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Research Paper

Protective Effect of Vanillic Acid Against Pentylenetetrazole -Induced Convulsions in Male Rats

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ABSTRACT

Epilepsy is a serious and widespread neurological disorder characterized by recurrent seizures caused by excessive electrical activity of the brain. The aim of this study was to investigate the effect of vanillic acid (VA) on generalized convulsions in rats. To evaluate the anticonvulsant effect of VA, we used pentylenetetrazol (PTZ), a standard method for analyzing convulsions. Fifty-six adult male Wistar rats (200±20 g) were randomly divided randomly into 7 groups: Control (received PTZ and VA as vehicle); PTZ (80 mg/kg, I.P); PTZ+VA25, 50, 100 and 200 (received VA at doses of 25, 50, 100 and 200 mg/kg, respectively) and PTZ+PHB (received phenobarbital 80 mg/kg). VA or normal saline was administered 30 minutes before induction of PTZ convulsion. Immediately after PTZ administration, the following was observed in the rats: (1) Latency to onset of convulsions, (2) Number of convulsions and (3) death for the period of 60 min and convulsion behavior. Pain, MDA, SOD and GPx were assessed in each group. The results of the present study showed that VA had an anticonvulsant and analgesic effect in rats with PTZ-induced convulsions. Also, MDA level decreased after VA administration, and GPx and SOD activities were increased by VA in PTZ-induced rats. The results of the current study suggest that VA may have an anticonvulsant effect by inhibiting and/or reducing PTZ -induced seizures in the rats used by increasing or somewhat impairing GABAergic neurotransmission.

Keywords Convulsion, Oxidative stress, Pain, Pentylenetetrazol, Vanillic acid

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Introduction

Epilepsy is a common chronic neurological disorder characterized by recurrent seizures caused by excessive electrical activity of the brain. The medical treatment of epilepsy consists mainly of the prevention of seizure activity by antiepileptic drugs. Antiepileptic drugs prevent seizures in about two thirds of patients [1,2].

The pharmacological treatment of seizures is primarily based on the modulation of voltage-gated ion channels, the enhancement of GABAergic inhibition and the reduction of glutamatergic excitation[3]. All currently available antiepileptic drugs are synthetic molecules[4]. Despite the optimal use of available antiepileptic drugs (AEDs), many epilepsy patients are unable to control their seizures. In addition, many patients suffer from the severe side effects of chronic treatment, which may include chronic toxicity, cognitive impairment, sedation and teratogenesis[5]. Therefore, the search for newer, more effective and safer neuroprotective agents for the treatment of epilepsy should continue. It has been scientifically proven that medicinal plants used in traditional medicine for the treatment of epilepsy have promising anticonvulsant effects in animal models, so screening for anticonvulsant effects can be performed[6].

VA (4-hydroxy-3-methoxybenzoic acid) is a phenolic derivative from edible plants and fruits and has antibacterial[7], antimicrobial[8], antifilarial[9] and hepatoprotective properties[10]. VA is a benzoic acid derivative used as a flavoring agent. It is an oxidized form of vanillin, which is formed during the conversion of vanillin to ferulic acid [11, 12]. The largest amount of VA in plants has been found in the roots of Angelica sinensis, which is used in traditional Chinese medicine [13].

In this study, we aimed to evaluate the efficacy of VA on generalized seizures in rats. To evaluate the anticonvulsant activity of VA, we used PTZ, a standard method for analyzing the efficacy of antiepileptic drugs in generalized absence type epilepsy.

Materials and Methods

Drugs and test kits

Pentylenetetrazole (PTZ) and vanillic acid (VA) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Phenobarbital sodium was purchased from Daroupakhsh Company (Tehran, Iran). Ketamine and xylazine were purchased from Alfasan Company (Woerden, Holland). Kits for glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) were purchased from Zellbio (Zellbio, Germany).

Experimental design

Fifty-six adult male Wistar rats (200±20 g) were obtained from the central animal laboratory of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. The rats were maintained in an animal house at constant temperature (22 ± 2°C) and humidity (55-60%) in a 12:12h light-dark cycle and were fed food pellets and water, ad libitum. All experiments were conducted during the light phase of the cycle (between 8:00 am and 5:00 pm) and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with the approval of the AJUMS Animal Care and Use Committee (ethics code: IR.AJUMS.REC.1394.668). We attempted to minimize stress to the animals and reduce the number of rats used in this study. All animals were treated for 1 day (5 min) prior to the experiments. The animals were randomly divided into 7 groups (n = 8):

1. Control; rats received vehicle of PTZ (0.5 ml normal saline, i.p.) and vehicle of VA (0.5 ml normal saline containing DMSO, i.p.).
2. PTZ; rats injected PTZ (80 mg/kg, I.P) and vehicle of VA (normal saline containing DMSO, i.p.).
3. PTZ+VA25; rats injected PTZ (80 mg/kg, i.p.) and VA (25 mg/kg, i.p.).
4. PTZ+VA50; rats injected PTZ (80 mg/kg, i.p.) and VA (50 mg/kg, i.p.).
5. PTZ+VA100; rats injected PTZ (80 mg/kg, i.p.) and VA (100 mg/kg, i.p.).
6. PTZ+VA200; rats injected PTZ (80 mg/kg, i.p.) and VA (200 mg/kg, i.p.).
7. PTZ+PHB (Positive control); rats injected PTZ (80 mg/kg, i.p.) and Phenobarbital (80 mg/kg, i.p.).

