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NUTRITIONAL SUPPLEMENTS CO-ENZYMEQ10 AND OMEGA3 FATTY-ACID CO-ADMINISTRATION RELIEVES NEUTRITIC PLAQUE, DYSKINESIA AND APOPTOSIS IN HALOPERIDOL INDUCED DOPAMINE DEPLETION IN MICE

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ABSTRACT

Introduction: Dopamine is crucial in executive functions and motor system control. Its depletion appears to be the patho-physiological hallmark of several neurodegenerative diseases including but not limited to Parkinson's, schizophrenia, addiction and attention deficit disorders. This study examined the effects of Co-enzyme Q10 (CQ10) and omega-3 fatty acid co-supplementation in haloperidol induced dopamine depletion in mice. **Materials and Method:** Adult male mice weighing 30-35g each were grouped into six (A-F) of 10animals each. Group A control, fed standard diet, Group B, ip haloperidol (HAL) while groups C, 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 administrations were daily and lasted 28 days. Behaviours of mice in the open field apparatus, radial arm maze and anxiety related elevated plus maze were assessed, following which animals were sacrificed. Prefrontal cortex sections were homogenized for malondialdehyde (MDA), Interleukin -10 (IL-10), caspase-3 and dopamine and also processed for histological and immunohistochemical studies. **Results:** Result showed a significant [F (9, 90) = 21.9, $p < 0.001$] decrease in locomotor activity with HAL, HAL&LD and a significant increase with HAL&CQ_{10}&Ω-3F compared to control. An increase in radial arm 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&LD, there was an increase in open arm with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F and a decrease in closed arm with HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Lipid peroxidation measured as MDA concentration [F (9, 90) = 22.10, $p < 0.001$] increased significantly with HAL, HAL&LD, and decreased with HAL&CQ_{10}, HAL&Ω-3F, HAL&CQ_{10}&Ω-3F compared to control. Compared to HAL&LD, there was an increase in the IL-10levels with HAL&CQ_{10}, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively. Compared to HAL, dopamine increases 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 Caspase-3activity with HAL&$CQ_{10}$and HAL&CQ_{10}&Ω-3F respectively. Normal pyramidal cells with normal cytoplasmic distribution of nissl bodies were seen in HAL&CQ_{10}&Ω-3F, while groups HAL and HAL&LD showed comparatively reduced staining intensities with poor distribution of nissl bodies within their cytoplasm. Bielschowsky silver staining revealed neuritic plaques and argyrophilic structures in the HAL treated group. These fibrillary amyloid deposits were absent in the supplemented groups. Neuron Specific Enolase immunoreactivity is present HAL and HAL&LD while absent in HAL&$CQ_{10}$and HAL&CQ_{10}&Ω-3F. Nil astrocytic reaction to dopamine depletion also seen in the prefrontal cortex of HAL&Ω-3F, HAL&CQ10 and HAL&CQ_{10}&Ω-3F. **Conclusion:** The study concluded that CQ10 and omega3 fatty acid co-supplementation ameliorates neuroinflammation, lipid peroxidation, neutritic plaque and apoptosis in haloperidol induced dopamine depletion in mice.

KEYWORDS: Dopamine depletion; Neuroinflammation; CoQ10; Omega3 fatty- acid.

1.0. INTRODUCTION

Dopamine is crucial in executive functions, motor control, motivation, sexual arousal, reinforcement, and reward, including lower-level functions such as lactation, sexual gratification, and nausea. The dopaminergic cell groups and pathways constitute the dopamine system which is neuromodulatory. Dopamine plays a role in the decision-making process for choosing an action in at

least two significant ways. Firstly, it establishes the "threshold" for taking an action. The fewer stimuli needed to trigger a particular behavior, the higher the level of dopamine activity.[1] Because of this, high amounts of dopamine cause impulsive behavior and high levels of motor activity, whereas low levels of dopamine cause torpor and slower reactions. The role of dopamine as a "teaching" signal is its second significant consequence.[1] The basal ganglia circuit is changed in a way that makes the same response simpler to trigger when related situations occur in the future when an action is followed by an increase in dopamine activity. Dopamine serves as a reward signal in this instance of operant training.[2] While studies have demonstrated that dopamine cannot simply be equated with hedonic "liking" or pleasure, as shown in the consummatory behavioral response, dopamine does play a crucial role in inducing "wanting," linked with the appetitive or approach behavioral reactions to rewarding stimuli.[3] Since pleasure centers have been identified both inside the dopamine system (e.g., nucleus accumbens shell) and outside the dopamine system (e.g., ventral pallidum and parabrachial nucleus), dopamine neurotransmission is implicated in some but not all aspects of pleasure-related cognition.[4]

Dopamine depletion appears to be the patho-physiological hallmark of several neurodegenerative diseases including but not limited to Parkinson's, schizophrenia, addiction and attention deficit hyperactivity disorder (ADHD). Studies have documented a reduction in dopamine production and dopamine receptor density, or the total number of receptors, as people advance in age.[5] The striatum and extrastriatal areas have also been found to experience this decrease.[6] The D1, D2, and D3 receptors are all decreasing, as it is generally known.[7] Many neurological symptoms, such as reduced arm swing and increased rigidity, are thought to be caused by the dopamine loss that occurs with aging. Cognitive flexibility may age-related changes brought on by variations in dopamine levels.[8] Parkinson's disease, which is marked by stiffness and difficulties beginning movement, is caused by significantly decreased dopamine levels in the substantia nigra circuit. ADHD is a condition associated with impaired cognitive control, which in turn causes issues with controlling attention, inhibiting behaviour (inhibitory control), and forgetting things or missing details (working memory), among other issues. ADHD is caused by altered dopamine neurotransmission.[9] Along with connections to other neurotransmitter receptors and transporters, dopamine receptors and the dopamine transporter have been linked to ADHD genetically.[10] The medicines that are used to treat ADHD are where the association between dopamine and ADHD is most significant. Psychostimulants like methylphenidate (Ritalin, Concerta) and amphetamine (Evekeo, Adderall, Dexedrine), which raise dopamine and norepinephrine levels in the brain, are some of the most effective treatment options for ADHD.[11] The indirect stimulation of dopamine and norepinephrine receptors, most especially dopamine receptor D1 and adrenoceptor 2 in the prefrontal cortex, mediates the therapeutic benefits of these psychostimulants in treating ADHD.[12][13]

Coenzyme Q10 (CoQ10), also known as ubiquinone, an endogenously produced lipid is a member of the electron transport chain (ETC). CoQ10 transports electrons to complex III of the mitochondrial electron transport chain, or ETC, from complexes I and II as well as from the oxidation of fatty acids and branched-chain aminoacids (through flavin-linked dehydrogenases).[14] Because reduced CoQ10 also possesses antioxidant characteristics, it may be able to prevent oxidative damage to membrane lipids, proteins, and mitochondrial DNA (mtDNA). CoQ10 has also been used extensively in the treatment of mitochondrial disorders (MD) and other neurodegenerative disorders, as well as its analogue idebenone, which shares an identical modified parahydroxybenzoate ring with CoQ10 but has a short 10-carbon tail. Other potential management indications for the use of CoQ10 include migraine[15;16], chronic tinnitus[17] hypertension[18] heart failure, and atherosclerosis.[19] The effect of CoQ10 as a neuroprotective agent against reactive oxygen species (ROS) damage and apoptotic cell death was examined in a number of research. In the event that oxidative stress is applied to neural cells, CoQ10 may operate by stabilizing the mitochondrial membrane.[20] Pretreatment with water-soluble CoQ10 reduced the amount of mitochondrial ROS production and preserved mitochondrial membrane potential during oxidative stress. The possibility that CoQ10 may play a preventive effect in neurodegenerative illnesses was supported by the evidence of mitochondrial involvement in these conditions.[21]

