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Effect of *Capparis spinosa* L. on cognitive impairment induced by D-galactose in mice via inhibition of oxidative stress

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Background/aim: To determine the phenolic acid levels and DNA damage protection potential of *Capparis spinosa* L. seed extract and to investigate the effect of the extract on cognitive impairment and oxidative stress in an Alzheimer disease mice model.

Materials and methods: Thirty BALB/c mice divided into 5 groups (control, D-galactose, D-galactose + *C. spinosa* 50, D-galactose + *C. spinosa* 100, D-galactose + *C. spinosa* 200) were used. Mice were administered an injection of D-galactose (100 mg/kg, subcutaneous) and orally administered *C. spinosa* (50, 100, or 200 mg/kg) daily for 8 weeks.

Results: Syringic acid was detected and the total amount was 204.629 μg/g. Addition of 0.05 mg/mL *C. spinosa* extract provided significant protection against the damage of DNA bands. *C. spinosa* attenuated D-galactose-induced learning dysfunctions in mice and significantly increased memory retention. Malondialdehyde (MDA) levels increased and superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities decreased in the D-galactose group. *C. spinosa* (200 mg/kg body weight) significantly decreased MDA level and increased SOD, GPx, and CAT activities.

Conclusion: These results show that *C. spinosa* has the potential in ameliorating cognitive deficits induced by D-galactose in mice and the antioxidant activity may partially account for the improvement of learning and memory function.

Key words: Alzheimer disease, *Capparis spinosa* L., D-galactose, oxidative stress, cognitive impairment

1. Introduction

Alzheimer disease (AD) is neurodegenerative disease, characterized by the gradual deterioration of memory and other cognitive functions. Increase in age is a major risk factor for this disease and the prevalence of AD has increased worldwide as the elderly population of the world is increasing (1). The neuropathological hallmarks of AD brains are amyloid beta fibrils in senile plaques, neurofibrillary tangles, and neuronal loss (2). The etiopathogenesis of AD is multifactorial and it is suggested that oxidative stress has a significant role in the beginning and progression of this disease, yet the sources of free radicals and the mechanisms disrupting the redox balance remain elusive (3–5).

D-Galactose is a reducing sugar found in the body. When it is present at higher levels than normal, it can be converted to galactitol by galactose oxidase, resulting in the generation of oxygen-derived free radicals (6). Chronic systemic exposure to D-galactose induces aging-related changes such as corruption of spatial learning, memory loss, neurodegeneration, and diminishing activities of antioxidant enzymes and increased production of free radicals (7–9).

Capparis spinosa L. is a long-lasting shrubby plant that belongs to the family Capparidaceae and grows naturally throughout the world, especially widely in the Mediterranean basin. It has been used since ancient times for aromatic properties in cooking, and besides its use as flavoring, *C. spinosa* has also been used as a traditional herbal medicine for its antihypertensive, poultice, tonic, and diuretic characteristics (10). This plant has been investigated for several pharmacological effects. The bud extract of *C. spinosa* inhibits the replication of herpes simplex virus type 2 and upregulates the expression of proinflammatory cytokines (11). It was demonstrated that the methanol extracts of *C. spinosa* flowering buds have antiallergic effectiveness (12), and the lyophilized extract of *C. spinosa* buds shows in vitro antioxidant effectiveness

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in various models (13,14). Recently it was shown that *C. spinosa* fruit extract may be a safe antihyperglycemic agent for type 2 diabetic patients (15). It was demonstrated that the aqueous extract of *C. spinosa* has potent lipid-lowering activity in hyperglycemic rats (16). Other activities are chondrocyte protective activity (17) and a protective effect against oxidative stress in systemic sclerosis dermal fibroblasts (18).

Previous studies for *C. spinosa* have reported flavonoids, alkaloids, lipids, polyphenols, indole, and glucosinolates (10,19). However, there are only few reports on the seeds of *C. spinosa*. Seed oils of *Capparis ovata* and *C. spinosa* from different parts of Turkey were analyzed, showing the composition of fatty acids, tocopherols, and sterols and the content of glucosinolates (20). Lam et al. isolated and characterized a protein from *C. spinosa* seeds with mitogenic activity, potent HIV-1 reverse transcriptase activity, antifungal activity, and antiproliferative activity (21). Recently, Duman and Ozcan reported the seed and seed oil contents of *C. spinosa* species growing in Turkey (22), and in another study they showed the phenolic matter content and radical activity values of seeds of *Capparis* species in Turkey (10). In that study high radical activity was found for seeds of *C. spinosa* (10).

To the best of our knowledge there has been no study that has investigated the effect of *C. spinosa* on cognitive impairment. Therefore, in light of these considerations, the current study was undertaken to investigate the effect of *C. spinosa* in oxidative stress and cognitive impairment in an AD mice model.

2. Materials and methods

2.1. Drug and chemicals

D-Galactose and the agents used for diagnostic purposes were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Commercial kits used for the determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The purity of all chemical reagents was of at least analytical grade.

