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Preventive Effect of Grape Seed Hydroalcholic Extract on Dementia Type of Alzheimer's Disease in Aged Male Rats

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Abstract: Aim of this study was to investigate the effects of Grape Seed Extract (GSE), as a potent antioxidant on spatial memory in rats with Alzheimer's Disease (AD). Alzheimer's disease is a progressive neurodegenerative disease clinically characterized by dementia and neurobehavioral deterioration. Forty five aged male wistar rats were divided randomly into three equal number groups (n = 15). Control; AD and GSE+AD. The rat model of Alzheimer's disease was induced by local injection of Ibotenic acid (Ibo) into brain Nucleus Basalis Magnocellularis (NBM) or meynert bilaterally (Ibo, 6 μg μL^{-1} each site) under stereotaxic surgery. The spatial memory performance was evaluated by Morris Water Maze task. The results show that injection of Ibo into NBM of rats could impair spatial memory capacity. The GSE supplementation (100 mg kg^{-1}, by gavages) for 30 days before NBM lesion could be alleviating pre-lesion memory impairment. Present results showed that GSE could be a useful agent to prevent neurodegenerative disorders such as AD.

Key words: Alzheimer's disease, ibotenic acid, grape seed extract, morris water maze, rat

INTRODUCTION

Alzheimer's Disease (AD) is an age-related and progressive neurodegenerative disease characterized by dementia and the loss of neuronal cells in the brain (Marcus *et al*., 1998). Although, the exact etiologic factor of AD is unknown, evidence has been mounting that supports the hypothesis that free radical-induced oxidative damage may play a role in development of AD (Savla and Palmer, 2005). Although, it is unknown whether oxidative damage is primary for AD process, however, it is a part of the pathologic process (Praticó and Delanty, 2000). Some investigators have focused on the efficacy of using antioxidants in prevent of dementia and probable AD. This is based on the premise that if antioxidative stress occurs due to imbalance between free radical generation and antioxidant availability, then antioxidant supplementation may be used to scavenge excess free radicals and alter the development of the disease, or both. Some of the antioxidants that have been studied include vitamin E, estrogens, phytoestrogens and Ginkgo biloba (Tuppo and Forman, 2001). Antioxidants that accumulate in neural tissue are potential candidates for prevention or treatment of disorders involving oxidative damage. Animal models have provided a wealth of information on the biological effects of phytochemicals from vegetables and fruits on the oxidative damage during aging (Joseph *et al*., 1999). Grape seeds are rich source of monomeric phenolic compounds such as catachin, epicatechin and dimeric, trimeric and tetrameric proanthocyanidins.

Grape seed extract have long been recognized to posses myriads of properties, including antioxidant, anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating capabilities, etc., (Bagchi *et al*., 1998). Yamakoshi *et al*. (2002) showed that grape seed extracts are non-toxic to rats. Interestingly, epidemiological studies have pointed out that moderate consumption of red wine, an alcoholic beverage containing a huge amount of polyphenols (reservatrol, proanthocyanidin) reduces the incidence of certain age related neurological disorders including macular degeneration and dementia (Bastianetto and Quirion, 2002). Various reports have also shown that long term dietary supplementation of polyphenols improved the cognitive performance in aged rats (Balu *et al*., 2005). If Oxidative Stress (OS) is a major factor in brain aging and in age-related neurodegenerative disease, it would seem that some of its deleterious effects could be retarded or even reversed by increasing antioxidant levels and that the putative synergistic effects of combinations of antioxidants might be particularly effective in this regard (Joseph *et al*., 1999). In the earlier study we examined whether long-term feeding with grape seed

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extract (100 mg kg^{-1} for 30 days) would forestall the improvement effects of spatial memory in healthy aged rats. Studies indicated that GSE could prevent the onset of age-related deficits in cognitive behavior (e.g., Morris water maze performance). Their results suggested that antioxidant-rich foods could be beneficial in forestalling functional age-related deficits (Sarkaki *et al*., 2007). Thus, the purpose of the present experiment was to examine whether supplementations with GSE would be effective in preventing memory deficits in animal model of dementia type of AD in rats.

MATERIALS AND METHODS

Animals: Forty five aged male Wistar rats weighing 300±50 g, 22±2 months were used. Animals were obtained from Ahwaz Jondishapour University of Medical Sciences Animal House and maintained in a clean rodent room. This study was done from March 20, 2006 to July 13, 2008 in Ahwaz Physiology Research Center, Neuroscience Lab., Ahwaz-Iran. Each animal was housed under controlled conditions of temperature (20±2°C) and 12 h light/dark cycle (07:00 am to 07:00 pm) with food and water *ad libitum* before and after surgery throughout the experiments. Experimental animals were handled according to the regulations of the University and Institutional legislation, controlled by the Local Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals. The animals were randomly divided into three equal number groups (n = 15); Control group; AD (with NBM lesion) and GSE+AD that received grape seed extract by oral gavages daily (for 30 days, 100 mg kg^{-1}) before NBM lesioning.

