Melissa officinalis Extract Improves Spatial Memory and Cognitive Flexibility in a Valproic Acid-Induced Rat Model of Autism Spectrum Disorder: Evidence from Y-Maze Performance

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Abstract

Cognitive impairments, especially spatial memory and cognitive flexibility deficits are core yet therapeutically disregarded aspects of autism spectrum disorder (ASD), and there are no approved drugs that specifically address those areas even though they have a significant effect on adaptive functioning. This experiment assessed the therapeutic value of ethanolic M. officinalis extract on hippocampal-dependent cognitive function in a validated prenatal rat model of ASD with valproic acid (VPA) induced ASD. On gestational day 12.5, pregnant Wistar rats were injected with VPA (600mg/kg, subcutaneously) or saline, and male offspring were injected daily with M. officinalis extract (100mg/kg, oral) or vehicle between postnatal days 35-82. Complementary Y-maze paradigms were used to determine cognitive performance as PND 83, including spontaneous alternation (spatial working memory and cognitive flexibility) and spatial reference memory (60 min retention interval). The exposure to prenatal VPA resulted in severe cognitive impairments where spontaneous alternation has dropped to 22.41% and discrimination index dropped to 0.288. M. officinalis daily treatment led to spectacular cognitive restoration with alternation performance increasing to 41.89% almost twice and discrimination index increasing to 0.708, an improvement of 145% and bettering no-exposed controls by nearly 72%. Intrinsic cognitive-enhancing properties were also supported by the fact that extract-treated control animals also showed better results. According to GC-MS, a-terpineol was the major constituent (29.898%). These results indicate that M. officinalis extract plays a significant part in reducing VPA-induced impairments in spatial working memory and cognitive flexibility, which justifies its translational prospects as a multi-target botanical intervention that targets the critically underrepresented area of cognition impairment in ASD.

Keywords: Autism; Cognitive flexibility; Spatial memory; Y-maze; Melissa officinalis

1. Introduction

Autism spectrum disorder ASD is a complicated neurodevelopmental disorder that occurs in 1 in 36 children in the United States and is not only associated with the lack of social communication and repetitive behaviors but also with severe cognitive deficits that have a major effect on everyday functioning (Hodis et al., 2025). Although diagnostic criteria have historically focused on the social and behavioral aspects, growing evidence is identifying cognitive malfunction, especially the impairment of executive functioning, behavioral flexibility, and spatial working memory, as equally defining traits that decisively define long-term outcomes and quality of life (Banker et al., 2021). Spatial working memory, allowing temporary storage and manipulation of visuospatial information necessary to perform navigation and solve problems, depends on complex hippocampal-prefrontal neural networks(Patai & Spiers, 2021; Wirt & Hyman, 2017), which show consistent abnormalities in ASD populations. Neuroimaging data demonstrate volumetric changes, abnormal activation patterns, and impaired connectivity in these circuits, which are converted into spatial navigation, cognitive flexibility, and adaptive functioning deficits that persist throughout life (Duan & Chen, 2022). The prenatal valproic acid exposure paradigm is one of the most thoroughly tested experimental models of studying autism-like phenotypes in rodents, with a high level of construct and face validity (Mehra et al., 2022). Gestational day 12.5 VPA treatment during neural tube closure leads to behavioral, neuroanatomical, and neurochemical changes that replicate the main ASD characteristics(Ornoy et al., 2023). In addition to social impairments and repetitive behaviors, VPA-exposed offspring always have severe cognitive deficits such as dysfunction of working memory, poor behavioral flexibility, and impaired spatial learning (Taheri et al., 2024). These cognitive phenotypes are caused by inherent impairments in the hippocampal neurogenesis, disturbed GABAergic-glutamatergic ratio, and impaired synaptic structure of hippocampal sub regions, which makes this model an especially useful tool in assessing cognitive interventions (Koch, 2023). The existing pharmacological treatment is deeply unsatisfactory, and only risperidone and aripiprazole have been approved to treat irritability but not core symptoms or cognitive impairments. These drugs have significant side effects such as metabolic imbalance, weight gain, and drowsiness which are especially troublesome in children(Davico et al., 2023; Fieiras et al., 2023; Tomiyama et al., 2025). This clinical vacuum has given rise to significant interest in phytochemical interventions that possess cognitive effects and have good safety profiles. Melissa officinalis or lemon balm (*M. officinalis*), a family member of Lamiaceae that has been widely used traditionally to promote cognitive functions, also has bioactive compounds such as rosmarinic acid, flavonoids, and terpenoids. Preclinical studies report anxiolytic effects, memory enhancement, and acetylcholinesterase inhibition (Mathews et al., 2024) (Mathews et al., 2024), alongside potential GABAergic modulation and antioxidant effects that may correct the excitatory-inhibitory unbalance and oxidative stress of autism pathophysiology (Di Risola, 2023; Roy et al., 2025). The Y-maze apparatus offers a very sensitive paradigm of hippocampal-dependent cognition, by two complementary protocols: spontaneous alternation behavior which evaluates the working memory and behavioral flexibility (Kim et al., 2023), and spatial reference memory which evaluates the formation and retrieval of spatial representations. Spontaneous alternation requires maintaining dynamic representations of those locations that have been recently visited and inhibit perseverative responses, processes reliant on the hippocampal-prefrontal integration (Grgurich, 2025). Successful performance requires functional coordination of spatial working memory, attention and response inhibition is required to achieve successful performance with alternation percentage serving as a sensitive index of cognitive flexibility and circuit integrity (Cleal et al., 2021). Although cognitive impairments are considered as fundamental

