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A nutritional supplement protects substantia nigra neurons against neurotoxicity induced by rotenone

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ABSTRACT

Oxidative stress is major contributing factor to dopaminergic cell death in Parkinson's disease (PD). In this study, the effect of the multivitamin and polyunsaturated fatty acids (PUFAs) containing nutritional supplement "Ensure" on oxidative stress and neuronal damage in the rotenone-induced PD was studied. Mice were treated with rotenone (1.5 mg/kg, subcutaneously), every other day for two weeks together with the vehicle, Ensure (1 or 2 g/kg) or L-dopa (25 mg/kg) orally once a day. Biomarkers of oxidative stress, namely, malondialdehyde (MDA), nitric oxide and reduced glutathione were measured in brain homogenates. Testing for neuromuscular strength, motor incoordination and imbalance was done as well as histological study of the brain. Results showed that the level of MDA, an index of lipid peroxidation and nitric oxide were significantly increased along with marked decrease in reduced glutathione content in brain after rotenone injection. Rotenone-treated mice exhibited weakness of grip strength, and impairment of motor balance and coordination. The histological study demonstrated a reduction in size and number of substania nigra pigmented cells and deeply stained degenerated neurons in cerebral cortex and hippocampus. Ensure treatment reduced lipid peroxidation (MDA), nitric oxide, and increased brain reduced glutathione content. It also improved motor deficits in rotenone groups. Ensure reduced histological damage evidenced by marked amelioration of the rotenone-induced neurodegenerative changes in a dose-dependent manner. These results suggest that multivitamin and PUFAs supplementation may be useful adjunctive therapy in patients with PD by virtue of an antioxidant action.

Key words: Parkinson's disease; oxidative damage; rotenone; multivitamins; nutritional supplements

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder, the cardinal features being slowness of voluntary movement (bradykinesia), muscular rigidity, postural instability, gait disturbances and a resting tremor (Beitz, 2014). Non-motor features also occur in PD and include autonomic

dysfunctions, sleep disturbance, apathy, depression and neuropsychiatric symptoms (Reichmann et al., 2009). Parkinson's disease is the result of a preferential and continued death of dopaminergic neurons of the substantia nigra pars compacta (SNc) of midbrain basal ganglia and the consequent marked decrease in striatal dopamine content (Hughes et al., 1992). The disease affects approximately 1% of the population above the age of 65y and the incidence increases rapidly with advancing age (Van Den Eeden et al., 2003; Benninger et al., 2009). In most cases of PD (over 95%) there is no obvious cause (idiopathic PD) and it is widely accepted that the disease is the result of the effect of environmental factors eg. toxic insecticides in genetically susceptible subjects. In this context, there is evidence from epidemiological studies that suggested exposure to insecticides and pesticides as a factor which increases the risk of developing PD (Freire and Koifman, 2012; Narayan et al., 2013). In addition, animal experiments have shown that systemic or intrastriatal injection of the pesticide rotenone results in nigrostriatal dopaminergic neurodegeneration (Sherer et al., 2003; Abdel-Salam et al., 2014a).

In PD, there is ample evidence to implicate oxidative stress, mitochondrial damage and neuroinflammation as the leading pathogenetic mechanisms that underlie the death of SNc dopaminergic cells (Miller et al., 2009; Blesa et al., 2015). Oxidative stress occurs when oxidant free radicals are produced at a rate that exceeds the capacity of the cell's antioxidant defense leading to oxidative damage to cell biomolecules (Halliwell, 2001). The brain tissue is in particular vulnerable to oxidative stress mainly due to (i) the high rate of oxygen utilization with an increase in generation of reactive oxygen metabolites such as superoxide and hydrogen peroxide by the mitochondrial electron transport chain (ii) insufficient antioxidants; and (iii) the rich content of PUFAs, the preferred target for reactive oxygen radicals (Floyd et al., 1999; Halliwell, 2001). Other factors favoring oxidative stress in the PD brain are the increase in SNc iron, a redox-active transition metal (Sofic et al., 1988; Salazar et al., 2006) capable of catalyzing damaging cellular reactions (McCord, 1998) and the decrease in glutathione content (Sian et al., 1994). In PD brain, there is an increase in oxidatively damaged proteins (Alam et al., 1997), membrane lipids (Dexter et al., 1994) and nucleic acids (Zhang et al., 1999) as well as misassembly and reduced catalytic activity of the mitochondrial complex I (Keeney et al., 2006). Hence, therapeutic agents able to interfere with free-radical mediated cell injury represents a useful approach to maintain the integrity of SNc dopaminergic neurons. Keeping with this is the fact that none of the existing dopamine replacing (L-dopa) or other dopminergic drugs alter the natural history of PD. Rather, these drugs provide only symptomatic improvement that is followed several years later by decreased efficacy or emergence of motor complications in case of L-dopa (Rascol et al., 2011).

