

Original Article

# Evaluation of the GABA<sub>A</sub> receptor on pain sensitivity in male rat pretreated with *Valeriana officinalis* extract using formalin test

Mahnaz Taherianfard\* , Mehdi Karamifard

Physiology Division of Basic Science Department, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

## Abstract

**Introduction:** It seems that *Valeriana officinalis* (valerian) extract through gamma-amino-butyric acid A (GABA<sub>A</sub>) receptor possesses analgesic effect. The aim of the present study was to investigate the effect of muscimol and picrotoxin on pain sensitivity in male rats pretreated with valerian extract using the formalin test.

**Methods:** Thirty-five male rats weighing 200-250g in standard temperature 20±2 °C and light cycle of 12/12h used. Animals were randomly divided to 7 groups: sham 1 (injection of saline); sham 2 (pretreated with valerian + ICV injection of artificial cerebro spinal fluid); experimental1 (injection of valerian extract); experimental 2 or 3 (pretreated with valerian extract + ICV injection of muscimol 250 and 500 ng/rat); experimental 4 or 5 (pretreated with valerian extract + ICV injection of picrotoxin 250 and 500 ng/rat). Valerian extract 400 mg/kg was administrated by intraperitoneal injection. Pain evaluation was done by the formalin test. Lateral ventricles cannulated unilaterally by the stereotaxic procedure.

**Results:** Data showed that valerian extract significantly decreased pain sensitivity in the late phase of the formalin test in comparison to sham 1 group. Muscimol in both doses significantly decreased pain in comparison to sham 1, while at the dose of 500 ng/rat significantly increased pain sensitivity in comparison to sham 2 at late phases of formalin test. Picrotoxin at both doses significantly decreased pain sensitivity in comparison to sham 1, while significantly increased pain sensitivity in comparison to the sham 2 at late phases of formalin test.

**Conclusion:** According to present results, valerian extract had analgesic effect through the GABA<sub>A</sub> receptor.

## Keywords:

*Valeriana officinalis*;  
Muscimol;  
Picrotoxin;  
Pain;  
Rat

**Received:** 3 Feb 2018

**Accepted:** 8 Jun 2018

## \*Correspondence to:

M. Taherianfard

**Tel:** +98-7132296950

**Fax:** +98-7132296940

## Email:

taherian@shirazu.ac.ir

## Introduction

Pain is an unpleasant sense directly due to tissues damage. Nociceptor stimulated by chemical mediators such as prostaglandin, histamine, dopamine, serotonin and gamma amino butyric acid (GABA) (Mertens et al., 2001). Many synthetic

analgesic drugs used for the pain management. It has well established that the synthetic analgesic drugs have side effects in the clinical practice, so researchers have focused on herbal medicines as more appropriate analgesic agents. *Valeriana officinalis* (valerian) extract belongs to valerianaceae family that mainly grows in the areas of the America, Europe, and Asia (Yuan et al., 2004). This herb used

in medicine in different countries (Benke et al., 2009; Khayat Nouri and Namvaran Abbas Abad, 2011). Several studies have reported anticonvulsant effects of valerian extract and they have mentioned its effects mediated through the GABA system (Khayat Nouri and Namvaran Abbas Abad, 2011; Torres-Hernandez et al., 2015).

Furthermore, valerian extract possesses sedative, hypnotic and anxiolytic properties (Dietz et al., 2005; Fernandez et al., 2004; Heidari and Razban, 2004; Solati and Sanaguye Motlagh, 2008). Phytochemical studies have shown valerian contains valepotriates, alkaloids, sesquiterpenes, alcohols, volatile oils, aromatic compounds and other polar and non-polar organic compounds. These are responsible for mentioned effects of valerian extract (Fernandez et al., 2004; Torres-Hernandez et al., 2015). Several potential mechanisms have proposed for valerian effects. Antianxiety properties of valerian relates to the GABA system function (Khayat Nouri and Namvaran Abbas Abad, 2011; Yuan et al., 2004; Murphy et al., 2010).