Docosahexaenoic acid (DHA) is essential for supporting a variety of cell membrane functions in the diet, especially in the membrane-rich grey matter, which is crucial for brain function and vision.[22;23] DHA is the most prevalent omega-3 fatty acid in the brain and a significant structural component of the mammalian brain.[24] It is being investigated as a potential essential nutrient with potential benefits for problems of neurodevelopment, cognition, and neurodegeneration.[22] There are three types of omega-3 fatty acids involved in human physiology, they are alpha-linolenic acid (ALA), which is found in plant oils, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are both frequently found in fish oil and which are derived from microalgae that are later consumed by phytoplankton, a source of food for fish. Omega-3 fatty acids are accessible as daily supplements and are essential for cellular function and metabolism. The scientific data supporting the potential effectiveness of PUFA supplementation in treating neurodegenerative diseases including Parkinson's (PD) and Alzheimer's (AD) is growing.[25;26;27;28] Although dietary suggestions are not a

cure for Parkinson's disease or Alzheimer's disease, they could be able to ease some of the symptoms or delay the physical and mental decline. The aim of this study is to evaluate the effects of co-administration of Co-enzyme Q10 and Omega3 fatty acid on the prefrontal cortex in haloperidol induced dopamine depletion in mice.

2.0. MATERIALS AND METHOD

2.1. 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).

2.2. Experimental Animals

Healthy 24 month old Swiss mice were procured from the animal house of Empire Breeders, Osogbo, Osun State, Nigeria and used for this experiment. 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.

2.3. Animal Diet

All animals were fed standard rodent diet until the beginning of the study. At the onset of the experiment mice 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.

2.4. 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. Administration was daily and for 28 days. Behaviours of mice in the open field, elevated plus maze and radial arm maze were assessed, following which animals were sacrificed. Prefrontal cortex sections were homogenized for malondialdehyde (MDA), Interleukin -10 (IL-10), caspase-3 and dopamine while the prefrontal cortex was processed for histological and immunohistochemical studies.

2.5. 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 open field apparatus, radial arm maze and the elevated plus maze. 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.

2.5.1. Open field test

Animals were exposed to the open field arena for ten minutes during which the following behaviours were observed and scored; Horizontal locomotion, Rearing and Grooming. The open field arena is a rectangular area made of painted hard wood. The floor measured 36 x 36x 26 cm and was made of made of white painted wood. The floor was then divided into 16 equal squares at the bottom. Generally, spontaneous motor activity was monitored for 30 minutes in the open field as described by Ajayi and Ukponmwan (1994). After treatment, each mouse was introduced into the field and the total locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind legs or with its fore arms against the walls of the observation cage or free in the air) and frequency of grooming (number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions) within each 10 minute interval were recorded. The arena is cleaned with 70 % alcohol to eliminate olfactory bias and allowed to dry before introducing a fresh animal. Behavioural tests were done between 7.00 am and 3.00 pm.

2.5.2 Memory test (Radial arm -maze)

Spontaneous alternation is a measure of spatial working memory. The 8-arm radial arm maze apparatus was also used to score working-memory. Each arm measured 33 cm in length and all arms radiate from a small circular central platform. Each mouse is allowed to explore all arms and is scored.

2.5.3. Anxiety model (elevated plus maze)

The elevated plus maze is a plus shaped apparatus with four arms at right angles to each other. The two open arms lie across from each other measuring 25 x 5 x 5 cm and perpendicular to two closed arms measuring 25 x 5 x 16 cm with a centre platform (5 x 5 x 0.5 cm). The closed arms have a high wall (16 cm) to enclose the arms whereas the open arms have no side wall only a latch that

prevents animals' from falling. Following administration of drugs or vehicle mice were placed in the central platform facing the closed arm and their behaviour recorded for 5 minutes. The criterion for arm visit was considered only when the animal decisively moved all its four limbs into an arm. The maze was cleaned with 70 % ethanol after each trial. The percentage of time spent in the arms was calculated as time in open arms or closed arm/total time x100, the number of entries into the arms was calculated using number of entries into open or closed arms/total number of entries.

2.6. 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 of the prefrontal cortex were cut and stained with haematoxylin and eosin for general histological study, cresyl violet staining protocol to demonstrate Nissl substance, Bielschwosky's silver staining method was used to determine the formation of neuritic plaques and neurodegeneration; and immunohistochemical studies for Glial Fibrillary Acid Protein (GFAP) and Neuron Specific Enolase (NSE).

2.7. 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 malondialdehyde (MDA) content, anti-inflammatory marker (Interleukin -10), dopamine and Caspase 3 respectively.

2.8. Biochemical assays

2.8.1. Estimation of malondialdehyde content (Lipid peroxidation)

Aqueous hydroperoxide levels of each sample of the brain homogenate were estimated with a PeroxiDetect™ kit (Sigma MO, USA), *tert*-Butyl peroxide served as standard, and absorbance was read at 560mm in a spectrometer (Pharmacia Biotech, Uppsala, Sweden).

2.8.2. Determination of interleukin 10

IL-10 receptor functions screen by flow. This functional test evaluated the ability of IL-10 to induce tyrosine phosphorylation of STAT3 in cells. If the IL-10 receptors are absent or non-functional, no STAT3 phosphorylation is observed.

2.8.3. Determination of Dopamine

Determination of dopamine hydrochloride using potassium ferricyanide-Fe(III) by spectrophotometry. Fe (III) is deoxidized to Fe(II) by dopamine hydrochloride at pH 4.0, and then Fe(II) reacted with potassium ferricyanide to form a soluble prussian blue (KFeIII[FeII(CN)6]). The absorbance of this product was monitored over time using a spectrophotometer at an absorption maximum of 735 nm, and the amount of dopamine hydrochloride could be calculated based on the absorbance. A good linear relationship of the concentration of dopamine hydrochloride versus absorbance was observed, and a linear regression equation of A = 0.022 + 0.16921C (μg mL–1) was obtained. Moreover, the apparent molar absorption coefficient for the indirect determination of dopamine hydrochloride was 3.2 × 104 L mol–1 cm–1.

2.8.4. Determination of Caspase 3

Caspase-3 Assay Kit (Colorimetric) ab39401 provides a simple and convenient means for assaying the activity of caspases that recognize the sequence DEVD. The Caspase-3 assay protocol is based on the formation of the chromophore p-nitroaniline (p-NA) by cleavage from the labeled substrate DEVD-pNA. The p-NA can be quantified using a spectrophotometer or a microtiter plate reader reading absorbance at 400 or 405 nm. Comparison of the absorbance of p-NA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-3 activity. Caspase-3 assay protocol summary: add samples to wells add reaction buffer and DEVD-p-NA substrate and incubate for 60-120 min at 37ºC, finally, analyze with microplate reader.

2.8.5. 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.

