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

Abstract: *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 have had a special place in traditional Chinese, Indian and Iranian medicine. The present study is an experimental study with control and experimental groups. This study tended to compare anxiolytic effect of aqueous extract of Valeriana officinalis root with Buspirone in mice. The studied population included all mature male mice. 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 Valeriana 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 used to analyze the data. The results showed a significant difference in anxiolytic effect of 300 mg/kg aqueous extract of valeriana officinalis root with 30 mg/kg buspirone in mice (P<0.05). Therefore, it can be concluded that valeriana officinalis is more effective in reducing anxiety compared to buspirone.*

Keywords: anxiety, valeriana officinalis, buspirone

Introduction

With industrialization of countries, anxiety is currently rising in people. Anxiety is a natural and unavoidable element of everyday life and includes physical, psychological and behavioral changes which occur automatically when encountering threats and danger. These changes include a diffuse, unpleasant and vague feelings of panic, along with autonomic symptoms such as confusion, sweating, diarrhea, increased blood pressure, palpitations, mydriasis, restlessness, tremor, increased heart rate, frequent urination, numbness and syncope. Anxiety is considered as a disorder when it occurs in situations where there is no real risk or lasts more than usual after overcoming the risky situation (Tiwari et al., 2012).

According to the American Psychological Association (2013), anxiety is a completely natural behavior which helps us act correctly in a difficult situation, but sometimes anxiety becomes so severe that it becomes problematic; this is where anxiety disorders are likely to develop. 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: 1) the amount of fear more than the risk, 2) the person is constantly in a state of fear and concern without any specific cause, 3) there is chronic fear and concern and constantly afflicts the person to the extent that he cannot live his everyday life (Ganji, 2014).

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 (Kaplan & Sadock, 1988).

Basically, biological process is associated with undeniable anxiety. Biological assumptions usually rely on objective criteria which compare brain function in patients with anxiety disorder with normal people. The question that arises is whether anxiety pathology is secondary process on primary psychological mechanism, or various experiences of anxiety represent biological function and brain function. It is believed that the state of anxiety increases the activity of gamma-Aminobutyric acid (GABA), the main inhibitor of the brain.

In other words, anxiety behaviors are the result of a complex reaction which begins with changes in brain function and neuroendocrine processes. In emotional and anxiety conditions, changes in limbic system function and activation of hypothalamic-pituitary axis is followed by change in 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 evidences which suggest that different neurotransmitter systems and neuronal circuits in brain regions, including the components of limbic system, play an important role in mediation of anxiety behaviors (Vafai, et al., 2009).

One of these neurotransmitters is GABA (gamma-Aminobutyric acid), which has important inhibitory effects in the central nervous system and it is necessary to balance the states of stimulation and neural inhibition in normal brain function. 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 (Mohler, 2012). 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).

Various herbs and herbal compounds are traditionally used in conjunction with chemical drugs to control and treat anxiety in different parts of the world. Medicinal herbs have been used for 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 estimates that about 80% of the world's population uses medicinal herbs for aspects of their health care (Moerman, 1996; Cowan, 1999).

On this basis, medicinal plants currently form an important part of traditional medicine in many countries and they also have a special value in new therapeutic approaches. In the meantime, the Valeriana officinalis, scientifically called Nardostachy jatamansi, belonging to the Valerianceae family, grows in temperate regions of the Northern Hemisphere, including Iran (Zargari, 2004). The roots and rhizomes of this plant are used in traditional medicine to treat various discomforts, including neurological disorders such as epilepsy, insomnia, dizziness, palpitations, and sedation (Haji Akhundi & Baligh, 2004). 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 Valeriana officinalis are attributed to its volatile oils, including valerenal and valerenic acid. Sedative effects of Valeriana 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 (Zargari, 2004). Valeriana 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 mouse brain. In 1982, Hazel Huff showed that valerate and isovalerate present in N. jatamansi result in loosening of muscle cells. In 2001, Crystal proposed that long-term administration of N. jatamansi has a lower side effect than benzodiazepines. According to recent studies on cerebral ischemia, this plant is known as GABA (GABA) receptor agonists. 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 (Amann & Pasker, 2002).