ASD characteristics and interest in botanical therapeutics is increasing (Aldekhail et al., 2025), the systematic studies that would investigate the effects of *M. officinalis* on the particular cognitive domains in the validated autism models are still lacking. Although previous studies had determined social behavioral advantages and antioxidant effects, the possibility of improving working memory impairment and cognitive flexibility has not been investigated (Piri & Sepehri, 2025). The aim of the study was to assess therapeutic potential of ethanolic extract of *M. officinalis* in reversing cognitive impairment using prenatal VPA-induced rat model of ASD. This study used Y-maze behavioral paradigms and a detailed phytochemical profiling to describe the effects of extracts on hippocampal-dependent cognitive functions and to offer mechanistic understanding to support translational development in this underserved therapeutic field, specifically targeting spatial working memory and cognitive flexibility.

2. Materials and Methods

2.1. Experimental Animals

Adult Wistar rats (males and females with weights 178-268 g) were used as sources, which is produced at from University of Karbala's College of Pharmacy animal facility. The animal room maintained environmental stability: 12:12 h photoperiod, 22 ± 2°C, and 50-60% humidity. Standard rodent chow standard rodent chow and water ad libitum. A 10 days adaptation period preceded any manipulations.

2.2. Breeding Protocol and Gestational Timing

Overnight, two females were put in a cage with one male. Pregnancy diagnosis was done using vaginal cytology: swabs taken in the morning and examined under the microscope to determine the presence of sperm. Gestational day 0.5 was defined as sperm detection. Pregnancy confirmation was followed by isolation of the pregnant females where they were fed normally during gestation.

2.3. Prenatal VPA Induction Protocol

Sodium valproate (Santa Cruz Biotechnology, SC-202378, USA) at 600 mg/kg was dissolved in 2.5 mL sterile saline immediately before use. Pregnant rats received subcutaneous injection at GD 12.5 a critical neurodevelopmental window. Control animals received equivalent saline volumes.

2.4. Plant Material Sourcing and Authentication

The leaves of *Melissa officinalis* were purchased in the period between January-April 2025 through the Iranian suppliers of herbs. Shade dried in room temperature (7-10 days).

2.5. Ethanolic Extract Preparation and Standardization

Cold maceration extraction procedure: 100 g of powdered leaves were soaked in 400 mL of 70% ethanol (v/v) in darkness. Whatman No. 1 paper filtration was followed by rotary evaporation under vacuum. Oven-drying (40°C, 72 hours) was done on concentrated material. Final product was reconstituted in distilled water, up to 100 mg/mL, and refrigerated at 4°C.

