Available Online at www.ijpscr.info
International Journal of Pharmaceutical Sciences and Clinical Research 2021; 1(1):79-94

CASE STUDY

Therapeutic and Prophylactic Effect of some Agents on Experimental Induced Parkinson Disease Models Review

L. Sarvananda[1], Amal D. Premarathna[2]

[1]Department of Farm Animal Production and Health, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka, [2]Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka

Received: 15-12-2020; Revised: 10-01-2021; Accepted: 01-02-2021

ABSTRACT

Neurodegenerative movement disorder of the central nervous system (CNS) (Parkinson's disease [PD]) is characterized by necrosis of dopaminergic neurons in the substantia nigra pars compacta region of the midbrain. The etiology of PD is still unknown and is believed to be multifactorial, oxidative stress and mitochondrial dysfunction are widely considered major consequences, which provide important clues to the disease mechanisms. Studies have showed the role of free radicals and oxidative stress that contributes to the cascade of events leading to dopamine cell degeneration in PD. In general, in-built protective mechanisms consisting of enzymatic and non-enzymatic antioxidants in the CNS play decisive roles in preventing neuronal cell loss due to free radicals. However, the ability to produce these antioxidants decreases with aging. Therefore, antioxidant therapy alone or in combination with current treatment methods may represent an attractive strategy for treating or preventing the neurodegeneration seen in PD. Here, we summarize the recent discoveries of potential antioxidant compounds for modulating free-radical mediated oxidative stress leading to neurotoxicity in PD.

Keywords: Antioxidants, Free radicals, Neurodegerative, Neuroprotection, Oxidative stress, Parkinson's disease

INTRODUCTION

Neurodegenerative disorders are chronically progressive group of diseases involving nerve cells death. Majority of these diseases including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and cerebral ischemia are result of oxidative stress.[1] The brain is a very active organ of human body weighing only 2% of the body weight but it consumes 20% of body oxygen and 25% of body glucose at rest.[2] Since reactive oxygen species (ROS) produced in any tissue is directly proportional to its oxygen consumption which further increases with intellectual process such as thinking planning and reasoning,[3] the brain is continuously under oxidation/antioxidation process which makes it prone to oxidative damage. Several antioxidant mechanisms are available within brain to combat ROS. Brain cells have catalase and glutathione peroxidase in their cytosol which hydrolyzes H_2O_2 and reduces organic hydroperoxides respectively. Neuronal mitochondria have superoxide dismutase (SOD) for converting $.O_2$- to H_2O_2 which is further metabolized by catalase and thus preventing the formation of neurotoxic and inflammatory cytokine-inducing .ONOO- from $.O_2$ - and .NO.[4] Although, brain cells have defense mechanisms for

***Corresponding Author:**
L. Sarvananda,
E-mail: sarvacool18@gmail.com

dealing with ROS, yet it has been practically found that when level of ROS goes unusually high or antioxidant defense goes low, cells bear oxidative damage ultimately leading to neurodegenerative disorders.[5] Exogenous H_2O_2 may also produce ROS beyond the capacity of cellular defense system leading to apoptotic cell death.[6] So far, there are no effective drugs in conventional system which can effectively combat or check the onset or progression of neurodegenerative diseases.[7] However, Ayurveda has used many herbs for centuries to successfully treat and prevent neurodegenerative diseases.[8] There is very few scientific studies available showing neuroprotective effect of these plants.[9] Therefore, search for novel therapies with little or no side effects is increasing day by day. Medhya Rasayana is Ayurvedic drugs known to improve physical and mental health and immunity of the body.[10]

Antioxidants are widely discussed in both the lay press and the scientific literature as health promoting agents that may protect against various age-related diseases.[11] Antioxidants are exogenous or endogenous molecules that act against any form of oxidative stress and its associated ill effects on cellular systems.[12] The state of oxidative imbalance found during neurodegenerative processes is triggered by one or more factors such as brain aging, genetic predisposition, mitochondrial dysfunction, free-radical production, and environmental toxins.[13] To overcome free-radical-mediated consequences of disease processes and drug therapies, antioxidants are now being looked on as persuasive therapeutics against neuronal loss, as they have the capability to neutralize free radicals.[14] Therefore, the aim of this study is to review the recent study on the neuroprotective potential of antioxidants agents in Parkinson Disease experimental models.

MATERIALS AND METHODS

Chemicals and reagents

Immunocyto-fluorescence used in Western blotting, primary antibodies were monoclonal anti-HSP70 (Clone BRM-22, Sigma-Aldrich), anti-Grp 75 (Mortalin) (Abcam), and anti-α-tubulin (Clone AA13, Sigma-Aldrich). Anti-mouse IgG:HRP (Bangalore Genei) and anti-mouse Alexa Fluor 568 (Invitrogen) were used as secondary antibodies. The 3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyl tetrazolium bromide (MTT), Quercetin, and 1'-1" Diphenyl-2'-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich. The PCR reagents including dNTP Mix, Random Hexamer Primer, 100bp ladder, Reverse Transcriptase, and Taq DNA Polymerase were purchased from Fermentas, Thermo Fisher Scientific. Primers for synthesis of cDNA for α-tubulin, HSP, and Mortalin were prepared from Biolink, India. All other chemicals and reagents were procured in their purest form available commercially from Indian companies.[15]

Preparation of CP-MEx, CP-EEx, and CP-WEx

Dried whole plants of *C. pluricaulis* were procured from local Ayurvedic Merchants and got identified from the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, India. These were then powdered and 10 g of dry rhizome powder each was suspended separately in 100 ml of methanol/ethanol/distilled water and kept stirring for 48 h at 30 ± 5°C, followed by filtration under sterile conditions. The filtrates, thus, obtained were concentrated with a vacuum rotary evaporator (Buchi, Switzerland) at temperature of 35°C and pressures 280, 170, and 60 mbar for methanolic, ethanolic, and water extracts, respectively. The concentrated extracts, thus, obtained are air dried to make powder. These were further diluted in respective solvent to give final concentration of 50 μg/ml each for CPMEx, CP-EEx, and CP-WEx.[15]

Effect of curcumin on rotenone toxicity, the LC_{50}

To test effect of curcumin on rotenone toxicity, the LC_{50} (the concentration causing 80% cell death) of rotenone was used. Pretreatment cells with curcumin at 0, 0.1, 05, 1, and 5 μM concentrations for 1 h, and then exposed them to LC80 of rotenone for 24 h was done. A pan caspase inhibitor, z-VAD (100 μM) as a positive control for curcumin protective experiments

was used. Trypan blue exclusion was used to measure cell death by counting the number of dead (blue) and live cells in the cultures after rotenone exposure and/or curcumin treatment.

2.2.3 In Experiment I, cells were incubated with different concentrations of rotenone (2.5, 5, 50, 100, and 200 nM) for 24 h, and MTT assay was performed to detect IC_{50} value of rotenone. In Experiment II, cells were pretreated with different concentrations of hesperidin (2.5, 5, 10, 20, and 40 μg) for 4 h and then incubated with rotenone (effective dose) for 24 h. The effective dose of hesperidin was used to identify potential neuroprotective effects against rotenone toxicity.[16]

Cell culture and treatments

Human neuroblastoma cell line was obtained from NCCS, Pune, India, and maintained on Dulbecco's Modified Eagle's Medium (DMEM) supplemented with streptomycin (100 U/ml), gentamycin (100 μg/ml), 10% FCS (Life Technologies) at 37°C, and humid environment containing 5% CO_2. The H_2O_2 dose (IC_{50}) for neuroprotection studies was calculated by treating cells with H_2O_2 (7.5 μM–1000 μM diluted in medium) at 50% confluency for 24 h in serum free medium. To obtain the cytotoxicity profile and non-toxic dose of *C. pluricaulis* extracts was tested at higher doses from 25 to 2000 μg/ml. The human neuroblastoma cells were treated with CP-MEx, CP-EEx, CP-WEx, and quercetin at concentration from 1.5 μg/ml to 50 μg/ml diluted in medium for 24 h at 30–40% confluency and then subjected to H_2O_2 (IC_{50} concentration, i.e., 250 μM) treatment for 24 h in serum free medium. The medium of control culture without H_2O_2 and without extract was replaced with a fresh one.[17]

Cell viability assay

MTT was used to assess cell integrity and potential cytotoxicity of the plant extract by monitoring the uptake of the vital mitochondrial dye, 3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyl tetrazolium bromide (MTT) by cell mitochondria.[18]

Chemical standardization of CP-MEX and nature of active components

CP-MEx was subjected to preliminary phytochemical screening for alkaloids, amino acids, anthraquinones, flavonoids, phytosterols, saponins, steroids, tannins, triterpenoids, and reducing sugars following the methods of Harborne. It was further subjected to thin-layer chromatography (TLC) using chloroform:methanol (19:1) as solvent front. TLC plate was subjected to iodine vapors for observation.[19]

Estimation of activities of antioxidant enzymes and levels of antioxidants

Catalase

Catalase activity was measured according to the method of Aebi. The rate of decomposition of H_2O_2 by catalase was measured spectrophotometrically at 240 nm. The reaction mixture (1 ml) contained 0.8 ml phosphate buffer (0.2 M, pH 7.0) containing 12 mM H_2O_2 as substrate, 100 μl enzyme sample and distilled water to make up the volume. The decrease in absorbance/minute at 240 nm was recorded against H_2O_2-phosphate buffer as blank.[20]

