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Protective Effect of Pomegranate Seed Oil against D-Galactose-Induced Aging in Rats

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ABSTRACT

Background & Objective: Aging is a natural phenomenon which can cause changes in most organs and cells. Numerous mechanisms including oxidative stress and free radical generation is involved in the progression of the aging process. Pomegranate seed oil (PSO), has different therapeutic properties including anti-oxidant and anti-inflammatory effects. In this research, the effect of PSO against D-galactose-induced aging is investigated.

Materials & Methods: D-galactose, 500 mg/kg, injected subcutaneously (S.C.) to induce aging in rats. Animals in treatment groups received PSO, 0.4 and 0.8 ml/kg intraperitoneally (i.p.). After 42 days, behavioral test was evaluated by passive avoidance (PA). Then animals killed, blood samples collected by cardiac puncture, and brain and liver removed. Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measured in serum. Malondialdehyde (MDA) and thiol contents of brain and liver homogenized tissue samples were determined.

Results: D-galactose increased lipid-peroxidation in liver and brain tissues as well as elevation of ALT, AST, but the level of thiol contents decreased in homogenized tissues. Both doses of PSO attenuated d-galactose-induced injury in liver and brain by decreasing ALT, AST, MDA and elevation of thiol content. The PA test showed that PSO increased the latency time to enter the dark chamber compared to the control group.

Conclusion: PSO decreased D-galactose-induced aging in rats via prevention of oxidative stress. This effect may be related to the presence of various compounds and their anti-oxidant properties, which is found in PSO.

Keywords: Aging, Lipid peroxidation, Oxidative stress, Punica granatum

Introduction

Aging is identified as a natural phenomenon, which can cause changes in most cells, tissues, molecules, and organs (1). Some diseases, such as atherosclerosis, cardiovascular disease, neurodegenerative diseases, and pulmonary and renal disorders, are associated with aging. D-galactose, which is found in different types of food, including cherries, plums, honey, yogurt, and butter, is metabolized to glucose by galactokinase and uridyltransferase at normal concentrations (2). At higher concentrations, D-galactose leads to aging via reactive oxygen species (ROS) production, inflammation, and apoptosis in different cells (3). The combination of D-galactose with free amines produces advanced glycation end products (AGEs), and its binding to their receptors (RAGE) increases the level of inflammatory agents, influencing cognitive activity (4).

Some recent studies have proposed that the function of D-galactose is similar to the natural aging process in humans. Therefore, to evaluate the protective effects of different compounds against aging, D-galactose can be used in animal models to induce aging (5). It is known that chronic administration of D-galactose for 6-10 weeks leads to neuroinflammation, memory dysfunction, apoptosis, and oxidative stress (6). Different studies have reported that medicinal herbs play a role in the attenuation of tissue injury related to the aging phenomenon (7).

Punica granatum, which is widely cultivated in the Middle East, has different pharmacological properties, including anti-proliferative, antioxidant, anti-inflammatory, anti-tumor, and antimicrobial effects. Fatty acids comprise nearly 12-20% of pomegranate seeds, the most important of which is punicic acid. Other isomers include linoleic acid, catalpic acid, and α-eleostearic acid

(8). According to different studies, pomegranate seed oil (PSO) can reduce the side effects of toxic agents, such as diazinon (9), cisplatin (10), mercuric chloride (11), gentamicin (12), and hexachlorobutadiene (13). On the other hand, it is known that oxidative stress and inflammation play a role in the aging process; therefore, consumption of natural products may be effective in decelerating this process. The present study aimed to investigate the protective effects of PSO against D-galactose-induced aging.

Materials and Methods

In this study, which was carried out during 2020, D-galactose (>99% purity) was purchased from Samchun Chemical Co. (South Korea). PSO, extracted from *Punica granatum* seeds by cold pressing (d=0.81 g/mL at 25°C) based on the Production License No. 11616/12, was kindly gifted by Urom Narin Co. (Urmia, Iran). Moreover, 2,20-dinitro-5,50-dithiodibenzoic acid (DTNB) and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (USA), and potassium chloride (KCl), thiobarbituric acid (TBA), and phosphoric acid were purchased from Merck Co. (Germany). Finally, the glutathione peroxidase assay kit and the total antioxidant capacity kit were purchased from Zellbio GmbH (Germany).

