IJMS
Vol 44, No 3, May 2019

Original Article

Anticonvulsant Activity of *Viola tricolor* against Seizures Induced by Pentylenetetrazol and Maximal Electroshock in Mice

Vafa Baradaran Rahimi[1], PhD;
Vahid Reza Askari[1], PhD;
Mahmoud Hosseini[2], PhD;
Bahareh Sadat Yousefsani[3], PhD;
Hamid Reza Sadeghnia[3], PhD

[1]Student Research Committee, Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;
[2]Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran;
[3]Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondence:
Hamid Reza Sadeghnia, PhD;
Pharmacological Research Center of Medicinal Plants, Faculty of Medicine, Postal code: 91779-48564, Mashhad, Iran
Tel:+98 51 38828566
Fax:+98 51 38828567
Email: sadeghniahr@mums.ac.ir
Received: 25 September 2017
Revised: 1 December 2017
Accepted: 10 December 2017

Abstract

Background: Recently, there has been much more interest in the use of medicinal plants in search of novel therapies for human neurodegenerative diseases such as epilepsy. In the present study, we investigated the anticonvulsant effects of *Viola tricolor (V. tricolor)* on seizure models induced by pentylenetetrazol (PTZ) and maximal electroshock stimulation (MES).

Methods: Totally, 260 mice were divided into 26 groups (n=10). Thirty minutes after treatment with the hydroalcoholic extract of *V. tricolor* (VHE 100, 200, and 400 mg/kg) and its ethyl acetate (EAF 50, 100, and 200 mg/kg) and *n*-butanol (NBF 50, 100, and 200 mg/kg) fractions as well as diazepam (3 mg/kg), seizure was induced by PTZ (100 mg/kg) or by MES (50 Hz, 1 s and 50 mA). Analysis was performed via ANOVA with the Tukey–Kramer post-hoc test using GraphPad Prism 6.01 (La Jolla, CA).

Results: The VHE (400 mg/kg) significantly enhanced latency to the first generalized tonic-clonic seizures (GTCs) induced by PTZ in comparison to the control group ($P<0.001$). All 3 concentrations of the EAF (50, 100, and 200 mg/kg) significantly prolonged the latency of PTZ-induced seizures compared to the control group. Additionally, all the concentrations of the NBF (50, 100, and 200 mg/kg) made a significant increment in GTCs latency induced by PTZ in comparison to the control group. On the other hand, all the concentrations of the VHE, EAF, and NBF significantly reduced the incidence of hind-limb tonic extension (HLTE) induced by MES, when compared to the control group.

Conclusion: The present study showed that *V. tricolor* and its ethyl acetate and *n*-butanol fractions possessed anticonvulsant effects as confirmed by the prolongation of latency to the first GTCs induced by PTZ and decrement in the incidence of HLTE induced by MES.

Please cite this article as: Baradaran Rahimi V, Askari VR, Hosseini M, Yousefsani BS, Sadeghnia HR. Anticonvulsant Activity of *Viola tricolor* against Seizures Induced by Pentylenetetrazol and Maximal Electroshock in Mice. Iran J Med Sci. 2019;44(3):220-226. doi: 10.30476/IJMS.2019.44977.

Keywords ● Seizures ● Viola ● Pentylenetetrazol ●Electroshock ● Mice

What's Known

- Approximately 70% of seizures could be controlled with the current anticonvulsant drugs. However, they cause several side effects, which some patients may find intolerable. Therefore, new anticonvulsant drugs with fewer side effects are required.

What's New

- The present study showed that the hydroalcoholic extract of *V. tricolor* and its ethyl acetate and *n*-butanol fractions possessed anticonvulsant effects as confirmed by the prolongation of latency to the first GTCs induced by PTZ and decrement in the incidence of HLTE induced by MES.

