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Coenzyme-Q10 and Omega-3 Fatty-Acid Ameliorates Catalepsy and Neuroinflammation in Haloperidol Induced Parkinson's disease Mouse Model

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ABSTRACT

The benefits of dietary supplements like co-enzyme Q_{10} (CQ10) and omega-3 fatty acids in reducing the burden of neurodegenerative diseases including Parkinson's disease (PD) has recently become the focus of research. Also, because mitochondrial dysfunction and oxidative damage have been linked to the development of Parkinsonism, the use of coenzyme-Q10 and omega-3 fatty acid has been suggested. This study examined the effects of CQ10 and omega-3 fatty acid in haloperidol induced mouse model of PD. Sixty Adult male mice weighing 30-35g each were assigned into six groups (A-F) of 10animals each. Group A control, fed standard diet, Group B, ip haloperidol (HAL) while groups C, oral Levodopa Cabidopa (LD) plus ip haloperidol, D and E, CQ1O diet (120 mg/kg of feed) and omega-3 fatty acid (500 mg/kg of feed) diet respectively, following ip haloperidol. Group F co-administered CQ10 and omega-3 fatty acid diet with ip haloperidol. All were administered daily for 28 days. Behaviour of mice in catalepsy bar test was assessed, following which animals were sacrificed by cervical dislocation. Cerebral sections were homogenized for tumour necrotic factor α (TNF-α) and cerebellar cortex also processed for histological study. Result showed a significant decrease in catalepsy score with HAL.CQ_{10}.Ω-3 (p= 0.001) and an increase with HAL and HAL.LD. TNF-α decreased significantly with HAL.Ω-3, HAL.CQ_{10}.Ω-3 and increased with HAL and HAL.LD. Haematoxylin and Eosin of the cerebellar cortex showed moderately depleted and cromatolyzed purkinje cells with HAL and HAL.LD, which appeared ameliorated in CQ_{10}.Ω-3F.HAL and HAL.CQ_{10} groups. The study concluded that CQ10 or omega3 fatty acid alone or as adjunct, exerts ameliorative effects on neuroinflammation in haloperidol induced Parkinsonism in mice.

Keywords: Parkinson's disease; Neuroinflammation; CoQ10; Omega3 fatty- acid

INTRODUCTION

In the last decade, the benefits of nutritional status and dietary supplements like co-enzyme Q_{10} and omega-3 fatty acids in reducing the burden of neurodegerative diseases, including Parkinson's Disease (PD) has been the focus of research [1,2]. Also, because mitochondrial dysfunction, oxidative damage and neuroinflammation have been linked to the development of Parkinsonism [3], the use of supplements like coenzyme-Q10 and omega-3 fatty acid has been suggested.

Coenzyme Q_{10} (CoQ10), a fat-soluble compound plays a pivotal role in mitochondrial oxidative phosphorylation and the production of adenosine triphosphate (ATP). It as well functions as an antioxidant in cell membranes and lipoproteins [4]. Investigations into the role of CoQ10 as a neuroprotective agent against reactive oxygen species damage and apoptotic cell death suggests CoQ10 may act by stabilizing the mitochondrial membrane when neuronal cells are subjected to oxidative stress [5]. Nutrient such as omega-3 polyunsaturated fatty acids (PUFAs) is believed to enhance several cerebral functions [6], as a part of the plasma membrane, it is involved in several processes, including increased synaptic development and functionality (Cansev *et al.,* 2008), synaptic integrity and plasticity [7], contributing to neuroplasticity and subsequent enhancement of cognitive activity [8].

Three types of omega-3 are involved in human physiology are: α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Weiser *et al.*, 2016). DHA makes up over 90% of the ω-3 PUFAs and 10–20% of total lipids in the brain [9]. It is said to be able to modulate cellular properties and physiological processes such as membrane fluidity, release of neurotransmitters, gene expression, myelination, neuroinflammation and neuronal growth [10].

