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Full Length Research Paper

The effects of grape seed and grape pomace extracts on spatial memory impairment induced by hyoscine in mice

Akram Jamshidzadeh[1]*, Batool Faeghe Baha-al-dini Baigi[2] and Mehrdad Aram[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Fars, Iran.

[2]Department of Pharmacology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Fars, Iran.

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The cholinergic system and its receptors play a role in memory function. Hyoscine, as an anticholinergic agent causes dysfunction to the spatial memory and learning. The present study aims to evaluate the effects of two different extracts of grape seed (25, 50, 75 mg/kg) and grape pomace (25, 50,100 mg/kg) on the spatial memory impairment induced by hyoscine (1 mg/kg) in male mice. Spatial memory was evaluated using Morris water maze test. Each animal received three trials per day for four days and day five was the probe day (without platform). Speed, distance and time spent in each quadrant were recorded. The percentage of time spent in the target quadrant (Q2) on the probe day was used as an index of memory. The extracts were injected 5 min prior to hyoscine which was injected 20 min before the trial. Pretreatment with different doses of grape seed extract and grape pomace extract 5 min prior to hyoscine prevented the memory impairment effects of hyoscine, and the time for searching platform in Q2 was significantly increased ($p<0.05$), compared to that of hyoscine treated group. Extracts alone did not show any effects on the memory. Grape seed in doses up to 75 mg/kg and grape pomace in doses up to 100 mg/kg extract in doses that did not show any effect on the memory and motor activities could prevent memory impairment effects of hyoscine.

Key words: Grape extracts, hyoscine, spatial memory, mice.

INTRODUCTION

Dementia may be associated with a variety of conditions including Alzheimer and cerebrovascular disorders. Neurodegeneration in such cases is followed by cognitive impairment and chiefly memory loss. Alzheimer's disease is most prevalent among those over 65 years of age, and some acetyl cholinesterase inhibitors (ACHEIs), estrogens and non steroidal anti-inflammatory drugs (NSAIDs) have been used during the past decades to treat and prevent this disease (Musial et al., 2007; Raina et al., 2008). In addition to the synthetic drugs, medicinal plants are also used as alternative treatment in different parts of world especially in developing countries and some of them have been experimentally investigated (Lee et al., 2003; Rubio et al., 2007). Nevertheless, no effective treatment has been found yet for dementia and Alzheimer's disease.

Epidemiologic studies have pointed out that moderate consumption of red wine, containing a huge amount of polyphenols, reduces the incidence of certain age-related neurological disorders including macular degeneration and dementia (Truelsen et al., 2002; Letenneur, 2004). A study showed that red wine with/without alcohol produces antioxidant effects due to its great amount of polyphenols (Arendit et al., 2005). Various reports have also shown that long term dietary supplementation of polyphenols improved the cognitive performance in the aged rats (Joseph et al., 2005; Wang et al., 2008).

The memory enhancing effects of grape seed extract (GSE) on the aged rats has also been examined (Sarkaki et al., 2007). Concord grape juice supplementation has

*Corresponding author. E-mail: ajamshid@sums.ac.ir. Tel: #98 711 2424126. Fax: #98 711 2426070.

Table 1. Yield and total phenolic content of grape seed and pomace extracts.

Extract	Yield (%)	Total phenolic content (mg gallic acid /g)
GSE	8.8	46.04
GPE	3.2	10.93

GSE: grape seed extract. GPE: grape pomace extract.

been shown to enhance cognitive function in older adults with early memory decline (Krikorian et al., 2010). Grape seed and bagasse (berry without juice and seed) contain different levels of polyphenols, which is reported to be effective as antioxidant (Gokturk et al., 2006). To the best of our knowledge, there has not been any comparative study addressing the effects of seed and pomace (berry without seed) of grapes extracts on cognitive impairment. Thus, this study seeks to investigate the efficacy of grape seed extract and grape pomace extract (GPE) on the memory impairment induced by hyoscine.