Introduction

Epilepsy is a neurological disease characterized by the extreme discharge of electrical activity in neurons, and it affects about 1% of the human general population.[1] This neurological disorder causes many medical complications and seriously deteriorates life quality. Patients with epilepsy frequently suffer from cognitive impairment and memory function.[2]

Many animal models have been used to study the antiepileptic properties of pentylenetetrazol (PTZ) and maximal electroshock stimulation (MES).[3] PTZ is a gamma-aminobutyric acid type A ($GABA_A$) receptor antagonist that selectively blocks the chloride channel.[4] It is well known that PTZ, at doses of 90 to 100 mg/kg, creates generalized tonic-clonic seizures (GTCs) in rodents which progress from mild myoclonic jerks to the clonus of the face and forelimbs without righting reflex loss, to the clonic seizures of the limbs with righting reflex loss, to the full tonic extension of both forelimbs and hind limbs.[5] On the other hand, electroconvulsive shock induces hind-limb tonic extension (HLTE) in approximately 99.9% of animals.[6]

Recent years have witnessed intensified focus on the use of medicinal plants with a view to finding novel therapies for human neurodegenerative diseases such as epilepsy, Alzheimer's, and Parkinson's. *Viola tricolor* L. (*V. tricolor*) is a traditional medicinal plant belonging to the Violaceae family.[7] *V. tricolor* consists of flavonoids, saponins, anthocyanins, coumarins, carotenoids, tannins, salicylic acid, and other phenolic acids.[8] Previous research has shown that *V. tricolor* possesses many pharmacological properties such as antioxidant,[9,10] anti-inflammatory,[11] anti-microbial,[12] anticancer,[13] neuroprotective, and anti-ischemic[14] activities. Furthermore, it possesses good activity to the benzodiazepine-site of the GABA receptor. Rutin, a major flavonoid component in *V. tricolor*,[7] shows sedative effects through modulating the $GABA_A$ receptor.[15] In addition, the hydroalcoholic extract of *V. tricolor* (VHE) is reported to have sleep-prolonging effects through positive allosteric modulation of the $GABA_A$ receptor.[16] There is, however, no pharmacological research in the existing literature evaluating the anticonvulsant properties of *V. tricolor.*

In the present study, we investigated the anticonvulsant effects of the VHE and its ethyl acetate (EAF) and *n*-butanol (NBF) fractions on seizure models induced by PTZ and MES.

Materials and Methods

Animals

Male albino mice weighing between 22 and 28 g were used in this study. Before the commencement of the experiments, the mice were maintained in separated standard cages in a silent and ventilated laboratory with 12-hour light/dark cycles, humidity of 61±3%, and a temperature of 22±2 ˚C. The animals were given free access to lab chow and water. Each animal was tested once. The study was executed in accordance with ethical guidelines approved by the Animal Care Use Committee of Mashhad University of Medical Sciences.

Plant Collection and Extraction

V. tricolor was collected from the Iranian province of Khorasan in July 2013 and authenticated by the Herbarium Department, Ferdowsi University of Mashhad (herbarium number: V16-2013). The VHE was prepared using the Soxhlet method. The leaves were dried in shadow and ground to fine powder with a blender. Thereafter, 100 g of the leaf powder was incubated with 70% ethanol (Merck, Darmstadt, Germany) for 48 hours.[17] The VHE was dried in a rotary evaporator apparatus at 35˚C (yield 20% w/w).

For the preparation of different fractions from the VHE, 10 g of the dried extract was suspended in 400 mL of distilled water and transferred to a separator funnel. The EAF (Merck, Darmstadt, Germany) or the NBF (Merck, Darmstadt, Germany) was isolated using solvent-solvent extraction.[18] All of the fractions were dried in the rotary evaporator apparatus at 35˚C and kept at -20˚C until use. Subsequently, the EAF was dissolved in saline containing 1% Tween 20 (Sigma-Aldrich, St. Louis, MO) and the NBF was dissolved in saline containing 1% DMSO (Sigma-Aldrich, St. Louis, MO).

Study Design

A total of 260 male albino mice were divided into 26 groups (n=10 each). All the experiments were designed to include saline as the control group, 3 single doses of VHE groups (100, 200, and 400 mg/kg), 3 single doses of EAF groups (50, 100, and 200 mg/kg), 3 single doses of NBF groups (50, 100, and 200 mg/kg), and diazepam (DZP, Tolidaru Pharmaceuticals, Tehran, Iran) (3 mg/kg) as the positive control all via intraperitoneal administration (table 1).

PTZ Seizure Model

Thirty minutes after treatment with saline, VHE, EAF, NBF, and diazepam, the induction of seizure was done via an intraperitoneal administration of PTZ (Sigma-Aldrich, St. Louis, MO) (100 mg/kg) and latency to the first GTCs with righting reflex loss was recorded within the next 1 hour (table 1).

