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The antioxidant role of decaffeinated green coffee extract against rotenone induced parkinsonism in rats

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Abstract- Caffeine offered to have anti-cancer, neuroprotective, anti-atherosclerosis, renoprotective and hepatoprotective properties due to their mitochondrial protective, antioxidant, anti-inflammatory and anti-apoptoptic functions. But it is reported adverse effect, so the presence study aimed to study the neuroprotective role of decaffeinated green coffee extract (dGCE) rotenone induced animal model of PD by analysing TBARS and antioxidant such as SOD, catalase, GPx and GSH. Rotenone injected rat showed enhanced levels of TBARS and diminished levels and activities GSH and SOD, catalase and GPx. The study confirms the neuroprotective/antioxidant property of dGCE mainly due to the presence of chlorogenic acid (CGA) and other components of the extract.

1. INTRODUCTION

Coffee is one among the frequently consumed beverages globally and its annual intake has been increased to ~500 billion cups/year [1]. Low or moderate coffee intake offered protection against the risk of Parkinson's disease (PD), Alzheimer's disease (AD), anti-analgesic agent, obesity, tachycardia, increase of blood pressure, anxiety, insomnia, metabolic disorders including type 2 diabetes and various forms of cancer as indicated by

several epidemiologic studies [2,3,4]. The pharmacological activities of coffee depend upon the factors such as alcohol consumption, ethnicity, smoking, gender and mainly the metabolism of caffeine [5]. The beneficiary role of caffeine is mainly depends upon its inhibitory action against adenosine receptor and interference with cholinergic, serotonin, opioid, and γ-aminobutyric acid receptors [6].

However, more consumption of coffee may leads to hypokalemia, sleep disorders and cardiac diseases [7, 8, 9]. Decaffeination process is employed by soaking unroasted green coffee beans in several organic solvents before the roasting process. During this process the content of the caffeine in the coffee is reduced from 2.1% to 0.02-0.3% [10]. About 10% of the entire coffee consumers is changed into the habit of intaking decaffeinated coffee [11]. Green coffee extract becomes a potent source of chlorogenic acid (CGA) during the decaffeination process by eliminating the adverse reactions of caffeine [12, 13]. Toxicological studies indicated that there is no evidence on adverse reactions of dGCE. The potent antioxidant capacity of dGCE belongs to the levels of phenolic substances particularly CGA that is reported to have both in vitro and in vivo antioxidant properties [14]. Moreover it possesses other pharmaceutical activities such as antidiabetic, anti-hyperlipidimic, anti-inflammatory and neuroprotective properties [15, 16, 17, 18].

Parkinson's disease is the common neurodegenerative disorder that is characterized by degeneration of dopominergic neurons in the brain resulting in the symptoms such as hypokinesia, akinesia, neurographia, bradykinesia and tremor. Oxidative stress is mainly correlated with other pathological processes such as excitotoxicity, inflammation, nitric oxide toxicity, mitochondrial dysfunction and apoptosis that are the contributors of dopaminergic neuronal degeneration in PD [19]. There is always a debate arises between the scientist regarding whether the oxidative stress causes or it itself a outcome of, these pathological events. Oxidative stress was estimated by performing the assay of thiobarbituric acid reactive substances (TBARS) in terms of lipid peroxidation product and enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidise (GPx) and catalase and the levels of non enzymatic antioxidant like reduced

glutathione (GSH). Increased levels of TBARS and GSH and activities of enzymatic antioxidants were reported in experimental models of PD and in patients [20, 19].

The golden standard drug for PD is L-DOPA which, provides the symptomatic relief and its prolonged usage induces harsh adverse effects that worsens the course of the disease. Numerous pharmacological agents such as DA agonists calcium antagonists, MAO-B inhibitors, NMDA antagonists, glutamate release inhibitors, nitric oxide syntheses inhibitors, sulfhydryl drugs, dimethyl thiourea and immunosuppressants along with combination of L-DOPA was reported to offer neuroprotection against experimental model of PD with fewer side effects. Another important therapeutic implications is the usage of natural plant polyphenols which is reported to enter into the BBB and offer the neuroprotection owing to its iron chelating, mitochondrial protective, radical scavenging, anti-apoptotic and anti- inflammatory properties [21, 22]. As the anti-parkinsonic role of dGCE is not investigated till now the current experiment is designed. Numerous biochemical and pathological adverse processes such as oxidative stress, mitochondrial dysfunction, inflammation and apoptosis are reported to be involved in cause and progression of PD [23]. The concept of oxidative stress and antioxidants may be directly or indirectly involved in the pathogenesis of Parkinson's disease [24, 25, 26].

