ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

6-Aminoflavone Activates Nrf2 to Inhibit the Phospho-JNK/TNF-α Signaling Pathway to Reduce Amyloid Burden in an Aging Mouse Model

Published as part of the ACS Omega virtual special issue "Phytochemistry".

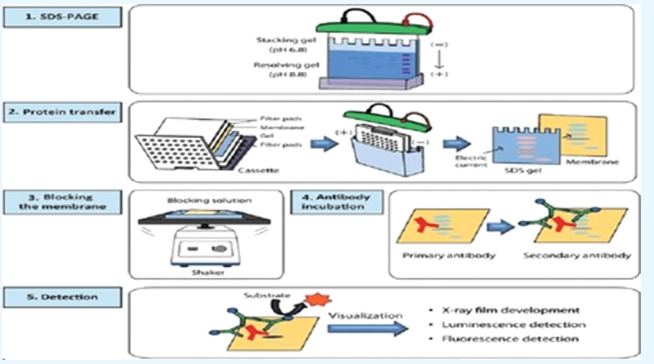
Shakeel Ahmad, Shahid Ali Shah, Umar Nishan, Naeem Khan,* Mikhlid H. Almutairi, Fozia Fozia,* Nargis Jamila, Bader O. Almutairi, and Zia Ullah

Cite This: *ACS Omega* 2023, 8, 26955−26964

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: In the current study, we examined the antioxidant activity and anti-amyloidogenic potential of 6-aminoflavone in an adult mice model of D-galactose-induced aging. Male albino eight-week-old mice were assigned into four groups: 1. the control group (saline-treated), 2. D-galactose-treated mice (100 mg/kg/day, intravenously) for eight weeks, 3. D-galactose-treated mice (100 mg/kg/day, intravenously for eight weeks) and 6-AF-treated mice (30 mg/kg/day, intravenously for the final four weeks), and 4. 6-AF-treated mice (30 mg/kg/day i.p. for four weeks). We conducted many assays for antioxidant enzymes, including lipid peroxidation, catalase, glutathione (GSH), peroxidase (POD), and sulfoxide dismutase (SOD) (LPO). Western blotting was used to assess protein expression while the Morris water maze (MWM) and Y-maze (YM) were used to study behavior. The findings show that 6-AF greatly improved neuronal synapse and memory impairment brought on by D-galactose and it significantly inhibited BACE1 to reduce the amyloidogenic pathway of A (both amyloid β production and aggregation) by upregulating Nrf2 proteins (validated through molecular docking studies) and suppressing phosphorylated JNK and TNF-α proteins in adult albino mice's brain homogenates. These findings suggest that 6-AF, through the Nrf2/p-JNK/TNF-α signaling pathway, can diminish the oxidative stress caused by D-galactose, as well as the amyloidogenic route of A formation and memory impairment.



1. INTRODUCTION

In addition to cell death, β amyloid clumps, and hyperphosphorylated tau protein tangles, the common aging illness known as Alzheimer's disease (AD) differs from other disorders in a number of ways.[1] According to some researchers, one of the biggest dangers to mitochondrial dysfunction, which contributes to the onset of Alzheimer's disease, is oxidative stress or the production of reactive oxygen species (ROS).[2] ROS may possibly be a factor in brain inflammation. Damage to proteins, DNA, and lipids occurs as a result of an excess of reactive oxygen species (ROS) inhibiting antioxidant defense mechanisms.[3] Neuroinflammation can also result in synaptotoxicity and memory impairment.[4] D-Galactose, a component of Gram-negative bacteria's polysaccharide, attaches to microglia's Toll-like receptor 4 (TLR4), activating an innate immune response.[5] TLR4 and D-galactose interact to activate and increase nuclear factor kappa-B (NF-B) and other kinases, which results in the creation of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF).[6] D-Galactose has thus been used for many years as an animal model to research the molecular causes of memory impairment.[7] D-Galactose-induced AD neuropathology is the most useful model for assessing the therapeutic efficacy of new and experimental drugs for the cure of Alzheimer's disease in animals. Polyphenols and flavonoids, which are the most important polyphenols and essential components of the human diet, are found in plants and are excellent sources of these compounds.[8−10] When used both in vitro and in vivo, 6-aminoflavone exhibits strong antiproliferative action against a number of human tumor cell lines.[11] The chosen molecule, 6-aminoflavone, may be used as a possible treatment against

Received: March 16, 2023
Accepted: July 3, 2023
Published: July 19, 2023

neurodegenerative illnesses because of its reported antiproliferative action.

The current work examined the protective effects of 6-AF against D-galactose-induced oxidative stress, neural synapse dysfunction, and memory impairment in adult male albino mice. Our most recent studies demonstrate that 6-AF can greatly reduce neuroinflammation, synaptic dysfunction, oxidative stress, and memory impairment brought on by D-galactose in AD animal models via boosting the phospho-Akt signaling pathway.

2. MATERIALS AND METHODS

2.1. Chemicals. PBS (phosphate buffer saline) tablets, acrylamide, Trizma base, D-galactose, ammonium per sulfate (APS), sodium dodecyl sulfate (SDS), bis-acrylamide, potassium chloride (KCl), and sodium chloride (NaCl) were purchased from Sigma-Aldrich Chemical Co. and Daejung Chemicals & Metals Co., Ltd.

2.2. Mice and Their Grouping. The National Institutes of Health (NIH) veterinary subdivision provided mature male Swiss albino mice, which were then transported to the NMMRC, Peshawar, to be used in these experiments. The mice were kept in cages that were clearly identified and had unrestricted entrance to food and water at Biobase in China. They were also given some time to become adjusted to their new environment. Later, the mice were separated into four groups (n = 10) at random, as shown below.

1. Control mice (0.9% saline-treated).
2. D-Galactose-administered mice (100 mg/kg).
3. D-Galactose-administered mice (100 mg/kg) + 6-AF-administered mice (30 mg/kg).
4. 6-AF-administered mice (30 mg/kg).

Then, the male mice, who were on average 30−32 g in weight, were put in appropriate cages in an animal home with a controlled environment that had a 12 h light and dark cycle, a temperature of 25 °C, and an endless supply of water and standardized food. The NMMRC's animal ethics committee recommended that Peshawar and all other study animals be handled with the utmost care and consideration.

2.3. Behavioral Tests. To illustrate the therapeutic benefits of 6-AF on memory impairment brought on by D-Galactose, two well-known behavioral tests were employed. For 8 weeks, D-galactose was administered intraperitoneally (i.p.) to the mice in groups 2 and 3. In a similar manner, groups 3 and 4 each received 4 weeks of intravenous (6-AF) treatment. The behavioral tests were conducted as single-blind trials, while all of the research animals were thereafter tagged. The mice were randomly divided into four groups. The mice treatment groups and tags were kept a secret from the researcher conducting the behavioral assessments.

2.4. Morris Water Maze Test. The Morris water maze (MWM) test was used to examine mice's hippocampal-based long-term spatial learning ability. The design and specifications of the MWM test apparatus are specifically described in a recent study.[12] The mice underwent training to swim twice daily prior to the start of the actual testing in order to acclimatize to the water tank and platform for 3 days. The mean escape delay was later determined for each mouse over a duration of 60 s in order to locate the hidden platform. This procedure was carried out for a total of 05 days. If the mice did not discover the platform within the allotted time, they were physically guided and left on the platform for 10 s. The escape delay time in seconds for each day was recorded. The mice were given a two-day rest period before the final probe testing. This involved hiding the platform and counting how long each mouse spent in the target quadrant.

2.5. Y-Maze Test. As previously stated, the behavioral Y-maze test was conducted.[13] Three arms with the combined measurements of 50 × 10 × 20 cm^3 (L × W × H) make up the Y-maze device, which are joined at an angle of 120° degrees. Mice were given a total of 10 min per time to get used to this new habitat. After that, the mice were placed in the middle of the maze for 8 min to roam freely across its three arms. Software was used to calculate the total number of arm entries of each mouse and each subsequent triplet. The percentage of alternations was then calculated using the algorithm shown below

$$\text{Percentage of alternations} = \frac{\text{Total number of Successive triplets sets}}{\text{Total number of arm entries} - 2} \times 100$$

The degree of alternations was closely interrelated with the mice's capacity to retain spatial information.

