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Gemfibrozil, a lipid-lowering drug, reduces anxiety, enhances memory, and improves brain oxidative stress in D-galactose-induced aging mice

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Abstract

Gemfibrozil (GFZ) is a lipid-lowering drug with several other effects, such as antioxidant and anti-inflammatory activities. In the current study, chronic D-galactose treatment (D-gal, 150 mg/kg/day; i.p., 6 weeks) induced a model of accelerated aging in male mice and was used to study the behavioral, anti-oxidative, and neuroprotective effects of GFZ (100 mg/kg/day; p.o.). Anxiety-like behaviors were assessed using the elevated plus-maze while working memory was measured by spontaneous alternation in a Y-maze. Brain oxidative stress was determined by measuring malondialdehyde (MDA) levels, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities. Neuropathological evaluation of the brain with hematoxylin–eosin and Masson's trichrome staining was also performed. The results demonstrated that the anxious-like phenotype and the cognitive impairments observed in D-gal-treated mice could be prevented in those animals coadministered with GFZ. Besides, the decrease in SOD and GPx antioxidant enzymatic activities and increase of MDA levels were also prevented in the brains of D-gal plus GFZ treated mice. Preliminary hematoxylin–eosin staining also suggested neuroprotective effects of GFZ. The results of Masson's trichrome staining showed no evidence of fibrosis in brain sections of different experimental groups. The current data provide novel insights into GFZ in the D-galactose-induced aging mouse model that open promising future research lines to determine inflammatory mediators and cell signaling underlying these effects.

Abbreviations: %OAE, percentage of open arm entries; %OAT, percentage of open arm time; D-gal, D-galactose; EPM, elevated plus maze; GFZ, gemfibrozil; GPx, glutathione peroxidase; MDA, malondialdehyde; PPAR-α, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; SOD, superoxide dismutase.

KEYWORDS
aging, D-galactose, gemfibrozil, mice, oxidative stress

1 | INTRODUCTION

Aging triggers a slow and complex biological phenomenon associated with molecular and cellular dysfunction in all tissues and organs via different mechanisms [1]. Out of the different theories of aging, the oxidative stress theory is more accepted among scientists [2]. This hypothesis suggests that with the increase of age, the production of reactive oxygen species (ROS) increases, and on the other hand, the capacity of the body to eliminate these compounds decreases [3, 4]. The oxidative alterations in the brain increase the incidence of various pathological conditions such as cognitive impairment and anxiety in aged individuals [5, 6]. These conditions are a matter of great concern, and important medical gerontology efforts are made to screen for preventive/therapeutical agents [7, 8].

At translational level, models to mimic the aging scenario are used so that they can serve to assess the anti-oxidant, anti-inflammatory, and neuroprotective properties of different compounds. A great body of evidence has shown that chronic administration of D-galactose (D-gal) triggers aging processes similar to natural aging in experimental animals [9–11]. It is well established that D-gal induces aging via the overproduction of ROS, antioxidant enzyme downregulation and affects cognition and behavior [9, 10, 12, 13]. The dose of D-gal 150 mg/kg allows reproducing these effects within 6 weeks and males are more prone to show its impact [12, 13].

Gemfibrozil (GFZ) is a common drug from the fibrate family. This drug is prescribed to reduce the plasma levels of triglyceride in patients with hypertriglyceridemia and mixed dyslipidemia [14]. The exact mechanism of GFZ is not clearly understood, but it has been shown that interacting with peroxisome proliferator-activated receptor-α (PPAR-α) is responsible for its effects [15]. Moreover, GFZ exerts other effects such as antioxidant and anti-inflammatory properties which are independent of PPAR-α [16, 17]. GFZ can reduce oxidative stress and inflammation in atherosclerosis in experimental diabetes [17]. Moreover, GFZ inhibits the production and release of inflammatory parameters in various pathological conditions such as sepsis and nephrotoxicity of cisplatin [16, 18]. Furthermore, few studies have shown that GFZ has neuroprotective effects in Alzheimer's disease [19] and permanent middle cerebral artery occlusion [20].

In the current study, we assessed the anxiolytic, antioxidant, and neuroprotective effects of GFZ in normal adult mice and in D-gal-induced aging. Thus, its ability to prevent brain hallmarks associated to aging induced by D-gal, based on behavioral indices, oxidative stress parameters, and neuropathological alterations.