Animals

Forty adult male Wistar rats (220±250 g) were purchased from the Animal House of the Faculty of Medicine of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were housed in a pathogen-free facility in a 12:12 h light/dark cycle at 21±2°C (40-50% humidity), with *ad libitum* access to food and water. All animal procedures were approved by the Ethics Committee of Mashhad University of Medical Sciences and were in compliance with the national regulations and the National Institutes of Health (NIH) guidelines for the use and care of laboratory animals (IR.MUMS.MEDICAL.REC.1399.163).

Experimental protocol

After acclimatization, the animals were randomly divided into four groups (10 per group): group 1 (control group), receiving saline (i.p.) for 42 days; group 2, receiving D-galactose (500 mg/kg, s.c.) for 42 days; group 3, receiving D-galactose (500 mg/kg/day, s.c.) plus PSO at 0.4 mL/kg (i.p.) for 42 days; and group 4, receiving D-galactose (500 mg/kg/day, s.c.) plus PSO at 0.8 mL/kg (i.p.) for 42 days.

After the end of treatment, a passive avoidance (PA) test was carried out to evaluate the animals' behaviors. Next, blood samples were collected by cardiac puncture and centrifuged to separate the serum; they were kept in a freezer to measure the aspartate transaminase (AST) and alanine aminotransferase (ALT) levels. Besides, the animals' brain and liver tissues were removed, homogenized, and stored in a freezer to determine the level of malondialdehyde (MDA) and thiol content.

PA test

The PA test is generally used to evaluate memory and learning in rodent models with the central nervous system (CNS) disorders. The test chamber is divided into dark and light compartments, separated by a gate. On the first day, the animals were allowed to find both compartments. Next they were exposed to the light chamber for 60 seconds. The initial latency to enter the dark compartment was recorded after the guillotine opening. The guillotine door was closed when the rat entered the dark chamber, and an electric foot shock (1 mA, 2 sec) was delivered through the grid floor. Next, the rats were removed from the dark chamber and returned to the cage. Twenty-four hours later, the latency time was measured in the same manner without foot shock, and the latency time to enter the dark chamber was recorded up to 300 seconds (14).

Measurement of MDA and thiol levels

Lipid peroxidation was evaluated by the measurement of MDA. For this purpose, 0.5 cc of homogenized tissue was mixed with phosphoric acid and TBA and added to boiling water for 45 minutes. After cooling, n-butanol was added to the mixture, vortexed, and centrifuged for 10 minutes. Finally, 200 µL of the supernatant was transferred to a plate, and absorbance was read at 530 nm (MDA level [nmol/mg tissue]= absorbance/1.56×10^5).

Measurement of thiol content

The homogenized tissue was mixed with TCA, vortexed, and centrifuged for 10 minutes. Tris-EDTA was then added to the mixture, and absorbance was read at 412 nm. Next, 20 µL of DTNB was added, and absorbance was measured after 15 minutes; DTNB was used as a blank.

Evaluation of liver enzymes in the serum

The levels of ALT and AST were determined in the serum using Pars Azmoon kits.

Statically analysis

Data expressed as mean ± SEM. Statistical analysis was carried out by Prism 6 software (La Jolla, CA). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test for comparison between groups. The p-values less than 0.05 were considered to be statistically significant.

Results

Effect of PSO on Passive avoidance test

Effect of PSO on Latency time

As shown in Figure1, d-galactose group showed lower time latency to enter the dark chamber during 1, 24, and 72 hours after shock compared to control group ($P<0.001$). PSO with dose of 0.8ml/kg increased the latency time after shock in all mentioned times ($P<0.001$) but lower dose of PSO (0.4ml/kg) increased latency time 1h ($P<0.05$) and 24h ($P<0.01$) after shock. There was no significant difference in the time latency

72 hours after shock between the d-galactose group and low dose - administered PSO group.

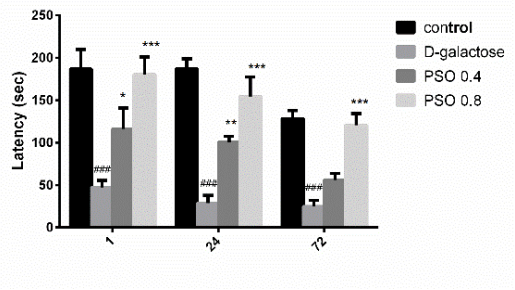

Figure 1. Effect of PSO on delay time for entrance to dark chamber following d-galactose-induced aging. Data expressed as mean ± SEM (n=10).###p<0.001 vs control, * p<0.05, ** p<0.01 and *** p<0.001 vs d-galastose treated animals.