In this regard, studies on experimental animals have shown that sedative and anxiolytic effects of Valeriana 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 nervous system with its inhibitory effect, resulting in sedation, anticonvulsant and anti-anxiety effects (Solati & Sanagouye Motlagh, 2008).

On the other hand, Buspirone is an anti-anxiety drug which has 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 Valeriana 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 and efficiently used instead of or in combination with other sedative drugs, the present study tends to test the following questions: Is there a relationship between anxiolytic effects of Valeriana officinalis and buspirone administered in treatment of anxiety?

Studying the effect of hydroalcoholic extract of Valeriana officinalis on astrocyteos of hippocampus formations of Rats, Roozbehi et al. (2015) concluded that this extract or effective compounds such as phenolic acid, esters, flavonoids, monoterpenes and sesquiterpenes, and antioxidant properties can affect extracellular neuronal environment and proliferate astrocyte cells.

Khajehpour et al. (2014) studied adrenergic interference in anxiolytic effect of hydroalcoholic extract of Valeriana officinalis root and showed that injection of this extract reduces anxiety behavior by increasing the elapsed time percentage in the open arm and percentage of arrival into this arm. Moreover, injection of epinephrine prior to Valeriana 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 reduction of anxiety behaviors due to hydroalcoholic extract of this plant.

Ekbatani et al. (2011) evaluated the effect of Valeriana 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 affects six out of seven areas of sleep disorder. For this reason, healthcare providers need to know this medicinal herb.

Kafash Elahi et al. (2011) compared the weakening effects of the extract of Valeriana officinalis root, diazepam and ketamine on central nervous system in cat and reported that clinical signs of

this extract depend on effects of substances on 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. (2014) 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 efficient in improving the educational subject. MinNam et al (2013) studied 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. Valeriana officinalis is commonly used in traditional and herbal medicine in many cultures. It seems that Valeriana 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 (2012) concluded that this plant is widely used in treatment of insomnia and anxiety and it is significantly effective in decreasing anxiolytic activities, and this effectiveness is also more pronounced than valerenic acid.

Surjit et al. (2012) 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.

Materials and Methods

- Mature male mice
- Mouse food (purchased from Javaneh Khorasan Company)
- Valeriana officinalis aqueous extract
- 5 mg Buspirone (Loghman Pharmacy Company)
- Alcohol (Kimia Alcohol Zanjan Company)
- Distilled water
- Cotton
- Plus maze
- Incubator

- Oven
- Erlenmeyer flask
- Animal cage
- Digital scale
- Refrigerator
- Computer
- Beaker
- Filter paper
- Electric mills
- Incubator
- Büchner funnel
- Vacuum pump

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 animal room of the Faculty of Basic Sciences, Azad University of Nishabur, 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, Valeriana 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 ordinary 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 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 Valeriana officinalis and compare with buspirone, the mice were randomly assigned to 5 groups of 6 after 10 days. The groups included:

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 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.

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

To make an aqueous extract of Valeriana officinalis root (100 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.

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

To make an aqueous extract of Valeriana 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.

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

To make an aqueous extract of Valeriana officinalis root (300 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.

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

An elevated plus maze which is a standard model for investigating the anxiety behavior in rodents was used to assess anxiety. This assessment was based on a model first proposed by Peloow et al; this experimental model measures unconditional anxiety and does not need the animal to train and learn (Tavakoli, 2012). 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 a 100 watt lamp located at an altitude of 120 cm from the plus maze (Solati, et al., 2009). 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 center of gravity of the body) on closed arms was an anxiety marker (Hashemi Firoozi, et al., 2012).

For 10 days, buspirone and aqueous extract of Valeriana 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 behavior 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 SPSS19 statistical program was used and charts were drawn by Excel software.

Table 1 shows the mean and standard deviation of the anxiety scores in the groups SH, A, B, C and BUS.

Table 1: 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 1 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.

