Original Article

Investigation of the Effects of *Satureja edmondi* on Memory Impairment Caused by Chemical Kindling in Adult Male Rats

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Abstract

Introduction: Epilepsy causes neuronal damage that disturbs normal brain functioning, especially in the hippocampal formation. In addition, it has been shown that cognitive inconsistencies, changes in emotional behavior, and neuronal loss in the hippocampus occur during pentylenetetrazole (PTZ)-induced kindling. So, the purpose of the present research was to investigate whether administration of *Satureja edmoni* is able to prevent memory impairment, caused by PTZ-induced kindling in adult male rats. **Materials and Methods:** In this study, male rats were kindled by repeated (two or three) injection of PTZ intraperitoneally (i.p.) (25 mg/kg); then all animals in the extract groups were treated with 100, 200, or 400 mg/kg of *S. edmondi*. For behavior assessment, an inhibitory passive avoidance task was used. **Results:** Our results showed that animals in the kindled group took less time to enter dim hutch than control rats. There was a significant difference in step-through latency (STL) recorded from group of rats with PTZ-induced kindling treated with *S. edmondi* at concentrations 100 and 200 mg/kg and control rats, but differences between STL of PTZ-induced kindling animals treated with *S. edmondi* 400 mg/kg vs. control rats were not significant. **Conclusion:** In this study, we observed that PTZ induced impairing effects on passive avoidance memory; in contrast, administration of *S. edmondi* could abolish the impairment effect of epilepsy on memory.

Keywords: *Kindling, memory, rat, Satureja edmondi*

Introduction

Epilepsy is one of the most common and chronic brain disorders that affect about 1% of the general population.[1] Epilepsy causes neuronal damage that disturbs normal brain functioning, especially in the hippocampal formation.[2]

Pentylenetetrazol (PTZ), as a non-competitive chloride ionophore complex of the gamma-amino-butyric-acid (GABA) antagonist, is used as seizure chemical stimulus in the experimental study in animals.[3] A periodic systemic injection of PTZ induces an acute, severe seizure in animals.[4]

During PTZ-induced kindling, behavioral, neurophysiological, and neurochemical symptoms are changeable from person to person.[5]

PTZ has the inhibitory effect on some neurotransmitters such as the GABAergic and glutamatergic systems.[6] Control of neuronal excitability and epileptogenesis are also affected by PTZ.

Research findings indicate that kindling-induced seizures caused cognitive inconsistencies, changes in emotional behavior, and neuronal loss in the hippocampus[7,8] that cause impairment in cognitive process in the hippocampus.[9] According to Lamberty and Klitgaard,[10] PTZ-induced kindling interferes in spatial memory.

Twelve 12 species of the genus *Satureja* are known; some of them are endemic to Iran, which includes *S. edmondi, S. intermedia, S. sahendica, S. isophylla, S. kallarica, S. atropatana, S.bachtiarica,* and *S. khuzistanica.* This plant is a perennial and bushy aromatic herb that grows 50 cm high with yellow-colored flowers, dense white villous hairs, and a dense covering of punctuate glands on both leaf surfaces. Flowering occurs in autumn at late September and October.[11] *S. edmondi* is grown in the west of Iran, Kermanshah and usually grows on rock. *S. edmondi* is often used to treat digestive and respiratory disorders such as state of enteritis, chronic ulcer, and asthma.[12]

According to the study carried out by Ghorbanpour *et al.*,[13] essential oils of *Satureja*

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species such as *S. edmondi* include predominant compounds such as p-cymene, carvacrol, p-cymene, thymol, and their precursors and γ-terpinene.

Various activities of carvacrol are known, which include antimicrobial, antioxidant, anticandidal, and anti-inflammatory properties.[14,15] The hydrophobic terpenoid carvacrol suppresses L-type Ca2+in a concentration-dependent way.[16]

The aim of the present study was to determine whether administration of *S. edmondi* is able to prevent memory impairment, caused by PTZ-induced kindling in adult male rats.

Materials and Methods

Animals and treatment

For this experimental study, adult Wistar male rats (250–300 g) (Razi Institute, Tehran, Iran) were housed in a Plexiglass hutch. They had degage disposal to food and water and kept at the standard animal room. The animals were kindled by transfusion of 25 mg/kg (i.p.) PTZ (Sigma, St. Louis, MO, USA), 1 mL/kg for every 15 min until seizures happened (two or three transfusion). After injection, seizure activity was seen for 45 min and recorded; briefly, stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic jerks, without rearing; stage 3, myoclonic jerks, rearing; stage 4, turn over into side position, clonic-tonic seizures; stage 5, turn over into back position, generalized tonic-clonic seizures. The rats that reached stage 4/5 according to the scale change by Becker *et al.*[17,18] were considered as kindled animals. In the extract groups, all animals received *Satureja* hydroalcoholic extract three times (first time: 1 h after kindling, second time: 24 h after kindling, and third time: 1 h before training session). Control animals received saline instead.

