TheprophylacticroleofChrysinagainstClonazepaminducedbraintoxicityinmale albinorats

Abstract

Background: The bioactive substance chyrus 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/kgb.wt/day)wereinvestigatedbymeasuringNaKATPase,neuronaloxidativestress,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 groupsoftenratsapiece:Group 1:thecontrolgroupwhich treatedavehicle 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 Tween80was given atarateof50mg/kg b.wt/day. Group4:Clonazepam(CZP)andChrysin 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, nitricoxide,DNAfragmentation, sodiumoxide dismutase, catalase,andNa-KATPase contents were estimated.

Results: According to the biochemical analysis, after the Clonazepam therapy, the brain's contents of malondialdehyde (MDA),nitricoxide(NO),andDNAfragmentationincreased,while thoseof superoxidedismutase (SOD), catalase (CAT), and NaK ATPase activities declined.

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

Keywords:Chrysin,Clonazepam,malealbinorats,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 Systemandaderivativeofnitrobenzodiazepineswhicharepsychoactive drugsusedinepilepsycontrolandascentral nervoussystem depressantstodeclineseizureactivity.Itcausesmany sideeffects suchasataxia,sedation,amnesia, andmyo-relaxation. NitrobenzodiazepineworksbymodulatingGABAreceptorstoproducesedative,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]

Asanantiepilepticmedication, clonazepamisfrequentlyrecommended for differentneurologicalandpsychiatric circumstances, as well as drug addictiontreatment. 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-termeffectsonpatients[2],sothisstudyexamineditsdailylong-termeffectsonrats.Clonazepamisreleaseddirectly intotheblood-brainbarrieranddistributedtomany bodysiteswhich causesalotofsideeffectssuch asanorexiaand palpitation [2]. Therefore, we aimed to study some of these side effects.

Theprimaryplantmetabolites, flavonoidsarepolyphenolicingredients with awide rangeofbiologicalactivities, 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].

Anaturallyoccurringflavonoidcalledchyrosin(5,7-dihydroxyflavone)hasbeenextractedfromavarietyofplants, including propolisand honey [11],chamomile and mushrooms [12],and afew 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 itsbeneficialeffects.IntestinalabsorptionofChrysin islow,withamaximum concentrationof12to64nMinserum. 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].

RecentresearchrecordedthatnaturalandsyntheticflavonoidshaveactivityonGABAtypeAreceptorsandmental disorders treatment [5,43-45].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. MaterialandMethods

2.1. Theexperimentalanimals

Inthecurrentinvestigation, fortyWistermaleratsat4weeks ofageandweighing 60±5 g,wereused.Theratswere accommodatedincagesofplasticwithtenanimalspercage,acquiredfromtheNationalOrganizationforDrugControl 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.Beforethestudybegan,theanimals were allowedaweek tobecomeacclimatedtothelabenvironment. 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. Thedrugs

Alfa Aesar Germany produced 5,7-dihydroxyflavone, or chysin, in a 98% concentration. Generic versions of clonazepam 2mg oraltablets aremarketed under the brandname Apetryl. Multi-Apex manufactures Apetryl tablets for the pharmaceutical industry in S.A.E. Badr City, Egypt.

2.3. Theexperimentaldesign

Theanimalssplitupintothefollowing 4groups,eachcontainingtenrats:

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].–Group3 [21]was given 50mg/kg b.wt./day ofChrysin suspended in 1%w/v Tween 80 [18].Thesame 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. Preparationoftissuehomogenate:

Following animal sacrifice, brain tissue was thoroughly cleaned and then rinsed with ice. After lightly blotting each tissueusingfilterpaper,abalancewasusedtoweigheachone.Usingapolytronhomogenizerat40°C,wecreated 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. Biochemicalassays:

Nitric oxide (NO) in brain homogenate was measured using the colorimetric method of Nimset al.[23], and brain malondialdehyde was identifiedasthiobarbituricacid(TBA) byOhkawa [24].ThecolorimetricmethodofBiovision, 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±0.04nmol/mg tissue protein). As known, the ADA did not change in the chrysin group (Table 1).