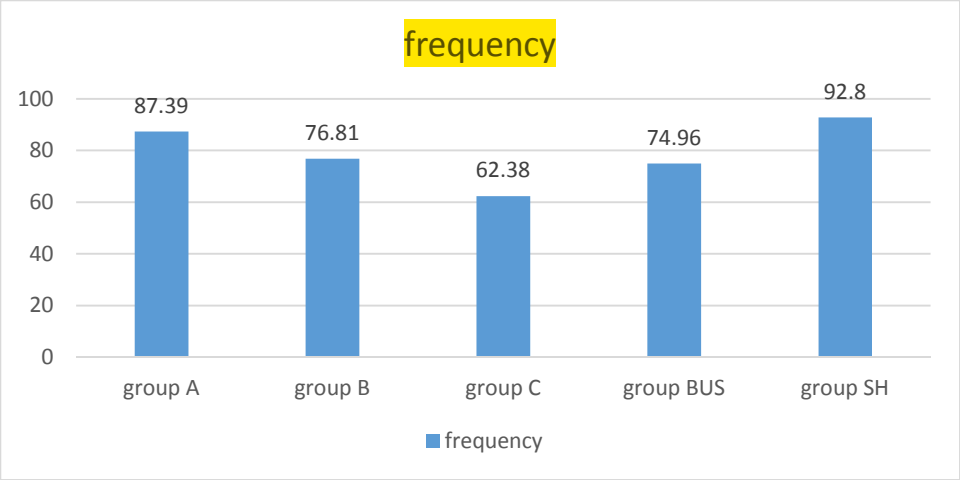

Figure 2: column chart of anxiety score in the groups

Testing normality of variables and homogeneity of variances

Shapiro-wilk test was used to test normality of data (Table 2) and Levene test was used to test homogeneity of anxiety in the studied groups (Table 3).

Table 2: 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 2 shows, data is normally distributed ($p<0.05$).

Table 3: homogeneity of variances (Levene)

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

As Table 3 shows, Levene's W=1.66; thus, the variances are homogenous ($p>0.05$).

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 4.

Table 4: 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 4, F-value=2.91 and P-value=0.04 supports the hypothesis; it can be claimed that there is a significant difference in anxiolytic effect of aqueous extract of Valeriana officinalis root and Buspirone in mice.

Sub-hypothesis 1

There is a significant difference in 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 5).

Table 5: 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 6).

Table 6: 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 anxiolytic effect of 300 mg/kg aqueous extract of Valeriana officinalis root and 30 mg/kg Buspirone in mice.

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

Table 7: 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 Valeriana officinalis root and 30 mg/kg Buspirone in mice ($p<0.05$).

Discussion and Conclusion

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 4, ANOVA and T-test results showed a significant relationship between anxiolytic effect of aqueous extract of Valeriana officinalis root and Buspirone in mice. The results show that aqueous extract of Valeriana 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 Valeriana 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 Valeriana officinalis root and 10 mg/kg buspirone in mice. According to the results listed in Table 5, 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 Valeriana 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 Valeriana officinalis root and 30 mg/kg buspirone in mice. In other words, 100 mg/kg aqueous extract of Valeriana officinalis root was weaker in reducing the anxiety compared to 30 mg/kg Buspirone.

The second sub-hypothesis indicated a significant difference in anxiolytic effect of 200 mg/kg aqueous extract of Valeriana officinalis root and 10 mg/kg buspirone in mice. According to the results listed in Table 6, 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 Valeriana 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 Valeriana officinalis root and 30 mg/kg buspirone in mice. In other words, 200 mg/kg aqueous extract of Valeriana 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 Valeriana officinalis root and 30 mg/kg buspirone in mice. According to the results listed in Table 7, 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 Valeriana officinalis root causes 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 Valeriana officinalis root and 30 mg/kg buspirone in mice. In other words, 300 mg/kg aqueous extract of Valeriana 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.

References

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

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

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

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.

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

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.

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

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.

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.

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

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

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.

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

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

Rezai, A. et al., 2011. Comparison of palliative and anti-anxiety effects of herbal tea extract with diazepam in rat. journal of zahedan medical sciences, 13(8), pp. 8-11.

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.

Rosen, JB ;Schulkin, J.)1998,(From normal fear to pathological anxiety .Psychol Rev; 105(2): 325-50.

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.

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.

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.

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).

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.

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.

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