SOD

SOD was estimated according to the method of Kono. This method is based on the principle of the inhibitory effects of SOD on the reduction of nitrobluetetrazolium (NBT) dye by superoxide radicals, which are generated by the autoxidation of hydroxylamine hydrochloride. The reduction of NBT was followed by an absorbance increase at 540 nm. In the test cuvette, the reaction mixture contained the following: 1.3 ml sodium carbonate buffer (50 mM), pH 10.0, 500 μl NBT (96 μM), and 100 μl triton X-100 (0.6%). The reaction was initiated by addition of 100 μl of hydroxylamine hydrochloride (20 mM), pH 6.0. After 2 min, 50 μl enzyme samples were added and the percentage inhibition in the rate of NBT reduction was recorded.[21]

Reduced glutathione (GSH) and glutathione peroxidase (GPx)

Total glutathione was measured as described by Sedlak and Lindsay. In brief, 100 µl samples were mixed with 4.4 ml of 10 mM EDTA and 500 µl of trichloroacetic acid (50% w/v). Contents were centrifuged at 3000×*g* for 15 min. The supernatant so obtained was mixed with 50 µl of 5,5-dithiobis(2-nitrobenzoic acid) (10 mM) and absorbance was measured at 540 nm. Standard curve was prepared using pure glutathione. Glutathione peroxidase activity was measured indirectly by monitoring the oxidation of NADPH. The reaction mixture (1 ml) containing 100 mM GSH, 15nM NADPH, and 15nM H_2O_2 in potassium phosphate buffer (50 mM, pH 7.5) was mixed with sample (50 µl) and the change in absorbance was monitored at 340 nm. Glutathione peroxidase activity is defined as 1 µmol of NADPH oxidized per min at pH 7.5 at 25°C using purified GPx enzyme.[21]

Lipid peroxidation (LPx)

Method of Beuge and Aust was followed to measure the lipid peroxidation level. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate melondialdehyde (MDA) on decomposition. MDA forms a 1:2 adduct with thiobarbituric acid (TBA) that gives a red colored product having absorption maximum at 532 nm. 100 µl samples were incubated with 100 µl each of FeSO4 (1 mM), ascorbic acid (1.5 mM), and Tris- HCl Buffer (150 mM, pH 7.1) in a final volume made of 1 ml, made up by DDW, for 15 min at 37°C. The reaction was stopped by adding 1 ml of trichloroacetic acid (10% w/v). This was followed by addition of 2 ml thiobarbituric acid (0.375% w/v). After keeping in boiling water-bath for 15 min, contents were cooled off and then centrifuged. The absorbance of supernatant so obtained was measured at 532 nm.[22]

Immunocytochemistry

All cells, control, and treated were rinsed 3 times with ice cold 0.1M PBS and fixed with Paraformaldehyde (4%) for 30 min. Permeabilization was carried out with 0.32% PBST for 15 min. Coverslips were washed thrice with 0.1%PBST followed by blocking with 5% NGS (Normal Goat Serum) prepared in 0.1% PBST for 1 h at room temperature. Cells were incubated with mouse anti-NF-200, mouse anti-HSP70, and mouse anti-Mortalin and, diluted in 0.1% PBST, for 24 h at 4°C in humid chamber. Coverslips were then washed with 0.1% PBST thrice. Secondary antibody (anti-mouse Alexa Fluor 488, anti-mouse Alexa Fluor 568 and anti-rabbit Alexa Fluor 488) was applied diluted (1:200) in 0.32% PBST for 2 h at room temperature. Coverslips were washed three times with 0.1% PBST and final washing was given with 0.1 M PBS. These were on the slides with anti-fading mounting media (Sigma) and were observed under fluorescent microscope Nikon E600. Images were captured using Cool Snap CCD camera and the pictures were analyzed using ImageJ 1.44p, NIH, USA.[23]

Reverse transcription-PCR

Human Neuroblastoma cell line cells from 25 cm^2 culture flask were homogenized in TRI Reagent (Sigma). Total RNA was extracted and reverse transcriptized according to the manufacturer's instruction.[24]

Data availability

The datasets analyzed during the current study are available in the PubMed, MEDLINE, DOAJ, Google Scholar, and Search Engine repository. These datasets were derived from the following public domain resources PubMed: https://pubmed.ncbi.nlm.nih.gov/, Google Scholar SEO: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Human+Neuroblastoma+cell+line+cells+from+25+cm2+culture+flask+were+homogenized+in+TRI+Reagent+%28Sigma%29.+&btnG=andDOAJ: https://doaj.org/

RESULTS AND DISCUSSION

Antioxidant compounds in experimental models of PD

Curcumin, the well-known component of yellow curry spice [Figure 1A and B] derived from turmeric,

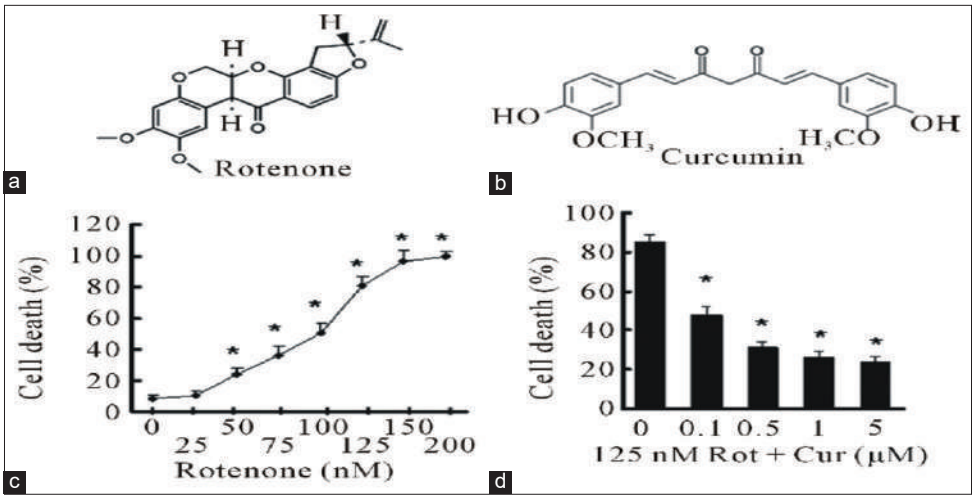

Figure 1A: Curcumin protected against Rotenone caused cell death *in vitro*. (a) Chemical structure of Rotenone; (b) Dose response curve of Rotenone-induced cell death in SH-SY5Y cells. SH-SY5Y cells were treated with Rotenone for 24 h in 2% FBS OPTI-1 media. Cell death was measured with Trypan blue assay. Values are expressed as means ± SEM. Ratio of cell death in multiple groups was significantly higher than that of control group with no Rotenone exposure (*$P < 0.05$); (c) chemical structure of curcumin; (d) SH-SY5Y cells were pre-treated with curcumin at the indicated concentrations for 1 h, then either left untreated (control) or treated with 125 nM rote- none for 24 h. Cell death was measured with Trypan blue exclusion assay. Curcumin treated cells showed significant lower cell death rate when compared to those cells with rotenone exposure but no curcumin treatment (*$P < 0.05$).