GABA is a main inhibitory neurotransmitter in central nervous system of mammals (Froestl, 2011). Activation of GABA<sub>A</sub> receptor opens chloride channel and makes intracellular hyperpolarization (Farrant and Nusser, 2005). Muscimol is an agonist of GABA<sub>A</sub> receptor and causes pain reduction (Reis et al., 2007). Studies have shown that administration of muscimol reduced pain in different pain tests (Gilbert and Franklin, 2001; Mahmoudi and Zarrindast, 2002). Picrotoxin is a competitive antagonist of GABA<sub>A</sub> receptor and changes pain response in the formalin test (Heidari et al., 1996).

There are many investigations on GABA<sub>A</sub> receptor and pain, on the other hand, relation between valerian and pain was investigated; but the analgesic effect of these two factor simultaneously was not evaluated. Therefore, the aim of the present investigation was to evaluate analgesic effect of muscimol (GABA<sub>A</sub> agonist) and picrotoxin (GABA<sub>A</sub> antagonist) in adult male rats pretreated with valerian extract using formalin test.

## Materials and methods

### Animals

Thirty-five adults' male Sprague Dawley rats weighing 200-250g were used. Animals housed in standard

condition and maintained under controlled-temperature  $20\pm 2$  °C and light-dark cycle as 12/12h. Rats were fed standard food and water ad libitum. The animals randomly divided into 7 groups (5 rats per group): sham 1, intraperitoneal (ip) injection of saline; sham 2, pretreated with ip injection of valerian 400 mg/kg + intracerebroventricular (ICV) injection of artificial cerebro spinal fluid (ACSF); experimental 1, ip injection of valerian 400 mg/kg; experimental 2 and 3, pretreated with ip injection of valerian 400 mg/kg + ICV injection of muscimol 250 or 500 ng/rat and experimental 4 and 5; pretreated with ip injection of valerian 400 mg/kg + ICV injection of picrotoxin 250 or 500 ng/rat. Intraperitoneal injection of valerian 400 mg/kg at single dose and ICV injection of ACSF, muscimol and picrotoxin performed 15 and 30 minutes before formalin test, respectively.

### Preparation of valerian extract

To prepare the valerian extract, 500g of dried valerian rhizome was ground and then added to the solvent 70% ethanol. Every 100 grams of powdered rhizome dissolved in 400ml of the above solution. The solution stirred for one hour on shaker to obtain almost uniform solution then placed at room temperature for 48 hours (25°C). After 48 hours, the solution filtered through whatman filter paper. Evaporator and lyophilized devices used for condensing of the final solution. After this process, the dried powder well mixed, in a fit ratio with distilled water on shaker until being dissolved completely and ip injectable solution (400mg/kg; LD50= 3300 mg/kg) was prepared (Patočka and Jakl, 2010).

### Stereotaxic procedure

Animals anesthetized with ip injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Rats fixed in the stereotaxic apparatus using blunt ear bars. The skull was carefully exposed and stainless steel guide cannula (23-gauge needle), were inserted bilaterally 3.5 mm above the lateral ventricle. The coordinates for lateral ventricle were 0.5 mm anterior to Bregma and 1.5 mm lateral to midline. The guide cannula fixed to the skull via dental acrylic cement and two tiny stainless steel screws. At the end, animals were given a 7-day recovery period.

### Formalin test

Thirty minutes after drug injections, 50μl formalin

solutions (2.5% in normal saline) was subcutaneously microinjected into the dorsal surface of the animal's right hind paw. The pain score every 15 seconds recorded as follows: if the animal showed no reaction, the score would be (0); if the animal did not rely on the injected paw, the score would be (1); if the animal holds its paw up, the score would be (2) and finally if the rat licks and/or bites the injected paw, the score would be (3). This evaluation was performed for 60 minutes. The obtained results in every 15 seconds were averaged every 5 minutes.

All animal procedures performed according to the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University.

### Statistical analysis

For data analysis, SPSS (version 21) used. The data analyzed by the one-way ANOVA, repeated measure ANOVA and post-hoc test was Tuckey.  $P < 0.05$  considered as statistically significant.

