Original Article

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The Effect of Hydro-Alcoholic Extraction of Valerian on Number and Morphology of Raphe Magnus Nucleus in Astrocytes in Rat Model

Animal Research Section

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ABSTRACT

Introduction: The valerian root extract has been used to treat sleep disorders, stress, depression, anxiety, muscle stiffness and tension. Astrocyte cells are involved in neural support, nutrition and protection. They regulate the transmission of electrical impulses within the brain.

Aim: The aim of the present study was to evaluate the effect(s) of valerian extract on the number and size of the astrocyte cells in raphe magnus nucleus of rats.

Materials and Methods: In the present experimental study, 40 male Wistar rats weighing 170-250 gm were randomly divided into four groups as follows: one control and three experimental groups. The control group received distilled water while animals in Group 2, 3 and 4 were gavaged by 300, 400 and 600 mg valerian root extract daily, respectively for two weeks. Astrocyte cells were stained with phosphotungstic acid. The size and number of astrocyte cells were calculated using the LS starter software. The collected data were analysed using SPSS statistical analysis with ANOVA and LSD tests.

Results: The mean number of astrocytes in experimental Groups 3 and 4 showed a significant increase in comparison with the control and experimental Group 2 (p-value <0.05). The mean diameter of astrocytes in all groups compared with the control group showed a significant decrease. Moreover, in Group 2, compared with Groups 3 and 4, the difference was statistically significant (p-value <0.05).

Conclusion: Aqueous extracts of valerian taken orally will increase the number of astrocytes in the raphe magnus.

In addition, administration of this extract reduced the diameter of astrocytes in nucleus raphe magnus, which is indicative of cell proliferation in the nucleus raphe magnus.

Keywords: Animal model, Astrocyte cells, Valeriana root extact

INTRODUCTION

Reticular formation is a wide spread network in medulla oblongata and the brain stem. The raphe magnus nuclei are a moderate size cluster of nuclei found in the brain stem (interpeduncular anterior nucleus and medulla oblongata). Their axons are extended into rostral and caudal direction sporadically [1]. Raphe nuclei system is admitted as the most extensive and complex anatomical and neurochemical system in the mammalian brain [2,3]. The special feature of this nuclei is that the significant ratio of its neuron population is serotonergic; However, research shows that dopamine, neurotensin, substance P and enkephalins as neurotransmitters are released in the raphe nucleus. Neurons in the raphe nucleus are the main sources of the release of serotonin in the brain [4]. There are seven or eight raphe nuclei, that are concentrated in the center and around the brain stem. Axons of neurons in the raphe nuclei form a neurotransmitter system, reaching almost every part of the central nervous system. Axons of neurons in the raphe nuclei in the cerebellum and spinal cord ends lower, while higher nuclei axons in the brain are broadcasted. The nucleus raphe magnus releases serotonin when stimulated. Serotonergic action is terminated primarily via uptake of 5-hydroxytryptamine (5-HT) from the synapse [5-9]. The operation identified through a monoamine neurotransmitter called Serotonin Transporter (SERT) on parasympathetic neurons. Factors such as ecstasy {3,4-Methylenedioxymethamphetamine (MDMA)}, amphetamines, cocaine, dextromethorphan (a cough syrup), tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs) prevent the reuptake of 5-HT. The 5-HT regulated activities such as sleep, appetite and libido. Hypothalamic suprachiasmatic nucleus serotonergic neurons projected along with norepinephrine and dopamine plays a role in regulating behavior. Acute stress increases serotonin temporarily, but chronic stress can drain reserves of serotonin [10,11].

Nervous tissue is composed of two types of cells: neurons responsible for receiving, transmitting and processing the message and release neurotransmitter and neuroglia responsible for nutrition, protection and supporting nerve cells, including astrocytes, oligodendrocytes, microglia and ependymal [12,13]. Astrocyte have important role in neurometabolism, maintaining consistency and rigidity of the nervous system, mediator uptake and prevention of electrical spreading and protection of brain and spinal cord from injury [14,15].

The valerian plant with the scientific name *Valeriana officinalis* is native to Europe, Asia and North America. The oily extract obtained from the dried root of this plant is useful in treating sleep disorder, stress, depression, anxiety disorder, muscle stiffness and seizure. Effective compounds in the plant including valepotriat, hydrovalepotriate and isovalepotriate which have a lot of benefits in pharmaceutical industry and are used as a sedative, anticonvulsant, hypnotic and also treatment of depression [16-18].

It has been reported that the hydroalcoholic extract of valerian root has no significant effect on number of neurons significantly [18].

Valeriana officinalis is used in the treatment of sleep disturbances, stress, depression, anxiety, muscle relaxant and also as an anticonvulsant, but no study has been conducted on the effect of valerian on the morphology of astrocytes, so the goal of present study was to evaluate the regulatory effects of hydroalcoholic extract of valerian root on the size and number of raphe magnus nuclei astrocytes in rat brain.

