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Research Article

Gas Chromatography–Mass Spectrometry Analysis and Memory-enhancing Effect of Hydroalcoholic Extract of a Polyherbal Unani Formulation' *Majoon Vaj*' in Scopolamine and Diazepam-induced Amnesic Mice

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ABSTRACT

Majoon (M) Vaj is a polyherbal Unani formulation possessing multiple neuropharmacological activities. It is used to treat *Nisyān* (dementia) for centuries. It has been reported as improving memory and overall mental faculties. The current study was carried out to assess the nootropic and anti-amnesic activity of *M. Vaj* in mice to validate its use in Unani medicine. The exteroceptive behavior model elevated plus maze (EPM) and morris water maze (MWM) and interoceptive behavior model (scopolamine and diazepam) were used to evaluate nootropic activity in mice. Pre-treatment with *M. Vaj* extract (220 and 440 mg/kg, p.o.) for 15 days showed a significant decrease transfer latency (TL) in EPM and decrease escape latency (EL) during training and increase time spent in the target quadrant (TSTQ) during retrieval in the MWM test, indicating nootropic activity. *M. Vaj* also significantly reversed scopolamine and diazepam-induced amnesia, as observed by decreased TL in EPM and increased TSQT in MWM, indicating anti-amnesic activity. Also, both doses of the test drug showed a similar response, which was almost equal to that of the standard drug piracetam. Reversal of scopolamine and diazepam-induced amnesia by *M. Vaj* indicated the possible facilitation of cholinergic transmission or GABA benzodiazepine pathway. GC-MS of *M. Vaj* led to the identification of 141 compounds. α-asarone (72.12%) and β-asarone (72.12%) were found in the highest amount as compared to other compounds.

Introduction

Dementia is a clinical syndrome caused by brain disorders that impair memory, learning, comprehension, and judgment.[1,2] It became more common in older persons. It affects about 50 million people worldwide.[3–5] Alzheimer's disease (AD) is the most common cause of dementia, accounting for 60–70% of all cases. It is distinguished by the deposition of βA plaques between neurons, which interact with cell membranes, causing oxidative stress and finally neuronal cell Memory.[6,7] Memory impairment may also be due to decrease levels of acetylcholine in the brain, hypoxia and increase oxidative stress. Inhibitors of acetylcholinesterase (AChE) are used to treat dementia because they increased the acetylcholine level at the cholinergic synapse.[8–10] However, there are several issues with these medications' tolerability and side effects.[11,12] *M. Vaj* is a semisolid poly-herbal Unani formulation containing *Acorus calamus*, *Piper nigrum, Zingiber officinalis, Nigella sativa, Carum carvi* and *Plumbago zeylanica*. In different *Qarabadeens* (unani pharmacopoeias), it has been described to be useful in *Nisyān* (dementia).[13,14] It has been reported as improving memory and overall mental faculties. Even retrieval of long-term memory loss has been mentioned in many books after the use of this drug, therefore it was selected to test the nootropic activity.[13–15] The ingredients of *M. Vaj* already showed

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neuroprotective, anti-neuroinflammatory, antioxidant, anticonvulsant, central nervous system (CNS) stimulant, and memory-enhancing properties.[16–21] The development of herbal nootropics as a substitute for chemical nootropics will significantly improve the healthcare system.

Materials And Methods

Experimental Animals

Swiss male mice (2–3 months, 20–25 gm) were used for the study. Mice were acquired from Sri Raghavendra enterprises, Bangalore-560040. The animals were kept in groups of six under standard laboratory conditions (23° ± 2°C temperature, 12 hours lighting) and had free access to a standard diet and water. They were acclimatised for 10 days. The drugs were given to the animals in the morning and all the behavioral tests were carried out under the same settings and at the same time. The Institutional Animals Ethics Committee (IAEC), NIUM, Bangalore, Karnataka, approved the experimental protocol vide Reg. No IAEC/6/17/IA/04 dated 29/02/2020. The experiments on animals were conducted in the Animal House, NIUM, Bangalore-91.