MES Model

Thirty minutes after treatment with saline, VHE, EAF, NBF, and diazepam, the induction of seizure was performed via electrical stimuli (50 Hz, 1 s and 50 mA) through ear-clip electrodes using a stimulator apparatus

Table 1: Study design for evaluating the anticonvulsant activity of *V. tricolor* against seizures induced by PTZ and MES in mice

Group	Treatment
1	Saline (Control)+PTZ (100 mg/kg)
2	Saline containing 1% Tween 20+PTZ (100 mg/kg)
3	Saline containing 1% DMSO+PTZ (100 mg/kg)
4-6	VHE (100, 200 and 400 mg/kg)+PTZ (100 mg/kg)
7-9	EAF (50, 100 and 200 mg/kg)+PTZ (100 mg/kg)
10-12	NBF (50, 100 and 200 mg/kg)+PTZ (100 mg/kg)
13	DZP (5 mg/kg)+PTZ (100 mg/kg)
14	Saline (Control)+MES
15	Saline containing 1% Tween 20+MES
16	Saline containing 1% DMSO+MES
17-19	VHE (100, 200 and 400 mg/kg)+MES
20-22	EAF (50, 100 and 200 mg/kg)+MES
23-25	NBF (50, 100 and 200 mg/kg)+MES
26	DZP (5 mg/kg)+MES

PTZ: Pentylenetetrazol; VHE: Hydroalcoholic extract of *V. tricolor*; EAF: Ethyl acetate extract of *V. tricolor*; NBF: *n*-butanol Extract of *V. tricolor*; DZP: Diazepam; MES: Maximal electroshock stimulation

(MGH-777, Development of Electronic Industry, Iran). The incidence of the animals exhibiting HLTE in each group was recorded within the next 1 hour (table 1).[19]

Statistical Analysis

The statistical analyses were performed via one-way analysis of variance (ANOVA) with the Tukey–Kramer post-hoc test and the Fisher exact probability test using GraphPad Prism 6.01 (La Jolla, CA). A P value less than 0.05 was considered statistically significant. Moreover, the homogeneity and normality were checked using both the Brown–Forsythe test and the Bartlett test.

Results

Homogeneity and Normality of the Data

Regarding the evaluation of the normality and homogeneity of the data, both of the Brown–Forsythe test and the Bartlett test showed no significantly different standard deviation across the groups, which meant that the assumption of equal variances was true (data not shown).

V. tricolor and Its Fractions Significantly Increased GTCs Latency Induced by PTZ

Diazepam (5 mg/kg) significantly increased GTCs latency induced by PTZ when compared to the control group (P<0.001) (figure 1A). As is illustrated in figure 1A, the VHE (400 mg/kg) significantly enhanced GTCs latency induced by PTZ in comparison to the control group (P<0.001). The anticonvulsant effects at all 3 concentrations of the VHE (100, 200, and 400 mg/kg) were significantly lower than those of diazepam (P<0.001).

All 3 concentrations of the EAF (50, 100, and 200 mg/kg) significantly prolonged the latency of PTZ-induced seizures compared to the control group (P=0.035, P<0.001, and P<0.001, respectively) (figure 1B). The effects of diazepam on GTCs latency were markedly greater than those of the EAF concentrations (50, 100, and 200 mg/kg) (P<0.001) (figure 1B). The control group and the control+Tween group did not show any significant differences (figure 1B).

As is shown in figure 1C, all 3 concentrations of the NBF (50, 100, and 200 mg/kg) made a significant increment in GTCs latency induced by PTZ, in comparison to the control group (P=0.0441, P<0.001, and P<0.001, correspondingly). In comparison to the diazepam group, latency to PTZ-induced seizure was significantly lower in the NBF groups (50, 100 and 200, mg/kg) (P<0.001). The control group and the control+DMSO group did not represent any significant differences (P=0.998) (figure 1C).

V. tricolor and Its Fractions Significantly Reduced the HLTE Incidence Induced by MES

As is illustrated in figure 2, diazepam conferred complete protection against MES-induced seizure (P<0.001). The VHE (100, 200, and 400 mg/kg) significantly diminished the HLTE incidence when compared to the control group (P=0.034, P<0.001, and P<0.001, respectively) and these effects were significantly lower than those of diazepam (P<0.001) (figure 2A).