MATERIALS AND METHODS

This experimental research was conducted under the approved conditions by the Institutional Animal Care and Use Committee (IACUC) and Ethics Committee of Yasuj University of Medical Science which conforms to the provisions of the Declaration of Helsinki (DOH,

2013). Fourty male Wistar rats with an average weight of 170-250 gm and age 2-2.5 months were collected randomly from medical science animal house. Animals were handled according to the guide for care and use of laboratory animals (Yasuj University of medical sciences) and maintained in a 12 hrs light: 12 hrs dark photoperiod under constant temperature (22±2°C) and relative humidity for two weeks. The rats were divided into four different subgroups as following:

Group 1 (control): were gavaged with distilled water during the study,

Group 2: were gavaged with 300 mg/kg,

Group 3: were gavaged with 400 mg/kg and

Group 4: were gavaged with 600 mg/kg of extract every day.

Valerian roots were prepared and dried under indirect sunlight and and then grinded. The 500 gm of the dried extract was soaked in water and ethanol (1:1 ratio) for 24 hours, then filtered two times, transferred to rotary machine for concentrating and separating additional solvent. Next, the extract was dried in an incubator, under vacuum pressure at 50°C. Afterwards, it was weighed and dissolved in distilled water two times to the final volume of 500 mL. Rats were gavaged by water or hydroalcoholic extract of valerian root daily for 14 days. Finally, the rats were anaesthetised using Ketamine injection and a solution of Heparin, normal saline, Paraformaldehyde (5%) and Glutaric acid (5%) were perfused through the animal's heart. Rat brain was removed from the skull and transferred to plates containing formalin (10%). The brain tissue were processed by the tissue processing and paraffin embedded. ten micron sections were prepared from the blocks by microtome. The specimens were serially cut and one piece selected from each 10 pieces separately and stained with phosphotungistic acid. The Number and diameter of astrocytes were measured by a Olympus BX51 microscope and Olysia software.

STATISTICAL ANALYSIS

Data were analysed by SPSS (version 13.0). One way ANOVA test and LSD spatial test was used to compare the groups. Table data was presented based on the mean and standard deviation for each group. The differences in the proportions were considered signifcant when p-value <0.05.

RESULTS

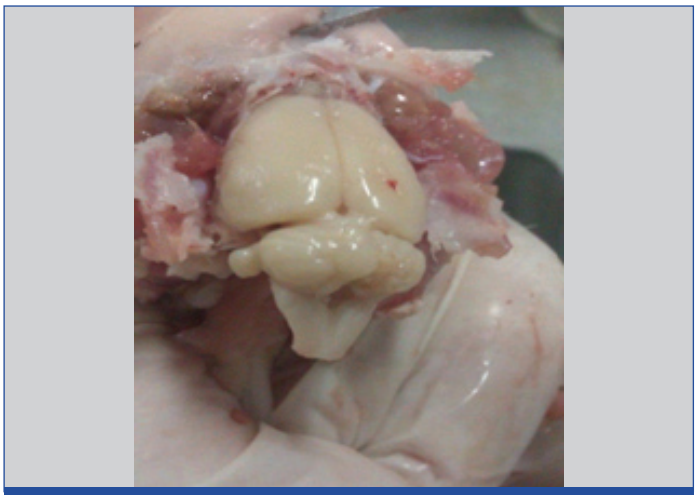
The mean number of astrocyte cells in the Group 3 and 4 which were gavaged by 400 and 600 mg/kg/day of valerian extract, compared with the Group 1 (control) and second Group 2 (gavaged by 300 mg/kg/day of valerian extract), indicated a statistically significant increase (p<0.05). The mean number of astrocyte cells in the Group 2 (gavaged by 300 mg/kg/day of valerian extract) did not indicate a significant difference compared to the Group 1 (control) (p>0.05) [Table/Fig-1]. The mean diameter of astrocytes in the Group 2,3,4 and in Group 1 (control) indicated a significant decrease. This reduced diameter was more in the Groups 3 and 4. Thereby, reducing the diameter of astrocytes in Group 4 compared with the Group 3 did not show a significant difference (p-value >0.05). The mean diameter of astrocytes in the Group 3 and 4 compared to the Group 2 indicated a significant decrease [Table/Fig-2,3]. Results show that astrocyte fibrils were blue and the neuronal cytoplasm turned yellow. Thus, increasing the number of astrocytes confirmed in Group 3 and 4 compared with the Group 1 (control). [Table/Fig-4].