2.6. Phytochemical Profiling by GC-MS Analysis

Chemical composition analysis utilized Scion 436 GC-MS (Scion Instruments, USA). Operational settings: helium carrier gas, 250°C injector temperature. Temperature gradient: 40°C (1 min hold) → 5°C/min ramp → 130°C (3 min) → 5°C/min → 150°C (3 min) → 5°C/min → 190°C (5 min). Compounds were identified using NIST library matching.

2.7. Experimental Design and Treatment Regimens

2.7.1. Prenatal Treatment Groups

Following mating confirmation (GD 0.5), dams were randomized (n=5/group):

- **VPA Group**: 600 mg/kg valproate, subcutaneous, GD 12.5
- **Control Group**: Saline, subcutaneous, GD 12.5

Offspring remained with mothers until PND 24 weaning.

2.7.2. Postnatal Treatment Protocol

At PND 35, male offspring from each prenatal group were subdivided (n=5/group), creating four experimental conditions:

a. Control + Vehicle: Saline-exposed, receiving tap water orally
b. VPA + Vehicle: Valproate-exposed, receiving tap water orally
c. VPA + M.O.: Valproate-exposed, receiving extract (100 mg/kg) orally
d. Control + M.O.: Saline-exposed, receiving extract (100 mg/kg) orally

Daily oral gavage (PND 35-82) delivered treatments. Morning administration (09:00-11:00) maintained consistency.

2.8. Y-Maze Cognitive Assessment Protocols

2.8.1. Apparatus Specifications and Environmental Control

Testing occurred at PND 83 in a Y-maze apparatus. Three identical arms (60 cm long × 12 cm wide × 20 cm high) radiated at 120° angles, labeled A, B, C. Testing room conditions: 22 ± 2°C, dim lighting (30-40 lux), minimal noise. Overhead camera captured all sessions.

2.8.2. Spontaneous Alternation Paradigm

Rats were placed individually at one arm's end, facing center, for 8-minute free exploration. Arm entry: all four paws crossing threshold. Entry sequences were recorded by observers blind to treatment groups. Total entries measured locomotor activity. Alternation triads (three successive entries, all different arms: A-B-C, B-C-A, etc.) indicated working memory. Correct alternations required non-repeating sequences; incorrect sequences contained repetitions (A-B-A, C-C-B).

2.8.3. Spatial Reference Memory Assessment

Two-phase protocol with retention interval tested spatial discrimination. **Acquisition (15 min):** One arm blocked; rat explored two accessible arms freely, forming spatial memory. **Retention**

interval: 60 minutes. **Test phase (5 min)**: Barrier removed; all three arms accessible. Previously blocked arm now novel. Intact memory produces novel arm preference. Video analysis quantified time per arm, entry frequency.

2.8.4. Behavioral Parameters and Calculation Methods

Spontaneous Alternation:
Alternation % = [Correct Alternations ÷ (Total Entries - 2)] × 100
Values >50% indicate functional working memory; ≤50% suggests impairment.
Spatial Reference Memory:
Discrimination Index = Novel Arm Time ÷ (Novel Arm Time + Mean Familiar Arms Time)
Values approaching 1.0 demonstrate intact spatial memory; ~0.33 indicates chance performance (impairment).

3. RESULTS

3.1 Phytochemical Composition of *Melissa officinalis* Extract

The ethanolic M. officinalis extract was analyzed using gas chromatography-mass spectrometry to reveal eight different phytochemical compounds with retention times of between 4.823 and 23.823 minutes (Table 1). a-terpineol was the most common one with 29.898% of the total area of its peak and with high identification confidence (98% probability match). Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester was the second most common constituent and had a probability of 73% and a percentage of 22.270 (22.27013.631). Cyclohexene derivatives (18.362% of the total composition) were 3-methyl-6-(1-methyletylidene) (12.402%, 75% probability), and 1-methyl-4-(1-methylethenyl)-, (S)- (5.960% 70 probability). Minor components were 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (9.148%, 60% probability), 3-buten-2-one, 4 (2,5,6,6-tetramethyl-1-cyclohexen-1-yl)- (7.703%, 80% probability) and tonalid (5.149%). The likelihood was consistent between 60-98 with major constituents being identified reliably. This chemical profile validates the fact that there is a variety of bioactive compounds that can actually work synergistically to produce the observed therapeutic effects.