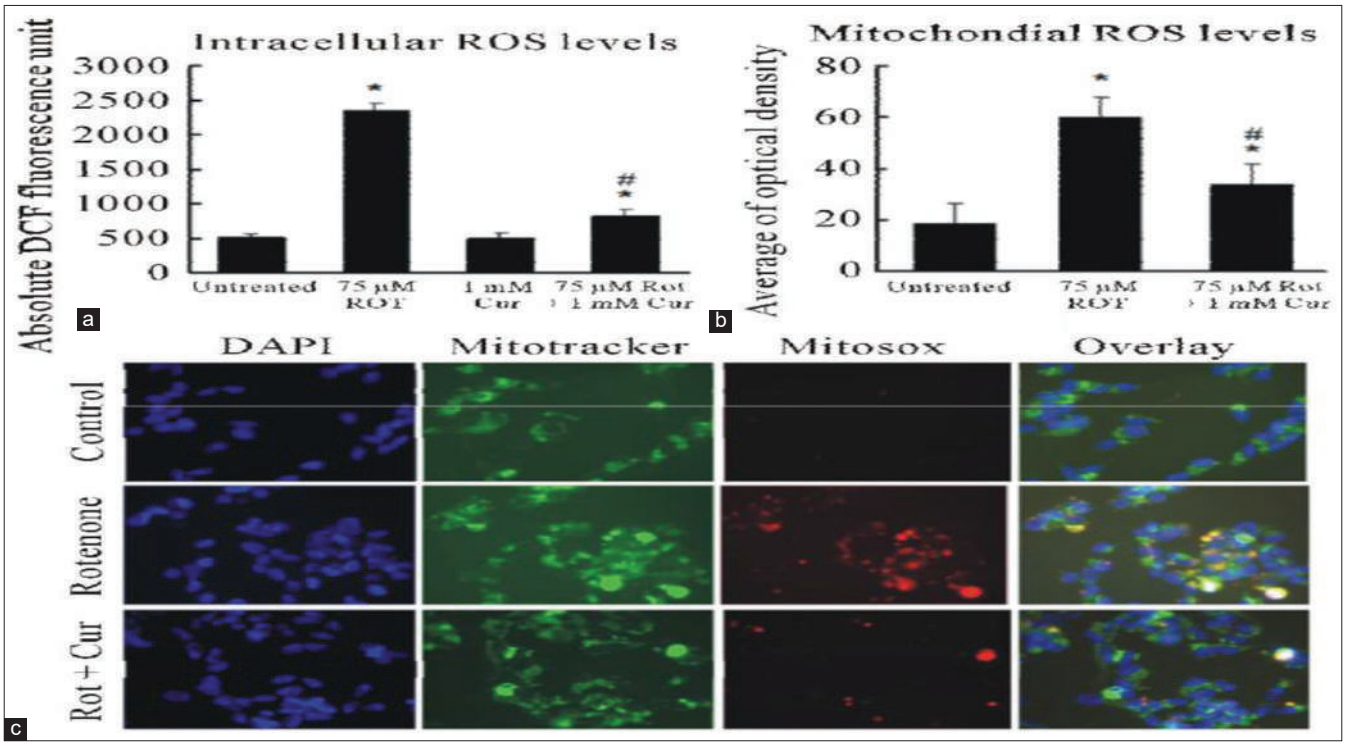

Figure 1B: Curcumin reduced intracellular and mitochondrial ROS levels. (a) Quantification of DCF fluorescence in SH-SY5Y cells. Curcumin significantly reduced rotenone-induced intracellular ROS increase; (b and c) curcumin significantly reduced rotenone-induced ROS in mitochondria. Digital photomicrograph under fluorescent illumination showing mitochondria superoxide signal is shown in (c) and the average optical density of MitoSOX is summarized in (b). The ROS level is indicated by the average value of cellular MitoSOX optical density in ten random fields in each condition. Data are shown as the mean ± SEM for three separate experiments. (*$P < 0.05$, ROS level was significantly different when compared with the cells with no rotenone and no curcumin treatment. $^{\#}P < 0.05$, ROS level was significantly different when compared to cells with rotenone exposure).

has been used as a food preservative and herbal medicine in India for hundreds of years.[25] In a recent study conducted; chronic dietary supplementation with turmeric protected against MPTP-mediated neurotoxicity *in vivo* in a mice model of PD.[26] In another study to explored the protective effects of curcumin against rotenone-induced toxicity in SH-SY5Y cells, scientist also showed that curcumin administration (1 mM) protected against rotenen-induced cell death in a dose-dependent manner by reducing the intracellular ROS levels, mitochondrial depolarization, cytochrome c release, and caspase-9 and caspase-3 activation.[27] Figure 2A and B convolvulus pluricaulis, is among the frequently prescribed rasayanas for improvement of learning and memory and also treatment of mental health problem. CP-MEX, CP-EEX, and CP-WEX have been reported to protect the IMR32 neuroblastoma cell from induce toxic effect of H_2O_2 at dose-dependent manner (Kshitija *et al*., 2012). The authors show that CP-MEX 25 μM yield better result compare to CP-EEX and CP-WEX.

Hesperidin [Figure 1], a naturally occurring flavonone major flavanone that exist in citrus and other plants and can isolated in large amount from peels of *Citrus aurantium* (bitter orange).[28] It has been reported that, this plant exert wide range of pharmacological effects such as antioxidant, anti-inflammatory, anti-hypercholesterolemic, and anticarcinogenic.[29] Antioxidant activity of Hesperidin has been tested and reported to protect SK-N-SH human neuroblastoma cells against cytotoxicity induce by rotenone at the dose of 20 μg.[30]

Quercetin [Figure 1B], a major flavonoid, deserves attention because of its beneficial effects observed in various *in vitro* and *in vivo* neural damage models. In a recent study, neuroprotective effects

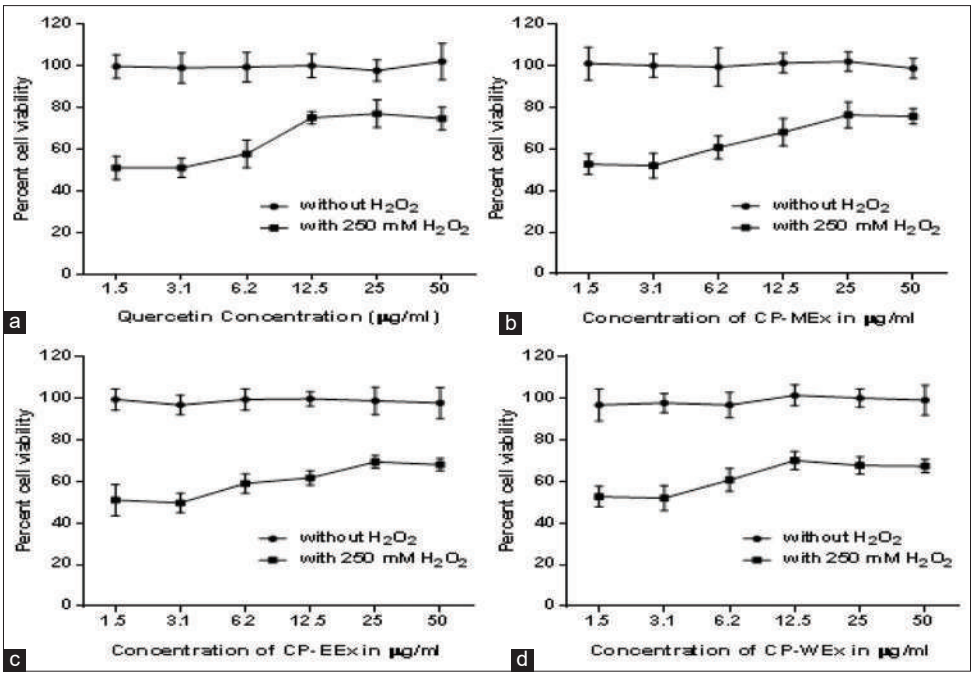

Figure 2A: The effects of quercetin on IMR32 neuroblastoma cell viability in the absence or presence of H_2O_2. (a) Incubation of quercetin with IMR32 cells for 24 h protected against the cytotoxicity elicited by H_2O_2 in a concentration-dependent manner. No cytotoxicity was observed in the cells after incubation with quercetin alone for 24 h in the concentration range 1.5–50 μg/ml. (b) The effects of CP-MEx on IMR32 cell viability in the absence or presence of H_2O_2. Incubation of the extract with HepG2 cells for 24 h protected against the cytotoxicity elicited by H_2O_2 in a concentration-dependent manner. No cytotoxicity was observed in the cells after incubation with the extract alone for 24 h in the concentration range 1.5–50 μg/ml. (c) The effects of CP-EEx on IMR32 cell viability in the absence or presence of H_2O_2. Incubation of the extract with HepG2 cells for 24 h protected against the cytotoxicity elicited by H_2O_2 in a concentration-dependent manner. No cytotoxicity was observed in the cells after incubation with the extract alone for 24 h in the concentration range 1.5–50 μg/ml. (c and d) The effects of CP-WEx on IMR32 cell viability in the absence or presence of H_2O_2. Incubation of the extract with HepG2 cells for 24 h protected against the cytotoxicity elicited by H_2O_2 in a concentration-dependent manner. No cytotoxicity was observed in the cells after incubation with the extract alone for 24 h in the concentration range 1.5–50 μg/ml.