2 | MATERIALS AND METHODS

2.1 | Animals and treatments

Twenty-eight 2–3 months old male mice (27 ± 2 g) were housed in Plexiglas cages (seven mice per cage) at the Animal House of the Rafsanjan University of Medical Sciences. They were kept at constant temperature (22 ± 2°C) and humidity (60%), and at 12-h light/dark cycle (lights on at 8:00 a.m.) with free access to food (standard pellet chow; Pars Dam, Tehran, Iran) and water.

Fifteen days after arrival, the animals were randomly divided into four experimental groups (seven animals per group). The naïve group received no intervention and received standard drinking water (10 ml/kg). D-gal was purchased from Sigma-Aldrich Company (Germany) and GFZ was obtained from Abidi Pharmaceutical Company (Iran). The GFZ group received GFZ (100 mg/kg; orally). D-gal group received D-gal (150 mg/kg; i.p.) for 6 weeks [21–23]. The D-gal + GFZ group received GFZ (100 mg/kg; orally) [24] for 6 weeks concomitantly with D-gal treatment (150 mg/kg; i.p.).

The Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC, National Academy Press, no. 85-23, revised 1996) was followed. The study was approved by the Ethics Committee of the Rafsanjan University of Medical Sciences (IR.RUMS.REC.1399.030) and Kerman University of Medical Sciences (IR.KMU.REC.1399.233).

2.2 | Behavioral assessment

Twenty-four hours after the last drug administration, the behavioral tests were performed. All the behavioral experiments were done by a blinded observer, in the morning between 9:00 and 12:00 a.m. Animals were acclimated 1 h to the test room before the behavioral assessment. After each experiment, the maze was thoroughly cleaned with diluted ethanol (10%) to prevent the adverse effects of olfactory cues.

The elevated plus maze (EPM) test to evaluate rodent anxiety-like behaviors was used according to the protocol described in our previous studies [25].

Briefly, the maze consisted of two open (50 × 10 cm) and two closed arms (50 × 10 × 40 cm), representing a plus sign. Each animal was put in the central area of the EPM facing an open arm and recorded for 5 min with a digital camera. The percentage of open arm entries (%OAE) and the percentage of time spent into the maze's open arms (%OAT) were calculated. Locomotor activity (total number of entrances) was also measured to confirm no differences in this behavioral aspect [12].

The Y-maze to evaluate the working memory [26] consisted of three arms (15 × 30 × 40 cm with equal angles between arms). Briefly, the animals were put in the maze center and observed for 8 min with a digital camera. Correct spontaneous alternation of arms, defined as not revisiting the same arm twice before visiting another, was measured. The percentage of correct alternations was calculated as the index: the number of alternations/total arm visits (minus 2) × 100.

2.3 | Biochemical and histopathological analysis

2.3.1 | Tissue preparation

Twenty-four hours after behavioral tests, mice of each group were sacrificed. Brains were immediately removed and divided into two hemispheres: one hemisphere was fixed in 10% phosphate-buffered formalin for histological assessment, and the other one was homogenized (1/10 w/v) in ice-cold Tris–HCl buffer (100 mM, pH 7.4), centrifuged 4427 ×*g* for 20 min, and the supernatant was collected and stored at −80°C for the biochemical analyses [27].

2.4 | Oxidative stress evaluation

The lipid peroxidation was measured with MDA levels by a commercially available kit (ZellBio, Lonsee, Germany; Catalog Number: ZB-MDA-96A) according to the manufacturer's protocol (the detection limit: 0.1 μM and the detection range: 0.78–50 μM).

SOD activity was measured by a commercially available kit (ZellBio, Germany; Catalog Number: ZB-SOD-96A), according to the manufacturer's protocol (the detection limit: 1 U/ml and the detection range: 5–100 U/ml).

GPx activity was evaluated by a commercially available kit (ZellBio, Germany; Catalog Number: ZB-GPX-96A), according to the manufacturer's protocol (the detection limit: 5 U/ml and the detection range: 20–500 U/ml).

The light absorption was read by the ELISA Microplate Reader (Rayto, Shenzhen, China). All samples were analyzed according to the manufacture's protocol in duplicate, and the results were presented as a percentage of the naïve group.

