



Research Paper: Anxiolytic Effect of the Hydro-alcoholic Extract of Anethum Graveolens Seed in Adult Female Wistar Rats: Modulation of GABA Receptors



Rana Shahabi¹, Mohammad Rostampour^{2,3*}, Behrooz Khakpour^{2,3}, Bahram Soltani^{2,4}, Farshid Saadat⁵

1. Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

2. Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

3. Department of Physiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

4. Department of Pharmacology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

5. Department of Immunology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.



Citation Shahabi R, Rostampour M, Khakpour B, Soltani B, Saadat F. Anxiolytic Effect of the Hydro-alcoholic Extract of Anethum Graveolens Seed in Adult Female Wistar Rats: Modulation of GABA Receptors. Caspian J Neurol Sci. 2022; 8(1):1-6. <https://doi.org/10.32598/CJNS.8.28.302.1>

Running Title Anxiolytic Effect of Anethum Graveolens Seed
<https://doi.org/10.32598/CJNS.8.28.302.1>



© 2018 The Authors. This is an open access article under the CC-BY-NC license.

ABSTRACT

Background: Along with industrial development and the increasing social complexity of societies, anxiety is one of the most prevalent psychological disorders. Medicinal plants are considered as an enrichment source of ingredients with biological activity.

Objectives: The aim of this study was to evaluate the anxiolytic effect of Anethum Graveolens seed (AGS) and the possible involvement of Gamma-Aminobutyric Acid (GABA)-ergic system in the AGS effect.

Materials & Methods: In the present experimental study, 64 female Wistar rats were divided into eight groups and received various concentrations of hydroalcoholic extract of AGS. To measure the level of anxiety, an elevated plus maze was used in a way that the animal's head turned to an open arm. Prior to the injections of AGS extract, the GABA receptor antagonist was used. The results were analyzed by one-way analysis of variance using IBM SPSS v. 16.

Results: Dose-response experiments showed that the AGS extract significantly decreased the anxiety indices compared to the control group ($P < 0.05$). To analyze locomotor activity, our data showed that AGS extract at 0.1, 1, and 10 mg/kg could significantly increase locomotor activity compared to the control group ($P < 0.001$). Pentylentetrazol (PTZ) + extract significantly decreased the anxiolytic effect of AGS extract ($P < 0.01$).

Conclusion: Considering the anti-anxiety effects of AGS extract and a reduction in this effect caused by PTZ, part of the anti-anxiety effect of extract might be assumed via its interaction with GABA-ergic receptors. Further experimental trials; however, are required for the establishment of the anti-anxiety impact of AGS.

Keywords: Anxiety, Anethum Graveolens, Pentylentetrazole, Gamma-aminobutyric Acid

Article info:

Received: 25 Jul 2021

First Revision: 10 Aug 2021

Accepted: 13 Sep 2021

Published: 01 Jan 2022

* Corresponding Author:

Mohammad Rostampour

Address: Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

Tel: +98 (13) 33690099, **Fax:** +98 (13) 33690036

E-mail: rostampour@gums.ac.ir; rost_v@yahoo.com

Highlights

- The hydro-alcoholic extract of *Anethum Graveolens* Seed (AGS) reduced anxiety indices in the model of elevated plus-maze.
- Part of the anti-anxiety effect of AGS extract can be via GABA-ergic receptor interaction.

Introduction

Anxiety is one of the psychological disorders, which might be experienced by a lot of people in their life span. Physiologically, anxiety is a complex behavior in response to physical or environmental stress due to uncertainty or potentially negative future events [1, 2]. This disorder affects about 10-30% of people in different societies [3].

By introducing benzodiazepines as an enhancer of the effect of the neurotransmitter Gamma-Aminobutyric Acid (GABA) about 50 years ago, treatment of anxiety was revolved. GABA as a main inhibitory neurotransmitter in the brain exerts its effect via GABAA and GABAB receptors through ligand-gated ion channels and G protein-coupled receptors, respectively [4]. Changes in GABA level cause an imbalance between excitatory and inhibitory systems and provokes neuropsychiatric disorders, such as anxiety [5]. Pentylentetrazol (PTZ) is a prototypical anxiogenic drug that acts via selective blockage of GABAA receptor channels [6].