MATERIALS AND METHODS

Preparing seed and pomace extracts

Black grapes (*Vitis vinifera* Cv. Rishbaba siah) were purchased from a local grocery late summer 2008 in Shiraz, southern Iran. Then, the intact ripe berries were detached from the branches and were pitted manually. Both the seeds and the pomace were air dried in a dark room, afterwards, pulverized into powder. Seed powder was defatted with hexane (one part powder to 10 parts hexane, w/v). The powdered seed and pomace (50 gr) was macerated with 70% methanol in separate beakers. The two beakers were then sonicated for 15 min and stirred for 30 min, then stored in dark for 24 h.

After which it was filtered and concentrated in vacuo under reduced pressure and the residue was air dried for 72 h. When it was completely dried it was transferred to a dessicator and placed in a refrigerator (Yilmaz and Toledo, 2006). Total phenol contents of the extracts were determined using Folin-Ciocalteu method (Vernon et al., 1999).

Experimental animals

Male “Imprinting Control Region” mice (25-35 g) were obtained from the Laboratory of Animals Research Center, Shiraz University of Medical Sciences. The mice were stabilized under laboratory condition for one week before the onset of the experiments. The research protocol complied with the guidelines for animal care of our institution. The animals were divided into 14 groups (6 animals/group). Group 1 (water, control), group 2 (hyoscine, 1 mg/kg/day i.p.), groups 3-5 pomace extract (25, 50 and 100 mg/kg/day i.p.), groups 6-8 grape seed extract (25, 50 and 75 mg/kg/day i.p.), groups 9-11 pomace extract (25, 50 and 100 mg/kg/day i.p. + hyoscine, 1 mg/kg/day i.p.), groups 12-14 grape seed extract (25, 50 and 75 mg/kg/day i.p. + hyoscine, 1 mg/kg/day i.p.).

The extracts were injected 5 min prior to hyoscine which was injected 20 min before the trial. The extracts alone were injected 25 min before the trial (all injections were interaperitoneally and daily for 4 days); experimental models were previously described by Yamada et al. (2004).

Morris water maze test

To investigate the effects of the above agents on the memory and spatial learning, Morris water maze test was used (Morris, 1984). The maze consists of a circular metal tank with black inner lining and a diameter of 145 cm. The tank was filled with 19-26°C water. The pool was divided into 4 quadrants (Q1, Q2, Q3, and Q4) of equal surface areas. A metal escape platform with dark color and 11 cm diameter was placed in a fixed location in the tank, 1 cm below the water surface. The platform was not visible from just above the water level. Many extra-maze cues surrounded the maze were available for the animals to use in locating the escape platform. On the training trials, the platform remained in a constant location in the center of one quadrant (Q2) equidistant from the center and the edge of the pool.

Each animal was trained four days and three trials per day for swimming in the pool to reach the platform. Each training trial involved placing the animal into the pool facing the wall in one of the four quadrants. A different starting point was randomly used on each trial. The animals were allowed to swim freely until they found the escape platform. The latency to find the hidden platform was recorded and used as a measure of the acquisition of the task. If an animal failed to locate the platform within 100 s it was then manually guided to the escape platform by the experimenter. The intertrial interval was 20 s during which the animal remained on the platform. Twenty hours after the last training trial on the probe day, the platform was removed from the pool, the animals were allowed to swim for 60 s in the pool and the time spent in the target quadrant Q2 (the quadrant in which the platform was placed during training) was recorded. The percentage of time spent in the previous training quadrant Q2 was used as an index of memory (Chopin et al., 2002).

Statistical analysis

All values were expressed in mean ± SEM of 6 animals. Analysis of variance (ANOVA), followed by Tukey test was used to evaluate the significance of the results obtained and $p<0.05$ was considered significant. All the computations were performed using SPSS (version 16) software.

RESULTS

The yields and total phenolic content of the grape seed and pomace extracts are presented in Table 1. Grape seed extract had higher extract yield and total phenolic content than the pomace extract. The data in Table 2 indicate the mean escape latency time to reach the hidden platform, for the mice with memory impairment induced by hyoscine

Table 2. The effects of grape seed extract, grape pomace extract and hyoscine on the escape latency time in finding the platform on days 1 - 4.