2.2. Plant material and sample preparation

The seeds of *C. spinosa* were collected from the southeastern region of Turkey (Gaziantep) in June 2013. Plant material was authenticated by Assistant Professor Erol Dönmez, a member of the Cumhuriyet University Faculty of Science's Department of Biology. After collection, the plant material was dried by removing the water content in an environment with no direct sunlight and good air flow. It was then ground to a powder with the help of a blender. The air-dried and finely ground samples were extracted by using a method described elsewhere (23). Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol (MeOH) at 60 °C for 6 h. Due to the aqueous polar characteristics of the phenolic acids, the extract was further fractionated with chloroform and distilled water. Finally, the extracts were then lyophilized and kept in the dark at 4 °C until tested.

2.3. Determination of phenolic acid levels of extracts

The analysis of phenolic acids was employed according to the method described by Öztürk et al. (24) with a slight modification using an Agilent HPLC series 1200 (Agilent, Waldbronn, Germany). The separation of gallic, protocatechuic, *p*-hydroxy benzoic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, ferulic, *o*-coumaric, rosmarinic, and *trans*-cinnamic acids was performed on an Agilent Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm i.d., 5 µm particle size) (Figure 1). The chromatographic conditions were: flow rate of 1 mL/min, sample injection volume of 5 µL, operation temperature of 23 °C, UV detection at 280 nm, and mobile phases A (methanol : water : formic acid (10 : 88 : 2, v/v)) and B (methanol : water : formic acid (90 : 8 : 2, v/v)). A gradient program was used as follows: 100% A, 0–20 min; changed to 80% A, 25–50 min, then to 50% A, 50–54 min; followed by isocratic elutions of 50% A, 54–64 min, 0% A, and 64–70 min, 100% A. The results were evaluated with regard to the areas of the peaks and their retention times. Quantitation was based on calibration curves built for each of the compounds identified in the samples.

2.4. Determination of DNA damage protection potential of extracts

DNA damage protection activity of the extract was evaluated on pBR322 plasmid DNA (Vivantis, Subang Jaya, Malaysia). Plasmid DNA was oxidized with H_2O_2 + UV treatment in the presence of extracts and checked on 1% agarose gels according to Russo et al. after some modifications (25). In brief, the experiments were performed in a volume of 10 µL in a microfuge tube containing 3 µL of pBR322 plasmid DNA (172 ng/µL), 1 µL of 30% H_2O_2, and 5 µL of extract in the concentrations of 5, 10, 20, 40, and 50 µg/mL, respectively. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (DNR-IS) with an intensity of 8000 µW/cm^2 at 302 nm at room temperature. After irradiation, the reaction mixture (10 µL) along with gel loading dye (6X) was loaded on a 1% agarose gel for electrophoresis. Untreated pBR322 plasmid DNA was used as a control in each run of gel electrophoresis along with partially treated plasmid, i.e. only UV or only H_2O_2 treatment. Gels were stained with EtBr and photographed with the gel documentation system (MiniBIS Pro, DNR-IS, Jerusalem, Israel).

2.5. Animals and drug administration

Male 8-week-old BALB/c mice (n = 30) were used. All experiments were conducted at Cumhuriyet University. Six mice were housed per cage under standardized conditions

(12-h light/dark cycle, 24 ± 2 °C, 35% to 60% humidity) and allowed a commercial standard mice diet and water ad libitum. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the Animal Ethics Committee of Cumhuriyet University (Turkey). After 1 week of adaptation the mice were randomly divided into 5 groups (n = 6 in each group): control, D-galactose, D-galactose + *C. spinosa* 50, D-galactose + *C. spinosa* 100, and D-galactose + *C. spinosa* 200. D-galactose was dissolved in sterile saline (0.3 mL) and injected subcutaneously (100 mg/kg daily) for 60 continuous days (26,27) while mice in the control group were treated with the same volume of sterile saline. D-galactose + *C. spinosa* mice were orally administered lyophilized *C. spinosa* extract at the doses of 50, 100, and 200 mg/kg per day respectively after subcutaneous injection of D-galactose (100 mg/kg daily). At the end of the drug administration period, behavioral tests and biochemical analysis were performed. During the entire experimental process the mice were weighed each week.

2.6. Morris water maze

The Morris water maze test was performed according to the methods described previously (28). The procedure of this test consisted of 2 learning and memory training trials per day for 4 consecutive days and after that a probe trial was performed on day 5 for each mouse. The experimental apparatus consisted of a circular pool 150 cm in diameter and 35 cm deep, filled with water kept at 24 ± 1 °C. The pool was divided into 4 quadrants of equal areas. A platform (4.5 cm in diameter) was placed 1 cm below the water surface at the midpoint of 1 quadrant.

2.6.1. Spatial navigation task

In each daily trial, a mouse was placed in the water maze at a determined location and the animal was allowed to find the hidden platform. The time needed for each mouse to find the hidden platform was recorded for all trials. A mouse that found the platform was allowed to stay on the platform for 15 s to memorize its location and was then returned to its cage for an interval. If it failed to find the platform within 60 s, escape latency was recorded as 60 s. Latency to escape (finding the hidden platform) was recorded in each trial. The extent of learning was assessed with this test.

2.6.2. Probe test

The spatial probe test was performed on day 5 by removing the platform. Each mouse was allowed to swim for 60 s. The time required for the mouse to reach the target quadrant, the time that mouse spent swimming in the target quadrant, and the number of crossings over the platform zone were recorded. Memory consolidation was assessed with this test.