2.8.6. Cresyl Violet Staining Protocol

Cresyl Violet solution was used to stain Nissl substance in the cytoplasm of neurons in formalin-fixed tissue. The neuropil would be stained a granular purple-blue. The Cresyl Violet method uses basic aniline dye to stain RNA blue, and is used to highlight important structural features of neurons. The Nissl substance (rough endoplasmic reticulum) appears dark blue due to the staining of ribosomal RNA, giving the cytoplasm a mottled appearance. Individual granules of extra-nuclear RNA are named Nissl granules (ribosomes). DNA present in the nucleus stains a similar colour. Deparaffinized tissue was rehydrated in descending concentrations of alcohol for 2 minutes each, washed in distilled water three times, and stained with 0.1% Cresyl Violet for 10 minutes; sections were rinsed with running

tap water for 5 minutes to remove excess stain following which they were washed in 70 % alcohol and differentiated in 1 % acid alcohol for 10 seconds. Excess acid was removed by rinsing adequately under running water. Sections were then dehydrated through ascending grades of ethanol (50 % through to absolute ethanol), cleared in xylene and mounted on dibutyl phathalate xylene (DPX) medium for microscopic examination.

2.8.7. Bielschowsky Silver Staining Protocol

The Bielschowsky silver staining method is used to demonstrate neurofibrillary tangles, nerve fibres and senile plaques in tissue samples. It works on the principle that the nerve fibres are sensitized with a silver solution. Sections are treated with ammoniacal silver, and then reduced to visible metallic silver. Deparaffinized and rehydrated sections were incubated in pre-warmed (37°C) 20% silver nitrate solution for 15 minutes, washed and then placed in ammonium silver nitrate solution at 40°C for a further 30 minutes. Sections were subsequently developed for 1 minute and then transferred to 1% ammonium hydroxide solution for 1 minute to stop the reaction. Sections were then washed in distilled H2O, placed in 5% sodium thiosulphate solution for 5 minutes, washed, cleared and mounted in dibutyl phathalate xylene (DPX) medium. Axons, plaque neurites and tangles stain black, in a yellow to brown background whilst plaque and vascular amyloid generally stain brown to dark brown.

2.8.8. Immunohistochemistry Protocol

Sections are taken to water; following which antigen retrieval is performed in Novocastra proteinase K solution for 15 minutesandequilibrated in running water. Sections are blocked in Novolink^{TM} peroxidase block for 15 minutes, and then washed in phosphate buffered saline (PBS). Tissue protein is blocked by immersion in novolink protein block for 15 minutes, and then sections are washed in PBS, incubated for 15 minutes in Novolink^{TM} post primary block**,** washed twice in PBS and then incubated for 15 minutes in Novolink ^{TM} polymer. Treatment with Novolink ^{TM} DAB working solution is carried out 5 minutes, sections washed twice in PBS, rinsed in water, Sections were counter stained with haematoxylin for 2 minutes, rinsed in water, dehydrated and mounted on synthetic resin medium (DPX) using glass cover slips. All antibodies used were diluted by 1/50 using standard antibody diluents and procedure carried out in a humidity chamber.

2.8.9. Immunohistochemical Staining

Immunohistochemical tests for glial fibrillary acid protein (GFAP) and NSE were performed using the Novocastra^{TM} and Novolink^{DM} polymer detection system (Leica Biosystems, UK) and appropriate primary monoclonal antibodies. The Novolink polymer detection system (PDS) utilizes an innovative controlled polymerization technology to prepare polymer horseradish peroxidase (HRP) linker antibody conjugates, hence overcoming the problem of non specific staining as seen with the Streptavidin/Biotin detection systems secondary to exogenous biotin. The Novolink and Novocastra sytems allow for identification of small quantities of antigen in sections of formalin-fixed, paraffin- embedded tissue in a sequence of steps. Endogenous peroxidase activity in the tissues are neutralized using the Novocastra^{TM} peroxidase block, followed by the application of the Novocastra protein block which helps reduce non specific binding between primary antibody and polymer. Sections are sequentially incubated in optimally diluted quantities of primary antibody; penetration of the polymer reagents is improved by using Novocastra post primary block. Sections are further incubated with the substrate chromogen 3'3 diaminobenzadine (DAB) prepared from Novocastra DAB chromogen and Novolink DAB substrate buffer (polymer). Reaction with the peroxidase produces a visible brown precipitate at the antigen site. Monoclonal antibodies (Leica Biosystem) were used for the appropriate antigen glial fibrillary acid protein (GFAP) and neuron specific enolase (NSE).

2.8.10. Photomicrography

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

2.8.11. Statistical analysis

Data was analyzed 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.

3.0. RESULTS

3.1. Neurobehavioural Results

3.1.1. CQ_{10}or Omega-3 fatty acid on locomotor activity

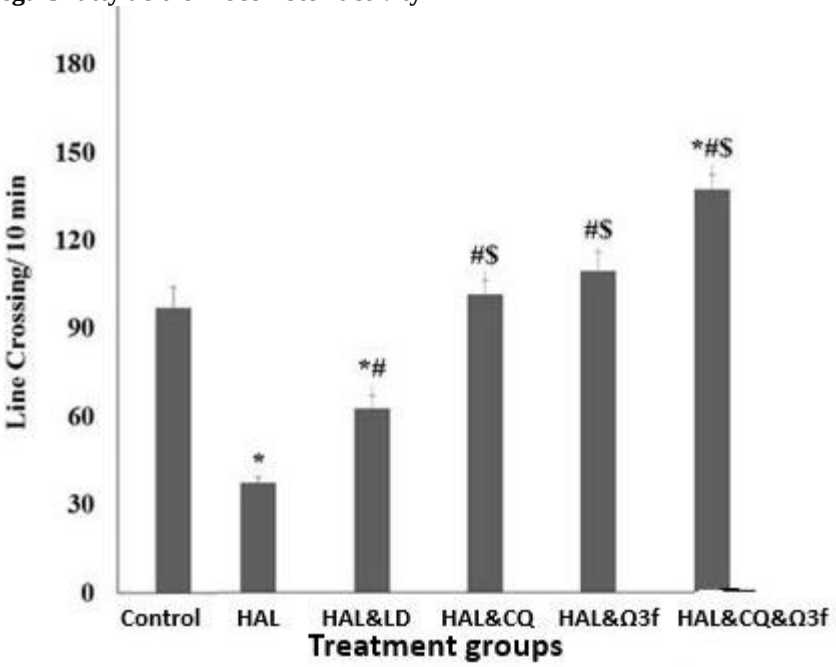


Figure 1: CQ_{10} or Omega-3 fatty acid on locomotor activity in the open field box.

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.