Preparation of plant extract

S. edmondi was obtained from Dallaho Mountains in the west of Kermanshah, Iran. The plant was identified and authenticated by Dr Sharifi, Assistant Professor, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran. *S. edmondi* leaves were dried and powdered. About 200 g of powdered plant was added to 400 cc 70% ethanol and were left to macerate at room temperature for 4 h. Then, the soaked plant was extracted by the percolation method; it was removed from the percolator, filtered by a Whatman filter paper (No. 4), and then dried under reduced pressure at 37°C with a rotator evaporator.

Behavioral experiment

The passive avoidance memory was assessed in the shuttle box that includes one light and one dim hutch of the same size portioned by a moveable tumbrel door. In the dim hutch, the floor had stainless-steel rods with 0.5 cm diameter, parted with 1 cm space. Using an isolated stimulator, the alternative electric shocks (50 Hz, 3 s, 0.7 mA) intensity acrimony was transferred to the floor of the dim hutch.

Training

The training protocol was the same as our previous research.[19] The animals were permitted to become habituated to the lab milieu at least 30 min before the training phase. Then, each animal was gently placed in the light hutch; 10 s later, by opening the tumbrel door, animal was allowed to go to the dim hutch. Each rat which waited more than 100 s in the light hutch was removed. For the animal that passed with all four paws to another compartment, the door was closed and a foot shock (50 Hz, 0.7 s, 1 mA) was immediately delivered to the grid floor of the dim hutch. Formerly, the rat was removed from the apparatus and the process was repeated after 2 min. When the rat stayed in the light hutch for 120 s, the training was finished; after the training session, rats were placed temporarily into its home cage. The maximum trials of training session for each rat were three times.

Retention test

Twenty-four hours after final training, retention testing was implemented. Each rat was quietly positioned in the light hutch and after 10 s the door was opened. The latency time of animal to enter the dim hutch step-through latency (STL) was recorded, and the takeoff point was 600 s. No electric shock was applied in the testing day.

Statistical analysis

The results were statistically estimated by one-way analysis of variance (ANOVA) and were presented as means ± SEM. Using *post hoc* Tukey's test, additional analysis for multiple comparisons was carried out. In all evaluations, $P < 0.05$ was considered the level of statistical significance.

Results

Effect of PTZ-induced kindling on passive avoidance memory

As shown in Figure 1A, the result of our experiment indicated that the kindled group required less time to enter the dark chamber than those in the control group ($t_{14} = 3.1$, $P = 0.00$) [Figure 1A]. Figure 1B shows that time spent in the dark chamber in the kindled group was higher than that spent in the control group ($t_{14} = 6.7$, $P = 0.00$).

Administration of *S. edmondi* eliminates the impairment effect of PTZ-induced kindling on passive avoidance memory

One-way ANOVA showed that there was a significant difference in STL recorded from group of rats with PTZ-induced kindling treated with *S. edmondi* at concentrations 100 and 200 mg/kg and control rats, but it did not show significant differences. There were no significant differences between STL recorded from PTZ-induced kindling treated with *S. edmondi* 400 mg/kg vs. control rats ($F_{4, 39} = 7.88$; $P = 0.00$; Figure 2A). Similar results were observed for spending time in the dark chamber ($F_{4, 39} = 13.83$; $P = 0.00$; Figure 2B). The results show that

