Original Article

THE EFFECT OF GRAPE SEED EXTRACT (GSE) ON SPATIAL MEMORY IN AGED MALE RATS

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ABSTRACT

Objective: To test the effect of GSE on spatial memory in aged male rats.

Methodology: We hypothesized that ingestion of polyphenols-enriched preparation such as GSE would be manifested as cognitive-enhancement that would be consistent with neuroprotection in aged rats. Aged male rats were divided into two groups (control and GSE treated). Control rats received normal saline and treated rats received GSE (100mg/kg) by oral gavage for 30 days. They trained in Morris water maze.

Results: Our data showed that administration of grape seed extract to aged rats improves memory retention in water maze performance.

Conclusion: Based on the results of the Morris water maze tests using aged rats, we found that GSE had remarkable cognitive-enhancing effect.

KEY WORDS: GSE, memory, aging, Water maze, Rat.

INTRODUCTION

Grape seeds are the waste products of the winery and grape juice industry. Grape seed extract is known as a powerful antioxidant that protects the body from premature aging, disease, and decay.

Grape seed contains mainly phenols such as proanthocyanidin (oligomeric proanthocyanidin). The beneficial effects of grape seed polyphenols are due to their free radical scavenging capability, but the antioxidant activity of grape seed polyphenols is superior to other well-known antioxidants, such as vitamin C, vitamin E, and beta-carotene.[1,2]

Antioxidants that accumulate in neuronal tissue are potential candidates for prevention or treatment of disorders involving oxidative stress.[3] Grape seeds are rich source of monomeric phenolic compounds such as catechin, epicatechin, and dimeric and tetrameric proanthocyanidin. Structurally, the proanthocyanidins are a group of polyhydroxyfavan-3-ol monomer units.[4] The grape seed extract is also used in Japan as an additive for various food applications.[5]

Aging affect learning and memory in rodents, and in humans. Compared with young rats, aged rats exhibit learning and memory deficits in the Morris water maze task, radial arm maze task, tunnel maze task, and the delayed non-matching to place task in water.[6] Aging is often associated with a storage decrease of cognitive functions. With the actual increase of human lifespan, the study of aging memory impairment has become predominant in neurobiology.[8]

Oxidative stress is considered as a risk factor and contributing to age-related increase in oxi-

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dized lipids and proteins in central nervous system during aging that ultimately leads to cellular damage.[9] Cognitive deficits such as learning and memory impairment and delayed amnesia are the debilitating consequences of aging.[10]

In recent years, the "French paradox" has stimulated new interest to investigate weather grape seed polyphenols offer antioxidant benefit to aging brain.[11] Despite the considerable works done on grape seed extract (GSE), its effect on memory remains to be elucidated. Therefore in this study, we have investigated the effects of GSE on memory in retired aged male rats, and on the base of some experiments in other fields we hypothesized that, 100mg/kg of GSE would improve spatial memory in aged rats.

MATERIAL AND METHODS

Animals: Healthy aged male albino rats of Wister strain were used in this study. The animals were housed in standard conditions. Experimental animals were handled according to the regulations of the university and institutional legislation. The animals were divided into two groups consisting of 15 animals each. Group I rats served as vehicle-treated controls (2 years old; body weight >300g). Group II rats (2 years old; body weight >300g) received grape seed extract (100mg/kg body weight)[8] dissolved in saline by oral gavage for 30 days. The observations were continued for 30 days.

Extract preparation: Grape, as large clusters with red berries, was bought from a local super market in 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 < 0.4mm). The grape seed powder was macerated in 75% ethanol for 72h at room temperature. The ethanol extract evaporated to remove ethanol, and grape seed extract was obtained as a lyophilized powder.[12]

Morris water maze task: Animals were tested for their spatial memory abilities using the Morris Water Maze (MWM) as described elsewhere.[13] Abreviately the protocol consist of four days (4 trials daily) and a probe trial in fifth day. Each trial was 60 seconds and inter trial time was 60 seconds.

Data from the water maze included escape latencies to find the platform, the velocity (cm/s) during trials, the swimming distances before reaching the platform and percent of time that rats over the place where the platform was previously situated (the probe trial). *Statistical Analysis:* The data are presented as Mean ± SEM. Statistical analysis of the data was carried out using student t-test. The statistical analysis was performed using a computer program (SPSS, VER.13). The data were considered to be significant statistically if probability had a value of 0.05 or less.