Figure 1. Shows the effect of CQ_{10}or Omega-3 fatty acid on locomotor activity measured as line crossing in the open field arena. There was a significant [F (9, 90) = 21.9, p < 0.001] decrease in locomotor activity with HAL, HAL&LD and a significant increase with HAL&CQ_{10}&Ω-3F compared to control. Compared to HAL control, locomotor activity 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 locomotor activity with HAL&CQ_{10}, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.1.2. CQ_{10}or Omega-3 fatty acid on rearing

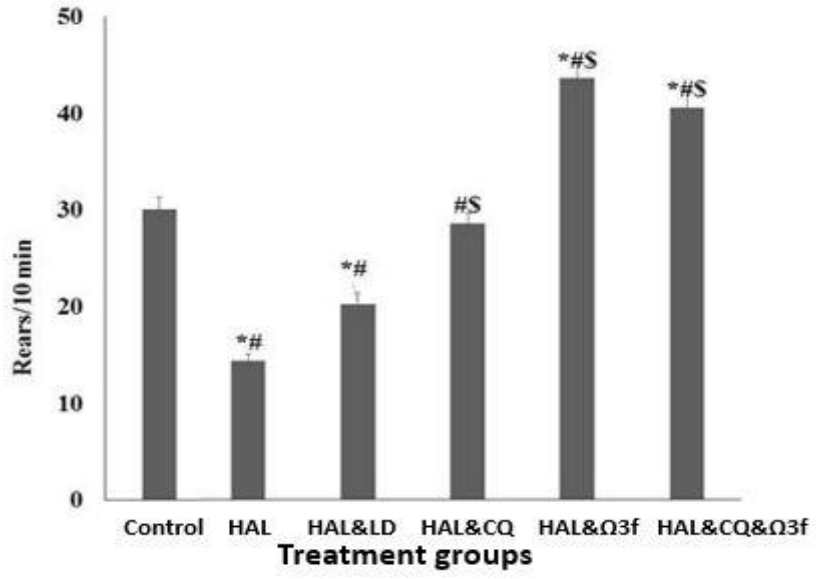


Figure 2: CQ_{10} or Omega-3 fatty acid on rearing activity in the open field arena.

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.

Figure 2 shows the effect of CQ_{10}or Omega -3 fatty acid on rearing activity in the open field arena. There was a significant [F (9, 90) = 12.6, p < 0.001] decrease in rearing activity with HAL, HAL&LD and a significant increase with HAL&Ω-3F and HAL&CQ_{10}&Ω-3F compared to control. Compared to HAL control, rearing activity 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 rearing activity with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.1.3. CQ_{10}or Omega-3 fatty acid on grooming

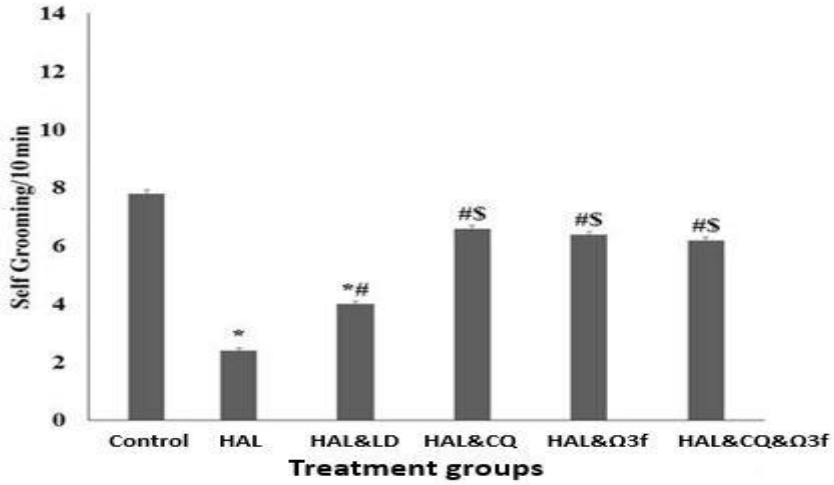


Figure 3: CQ_{10} or Omega-3 fatty acid on self-grooming behaviour in the open field arena.

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.

Figure 3 shows the effect of CQ_{10}or Omega-3 fatty acid on self-grooming behaviour in the open field arena. There was a significant [F (9, 90) = 22.5, p < 0.001] decrease in self-grooming behaviour with HAL and HAL&LD compared to control. Compared to HAL, self-grooming behaviour 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 self-grooming behaviour with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.1.4. CQ_{10}or Omega-3 fatty acid on radial-arm maze spatial working-memory

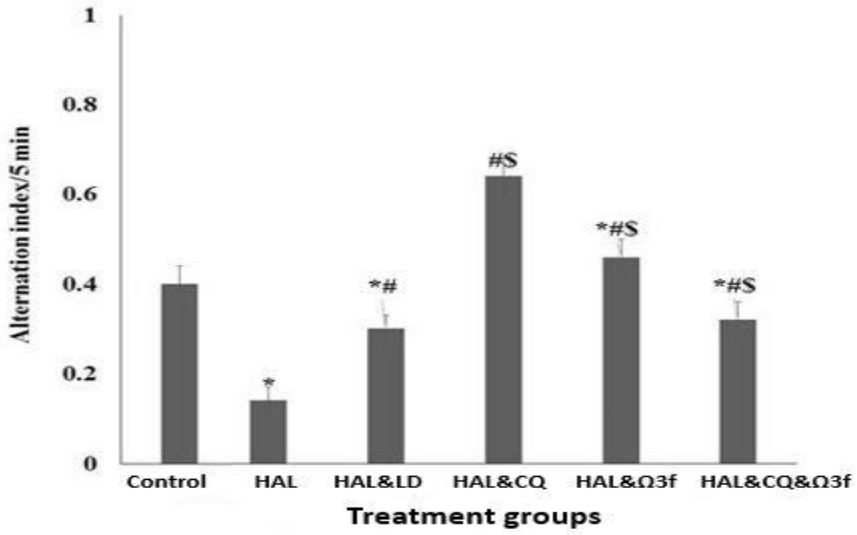


Figure 4: CQ_{10} or Omega-3 fatty acid on spatial working memory in the radial arm maze.

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.

Figure 4 shows the effect of CQ_{10}or Omega-3 fatty acid on radial-arm spatial working-memory measured as alternation index. There was a significant [F (9, 90) = 18.4, p < 0.001] increase in radial arm maze spatial working memory with HAL&$CQ_{10,}$ HAL&Ω-3 and a decrease with HAL, HAL&LD and HAL&CQ_{10}&Ω-3F compared to control. Compared to HAL, radial arm 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 radial arm maze spatial working memory with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.1.5. CQ_{10} or Omega-3 fatty acid on time spent in the open arm

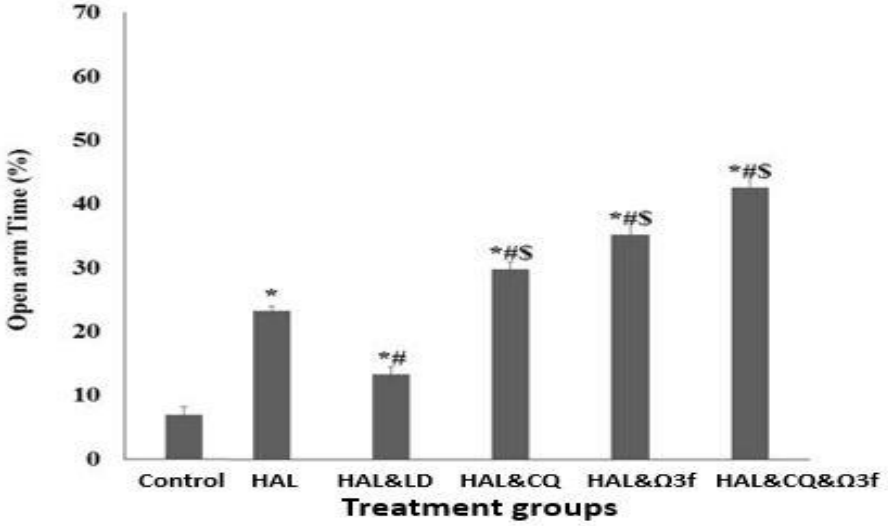


Figure 5: CQ_{10} or Omega-3 fatty acid on open arm time (%) in the elevated plus maze.

