Comparison of Anxiolytic Effect of Aqueous Extract of Root of *Valeriana officinalis* with Buspirone in Mature Male Mice

Abstract

Background: Anxiety is the most common psychiatric disorder which can be cured by herbal and chemical medicine. Herbal medicine and complementary medicine are widely used among people suffering from anxiety disorder. *Valeriana officinalis* known to cause anxiolytic and sedative effects to have a special place in traditional Chinese, Indian and Iranian medicine.

Aims: This study aims to compare the anxiolytic effect of aqueous extract of root of *V. officinalis* root with Buspirone in mature male mice.

Study design: Study was conducted in the Islamic Azad University of Mashhad to an animal room of the Faculty of Basic Sciences, Azad University of Nishabur, under standard conditions. This experimental study has been demonstrated by control and experimental groups. The sample consisted of 30 mature male mice assigned into five groups of six: group A receiving distilled water (control group), group B, group C and group D receiving 100 mg/kg, 200 mg/kg and 300 mg/kg aqueous extract of *V. officinalis*, respectively, and group E receiving 30 mg/kg buspirone orally in drinking water. Anxiolytic effects were evaluated 10 days after receiving by using maze. One-way ANOVA and T-test were analyzed by using SPSS 19 software package.

Results and discussion: The results show that the aqueous extract of *V. officinalis* root causes a significant increase in the number of arrivals and elapsed time in open arms compared to Buspirone. Medicinal plants currently form an important part of traditional medicine in many countries and they have a special place in new therapeutic approaches. ANOVA and T-test results showed a significant ($P<0.05$) relationship between the anxiolytic effect of aqueous extract of *V. officinalis* root and Buspirone in mice.

Conclusion: It can be concluded that *V. officinalis* are more effective in reducing anxiety compared to buspirone.

Keywords: Anxiolytic Effect, aqueous root extract, psychiatric disorder, *Valeriana officinalis*, buspirone, mice

Introduction

With the industrialization of countries, anxiety is currently rising in people. Anxiety disorders affect 40 million people in the United States (U.S.). It is the most common group of mental illnesses in the country. However, only 36.9 percent of people with the condition receive treatment. These changes include diffuse, unpleasant and vague feelings of panic, along with

autonomic symptoms such as confusion, sweating, diarrhoea, increased blood pressure, palpitations, mydriasis, restlessness, tremor, increased heart rate, frequent urination, numbness and syncope. Anxiety is considered a disorder when it occurs in situations where there is no real risk or lasts more than usual after overcoming the risky situation [1].

The American Psychological Association (APA) defines anxiety as "an emotion characterised by feelings of tension, worried thoughts and physical changes like increased blood pressure." Anxiety disorder is a mental state or severe arousal, the main characteristic of which is fear, doubt and excessive worry. The characteristics of anxiety disorders are: the amount of fear more than the risk, the person is constantly in a state of fear and concern without any specific cause, there are chronic fear and concern and constantly afflicts the person to the extent that he cannot live his everyday life [2].

In other words, anxiety refers to a state of distress and unpleasant inner feeling, which appears on physiological, psychological and cognitive levels and disrupts normal activities [3]. Biological assumptions usually rely on objective criteria which compare brain function in patients with anxiety disorder with normal people. It is believed that the state of anxiety increases the activity of gamma-Aminobutyric acid (GABA), the main inhibitor of the brain. In emotional and anxiety conditions, changes in limbic system function and activation of hypothalamic-pituitary axis is followed by a change in the adrenal cortex of plasma level of glucocorticoid hormone. These hormones can intervene in brain functions and produce the necessary responses to anxiety. On the other hand, there are many pieces of evidence which suggest that different neurotransmitter systems and neuronal circuits in brain regions, including the components of limbic system, play an important role in the mediation of anxiety behaviours [4]. There is a significant relationship between the levels of GABA in the brain and anxiety, as benzodiazepine used as a sedative drug for the past decades, emulates GABA [5]. Buspirone is not chemically and pharmaceutically similar to benzodiazepines, barbiturates and other sedative agents. Unlike benzodiazepine sedatives, Buspirone has no effect on muscle relaxation and drowsiness. Buspirone is thought to have a strong tendency for serotonin receptors (5-HT1A), but it does not have a significant tendency for benzodiazepine receptors. Buspirone also has a moderate tendency for brain dopamine receptors (D2).