Drugs and Chemicals

Piracetam (Nootropil®): Dr Reddy Laboratories Ltd. (Batch no. DK0005); Diazepam (LORI®): NEON Laboratories Ltd, (Batch no. 143296) and Scopolamine hydrobromide from Yarrow chem products, Mumbai, India (Batch no. 11301220V).

Collection and Authentication of the Raw Drugs

All the ingredients of *M. Vaj* were obtained from the pharmacy of NIUM, Bangalore except *A. calamus, N. sativa,* and *P. zeylanica*, which were purchased from the herbal market of Bangalore. All the drugs were identified and authenticated by Dr V Rama Rao (Botanist at Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore with reference No. Authentication/SMPU/RARIMD/BNG/2020-21/865. The voucher specimens have been deposited in the herbarium of the institute (voucher specimen no. 96/IA/Res/2021).

Preparation of Extract

All the components of *M. Vaj* were processed into a coarse powder separately using an electric grinder and were passed through sieve number 80 separately. Powders were mixed according to the ratio specified in *Qarabadeen Azam* and *Al-Qarabadeen*.[13,14] The extract of *M. Vaj* was prepared using the soxhlet apparatus. A 100 gm of the drug was extracted in 50% ethanol (400 mL) for 6 hours at 65–80°C. After extraction, the extract was filtered through Whatman filter paper 44 before being placed in a water bath to evaporate the entire solvent. The extract was weighed and the yield percentage was calculated regarding the crude drug and was found to be 22%.

Drug Preparation and Administration

M. Vaj was dissolved in distilled water and administered orally with the help of a feeding needle while piracetam, diazepam and scopolamine hydrobromide were administered intraperitoneally. All of the treatments were administered in the form of a freshly prepared suspension at the time of administration.

Behavioural Tests Employed for Evaluation of Nootropic Activity

Elevated Plus Maze Test (EPM)

The EPM was developed to investigate learning and memory. It had two open arms (16×5 cm) and two covered arms (16×5×12 cm). Both were linked with a central platform (5×5 cm), and the maze was raised 25 cm from the floor. The strategy and method for testing memory were carried out by the specifications established by earlier researchers.[22,23] Transfer latency (TL) was measured by placing the mouse at the end of an open arm towards the central platform. TL is the time taken by the mouse to enter into one of the enclosed. If the mouse did not enter within 90 seconds, the TL was assigned as 90 seconds. On the first day (*i.e.,* the 15th day of treatment), TL was observed in both (Exteroceptive and Interoceptive) tests and retention of the learned task was tested again the next day i.e., on the 16th day.

Morris Water Maze Test (MWM)

It has been frequently used to assess spatial learning and memory in mice. The strategy and method for testing memory were carried out by the criteria previously described.[24,25] It contains a circular pool (60×25 cm) which was filled with water at 20 cm. The pool was kept in the same location throughout the trial. To make the pool water opaque, milk powder was added. Threads divided the pool into four quadrants of equal size (T1, T2, T3, and T4). A submerged white platform (6×6 cm), was situated inside the target quadrant (T_4), 1.2 cm below the surface of the water. During training, the mouse was put gently in the centre of each quadrant and given 120 seconds to identify the underwater platform. If the mouse didn't discover the platform within 120 seconds, it was pushed gently into the platform and allowed to stay there for 20 seconds. Each mouse was subjected to four consecutive trials with a 5 minutes gap between them for four successive days (11th to 14th day). Escape latency (EL) was recorded from the 11th to the 14th day of drug administration. EL is the time taken by the mouse to move from the initial quadrant to locate the submerged platform in the target quadrant. Throughout the training phase, the target quadrant (T4) remained unchanged. During the training, each animal began in the following position:

Day1 - T_1, T_2, T_3, and T_4
Day2- T_2, T_3, T_4, and T_1

Day3- T_3, T_4, T_1, and T_2
Day4- T_4, T_1, T_2, and T_3

On the 15th day, the platform was taken out from the pool and the mouse was kept back in any of the quadrants (T_1, T_2 and T_3) and allowed to explore the target quadrant (T_4) for 300 second. The time spent in the target Quadrant (TSTQ) in search of the missing platform was recorded as a memory retrieval index. During the trials, the observer remained in the same location.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Bioactive constituents like α-asarone, β-asarone, β-pinene, β-phellandrene, thymoquinone and phenolic compound from *M. Vaj* was determinutesed using GC-MS analysis. The agilent GC5975MSD model was used for the GC-MS. The software required for analytical studies is Turbo mass ver. 5.5. The chromatogram was matched to the NIST-11 library to identify bioactive chemicals.

Experimental Design

Groups for EPM

Group 1 to 4: Distilled water (10 mL/kg p.o.) (Plain control), Piracetam (400 mg/kg i.p.) (Standard control), and *M. Vaj* (220 and 440 mg/kg p.o.), respectively, were given for 15 consecutive days. TL was recorded 45 minutes after the drug administration on the 15th day and retention was observed on the 16th day.

Group 5: Single dose of scopolamine (0.4 mg/kg, i.p.) and served as a negative control. TL was recorded after 45 minutes of injection and again after 24 hours.

Group 6 to 8: Piracetam (400 mg/kg, i.p.) and *M. Vaj* (220 and 440 mg/kg p.o.) were given for 15 successive days. On the 15th day, scopolamine (0.4 mg/kg i.p.) was given after 90 minutes of administration of the respective treatments. TL was recorded after 45 minutes of administration of scopolamine and again after 24 hours.

Group 9: Single dose of diazepam (1-mg/kg, i.p.) and served as a negative control. TL was recorded after 45 minutes of injection and again after 24 hours.

Group 10 to 12: Piracetam (400 mg/kg, i.p.) and *M. Vaj* (220 and 440 mg/kg p.o.) were given for 15 successive days. On the 15th day, diazepam (1-mg/kg, i.p.) was given after 90 minutes of administration of the respective treatments. TL was noted after 45 minutes of administration of diazepam and again after 24 hours.

Groups for MWM

Group 13 to 16: Distilled water (10 mL/kg p.o.) (plain control), piracetam (400 mg/kg i.p.) (standard control) and *M. Vaj* (220 and 440 mg/kg p.o.), respectively, were given for 15 successive days. EL was noted 45 minutes after administration of respective treatment from the 11th to the 14th day. On the 15th day, TSTQ was recorded 45 minutes after administration of the respective drug.

Group 17: It served as a negative control group. EL was recorded from the 11th to the 14th day. On the 15th day, scopolamine (0.4 mg/kg, i.p.) was injected and TSTQ was noted 45 minutes after the administration of scopolamine.

Group 18 to 20: Piracetam (400 mg/kg, i.p.) and *M. Vaj* (220 and 440 mg/kg p.o.), respectively, were administration for 15 consecutive days. EL was recorded 45 minutes after administration of respective treatment from the 11th to 14th day. On the 15th day, scopolamine (0.4 mg/kg, i.p.) was injected 30 minutes after administration of the respective drug and TSTQ was recorded 45 minutes after the administration of scopolamine.

Statistical Analysis

The data obtained from different groups were statistically analysed by using one-way ANOVA followed by Tukey-Kramer multiple comparison test using GraphPad Prism version 9.1.0 (221) and presented as mean ± SEM. A *p*-value less than 0.05 were considered statistically significant.