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.

Figure 5 shows the effect of CQ_{10}or Omega-3 fatty acid on percentage time spent in the open arm of the EPM. There was a significant [F (9, 90) = 50.5, p < 0.001] increase in open arm time with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3Fcompared to control. Compared to HAL, open arm time decreased significantly with HAL&LD, and increased with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Compared to HAL&LD, there was an increase in open arm with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.1.6. CQ_{10} or Omega-3 fatty acid on time spent in the closed arm

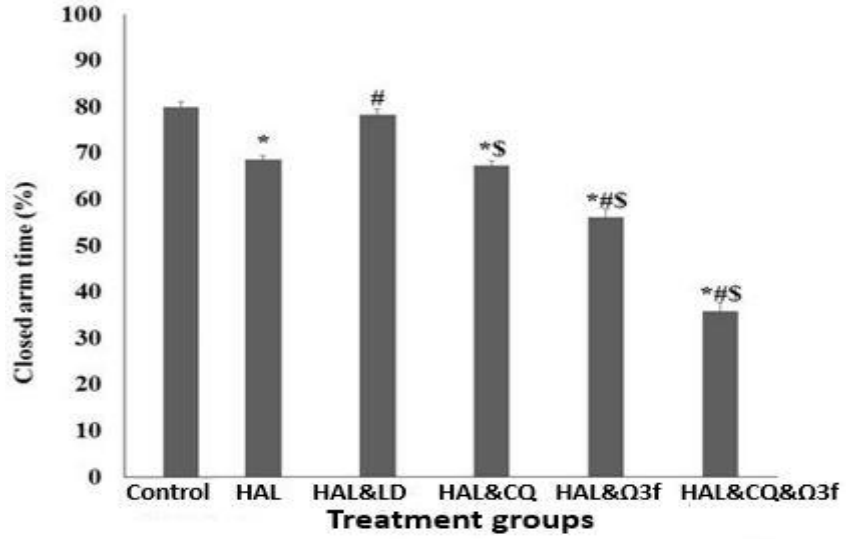


Figure 6: CQ_{10} or Omega-3 fatty acid on closed arm time (%) in the elevated plus maze.

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.

Figure 6 shows the effect of CQ_{10}or Omega-3 fatty acid on percentage time spent in the closed arm of the EPM. There was a significant [F (9, 90) = 1248, p < 0.001] decrease in closed arm time with CQ_{10}&Ω-3F, HAL, HAL&CQ_{10} and HAL&CQ_{10}&Ω-3Fcompared to control. Compared to HAL, closed arm time increased significantly with HAL&LD, HAL&Ω-3F and HAL&CQ_{10}&Ω-3F. Compared to HAL&LD, there was a decrease in closed arm with HAL&Ω-3F and HAL&CQ_{10}&Ω-3F.

3.2. CQ_{10} or Omega-3 fatty acid alone or in combination on biochemical assays

Table 1 shows the effect of CQ_{10} or Omega-3fatty acid on lipid peroxidation, interleukin-10, dopamine and caspase-3 levels in the brain. Lipid peroxidation measured as MDA concentration [F (9, 90) = 22.10, p < 0.001] increased significantly with HAL, HAL&LD, and decreased with CQ_{10}&Ω-3F, HAL&$CQ_{10,}$ HAL&Ω-3F, HAL&CQ_{10}&Ω-3F compared to control. Compared to HAL control, levels of MDA 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 MDA levels with HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively.

Compared to HAL control, levels of IL-10 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 IL-10levels with HAL&CQ_{10}, HAL&Ω-3Fand HAL&CQ_{10}&Ω-3Frespectively. Dopamine p < 0.001 decreased significantly with HAL, HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F compared to control. Compared to HAL control, dopamine increases significantly with HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3F respectively. Compared to HAL&LD, there was increase in the Caspase-3activity with HAL&$CQ_{10}$and HAL&CQ_{10}&Ω-3F respectively.Caspase-3activity [F (9, 90) = 13.32, p < 0.001] decreased significantly with HAL, HAL&LD, HAL&$CQ_{10,}$ HAL&Ω-3F and HAL&CQ_{10}&Ω-3Fcompared to control. Compared to HAL control, caspase-3activity 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 Caspase-3activity with HAL&$CQ_{10}$and HAL&CQ_{10}&Ω-3F respectively.

Table 1: Data Showing Biochemical Assays Results

Groups	MDA (uM)	Il-10 (pg/ml)	Caspase-3 (µmolpNA/min/ml)	Dopamine(pg/ml)
Control	16.59±1.10	252.60±1.90	3.97±0.22	24.48±1.10
HAL	24.69±1.20*	148.194±1.10*	3.64±0.10*	07.21±1.20*
HAL&LD	16.34±0.60$^{*\#}$	171.33±1.20*	2.65±0.10$^{*\#}$	14.34±0.60$^{*\#}$
HAL&CQ_{10}	6.49±1.10$^{*\#\$}$	321.659±1.22$^{*\#\$}$	0.04±0.01$^{*\#\$}$	17.26±1.10$^{*\#\$}$
HAL&Ω-3F	9.91±0.50$^{\#\$}$	474.81±1.30$^{*\#\$}$	2.63±0.10$^{*\#\$}$	22.51±0.50$^{\#\$}$
HAL&CQ_{10}&Ω-3F	8.02±0.50$^{*\#\$}$	403.10±1.50$^{*\#\$}$	1.65±0.10$^{*\#\$}$	27.08±0.50$^{*\#\$}$

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; Dopamine. TE: trollox equivalent.

3.3 Histological Results

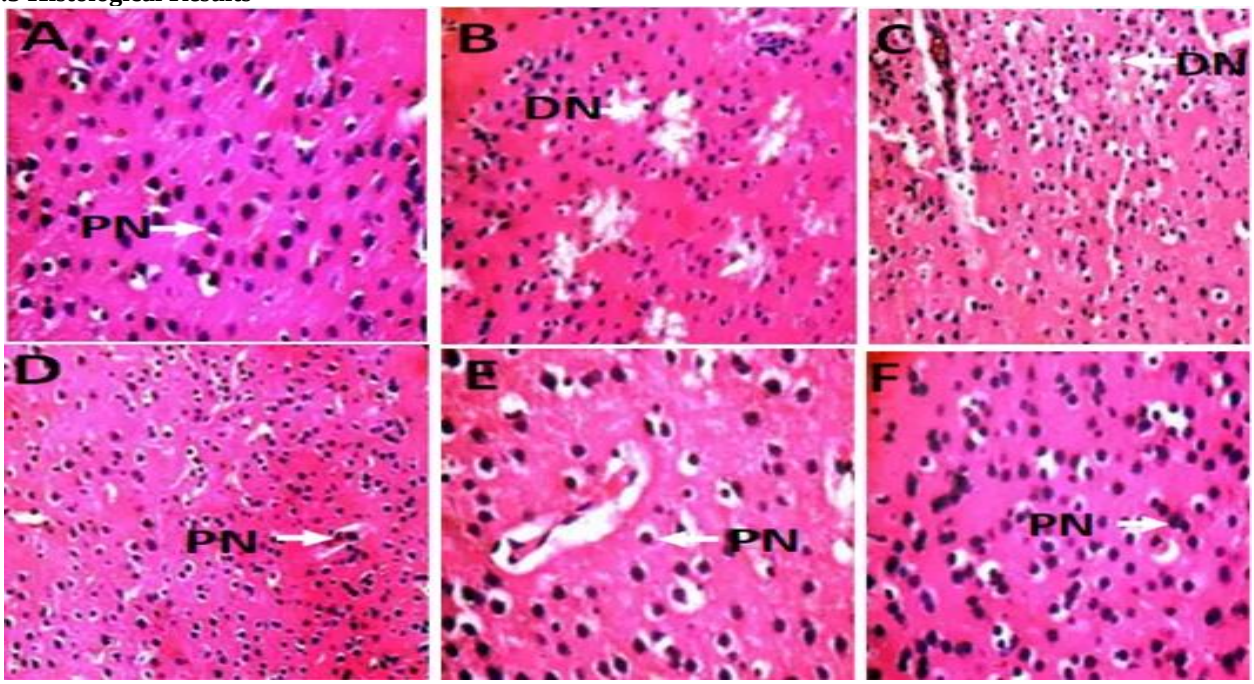


Plate 1: Photomicrograph of a pre-frontal cortex section stained by Haematoxylin and Eosin.