2.5 | Neuropathological assessment

For the neuropathological studies, the samples fixed in formalin were dehydrated with a sequence of ethanol solutions and then were embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E) for light microscopic examinations (OLYMPUS Microscope, Tokyo, Japan). Six slides per animal and six fields per slide were assessed for neuropathological alternations such as apoptosis and inflammation [28]. Different regions of the brain including the cortex, white matter and hippocampus were evaluated in a blind manner by an expert pathologist. Moreover, the brain sections were stained with Masson's trichrome method for analyzing brain fibrosis and detecting the collagen fibers (blue staining).

2.6 | Statistical analysis

Statistical analysis was done by GraphPad Prism software version 6.01 for Windows (San Diego, California, USA). Data were presented as mean ± SEM. For parametric variables, the differences between the groups were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. For nonparametric variables, the groups' differences were analyzed with Kruskal–Wallis followed by the Dunn post hoc test. A two-way repeated-measures ANOVA (RMA) was used for comparison of body weight changes among experimental groups. Differences were considered statistically significant when $P < 0.05$.

3 | RESULTS

3.1 | Anxiety-like behavior and locomotion in the EPM

The development of anxiety-like behavior was evaluated in D-gal-treated mice by the EPM test. The results of the EPM test revealed that administration of D-gal decreased %OAT ($P < 0.001$) and %OAE ($P < 0.001$) in comparison with the naïve group (Figure 1a,b). GFZ slightly (10%) increased the % OAE in adult animals albeit did not reached statistical significance. However, when coadministered with D-gal, GFZ prevented the decrease of %OAT and %OAE ($P < 0.001$) in a significant manner ($P < 0.001$, in comparison with the D-gal group). The locomotor activity did not differ significantly between the experimental groups (Figure 1c).

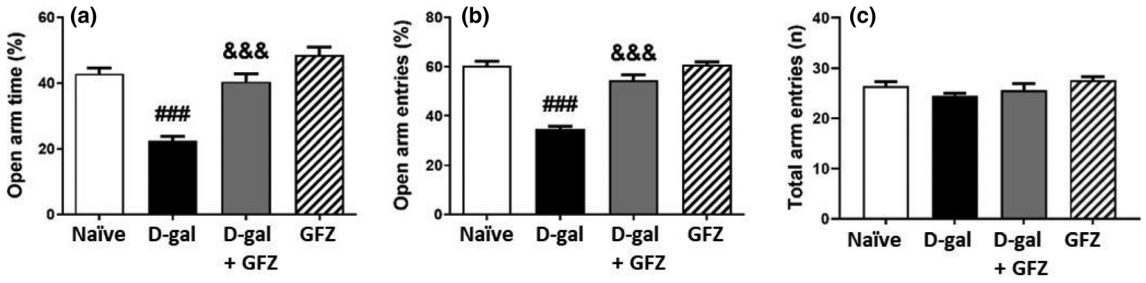


FIGURE 1 The effect of GFZ on anxiety-like behaviors in adult mice or D-gal-induced aging. The values are expressed as mean ± SEM ($n = 7$ in each group). ###$P < 0.001$ compared to the normal group. &&& $P < 0.001$ compared to the D-gal group

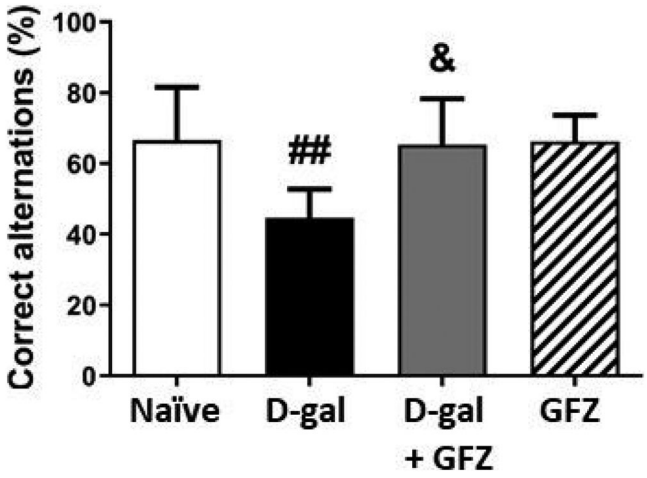


FIGURE 2 The effect of GFZ on memory performance in adult mice and D-gal-induced aging. The values are expressed as mean ± SEM ($n = 7$ in each group). ##$P < 0.01$ compared to the normal group; & $P < 0.05$ compared to the D-gal group

3.2 | Working memory in the Y-maze

The percentage of correct alternations in the animals with D-gal-induced aging was found significantly reduced compared to normal animals ($P < 0.01$) (Figure 2). While GFZ alone did not affect the Y-maze index in naïve animals, its coadministration with D-gal increased their percentage of correct alternations ($P < 0.05$, as compared with the D-gal group).

