Studying the Effect of Hydro- alcoholic Extract of Valeriana officinalis on the Number and Size of Raphe Magnus Neurons in Mature Rats

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ABSTRACT:
Introduction & Objective: Effective materials from Valerian officinalis L. have too much usage in the pharmacological industry. It is used as a sedative, anticonvulision, and antidepressant drug. Serotonin has a widespread role in vital function such as sleep, awareness and calmness. In this study we evaluated the effect of hydrochloric extract of valerian on number and size of raphe magnus neurons in adult rat.

Materials & Methods: In this experimental study, which was conducted at Yasuj University of Medical Sciences in 2009, forty adult Wistar rats, each 170-250 gr, were divided randomly into four groups (one control group and three experimental groups). The animals were injected daily for one month with doses of 300, 400 and 600 mg/kg of the extract. The control group just received distilled water. After transcardial perfusion, the whole brain was separated, then 10 µm sections of the brain stem were prepared, and hematoxylin and eosin (H&E) staining were done. Number and size of raphe magna neurons were observed under light microscope. The gathered data were analyzed by the SPSS software using One-way ANOVA and LSD.

Results: The control group did not statistically show significant changes in number of raphe magna neurons. Comparison of the means of long and short diameter neurons showed significant increases in experimental groups with control group (P<0.05). In experimental groups the neuron nucleuses were more euchromatic than the control group.

Conclusion: Hydrochloric extract of valerian has no effect on raphe magnus neurons, but it is effective on neurons’ size. It can be concluded that the extract increases both neurons activity and serotonin secretion.

Keywords: Valerian, Extraction, Raphemagnus, Reticular Formation.

INTRODUCTION
The reticular network takes place in the medulla oblongata, and pons, and midbrain as frills and branches. These branches form the raphe nuclei as the interconnected cell densities at the midlines. The nuclei take place in a symmetrical and regular state with tail head position in the brainstem. The characteristic of reticular formation of neurons is that they have no special arrangements while their axons have extended sporadically in two head and tail orientations.
(Rostral and Caudal) (1). The formations of these neurons include large multi-dimensional, the spindle-shaped, and the small, pear-shaped (2 and 3). According to the previous studies, Raphe nuclei and reticular formation are the regions rich in serotonin (4). The changes of serotonin content in the central nervous system (CNS) lead to change the vital functions such as sleep, appetite, sexual activity, motion reflexes, body temperature, Adrenocorticotropic hormone secretion (1), Prolactin secretion, and the growth (6 and 5). The effect of serotonin in the disorders such as Parkinson’s, Korea’s, Huntington’s, depression, schizophrenia and epilepsy has been proved (7-9).

Chemical or electrical stimulation on the Raphe nucleus especially on the raphe magnus leads to release the serotonin in the spinal cord (10). Most numbers of the serotonergic cells which forward their fibersto the spinal cord take place in the lower pons, midbrain, and medulla oblongata, so that 77.5 % of serotonergic neurons of medulla oblongata are in the raphe nucleus. Therefore it is found that the density of serotonergic cell in the raphe nucleus is very high (11). In the nucleus raphe magnus, serotonergic neurons take place in the formation of the pyramid as a dome so that two sides of them end with the regions where there are Reticular formation nuclei (Gigantocellularis (Gi) and Para- gigantocellularis (PGi)). Due to the high density of serotonergic neurons in the nucleus of raphe magnus, the spinal – raphe pathway has been known as a serotonergic pathway for a long time (11).

Valeriana officinalis is an herbaceous plant which belongs to Magnoliopsida class, Asteridae subclass, Dipsacales order, Valerianaceae family, Valeriana L. Gender, and Valeriana officinalis L. species which is found in the temperate regions of Asia, Europe and United states while its Rhizome and branched roots are the usable parts (13 and 12). Noteworthy the active substances of Valeriana officinalis including Valepotriate (8), Didrovaltrate (9) and Isovaltrate (10) are mostly used in the pharmaceutical industry and they are also applied as sedative, anticonvulsants, hypnotics, and also for the treatment of depression (14). Anticonvulsant effects of Valeriana officinalis extract (16, 15, and 12) and also the effect of this plant on increasing the quality of human sleep and shortening the time to fall asleep in mice have been proven in the various studies (17). Therefore the aim of this study was to investigate the effect of hydro-alcoholic extract of Valeriana officinalis L. on the number and size of raphe magnus neurons in mature rats.

