

Investigating the effect of *Melissa officinalis* plant extract on pentylenetetrazol-induced seizures in Autistic rat

Running title: Effect of *Melissa officinalis* Extract on Seizures in Autistic Rats

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Abstract

Background: Autism is a disorder characterized by deficits in speech, social interaction, and the presence of repetitive, purposeless behaviors. Seizure prevalence in individuals with autism ranges widely from 5% to 40%. *Melissa officinalis* is used in Iranian traditional medicine as a sedative, antipyretic, antispasmodic, and anticonvulsant. This study investigates the potential effects of the plant's extract on PTZ-induced seizures in a male Wistar rat model of autism.

Methods: Healthy male and female Wistar rats were bred. Pregnant females were fed a standard diet with water until day 12.5 of gestation. Dams were assigned to four groups: (1) control, (2) autism, (3) autism + *Melissa officinalis* extract 100 mg/kg, (4) autism + *Melissa officinalis* extract 200 mg/kg. Two weeks after birth, PTZ (80 mg/kg) was administered to all groups. Animals were observed for 60 minutes post-injection. Ricin criterion was used to evaluate seizure severity.

Result: The anticonvulsant effect showed a clear dose-response relationship, with the 200 mg/kg dose producing a significant reduction in seizure duration compared to the 100 mg/kg dose ($p < 0.05$), indicating dose-dependent efficacy.

Conclusion: Administration of *Melissa officinalis* extract to male rats with autism reduced PTZ-induced seizure activity. The *Melissa officinalis* extract appears to exert inhibitory effects on PTZ-induced seizures in this autism model.

Key words: Autism, *Melissa officinalis* plant, Seizure, PTZ

Introduction

Autism is a type of neurodevelopmental disorder characterized by deficits in social interactions, reduced verbal communication, repetitive stereotypical behaviors, and limited interests (1). The pathology of autism is not completely understood; however, both genetic risk factors (35% to 40%) and environmental factors (60%) play a role in the development and exacerbation of autism (2). Among environmental factors, valproic acid plays a role in causing autism, particularly during pregnancy, and is currently used to create autistic models because valproic acid inhibits histone deacetylase enzymes, resulting in increased acetylation in histone proteins, chromosomal structural changes, and changes in gene expression. Due to exposure to valproic acid during early development, the developing neural circuitry is disrupted, leading to autistic behavioral phenotypes in animal models. Research studies indicate that 15 to 30 percent of individuals with autism experience general seizures. Over 100 genetic and genomic disorders have been considered for autism, among which many can be related, at least in some individuals with autism, to epilepsy and intellectual disabilities (3). Evidence for the causes of autism based on heredity exists, such as de novo mutations, which can include various copy number variations, single nucleotide polymorphisms, and epigenetic changes. Many of these genes are involved in synapse formation, neurotransmitter function, and neural plasticity, which likely explain the simultaneous connection with the development of neurological disorders, including epilepsy (4). Several pieces of evidence suggest that the risk of epilepsy increases with age in autistic patients, and its prevalence can double during adolescence compared to childhood (5). In various experiments, the animal seizure model is induced by pentylenetetrazol. Pentylenetetrazol, also known as Metrazol, Pentetrazol, Cardiazol, and PTZ, is a drug that stimulates circulation and respiration and, in high doses, leads to convulsive movements. Researchers believe that pentylenetetrazol acts by blocking the GABA system, especially inhibiting postsynaptic GABA receptors (6). Administering PTZ in high doses is a quick and effective method to investigate susceptibility to seizures and select new anticonvulsant drugs (7). Before the discovery of anticonvulsant drugs, epilepsy patients were treated with brain surgery, cupping, and herbal medicine. Nowadays, given the adverse side effects of synthetic drugs, there is a positive trend towards herbal medicines (8). One of the plants involved in this study is lemon balm (*Melissa officinalis*), which, in traditional Iranian medicine, is used as a tranquilizer, antispasmodic, and anticonvulsant. This plant is also employed for the treatment of insomnia, sleep disturbances, anxiety, depression, and neurological diseases (9). Given that traditional medicine notes the calming and anticonvulsant properties of lemon balm, this research project will investigate the potential effects of its extract on pentylenetetrazol-induced seizures in male Wistar rats. The aim of the study is to evaluate the possible impact of the extract on the onset and duration of seizure stages and to determine whether the effects of the lemon balm extract are dose-dependent.