Nutritional supplements or therapies are widely used in the elderly to provide nutritional support and/or improve health and well-being (Dwyer et al., 2015). Patients with PD often have nutritional deficiencies related to gastric and intestinal dysfunction eg, gastroparesis, dysphagia, anorexia, and constipation (Rozenberg et al., 2013; Zilli Canedo Silva et al., 2015). Therefore, adjustment of nutritional intake and providing vitamins and micronutrients may help to improve symptoms and quality of life (Barichella et al., 2009). Moreover, there is evidence to indicate that some dietary patterns or specific nutrients may be able to lower the risk of PD eg., diet rich in polyphenols, antioxidant vitamins such as ascorbic acid and α-tocopherol, and unsaturated fatty acids (Abdel-Salam and Gaafar, 2016). In PD, there is an immense need to maintain neuronal health and slow down or at the best stop the progress of the disease. The aim of this study was therefore to investigate the effect of a commercially available nutritional supplement "Ensure" in an experimental model of PD induced in mice by the pesticide rotenone.

2. MATERIALS AND METHODS

Animals

Swiss Male mice weighing 27-30 g were used in the study. Mice obtained from the Animal House Colony of the National Research Centre, were kept under temperature- and light-controlled conditions (20–22 °C and 12 h/12 h light/dark cycle) and given free access to standard laboratory rodent chow and tap water during the study. Animal procedures followed the guidelines of the institute ethics committee for the use of animals in experimental studies and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and Chemicals

Rotenone was purchased from Sigma-Aldrich (St Louis, MO, USA) and freshly prepared in 100% dimethyl sulfoxide (DMSO). Ensure powder milk was used. This nutritional supplement contains the fatty acids linoleic and linolenic acids, soy oil, soy protein isolate and the vitamins: choline chloride, ascorbic acid, ascorbyl palmitate, ascorbyl palmitate dl-alpha tocopheryl acetate, niacinamide, calcium pantothenate, mixed tocopherol, pyridoxine hydrochloride, thiamine hydrochloride, riboflavin, vitamin A palmitate, folic acid, biotin, beta-carotene, phylloquinone, cyanocobalamin, and vitamin D3 (Abbott Laboratories BV Zwolle.,

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Holland). Ensure was dissolved in distilled water and freshly prepared before oral administration. Remaining chemicals and reagents in the present study were of analytical grade and purchased from Sigma-Aldrich.

Experimental Design

Mice were randomly assigned to equal treatment groups (6 animals each).

Group 1 received the vehicle (DMSO) three times a week and served as negative control.

Groups 2 was treated with rotenone at 1.5 mg/kg, subcutaneously every other day for two weeks, together with the vehicle and served as positive control.

Group 3 was treated with rotenone at 1.5 mg/kg, subcutaneously every other day for two weeks, and at the same time received L-dopa (25 mg/kg) orally once a day.

Groups 4 & 5: these two groups received rotenone injections (1.5 mg/kg), subcutaneously every other day for two weeks and were treated at the same time with Ensure (1 or 2 g/kg), orally once a day.

At the end of the study, mice were euthanized by cervical dislocation and each brain was quickly removed, washed with ice-cold phosphate-buffered saline (PBS, pH 7.4), placed on ice-cold plate, dissected, weighed, and stored at −80°C for the biochemical studies. Tissues were homogenized in 0.1 M phosphate-buffered saline at pH 7.4 to give a final concentration of 10 % w/v. Homogenization was performed using a homogenizer (ULTRA-TURAX, IKA T10 basic, Germany) at speed 5000 rpm for 30 seconds. The histopathological study was carried out on separate groups of mice (n = 4/group).

Biochemical Assays

Lipid peroxidation assay

Lipid peroxidation was measured in brain homogenates by determining malondialdehyde (MDA) according to Nair and Turne (1984). In this assay 2-thiobarbituric acid reacts with MDA at 25°C to yield a red colored complex with a peak absorbance at 532 nm.

Reduced glutathione assay

Reduced glutathione was determined in brain homogenates according to Ellman (1959). Ellman´s reagent (DTNB; 5, 5′-dithiobis (2-nitrobenzoic acid)) reacts with the free thiol group of GSH to form 2-nitro-s-mercaptobenzoic acid. The chromophore has yellow color and the absorption is measured with spectrophotometer at 412 nm.

Nitric oxide assay

Nitric oxide was determined using Griess reagent. Nitrate is converted to nitrite with by the enzyme nitrate reductase. Nitrite then reacts with the Griess reagent to form a purple azo compound, and its absorbance is measured at 540 nm with spectrophotometer (Archer, 1993).

Behavioral testing

Stair test

In order to test skilled reaching, mice were made to ascend a stair, placed at an angle of 55° above the bench, and the time it took to climb the stair is recorded using a stop watch (Baird et al., 2001).

Wire hanging test

This test is used for the measurement of neuromuscular strength where mice were allowed to hang by their forelimbs from a steel rod above the bench. The latency to fall was counted for three trials with a cutoff time of 180 s (Crawley, 2017).

Wood walking test

To assess motor coordination and balance, mice were made to cross over a wooden stick (~1 m in length, 1 cm in width and elevated 30 cm from the ground) and the time each mouse spent to reach the end is recorded (Rogers et al., 1997).

Histopathological Studies

Brain samples were fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in paraffin using standard procedures. Sections of 5 µm thickness were stained with hematoxylin and eosin (Hx & E) for the histopathological study using light microscope: Olympus Cx 41 with DP12 Olympous digital camera (Olympous optical Co. Ltd, Tokyo. Japan).

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Statistical Analysis

Results are expressed as mean ± SE. Data were statistically analyzed using one way analysis of variance (ANOVA) followed Tukey's multiple comparisons test. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was used. Differences were considered statistically significant at a probability value of less than 0.05.

