Assessment of Anxiolytic Activity of Nuts of Prunus Amygdalus Dulcis (Almond) in Mice

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Abstract
The incidence of anxiety in the community is very high and is associated with a lot of morbidity. The most widely prescribed medications for anxiety disorders are the benzodiazepines in which their use is limited by their side effects, hence many herbs employed in traditional and alternate medicine for sleep disorders and related diseases. The nuts of Prunus amygdalus (almond) are found to possess anti-stress properties, also is an effective health building food, both for the body and the mind.

Anxiolytic activity of Prunus amygdalus dulcis were studied using open-field tests in mice. The efficacy of two doses of Prunus amygdalus dulcis 800 and 1600 mg/kg was compared with standard anxiolytic drug the diazepam (1mg/kg). Both the diazepam and almond (1600 mg/kg) treated groups in this study showed extremely significant increased (p<0.001) in both the number of rearing against the wall and the time spent in central squares with significant increase in the number of crossed squares (p<0.01) and (p<0.05) respectively. The number of grooming was extremely significant decreased (p<0.001) while the duration of grooming showed no significant difference (p>0.05) in compare to the control group. The group that given 800mg/kg of almond showed highly significant decrease (p<0.01) in the number of grooming while the duration of grooming showed no significant difference (p>0.05) in the number of rearing against the wall, the time spend in central squares and the number of squares crossed in compare to the control group.

These findings suggest that Prunus amygdalus dulcis in a dose 1600 mg/kg possess anxiolytic-like properties equal to that of diazepam while 800mg/kg Prunus amygdalus dulcis has less effect.

Keywords: anxiolytic effect, Prunus amygdalus dulcis, open field test, mice, diazepam.
Introduction

Anxiety is the displeasing feeling of fear and concern. When anxiety becomes excessive, it may be considered as an anxiety disorder (1). Anxiety disorders such as generalized anxiety, panic and obsessive-compulsive disorders, phobias or post traumatic stress disorders are common and major cause of disability (2,3). Anxiety is affecting 1/8th of the total population worldwide and is a very important area of research interest in psychopharmacology during this decade (4). The complexity of daily life in modern society frequently leads to varying degree of anxiety. Mood and anxiety disorders have been found to be associated with chronic pain among medical patients in both developed and developing countries (5). Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopamanergic system have also been shown to play a role in anxiety (6). Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines (BZDs). However, the clinical uses of BZDs are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability (7). Buspirone, the non-sedating anxiolytic agent, which gave rise to much optimism, is slowing acting and not effective in a high percentage of patients, and can results in tachycardia (8). Though selective serotonin reuptake inhibitors are considered as one of the important group of anxiolytic agents, they are also associated with significant side-effects such as insomnia, decreased libido, sexual dysfunction and are only effective in approximately 50% to 60% of patients (9). It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases (10).

Almonds are prunes that belong to the rose family, the Rosaceae. They were traditionally placed in a sub-family, the Prunoideae (or Amygdaloideae), but sometimes, they are placed in their own family, the Prunaceae (or Amygdalaceae) (11). The almond tree is a small deciduous tree which grows to between 4 and 10 meters in height, with a trunk of up to 30 centimeters in diameter. The flowers are pale pink and 3-5 cm in diameter with five petals (12,13). There are three varieties of almonds, all of which produce nuts, but some are edible and some are not. One almond variety produces the sweet nuts we eat, one produces poisonous, bitter nuts and a third variety produces a mixture of bitter and sweet nuts. Two major types of almonds are grown commercially, which can be categorized as sweet almonds (Prunus amygdalus dulcis) and bitter almonds (Prunus Amygdalus amara) (14).
The edible portion of the Prunus amygdalus is its nuts, which are commonly known as almonds or badam, and it is a popular, nutritious food (15). In addition to its nutritional values, it has some medicinal values that may be helpful for treating certain diseases and health problems.(16) The almond is an effective health building food, both for the body and the mind; it is also a valuable food remedy for several common ailments like anaemia, as they contain copper, iron and vitamins. (17) The nuts of Prunus amygdalus are found to possess various pharmacological properties, such as anti-stress (18), antioxidant (19), immunostimulant (20), and lipid lowering (21). The almond is highly beneficial in preserving the vitality of the brain, strengthening the muscles and prolonging life. (16,17) Almonds are a good source of nutrients which are associated with the health of the heart, such as vitamin E, mono unsaturated fatty acids, poly-unsaturated fatty acids, arginine, and potassium (17). The active constituents of almonds are globulins such as amandine and albumin and amino acids such as arginine, histidine, lysine, phenylalanine, leucine, valine, tryptophan, methionine and cystine (22). Almonds also contain a variety of phenolic compounds which are localized principally in their skin, including flavonols (isorhamnetin, kaempferol, quercetin, catechin and epicatechin), flavanones (naringenin), anthocyanins (cyanidins and delohinidin), procyanidins, and phenolic acids (caffeic acid, ferulic acid, P-coumaric acid and Vanillic acid). (23) Almonds contain proteins and certain minerals such as calcium and magnesium. Its also contain phytosterols which are associated with cholesterol-lowering properties. The phytosterol content of almonds is 187 mg/100mg (22). Almonds contain approximately 49% oils , of which 62% is mono-unsaturated oleic acid (an omega-9 fatty acid), 24% is linoleic acid (a poly unsaturated omega 6 essential fatty acid) and 6% is palmitic acid (a saturated fatty acid).(24) Despite the numerous scientifically proven pharmacological activities of Prunus amygdalus dulcis there was no scientific data on its potential as an anxiolytic agent. Hence present study was done to assess anxiolytic activities of Prunus amygdalus dulcis.