Methods

In this research, healthy male and female Wistar rats weighing 230-250 grams were used (Ethical code: IR.GOUMS.AEC.1402.013). They were kept in separate cages for mating. The pregnancy of female rats was diagnosed by vaginal plug observation method. Pregnant rats were isolated and received water and normal food until the 12th day of pregnancy. From the 12th day of pregnancy, female rats are divided into 4 groups: Group 1, control: male offspring of untreated mothers who received only normal food and water. Group 2, autism: male offspring of pregnant mothers who received 500 mg/kg of valproic acid via IP on day 12/5 of pregnancy (Autism model) (10). Group 3, autism + *Melissa officinalis* plant extract with a dose of 100 mg/kg: receiving *Melissa officinalis*

plant extract with a dose of 100 mg/kg by gavage for two weeks by 30-day-old male children of pregnant mothers who received 500 mg/kg of valproic acid via IP on day 12.5 of pregnancy. Group 4: autism + *Melissa officinalis* plant extract with a dose of 200 mg/kg: receiving *Melissa officinalis* plant extract with a dose of 200 mg/kg by gavage for two weeks by 30-day-old male children of pregnant mothers who received 500 mg/kg of valproic acid via IP on day 12.5 of pregnancy. (10,11) (*Melissa officinalis* extract from Dr. Zarghani's Pearl Farm Company is prepared from the young leaves and stems of this plant, which have a high concentration of active compounds.) After two weeks of drug administration, PTZ was injected intraperitoneally to the groups at a dose of 80 mg/kg to cause seizures and the behaviors of the mice were recorded for 60 minutes (12). In convulsive models, the Racine criterion was used to evaluate the severity of seizures. The Racine criterion is one of the established scales for evaluating the severity and stages of seizures in animal models, particularly in studies related to drug examination and therapies. This assessment is specifically designed for mice and includes the evaluation of various stages of seizures, from the earliest symptoms to the end of the seizure. (13).

Racine Stages

Stage 1: Facial and ear muscle contractions and writhing or stretching of the body and forelimbs (single myoclonic seizures of the head). Stage 2: Convulsive shock-like muscle twitches without jumping (repeated head seizures with sudden forelimb jerks). Stage 3: Convulsive shock-like muscle twitches with whole-body and forelimb jumping (typically legs apart, tail straightened). Stage 4: Falling sideways with generalized clonic-tonic seizures, sometimes standing on two feet. Stage 5: Falling backward with generalized clonic-tonic seizures. Stage 6: Death.

Results

1-The effect of *Melissa officinalis* extracts the duration of the first stage of seizures (Stage 1: contraction of facial muscles and ears and pulling the body) in the autism model

According to Figure 1 ANOVA analysis shows that there are significant differences among the groups VPA, VPA + 100 mg/kg, and VPA + 200 mg/kg in comparison with the control group, with p-values of $p < 0.0001$, $p = 0.006$, and $p < 0.0001$, respectively. Additionally, the groups receiving 100 mg/kg and 200 mg/kg of *Melissa officinalis* extract show a significant difference compared to the autism group ($p < 0.0001$), indicating a reduction in seizure duration relative to the autism group.