2.6. Western Blotting Analysis. After receiving the previously described therapy, all of the animals were killed.[12] The hippocampus region of the mice's brain was carefully removed after they had been beheaded and was then promptly transferred to RNA later solution and PBS (1:1) on ice. After homogenizing the hippocampus brain in a solution of total protein extraction reagent, the tissue supernatants were unruffled and kept at −20 °C for further investigations. The protein concentration was determined using the Bio-Rad protein estimation assay, and the absorbance at 595 nm was measured. Gel electrophoresis employing SDS-PAGE 12−15% was carried out after normalizing all sample proteins to 30 g/group. The run's operation conditions were kept at 50 mA for the first 20−30 min. For the following 1.5−2 h, until the run was finished, they were switched to 120 V. Proteins from the gel were then transferred to a poly(vinylidene fluoride) (PVDF) membrane using semi-dry transblotting, according to Santa Cruz Biotechnology in the United States (Bio-Rad). Among the mouse-derived primary antibodies used were those against SYP, PSD-95, p-Akt, NF-kB, β-actin, IL-1, and TNF-α from Santa Cruz, California, in the United States. Anti-mouse secondary antibodies from Santa Cruz, California, in the USA, were used in conjunction with these primary antibodies. The results were used to produce X-ray films.[12]

2.7. Evaluation of Brain Homogenates for Antioxidants. *2.7.1. Catalase Assay (CAT).* Catalase activity (CAT) was determined using an older approach with little changes.[14] 400 mL of H_2O_2 (5.9 mM), 2500 mL of phosphate buffer (50 mM) at pH 5.0, and 100 mL of brain supernatant were all included in 3 mL of the reaction mixture. At 1 min intervals, the reaction mixture's change in absorbance was measured at 240 nm. A 0.01 unit/minute change in absorbance was regarded as one unit of activity.

2.7.2. Peroxidase Assay (POD). The peroxidase assay was determined using a previous method with minute changes in order to quantify the peroxidase activity.[14] The peroxidase test's reaction mixture is composed of 300 mL of H_2O_2 (40 mM), 100 mL of guaiacol (20 mM), 2500 mL of phosphate buffer (50 mM, pH 5.0), and 1000 mL of brain homogenate supernatant. Measurements of the reaction mixture's absorbance at 470 nm were taken for each minute. The alteration in

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

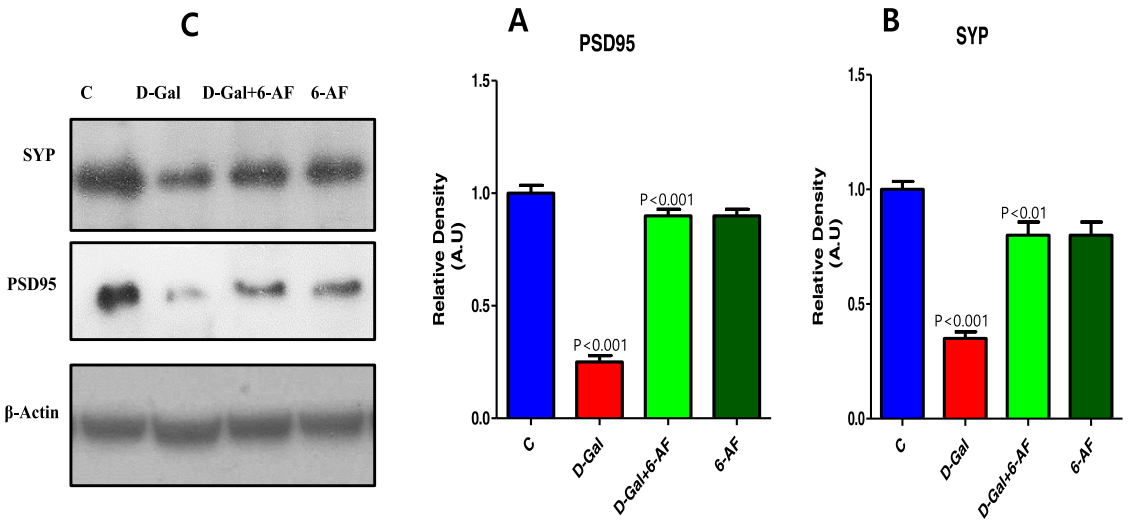


Figure 1. Both pre-and post-synaptic protein expression enhanced by 6-AF against D-galactose in mice. (A, B). The findings of the western blotting method of pre- and post-synapse protein expression of synaptophysin and PSD-95 and SYP in the brain supernatant of homogenates treated with either D-galactose alone or in combination with 6-AF are shown. (C) The histograms of respective relative densities of both SYP and PSD-95. ImageJ software was used to know the densities and to make graphs. The findings were calculated using an arbitrary unit (A.U.) and a histogram that shows the mean in A.U. SEM. $p \leq 0.001$ and $p \leq 0.01$ are significant.

absorbance of less than 0.01/min was measured to be a single unit of peroxidase activity (POD).

2.7.3. Superoxide Dismutase Assay (SOD). As described, a slight adjustment to this test was made.[15] The reaction mixture for determining the superoxide dismutase (SOD) activity included 300 mL of brain homogenate supernatant, 100 mL of sodium pyrophosphate buffer (0.052 mM), and 100 mL of phenazine methosulphate (186 M). The enzymatic process was started by adding 200 L of NADH (780 M) to the mixture. The reversing agent, 1000 L of glacial acetic acid, was introduced after 1 min. The yield of chromogen per mg of protein was determined by measuring the absorbance of the reaction at 560 nm.

2.7.4. Reduced Glutathione Assay (GSH). In a previous investigation, 1000 L of the brain homogenate's proteins were precipitated by the addition of an equal amount of a solution of 4% sulfosalicylic acid, to identify decreased glutathione levels.[16] The reaction mixture was first heated for an hour to 4 °C, followed by 20 min centrifugation at 1200*g*. 200 L of 100 mM DTNB and 2700 L of a phosphate buffer solution (0.1 M and pH 7.4) were combined to create the reaction mixture. Then, the absorbance at 412 nm of the reaction mixture was measured. Findings related to decreased glutathione were shown as M/g tissue.

2.7.5. Estimation of Lipid Peroxidation (TBARS). A little modification to a previously described technique was used to conduct the lipid peroxidation (TBARS) experiment.[17] For the experiment, 1000 L of a mixture for the reaction including 580 L of pH 7.4 0.1 M phosphate buffer, 200 L of 100 mM ascorbic acid, 200 L of brain homogenate supernatant, and 20 L of 100 mM ferric chloride was created. For an hour, the reaction mixture was incubated in a stirred water bath that was kept at 37 °C. With the addition of 1000 mL of 10% trichloroacetic acid solution, the process was stopped. Following the addition of 1000 L of 0.67% thiobarbituric acid, the tubes were immediately placed in a cold bath and centrifuged at 2500*g* for 10 min. Using a spectrophotometer to measure the supernatant's absorbance at 535 nm, the quantity of lipid peroxidation (TBARS) generated in each sample was calculated. Information was presented in terms of nM TBARS/min/mg of tissue at 37 °C. 1.561 105 $M^{-1}cm^{-1}$ is the molar extinction coefficient of TBARS.

2.7.6. Statistical Analysis. A specific piece of computer software was used to scan, gather, and statistically analyze all of the results' X-rays. They consisted of ImageJ, Adobe Photoshop, and Prism 5, among others. Mean scanning electron microscopy (SEM) represents the density of the proteins by using arbitrary units (A.U.s), and *p* 0.05, *p* 0.01, and *p* 0.001 were used to represent that the mice administered with D-galactose were significantly different from the animals treated with normal saline.

2.7.7. All-Atom Molecular Dynamics Simulation. This study looked at the therapeutic effectiveness of 6-AF against D-galactose-caused memory impairment and oxidative stress-mediated neuronal synapse in normal adult male albino mice. In an AD animal model, 6-AF can significantly lessen D-galactose-caused oxidative stress, synapse dysfunction, neuroinflammation, and memory impairment via activating the phospho-Akt (p-Akt) signaling pathway. To examine the activating mechanism of p-Akt by 6-AF, we used comprehensive molecular dynamics (MD) and molecular docking modeling with the molecular operating environment (MOE) and the AMBER software package.