Medicinal plants are safe, effective, and inexpensive resources that are screened by different communities for the isolation of bioactive compounds and new drug inventions [7]. *Anethum graveolens* (dill) as a one-year-old plant grows mainly in the southwest of Asia and southeast of Europe and traditionally has been used as a sedative and an appetizer [8, 9]. Also, anticonvulsant activity of AGS hydro-alcoholic extract against PTZ-induced seizure has been reported [10]. Various species of dill comprise different kinds of steroids and phytoestrogen, which could have anti-inflammatory and tranquilizing effects [11, 12]. Moreover, various components, such as monoterpenes (carvone, limonene, linalool, etc.) have been isolated from *A. graveolens* according to phytochemical screening [13].

Little is known about the therapeutic effect of dill on anxiety. In the present report, we studied the anxiolytic effect of dill seed extract on the behavior of female rats.

We further searched for the involvement of GABAA receptors using an antagonist of these mentioned receptors.

Materials and Methods

Animals and housing

In this experimental study, 64 mature female Wistar rats (180±20 g) were obtained from the animal house of Guilan University of Medical Sciences (GUMS). Animals were kept in clear plastic cages (15×26×42 cm) under 12:12h light: dark cycle and controlled temperature (22±2°C). They were fed freely except for during the tests. There were eight rats in each group. Animals received the hydro-alcoholic extract of *A. Graveolens* Seed (AGS) (0.1, 1, 10, 100, and 1000 mg/kg), saline (10 ml/kg, negative control group), diazepam (0.6 mg/kg, positive control group), and PTZ (20 mg/kg) + hydroalcoholic extract of AGS (10 mg/kg). After determination of the estrous cycle in rats based on their sexual features, vaginal secretion and its cellular contents were tested by a light microscope [14]. All rats were injected intraperitoneally in a volume of 10 ml/kg and tested after 30 min.

Chemicals and preparation of AGS hydro-alcoholic extract

Pentylentetrazol and diazepam were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Caspian Company (Rasht, Iran), respectively. Both chemicals were dissolved in saline. The seed of *A. graveolens* was purchased from a local medicinal plants market. A voucher specimen was deposited in the herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran (no. MPH-1966). After cleaning and drying the plant seeds at room temperature, the seed powder (100 g) was macerated in ethanol 80% for 24 h at dark. Subsequently, the mixture was filtered and concentrated under reduced pressure at 40°C by a rotary evaporator. The yield of hydro-alcoholic extract was 6.4% (g/g). According to the GC/MS analysis of AGS, D-carvone, D-limonene and apiol were about 88% of AGS constituents [10].

Behavioral assay (elevated plus maze)

The Elevated Plus Maze (EPM) apparatus was purchased from Borj Sanat Company, Tehran, Iran. The apparatus was a cross symbol and comprised two open arms (5 x 10 cm) across from each other and perpendicular to two closed arms (39.5x10 x 49.5cm) with a center platform (10 x 10cm) elevated to a height of 60 cm above the floor. Each rat received one trial in our test. Rats were given access to all arms and allowed to move freely between them for 5 min. Animals were placed in the apparatus so that the head turned to an open arm. The number of entries into the Open Arms (OAE) and the time spent in them (OAT) were used as indices of open space-induced anxiety in rats. Locomotor activity was calculated by the addition of the total number of entries into the open arms (OAE) and the total number of entries into the close arms (CAE) based on the below formula:

$$\text{Locomotor activity} = \text{OAE} + \text{CAE}$$

After each trial, all arms and the center area were cleaned with super-hypochlorous water [15].

Statistical analysis

All data are expressed as Mean±SEM and were analyzed using software SPSS v. 16 (SPSS, Chicago, IL). One-way ANOVA followed by Tukey's test for multiple comparisons were used for statistical evaluation. A P-value less than 0.05 was considered significant.

Results

Effect of AGS extract on %OAT

Different doses of AGS extract (0.1, 1, 10, 100, and 1000 mg/kg) were injected into the female Wistar rats. Behavioral activities of the rats after facing environmental stress were measured by EPM test 30 min after the injections. Animals that received 10 mg/kg of the extract showed a significant increase in the percentage of OAT ($P < 0.01$). Other doses of AGS extract had also a similar effect compared to the control group (saline-injected animals), but these differences were not significant (Table 1).