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.

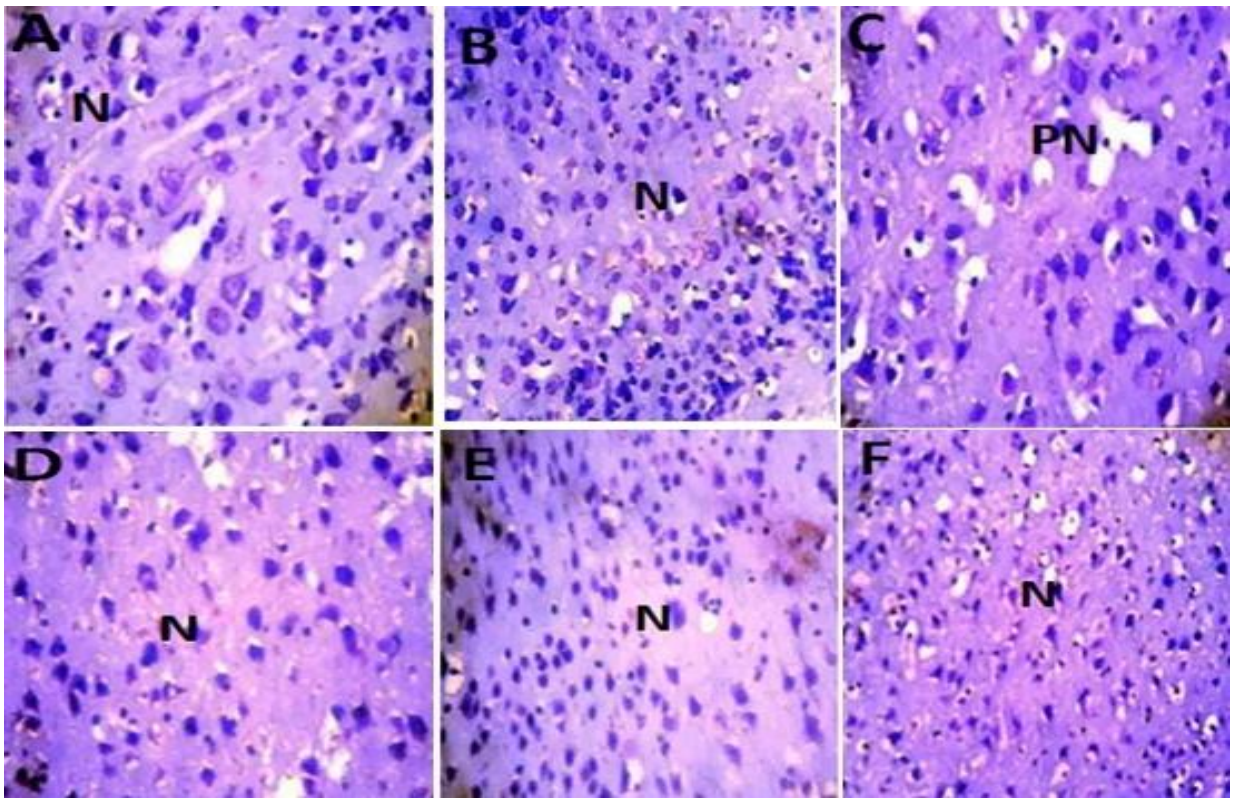


Plate 2: Photomicrograph of a prefrontal cortex section stained by cresyl violet.

(A, C &F) normal pyramidal cells with normal cytoplasmic distribution of nissl bodies (N), however, groups (B, D and E) comparatively reduced staining intensities with poor distribution of nissl bodies within their cytoplasm (PN). Mag X 400.

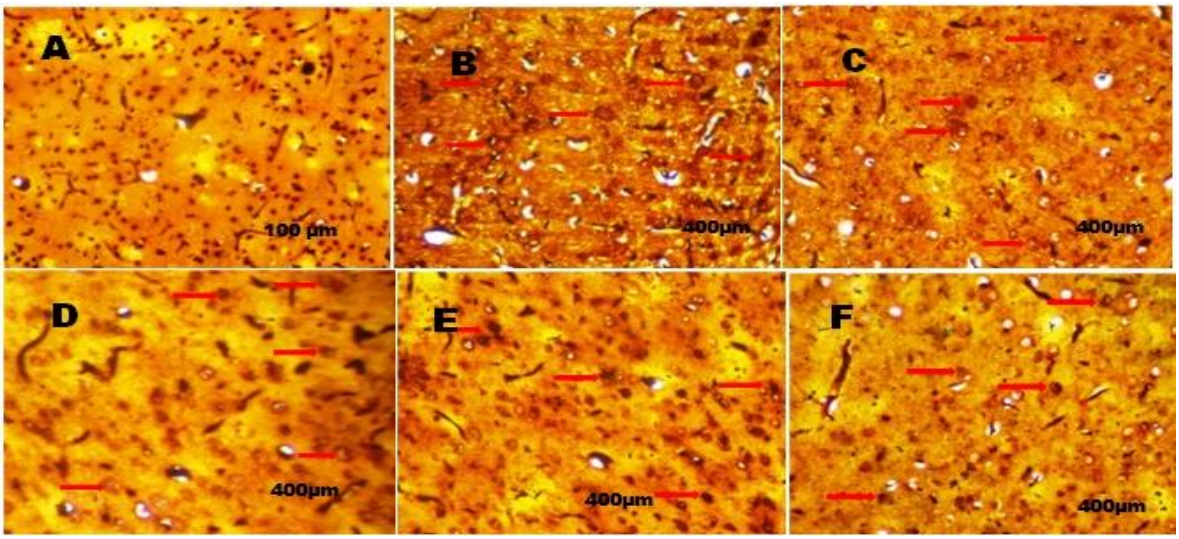
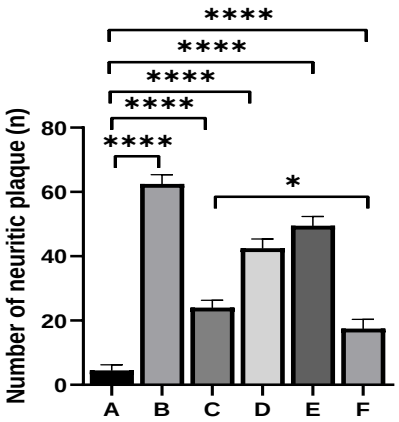


Plate 3: Photomicrograph of a prefrontal cortex stained by Bielschowsky.



