



## INVESTIGATE A PLANT PRODUCT AS AN ANXIOLYTIC AGENT

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### ARTICLE INFO

#### Article History:

Received 18<sup>th</sup> November, 2016

Received in revised form 7<sup>th</sup>  
December, 2016

Accepted 16<sup>th</sup> January, 2017

Published online 28<sup>th</sup> February, 2017

#### Key words:

*Pistachio khinjuk*, anxiolytic properties, Hyponeophagia test.

### ABSTRACT

In spite of the presence of known anxiolytic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat anxiety disorder. In this study, authors describe effects of essential oil of PK on anxiety markers in male mice using the hyponeophagia test. Groups of 6 mice each were given 200, 400 or 600 µg/kg essential oil of PK *per os*. Another 6 mice were applied as saline controls. A plastic jug was used as a hyponeophagia unit with a spout with food to direct mice. Mice were allowed to roam freely in the jug for 120 seconds at day 15, 20 and 25 after treatments. In the test, the time spent until mice began to eat food and number of stools of mice were measured. After each trial the recipient was cleaned with ethanol to prevent a bias based on olfactory cues. The results show that mice in group 2, 3, and 4 to group 1 found food faster, so amount of anxiety in group 2, 3, and 4 to group 1 was fewer. The present research indicated the anxiolytic activities of the medical plant, offering to use as an anxiolytic supplement.

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### INTRODUCTION

Anxiety is the one of the most usual problems before the surgery<sup>1</sup>. Its rampancy rate ranged from 11 to 80 percent in all the adult patients. Increasing use of narcotics, anesthetics and retardation in wound healing are some of the other accompanying problems<sup>2,3</sup>. Antidepressants are used to treat anxiety, but most of these drugs have many side effects<sup>4</sup>.

Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost<sup>5-7</sup>. Many traditional plant are used for treatments harmful diseases to the body throughout the world<sup>8,9</sup>. Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants<sup>10-12</sup>. Essential oils could be extracted from different parts like leaves, stems, flowers, roots including bushes and trees through distillation<sup>13-15</sup>. It is applied by special type of essential oil extracted from aromatic plants for medical objectives such as cure anxiety<sup>16</sup>.

*Pistachio khinjuk* (PK), a plant belonging to the Sapindales order, Anacardiaceae family and *Pistaciagenus*, is one of the oldest medicinal plants. It was reported that PK had several therapeutic effects such as bacterial disease, fungal illness, and inflammatory sickness<sup>17,18</sup>. PK has a long history of use in traditional medicine, but there is no evidence that it is useful to treat anxiety disorder<sup>19</sup>. The Hyponeophagia test is one of the most widely used tests to measure anxiety-like behavior in mice<sup>20</sup>. There is no study on effects of PK in Hyponeophagia test (anxiety test) in mice. Therefore, the aim of the study was determination of properties of PK on anxiety in mice.

### MATERIAL AND METHODS

#### Plant sample collection

In the tentative study, plant collected from Kermanshah. The sample was purified from any strange, plants, dust, or any other contaminants.

### Essential oil extraction

For preparation of essential oil from aerial part PK by hydro-steam distillation using the Clevenger device, 100 to 150 g of plant was introduced in the distillation flask (1L). Aromatic molecules of the essential oil was assailed from the plant material and evaporated into hot steam. The hot steam forced the plant material to liberate the essential oil without burning the plant material itself. Then, hot steam containing the essential oil was elapsed through a cooling system in order to compress the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was cleaned up through anhydrous Na<sub>2</sub>SO<sub>4</sub> to desiccate the yielded essential oil. Then, the essential oil was collected in tightened vials and stored in a refrigerator.

### Animal

24 male mice weighing 35–40 g were used. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/dark cycle) and maintained with free access to water and ad libitum standard pelleted food.

### Study design

Mice divided in four group: Group 1 (control group): In the group, mice received saline. Group 2: Treatment mice received received essential oil of PK (200 µg/kg, p.o.). Group 3: Treatment mice received received essential oil of PK (400 µg/kg, p.o.). Group 4: Treatment mice received received essential oil of PK (600 µg/kg, p.o.). A plastic domestic jug converted for avail as a Hyponeophagia testing unit. Food located inside the spout, which was to be the vehicle for the toxin, in the infested area and conveniently directs the attention of the mouse straight to it. Mice are allowed to move freely under this plastic for 120 seconds in days 15, 20, 25. The time spent until mice began to eat food and number of stools were measured. After each trial, plastic and under it cleaned with ethanol to inhibit a bias based on olfactory cues<sup>20</sup>.