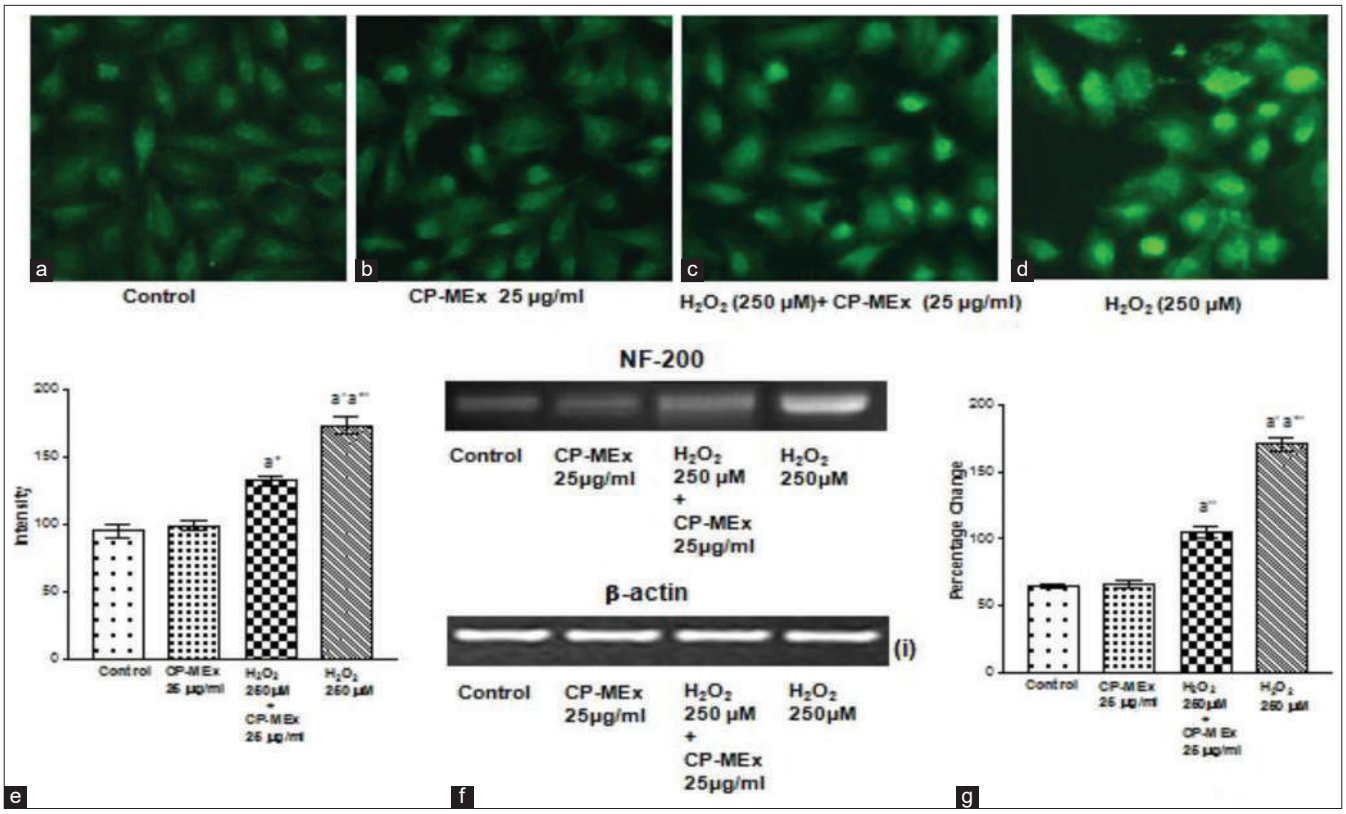

Figure 2B: Localization of NF-200 in IMR 32 neuroblastoma cells (a), untreated control (b), CP-MEx treated (c) CP-MEx+H_2O_2 treated (d) H_2O_2 treated. Cells grown on coverslips (n = 5) for 4 days were fixed and stained for NF-200 (Alexa Fluor 488) immunoreactivity. (e) Relative intensity measurement of NF-200 immunofluorescence performed by ImageJ 1.44p. (f) Representative reverse transcription-polymerase chain reaction (RT-PCR) showing NF-200 and β-actin expression in untreated control, CPMEx treated, CP-MEx+H_2O_2 treated, H_2O_2 treated IMR 32 neuroblastoma cells. (g) Relative optical density measurement of mean values of NF-200 expression in RT-PCR for each group expressed as percentage of β-actin. The values having $P < 0.05$ are considered significant. a', statistically significant change in H_2O_2 treated cultures with respect to the control cultures; a", statistically significant change in CP-MEx + H_2O_2 treated cultures with respect to the CP-MEx treated cultures; a'", statistically significant change in H_2O_2 treated cultures with respect to the CP-MEx + H_2O_2 treated cultures.

of quercetin in PC12 cells and in a zebrafish model were investigated.[31] Quercetin at 25, 50, and 100 µM prevented 6-OHDA-induced PC12 cell apoptosis. In the zebrafish model, pretreatment with quercetin at 6 and 12 µM significantly attenuated 6-OHDA-stimulated DAergic neuron loss leading to its development as an effective therapeutic agent for treating PD.[32] In addition, new roles for quercetin in hypoxia and ischemia-induced neuroprotection in relation to suppression of oxidative stress, improvement in behavioral function, reduction in infarct volume, brain swelling, and cellular injury in both *in vivo* and *in vitro* models based on its antioxidant functions are also well studied.[33]

Coenzyme Q10 [CoQ10 Figure 1C] is a key component of the electron transport chain and plays an essential role in ATP production. In a recent study, the therapeutic effect of CoQ10 and reduced CoQ10 in the MPTP model of Parkinsonism mice was studied.[34] CoQ10 administered at 1600 mg/kg/day resulted in significant protection against loss of DA induced by MPTP treatment (10 mg/kg, i.p., each 2 h × 3 doses), which was accompanied by a marked increase in plasma concentrations of CoQ10. In a chronic MPTP model (40 mg/kg/day for 1 month), CoQ10 treatment at 1600 mg/kg/day in the diet also showed excellent therapeutic effects by significantly inhibiting striatal DA depletion, loss of dopaminergic neurons in the SNpc, and the formation of SNCA aggregate in the dopaminergic neurons of mice.

CoQ10 and creatine [Figure 1D] combination therapy has been investigated in a MPTP mouse

model of PD.[35] The researchers show that supplementation with these combined agents in mice through a diet with 2% creatine and 1% CoQ10 for 1 week before MPTP treatment (40 mg/kg body weight daily for 28 days through osmotic pumps) produced additive neuroprotective effects against dopamine depletion in the striatum and loss of tyrosine hydroxylase (TH) neurons in the SNpc, reduced lipid peroxidation and pathologic SNCA accumulation in SNpc neurons, and loss of DAergic neurons.

Resveratrol [Figure 1E] is a well-known antioxidant that exerts extensive pharmacological effects including anti-inflammatory, anti-mutation, anti-tumor, and blood fat regulatory functions.[36] Oral treatment with resveratrol or a resveratrol liposome (20 mg/kg/day) for 14 days protected DAergic neurons in PD rats. The levels of total ROS decreased markedly, and the total antioxidant capability of nigral tissues improved significantly. The scientist concluded that the radical scavenging ability and antioxidant properties of resveratrol may contribute to its potent neuroprotection in PD. Further, a single dose of up to 5 g of resveratrol caused no serious adverse effects in healthy volunteers in clinical studies due to its safe use for neuroprotection.[37]

Luteolin [Figure 2A] is a polyphenolic compound found in many foods including peanut shells, parsley, artichoke leaves, celery, peppers, olive oil, rosemary, lemons, peppermint, sage, and thyme. Luteolin (5, 10, and 20 μM) significantly attenuated the increase in ROS production and prevented decreases in activities of mitochondria, CAT, and GSH in ROS-insulted primary neurons. That study indicated that the neuroprotection exerted by luteolin in ROS-insulted primary neurons might occur through a rebalancing of pro-oxidant-antioxidant status.[38]

Brassinosteroids (BRs) [Figure 2] are highly oxygenated steroids isolated from several vegetables, including Vicia faba seeds and pollen.[39] In a recent study, two natural BRs and five synthetic analogs were synthesized and evaluated for their neuroprotective actions against MPP+-induced neuronal PC12 cells. The researchers suggested that selected BRs and analogs protected neuronal PC12 cells against MPP+ toxicity and exerted neuroprotective effects derived from their antioxidative properties. In addition, they reported that the steroid B-ring and lateral chain play an important antioxidative role in the neuroprotective action and further research on *in vivo* animal models of PD should be conducted.[40]

It has been explored that, the compound idebenone [Figure 2B] to extend lifespan and improve motor function in HtrA2 knockout mice.[41] Feeding HtrA2 knockout mice with idebenone (500 mg/kg body weight/day orally) extended lifespan and delayed worsening of the motor phenotype. Experiments conducted in cell culture and on brain tissue of mice revealed that idebenone acts by downregulating the integrated stress response. The researchers reported that idebenone ameliorates disease symptoms in HtrA2 knockout mice indicating that antioxidants might delay neuronal degeneration in the striata of these mice. This result illustrates the potential of idebenone for treating neurodegenerative diseases including PD.[42]

3α-acetoxyeudesma-1,4(15),11(13)-trien-12,6a-olide [AETO, Figure 2C] is a compound isolated from the leaves of Laurusnobilis L., AETO (0.4, 2, and 10 μM) decreases the active form of caspase-3 and the levels of p53, which were accompanied by increased levels of Bcl-2 in a dose-dependent manner. Flow cytometry and Western blot analyses showed that AETO significantly inhibits DA-induced apoptosis and suppresses intracellular tyrosinase activity, ROS generation, quinoprotein, and SNCA formation. These results indicate that AETO inhibits DA-induced apoptosis, which is closely related to the suppression of intracellular tyrosinase activity and the formation of α-syn, ROS, and quinoprotein in SH-SY5Y cells.[43]

S-allylcysteine [SAC, Figure 2D] is sulfur-containing compounds derived from garlic have various biological actions. The most abundant organosulfur compound in aged garlic extracts has been evaluated for its protective actions against oxidative stress induced by MPP+ in the striatum of C57BL/6J mice. Pretreatment with SAC (125 mg/kg i.p.) daily for 17 days, followed by administration of MPP+ (0.72 mg/kg i.c.v.), significantly attenuates MPP+-induced loss of striatal DA levels (32%).