Omega-3 fatty acids mediate anti-inflammatory effects and increased levels of EPA or DHA has shown to decrease the levels of PGE2 and 4 series- leukotrienes. Eicosapentaenoic acids compete with constitutive levels of arachidonic acid in cell membranes for the same desaturation enzymes and produce 3-series prostaglandins and thromboxanes, and 5-series leukotrienes which have low pro-inflammatory potential. The alteration in leukotriene biosynthesis due to higher concentration of omega-3 fatty acids compared to arachidonic acid underlies the anti-inflammatory effects. The aim of this study is thus to evaluate the role of Coenzyme-Q10 and Omega-3 Fatty-Acid in haloperidol induced Parkinson's disease Mouse Model

MATERIALS AND METHOD

Drugs and Chemicals: Coenzyme Q10, Omega-3 fatty acids, Levodopa/Carbipoda and haloperidol were obtained from MedPlus Pharmacy, Osun Mall, Osogbo. Nature's field Coenzyme Q10 100mg capsule (Bactolac Pharmaceutical Inc, USA), Asoj Omega-3 capsule (Eibe Pharma Nigeria LTD), Haloperidol Injection I.P.; HALOP(R) (Tripada HealthCare PVT. LTD. India), ELISA TNF-α kit (RayBio(R)) and Levodopa/Carbipoda (SINAMET 250mg +25mg; Drug International Limited, Bangladesh)

Experimental Animals: Healthy 24-month-old Swiss mice were obtained from the animal house of Empire Breeders, Osogbo, Osun State, Nigeria and used for this study. Mice were housed in temperature-controlled (22.5°C ±2.5°C) quarters in plastic cages measuring 12 x 9 x 6 inches. A 12 hour light –dark cycle was maintained with lights on at 7.00 a.m. Animals were allowed easy access to food and water ad libitum. All procedures were carried out in accordance with approved protocols of the Research Ethical Committee of College of Health Sciences, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso.

Animal Diet: All animals were fed standard rodent chow until the commencement of the study. At the beginning of the study animals were fed standard diet (SD, commercially available rodent feed containing 29% protein, 11% fat, and 58% carbohydrate), co-enzyme Q10 supplemented diet (CQ10 was incorporated into SD at 120 mg/kg of feed) or Omega-3 fatty acid supplemented diet at 500 mg/kg of feed daily for a period 28 days. Concentration of co-enzyme Q10 and Omefa-3 fatty acid concentrations was determined from Abdin and Hamouda, 2008; Onaolapo *et al.,* 2020.

EXPERIMENTAL METHODOLOGY

Adult male mice weighing 30-35g each were assigned into six groups (A-F) of 10animals each. Group A control, fed standard diet, Group B, ip haloperidol (HAL) while groups C, ip haloperidol plus oral Levodopa Cabidopa (LD), D and E, CQ1O diet (120 mg/kg of feed) and omega-3 fatty acid (500 mg/kg of feed) diet respectively, following ip haloperidol. Group F co-administered CQ10 and omega-3 fatty acid diet with ip haloperidol. All were administered daily for 28 days. Behaviours of mice in the Y-maze and catalepsy bar test were assessed, following which animals were sacrificed. Brain sections were homogenized for total antioxidant capacity (TAC) and tumour necrotic factor α (TNF-α) while the hippocampus, prefrontal and cerebellar cortexes processed for histological study.

Behavioural tests: On test days, animals in the respective groups were transported in their home-cages to the laboratory, following which they were allowed 30 minutes to acclimatize before commencement of behavioural tests. Animals were exposed to Y-maze, and the catalepsy bar test. At the beginning of the behavioural tests, each animal was placed in the behavioural apparatus and allowed to explore freely while its behaviour was recorded. Each mouse was then removed from the maze and returned to its home cage. The interior surfaces of the maze was cleaned with 70 % ethanol, and wiped dry to remove traces of nonspecific odour. The behavioural parameters were later scored by two observers, who were blind to the groupings.

Y maze Memory Test: Spontaneous alternation is a measure of spatial working memory. The Y-maze can be used as a measure of short term memory, general locomotor activity and stereotypic behaviour. Y- Maze is composed of three equally spaced arms (120°, 41 cm long and 15 cm high). The floor of each arm is made of wood and is 5 cm wide. For each animal, the Y-maze testing was carried out for 5 minutes.

Catalepsy measurements: Catalepsy was observed using the bar test as described by Fink-Jensen *et al.* 2003. On day 28, animals were administered last dose of their drugs and food following which they were returned to their home cages until testing. The mice were tested for cataleptic responses 30 and 60 minutes after the injection of haloperidol. This was done by lifting the mouse and placing it with its front paws on a steel bar (15 cm long, 0.5 mm diameter, and 5.5 cm above the horizontal surface) with the hind legs on the plane surface. Markings were made at the centre of the steel bar to ensure mice were placed at the same spot all through the experiment. For any mouse that did not stay in position, the procedure was repeated a total of three times, and if the mouse was still unable to remain in place for any length of time, the cataleptic response time was recorded as zero seconds. Time spent in the specified position was measured with a cut-off at 60 seconds. For animals that removed one or more paw(s) from the bar, the time this event occurred was noted and recorded. After each testing, the animal was returned to its home cage until next test.