2.7. Biochemical analysis

Mice were sacrificed 24 h after Morris water maze test. The whole brain tissue was excised immediately in ice-cold conditions, blotted free of blood and tissue fluids, weighed, and stored at –80 °C for further analysis.

2.7.1. Estimation of lipid peroxidation

Lipid peroxidation was monitored in terms of malondialdehyde (MDA) by the method of Ohkawa et al. (29). MDA level was determined by thiobarbituric acid reactive substances (TBARS) in brain tissue homogenate, based on the reaction between MDA and thiobarbituric acid. Thiobarbituric acid when allowed to react with MDA aerobically formed a colored complex [MDA-(TBA) 2 complex], which was measured by spectrophotometer (Shimadzu UV-1700, Shimadzu, Kyoto, Japan) at 532 nm. MDA concentration (measured as TBARS) was calculated as nmol/mL. Absorbance values were compared with a series of standard solutions of 1,1,3,3-tetraethoxypropane.

2.7.2. Measurement of SOD activity

SOD was determined by using a commercially available standard enzymatic kit (Cayman Chemical Company) and following the manufacturer's instructions. Brain tissue was homogenized in cold 20 mM HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose per gram of tissue and was centrifuged at 1500 × *g* for 5 min at 4 °C. The supernatant was removed and stored on ice for total SOD activity (cytosolic and mitochondrial) assay according to the protocol provided by the assay kit manufacturer. The absorbance was read at 440–460 nm using a plate reader. The SOD activity was expressed as U/mg protein. This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The SOD assay measures all 3 types of SOD (CU/Zn, Mn, and Fe SOD). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

2.7.3. Measurement of GPx activity

GPx activity was measured using a commercially available kit (Cayman Chemical Company) and following the manufacturer's instructions. Brain tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.5), containing 5 mM EDTA and 1 mM DTT per gram of tissue and centrifuged at 10,000 × *g* for 15 min at 4 °C to obtain supernatant for GPx analysis. The removed supernatant was stored on ice and the absorbance was read once every minute at 340 nm using a plate reader to obtain at least 5 time points. Levels of GPx activity were expressed as nmol min^{-1} mg $protein^{-1}$ in tissue. The measurement of GPx activity is based on the principle of a coupled reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after the reduction of hydroperoxide by GPx is recycled to its reduced state by GR in the presence of NADPH accompanied by a decrease in absorbance at 340 nm. One unit of GPx is defined as the

amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25 °C.

2.7.4. Measurement of CAT activity

CAT activity was measured using a commercially available kit (Cayman Chemical Company) and following the manufacturer's instructions. Brain tissue was homogenized in 50 mM potassium phosphate (pH 7.0), containing 1 mM EDTA per gram of tissue and centrifuged at 10,000 × *g* for 15 min at 4 °C to obtain supernatant for CAT analysis. The removed supernatant was stored on ice and the absorbance was read at 540 nm. Levels of CAT activity were expressed as nmol min^{-1} mg $protein^{-1}$ in tissue. The measurement of CAT activity is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald forms a bicyclic heterocycle with aldehydes that after oxidation changes from colorless to purple. One unit of CAT is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25 °C.

2.7.5. Protein assay

The total protein content was estimated by the method of Bradford (30). Bovine serum albumin was used as the standard.

2.8. Statistical analyses

Data were analyzed using the SPSS 15 for Windows (SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviation (SD). The data obtained from the spatial navigation task were analyzed using 2-way ANOVA followed by Tukey–Kramer multiple comparison tests as a post hoc test with repeated measures and the factors were groups and training days. The other data were analyzed with 1-way ANOVA followed by Newman–Keuls or Tukey–Kramer multiple comparison tests as the post hoc test. $P < 0.05$ was regarded as significant.

3. Results

3.1. Phenolic acid levels of extracts

In this study, amounts of gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, ferulic, *o*-coumaric, rosmarinic, and *trans*-cinnamic acids were quantified in polar methanol extracts of *C. spinosa*. Due to the polar characteristics of phenolic acids, as mentioned in Section 2, methanol extracts obtained from the plants were also further fractionated with equal amounts of chloroform and distilled water. In this way, nonpolar phytochemicals were eliminated from the target extract. Polar phytochemicals brought together within the water fraction were lyophilized and stored at 4 °C until tested and analyzed. In *C. spinosa* extract, only syringic acid was detected, and the total amount was 204.629 µg/g (Figure 1).

3.2. DNA damage protection potential of extracts

Figure 2 shows the electrophoretic pattern of DNA after UV photolysis of H_2O_2 in the absence and presence of the aqueous extract of *C. spinosa*. DNA derived from pBR322 plasmid showed 2 bands upon agarose gel electrophoresis (column 1); the faster moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving band was the open circular form (ocDNA). The UV irradiation of DNA in the presence of H_2O_2 (column 3) resulted in the cleavage of ocDNA to a faint linear DNA and smears on the agarose gel, indicating that the OH· generated from UV photolysis of H_2O_2 produced DNA strand scission.