SAC significantly blocks (100% of protection) lipid peroxidation and reduction of superoxide radical production indicated by upregulation of Cu-Zn-superoxide dismutase activity in MPP+-induced mice. Behavioral analyses showed that SAC improves MPP+-induced impairment of locomotion (35%). These findings suggest that SAC attenuates MPP+-induced neurotoxicity in the striatum of mice through its potent antioxidant effect against oxidative stress induced by MPP+.[44]

Organosulfides Ebselen and diphenyldiselenide [Figure 5] were evaluated for neuroprotective effects on differentiated human neuroblastoma SH-SY5Y cell line challenged with 6-OHDA has been investigated.[45] Screening of several organoselenides to investigate their antioxidant potential at 3 μM concentrations showed neuroprotective potential in differentiated human neuroblastoma SH-SY5Y cells challenged with 6-OHDA. The researchers indicated that these selected organoselenium molecules could be further developed as potential pharmacological and therapeutic drugs to treat PD.[46]

Deprenyl is a selective MAO-B inhibitor [Figure 3A] used in clinics to slow the progression of symptoms in patients with PD. In a recent study, deprenyl (10, 20, 50, and 100 μM) upregulated NQO1 expression and activity, attenuated the increase in quinoprotein levels in MPP+-treated PC12 cells, and protected against oxidative damage by triggering the Nrf2/ARE pathway.[47] Moreover, its effect on NQO1 upregulation was greatly attenuated in Nrf2 siRNA transfected cells. Activation of Nrf2/ARE signaling by deprenyl in PC12 cells is independent of MAO-B inhibition.[48]

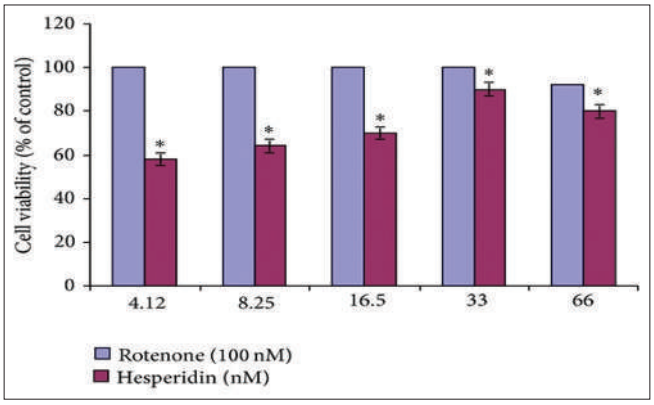

Figure 3: Effect of hesperidin on rotenone-induced reduction in cell proliferation in SK-N-SH neuroblastoma cells. Treatment with hesperidin alone (blue column) (2.5, 5, 10, 20, and 40 μg) did not affect cell proliferation. Hesperidin (2.5, 5, 10, and 20 μg) pre-treatment dose dependently enhanced cell proliferation against rotenone toxicity.

SCM198 [4-guanidino-n-butyl syringate, Figure 3B] is a chemically synthesized compound that exhibits cardioprotective effects in myocardial infarction models[49] as well as neuroprotective effects on middle cerebral artery occluded rats.[50] Pretreatment with SCM198 (0.1, 1, and 10 mM) significantly increased SOD activity, ameliorated intracellular ROS generation, prevented the dissipation of mitochondrial membrane potential, decreased apoptotic cell death, downregulated Bax, and upregulated Bcl-2 mRNA and protein levels compared with those in 6-OHDA damaged cells. Intragastric administration of SCM198 at 18 or 60 mg/kg/day for 4 weeks significantly ameliorated apomorphine-induced contralateral rotations in 6-OHDA-lesioned rats. The study indicated that the underlying mechanisms of SCM198 for delivering potent neuroprotective effects against 6-OHDA-induced toxicity both *in vivo* and *in vitro* might be by inhibiting oxidative stress and apoptosis.[51]

Phenothiazine [Figure 3C] is an organic compound that occurs in various anti-psychotic and antihistaminic drugs. It was reported that 500 nM phenothiazine exerts strong neuroprotective effects at the cellular level and results in better performance on behavioral assays. Thus, chain-breaking agents such as phenothiazine can be developed as therapeutic agents for PD as they rescue DAergic toxicity *in vivo* at nanomolar concentrations based on potent antioxidant properties.[52] Although the doses tested *in vitro* and *in vivo* in PD models are far below the toxic level, side effects such as extrapyramidal symptoms including akathisia and tardive dyskinesia, hyperprolactinemia, neuroleptic malignant syndrome, as well as substantial weight gain need to be addressed.[53]

The dl-3n-butylphthalide [NBP, Figure 4D] evaluated the therapeutic potential by treating clinically affected PD patient. NBP (0.1, 1.0, and 10 μM) reduces MPP+ cytotoxicity by suppressing the mitochondrial permeability transition, reducing oxidative stress, and increasing cellular GSH

content in MPP+-treated PC12 cells. Moreover, NBP also reduces accumulation of SNCA, the main component of Lewy bodies.[54]

Another novel antioxidant, SUN N8075 [Figure 6], is currently in clinical trials for patients suffering from stroke.[49] The previous studies have revealed a potent neuroprotective activity of this agent in an *in vivo* transient middle cerebral artery occlusion model. This study suggested that the underlying neuroprotective mechanism might partly involve protection against oxidative stress.[55] The same scientist investigated the neuroprotective effects of SUNN8075 *in vitro* on both H_2O_2-induced ROS production and 6-OHDA-induced cell death in human neuroblastoma SH-SY5Y cells. They also evaluated its putative neuroprotective effects on MPTP-induced neurotoxicity in an *in vivo* mouse model of PD. SUNN8075 treatment at micromolar concentrations significantly decreased the H_2O_2-induced production of ROS and protected against 6-OHDA-induced cell death. Intra-peritoneal injections of SUNN8075 (30 mg/kg, twice with a 5 h interval) inhibited lipid peroxidation in the mouse forebrain *in vivo*. Moreover, SUN N8075 (10 and 30 mg/kg i.p., twice) exhibited significant protective effects against the MPTP-induced decrease in TH-positive cells in the substantianigra. The researchers concluded that the protective effects of SUN N8075 in experimental PD models were, at least in part, through an anti-oxidation mechanism [Figure 3].[56]

N-acetyl-l-cysteine [NAC, Figure 4A] is a pharmaceutical drug and nutritional supplement used primarily as a mucolytic agent and in the management of paracetamol overdose. Recently, it was hypothesized that NAC supplementation in drinking water (40 mM) protects against SNCA toxicity.[57] Oxidative stress may increase the accumulation of toxic forms of SNCA in a DA-dependent manner.[58] Transgenic mice over-expressing wild-type human SNCA drank water supplemented with NAC from ages 6 weeks to 1 year. As a result, NAC increased SN levels of GSH within 5–7 weeks of treatment. Researchers reported that the loss of DAergic terminals at 1 year associated with SNCA over-expression was significantly attenuated by NAC supplementation.[59] Furthermore, NAC significantly decreased the levels of human SNCA in the brains of PDGFb-SNCA transgenic mice compared to those in controls.[60] The scientist suggested that increased oxidative stress due to early GSH deficiency in the SN may lead to enhanced toxicity of SNCA in DAergic SN neurons, suggesting that strategies to increase GSH or to block oxidative stress by NAC may protect against the SNCA toxicity seen in PD.[61]

Oleanolic acid is a triterpenoid, which has been used for centuries in Asian medicine, due to its anti-inflammatory activity. Some workers investigated the synthetic triterpenoid, CDDO-methyl amide (2-cyano-N-methyl-3,12-dioxooleana-1,9(11)-dien-28 amide; CDDO-MA) [Figure 4B], which is at least 200,000 times more potent than its naturally occurring distant parent.[62] Oleanolic acid induced NQO-1. CDDO-MA (800 mg/kg of diet) exerted profound neuroprotective effects against MPTP and 3-nitropropionic acid neurotoxicity. It was showed that the neuroprotective effects were due to its antioxidant effects, caused by induction of pathways known to be regulated through the Nrf2/antioxidant response element (ARE) signaling pathway, such as GSH synthesis [Figure 4].[63]

NP7 [Figure 4C] is a new marine derived antioxidant, with a different chemical molecular structure than that of classic phytochemicals, obtained by lead optimization system from Streptomyces spp. Study shows that protective effects of NP7 on cell death induced by oxidative stress in neuronal and glial midbrain cultures from Parkin null mice (PK-KO).[64] NP7 (5–10 μM) prevented H_2O_2-induced apoptosis and necrosis of midbrain neuronal and glial cultures from wild type and PK-KO mice. NP7 suppressed microglial activation and the H_2O_2-induced dropout of DA neurons. Result indicated that NP7 might be a promising neuroprotecting agent against oxidative stress in PD.[65]

Bromocriptine, a DA agonist [Figure 7], has been widely used in PD clinics since 1974 to delay and minimize deleterious motor fluctuations after long-term l-dopa treatment.[66] Recent observations suggest that bromocriptine is a free-radical scavenger that scavenges hydroxyl and superoxide radicals *in vitro* and acts as an antioxidant that

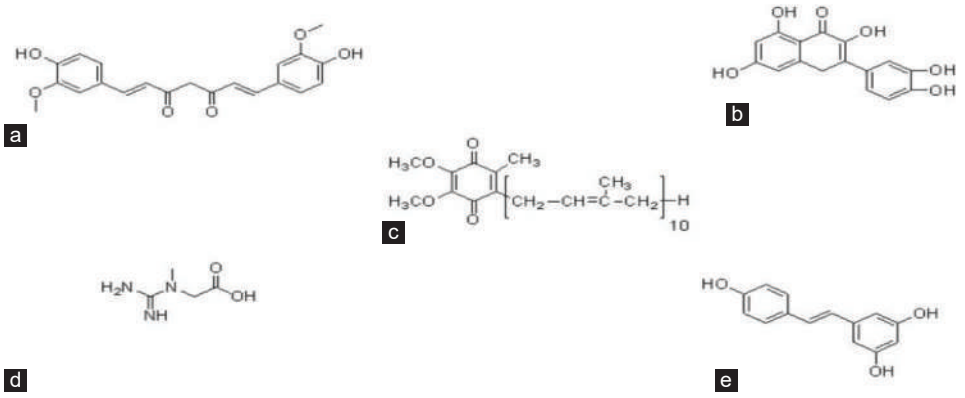

Figure 4: The molecular structure of curcumin (a); quercetin (b); coenzyme Q10 (c); creatine (d); and resveratrol (e).

