NOOTROPIC ACTIVITY OF *VITIS VINIFERA* JUICE IN NORMAL AND MEMORY-IMPAIRED MICE USING SPATIAL LEARNING AND RECOGNITION MEMORY PARADIGMS

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ABSTRACT

Vitisvinifera Linn. (Grape) contains a variety of bioactive components including polyphenols. Flavonoids are the major phenolic compounds (65–76%) in grapes. Grapephenolicspossessseveralhealthpromotingpropertiesdue to their antioxidant potential. It is thought that antioxidants have memory-enhancing potential. Therefore, in thisstudy, weevaluatedthenootropicactivity of the*Vitisvinifera* fruit juice in normal and memory-impaired mice using the Morris water maze model and object recognition test. In the Morris water maze model, there was a significant decrease in escape latency (EL) and a significant increase in time spent in the target quadrant (TSTQ) as compared with normal control and memory-impaired mice ($P < 0.001$). In the object recognition test, there was a significant increase in the discrimination index ($P < 0.001$). Thesefindingssuggestedthepossibleuse of *Vitisvinifera*juicetowards a widerange of cognitivedisabilityandproposedthenootropicactivity of the*Vitisvinifera*juice.

Key words:*Vitisvinifera,* Memory-enhancing, Dementia, Nootropic, Morris water maze, Object recognition test

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INTRODUCTION

Memory constitutes one of the fundamental functions of the brain. Brain uses the process of memory to record the experiences that can be utilized to adapt their responses to the environment (1,2) Central cholinergic system plays a pivotal role in the regulation of cognitive functions (3,4). Alzheimer's disease is one of the most important dementing conditions and it has gained the utmost attention in the past decade. The impairment of cognitive functions is the primary characteristic of Alzheimer's disease (AD) (5,6). Degeneration of cholinergic neurons in nucleus basalismagnocellularis of the cortex is regarded as one of the most distinguished feature of AD, mainly accounting for loss of memory (7,8). Scopolamine impairs the process of learning and memory through its central cholinergic action (9,10). The drugs that increase cholinergic neurotransmission improve impaired cognitive performance in AD and other dementing diseases. (11,12). Herbal drugs are used as main therapeutic agents in different diseases. Herbal drugs are also used for prevention of different diseases.(13,14). Medicinal herbs are considered as a safer alternative of modern synthetic drugs (15)

Vitisvinifera Linn (Grape) family Vitaceae (16) besides being a member of the world's largest fruit crops is also one of the highly consumed fruits in the world (17-20). Phytochemical screening of the *Vitisvinifera* revealed that it contains a number of important constituents such as glycosides, saponins, alkaloids, phenolics, terpenes, resins, cardiac tannins, sterols, and volatile

oils (20,21). It is believed that the beneficial effects of *Vitisvinifera* are related to a variety of bioactive components, especially to polyphenols (22-24). Flavonoids are the major phenolic compounds (65–76%) in grapes (20). Grape phenolicspossess several health promoting properties owing to their antioxidant potential. Based on the notion that antioxidants have memory-enhancing potential, herbal practitioners in interior Sindh, recommend the use of fresh grape juice in the management of dementing conditions and as a memory booster. There has been no scientific study to validate such a practice.Therefore, in this study, we evaluated the nootropic activity of the *Vitisvinifera* juice in normal and memory-impaired mice using spatial learning and recognition memory paradigms.

MATERIALS AND METHODS

The Collection of Plant Material

Fresh fruits of *Vitisvinifera*, White Kishmish variety,were purchased from local markets, Karachi, Pakistan. A pharmacognosist of the Department of Pharmacognosy, Ziauddin University, Pakistan, authenticated the sample. Voucher specimen (P/PHL1390) was deposited in the institute for future reference.

Preparation of juice:

Vitisvinifera fruits were squeezed by hand in a muslin cloth to yield fresh juice. Fresh fruit was used every day to obtain the juice. The yield was approximately 80-100 mL/100 g.