Groups	Number of member	Mean number of astrocyte	p-value
Experimental Group-1 (Control)	10	32.33±1.95	0.26
Experimental Group-2	10	34.46±2.77	0.18
Experimental Group-3	10	67.13±3.37*	0.021
Experimental Group-4	10	75.13±2.93*	0.014

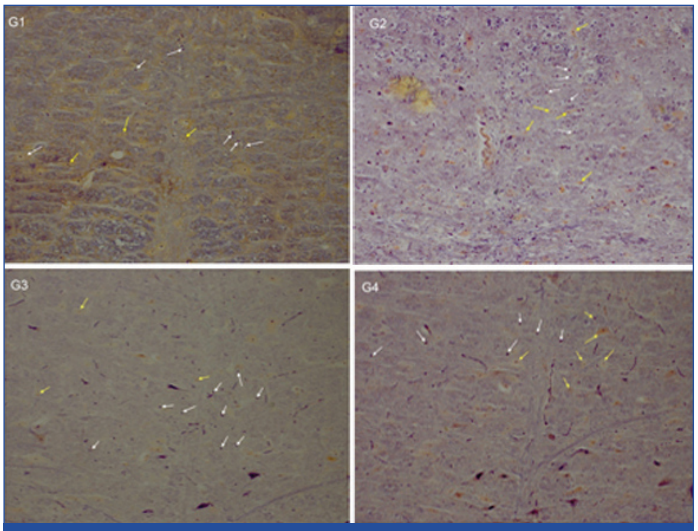
[Table/Fig-1]: Mean number of astrocyte cells in raphe magnus nuclei in different groups.
*Represent significant statistic different in compare with control group (p-value <0.05)

Groups	Number of member	Mean diameter of astrocyte	p-value
Experimental Group-1 (Control)	10	10.25±0.63	0.17
Experimental Group-2	10	6.45±0.37*	0.042
Experimental Group-3	10	4.53±0.9*	0.021
Experimental Group-4	10	3.9±0.34*	0.013

[Table/Fig-2]: Mean diameter of astrocyte cells in raphe magnus nuclei in different groups.
*Represent significant statistic different in compare with control group (p-value <0.05)



[Table/Fig-3]: Rat brain.



[Table/Fig-4]: Slide microscopic show neroun and astrocyte cells in raphe magnus nuclei in different groups (20X).
Neuron (yellow arrow), astrocyte (white arrow), phosphotungistic acid stain

DISCUSSION

The mean number of astrocyte cells in the Groups 3 and 4 which were gavaged by 400 and 600 mg/kg/day of valerian extract, compared with the Group 1 (control) and Group 2 (gavaged by 300 mg/kg/day of valerian extract), indicated a statistically significant increase.

Much of the neurons of the raphe magnus nuclei are serotonergic. However, there were evidence of presence of neurotransmitters such as dopamine, neurotensin, substance P and enkephalin existence [5-9]. The anatomical differences between these cells in the various animals are very low, so that the data obtained from this study of rats can generalise to other animals and human [18,19].

The number of serotonergic cells in the raphe magnus nucleus was

abundant [20,21]. Chemical or electrical stimulation of the raphe magnus nucleus caused the release of serotonin in the spinal cord. *Valeriana officinalis* are active ingredients in pharmaceutical industry and used as sedatives, anticonvulsants, hypnotics and also to treat depression and anxiety. Research shows that valerian affects the neurotransmitters and receptors [22,23].

In the present study, the mean number of astrocytes in the experimental Groups 3 and 4 compared to the Group1 (control) and the experimental group compared to the number of astrocytes in these two groups indicated no significant differences. In the present study, the mean number of astrocytes in the experimental Groups 3 and 4 compared to the Group 1 (control) and the experimental group 2 indicated significant differences. But the control group compared with the second group and the third experimental group compared with the fourth group indicated no statistical significant difference. Teaching and spatial learning can increase astrocyte cells in dental gyrus and this increase can be related to the duration of learning [24-26].

The researchers examined the effect of spatial teaching in the number of astrocytes in different area of the rat hippocampus and it has been reported that the spatial teaching may increase the number of astrocytes in different areas of the hippocampus [27-29]. The mean diameter of astrocytes in nucleus raphe magnus in the experimental group second, third and fourth in compared to the control group were not showing a statistically significant difference.

The mean diameter of astrocytes in nucleus raphe magnus in the third and fourth experimental groups compared to the second experimental group showed a significant decrease, but the third experimental group compared with the fourth group did not show a statistically significant difference. It can be concluded that valerian extract increases the proliferation of astrocytes in the raphe magnus [30-32].

Because the astrocytes population of this area were younger and therefore smaller, according to the findings, related to the number of astrocytes, the increase was justified. Astrocytes are positioned between the neurons and the capillaries and plays an important role in neuron energy metabolism [29-33].

Glycogen in the brain tissue is stored in large quantities in astrocytes. Astrocytes are neuroglia cells in the central neural tissue, which play an important role in the metabolism of materials, nutrition, waste removal and axonal neuronal conduction [28,29]. Recent studies have shown that astrocytes have active role in neuronal activity such as ion exchange, energy production, release of neurotransmitters and producing synapses [34]. Studies conducted so far have only supported the role of astrocytes to neurons in the central nervous system, but a recent study has reported a greater role for astrocytes in information processing [35]. This study indicated that astrocytes not only receive login information, but also transmit signals between neurons. The secretion of neurotransmitters such as serotonin interactions and GABA valerian is due to this fact [19-21].

CONCLUSION

Proliferation of astrocytes is to support the activity of neurons. So, this extract reduces the size of astrocytes in this area, all of which show the increased effect of this extract in astrocytes. Histochemistry for testing examination raphe magnus nucleus did not have sufficient financial means.

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