Bielschowsky silver staining after intervention in the studied groups reveals neuritic plaques and argyrophilic structures in the HAL induced and treated group. These fibrillary amyloid deposits are absent in the non-induced control mice while varying degrees of neuritic plaques and argyrophilic structures (red arrows) are seen across other groups. **A.**control group. **B.** HAL group. **C.** LD treated group **D.** CQ1O treated group. **E.**Omega-3 fatty acid diet treated group **F.**CQ10 and omega-3 fatty acid diet treated group. Histomorphometry of Bielschowsky silver staining in the brain of HAL exposed mice; Image J count of neuritic plaques. Data are expressed as mean ± SD. *p<0.05, **p<0.01,***p<0.001 ****p<0.0001. One-way ANOVA followed by Turkey post hoc test. Mag. X400.

3.4. Immunohistochemical Results

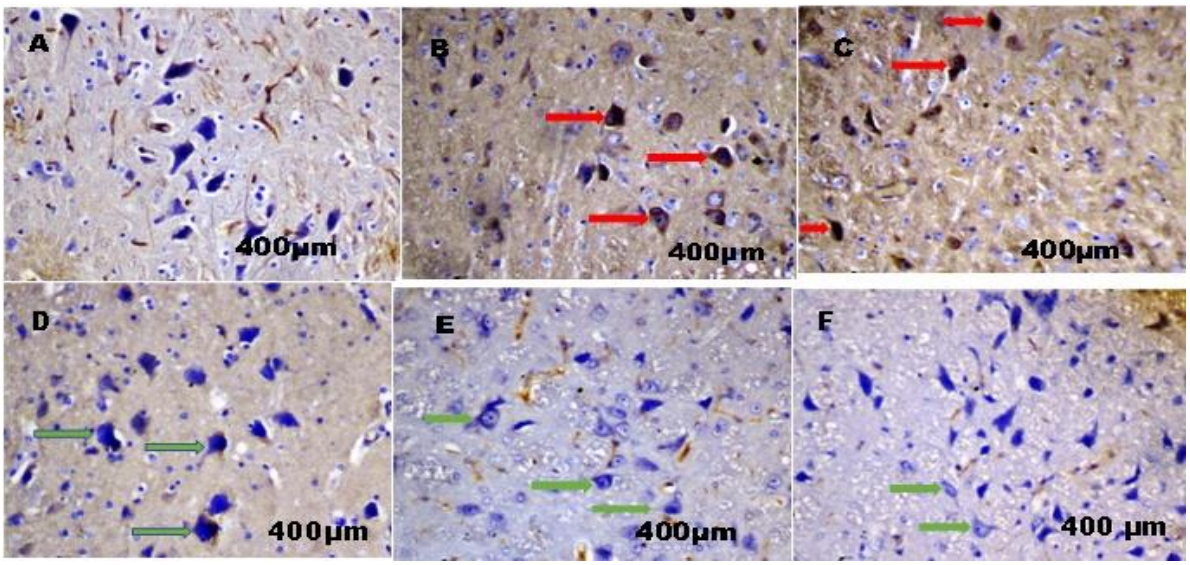
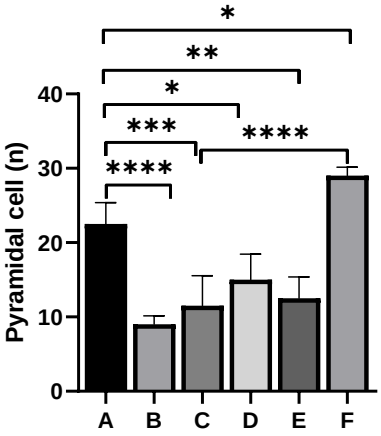


Plate 4: Photomicrograph of a prefrontal cortex stained by neuron-specific enolase (NSE).

Immunohistochemical localization of neuron-specific enolase (NSE) with immunoreactivity in neurons in prefrontal cortex of HAL exposed mice. NSE immunoreactivity is present B and C (Red arrow). Note the absence of immunoreactivity in D, E and F (Green arrow).**A.** control group (Negative). **B.** HAL group. **C.** LD treated group **D.** CQ1O treated group. **E.** Omega-3 fatty acid diet treated group **F.** CQ10 and omega-3 fatty acid diet treated group. **G.** NSE immunoreactivity in prefrontal cortex of HAL exposed mice; Image J count of pyramidal cells in the brain. Data are expressed as mean ± SD.*p<0.05, **p<0.01,***p<0.001 ****p<0.0001. One-way ANOVA followed by Turkey post hoc test. MagX 400.



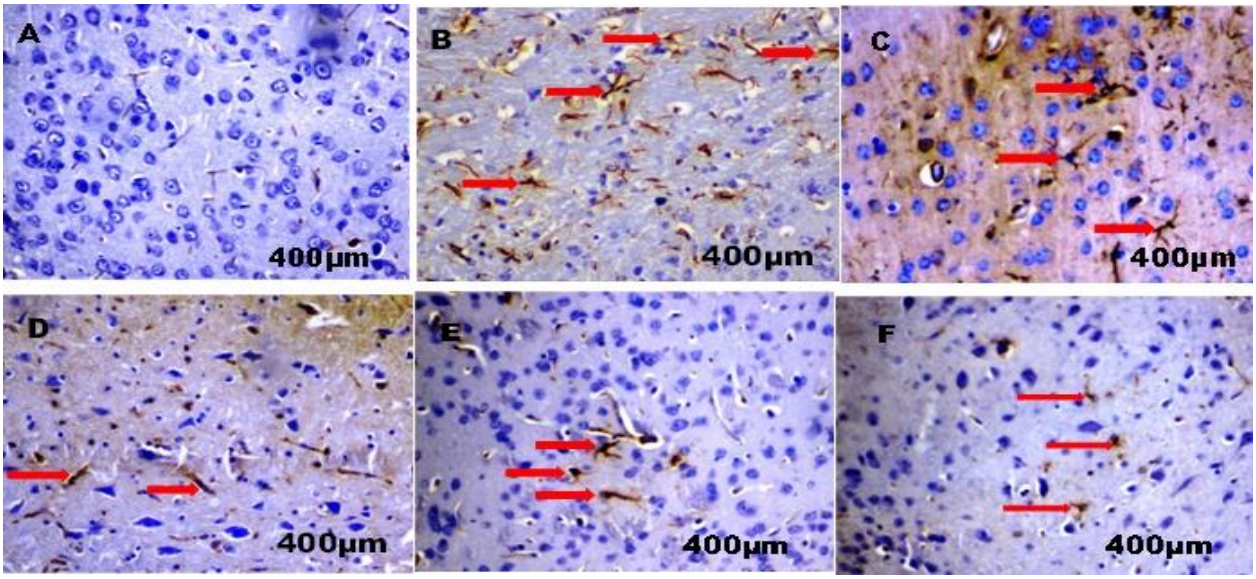
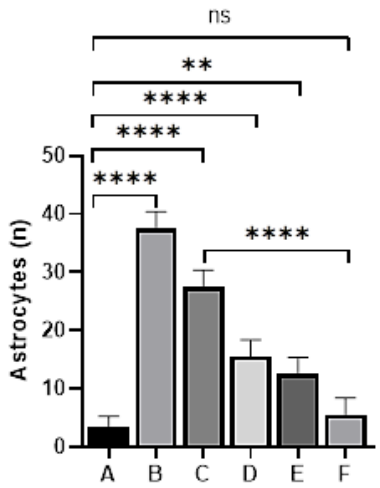


Plate 5: Photomicrograph of a prefrontal cortex stained by G-FAP.