Effect of PSO on retention time in dark room

Data showed that d-galactose increased the retention latency time significantly in dark room compare to control group during 3, 24 and 72hours (P<0.001). PSO by high and low dose decreased the retention time 1, 24 and 72h after shock (P<0.001). (Figure2).

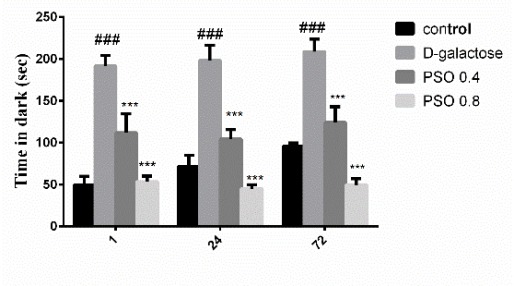

Figure2. Effect of PSO on retention time in dark chamber following d-galactose-induced aging. Data expressed as mean ± SEM (n=10). ###p<0.001 vs control, *** p<0.001 vs d-galastose treated animals.

Effect of PSO on lipid-peroxidation in brain and liver in aging model

The amount of MDA was measured as an index of lipid-peroxidation in liver and brain. D-galactose increased the level of MDA in both tissues significantly compare to control group (P<0.001). PSO decreased MDA significantly by 0.4ml/kg p<0.01 and 0.8ml/kg p<0.001) compared to D-galactose administered group (Figure 3).

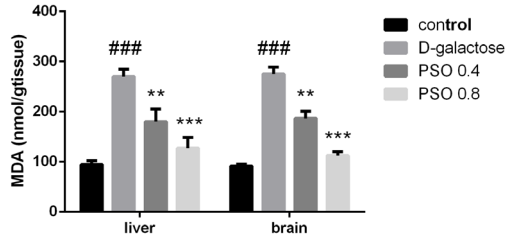

Figure3. Effect of PSO on lipidperoxidation following d-galactose-induced aging. Data expressed as mean ± SEM (n=10). ###p<0.001 vs control, ** p<0.01 and *** p<0.001 vs d-galastose treated animals.

Effect of PSO on thiol content in brain and liver in aging model

The thiol content was determined in liver and brain homogenized tissues. D-galactose led to attenuation of thiol following aging (p<0.001). PSO, 0.4 (P<0.05) and 0.8ml/kg (p<00.01) elevated the amount of thiol significantly (Figure 4).

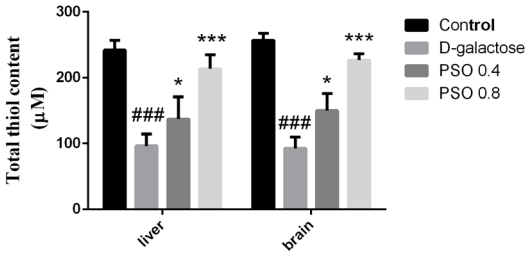

Figure 4. Effect of PSO on thiol content following d-galactose-induced aging. Data expressed as mean ± SEM (n=10). ###p<0.001 vs control, * p<0.05 and *** p<0.001 vs d-galastose treated animals.

Effect of PSO on liver enzymes

D-galactose increased ALT and AST compare to control group (p<0.01). PSO, 0.4 (P<0.05) and 0.8ml/kg reduced the level of AST and ALT p<0.001) compare to d-galactose group (Figure 5).

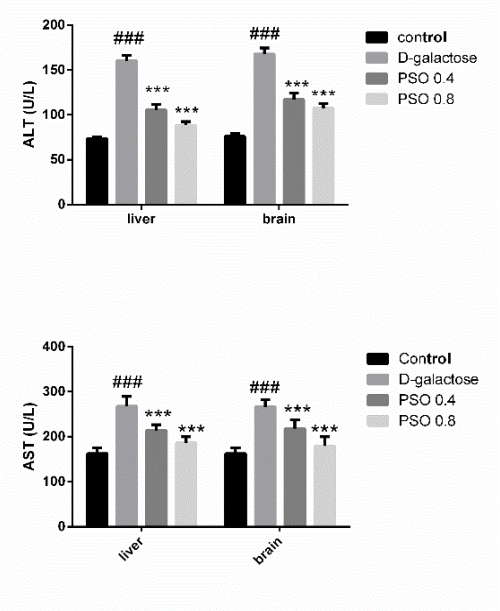

Figure 5. Effect of PSO on liver enzymes ALT (top) and AST (down), following d-galactose-induced aging. Data expressed as mean ± SEM (n=10). ###p<0.001 vs control, *** p<0.001 vs d-galastose treated animals.