2.7.8. Molecular Docking. To forecast potential molecular interactions between the investigated chemical and the target Nrf2 receptor protein, molecular docking was carried out. Initially, the two-dimensional (2D) structure of 6-aminoflavones was acquired from the PubChem database and the structure of Nrf2 was retrieved using the PDB database with ID 6 QMK.[18] Additionally, molecular docking calculations, energy minimization, and molecular visualization of docking data were carried out using the Molecular Operating Environment software package. Ten different positions were created for the six previously supported aminoflavones.[19] The docking was determined using the London dG score method. The London dG scoring function is used to calculate the binding free energy of a ligand at a specific location in a target

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

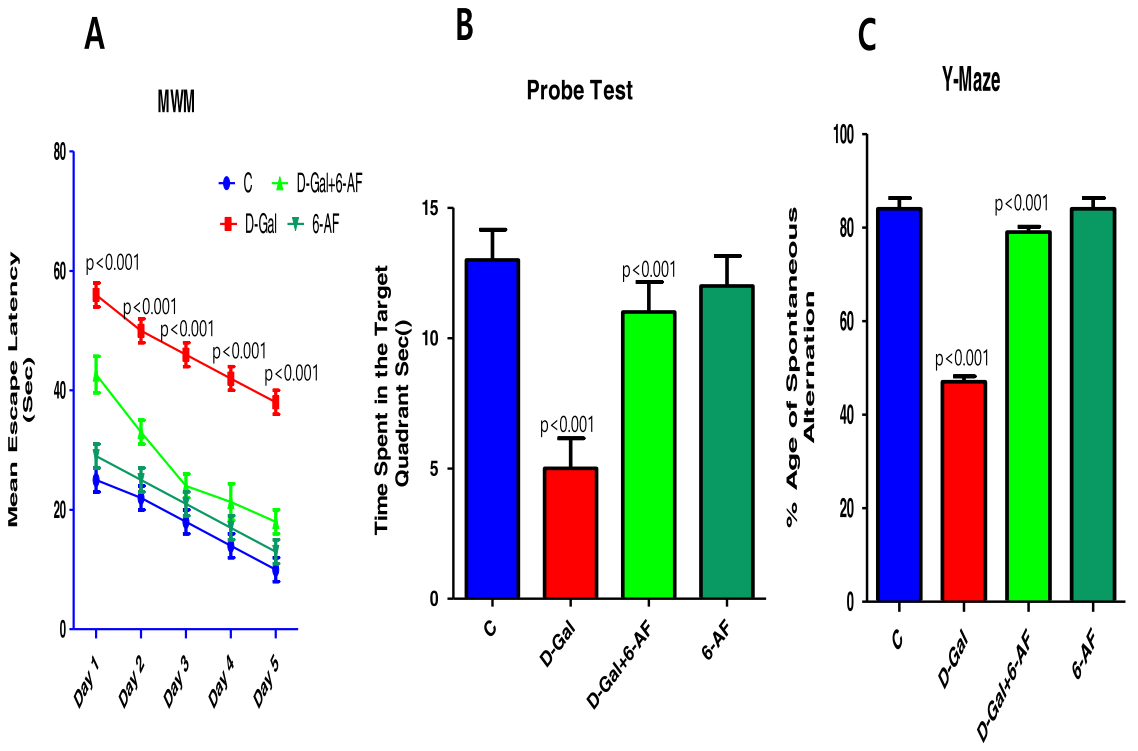


Figure 2. 6-AF boosted memory in adult mice's brains. The results of behavioral tests are presented as follows: (A) the Morris water maze test's average escape latency from day 1 to day 5, (B) the probe test, and (C) the percentage of spontaneous modification in the Y-maze test. All verified data are shown as a mean SEM. $p \leq 0.01$ and $p \leq 0.001$ are significant.

structure. The final docking scores were acquired using the GBVI/WSA G scoring algorithm.[20]

3. RESULTS AND DISCUSSION

3.1. 6-AF Therapies Increased the Expression of Pre- and Post-Synapse Proteins in D-Galactose-Administered Adult Mice. The genes and proteins of neuronal synapses are reportedly negatively impacted by D-galactose.[21] Thus, the western blotting technique was used to analyze pre- and post-synapse proteins in all experimental groups. According to results from a western blot investigation, D-galactose reduced the expression of proteins important for brain synapses. D-Galactose (120 mg/kg) was chronically administered to adult male albino mice for a period of eight weeks. In the homogenates of the mice's brains, SYP (synaptophysin), a pre-synapse protein, and PSD-95, a post-synapse protein, are both expressed. Figure 1A−C demonstrates that 6-AF significantly (p 0.01) improves the protein expressions of both SYP and PSD-95 proteins in the third group of rat brain homogenates, which was injected with 6-AF three times per week along with 120 mg/kg D-galactose for four week

3.2. 6-AF Restored Memory Deficits in D-Galactose-Treated Mice. In the aging model used by D-galactose, the effects of 6-AF on spatial learning and memory were examined using the Morris water maze and the Y-maze. These mice were taught and given a break, and then their data were gathered from the first to the fifth day. Our results show that over the training days in all experimental groups, the mean latency to detect the hidden platform steadily reduced. On the first day, the 6-AF-injected mice found the submerged platform quickly, but the D-galactose-injected mice took longer to find it and were able to get away. Despite having a significant amount of escape delay, the D-galactose-injected mice identified the hidden platform successfully on days 2 and 3 (seconds). It is interesting to note that mice treated with vitamin D showed extraordinary learning abilities and that from days 2 to 5, the escape latencies time were dramatically (p 0.01) reduced while trying to recognize the submerged platform, practically matching the performance of untreated mice. Figure 2A shows how this delay significantly reduced from day 1 to day 5 of the experiment in normal male albino mice, who were shown to have very short escape latency. The latencies to the submerged platform were simply greater in the D-galactose-treated animals than those in the untreated mice, as shown in Figure 2A. Figure 2A shows that the mice in the fourth group, which only get the 6-AF therapy, also show better behavior as the escape latency regularly decreases from day 1 to day 5. According to these findings, mice treated with D-galactose appear to have memory issues and poor spatial learning. On the other hand, mice treated with D-galactose and 6-AF injections have considerably (p 0.01) reduced elevated latencies to the platform (Figure 2A).

We recorded how much time each of the three groups spent in the target quadrant on the 6th day, following the removal of the submerged platform. The adult mice were set free to find the concealed platform beneath the water. As a result, D-galactose-administered mice depleted less time (p 0.001) in the target quadrant than untreated mice, although control mice spent more time there. The mice who also got 6-AF to D-galactose depleted considerably (p 0.001) more time (compared to D-galactose-administered animals) in the target quadrant, but less time (compared to control mice) there (Figure 2B). The time that the 6-AF-treated mice spent at this place was almost equal to the time that the usual control animals spent there.