3.3 | Biochemical analysis of the MDA levels and GPx and SOD activities

The level of MDA in animals with D-gal induced aging was significantly increased in comparison with naïve animals ($P < 0.001$) (Figure 3A). GFZ alone did not significantly affect the level of MDA in adult animals but when coadministered with D-gal, it prevented the increase of the MDA levels ($P < 0.001$, as compared with the D-gal group).

The activity of GPx and SOD was significantly decreased in the D-gal aged animals in comparison with the naïve group (all $P < 0.001$) (Figure 3b,c). Administration of GFZ to adults did not modify the activity of GPx and SOD shown in naïve animals (all $P > 0.05$) but significantly prevented the reduction when coadministered with D-gal (all $P < 0.001$, as compared with the D-gal group).

3.4 | Histopathological alterations

In the naïve group, the histopathological morphologies of the white matter, cortex, and hippocampus were normal, and the neurons had clear nucleoli and cytoplasm (Figure 4a–c). In the D-gal group, the extent of injuries such as inflammation and necrosis were revealed in the white matter, cortex, and hippocampus (Figure 4d–f). Moreover, coadministration of GFZ with D-gal mitigated these histopathological lesions in the white matter, cortex, and hippocampus (Figure 4g–h). GFZ alone did not induce pathological lesions in naïve animals (not shown).

Moreover, the Masson's trichrome staining results showed no evidence of fibrosis in brain sections of different experimental groups (Figure 5a–c). GFZ alone did not induce fibrotic lesions in naïve animals (not shown).

4 | DISCUSSION

The current study showed that the administration of D-gal (150 mg/kg, i.p.) caused severe aging-related manifestations such as increased anxiety-like behavior and impairment in working memory in the adult experimental animals. Moreover, D-gal increased the MDA levels in the brain and decreased the GPx and SOD antioxidant enzymes activities. Neuropathological studies confirmed all these behavioral and biochemical results. However, the main finding reported is that the coadministration of an oral dose of GFZ 100 mg/kg prevented the deteriorating effects of D-gal to normal levels.

Memory impairment is one of the most important clinical manifestations of aging and age-related

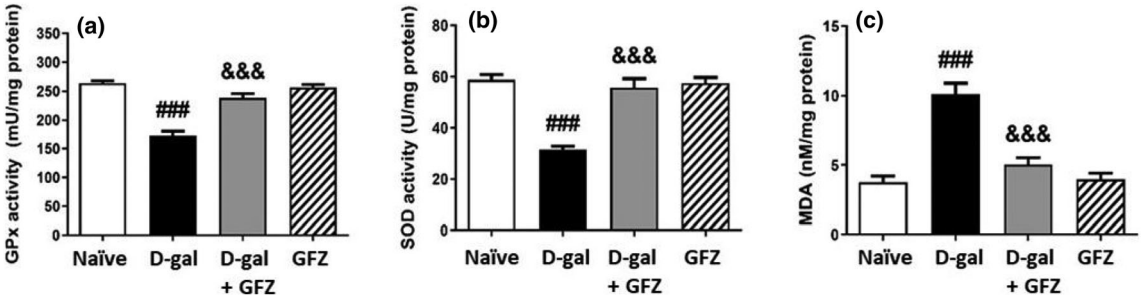


FIGURE 3 The effect of GFZ on GPx and SOD activities as well as MDA level in adult mice and D-gal induced-aging. The values are expressed as mean ± SEM ($n = 7$ in each group). ###$P < 0.001$ compared to the normal group. &&& $P < 0.001$ compared to the DGL group