All 3 concentrations of the EAF (50, 100, and 200 mg/kg) significantly reduced the HLTE incidence in comparison with the control group (P=0.038, P<0.001, and P<0.001, respectively) (figure 2B). The effects of diazepam on the HLTE incidence were markedly greater than those of the EAF concentrations (50, 100, and 200 mg/kg) (P<0.001) (figure 2B). The control group and the control+Tween group showed no significant differences (figure 2B).

As is illustrated in figure 2C, all 3 concentrations of the NBF (50, 100, and 200 mg/kg) made a significant attenuation in the HLTE incidence induced by MES when compared to the control group (P<0.001). The anticonvulsant effects of the NBF (50, 100, and 200 mg/kg) were significantly lower than those in the diazepam group (P<0.001, P=0.0045, and P=0.047, correspondingly). The control group and the control+DMSO group did not represent any significant differences (P>0.999) (figure 2C).

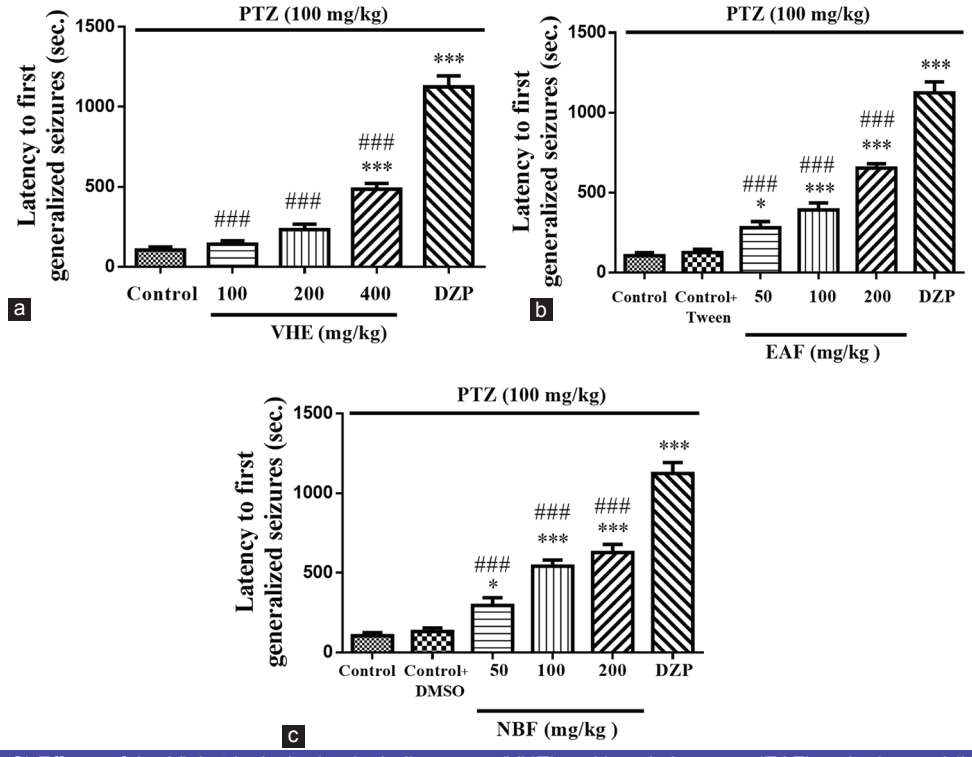

Figure 1: (A-C) Effects of the *Viola tricolor* hydroalcoholic extract (VHE) and its ethyl acetate (EAF) and *n*-butanol (NBF) fractions on latency to the first generalized tonic-clonic seizures (GTCs) induced by pentylenetetrazol (PTZ) (100 mg/kg). Data are presented as mean±SEM. *P<0.05, ***P<0.001 as compared to the control group, ###P<0.001 as compared to the diazepam group.