The Selection of Animals

This study was conducted utilizing Swiss male albino mice weighing between 20 – 25 g. The specifications given in Helsinki Resolution 1964 were followed during animal handling. This

research was approved by the Board of Advanced Studies and Research, University of Karachi vide BASR resol. No. 16 dated 26-08-2013.

Dosing

The dose of the juice was calculated according to the body weight of the mice. The juice was administered in mice at two different doses, i.e., 4 mL/kg and 8 mL/kg. The dosing of the juice was done once daily according to the body weight of the animals.

Experimental design

A total number of 80 healthy Swiss male albino mice weighing between 20 – 25 g were procured from the animal house of University of Karachi, Pakistan. The animals were kept in polypropylene cages with a layer of sawdust litter under controlled conditions at room temperature 25–30 °C, relative humidity 45–55%, and 12/12 hours light/dark cycle. The mice were given standard pellets and water *ad libitum*. The mice were divided into eight groups *viz*.

Group I: Normal control, given normal saline 8 mL/kg, p.o.

Group II: Treatment group, given VVJ 4 mL/kg, p.o;

Group III: Treatment group, given VVJ 8 mL/kg, p.o;

Group IV: Positive control, given piracetam 200 mg/kg, p.o;

Group V: Negative control, given scopolamine 0.4 mg/kg, i.p.

Group VI: Treatment group, given piracetam 200 mg/kg, p.o + scopolamine 0.4 mg/kg, i.p.

Group VII: Treatment group, VVJ 4 mL/kg, p.o + scopolamine 0.4 mg/kg, i.p.

Group VIII: Treatment group, VVJ 8 mL/kg, p.o + scopolamine 0.4 mg/kg, i.p.

The drugs were given to all animals by oral gavage once a day for 60 days except scopolamine. Scopolamine was administered only on the day of the experiment. The experiment was carried out after 60 minutes of scopolamine administration on the 7^{th}, 15^{th}, 30^{th} and 60^{th} day of the drug treatment.

Morris water maze model

For the evaluation of the effect of the *Vitisvinifera* juice on memory of mice, Morris water maze test was utilized. The test was conducted in accordance with the procedure and the parameters followed by earlier studies (25). Precisely, Morris water maze-(MWM) for mice comprised of a roundabout pool (60 cm in breadth, 25 cm in height) filled to a depth of 20 cm with water kept up at 25 °C. The water was made opaque with nontoxic white coloured dye. The tank was separated into four quadrants of equal size with the assistance of two strings, settled at the right angle to one another on the edge of the pool. A submerged platform (with top surface 6 cm × 6 cm and painted in white) was put inside the target quadrants (Q4 in the present study) of this pool 1 cm beneath the surface of water. The position of the platform was kept unaltered all through the training session. Every mouse was subjected to four continuous trials every day with a gap of 5 minutes, during which the mice were permitted to escape onto the hidden platform and to stay there for 20 seconds. Amid the training session, the mouse was gently put in the water between quadrants, facing the wall of pool with drop area changing for every trial, and permitted 120 seconds to find submerged platform. In case the mouse could not locate the platform in 120 seconds, it was guided delicately onto the platform and was allowed to stay there for 20 seconds. The scored parameters were escape latency (EL), the time taken by the mouse to move from the beginning quadrant to discover the hidden platform in the target quadrant, and time spent in the target quadrant (TSTQ) (26,27).

Object recognition (ORT) model

The apparatus was comprised of a white coloured plywood box (70 × 60 × 30 cm) with a network floors that could be effortlessly cleaned with hydrogen peroxide after every trial. The apparatus was enlightened by a 60 W light suspended 50 cm over the crate. The item to be discriminated was likewise made of plywood in two separate states of 8 cm height and colourеddark. On the day preceding the test, mice were permitted to investigate the case (without any object) for 2 minutes. On the day of the test in the first trial (T1), two indistinguishable objects were exhibited in two inverse corners of the container, and the measure of the time taken by each one mouse to finish 20 seconds of object investigation was recorded. The investigation was considered as guiding the nose at a separation short of what 2 cm to the object and/or touching with the nose. Amid the second trial (T2, 90 minutes after T1), another object supplanted one of the objects introduced in T1, and mice were left individually in the container for 5 minutes. The time used for investigating the natural (F) and the new protest (N) was recorded separately, and discrimination index (DI) was calculated as (N - F) / (N + F). Consideration was taken to dodge place preference and the impact of olfactory stimuli by haphazardly changing the part (familiar or new object) and the position of the two objects amid T2 and cleaning the apparatus with hydrogen peroxide. The first trial (T1) was carried 60 minutes after the last treatment on the 8^{th} day and the second trial (T2) was carried 90 minutes after the first trial (T1) (28,29).