As can be seen from the Figure 2, columns 6–9 show the DNA damage protection potential of the aqueous extract of

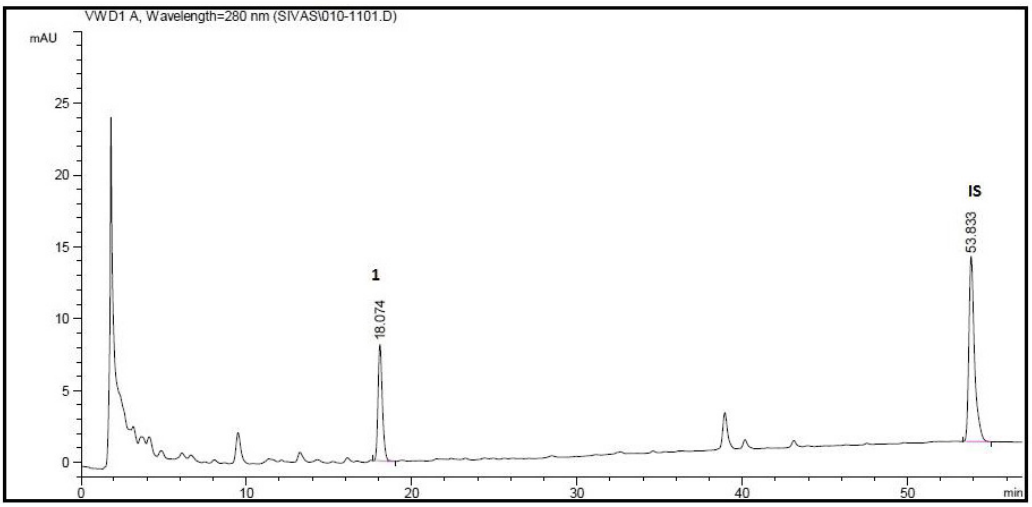

Figure 1. HPLC chromatogram showing phenolic acids available in *C. spinosa* [1: Syringic acid, Rt (minutes): 18.074, source of confirmation: UV, IS: internal standard, Rt (minutes): 53.833].

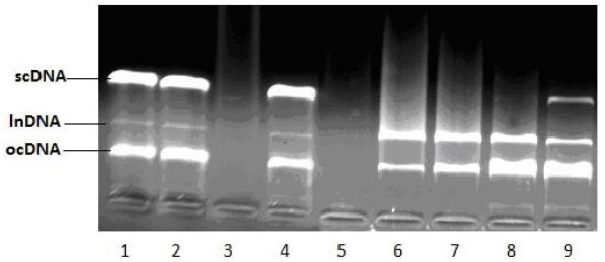

Figure 2. Electrophoretic pattern of pBR322 plasmid DNA after treatment with UV and H_2O_2 in the presence of *C. spinosa* aqueous extract. Column 1: Plasmid DNA (3 µL) + dH_2O (6 µL), Column 2: plasmid DNA (3 µL) + dH_2O (6 µL) + UV, Column 3: plasmid DNA (3 µL) + dH_2O (6 µL) + UV + H_2O_2 (1 µL), Column 4: plasmid DNA (3 µL) + dH_2O (6 µL) + H_2O_2 (1 µL), Column 5: plasmid DNA (3 µL) + aqueous extract (0.005 mg/mL, 5 µL) + UV + H_2O_2 (1 µL), Column 6: plasmid DNA (3 µL) + aqueous extract (0.01 mg/mL, 5 µL) + UV + H_2O_2 (1 µL), Column 7: plasmid DNA (3 µL) + aqueous extract (0.02 mg/mL, 5 µL) + UV + H_2O_2 (1 µL), Column 8: plasmid DNA (3 µL) + aqueous extract (0.04 mg/mL, 5 µL) + UV + H_2O_2 (1 µL), Column 9: plasmid DNA (3 µL) + aqueous extract (0.05 mg/mL, 5 µL) + UV + H_2O_2 (1 µL).

C. spinosa. In columns 6, 7, and 8, 2 major bands labeled as ocDNA and scDNA are protected by the presence of 0.01, 0.02, and 0.04 mg/mL extract concentration, respectively. The addition of 0.05 mg/mL *C. spinosa* extract to the reaction mixture conferred significant protection against damage of all DNA bands (column 9), while the addition of lowest extract concentration (0.005 mg/mL) did not show any protection against DNA damage (column 5).

3.3. Effect of *C. spinosa* on learning and memory performance

3.3.1. Spatial navigation task

To investigate the effect of *C. spinosa* on D-galactose-induced cognitive impairment, we used the Morris water maze test. This test is a sensitive test for determining the impairment of spatial learning and memory (26). Two-way repeated measures ANOVA (day × group) showed that there was a significant difference in mean latency between groups ($F = 33.76$, $P = 0.001$, $P < 0.01$) and also that there was a significant difference in mean latency between days ($F = 48.68$, $P = 0.001$, $P < 0.01$). The D-galactose mice spent a longer time finding the platform than control group mice on training days (day 1, $P > 0.05$; day 2, $P < 0.01$; day 3, $P < 0.01$; day 4, $P < 0.01$). This showed that D-galactose mice had significant cognitive impairment. On the other hand, mice treated daily with *C. spinosa* at 200 mg/kg decreased the time for escape latency significantly when compared to D-galactose mice from the second day onwards ($P < 0.01$, $P < 0.01$, and $P < 0.01$). *C. spinosa* treatment at 50 mg/kg and 100 mg/kg daily had similar effects ($P < 0.05$, $P < 0.05$, and $P < 0.05$) (Figure 3). These results show that both day