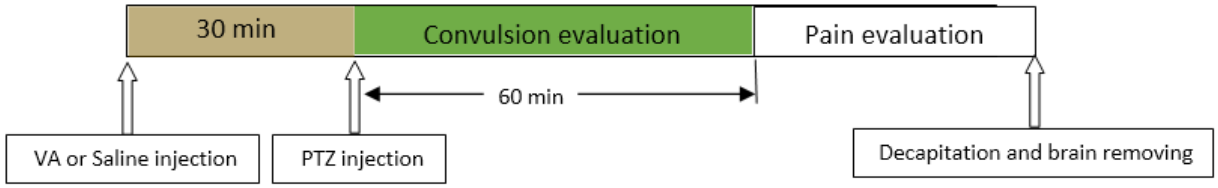

Figure 1. Timing of the study

In the PTZ-treated groups VA or normal saline was administered 30 minutes before the induction of the PTZ-convulsion. The treatment schedule and the intervals for estimating the different parameters are shown in Figure 1.

VCollection of brain samples

At the end of the experiments, the rats were deeply anesthetized with ketamine and xylazine (90 mg/kg and 20 mg/kg, respectively) and then decapitated. The brain was quickly removed, rinsed with normal saline and frozen. All samples were stored at -80 ℃ until further processing.

Lipid peroxidation (LPO) assay

Tissues were homogenized in cold KCl solution (1.5%) to obtain a 10% homogenate suspension, which was used to measure thiobarbituric acid reactive substances (TBARS) values expressed as malondialdehyde (MDA) equivalents. TBARS levels, an index of LPO generated by free radicals, were measured. MDA reacts with TBA to form a red colored complex that has the highest absorbance at 532 nm. Briefly, 3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) were added to 0.5 ml homogenate in a centrifuge tube and the mixture was heated in a boiling water bath for 45 minutes. After cooling, 4 ml of n-butanol was added to the mixture and mixed with the shaker for 1 min. Centrifugation was then carried out at 2000×g for 20 min. The colored supernatant was transferred to a new tube and its absorbance was read at 532 nm. The TBARS content was determined using 1,1,3,3-tetramethoxypropane as a standard. The standard curve of MDA was created for the concentration range of 0–20 µM[14].

Glutathione peroxidase (GPx) activity assay

GPx activity was measured using the colorimetric assay kit for glutathione peroxidase activity (Zellbio, Germany). One unit is defined as the amount of enzyme that oxidizes 1 µmol of NADPH to $NADP^+$ per ^{minute} under the conditions of the assay kit at 25℃.

Superoxide dismutase (SOD) activity assay

SOD activity was measured using the superoxide dismutase (SOD) activity assay kit (Zellbio, Germany).

Tail-flick test

The latency for tail-flick (tail retraction) was determined using the standard tail-flick test [15]. Rats were placed on the analgesia meter (M.T 9500, Borj Sanat Co. Tehran-Iran) equipped with an infrared heat source directed at three different parts of the tail 4 to 7 cm from the distal end (at 3-min intervals), and the mean value was chosen as the latency. When the animal wagged its tail, a photocell was activated, and the time between activation of the heat source and the tail wagging latency was recorded as an index of pain sensitivity. The stimulus intensity was adjusted so that the latency for tail flicking was 3 to 4 sec; to avoid tissue injury, a cut-off time of 10 sec was used. One day before the test, the animals were allowed to habituate to the apparatus, for two min[16].

PTZ test

Convulsions were induced by intraperitoneal administration of 80 mg/kg PTZ. The maximum seizure was precisely defined by tonic extension of the hind limbs at an angle of nearly 180° to the plane of the body axis. VA was administered at doses of 25, 50,100 and 200 mg/kg 30 minutes prior to PTZ injection. Immediately after PTZ administration , the following was observed in the rats: (1) onset of convulsions (elapsed time from PTZ injection to onset of convulsion), (2) duration of convulsion (number of rats showing convulsions), and (3) death during the 60 -minute period and convulsive behavior[17].