Table 1: Phytochemical compounds detected in the ethanolic extract of *Melissa officinalis* using GC-MS. Performance in Spatial Reference Memory

Peak	Retention Time (min)	Area (%)	Probability (%)	Compound Name
1	4.823	5.960	70	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)
2	8.754	29.898	98	α-Terpineol
3	8.867	12.402	75	Cyclohexene, 3-methyl-6-(1-methylethylidene)
4	19.715	22.270	73	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester
5	19.827	7.703	80	3-Buten-2-one, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)
6	21.471	13.631	82	Salicylic acid, tert.-butyl ester
7	23.630	9.148	60	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin
8	23.823	5.149	-	Tonalid

Values represent relative abundance as percentage of total peak area. Probability indicates NIST library match confidence

3.2 Effects on Spontaneous Alternation Behavior

3.2.1 Exploratory Activity and Arm Entry Patterns

Analysis of general exploratory behavior revealed variable locomotor activity across experimental groups (Table 2). Total arm entries during the 8-minute testing period ranged from 8 to 22 entries per animal, with individual variability observed within groups. Control animals (Normal Saline + Vehicle) exhibited mean total entries ranging from 8 to 22 (mean ± SE calculated across n=5: 15.2 ± 2.32 entries), while VPA-exposed animals showed comparable activity levels (12-18 entries; 15.6 ± 1.08), indicating that prenatal VPA exposure did not significantly alter baseline locomotor function or exploratory drive. Animals receiving extract treatment (VPA + Extract group) demonstrated similar entry patterns (10-18 entries; 15.2 ± 1.36), as did the Normal Saline + Extract group (8-18 entries; 14.4 ± 1.91). The absence of significant between-group differences in total arm entries confirmed that observed cognitive deficits could not be attributed to motor impairments, sedation, or altered exploratory motivation.

3.2.2 Spontaneous Alternation Performance

Spontaneous alternation percentage, the primary index of spatial working memory and cognitive flexibility, revealed profound group differences (Table 2, Figure 1). Control animals (Normal Saline + Vehicle) displayed highly variable performance, with alternation percentages ranging from 12.50% to 41.67% (mean: 21.87 ± 5.40%), surprisingly falling below the theoretical chance level of 50% and suggesting potential stress-related performance decrements even in non-VPA-exposed animals. Prenatal VPA exposure without therapeutic intervention (VPA + Vehicle group) produced even more severe impairment, with alternation percentages ranging from 8.33% to 31.25% (mean: 22.41 ± 4.23%), indicating profound working memory dysfunction. Individual animals in this group demonstrated marked perseverative behaviors and reduced ability to systematically explore the maze environment.

Strikingly, *M. officinalis* extract treatment in VPA-exposed offspring (VPA + Extract group) produced substantial cognitive enhancement, elevating alternation percentages to a range of 37.50-50.00% (mean: 41.89 ± 2.62%). This represented nearly a doubling of performance relative to untreated VPA animals and approached normal functional levels. The therapeutic effect was consistent across individual animals within the group, demonstrating reliability of the cognitive enhancement. Most remarkably, extract administration to non-VPA-exposed controls (Normal Saline + Extract group) yielded alternation percentages of 33.33-50.00% (mean: 40.06 ± 3.29%), significantly exceeding untreated controls and indicating cognitive-enhancing properties independent of pathological reversal.