RESULTS

Acquisition Trials:

Path length: The results indicate that mean path length(cm) was decreased across days in both aged controls and grape seed extract (GSE) treated groups; but it was shorter in treated animals than control significantly across 4 consecutive training sessions and for total acquisition trials (Fig. 1A and B, *$p<0.05$, **$p<0.01$, n=15). As illustrated in figure 1 the mean path length to locate the escape platform was decreased in GSE treated animals significantly. *Latency time:* As illustrated in figure 2, the mean latency time (second) to find and locate on hidden platform (maximum 60 seconds for each trial) in GSE treated aged rats was decreased significantly (*$p<0.01$) even from first day of training across 4 consecutive training sessions (A) and total acquisition trials (B) into water maze when compare with controls (Fig. 2 A and B, *$p<0.01$, n=15). *Swimming speed:* As shown in figure 3, the mean speed (cm/s) of GSE treated aged rats was increased significantly for total acquisition trials into water maze during 4 consecutive training sessions when compared with controls (*$p<0.01$). *Probe trial:* The percent of total time (second) 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
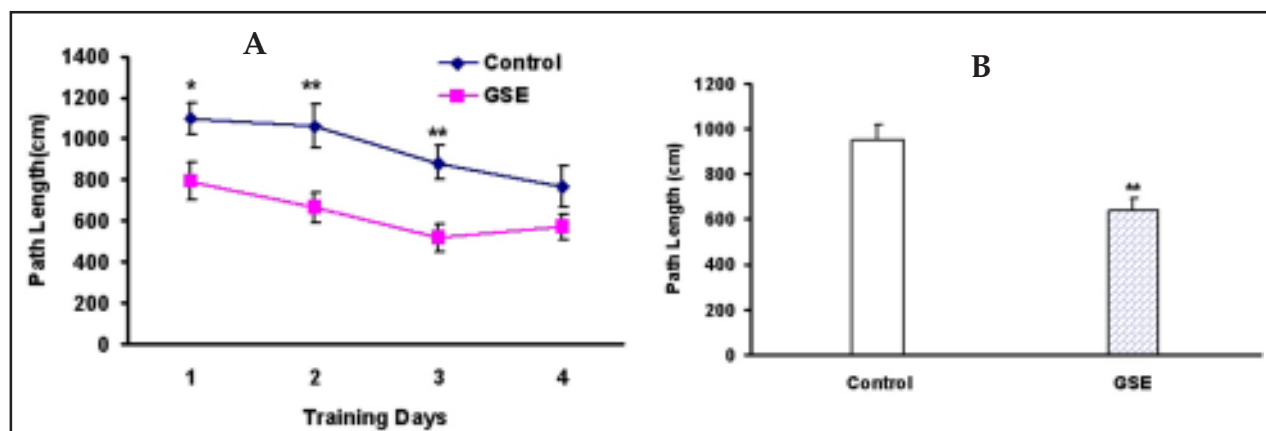
Figure-1: Path length (Mean ± SEM) to locate on the hidden escape platform for each day (A) and total acquisition trials (B) into water maze for aged control and treated rats during 4 consecutive training days (*p<0.05, **p<0.01, n=15, GSE treated (â-) vs control (â^{TM1}_1), student t-test).

removed was increased significantly in GSE treated aged rats vs controls (*P<0.001, n=15, fig.4).

DISCUSSION

The path length was decreased in GSE treated aged rats when compared with control rats (figure 1). A significant decrease of escape latency time was observed in grape seed extract-administered aged male rats (figure 2). The learning and memory process in GSE treated aged rats are very faster than control aged rats (figure 2). The swimming speed also was higher in GSE treated rats than control (figure 3). As well as the percent of time that rats spent in goal quarter during probe trial was increased significantly in GSE treated aged rats (figure 4). These results imply that supple-

mentation of aged rats with grape seed extract show increased spatial memory performance. These findings have indicated that grape seed extract enhanced spatial memory in aged male rats thereby protecting the central nervous system from the memory impairment. These results are in agreement with Bickford et al, which reported that chronic administrations of antioxidants alleviate age-associated cognitive deficits in animals.[14]

The free radical theory of aging is one of the most popular, single mechanistic theories of aging, which discloses increased generation of free radical as the major cause of cellular damage. Such free radical-mediated damages are prevalent during aging which leads to age associated diseases as like Alzheimer's disease (AD) and Parkinson's disease (PD).[15]
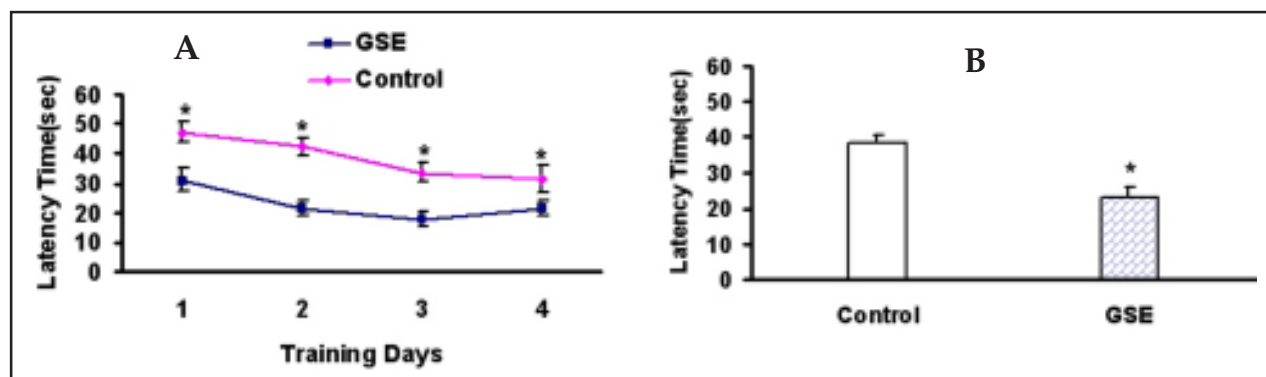