3. RESULTS

Biochemical Results

Lipid Peroxidation

Mice treated with only rotenone exhibited significantly increased level of MDA by 70.8% (28.85 ± 0.89 *vs.* 16.89 ± 0.71 nmol/g. tissue) compared with the vehicle group. L-dopa given to rotenone-treated animals caused 23.2% decrease in MDA level compared with the rotenone control (22.15 ± 0.82 *vs.* 28.85 ± 0.89 nmol/g. tissue). The administration of Ensure at 1 and 2 g/kg, resulted in significantly decreased MDA levels by 30.2% and 34.6% (from 28.85 ± 0.89 in the rotenone only group to 20.13 ± 0.95 and 18.86 ± 0.67 nmol/g. tissue in the rotenone/Ensure- treated groups).

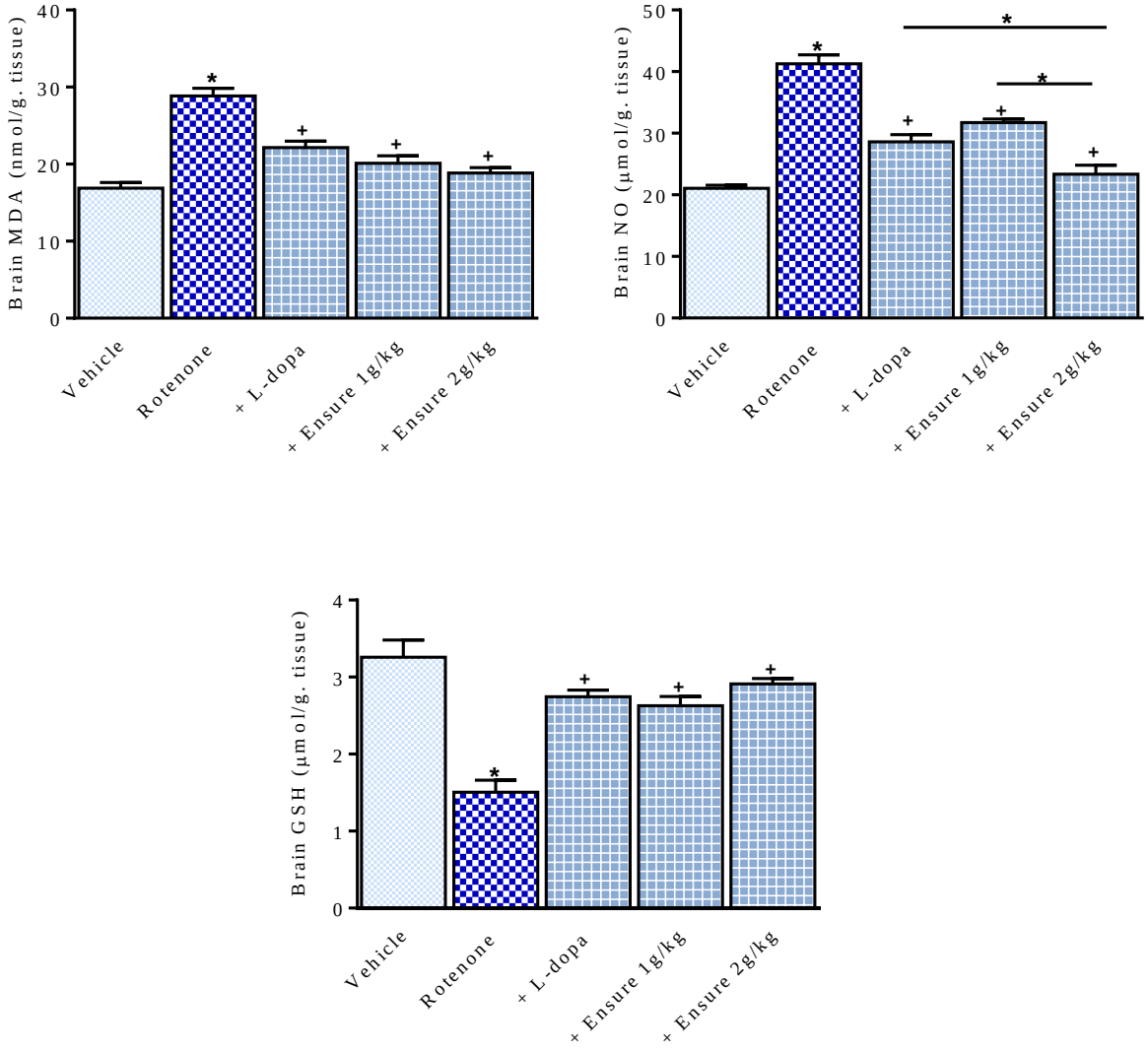

Figure 1. Effect of Ensure or L-dopa on brain oxidative stress in rotenone-treated mice. Values are means ± SEM. *: P<0.05 *vs.* vehicle and between different groups as indicated in the graph. +: P<0.05 *vs.* rotenone control.