Sacrifice of Animals: At the end of the experimental period, mice were observed for changes in their physical characteristics. Sacrifice was by cervical dislocation (Lin *et al*., 2014) and the brain of each of the animals dissected out. The brain was observed grossly and then fixed in 10 % neutral buffered formalin for histological studies. Paraffin sections were cut and stained with haematoxylin and eosin for general histological study.

Brain homogenate: Mice were euthanized, whole brains were weighed, and sections of the prefrontal cortex and midbrain were homogenized in ice-cold phosphate-buffered saline. The homogenate was centrifuged at 5000 r/min, 4°C, for 15 minutes. The supernatant was used for the estimation of antioxidant status (total antioxidant capacity), anti-inflammatory marker (Tumor Necrotic Factor –Alpha).

Determination of total antioxidant capacity: The TAC of homogenate was measured by a method based on 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) (Erel, 2004). In this assay, ABTS is incubated with potassium persulfate to produce oxidation of ABTS. Briefly, an amount of 10mg of ABTS was dissolved in 10mL of an aqueous solution containing 2.5mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for one to four hours before use. For the study of samples, ABTS oxidized stock solution was diluted with deionized water to an absorbance of 0.70 at 734m. After addition of 1mL of diluted ABTS oxidized to 10µL of serum, the absorbance reading was taken ten minutes after initial mixing. The TAC was calculated using Trolox® as standard and the result was expressed as mEq Trolox®/L.

Determination of Tumour Necrotic Factor Alpha: Each tissue region was homogenized in 5× volume of 1X PBS. Homogenates were centrifuged at 14000g for 30 min and supernatant was retained. Protein concentration was measured using the BCA assay (Pierce). TNF-α was measured by enzyme-linked immunosorbent assay (ELISA TNF-α kit, R&D Systems) according to manufacturer's instructions. The final concentrations were then adjusted for the protein content in the sample.

Haematoxylin and Eosin Staining Protocol (H&E): H&E staining was carried out as described by Drury and Wallington (1980). Deparaffinized tissue was rehydrated in descending concentrations of alcohol for 2 minutes each, washed in distilled water three times, and stained with haematoxylin for 20 minutes, and then rinsed with running tap water for 5 minutes. They were subsequently differentiated in 1 % acid alcohol for 10 seconds, rinsed adequately under running water to remove excess acid. The nuclear staining was followed by counterstaining with eosin for 2 minutes, dehydrated through 95 % alcohol, cleared in xylene and mounted on dibutyl phathalate xylene (DPX) medium for microscopic examination.

Photomicrography

Brain sections were examined under an Olympus trinocular microscope (XSZ-107 E, Japan) with a digital camera (Canon powershot 2500) attached.

Statistical analysis: Data was analysed using Chris Rorden's ezANOVA for windows (version 0.98). One-factor ANOVA was used for analysis. Tukey's honest significant difference (HSD) test was used for within and between-group comparisons. Results were expressed as mean ± S.E.M and p values less than 0.05 were considered statistically significant.

RESULTS

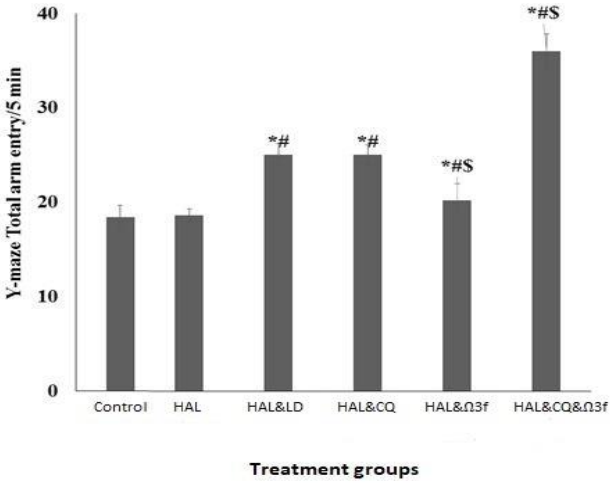

Figure 1: Effect of CQ_{10} and Omega-3 fatty acid on total arm entry in the Y-maze.

Each bar represents Mean ± S.E.M, $*p<$ 0.05 vs. control, $^{\&}p<$ 0.05 vs. LD, $^{\#}p<$ 0.05 vs. HAL, $^{\$}p<$ 0.05 vs. HAL/LD. Number of mice per treatment group=10. CQ_{10}: Co-enzyme Q*10*, HAL: Haloperidol, LD: Levodopa-carbidopa, Ω-3F: omega-3 fatty acid.