Astrocytic reaction to dopamine depletion in the prefrontal cortex of experimental mice. Dopamine depletion increases G-FAP immunostaining in the prefrontal cortex sections across the HAL exposed/ treatment groups. **A.** control group (Negative). **B.** HAL group (positive +++). **C.** LD treated group (++) **D.** CQ1O treated group (+). **E.** Omega-3 fatty acid diet treated group (+) **F.** CQ10 and omega-3 fatty acid diet treated group (+). **G**. Histomorphometry of the astrocytic reaction to dopamine depletion in the prefrontal cortex of experimental mice.Image J count of astrocyte in the brain. n = number of astrocytes; *p<0.05, **p<0.01, ****p<0.0001. Values are expressed as mean ± SD. One-way ANOVA followed by Turkey post hoc test. Mag X400.



4.0. DISCUSSION

Haloperidol induces dopamine depletion by blocking central dopamine receptors. Dopamine receptors have been demonstrated to be equally distributed postsynaptically on striatal neurones and presynaptically

on cortico-striatal terminals, as indicated by their particular, high-affinity binding to the potent neuroleptic haloperiodol. Haloperidol relieves the delusions and hallucinations that are frequently connected to psychosis by competitively blocking post-synaptic dopamine (D2) receptors in the brain, blocking dopamine neurotransmission and eradicating dopamine neurotransmission. Classical of dopamine depletion are various neurodegenerative diseases including Parkinson's, schizophrenia, addiction, attention difficulties, hallucination and delusions, attention deficit hyperactivity disorder (ADHD), also including low sex drive and weight fluctuation. Haloperidol also caused low line crossing, rearing and mouse grooming as locomotor activities parameters, reduced spatial working memory and evidence of reduced open- arm time (suggestive of anxiety) in this study, dyskinesia caused by inhibiting post-synaptic dopamine receptors in the brain. Comparative assessment of L-Dopa; an exogenous dopamine with co-administration of CoQ10 and omega3 fatty acid showed a more enhanced locomotor and memory performances in the latter. CoQ10 via mitochondrial oxidative phosphorylation and resultant generation of ATP in the inner mitochondrial membrane most presumably posed a synergistic reaction with augmented neuronal plasticity typical of omega3 fatty acid. Although, there has been increased research attention on nutritional supplements in the management of neurodegenerations, partly because they have lesser side effects unlike standard medications, the mechanism behind the synergy and comparatively more desired outcome with the duo of CoQ10 and Omega 3, against L-Dopa, may be astonishing. Higher anti-inflammatory and anti apoptotic activities were seen with CoQ10 and Omega 3 co-administration via interleukin 10 and caspase3. CoQ10 and Omega 3 co-administration also demonstrated greater suppression of lipid peroxidation in this research. The summation of these could possibly explain why they delivered more exogenous dopamine level in mice used in this study, than levodopa cabidopa. Earlier study suggests that the anti-inflammatory effect of CoQ10 may be associated with adiponectin; by causing an increase in CoQ10 levels, supplementation leads to a rise in adiponectin levels, which then leads to a decrease in the inflammatory response mediated by TNF-α.[29] Fatty acids are incorporated into cell membrane phospholipids.[30] PUFAs exert actions within the membrane itself or following controlled release from the membrane by phospholipase enzymes.[31] The anti-inflammatory actions of the omega -3 fatty acids are known to be at least partly mediated by cell surface and intracellular receptors, GPR120 and NR1C3 (i.e. PPAR-γ), respectively. Both these receptors appear to be involved in inhibiting activation of NFκB, the prototypical pro-inflammatory transcription factor. PPAR-γ acts by a physical interaction while GPR120 inhibits signalling upstream of phosphorylation of IκB.[32]

Neuronal death and degeneration causes poor distribution of nissl bodies and reduced staining ability with cresyl violet. This is consistent with the haloperidol group in this study with reduced stain-ability possibly because of more severely degenerated pyramidal neurons of the prefrontal cortex, present in the group. L-Dopa intervention also showed moderate degeneration of pyramidal, cells exhibiting cytoplasmic vacuolation. CoQ10 and Omega 3 co-administration relieves the neutritic plagues and neurofibrillary tangles induced by haloperidol most likely by reducing the production of pro-inflammatory cytokines by limiting the expression of the gene encoding nuclear factor kappa B (NF-Kb), lipoperoxides caused by dopamine depletion and oxidizing agents produced during the infectious process induce the signalling pathway in monocytes that activates the NF-kB factor, resulting in the release of TNF-alpha being favoured.[29] Neuron specific enolase (NSE), is an enzyme involved in glycolytic energy metabolism in the brain, which is released from neurons during injury, found in the cytoplasm of the neurons (isoenzyme γγ) and neuroendocrine cells (isoenzyme αγ) and is considered a biomarker for neuronal injury. NSE as a major biomarker that can directly assess functional damage to neurons was also found to be high in concentrations in traumatic brain injuries corresponding to the degree of severity of the injury. Findings in this study showed significant immunoreactivity of NSE in the haloperidol administered group, suggestive of a prefrontal cortex injury, orchestrated by dopamine depletion. CoQ10 and Omega 3 fatty acid co-administration intervention groups were devoid of NSE immunoreactivity rather enhanced pyramidal cell count.

First isolated in 1971, Glial fibrillary acidic protein (GFAP) is specifically found in glial cells of the central nervous system, constituting the major part of the cytoskeleton of astrocytes. GFAP is a biomarker for astroglial injury, gliosis that upregulates GFAP makes GFAP an attractive candidate biomarker for brain injury screening. Mouse model studies have been conducted to assess functional outcomes after brain injury and observed increment in GFAP level correlated with inhibited spatial learning, as evidenced by decreased performance in maze model trials. Haloperidol administration in this current research revealed significant GFAP immunoreactivity and high astrocytic counts, L-Dopa intervention post haloperidol, also exhibited similarly high GFAP immunoreactivity. These rates of upsurge in reactive astrocytes were consistent with inhibited mouse radial-arm maze exposure and reduced locomotor parameters (line crossing, rearing and grooming) displayed in open field apparatus. CoQ10 and Omega 3 fatty acid co-supplementation post haloperidol showed comparatively lower reactive astrocytic count on GFAP, enhanced locomotor activities and reduced anxiety as evidenced by time spent in closed arm of elevated plus maze.

5.0. CONCLUSION

Haloperidol induced dopamine depletion in mouse with attendant dyskinesia, anxiety-related behaviours, reduced memory on radial-arm maze, neuroinflammation, lipid peroxidation, neutritic plaque formation in its prefrontal cortex as well as NSE immunoreactivities and elevated astrocytic counts on GFAP. Comparative assessments of L-Dopa intervention to CoQ10 and Omega 3 fatty acid co-supplementations showed a better amelioration of the prefrontal cortex injury, increased dopamine counts and subsequent excitation in the behavioural models, in the latter than L-Dopa.

6.0. Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments are as examined and approved by the Research Ethical Committee of College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. All authors hereby declare that all experiments have been examined and approved by the said ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

7.0. Competing Interests

Authors have declared that no competing interests exist.

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