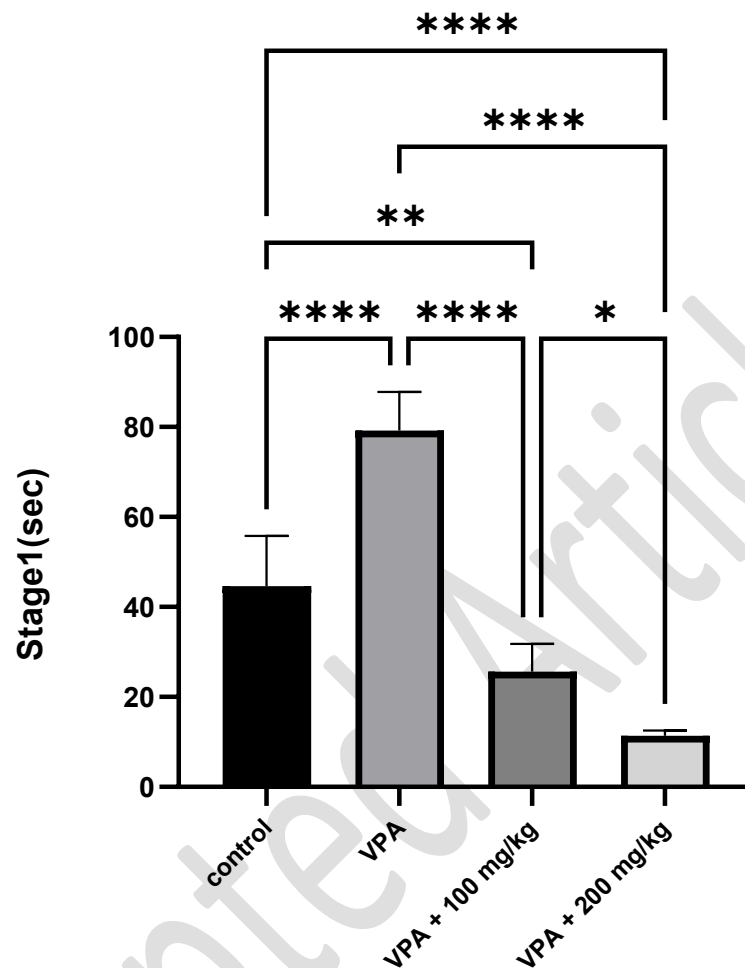


Figure 1. The effect of *Melissa officinalis* extract on the duration of the first stage of seizures. Data are expressed as SEM±Mean for rats in each group. (n=5), * p=0/0460, ** p=0/0065, **** p<0/0001.

2-The effect of *Melissa officinalis* plant extracts the duration of the second stage of seizures (contractile movements of the muscular cheek shock without jumping)

Based on Figure 2, ANOVA analysis shows that there is a significant difference between the VPA, VPA + 100 mg/kg, and VPA + 200 mg/kg groups compared with the control group, with p-values of $p = 0.0005$, $p < 0.0001$, and $p < 0.0001$, respectively. Additionally, the groups receiving 100 mg/kg and 200 mg/kg of *Melissa officinalis* extract show a significant difference compared to the autism group ($p < 0.0001$), indicating a reduction in the duration of the second stage of seizures.

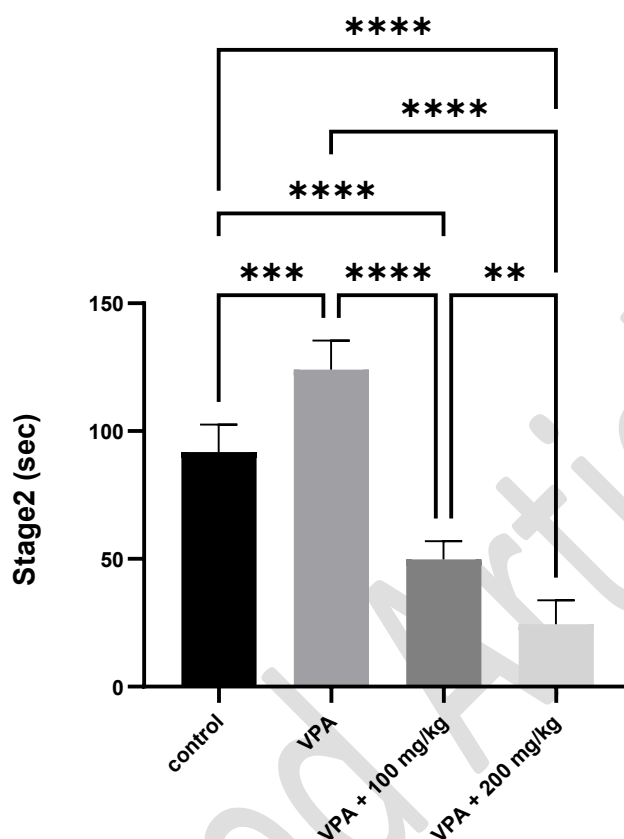


Figure 2. The effect of *Melissa officinalis* plant extract on the duration of the second stage of seizures. Data are expressed as SEM±Mean for rats in each group. (n=5). ** p=0/0042, ***p=0/0005, ****p<0/0001.

3-The effect of *Melissa officinalis* plant extract on the duration of the third stage of seizures (contractile movements of muscle shock with jumping)

According to Figure3, ANOVA analysis shows that there is a significant difference between the VPA, VPA+100mg/kg and VPA+200mg/kg groups compared to the control group, p=0.001, p<0.0002 and p<0.0001, respectively. In addition, the groups receiving 100 mg/kg and 200 mg/kg of *Melissa* extract have a significant difference compared to the autism group (p<0.0001), which indicates a reduction in the duration of the third stage of seizures.