## Results

Present data showed that ip injection of valerian extract 30 minutes before formalin test had significant ( $P < 0.001$ ) analgesic effect during the late phase of the formalin test in comparison to sham 1; but not in the early phase of formalin test. Analgesic effect of valerian extract was similar to sham 2 (Fig. 1).

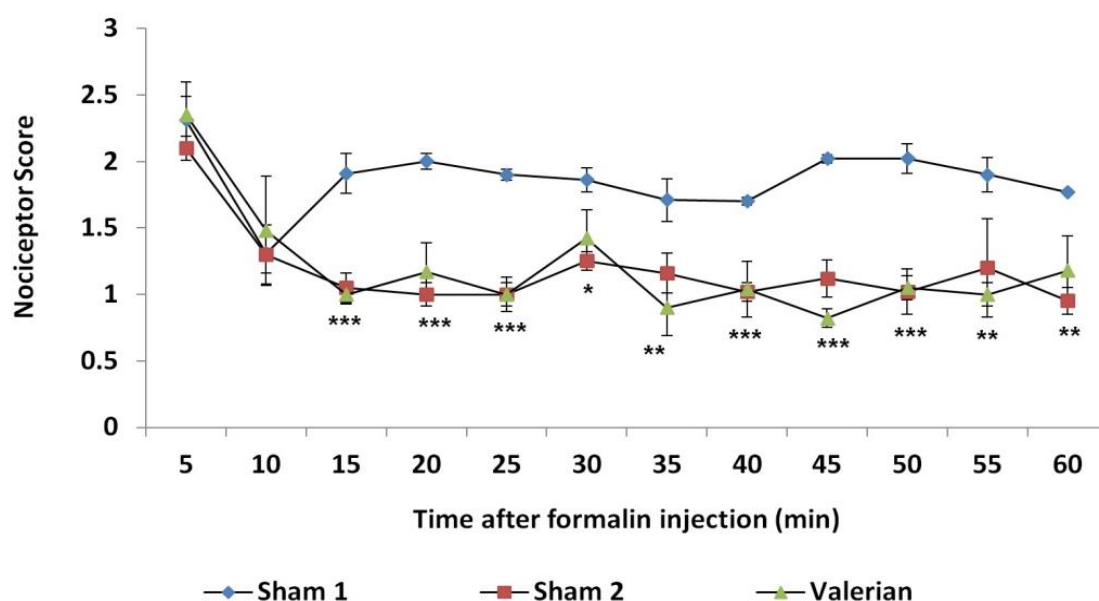
In rat pretreated with ip injection of valerian, ICV

injection of muscimol at both doses didn't have significant effect in the early phase of the formalin test ( $P > 0.05$ ) in comparison to sham 1 and sham 2. Nevertheless, muscimol at both doses in the late phase of the formalin test significantly ( $P < 0.05$ ) decreased pain sensitivity in comparison to sham 1. In the late phase of the formalin test muscimol 500 ng/rat significantly ( $P < 0.05$ ) increased pain sensitivity relative to sham 2 (Fig. 2).