Epileptic behaviors

Behavioral convulsions were categorized as follows: Class I, hypoactivity and oral and facial automatisms; Class II, head nodding and chewing; Class III, forelimb

clonus without rearing; Class IV, bilateral forelimb clonus and rearing; Class V, rearing and loss of posture. The rats were observed constantly for 60 minutes after PTZ injection for the occurrence of limbic seizures and status epilepticus. The latency to the first occurrence of convulsive behavior (forelimb clonus) and the latency to the onset of status epilepticus were also recorded[18].

Tail flick test

Latencies to respond to a heat stimulus were measured using Tail flick apparatus (Borj Sanat, Tehran, Iran, 2012). The animals were kept in Plexiglas restraints throughout the experiment. A medium intensity light beam (40% of maximum intensity) was then shone on the rat's tail when the start button was pressed. The irradiation was quickly stopped by a flexor reflex of the tail over a light sensor. The interval between the resumption of light emission and the flexor reflex of the tail was recorded by the automatic timer as tail flick latency. Irradiation was automatically terminated after 10 seconds (cut-off time) when the animal no longer responded to the pain stimulus. The tail flick test was performed three times for each animal with an interval of 5 minutes between the two tests. The average of the latencies for each rat was calculated[19].

Statistics

The results were expressed as mean values ± SEM. Statistical analysis was performed using Graphpad Prism software (Ver. 6) and one-way ANOVA followed by Tukey's post hoc test for comparisons between groups. The Kruskal-Wallis test was used for the convulsion scale data. A p-value of less than 0.05 was considered a significant difference.

Results

Epileptic behaviors

VA at all doses significantly reduced the duration of convulsions compared to the PTZ group [Figure 2, p<0.001]. The standard antiepileptic drug phenobarbital sodium (80 mg/kg) was completely effective against PTZ-induced seizures compared to the PTZ group [Figure 2, p<.001]. VA at a dose of 200 mg/kg significantly delayed the onset of convulsions compared to the PTZ group [Figure 3, p<0.01]. The administration of VA had no effect on the extent of convulsions in the different groups compared to the PTZ group [Figure 4]. In addition, VA at a dose of 50 mg/kg reduced the number of convulsions compared to the PTZ group (Figure 5, p<0.01). Other VA doses had no effect on the number of convulsions [Figure 5].

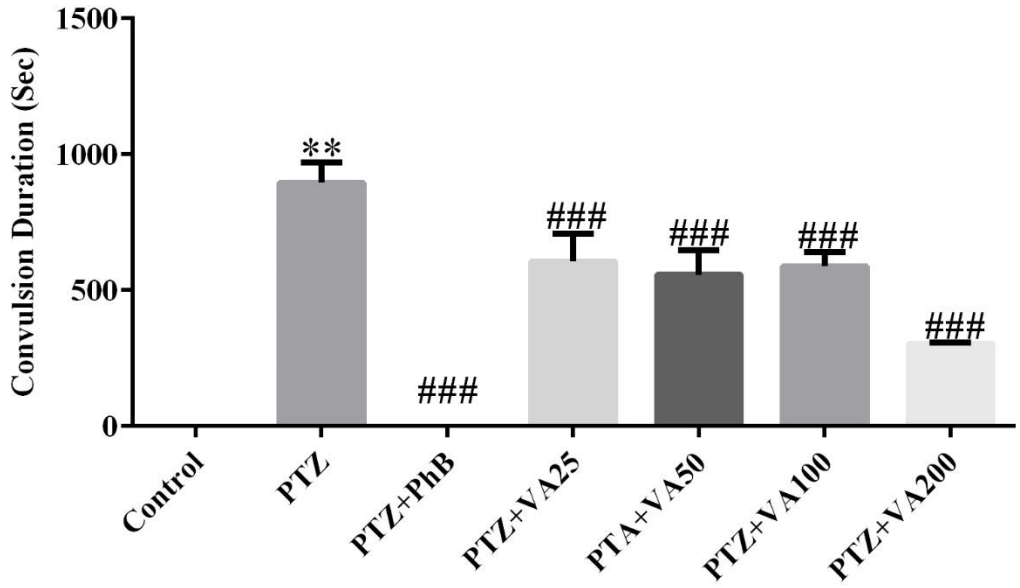

Fig. 2. Effects of VA on convulsion duration in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. **p<0.05 vs. control group, ###p<0.001 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

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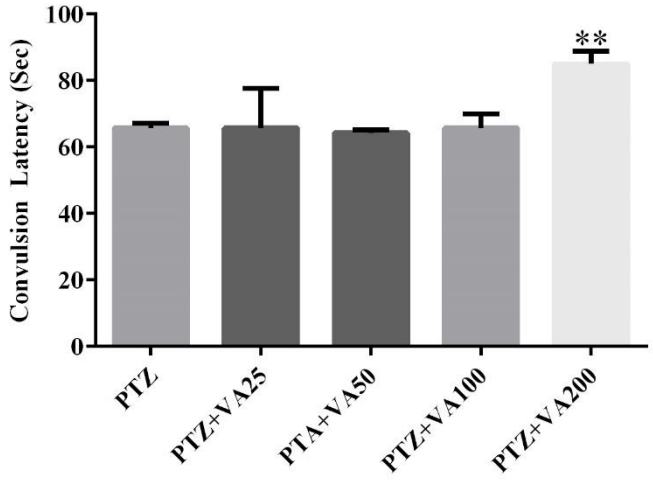