Effect of AGS extract on %OAE

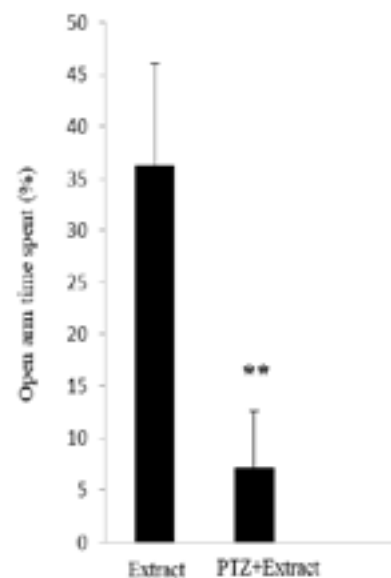
Analysis of OAE values showed that the injection of 10 mg/kg of the extract resulted in a significant increase in the number of entrances to the open arms compared to the control group ($P < 0.05$) (Table 1). These data confirmed the results obtained from the OAT assay.

Effect of AGS extract on locomotor activity

In order to analyze locomotor activity, we calculated CAE+OAE results. Our data showed that the AGS extract at 0.1, 1, and 10 mg/kg could significantly increase locomotor activity compared to the control group ($P < 0.001$) (Table 1). Because the extract at 10 mg/kg had a significant effect on %OAT and %OAE and locomotor activity; hence, we used it as an effective dose for further studies. Effect of AGS extract on %OAT, %OAE, and locomotor activity compared to the group receiving the antagonist plus extract %OAT, %OAE, and locomotor activity significantly decreased in the PTZ+extract group compared to the extract group ($P < 0.01$, $P < 0.001$, and $P < 0.01$, respectively) (Figure 1, 2 & 3).

Discussion

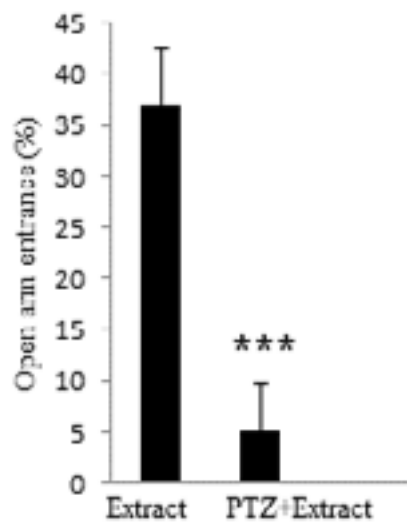
Due to technology improvements and lifestyle changes, anxiety has influenced individuals' characteristics, including decreased quality of life and an increase in the rate of neurodegenerative disorders [16]. Although efforts have been made to treat anxiety disorders using pharmaceutical treatments have shown hopeful results, they also have many side effects on patients. Recently, due to improving cellular and molecular techniques along with plant biology, scientists have used traditional plants for treating anxiety [17, 18].



CJNS

Figure 1. The effects of Anethum graveolens seed (AGS) extract and Pentylentetrazol (PTZ)+extract on the percentage of time spent in open arms (%OAT)

** $P < 0.01$ compared to the extract group (Mean±SEM, n=8).



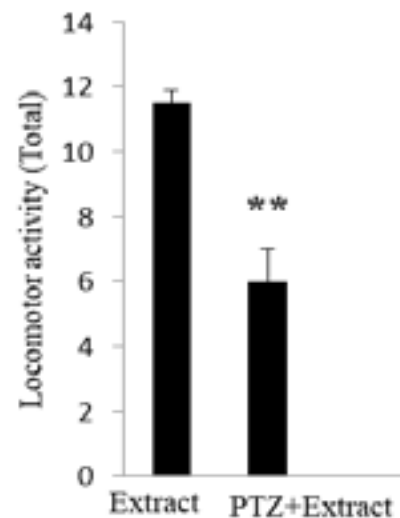
CJNS

Figure 2. The effects of Anethum Graveolens Seed (AGS) extract and Pentylene tetrazol (PTZ)+extract on the percentage of open arms entries (%OAE)

***P<0.001 compared to the extract group (Mean±SEM, n=8).

Dill has long been used to decrease different types of pain and constipation problems [19]. However, the effect of this plant on decreasing anxiety has not yet been understood. In this report, we studied the effect of different doses of dill seed extract on behavioral hallmarks of anxiety in female rats synchronized in the estrous cycle through examining various factors, including OAT, OAE, and locomotor activity in the EPM.