Figure-2: Latency time (Mean ± SEM) to find and locate on the hidden platform for each day (A) and total acquisition trials (B) into water maze for aged control and treated rats during 4 consecutive training days (*p<0.01, n=15, GSE treated (â-) vs control (â^{TM1}_1), student t-test).
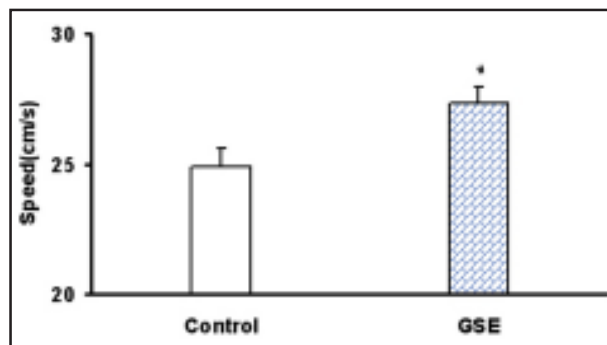
Figure-3: Speed (Mean±SEM, cm/s) of aged control and GSE treated rats for total acquisition trials into water maze during 4 consecutive training sessions (*p<0.05, n=15, student t-test).

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.[16] Proanthocyanidins are the major polyphenols in red wine as well as in grape seeds, and they have potent antioxidant activity, inhibit low density lipoprotein oxidation, as well as a variety of biological activities.[17,18]

Yamakoshi et al. showed that grape seed extract are non toxic to rats.[19] Balu M. et al. showed that grape seed extract enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation in the central nervous system of aged rats.[11] Interestingly, epidemiological studies pointed out that moderate consumption of red wine, an alcoholic beverage, containing huge amount of
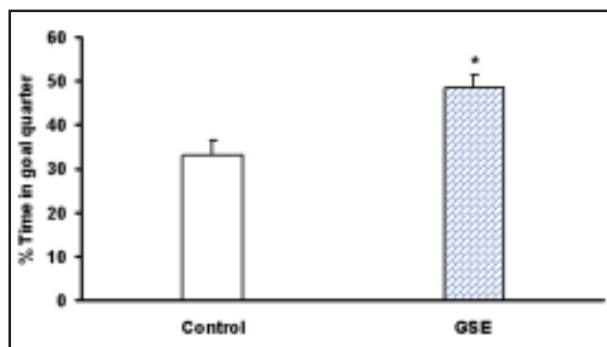

Figure-4: Percent of total time (Mean ± SEM, second) spent by rats in goal quarter for probe trials. Total time that spent in goal quarter was increased significantly in GSE treated aged rats vs controls (*p<0.001, n=15, student t-test).

polyphenols (proanthocyanidin, reservatol) reduces the incidence of certain age-related neurological disorders including macular degeneration and dementia.[20] Various reports have also shown that long-term dietary supplementation of polyphenols improved cognitive performance in aged rats.[21] Similar polyphenol-enriched preparations from blueberries and soy have shown protection against ovariectomy- induced or age-related cognitive deficits, suggesting that the molecular changes induced by these polyphenol preparations correlated with behavioral benefit.[16]

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.[22] Epidemiological data have shown that red wine may reduce the mortality rate from coronary heart disease, the so called "French paradox".[23] Recent findings have also suggested that grape seed polyphenols have neuroprotective roles to play against â-amyloid peptide (âAP), one of the specific causes of Alzheimer's disease.[24]

Grape seed extract has many possible mechanisms for neuroprotection. Grape seed extract is an effective free radical scavenger that reduces lipid peroxidation. Grape seed extract provides superior antioxidant efficacy as compared to vitamin C, and E at equal doses by weight. Grape seed extract inhibit DNA oxidative damage in the gerbil forebrain ischemia model. Grape seed extract can block cell death signaling mediated through the pro-apoptotic transcription factors and genes such as JNK-1 and c-JUN. Grape seed extract also has anti-inflammatory action in association with its oxygen free radical scavenging, and anti-lipid peroxodation activity. Grape seed extract reduces production of proinflammatory cytokines.[25] The observed improvement in water maze performance may be due to the antioxidant property of polyphenols present in the grape seed extract. In conclusion, based on the results of the Morris water maze tests using aged rats, we found that GSE had remarkable cognitive-enhancing activity.

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