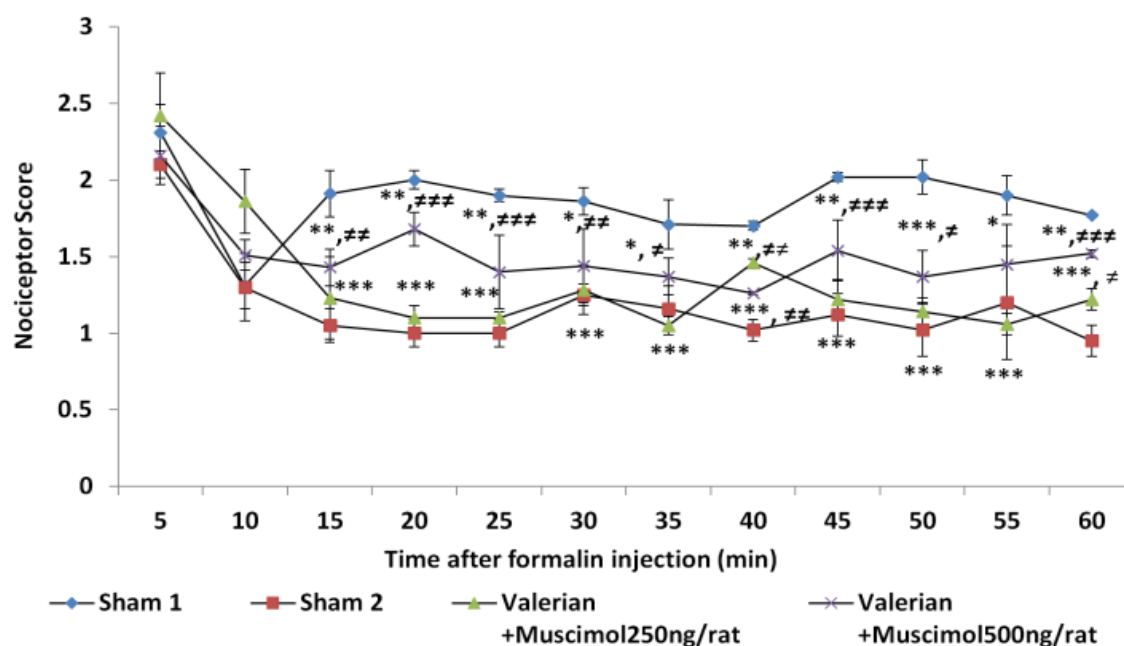
In rat pretreated with ip injection of valerian, ICV injection of picrotoxin at both didn't have significant effect in the early phase of the formalin test ( $P > 0.05$ ) in comparison to sham 1 and sham 2. In late phase of formalin test, picrotoxin at both doses significantly ( $P < 0.05$ ) decreased pain sensitivity in comparison to sham 1; but picrotoxin at both doses significantly ( $P < 0.05$ ) increased pain sensitivity in comparison to sham 2 (Fig. 3).

## Discussion

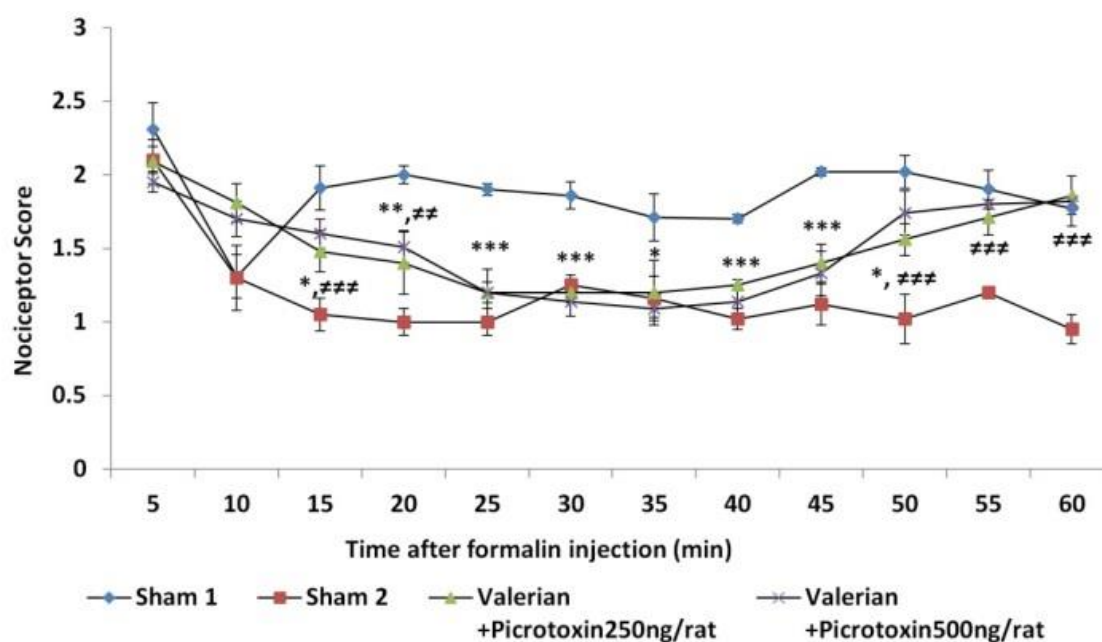
In the present study, data showed that valerian extract decreased pain sensitivity during the late phase of the formalin test. Valerian extract has been used for relieving rheumatic pain (Khayat Nouri and Namvaran Abbas Abad, 2011); however, mechanism of antinociceptive effect of the valerian extract is unknown. Several studies have shown valerian extract had sedative effect and improved sleep quality (Carlini, 2003). Fernandez et al. (2004) have