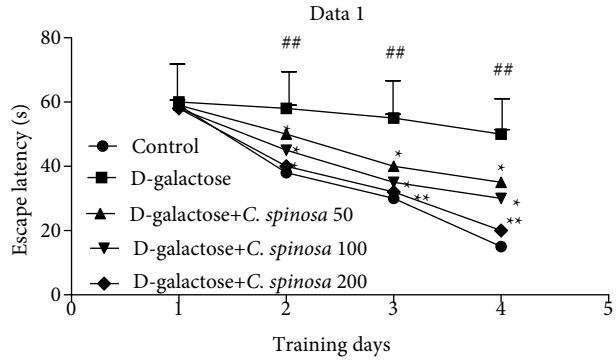

Figure 3. Effect of *C. spinosa* on the escape latency of D-galactose-treated mice in Morris water maze test. Escape latencies to find a hidden platform in the water maze were measured over 4 consecutive days of training. D-galactose was dissolved in sterile saline (0.3 mL) and injected subcutaneously (100 mg/kg daily) for 60 continuous days and control group mice were treated with the same volume of sterile saline. D-galactose + *C. spinosa* groups were orally administered lyophilized *C. spinosa* extract at the doses of 50, 100, or 200 mg/kg per day respectively after subcutaneous injection of D-galactose (100 mg/kg daily). Data are expressed as mean ± SD (n = 6 in each group). ##: $P < 0.01$ compared with control group; **: $P < 0.01$ and *: $P < 0.05$ compared to D-galactose group.

and group had significant effect on the escape latency and all groups of rats improved over the training trials. There was a significant interaction between day and group factors in escape latency. These results indicate that *C. spinosa* in a dose-dependent manner prevented D-galactose-induced learning dysfunction in mice.

3.3.2. Probe test

On day 5, data were obtained from 3 measurements of probe test performance. In the probe test, for mice in the control and the 100 and 200 mg/kg *C. spinosa* groups, the time spent to find the target quadrant was shorter than in the D-galactose group ($P < 0.01$ versus control group; $P < 0.01$ versus *C. spinosa* 100 group; $P < 0.01$ versus *C. spinosa* 200 group) (Figure 4A). Mice in the control and *C. spinosa* 200 groups spent a longer time swimming in the target quadrant than the mice in the D-galactose group ($P < 0.01$ versus control group; $P < 0.01$ versus *C. spinosa* 200 group) (Figure 4B). Similar results were obtained for the number of times the mice crossed the platform location. Mice treated with D-galactose crossed the platform location less frequently than mice in the control ($P < 0.01$) and *C. spinosa* 100 ($P < 0.05$) and 200 ($P < 0.01$) groups (Figure 4C). These results indicated that *C. spinosa* treatment at a daily dose of 200 mg/kg significantly increased memory retention compared to D-galactose-treated mice.

3.4. Effect of *C. spinosa* on MDA contents in the brain and SOD, GPx, and CAT activities

MDA levels, a main index of lipid peroxidation, were significantly increased in mice given D-galactose