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

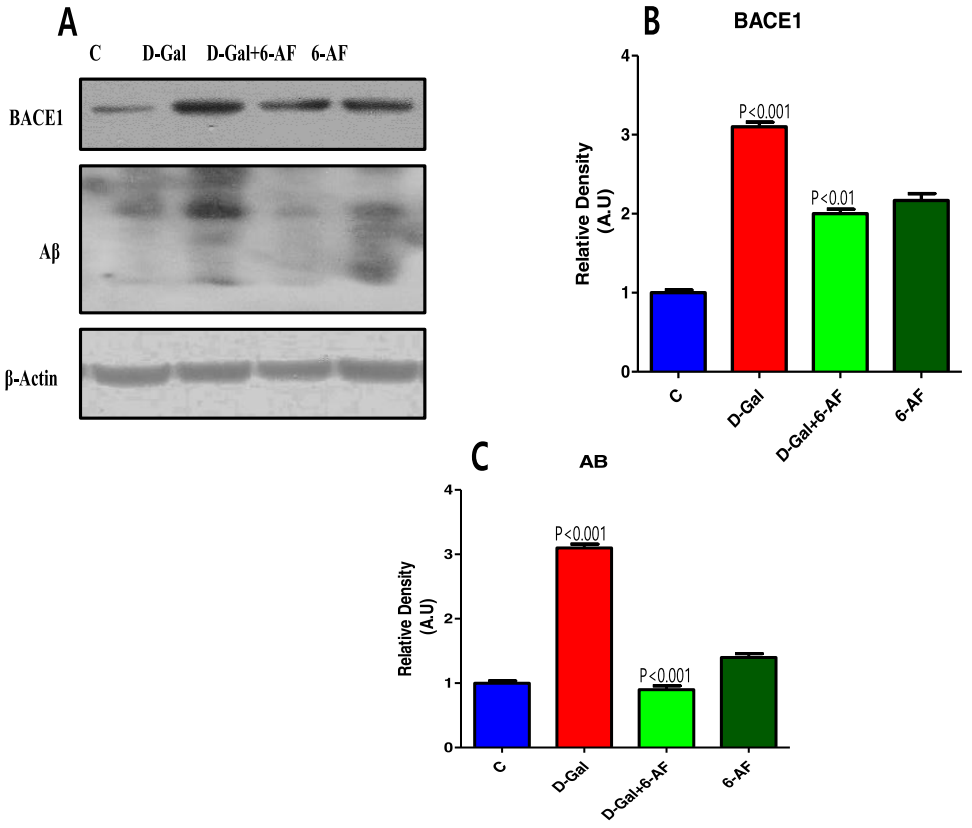


Figure 3. 6-AF reduced the concentration of BACE1 and Aβ proteins against D-galactose in the mice brain. The histograms of the respective relative densities from the western blot analysis of BACE1 and Aβ (A). The immunoblots of BACE1 and Aβ (B, C) with their respective histogram are given. β-Actin was employed as a standard (loading control). The findings were calculated with ImageJ software and a histogram exhibiting the mean in A.U. SEM and given in arbitrary units (A.U.s). $p \leq 0.01$ and $p \leq 0.001$ are the statistical significance levels.

The experimental mice were put through a Y-maze test to see how well they could recognize objects in their short-term memory. The correct methodology was employed in this experiment to determine the amount of spontaneous alternation present in the three mice groups. The Y-maze results revealed that the proportion of spontaneous alternation was exceedingly low in D-galactose mice and extraordinarily high in control animals (p 0.001). It was then followed by mice treated with 6-AF alone. The third group of mice who got 6-AF also showed a noticeably (p 0.001) higher percentage of spontaneous alternation than animals just getting D-galactose therapy, which is interesting (Figure 2C).

3.3. 6-AF Reduced the Amyloidogenic Pathway of Aβ-Synthesis. Oxidative stress load can increase the activity of the enzyme that degrades the β-site amyloid precursor protein (APP) in AD animal models and in human subjects exposed to D-galactose for an extended period of time.[22,23] For this reason, western blotting was employed to evaluate BACE1 and A protein expression. The results show that continuous treatment of D-galactose to the mice considerably (p 0.001) boosted BACE1 and Aβ activities in the brain. Figure 3A–C demonstrates that animals administered with D-galactose and 6-AF show dramatically decreased (p 0.001) BACE1 activity, hindering (p 0.001) the amyloidogenic pathway of A formation. Figure X depicts the nonconsiderable effect on BACE1 and Aβ proteins in the 6-AF alone-treated mice.

3.4. 6-AF Stimulated Nrf2 to Inhibit p-JNK and TNF-α against Oxidative Stress Caused by D-Galactose. It is already well established that persistent D-galactose injections into mice or rats result in oxidative damage.[21,24] As described in Figure 4A–E, we found that D-galactose dramatically increased LPO while reducing SOD, POD, CAT, and GSH in the current study. However, 6-AF greatly increased the antioxidant enzyme's activity and reduced oxidative stress.

The body's antioxidant defense molecules Nrf2 are repressed as a result of the burden of oxidative stress and are unable to operate normally.[12] Due to this, we used western blotting to assess the levels of Nrf2, p-JNK, and TNF-α expression. Nrf2, SIRT1, and HO-1 protein levels in the brain homogenates of the experimental animals were considerably (p 0.001) reduced as a result of prolonged D-galactose administration in mice, according to the western blot data. It is noteworthy to note that, as shown in Figure 5A–D, administering mice with vitamin D combined with D-galactose enhanced and activated antioxidant proteins such as SIRT1 (p 0.01), Nrf2 (p 0.01), and HO-1 (p 0.01) against D-galactose-induced oxidative stress.

3.5. Vitamin D Abrogated D-Galactose-Induced Neuroinflammation. A neuroinflammatory cytokine storm was triggered in the hippocampus by D-galactose, according to earlier findings, which also activated NF-kB.[25] The activation of NF-kB in mouse brain homogenates was likewise considerably (p 0.001) boosted by prolonged D-galactose administration of mice, according to our findings. This is followed by the considerably (p 0.001) increased expression levels of its downstream signaling molecules, including the TNF-α and IL-1 proteins. In the brain homogenates of the mice exposed to both D-galactose and vitamin D combination,

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

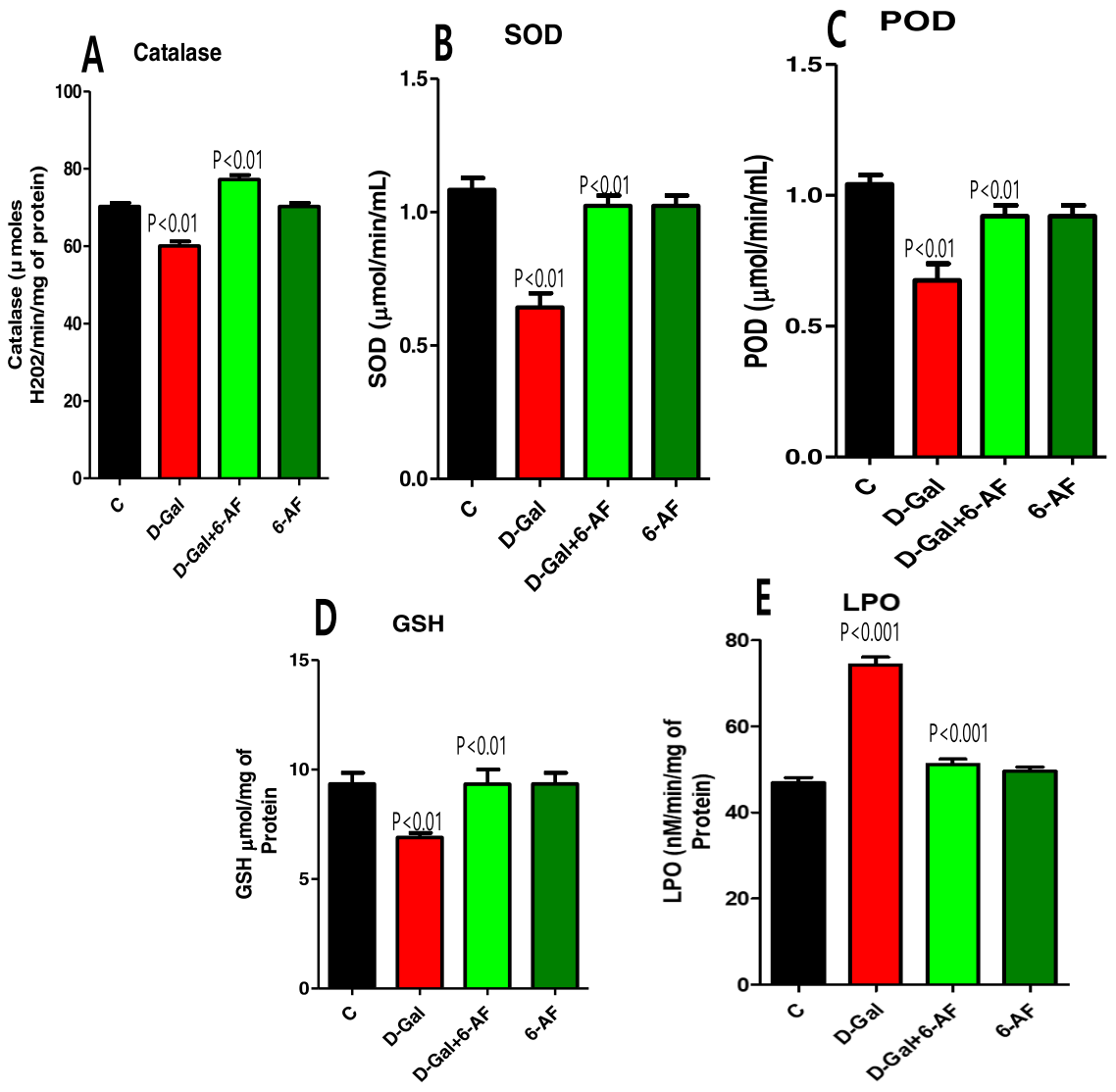


Figure 4. 6-AF restored the activities of D-Gal-suppressed antioxidant enzymes in the adult mice brain. The activities of antioxidant enzymes (A) catalase, (B) superoxide dismutase, (C) peroxidase, (D) glutathione (GSH), and (E) lipid peroxidase (TBARS) were measured in experimental mice brain supernatant homogenates treated with either d-galactose alone or in combination with 6-AF. The technique is detailed in the Materials and Methods section. Each group's findings are expressed as the mean SEM of (n = 5) mice. $p \leq 0.01$ and $p \leq 0.001$ are significant.

vitamin D dramatically (p 0.001) decreased the expression of NF-kB and signaling molecules, including TNF-α and IL-1 proteins. This demonstrates how vitamin D works to reduce inflammation.