InthedataillustratedinTable(1)Clonazepaminducedasignificantincreaseinnitricoxidewhencomparedtothe control group (27.08±0.99 µmol/mg tissue protein) and the association of chrysin with Clonazepam reversed this effect(18.85±0.20µmol/mg tissueprotein).Themeasurements ofbrainantioxidantstatusparametersarerepresented 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) andreversedafterthechrysintreatment(SOD=2.05±0.09U/mgtissueprotein&CAT=3.53±0.06nmol/min/mgtissue protein).

Table(1)showedtheDNAfragmentation contentsofallratsafterthetreatmentofClonazepam(1.70±0.04ODat 260nm) and chrysin, administration revealed ameliorative effect (0.99±0.02OD 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.

Table1:Malondialdehyde(MDA)content(nmol/mgtissueprotein),NOcontent(µmol/mgtissueprotein),DNA fragmentation(ODat260nm),CATactivity(nmol/min/mgtissueprotein),SODactivity(U/mgtissue protein), and NaK ATPase (mU/mg tissue protein) are shown for the various treated groups.

Parameters Groups	MDA	NO	DNA fragmentation	CAT	SOD	NaKA TPase
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]

Valuesarethemeansof8rats±SEatp<0.05.'a'representsa significantchangefromthecontrolgroup(G1), and 'b' represents a significant change from the CLONZ (G3) group.

Discussion and conclusion

As an anti-anxiety drug, clonazepam influences gamma-aminobutyric acid (GABA) (Mikulecká et al., 2014). Immoderatebrainactivityisprobabletocontributetoanxietyandotherpsychologicaldisorders.Clonazepam isused totreatpanicdiseaseandtoprevent some formsofconvulsions(Morishita,2009).Ontheotherhand,itcauses 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 asource ofoxidativedamagebecausetheyincreaselevelsofreactiveoxygenspecies(ROS),promptoxidationofproteinsand lipids, and depletion of the total antioxidant systems within cells [25, 26].

Inourstudy,theMDAcontentincreased significantly intheclonazepamtissuehomogenates.However,theSOD andCATshowedsignificantdecreasesascomparedtothecontrolrats.Sabryetal.statedthattheincrementinreactive species accompanied by the antioxidant defense system decreased [27].

LakshmietalandNixonetal statedthattheincrementofMDAinnervoustissuecausesadecreaseinantioxidant 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].

Asignificantincrementinnitricoxideasaresultofclonazepaminjectionsdailyfor6weeks.Animalstreatedwith diazepam had increased inflammatory cellular infiltrate and mild expression of p53 identified in the nuclei of some neurons [33].

InthepresentstudyDNAfragmentationincreasedaftertheclonazepamtreatmentandourresultsagreewithGirgis etal.,whoexaminedtheclonazepamabuseandchangesintheneuronsandDNAinratbrainsandrecordedthatthere 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,Bittigauetal.testedtheeffectsofmanyantiepilepticdrugs(AEDs)on7-day-oldrats,suchasdiazepam (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 severalmarketedmedicines.AccordingtoKhanetalandNadanacivaetal.,mitochondriaarethoughttobetheprimary 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].

Chrysinstoppedthereleaseofnitricoxide,pro-inflammatorycytokineslikeTNF-aandIL-1b,andtheexpressions 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 bythe studyof Gresa-Arribas et al., Also, chrysin decreases oxidative stress and inflammation, which decreases age-related cognitive decline [21, 41, 42].