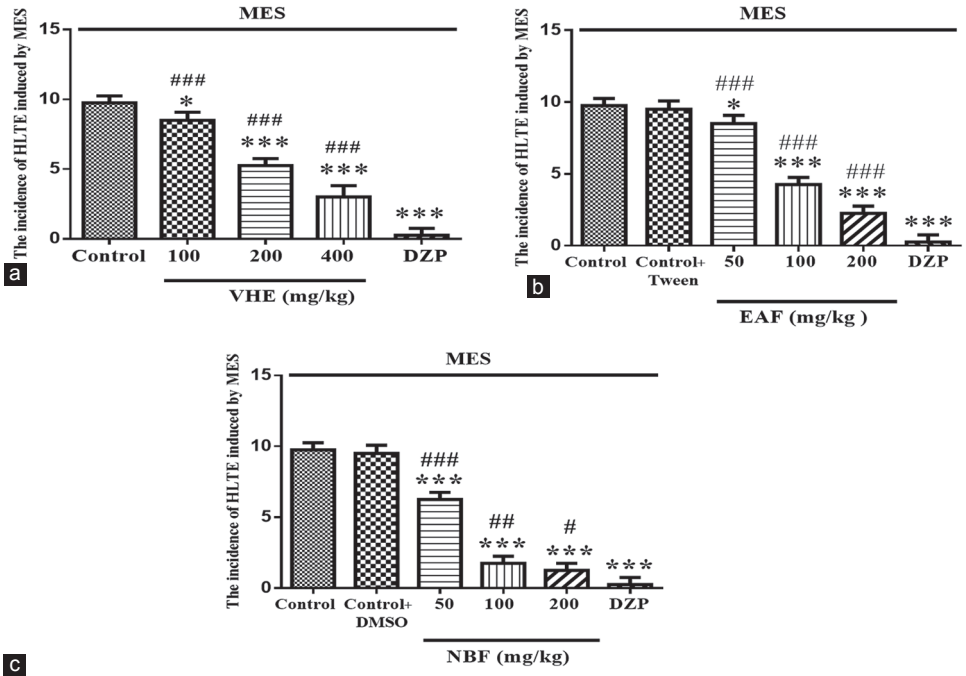

Figure 2: (A-C) Effects of the *Viola tricolor* hydroalcoholic extract (VHE), and its ethyl acetate (EAF) and n-butanol (NBF) fractions on the incidence of hind-limb tonic extension (HLTE) induced by maximal electroshock stimulation (MES) at 1 hour after treatment. Results are expressed as mean±SEM. *** P<0.001 as compared to the control group, ###P<0.001 as compared to the diazepam group.

Discussion

To our knowledge, this is the first study on the anticonvulsant activity of *V. tricolor*. In the present study, we determined the effects of the aerial parts of the VHE and its fractions, namely the EAF and the NBF, on PTZ- and MES-induced seizure in mice. The results revealed that the

VHE ameliorated seizure induced by PTZ and MES by the prolongation of the latency to the first GTCs and decrement in the incidence of HLTE.

Approximately 70% of seizures could be controlled with the current anticonvulsant drugs. Nevertheless, they cause several side effects, which some patients may find intolerable.[20]

Consequently, new anticonvulsant drugs with fewer side effects are required. There are 2 groups of drugs that prevent tonic extension induced by MES: (1) voltage-dependent Na channel inhibitors such as phenytoin, sodium valproate, felbamate, and lamotrigine and (2) glutaminergic receptor blockers such as felbamate. On the other hand, PTZ-induced seizures can be prevented by 2 other groups of drugs: (1) $GABA_A$ agonists including benzodiazepines and phenobarbital and (2) drugs that reduce T-type Ca^{2+}currents such as ethosuximide.[19]

Seizures are caused by an imbalance between the excitatory and inhibitory neurotransmitter systems such as glutamate and GABA, respectively. GABA is a major inhibitory neurotransmitter in the brain, which can be modulated by benzodiazepine allosteric modulators such as diazepam, and PTZ inhibits the neurotransmitter activity through opposing the function of the $GABA_A$ receptors.[21] According to our results, diazepam (5 mg/kg) significantly increased latency to the first GTCs induced by PTZ and decreased the incidence of HLTE induced following MES, when compared to the control group. The incidence of HLTE for the diazepam group was approximately zero, which meant that diazepam was able to completely inhibit seizures induced by MES. Many other studies have also shown the mitigating effects of benzodiazepines on seizures,[22,23] which supports our results.