NBM lesion: Animals were anesthetized with Ketamine (50 mg kg^{-1}, Rotex Medica, Germany) and Xylazine (20 mg kg^{-1}, Bayer, Germany). NBM lesioning induced by the method of Kwo-On-Yuen *et al*. (1990) with some modifications (Sarkaki *et al*., 2008). In brief, after anesthesia, each animal was placed on a stereotaxic apparatus (Narishige, Tokyo, Japan) and Ibotenic acid (Sigma, USA) dissolved in normal saline (6 μg μL^{-1}, in each site) was infused at a rate of 0.2 μL min^{-1} using a 10 μL syringe connected to an infusion pump (WPI 101i, USA). Infusion was done for 5 min at AP −1.4, ML±3.2, DV-6.8 from the bregma, according to the atlas of Paxinos and Watson (2006). After infusion, the cannula was left in place for 5 min to allow the diffusion. In the control group, an equal volume of normal saline was injected at the same positions (Shinoda *et al*., 1999).

GSE preparation: Grapes, as large clusters with red berries, were collected from southwest area, Ahwaz-Iran as *Vitis vinifera* Linn. Grape seeds were removed from the grapes, air dried (in shade) for one week and milled to fine powder (a particle size of <0.4 mm). The grape seed powder was macerated in 75% ethanol for 72 h at room temperature. The ethanol extract evaporated to remove ethanol and GSE was obtained as a lyophilized powder (Hwang *et al*., 2004).

Morris water maze task: Training in the maze took place during the light phase of the cycle between 9:00 and 15:00 h. A circular pool was used as described by Morris with some modification (Widy-Tyszekoeiwecz *et al*., 2002). The pool was made of metal and painted with black color, 1.20 m in diameter, 0.8 m high and was filled with 25°C tap water (0.6 m). It was positioned in the middle of a dimly light testing room enriched with distal visual stimuli. Four points, equally spaced along the circumference of the pool, were arbitrarily designated as N, E, S and W. The area of the pool was also conceptually divided into four equal size quadrants (NE, SE, SW and NW) two imaginary diagonal lines running through the center of the pool.

Rats were trained to locate a hidden black platform (10 cm diameter) maintained at a fixed location. To render it invisible to the rat, platform was submerged 1 cm below the surface of the water. The day before training, each rat was allowed to swim freely for 60 sec, allowed to climb the platform three times and rest on it for 30 sec. All rats were subjected to one session of four trials daily for 4 consecutive days. For each trial, the rat was placed in the water facing the wall of the pool at one of four equally spaced starting points. The order in which these starting points were used was determined randomly by computer for each trial and changed each day to prevent the use of a simple taxis strategy, but the location of the escape platform was always centered in the North East (NE) quadrant. A trial began when the rat was manually placed in the water facing the wall of the pool and was terminated when the rat reached out and got on the platform. All rats were left on the platform for 30 sec and then were removed and towel dried for 60 sec.

Rats that failed to find the platform within 60 sec were guided to find the platform by experimenter. The inter-trial interval was 60 sec. At the end of the day session the rat was wiped in cloth to dry and was returned to its home cage. In the probe trial on the 5th day of testing, the escape platform was removed from the pool and the rats were allowed to swim for a 60 sec trial. A video camera, connected to an image analysis system (Tivanic, Tivanic Co., Tehran, Iran), which in turn was connected to computer running the software, was mounted above the center of the water maze. The swimming path of the animal was tracked, digitized and stored for subsequent analysis using the same software.

Data from the water maze included escape latencies to find the platform, the swimming speed (m sec^{-1}) during trials, the swimming path length before reaching the platform and percent of time that rats spent over the place where the platform was previously situated (The probe trial) (Sarkaki *et al*., 2007; Alaeia *et al*., 2008).

Statistical analysis: The results are expressed as mean±SEM. Differences between groups were assessed by ANOVA using the SPSS software package for windows (ver.15.0). Post hoc testing was performed for inter-group comparisons using the Least Significance Difference (LSD) test; statistical significance at p-values <0.01, <0.05 have been given respective symbols in the figures (Balu *et al*., 2005).

RESULTS

Latency time: The mean latency time in four consecutive days to find and locate the hidden platform (maximum 60 sec for each trial) in AD group (rats with NBM lesion) was increased significantly ($p<0.01$) when compared with control group and in GSE+AD rats was not different with control group across 4 consecutive training sessions into water maze (Fig. 1).