According to our findings, all doses of AGS from the minimum of 0.1 mg/kg to the maximum of 1 g/kg were tolerated. In some time-limited experimental models,



CJNS

Figure 3. The effects of Anethum Graveolens Seed (AGS) extract and Pentylene tetrazol (PTZ)+extract on locomotor activity (total)

**P<0.01 compared to the extract group (Mean±SEM, n=8).

doses of AGS up to 2 g/kg did not cause any death [20]. Our dose-response study showed that 10 mg/kg of the AGS extract is the most effective dose in reducing anxiety in rats. These findings are in accordance with those of El Mansouri et al. (2016) who indicated that the lower dose of AGS showed the best antidepressant and analgesic properties than its higher dose [21].

Here, the EPM test was used for measuring anxiety-like behavior. It has been reported that anxiolytic agents increase locomotor activity on the open arms [22]. In this

Table 1. The effects of different doses of the extract on %OAT, %OAE, and locomotor activity (Mean±SEM) (n=8)

Groups	Mean±SEM		
	%OAT	%OAE	Locomotor activity (total)
Saline	3.92±1.5	13.68±4.9	5±0.9
AGS (0.1 mg/Kg)	14.34±6.5	14.83±4.5	11.62±1.3***
AGS (1 mg/Kg)	23.2±3.1	23.65±1.3	11.25±1.2***
AGS (10 mg/Kg)	36.26±9.8**	36.99±5.6*	11.5±0.4***
AGS (100 mg/Kg)	11.19±3.4	17.75±4.8	6.62±0.9
AGS (1000 mg/Kg)	14.95±4.7	21.35±5.4	5.37±0.6
Diazepam (0.6 mg/Kg)	63.21±5.8***	54.9±3.2***	8.88±1.02
AGS Extract (10 mg/Kg)+ PTZ (20 mg/Kg)	7.12±5.5	5.12±4.6	6±1.02

CJNS

*P<0.05; **P<0.0; ***P<0.001 compared to the saline control group; AGS: Anethum Graveolens Seed; OAT: Time Spent in Open Arms; OAE: Open Arms Entries; PTZ: Pentylene tetrazol.

experiment, the incremental effects of 0.1, 1, and 10 mg/kg of AGS extract on locomotor activity were more than diazepam as a standard drug. AGS had an anxiolytic effect and significantly increased locomotor activity compared to the saline control group; nevertheless, the incremental effect of diazepam on locomotor activity was not significant. This effect of AGS significantly was reduced by AGS+PTZ. Because PTZ is a noncompetitive antagonism of the GABAA receptor complex, its ability to significantly inhibit the anxiolytic effect of AGS extract might be postulated via the GABAA receptor [23]. Another possibility is related to the benzodiazepine-like effect of flavonoids on GABAA receptors, which might have anxiolytic consequences [24]. Moreover, monoterpenes isolated from *A. graveolens* have an anxiolytic-like effect [22].

In this study, we showed that blockade of the GABAA receptor by PTZ significantly decreased the anxiolytic effect of AGS extract. It could be assumed that the extract effect may be mediated via the GABAA receptors. In agreement with our study, Pourabbas, et al. showed that co-administration of PTZ with fennel extract decreased its anxiolytic effect [21]. Besides, the reducing effect of *Hypericum perforatum* extract containing flavonoid on fear behavior has been suppressed by co-administration with PTZ [25]. Recently, Hekmatzadeh et al. showed the anxiolytic effect of the dill seed on the labor women that was in line with our study [26]. It seems that PTZ with binding to the benzodiazepine site of the GABAA receptors prevents the anxiolytic effect of extract containing flavonoids.

Conclusion

We found that the mixture we extracted from dill seed could decrease anxiety symptoms in female rats perhaps through acting on the GABAA receptors. However, more cellular and molecular tests should be done to unravel the exact mechanism of the anxiolytic effect of dill seed extract and the possible involvement of GABA receptors. Further experimental studies are required for the establishment of the anti-anxiety impact of AGS.

Ethical Considerations

Compliance with ethical guidelines

All study procedures complied with the ethical guidelines of the Declaration of Helsinki 2013. All animal experiments were carried out following the national institute of health guide for the care and use of laboratory animals' publication no.8023 revised 1978. The approval date of the research proposal was July 18, 2020. The Guilan Uni-

versity also approved all Medical Sciences Ethical Committee (No.: P.3.132.1104).

Funding

This project was approved as a master's degree thesis of Rana Shahabi in physiology (No.:112).

