RESEARCH ARTICLE

Effect of Grape Seeds Ethanolic Extract on Spatial Memory in Mice

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Abstract

Grape (Vitis vinifera) is a member of vitaceae family. The seeds are rich in polyphenols and phenolic acids that are not only safe to be consumed, but also recognized to produce several and meaningful health benefits. We aimed here to investigate the memory and learning processes (spatial memory) activity of the ethanolic extract of the grape seeds by using Morris Water Maze (MWM) test in laboratory mice. The present study revealed that the administration of ethanolic extract of Vitis vinifera (50 mg/kg, IP for 3 days) showed significant reduction to the escape latency using the MWM especially in the 4th and 5th day of experiment when the results were compared to the control group. Conclusion: grape seed extract may behave as a memory stimulant that opens doors to use it medically as a supportive therapy in case of impaired memory functions.

Keywords: *Vitis vinifera, Spatial memory, Morris Water Maze.*

Introduction

Learning and memory are closely associated at both cellular and behavioral levels [1]. Memory is caused by changes in synaptic conductivity from one neuron to another leading to emergence of new or facilitating pathways to control messages in the neural circuit of the brain [2]. It is well known that the hippocampus and the cortex of the cerebrum are the substantial framework of memory figuration especially in the integration of spatial information [1].

For this reason, learning ability reduction can be stimulated by the hippocampus function drop [3]. Memory and learning are the most important functional levels of the CNS. The CNS helps to encode, store, retain, and recall information in the brain. Aging and stress are the most important causes of disturbed memory and poor learning [4].

Cholinergic drugs have positive effects on memory [5], while other drugs such as anti-cholinergic, antipsychotics, and anesthetics have negative effects on memory [6]. Along the history, people have discovered valuable medicinal plants and the importance of phytotherapy in curing or mitigating poor learning and memory loss [7]. It was found that supplementation with grape juice improves the spatial memory and mood in human young adults [8]. In aged lab animals, grape was found to mitigate the cognitive reduction after long period of high sugar/ high fat diet [9]. tudies have also found that grape juice enhances mild memory loss and driving ability in mothers of preteen children when the juice consumed for three to four months [10]. Also the role of the medicinal plants in learning and memory has recently attracted the attention of many researchers. So in this study, our goal was to explore the beneficial role of the grape seeds ethanolic extract (GSE) in the improvement of the memory skills in experimental albino mice.

Materials & Methods

Plant Material and Preparation of Extracts

Fresh seeds of grape were obtained from local market in Al-Diwanyia city/ Iraq. The seeds were washed and dried under shade for one week, then they were powdered and 200g of seeds plant material were extracted with ethanol (95% v/v) using Soxhlet apparatus by continuous heat extraction. Then, the resulted extract was condensed using rotary flash evaporator at a temperature of less

than 50°C [11]. The percentage yield of the ethanolic extract of seeds that are used in the present study was 28% from the crude material. The yield was dark brown in color with sticky consistency.

Animals

For this study, we used Swiss albino mice of weight 20-25 g of either sex were used. The animal house that belongs to the college of Veterinary Medicine / University of Al-Qadisiyah, where the mice were acclimated before the experiment and then kept during the study. They were maintained at 20 ± 2°C temperature with 12:12 hour cycle and ad lip supply of food and water.

MWM Test

We checked the influence of the above plant on spatial learning and memory using the MWM test [12, 13]. Mice were grouped as follow:

Control group (C): five mice received vehicle only (distilled water injected intraperitoneally).

Treatment group (GSE): five mice received alcoholic extract of grape seeds 50 mg/kg dissolved in adequate amount of distilled water intraperitoneally and for three consecutive days.

The maze that was used here is a water tank that made of metal with circle shape is a (100 cm in diameter). A depth of 15 cm of water (20-25°C) was poured in the tank that was divided arbitrarily into four equal quadrants designated as (N, E, S and W) of equal surface areas. As starting positions, we assigned 4 equally distributed points along the perimeter of the tank. A small platform with dark color (8 cm width) was put at 1 cm below the water surface in the center of one of the quadrants so that it was invisible from just above the water level. The platform remained in the same location during the training days. Before the training by one day, each animal of the above groups was allowed to swim freely for 60 seconds, climb the platform 3 times and also to rest on it for 30 second. The mice were subjected to training trials for 5 days; 3 trials each day with 20 minutes inter-trail time to allow swimming to reach the platform. Each training trail included locating the animal into the tank facing the wall in a quadrant. An animal was manually guided to the escape platform if it fails to locate the platform within 100 seconds.