There was a significant [F (9, 90) = 8.86, $p < 0.001$] increase in Y-maze total arm entry with HAL&LD,HAL&$CQ_{10,}$ HAL&CQ_{10}&Ω-3F and a decrease with HAL&Ω-3Fcompared to control. Compared to HAL control, Y-maze total arm entry increased significantly with HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Compared to HAL&LD, there was a decrease in Y-maze total arm entry with, HAL&Ω-3F and an increase with HAL&CQ_{10}&Ω-3F.

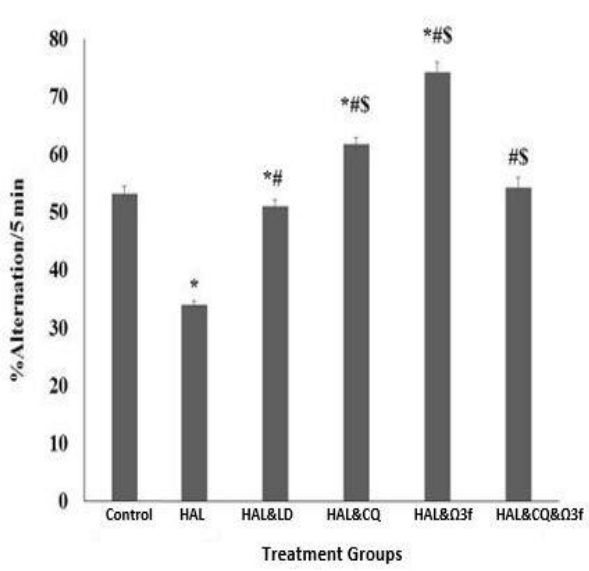

Figure 2: CQ_{10} or Omega-3 fatty acid on spatial working memory in the Y-maze.

Each bar represents Mean ± S.E.M, *$p<$ 0.05 vs. control, $^{\&}p<$ 0.05 vs. LD, $^{\#}p<$ 0.05 vs. HAL, $^{\$}p<$ 0.05 vs. HAL&LD. Number of mice per treatment group=10. CQ_{10}: Co-enzyme Q*10*, HAL: Haloperidol, LD: Levodopa-carbidopa, Ω-3F: omega-3 fatty acid.

Effect of CQ_{10}or Omega-3 fatty acid on spatial working-memory in the Y-maze measured as % alternation. There was a significant [F (9, 90) = 9.53, $p < 0.001$] increase in Y-maze spatial working memory with HAL&$CQ_{10,}$ HAL&Ω-3 and a decrease with HAL and HAL&LD compared to control. Compared to HAL control, Y-maze spatial working memory increased significantly with HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Compared to HAL&LD, there was an increase in Y-maze spatial working memory with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

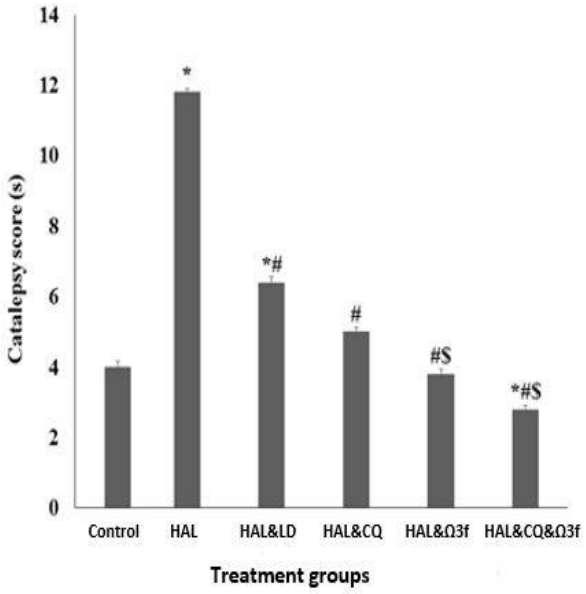

Figure 3: CQ_{10} and Omega-3 fatty acid alone or in combination on catalepsy scores

Each bar represents Mean ± S.E.M, *$p<$ 0.05 vs. control, $^{\&}p<$ 0.05 vs. LD, $^{\#}p<$ 0.05 vs. HAL, $^{\$}p<$ 0.05 vs. HAL/LD. Number of mice per treatment group=10. CQ_{10}: Co-enzyme Q*10*, HAL: Haloperidol, LD: Levodopa-carbidopa, Ω-3F: omega-3 fatty acid.