The aetiology of PD may arise owing to the exposure to infectious agents, exogenous or endogenous neurotoxins, mitochondrial dysfunction or genetic basis [24, 26]. Environmental exposure of pesticides and herbicides to humans leads to sporadic PD. Specifically, rotenone, paraquat and maneb have been associated with the PD incidence [27, 28]. Rotenone is reported to cause ROS generation, ATP depletion and cell death in neurons due to its inhibitory action on mitochondrial complex I. Rotenone toxicity mimics many pathological hallmarks of PD, including loss of dopaminergic neurons in SN and formation of Lewy bodies which is presumably due to oxidative damage, mitochondrial dysfunction and disruption of axonal transport [29, 30].

2. MATERIALS AND METHODS

2.1 Measurement of GSH

The method used by Jollow et al. was used to measure the amount of GSH in brain homogenate (1974). The homogenate of brain tissue was centrifuged at 16,000 g for 15 min. at 4 °C. DTNB (dithiobis-2-nitrobenzoic acid) was dissolved in 4 ml of ice-cold, 0.1 mM solution in 1 M phosphate buffer, and the supernatant (0.5 ml) was added (pH 8). In a spectrophotometer, the optical density was read at 412 nm. As a standard, a calibration curve was developed using reduced glutathione [31].

2.2 Measurement of GPx

The GSH reductase solution (10 units/ml), 100 µl of 0.1M GSH, 100 µl of 2mM NADPH, 100 µl of 1M Tris-HCl (pH 8.0), 650 µl of distilled water, 10 µl of 7mM tert-butyl hydroperoxide, and 10 µl of brain supernatant made up the GPx assay mixture. At 340 nm, spectrophotometry was used to monitor the oxidation of NADPH at 37 °C. The quantity of GPX needed to oxidise 1 µmol of NADPH per minute was used to define one unit of activity [32].

2.3 Measurement of TBARS

As discussed previously, the activity of TBARS was determined [33]. In an essence, tissue extracts were incubated with 0.2 ml of phenyl methosulphate at 37 °C in a metabolic water bath shaker. 0.4 ml of 5% tricarboxylic acid and 0.4 ml of 0.67% thiobarbituric acid were added after 1 hour of incubation. After centrifuging the reaction mixture for 15 minutes at 4000 rpm, the supernatant was heated for 10 minutes. Samples were read at 535 nm after cooling. Nanomole of TBARS formed/h/g tissue was used to express the rate of lipid peroxidation.

2.4 Measurement of Catalase

1.95 ml of phosphate buffer (0.1 M, pH 7.4), 1 ml of H_2O_2 (0.09 M), and 0.05 ml of PMS were added. Calculations of catalase activity were done using the formula nmol H_2O_2 consumed minutes/mg protein at 240 nm [35].

2.5 Measurement of SOD

Xanthine and xanthine oxidase are utilised as a superoxide producer and Nitro blue tetrazolium (NBT) is used as a superoxide indicator in an indirect inhibition experiment to measure the activity of superoxide dismutase. 20 µL of xanthine oxidase and 960 µL of a 50 mM sodium carbonate buffer (pH 10.2) containing 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA were added to 20 µL of PMS. By using spectrophotometry, changes in absorbance were seen at 560 nm. Units/min/mg protein was used to express the activity [35].

3. RESULTS

The nigral levels of TBARS and non-enzymatic antioxidants like GSH and the activities of enzymatic antioxidants such as SOD, catalase, and GPx were quantified in control, rotenone and/or dGCE treated rats to assess the antioxidant effect of dGCE.

3.1 Levels of lipid peroxidation in the rotenone induced rat

In the rotenone treated rats, the levels of TBARS and GSH were enhanced and diminished significantly as compared to control animals. The co-administration of dGCE in rotenone injected rats significantly diminished and enhanced the levels of TBARS and GSH levels as compared to rotenone alone injected rats. There is no significant alterations found in the levels of TBARS and GSH bewteen the dGCE alone administered and control rats (Fig 1 and 2).