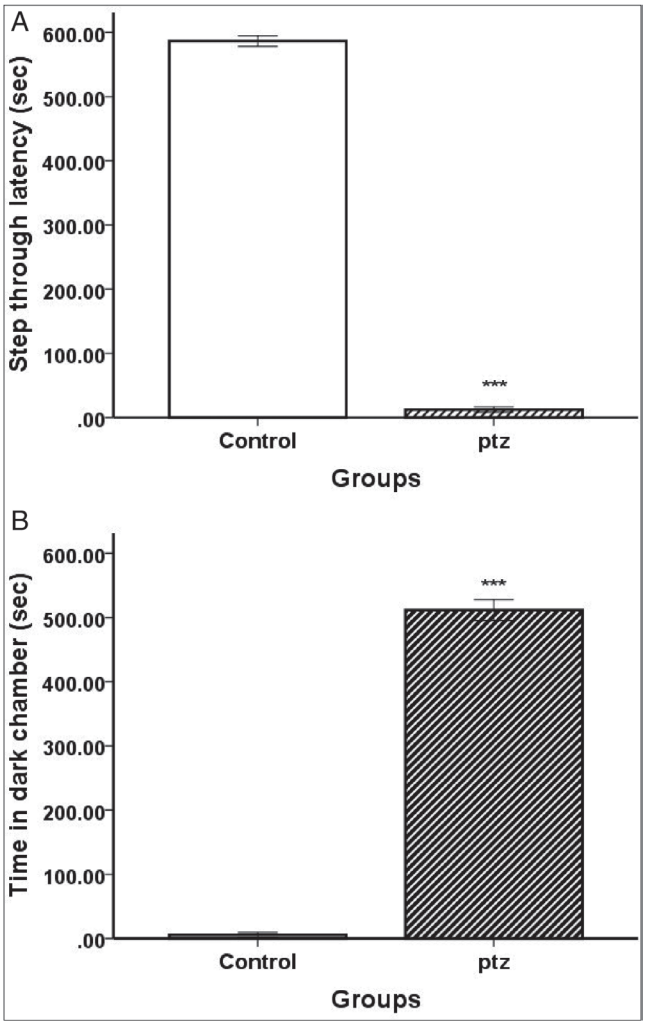

Figure 1: (A) The effect of PTZ-induced kindling on STL. The retention latencies of kindled rats were significantly reduced compared with control rats. Each bar represents mean± SEM, eight rats were in each group.****P*<0.001. (B) The effect of PTZ kindling on time spent in the dark chamber. Time spent in the dark chamber in the kindled group was significantly increased compared with the control group. All rats were tested 24 h after the training session. Each bar represents mean± SEM, *n*=8 in each group, ******P*<0.001**

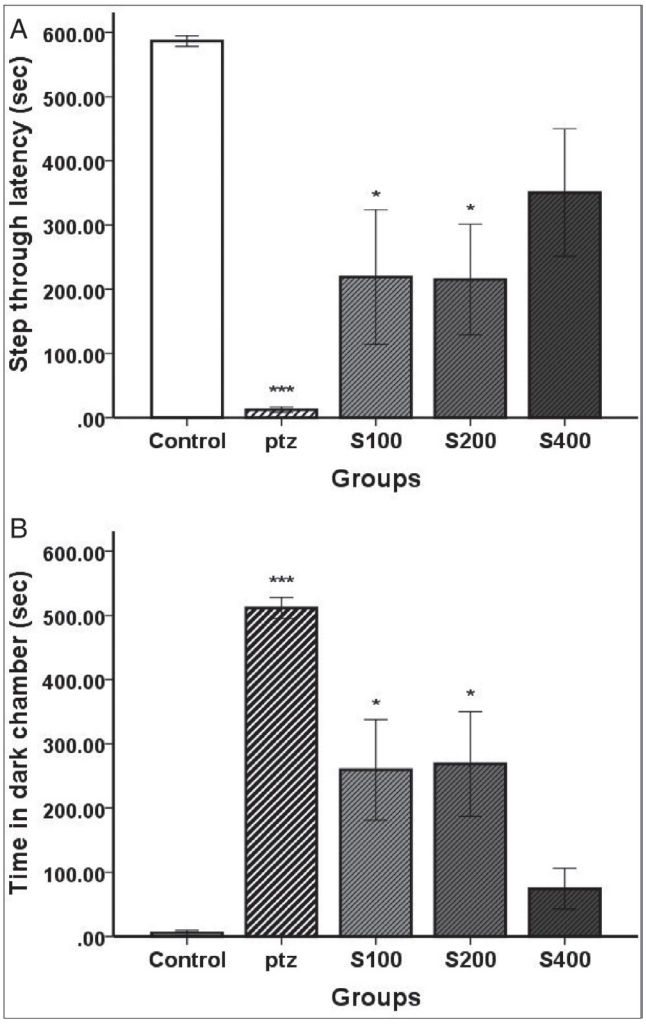

Figure 2: (A) The effect of i.p. administration of *S. edmondi* on inhibitory avoidance response in control and kindled rats that received different doses (0,100, 200, or 400 mg/kg) of *Satureja* extract. Three groups of rats (*n*=8 each) received *Satureja* extract (100, 200, or 400 mg/kg) for three times (after 1 and 24 h after kindling and also 1 h before training). All animals were tested 24 h after the training session. Each bar represents mean± SEM. ****P*<0.05, ******P*<0.001. (B) The effect of i.p. administration of *S. edmondi* on the time spent in the dark chamber. Three groups of rats received *Satureja* extract (100, 200, or 400 mg/kg) for three times (after 1 and 24 h after kindling and also 1 h before training). All rats were tested 24 h after the training session. Each bar represents mean± SEM, *n*=8 in each group. ****P*<0.05 and ******P*<0.001