There was a significant [F (9, 90) = 58.1, $p < 0.001$] decrease in catalepsy score with HAL&CQ_{10}&Ω-3 and an increase with HAL and HAL&LD compared to control. Compared to HAL control, catalepsy score decreased significantly with HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Compared to HAL&LD, there was a decrease in the catalepsy score with HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

Table 1: CQ_{10} and Omega-3 fatty acid alone or in combination on Total Antioxidant capacity and Tumour Necrotic Factor

Groups	TAC(MmTE)	TNF-α (ng/L)
Control	5.23±0.20	103.44±1.40
HAL	2.48±0.24*	124.604±2.10*
HAL&LD	3.40±0.22$^{*\#\$}$	121.77±1.10$^{*\#}$
HAL&CQ_{10}	7.91±0.32$^{*\#\$}$	102.84±1.10$^{\#\$}$
HAL&Ω-3F	6.45±0.25$^{\#\$}$	99.75±1.10$^{*\#\$}$
HAL&CQ_{10}&Ω3F	3.40±0.10$^{*\#}$	46.94±0.60$^{*\#\$}$

Data presented as Mean ± S.E.M, *p< 0.05 vs. control, $^{\&}p$< 0.05 vs. LD, $^{\#}p$< 0.05 vs. HAL, $^{\$}p$< 0.05 vs. HAL/LD. Number of mice per treatment group=10. CQ_{10}: Co-enzyme Q*10*, HAL: Haloperidol, LD: Levodopa-carbidopa, Ω-3F: omega-3 fatty acid, MDA: malondialdehyde, TAC; total antioxidant capacity, IL-10: Interleukin -10, TNF-α; Tumour necrosis factor-alpha, TE: trollox equivalent.

Total antioxidant capacity (TAC) [F (9, 90) = 9.23, p < 0.001] decreased significantly with HAL, HAL&LD, HAL&CQ_{10}&Ω-3 and increased with HAL&CQ_{10} compared to control. Compared to HAL, levels of TAC increased significantly with HAL&LD, HAL&CQ_{10}, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively. Compared to HAL&LD, there was an increase in the TAC levels with HAL&$CQ_{10}$and HAL&Ω-3 F respectively. Tumour necrosis factor-α (TNF-α) [F (9, 90) = 15.20, p < 0.001] decreased significantly with HAL&Ω-3, HAL&CQ_{10}&Ω-3 and increased with HAL and HAL&LD compared to control. Compared to HAL control, levels of TNF-α decreased significantly with HAL&LD, HAL&CQ_{10}, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively. Compared to HAL&LD, there was a decrease in the TNF-αlevels with HAL&CQ_{10}, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively.