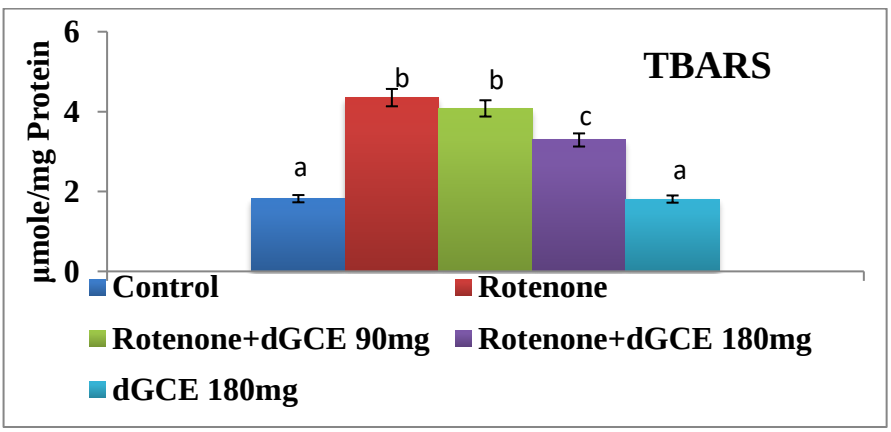

Fig. 1 demonstrates the changes in the levels of TBARS in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

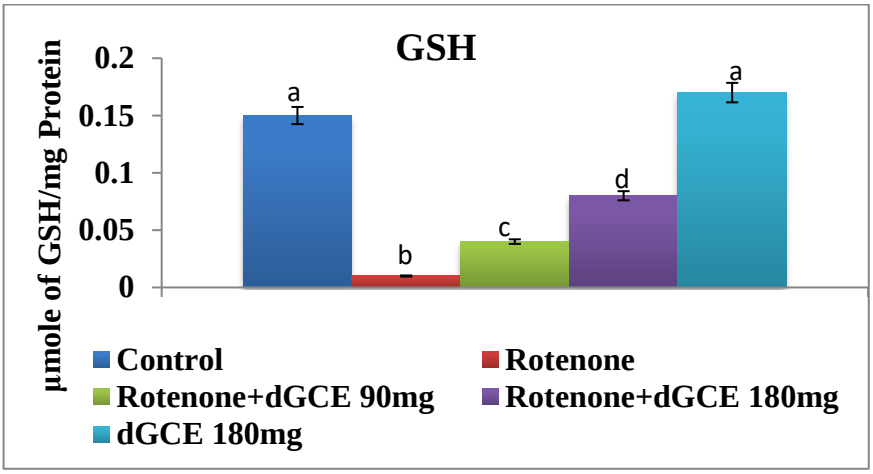

Fig. 2 illustrates the changes in the level of GSH in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

3.2 Levels of antioxidant enzymes in the rotenone induced rat

In the rotenone treated rats, the activities of SOD, catalase and GPx were significantly diminished as compared to control animals. The co-administration of dGCE to rotenone injected rats enhanced rhe activity of enzymatic antioxidants as compared to rotenone alone injected rats. There is no significant alteration found in the activities of enzymatic antioxidant bewteen the dGCE alone administered and control rats (Fig 3, 4 and 5).

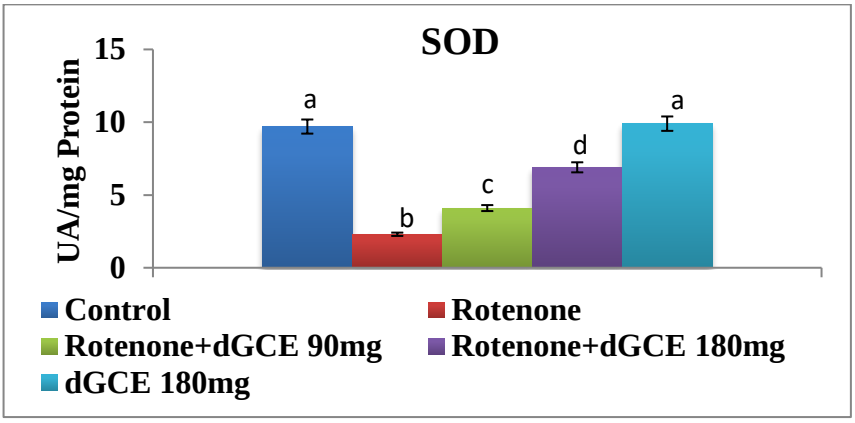

Fig. 3 indicates the changes in the activity of SOD in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