Statistical Analysis

Data expressed are mean ± standard error of mean (SEM). Data were analysed by one-way ANOVA followed by Newman-Keuls*post hoc* test. All statistical analyses were performed by

using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, San Diego, CA, USA. Statistical significance was accepted at a probability level of 0.01 or less.

RESULTS

Newman-Keuls*Post hoc* test revealed that, in normal mice, administration of *Vitisvinifera* juice (VVJ) at the dose of 4 mL/kg and 8 mL/kg produced a significant decrease in escape latency (EL) and a significant increase in time spent in the target quadrant (TSTQ) as compared with the normal control group ($P < 0.001$ and $P < 0.001$). Moreover, administration of piracetam 200 mg/kg in normal mice exhibited a significant decrease in EL and a significant increase in TSTQ as compared with the normal control group ($P < 0.001$ and $P < 0.001$) (Table 1 and Table 2).

Table 1: Effect of *Vitisvinifera* on escape latency (EL) of normal mice in Morris water maze test

Treatment	EL (Sec.) on day 7	EL (Sec.) on day 15	EL (Sec.) on day 30	EL (Sec.) on day 60
Normal Control (Saline 8 mL/kg)	78.1 ± 3.63	74.1 ± 3.66	78.9 ± 2.76	78.1 ± 3.62
Vitisvinifera juice 4 mL/kg	73.1 ± 2.97 (6.4% decrease)	66.5 ± 2.04 (10.3% decrease)	51.4 ± 3.14*** (34.9% decrease)	47.6 ± 3.08*** (39.1% decrease)
Vitisvinifera juice 8 mL/kg	49.2 ± 4.56*** (37% decrease)	38.9 ± 2.54*** (47.5% decrease)	39.9 ± 2.72*** (49.4% decrease)	35.2 ± 1.92*** (54.9% decrease)
Piracetam 200 mg/kg	38.4 ± 2.85*** (50.8% decrease)	33.8 ± 1.40*** (54.3% decrease)	24.1 ± 2.11*** (69.5% decrease)	25.4 ± 1.57*** (67.5% decrease)

Number of animals (n) = 10.

The values are mean ± S.E.M.

*P< 0.05, **P< 0.01, ***P < 0.001 when compared with the control group (One-way ANOVA followed by Newman-Keuls*post hoc* test).

Table 2: Effect of *Vitisvinifera* on time spent in target quadrant (TSTQ) of normal mice in Morris water maze test

Treatment	TSTQ (Sec.) on day 7	TSTQ (Sec.) on day 15	TSTQ (Sec.) on day 30	TSTQ (Sec.) on day 60
Normal Control (Saline 8 mL/kg)	91.7 ± 7.28	94.7 ± 10.78	105.6 ± 9.09	92.1 ± 7.57
Vitisvinifera juice 4 mL/kg	102.3 ± 6.62 (11.6%increase)	126.10 ± 8.13** (33.2% increase)	139.5 ± 6.36** (32.2% increase)	143.0 ± 8.93*** (55.3% increase)
Vitisvinifera juice 8 mL/kg	120.3 ± 9.60* (31.2% increase)	123.7 ± 7.36** (30.6% increase)	139.9 ± 7.59** (32.5% increase)	150.3 ± 9.19*** (63.2% increase)
Piracetam 200 mg/kg	127.4 ± 6.11** (38.9% increase)	141.1 ± 4.68*** (49% increase)	148.4 ± 8.23*** (40.5% increase)	165.2 ± 12.05*** (79.4% increase)