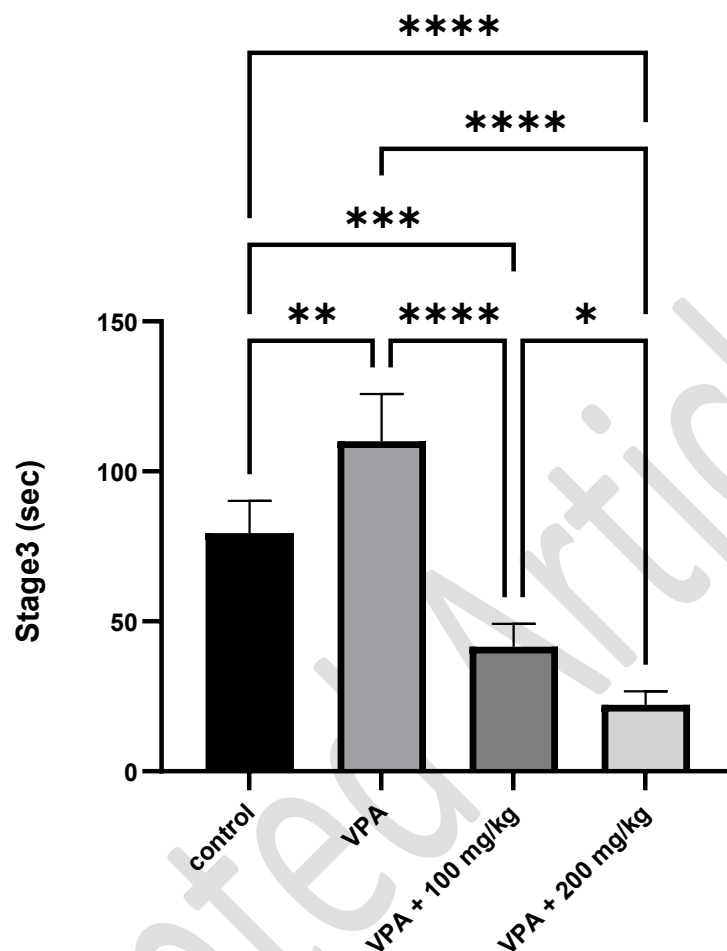


Figure 3. The effect of *Melissa officinalis* plant extract on the duration of the third stage of seizures. Data are expressed as SEM±Mean for rats in each group. (n=5). * p=0/0456, ** p=0/0016, *** p=0/0002, **** p<0/0001.

4-The effect of lemon balm plant extract on the duration of the fourth stage of seizures (falling on the side with generalized tonic-clonic seizures)

According to Figure 4, ANOVA analysis showed that there was a significant difference between the VPA, VPA+100mg/kg and VPA+200mg/kg groups compared to the control group ($p<0.0001$). In addition, the groups receiving 100 mg/kg and 200 mg/kg of *Melissa* extract had a significant difference compared to the autism group ($p<0.0001$), indicating a reduction in the duration of the fourth stage of seizures.