Results

Effect of *M. Vaj* on TL using EPM

M. Vaj (220 and 440 mg/kg, p.o.) and piracetam (400 mg/kg, i.p.) administered for 15 successive days did not significantly affect the TL of mice on the 15th day as compared to the plain control group but both doses of *M. Vaj* and piracetam significantly decreased TL of mice on the 16th day as compared to the plain control group indicating significant memory enhancing activity. Scopolamine (0.4 mg/kg, i.p.) and diazepam (1-mg/kg, i.p.) significantly increased TL in mice, indicating its amnesic effect. *M. Vaj* (220 and 440 mg/kg, p.o.) and piracetam (400 mg/kg i.p.) significantly reversed scopolamine (0.4 mg/kg, i.p.)- and diazepam (1-mg/kg, i.p.) induced memory impairment in mice as compared to respective scopolamine and diazepam treated groups. Thus, the findings suggested that both doses of the test drug produced significant nootropic and memory-protective activity, which was comparable to Piracetam (Table 1, Fig.1).

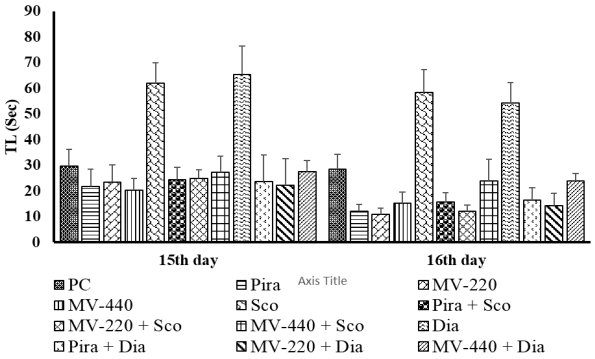

Fig. 1: Effect of *M. Vaj* on TL.

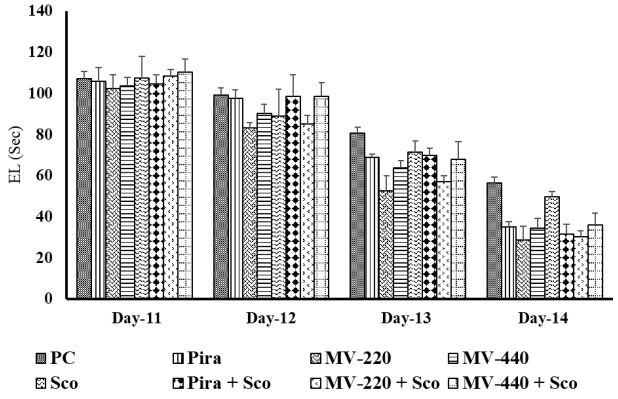

Fig. 2: Effect of *Majoon Vaj* on EL.

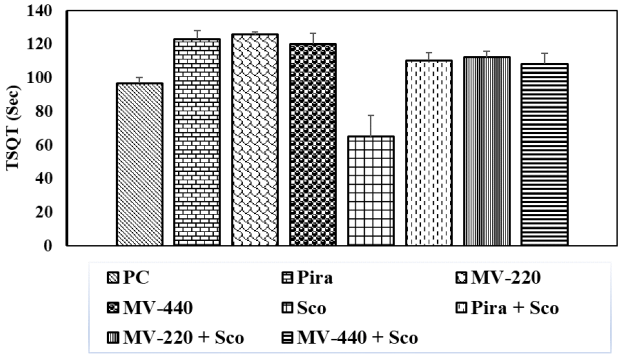

Fig. 3: Effect of *M. Vaj* on TSTQ

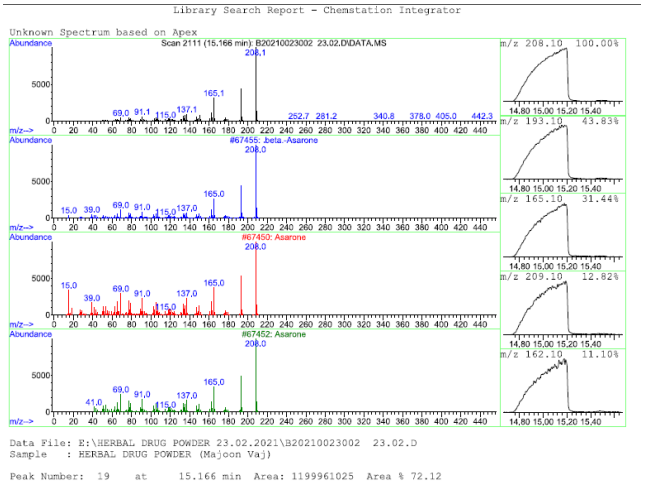

Fig. 4: Mass spectrum of the extract of *M. Vaj*: α-asarone and β-asarone.

