves have been used for medicinal purposes and are considered as a symbol of peace. Therefore, it is believed that olive leaf tea imparts a soothing and relaxing effect. Nowadays, olive leaf tea is preferred over black tea and green tea due to presence of zero amount of caffeine.¹⁹ Initially, when the animals were placed in the apparatus they made attempts to run off due to stressful reaction,²⁰ which was further confirmed by the literature survey suggesting high levels of corticosteroids when subjected to experimental conditions.²¹ It can be said that anxiety develops due to dread when exposed to the apparatus, so the reduction in number of head dips can be considered as the reduction in anxiety and fear. Our results showed reduction in head dip from 20 (baseline) to 6 on 28th day of dosing,²² this presumption supports our result that olive leaf tea has anxiolytic properties. Oleuropein is the main phenolic component that is responsible for the marked therapeutic effects (Table 1)(Figure 1).¹¹

Table 1 Effect on olive leaf tea on head dip

Solutions	Baseline	7 Days	14 days	21 days	28 days
Control 0.2mL Distilled water	18 \pm 1.41	19 \pm 1.04	18 \pm 1.36	19 \pm 1.03	19 \pm 0.81
Olive Leaf Tea (0.2mL)	20 \pm 1.37	15 \pm 0.75***	12 \pm 0.83***	9 \pm 0.82***	6 \pm 0.79***

Values are Mean \pm SD, N=6= number of animals, *** p <0.001=highly significant

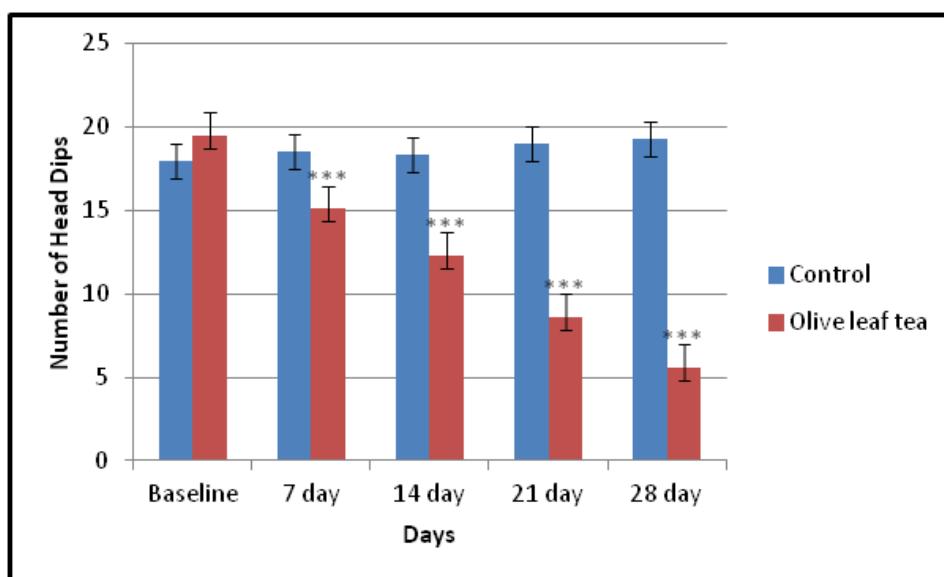


Figure 1 Effect of olive leaf tea on head dip.

On exposure to cage crossing apparatus, mice crossed the cage numerous times due to the resulting state of fear. Though after administration of Olive leaf tea number of cage crossing decreased significantly.²³ Olive leaf tea was administered to mice on daily basis, according to Table 2 which showed a marked reduction in the number of cage crossings on day 21st,¹⁵ which were further reduced on day 28th.¹⁰ This showed that anxiety levels of mice were significantly reduced. Anxiety produces stress which causes increase

production of adrenal hormones as well as increase in the utilization of vitamin C, E and other micronutrients which enhance metabolism of fats, carbohydrates and proteins to produce energy to overcome stress.²⁴ Biochemical changes are induced due to stresses which are normally overcome by body's biological anti-oxidant system, including enzymatic anti-oxidants such as glutathione peroxidase and superoxide dismutase etc. Non-enzymatic anti-oxidants such as vitamin C, E and trace minerals provide secondary defense.²⁵

Table 2 Effect of olive leaf tea on cage crossing

Solutions	Baseline	7 Days	14 days	21 days	28 days
Control 0.2mL Distilled water	41±1.21	41±0.63	42±0.89	41±0.86	42±0.90
Olive Tea (0.2mL)	42±1.32	34±0.75***	27±0.81***	15±1.21***	10±0.98***

Values are Mean±SD, N=6= number of animals, ***p<0.001=highly significant

Oleuropein possesses potent antioxidant and anti-inflammatory properties. Chelation of metal ions such as Cu and Fe with Oleuropein prevents free radical formation.²⁶ Furthermore, it inhibits several inflammatory enzymes.²⁷ Both Oleuropein and hydroxytyrosol have been shown to be scavengers of superoxide anion, contributing to its anti-oxidant nature (Table 2) (Figure 2).²⁸

