Research Article

Quail (*Coturnix japonica*) Egg modulated Cerebral Oxidative Stress, Autophagy, Inflammasomes, Apoptosis and PI3K-AKT-mTOR Signaling Pathways impaired by Rotenone in Rats

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Abstract | Trado-medicinal and nutritional therapies are currently acceptable, compared to synthetic orthodox drugs with consequent side effects and worrisome dose-prescription. The aim of the study was to investigate the mechanism of protection of quail egg mixture against rotenone-induced cerebral oxidative stress, autophagy, inflammasomes, apoptosis, and PI3K-AKT-mTOR signaling pathways as potent in reversing the deleterious effects of this neurotoxicant against cognitive and on cognitive function. The constituents of the quail egg mixture were characterized to determine vitamins, mineral elements and amino acids present therein. Wistar rats were exposed to 5 mg/kg body weight (BWT) of rotenone intraperitoneally. 1000 mg/kg BWT of quail egg mixture (ROTQ) and 5 mg/kg BWT of ubiquinone (ROTU) were administered to mitigate for 5 weeks. Behavioral indices using Y-maze was conducted to determine the spatial working memory of the rats were conducted After the treatments, the gene expression of some endogenous biomarkers such as LC3, Beclin-1, NLRP3, IL-1β, Cas-3, Bax, Bcl-2, PI3K, AKT, and mTOR were evaluated. The results revealed the effects of rotenone and quail egg mixture on neuronal autophagy, inflammasome, apoptosis, and a major signaling pathway mechanism. The bioactive peptide in the quail egg mixture demonstrated neuroprotection by supplying amino acids that are precursors to dopamine, acetylcholine and GABA, mitigating neuronal cell death. The quail egg mixture is rich in vitamins that are bioactive in the protection of neurons and restoring of neuronal functions. Quail egg bioactive compounds challenged rotenone-induced neurotoxicity favorably with the significant restoration of the functions of the neuron.

Keywords | Inflammasome, Autophagy, Neuronal signaling, Parkinson's disease, Quail egg, Apoptosis, Rotenone

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INTRODUCTION

Many insecticides, piscicide and pesticides are used in both large and small scale fumigation by individuals. Workers in industries where these chemicals are manufactured are not exempted from the deleterious effects of exposure to these products which contain rotenone, an isoflavone lipophilic pesticide, which is a

neurotoxic substance (Narongchai *et al*., 2005) Rotenone has been shown to inhibit complex I (NADH:ubiquinone oxidoreductase) of the mitochondrial electron transport chain, leading to ATP depletion and electron leakage. This dysfunction contributes to the generation of reactive oxygen species (ROS) and oxidative stress. Intraperitoneal administration of rotenone in experimental models replicates key pathological hallmarks of Parkinson's disease, including dopaminergic neuronal loss and motor impairments (Tanner *et al*., 2011; Zhang *et al*., 2019) coupled the toxicity of the dopaminergic neurons with the of the It has been implicated in the manifestation of neurodegenerative features majorly similar to Parkinson's disease (Venkateshgobi *et al*., 2018) and the potential risk of Alzheimer's disease (Alam and Schmidt, 2002).

At the cellular level, rotenone disrupts mitochondrial function by blocking the transfer of electrons from the iron-sulfur (Fe-S) clusters in complex I to ubiquinone, effectively inhibiting oxidative phosphorylation. This impairment results in a decline in mitochondrial membrane potential and subsequent energy crisis within cells (Yarmohammadi *et al*., 2020). In addition to mitochondrial dysfunction, rotenone modulates key molecular pathways, altering the expression of inflammasomes and apoptotic, anti-apoptotic, and autophagic proteins such as Beclin-1, LC3, NLRP3, IL-1β, caspase-3, Bax, Bcl-2, PI3K, Akt, and mTOR (Hong *et al*., 2010; Venkateshgobi *et al*., 2018; Priyanka *et al*., 2020; Yarmohammadi *et al*., 2020). The PI3K/Akt/mTOR pathway, a critical intracellular signaling cascade, regulates diverse cellular processes including growth, proliferation, apoptosis, and autophagy. Activation of PI3K leads to Akt phosphorylation and recruitment to the plasma membrane, promoting downstream signaling (Fulda, 2009; Priyanka *et al*., 2020).