Medicinal herbs have been used for the treatment of diseases in humans. It is estimated that more than 10% of thousands of well-known plant species have drug use. The World Health

Organization (WHO) estimates that about 80% of the world's population uses medicinal herbs for aspects of their health care [6, 7]. *Valeriana officinalis*, scientifically called *Nardostachy jatamansi*, belonging to the Valerianceae family, grows in temperate regions of the Northern Hemisphere, including Iran [8].

The roots and rhizomes of this plant are used in traditional medicine to treat various discomforts, including neurological disorders such as epilepsy, insomnia (Valerian is most commonly used for sleep disorders, especially the inability to sleep (insomnia), dizziness, palpitations, and sedation [9]. Many compounds have been identified in the extract of this plant. The most important of these are valproates, isovalproates and divalproates. Other components make up only 0.4% of the extract. Recently, sedative effects of *V. officinalis* are attributed to its volatile oils, including valerenal and valerenic acid. Sedative effects of *V. officinalis* have been noted in the ancient Greek books, including Hippocrates and trials conducted confirm these effects; in traditional medicine, the use of this plant dates back to thousands of years [8]. *V. officinalis* has been known for its anticonvulsant, sedative, anti-hysteria, and palpitation reducing effects. In 1981, Dell Logia stated that root and rhizome extract of *N. jatamansi* had a weakening effect on the mouse brain. Biochemical studies show that valerenic acid inhibits the enzyme responsible for GABA catabolism and increases GABA concentration in the brain tissue. Increasing the GABA concentration in the brain reduces the activity of various brain nuclei and causes sedative effects [10]. In this regard, studies on experimental animals have shown that sedative and anxiolytic effects of *V. officinalis* extract are due to compounds such as valerenic acid and valepotriate; moreover, valerian extracts increase by GABA neurotransmitters in the brain. GABA reduces the activity of the nervous system with its inhibitory effect, resulting in sedation, anticonvulsant and anti-anxiety effects [11]. Buspirone is an anti-anxiety drug which has a high tendency for serotonin receptor type (1 A) and a moderate effect on dopaminergic system and a relative agonist effect on alpha-adrenergic receptors.

According to the studies on sedative effects of *V. officinalis* and taking steps to develop utilization of medicinal plants with fewer complications for patients, and moreover, given the need to introduce an agent which can be effectively used instead of or in combination with other sedative drugs. The present study tends to test the relationship between anxiolytic effects of the root of *V. officinalis* and buspirone administered in the treatment of anxiety. Studying the effect of hydroalcoholic extract of *V. officinalis* on astrocyteos of hippocampus formations of Rats,

Roozbehi et al. [12] concluded that this extracted compounds such as phenolic acid, esters, flavonoids, monoterpenes and sesquiterpenes, and antioxidant properties can affect the extracellular neuronal environment and proliferate astrocyte cells.

Khajehpour et al. [13] studied adrenergic interference in anxiolytic effect of hydroalcoholic extract of *V. officinalis* root and showed that injection of this extract reduces anxiety behaviour by increasing the elapsed time percentage in the open arm and percentage of arrival into this arm. Moreover, injection of epinephrine prior to *V. officinalis* extract reduced its anxiolytic effect, while the same dose of epinephrine alone did not affect anxiety. As a result, it can be argued that central adrenergic mechanisms may be involved in the reduction of anxiety behaviours due to hydroalcoholic extract of this plant.

Ekbatani et al. [14] evaluated the effect of *V. officinalis* on sleep disorders in postmenopausal women and concluded that this herb could improve various areas of sleep disorders. Half of the postmenopausal women have sleeping problems which can lead to a decline in their quality of life. Meanwhile, herbal medicines positively affect six out of the seven areas of sleep disorder. For this reason, healthcare providers need to know this medicinal herb.

Kafash Elahi et al. [15] compared the weakening effects of the extract of *V. officinalis* root, diazepam and ketamine on central nervous system (CNS) in cat and reported that clinical signs of this extract depend on effects of substances on the activity of gamma aminobutyric acid or GABA and its receptors. This confirms the weakening effect of this plant on central nervous system.