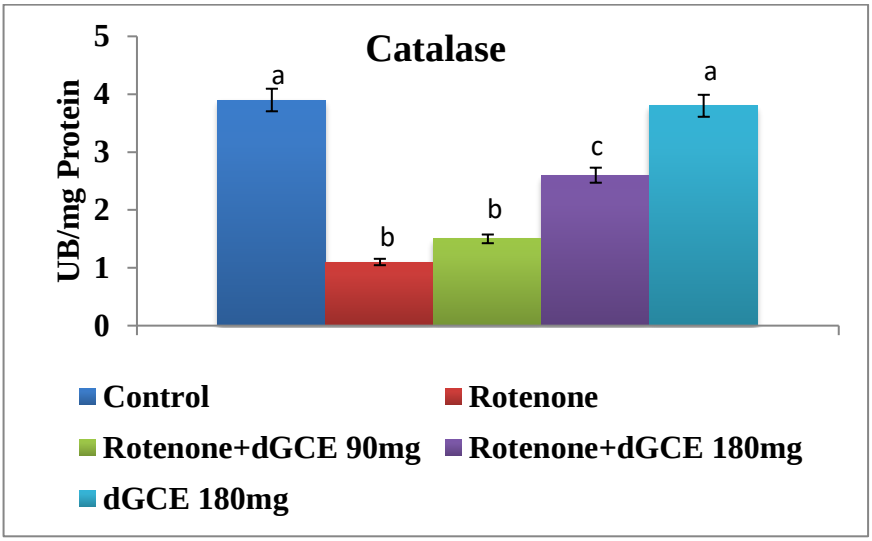

Fig. 4 shows the changes in the activity of catalase in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p< 0.05$) with each other.

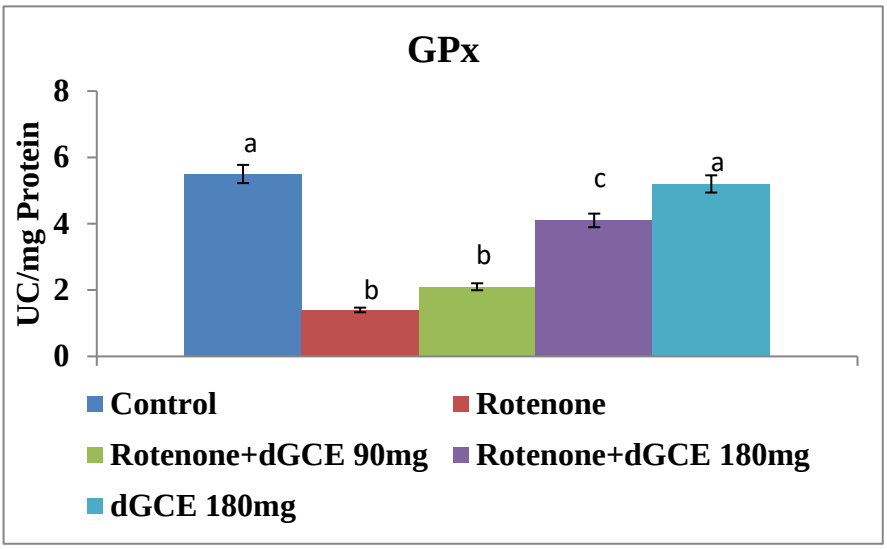

Fig. 5 demonstrates the changes in the activity of GPx in SN of control and experimental rats. Data are shown as mean±SEM for six rats in each group. Values not sharing common alphabet differed significantly ($p<0.05$) with each other.