Effect of *M. Vaj* on EL and TSTQ using MWM

M. Vaj (220 and 440 mg/kg, p.o.) and piracetam (400 mg/kg, i.p.) significantly decreased EL of mice on the 14th day and increased TSTQ on the 15th day as compared to the control group, showing striking memory-enhancing activity of standard and test drugs. However, on the 11th day, *M. Vaj* and piracetam did not significantly affect EL

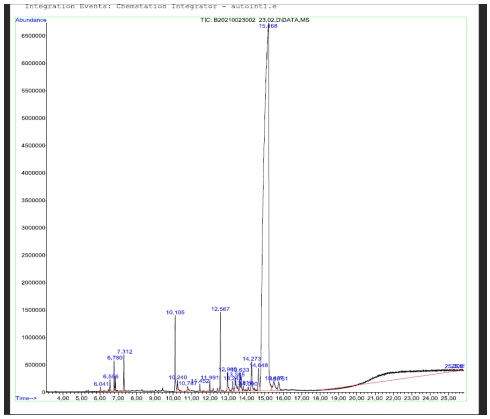

Fig. 5: GC-MS Chromatogram.

as compared to the control. Further, on the 12th and 13th days, *M. Vaj* (220 mg/kg p.o.) significantly reduced EL as compared to the control. The single dose of scopolamine (0.4 mg/kg, i.p.) when administered in the negative control group 45 minutes before recording TSTQ on the 15th day, significantly decreased TSTQ by mice as compared to plain control, indicating their amnesic activities. *M. Vaj* (220 and 440 mg/kg, p.o.) and piracetam (400 mg/kg i.p.) treatment for 15 successive days significantly reversed the scopolamine-induced decreased TSTQ by mice, indicating a reversal of scopolamine-induced amnesia (Tables 2 and 3, Figs. 2 and 3).

GC-MS Analysis

The GC-MS analysis of *M. Vaj* showed 23 peaks (Fig. 5) and its interpretation using the database of the NIST-11 library revealed 141 compounds. Out of this, 17 compounds have high antioxidant activity *viz.,* α-asarone, β-asarone, β-pinene, β-phellandrene, thymoquinone, duroquinone, g-terpinene, 3-carene, o-cymene, p-cymene, α-copaene, α-cubebene, cedrene, caryophyllene, methyleugenol, geranyl-p-cymene, trans-α-bergamotene. The α-asarone (72.12%) and β-asarone (72.12%) were found in the highest amount as compared to other compounds (Fig. 4 and 5).

Discussion and Conclusion

AD is a neurodegenerative disorder and the most common cause of dementia, characterized by cognitive dysfunction, memory impairment, and dan due to the rise of the ageing population and life expectancy. The development of herbal nootropics as a substitute for chemical nootropics will significantly improve the healthcare system.[27] In this study, we report for the first time that a hydroalcoholic extract of *M. Vaj* showed significant nootropic and anti-amnestic effects, as determined by behavioral tests using the EPM and MWM, as well as scopolamine and diazepam-induced amnestic models. These models are often used to study the effects of drugs on learning and memory.[28]