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Nitric Oxide

Rotenone treatment caused significantly increased brain nitric oxide by 95.5% (41.26 ± 1.4 *vs.* 21.1 ± 0.50 µmol/g. tissue). Nitric oxide showed significant decrease by 30.7% in rotenone/L-dopa group compared to rotenone control (28.59 ± 1.18 *vs.* 41.26 ± 1.4 µmol/g. tissue). The level of nitric oxide was also significantly decreased after treatment with Ensure at 1 and 2 g/kg by 23.1% and 43.4%, respectively (from control value of 41.26 ± 1.4 to 31.74 ± 0.58 and 23.37± 1.45 µmol/g. tissue).

Reduced Glutathione

Brain reduced glutathione showed 54% decrease in rotenone-treated mice compared with that of the vehicle control (1.50 ± 0.16 *vs.* 3.26 ± 0.22 µmol/g. tissue). The level of reduced glutathione significantly increased by 83.3% by L-dopa compared with the rotenone control value (2.75 ± 0.08 *vs.* 1.5 ± 0.16 µmol/g. tissue). Significant increments of reduced glutathione by 75.3% and 94% were also observed after treatment with Ensure given at 1 and 2 g/kg (from rotenone control value of 1.50 ± 0.16 to 2.63 ± 0.11 and 2.91 ± 0.07 for 1 and 2 g/kg Ensure).

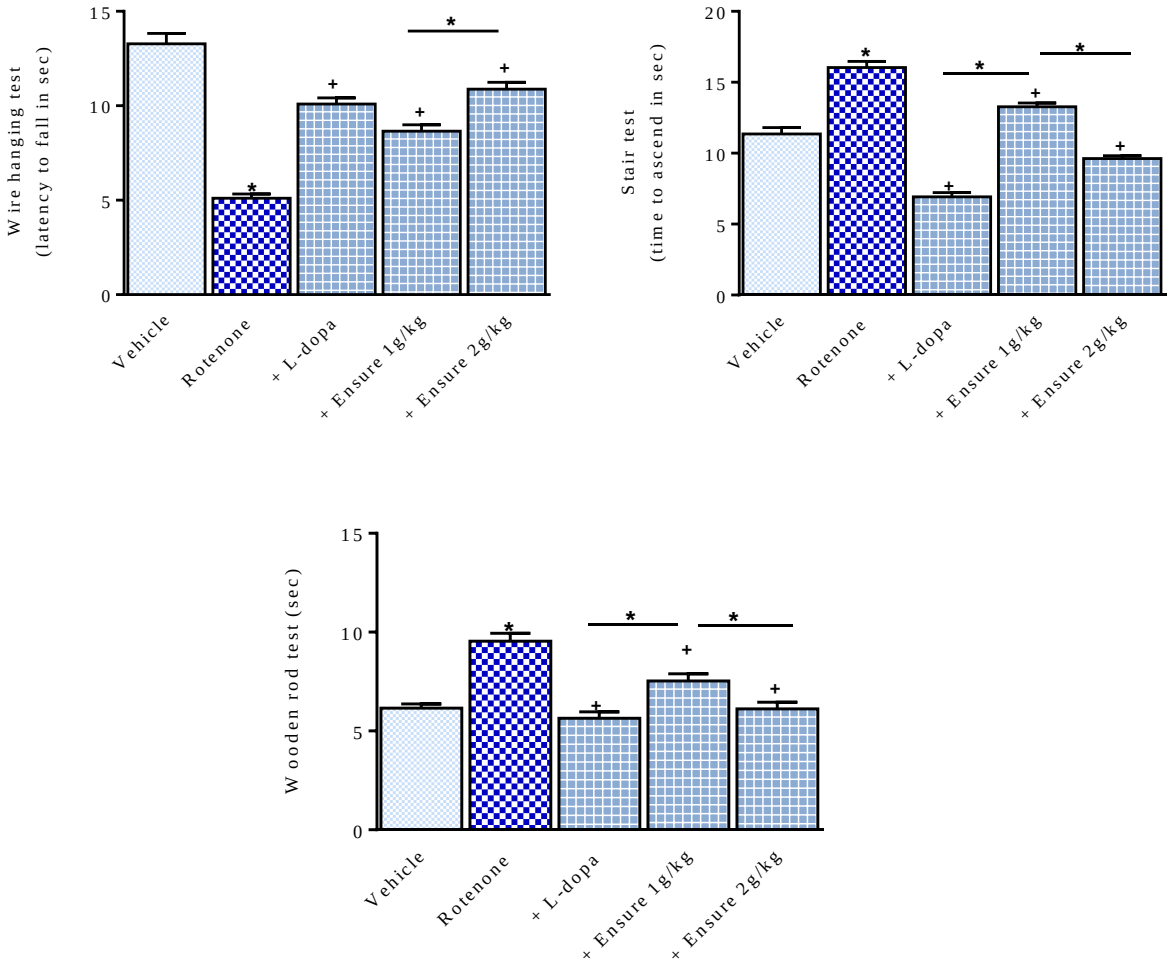

Figure 2. Effect of Ensure or L-dopa on motor tests in mice treated with rotenone. Values are means ± SEM. *: P<0.05 *vs.* vehicle and between different groups as indicated in the graph. +: P<0.05 *vs.* rotenone control.