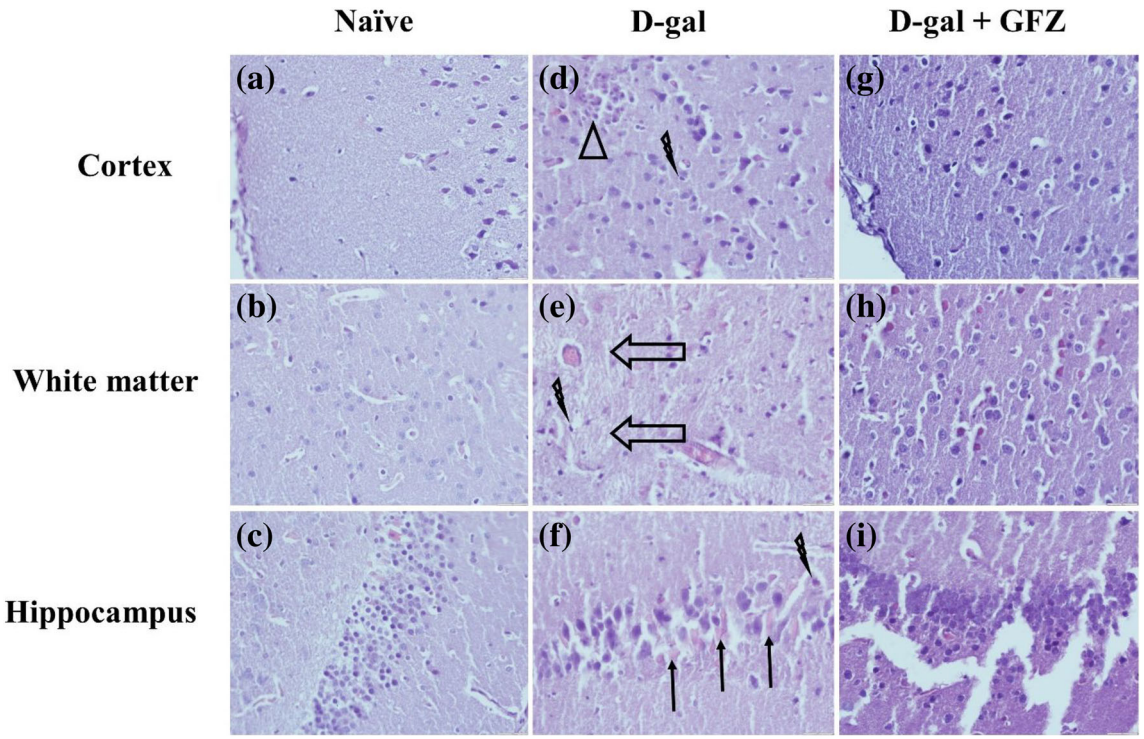
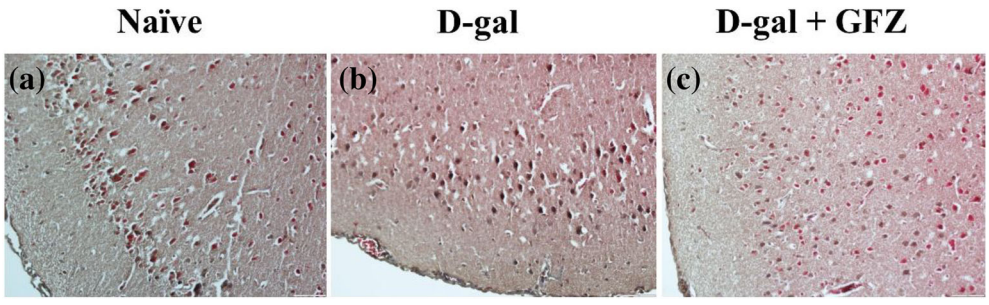


FIGURE 4 The effect of GFZ on the pathological lesions of D-gal induced-aging revealed by H&E and staining (×400). (a–c) Cortex, white matter, and hippocampus of the naïve group; (d–f) cortex, white matter, and hippocampus of the D-gal group; (g–i) cortex, white matter, and hippocampus of the D-gal + GFZ. Arrow head: inflammation; line arrow: apoptosis; arrow: degeneration; lighting bolt: pyknotic nuclei

FIGURE 5 The effect of GFZ on brain fibrosis in D-gal induced-aging revealed by Masson's trichrome staining (×200). (a) Cortex and white matter of the naïve group; (b) cortex and white matter of the D-gal group; (c) cortex and white matter of the D-gal + GFZ



neurological disorders [29]. Previous experimental reports have revealed that memory impairment is observed in both natural and D-gal-induced aging animals [28, 30]. It is well established that the overproduction of ROS in aged humans and other animals is mainly responsible for memory performance decline [31]. Excessive ROS could lead to the activation of iNOS and astroglia in the brain. In agreement,

the present work showed that the induction of aging with D-gal worsened working memory performances compared to naïve adult animals. More importantly, we found that GFZ was able to prevent this cognitive impairment since when coadministered with D-gal, the performance of aged animals in the Y-maze test was normal. Previous studies have shown the beneficial effects of GFZ on cognitive functions in Alzheimer's disease [19, 32]. Those studies demonstrated that the effects of GFZ on learning and memory performance were due to the neuroprotective activity of GFZ via reducing the iNOS levels in the brain and decreasing the activation of astroglia.