Table 1: Effect of *M. Vaj* on TL using EPM

Treatments	*Dose/kg*	*TL (sec) on the 15th day*	*TL (sec) on the 16th day*
Plain control (Distilled water) for 15 days	10 mL	29.66 ± 3.86	28.5 ± 3.45
Piracetam for 15 days	400 mg	21.83 ± 6.71	12.16 ± 2.68 a1
M. Vaj for 15 days	220 mg	23.5 ± 6.62	10.83 ± 2.49 a2
M. Vaj for 15 days	440 mg	20.33 ± 4.66	15.33 ± 4.31a1
Scopolamine only one day	0.4 mg	62.00 ± 7.90 a1	58.33 ± 9.09 a1
Piracetam for 15 days + Scopolamine on 15th day	400 mg + 0.4 mg	24.50 ± 4.66 b2	15.66 ± 3.68 b3
M. Vaj for 15 days + Scopolamine on 15th day	220 mg + 0.4 mg	24.83 ± 3.43 b2	12.16 ± 2.33 b3
M. Vaj for 15 days + Scopolamine on 15th day	440 mg + 0.4 mg	27.33 ± 6.31 b1	23.83 ± 8.54 b2
Diazepam only one day	1 g	65.33 ± 11.17 a1	54.33 ± 7.86 a1
Piracetam for 15 days + Diazepam on 15th	400 mg + 1mg	23.66 ± 10.45 c2	16.5 ± 4.66 c3
M. Vaj for 15 days + Diazepam on 15th day	220 mg + 1 mg	22.16 ± 4.17 c2	14.33 ± 2.89 c3
M. Vaj for 15 days + Diazepam on 15th day	440 mg + 1 mg	27.66 ± 6.57 c1	24.00 ± 5.75 c2

n = 6. Values are expressed as Mean ± SEM. Data were analysed by one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test. Where a, b and c = comparison with Plain control, Scopolamine and Diazepam respectively and 1, 2 and 3 = $p < 0.05$, $p < 0.01$, $p < 0.001$

Table 2: Effect of *M. Vaj* on EL using MWM

Treatments	*Dose/kg*	*EL (sec) Day-11*	*EL (sec) Day-12*	*EL (sec) Day-13*	*EL (sec) Day-14*
Plain control (Distilled water) for 15 days	10 mL	107.0 ± 3.57	99.0 ± 3.67	80.67 ± 2.77	56.33 ± 2.89
Piracetam for 15 days	400 mg	105.8 ± 6.71	97.50 ± 4.12	68.83 ± 1.77	35.17 ± 2.33 a1
M. Vaj for 15 days	220 mg	102.5 ± 6.62	83.33 ± 2.30 a1	52.67 ± 7.14 a2	28.67 ± 6.57 a2
M. Vaj for 15 days	440 mg	103.7 ± 3.98	90.33 ± 4.31	63.67 ± 3.68	34.50 ± 4.65 a1
Scopolamine only on 15th day	0.4 mg	107.3 ± 10.76	89.0 ± 12.88	71.33 ± 5.63	49.83 ± 2.40
Piracetam for 15 days + Scopolamine on 15th day	400 mg + 0.4 mg	104.5 ± 4.66	98.67 ± 10.47	69.67 ± 3.68	31.50 ± 4.66 a2,b1
*M. Vaj*for 15 days + Scopolamine on 15th day	220 mg + 0.4 mg	108.3 ± 3.38	85.17 ± 4.17	57.0 ± 2.95 a1	30.33 ± 2.89 a3,b1
M. Vaj for 15 days + Scopolamine on 15th day	440 mg + 0.4 mg	110.3 ± 6.31	98.67 ± 6.57	67.83 ± 8.54	36.00 ± 5.75 a2

n = 6. Values are expressed as Mean ± SEM. Data were analysed by one-way ANOVA followed by Tukey-Kramer multiple comparisons test. Where a and b = comparison with Plain control and Scopolamine respectively and 1, 2 and 3 = $p < 0.05$, $p < 0.01$, $p < 0.001$.