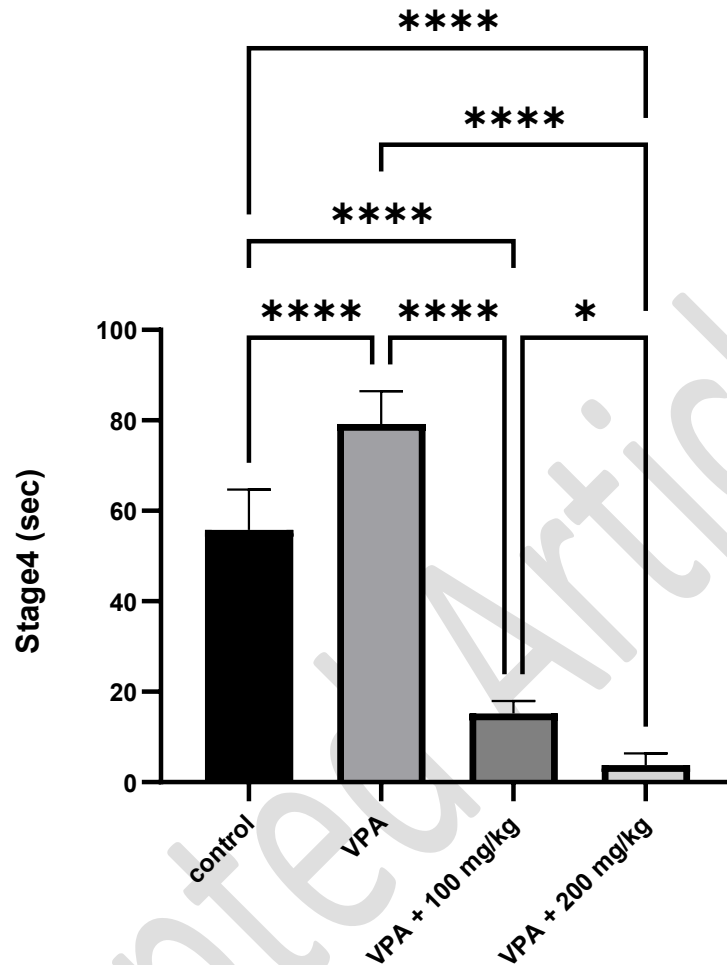


Figure 4. The effect of *Melissa officinalis* plant extract on the duration of the fourth stage of seizures. Data are expressed as SEM±Mean for rats in each group. (n=5). * $p=0/0401$, **** $p<0/0001$.

5-The effect of lemon balm plant extract on the duration of the fifth stage of seizures (falling on the back with generalized clonic-tonic seizures)

Figure 5 shows that ANOVA analysis reveals significant differences among the VPA, VPA + 100 mg/kg, and VPA + 200 mg/kg groups compared with the control group, with p-values of $p = 0.002$, $p = 0.0003$, and $p < 0.0001$, respectively. Additionally, the groups receiving 100 mg/kg and 200 mg/kg of *Melissa* extract show a significant difference compared to the autism group ($p < 0.0001$), indicating a reduction in the duration of the fifth stage of seizures.

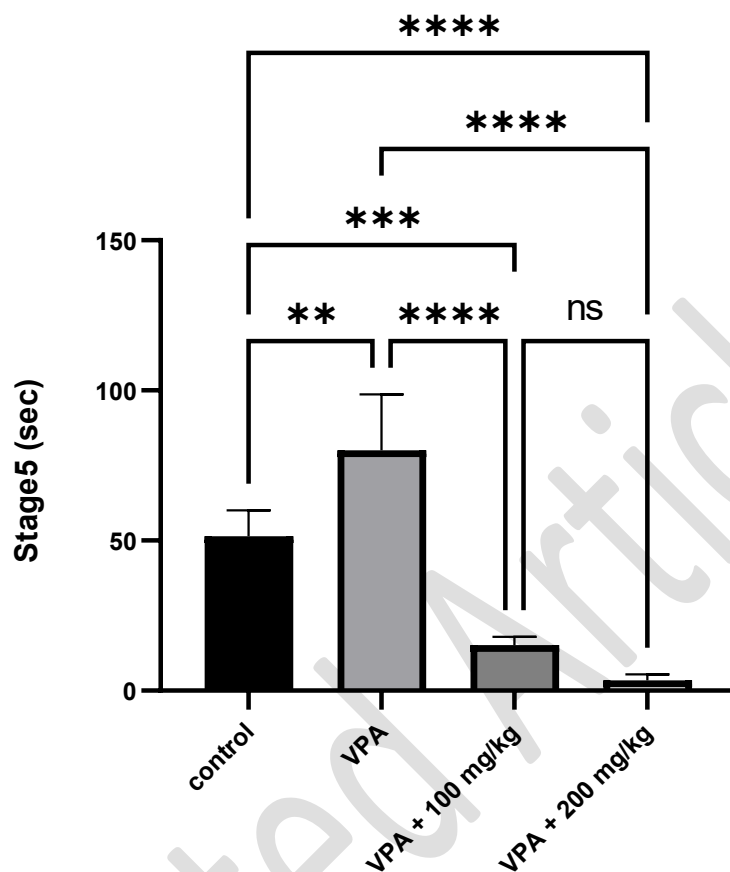


Figure 5. The effect of *Melissa officinalis* plant extract on the duration of the fifth stage of seizures. Data are expressed as SEM±Mean for rats in each group. (n=5). * p=0/3157, ** p=0/0026, *** p=0/0003, **** p<0/0001.