Fig. 3. Effects of VA on convulsion latency in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. **p<0.05 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

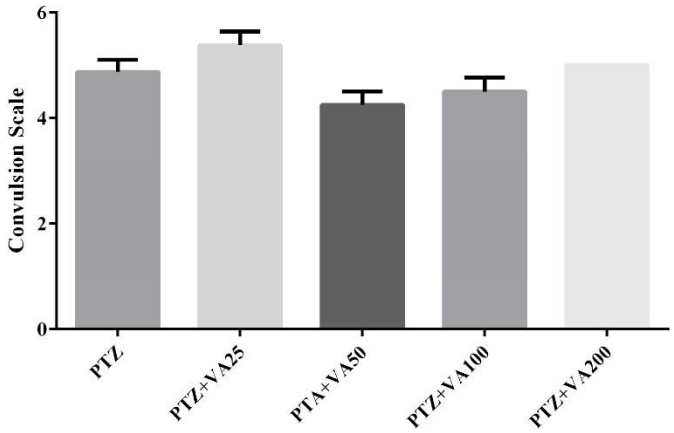

Fig. 4. Effects of VA on convulsion scale in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by Kruskal-Wallis test. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

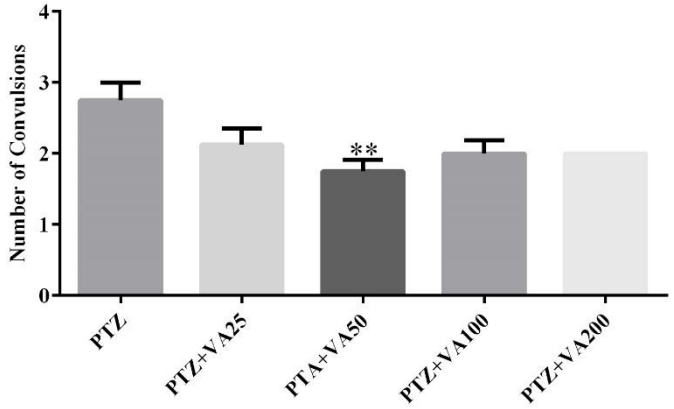

Fig. 5. Effects of VA on number of convulsions in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. **p<0.05 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

Tail-flick test

Figure 6 shows a significant reduction in the latency of tail flicking in the PTZ group compared to the control group (P<0.01). in contrast, treatment of the animals with phenobarbital or various doses of VA significantly increased the latency of tail flick latency compared to the PTZ group (p<0.001).

Biochemical analysis

PTZ-induced seizures significantly increased the MDA content, and treatment with VA at a dose of 100 and 200 mg/kg reduced the MDA content compared to the PTZ group [p<0.001, Figure 7]. Animals treated with PTZ showed a decrease in GPx activity, which was restored by VA at a dose of 100 mg/kg [p<0.05, Figure 8]. Other VA doses (25, 50 and 200 mg/kg) had no effect on GPx activity compared to the PTZ group [Figure 8]. PTZ also caused a decrease in SOD activity [p<0.05, Figure 9], and VA at a dose of 200 mg/kg prevented this effect [P <0.001, Figure 9]. VA at a dose of 25, 50 and 100 mg/kg failed to restore SOD activity [Figure 9].

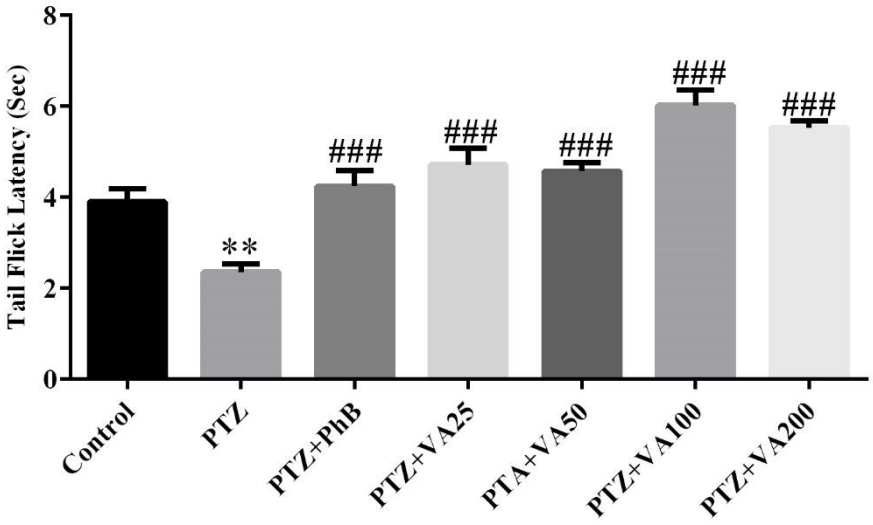

Fig. 6. Effects of VA on tail flick latency in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. **p<0.01 vs. control group, ###p<0.001 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