Histology Results

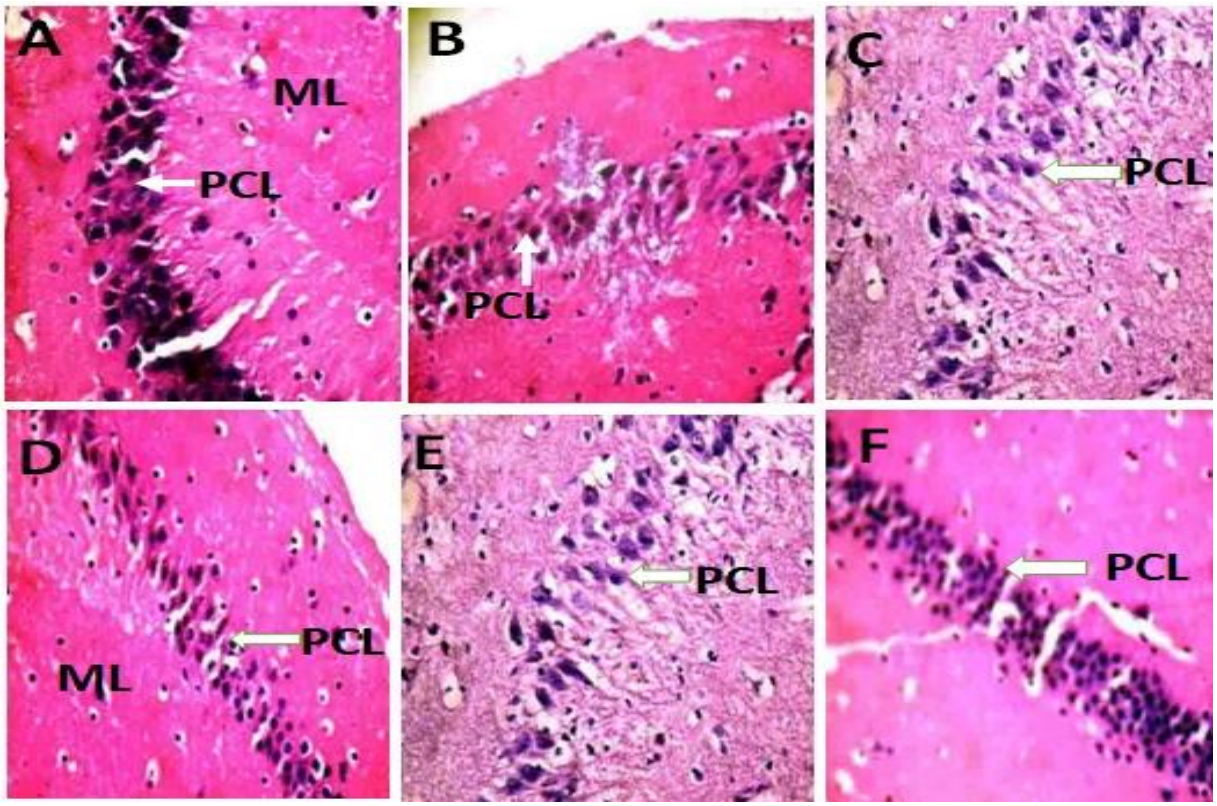

Plate 1: Photomicrograph of a hippocampus section stained by Haematoxylin and Eosin (A-F)

Normal Hippocampus with normal neuronal cells, the structural organization of the CA1, CA2 AND CA3 seen appear normal (white arrow). **(B & C)** The CA2 show area of mild to moderate neuronal depletion and pyramidal cells shrinkage (white arrow). Mag X 400

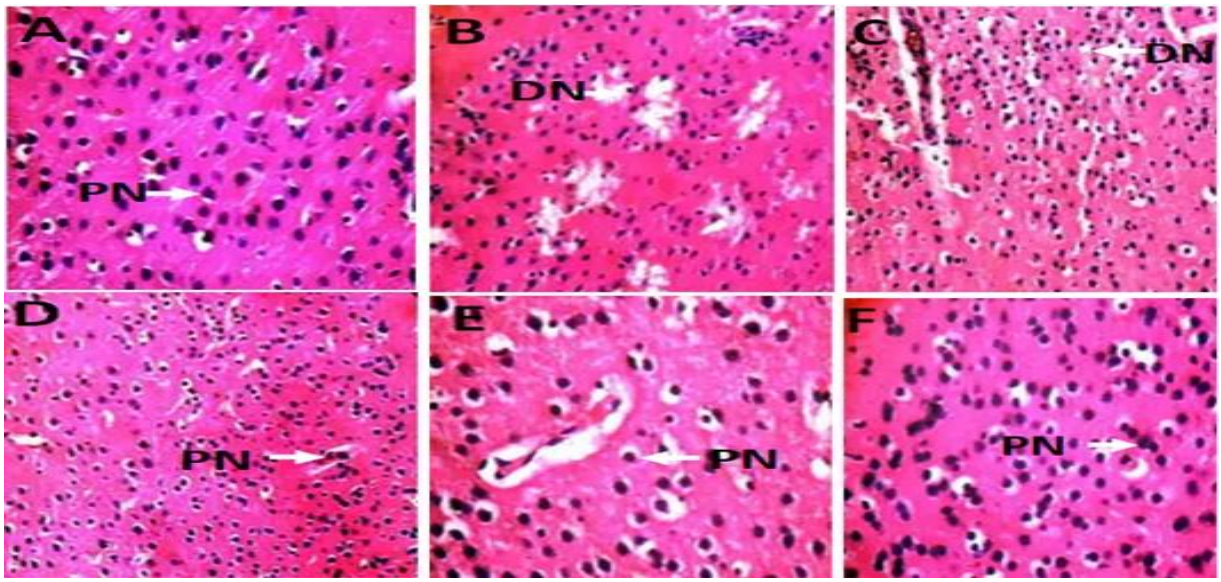

Plate 2: Photomicrograph of prefrontal cortex section stained by Haematoxylin and Eosin. Mag X 400

The pre frontal cortex; showing **(A, E & F)** normal laminae, the pyramidal neuronal cells (PN) appear normal (white arrow) and the stroma appear normal. **(B)** Focal areas of severe degeneration of neuronal cells (DN) exhibiting cytoplasmic vacuolation and the stroma appear normal. **(C)** Moderate to severe degeneration of neuronal cells exhibiting cytoplasmic vacuolation (white arrow) and the stroma appear normal. **(D)** Several normal neuronal cells exhibiting cytoplasmic vacuolation (white arrow), the stroma appear normal. Mag X 400