S. edmondi could abolish the impairment effects of PTZ-induced kindling on passive avoidance learning.

Discussion

In this present study, it was observed that administration of *S. edmondi* accomplishment could prevent/restrain the impairment effect of epilepsy on memory. This result is consistent with another study reporting that *S. hortensis* has anticonvulsant activity in the PTZ model in mice.[20]

Another study reported that PTZ through altering in some neurotransmitter systems such as the GABAergic, glutamatergic, and glycine is able to induce epilepsy.[21,22] Kindling causes a decrease in the release of GABA in the hippocampus and a decrease in GABA receptor sensitivity.[23] It is reported that an enhancement in glutamate neurotransmitter at the hippocampal region occurs during PTZ kindling.[24] PTZ increases the density of glutamate receptor and activates N-methyl-D-aspartate (NMDA) receptors.[25] It is suggested that PTZ induced seizures by glutamate receptor activation and inhibition of GABA neurotransmitters.[23,26]

Carvacrol is abundantly found in the essential oils of the Lamiaceae family. In addition, it was reported that carvacrol effectively suppresses Ca2+ current in neuronal membranes.[27,28] The blocking effect of the carvacrol on Ca2+ current resembles that of many conservative Ca2+-entry blocker agents, like verapamil or nifedipine.[16] So it may be concluded that the protective effect of *S. edmondi* on PTZ-induced seizures is at least relatively due to the presence of carvacrol.

Quintans-Júnior *et al.*[29] reported that carvacrol may reduce PTZ-induced seizures via inhibition of GABAergic transmission.

PTZ may activate NMDA receptor, leading to increased intracellular Ca^{2+}. Ca^{2+} activates calcium-dependent proteins such as calcineurin, which ultimately impair learning and memory.[30]

Therefore, with regard to the above, probably *S. edmondi* treatment by blocking Ca2+ current of calcium channels prevents the impairment effect of PTZ-induced kindling on passive avoidance memory.