Every single mouse was left on the platform for 30 seconds, after that they were removed from the platform and towel dried for 60 seconds. The time that was spent by each mouse to find the hidden object is considered as escape latency. Leaving the platform in the same spot of the water maze throughout the study is critical to assess the outcome of GSE on spatial reference memory [12, 13].

Statistical Analysis

All the recorded values were analyzed statistically by one-way ANOVA and then by least significant differences (LSD). Differences were considered significant at a $p < 0.05$. All values are expressed as mean ± standard error.

Results

MWM Results

Improvement in the spatial learning and memory processes due to the GSE effect was assessed, and the time that was needed by the mice to reach the hidden platform in the MWM is illustrated in Table (1). There were significant differences between the group of mice that administered with GSE extract (50 mg/kg intrapertonally) compared to the vehicle-treated animals on days four and five from the beginning of experiment. While days 1, 2 and 3 data did not show significant differences in the time that was required to reach to the hidden platform. In another word, the mice that were treated with GSE exhibited a significant decline in the escape latency comparing to the C group as showed in Table (1).

Table 1: effect of the GSE on the spatial memory in albino mice

Groups of experiment / Days	C (escape latency time in second)	GSE (escape latency time in second)
First day	74.2 ± 1.32	72.6 ± 2.28
Second day	71.5 ± 1.98	67.2 ± 4.2
Third day	64.2 ± 1.32	60.36 ± 3.1
Fourth day	60.1 ± 2.4	51.45 ± 1.68
Fifth day	54.2 ± 1.11	42.12 ± 0.94
Average	64.84 ± 3.66	58.74 ± 5.45

Value represent mean of escape latency time in the second ± standard error

Number of animals = 5 mice per group

LSD value = 4.86

Discussion

Grape seeds influences on health are mainly detected by in vitro studies to show anti-inflammatory,cardio-protective, antimicrobial and anticancer [14] properties. Those effects might be through the ability of some grape seeds components to affect some cellular and molecular pathways [15]. The MWM is believed to be able to effectively distinguish spatial memory from sensational, motivational and retrieval processes of brain [13].

It is and often used to test the capabilities of spatial memory, which is considered closely associated with septo-hippocampal cholinergic activity [16]. Rodent models have been successfully used to study the behaviors associated with sensory, locomotive and cognitive abilities [17].

In this study, the ethanolic extract of grape seed significantly reduced the escape latency comparing to the control group specifically on day fourth and fifth of the beginning of the experiment. In addition, the treated group showed a trend to lower the escape latency in the first three days of the experiment. This fundamental influence might be related to the polyphenols content of the grape seeds as was described previously in different work [18, 19].Purified grape polyphenols were found to have a significant influence on selected subpopulations of neurons that are responsible for contextual memory functions in an optogenetic model [20]. An earlier study suggested that grape may reduce incidence of Alzheimer disease (AD) and attenuate AD-type cognitive deterioration and amyloid neuropathy [21]. This effect

might be due to the presence polyphenol compounds in the grape that may play substantial role in reducing brain amyloid neuropathy and improving cognitive function by promoting non-myloidogenic secretase activity [22]. Data have shown that the compounds procyanidin oligoruners that present in the grape seeds are much more potent than vitamin C and vitamin E as antioxidants and can play role in the protection of the brain from the effects of stress conditions that the animals may expose to during swimming [23]. Also *in vitro* studies mentioned that resveratrol that mainly present in the grape seeds, have ability to reduce Aß- peptides by promoting intracellular Aß peptides degradation [24].

Another *in vitro* study also found that polyphenolic compounds of grape have a significant effect in reducing the aggregation of synthetic Aß peptides [25]. In other study, grape seeds polyphenolic extract demonstrated an *in vitro* efficacy to attenuate AD-type Aß peptides neuropathy in TG2576 AD mouse model [25].

As detailed above, GSE studies have revealed possible mechanism to improve neuropathies including free radical scavenging property that reduces lipid peroxidation. Our study suggests that the observed enhancement in water maze performance in mice following GSE administration might be related to the antioxidant and cholinergic effect of polyphenolic compounds that present in grape seeds. In conclusion, grape plant has a beneficial impact in enhancing spatial memory and learning ability in experimental albino mice according to the MWM results.

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