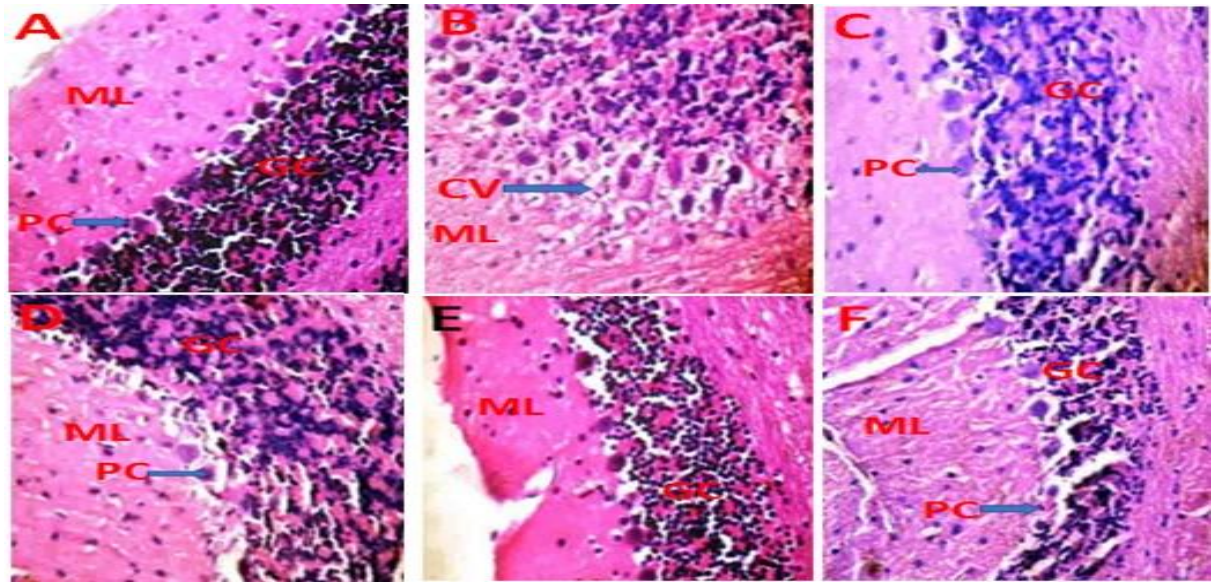

Plate 3: Photomicrograph of a cerebellar cortex stained by Haematoxylin and Eosin.

(A-F); Photomicrograph of a cerebellar cortex stained by Haematoxylin and Eosin Showing normal folia, the Molecular cell layer (ML), the purkinje cells layer (PC), and granular layer appear normal (GC). B- Shows cytosplamic vacuolation (CV) and nuclei shrinkage. Mag X 400

DISCUSSION

There have been several useful animal models developed for the study of Parkinson's disease. Motor deficit in rodent models is evaluated as Haloperidol-induced catalepsy, in which the animal is not able to correct an externally imposed posture, especially for the screening of potential antiparkinsonism compounds. Haloperidol induces catalepsy by blocking of dopamine (D2) receptors and reducing dopaminergic transmission [(11)]. Similarly, an enhanced stimulation of the intrinsic central cholinergic system has also been suggested in haloperidol-induced catalepsy as it has been reported to be intensified and antagonized by pilocarpine and atropine, respectively [(12).] Evidence also suggests that the central serotonergic system modulates nigrostriatal dopaminergic transmission with 5-HT_3 antagonists reported to alleviate neuroleptic-induced catalepsy [(13).]

Pathophysiology of Parkinsonism can be traced to death of dopaminergic neurons as a result of changes in biological activity in the brain. Such activities are believed to include either, changes in cell metabolism or mitochondrial function, neuroinflammation, protein aggregation in Lewy bodies, disruption of autophagy, and blood-brain barrier breakdown resulting in vascular accident [(14).]

The role of neuroinflammation in neurodegenerative diseases is generally understood. Microglia cells are recognized as the innate immune cells of the central nervous system. Acute inflammation in the brain is typically characterized by rapid activation of microglia. During this period, there is no peripheral immune response. Over time, however, chronic inflammation causes the degradation of tissue and of the blood–brain barrier. During this time, microglia generates reactive oxygen species and release signals to recruit peripheral immune cells for an inflammatory response [(15).] Microglia similarly has two major states: M1, a state in which cells are activated and secrete pro-inflammatory factors; and M2, a state in which cells are deactivated and secrete anti-inflammatory factors [(16).] Microglia is usually in a resting state (M2), but in Parkinson's disease can enter M1 due to the presence of α-synuclein aggregates. The M1 microglia release pro-inflammatory factors which can cause motor neurons to die. In this case, dying cells can release factors to increase the activation of M1 microglia, leading to a positive feedback loop which causes continually increasing cell death [(15).]

