

The prophylactic role of Chrysin against Clonazepam induced brain toxicity in male albino rats

Abstract

Background: The bioactive substance chryrus is found in bee propolis and all plants of the species *Passiflora*. It is well recognized to have neuroprotective properties and a wide range of pharmacological activity.

Purpose: In this work, the consequence of Chrysin administration (50 mg/kg b.wt/day) on brain toxicity caused by Clonazepam (CZP, 2mg/kg b.wt/day) were investigated by measuring NaK ATPase, neuronal oxidative stress, neuro-inflammation, and DNA fragmentation.

Study design: In our investigation, we used male albino rats 4 weeks old and weighing 60 ± 5 g. There were four groups of ten rats apiece: Group 1: the control group which treated a vehicle with 1% w/v Tween 80. Group 2: given 1% w/v Tween 80-suspended Clonazepam (CZP) at a dose of 2 mg/kg b.wt./day. In Group 3: Chrysin suspended in 1% w/v Tween 80 was given at a rate of 50 mg/kg b.wt/day. Group 4: Clonazepam (CZP) and Chrysin were given at the same prior dosages as before (2 mg/kg b.wt/day for Clonazepam (CZP) and 50 mg/kg b.wt/day for Chrysin).

Methods: Malondialdehyde, nitric oxide, DNA fragmentation, sodium oxide dismutase, catalase, and Na-K ATPase contents were estimated.

Results: According to the biochemical analysis, after the Clonazepam therapy, the brain's contents of malondialdehyde (MDA), nitric oxide (NO), and DNA fragmentation increased, while those of superoxide dismutase (SOD), catalase (CAT), and NaK ATPase activities declined.

Conversely, the biochemical screening of animal brain tissue administered with CZP+ Chrysin revealed an improvement in the brain tissue's ability to withstand the damage caused by CZP.

Keywords: Chrysin, Clonazepam, male albino rats, neurotoxicity

1. Introduction

Drug addiction and drug misuse are major causes of brain diseases through oxidative stress, mitochondrial malfunction, apoptosis, and a reduction in neurogenesis. Drug-induced neurotoxicity and neuronal dysfunction are reflected in the neural deterioration found in drug users [1].

A long-term therapy medicine, clonazepam is a class II as maintained by the Biopharmaceutical Classification System and a derivative of nitrobenzodiazepines which are psychoactive drugs used in epilepsy control and as central nervous system depressants to decline seizure activity. It causes many side effects such as ataxia, sedation, amnesia, and myo-relaxation. Nitrobenzodiazepine works by modulating GABA receptors to produce sedative, anticonvulsant, muscle relaxant, and anxiolytic effects and regulate chloride transportation through the ion channel complex. Clonazepam has a euphoric effect that makes it a popular drug of abuse. It affects d-aminobutyric acid (GABA), causing retrograde amnesia and impairment of memory [2-5]

As an antiepileptic medication, clonazepam is frequently recommended for different neurological and psychiatric circumstances, as well as drug addiction treatment. Despite being regarded as safe; clonazepam is abused as a street drug [6]. Moreover, clonazepam used with another antiepileptic drug is considered an obstacle in studying its long-term effects on patients [2], so this study examined its daily long-term effects on rats. Clonazepam is released directly into the blood-brain barrier and distributed to many body sites which causes a lot of side effects such as anorexia and palpitation [2]. Therefore, we aimed to study some of these side effects.

The primary plant metabolites, flavonoids are polyphenolic ingredients with a wide range of biological activities, including those that are anti-inflammatory, antithrombotic, antioxidant, antiallergic, antibacterial, analgesic, and vasodilatory. These effects are mediated by numerous signaling pathways and physiological mechanisms that are involved in a number of medical disorders [7-10].

A naturally occurring flavonoid called chrysin (5,7-dihydroxyflavone) has been extracted from a variety of plants, including propolis and honey [11], chamomile and mushrooms [12], and a few other medicinal plants such as *Radix scutellariae* [13]. Moreover, Chrysin has been utilized in traditional medicine since ancient times. Research using various animal models examined and reported its numerous biological activities, such as anti-inflammatory, antioxidant, vasorelaxant, and neuroprotective effects [14-17]. Chrysin's solubility and bioavailability are related to its beneficial effects. Intestinal absorption of Chrysin is low, with a maximum concentration of 12 to 64 nM in serum. Among the many pharmacological properties of chrysin is antiasthmatic action. It inhibits DNA topoisomerases, histone deacetylase, nuclear factor-kB (NF-kB), and inducible nitric oxide synthase (iNOS) [7, 8].