Ethicalapproval

The protocols and investigational methods were approved by the authorities of Ain Shams University and adhere to Egyptianstandardsforanimalprotection,aspertheAnimals(ScientificProcedures)Act,1986intheUnitedKingdom, 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.,etal.,*Neurotoxicityofheroin–cocainecombinationsinratcorticalneurons.*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.,etal.,*Eliminationof7-aminoclonazepaminurineafterasingledoseofclonazepam.*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.andB.Johnson,*Addiction:PartI.Benzodiazepines—sideeffects,abuseriskandalternatives.* Americanfamilyphysician,2000.**61**(7):p.2121-2128.
7. Farkhondeh,T.,etal.,*Effectofchrysinonnociceptioninformalintestandserumlevelsofnoradrenalinand corticosterone in rats.* International journal of clinical and experimental medicine, 2015. **8**(2): p. 2465.
8. Samarghandian,S.,etal., *Assessmenttheeffectofsaffronethanolicextract(CrocussativusL.)onoxidative 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.* Canadianjournal ofphysiologyand 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.,etal.,*Chrysinandotherleafexudateflavonoidsinthegenus Pelargonium.*Phytochemistry, 1997. **46**(8): p. 1349-1353.

12. Anandhi,R.,etal.,*Antihypercholesterolemicandantioxidativeeffectsofanextractoftheoystermushroom, 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. ARashed,L.,H.FAl-Saeed,andM.MEl-Shawwa,*Neuroprotectiveeffectsofchrysinonadultmalealbino 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.,etal.,*Chrysinreducedacrylamide-inducedneurotoxicityinbothinvitroandinvivoassessments.* IranianBiomedicalJournal,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,andL.K.Rogers,*Theroleofoxidativestressintoxicitiesduetodrugsofabuse.* CurrentOpinioninToxicology,2020.**20**:p.29-35.

26. Cunha-Oliveira,T.,A.CristinaRego,andC.ROliveira,*Oxidativestressanddrugsofabuse:anupdate.* Mini-ReviewsinOrganicChemistry,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.,etal.,*ChronicexposuretoacrylamideinducesDNAdamageinmalegermcellsofmice.* ToxicologicalSciences,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., *Theroleofnitricoxide(NO)donorsin anxiety.Lightsandshadows.* NitricOxide, 2018. **77**:p. 6-11.

32. Papi,S.,F.Ahmadizar,andA.Hasanvand,*Theroleofnitricoxideininflammationandoxidativestress.* ImmunopathologiaPersa,2019.**5**(1):p.e08-e08.

33. Badawy,S.,etal.,*Effectsofdependenceoftramadol,diazepamandtheircombinationonthebrainofAlbino 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 clonidineduringwithdrawalperiod.*MansouraJournalofForensicMedicineandClinicalToxicology,2010. **18**(1): p. 25-49.

35. Bittigau,P.,etal.,*Antiepilepticdrugsandapoptoticneurodegenerationinthedevelopingbrain.*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 immunocapturedOXPHOScomplexactivitiesandrespiration.*Toxicologyandappliedpharmacology,2007. **223**(3): p. 277-287.

38. Patel,M.,*Mitochondrialdysfunctionandoxidativestress:causeandconsequenceofepilepticseizures.*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 attenuationofoxidativestressandinflammation.*InternationalJournalofMolecularSciences,2014.**15**(11): p.20913-20926.

41. Gresa□Arribas,N.,etal.,*InhibitionofCCAAT/enhancerbindingproteinδexpressionbychrysininmicroglial 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 stressandmodulationofBDNFlevelsinagedmousebrain.*PharmacologyBiochemistryandBehavior,2015. **134**: p. 22-30.

43. Ahmed, F. H., Baker, K. E. and Gabal, A. M. S. (2021) "Comparative Study between Saffron (Crocus sativus L.) and / or Turmeric (Curcuma longa L.) Extracts on D-galactose Deleterious Brain Effects in Rats", Journal of Pharmaceutical Research International, 33(33B), pp. 33–57. doi: 10.9734/jpri/2021/v33i33B31796.

44. Seif, M. M., Ahmed-Farid, O. A.-H., &Aboulthana, W. M. (2017). Evaluation of the Protective Effect of Acacia senegal Extract against di-(2-ethylhexyl phthalate) Induced Hepato- and Neurotoxicity in Rats. Annual Research & Review in Biology, 19(2), 1–17. https://doi.org/10.9734/ARRB/2017/35608

45. Ghanem CI, Pérez MJ, Manautou JE, Mottino AD. Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. Pharmacological research. 2016 Jul 1;109:119-31.