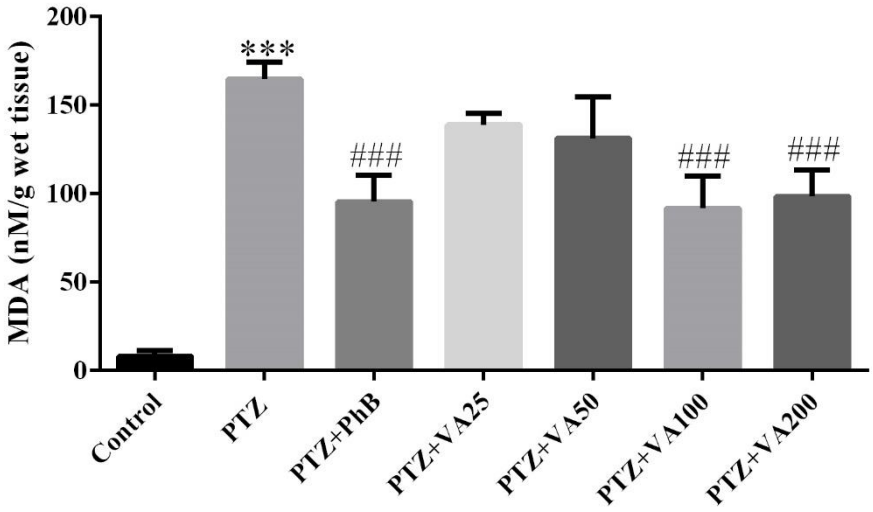

Fig. 7. Effects of VA on MDA level in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***p<0.001 vs. control group, ###p<0.001 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

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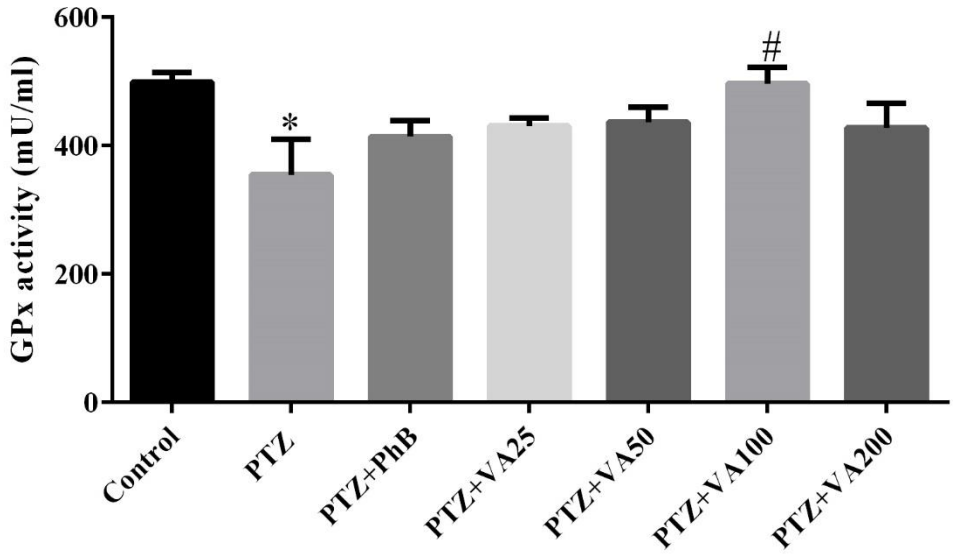

Fig. 8. Effects of VA on GPx activity in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. *p<0.05 vs. control group, #p<0.05 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