Neurodegenerative disorders affect all ages, however it predominantly affect the elderly, and are characterized by progressive neuronal loss and associated symptoms such as motor tremors, postural instability, rigidity, bradykinesia, and cognitive decline (Wenyu *et al*., 2015). In Parkinson's disease, the degeneration of dopaminergic neurons in the substantia nigra leads to dopamine deficiency and the formation of Lewy bodies, cytoplasmic inclusions composed mainly of α-synuclein and ubiquitin (Comoglu *et al*., 2013). Similarly, cognitive deficits arise from the degeneration of cholinergic neurons in the basal forebrain (Rizzi and Tan, 2017).

The Japanese quail (*Coturnix japonica*) egg has been traditionally valued for its therapeutic properties in conditions associated with oxidative stress, inflammation, and apoptosis (Howard *et al*., 2006; Ibukun and Oladipo, 2016; Oladipo *et al*., 2020). Its beneficial effects are attributed to its rich content of amino acids, essential fatty acids, vitamins, and minerals, which support cellular growth and function. Vitamins such as the B-complex group are crucial for maintaining normal brain activity, while antioxidants like vitamin E protect neurons from oxidative damage. Zinc, an important trace element in quail eggs, exhibits anti-peroxidative effects and protects cells against free radicals. Moreover, zinc modulates the activity of enzymes such as carbonic anhydrase (Oladipo *et al*., 2020), DNA-dependent RNA polymerase (King *et al*., 2004), and thymidine kinase (Prasad and Oberleas, 1970), mitigating their potential deleterious effects.

Given these neuroprotective properties, the present study investigates the effects of quail egg constituents in counteracting rotenone-induced neuronal dysfunction and exploring their potential in modulating oxidative stress and apoptotic pathways.

MATERIALS AND METHODS

Rotenone (1g) used was manufactured by Sigma-Aldrich (CAS number 83-79-4) and obtained from Pascal Scientific Limited, Akure, Nigeria. Other chemicals used were of A.R. grade of research lab brand and were also procured from Pascal Scientific Limited, Akure, Nigeria.

Sample collection

Quail eggs (100 pieces) were obtained at a local market in Akure-South Local Government Area, Ondo State. Nigeria. The contents of the eggs were transferred into a petri dish, vortexed to homogeneity and freeze-dried using a Bioevopeak Benchtop Freeze Dryer (LYO06B-1S, Seattle, United States). The freeze-dried sample was stored at 4 °C until use.

Characterization of components

HPLC-3100 (Seattle, United States) was used to characterize vitamins in the mixture as described by Oladipo *et al*. (2020). The mineral elements in the quail egg mixture were determined using a Flame Photometer and Atomic Absorption Spectrophotometry following standard operating procedures.

Experimental intoxication by rotenone

Adult male Wistar rats, weighing 180±20 g were acquired from Experimental Animal Farm, Ogbomoso, Nigeria. Rats were housed under controlled conditions of temperature (22±1 °C), humidity (50-55 %), and light (12 h light/12 h dark cycle). Experimental procedures were approved by the Centre for Research and Development, the Federal University of Technology, Akure, Nigeria (FUTA/SOS/2015/331). Carboxymethylcellulose (4%) and chloroform (1.25%) were used as the vehicle for dissolving 5mg/kg BWT of rotenone and administered

intraperitoneally. The treatments lasted for 5 weeks.

The experiment was designed with five rats in each group and the mixture was administered orally:

NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; ROT: Rotenone (5mg/kg BWT i.p).

Y-maze test

The spatial working memory of the rats was evaluated using Y-maze. The frequencies of alteration between the arms were recorded and plotted to determine the memory index (percentage spontaneous alternation) (Kraeuter *et al*., 2018).

Tissue preparation

Rats were weighed and sacrificed by cervical dislocation. The whole brain tissue was obtained after decapitation and careful dissection. Each brain was weighed, immersed in ice-cold 1.15% potassium chloride solution, and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) in ratio 1:5 (w/v) using a Teflon homogenizer. The homogenized tissue was centrifuged at 3000 x g in a high-speed refrigerated centrifuge (CFGR-16DR, Seattle, United States) for 10 min at 4 ºC to separate the supernatant. A portion of the brain was processed into a 4-5 µm cross-sectional diameter stained with hematoxylin-eosin and examined under a microscope and a magnification of x100.

mRNA genes of neuronal autophagy, inflammasome, apoptosis and anti-apoptosis, and signalling pathway proteins

The mRNA expression was determined by extraction using a total RNA extraction kit (Sangon Biotech Co., Ltd, China) for the manufacturer's protocol by RT-PCR. A wavelength of 260/280 nm was used in the determination of the purity of the total RNA. The reverse transcription of cDNA from RNA was conducted using cDNA biosynthesis kit (Sangon Biotech Co., Ltd) SYBR® Premix Ex TaqTM coupled with Applied Biosystems 7900 Fast RT-PCR System (Applied Biosystems, Stadt, California) was used to subject the cDNA samples to PCR. The gene expression formula 2-ΔΔCt was used in the calculation of the RT-PCR data. All values were normalized by β-actin. The primer sequences of the target genes are as follows:

β-actin- F: 5'GCCATGTACGTAGCCATCCA3'
R: 5'GAACCGCTCATTGCCGATAG3'
LC3 gene- F: 5'CCTGCTGCTGGCCGTAGT3'
R: 5'TGATGAAGTCTTCCTGCCAAAA3'
Beclin-1 F: 5'AGCACGCCATGTATAGCAAAGA3'
R: 5'GGAAGAGGGAAAGGACAGCAT3'
NLRP3 F: 5'TGCTCTTCACTGCTATCAAGCCCT3'
R: 5'ACAAGCCTTTGCTCCAGACCCTAT3'
IL-1β F: 5'TGGAAAAGCGGTTTGTCT3'
R: 5'ATAAATAGGTAAGTGGTTGCC3'
Caspase-3 F: 5'GTGGAACTGACGATGATATGGC3'
R: 5'CGCAAAGTGACTGGATGAACC3'
Bax F: 5'CGGCGAATTGGAGATGAACTGG3'
R: 5'CTAGCAAAGTAGAAGAGGGCAACC3'
Bcl-2 F: 5'TGTGGATGACTGACTACCTGAACC3'
R: 5'CAGCCAGGAGAAATCAAACAGAGG3'
PI3K F: 5'ACACCACGGTTTGGACTATGG3'
R: 5'GGCTACAGTAGTGGGCTTGG3'
AKT F: 5'ATGTCCGAGATCCTACCCTACG3'
R: 5'AGCGAAGAAGGAGTTGGTGTC3'
mTOR F: 5'GGTGGACGAGCTCTTTGTCA3'
R: 5'AGGAGCCCTAACACTCGGAT3'

Western blot analysis

Cold homogenization and centrifugation at 10,000 x g (5 min) of brain tissue before subjecting to T-PER (Tissue-protein extraction lysis solution) (50 mg/500 µl lysing solution) along with 10 µl protease inhibitor cocktail and 10 µl phosphatase inhibitor generated the protein lysates. Bradford assay was used to quantify protein and 35 µg of protein was loaded into the SDS-PAGE. The protein samples that were separated were then transferred to the nitrocellulose membrane by using Bio-Rad Transblot turbo transfer system. The membrane was sealed with 5 % Bio-Rad sealing reagent. The sealed membrane was then incubated with 1:1000 dilution primary rabbit antibodies (Cell Signaling Technology, USA) for 12 h at 4 °C. Thereafter, the membrane was washed and incubated with secondary antibody (1:20,000) treatment for 1 h. Bands were revealed and observed in the chemiluminescence document reader, on the addition of Immobilon Western chemiluminescent HRP substrate at standardized exposure time. Densitometry analysis was used to analyze bands of the two groups and compared to β-Actin (Major Science image analysis software) (Bathina and Das, 2018).

Statistical analysis

All values are expressed as mean ± standard deviation. Statistical evaluation was done using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The significance level was set at $p<0.05$.

Ethics approval and consent to participate

The experiment and animal handling inclined with the Ethical Regulation and Guide for the Care and Use of Laboratory Animals of the Centre for Research and Development (CERAD), the Federal University of Technology, Akure, Nigeria (FUTA/SOS/2015/331).

RESULTS

Biochemical effects of treatments on the brain

The mRNA expression levels of LC3 and Beclin-1 are presented in Figure 1a, b, respectively. Rotenone intoxication significantly upregulated the expression of both LC3 and Beclin-1 ($p < 0.05$) compared to the quail egg mixture (QEM) and ubiquinone treatment groups. Although QEM treatment did not significantly reduce LC3 expression compared to the rotenone group, a lower expression was observed in QEM-treated animals relative to those treated with ubiquinone alone ($p < 0.05$).