Table 2: Individual animal performance in spontaneous alternation paradigm

No	Group	Total Arm Entries	Correct Alternations	Incorrect Alternations	Alternation %
1	VPA + Extract	16	6	3	37.50
2	VPA + Extract	16	8	1	50.00
3	VPA + Extract	18	8	2	44.44
4	VPA + Extract	16	6	3	37.50
5	VPA + Extract	10	4	2	40.00
6	VPA + Vehicle	16	5	4	31.25
7	VPA + Vehicle	12	1	5	8.33

8	VPA + Vehicle	16	5	4	31.25
9	VPA + Vehicle	18	4	6	22.22
10	VPA + Vehicle	16	3	6	18.75
11	Normal Saline + Extract	16	7	2	43.75
12	Normal Saline + Extract	18	6	4	33.33
13	Normal Saline + Extract	8	4	1	50.00
14	Normal Saline + Extract	14	5	3	35.71
15	Normal Saline + Extract	16	6	3	37.50
16	Normal Saline + Vehicle	22	9	3	40.91
17	Normal Saline + Vehicle	12	5	2	41.67
18	Normal Saline + Vehicle	8	4	1	50.00
19	Normal Saline + Vehicle	14	4	3	28.57
20	Normal Saline + Vehicle	20	7	4	35.00

Each row represents individual animal performance. Alternation % calculated as: [Correct Alternations ÷ (Total Entries - 2)] × 100.

3.2.3 Analysis of Correct versus Incorrect Alternations

Close analysis of altering patterns gave mechanistic understanding of cognitive impairment. Untreated VPA-exposed animals made significantly more errors (mean: 5.0 ± 0.45 errors) than they made correct alternation triads (mean: 3.6 ± 0.81), which is perseverative responding, and the failure to sustain dynamic spatial working memory. Such a pattern of errors showed that the recently visited locations could not be inhibited and was an indication of dysfunction in the prefrontal-hippocampal circuitry. Extract treatment minimized erroneous alternations (VPA + Extract: mean 2.2 ± 0.37 errors) and maximized correct alternations (mean: 6.4 ± 0.81) and indicated not only greater working memory ability but also better response inhibition. Similarly, the patterns of alternation of the Normal Saline + Extract group were favorable (correct: 5.6 ±0.60; incorrect: 2.4 ± 0.51), which validated cognitive-enhancing effects between phenotypes.

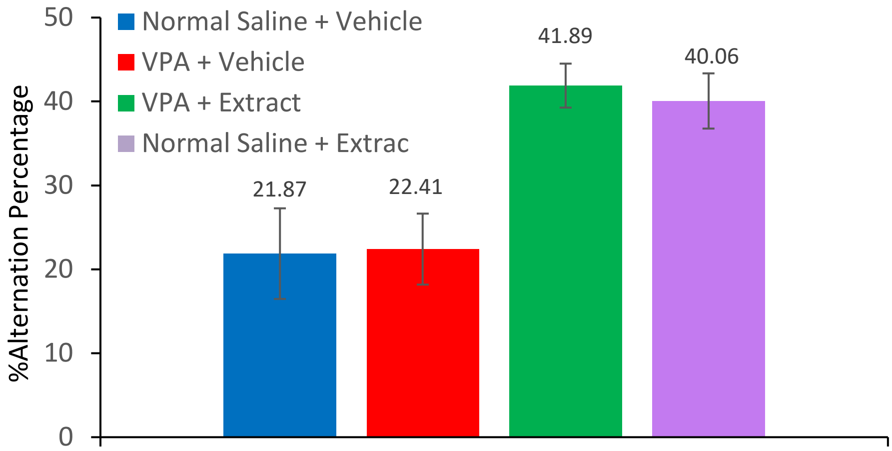

Figure 1: *Melissa officinalis* extract ameliorates working memory deficits in spontaneous alternation performance