Day groups	Latency time 1	Latency time 2	Latency time 3	Latency time 4
Control	82.17±10.67	82.07±4.57	70.52±6.73	66.74±5.33
H	93.2±4.03	85.57±4.07	87.54±4.27 †	84.88±4.68 † †
GSE 25	88.78±5.06	67.22±6.65	61.32±6.65	41.95±3.39
GSE 50	83.56±4.72	58.62±5.78	56.67±6.19	42.91±4.92
GSE 75	86.05±6.22	54.34±9.19	66.09±9.00	56.65±5.63
GSE25+H	76.35±3.62	71.93±4.57	49.64±6.16 ***	49.36±6.49 ***
GSE50+H	80.89±5.82	62.09±6.92	65.8±3.43 *	49.94±6.87 ***
GSE75+H	90.77±4.62	59.97±8.27	59.55±4.97 ***	44.04±4.38 ***
GPE 25	82.27±10.58	73.45±4.72	70.23±3.41	57.49±5.61
GPE 50	94.31±3.19	76.33±3.63	67.61±4.79	61.71±4.95
GPE 100	79.96±5.14	72.19±2.35	67.81±4.79	64.55±2.59
GPE25+H	93.63±3.91	93.47±3.93	84.59±4.25	89.15±1.74
GPE50+H	85.92±5.98	81.83±8.39	89.52±1.24	87.62±2.25
GPE 100 + H	81.73±5.47	76.29±5.53	62.33±3.52 **	59.59±4.62***

(Sec, Mean ± SEM) to find and locate the hidden platform for the days 1-4 in Morris water maze for the controls, hyoscine (H) (1 mg/kg/day i.p.), grape seed extract (GSE) (25, 50 and 75 mg/kg/day i.p.) and grape pomace extracts (GPE) (25, 50 and 100 mg/kg/day i.p.) during 4 consecutive training days, †: Significantly different from control group in related day ($p<0.05$). † †: Significantly different from control group in related day ($p<0.01$). *: Significantly different from hyoscine -treated group in related day ($p<0.05$). **: Significantly different from hyoscine -treated group in related day ($p<0.01$). ***: Significantly different from hyoscine -treated group in related day ($p<0.001$).

(1 mg/kg/day i.p.) and pretreated with GSE (25, 50 and 75 mg/kg/day i.p.) and GPE (25, 50 and 100 mg/kg/day i.p.). On days 1 and 2, the mean escape latency time in finding the platform between the groups was not significantly different. However, the mean values on days 3 and 4 in the group receiving hyoscine were significantly increased, compared to the controls ($p< 0.05$). On the other hand, the mean on days 3 and 4 for the groups receiving three doses of GSE 5 min prior to hyoscine was significantly lower than the mean in those receiving only hyoscine. No differences were observed in escape latency time in the lower doses of GPE (25 and 50 mg/kg), however, the higher dose (100 mg/kg) reduced escape latency time, compared to the controls ($p<0.05$). The mean latencies to reach the escape platform in the GSE and GPE cases (when administrated alone), were not significantly different from that in the control group.

Table 3 illustrates the swimming speed to reach the platform on days 1, 4 and 5 and the distance traveled on day 5 for the mice with memory impairment induced by hyoscine and pretreated with GSE and GPE. As shown, the mean of swimming speed on days 1, 4 and 5 was not significantly different between the groups. No significant difference was observed in the distance traveled by the groups, either. The percentage of swimming time spent within the training quadrant during the probe trial is shown in Figure 1. The hyoscine treated group spent significantly less time in the training quadrant ($p< 0.01$) than the control group. GSE-treated groups spent significantly more time in the training quadrant than the hyoscine group, but only the higher dose of GPE (100 mg/kg) reversed the effect of hyoscine on the percentage of swimming time.

DISCUSSION

Alzheimer's disease (AD) is clinically characterized by a progressive loss of cognitive abilities. The pathophysiology of this disease is complex and involves several different biochemical pathways (Souder, 2005). The key symptoms of Alzheimer's disease are primarily caused by cholinergic system dysfunction. It is known that acetylcholine (ACh) is an important neurotransmitter related to memory and learning. Acetylcholinesterase (AChE) modulates ACh to proper levels by degradation; accordingly, excessive AChE activity leads to constant ACh deficiency, memory, and cognitive impairments (Musial et al., 2007). Currently, AChE inhibitors (AChEI) are the first line of treatment for AD patient.