Gromball et al. [16] studied improvement in hyperactivity, concentration difficulties and impulsiveness during a seven-week treatment with valerian root and lemon balm extracts in primary school children. They concluded that this extract hopefully can lead to durable treatment in children. In addition, this method is very effective in improving the educational subject.

MinNam et al. [17] studied *V. officinalis* extract and its main component, valerenic acid, ameliorate D-galactose-induced reductions in memory, cell proliferation and neuroblast differentiation by reducing corticosterone levels and lipid peroxidation. *V. officinalis* is commonly used in traditional and herbal medicine in many cultures. It seems that *V. officinalis* root extract and valerenic acid enhance cognitive function, promote cell proliferation and neuroblast differentiation, and also decrease serum corticosterone and lipid peroxidation in elderly mice.

Evaluating the effectiveness of high valerenic acid and low acetoxy valerenic acid contents in reducing anxiolytic activity, Felgentreff et al. [18] concluded that this plant is widely used in the treatment of insomnia and anxiety and it is significantly effective in decreasing anxiolytic activities, and this effect is also more pronounced than valerenic acid.

Surajit et al. [19] evaluated effectiveness of *Valeriana wallichii* root extract in improving sleep quality and modulating brain monoamine levels in rats and showed that this extract can positively improve sleep quality on two levels of the brain, cortex and stem. This present study aims to compare the anxiolytic effect of aqueous extract of *Valeriana officinalis* root with Buspirone in mature male mice.

Materials and Methods

In this study, 30 mature male mice were randomly divided into 5 groups of 6. A total of 30 mature male mice with a weight range of 25-35 g were moved from Islamic Azad University of Mashhad to an animal room of the Faculty of Basic Sciences, Azad University of Nishabur, Northeast Iran, under standard conditions (Figure 1). The mice were kept under controlled light conditions (12 hours of light and 12 hours of darkness), 70% relative humidity and 22 ± 3°C for 10 days. Standard plates were used to feed the mice by sufficient mouse feed (Javaneh Khorasan Company) and drinking water.

Figure 1: animal room of the Faculty of Basic Sciences, Azad University of Nishabur

To make extracts, *V. officinalis* root was purchased from Apothicaire; genus and species were determined by the Department of Botany of the Faculty of Basic Sciences, Islamic Azad University of Neishabour. The plant was then powdered by an electric mill; 500 mg dry powder was moved to a 5000 ml beaker and soaked in 1 lit distilled water. It was incubated at 35°C for 72 h and stirred every day. Then, it was filtered by using filter paper and vacuum pump and Büchner funnel. The filtered liquid was poured into a large tray and placed in an oven at 30-35°C for 24 h to remove the solvent to collect the condensed solution and the honey extract and keep it in the freezer.

The mice were randomly placed in cages in order; to study anxiolytic effects of aqueous extract of *V. officinalis* and compare with buspirone, the mice were randomly assigned to 5 groups of 6 after 10 days. List of the treatment group and its corresponding experimental design has been depicted in Table 1.

Table 1: List of the treatment group and its corresponding experimental design

Sl No.	Treatment group	Experimental design
1.	Healthy control group SH	1% distilled water was added to drinking water from day one to end of experiment
2.	Treatment group A	100 mg/kg aqueous extract of *Valeriana officinalis* was added to drinking water from day one to end of experiment
3.	Treatment group B	200 mg/kg aqueous extract of *Valeriana officinalis* was added to drinking water from day one to end of the experiment
4.	Treatment group C	300 mg/kg aqueous extract of *Valeriana officinalis* was added to drinking water from day one to end of experiment
5.	Treatment group BUS	30 mg/kg Buspirone was added to drinking water from day one to end of experiment

A 5 mg tablet of Buspirone was purchased from the pharmacy; given that the consumed dose is 30 mg/kg, the required dose for each mouse was first calculated according to its weight.

$$\frac{\text{30 mg dosage}}{\text{X dosage per mice}} \;\Big|\; \frac{\text{1000 g}}{\text{30 g weight of each mouse}} \qquad X=0.9$$