3.3 Spatial Reference Memory Performance

The index of spatial discrimination of 1 versus 2 of preferential exploration of novel versus familiar location following 60 min retention interval showed the difference between the groups in terms of long-term spatial memory consolidation ability (Table 3, Figure 2). The control animals (Normal Saline + Vehicle) showed moderate discrimination ability (DI: 0.412 ± 0.137), which means that the exploration of the novel arm was selective but not entirely selective. The result of this intermediate performance was an indication that even non-pathological animals had a difficulty with the consolidation of spatial memory under the conditions of testing that were used. Prenatal exposure to VPA significantly affected the spatial reference memory with the VPA + Vehicle group having significantly lower discrimination indices (0.288 ± 0.095, $p<0.05$ vs. control) close to the chance (0.33) and showing severe impairments in the ability to form stable spatial representations of the acquisition phase or recalling the representations following the retention interval. Single animals within the group exhibited little preference to novel arms and spent an equal amount of time on each of the three arms indicatives of a significant dysfunction of hippocampal-dependent memory. The administration of *M. officinalis* extract resulted in impressive recovery of the spatial reference memory on VPA-exposed offspring. Discrimination indices were found to be greater in VPA + Extract group of 0.708 ±0.083, which is a 145 % better result than untreated VPA animals ($p<0.05$), and greatly exceedingly even control performance by 72 %, this dramatic effect indicated not only VPA induced spatial memory deficits were reversed by extract treatment, but in addition this treatment was effective in improving performance even above control levels of performance. The Normal Saline + Extract group also showed excellent performance of spatial discrimination (0.726 ± 0.077) the best of all groups and this confirmed intrinsic memory-enhancing ability of *M. officinalis* irrespective of disease condition.

Statistical analysis revealed no significant difference between two extract-treated groups ($p>0.05$), but both differed significantly from VPA + Vehicle ($p<0.05$), the Normal Saline + Extract group showed significant enhancement compared to untreated controls ($p<0.05$), while VPA + Vehicle and Normal Saline + Vehicle groups shared statistical homogeneity in their suboptimal performance.

Table 3: Spatial discrimination index across experimental groups

Group	Discrimination Index (Mean ± SE)	Statistical Grouping
Normal Saline + Vehicle	0.412 ± 0.137	AB
VPA + Vehicle	0.288 ± 0.095	B
VPA + Extract	0.708 ± 0.083	A
Normal Saline + Extract	0.726 ± 0.077	A

Values represent mean ± SE (n=5 per group). Different letters indicate significant differences at p<0.05 (LSD post-hoc test). DI calculated as: Novel Arm Time ÷ (Novel Arm Time + Mean Familiar Arms Time). Chance level = 0.33.