**Fig.1.** Effect of ip injection of valerian 400 mg/kg on pain sensitivity by formalin test. Each value represents the mean±S.E. \* compared to the sham 1 group. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig.2.** Effect of ICV injection of muscimol (250 and 500ng/rat) in rats were pretreated with ip injection of valerian 400 mg/kg on pain sensitivity by formalin test. Each value represents the mean  $\pm$  S.E. \* represented significant difference of muscimol in both doses compared to the sham 1 group. # represented significant difference of muscimol in both doses compared to the sham 2 group. \* $P$ <0.05; \*\* $P$ <0.01; \*\*\* $P$ <0.001. # $P$ <0.05; ## $P$ <0.01; ### $P$ <0.001.



**Fig.3.** Effect of ICV injection of picrotoxin (250 and 500ng/rat) in rats were pretreated with ip injection of valerian 400 mg/kg on pain sensitivity by formalin test. Each value represents the mean  $\pm$  S.E. \* represented significant difference of picrotoxin in both doses compared to the sham 1 group. # represented significant difference of picrotoxin in both doses compared to the sham 2 group. \* $P$ <0.05; \*\* $P$ <0.01; \*\*\* $P$ <0.001. # $P$ <0.05; ## $P$ <0.01; ### $P$ <0.001.

reported some constituents of valerian extract including hesperidin, methylapigenin, linarin and valerenic acid contributed to its sedative and hypnotic effects (Fernandez et al., 2004). Cavadas et al. (1995) reported that valerian extract contain GABA. In the present study, pretreatment with ip injection of

valerian extract 400 mg/kg and ICV injection of muscimol at both doses decreased pain sensitivity in comparison to sham 1 group. Several studies have shown the analgesic effect of muscimol (Gilbert and Franklin, 2001; Mahmoudi and Zarrindast, 2002). Taherianfard et al. showed that ICV (2009) and intra

hippocampal (2011) injection of muscimol at doses of 250 and 500 ng/rat induced significant analgesia (Taherianfard et al., 2009; Taherianfard and Mosavi, 2011). Yuan et al. (2004) have shown sedative effect of valerian extract and valerenic acid mediated via GABA<sub>A</sub> receptor. Furthermore, several studies have shown valerian had anticonvulsant properties as same as benzodiazepine drugs that act via GABA<sub>A</sub> receptor (Khayat Nouri and Namvaran Abbas Abad, 2011; Torres-Hernandez et al., 2015).

In the present study, pretreatment with valerian extract and muscimol had higher analgesic effect at dose 250 ng/rat of muscimol than that at dose 500 ng/rat of muscimol. In rats pretreated with valerian, muscimol with a higher dose (500ng/rat) had less analgesic effect in comparison to sham 2. Yuan et al. reported that pretreatment with valerian and valerenic acid decreased muscimol inhibitory effects in brainstem and this effect is dose dependent (Yuan et al., 2004). Muscimol 500ng/rat in the present study seems to have a similar mechanism of action; so it had lower analgesic effect relative to sham 2 and muscimol 250ng/rat. Although GABA<sub>A</sub> receptor is postsynaptic receptor, it also exists in presynaptic membrane (Belenky et al., 2003), so it might be in high dose stimulated the presynaptic receptor and has hyperalgesic effect. On the other hand, it seems that the release and reuptake of GABA are affected by valerian (Santos et al., 1994a; Santos et al., 1994b). The various extracts of valerian contained GABA that stimulated GABA receptors (Murphy et al., 2010; Yuan et al., 2004). Valerenic acid, the main substance of valerian extract, bound to GABA<sub>A</sub> receptor with high affinity (Murphy et al., 2010). Another study has shown a point mutation in GABA<sub>A</sub> receptor decreased valerenic acid effects (Benke et al., 2009). Ethanolic extract of valerian stimulated glutamic acid decarboxylase, an enzyme that synthesizes GABA neurotransmitter in brain, and furthermore, some derivatives of valerian extract inhibited GABAase that catabolizes GABA neurotransmitter (Murphy et al., 2010; Yuan et al., 2004).