To make an aqueous extract of *V. officinalis* root (100 mg/kg), the dose per mice was calculated according to its weight. Then, the calculated value was accurately measured by a digital scale and solved in drinking water of the mice.

$$\frac{100\ \text{Mg}}{X} \Big| \frac{1000\ \text{g}}{30\ \text{g weight of each mouse}} \qquad X=3$$

To make an aqueous extract of *V. officinalis* root (200 mg/kg), the dose per mice was calculated according to its weight. Then, the calculated value was accurately measured by digital scale and solved in drinking water of the mice.

$$\frac{200\ \text{Mg}}{X} \Big| \frac{1000\ \text{g}}{30\ \text{g weight of each mouse}} \qquad X=6$$

To make an aqueous extract of *V. officinalis* root (300 mg/kg), the dose per mice was calculated according to its weight. Then, the calculated value was accurately measured by a digital scale and solved in drinking water of the mice.

$$\frac{300\ \text{Mg}}{X} \Big| \frac{1000\ \text{g}}{30\ \text{g weight of each mouse}} \qquad X=9$$

An elevated plus maze which is a standard model for investigating the anxiety behaviour in rodents was used to assess anxiety. This experimental model measures unconditional anxiety and does not need the animal to train and learn [20]. The metal maze consisted of two open arms (each 10×50 cm) and two closed arms (each 10×50 cm) and height of the closed arms was 10 cm. The junction of two arms was a square-shaped space (10×10 cm). The device was about 50 cm above the floor. The light was provided by a 100 watt lamp located at an altitude of 120 cm from the plus maze [21]. Duration of the presence of mice on open arms was a marker of non-anxiety and duration of the presence of mice (arrival to each arm was the time when the head and both hands reach the arm to the center of gravity of the body) on closed arms was an anxiety marker [22].

For 10 days, buspirone and aqueous extract of *V. officinalis* were prepared daily and added to drinking water and enough food was placed; moisture, temperature and light were controlled every day. Ten days after receiving the drug, the mice were moved from the animal room to the anxiety testing laboratory (Animal Laboratory of the Faculty of Basic Sciences, Islamic Azad University, Neishabour Branch) one hour before the test (in order to adapt to the environment); before and after the experiment, it was attempted to provide a low-stress environment.

Each animal was placed in the middle of the central chassis in front of an open arm; for 5 min, movements and behaviour of the animal were recorded by a camera mounted on top of the maze and the video was automatically stored on the computer system connected to the maze and the camera. Each animal was used only once in the experiment. After completing each test, all parts of the device which was in contact with the mouse were cleaned and dried with cotton and alcohol; then the next mouse was placed in the device. All experiments were carried out within a time interval of 8-14. In all experiments, the ethics guidelines recommended by the International Veterinary Academy of Pain Management were observed.

Results

In order to describe the data, descriptive statistical method (mean, median, mode and standard deviation) was performed by software which was installed on the computer system connected to the camera and the maze system. In order to test the hypotheses, inferential statistics of one way ANOVA and T-Test were used; for analyzing the data, the SPSS 19 statistical program was used and charts were drawn by Excel software (2010).

Table 2 shows the mean and standard deviation of the anxiety scores in the groups SH, A, B, C and BUS.

Table 2: mean and standard deviation of anxiety scores in groups

Variable	Group	N	Mean	SD
Anxiety	A	6	87.39	8.52
	B	6	76.81	15.03
	SH	6	92.80	6.38
	BUS	6	74.96	9.55
	C	6	62.38	5.08
	Sum	30	83.76	11.69

As Table 2 shows, mean of the groups A, B, BUS, C and SH are 87.39, 76.81, 74.96, 62.38 and 92.80, respectively; moreover, standard deviation of the groups A, B, BUS, C and SH are 8.52, 15.03, 6.38, 9.55 and 11.96, respectively. Figure 2 shows column chart of anxiety score in the groups A, B, BUS, C and SH.