The VHE prolonged latency to the first GTCs induced by PTZ in a concentration-dependent manner. Only 400 mg/kg of the VHE conferred significant protection. Nevertheless, all 3 concentrations of the VHE significantly diminished the incidence of HLTE induced by MES. The anticonvulsant effects of diazepam were stronger than those of the VHE. To further investigate the composition responsible for the anticonvulsant effects of *V. tricolor*, we prepared 2 fractions from the hydroalcoholic extract: (1) the EAF, containing intermediate polarity constituents such as flavonoids, and (2) the NBF, accumulating non-polar agents such as alkanes, sterols, and some terpenoids.[24] Our results illustrated that the EAF and the NBF both possessed good anticonvulsant effects. The EAF and the NBF at all 3 concentrations significantly raised latency to the first GTCs and also reduced the incidence of HLTE induced by MES. However, the anticonvulsant effects of diazepam were markedly superior to those of the EAF and the NBF. Both of the fractions, especially the NBF, showed better results than the VHE. Therefore, it seems that intermediate polar and mostly non-polar compounds of *V. tricolor* are responsible for the anticonvulsant effects of this plant. The presence of flavonoids and phenolic compounds such as kaempferol, luteolin, violanthin, quercetin, and rutin in *V. tricolor* has been reported previously.[25] In addition, it has been demonstrated that flavonoids and flavonoid-enriched extracts can possess anticonvulsant, sedative, or anxiolytic effects via the modulation of $GABA_A$ receptors.[24, 26] Moreover, it has been determined that intra-cerebroventricular administration of rutin mitigates minimal clonic seizures and GTCs induced by PTZ in a concentration-dependent manner. Furthermore, the anticonvulsant effects of rutin can be reversed by flumazenil. It has been assumed that rutin has anticonvulsant effects in the brain, possibly through positive allosteric modulation of the $GABA_A$ receptor complex.[15] A previous study demonstrated that quercetin (50 mg/kg), as one of the constituents of *V. tricolor*, was able to reduce seizure severity in a PTZ-induced model through a decrement in the mean seizure stages.[27] Both acute and chronic administrations of quercetin (25 or 50 mg/kg) attenuate the ethanol withdrawal-induced reduction in PTZ seizure threshold in mice.[28] It has also been demonstrated that *V. betonicifolia* possesses good anticonvulsant activity. A previous investigation reported that the *n*-hexane fraction of *V. betonicifolia* (400 and 500 mg/kg i.p.) significantly increased the latency to the first GTCs induced by PTZ.[29]

The current study is the first investigation of its kind to evaluate the anticonvulsive effects of *V. tricolor* through a simple method using PTZ and MES; nonetheless, we suggest that future studies be conducted with accurate procedures to confirm the antiepileptic and anticonvulsive effects of the plant. A salient limitation of the present study is that we did not examine the cytotoxicity of the plant. (It should be noted, however, that we performed this examination in our previous study.)[13] Another drawback is that we performed no standardization with high performance liquid chromatography or total phenolic content.

Conclusion

The present study showed that the VHE and its fractions, namely the EAF and the NBF, possessed anticonvulsant effects through the

prolongation of latency to the first GTCs induced by PTZ and decrement in the incidence of HLTE induced by MES. The isolation of the accurate active compound(s) from these fractions may yield a novel anticonvulsant agent.

Acknowledgment

This study was financially supported by the Research Council of Mashhad University of Medical Sciences.

Conflict of Interest: None declared.