The open field test was primarily introduced to measure the emotional behavior in rats but it has proven to be equally successful with mice as well.^{29,30} The experiment contributes an exclusive probability to systematically determine new environmental exploration, usual locomotors activity, and contributes in basic screening for anxiety-related behavior in rodents.³¹ Social isolation and stress due to bright light exposure posed an unfamiliar test environment to the rodents. These two factors had an impact in production of anxiety like behavior in an open field apparatus.³²

(Table 3) (Figure 3) results showed a significant reduction in the number of peripheral boxes crossed by the test mice as compared to the control mice. Meanwhile, a slight increase in the number of central squares crossed by the test mice was shown. The baseline reading was 3 which were increased to 7 on 28th day of dosing. The increased frequency showed reduced anxiety and augmented exploratory actions.⁵

Oleuropein (OLE) improves various physiological functions, produces multiple pharmacological actions in the central nervous system, and has in vitro and in vivo neuro protective effects.^{33,34} In the literature study on an animal model of anxiety, involving elevated plus maze OLE administration potentially reduced anxiety-like behavior by more entries and exploratory activities, and less open arm avoidance, resulting in reduced index of anxiety (Table 4) (Figure 4).³⁵

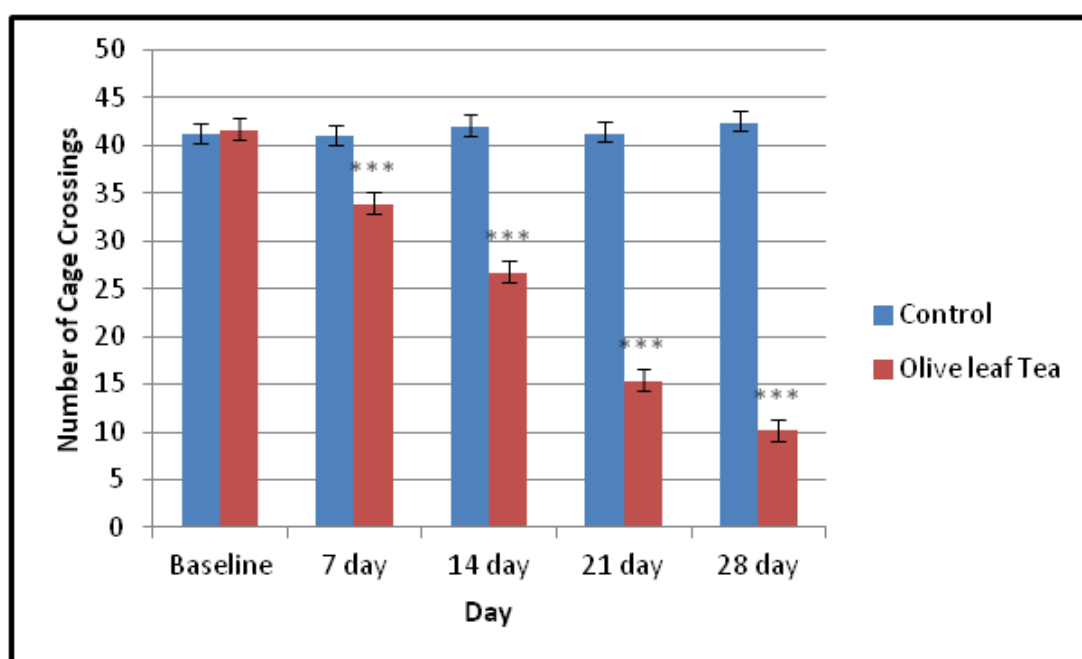


Figure 2 Effect of olive leaf tea on cage crossings.

Table 3 Effect of olive leaf tea on open field test (central crossings)

Solutions	Baseline	7 Days	14 days	21 days	28 days
Control 0.2mL Distilled water	3±0.75	3±1.09	4±0.81	3±1.26	3±0.74
Olive Tea (0.2mL)	3±0.89	5±0.51***	6±0.54***	6±0.51***	7±0.52***