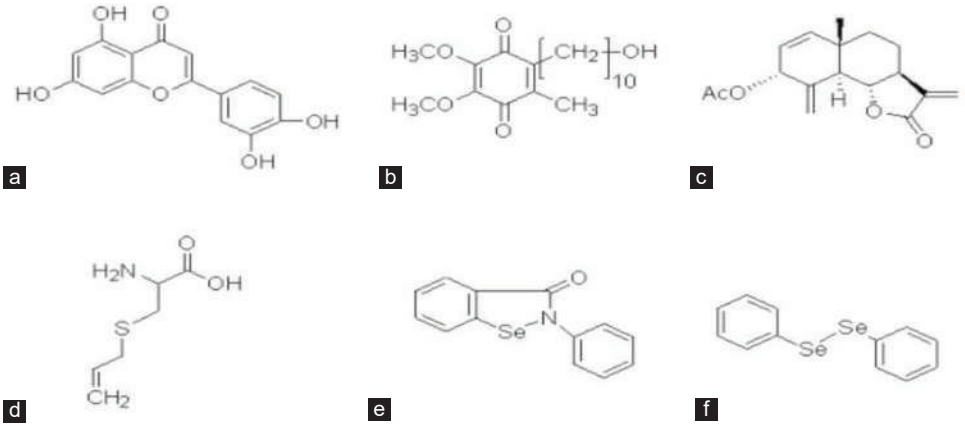

Figure 5: The molecular structure of Luteolin (a); Idebenone (b); 3α-acetoxyeudesma-1,4(15),11(13)-trien-12,6a-olide (c); S-Allylcysteine (d); Ebselen (e); and Diphenyldiselenide (f).

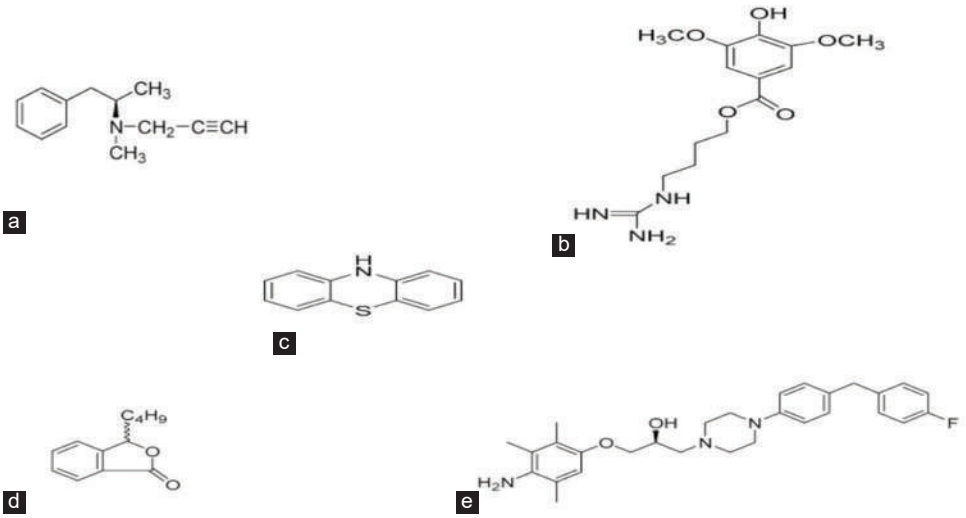

Figure 6: The molecular structure of Deprenyl (a); SCM198 (b); Phenothiazine (c); dl-3n-Butylphthalide (d); and SUN N8075 (e).

inhibits free-radical formation.[67] Based on these properties, it was reported that cytoprotective mechanism of bromocriptine against oxidative damage in H_2O_2-treated PC12 cells. Bromocriptine (5 μM) upregulated the expression and activity of the antioxidant enzyme NQO1, attenuated the increase in the protein-bound quinone in H_2O_2-treated PC12 cells. Protected PC12 cells against

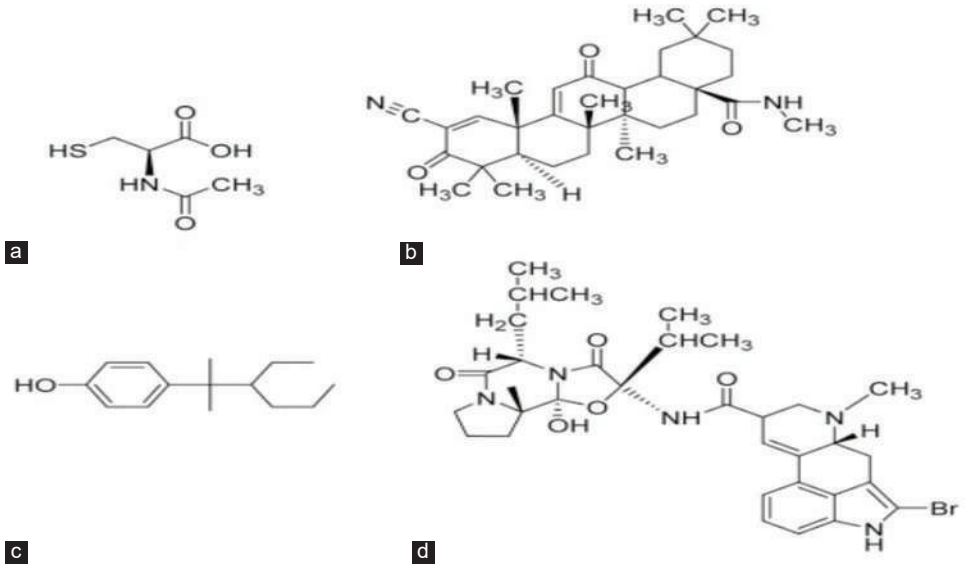

Figure 7: The molecular structure of N-acetyl-l-cysteine (a); CDDO-methyl amide (b); NP7 (c); Bromocriptine (d).

oxidative damage, and increased the expression and nuclear translocation of Nrf2. The Nrf2-related cytoprotective and antioxidative effects of bromocriptine are independent of DA receptor activation.[68]

Synthetic compounds, such as selenium,[69] R-alpha-lipoic acid,[70] rosmarinic acid,[71] eugenol[72] isoborneol,[73] melatonin,[74] metalloporphryins compounds, and metal ion chelators,[75] have neuroprotective effects in PD models based on their antioxidative properties.

CONCLUSIONS

Despite the availability of many drug classes such as l-dopa, DA agonists, monoamine oxidase inhibitors, catechol-O-methyltransferase inhibitors, and anticholinergic agents for the symptomatic treatment of PD, a cure remains elusive. Although the exact nature of mechanism that involves neurodegeneration in PD is not well understood, oxidative stress is one of the major risk factors that could initiate and/or promote degeneration of DA neurons. Therefore, antioxidant therapy could prevent or reduce the rate of progression of this disease. It has been demonstrated that antioxidant compounds are able to protect neuronal cells by scavenging free radicals or activating the antioxidant mechanisms. Numerous *in vitro* and *in vivo* animal studies reported during the past 5 years centered on oxidative stress and ROS mediated mechanisms such as radical scavenging, metal chelating, and/or regulation of antioxidant enzymes. However, experimental evidence also showed that oxidative stress is not the sole deleterious factor implicated in the death of DAergic neurons and other mechanisms involving modulatory effects on signal transduction pathways and gene expression may also play key roles in the neuroprotection of progressive PD. The compounds discussed in this review may also act by regulating these pathways along with antioxidative mechanisms, which may be synergistic for delivering beneficial effects in PD. In addition, combination therapy with antioxidants and existing drugs might also be beneficial and enhance the efficacy of standard therapy in the treatment of PD. Various types of free radicals are produced, and antioxidants vary in their ability to quench these different free radicals, therefore supplementation with multiple antioxidants and relying on a cocktail of agents, each specifically targeting one aspect of the degenerative mechanism in the correct time frame and dose, may provide better results to achieve promising clinical effects. However, examining the critical factors including the optimum concentrations required, what biologically active forms are needed and crossing of these agents into blood–brain barrier to exert potential therapeutic benefits are indeed essential. A complete understanding of the molecular

mechanisms of the ROS specificities in PD, and larger studies, both epidemiologic and randomized clinical trials in humans, as well as animal studies, are urgently needed to confirm these findings for delivering beneficial effects in the treatment of PD.

ACKNOWLEDGMENT

The authors want to thank the technical officer in the Department of Farm Animal Production and Health, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka, for providing necessary facilities and good guidance to write this review article.