Behavioral Results

Wire Hanging Test

Rotenone impaired motor strength of mice, significantly decreasing the latency to fall from a steel rod by 61.6% compared with the vehicle control (5.11 ± 0.23 *vs.* 13.28 ± 0.54 sec). The administration of L-dopa was associated with a significant increase in the

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latency time by 97.6% (10.10 ± 0.31 *vs. vs.* 5.11 ± 0.23 sec) compared with the rotenone control. In rotenone/Ensure-treated groups, the latency to fall increased by 69.3% and 112.9% respectively, compared with the rotenone control value (8.66 ± 0.34 and 10.88 ± 36 vs. 5.11 ± 0.23 sec).

Wood Walking Test

Rotenone-treated mice showed significantly longer time to traverse a wooden stick compared to their vehicle controls (35.5% increase in time; 9.54 ± 0.39 vs. 6.15 ± 0.21 sec). In mice treated with L-dopa, the time raken to traverse the stick was significantly decreased by 41% compared with the rotenone control value (5.64 ± 0.32 *vs.* 9.54 ± 0.39 sec). Mice administered Ensure at 1 and 2 g/kg also took shorter time to traverse the stick by 21.1% and 35.8%, respectively compared with the rotenone control (7.53 ± 0.40 and 6.12 ± 0.33 *vs.* 9.54 ± 0.39 sec).

Stair Test

The time mice took to ascend the stair was significantly increased by rotenone by 41.7 % compared to the vehicle controls (16.1 ± 0.42 *vs.* 11.36 ± 0.45 sec). L-dopa resulted in significant decrease in time to ascend by 57% compared with the rotenone control (6.92 ± 0.27 *vs.* 16.1 ± 0.42 sec). In the rotenone/Ensure groups, the time to ascend the stair was significantly decreased by 17.6% and 40.2% compared to the rotenone only group (13.27 ± 0.21 and 9.62 ± 0.20 *vs.* 16.1 ± 0.42 sec).

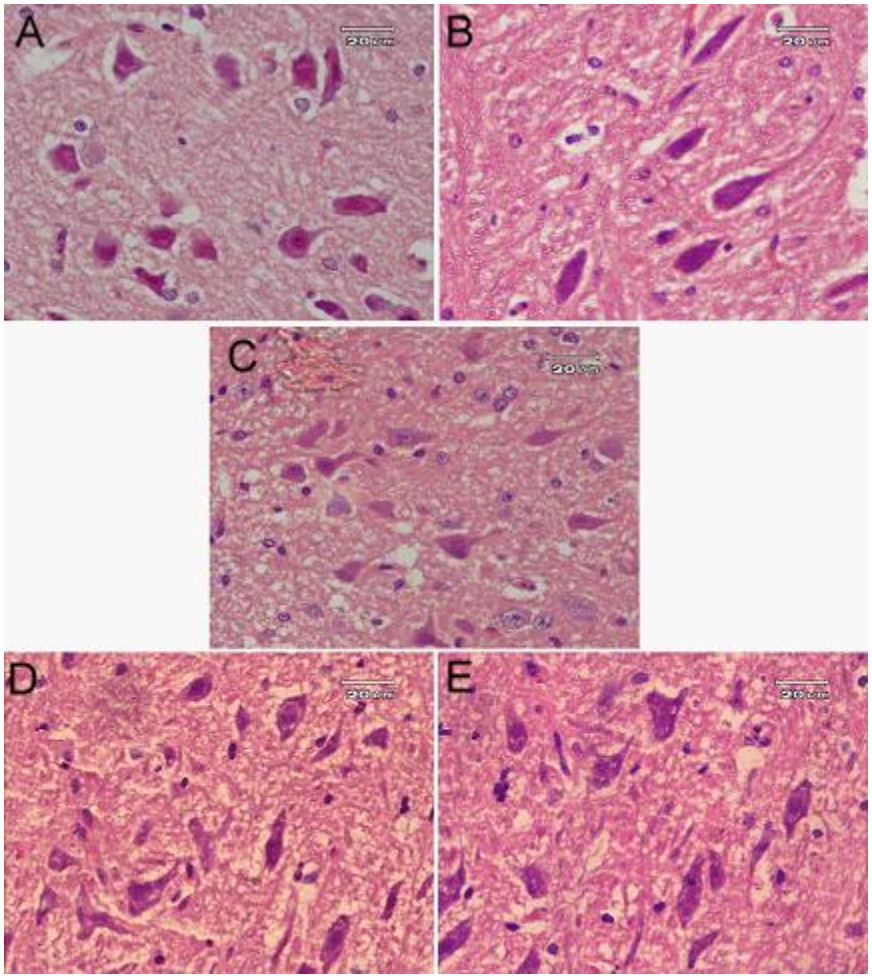

Figure 3. Representative photomicrographs of sections of the substantia nigra after treatment with: **(A)** Vehicle showing the normal pigmented neurons. **(B)** Rotenone showing reduction in size and number of pigmented cells. **(C)** Rotenone + L-dopa showing mild amelioration of the decrease in size and number of pigmented neurons. **(D)** Rotenone + Ensure 1 g/kg showing slight decrease of pigmented cells. **(E)** Rotenone + Ensure 2 g/kg showing normalization of this area structure (Hx & E).