### Statistical analysis

Descriptive statistics including the mean, standard error, median, minimum and maximum were calculated for all variables. The one-way ANOVA followed by Tukey post hoc test were used for comparison of different parameters. The data were analyzed by SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA) and P<0.05 was accepted as statistically significant.

## RESULTS

The time spent until mice began to eat food shown in following tables. Also, number of stools in 15, 20, and 25 days indicated underneath.

**Table 1** The time spent until mice began to eat food and number of stools of mice in 15 day.

Day	Group	The time spent until mice began to eat food	Number of stools
15	1	105.40±1.41 <sup>a</sup>	0.60±0.05 <sup>A</sup>
	2	96.50±1.51 <sup>ab</sup>	0.40±0.05 <sup>B</sup>
	3	90.33±2.06 <sup>b</sup>	0.00±0.05 <sup>C</sup>
	4	86.66±1.36 <sup>b</sup>	0.40±0.05 <sup>B</sup>

**Table 2** The time spent until mice began to eat food and number of stools of mice in 20 day.

Day	Group	The time spent until mice began to eat food	Number of stools
20	1	57.80±4.05 <sup>a</sup>	0.60±0.05 <sup>A</sup>
	2	55.33±2.42 <sup>a</sup>	0.40±0.05 <sup>B</sup>
	3	43.33±3.01 <sup>b</sup>	0.40±0.05 <sup>B</sup>
	4	39.00±0.89 <sup>b</sup>	0.40±0.05 <sup>B</sup>

**Table 3** The time spent until mice began to eat food and number of stools of mice in 25 day.

Day	Group	The time spent until mice began to eat food	Number of stools
25	1	84.25±4.31 <sup>a</sup>	0.8±0.04 <sup>A</sup>
	2	78.66±2.06 <sup>a</sup>	0.60±0.09 <sup>A</sup>
	3	68.00±2.09 <sup>b</sup>	0.40±0.05 <sup>B</sup>
	4	55.66±2.58 <sup>c</sup>	0.40±0.04 <sup>B</sup>

## DISCUSSION

Almost all of human nutrition depends on plants, either directly through foods used by people, or indirectly as feed for animals or the flavoring of foods<sup>21-25</sup>. Also, because of their security and low cost as well as their effects on all disorders of body in traditional ethnomedicine<sup>26-29</sup>. The use of plant compounds to treat diseases is an old practice in a large part of the world<sup>30, 31</sup>. Interest in plants with anxiolytic effects has revived as a result of popular problems associated with the use of antidepressant drugs. In Iranian traditional medicine used PK as anxiolytic plant.

The Hyponeophagia test is one of the most widely used tests to appraise anxiety-like behavior in mice. The test estimates stress-induced anxiety and as such can be used to determine compounds that may have anxiolytic effects. Hippocampal lesions produce an abundant decline in Hyponeophagia in mice<sup>20</sup>. In a study shown that privilege of Hyponeophagia over anxiety tests such as the plus-maze, is that if the results of the first test fail to attain statistical significance, another test can be run using a several novel test environment and food, as the type of anxiety being measured appears to be the similar and not qualitatively changed by the formerly run tests, unlike in the plus-maze<sup>32</sup>.

Findings from the current study revealed that in 15, 20, and 25 days, are seen the time spent until mice began to eat food and number of stools of mice reduced in group 2, 3, 4 to 1, and in group 3 in 20 day is least. Overall, these results demonstrated that mice in group 2, 3, 4 to group 1 found food faster.

Our results support the use of the plant in traditional medicine and offer that essential oil of PK possess good anxiolytic properties. It can be used as anxiolytic supplement in the developing countries towards the development of recent remedial agent. Additional *in vivo* studies and clinical trials would be needed to justify.

### Acknowledgment

We, the authors wish to thank Medical Sciences University of Kermanshah, Iran for the financial support of this work.

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