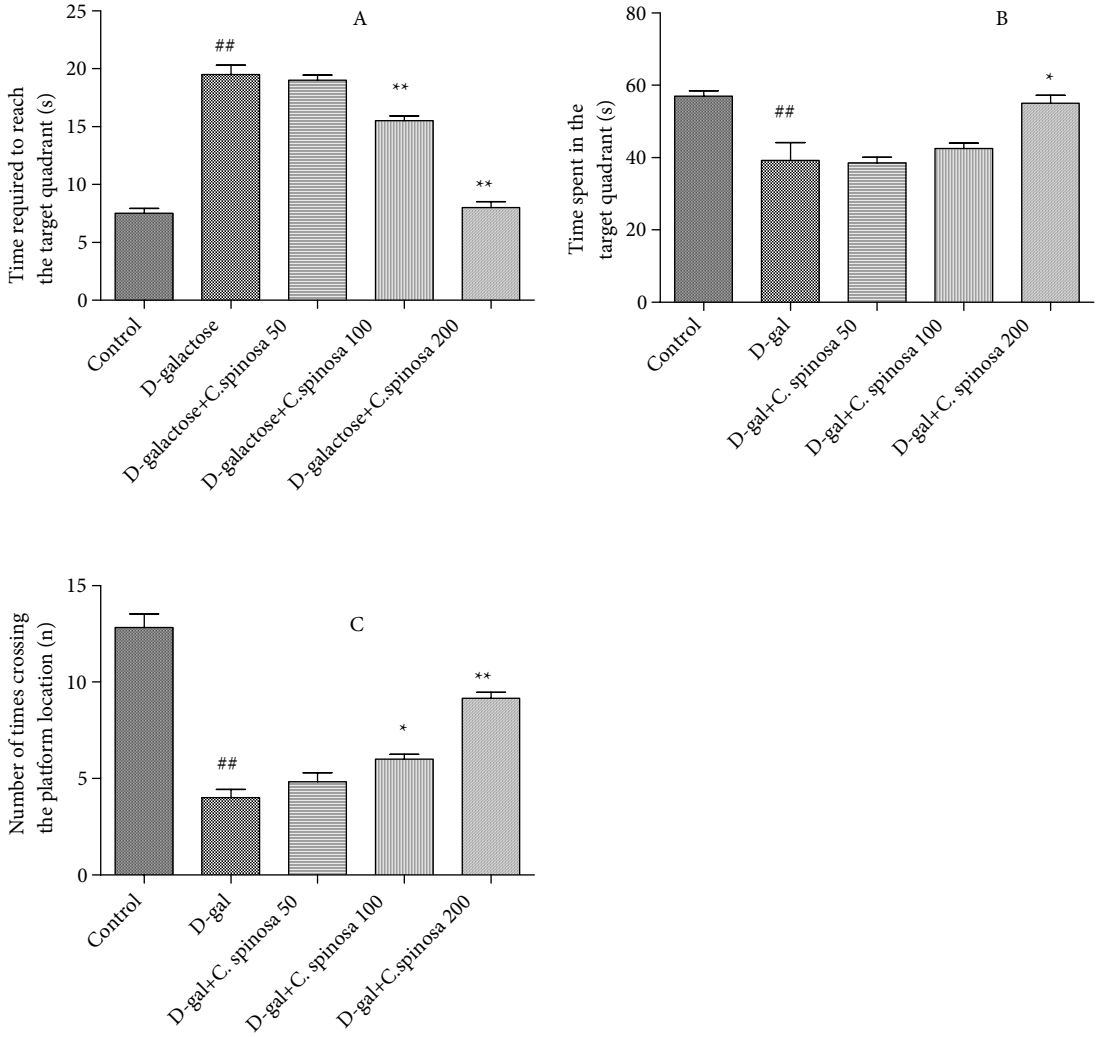

Figure 4. Effect of *C. spinosa* on behavior of D-galactose (D-gal)-treated mice in Morris water maze test. D-galactose was dissolved in sterile saline (0.3 mL) and injected subcutaneously (100 mg/kg daily) for 60 continuous days and control group mice were treated with the same volume of sterile saline. D-galactose + *C. spinosa* groups were orally administered lyophilized *C. spinosa* extract at the doses of 50, 100, or 200 mg/kg per day respectively after subcutaneous injection of D-galactose (100 mg/kg daily). Data are expressed as mean ± SD (n = 6 in each group). (A) Comparison of the time required to reach the target quadrant on day 5, (B) comparison of the time spent in the target quadrant on day 5, (C) comparison of number of times crossing over the platform site on day 6. ##: $P < 0.01$ compared with control group; **: $P < 0.01$ and *: $P < 0.05$ compared to D-galactose group.

compared to the control group ($P < 0.05$). Treating mice with *C. spinosa* depressed MDA enhancement in brains of D-galactose-treated mice and the effect of *C. spinosa* was dose-dependent ($P < 0.01$ or $P < 0.05$) (Figure 5).

A significant decrease ($P < 0.05$) in the levels of SOD, GPx, and CAT were noticed in D-galactose mice when compared to control mice. Administration of *C. spinosa* dose-dependently prevented the D-galactose-induced decrease in brain SOD and GPx and CAT activities. *C. spinosa* showed the strongest effect at improving the SOD, GPx, and CAT activities in the brain at a dose of 200 mg/kg per day (Figure 6).

4. Discussion

AD is a neurodegenerative disease that causes dementia; after diagnosis, it leads to a complete incapacity and death within 3 to 9 years. For preventing or retarding the disease process there is a need to develop new agents as current therapies against AD have limited effectiveness. The exact cause and pathogenesis of AD is not completely understood. It is accepted that oxidative stress plays a key role in many aging-related diseases such as Parkinson disease and AD (31). A lipid peroxidation product, MDA is increased in cerebrospinal fluid and brain regions of patients with AD (32,33), and reduction of antioxidant

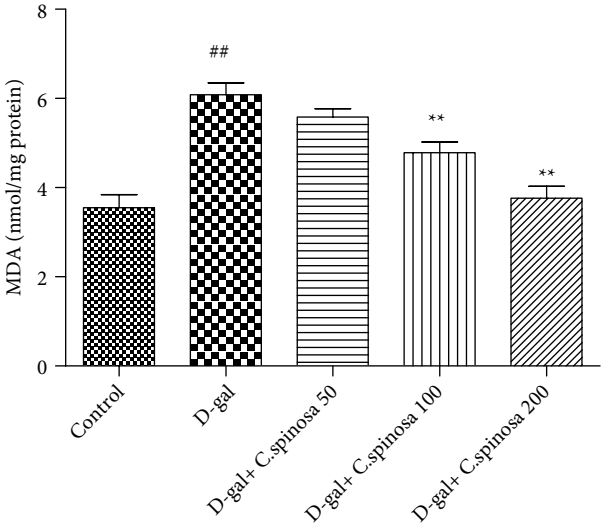

Figure 5. Effect of *C. spinosa* on the level of MDA in brain tissue of D-galactose (D-gal)-treated mice. D-galactose was dissolved in sterile saline (0.3 mL) and injected subcutaneously (100 mg/kg daily) for 60 continuous days and control group mice were treated with the same volume of sterile saline. D-galactose + *C. spinosa* groups were orally administered lyophilized *C. spinosa* extract at the doses of 50, 100, or 200 mg/kg per day respectively after subcutaneous injection of D-galactose (100 mg/kg daily). Data are expressed as mean ± SD (n = 6 in each group). ##: P < 0.01 compared with control group; **: P < 0.01 compared to D-galactose group.