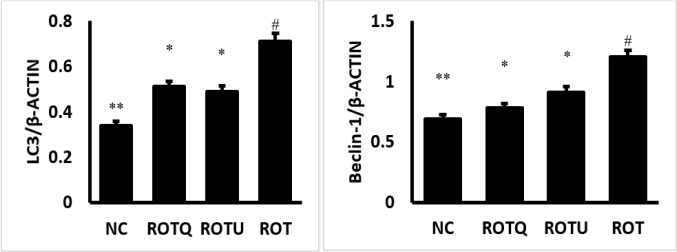

Figure 1: mRNA expression levels of LC3 (a) and Beclin-1 (b) measured using RT-qPCR. Data are presented as the mean ± standard deviation (n=5/group). Superscripts indicate significant difference at $p<0.05$. NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; and ROT: Rotenone (5mg/kg BWT i.p)

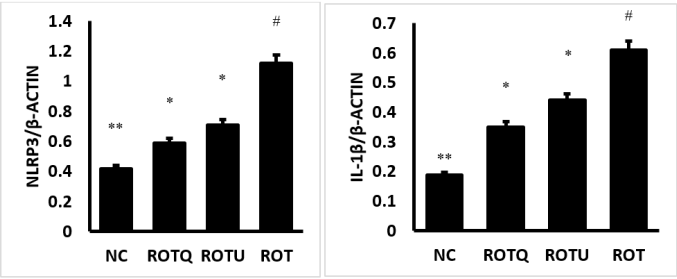

Figure 2: mRNA expression levels of NLRP3 (a) and IL-1β (b) measured using RT-qPCR. Data are presented as the mean ± standard deviation (n=5/group). Superscripts indicate significant difference at $p<0.05$. NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; and ROT: Rotenone (5mg/kg BWT i.p)

The effect of QEM on the expression of NLRP3 (Figure 2a) and IL-1β (Figure 2b) showed a significant reduction in mRNA levels compared to both the untreated rotenone group (ROT) and the ubiquinone-treated group (ROTU) ($p < 0.05$). These findings suggest that QEM administration attenuated the expression of inflammasome-related genes.

Similarly, the mRNA expression of caspase-3 (Casp-3) (Figure 3a) was significantly reduced in the QEM-treated group. Comparable trends were observed for Bax (Figure 3b) and Bcl-2 (Figure 3c), where QEM administration led to significantly lower expression levels of both pro- and anti-apoptotic markers ($p < 0.05$) compared to the ROT and ROTU groups.

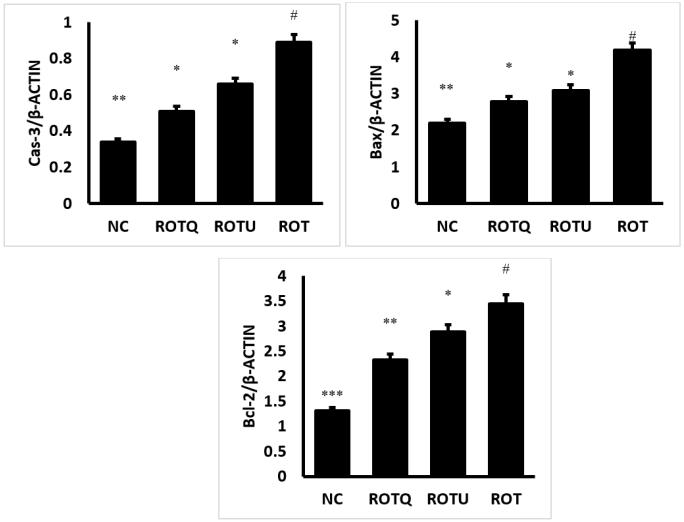

Figure 3: mRNA expression levels of Cas-3 (a), Bax (b), and Bcl-2 (c) measured using RT-qPCR. Data are presented as the mean ± standard deviation (n=5/group). Superscripts indicate significant difference at $p<0.05$. NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; and ROT: Rotenone (5mg/kg BWT i.p).

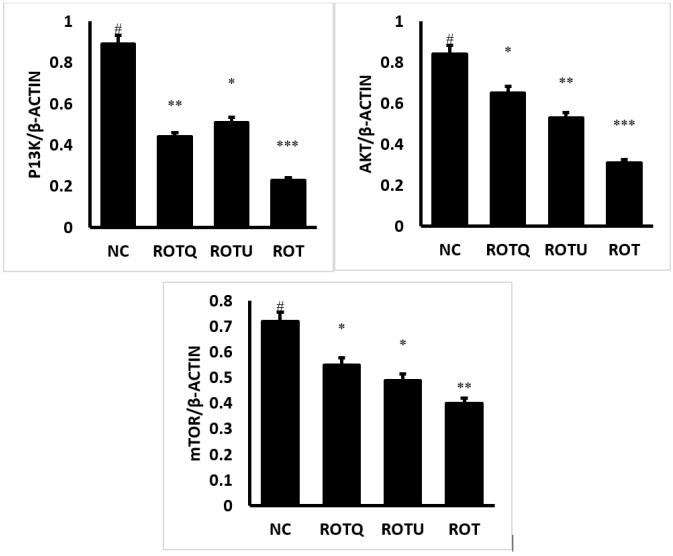

Figure 4: mRNA expression levels of P13K (a), Akt (b), and mTOR (c) measured using RT-qPCR. Data are presented as the mean ± standard deviation (n=5/group). Superscripts indicate significant difference at $p<0.05$. NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; and ROT: Rotenone (5mg/kg BWT i.p).