Path length: The results indicate that mean path length was increased across days in AD rats when compared with control group and was decreased in GSE+AD rats when compared with AD group across 4 consecutive training sessions (Fig. 2, $p<0.05$ compared with AD group and $p<0.01$ compared with GSE +AD group). As shown in Fig. 2 the mean path length to locate the escape platform was decreased in pre-lesion GSE treated (GSE+AD) animals significantly ($p<0.01$).

Swimming speed: The mean speed of AD rats was decreased significantly for total acquisition trials into water maze during 4 consecutive training sessions when compared with control group ($p<0.05$), while it was increase significantly in GSE+AD group ($p<0.01$, Fig. 3).

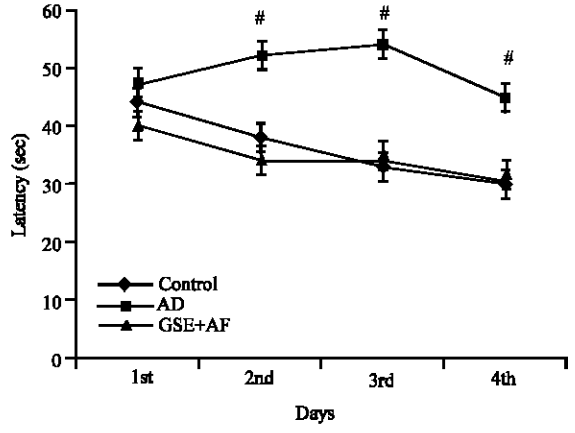

Fig. 1: Mean±SEM of latency time (sec) to find and locate on the hidden platform into water maze for control, AD and GSE+AD groups during 4 consecutive training days ($^{\#}p<0.01$ AD compared with control and GSE+AD groups. n = 15, one way ANOVA and LSD post hoc test)

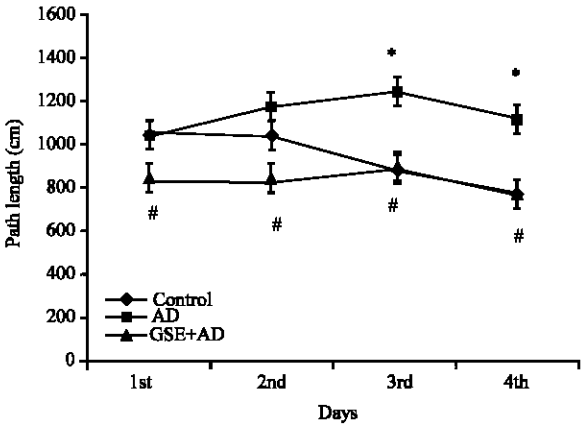

Fig. 2: Mean±SEM of path length (cm) to locate on the hidden escape platform for each day into water maze for control, AD and GSE+AD groups during 4 consecutive training days ($^{*}p<0.05$ for AD group VS control group, $^{\#}p<0.01$ for GSE+AD group VS AD group, n = 15, one way ANOVA and LSD post hoc test

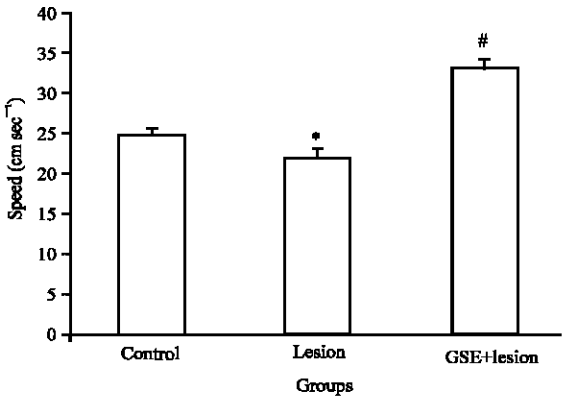

Fig. 3: Mean±SEM of speed (cm sec^{-1}) of control, AD and GSE+AD groups for total acquisition trials into water maze during 4 consecutive training sessions (n = 15, one way ANOVA and LSD post hoc test). Speed was reduced in AD VS control ($^{*}p<0.05$) and increased in GSE+AD ($^{\#}p<0.01$)