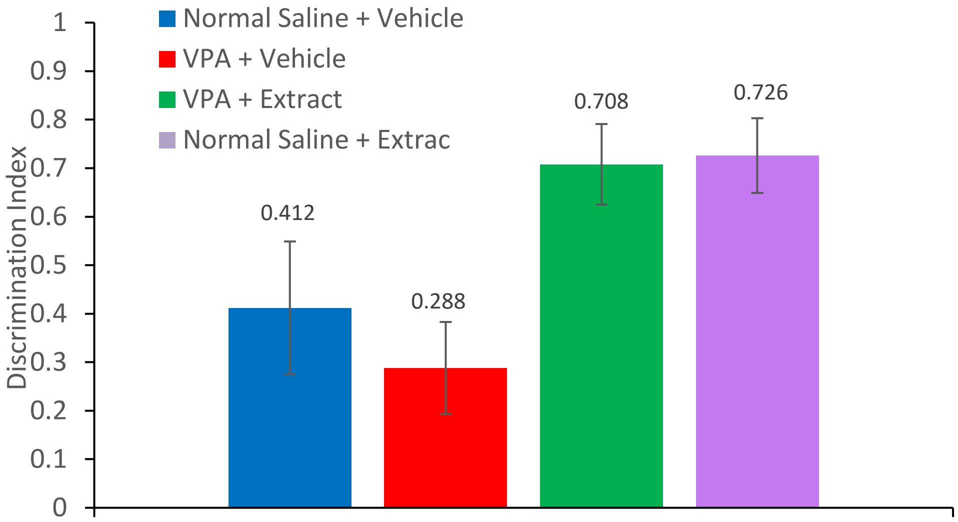

Figure 2: Spatial reference memory performance across treatment groups

4. Discussion

This paper shows that ethanol M. officinalis extract is able to alleviate learning deficits in a rat subject of autism spectrum disorder induced by prenatal exposure to valproic acid significantly. The VPA-exposed rats exhibited significant impairment of spatial working memory (spontaneous alternation: 22.41), and reference memory (discrimination index: 0.288) but daily oral (100 mg/kg) M. officinalis extract (PND 35-82) generated significant effects (alternation: 41.89; discrimination index: 0.708). These results are specifically applicable since cognitive dysfunction, particularly, executive function impairment, behavioural flexibility, and spatial memory is an underutilized treatment group in autism with no FDA-approved pharmacological interventions directly addressing these impairments to date (Salloum-Asfar et al., 2024) (Salloum-Asfar et al., 2024). The behavioural phenotype is in line with the literature suggesting that exposure to prenatal VPA at GD 12.5 triggers cascading developmental neuroeffects, which are reflected by structural and functional impairments in cognitive brain areas (Zarate-Lopez et al., 2024) (Zarate-Lopez et al., 2024). The spontaneous alternation result, which was significantly lower than the 50% chance level, implies that the working memory was severely damaged and that perseveration was also observed as perfrontal-hippocampal circuit dysfunction. This is consistent with the literature showing that VPA exposure impairs the integrity of the hippocampal subregions, specifically, dentate gyrus and CA3 fields, where synaptic protein expression is inhibited and neurogenesis is postponed (Long et al., 2024) (Long et al., 2024).

Neurochemical balance and synaptic plasticity are some of the mechanistic underpinnings of VPA-induced cognitive impairment. Exposure to VPA interferes with the GABAergic interneuron migration and maturation, which affect inhibitory tone required to operate working memory processes (Tzilivaki et al., 2023) (Tzilivaki et al., 2023). Moreover, VPA inhibits the expression of brain-derived neurotrophic factor (BDNF) and affects the synaptic plasticity, long-term potentiation, and dendritic spine formation due to disturbed TrkB-CREB signalling (Mohamm(Tzilivaki et al., 2023)adkhani et al., 2022). (Mohammadkhani et al., 2022). They include oxidative stress and neuroinflammation, which damage synapses and promote microglia (Bjørklund et al., 2020) (Bjorklund et al., 2020). The pronounced cognitive enhancement of the M. officinalis-treated group implies the use of various therapeutic processes. First, the cholinergic effects caused by acetylcholinesterase inhibition enhance the availability of the acetylcholine required in attention, working memory encoding, and theta oscillations in the course of the spatial navigation (Ghazizadeh et al., 2021). (Ghazizadeh et al., 2021). Second, M. officinalis components regulate GABAergic neurotransmission, which could be a normalizer of excitatory-inhibitory imbalance (Mathews et al., 2024). (Mathews et al., 2024). Third, phytochemicals (particularly Rosmarinus acid and flavonoids) enhance the BDNF expression and TrkB-CREB signalling, which favor the development of dendritic spines, protein synthesis at synapses, and long-term potentiation (Shi et al., 2025). (Shi et al., 2025). Controlled by BDNF/TrkB interaction with cAMP/PKA/CREB signalling, gene transcription programs that transform short-term alterations into long-term memory traces (Gupta et al., 2025) (Gupta et al., 2025) probably control the reported 60-minute spatial consolidation of memory. The phytochemistries found a-terpineol in the highest amount (29.898%), which is a monoterpenoid, possessing neuroprotective and cognitive enhancing effects such as antioxidant activity, anti-inflammatory effects, and neurotransmitter modulation (Vieira et al., 2020) (Vieira et al., 2020). Non-volatile phenolic acids and flavonoids, however, are not detected by GC-MS methodology, which limits its use in identifying bioactive classes that are likely an important contribution to therapeutic responses. Complementary HPLC-MS techniques should be used in future standardization in order to fully characterize it. The functional implications of the spatial reference memory improvement are great. Reference memory is a multi-stage processing that requires sequential hippocampal processing such as dentate gyrus pattern separation, CA3 associative encoding and CA1-entorhinal consolidation (Long et al., 2024). (Long et al., 2024). The high improvement in the discrimination index is a sign of recovery at these phases with direct translational applicability to navigation, environmental learning, and spatial problem-solving- areas often impaired in autism that limit the ability to become independent. Recent ASD pharmacotherapy, which is confined to risperidone and aripiprazole to treat irritability, has severe adverse effects without curing fundamental symptoms and cognitive deficiency (Yang et al., 2025) (Yang et al., 2025). There is a potential benefit in M. officinalis: a long history of traditional use with reported safety, anxiolytic properties that show benefits in anxiety comorbidities, multi-target effects, and addressing the complex pathophysiology, as well as its availability as a dietary supplement with generally good tolerability (Mathews et al., 2024) (Mathews et al., 2024). Nonetheless, translation will be faced with a standardization issue since phytochemical composition differs due to the cultivation, harvesting, and extraction processes. Verified bioactivity Pharmaceutical grade preparations are required. The optimal human dosing is not determined, rodent dose of 100 mg/kg is approximated to be 16 mg/kg in humans (1000-1200mg/70-kg adult), but due to age-dependent pharmacokinetics, it is recommended to determine dose carefully. There is the issue of some methodological considerations. Y-maze paradigms only test hippocampal-dependent cognition which are just a part of the cognitive processes which are impaired in autism. The sample (n=5/group) should be replicated by bigger cohorts. Use of male offspring only restricts generalizability but it had to be done to minimize variance. The hypothesized mechanisms should be confirmed by research on neurochemical changes, synaptic proteins, neuroinflammatory markers and the levels of BDNF. Research in the future needs to be comprehensive in characterization of mechanistic interactions, investigate the full autism spectrum of phenotypes, explore combinatorial approaches to behavioral interventions, identify the most effective treatment windows and identify biomarkers to be used in personalized therapies. Conclusively, this paper offers preclinical results that M. officinalis extract can generate significant positive effects on hippocampal-dependent cognitive damage in the VPA autism model using multi-targeting. Cognitive changes that were observed make M. officinalis a translational development candidate. Strict clinical research should be enabled to discover whether these results are applicable to meaningful cognitive changes in the individuals with autism spectrum disorder, which may fill a grossly underinvested area of therapeutic intervention.