Table 1: Mineral elements.

	K	Na	Ca	Mn	Fe	Cu	Zn	P	Mg	N	Se
Quail egg mixture	1825	3465	109850	ND	83	2	88	1361.2	51252	69768	1932

The gene expression of PI3K (Figure 4a), Akt (Figure 4b), and mTOR (Figure 4c) key molecules in cell survival signal transduction was markedly suppressed by rotenone treatment. However, QEM significantly reversed this suppression. While the effect of QEM on PI3K was significantly lower than that of ubiquinone ($p < 0.05$), its administration led to significantly higher expression levels of Akt and mTOR compared to the ubiquinone-treated group ($p < 0.05$).

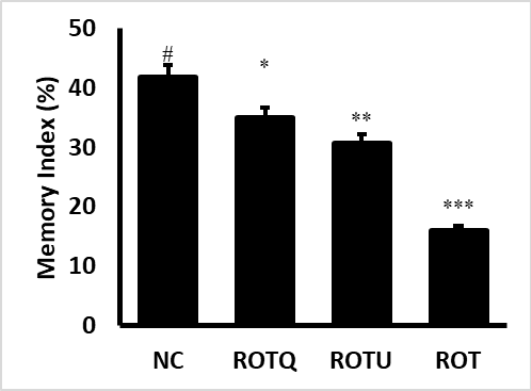

Figure 5: Spontaneous alternation test (memory index). Data are presented as the mean ± standard deviation (n=5/group). Superscripts indicate significant difference at $p<0.05$. NC: Negative control; ROTQ: Rotenone (5mg/kg BWT i.p) and 1000mg/kg BWT of quail egg mixture (QEM); ROTU: Rotenone (5mg/kg BWT i.p) with ubiquinone 5mg/kg BWT; and ROT: Rotenone (5mg/kg BWT i.p)

Cognitive performance, assessed using the Y-maze spontaneous alternation test, is shown in Figure 5. Rats in the ROT group exhibited a significant reduction in spatial memory index relative to the normal control (NC) group ($p < 0.05$), indicating rotenone-induced memory impairment. Conversely, the ROTQ group (rotenone + QEM) demonstrated a significantly higher memory index than both the ROT and ROTU groups ($p < 0.05$), indicating a substantial recovery of cognitive function due to QEM administration.

Quantification of bioactive components in QEM

As shown in Figure 6, chromatography analysis of QEM yielded eleven peaks on the chromatogram. These peaks showed the presence of vitamins A, D, E, K, B3, B6, C, B1, B2, B9, and B5. Figure 7 shows the amount of amino acids present in the QEM. Aspartic acid was the most predominant amino acid, other amino acids in significant amounts were isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and alanine. Amino acids such as serine, tyrosine, proline, histidine, glycine, and arginine existed in concentrations less than 600 mg/100g. Other amino acids such as cysteine, glutamic acid, hydroxyproline and tryptophan existed in concentrations less than 200 mg/100g. The non-essential amino acid (NEAA) aspartic acid was the most abundant, accounting for 1543.3 mg/100g. Table 1 shows the amount of some mineral elements found in QEM. Mineral elements such as K, Na, Ca, Fe, Cu, Zn, P, Mg, N and Se were present.

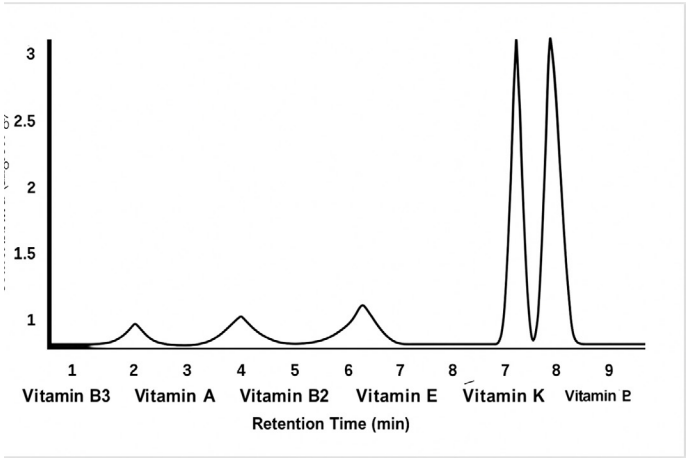

Figure 6: Vitamins composition of quail egg mixture (mg/100g).