AUTHOR CONTRIBUTION

LS and AD conceived the study design, interpreted, and manuscript preparation. Both are drafting of the article details with an edit the manuscripts. All authors read and approved the final manuscript

REFERENCES

1. Chen D, Zhang T, Lee TH. Cellular mechanisms of melatonin: Insight from neurodegenerative diseases. Biomolecules 2020;10:1158.
2. Maragkoudaki X, Naylor M, Papacleovoulou G, Stolarczyk E, Rees D, Pombo JM, *et al*. Supplementation with a prebiotic (polydextrose) in obese mouse pregnancy improves maternal glucose homeostasis and protects against offspring obesity. Int J Obes 2020;44:2382-93.
3. Catanzaro E, Bishayee A, Fimognari C. On a beam of light: Photoprotective activities of the marine carotenoids astaxanthin and fucoxanthin in suppression of inflammation and cancer. Mar Drugs 2020;18:544.
4. You Z, Zhang Z, Blagg BS, Dobrowsky RT. KU-596 decreases mitochondrial superoxide and improves bioenergetics following downregulation of manganese superoxide dismutase in diabetic sensory neurons. Exp Neurol 2019;313:88-97.
5. Durdik M, Kosik P, Markova E, Somsedikova A, Gajdosechova B, Nikitina E, Belyaev I. Microwaves from mobile phone induce reactive oxygen species but not DNA damage, preleukemic fusion genes and apoptosis in hematopoietic stem/progenitor cells. Sci Rep 2019;9:1-12.
6. Zhou DR, Eid R, Miller KA, Boucher E, Mandato CA, Greenwood MT. Intracellular second messengers mediate stress inducible hormesis and programmed cell death: A review. Biochim Biophys Acta Mol Cell Res 2019;1866:773-92.
7. Teixeira MI, Lopes CM, Amaral MH, Costa PC. Current insights on lipid nanocarrier-assisted drug delivery in the treatment of neurodegenerative diseases. Eur J Pharm Biopharm 2020;149:192-217.
8. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. Metabolites 2019;9:258.
9. D'Angelo S. Current evidence on the effect of dietary polyphenols intake on brain health. Curr Nutr Food Sci 2020;16:1170-82.
10. Bhatt KL, Khader A. Conceptual and clinical approach of immunology in Ayurveda. J Ayurveda Integr Med Sci 2020;5:164-8.
11. Ribaudo G, Bortoli M, Pavan C, Zagotto G, Orian L. Antioxidant potential of psychotropic drugs: From clinical evidence to in vitro and in vivo assessment and toward a new challenge for *in silico* molecular design. Antioxidants 2020;9:714.
12. Prasad S, Srivastava SK. Oxidative stress and cancer: Chemopreventive and therapeutic role of triphala. Antioxidants 2020;9:72.
13. Agnihotri A, Aruoma OI. Alzheimer's disease and Parkinson's disease: A nutritional toxicology perspective of the impact of oxidative stress, mitochondrial dysfunction, nutrigenomics and environmental chemicals. J Am Coll Nutr 2020;39:16-27.
14. Sali VK, Vasanthi HR. Protective effect of rutin isolated from *Spermococe hispida* against cobalt chloride-induced hypoxic injury in H9c2 cells by inhibiting oxidative stress and inducing apoptosis. Phytomedicine 2018;51:196-204.
15. Dhuna K, Dhuna V, Bhatia G, Singh J, Kamboj SS. Neuroprotective effect of *Convolvulus pluricaulis* methanol extract on hydrogen peroxide induced oxidative stress in human IMR32 neuroblastoma cell line. Biotechnol J Intern 2012;2:192-210.
16. Umar NM, Parumasivam T, Aminu N, Toh S M. Phytochemical and pharmacological properties of *Curcuma aromatic* Salisb (wild turmeric). J Appl Pharm Sci 2020;10:180-94.
17. Pranda MA, Murugesan BJ, Knoll AJ, Oehrlein GS, Stroka KM. Sensitivity of tumor versus normal cell migration and morphology to cold atmospheric plasma-treated media in varying culture conditions. Plasma Proc Polym 2020;17:1900103.
18. Verdoodt F, Dehlendorff C, Jäättelä M, Strauss R, Pottegård A, Hallas J, *et al*. Antihistamines and ovarian cancer survival: Nationwide cohort study and *in vitro* cell viability assay. J Natl Cancer Inst 2020;112:964-7.
19. Dhuna K, Dhuna V, Bhatia G, Singh J, Kamboj SS. Cytoprotective effect of methanolic extract of *Nardostachys jatamansi* against hydrogen peroxide induced oxidative damage in C6 glioma cells. Acta Biochim Pol 2013;60:21-31.
20. Saadi S, Hussein RH, Mohammed S. Estimation some antioxidants enzymes in stress patients in Babylon city.

Indian J Forensic Med Toxicol 2020;14:2463-6.
21. Irawan B, Labeda I, Lusikooy RE, Sampetoding S, Kusuma MI, Uwuratuw JA, *et al*. Association of superoxide dismutase enzyme with staging and grade of differentiation colorectal cancer: A cross-sectional study. Ann Med Surg 2020;58:194-9.
22. Shamsher N, Prabhu C. QuitPuff: A simple method using lipid peroxidative changes in saliva to assess the risk of oral precancerous lesions and oral squamous cell carcinoma in chronic smokers. Indian J Med Paediatr Oncol 2020;41:670.
23. Lin Z, Wang Z, Zhou X, Zhang M, Gao D, Zhang L, *et al*. Discovery of new fluorescent thiazole-pyrazoline derivatives as autophagy inducers by inhibiting mTOR activity in A549 human lung cancer cells. Cell Death Dis 2020;11:1-12.
24. Zhang T, Tian C, Wu J, Zhang Y, Wang J, Kong Q, *et al*. MicroRNA-182 exacerbates blood-brain barrier (BBB) disruption by downregulating the mTOR/FOXO1 pathway in cerebral ischemia. FASEB J 2020;34:13762-75.
25. Sharifi-Rad J, El Rayess Y, Abi-Rizk A, Sadaka C, Zgheib R, Zam W, *et al*. Curcumin and Heath: A devoted review to bioactive effects and safety profiles for food, biotechnological and medicinal applications. Front Pharmacol 2020;11:1021.
26. Makkar R, Behl T, Bungau S, Zengin G, Mehta V, Kumar A, *et al*. Nutraceuticals in neurological disorders. Int J Mol Sci 2020;21:4424.
27. Liu Z, Li T, Yang D, Smith, W. Curcumin protects against rotenone-induced neurotoxicity in cell and drosophila models of Parkinson's disease. Advances in Parkinson's Disease, 2013;2,18-27.
28. Zhao C, Wang F, Lian Y, Xiao H, Zheng J. Biosynthesis of citrus flavonoids and their health effects. Crit Rev Food Sci Nutr 2020;60:566-83.
29. Slanina T, Petrovičová L, Massányi P. Role of natural substances and vitamin supplementation in tinnitus prevention and treatment. J Microbiol Biotechnol Food Sci 2020;9:987-94.
30. Khan A, Jahan S, Imtiyaz Z, Alshahrani S, Makeen HA, Alshehri BM, *et al*. Neuroprotection: Targeting multiple pathways by naturally occurring phytochemicals. Biomedicines 2020;8:284.
31. Wu D, Zhang S, Sun N, Zhu B, Lin S. Neuroprotective function of a novel hexapeptide QMDDQ from shrimp via activation of the PKA/CREB/BNDF signaling pathway and its structure-activity relationship. J Agric Food Chem 2020;68:6759-69.
32. Mohamed ZA, Eliaser EM, Mazzon E, Rollin P, LianEe GC, Razis AF. Neuroprotective potential of secondary metabolites from *Melicope lunu-ankenda* (*Rutaceae*). Molecules 2019;24:3109.
33. Bazmandegan G, Shamsizadeh A, Najafi MF, Assadollahi Z Allahtavakoli M, Kamiab Z, *et al*. Iranian brown propolis possesses neuroprotective effect against ischemic neuronal damage in mice. J Herb Med Pharmacol 2020;9:121-9.
34. Park HW, Park CG, Park M, Lee SH, Park HR, Lim J, *et al*. Intrastriatal administration of coenzyme Q10 enhances neuroprotection in a Parkinson's disease rat model. Sci Rep 2020;10:9572.
35. Jeong JS, Piao Y, Kang S, Son M, Kang YC, Du XF, *et al*. Triple herbal extract DA-9805 exerts a neuroprotective effect via amelioration of mitochondrial damage in experimental models of Parkinson's disease. Sci Rep 2018;8:15953.
36. D'Angelo S, Scafuro M, Meccariello R. BPA and nutraceuticals, simultaneous effects on endocrine functions. Endocr Metab Immune Disord Drug Targets 2019;19:594-604.
37. Singh AP, Singh R, Verma SS, Rai V, Kaschula CH, Maiti P, *et al*. Health benefits of resveratrol: Evidence from clinical studies. Med Res Rev 2019;39:1851-91.
38. Ikram M, Park TJ, Ali T, Kim MO. Antioxidant and neuroprotective effects of caffeine against Alzheimer's and Parkinson's disease: Insight into the role of Nrf-2 and A2AR signaling. Antioxidants 2020;9:902.
39. Venema JH, Giuffrida FR, Paponov IV, Albacete AL, Perez-Alfocea FR, Dodd IC, Colla G. Rootstock-scion signalling: Key factors mediating scion performance. In: Vegetable Grafting: Principles and Practices. Wallingford, UK: Centre for Agriculture and Bioscience International; 2017. p. 94-131.
40. Cichon N, Saluk-Bijak J, Gorniak L, Przyslo L, Bijak M. Flavonoids as a natural enhancer of neuroplasticity an overview of the mechanism of neurorestorative action. Antioxidants 2020;9:1035.
41. Blanco LP, Pedersen HL, Wang X, Lightfoot YL, Seto N, Carmona-Rivera C, *et al*. Improved mitochondrial metabolism and reduced inflammation following attenuation of murine lupus with coenzyme Q10 analog idebenone. Arthritis Rheumatol 2020;72:454-64.
42. Ahmadian-Moghadam H, Sadat-Shirazi MS, Zarrindast MR. Therapeutic potential of stem cells for treatment of neurodegenerative diseases. Biotechnol Lett 2020;42:1073-101.
43. Koo U, Nam KW, Ham A, Lyu D, Kim B, Lee SJ, *et al*. Neuroprotective effects of 3α-acetoxyeudesma-1, 4 (15), 11 (13)-trien-12, 6α-olide against dopamine-induced apoptosis in the human neuroblastoma SH-SY5Y cell line. Neurochem Res 2011;36:1991-2001.
44. Zhong J, Xie J, Xiao J, Li D, Xu B, Wang X, *et al*. Inhibition of PDE4 by FCPR16 induces AMPK-dependent autophagy and confers neuroprotection in SH-SY5Y cells and neurons exposed to MPP+-induced oxidative insult. Free Radic Biol Med 2019;135:87-101.
45. Yoshitomi R, Nakayama K, Yamashita S, Kumazoe M, Lin TA, Mei CY, *et al*. Plasma homocysteine concentration is associated with the expression level of folate receptor 3. Sci Rep 2020;10:1-8.
46. Radenković N, Kostić M, Đorđević N, Doličanin Z, Soldatović T, Živanović M, *et al*. Synthesis of new