5. Conclusion

This paper has shown that ethanolic extract of M. officinalis has substantial cognitive advantages on a rat model of prenatal valproic acid-induced autism spectrum disorder as indicated by an extensive Y-maze behavioral analysis. There were severe deficits in spatial working memory (spontaneous alternation: 22.41%) and spatial reference memory (discrimination index: 0.288) in VPA-exposed offspring, which are hippocampal circuit dysfunctional indicators of autism-related cognitive impairment. Administration of extract of M. officinalis (100 mg/kg), in juvenile-adolescent phase (PND 35-82) led to a significant recovery in the two domains of cognition, with alternation performance reaching 41.89 percent and discrimination index reaching 0.708 or above, significantly over the non-exposed controls. The phytochemical analysis have revealed a-terpineol as the most common constituent (29.898%), other bioactive compounds may also provide therapy as a multi-target activator such as cholinergic, GABAergic, BDNF, and antioxidant and anti-inflammatory effects. The cognitive gains are directly proportional to fundamental weaknesses in autism populations: working memory, behavioral flexibility and spatial cognition which are substantial predictors of education, social adjustment and independent living. Such cognitive domains are not addressed by the current pharmaceutical possibilities which underlines the necessity to use some alternative to the drugs with good safety profiles. Having established traditional use, known tolerability, and polypharmacological neurobiological effects, M. officinalis is a promising translation to clinical use. Future studies need to examine rigorous mechanistic characterization, dose-optimization, identification of treatment window, and appropriately designed clinical trials to determine whether these preclinical results can be applied to meaningful cognitive change in individuals with autism spectrum disorder, and have the potential to fill a very underserved area of research in therapeutics.

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