6- In step 6, which was related to the death of the groups due to the injection of PTZ, no deaths were observed between the groups.

Discussion

Autism is a neurodevelopmental disorder characterized by impairments in communication, social interaction, and the emergence of repetitive, stereotyped movements and purposeless behaviors (1). Among individuals with autism, seizures affect approximately 15-30% of cases, though lifetime epilepsy prevalence in autism has been reported to range from 5% to 40% (3). Long-term use of antiepileptic drugs is often limited by adverse effects; conversely, inadequate seizure control can impede multiple functional abilities. Despite progress in anticonvulsant drug discovery, epilepsy remains not fully curable, underscoring the need for exploring herbal alternatives that may offer safer and more effective options (14). Recently, interest has grown in plant-derived medicines due to potential efficacy with fewer side effects. *Melissa officinalis* (Lemon balm) is traditionally used in Iranian medicine as a sedative, antispasmodic, and anticonvulsant, and it is also employed for insomnia, sleep disturbances, anxiety, depression, and various neurological conditions (9). Given traditional claims about its calming and anticonvulsant properties, this study

investigates the effects of *Melissa officinalis* extract on PTZ-induced seizures in male Wistar rats modeled to exhibit autistic traits. PTZ is used to induce seizures because it disrupts the balance between inhibitory and excitatory neurotransmission; as a GABA_A receptor antagonist, PTZ interferes with chloride conductance, while also activating glutamatergic circuits linked to the Calcium–calmodulin/NOS/NO pathway (15). Based on a dose of 80 mg/kg, PTZ reliably elicits myoclonic jerks and generalized tonic–clonic seizures, which justifies its use in this experimental paradigm (16).

Angel A and colleagues in 2016 conducted a study to analyze seizure sensitivity in autistic model mice using valproic acid. Seizures in 14-day-old mice were induced using two models: pentylenetetrazol (PTZ) and lithium pilocarpine (Li-Pilo). They assessed the sensitivity of male and female mice in each group to seizures with these two models. The results showed no difference in the severity of seizures induced by Li-Pilo between VPA and control animals, but prenatal VPA altered sensitivity to PTZ-induced seizures in developing mice, possibly associated with changes in GABAergic transmission (17).

Volkmar and colleagues in 1990 examined seizures in autistic individuals. In this study, the prevalence of seizures in a series of 192 autistic individuals was investigated. 21% of the cases showed seizure disorders. Seizure disorders were more common among individuals with lower IQ. A specific age-related increase in the risk of seizures was 3 to 22 times greater than the general population. Contrary to previous studies, the risk of seizures was higher in early childhood, although it also increased in early adolescence (18). Our research results show that the duration of seizure stages in autistic groups is longer than in other groups, which aligns with previous studies indicating that autistic offspring exhibit more seizure disorders than other groups. In a study conducted by Ghayour et al. in 2011, the antiepileptic effects of lemon balm extract were investigated in pentylenetetrazole-induced seizures at doses of 50, 80, and 120 mg/kg. In this study, the extract was injected into mice and then PTZ was injected intraperitoneally at a dose of 80 mg/kg to observe seizure behaviors. The results showed that lemon balm extract has potential sedative and antiepileptic effects that most likely act through the GABAergic system (8). In our research, the findings show that lemon balm extract, especially at a dose of 200 mg/kg, has greater antiepileptic effects than the dose of 100 mg/kg, and the data also show that lemon balm extract significantly reduces the duration of different stages of seizures compared to the control and autism groups. In addition, compared to the control group, in all experimental groups, the latency period of generalized tonic-clonic seizures was significantly increased ($P < 0.05$). These results are similar to the findings of Ghayour et al., except that different doses of the extract were used and different effects were observed on seizure duration and severity. Finally, it can be said that the hydroalcoholic extract of *Melissa officinalis* has potential sedative and antiepileptic effects and most likely exerts its effects through the GABAergic system. These results indicate the importance of the dose and mode of administration of the extract in the efficacy of herbal treatments for seizure management. Studies have shown that the cholinergic conduction system is intricately linked to epilepsy, with multiple studies revealing its role in the generation and spread of epileptiform discharges (19). Lemon balm, due to its monoterpenes, inhibits acetylcholinesterase and also binds to both nicotinic and muscarinic acetylcholine receptors, thereby modulating the cholinergic system involved in cognitive processes (20). Conversely, enhancing GABAergic transmission—though it can intensify seizures in some contexts—has been shown to suppress pentylenetetrazol-induced tonic-clonic seizures (21). Based on this, a possible mechanism for the sedative and calming effects of lemon balm may be the GABAergic inhibition in the central nervous system, wherein active constituents may interact with the benzodiazepine