Pt (II) complex bearing organoselenium ligands and evaluation of cytotoxic activity of some structurally related Pd (II) complexes. Maced J Chem Chem Eng 2020;39:59-64.
47. Lai TT, Yang CM, Yang CH. Astaxanthin protects retinal photoreceptor cells against high glucose-induced oxidative stress by induction of antioxidant enzymes via the PI3K/Akt/Nrf2 pathway. Antioxidants 2020;9:729.
48. Sanders O, Rajagopal L. Phosphodiesterase inhibitors for Alzheimer's disease: A systematic review of clinical trials and epidemiology with a mechanistic rationale. J Alzheimers Dis Rep 2020;4:185-215.
49. Koppula S, Kumar H, More SV, Kim BW, Kim IS, Choi DK. Recent advances on the neuroprotective potential of antioxidants in experimental models of Parkinson's disease. Int J Mol Sci 2012;13:10608-29.
50. Ning K, Wang MJ, Lin G, Zhang YL, Li MY, Yang BF, *et al*. eNOS-nitric oxide system contributes to a novel antiatherogenic effect of leonurine via inflammation inhibition and plaque stabilization. J Pharmacol Exp Ther 2020;373:463-75.
51. Benfeito S, Oliveira C, Fernandes C, Cagide F, Teixeira J, Amorim R, *et al*. Fine-tuning the neuroprotective and blood-brain barrier permeability profile of multi-target agents designed to prevent progressive mitochondrial dysfunction. Eur J Med Chem 2019;167:525-45.
52. Xie J, Liang R, Wang Y, Huang J, Cao X, Niu B. Progress in target drug molecules for Alzheimer's disease. Curr Top Med Chem 2020;20:4-36.
53. Bangwal R, Bisht S, Saklani S, Garg S, Dhayani M. Psychotic disorders, definition, sign and symptoms, antipsychotic drugs, mechanism of action, pharmacokinetics and pharmacodynamics with side effects and adverse drug reactions: Updated systematic review article. J Drug Deliv Ther 2020;10:163-72.
54. Bieri G, Brahic M, Bousset L, Couthouis J, Kramer NJ, Ma R, *et al*. LRRK2 modifies α-syn pathology and spread in mouse models and human neurons. Actaneuropathologica 2019;137:961-80.
55. Jantas D, Chwastek J, Grygier B, Lasoń W. Neuroprotective effects of necrostatin-1 against oxidative stress-induced cell damage: An involvement of cathepsin D inhibition. Neurotox Res 2020;37:525-42.
56. Noda Y, Motoyama S, Nakamura S, Shimazawa M, Hara H. Neuropeptide VGF-derived peptide LQEQ-19 has neuroprotective effects in an *in vitro* model of amyotrophic lateral sclerosis. Neurochem Res 2019;44:897-904.
57. Fazary AE, Awwad NS, Ibrahium HA, Shati AA, Alfaifi MY, Ju YH. Protonation equilibria of N-acetylcysteine. ACS Omega 2020;5:19598-605.
58. Wong YC, Luk K, Purtell K, Nanni SB, Stoessl AJ, Trudeau LE, *et al*. Neuronal vulnerability in Parkinson disease: Should the focus be on axons and synaptic terminals? Mov Disord 2019;34:1406-22.
59. Srivastava AK, Choudhury SR, Karmakar S. Melatonin/polydopamine nanostructures for collective neuroprotection-based Parkinson's disease therapy. Biomater Sci 2020;8:1345-63.
60. Henchcliffe C, Beal MF. Potential therapies for mitochondrial dysfunction. In: Mitochondrial Dysfunction in Neurodegenerative Disorders. London: Springer; 2012. p. 215-30.
61. Surinlert P, Kongthong N, Watthanard M, Sae-Lao T, Sookbangnop P, Pholpramool C, *et al*. Styrene oxide caused cell cycle arrest and abolished myogenic differentiation of C2C12 myoblasts. J Toxicol 2020;2020:1807126.
62. Son J, Lee SY. Therapeutic potential of ursonic acid: Comparison with ursolic acid. Biomolecules 2020;10:1505.
63. Luo YH, Cheng HJ, Tsai FY, Tsou TC, Lin SY, Lin P. Primary amine modified gold nanodots regulate macrophage function and antioxidant response: Potential therapeutics targeting of Nrf2. Int J Nanomed 2020;15:8411.
64. Mena MA, Casarejos MJ, Solano R, Rodríguez-Navarro JA, Gómez A, Rodal I, *et al*. NP7 protects from cell death induced by oxidative stress in neuronal and glial midbrain cultures from parkin null mice. FEBS Lett 2009;583:168-74.
65. Ahmed SS, Pallavi K, Aiswarya PS, Ramakrishnan V, Husain RA. Neuroprotective compounds from marine microorganisms. Encyclopedia Mar Biotechnol 2020;3:1559-79.
66. Tábi T, Vécsei L, Youdim MB, Riederer P, Szökő É. Selegiline: A molecule with innovative potential. J Neural Transmiss 2020;127:831-42.
67. Castro-González LM, Alvarez-Idaboy JR, Galano A. Computationally designed sesamol derivatives proposed as potent antioxidants. ACS Omega 2020;5:9566-75.
68. Vasconcelos AR, dos Santos NB, Scavone C, Munhoz CD. Nrf2/ARE pathway modulation by dietary energy regulation in neurological disorders. Front Pharmacol 2019;10:33.
69. Liu H, Yu Q, Fang C, Chen S, Tang X, Ajuwon KM, *et al*. Effect of selenium source and level on performance, egg quality, egg selenium content, and serum biochemical parameters in laying hens. Foods 2020;9:68.
70. Liu Y, Zhu W, Ni D, Zhou Z, Gu JH, Zhang W, *et al*. Alpha lipoic acid antagonizes cytotoxicity of cobalt nanoparticles by inhibiting ferroptosis-like cell death. J Nanobiotechnol 2020;18:1-14.
71. Lv R, Du L, Zhou F, Yuan X, Liu X, Zhang L. Rosmarinic acid alleviates inflammation, apoptosis, and oxidative stress through regulating miR-155-5p in a mice model of Parkinson's disease. ACS Chem Neurosci 2020;11:3259-66.
72. Fekrirad Z, Gattali B, Kashef N. Quorum sensing-regulated functions of *Serratia marcescens* are reduced by eugenol. Iran J Microbiol 2020;12:451-9.
73. Rosato A, Sblano S, Salvagno L, Carocci A,

Clodoveo ML, Corbo F, *et al*. Anti-Biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. Antibiotics 2020;9:637.

74. Lissoni P, Messina G, Borsotti G, Tosatto A, Frigerio S, Tassoni S, Fede DG. Modulation of immune and anti-tumor effects of cancer immunotherapy with anti-Pd-1 monoclonal antibodies by the pineal hormone melatonin: Preliminary clinical results. J Immunol Allergy 2020;1:1-6.
75. Tosato M, Di Marco V. Metal chelation therapy and Parkinson's disease: A critical review on the thermodynamics of complex formation between relevant metal ions and promising or established drugs. Biomolecules 2019;9:269.