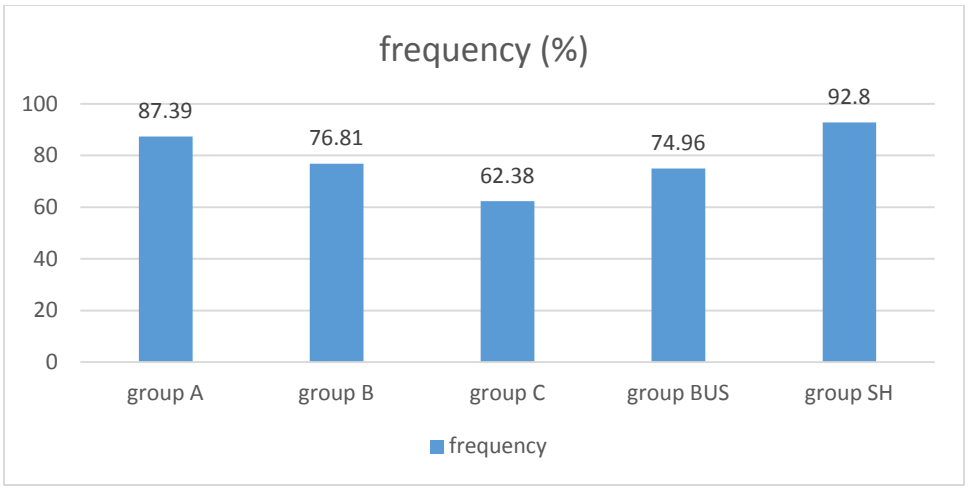

Figure 2: Graphical representation of anxiety score in the groups

Testing normality of variables and homogeneity of variances

Shapiro-wilk test was used to test the normality of data (Table 3) and Levene test was used to test the homogeneity of anxiety in the studied groups (Table 3).

Table 3: Shapiro-wilk test for testing normality

Variable	Group	N	W	p-value
Anxiety	A	6	0.90	0.42
	B	6	0.83	0.11
	BUS	6	0.87	0.25
	C	6	0.98	0.95
	SH	6	0.92	0.53

As Table 3 shows, data is normally distributed ($p<0.05$).

Table 4: homogeneity of variances (Levene)

Variable	Df 1	Df 2	Levene's W	p-value
Anxiety	4	25	1.66	0.18

As Table 4 shows, Levene's W=1.66; thus, the variances are homogeneous ($p>0.05$).

Hypothesis testing

There is a significant difference in anxiolytic effect of aqueous extract of *Valeriana officinalis* root and Buspirone in mice.

To test this hypothesis, ANOVA was used as shown in Table 5.

Table 5: one-way ANOVA

Variable	Source of variations	Sum of squares	Df	Mean of squares	F-value	p-value
Anxiety	Group	1262.19	4	315.54	2.91	0.04
	Error	2706.76	25	108.23		

Total	3967.96	29	

As shown in Table 5, F-value=2.91 and P-value=0.04 supports the hypothesis; it can be claimed that there is a significant difference in the anxiolytic effect of aqueous extract of *Valeriana officinalis* root and Buspirone in mice.

Sub-hypothesis 1

There is a significant difference in the anxiolytic effect of 100 mg/kg aqueous extract of *Valeriana officinalis* root and 30 mg/kg Buspirone in mice.

T-test was used to examine the first sub-hypothesis (Table 6).

Table 6: t-test results for sub-hypothesis 1

Variable	t-value	Df	p-value	Mean difference	Standard error
Anxiety	-1.39	10	0.35	-1.37	2.21

As the table above shows, the first sub-hypothesis is rejected (p-value=0.35).

Sub-hypothesis 2

There is a significant difference in anxiolytic effect of 200 mg/kg aqueous extract of *Valeriana officinalis* root and 30 mg/kg Buspirone in mice.

T-test was used to examine the second sub-hypothesis (Table 7).

Table 7: t-test results for sub-hypothesis 2

Variable	t-value	Df	p-value	Mean difference	Standard error
Anxiety	-1.85	10	0.89	-1.93	3.56

As the table above shows, the second sub-hypothesis is rejected (p-value=0.89).

Sub-hypothesis 3

There is a significant difference in the anxiolytic effect of 300 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg Buspirone in mice.

T-test was used to examine the third sub-hypothesis (Table 8).