3.6. Molecular Docking. 6-Aminoflavone had a docking score of −7.50. According to Figure 6, the substance demonstrates a strong binding interaction with the Nrf2 receptor. The substance 6-aminoflavone interacted with the receptor through two pi−pi interactions and one h-bond donor (Table 1).

Vitamins have been suggested as AD treatment substances. Among these, vitamins C, E, and D have generated a lot of attention. According to in vivo studies, vitamin C's powerful antioxidant action[26] reduced neuroinflammation and brain oxidative damage. Additionally, it was shown that vitamin C improved behavioral deterioration in an AD mice model by reducing the production of Aβ oligomers and tau phosphorylation. In vivo, the decrease in Aβ levels[27] and Aβ plaque load[28] was also noted. The antioxidant and anti-inflammatory properties of vitamin E, which is found in many fruits and vegetables, were also demonstrated in vivo.[29] Another in vivo experiment showed that vitamin E decreased Aβ levels.[30] Deficit in vitamin B12 has long been linked to neurological issues and a higher risk of Alzheimer's disease (AD), according to certain research. Elevated homocysteine levels are a particular symptom of vitamin B12 deficiency and can be detrimental to the brain through oxidative stress, increased calcium influx, and apoptosis. Complete blood counts, measures of serum homocysteine levels, and tests for vitamin B12 insufficiency can all be used to identify the condition.[31,32]

Ginkgo biloba has been studied as a possible therapy for Alzheimer's disease and other neurological conditions (Ginkgo biloba L., Ginkgoaceae). In vitro studies have shown that ginkgo biloba extract can prevent Aβ aggregation, lessen Aβ fibrillogenesis, and destabilize existent fibrils.[33] The cognitive and memory impairment in an AD rat model is ameliorated by ginkgo biloba, according to strong in vivo experimental data.[34,35] Ginkgo biloba also possesses anti-inflammatory and

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

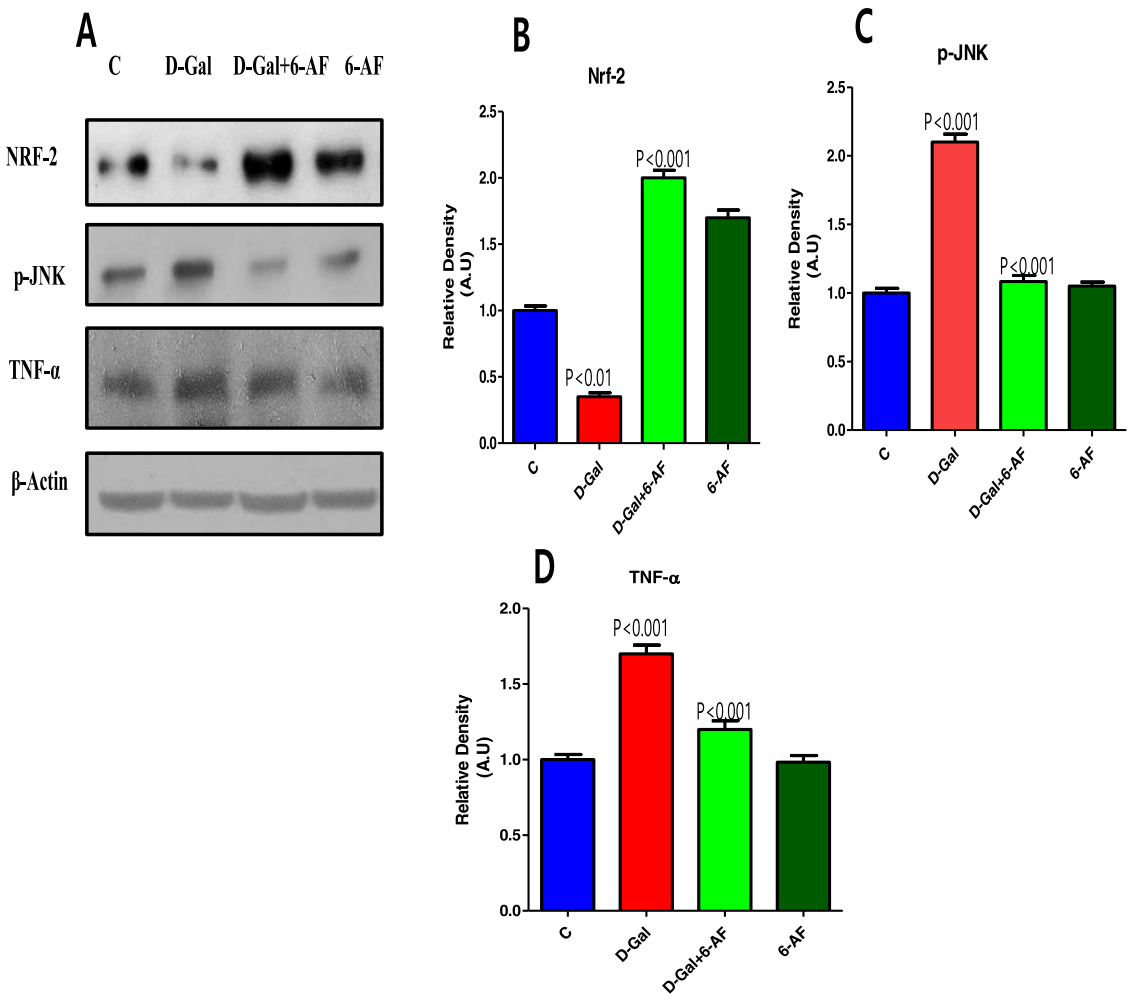


Figure 5. 6-AF inhibited Nrf2 and reduced D-galactose-induced neuroinflammation in adult mice. The histograms of the respective relative densities from the western blotting investigation of the markers of neuroinflammation Nrf2, p-JNK, and TNF-α (A) are displayed in (B–D). We used β-actin as the loading control. With ImageJ software, the data were computed, and a histogram showing the mean in A.U. SEM in arbitrary units was created (A.U.). Both $p \leq 0.01$ and $p \leq 0.001$ are significant.

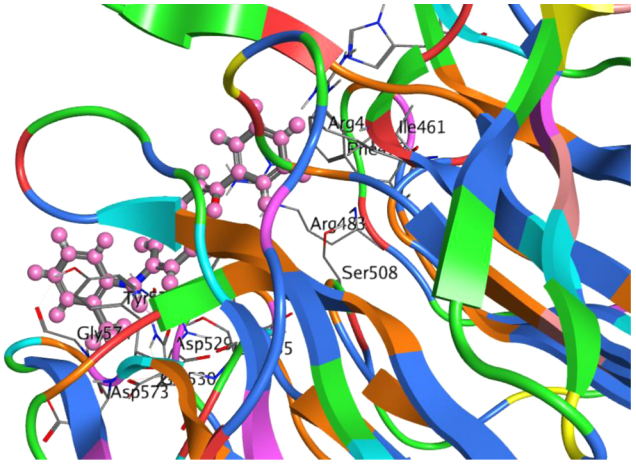


Figure 6. Binding interaction of the complex of 6-aminoflavone with the Nrf2 receptor.

antioxidant effects. Ginkgo biloba supports the nonamyloidogenic pathway of APP by enhancing β-secretase activity and reducing the synthesis of A, according to in vivo studies.[36,37] *Crocus sativus* L., a plant in the Iridaceae family, has anti-