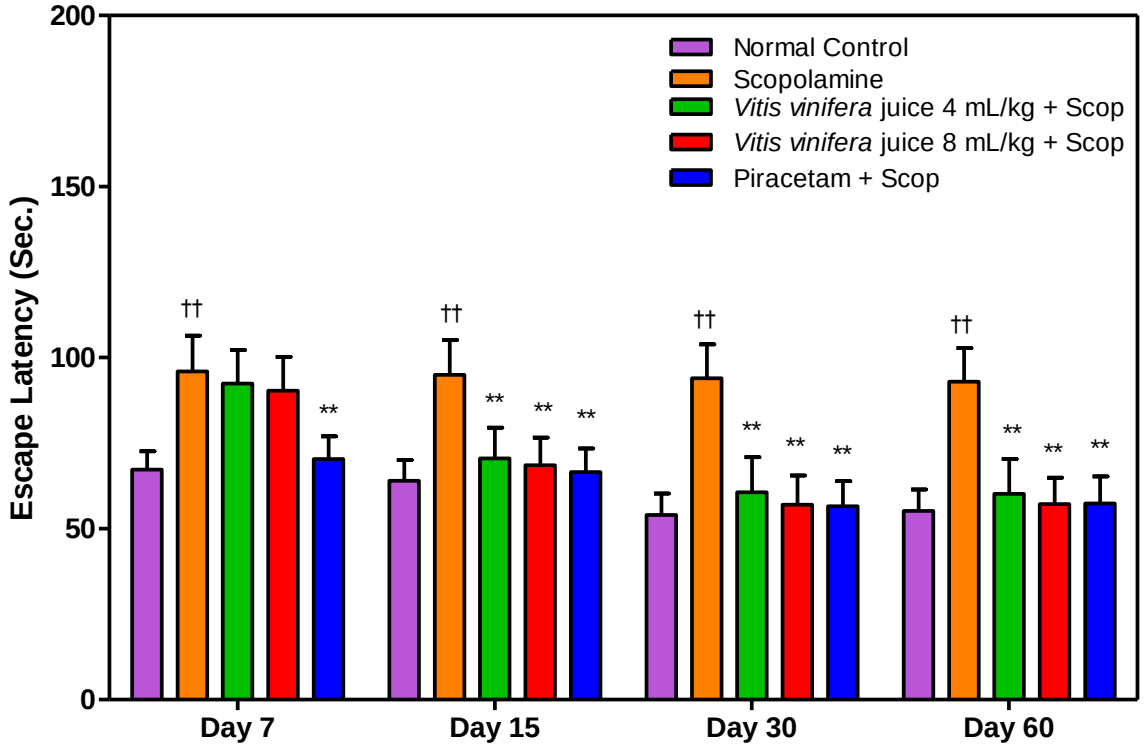
Number of animals (n) = 10.

The values are mean ± S.E.M.

$^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P < 0.001$ when compared with the control group (One-way ANOVA followed by Newman-Keuls*post hoc* test).

Administration of scopolamine (0.4 mg/kg) induced memory impairment in the control group, as indicated by a significant increase in EL and significant decrease in TSTQ as compared with the normal control group ($P< 0.01$ and $P< 0.01$). In scopolamine-induced memory-impaired mice, administration of VVJ (4 mL/kg and 8 mL/kg) showed a significant decrease in EL and a significant increase in TSTQ as compared with control group ($P< 0.01$ and $P< 0.01$). Moreover, administration of piracetam 200 mg/kg showed a significant decrease in EL and a significant increase in TSTQ as compared with control group ($P< 0.01$ and $P< 0.01$) (Figure 1 and Figure 2).These effects were observed on the 7^{th}, 15^{th}, 30^{th} and 60^{th} day of the drug treatment. The decrease in the EL and increase in TSTQ produced by VVJ was comparable to piracetam, the positive control.

Figure 1: Effect of *Vitis vinifera* on escape latency (EL) in memory-impaired mice in Morris water maze test



Number of animals (n) = 10.