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Histopathological Results

Figures 3, 4 and 5 show the brain regions examined in the study. Following rotenone injection, there were marked reduction in both the size and number of pigmented cells in SN as well as many deeply stained (degenerated) neurons having karryorhectic nuclei in the cerebral cortex and hippocampus. After treatment with the dietary supplement Ensure there was dose-dependent amelioration of these pathological changes with the dose of 2 g/kg conferring almost total protection against the rotenone-induced neurodegeneration.

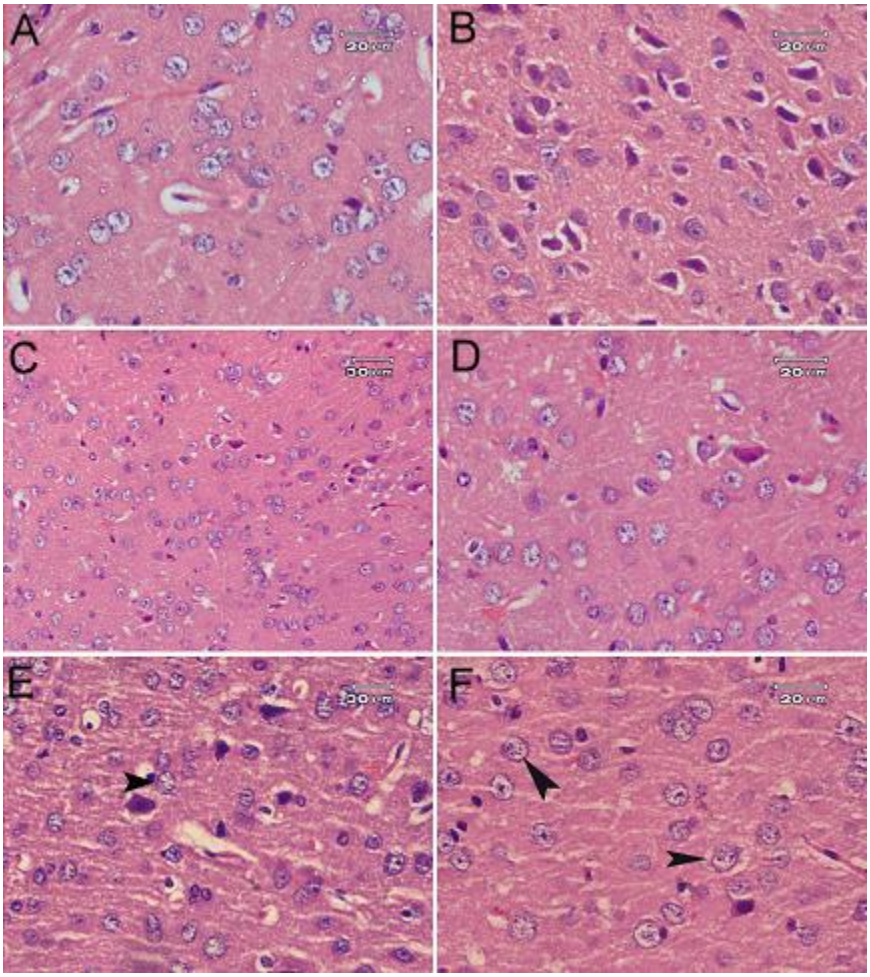

Figure 4. Representative photomicrographs of sections of the cerebral cortex after treatment with: **(A)** Vehicle showing the shape of normal neurons. **(B)** Rotenone showing many deeply stained neurons (arrow). **(C)** Rotenone + L-dopa showing marked decrease of darkly stained neurons. (D) A higher magnification for the previous section shows neurons with karyorrhectic nuclei (arrowhead). Most of neurons appear normal (arrow). **(E)** Rotenone + Ensure 1 g/kg showing a noticeable decrease of deeply stained cells. Some neurons with karyorrhexis are still observed. **(F)** Rotenone + Ensure 2 g/kg showing no deeply stained neurons, but some cells with karyorrhexis are still noticed (arrowhead) (Hx & E).

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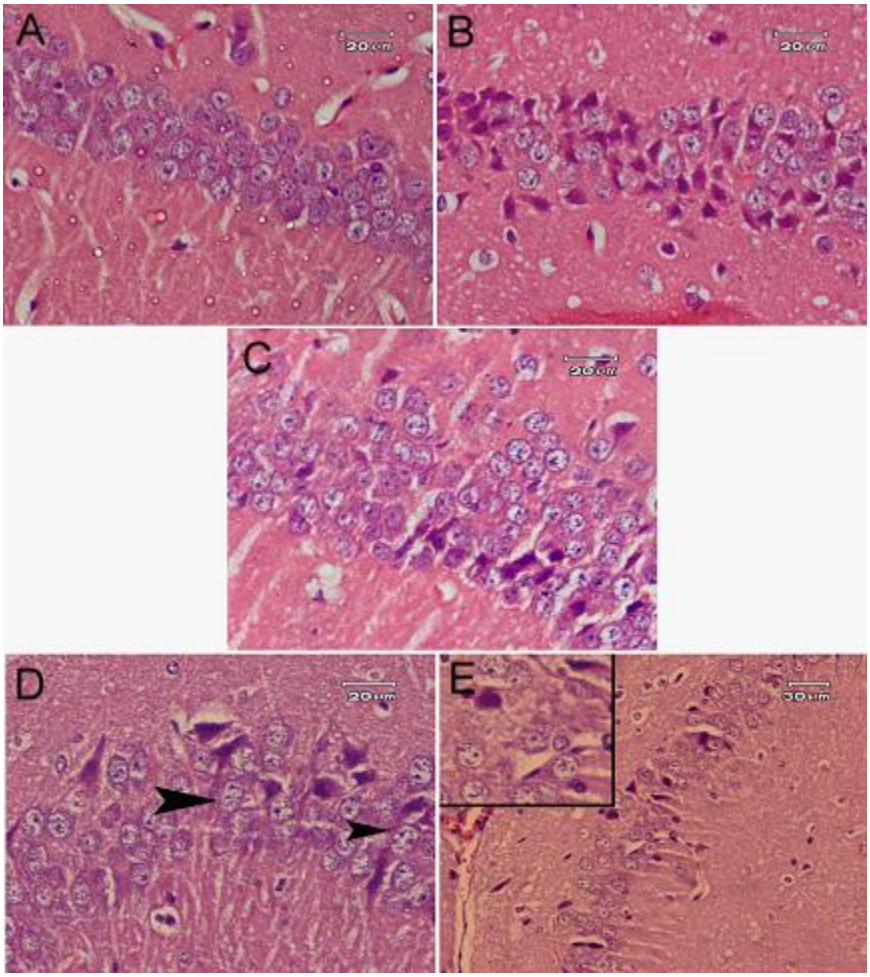