Discussion

D-galactose, which is a simple sugar found in most foods, is converted to glucose by galactokinase and uridylyltransferase enzymes **(15)**. At high concentrations, D-galactose is converted to galacto-hexo-di-aldose and hydrogen peroxide by galactose oxidase, leading to oxidative stress, inflammation, and apoptosis. Therefore, chronic administration of D-galactose for 6-8 weeks leads to aging in laboratory models similar to humans **(2)**. In the present study, the effect of PSO on D-galactose-induced aging was investigated in rats for the first time. Administration of 500 mg of D-galactose for six weeks led to learning and memory dysfunctions in rats. In the PA test, the main indicator of memory was the latency to enter the dark compartment. Some studies have reported that an increase in latency to enter the dark compartment is a sign of improved memory performance **(16)**. In this study, the latency to enter the dark compartment was shorter in the D-galactose group compared to the control group, which confirms the impairment of memory and learning. This finding is consistent with the results of other studies, which showed that D-galactose causes memory dysfunction **(17, 18)**.

In this regard, Qu et al. showed that subcutaneous administration of D-galactose (100 mg/kg) for eight weeks led to the impairment of memory and elevation of oxidative parameters **(19)**. Moreover, a clinical study revealed that administration of pomegranate juice augmented memory in middle-aged and older adults with mild memory complaints **(20)**. Another study revealed that pomegranate flower improved STZ-induced memory deficiency by decreasing lipid peroxidation and elevating glutathione in the brain **(21)**. Additionally, passive and active avoidance tests showed that administration of the hydroalcoholic extract of pomegranate seed led to the improvement of memory in a hypoperfusion-induced ischemia model of female and male rats **(22, 23)**.

Additionally, in a previous study, pomegranate extracts caused an improvement in memory function via modulation of amyloid-beta in an age-related Alzheimer's disease animal model **(24)**. Ellagic acid, as an active component of pomegranate, can attenuate memory dysfunctions in D-galactose-induced aging via antioxidant and anti-inflammatory properties **(15)**. Free radicals can attack lipids and lead to the generation of MDA, which is identified as an oxidative stress marker **(25)**. Also, oxidative stress decreases the thiol content, which plays an important role in antioxidant properties **(26)**.

In the present study, administration of PSO decreased the level of MDA and elevated the thiol content in rats in a D-galactose-induced aging model. This finding agrees with the results reported by Bihamta et al., which indicated the protective effects of PSO against H_2O_2-induced oxidative stress in H9C2 cells **(27)**. Also, PSO caused a reduction in MDA and improved the thiol content in STZ-induced diabetic rats **(28)** and a model of $HgCl_2$-induced nephrotoxicity **(11)**.

The present results revealed that D-galactose increased the levels of AST and ALT in the serum, while PSO significantly decreased the levels of these enzymes in the serum. Other studies suggest that D-galactose damages the liver and leads to the elevation of liver enzymes in the serum **(29)**; besides, PSO plays a prominent role in the modulation of liver enzymes **(11, 30)**. Ellagic acid, as an active component of PSO, reduces AST and ALT in the serum following D-galactose-induced aging **(31)**. Considering the role of oxidative stress and inflammation in the aging process, the anti-aging effects of PSO may be related to its antioxidant activity due to the presence of active compounds.

Conclusion

In conclusion, data revealed that PSO decreases D-galactose induced aging process by attenuation of the free radicals formation, lipid peroxidation and elevation of thiol content. Therefore, PSO may alleviates brain and liver damage due to aging via modulation of stress oxidative. The protective effect may relates to the presence of anti-oxidant ingredients. Evaluation of the anti-aging effect of PSO needs more investigation as in vitro and in vivo models.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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