Recent research recorded that natural and synthetic flavonoids have activity on GABA type A receptors and mental disorders treatment [5]. Also, they mentioned that chrysin is a fixed ligand for benzodiazepine in which it inhibits flunitrazepam binding to the benzodiazepine binding site. Therefore, we aimed to study the effect of chrysin on Clonazepam-induced oxidative stress. As there is a high concentration of lipids in the brain, we also aimed to investigate oxidative stress, antioxidant status, and its hazard effects on DNA content and Na-K ATPase activity.

2. Material and Methods

2.1. The experimental animals

In the current investigation, forty Wister male rats at 4 weeks of age and weighing 60 ± 5 g, were used. The rats were accommodated in cages of plastic with ten animals per cage, acquired from the National Organization for Drug Control and Research's (NODCAR) animal shelter. Throughout the experiment, animals were housed at a consistent temperature of 25 ± 2 °C and subjected to a 12-hour light-and-dark cycle. For the study, a pellet diet billboard was utilized. Before the study began, the animals were allowed a week to become acclimated to the lab environment. The experimental procedures managed in agreement with the guidelines put forth by the Ain Shams University Ethics Committee of Scientific Research in Cairo, Egypt.

2.2. The drugs

Alfa Aesar Germany produced 5,7-dihydroxyflavone, or chrysin, in a 98% concentration. Generic versions of clonazepam 2 mg oral tablets are marketed under the brand name Apetryl. Multi-Apex manufactures Apetryl tablets for the pharmaceutical industry in S.A.E. Badr City, Egypt.

2.3. The experimental design

The animals split up into the following 4 groups, each containing ten rats:

El Khashab et al. report that Group 1 is the control group that given a vehicle containing 1% w/v Tween 80. [18]. Group 2 was administered Clonazepam (CZP) [19] at a dose of 2 mg/kg b.wt./day, suspended in 1% w/v Tween 80 [20]. – Group 3 [21] was given 50 mg/kg b.wt./day of Chrysin suspended in 1% w/v Tween 80 [18]. The same prior dosages of CZP (2 mg/kg b.wt./day) and Chrysin (50 mg/kg b.wt./day) were administered to Group 4 (Clonazepam and Chrysin). daily and for six weeks, all animal groups received oral gavage treatment. Reagan-Shaw et al., noted that the chosen doses are pharmaceutical doses that are equivalent to human body weight [22].

2.4. Preparation of tissue homogenate:

Following animal sacrifice, brain tissue was thoroughly cleaned and then rinsed with ice. After lightly blotting each tissue using filter paper, a balance was used to weigh each one. Using a polytron homogenizer at 40 °C, we created 0.05 M phosphate buffer (pH 7) for 10% of the tissue homogenate. To exclude unbroken cells, mitochondria, erythrocytes, nuclei, and cell debris, the homogenate samples were centrifuged for 20 minutes at 10,000 rpm. The following enzymes were measured in the supernatant: catalase, sodium oxide dismutase, nitric oxide (NO), fragmentation of DNA, and malondialdehyde (MDA).

2.5. Biochemical assays:

Nitric oxide (NO) in brain homogenate was measured using the colorimetric method of Nims et al.[23], and brain malondialdehyde was identified as thiobarbituric acid (TBA) by Ohkawa [24]. The colorimetric method of Biovision, USA was utilized to assess the activities of catalase (CAT), superoxide dismutase (SOD), and sodium-potassium ATPase. DNA fragmentation in the brain was identified using Agarose Gel Electrophoresis

3. Results:

Malondialdehyde contents in brain rats of different groups were measured after 6 weeks of different administrations. It recorded a significant increment (2.90 ± 0.05 nmol/mg tissue protein) after the treatment of Clonazepam, and there was a significant reduction after chrysin administration (1.71 ± 0.04 nmol/mg tissue protein). As known, the ADA did not change in the chrysin group (Table 1).