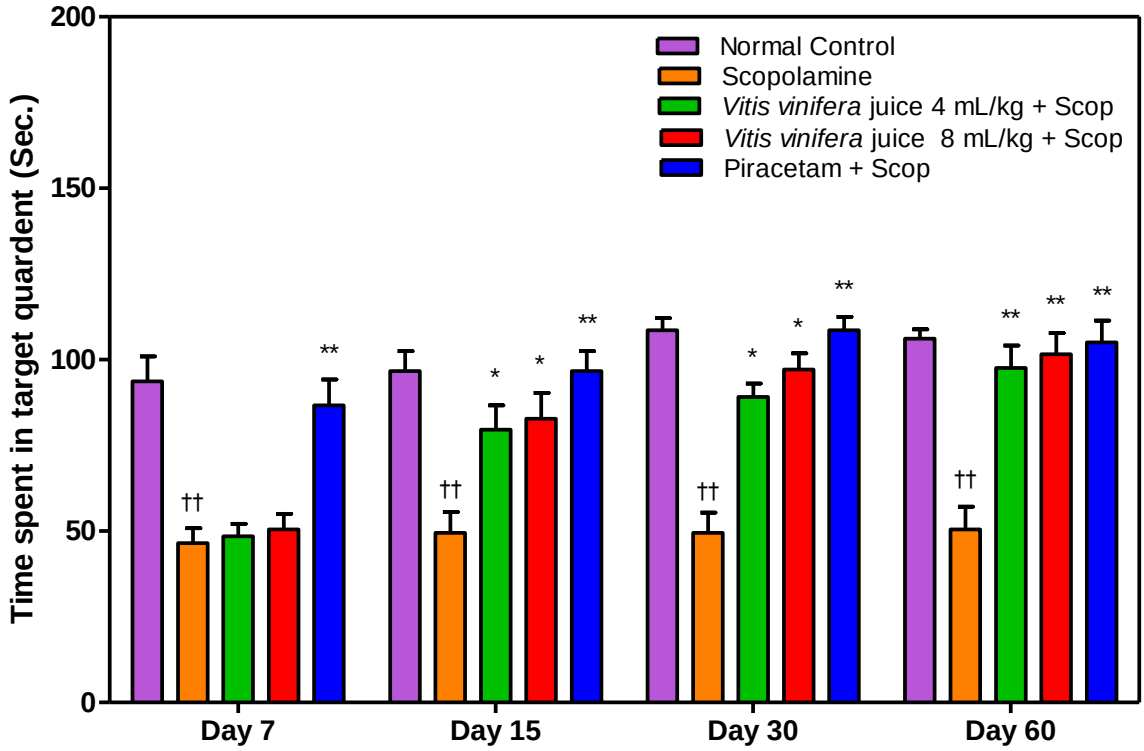
The values are mean ± S.E.M.

$^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$, $^{\dagger\dagger\dagger}P< 0.001$ when compared with the normal control group

$^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ when compared with control (scopolamine-treated) group,

(One-way ANOVA followed by Newman-Keuls*post hoc* test).

Figure 2: Effect of *Vitisvinifera* on time spent in target quadrant (TSTQ) in memory-impaired mice in Morris water maze test



Number of animals (n) = 10.

The values are mean ± S.E.M.

$^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$, $^{\dagger\dagger\dagger}P< 0.001$ when compared with the normal control group

$^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ when compared with control (scopolamine-treated) group,

(One-way ANOVA followed by Newman-Keuls*post hoc* test).

In Object Recognition Test (ORT),Newman-Keuls*post hoc* test revealed that, in normal mice, administration of *Vitisvinifera* juice (VVJ) at the dose of 4 mL/kg and 8 mL/kg produced a significant increase in discrimination index (DI) as compared with the normal control group($P < 0.001$). Moreover, administration of piracetam 200 mg/kg in normal mice exhibited a significant increase in DI as compared with the normal control group ($P < 0.001$) (Table 3).