4. DISCUSSION

In this study, rotenone treatment significantly elevated the levels of TBARS and diminished the activities of enzymatic antioxidants such as SOD, catalase and GPx and decreased the levels of GSH, which corroborate with early finding [20]. Brain is cruelly prone to oxidant-antioxidant imbalance due to its (i) high metabolic activity (because of lengthy axons and millions of synapses that requires more energy [36] and utility of more oxygen (~ 1-3% of mitochondrial oxygen consumption being converted to ROS in normal conditions) [37], (ii) abundance of ROS-synthesising enzyme like as monoamine oxidase and tyrosine hydroxylase in SN [38] (iii) auto-oxidation of DA and its metabolites and (iv) presence of enhanced iron levels in the SN leading to the reduction of H_2O_2 to form the highly reactive hydroxyl radical, (.OH), (v) depleted levels of antioxidants, (vi) enhanced protein folding found in endoplasmic reticulum and cellular Ca^{2+} levels and (vii) increased neuroinflammation leading to over/continuous activation of microglia resulting in more and unregulated discharge of ROS [39, 40]. In the post mortem studies particularly SN of PD patients, increased levels of

oxidative stress and iron concentrations [41], diminished level of GSH [42], enhanced lipid peroxidation products [43] and oxidation of DNA and protein [44] were found. Elevated levels of the lipid peroxidation products like malondialdehyde, TBARS and lipid hydro peroxide were found in the SN [45,46].

Rotenone is the potent inhibitor of mitochondrial electron transport chain (ETC) complex I, thereby preventing the flow of electrons and leading to its accumulation in the ETC-I. As the majority of inhaled oxygen (20%) enters into brain, this accumulated electron directly may to O2 and forms superoxide anion which is the most reactive ROS [47]. The ETC-I is the primary site of ROS synthesis because of its slight inhibition may leads to ROS formation [48]. If the electrons are stayed more than stipulated period they react vigorously with the abundant oxygen molecule and form the ROS molecule in the mitochondria. Enhanced production of ROS molecule leads to depletion of antioxidants which favours the lipid peroxidation process. Bashkatova et al., showed increased NO and TBARS level in the ST and cortex following the chronic rotenone treatment, which corroborates with our study [49]. The antioxidant activity of dGCE is greater than the roasted coffee extract [50, 51] because dGCE is reported to consist of more amount of phenolic acid [52]. The major phenolic compound found in dGCE is CGA which is responsible for its potent antioxidant activity [50]. Co-treatment of dGCE diminished the levels of rotenone induced TBARS levels due to the abundance of CGA, a potent antioxidant compound.

Lowered activities of SOD, catalase and GPx and the levels of GSH found in rotenone injected animals maybe because of bodily response to enhanced levels of ROS and its consequence lipid peroxidation process. Saravanan et al., demonstrated that diminished levels of GSH and activities of SOD, enhanced DA metabolism along with elevated levels of nitric oxide and TBARS which could contribute to dopaminergic neuronal death [53]. CGA consist of one carboxyl group and five active hydroxyl groups in which the phenolic OH group converts free radicals into hydrogen radicals. These hydrogen radicals and superoxide anions are eliminated by its potent antioxidant activity. Hu et al., 2006 reported the superoxide anions, hydrogen radicals and hydrogen peroxide scavenging potential of CGA [54]. The co-administration of dGCE

enhanced the activities of SOD, catalase and GPx and the levels of GSH. Another study by Kim et al., stated that there is no enhanced activity of SOD and catalase, were found in the wound of diabetic rats treated with CGA [55]. But in our study we found that the administration of dGCE enhances the levels and activities of SOD, catalase, GSH and GPx, may be due to the presence of other active components of dGCE. Therefore it is concluded that the antioxidant potential of dGCE found in rotenone induced PD rats were mainly due to the abundant presence of CGA and the other minor active components. This study concludes that the administration of dGCE i.e, even in the absence of caffeine showed potent neuroprotective effect due to the presence of other components.