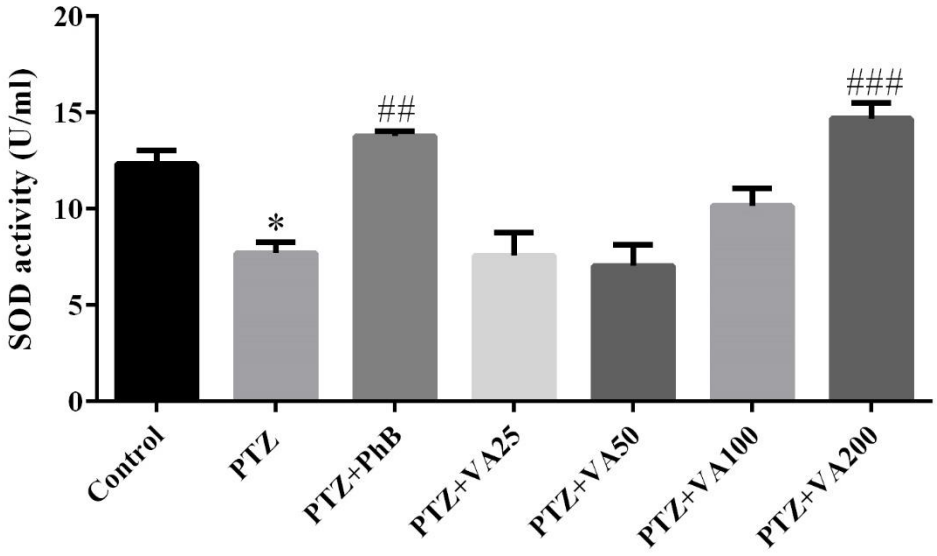

Fig. 9. Effects of VA on SOD activity in different groups. Values are as mean ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. *p<0.05 vs. control group; ##p<0.01 and ###p<0.001 vs. PTZ group. PTZ = Pentylenetetrazol, PhB = Phenobarbital, VA= Vanillic acid.

Discussion

The results of the present study showed that VA had an anticonvulsant and analgesic effect in rats with PTZ-induced convulsions. VA had no effect on passive avoidance memory at different doses. In addition, MDA levels decreased after VA administration and GPx and SOD activities were increased by VA in PTZ-induced rats.

PTZ may have an anticonvulsant effect by inhibiting the action of GABA at GABA-A receptors[20]. GABA is an important inhibitory neurotransmitter in the brain, and inhibition of its neurotransmission is thought to be the main factor in epilepsy[21]. Increasing GABA-ergic neurotransmission inhibits seizures, while inhibiting neurotransmission stimulates seizures[22]. The resistance of mice to PTZ-induced seizures by the standard anticonvulsants, phenobarbitone and

diazepam is predictable, subsequently many authors have shown that they exert their anticonvulsant effects by enhancing GABA-mediated inhibition[23]. CNS effects have been described for various phytochemicals. The antispasmodic effect has been recognized , alkaloids [24], essential oils[25], flavonoids [26], triterpenic steroids and triterpenoid saponins are described to have anticonvulsant action in several experimental seizure models for example PTZ [27, 28]. In addition, various flavonoids have been shown to act as benzodiazepine-like molecules in the central nervous system and can alter GABA-generated chloride currents in animal models of anxiety, sedation and convulsions [26].

Free radicals are considered the most likely candidates responsible for the neuronal changes that mediate the behavioral deficits in neurodegenerative disorders. Antioxidants are effective in rodent models of epilepsy, stroke and Alzheimer's disease [29]. The results of the current study suggests that VA may have an anticonvulsant effect by inhibiting and/or reducing PTZ -induced seizures in the rats used by increasing or somewhat impairing GABAergic neurotransmission.

This study also investigated the effect of VA on oxidative stress in PTZ-induced convulsion. Rats pretreated with VA at doses of 100 and 200 mg/kg showed a significant decrease in brain MDA content compared to the PTZ group. GPx is an endogenous antioxidant within the free radicals and prevents the formation of hydroxyl radicals, the most toxic form of free radicals [29]. The decrease in GPx and SOD in rats of the PTZ group found in the present study indicates that free radical formation was increased and that the reduced glutathione and SOD were depleted during the process of combating oxidative stress [30]. The decrease in MDA and the increase in GPx and SOD levels in rats of the PTZ+VA group indicates that VA had good antioxidant effect. Several studies have also shown that the antioxidant effect of medicinal plants is due to the presence of polyphenols and flavonoids [31]. VA is a significant antioxidant due to the presence of the carboxyl group [32].

The results of the present study that VA has an analgesic effect in rats with PTZ-induced convulsions. Kim et al. showed that VA significantly suppressed the expression of cyclooxygenase-2 in dextran sulfate sodium (DSS)-induced ulcerative colitis. In addition, they observed that plasma levels of interleukin (IL)-6 were higher in the DSS-treated group than in the control group, but these elevated levels were reduced by the administration of VA [33]. VA had an inhibitory effect on inflammatory mediators by suppressing NF-κB in lipopolysaccharide-stimulated mice[34].

Various studies have demonstrated the efficacy of VA in the treatment of immune or inflammatory reactions. For instance, VA increased the activity of human lymphocyte proliferation and the secretion of interferon-gamma in human peripheral blood mononuclear cells [35].