Conclusion

Taken together, in this study, we demonstrated that *S. edmondi* could abolish the negative effect of epilepsy on memory. However, the exact mechanisms involved in the learning and memory prevention due to *S. edmondi* are not well known and may need further investigations.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bernhardt BC, Hong S, Bernasconi A, Bernasconi N. Imaging structural and functional brain networks in temporal lobe epilepsy. Front Hum Neurosci 2013;7:624.
2. Bertram EH, Lothman EW, Lenn NJ. The hippocampus in experimental chronic epilepsy. Ann Neurol 1990;27:43-8.
3. Chen Z, Li Z, Sakurai E, Izadi Mobarakeh J, Ohtsu H, Watanabe T, *et al*. Chemical kindling induced by pentylenetetrazol in histamine H(1) receptor gene knockout mice (H(1)KO), histidine decarboxylase-deficient mice (HDC(-/-)) and mast cell-deficient W/W(v) mice. Brain Res 2003;968:162-6.
4. Kilbey MM, Ellinwood EH, Easler ME. The effects of chronic cocaine pretreatment on kindled seizures and behavioral stereotypies. Exp Neurol 1979;64:306-14.
5. Pavlova T, Stepanichev M, Gulyaeva N. Pentylenetetrazole kindling induces neuronal cyclin B1 expression in rat hippocampus. Neurosci Lett 2006;392:154-8.
6. Naseer MI, Shupeng L, Kim MO. Maternal epileptic seizure induced by pentylenetetrazol: Apoptotic neurodegeneration and decreased GABAB1 receptor expression in prenatal rat brain. Mol Brain 2009;2:20.
7. Naseer MI, Lee HY, Ullah N, Ullah I, Park MS, Kim SH, *et al*. Ethanol and PTZ effects on siRNA-mediated GABAB1 receptor: Down regulation of intracellular signaling pathway in prenatal rat cortical and hippocampal neurons. Synapse 2010;64:181-90.
8. Pitkänen A, Tuunanen J, Kälviäinen R, Partanen K, Salmenperä T. Amygdala damage in experimental and human temporal lobe epilepsy. Epilepsy Res 1998;32:233-53.
9. Gilbert TH, Hannesson DK, Corcoran ME. Hippocampal kindled seizures impair spatial cognition in the Morris water maze. Epilepsy Res 2000;38:115-25.
10. Lamberty Y, Klitgaard H. Consequences of pentylenetetrazole kindling on spatial memory and emotional responding in the rat. Epilepsy Behav 2000;1:256-61.
11. Jamzad Z. Thymus and *Satureja* species of Iran. Tehran: Research Institute of Forests and Rangelands; 2010. p. 171.
12. Moradi S, Sadeghi E. Study of the antimicrobial effects of essential oil of Satureja *edmondi* and nisin on *Staphylococcus aureus* in commercial soup. J Food Process Preserv 2017;41:e13337.
13. Ghorbanpour M, Hadian J, Hatami M, Salehi-Arjomand H, Aliahmadi A. Comparison of chemical compounds and antioxidant and antibacterial properties of various *Satureja* species growing wild in Iran. J Med Plants 2016;15:58-72.
14. De Vincenzi M, Stammati A, De Vincenzi A, Silano M. Constituents of aromatic plants: Carvacrol. Fitoterapia 2004;75:801-4.
15. Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, G M. Membrane toxicity of antimicrobial compounds from essential oils. J Agric Food Chem 2007;55:4863.
16. Magyar J, Szentandrássy N, Bányász T, Fülöp L, Varró A, Nánási PP. Effects of terpenoid phenol derivatives on calcium current in canine and human ventricular cardiomyocytes. Eur J Pharmacol 2004;487:29-36.
17. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281-94.
18. Becker A, Grecksch G, Rüthrich HL, Pohle W, Marx B, Matthies H. Kindling and its consequences on learning in rats. Behav Neural Biol 1992;57:37-43.
19. Pourmotabbed A, Mahmoodi G, Mahmoodi S, Mohammadi-Farani A, Nedaei SE, Pourmotabbed T, *et al*. Effect of central muscarinic receptors on passive-avoidance learning deficits induced by prenatal pentylenetetrazol kindling in male offspring. Neuroscience 2014;279:232-7.
20. Zolfagharian F, Razavi BM, Hosseinzadeh H. Anticonvulsant effect of *Satureja hortensis* aerial parts extracts in mice. Avicenna J Phytomed 2016;6:305-12.
21. Engelborghs S, D'Hooge R, De Deyn PP. Pathophysiology of epilepsy. Acta Neurol Belg 2000;100:201-13.
22. Ure JA, Perassolo M. Update on the pathophysiology of the epilepsies. J Neurol Sci 2000;177:1-17.
23. Eraković V, Zupan G, Varljen J, Laginja J, Simonić A. Altered activities of rat brain metabolic enzymes caused by pentylenetetrazol kindling and pentylenetetrazol-induced seizures. Epilepsy Res 2001;43:165-73.
24. Schroeder H, Becker A, Hoellt V. Sensitivity and density of glutamate receptor subtypes in the hippocampal formation are altered in pentylenetetrazole-kindled rats. Exp Brain Res 1998;120:527-30.
25. Schroeder H, Becker A, Grecksch G, Schroeder U, Hoellt V. The effect of pentylenetetrazol kindling on synaptic mechanisms of interacting glutamatergic and opioid system in the hippocampus of rats. Brain Res 1998;811:40-6.
26. Morimoto K. Seizure-triggering mechanisms in the kindling model of epilepsy: Collapse of GABA-mediated inhibition and activation of NMDA receptors. Neurosci Biobehav Rev 1989;13:253-60.
27. Erdélyi L. Guaiacol and vanilloid compounds modulate the A-type potassium currents in molluscan neurones. Acta Biol Hung 1999;50:65-79.
28. Györi J, Kiss T, Shcherbatko AD, Belan PV, Tepikin AV, Osipenko ON, *et al*. Effect of Ag+ on membrane permeability of perfused helix pomatia neurons. J Physiol 1991;442:1-13.
29. Quintans-Júnior L, Guimarães A, Araújo B, Oliveira G, Santana M, Moreira FV. Carvacrol, (-)-borneol and citral reduce convulsant activity in rodents. Afr J Biotechnol 2010;9:6566-72.
30. Moradpour F, Moazedi AA, Pourmotabbed A, Zarei F. Calcineurin is involved in retrieval of passive avoidance memory and synaptic plasticity impairment induced by nandrolone administration in adolescent male rats. Neurobiol Learn Mem 2019;163:107032.