In the data illustrated in Table (1) Clonazepam induced a significant increase in nitric oxide when compared to the control group (27.08 ± 0.99 μ mol/mg tissue protein) and the association of chrysin with Clonazepam reversed this effect (18.85 ± 0.20 μ mol/mg tissue protein). The measurements of brain antioxidant status parameters are represented in Table (1). It was SOD and CAT activates in the brain tissues and were significantly increased as a result of Clonazepam administration (SOD= 0.68 ± 0.04 U/mg tissue protein & CAT= 1.35 ± 0.06 nmol/min/mg tissue protein) and reversed after the chrysin treatment (SOD= 2.05 ± 0.09 U/mg tissue protein & CAT= 3.53 ± 0.06 nmol/min/mg tissue protein).

Table (1) showed the DNA fragmentation contents of all rats after the treatment of Clonazepam (1.70 ± 0.04 OD at 260nm) and chrysin, administration revealed ameliorative effect (0.99 ± 0.02 OD at 260nm). Also, the Na-K ATPase activity decreased after the treatment of Clonazepam (1.89 ± 0.10 mU/mg tissue protein) and a significant increment after the chrysin (3.90 ± 0.11 mU/mg tissue protein) treatment as compared to the control group.

Table 1: Malondialdehyde (MDA) content (nmol/mg tissue protein), NO content (μ mol/mg tissue protein), DNA fragmentation (OD at 260nm), CAT activity (nmol/min/mg tissue protein), SOD activity (U/mg tissue protein), and NaK ATPase (mU/mg tissue protein) are shown for the various treated groups.

Parameters Groups	MDA	NO	DNA fragmentation	CAT	SOD	NaK ATPase
CONT	0.87 ± 0.05	17.10 ± 0.91	0.81 ± 0.02	4.70 ± 0.08	2.53 ± 0.08	4.98 ± 0.15
CHRY	0.91 ± 0.10	16.18 ± 0.45	0.80 ± 0.07	4.88 ± 0.12	2.35 ± 0.06	5.03 ± 0.23
CLONZ	2.90 ± 0.05^a	27.08 ± 0.99^a	1.70 ± 0.04^a	1.35 ± 0.06^a	0.68 ± 0.04^a	1.89 ± 0.10^a
CLONZ+ CHRY	1.71 ± 0.04^{ab}	18.85 ± 0.20^b	0.99 ± 0.02^{ab}	3.53 ± 0.06^{ab}	2.05 ± 0.09^{ab}	3.90 ± 0.11^{ab}

Values are the means of 8 rats \pm SE at $p < 0.05$. 'a' represents a significant change from the control group (G1), and 'b' represents a significant change from the CLONZ (G3) group.

Discussion

As an anti-anxiety drug, clonazepam influences gamma-aminobutyric acid (GABA) (Mikulecká et al., 2014). Immoderate brain activity is probable to contribute to anxiety and other psychological disorders. Clonazepam is used to treat panic disease and to prevent some forms of convulsions (Morishita, 2009). On the other hand, it causes many troubles in the brain processes causing neurological impairments like in drug abusers [1].

Drug addiction induced neurotoxicity which is related to oxidative stress. In general, drug of misuse are a source of oxidative damage because they increase levels of reactive oxygen species (ROS), prompt oxidation of proteins and lipids, and depletion of the total antioxidant systems within cells [25, 26].

In our study, the MDA content increased significantly in the clonazepam tissue homogenates. However, the SOD and CAT showed significant decreases as compared to the control rats. Sabry et al. stated that the increment in reactive species accompanied by the antioxidant defense system decreased [27].

Lakshmi et al and Nixon et al stated that the increment of MDA in nervous tissue causes a decrease in antioxidant capacity and causes apoptosis in the cerebral cortex of animals. Moreover, the peroxidation of lipids and proteins causes neurological conditions like Alzheimer's [15, 28, 29].

Nitric oxide plays an important role in CNS physiology as the regulation of neurotransmitter biosynthesis. However, it reacts with the reactive oxygen species and forms peroxynitrite (ONOO⁻). ONOO⁻ nitrosylate protein, DNA, RNA, lipids, and other cellular molecules [30].