Until now, some AChEI including: tacrine, donepezil, rivastigmine, and galantamine have been approved by the FDA for the treatment of AD. However, AChEIs present some limitations such as their short half lives and excessive side effects caused by the activation of peripheral cholinergic systems, as well as hepatotoxicity, which is the most frequent and important side effect of

Table 3. The effects of grape seed extract, grape pomace extract and hyoscine on the swimming speed and traveled distance.

Day groups	Speed (cm/sec) day 1	Speed (cm/sec) day 4	Speed (cm/sec) day 5	Distance (cm) day 5
Control	14.62±.37	14.71±.23	14.86±.34	912.86±20.44
H	14.39±.34	14.94±.18	16±.36	987.33±22.62
GSE 25	14.13±.37	14.13±.23	16±.55	985.6±30.3
GSE 50	14.2±.23	14.47±.45	15±.63	923±32.16
GSE 75	14±.30	14.87±.31	14.6±.68	888.4±38.67
GSE25+H	14.61±.13	15.28±.31	15.83±.40	977.5±24.01
GSE50+H	15.05±.20	14.95±.19	16.14±.51	997.28±29.48
GSE75+H	14.28±.20	14.61±.29	15.67±.49	970.83±35.12
GPE 25	15.07±.37	14.93±.19	15.6±.51	943.4±29.81
GPE 50	15.6±.29	15±.18	15.6±.24	944.4±14.08
GPE 100	14.93±.27	15.13±.25	15±.45	914.8±25.09
GPE25+H	15.27±.19	14.8±.17	15.6±.20	939.6±13.95
GPE50+H	15.07±.40	14.4±.07	15.2±.20	941.2±11.45
GPE 100 + H	15.13±.25	15.6±.22	15.4±.40	941.2±26.63

Swimming speed and distance (Mean ± SEM) to find and locate the hidden platform for the days 1,4 and 5 in Morris water maze for the controls, hyoscine (H) (1 mg/kg/day i.p.), grape seed extract (GSE) (25, 50 and 75 mg/kg/day i.p.) and grape pomace extracts (GPE) (25, 50 and 100 mg/kg/day i.p.) during 4 consecutive training days and probe trial (day 5)