Table 1. Docking Scores of Interacting Residues of the Nrf2 Receptor with the Compound 6-Aminoflavone

interacting residues	interaction	distance	*E* (kcal/mol)
GLN	H-donor	2.92	−1.8
TYR	pi−pi	3.76	−0.0
TYR	pi−pi	3.81	−0.0

inflammatory and antioxidant properties in vivo.[38,39] A aggregation and fibrillogenesis were reduced by this substance in vitro.[40] In vitro tests have shown that the European native lemon balm (*Melissa officinalis* L., Lamiaceae) has antioxidant properties. Because the β-secretase activity is inhibited, in vivo investigations have demonstrated the efficacy of lemon balm extract to enhance memory in an AD model.[41] In vivo investigations have shown that green tea, or *Camellia sinensis* (L.) Kuntze, Theaceae, produced by boiling and drying plant leaves, is a significant source of antioxidants.[42] By lowering levels of Aβ oligomers[43] and the hyperphosphorylated tau protein,[44] green tea also reversed the loss of spatial learning and memory in an AD mouse model. Due to its in vivo anti-inflammatory and antioxidant effects, sage (*Salvia officinalis* L., Lamiaceae) has a long history of use in European herbal medicine.[45] Szaniszlo et al. (2009)[46] conducted a clinical

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

experiment to examine the impact of colostrinin on AD patients. According to the findings, colostrinin treatment for AD patients improved their everyday functioning and cognitive abilities. Therefore, using this chemical as a treatment for AD may be appropriate.

Levodopa and dopaminergic agonists are now being used to address the fundamental pathophysiology of Parkinson's disease (PD); however, this only alleviates the motor symptoms by reestablishing neurotransmission.[47] Levodopa is used with other dopaminergic medications, such as agonists of the dopamine receptors, monoamine oxidase type B inhibitors, and amantadine, an antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, to treat the fluctuation's effects as they manifest.[48]

The pathophysiology of Huntington's disease is still poorly understood, and no effective treatments have been found, 20 years after the genetics of the condition were identified. The motor, mental, and cognitive aspects of HD are only partially addressed by available therapeutic options.[49] Tetrabenazine (TBZ), the exclusive treatment for chorea, is also infamous for its drawbacks, including adverse medication interactions and side effects.[50] It has also been discovered to be successful to treat early HD symptoms with mood stabilizers and selective serotonin reuptake inhibitors (SSRIs).[49] Due to the dearth of effective medicines to reduce cognitive impairment in HD, healthcare practitioners and families of HD patients have been encouraged to combine an evidence-based treatment plan with education and symptom alleviation methods.[49,51] Succinate dehydrogenase (SDH) modifications have been linked to HD, and 3-nitropropionic acid (3-NP), a mitochondrial complex II inhibitor and an irreversible SDH inhibitor, mimics neurological degeneration in animals and alterations in behavior, biochemistry, morphology, and neuropathology that are comparable to those seen in HD pathogenesis.[52]

Additional data showed that Nobiletin, i.e., 5,6,7,8,3′,4′-hexamethoxyflavone (NOB), inhibited inflammatory cytokines, oxidative stress, and pro-apoptotic JNK activation, which were all linked to the reversal of hepatocyte death. In fact, accumulating ROS cause cytochrome c release from mitochondria, which activates apoptotic caspases and JNK in hepatocytic cells. The JNK signal is very crucial in the process of liver pathogenesis, which includes inflammation, hepatocyte proliferation, and cell death, among the signals linked to acute liver damage. The crucial process connected to JNK activation and the subsequent death of hepatocytes is the ignition of proinflammatory TNF-α by a number of stimuli.[53]

Under typical circumstances, the oxidation of unsaturated lipids in human tissues may be efficiently avoided. Several enzymes are involved in in vivo redox homeostasis and keep intracellular ROS at low levels, including glutathione peroxidase and superoxide dismutase. With an accompanying disturbance of redox circuitry and macromolecular damage, oxidative stress in biological systems is characterized by an imbalance between the creation of ROS and their elimination by the antioxidant system.[54]

In the present study, 6-AF is used as a potent drug against neurodegenerative diseases like AD, PD, and HD because of the reported antiproliferative, antioxidant, and anti-inflammatory activities of 6-AF. As the concentration of D-galactose increases in the human body due to aging, oxidative stress is caused because of a decrease in the levels of SOD, POD, GSH, and catalase and the increased concentration of LPO, which are all antioxidant enzymes, so memory is affected and declined, which leads to neurodegenerative diseases. Similarly, Oxidative stress leads to disturbance in the p-Akt signaling pathway. The inflammatory markers' concentration increases more than normal, which causes neuroinflammation. 6-AF significantly inhibited BACE1 to reduce the amyloidogenic pathway of Aβ (both amyloid β production and aggregation) by upregulating Nrf2 proteins and suppressing phosphorylated JNK and TNF-α proteins in adult albino mice's brain homogenates. The administration of 6-AF regulated the p-Akt pathway by blocking the oxidative stress as well as neuroinflammation caused by D-galactose in normal adult male albino mice, revealing that 6-AF can be used as a potent drug against neurodegenerative diseases (Figure 7).

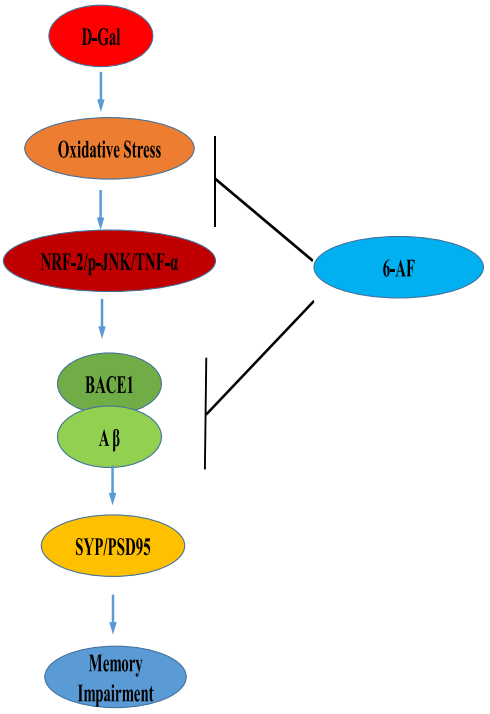


Figure 7. Suggested mechanism of 6-AF against memory impairment brought on by D-galactose in adult male albino mice.

The signaling pathway shows the theory behind 6-AF's defense against D-galactose-induced memory impairment in the male adult albino mouse brain. It shows how 6-AF in a p-Akt-dependent mechanism rescues D-galactose-induced multiple AD neuropathology in a mice model.

4. CONCLUSIONS

The overall results of this study show that 6-AF is a potent drug that reduces D-galactose-induced oxidative stress by upregulating antioxidant enzyme expression/activation, which reduces neuroinflammation. Additionally, by repairing the neuronal synapse, 6-AF restored memory deficiencies in adult albino mice. This work provides the first evidence that 6-AF is a viable therapeutic approach for addressing memory loss and compromised neuronal connections brought on by D-galactose. Additionally, it was discovered in this study that 6-AF reduced neuroinflammation by triggering the p-Akt pathway, which safeguarded male albino mice against D-galactose. Last but not least, 6-AF promotes the p-Akt signaling pathway to prevent D-galactose-induced neurotoxicity, which results in memory

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964

impairment. More research on the anti-inflammatory and antioxidative properties of 6-AF is needed.

■ ASSOCIATED CONTENT

Data Availability Statement

All of the data generated and analyzed have already been incorporated into the study.