References

1. Ullah I, Badshah H, Naseer MI, Lee HY, Kim MO. Thymoquinone and vitamin C attenuates pentylenetetrazole-induced seizures via activation of GABAB1 receptor in adult rats cortex and hippocampus. Neuromolecular Med. 2015;17:35-46. doi: 10.1007/s12017-014-8337-3. PubMed PMID: 25429759.
2. Realmuto S, Zummo L, Cerami C, Agro L, Dodich A, Canessa N, et al. Social cognition dysfunctions in patients with epilepsy: Evidence from patients with temporal lobe and idiopathic generalized epilepsies. Epilepsy Behav. 2015;47:98-103. doi: 10.1016/j.yebeh.2015.04.048. PubMed PMID: 25982884.
3. Cavarsan CF, Matsuo A, Blanco MM, Mello LE. Maximal electroshock-induced seizures are able to induce Homer1a mRNA expression but not pentylenetetrazole-induced seizures. Epilepsy Behav. 2015;44:90-5. doi: 10.1016/j.yebeh.2014.12.034. PubMed PMID: 25659045.
4. Khoshnoud MJ, Tanideh N, Namdarian S. Anticonvulsant activity of atorvastatin against seizure induced by pentylenetetrazole and maximal electroshock in mice. Trends in Pharmaceutical Sciences. 2015;1:44-7.
5. Anaeigoudari A, Hosseini M, Karami R, Vafaee F, Mohammadpour T, Ghorbani A, et al. The effects of different fractions of Coriandrum sativum on pentylenetetrazole-induced seizures and brain tissues oxidative damage in rats. Avicenna J Phytomed. 2016;6:223-35. PubMed PMID: 27222836; PubMed Central PMCID: PMCPMC4877964.
6. Sayyah M, Moaied S, Kamalinejad M. Anticonvulsant activity of Heracleum persicum seed. J Ethnopharmacol. 2005;98:209-11. doi: 10.1016/j.jep.2004.12.026. PubMed PMID: 15763386.
7. Vukics V, Kery A, Bonn GK, Guttman A. Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. Anal Bioanal Chem. 2008;390:1917-25. doi: 10.1007/s00216-008-1885-3. PubMed PMID: 18259733.
8. Gonçalves AFK, Friedrich RB, Boligon AA, Piana M, Beck RCR, Athayde ML. Anti-oxidant capacity, total phenolic contents and HPLC determination of rutin in Viola tricolor (L) flowers. Free Radicals and Antioxidants. 2012;2:32-7. doi: 10.5530/ax.2012.4.6.
9. Piana M, Zadra M, de Brum TF, Boligon AA, Goncalves AF, da Cruz RC, et al. Analysis of rutin in the extract and gel of Viola tricolor. J Chromatogr Sci. 2013;51:406-11. doi: 10.1093/chromsci/bms155. PubMed PMID: 23035206.
10. Vukics V, Kery A, Guttman A. Analysis of polar antioxidants in Heartsease (*Viola tricolor* L.) and Garden pansy (Viola x wittrockiana Gams.). J Chromatogr Sci. 2008;46:823-7. PubMed PMID: 19007486.
11. Hellinger R, Koehbach J, Fedchuk H, Sauer B, Huber R, Gruber CW, et al. Immunosuppressive activity of an aqueous Viola tricolor herbal extract. J Ethnopharmacol. 2014;151:299-306. doi: 10.1016/j.jep.2013.10.044. PubMed PMID: 24216163; PubMed Central PMCID: PMCPMC3918579.
12. Khoshkam Z, Zarrabi M, Sepehrizade Z, Keshavarzi M. The Study of Antimicrobial Activities of Partially Purified Cyclotide Content and Crude Extracts from Viola tricolor. J Med Bacteriol. 2016;5:29-35.
13. Sadeghnia HR, Ghorbani Hesari T, Mortazavian SM, Mousavi SH, Tayarani-Najaran Z, Ghorbani A. Viola tricolor induces apoptosis in cancer cells and exhibits antiangiogenic activity on chicken chorioallantoic membrane. Biomed Res Int. 2014;2014:625792. doi: 10.1155/2014/625792. PubMed PMID: 25243166; PubMed Central PMCID: PMCPMC4163403.
14. Mousavi SH, Naghizade B, Pourgonabadi S, Ghorbani A. Protective effect of *Viola tricolor* and Viola odorata extracts on serum/glucose deprivation-induced neurotoxicity: role of reactive oxygen species. Avicenna J Phytomed. 2016;6:434-41. PubMed PMID: 27516984; PubMed Central PMCID: PMCPMC4967839.
15. Nassiri-Asl M, Shariati-Rad S, Zamansoltani F. Anticonvulsive effects of intracerebroventricular administration of rutin in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32:989-93. doi:

10.1016/j.pnpbp.2008.01.011. PubMed PMID: 18262708.
16. Ghorbani A, Youssofabad NJ, Rakhsh H. Effect of Viola tricolor on pentobarbital-induced sleep in mice. Afr J Pharm Pharmacol. 2012;6:2503-9. doi: 10.5897/AJPP12.721.
17. Panday DR, Rauniar GP. Effect of root-extracts of Ficus benghalensis (Banyan) in memory, anxiety, muscle co-ordination and seizure in animal models. BMC Complement Altern Med. 2016;16:429. doi: 10.1186/s12906-016-1413-5. PubMed PMID: 27809820; PubMed Central PMCID: PMCPMC5094015.
18. Rahimi VB, Askari VR, Emami SA, Tayarani-Najaran Z. Anti-melanogenic activity of Viola odorata different extracts on B16F10 murine melanoma cells. Iran J Basic Med Sci. 2017;20:242-9. doi: 10.22038/ijbms.2017.8350. PubMed PMID: 28392894; PubMed Central PMCID: PMCPMC5378959.
19. Sayyah M, Mandgary A, Kamalinejad M. Evaluation of the anticonvulsant activity of the seed acetone extract of Ferula gummosa Boiss. against seizures induced by pentylenetetrazole and electroconvulsive shock in mice. J Ethnopharmacol. 2002;82:105-9. PubMed PMID: 12241984.
20. de Almeida RN, Agra Mde F, Maior FN, de Sousa DP. Essential oils and their constituents: anticonvulsant activity. Molecules. 2011;16:2726-42. doi: 10.3390/molecules16032726. PubMed PMID: 21441872.
21. Vollenweider F, Bendfeldt K, Maetzler W, Otten U, Nitsch C. GABA(B) receptor expression and cellular localization in gerbil hippocampus after transient global ischemia. Neurosci Lett. 2006;395:118-23. doi: 10.1016/j.neulet.2005.10.079. PubMed PMID: 16298486.
22. Kumar A, Lalitha S, Mishra J. Hesperidin potentiates the neuroprotective effects of diazepam and gabapentin against pentylenetetrazole-induced convulsions in mice: Possible behavioral, biochemical and mitochondrial alterations. Indian J Pharmacol. 2014;46:309-15. doi: 10.4103/0253-7613.132180. PubMed PMID: 24987179; PubMed Central PMCID: PMCPMC4071709.
23. Chweh A, Ulloque R, Swinyard E. Antipentylenetetrazol activity of diazepam: A site of action. Drug Dev Res. 1986;9:259-65. doi: 10.1002/ddr.430090403.
24. Askari VR, Baradaran Rahimi V, Ghorbani A, Rakhshandeh H. Hypnotic Effect of Ocimum basilicum on Pentobarbital-Induced Sleep in Mice. Iran Red Crescent Med J. 2016;18:e24261. doi: 10.5812/ircmj.24261. PubMed PMID: 27651944; PubMed Central PMCID: PMCPMC5020426.
25. Toiu A, Muntean E, Oniga I, Vostinaru O, Tamas M. Pharmacognostic research on Viola tricolor L. (Violaceae). Rev Med Chir Soc Med Nat Iasi. 2009;113:264-7. PubMed PMID: 21491816.
26. Jager AK, Saaby L. Flavonoids and the CNS. Molecules. 2011;16:1471-85. doi: 10.3390/molecules16021471. PubMed PMID: 21311414.
27. Nassiri-Asl M, Moghbelinejad S, Abbasi E, Yonesi F, Haghighi MR, Lotfizadeh M, et al. Effects of quercetin on oxidative stress and memory retrieval in kindled rats. Epilepsy Behav. 2013;28:151-5. doi: 10.1016/j.yebeh.2013.04.019. PubMed PMID: 23747498.
28. Joshi D, Naidu PS, Singh A, Kulkarni SK. Protective effect of quercetin on alcohol abstinence-induced anxiety and convulsions. J Med Food. 2005;8:392-6. doi: 10.1089/jmf.2005.8.392. PubMed PMID: 16176153.
29. Muhammad N, Saeed M, Khan H, Raziq N, Halimi SM, Abass M. Antipyretic and anticonvulsant activity of n-hexane fraction of Viola betonicifolia. Asian Pac J Trop Biomed. 2013;3:280-3. doi: 10.1016/S2221-1691(13)60063-5. PubMed PMID: 23620851; PubMed Central PMCID: PMCPMC3634924.