Table 8: t-test results for sub-hypothesis 3

Variable	t-value	Df	p-value	Mean difference	Standard error
Anxiety	-2.93	10	0.001	-6.84	4.69

As the table above shows, the third sub-hypothesis is accepted (t-value=-2.93; p-value=0.001). Thus, there is a significant difference in anxiolytic effect of 300 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg Buspirone in mice ($p<0.05$).

Discussion

Medicinal plants currently form an important part of traditional medicine in many countries and they have a special place in new therapeutic approaches. In order to explain the main hypothesis based on scientific documents after intervention on all experimental and control groups according to Table 5, ANOVA and T-test results showed a significant relationship between anxiolytic effect of aqueous extract of *V. officinalis* root and Buspirone in mice. The results show that aqueous extract of *V. officinalis* root causes a significant increase in the number of arrivals and elapsed time in open arms compared to Buspirone. Therefore, it can be concluded that *V. officinalis* is more effective than Buspirone in reducing anxiety.

The first sub-hypothesis indicated a significant difference in anxiolytic effect of 100 mg/kg aqueous extract of *V. officinalis* root and 10 mg/kg buspirone in mice. According to the results listed in Table 6, p-value=0.25 ($p<0.05$) supports the assumption of equal means between groups A and BUS. The results show that 100 mg/kg aqueous extract of *V. officinalis* root causes a significant difference in the number of arrivals and elapsed time of animals in open arms, compared to 10 mg/kg buspirone; therefore, there is a significant difference in anxiolytic effect of 100 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg buspirone in mice. In other words, 100 mg/kg aqueous extract of *V. officinalis* root was weaker in reducing the anxiety compared to 30 mg/kg Buspirone.

The second sub-hypothesis indicated a significant difference in the anxiolytic effect of 200 mg/kg aqueous extract of *V. officinalis* root and 10 mg/kg buspirone in mice. According to the results listed in Table 7, p-value=0.89 ($p>0.05$) supports the assumption of equal means between groups B and BUS. The results show that 200 mg/kg aqueous extract of *V. officinalis* root causes no significant difference in the number of arrivals and elapsed time of animals in open arms, compared to 30 mg/kg buspirone; therefore, there is no significant difference in anxiolytic effect of 200 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg buspirone in mice. In other words, 200 mg/kg aqueous extract of *V. officinalis* and 30 mg/kg Buspirone were equally effective in reducing the anxiety.

The third sub-hypothesis indicated a significant difference in anxiolytic effect of 300 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg buspirone in mice. According to the results listed in Table 8, p-value=0.001 ($p<0.05$) does not support the assumption of equal means between groups C and BUS. The results show that 300 mg/kg aqueous extract of *V. officinalis* root caused a significant increase in the number of arrivals and elapsed time of animals in open arms, compared to 30 mg/kg buspirone; therefore, there is a significant difference in anxiolytic effect of 300 mg/kg aqueous extract of *V. officinalis* root and 30 mg/kg buspirone in mice. In other words, 300 mg/kg aqueous extract of *V. officinalis* and 30 mg/kg Buspirone were not equally effective in reducing anxiety; instead, 300 mg/kg extract further reduced anxiety and had a higher effect.

Conclusion

This study tended to compare the anxiolytic effect of aqueous extract of *Valencia officinalis* root with Buspirone in mice. Medicinal plants currently form an important part of traditional medicine in many countries and they have a special place in new therapeutic approaches. The results showed that aqueous extract of *V. officinalis* root causes a significant increase in the number of arrivals and elapsed time in open arms compared to Buspirone. ANOVA and T-test results showed a significant relationship between the anxiolytic effect of aqueous extract of *V. officinalis* root and Buspirone in mice. Therefore, it can be concluded that *V. officinalis* is more effective than Buspirone in reducing anxiety.

It can be concluded that *V. officinalis* is more effective in reducing anxiety compared to buspirone.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent:

NA

References

1. Tiwari N, Baldwin DS. Yogic Breathing Techniques in the Management of Anxiety and Depression: Systematic Review of Evidence of Efficacy and Presumed Mechanism of Action. Anxiety. 2012;320: 228.