Table 3: Effect of *Vitisvinifera* on discrimination index in normal mice in object recognition test

Treatment	DI on day 7	DI on day 15	DI on day 30	DI on day 60
Normal control (Saline 8 mL/kg)	0.24 ± 0.013	0.24 ± 0.013	0.24 ± 0.014	0.24 ± 0.014
VVJ 4 mL/kg	0.25 ± 0.009 (4.2% increase)	0.43 ± 0.012*** (79.2% increase)	0.43 ± 0.013*** (79.2% increase)	0.44 ± 0.016*** (83.3% increase)
VVJ 8 mL/kg	0.26 ± 0.007 (8.3% increase)	0.48 ± 0.018*** (100% increase)	0.50 ± 0.011*** (108.3% increase)	0.50 ± 0.011*** (108.3% increase)
Piracetam 200 mg/kg	0.52 ± 0.016*** (116.5%increase)	0.52 ± 0.015*** (116.5% increase)	0.51 ± 0.016*** (112.5% increase)	0.52 ± 0.019*** (116.5% increase)

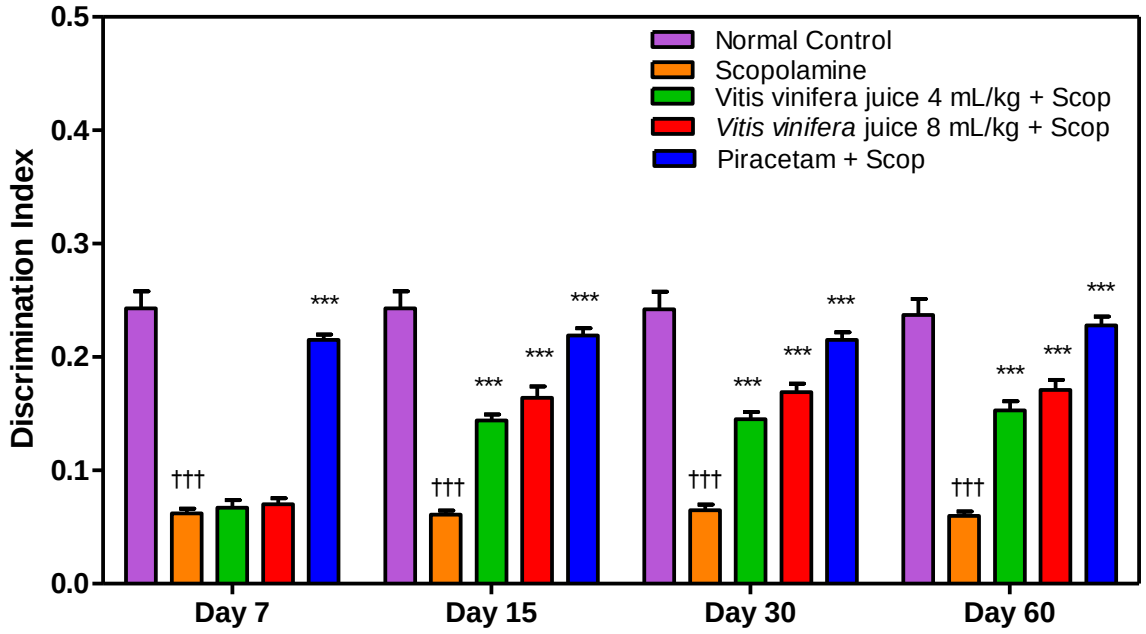
Number of animals (n) = 10.

The values are mean ± S.E.M.

*P < 0.05, **P < 0.01, ***P < 0.001, when compared with the control group (One-way ANOVA followed by Newman-Keuls*post hoc* test).

Administration of scopolamine (0.4 mg/kg) induced memory impairment in the control group, as indicated by a significant decrease in DI as compared with the normal control group ($P < 0.001$). In scopolamine-induced memory-impaired mice, administration of VVJ (4 mL/kg and 8 mL/kg) showed a significant increase in DI as compared with control group ($P < 0.001$). Moreover, administration of piracetam 200 mg/kg showed a significant increase in DI as compared with control group ($P < 0.001$) (Figure 2.15).These effects were observed on the 7^{th}, 15^{th}, 30^{th} and 60^{th} day of the drug treatment. The increase in the discrimination index (DI) produced by VVJ was comparable to piracetam, the standard drug.