Authors contributions

Conceptualization: Mohammad Roostampour; Methodology: Rana Shahabi, Mohammad Roostampour; Investigation, writing the original draft: All authors. Resources: Behrooz Khakpour, Bahram Soltani; Review and editing: Mohammad Roostampour, Farshid Saadat, Supervision: Mohammad Roostampour.

Conflict of interest

The authors declared no conflicts of interests.

Acknowledgements

The authors greatly appreciate the Guilan University of Medical Sciences for support of this study.

References

- [1] Clement Y, Chapouthier G. Biological basis of anxiety. *Neurosci Biobehav Rev.* 1998; 22(5):623-33. [DOI:10.1016/S0149-7634(97)00058-4]
- [2] Duval ER, Javanbakht A, Liberzon I. Neural circuit in anxiety and stress disorders: A focused review. *Ther Clin Risk Manag.* 2015; 11:115-26. [DOI:10.2147/TCRM.S48528] [PMID] [PMCID]
- [3] Hyman SE, Cohen JE. Disorders of mood and anxiety. In: Hudspeth AJ, Kandel ER, Schwartz JH, Mack S, Siegelbaum SA, Jessell TM, editors. *Principles of Neural Science.* New York: McGraw-Hill Education; 2013. p. 1403-24. https://www.google.com/books/edition/Principles_of_Neural_Science_Fifth_Editi/s64z-LdAIsEC?hl=en
- [4] Roostampour M, Hadipour E, Oryan Sh, Soltani B, Saadat F. Anxiolytic-like effect of hydro-alcoholic extract of ripe pistachio hulls in adult female Wistar rats and its possible mechanisms. *Res Pharm Sci.* 2016; 11(6):454-60. [DOI:10.4103/1735-5362.194870] [PMID] [PMCID]
- [5] Jembrek MJ, Vlacin J. GABA receptors: Pharmacological potential and pitfalls. *Curr Pharm Des.* 2015; 21(34):4943-59. [DOI:10.2174/1381612821666150914121624] [PMID]
- [6] Soares DCS, Portela JLR, Roos DH, Rodrigues NR, Gomes KK, Macedo GE, et al. Treatment with pentylenetetrazole (PTZ) and 4-aminopyridine (4-AP) differently affects survival, locomotor activity, and biochemical markers in *Dros-*