Figure 5. Representative photomicrographs of sections of the hippocampus after treatment with: **(A)** Vehicle showing the normal structure of this area. **(B)** Rotenone showing many darkly stained neurons (arrow). Karyorrhexis (arrow head) in some neurons is noticed. **(C)** Rotenone + L-dopa showing marked decrease of darkly stained neurons. Karyorrhexis is noticed (arrowhead) in some cells. **(D)** Rotenone + Ensure 1 g/kg showing slight decrease of deeply stained cells (arrow) and neurons with karyorrhexis (arrowhead). **(E)** Rotenone + Ensure 2 g/kg showing only a few deeply stained neurons, but cells with karyorrhexis are not detected (Hx & E).

4. DISCUSSION

The present study is the first to report that in the rotenone-induced model of PD in mice, the administration of a multivitamin and PUFAs containing nutritional supplement was associated with biochemical, behavioral and histologic protection. These results thus support the notion that the increase in the intake of antioxidant vitamins and PUFAs would help to preserve SNc neurons subjected to an oxidant milieu in PD.

Rotenone is a widely used toxin which induces experimental PD in laboratory animals. It causes dopamine neuronal cell death by virtue of its ability to increase the generation of intracellular reactive oxygen metabolites in brain with resultant depletion of the antioxidants such as reduced glutathione, superoxide dismutase, and total antioxidant capacity leading to oxidative injury to cell biomolecules such as membrane lipids, DNA and enzyme proteins (Li et al., 2003; Radad et al., 2006; Abdel-Salam et al., 2018b,c). Being a mitochondrial complex I inhibitor, rotenone impairs ATP production and increases the generation of superoxide radical by the mitochondria which results in oxidative mitochondrial damage, and initiation of cell apoptosis (Li et al., 2003; Grivennikova and Vinogradov, 2006). It is not surprising, therefore, that rotenone dopminergic neurotoxicity could be prevented by methylene blue (Abdel-Salam et al., 2014c) capable of bypassing complex I–III blockade (Wen et al., 2011) or by the use of yeast NADH--ubiquinone reductase (Marella et al., 2008). Moreover, antioxidants like α-tocopherol (Testa et al., 2005), vitamin C (Ibrahim et al., 2017) or the glutathione precursor *N*-acetyl-cysteine (Abdel-Salam et al., 2019) were shown to protect against rotenone induced neuronal cell death. Rotenone in addition increases the release of nitric oxide (Xiong et al., 2015) and the expression of the inducible

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form of nitric oxide synthase (iNOS) in the cerebral cortex, substantia nigra and striatum of treated animals (Abdel-Salam et al., 2014b, 2018b). This isoform is the source of excessive nitric oxide release by activated microglia and phagocytic cells following toxin or inflammatory mediated brain injury and which could result in exacerbation of the underling pathology and neurodegeneration. The latter is caused by the formation of more reactive nitric oxide-derived radicals, such as peroxynitrite ($ONOO^-$) from the reaction of nitric oxide and molecular oxygen or nigrogen oxides causing nitrosylation of thiols , oxidation and nitration of protein tyrosine residues (Brown, 2010). In this context, the ability of NOS inhibition in reducing dopaminergic cell apoptosis by rotenone provided support for the role of nitric oxide in the toxicant induced neuronal death (Gao et al., 2015).