It is known that brain NO dysfunction causes many diseases as anxiety and the etiology of MDD [31]. NO can make a reaction with superoxide and produce nitrite and nitrogen dioxide which cause DNA damage [32].

A significant increment in nitric oxide as a result of clonazepam injections daily for 6 weeks. Animals treated with diazepam had increased inflammatory cellular infiltrate and mild expression of p53 identified in the nuclei of some neurons [33].

In the present study DNA fragmentation increased after the clonazepam treatment and our results agree with Girgis et al., who examined the clonazepam abuse and changes in the neurons and DNA in rat brains and recorded that there is a significant degradation in brain DNA as compared to control rats [34]. Moreover, Bittigau et al. recorded that there is apoptotic neurodegeneration in the clonazepam brain rat tissues [35].

Moreover, Bittigau et al. tested the effects of many antiepileptic drugs (AEDs) on 7-day-old rats, such as diazepam (30 mg/kg) and clonazepam (0.5–4 mg/kg), and recorded that diazepam exerts wide-spread apoptotic neurodegeneration and clonazepam has neurotoxic effects. They returned these data to the apoptotic death of AEDs [35].

Our study recorded a significant depletion in Na-K ATPase content as a result of clonazepam administration. Numerous studies have shown that mitochondrial dysfunction and toxic consequences are common side effects of several marketed medicines. According to Khan et al and Nadanaciva et al., mitochondria are thought to be the primary target organelle for drug-induced toxicity [36, 37]. They can be injured through a variety of mechanisms, including oxidation of fatty acids, increased mitochondrial DNA damage, redox cycling, electron transport uncoupling from adenosine triphosphate (ATP) synthesis, and mitochondrial permeability transition pore opening. A variety of acute and chronic neurological disorders are influenced by mitochondrial impairment, and its involvement in epilepsy provides proof of respiratory chain action failure in the more recent sporadic forms [38].

Recent research stated that the natural flavonoids have activity on GABA type A receptors and mental disorders treatment and added that chrysin is a fixed ligand for benzodiazepine in which it inhibits flunitrazepam binding to benzodiazepine binding site [5].

Our study recorded the brain ameliorative effects of chrysin, which represented a reverse result in MDA, nitric oxide (NO), and DNA fragmentation in animals injected with clonazepam through increasing SOD and CAT. Moreover, it repaired the level of Na-K ATPase activity in the brain tissues.

In the brain tissue of mice treated with D-galactose, Anand et al found that chrysin has ameliorative effects on redox status, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione [39]. Additionally, it lessens lipid peroxidation, enhances memory impairment, and shields rats' brains from chronic cerebral hypoperfusion-induced neuronal death [16, 40].

Moreover, Chrysin has many pharmacological properties. It inhibits DNA topoisomerases, histone deacetylase, nuclear factor-kB (NF-kB), and inducible nitric oxide synthase (iNOS) [7, 8].

Chrysin stopped the release of nitric oxide, pro-inflammatory cytokines like TNF- α and IL-1 β , and the expressions of iNOS and COX-2 by preventing signaling molecules activations linked to neuroinflammation (c-Jun N-terminal kinase and NF-kB) [8].

Mehri et al. examined the effects of chrysin in acrylamide on increased TNF- α and stated that chrysin led to the decline of TNF- α , and this result is by the study of Gresa-Arribas et al., Also, chrysin decreases oxidative stress and inflammation, which decreases age-related cognitive decline [21, 41, 42].

Ethical approval

The protocols and investigational methods were approved by the authorities of Ain Shams University and adhere to Egyptian standards for animal protection, as per the Animals (Scientific Procedures) Act, 1986 in the United Kingdom, related guidelines, and the European Communities Council Directive of November 24, 1986 (86/609/EEC). Study Code# ASU/W/Sci-5R/23-2-27