enzymes such as GPx and SOD have been seen in central nervous system of AD patients (34). It has been demonstrated that in AD oxidative stress is linked with several pathological processes such as mitochondria dysfunction, amyloid beta-induced neurotoxicity, metal dyshomeostasis, and tau pathology (1). Because of this, using natural antioxidants that are capable of targeting different molecular events that are involved in the pathogenesis of AD may be a good approach in the prevention or treatment of this disease.

The present study was done to examine the effect of *C. spinosa* seed extracts on cognitive impairment induced by D-galactose in mice. To the best of our knowledge, this study shows for the first time that *C. spinosa* seed extracts have potential in ameliorating cognitive deficits induced by D-galactose in mice by inhibiting oxidative stress in a dose-dependent manner.

Mainly because of their redox properties, phenolic compounds have antioxidant activity. This activity allows them to reduce free radicals and metal chelators; thus, they prevent many diseases including myocardial infarction, atherosclerosis, and cancer (35–37). In this study, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, chlorogenic, syringic, *p*-coumaric, ferulic, *o*-coumaric, rosmarinic, and *trans*-cinnamic acids were investigated in polar methanol extracts of *C. spinosa* and syringic acid was detected at a total amount of 204.629 μg/g. Syringic acid is a natural phenolic acid with a strong antioxidant effect that has multiple pharmacological properties such as anticancer effects and antihypertensive and hepatoprotective effects (38–40). DNA damage protection potential of extracts was also determined. The lowest extract concentration (0.005 mg/mL) showed no protection against DNA damage. In the presence of 0.01, 0.02, and 0.04 mg/mL extract concentration, 2 major bands, named ocDNA and scDNA, were protected while the application of 0.05 mg/mL *C. spinosa* extract showed significant protection against the damage of all DNA bands. These results show that *C. spinosa* extract has DNA damage protection potential at a significant level.

D-Galactose injection is used to induce the mimicking of some characters of cognitive dysfunction and oxidative damage in experimental animals that look like accelerated aging. In order to explain the mechanism of D-galactose, many hypotheses have been put forward; one of them is that chronic systemic exposure to D-galactose increases levels of MDA and lipofuscin and also decreases GPx and SOD activities (6,41). In our study, when the Morris water maze test was applied, we observed that long-term administration of D-galactose in mice caused cognitive deficit and showed significant impairment of learning and memory ability. This finding is consistent with other studies that reported cognitive impairment by D-galactose in rodents (8,26,41). Morris water maze tests suggested that *C. spinosa* attenuated D-galactose-induced cognitive impairment as detected by diminishing latency to reach the hidden platform. We also observed that *C. spinosa* significantly increased the time spent in the target quadrant and the number of times that mice crossed the platform location. These results indicate that *C. spinosa* in a dose-dependent manner prevented D-galactose-induced learning dysfunction in mice and also increased memory retention compared to D-galactose-treated mice.

Many experimental and human studies indicate that in the development and progression of AD oxidative stress is one of the important factors (1,42,43). Free radicals take part in neuronal degeneration and accelerating aging. In order to protect cells against oxidative damage induced by free radicals, endogenous antioxidant enzymes such as CAT, SOD, and GPx are activated in the body. As there is a strong correlation between oxidative stress and cognitive dysfunction, these antioxidant enzymes have important roles in fixing memory and learning deficits (7,8). In our study, decreased levels of SOD, GPx, and CAT were observed in D-galactose-treated mice. However, *C. spinosa* significantly increased the activities of SOD, GPx, and CAT, showing that *C. spinosa* improved antioxidant activity. The