Our results show that the administration of the nutritional supplement "Ensure" was associated with significant decrease in lipid peroxidation and the level of nitric oxide in the brain of rotenone-treated animals. This indicates an attenuating effect for the nutritional supplement on the release of reactive oxygen radicals and the development oxidative stress with sparing of the brain content of reduced glutathione. In addition, the supplement by restoring the raised level of nitric oxide can prevent the neurotoxic effects of excessively released nitric oxide. Oxidative stress is a major pathogenetic mechanism that drives dopminergic cell death in PD (Blesa et al., 2015). These subjects have oxidatively damaged cellular biomolecules in the SN (Dexter et al., 1994; Alam et al., 1997 Zhang et al., 1999). There is also evidence indicating the presence of systemic oxidative stress in PD. It was found that lipid hydroperoxides increase and the antioxidant system decreases in serum as the disease advances, independent of dopamine replacement therapy (Fedorova et al. 2017), thereby, strengthening the value of antioxidant supplementation in PD. The effects of dietary factors and antioxidants on the risk of PD have been the subject of extensive research. Thus, Mediterranean-type diet rich in phenolics and flavonoids, the antioxidant vitamins ascorbic acid and α-tocopherols derived from vegetables and fruits and unsaturated fatty acids eg., from olive oil was associated with reduced PD risk and preserved cognitive abilities (Abdel-Salam and Gaafar, 2016). Studies found that dietary intakes of beta-carotene, ascorbic acid, and vitamin E inversely correlated with PD (Hellenbrand et al., 1996; Yang et al., 2017).

Vitamin E and vitamin C are not synthesized in humans who depend on dietary intake. Supplementation with these chain breaking antioxidants was shown to increase plasma antioxidant capacity and decrease plasma markers of oxidative stress in obese men (Goralska et al., 2007). In brain, vitamin C (L- ascorbic acid) is an important water-soluble antioxidant with strong reducing properties. It is important in maintaining the survival of dopaminergic neurons and also in stimulating their differentiation of neural precursors (Bagga et al., 2008). Vitamin C has been shown to protect dopaminergic neurons against dopamine toxicity (Jiang et al., 2008). Vitamin E or α-tocopherol is a fat soluble vitamin and an efficient scavenger of lipid peroxyl radicals, thereby, protecting PUFA in biological membranes against the damaging effects of reactive oxygen metabolites (Ternay and Sorokin, 1997). It also acts to protect mitochondrial membranes from oxidative injury such as that mediated by peroxynitrite (Vatassery et al., 2004). This action of vitamin E is particular importance in view of the evidence of oxidative damage to mitochondrial complex I in PD (Keeney et al., 2006). In addition, it has been shown that rats supplemented with vitamin E in diet for two months exhibited a significant increase in α-tocopherol levels in different brain regions (Martin et al., 1999). Moreover, vitamin E may be of value in improving cognitive function (Zhao et al., 2022) which shows impairments in late advanced PD (Painous and Marti, 2020). Other vitamins of clinical relevance to PD is pantothenic acid or vitamin B5 which exerts neuroprotective effects (Penet et al., 2008) and is decreased in patients with Parkinson's disease dementia (Scholefield et al., 2021) and vitamin B6, the low intake of which was associated with an increased risk of the disease (Murakami et al. 2010).

Carotenoids are natural colored pigments which provide colors to flowers, seeds, fruits, and vegetables. Their color ranges from light yellow through orange to deep red and are used as colorants for human food and nutritional supplements (Namitha and Negi, 2010). Carotenoids are potent antioxidants; beta-carotene and lycopene as well as the oxycarotenoids zeaxanthin and lutein exert antioxidant functions in lipid phases by quenching of free-radicals or singlet oxygen (Sies et al., 1992; Sies and Stahl, 1995). Carotenoids in addition benefit cognition and supplementation of diet with lutein, zeaxanthin as well as meso-zeaxanthin was found to improve memory in healthy subjects (Power et al., 2018).

There is also evidence that the type of dietary fat intake is able to modulate the risk of PD. Thus, high intake of monounsaturated fatty acids, and polyunsaturated fatty acids (PUFAs) were significantly associated with a lower risk of PD (de Lau et al. 2005). In contrast, a higher consumption of saturated fat may be associated with an increased risk of the disease (Hantikainen et al. 2022). In vitro, linoleic acid protected against PC12 cell death induced with 1-methyl-4-phenylpyridinium (MPP+) (Tang et al., 2014). In SH-SY5Y cell line and mice treated with the dopaminergic neurotoxin 6-hydroxydopamine, linoleic acid showed anti-inflammatory and neuroprotective effects, possible through increasing the biogenesis of lipid droplets and improving the autophagy/lipophagy flux (Alarcon-Gil et al., 2022). Thus, the linoleic acid content of the dietary supplement "Ensure" may also be involved in its neuroprotective effects observed in the present study.

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5. CONCLUSIONS

In this study, treatment of rats having experimentally-induced PD with a commercially available multivitamin and linoleic acid containing nutritional supplement was found to exert protective effects. The degenerative changes in the pigmented cells in SN as well as in neurons in the cerebral cortex and hippocampus were markedly ameliorated by the supplement together with regaining of motor strength and balance. The protective effect was associated with decreased brain lipid peroxidation, nitric oxide besides increased brain reduced glutathione level, indicative of an antioxidant mechanism. These findings support the role of multivitamin and supplementation polyunsaturated fatty acids in preventing neurodegeneration in PD.

Author contribution

O.M.E.A.S. and M.E.E-S. designed the study and conducted the research work and biochemical studies. N.S. performed the histological study and its interpretation. O.M.E.A.S. prepared the manuscript. O.M.E.A.S., M.E.E-S. and N.S. approved the final version of the manuscript.

Ethical approval

Animal procedures followed the guidelines of the institute ethics committee for the use of animals in experimental studies and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Informed consent

Not applicable.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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