Anxiety is a prevalent problem in the late stages of life and may negatively affect the quality of life in older adults [33]. In agreement with previous reports, we also demonstrated that D-gal administration leads to anxiety-like behaviors, which are likely to elevate oxidative stress in the brain [23, 34]. In the present study, anxiety-like behaviors were found increased in the D-gal group in comparison with naïve animals. On the other hand, GFZ decreased these behaviors in the D-gal-treated animals. It is well established that GFZ has anxiolytic effects. GFZ shows protective effects against acute restraint stress in rats via neuroprotective, anti-inflammatory, and antioxidative activities [35]. Another study showed that GFZ reduced anxiety-like behaviors in mice with Alzheimer's disease via activating PPAR-α [19]. The study results demonstrated that GFZ reduces the beta-amyloid-stimulated expression of TNF-α and interleukin 6, which consequently enhances beta-amyloid clearance via autophagy through the participation of microglia and astrocytes. Accordingly, it seems that GFZ could decrease anxiety-like behaviors in aging mice via antioxidative effects and activating PPAR-α.

The beneficial effects of GFZ on potentiating the antioxidant defense system and reducing antioxidative parameters have been shown previously in other physiological systems. GFZ increased the activities of SOD and GPx and reduced MDA levels in renal and cardiac tissues of rats treated with doxorubicin [36, 37]. Moreover, it has been demonstrated that pretreatment with GFZ reduces the hippocampal level of MDA as well as the activity of SOD in rats with acute restraint stress [38]. Regarding the aging process, it is well established that the activities of antioxidant enzymes, such as SOD and GPx, decrease with the increase of age [39]. Meanwhile, the chain reaction of lipid peroxidation is accelerated during senile periods [35]. Therefore, the present study showed that the administration of D-gal mimicked the brain aging effects, as shown by the decreased antioxidant enzyme activity, namely SOD and GPx activities, as well as increased the MDA level in the brain tissues of D-gal-treated animals. Interestingly, for the first time, our work indicated that the coadministration of GFZ could reverse these changes in the brain of D-gal-treated mice. Therefore, GFZ should be further investigated for its potential effect on reducing neurological manifestations of aging, such as anxiety and memory impairments and underlying oxidative stress-associated processes.

In addition, the neuropathological observations were in parallel with the behavioral and biochemical results. The histopathological results showed that aging could induce pathological lesions such as apoptosis of neurons and inflammation in different parts of the brain (cortex, white matter, and hippocampus). Moreover, and despite quantification of neuronal loss was not done, gross observation indicated that GFZ administration had protective effects on the brain neurons in animals with D-gal-induced aging and that the severity of the pathological lesions was remarkably reduced. That would be in agreement with reports on GFZ exerting protective effects on hippocampal neurons in an animal model of acute restraint stress via reducing apoptosis and cellular death [38]. For the first time, we analyzed the brain fibrosis by Masson's trichrome staining in aging mice and we found that administration of D-gal (150 mg/kg; i.p.) for 6 weeks did not induce any fibrotic lesions in the brain.

5 | CONCLUSION

In conclusion, the current study revealed that administration of GFZ was effective in preventing hallmark behavioral manifestations of aging such as anxiety and impairment of working memory induced by D-gal. In addition, GFZ reduced the brain oxidative stress via increasing the activities of SOD and GPx as well as decreasing the levels of MDA to protect against D-gal-induced aging. Moreover, GFZ restored the survival of neurons in different parts of the brain such as the cortex/hippocampus and white matter in the accelerated aging model. Although further studies are needed to determine the exact underlying mechanisms of the anti-aging effects of GFZ, our results suggest that the administration of GFZ could be a promising approach to protect against age-related conditions. These data could be used to conduct a retrospective study on patients regularly prescribed with GFZ for the incidence of behavioral complications of aging with age-matched controls who did not consume this drug during the aging period.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Rafsanjan University of Medical Sciences (IR.RUMS.REC.1399.030) and Kerman University of Medical Sciences (IR.KMU.REC.1399.233).