Values are Mean±SD, N=6=number of animals, ***p<0.001=highly significant

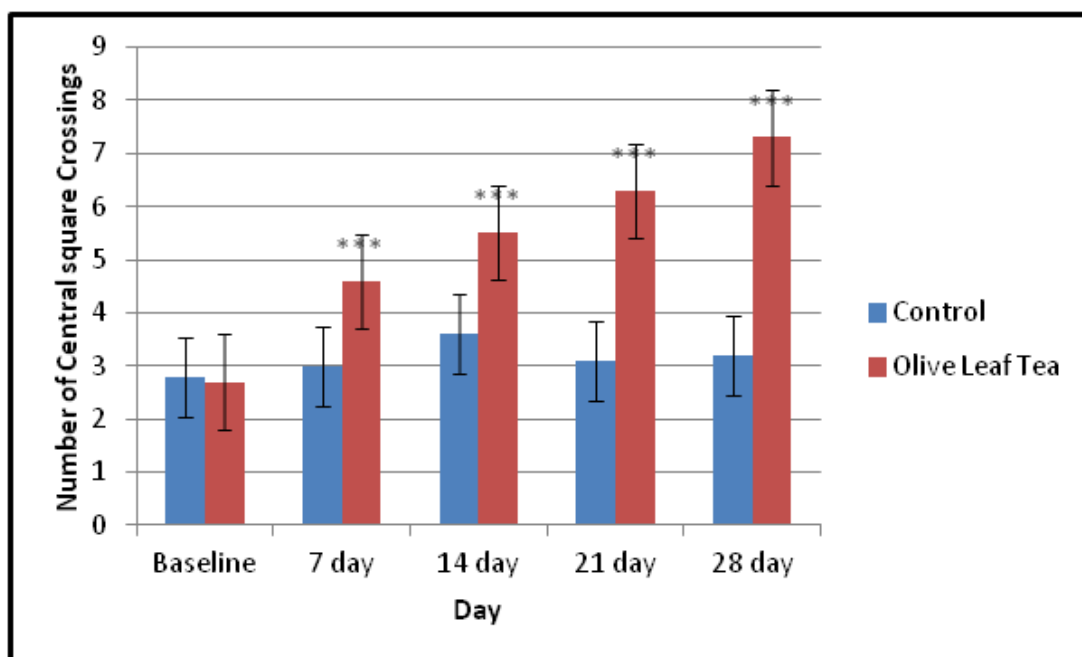
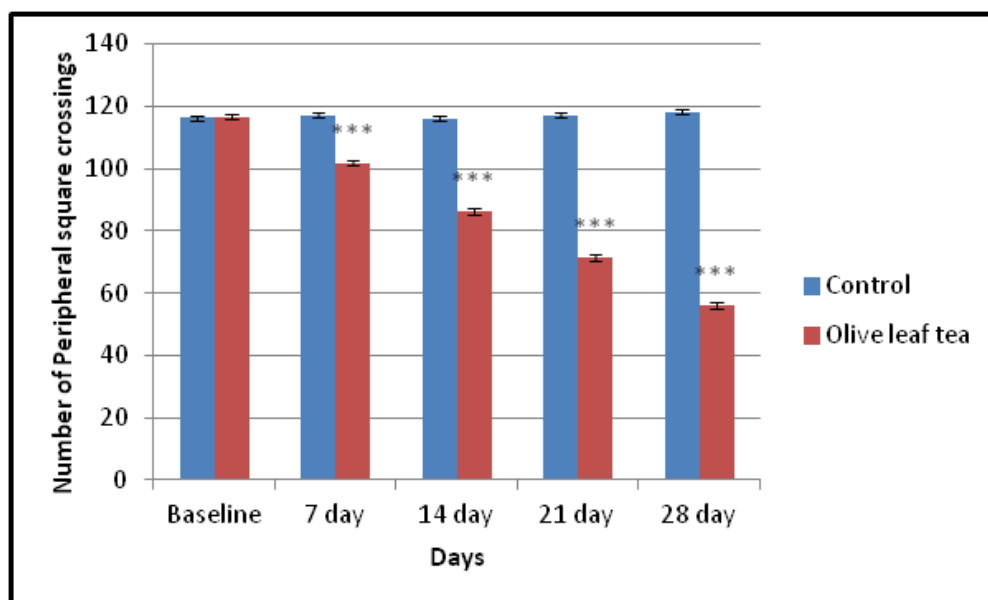


Figure 3 Effect of olive leaf tea on central square crossings.

Table 4 Effect of olive leaf tea on open field test (peripheral square crossings)

Solutions	Baseline	7 Days	14 days	21 days	28 days
Control 0.2mL Distilled water	116±0.81	117±0.82	116±1.21	117 ±0.75	118±0.69
Olive Tea (0.2mL)	117±1.03	102±1.16***	86±1.01***	71±0.98***	56±0.79***

Values are Mean±SD, N=6=number of animals, ***p<0.001=highly significant

**Figure 4** Effect of olive leaf tea on peripheral square crossings.

Conclusion

From our research study, it can be concluded that olive leaf tea plays a significant role in decreasing anxiety due to presence of Oleuropein which possesses anxiolytic, anti-oxidant as well as other pharmacological activities (anti-inflammatory). These pharmacological activities can also be beneficial for a healthy life style and other aspects of life if a normal individual were to consume olive leaf tea as part of a daily routine.

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None

Conflicts of interest

The author declares that there are no conflicts of interest.

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