2. Ganji, M., 2014. Complete Guide to DSM5 Changes and Essential Tips. Tehran: Savalan.

3. Kaplan, HI; Sadock, Bj. (1998). Synapsis of psichiatery. Newyork: Williams & wilkins.

4. Vafai, A., Taherian, A. & Sobhaninejad, M. S., 2009. Evaluation of interactions of corticosterone and verapamil on anxiety reactions in mice. Pharmaceutical Sciences, 11(3), pp. 219-226.

5. Mohler, H.)2012.(The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology; 62(1): 42-53.

6. Moerman DE. (1996). An analysis of the food plants and drug plants of native North. American Journal of Ethnopharmacology, 52: 1-2.

7. Cowan, MM. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4): 564 -582.

8. Zargari, A., 2004. medicinal plants. Tehran: Tehran university.

9. Haji Akhundi, A. & Baligh, N., 2004. Practical Guide to Medicinal Plants. Tehran: scientific publication center.

10. Amann, R; Peskar, B .(2002). Molecular pharmacology. Saunders, Philadelphia: 4 -121.

11. Solati, J. & Sanagouye Motlagh, H., 2008. Anti-anxiety effects of Valepotriates derived from *Valeriana officinalis* in rats. journal of medical sciences and healthcare services university, 12(48), pp. 64-67.

12. Roozbehi, A., 2015. effect of hydroalcoholic extract of *Valeriana officinalis* on astrocyteos of hippocampus formations of Rats. armaghan e danesh, 20(99), pp. 298-307.

13. Khajehpour, L., 2014. adrenergic interference in anxiolytic effect of hydroalcoholic extract of *Valeriana officinalis* root. feyz, 18(4), pp. 362-368.

14. Ekbatani, N. N., Taavoni, S. & Haghani, H., 2011. the effect of *Valeriana officinalis* on sleep disorders in postmenopausal women. 15(4), pp. 272-277.

15. Kafash Elahi, R., 2011. comparative study of the weakening effects of the extract of *Valeriana officinalis* root, diazepam and ketamine on central nervous system in cat. Veterinary Journal of islamic azad university of tabriz, 5(20), pp. 1411-1417.

16. Gromballa, J; Beschornerb, F; Wantzenc, C ; Paulsend, U, & Burkart,M .(2014). Hyperactivity, concentration difficulties and impulsiveness improveduring seven weeks' treatment with valerian root and lemon balmextracts in primary school children. Phytomedicine. 21: 1098-1103.

17. Min Nam, S ; Choi, J.H ;Yoo, D.Y; Kim, W; Jung, H.Y; Kim, J.W & et al. (2013). *Valeriana officinalis* extract and its main component, valerenic acid, ameliorate D-galactose-induced

reductions in memory, cell proliferation, and neuroblast differentiation by reducing corticosterone levels and lipid peroxidation. Experimental Gerontology. 48: 1369–1377.

18. Felgentreff, F; Becker, A ; Meier, B. & Brattstrom, A. (2012). Valerian extract characterized by high valerenic acid and low acetoxy valerenic acid contents demonstrates anxiolytic activity. Phytomedicine. 19: 1216-1222.

19. Surajit, S; Koushik, R ;Yogendra, K;Shilpa, G ;Hina, K ;Sanjeev, K; Kshipra, M, & Panjwani, U. (2012). *Valeriana wallichii* root extract improves sleep quality and modulates brain monoamine level in rats. Phytomedicine. 19: 924– 929.

20. Tavakoli, M., 2012. Effect of Vitex agnus-castus Extract on Anxiety-like Behaviors in Orectomized Rats. journal of babol university of medical sciences, 14(5).

21. Solati, J., Oryan, S., Zarindast, M. & Parivar, K., 2009. Effects of amygdala colonergic system on controlling the anxiety of rats in elevated plus Maze test. jrounal of kordestan university of medical sciences, Volume 4, pp. 65-74.

22. Hashemi Firoozi, N., Mahmoudi, M. & Shahidi, S., 2012. The Effect of Serotonin and Cyclic guanosine monophosphate androgens on Anxiety Level of Mice. journal of tabriz university of medical sciences, 34(5), pp. 41-45.