binding site on the GABA_A receptor. Lemon balm extract also exerts strong inhibition of GABA transaminase (GABA-T) (22). Some flavonoids within the extract appear to affect certain inhibitory GABAergic neurons, augmenting this inhibitory system. Additionally, lemon balm contains geraniol with sedative and anticonvulsant effects, caffeic acid with anticonvulsant properties, and aromatic compounds that reduce anxiety and psychological tension, contributing to calming effects on the nervous system (23). Therefore, the anticonvulsant effects of lemon balm extract are likely due to a combination of these constituents, and delineating the contribution of each requires further, separate investigations.

Given the results obtained from this study and the positive effects of the hydroalcoholic extract of **Melissa officinalis** on reducing seizures, it is essential to pay special attention to future research directions. Furthermore, investigations into the effects of lemon balm extract on cognitive and behavioral aspects may be particularly important. The findings of several studies indicate that **Melissa officinalis** can influence memory improvement, anxiety reduction, and enhancement of cognitive performance. For example, a study by Akhondzadeh et al. (2010) demonstrated that lemon balm extract has positive effects on cognitive performance in individuals with mood and anxiety disorders, and it may help improve attention and concentration (24). Additionally, another study showed that the consumption of lemon balm extract significantly enhances recall and attention performance in laboratory rats (25). Moreover, further research has indicated that lemon balm extract can have calming effects and enhance cognitive performance, assisting in the reduction of anxiety and stress among healthy individuals (20). Therefore, future studies should specifically examine the effects of **Melissa officinalis** extract on cognitive aspects in patients with autism and other neurological disorders. Such research may lead to a better understanding of the therapeutic potential of this plant in improving the quality of life for individuals facing cognitive and behavioral challenges, this work aims to contribute to the development of more effective and safer treatments.

Conclusion

The present work shows that *Melissa officinalis* extract reduces PTZ-induced seizure activity in male Wistar rats exhibiting autistic traits, suggesting a possible therapeutic role for the extract in controlling seizures among individuals with autism. Additional research is needed to elucidate the mechanisms involved and to assess long-term safety.

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Ethical Statement

This study was approved by the Ethics Committee of Golestan University of Medical Sciences with an approval code of (Ethical code: IR.GOUMS.AEC.1402.013.)

Conflicts of Interest

The authors declared no conflict of interest.

Author Contributions

This article is the result of a research project conducted in collaboration with Ms. Fatemeh Piri and Dr. Hamid Sepehri (project manager) at Golestan University of Medical Sciences.

Data Availability Statement

All the data have been included in the manuscript and will be made available upon publication of the manuscript