MATERIALS AND METHODS
This is an experimental study which was conducted at 2009 in the Yasuj University of Medical Sciences. 40 groups of rats from the Wistar race and the weights of 170 to 250 grams were used. All animals were kept in standard animal house and also for 12 hours of darkness and light. The animals were transferred in cages containing 5 animals and they had free access to the food and water.

The protocol of the present research was documented according to international law about the laboratory animals and it was approved in the Ethics Committee of the Yasuj University of Medical Sciences.

Valeriana officinalis L. was provided from the reputable centers in order to prepare the extract of this plants root. After identification and naming by the botanist, the extraction was performed. In this method, the root of Valeriana officinalis L. was powdered and then the solvent that was ethanol for this research was added. The prepared solution was soaked 24 hours, while the excess solvent was extracted and it was concentrated using the Rotary device after smoothing.

The animals were divided randomly into 4 groups; groups including 2, 3, and 4 were the experimental groups that received 300, 400 and 600 mg/ kg hydroalcoholic root extract of Valeriana officinalis L. by gavage daily, respectively. However aquapura was prescribed for Group 1 or control group at the same time. After one month, the mice were anesthetized with ether. Primarily the chests

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of anesthetized animals were opened for perfusion and cannulas were placed by the tip of the heart in their ascending aorta. Then the blood vessels of the brain was washed with 100 cc saline and then was fixed by 200 ml of fixative substance containing formaldehyde 10% in 0.1 molar phosphate buffer (pH = 7. 4). After exiting the brain from the skull and preparation of the blocks from the brain tissue, truncations with 10 micron diameter were prepared using a rotary microtome BRIGHT 504 model. According to the Paxinos Atlas, the length of the raphe magnus nucleus in the adult rats is 2440 micron. Since the thickness of truncations was 10 microns in this study, then 244 serial truncations were prepared from the head to the tail of nucleus. Afterward the truncations with the frequencies of one to ten and as many as 25 truncations for each animal were stained with hematoxylin and eosin. Neurons were photographed using Olympus microscope model TH-200. In the present research, neurons which had detectable nucleus were counted. The neurons in the magnus raphe nucleus were counted in a square with the area of 90,000 micrometers square using analytical software LS starter while their large and small diameters were measured (18). The number of counted neurons was multiplied in number 10 in order to obtain total approximate number of the neurons. The collected data was analyzed using SPSS, one-way and two-ways ANOVA and also LSD.

RESULTS
The results of this study showed that three types of morphologies are found in the magnus raphe nucleus with the following frequencies (figures 1 and 2): 53 % of spindle neurons, multipolar neurons with 28 % and triangular neurons 18%. The comparison of the average numbers of the multipolar, spindle, and triangular neurons between the experimental and control groups showed no significant difference. In the different groups, the numbers of multipolar neurons were counted between 240 and 265, spindle neurons was obtained between 451 and 501, and triangle neurons was counted between 151 and 172 (see Diagram1). Comparing the size of large diameter in the triangular neurons of the experimental group rather than the control group showed no significant difference, however, a relative increasing is obvious in the large diameter of the neurons in the experimental group 2 compared to the other groups. Comparison of the large diameter size in the spindle neurons in experimental groups 2 and 3 significantly increased compared to the control group (p < 0.001). The comparison of large multipolar size of the neurons demonstrated a stair increasing from the control groups to the experimental group 2 while this increase was significantly in all categories compared to the control group (p < 0.001) (Diagram2). The comparison of small triangular size of the neurons in the experimental group 3 revealed significant differences compared to the control group (p < 0.05). Comparing the size of small diameter of the spindle neurons in experimental groups 2 and 3 showed a significant difference compared to the control group (p < 0.001). Comparing the size of small diameter in the multipolar neurons in the experimental groups showed a significant difference compared to the control group (p < 0.001) (Diagram3).