- ophila melanogaster. *Mol Cell Biochem.* 2018; 442(1-2):129-42. [DOI:10.1007/s11010-017-3198-3] [PMID]
- [7] Buragohain J. Folk medicinal plants used in gynecological disorders in Tinsukia District, Assam, India. *Fitoterapia.* 2008; 79(5):388-92. [DOI:10.1016/j.fitote.2008.03.004] [PMID]
- [8] Jana S, Shekhawat GS. Anethum graveolens: An Indian traditional medicine herbal and spice. *Pharmacogn Rev.* 2010; 4(8):179-84. [DOI:10.4103/0973-7847.70915] [PMID] [PMCID]
- [9] Roohbakhsh A, Esmaeili H, Asami Z, Arab Baniasad F, Shamsizadeh A, Rezvani ME. [Effect of Anethum graveolens hydro-alcoholic extract on pentylenetetrazole-induced paroxysm (Persian)]. *Horizon of Med Sci.* 2013; 19(1):41-5. <http://hms.gmu.ac.ir/article-1-1466-en.html>.
- [10] Rostampour M, Ghaffari A, Salehi P, Saadat F. Effects of hydro-alcoholic extract of Anethum Graveolens seed on pentylenetetrazol-induced seizure in adult male mice. *Basic Clin Neurosci.* 2014; 5(3):199-204. [PMID] [PMCID]
- [11] Ahmadi Mahmoodabadi N. [The effects of hydroalcoholic extracts of dill (Anethum graveolens L.) and artichoke (Cynara scolymus L.) against type 1 diabetes mellitus (Persian)]. *Iran J Med Aromat Plants.* 2008; 24(3):333-41. https://ijmapr.areeo.ac.ir/article_117021.html?lang=en
- [12] Pourabbas S, Kesmati M, Rasekh A. [Study of the anxiolytic effects of fennel and possible roles of both GABAergic system and estrogen receptors in these effects in adult female rat (Persian)]. *Physiol Pharmacol.* 2010; 15(1):134-43. <http://ppj.phypha.ir/article-1-664-en.html>.
- [13] Taher M, Ghannadi A, Karimiyan R. [Effects of volatile oil extracts of Anethum graveolens L. and Apium graveolens L. seeds on activity of liver enzymes in rat (Persian)]. *J of Qazvin Univ of Med Sci.* 2007; 11(2):8-12. <http://journal.qums.ac.ir/article-1-69-en.html>
- [14] Jelodar GH, Askari K. Effect of Vitexagnus-castus fruits hydro-alcoholic extract on sex hormones in rat with induced Polycystic Ovary Syndrome (PCOS). *Physiol Pharmacol.* 2012; 16(1):62-9. <http://ppj.phypha.ir/article-1-816-en.html>
- [15] Gholamzadeh S, Zare S, Ilkhanipoor M. Evaluation of the anxiolytic effect of Echium Amoenum petals extract, during chronic treatment in rat. *Res Pharm Sci.* 2007; 2(2):91-5. <http://rps.mui.ac.ir/index.php/jrps/article/view/32>
- [16] Manafirad A, Farzadfar F, Habibi L, Azhdarzadeh M, Aghaverdi H, Tehrani KH, et al. Is amyloid- β an innocent bystander and marker in Alzheimer's disease? Is the liability of multivalent cation homeostasis and its influence on amyloid- β function the real mechanism? *J Alzheimers Dis.* 2014; 42(1):69-85. [DOI:10.3233/JAD-140321] [PMID]
- [17] Hanrahan JR, Chebib M, Johnston GA. Flavonoid modulation of GABAA receptors. *Br J Pharmacol.* 2011; 163(2):234-45. [DOI:10.1111/j.1476-5381.2011.01228.x] [PMID] [PMCID]
- [18] Jamil F, Behnam Rassouli M, Mahdavi Shahri N, Dehghani H. [Induction of differentiation and cell death of the derivative of 4-aryl-4H-Chromenes Family on NB4 human promyelocytic Leukemia cell line (Persian)]. *rat. J Cell & Tissue.* 2013; 4(2):149-57. http://jct.araku.ac.ir/article_3312.html?lang=en
- [19] Morrise RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist AP5. *Nature.* 1986; 319(6056):774-6. [DOI:10.1038/319774a0] [PMID]
- [20] Goodarzi MT, Khodadadi I, Tavilani H, Abbasi Oshaghi E. The role of anethum graveolens L. (Dill) in the management of diabetes. *J Trop Med.* 2016; 2016:1098916. [DOI:10.1155/2016/1098916] [PMID] [PMCID]
- [21] El Mansouri L, Bousta D, El Youbi-El Hamsas A, Boukhira S, Akdime H. Phytochemical screening, antidepressant and analgesic effects of aqueous extract of anethum graveolens L. From Southeast of Morocco. *Am J Ther.* 2016; 23(6):e1695-9. [DOI:10.1097/MJT.000000000000090] [PMID]
- [22] Yasumura M, Yoshida T, Yamazaki M, Abe M, Natsume R, Kanno K, et al. IL1RAPL1 knockout mice show spine density decrease, learning deficiency, hyperactivity and reduced anxiety-like behaviors. *Sci Rep.* 2014; 4:6613. [DOI:10.1038/srep06613] [PMID] [PMCID]
- [23] Rajabzadeh A, Ebrahimzadeh Bideskan AI, Fazel AI, Sankian M, Rafatpanah H, Haghiri H. The effect of PTZ-induced epileptic seizures on hippocampal expression of PSA-NCAM in offspring born to kindled rats. *J Biomed Sci.* 2012; 19(1):56. [DOI:10.1186/1423-0127-19-56] [PMID] [PMCID]
- [24] Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl).* 1987; 92(2):180-5. [DOI:10.1007/BF00177912] [PMID]
- [25] Gheitasi S, Vaezi G H, Keramati K. [Effects of intracerebroventricular injection of alcoholic extract of Hypericum perforatum on fear behavior in presence pentylenetetrazol (PTZ) in adult male rat (Persian)]. *J Ilam Univ Med Sci.* 2010; 17(4):36-44. <https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=168479>
- [26] Hekmatzadeh SF, Bazarganipour F, Allan H, Aramesh S, Mohammadi J. Affiliations effects of boiled dill seed on anxiety during labor: A randomized clinical Trial. *Chin J Integr Med.* 2020; 26(2):100-5. [DOI:10.1007/s11655-019-3222-4] [PMID]