Conclusion

The results of the present study show that VA has an anticonvulsant and analgesic effect in PTZ-induced rats with convulsion. In addition, VA decreased MDA levels and increased GPx and SOD activity in the PTZ groups receiving VA. The results of the current study suggest that VA has anticonvulsant and analgesic effects and could inhibit and/or reduce PTZ -induced seizures in rats through its antioxidant activity.

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Conflicts of interest

There are no conflicts of interest.

References

[1] Pollard JR, French J. Antiepileptic drugs in development. Lancet Neurol. 2006;5(12):1064-67. [DOI: 10.1016/S1474-4422(06)70627-5] [PMID]

[2] Hardman JG, Limbird LE, Gilman AG. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th ed. McGraw-Hill: New York; 2001. [Link]

[3] Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs. Nat Rev Neurosci. 2004;5(7):553-64. [DOI: 10.1038/nrn1430] [PMID]

[4] Hema B, Bhupendra S, Mohamed Saleem TS, Gauthaman K. Anticonvulsant effect of Drosera burmannii Vahl. Int J Applied Res Nat Prod. 2009;2(3):1-4. [link]

[5] Raza M, Shaheen F, Choudhary MI, Sombati S, Rafiq A, Suria, A, et al. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from Delphinium denudatum. J. Ethnopharmacol. 2001;78(1): 73– 78. [DOI: 10.1016/s0378-8741(01)00327-0] [PMID]

[6] Wannang NN, Anuka JA, Kwanashie HO, Gyang SS, Auta A. Anti-seizure activity of the aqueous leaf extract of Solanum nigrum L. (Solanaceae) in experimental animal. Afr Health Sci. 2008;8(2):74-79. [PMID]

[7] Rai R.P., Maurya M.S. Synthesis and evaluation of antibacterial activity of vanillin derivatives. J Sci Technol India. 1996;4: 275–276. [link]

[8] Delaquis P, Stanich K, Toivonen P. Effect of pH on the inhibition of Listera spp. by vanillin and vanillic acid. J Food Prot. 2005;68(7):1472–1476. [DOI: 10.4315/0362-028x-68.7.1472] [PMID]

[9] Varma R.S, Shukla A, Chatterjee R.K. Evaluation of vanillic acid analogues as a new class of antifilarial agents. Indian J Exp Biol. 1993;31(10): 819–821. [PMID]

[10] Singh M, Tiwari V, Jain A, Ghoshal S. Protective activity of picroliv on hepatic amoebiasis associated with carbon tetrachloride toxicity. Indian J Med Res. 2005;121(5): 676–682. [PMID]

[11] Lesage-Meessen L, Delattre M, Haon M, Thibault J.F, Ceccaldi B.C, Brunerie P, et al. A two-step bioconversion process for vanillin production from ferulic acid combining Aspergillus niger and Pycnoporus cinnabarinus. J. Biotechnol. 1996;50(2-3): 107–113. [DOI: 10.1016/0168-1656(96)01552-0] [PMID]

[12] Civolani C, Barghini P, Roncetti A.R, Ruzzi M, Schiesser A. Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of Pseudomonas fluorescens strain BF13. Appl. Environ. Microbiol. 2000;66(6):2311–2317. [DOI: 10.1128/AEM.66.6.2311-2317.2000] [PMID]

[13] Duke J.A. Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. 999th ed.; CRC Press: Boca Raton, FL, USA, 1992. [Link]

[14] Mansouri MT, Farbood Y, Sameri MJ, Sarkaki A, Naghizadeh B, Rafeirad M. Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. Food chemistry. 2013;138(2-3):1028-33. [DOI: 10.1016/j.foodchem.2012.11.022] [PMID]

[15] D'Amour FE, Smith DL. A method of determining loss of pain sensation. J Pharmacol Exp Ther. 1941;72(1):74–79. [Link]

[16] Dolatshahi-somehsofla M, Esmaeili-Mahani S, Motamedi F, Haeri A, Ahmadian A. Adrenalectomy potentiates the antinociceptive effects of calcium channel blockers. Pharmacol Biochem Behav. 2009;92(2):327–334. [DOI: 10.1016/j.pbb.2008.12.021] [PMID]

[17] Samra K, Okan A, Yildarim S, Rustu O, Sedef K, Ihsan C. Paeoniflorin Diminishes Maximal Electroshock and PTZ–induced Convulsions in Mice. Hacettepe University Journal of the Faculty of Pharmacy. 2012; 32(1):17-30. [Link]

[18] Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. Electroencephalogr Clin Neurophysiol. 1972;32(3):269-279. [DOI: 10.1016/0013-4694(72)90176-9] [PMID]

[19] Fadaei-Kenarsary M, Farbood Y, Mansouri S.M.T, Fathi Moghaddam H. Effects of Venlafaxine & Methadone Alone and in Combination with Spontaneous Morphine withdrawal Syndrome & Pain Sensation in Rats. Basic and Clinical Neuroscience. 2015;6(1):21-28. [PMID]