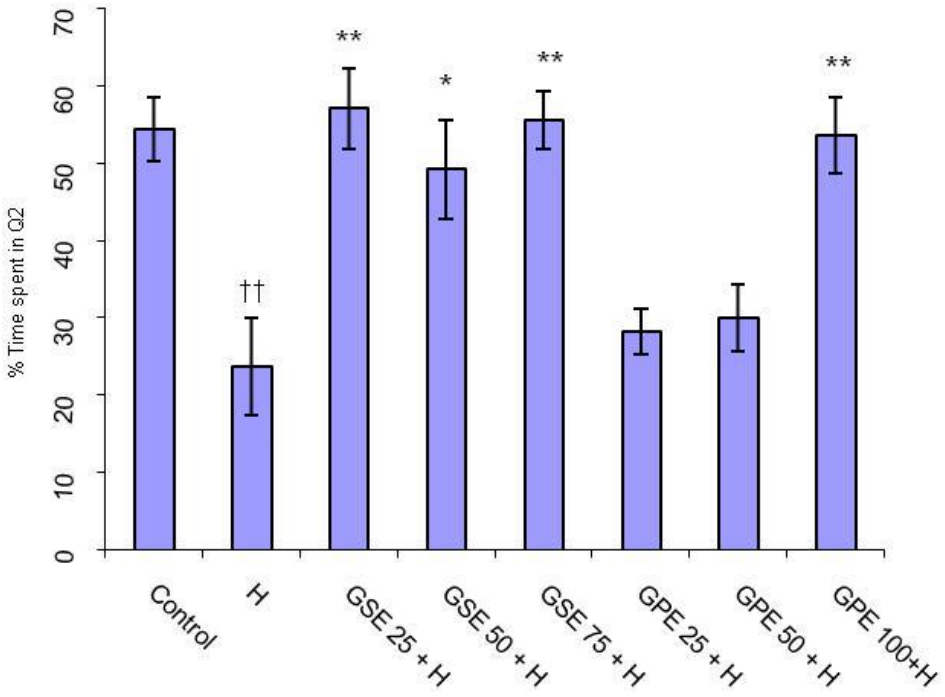

Figure 1. The percentage of time (Mean ± SEM) spent swimming within the training quadrant during the probe trial (day 5). Hyoscine (H) (1 mg/kg/day i.p.), grape seed extract (GSE) (25, 50 and 75 mg/kg/day i.p.) and grape pomace extract (GPE) (25, 50 and 100 mg/kg/day i.p.). † †: Significantly different from control group ($p<0.01$). *: Significantly different from hyoscine -treated group ($p<0.05$). **: Significantly different from hyoscine -treated group

tacrine therapy (Qizilbash et al., 2007; Raina et al., 2008). For this reason, alternative and complementary therapies are needed. Several studies have shown the neuro-protective and/or cognition-enhancing properties of natural products and their components using different animal models (Lee et al., 2003; Rubio et al., 2007; Wang et al.,

2008).

The present study aims at comparatively investigating separately the effects of 3 doses of GSE and GPE, on the memory impairment induced by hyoscine, using Morris water maze test. In this test, performance is dependent on the number of training days. In a probe trial without platform, 24 h after three or four training days, mice spend significantly more than 25% of their time in the quadrant that contained the platform during the training sessions (Q2), indicating that they have learned the location of the platform in this quadrant.

However, after a couple of training days, equal time length was spent in all the four quadrants of the pool, suggesting that animals have not yet learned the platform location. Chopin et al. (2002), recommended four days of training for learning about the hidden platform location. The present results showed that the total phenolic content of the grape seed extract is greater than that in the grape pomace extract. These findings are in agreement with those of (Murthy et al., 2002; Gokturk et al., 2007) who reported that although both the seed and bagasse contain rich amount of polyphenols, more can be extracted from the seeds than from the bagasse. Our results showed that the animals which received different doses of GSE prior to hyoscine spent longer time in Q2, compared to those which received hyoscine alone. These results indicated that the three doses of GSE were effective against the memory impairment caused by hyoscine.

In contrast, the groups receiving lower doses (25, 50 mg/kg) of pomace extract prior to hyoscine spent almost the same time length in all the quadrants, but, the mice which received higher dose of pomace extract (100 mg/kg) spent longer time in Q2, compared to the group receiving only hyoscine. This finding lends support to the efficacy of high dose of pomace extract against the memory impairment induced by hyoscine. Therefore, we can suggest that seed extract possesses greater protective potency than pomace extract against memory impairment. As mentioned in the results, the groups receiving seed and pomace extracts (without hyoscine), exhibited the same behaviors as the control group. So, these doses of our extracts have had no effects on the normal memory. Swimming speed is an indicator of changes in spontaneous locomotor activity and sedation (Turkmen et al., 2006).

In the present study, no change occurred to the swimming speed nor in the traveled distance in different groups of mice, which indicates that the extracts and hyoscine cause no change in motor activities and have no sedative effects. There are many possible mechanisms for neuroprotection. It is an effective free radical scavenger that reduces lipid per oxidation. It also has anti-inflammatory action in association with its oxygen free radical scavenging. Anti-lipid peroxodation activity and reduces production of pro-inflammatory cytokines (Feng et al., 2005; Sarkaki et al., 2007). Taking into account the present findings and some previously reported studies (Wang et al., 2008; Joseph et al., 2009; Krikorian et al., 2010) we could conclude that both GSE and GPE are effective against cognitive impairment. It is worth noting that GSE contain greater level of polyphenolic compounds than GPE. Meanwhile, the two extracts have no effect on the normal cognition or motor activities; therefore, they can be used as prophylactic agents in cases of memory deficits or similar conditions. Since GSE supplies higher level of antioxidants, it can be exploited into the daily consumption.

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