References

1. Cunha-Oliveira, T., et al., *Neurotoxicity of heroin–cocaine combinations in rat cortical neurons*. Toxicology, 2010. **276**(1): p. 11-17.
2. Ahmed, G.K., et al., *Effect of long-term administration of clonazepam, carbamazepine, and valproate on cognitive, psychological, and personality changes in adult epilepsy: a case–control study*. Middle East Current Psychiatry, 2021. **28**: p. 1-10.
3. E Nardi, A., et al., *Clonazepam for the treatment of panic disorder*. Current Drug Targets, 2013. **14**(3): p. 353-364.
4. Negrusz, A., et al., *Elimination of 7-aminoclonazepam in urine after a single dose of clonazepam*. Analytical and bioanalytical chemistry, 2003. **376**: p. 1198-1204.
5. Roberts, S.M., D.S. Bezinover, and P.K. Janicki, *Reappraisal of the role of dolasetron in prevention and treatment of nausea and vomiting associated with surgery or chemotherapy*. Cancer Management and Research, 2012: p. 67-73.
6. Longo, L.P. and B. Johnson, *Addiction: Part I. Benzodiazepines—side effects, abuse risk and alternatives*. American family physician, 2000. **61**(7): p. 2121-2128.
7. Farkhondeh, T., et al., *Effect of chrysin on nociception in formalin test and serum levels of noradrenalin and corticosterone in rats*. International journal of clinical and experimental medicine, 2015. **8**(2): p. 2465.
8. Samarghandian, S., et al., *Assessment the effect of saffron ethanolic extract (Crocus sativus L.) on oxidative damages in aged male rat liver*. Der Pharm Lett, 2016. **8**(3): p. 283-90.

9. Samarghandian, S., et al., *Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats*. Canadian journal of physiology and pharmacology, 2016. **94**(4): p. 388-393.
10. Samarghandian, S., et al., *Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney*. Biochemistry research international, 2016. **2016**.
11. Williams, C.A., et al., *Chrysin and other leaf exudate flavonoids in the genus Pelargonium*. Phytochemistry, 1997. **46**(8): p. 1349-1353.
12. Anandhi, R., et al., *Antihypercholesterolemic and antioxidative effects of an extract of the oyster mushroom, Pleurotus ostreatus, and its major constituent, chrysin, in Triton WR-1339-induced hypercholesterolemic rats*. Journal of Physiology and Biochemistry, 2013. **69**: p. 313-323.
13. Tong, L., et al., *Simultaneous determination of baicalin, wogonoside, baicalein, wogonin, oroxylin A and chrysin of Radix scutellariae extract in rat plasma by liquid chromatography tandem mass spectrometry*. Journal of pharmaceutical and biomedical analysis, 2012. **70**: p. 6-12.
14. Lapidot, T., M.D. Walker, and J. Kanner, *Antioxidant and prooxidant effects of phenolics on pancreatic β -cells in vitro*. Journal of agricultural and food chemistry, 2002. **50**(25): p. 7220-7225.
15. A Rashed, L., H. F Al-Saeed, and M. M El-Shawwa, *Neuroprotective effects of chrysin on adult male albino rats exposed to acrylamide and radiation*. Al-Azhar Medical Journal, 2016. **45**(3): p. 493-504.
16. He, X.-L., et al., *Chrysin improves cognitive deficits and brain damage induced by chronic cerebral hypoperfusion in rats*. European Journal of Pharmacology, 2012. **680**(1-3): p. 41-48.
17. Mercer, L.D., et al., *Dietary polyphenols protect dopamine neurons from oxidative insults and apoptosis: investigations in primary rat mesencephalic cultures*. Biochemical pharmacology, 2005. **69**(2): p. 339-345.
18. El Khashab, I.H., et al., *Chrysin attenuates global cerebral ischemic reperfusion injury via suppression of oxidative stress, inflammation and apoptosis*. Biomedicine & Pharmacotherapy, 2019. **112**: p. 108619.
19. Mohamed, T.M., H.M.A. Ghaffar, and R.M.R. El Husseiny, *Effects of tramadol, clonazepam, and their combination on brain mitochondrial complexes*. Toxicology and industrial health, 2015. **31**(12): p. 1325-1333.
20. Socała, K., et al., *Effect of tadalafil on seizure threshold and activity of antiepileptic drugs in three acute seizure tests in mice*. Neurotoxicity research, 2018. **34**: p. 333-346.
21. Mehri, S., et al., *Chrysin reduced acrylamide-induced neurotoxicity in both in vitro and in vivo assessments*. Iranian Biomedical Journal, 2014. **18**(2): p. 101.
22. Reagan-Shaw, S., M. Nihal, and N. Ahmad, *Dose translation from animal to human studies revisited*. The FASEB journal, 2008. **22**(3): p. 659-661.
23. Nims, R.W., et al., *Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions*. Methods, 1995. **7**(1): p. 48-54.
24. Ohkawa, H., *Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction*. Anal Biochem, 1979. **44**: p. 276-278.
25. Pavlek, L.R., J. Dillard, and L.K. Rogers, *The role of oxidative stress in toxicities due to drugs of abuse*. Current Opinion in Toxicology, 2020. **20**: p. 29-35.
26. Cunha-Oliveira, T., A. Cristina Rego, and C. R Oliveira, *Oxidative stress and drugs of abuse: an update*. Mini-Reviews in Organic Chemistry, 2013. **10**(4): p. 321-334.
27. Sabry, S.A., M.A. Ibrahim, and R.M. Mosaad, *Biochemical, histological and ultrastructural studies on the ameliorative effects of chrysin on the hepatotoxicity of clonazepam in developing male albino rats*. African Journal of Biological Sciences, 2022. **18**(2): p. 161-175.
28. Lakshmi, D., et al., *Ameliorating effect of fish oil on acrylamide induced oxidative stress and neuronal apoptosis in cerebral cortex*. Neurochemical Research, 2012. **37**: p. 1859-1867.