**Aim of the study**

To assess the anxiolytic activity of the nuts of Prunus amygdalus dulcis (almond).

**Material and methods**

**Animals:** Twenty eight Swiss adult mice (weighting 25-30 g) of either sex were used in this study. The animals were housed in standard cages in the animal house of Babylon Medical College, under controlled temperature around 25 °C and 12 hours light-dark cycles. They were supplied with a standard diet and tap water ad libitum.

**Plant:** Nuts of Prunus dulcis were purchased from the local market and added to the animals food as a single daily dose for 14 days. The Prunus dulcis nuts was added in two doses, 800 mg/kg and 1600 mg/kg daily.

**Experimental design:** After 2 weeks of adaptation, the animals were randomly divided into 4 groups (7 mice in each) as follows:

- **Group (1) control group:** They were maintained on standard chow diet for 14 days only. At day fifteen open field test(OFT) done.
- **Group (2):** The Prunus dulcis (800mg/kg) were added to standard chow diet as a daily dose for 14 days. At day fifteen OFT done.
- **Group (3):** The Prunus dulcis (1600mg/kg) were added to standard chow diet as a daily dose for 14 days. At day fifteen OFT done.
- **Group (4):** They were injected with 1 mg/kg intraperitonealy i.p. diazepam
Open field test
This test utilizes behavioral changes in rodents exposed to novel environments and is used to detect angiogenic and anxiolytic activity under identical situations. Various types of open field apparatus have been used to test the mice. An open field apparatus used comprising of a floor space of 100 cm x 100 cm and with 50 cm high walls. The floor was colored black and the floor area was divided into 100 equal squares by white lines. A mouse is placed at the center of the field and is left for 5 minutes with the aid of video camera.

The floor of the open field was cleaned with 70% ethanol and allowed to dry between tests. (25,26) Each mouse was monitored carefully and recorded by video camera (SONY/ Cyber-shot) for 5 minutes in order to record the main parameters mentioned above.

Statistical analysis:
SPSS version 17.0 was used for the statistical analysis, Analysis of variance (ANOVA) was used for multiple sample analysis. Results were expressed as mean ± SD.(27)

Results
The results in both the diazepam and Prunus dulcis 1600 mg/kg groups showed an extremely significant increase (p<0.001) in the number of rearing and time spend in the central squares with significant increase in the number of crossed squares(p<0.01) and (p<0.05) respectively, the number of grooming was extremely significantly decreased (p<0.001) while the duration of grooming showed no significant difference (p>0.05) in compare to the control group. While, the group that received 800mg/kg Prunus dulcis showed highly significant decrease (p<0.01) in number of grooming, and the duration of grooming showed extremely significant increase (p<0.001). But, with no significant difference (p>0.05) in the number of rearing, the time spend in central squares and number of squares crossed in compare to the control group. (Table 1).

Table (1) : Effects of nuts of Prunus dulcis on open field tests parameters, results expressed as mean ± SD.

<table>
<thead>
<tr>
<th>groups</th>
<th>Number of Rearings</th>
<th>Number of squares crossed</th>
<th>Time spend in central squares (seconds)</th>
<th>Number of grooming</th>
<th>Duration of grooming (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>25.5 ±3.7</td>
<td>294.2±20.4</td>
<td>8.5±3.3</td>
<td>17.71±7.49</td>
<td>12.1±5.8</td>
</tr>
<tr>
<td>Group (2)</td>
<td>24.4±3.1</td>
<td>261.4±39.5</td>
<td>11.5±6.0</td>
<td>9.2±3.4**</td>
<td>85.7±23.1***</td>
</tr>
<tr>
<td>Group (3)</td>
<td>33.1±4.3***</td>
<td>337.1±46.8*</td>
<td>90.7±28.6***</td>
<td>7.00±2.16***</td>
<td>15.0±5.7</td>
</tr>
<tr>
<td>Group (4)</td>
<td>33.4±3.4***</td>
<td>347.8±39.9**</td>
<td>152±30.3***</td>
<td>5.00±2.16***</td>
<td>11.4±2.35</td>
</tr>
</tbody>
</table>