■ AUTHOR INFORMATION

Corresponding Authors

Naeem Khan − *Department of Chemistry, Kohat University of Science & Technology, Kohat 26000 Khyber Pakhtunkhwa, Pakistan*; Email: nkhankust@yahoo.com

Fozia Fozia − *Department of Biochemistry, KMU Institute of Medical Sciences, Kohat 26000 KP, Pakistan*; orcid.org/0000-0002-4554-7427; Email: drfoziazeb@yahoo.com

Authors

Shakeel Ahmad − *Department of Chemistry, Kohat University of Science & Technology, Kohat 26000 Khyber Pakhtunkhwa, Pakistan*

Shahid Ali Shah − *Department of Biology, University of Haripur, Haripur 22620 Khyber Pakhtunkhwa, Pakistan*

Umar Nishan − *Department of Chemistry, Kohat University of Science & Technology, Kohat 26000 Khyber Pakhtunkhwa, Pakistan*; orcid.org/0000-0002-0106-3068

Mikhlid H. Almutairi − *Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia*; orcid.org/0000-0002-0337-6412

Nargis Jamila − *Department of Chemistry, Shaheed Benazir Bhutto Women University, Peshawar 25000 Khyber Pakhtunkhwa, Pakistan*

Bader O. Almutairi − *Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia*

Zia Ullah − *College of Professional Studies, Northeastern University, Boston, Massachusetts 02115, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c01781

Author Contributions

N.K., S.A.K., and U.N.: conceptualization; S.K.: data curation; S.K. and N.J.: formal analysis; M.H.A. and B.O.A.: funding acquisition; S.A. and F.F.: investigation; S.A.: methodology; N.K.: project administration; S.A.K. and F.F.: resources; N.K., S.A.S., and U.N.: supervision; N.J. and M.H.A.: visualization; S.A.: writing—original draft; and N.K., S.A.S., U.N., F.F., M.H.A., B.O.A., and Z.U.: writing—review & editing.

Funding

This research was funded through the Researchers Supporting Project (RSP2023R191), King Saud University, Riyadh, Saudi Arabia.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers Supporting Project number (RSP2023R191), King Saud University, Riyadh, Saudi Arabia.

■ REFERENCES

(1) Gella, A.; Nuria, D. Oxidative stress in Alzheimer disease. *Cell Adhes. Migr.* **2009**, *3*, 88−93.

(2) Yu, G. M.; Hirokazu, K.; Miki, O.; Teruo, M. The anti-inflammatory and antioxidant effects of melatonin on LPS-stimulated bovine mammary epithelial cells. *PLoS One* **2017**, *12*, No. e0178525.

(3) Khurana, N.; Suresh, C. S. Targeting crosstalk between Nrf-2, NF-κB and androgen receptor signaling in prostate cancer. *Cancers* **2018**, *10*, 352.

(4) Yang, L.; Renyuan, Z.; Yu, T.; Pengfei, C.; Yu, S.; Shuai, M.; Xiaoqiang, L. Neuroprotection by dihydrotestosterone in LPS-induced neuroinflammation. *Neurobiol. Dis.* **2020**, *140*, No. 104814.

(5) Tang, S.-C.; Justin, D. L.; Pradeep, K. S.; Dong, G. J.; Mohamed, R.; Mughal, A. C.; Dominic, A. S.; William, R. M.; Thiruma, V. A.; Mark, P. M. Toll-like receptor-4 mediates neuronal apoptosis induced by amyloid β-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. *Exp. Neurol.* **2008**, *213*, 114−121.

(6) He, P.; Shikai, Y.; Jiaojiao, Z.; Yuxing, G.; Shuhan, Z.; Zhigang, L.; Xuebo, L.; Chunxia, X. Eriodictyol attenuates LPS-induced neuroinflammation, amyloidogenesis, and cognitive impairments via the inhibition of NF-κB in male C57BL/6J mice and BV2 microglial cells. *J. Agric. Food Chem.* **2018**, *66*, 10205−10214.

(7) Jin, Y.; Jian, P.; Xiaona, W.; Dong, Z.; Tianyin, W. Ameliorative effect of ginsenoside Rg1 on lipopolysaccharide-induced cognitive impairment: role of cholinergic system. *Neurochem. Res.* **2017**, *42*, 1299−1307.

(8) Pandey, K. B.; Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longevity* **2009**, *2*, 270−278.

(9) Abbas, M.; Farhan, S.; Faqir; M, A.; Muhammad, A.; Tabussam, T.; Muhammad, S. B.; Adnan, I.; Shahzad, H.; Hafiz, A.; Rasul, S. Natural polyphenols: An overview. *Int. J. Food Prop.* **2017**, *20*, 1689−1699.

(10) Cintia, R. B.; Mario, R. M. Health benefits of flavonoids. Bioactive compounds. *Wood head Publ.* **2019**, 185−201.

(11) Haddad, A. Q.; Vaidyanathan, V.; Moawiya, A. H.; et al. Novel antiproliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. *Prostate Cancer Prostatic Dis.* **2006**, *9*, 68−76.

(12) Shahid, A. S.; Yoon, G. H.; Chung, S. S.; Abid, M. N.; Kim, T. H.; Lee, H. Y.; Kim, M. O.Novel osmotin inhibits SREBP2 via the AdipoR1/AMPK/SIRT1 pathway to improve Alzheimer's disease neuropathological deficits. *Mol. Psychiatry* **2017**, *22*, 407−416.

(13) El-Khadragy, M. F.; Manal, M. A.; Ebtesam, E.; Abdel, M. Neuroprotective effects of *Citrus reticulata* in scopolamine-induced dementia oxidative stress in rats. *CNS Neurol. Disord.: Drug Targets* **2014**, *13*, 684−690.

(14) Chance, B.; Maehly, A. C. [136] Assay of catalases and peroxidases. *Methods Enzymol.* **1955**, 764−775.

(15) Kakkar, P.; Ballabh, D.; Viswanathan, P. N. A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem. Biophys.* **1984**, *21*, 130−132.

(16) Salau, V. F.; Erukainure, O. L.; Ibeji, C. U.; Olasehinde, T. A.; Koorbanally, N. A.; Islam, M. S. Vanillin and vanillic acid modulate antioxidant defense system via amelioration of metabolic complications linked to Fe 2+-induced brain tissues damage. *Metab. Brain Dis.* **2020**, *35*, 727−738.

(17) De, L. J. A.; Borges, C. R. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *J. Visualized Exp.* **2020**, *12*, No. e61122.

(18) Liu, Y.; Ning, Z.; Chen, Y.; Guo, M.; Liu, Y.; Gali, N. K.; Sun, L.; Duan, Y.; Cai, J.; Westerdahl, D.; Liu, X.; et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* **2020**, *582*, 557−560.

(19) Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490−519.

(20) Gerber, P. R.; Müller, K. MAB, a generally applicable molecular force field for structure modelling in medicinal chemistry. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 251−268.

(21) Rehman, S. U.; Sarwar, T.; Mohammed, A. H.; Hassan, M. I.; Mohammad, T. Studying non-covalent drug−DNA interactions. *Arch. Biochem. Biophys.* **2015**, 49−60.