AUTHOR CONTRIBUTIONS

E. H., S. M., and V. E. performed the experiments and collected the data; A. K. and J. H. performed the graphical and statistical analyses and analyzed the data; M. Y. Z., F. K. wrote and drafted the article and revised it critically for important intellectual content; L. G. L. and V. V. B. edited and revised the article critically for important intellectual content. I. F. was responsible for the conception, design of research and supervised the experiment execution. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1. Shin KR, Kim MY, Kim YH. Study on the lived experience of aging. *Nurs Health Sci*. 2003;5(4):245-252.
2. Kennedy BK, Pennypacker JK. Drugs that modulate aging: the promising yet difficult path ahead. *Transl Res*. 2014;163(5):456-465.
3. Pak JW, Herbst A, Bua E, Gokey N, McKenzie D, Aiken JM. Mitochondrial DNA mutations as a fundamental mechanism in physiological declines associated with aging. *Aging Cell*. 2003;2(1):1-7.
4. van Velzen LS, Wijdeveld M, Black CN, et al. Oxidative stress and brain morphology in individuals with depression, anxiety and healthy controls. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2017;76:140-144.
5. Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc Natl Acad Sci U S A*. 1996;93(10):4765-4769.
6. Fatemi I, Heydari S, Kaeidi A, et al. Metformin ameliorates the age-related changes of d-galactose administration in ovariectomized mice. *Fundam Clin Pharmacol*. 2018;32(4):392-399.
7. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. *Nature*. 2019;571(7764):183-192.
8. Kennedy BK, Berger SL, Brunet A, et al. Geroscience: linking aging to chronic disease. *Cell*. 2014;159(4):709-713.
9. Li W, Wei F, Fan M, et al. Mimetic brain aging effect induced by D-galactose in mice. *Chinese J Pharmacol and Toxicol*. 1994;9(2):93-95.
10. Ho SC, Liu JH, Wu RY. Establishment of the mimetic aging effect in mice caused by D-galactose. *Biogerontology*. 2003;4(1):15-18.
11. Hakimizadeh E, Zamanian M, Giménez-Llort L, et al. Calcium dobesilate reverses cognitive deficits and anxiety-like behaviors in the D-galactose-induced aging mouse model through modulation of oxidative stress. *Antioxidants*. 2021;10(5). doi: 10.3390/antiox10050649
12. Fatemi I, Khaluoi A, Kaeidi A, Shamsizadeh A, Heydari S, Allahtavakoli MA. Protective effect of metformin on D-galactose-induced aging model in mice. *Iran J Basic Med Sci*. 2018;21(1):19-25.
13. Baeta-Corral R, Castro-Fuentes R, Giménez-Llort L. Sexual dimorphism in the behavioral responses and the immunoendocrine status in D-galactose-induced aging. *J Gerontol A Biol Sci Med Sci*. 2018;73(9):1147-1157.
14. Hammond DA, Finlay L. Treatment of hypertriglyceridemia-induced acute pancreatitis with insulin, heparin, and gemfibrozil: a case series. *Hosp Pharm*. 2017;52(10):675-678.
15. Pahan K, Jana M, Liu X, Taylor BS, Wood C, Fischer SM. Gemfibrozil, a lipid-lowering drug, inhibits the induction of nitric-oxide synthase in human astrocytes. *J Biol Chem*. 2002;277(48):45984-45991.
16. Camara-Lemarroy CR, Guzman DELAGFJ, Cordero-Perez P, Ibarra-Hernandez JM, Munoz-Espinosa LE, Fernandez-Garza NE. Gemfibrozil attenuates the inflammatory response and protects rats from abdominal sepsis. *Exp Ther Med*. 2015;9(3):1018-1022.
17. Calkin AC, Cooper ME, Jandeleit-Dahm KA, Allen TJ. Gemfibrozil decreases atherosclerosis in experimental diabetes in association with a reduction in oxidative stress and inflammation. *Diabetologia*. 2006;49(4):766-774.
18. Behiry S, Rabie A, Kora M, Ismail W, Sabry D, Zahran A. Effect of combination sildenafil and gemfibrozil on cisplatin-induced nephrotoxicity; role of heme oxygenase-1. *Ren Fail*. 2018;40(1):371-378.
19. Luo R, Su LY, Li G, et al. Activation of PPARA-mediated autophagy reduces Alzheimer disease-like pathology and cognitive decline in a murine model. *Autophagy*. 2020;16(1):52-69.
20. Guo Q, Wang G, Liu X, Namura S. Effects of gemfibrozil on outcome after permanent middle cerebral artery occlusion in mice. *Brain Res*. 2009;1279:121-130.
21. Cheng SM, Ho YJ, Yu SH, et al. Anti-apoptotic effects of diosgenin in D-galactose-induced aging brain. *Am J Chin Med*. 2020;48(2):391-406.
22. Chen HI, Ou HC, Chen CY, et al. Neuroprotective effect of rhodiola crenulata in D-galactose-induced aging model. *Am J Chin Med*. 2020;48(2):373-390.
23. Kaviani E, Rahmani M, Kaeidi A, et al. Protective effect of atorvastatin on D-galactose-induced aging model in mice. *Behav Brain Res*. 2017;334:55-60.
24. Ozansoy G, Akin B, Aktan F, Karasu C. Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. *Mol Cell Biochem*. 2001;216(1–2):59-63.
25. Zakeri M, Fatemi I, Kaeidi A, et al. Pro-neurocognitive and anti-sarcopenic benefits of one-year metformin therapy in ovariectomized aged mice. *Clin Exp Pharmacol Physiol*. 2019;46(12):1133-1140.
26. du Jardin KG, Jensen JB, Sanchez C, Pehrson AL. Vortioxetine dose-dependently reverses 5-HT depletion-induced deficits in spatial working and object recognition memory: a potential role for 5-HT1A receptor agonism and 5-HT3 receptor antagonism. *Eur Neuropsychopharmacol*. 2014;24(1):160-171.
27. Fatemi I, Delrobaee F, Bahmani M, Shamsizadeh A, Allahtavakoli M. The effect of the anti-diabetic drug metformin on behavioral manifestations associated with ovariectomy in mice. *Neurosci Lett*. 2019;690:95-98.
28. Hakimizadeh E, Kaeidi A, Taghipour Z, et al. Ceftriaxone improves senile neurocognition damages induced by D-galactose in mice. *Iran J Basic Med Sci*. 2020;23(3):368-375.