Figure 3: Effect of *Vitisvinifera*juiceon discrimination index in memory-impaired mice in object recognition test



Number of animals (n) = 10.

The values are mean ± S.E.M.

$^{\dagger}P< 0.05$, $^{\dagger\dagger}P< 0.01$, $^{\dagger\dagger\dagger}P< 0.001$ when compared with the normal control group

$^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ when compared with control (scopolamine-treated) group,

(One-way ANOVA followed by Newman-Keuls*post hoc* test).

DISCUSSION

The Morris water maze model is broadly used to evaluate the impacts of medications on learning and memory. In this model, a reduction in escape latency (EL) and an increment in time spent in the target quadrant (TSTQ) show change of learning and memory (27). *Vitisvinifera* juice (4 mL/kg and 8 mL/kg) demonstrated a significant reduction in EL and a huge increase in TSTQ in normal and memory-impaired mice. Object Recognition Test (ORT) measures nonspatial working memory with the characteristics of episodic memory lasting for at least 60 minutes (28-30). ORT is concentrated around the spontaneous affinity of rodents to contribute more time examining a novel object than a known object. The decision to study the novel object shows the utilization of learning and recognition memory. Object recognition depends on the integrity of the cholinergic framework as exhibited by its debilitation by scopolamine, injuries of the center basalis, and developing. The disability is restored by the utilization of aniracetam and oxiracetam with an inverse U-shaped dose-response curve (30).

The nootropic medications have a place in the class of psychotropic agents with specific facilitator impact on intellectual performance, memory, and learning. The elevation in DI in ORT by VVJ (4 mL/kg and 8 mL/kg) essentially demonstrated that VVJ had nootropic action. Subsequently, the VVJ meets a significant criterion for nootropic activity, i.e. enhancement in memory without cognitive impairment (31,32). These findings could be further inferred for possible use of VVJ in poor learner people.

An alternate prime focus for nootropics is their use in the management of the cognitive deficiency. It is a well-established fact that systems of learning and memory are very closely

attached with cholinergic pathways projecting to the cerebral cortex and hippocampus (30). Studies show that cholinergic inadequacy may represent a percentage of the cognitive debilitations in Alzheimer's Disease (AD) (33). Scopolamine, a cholinolytic agent causes impairment of learning and amnesia in rodents and humans via blockade of central muscarinic (M) receptors (34). An interoceptivebehavioural model, for example, scopolamine-induced amnesia in test subjects is a generally cited model that simulates human dementia as a rule and AD in specific (35,36). The administration of scopolamine results ina transient memory shortage when given shortly before the test. The effectiveness of various cholinomimeticmedications to reverse the amnesic effects of scopolamine is currently well described in animals and humans (37).

AchE inhibitors, which improve the access of acetylcholine (Ach) in the synaptic cleft, had the capacity to invert the scopolamine-induced cognitive deficiency, showing that the deficiencyin cognitionis cholinergic in nature (36).

In the present study, pretreatment with VVJ (4 mL/kg and 8 mL/kg) secured the mice from learning and memory impairment produced by scopolamine 0.4 mg/kg, as apparent from a significant decrease in EL and a significant increase in TSTQ and DI in Morris water maze and object recognition test. Antagonistic activity of VVJ against scopolamine-induced amnesia substantiates nootropic action of VVJ. This effect may be due to acetyl choline esterase inhibitory effect of *Vitis vinifera* constituents such as flavonoids e.g. anthocyanin (38). Literature shows that resveratrol has memory-enhancing effect (39) so nootropic activity of *Vitisvinifera*juicemay be duetograperesveratrol. The findings of this study suggested the possible use of the VVJ towards an extensive variety of cognitive disabilities connected with cholinergic

transmission and regulation in the CNS and in this way proposed the nootropic activity of *the Vitisvinifera* juice.

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