REFERENCE S

1. Shang F, Li X, Jiang X. Coffee consumption and risk of the metabolic syndrome: A meta-analysis. Diabetes & metabolism. 2016;42(2):80-7.
2. Rosso A, Mossey J, Lippa CF. Caffeine: neuroprotective functions in cognition and Alzheimer's disease. American Journal of Alzheimer's Disease & Other Dementias. 2008 Oct;23(5):417-22.
3. Van Dam RM, Hu FB. Coffee consumption and risk of type 2 diabetes: a systematic review. Jama. 2005;294(1):97-104.
4. Nehlig A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neuroscience & Biobehavioral Reviews. 1999;23(4):563-76.
5. Poole R, Kennedy OJ, Roderick P, Fallowfield JA, Hayes PC, Parkes J. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. bmj. 2017;359.
6. Hu ZF, Yu WL, Zhao YP. Study on the scavenging of ROS and anti-lipid peroxidation by chlorogenic acid. Food Sci. 2006;2:128
7. Smith A. Effects of caffeine on human behavior. Food and chemical toxicology. 2002;40(9):1243-55.
8. Appel CC, Myles TD. Caffeine-induced hypokalemic paralysis in pregnancy. Obstetrics & Gynecology. 2001;97(5):805-7.
9. Cornelis MC. Coffee intake. Progress in molecular biology and translational science. 2012;108:293-322.

10. Balyaya KJ, Clifford MN. Individual chlorogenic acids and caffeine contents in commercial grades of wet and dry processed Indian green Robusta coffee. JOURNAL OF FOOD SCIENCE AND TECHNOLOGY-MYSORE-. 1995;32:104-8.

11. Silvarolla MB, Mazzafera P, Fazuoli LC. A naturally decaffeinated arabica coffee. Nature. 2004;429(6994):826.

12. Statista. Coffee Report 2020. Available online: https://www.statista.com/study/48823/coffee-report/ (accessed on 12 October 2021).

13. Chindapan N, Soydok S, Devahastin S. Roasting kinetics and chemical composition changes of Robusta coffee beans during hot air and superheated steam roasting. Journal of food science. 2019;84(2):292-302.

14. Shahidi F, Chandrasekara A. Hydroxycinnamates and their in vitro and in vivo antioxidant activities. Phytochemistry Reviews. 2010;9(1):147-70.

15. McCarty MF. A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. Medical hypotheses. 2005;64(4):848-53.

16. Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food and chemical toxicology. 2010;48(3):937-43.

17. Dos Santos MD, Almeida MC, Lopes NP, De Souza GE. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biological and Pharmaceutical Bulletin. 2006;29(11):2236-40.

18. Li Y, Shi W, Li Y, Zhou Y, Hu X, Song C et al. Neuroprotective effects of chlorogenic acid against apoptosis of PC12 cells induced by methylmercury. Environmental Toxicology and Pharmacology. 2008;26(1):13-21.

19. Nikam S, Nikam P, Ahaley SK, Sontakke AV. Oxidative stress in Parkinson's disease. Indian Journal of Clinical Biochemistry. 2009;24(1):98-101.

20. Dhanalakshmi C, Janakiraman U, Manivasagam T, Justin Thenmozhi A, Essa MM, Kalandar A et al. Vanillin attenuated behavioural impairments, neurochemical deficts, oxidative stress and apoptosis against rotenone induced rat model of Parkinson's disease. Neurochemical research. 2016;41(8):1899-910.

21. Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. The American journal of clinical nutrition. 2005;81(1):313S-6S.

22. Hider RC, Liu ZD, Khodr HH. Metal chelation of polyphenols. InMethods in enzymology 2001 (Vol. 335, pp. 190-203). Academic Press.

23. Martin JB. Molecular basis of the neurodegenerative disorders. New England Journal of Medicine. 1999;340(25):1970-80.

24. Pioro EP. Antioxidant therapy in ALS. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders. 2000;1(sup4):S5-15.

25. Kowall NW, Ferrante RJ, Martin JB. Patterns of cell loss in Huntington's disease. Trends in Neurosciences. 1987;10(1):24-9.

26. Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Hasan K et al. Neuroinflammation, oxidative stress, and the pathogenesis of Parkinson's disease. Clinical neuroscience research. 2006;6(5):261-81.

27. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE et al. Pesticide exposure and risk for Parkinson's disease. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2006;60(2):197-203.

28. Ferraz HB, Bertolucci PH, Pereira JS, Lima JG, Andrade LA. Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology. 1988;38(4):550-.

29. Uversky VN. Neurotoxicant-induced animal models of Parkinson's disease: understanding the role of rotenone, maneb and paraquat in neurodegeneration. Cell and tissue research. 2004;318(1):225-41.