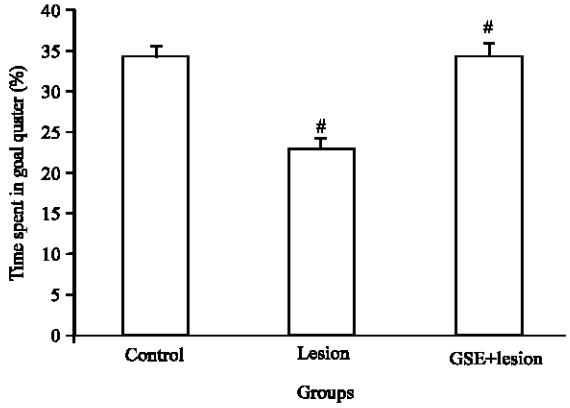

Fig. 4: Mean±SEM of percent of total time spent by rats in goal quarter for probe trials. Total time that spent in goal quarter was decreased significantly in AD group when compared with control group ($^{\#}p<0.01$, n = 15) and was increased significantly in GSE+AD group when compared with AD group ($^{\#}p<0.01$, n = 15, one way ANOVA and LSD post hoc test)

Probe trial: The percent of total time that rats spent in goal quarter (NE, location of removed platform) during probe trial on the fifth day of testing while the platform has been removed was decreased significantly ($p<0.01$) in AD group when compared with control group (Fig. 4) and was also increased significantly ($p<0.01$) in GSE+AD group when compared with AD group (Fig. 4).

DISCUSSION

Although, the results of this study showed a significant increase of escape latency and path length in AD rats but a significant decrease of escape latency was observed in GSE+AD group (Fig. 1). The swimming speed also was decreased in AD rats compare to control group, but it was increased significantly in GSE+AD groups to compare with other groups (Fig. 3). As well as the percent of time that rats spent in goal quarter during probe trial was decreased significantly in AD group compare to control group, but increased significantly in GSE+AD group with compare to AD group (Fig. 4).

These findings indicate that 100 mg kg^{-1}, daily by oral gavages grape seed extract before lesioning enhanced spatial memory in AD (NBM lesioned) aged male rats thereby protecting the central nervous system from the memory impairment. It is the first report of use grapes non-alcoholic extract on AD induced dementia. These results are consistent with the report of Bickford *et al.* (2000) that chronic administrations of antioxidants alleviate age-associated cognitive deficits in animals. Some clinical data have shown that procyanidin oligomers from grape seeds are 20 times more potent than vitamin C and 50 times more potent than vitamin E as antioxidant (Perry *et al.*, 2000). Grape Seed Extract (GSE) is a commonly available dietary supplement taken for the anti-oxidant activity that's attributed to its proanthocyanidin (oligomers of monomeric polyphenols) content (Kim *et al.*, 2005). Wang *et al.* (2006) found that moderate consumptions of the red wine cabernet sauvignon derived from *Vitis vinifera* promoted Aβ-lowering activity *in vivo* coincidentally with attenuation of spatial memory impairment in Tg2576 mice with AD. Earlier studies suggest that moderate consumption of red wine may reduce the incidence of AD and attenuate AD-type cognitive deterioration and amyloid neuropathology (Dorozynski, 1997; Orgogozo *et al.*, 1997; Luchsinger *et al.*, 2004). In this study, we administered an antioxidant dietary supplement to prevent AD induced dementia. Certain earlier studies have suggested that some polyphenolic compounds could reduce brain amyloid neuropathology and improve cognitive function by promoting non-amyloidogenic-secretase activity (Rezai-Zadeh *et al.*, 2005; Wang *et al.*, 2006). So, GSE may prevent the damage occurs by NBM lesion.

Marambaud *et al.* (2005) also reported that resveratrol, a natural polyphenol mainly found in grape and red wine, could reduce Aβ by promoting intracellular Aβ degradation *in vitro*. Another study also suggested that a few select grape-derived polyphenolics could reduce aggregations of synthetic Aβ peptides *in vitro* (Porat *et al.*, 2006).

Wang *et al.* (2008) used a naturally derived Grape Seed Polyphenolic Extract (GSPE) and demonstrate its *in vivo* efficacy to attenuate AD-type Aβ neuropathology in the Tg2576 AD mouse model. More importantly, they confirm that GSPE-mediated reduction of soluble high molecular weight (HMW) oligomeric Aβ peptide levels in the brains of Tg2576 mice significantly attenuated cognitive deterioration.

Grape Seed Extract (GSE) has many possible mechanisms for neuroprotection. It is an effective free radical scavenger that reduces lipid per oxidation. Grape seed extract provides superior antioxidant efficacy as compared to vitamin C and E at equal doses by weight. It also has anti-inflammatory action in association with its oxygen free radical scavenging, anti-lipid peroxoidation activity and reduces production of pro-inflammatory cytokines (McDonald *et al.*, 1991). The observed improvement in water maze performance in NBM-lesioned rats following GSE exposure may be due to the antioxidant property of existing polyphenols in the grape seed extract.

In conclusion, based on the results of the Morris water maze tests using NBM lesioned aged rats, present results indicate that GSE is an effective target on memory impairment in animal model of AD induced dementia and may be useful natural antioxidant in preventing of neurodegenerative diseases such as AD.

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