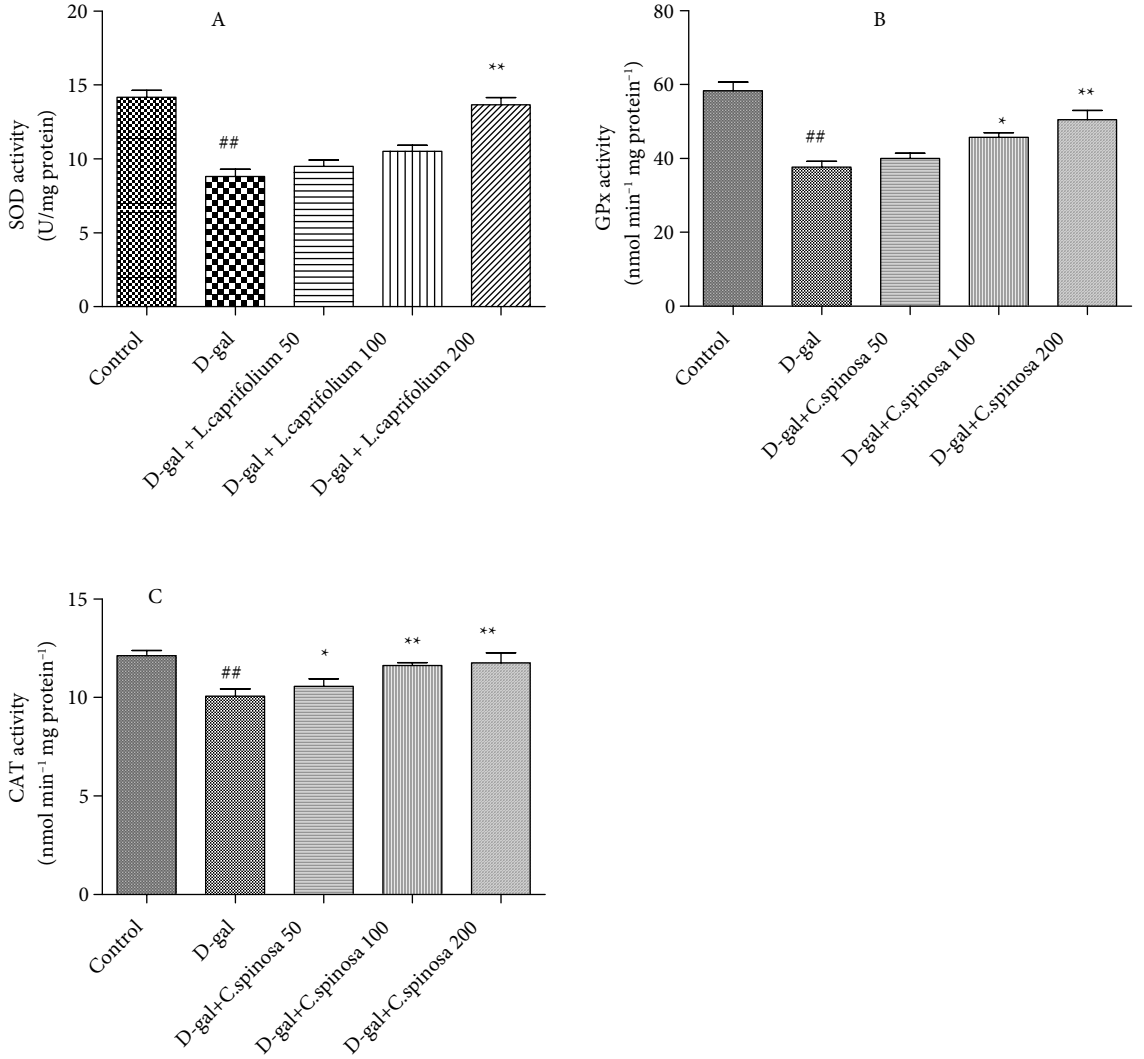

Figure 6. Effect of *C. spinosa* on the activity of SOD, GPx, and CAT in brain tissue of D-galactose (D-gal)-treated mice. D-galactose was dissolved in sterile saline (0.3 mL) and injected subcutaneously (100 mg/kg daily) for 60 continuous days and control group mice were treated with the same volume of sterile saline. D-galactose + *C. spinosa* groups were orally administered lyophilized *C. spinosa* extract at the doses of 50, 100, or 200 mg/kg per day respectively after subcutaneous injection of D-galactose (100 mg/kg daily). Data are expressed as mean ± SD (n = 6 in each group). ##: $P < 0.01$ compared with control group; **: $P < 0.01$ and *: $P < 0.05$ compared to D-galactose group.

enhanced activities of these antioxidant enzymes could be effective in eliminating different types of oxygen free radicals and their products, reversing D-galactose-induced cognitive deficits produced by oxidative damage.

Under conditions of oxidative stress MDA is an important indicator of membrane damage. Again in agreement with previous reports in brain tissue of mice treated with D-galactose, there was a significant increase in MDA levels, a product of lipid peroxidation (41,42). *C. spinosa* significantly decreased the level of MDA, showing that *C. spinosa* could decrease the production of MDA induced by D-galactose, and it improved the oxidative stress in the brain induced by D-galactose. The increased activities of SOD, GPx, and CAT and free radical scavenging activity in mice fed *C. spinosa* could contribute to its inhibitory effect on lipid peroxidation. The reduction of oxidative stress in the brain may be one mechanism by which *C. spinosa* improved learning and memory functions in D-galactose-treated rats.

In conclusion, our study demonstrated that *C. spinosa* significantly improved cognitive impairment induced by D-galactose injection in mice in a dose-dependent manner. We think that the syringic acid, a natural phenolic acid with a strong antioxidant ability detected in the *C.*

spinosa seed extracts, has an important role in this effect and this effect may be mediated partly through regulating the activities of antioxidant enzymes and scavenging free radicals. The effect of *C. spinosa* is likely through attenuating oxidative stress as demonstrated by increasing activities of SOD, GPx, and CAT enzymes and decreasing levels of MDA. These results suggest that *C. spinosa* may be a useful additive in addition to the current treatment of AD patients. Additional research with more detailed work is necessary to completely understand the molecular mechanisms behind the positive effect of *C. spinosa* against D-galactose-induced cognitive impairment.

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