Reference

1. Malow BA. Sleep disorders, epilepsy, and autism. *Mental Retardation and Developmental Disabilities Research Reviews*. 2004;10(2):122-5.
2. Williams LM. Precision psychiatry: a neural circuit taxonomy for depression and anxiety. *The Lancet Psychiatry*. 2016;3(5):472-80.
3. Amiet C, Gourfinkel-An I, Bouzamondo A, Tordjman S, Baulac M, Lechat P, et al. Epilepsy in autism is associated with intellectual disability and gender: evidence from a meta-analysis. *Biological psychiatry*. 2008;64(7):577-82.
4. De novo mutations in epileptic encephalopathies. *Nature*. 2013;501(7466):217-21.
5. Saemundsen E, Ludvigsson P, Hilmarsdottir I, Rafnsson V. Autism spectrum disorders in children with seizures in the first year of life-a population-based study. *Epilepsia*. 2007;48(9):1724-30.
6. Mandhane SN, Aavula K, Rajamannar T. Timed pentylenetetrazol infusion test: a comparative analysis with sc PTZ and MES models of anticonvulsant screening in mice. *Seizure*. 2007;16(7):636-44.
7. Fiske J, Boyle C. Epilepsy and oral care. *Dental Update*. 2002;29(4):180-7.
8. Ghayour M, Behnam-Rassouli M, Ghayour N, Tehranipour M, Kamyabi-Abkooh A. Investigating the anti-epileptic and sedative effects of hydroalcoholic extract of melissa officinalis (Lemon Balm) leaf on pentylenetetrazol induced epileptiform seizures in wistar rat. *Journal of Medicinal Plants*. 2012;11(41):64-73.
9. Syed FQ, Mirza MB, Elkady AI, Hakeem KR, Alkarim S. An insight of multitudinous and inveterate pharmacological applications of *Foeniculum vulgare* (fennel). *Plant and Human Health, Volume 3: Pharmacology and Therapeutic Uses*: Springer; 2019. p. 231-54.
10. Hara Y, Ago Y, Taruta A, Hasebe S, Kawase H, Tanabe W, et al. Risperidone and aripiprazole alleviate prenatal valproic acid-induced abnormalities in behaviors and dendritic spine density in mice. *Psychopharmacology*. 2017;234(21):3217-28.
11. Gorgij E, Komeili GR, Zakeri Z, Ebrahimi S. A Comparative Study on The Anticonvulsive Effect of The Hydroalcoholic Extract of The *Melissa Officinalis* Plant with Phenytoin on Wistar Rat. 2012.
12. Del-Bel EA, Oliveira PR, Oliveira JACd, Mishra PK, Jobe PC, Garcia-Cairasco N. Anticonvulsant and proconvulsant roles of nitric oxide in experimental epilepsy models. *Brazilian Journal of Medical and Biological Research*. 1997;30:971-9.
13. Rj R. Modification of seizure activity by electrical stimulation, II: motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972;32:281-93.
14. Zapata-Sudo G, Mendes TC, Kartnaller MA, Fortes TO, Freitas NF, Kaplan MAC, et al. Sedative and anticonvulsant activities of methanol extract of *Dorstenia arifolia* in mice. *Journal of Ethnopharmacology*. 2010;130(1):9-12.

15. Bikjdaouene L, Escames G, Camacho E, León J, Ferrer JM, Espinosa A, et al. Effects of some synthetic kynurenines on brain amino acids and nitric oxide after pentylenetetrazole administration to rats. *Journal of pineal research*. 2004;36(4):267-77.
16. Urbańska E, Drelewska E, Borowicz K, Błaszczak P, Kleinrok Z, Czuczwar S. NG-nitro-L-arginine, a nitric oxide synthase inhibitor, and seizure susceptibility in four seizure models in mice. *Journal of neural transmission*. 1996;103(10):1145-52.
17. Puig-Lagunes AA, Manzo J, Beltrán-Parrazal L, Morgado-Valle C, Toledo-Cárdenas R, Lopez-Meraz M-L. Pentylenetetrazole-induced seizures in developing rats prenatally exposed to valproic acid. *PeerJ*. 2016;4:e2709.
18. Volkmar FR, Nelson DS. Seizure disorders in autism. *Journal of the American Academy of Child & Adolescent Psychiatry*. 1990;29(1):127-9.
19. Khan GM, Smolders I, Ebinger G, Michotte Y. 2-Chloro-N6-cyclopentyladenosine-elicited attenuation of evoked glutamate release is not sufficient to give complete protection against pilocarpine-induced seizures in rats. *Neuropharmacology*. 2001;40(5):657-67.
20. Kennedy DO, Scholey AB. The psychopharmacology of European herbs with cognition-enhancing properties. *Current pharmaceutical design*. 2006;12(35):4613-23.
21. Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *Journal of Neuroscience*. 2002;22(15):6650-8.
22. Awad R, Levac D, Cybulska P, Merali Z, Trudeau V, Arnason J. Effects of traditionally used anxiolytic botanicals on enzymes of the γ -aminobutyric acid (GABA) system. *Canadian journal of physiology and pharmacology*. 2007;85(9):933-42.
23. Buchbauer G, Jirovetz L, Jager W, Plank C, Dietrich H. Fragrance compounds and essential oils with sedative effects upon inhalation. *Journal of pharmaceutical sciences*. 1993;82(6):660-4.
24. Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi A, Khani M. *Melissa officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. *Journal of Neurology, Neurosurgery & Psychiatry*. 2003;74(7):863-6.
25. Ozarowski M, Mikołajczak PL, Piasecka A, Kachlicki P, Kujawski R, Bogacz A, et al. Influence of the *Melissa officinalis* leaf extract on long-term memory in scopolamine animal model with assessment of mechanism of action. *Evidence-based Complementary and Alternative Medicine*. 2016;2016(1):9729818.