* = p<0.05, ** = p<0.01, *** = p<0.001.
Discussion

Anxiety is an emotional state experienced by people, and is not readily modeled in animals.(26) The incidence of pathologic anxiety in the community is very high and is associated with lot of morbidity. Life time prevalence anxiety in women is 30.5% and male is 19.2%.10 Hence, it is very important to address this problem of anxiety and find effective remedies.(28) In order to extend till now ethologically derived paradigms used in the evaluation of anxiety and fear in rodents, a modified open field was designed.(26) Open field method which is a standard screening procedure was used to screen the anxiolytics effect of of Prunus dulcis seeds in comparison with a standard anxiolytic drug like diazepam.(25,29)

The open field test is a paradigm used for evaluating the effect of drugs on gross general behavior and is used to measure the level of nervous excitability.(30) When removed from their acclimatized home cages and placed in a novel environment, animals express their anxiety and fear by showing decreased ambulation and exploration, immobilization or freezing, and reduction in normal rearing and grooming behavior. Increased micturation and defecation due to augmented autonomic activity is also observed. These paradigms are attenuated by classical anxiolytics and potentiated by anxiogenic agents.(31) It has been widely accepted that stress induces anxiety-like behavior expressed in the open-field as an inhibition of locomotion and time spent in the inner zone of the arena; the increase in locomotion resulting from the effects of anxiolytic drugs then is considered to reflect lowering of the anxiety level.(26,32)

This study showed an increase in locomotor and exploration activity expressed by the increase in number of rearing and time spend in central squares with increase in the number of squares crossed in both the diazepam and Prunus dulcis(1600 mg/kg) groups.

Grooming behavior is a displacement response expected to be displayed in a novel environment. Reduced grooming frequency and the increase in the duration may indicate reduced stress and anxiolytic-like effect(33). Add to that, it has been accepted that grooming appears in connection with the lowering arousal following the stressful event.(34) Yet, it is not quite clear if grooming appears as an indicator of de-arousal, or if it actively participates in the stress and arousal attenuation.(35) Number of grooming decreased in both the diazepam and Prunus dulcis (1600 mg/kg) groups. And This indicate that the Prunus dulcis (1600 mg/kg) has same anxiolytic effect like that of diazepam. The group that given Prunus dulcis 800mg/kg showed an increase in duration of grooming, while the number of grooming was decreased with no significant differences in the number of rearing, the time spend in central squares and the number of squares crossed. So the Prunus dulcis 800mg/kg has no effect on locomotor and exploration activity, but it was effective in grooming behaviors, and since that the reduction in grooming frequency and the increase in the duration indicate reduced stress and anxiolytic-like effect(33). Hence, it may has some anti-anxiety effect.

These effects of Prunus dulcis nut may be resulted from its phytochemical screening that showed presence of a variety of phenolic compounds including flavonols, flavanones (naringenin), anthocyanins (cyanidins and delohidinid), procyanidins, and phenolic acids.(23) Flavonoids (flavanones) have shown anti-anxiety activity in various studies. Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system and BZD receptors, as it was found that flavanones bind with high affinity BZD site of GABA A receptors.(36,37)

Anthocyanins, in vitro, possess MAO inhibitory activity for both MAO-A and MAO-B; MAO function is connected to
neurodegenerative diseases, depression, and anxiety.(38)

Moreover, tryptophan which is one of the active constituents of almonds,(22) has shown some promise as an antidepressant alone and as an "augmenter" of antidepressant drugs. (39) Therefore, flavanones, anthocyanins, and tryptophan may be responsible for the anti-anxiety activity of almond.

Up to knowledge there is no such study to compare with it.

In the present study the dose of 1 mg/kg diazepam posses anxiolytic rather than sedative effect which is consistent with a study that asses the anxiolytic and anticonvulsant effect of *alternanthera brasiliiana* in which they use 1 mg/kg diazepam i.p as standard drug. (40) While Zdeněk H., et al (2009), who evaluate different doses of diazepam conflict with this study, as they found that low doses 0.1, 0.3 mg/kg i.p have anxiolytic effect while the dose of 1 mg/kg have sedative effect presented by decreased locomotor activity and the time spend in central squares, in addition to reduced grooming after all three doses.(26) This may be due to the brand of diazepam used in the present study as it was Syrian and there was no other type in the local markets.

**Conclusions**

1- Sweet almond can be used as an alternative to the anxiolytic drugs.

2- Further studies are needed to study if the consumption of the *Prunus amygdalus dulcis* predisposes to interaction with anxiolytics on concurrent use.

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**References**

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