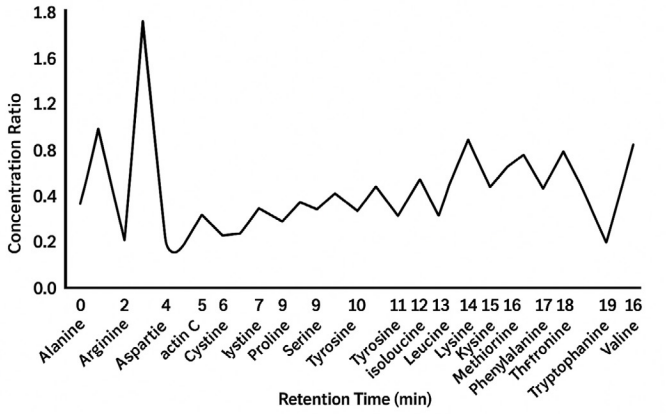

Figure 7: Amino acids composition in quail egg mixture.

DISCUSSION

Zinc plays an essential role in growth, collagen synthesis and DNA repair through the inhibiting the processes of loss of cholinergic and dopaminergic neurons vis a vis the upregulation the biosynthesis of genes of regulating enzymes and proteins of cognitive functions (Erdman *et al.*, 2011). Magnesium enhances brain function by

impeding the activity of the glutamate-induced excitatory neurotransmitter, thus minimizing calcium influx into the postsynaptic neurons, and relaxing smooth muscle to increase cerebral blood flow. It also plays important role in the homeostatic balance of the pathways involved in the secondary phase of brain injury. Selenium is another bioactive trace element with reported antioxidant properties which could function in the regulation of bio-energetic activities in the brain.

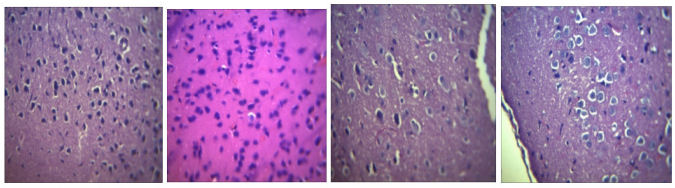

Plate 1: No visible lesions seen.

Plate 2: There is a mild spongiosis of the parenchyma.

Plate 3: There is a mild diffuse spongiosis of the parenchyma. Few neuronal cells are degenerate.

Plate 4: There is severe neuronal degeneration. The cells are rounded and faint staining. There is a mild spongiosis around the degenerate cells.

Figure 8: Plates 1-4: Histopathology of the brain in rotenone-induced brain disease model and effects of quail egg mixture in the attenuation.

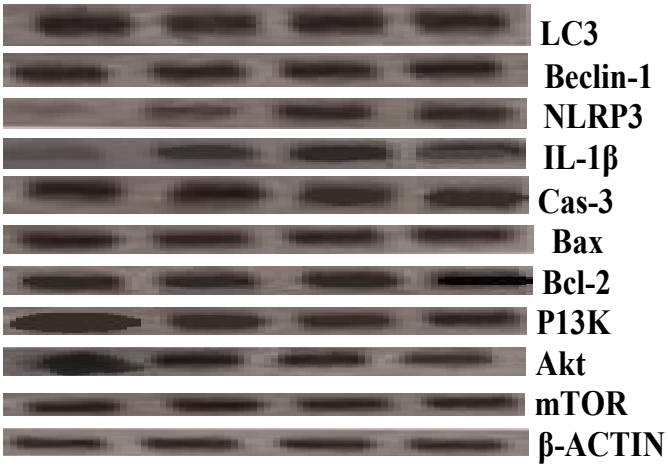

Figure 9: Western blot analysis of genes.

Previously published studies had scored quail egg as a nutritional source of anti-inflammatory, anti-apoptotic and anti-oxidant compounds (Prasad and Oberleas, 1970; Oladipo *et al*., 2020; Pan-Montojo *et al*., 2010). The bioactive compounds of the yolk demonstrated anti-inflammatory effects against TNF-α and IL-6 in streptozotocin-induced type I diabetes mellitus (Prasad and Oberleas, 1970). In the present study, rotenone, a known inducer of neurodegeneration was investigated for its effects on initiation of neuronal apoptosis, inflammation, and a disruptor of cell-cell signaling. The effects of rotenone were established in this study through the alterations or impairments of genetic expression that would translate to proteins which will regulate biochemical processes for cognitive function and memory loss, resulting to the loss of dopaminergic neurons.