Table 3: Effect of *M. Vaj* on TSTQ using MWM

Treatments	*Dose/kg*	*TSTQ (sec) 15th Day*
Plain control (Distilled water) for 15 days	10 mL	96.83 ± 3.32
Piracetam for 15 days	400 mg	123.2 ± 5.00 a2
M. Vaj for 15 days	220 mg	125.8 ± 1.62 a3
M. Vaj for 15 days	440 mg	120.3 ± 6.45 a2
Scopolamine only on the 15th day	0.4 mg	65.00 ± 12.88 a1
Piracetam for 15 days + Scopolamine on 15th day	400 mg + 0.4 mg	110.5 ± 4.66 b2
M. Vaj for 15 days + Scopolamine on 15th day	220 mg + 0.4 mg	112.3 ± 3.38 b3
M. Vaj for 15 days + Scopolamine on 15th day	440 mg + 0.4 mg	108.3 ± 6.31 b2

n = 6. Values are expressed as Mean ± SEM. Data were analysed by one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test. Where a and b = comparison with Plain control and scopolamine, respectively and 1, 2 and 3 = $p < 0.05$, $p < 0.01$, $p < 0.001$

Initially, the EPM was employed to assess anxiety[29] but some parameters of the EPM such as TL are used for the evaluation of memory. The MWM task has been extensively used to study spatial learning and memory in mice.[30] The present study explored the effect of M. VAJ (220 and 440 mg/kg, p.o.) administration for 15 successive days on the learning and memory of male swiss mice. In EPM, pre-treatment with *M. Vaj* (220 and 440 mg/kg, p.o.) improved learning and memory as evidenced by a significantly decreased TL on the second day (i.e., 24 hours after the first trial) (Table 1, Fig. 1). In MWM, pre-treatment with *M. Vaj* (220 and 440 mg/kg, p.o.) improved learning and

memory, as evidenced by a significantly decreased in EL time during training and a significantly increased in TSQT during retrieval, respectively; and *vice versa* (Table 2 and 3, Fig. 2 and 3). Scopolamine, a non-selective muscarinic cholinergic receptor antagonist, is a well-known centrally acting anti-cholinergic candidate that impairs both short- and long-term memory in both animals and humans.[31] Scopolamine has been found to block the binding sites of ACh muscarinic receptors in the cerebral cortex, resulting in an excess release of ACh that damages the hippocampal nerves and impairs learning and memory in mice.[24] It also increases oxidative stress and cholinergic dysfunction, both of which are critical factors in the aetiology of neurodegenerative diseases such as AD.[28] Diazepam is a standard aryl 1,4-benzodiazepine that has been demonstrated to cause anterograde amnesia. An amnesic effect of benzodiazepines is mediated by the activation of the γ-aminoisobutyric acid $(GABA)_A$ receptors in the CNS.[32] Thus, scopolamine and diazepam-induced memory impairment provides a suitable paradigm for assessing the anti-amnesic effects of novel drugs. In the current study, both scopolamine and diazepam significantly impaired mice memory, as seen by increased TL in EPM and decreased TSTQ in MWM. Pre-treatment with *M. Vaj* (220 and 440 mg/kg) for 15 successive days significantly reversed scopolamine and diazepam-induced amnesia as observed by a decrease in TL in EPM and an increase TSQT in MWM. Piracetam also reversed scopolamine and diazepam-induced amnesia.

Reversal of scopolamine and diazepam-induced amnesia by *M. Vaj* indicated the possible facilitation of cholinergic transmission or GABA benzodiazepine pathway. Acetylcholine is known to be an essential neurotransmitter responsible for the regulation of cognitive functioning. Cognitive dysfunction is associated with impaired cholinergic transmission and the facilitation of central cholinergic transmission resulting in improved memory.[25] Thus, the drugs which enhance cholinergic function can be used to treat dementia closely related to AD. The different ingredients of the M. Vaj exhibit anti-neuroinflammatory, antioxidant, cognitive-enhancing and neuroprotective properties.[16–19,33–36] GC-MS analysis of M. Vaj led to the identification of 141 compounds. Out of 141, 17 compounds have high antioxidant and other biological activities. α-asarone (72.12%) and β-asarone (72.12%) were found in the highest amount as compared to other compounds. α-asarone exhibits neuroprotective action through the blockade of N-methyl-D-aspartic acid (NMDA) receptor function. It improved spatial memory impairment and protected the hippocampal neurons from damage in Aβ-treated rats by inhibiting NO overproduction. It also ameliorates cognitive deficits due to the inhibition of pro-inflammatory cytokines and microglial activation in the hippocampus. α-asarone showed potent antioxidant activity by normalizing the ↑ superoxide dismutase (SOD), and LPO, ↓ catalase (CAT), glutathione peroxidase (GPx) GSH, in the hippocampus against noise stress.[37]