30. Maguire-Zeiss KA, Short DW, Federoff HJ. Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease?. Molecular Brain Research. 2005;134(1):18-23.

31. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151-69.

32. Yamamoto Y, Takahashi K. Glutathione peroxidase isolated from plasma reduces phospholipid hydroperoxides. Archives of Biochemistry and Biophysics. 1993;305(2):541-5.

33. Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of Withania somnifera glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. Journal of ethnopharmacology. 2001;74(1):1-6.

34. Claiborne AJ. Handbook of methods for oxygen radical research. Florida: CRC Press, Boca Raton. 1985:283-4.

35. Oberley LW, Spitz DR. [61] Assay of superoxide dismutase activity in tumor tissue. InMethods in enzymology 1984 (Vol. 105, pp. 457-464). Academic Press.

36. Ciulla M, Marinelli L, Cacciatore I, Stefano AD. Role of dietary supplements in the management of Parkinson's disease. Biomolecules. 2019;9(7):271.

37. Kirkinezos IG, Moraes CT. Reactive oxygen species and mitochondrial diseases. InSeminars in cell & developmental biology 2001 (Vol. 12, No. 6, pp. 449-457). Academic Press.

38. Spencer, J.P., 2007. The interactions of flavonoids within neuronal signalling pathways. Genes & Nutrition, 2(3), pp.257-273.

39. Puspita L, Chung SY, Shim JW. Oxidative stress and cellular pathologies in Parkinson's disease. Molecular brain. 2017;10(1):1-2.

40. Angelopoulou E, Piperi C, Papavassiliou AG. High-mobility group box 1 in Parkinson's disease: from pathogenesis to therapeutic approaches. Journal of neurochemistry. 2018;146(3):211-8.

41. Good PF, Olanow CW, Perl DP. Neuromelanin-containing neurons of the substantia nigra accumulate iron and aluminum in Parkinson's disease: a LAMMA study. Brain research. 1992;593(2):343-6.

42. Sian J, Dexter DT, Lees AJ, Daniel S, Agid Y, Javoy-Agid F et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 1994;36(3):348-55.

43. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, Mizuno Y. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proceedings of the National Academy of Sciences. 1996;93(7):2696-701.

44. Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B. A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. Journal of neurochemistry. 1997;69(3):1326-9.

45. Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A et al. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. Journal of neurochemistry. 1989;52(2):381-9.

46. Dexter DT, Holley AE, Flitter WD, Slater TF, Wells FR, Daniel SE et al. Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: an HPLC and ESR study. Movement Disorders. 1994;9(1):92-7.

47. Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proceedings of the Society for Experimental Biology and Medicine. 1999;222(3):236-45.

48. Seaton TA, Cooper JM, Schapira AH. Free radical scavengers protect dopaminergic cell lines from apoptosis induced by complex I inhibitors. Brain research. 1997 Nov 1;777(1-2):110-8.

49. Bashkatova V, Alam M, Vanin A, Schmidt WJ. Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. Experimental Neurology. 2004;186(2):235-41.

50. Górnaś P, Dwiecki K, Siger A, Tomaszewska-Gras J, Michalak M, Polewski K. Contribution of phenolic acids isolated from green and roasted boiled-type coffee brews to total coffee antioxidant capacity. European Food Research and Technology. 2016;242(5):641-53.

51. Priftis A, Stagos D, Konstantinopoulos K, Tsitsimpikou C, Spandidos DA, Tsatsakis AM et al. Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. Molecular medicine reports. 2015;12(5):7293-302.

52. Faraji M, Yamini Y, Gholami M. Recent advances and trends in applications of solid-phase extraction techniques in food and environmental analysis. Chromatographia. 2019;82(8):1207-49.

53. Saravanan KS, Sindhu KM, Mohanakumar KP. Melatonin protects against rotenone-induced oxidative stress in a hemiparkinsonian rat model. Journal of pineal research. 2007;42(3):247-53.

54. Hu ZF, Yu WL, Zhao YP. Study on the scavenging of ROS and anti-lipid peroxidation by chlorogenic acid. Food Sci. 2006;2:128-30.

55. Kim JK, Park SU. Chlorogenic acid and its role in biological functions: an up to date. EXCLI journal. 2019;18:310-6.