In the present study, pretreatment with ip injection of valerian and ICV injection of picrotoxin as GABA<sub>A</sub> receptor antagonist at both doses had more analgesic effect relative to sham 1, while it had less analgesic effect relative to sham 2 in late phase of formalin test. The previous studies have shown

methanolic extract of valerian significantly decreased incidence of clonic seizure induced by picrotoxin (Heidari and Razban, 2004). Picrotoxin increased formalin-induced licking in the formalin test via GABA<sub>A</sub> receptor blocking (Malan et al., 2002). Picrotoxin attached to GABA<sub>A</sub> receptor with a use-facilitation mechanism and it is not capable of binding to GABA<sub>A</sub> receptor when GABA chloride channels are closed (Dillon et al., 1995; Newland and Cull-Candy, 1992). Picrotoxin analgesic effect in the present study could be through the use-facilitation mechanism. Therefore, pretreatment with valerian as a GABA<sub>A</sub> agonist could open the chloride channel of GABA<sub>A</sub> receptor and reduce hyperalgesic effect of picrotoxin.

## Conclusion

According to present results, pretreatment with valerian extract and muscimol didn't improve pain responses. This effect can explain the same action site of valerian extract and muscimol. Picrotoxin effects are time- dependent and in pretreatment with valerian, picrotoxin reduced pain sensitivity, but analgesic effect of picrotoxin was significantly lower than sham 2 that received only valerian (Dillon et al., 1995; Newland and Cull-Candy, 1992;). Therefore, it seems that pretreatment with valerian disappeared hyperalgesic effect of picrotoxin and picrotoxin reduced analgesic effect of valerian.

## Acknowledgments

This was study financially supported by Shiraz University.

## Conflict of interest

The authors declare no conflict of interest.

## References

- Belenky MA, Sagiv N, Fritschy JM, Yarom Y. Presynaptic and postsynaptic gabaa receptors in rat suprachiasmatic nucleus. *Neuroscience* 2003; 118: 909-23.
- Benke D, Barberis A, Kopp S, Altmann KH, Schubiger M, Vogt KE, et al. Gaba a receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. *Neuropharmacology* 2009; 56: 174-81.
- Carlini EA. Plants and the central nervous system. *Pharmacol Biochem Behav* 2003; 75: 501-12.