β-asarone can pass the blood-brain barrier and shows anti-inflammatory effects on microglia-mediated neuroinflammation. It has been reported that β-asarone could attenuate neuronal apoptosis in the rat hippocampus. It significantly reduced focal cerebral ischemic/reperfusion injury by increasing antioxidant activity. It effectively suppressed the over-activation of microglial cells and the excessive production of pro-inflammatory mediators via the NF-kβ pathway. It improved the impairment of cognition and synaptic plasticity by modulating the excess release of pro-inflammatory cytokines and microglial activation in dizocilpine-treated mice. It alleviates the symptoms of AD by protecting astrocytes, inhibiting TNF-α, IL-1β secretion and downregulating aquapor in-4 expression. It also attenuates Aβ-induced neuronal apoptosis in the hippocampus by reversal down-regulation of Bcl-2, Bcl-w, caspase-3 activation, and c-JunN-termind kinase phosphorylation.[38] *M. Vaj* may possess neuroprotective and memory-enhancing properties due to its different ingredients' antioxidant[35,39–43] and neuro-anti-inflammatory[16,20,44–47] properties. Almost all the ingredients of *M. Vaj* contain flavonoids.[48–53] So, the effect of the drug may be due to the presence of flavonoids.

According to the Unani point of view, M Vaj has anti-amnesic effects by normalising *Sū' Mizāj* through a particular mechanism. As per the Unani pathophysiology, the temperament of the brain is disturbed by *Bārid* or *Raṭb Mādda,*[54,55] which leads to the derangement of mental faculties. Hence those drugs which have properties like *Muhallil-ī- Awram Balghamī* (Demulcent of phlegmatic swelling) and *Mulattif-ī- Akhlāṭ-ī-Ghalīẓa* (Resolvent of morbid matter)[54–56] can be used to rectify the *Sū' Mizāj* and consequently the *Nisyān*.

The test drug M. Vaj and its ingredients have been reported by various Unani physicians to possess properties like *Mulattif-ī-Akhlāṭ-ī-Ghalīẓa* (Resolvent of morbid matter), *Muhallil-ī-Awram Balghamī* (Demulcent of phlegmatic swelling), *Muqawwi-ī-A'ṣāb* (nervine tonic), *Muqawwi-ī-Hāfiẓa* (Memory enhancers), and *Muqawwi-ī-Dimāgh* (Brain tonics).[13,14,57] It is very helpful to correct the temperamental imbalance caused by *Bārid* or *Raṭb Mādda* by resolving the phlegmatic morbid matter. Also, the temperament of ingredients of *M. Vaj* is hot and dry so, it improves memory by altering the cold and moist temperament of the brain. In light of the findings of the study and the above discussion, it can be concluded that the test drug possesses significant nootropic activity. The nootropic activity of *M. Vaj* was largely comparable to piracetam but its margin of safety makes it superior to a conventional nootropic. *M. Vaj* seems to have great potential for therapeutic applications in the treatment of dementia and thus encourages more preclinical and clinical trials in this field. However further investigation is needed to elicit the specific mechanism of action.

Abbreviations

M: Majoon; TL: transfer latency; EL: escape latency; MWM: Morris water maze; EPM; elevated plus maze; TSTQ: time spent in target quadrant.

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Ethical Approval

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