[20] Vasconcelos SM, Lima NM, Sales GT, Cunha GM, Aguiar LM, Silveira ER. Anticonvulsant activity of hydroalcoholic extracts from Erythrina velutina and Erythrina mulungu. J Ethnopharmacol. 2007;110(2):271-274. [DOI: 10.1016/j.jep.2006.09.023] [PMID]

[21] Silambujanaki P, Chitra V, Kumari S, Sankari M, Raju D, Chandra CH. Anti- convulsant activity of Aqueous extract of Butea monosperma leaves. Res J Pharm Biol Chem Sci. 2010;1(2):431-435. [Link]

[22] Amabeokua GJ, Green I, Kabatende J. Anticonvulsant activity of Cotyledon orbiculata L. (Crassulaceae) leaf extract in mice. J Ethnopharmacol. 2007;112(1):101-107. [DOI: 10.1016/j.jep.2007.02.016] [PMID]

[23] Mahomed IM, Ojewole JA. Anticonvulsant activity of Harpagophytum procumbens DC. (Pedaliaceae) secondary root aqueous extract in mice. Brain Res Bull. 2006;69(1):57-62. [DOI: 10.1016/j.brainresbull.2005.10.010] [PMID]

[24] Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JC. Neuropharmacological activites of the crude alkaloidal fraction from stems of Tabernaemontana pandacaqui poir. J Ethnopharmacol. 1998;62(3):229-234. [DOI: 10.1016/s0378-8741(98)00081-6] [PMID]

[25] Dallmeier K, Carlini EA. Anesthetic, hypothermic, myorelaxant and anticonvulsant effects of synthetic eugenol derivatives and natural analogue. Pharmacol. 1981;22(2):113-127. [DOI: 10.1159/000137479] [PMID]

[26] Nassiri-Asl M, Shariati-Rad S, Zamansoltani F. Anticonvulsant effects of aerial parts of Passi flora incarnata extract in mice: involvement of benzodiazepine and opioid receptors. BMC Complement Altern Med. 2007;7:26. [DOI: 10.1186/1472-6882-7-26] [PMID] [PMCID]

[27] Chauhan AK, Dobhal MP, Joshi BC. A review of medicinal plants showing anticonvulsant activity. J Ethnopharmacol. 1988; 22(1):111-123. [DOI: 10.1016/0378-8741(88)90226-7] [PMID]

[28] Kasture VS, Kasture SB, Chopde CT. Anticonvulsive activity of Butea monosperma flowers in laboratory animals. Pharmacol Biochem Behav. 2002;72(4):965-972. [DOI: 10.1016/s0091-3057(02)00815-8] [PMID]

[29] Golechha M, Bhatia J, Arya DS. Hydroalcoholic extract of

Emblica officinalis Gaertn. afford protection against PTZ-induced sizure, oxidative stress and congnitive in rats. Ind J Exp Biol. 2010;48(5):474-478. [PMID]

[30] Schulz JB, Lindenau J, Seyfried J, Diechgans J. Glutathione, oxidative stress and neurodegeneration. Eur J Biochem. 2009;267(16):4904-4909. [DOI: 10.1046/j.1432-1327.2000.01595.x.] [PMID]

[31] Asuntha G, Prasannaraju Y, Prasad KV. Effect of ethanol extract of Indigofera tinctoria Linn (Fabaceae) on lithium/pilocarpine-induced status epilepticus and oxidative stress in wistar rats. Trop J Pharm Res. 2010;9(2):149-156. [DOI: 10.4314/tjpr.v9i2.53702]

[32] Chou TH, Ding HY, Hung WJ, Liang CH. Antioxidative characteristics and inhibition of alpha-melanocyte-stimulating hormone-stimulated melanogenesis of vanillin and vanillic acid from Origanumvulgare. Exp Dermatol. 2010;19(8):742–750. [DOI: 1111/j.1600-0625.2010.01091.x] [PMID]

[33] Kim SJ, Kim MC, Um JY, Hong SH. The beneficial effect of vanillic acid on ulcerative colitis. Molecules. 2010;15(10):7208-17. [DOI: 10.3390/molecules15107208] [PMID] [PMCID]

[34] Kim MC, Kim SJ, Kim DS, Jeon YD, Park SJ, Lee HS, et al. Vanillic acid inhibits inflammatory mediators by suppressing NF-B in lipopolysaccharide-stimulated mouse peritoneal macrophages. Immunopharmacol Immunotoxicol. 2011;33(3):525–532. [DOI: 10.3109/08923973.2010.547500] [PMID]

[35] Chiang LC, Ng LT, Chiang W, Chang MY, Lin CC. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. Planta Med. 2003;69(7):600-604. [DOI: 10.1055/s-2003-41113] [PMID]