- Cavadas C, Araujo I, Cotrim MD, Amaral T, Cunha AP, Macedo T, et al. In vitro study on the interaction of valeriana officinalis L. Extracts and their amino acids on gabaa receptor in rat brain. *Arzneimittelforschung* 1995; 45: 753-5.
- Dietz BM, Mahady GB, Pauli GF, Farnsworth NR. Valerian extract and valerenic acid are partial agonists of the 5-HT<sub>1A</sub> receptor in vitro. *Brain Res Mol Brain Res* 2005; 138: 191-7.
- Dillon GH, Im WB, Carter DB, McKinley DD. Enhancement by gaba of the association rate of picrotoxin and tert-butyl bi cyclophosphorothionate to the rat cloned alpha1 beta2 gamma2 gaba<sub>A</sub> receptor subtype. *Br J Pharmacol* 1995; 115: 539-45.
- Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of gaba(a) receptors. *Nat Rev Neurosci* 2005; 6: 215-29.
- Fernandez S, Wasowski C, Paladini AC, Marder M. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from valeriana officinalis. *Pharmacol Biochem Behav* 2004; 77: 399-404.
- Froestl W. An historical perspective on GABAergic drugs. *Future Med Chem* 2011; 3: 163-75.
- Gilbert AK, Franklin KB. Gabaergic modulation of descending inhibitory systems from the rostral ventromedial medulla (rvm). Dose-response analysis of nociception and neurological deficits. *Pain* 2001; 90: 25-36.
- Heidari MR, Khalili F, Ghazi-khansari M, Hashemi B, Zarrindast MR. Effect of picrotoxin on antinociception in the formalin test. *Pharmacol Toxicol* 1996; 78: 313-6.
- Heidari MR, Razban F. Effects of valeriana officinalis extract on the seizure induced by picrotoxin in mice. *J Kerman Univ Med* 2004; 11: 6-9.
- Khayat Nouri MH, Namvaran Abbas Abad A. Gabaergic system role in aqueous extract of valeriana officinalis L. Root on ptz-induced clonic seizure threshold in mice. *Afr J Pharm Pharmacol* 2011; 5: 1212-17.
- Mahmoudi M, Zarrindast MR. Effect of intracerebroventricular injection of gaba receptor agents on morphine-induced antinociception in the formalin test. *J Psychopharmacol* 2002; 16: 85-91.
- Malan TP, Mata HP, Porreca F. Spinal gaba(a) and gaba(b) receptor pharmacology in a rat model of neuropathic pain. *Anesthesiology* 2002; 96: 1161-7.
- Mertens P, Ghaemmaghami C, Bert L, Perret-Liaudet A, Guenot M, Naous H, et al. Microdialysis study of amino acid neurotransmitters in the spinal dorsal horn of patients undergoing microsurgical dorsal root entry zone lesioning. *J Neurosurg* 2001; 94: 165-73.
- Murphy K, Kubin ZJ, Shepherd JN, Ettinger RH. Valeriana officinalis root extracts have potent anxiolytic effects in laboratory rats. *Phytomedicine* 2010; 17: 674-8.
- Newland CF, Cull-Candy SG. On the mechanism of action of picrotoxin on gaba receptor channels in dissociated sympathetic neurones of the rat. *J Physiol* 1992; 447: 191-213.
- Patočka J, Jakl J. Biomedically relevant chemical constituents of valeriana officinalis. *J Appl Biomed* 2010; 8: 11-8.
- Reis G, Pacheco D, Francischi J, Castro M, Perez A, Duarte I. Involvement of gaba A receptor-associated chloride channels in the peripheral antinociceptive effect induced by gaba A receptor agonist muscimol. *Eur J Pharmacol* 2007; 564: 112-5.
- Santos MS, Ferreira F, Cunha AP, Carvalho AP, Macedo T. An aqueous extract of valerian influences the transport of gaba in synaptosomes. *Planta Med* 1994a; 60: 278-9.
- Santos MS, Ferreira F, Cunha AP, Carvalho AP, Ribeiro CF, Macedo T. Synaptosomal gaba release as influenced by valerian root extract--involvement of the gaba carrier. *Arch Int Pharmacodyn Ther* 1994b; 327: 220-31.
- Solati J, Sanaguye Motlagh H. Anxiolytic effects of valepotriates extracted from valeriana officinalis L. In rats. *J Qazvin Univ Med Sci* 2008; 12: 63-7.
- Taherianfard M, Goudarzi S, Fazeli M. Effect of intracerebro-ventricular injection of muscimol and picrotoxin on pain sensitivity during estrous cycle. *Sci Res Iran Vet J* 2009; 5: 40-9.
- Taherianfard M, Mosavi M. Hippocampal gaba(a) receptor and pain sensitivity during estrous cycle in the rat. *Iran J Med Sci* 2011; 36: 289-95.
- Torres-Hernandez BA, Del Valle-Mojica LM, Ortiz JG. Valerenic acid and valeriana officinalis extracts delay onset of pentylenetetrazole (ptz)-induced seizures in adult danio rerio (zebrafish). *BMC Complement Altern Med* 2015; 15: 228.
- Yuan CS, Mehendale S, Xiao Y, Aung HH, Xie JT, Ang-Lee MK. The gamma-aminobutyric acidergic effects of valerian and valerenic acid on rat brainstem neuronal activity. *Anesth Analg* 2004; 98: 353-8.