DISCUSSION AND CONCLUSION
Considering that active ingredients of Valeriana officinalis are used frequently in the pharmaceutical industry and also are applied as sedatives, anticonvulsants, hypnotics, and the treatment of the depression (14), therefore the present research aimed to the effect of hydro-alcoholic extract of Valeriana officinalis L. on the number and size of neurons located in the magnus raphe nucleus of the rats.

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1. Analysis LS Starter
2. Statistical Package for Social Sciences
3. Least significant differences
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Figure 1: Magnus raphe nucleus in the control group (magnification 400, Olympus microscope, stained with hematoxylin and eosin)

Figure 2: Magnus raphe nucleus in the experimental group 3 (magnification 400, Olympus microscope, stained with hematoxylin and eosin)

Diagram 1: Number of multipolar neurons, spindle and triangular nucleus raphe magnus in the studied groups.

Diagram 2: size large multipolar neurons, spindle and triangular nucleus raphe magnus in the studied groups.
The results of this study showed that there are three types of morphologies in the magnus raphe nucleus including spindle neurons with 53%, multipolar neurons with 28%, and triangular neurons with 18%. The achieved numbers of the neurons were same with the number of neurons that Jaghatai et al., (2004) reported (19).

Kordero et al (2001) reported the sizes of multipolar neurons (Greater than 40 microns), spindle neurons (larger than 2 microns), and neurons oval (larger than 15 microns) in the magnus raphe nucleus (20).

The results of the present research showed that hydro-alcoholic extract of Valeriana officinalis L. root has significant effect on the number of neurons in the magnus raphe nucleus of rats. Since neurons had not reproducibility, then if the extract of Valeriana officinalis has toxicity then it can induce the cell death while it didn’t occur. It is probably the reason for non-toxic effect of Valeriana officinalis L. that is compliance with the results of Zahedi and colleagues (2003) study.

The reason for increasing the size of neurons in the experimental groups of this research can be hypertrophy in organelles involved in the proteins synthesis including nucleus, rough and smooth endoplasmic reticulum, ribosomes and secretory granules.

Based on the conducted studies using the electron microscopy, Nissl bodies which are named conical bodies due to their special shapes in the cytoplasm and dendritic poles, are indeed the rough endoplasmic network that are involved in the protein synthesis while changes in the size and shape of them demonstrates the changes of the metabolic activity of cells (22, 19).

Several studies have shown that Tonic Formalin pain after one week could increase the long diameter of neurons by affecting the neurons of magnus raphe nucleus significantly (23). The opposite point of this event occurs in the aging process while their size is reduced along with the reduction of cell activity (19).

According to the results, increasing the small and large diameter neurons demonstrates increase of their activity for the secretion of serotonin that also demonstrates the impact of this plant in the treatment of depression, anxiety, and insomnia.

The results of the present research is consistent with the results of Houghton (1999) and Balderer & Borbely (1985). They showed that Valeriana officinalis L. has the calming effect and can cause insomnia treatment and sleep disturbances (25 and 24).

One of the most important ingredients in the root rhizome of the Valeriana officinalis L is valpotriates (26). In terms of chemical, these compounds are fatty esters with short chains including acetic acid, isovaleric acid, and beta stoxia isovaleric acid with a three factors acid. The alcohol component can contain a carbon skeleton and an epoxy ring (27). On the other hand, the
laboratory studies showed that valerenic acid involved in the complete extracts of valerian acts as a partial agonist for the receptor of 5-HT (5a) (28).

Different researches demonstrated that Valepotriates derived from the Valeriana officinalis L. increased the spent time in the high open arm and also raised the number of entry to the open arm. Therefore, it can be suggested that Valepotriates have the anti-anxiety effect (29).

Generally, it is concluded that hydro-alcoholic extract of Valeriana officinalis root has no effect on the number of neurons in the magnusraphe nucleus; however, it can affect the size of these neurons that demonstrates the increased activity of these neurons. Therefore the results can be the increase of serotonin secretion and the effect of this plant in the treatment of depression and anxiety.

Finally, it is suggested that a detailed research should be conducted using immunohistochemical techniques aimed to evaluate the morphology of neurons following the gavage of valerian.

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