29. Barnes CA, Nadel L, Honig WK. Spatial memory deficit in senescent rats. *Can J Psychol*. 1980;34(1):29-39.
30. Fatemi I, Saeed-Askari P, Hakimizadeh E, et al. Long-term metformin therapy improves neurobehavioral functions and antioxidative activity after cerebral ischemia/reperfusion injury in rats. *Brain Res Bull*. 2020;163:65-71.
31. Morgan JA, Singhal G, Corrigan F, Jaehne EJ, Jawahar MC, Baune BT. The effects of aerobic exercise on depression-like, anxiety-like, and cognition-like behaviours over the healthy adult lifespan of C57BL/6 mice. *Behav Brain Res*. 2018;337:193-203.
32. Chandra S, Pahan K. Gemfibrozil, a lipid-lowering drug, lowers amyloid plaque pathology and enhances memory in a mouse model of Alzheimer's disease via peroxisome proliferator-activated receptor α. *J Alzheimers Dis Rep*. 2019;3(1):149-168.
33. Wolitzky-Taylor KB, Castriotta N, Lenze EJ, Stanley MA, Craske MG. Anxiety disorders in older adults: a comprehensive review. *Depress Anxiety*. 2010;27(2):190-211.
34. Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety: relationship and cellular pathways. *Oxidative Med Cell Longev*. 2009;2(2):63-67.
35. Cui X, Zuo P, Zhang Q, et al. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: protective effects of R-alpha-lipoic acid. *J Neurosci Res*. 2006;84(3):647-654.
36. Haybar H, Goudarzi M, Mehrzadi S, et al. Effect of gemfibrozil on cardiotoxicity induced by doxorubicin in male experimental rats. *Biomed Pharmacother*. 2019;109:530-535.
37. Hosseinzadeh A, Goudarzi M, Fatemi I, et al. Gemfibrozil attenuates doxorubicin induced toxicity in renal tissues of male rats by reducing the oxidative insult and inflammation. *Biotech Histochem*. 2020;95(7):532-539.
38. Khalaj L, Nejad SC, Mohammadi M, et al. Gemfibrozil pretreatment proved protection against acute restraint stress-induced changes in the male rats' hippocampus. *Brain Res*. 2013;1527:117-130.
39. Rybka J, Kupczyk D, Kedziora-Kornatowska K, et al. Age-related changes in an antioxidant defense system in elderly patients with essential hypertension compared with healthy controls. *Redox Rep*. 2011;16(2):71-77.

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