(22) Tamagno, E.; Paola, B.; Alessandra, O.; Antonella, V.; Roberta, B.; Damiano, Z.; Maria, A. P.; et al. Oxidative stress increases expression and activity of BACE in NT2 neurons. *Neurobiol. Dis.* **2002**, *10*, 279−288.
(23) Lu, T.; Pan, Y.; Ying, P.; Shyan-Yuan Kao, C. L.; Li, C.; Isaac, K.; Kohane, I.; Jennifer, C.; Chan, J.; Bruce, A. Y. Gene regulation and DNA damage in the ageing human brain. *Nature* **2004**, *429*, 883−891.
(24) Khan, S.; Abbas, K.; Ashfaq, U. R.; Irfan, A.; Saif, U.; Abdul, A. K.; Syed, S. A.; Sahib, G. A.; Dong, Q. W. Immunoinformatics and structural vaccinology driven prediction of multi-epitope vaccine against Mayaro virus and validation through in-silico expression. *Infect., Genet. Evol.* **2019**, *73*, 390−400.
(25) Granic, I.; Amalia, M. D.; Ingrid, M. N.; Gertjan, vD.; Ulrich, L. El. Inflammation and NF-κB in Alzheimer's disease and diabetes. *J. Alzheimer's Dis.* **2009**, *16*, 809−821.
(26) Sil, S.; Tusharkanti, G.; Pritha, G.; Rupsa, G.; Syed, N. K.; Avishek, R. Dual role of vitamin C on the neuroinflammation mediated neurodegeneration and memory impairments in colchicine induced rat model of Alzheimer disease. *J. Mol. Neurosci.* **2016**, *60*, 421−435.
(27) Murakami, K.; Nakaba, M.; Yusuke, O.; Noriaki, K.; Kazuhiro, I.; Takuji, S.; Takahiko, S. Vitamin C restores behavioral deficits and amyloid-β oligomerization without affecting plaque formation in a mouse model of Alzheimer's disease. *J. Alzheimer's Dis.* **2011**, *26*, 7−18.
(28) Kook, S. Y.; Lee, K. M.; Kim, Y.; Cha, M. Y.; Kang, S.; Baik, S. H.; Lee, H.; Park, R.; Mook-Jung, I. High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice. *Cell Death Dis.* **2014**, *5*, e1083.
(29) Rosales-Corral, S.; Dun-Xian, T.; Russel, J. R.; Miguel, V. V.; Gabriela, M. B.; Pablo, A. M.; Genaro, G. O. Orally administered melatonin reduces oxidative stress and proinflammatory cytokines induced by amyloid-β peptide in rat brain: a comparative, in vivo study versus vitamin C and E. *J. Pineal Res.* **2003**, *35*, 80−84.
(30) Syuan, S.; Yuemang, Y.; Kunihiro, U.; Hengxuan, Y.; Virginia, M. Y. L.; John, Q. T.; Domenico, P. Early vitamin E supplementationin young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer'sdisease. *FASEB J.* **2004**, 323−325.
(31) Jatoi, S.; Hafeez, A.; Riaz, S. U.; Ali, A.; Ghauri, M. I.; Zehra, M. Low vitamin B12 levels: an underestimated cause of minimal cognitive impairment and dementia. *Cureus* **2020**, *12*, No. e6976.
(32) Cho, H. S.; Huang, L. K.; Lee, Y. T.; Chan, L.; Hong, C. T. Suboptimal baseline serum vitamin B12 is associated with cognitive decline in people with Alzheimer's disease undergoing cholinesterase inhibitor treatment. *Front. Neurol.* **2018**, *9*, 325.
(33) Xie, H.; Wang, J. R.; Yau, L. F.; Liu, Y.; Liu, L.; Han, Q. B.; Zhao, Z.; Jiang, Z. H. Catechins and procyanidins of *Ginkgo biloba* show potent activities towards the inhibition of β-amyloid peptide aggregation and destabilization of preformed fibrils. *Molecules* **2014**, *19*, 5119−5134.
(34) Belviranlı, M.; Nilsel, O. The effects of *Ginkgo biloba* extract on cognitive functions in aged female rats: Therole of oxidative stress and brain-derived neurotrophic factor. *Behav. Brain Res* **2015**, *278*, 453−461.
(35) Zhang, L. D.; Ma, L.; Zhang, L.; Dai, J. G.; Chang, L. G.; Huang, P. L.; Tian, X. Q. Hyperbaric oxygen and *Ginkgo biloba* extract ameliorate cognitive and memory impairment via nuclear factor kappa-b pathway in rat model of Alzheimer's disease. *Chin. Med. J.* **2015**, *128*, 3088−3093.
(36) Colciaghi, F.; Borroni, B.; Zimmermann, M.; Bellone, C.; Longhi, A.; Padovani, A.; Cattabeni, F.; Christen, Y.; Di, L. M. Amyloid precursor protein metabolism is regulated toward alpha-secretase pathway by *Ginkgo biloba* extracts. *Neurobiol. Dis.* **2004**, *16*, 454−460.
(37) Yao, Z. X.; Han, Z.; Drieu, K.; Papadopoulos, V. *Ginkgo biloba* extract (Egb 761) inhibits β-amyloid production by lowering free cholesterol levels. *J. Nutr. Biochem.* **2004**, *15*, 749−756.
(38) Samarghandian, S.; Azimi-Nezhad, M.; Samini, F.; Farkhondeh, T. The role of saffron in attenuating age-related oxidative damage in rat hippocampus. *Recent Pat. Food, Nutr. Agric.* **2016**, *8*, 183−189.
(39) Moallem, S. A.; Hariri, A. T.; Mahmoudi, M.; Hosseinzadeh, H. Effect of aqueous extract of *Crocus sativus* L.(saffron) stigma against subacute effect of diazinon on specific biomarkers in rats. *Toxicol. Ind. Health* **2014**, *30*, 141−146.
(40) Papandreou, M. A.; Kanakis, C. D.; Polissiou, M. G.; Efthimiopoulos, S.; Cordopatis, P.; Margarity, M.; Lamari, F. N. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of *Crocus sativusstigmas* extract and its crocin constituents. *J. Agric. Food Chem.* **2006**, *54*, 8762−8768.
(41) Ozarowski, M.; Mikolajczak, P. L.; Piasecka, A.; Kachlicki, P.; Kujawski, R.; Bogacz, A.; Bartkowiak, W. J.; Szulc, M.; Kaminska, E.; Kujawska, M.; Jodynis, L. J.; et al. Influence of the *Melissa officinalis* leaf extract on long-term memory in scopolamine animal model with assessment of mechanism of action. *J. Evidence-Based Complementary Altern. Med.* **2016**, *2016*, No. 9729818.
(42) Schimidt, H. L.; Garcia, A.; Martins, A.; Mello, C. P. B.; Carpes, F. P. Green tea supplementation produces better neuroprotective effects than red and black tea in Alzheimer-like rat model. *Food Res. Int.* **2017**, *100*, 442−448.
(43) Li, Q.; Zhao, H. F.; Zhang, Z. F.; Liu, Z. G.; Pei, X. R.; Wang, J. B.; Li, Y. Long-term green tea catechin administration prevents spatial learning and memory impairment in senescence-accelerated mouse prone-8 mice by decreasing Aβ1-42 oligomers and upregulating synaptic plasticity−related proteins in the hippocampus. *Neuroscience* **2009**, *163*, 741−749.
(44) Li, H.; Wu, X.; Wu, Q.; Gong, D.; Shi, M.; Guan, L.; Zhang, J.; Liu, J.; Yuan, B.; Han, G.; Zou, Y. Green tea polyphenols protect against okadaic acid-induced acute learning and memory impairments in rats. *Nutrition* **2014**, *30*, 337−342.
(45) Kolac, U. K.; Ustuner, M. C.; Tekin, N.; Ustuner, D.; Colak, E.; Entok, E. The anti-inflammatory and antioxidant effects of *Salvia officinalis* on lipopolysaccharide-induced inflammation in rats. *J. Med. Food* **2017**, *20*, 1193−1200.
(46) Szaniszlo, P.; German, P.; Hajas, G.; Saenz, D. N.; Kruzel, M.; Boldogh, I. New insights into clinical trial for colostrinin in Alzheimer's disease. *J. Nutr., Health Aging* **2009**, *13*, 235−241.
(47) Hirsch, E. C.; Hunot, S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol.* **2009**, *8*, 382−397.
(48) Schaeffer, E.; Pilotto, A.; Berg, D. Pharmacological strategies for the management of levodopa-induced dyskinesia in patients with Parkinson's disease. *CNS Drugs* **2014**, *28*, 1155−1184.
(49) Killoran, A.; Biglan, K. M. Current therapeutic options for Huntington's disease: good clinical practice versus evidence-based approaches? *Mov. Disord.* **2014**, *29*, 1404−1413.
(50) Lyon, R. P.; Setter, J. R.; Bovee, T. D.; Doronina, S. O.; Hunter, J. H.; Anderson, M. E.; Balasubramanian, C. L.; Duniho, S. M.; Leiske, C. I.; Li, F.; Senter, P. D. Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates. *Nat. Biotechnol.* **2014**, *32*, 1059−1062.
(51) Frank, S.; Jankovic, J. Advances in the pharmacological management of Huntington's disease. *Drugs* **2010**, *70*, 561−571.
(52) Patocka, J.; Bielavsky, J.; Cabal, J.; Fusek, J. 3-Nitropropionic acid and similar nitrotoxins. Metabolism of tryptophan in the liver: Interference with decarboxylation of other aromatic amino acids. *Acta Med. (Hradec Kralove, Czech Repub.)* **2000**, *43*, 9−13.
(53) Li, M.; Zhao, H.; Wu, J.; Wang, L.; Wang, J.; Lv, K.; Liu, S.; Wang, M.; Guan, W.; Liu, J.; Ho, C. T.; Li, S. Nobiletin protects against acute liver injury via targeting c-Jun N-terminal kinase (JNK)-induced apoptosis of hepatocytes. *J. Agric. Food Chem.* **2020**, *68*, 7112−7120.
(54) Lange, K. W.; Lange, K. M.; Nakamura, Y.; Li, S. Do natural antioxidants play a role in Alzheimer's disease? *J. Food Bioact.* **2020**, *11*, 2−10.

https://doi.org/10.1021/acsomega.3c01781
ACS Omega 2023, 8, 26955−26964