Our investigation into the effects of rotenone-induced neurodegeneration (Schober, 2004; Dhillon *et al*., 2008; Pan-Montojo *et al*.,2010; Xiong *et al*.,2013) on LC3 mRNA levels cum the influence of QEM bioactive components, the study revealed significant alterations in the gene expression. The administration of rotenone-induced oxidative damage and a pathology similar to Parkinson's disease (Schober, 2004; Dhillon *et al*., 2008; Pan-Montojo *et al*., 2010; Xiong *et al*., 2013). Rotenone increased the expression of this autophagosomal marker increasing autophagic activity in the neuronal tissue due to the accumulation of the LC3-II which is the lipidated form of LC3 (Cherra *et al*., 2010; Xiong *et al*., 2013). Although the neuroprotective effects of autophagy have been reported (Kouroku *et al*., 2007), the exact role requires elucidation. Our findings revealed that QEM bioactive demonstrate autophagy inhibition through the downregulation of the expression of LC3 gene. The upregulation of Beclin-1 advances autophagic response to neurotoxic stress vis a vis neuronal death. In this study, rotenone exacerbated Beclin-1 effects in the neuronal tissues. The administration of QEM mitigated the effects with a compensatory downregulation of the expression of Beclin-1 gene. Similar findings were reported in the investigation of the effects of autophagy inhibitor 3-methyladenine against the expression levels of LC3 and Beclin-1 (Hong *et al*., 2010). Vitamins C (Sangani *et al*., 2015) and D (Bhutia, 2022) had been reported to recruit autophagy in maintaining cellular homeostasis, these mechanisms are not without feedback and regulations in the instance of the occurrence of pathogenesis resulting from the autophagy. These vitamins and minerals present in QEM are potent as antioxidants and are inhibitors of autophagy as well (O'Connor *et al*., 2022).

The mechanism of signalling which involves PI_3K/Akt/mTOR is frequently altered in diseased states as they are implicated in cell cycle, cell survival, metabolism, motility, angiogenesis, chemoresistance and genomic instability (Fruman and Rommel, 2014; Pajarillo *et al*., 2019). PI_3K/Akt/mTOR cascade was deactivated due to rotenone intoxication (Zhang *et al*., 2019), this was confirmed in our investigation, however, QEM demonstrated a reversing effect and activation of PI_3K/Akt/mTOR. The increase in the expression level of PI_3K/Akt/mTOR is associated to increased phosphorylation of Akt which contributes to the recovery of dopaminergic neurons. Apoptosis is the most deleterious effect of rotenone in the neuroblastoma cell lines (Zhang *et al*., 2019). The expression of cas-3 which is the caspase-3 gene was depleted following the administration of QEM. This indicates a recovery of the cognitive and memory function loss due to neuronal apoptosis. Similar results were obtained for the expression

level of Bcl-2 and Bax. Contrary to some findings (Liu *et al*., 2018), the intoxication of rotenone upgraded the expression of Bcl-2, however, these findings revealed that rotenone administration depleted the expression. The justification for this could be the interaction of the proapoptotic Bax and the anti-apoptotic Bcl-2. Bcl-2 is a family of proteins that regulates the intrinsic pathway of apoptosis through outer mitochondrial permeability control (Liu *et al*., 2018). Bcl-2 forms a heterodimer with BECN1, which is a component of PtdIns3K complex.

In summary, the results presented rotenone as an initiator of apoptosis, inflammation, and oxidative degeneration in the brain. The markers identified were correspondingly reduced by the bioactive compounds in the quail egg components. Dietary intake of sources of selenium and vitamin E could mitigate apoptosis (Nunes *et al*., 2003). Another study revealed the repression of caspase-3 and caspase-8 by folic acid and vitamin B_{12} (Lv *et al*., 2013), vitamins A, C, and D (Yuksek *et al*., 2017). Quail egg yolk is a rich source of selenium, folic acid, and vitamins E (Oladipo *et al*. 2017), these bioactive elements and compounds conferred the inhibition of caspase-3 on quail egg, thus the quail egg components reversed the apoptotic effects of rotenone on the neurons. Impaired intestinal zinc absorption and cellular zinc uptake are the major causes of zinc deficiency.