29. Nixon, B.J., et al., *Chronic exposure to acrylamide induces DNA damage in male germ cells of mice*. Toxicological Sciences, 2012. **129**(1): p. 135-145.
30. Zhou, Q.-G., et al., *Neuronal nitric oxide synthase and affective disorders*. IBRO reports, 2018. **5**: p. 116-132.
31. Pitsikas, N., *The role of nitric oxide (NO) donors in anxiety. Lights and shadows*. Nitric Oxide, 2018. **77**: p. 6-11.
32. Papi, S., F. Ahmadizar, and A. Hasanvand, *The role of nitric oxide in inflammation and oxidative stress*. Immunopathologia Persa, 2019. **5**(1): p. e08-e08.
33. Badawy, S., et al., *Effects of dependence of tramadol, diazepam and their combination on the brain of Albino rats: Biochemical, histological and immunohistochemical study*. Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 2014. **23**(2): p. 139-147.
34. Girgis, N., et al., *Cellular and DNA changes due to clonazepam abuse in brains of albino rats and role of clonidine during withdrawal period*. Mansoura Journal of Forensic Medicine and Clinical Toxicology, 2010. **18**(1): p. 25-49.
35. Bittigau, P., et al., *Antiepileptic drugs and apoptotic neurodegeneration in the developing brain*. Proceedings of the National Academy of Sciences, 2002. **99**(23): p. 15089-15094.
36. Khan, T., et al., *Mitochondrial Dysfunction: Pathophysiology and Mitochondria-Targeted Drug Delivery Approaches*. Pharmaceutics, 2022. **14**(12): p. 2657.
37. Nadanaciva, S., et al., *Mitochondrial impairment by PPAR agonists and statins identified via immunocaptured OXPHOS complex activities and respiration*. Toxicology and applied pharmacology, 2007. **223**(3): p. 277-287.
38. Patel, M., *Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures*. Free Radical Biology and Medicine, 2004. **37**(12): p. 1951-1962.
39. Anand, K.V., et al., *Protective role of chrysin against oxidative stress in d-galactose-induced aging in an experimental rat model*. Geriatrics & gerontology international, 2012. **12**(4): p. 741-750.
40. Yao, Y., et al., *Chrysin protects against focal cerebral ischemia/reperfusion injury in mice through attenuation of oxidative stress and inflammation*. International Journal of Molecular Sciences, 2014. **15**(11): p. 20913-20926.
41. Gresa-Arribas, N., et al., *Inhibition of CCAAT/enhancer binding protein δ expression by chrysin in microglial cells results in anti-inflammatory and neuroprotective effects*. Journal of neurochemistry, 2010. **115**(2): p. 526-536.
42. Souza, L.C., et al., *Flavonoid Chrysin prevents age-related cognitive decline via attenuation of oxidative stress and modulation of BDNF levels in aged mouse brain*. Pharmacology Biochemistry and Behavior, 2015. **134**: p. 22-30.