Karem et al., (2019)[(17)] claimed omega-3 fatty acids administration protects against chronic Sleep-Deprivation-induced memory impairment probably via improving hippocampus antioxidant effects. Scientists including Sandhir et al., (2014)[(18)] found that coenzyme Q10 improves cognitive decline in post-menopausal mice by modulating mitochondrial functions and oxidative stress. McDonald et al., (2005)[(19)] also observed that coenzyme Q10-treated mice were likely to rapidly identify and remember the correct arm of the T maze compared to control. These are equally observed in this study as there was a significant increase in Y-maze total arm entry with $CQ_{10,}$ CQ_{10}&Ω-3F post haloperidol treatments compared to standard diet alone. When compared to haloperidol and L-Dopa (standard drug) treatment, there was a decrease in Y-maze total arm entry with, HAL&Ω-3F but an increase with

HAL&CQ_{10}&Ω-3F. Importantly, Y-maze total arm entry increased significant with co-administration of Coenzyme $Q_{10,}$ and Ω-3F compared to L-Dopa, suggesting a synergistic or buffering reaction between Coenzyme $Q_{10,}$ and Ω-3F.

In the pilocarpine model of epilepsy, CoQ10 reduced the severity of pilocarpine-induced seizures and complications of oxidative stress [(20)]. High dose of omega 3 oil altered the behaviour of the mice[(21)] in MPP+ induced
Parkinson's disease in mice. The MPP+ treated group demonstrated low cataleptic score compared to treated mice. In this study, Catalepsy score is significantly high in haloperidol administered group compared to control, however post haloperidol treatment with supplements showed a significantly low cataleptic score with Omega3 fatty acid, Coenzyme Q10 and their co-treatment compared to L-Dopa. Co-treatment of Omega3 fatty acid and Coenzyme Q10 exhibited lowest catalepsy score, compared to either alone.

Total antioxidant capacity (TAC) decreased significantly with haloperidol but increased with haloperidol treated CQ_{10} compared to control. Significant increase in TAC levels was observed with CQ_{10}&Ω-3Fadjunct treatment compared to HAL&L-Dopa which is indicative of a powerful antioxidative effect of CQ_{10}&Ω-3F co-administrations. Coenzyme Q10 is a strong antioxidant. It protects cells against free radical damage, suggesting that it is beneficial in brain health. Previous studies in mice show that coenzyme Q10 exerts a neuroprotective effect that is necessary for the improvement of cognitive function and prevention of age-related learning and memory deficits [(19)].

Tumour Necrosis Factor alpha (TNF alpha), is an inflammatory cytokine produced by macrophages or monocytes during acute inflammatory processes and responsible for a wide range of signaling events within cells, resulting to necrosis or apoptosis. The protein is also sacrosanct for resistance to infection and cancers. TNF alpha exerts many of its effects by binding, as a trimer, to either a 55 kDa cell membrane receptor termed TNFR-1 or a 75 kDa cell membrane receptor termed TNFR-2. Both these receptors belong to the so-called TNF receptor superfamily. In this study, TNF-α significantly increased with haloperidol compared to control. A significant decrease in TNF-α is thus noted with co-administrative treatment with CQ_{10}&Ω-3F compared to HAL&L-Dopa. The decrease in TNF-α in the treatment group is an indication of the anti-inflammatory benefits of the nutritional supplements. Co-administration of CQ_{10}&Ω-3F has a higher tumour suppressing capacity than L-Dopa.

The structural organization of the CA1, CA2 and CA3 were seen to appear normal across all groups, however in HAL group, CA2 shows area of mild pyramidal cells depletion following 28days administration. Despite demonstrable deficits in neurobehavioural and biochemical parameters, structural hippocampal alterations are not so evident. This may likely suggest that short term memory loss in haloperidol induced Parkinson's disease maybe mainly dopaminagic aberrations rather than hippocampal dysmorphology. Focal areas of severe degeneration of neuronal cells exhibiting cytoplasmic vacuolation were seen in haloperidol administered group L-Dopa intervention demonstrated moderate degeneration of neuronal cells exhibiting cytoplasmic

vacuolation. These observations suggest haloperidol induced morphological deficits in the prefrontal cortex, however, significant amelioration could be observed with groups CQ10, Omega3 and CQ10&Omega3 treatments. No dysmorphology observed in cerebellar cortex. Molecular cell layer, the purkinje cells layer, and granular layer appear preserved across all groups.

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