In quail egg yolk and albumen, there are neuro-functional bioactive substances, primarily vitamins, and amino acids, that are active in downregulating inflammatory responses. Retinoids which are derivatives of retinol (vitamin A) mitigates neuronal proliferation and differentiation through the activation of retinoid-associated signaling molecular pathways against neurodegeneration. Retinoids played role in synaptic plasticity, and cognitive and memory impairments (Misner *et al*., 2001; Etchamendy *et al*., 2003). Vitamins B derivatives such as B_1, B_6, B_9, and B_{12} have been recognized as contributors to cognitive and memory improvement (Miodownik and Lener, 2010). Vitamin C scavenges free radicals and thus exhibits antioxidant property, protecting neurons from the pathophysiological effects of pro-oxidants. These vitamins which are present in the quail egg yolk can mitigate the bioenergetic defects induced by rotenone on the neurons.

Peptides containing proline, methionine, and lysine have anti-inflammatory activity associated with decreased neurodegenerative disease and therapies for cognitive and memory defects of Parkinson's disease, these are referred to as bioactive peptides. The amino acids constituents of the hydrolysates or peptides with neuroprotective effects interact with factors relating to cell death. The amino acids residues of the hydrolysates/peptide are a potent inhibitor of cholinergic loss, suppressing neuronal cell death or neuronal apoptosis. As a result of this, neuroprotective peptides are linked to the regulation of cholinergic breakdown, oxidative stress, and apoptotic factors (Lee and Hur, 2019). Serine is important as a major constituent of phosphatidylserine which is a vital part of the cell membrane of the neuron, this facilitates the efficient release of neurotransmitters that help to improve brain functions (de Koning and Klomp, 2004; Madeira *et al*., 2015). Aromatic amino acids like tryptophan and phenylalanine are not synthesized de novo therefore must be obtained via a protein-rich diet (Barazzoni *et al*., 1998). Tyrosine is a conditionally necessary amino acid that is the precursor of the catecholamine neurotransmitters-dopamine, norepinephrine, and adrenaline, and tryptophan is the primary precursor of serotonin (Fernstrom and Fernstrom, 2007), these directly play signaling effects by stimulating the brain. The quail egg mixture adopted the rich functional peptides present in it to inhibit the activity of caspase-3, enhancing cholinergic, GABAergic and dopaminergic functions.

CONCLUSION

This study highlights the diverse mitigating potentials of quail egg mixture against neurodegeneration, contributed by its bioactive compounds, which include: vitamins, amino acids and essential minerals. Rotenone caused neuronal autophagy, apoptosis-inflammasome activation, and disruption of key signaling pathways through variable pathological mechanisms. Nutritional intervention with the quail egg mixture effectively countered these effects, as evident by significant changes in gene expression, which include: LC3, Beclin-1, NLRP3, IL-1β, Caspase-3, Bax, Bcl-2, PI3K, Akt, and mTOR. The neuroprotective effect is further demonstrated by the amino acids and dipeptides in the quail egg mixture, which serve as precursors to essential neurotransmitters such as: dopamine, acetylcholine, and GABA thereby mitigating neuronal cell death and promoting recovery of dopaminergic, cholinergic, and GABAergic neuronal pathways. Additionally, the mixture's vitamins contents play bioactive roles in restoring neuronal integrity and function through inhibition of pathological mechanisms and upregulation of antioxidant status and down-regulation of apoptosis in the neuronal hemisphere. Generally, the findings established that quail egg mixture causes significant neuroprotective interventions against rotenone-induced neurotoxicity and holds promise as a dietary neurotherapeutic agent.

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NOVELTY STATEMENT

This study provides the first mechanistic evidence that *Coturnix japonica* egg mitigates rotenone-induced cerebral injury by simultaneously regulating oxidative stress, autophagy, inflammasome activation, apoptosis, and the PI3K–AKT–mTOR signaling pathway, revealing a novel dietary-based neuroprotective strategy against neurodegenerative damage.

AUTHOR'S CONTRIBUTION

OGO: Formal analysis, conceptualization, methodology, supervision. POO, OT, OFB: Writing, editing. BFB: Fund acquisition, project administration. OMC, EO: Resources, visualization, supervision. MLO, AIA: Writing, editing, investigation. IOE: Supervision, conceptualization.

Ethics approval and consent to participate

The study was carried out in accordance with the Federal University of Technology, Akure, Nigeria's Ethical Regulation and Guide for the Care and Use of Laboratory Animals.

Consent for publication

The Authors declare consent to publish the article and the corresponding author to oversee the publication.

Availability of data and materials

The data used in the publication are owned by the Authors and are included in the manuscript.

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The authors used generative artificial intelligence tools solely to improve the clarity and language of the manuscript. These tools were not used for data analysis, interpretation, or generation of scientific content. All scientific conclusions and responsibility for the manuscript remain with the authors.

Conflict of interest

The authors have declared no conflict of interest.

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