Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of mice treatment with aluminium chloride in rotenone model of Parkinson's disease

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Abstract

This study aimed to investigate the effect of induced dementia due to low dose of aluminium chloride ($AlCl_3$) in rotenone model of Parkinson's disease. Male mice were treated for two weeks with one of the following: $AlCl_3$ (2 mg/kg, i.p.); rotenone (1.5 mg/kg, s.c.); $AlCl_3$ + rotenone; vehicle. Behavioral tests as: rearing activity, wire hanging, stair tests and Morris water maze (WMZ) together with histopathological examinations were done. The brain malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), paraoxonase-1 (PON-1) and butyrylcholinesterase were also determined. Results indicated that administration of $AlCl_3$, rotenone or their combination caused elevation in brain MDA and nitric oxide and decreased GSH content. Cortical PON-1 activity decreased by $AlCl_3$ or rotenone and showed more decrease after $AlCl_3$/rotenone co-treatment. Butyrylcholinesterase activity in cortex decreased by rotenone, but increased after $AlCl_3$ and $AlCl_3$/rotenone co-treatment. Both $AlCl_3$ and rotenone impaired performance in WMZ, wire hanging and stair tests and decreased rearing behavior. $AlCl_3$/rotenone co-treatment resulted in higher impairment in behavioral tests compared with that caused by either $AlCl_3$ or rotenone. These data indicate that oxidative stress is involved in neurotoxicity caused by either $AlCl_3$ or rotenone; $AlCl_3$ administration didn't enhance oxidative stress and neurodegeneration caused by rotenone although a low dose of $AlCl_3$ worsened rotenone-induced motor and memory impairment. Increased intake of this metal therefore is likely to worsen motor and cognitive symptoms in idiopathic Parkinson's disease (PD).

Keywords: *Parkinson's disease; Alzheimer's disease; rotenone;* Parkinson's disease dementia*; oxidative stress; neurodegeneration*.

1. Introduction

Parkinson's disease (PD), one of the most progressive chronic neurodegenerative illnesses among the elderly worldwide, is caused by the death of the dopaminergic neurons of the substantia nigra pars compatica (SNpc) of the midbrain basal ganglia due to accumulation of *alpha synuclein* protein forming "Lewy bodies." [1]. The consequent deficit in the dopamine content in the striatum results in disruption of basal ganglia circuity and functioning causing permanent motor system impairment. Through parallel loops which connect the basal ganglia and thalamus with the cerebral cortex and the brain stem motor nuclei, the basal ganglia control voluntary motor movements [2]. The cardinal manifestations of PD are those of slowing of motor activity i.e., bradykinesia or akinesia, muscular rigidity, postural instability, and a resting tremor [3]. Also, these patients develop non-motor symptoms such as cognitive decline, depression, anxiety, apathy, sleeping disturbances, likely due to the affection of other neuronal populations/neurotransmitters eg., cholinergic, serotonergic, GABAergic, and glutamatergic neurons by the neurodegenerative process [4,5].

Parkinson's disease dementia (PDD) is known as the changes in memory and behavior in someone already diagnosed with PD. Up to 75-80% of PD patients develop dementia. The development of dementia took about 10 years after the onset of movement problems. Early in PD, the brain parts important for movement are affected, by time due to disease progression; other brain parts that are responsible for mental functions such as memory and thinking become injured [6]. Parkinson's disease is a largely sporadic one occurring in people above 65 years with only about 5% of cases with identified genetic origin [6,7]. The cause for idiopathic PD is not known but increasing evidence suggest a role for environmental toxins such as pesticides and fungicides

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Received date 15 November 2023; revised date 04 February 2024; accepted date 03 March 2024
DOI: 10.21608/EJCHEM.2024.248072.8856

e.g., rotenone, paraquat and maneb and insecticides of the chlorpyrifos class [8]. Additional evidence suggests a role for aluminum in the pathogenesis of PD [9].

Another neurodegenerative disease of old age is Alzheimer's disease (AD) which is the common cause of dementia in elder people. The disease is mainly a sporadic one but can occur in rare familial forms [10]. Severe memory loss occurs that synchronizes with a progressive decline of functions, word finding, visuospatial skills and perception [11]. Degeneration of central cholinergic neurons, synaptic loss, atrophic neuritis, neurofibrillary tangles, senile plaques of amyloid beta peptides (Aβ) and extensive neuroinflammation are the pathological hallmarks of this disease [12-14]. Evidence have implicated increased aluminum brain levels in the development of AD [15,16]. Higher amounts of aluminum in drinking water or increased exposure to the metal were also found to be associated with greater cognitive decline with time [17]. Moreover, high levels of aluminum were detected in the brain of patients with familial AD [18]. This metal is the most abundant metal in the earth's crust and is widely available in the environment in building construction, electrical conductors and equipments, containers and packaging [16].

During normal cellular metabolism reactive nitrogen and oxygen species are produced. In the mitochondrial electron transport chain, electron leakage of superoxide radical ($O_2^{\bullet -}$) from molecular oxygen (O_2) occurs. $O_2^{\bullet -}$ reacts with NO producing the strong oxidant peroxynitrite ($ONOO^-$) or dismutated by the superoxide dismutase enzyme to O_2 and H_2O_2 which can be reduced by the transition metal ions to form $OH^{\bullet}$ via the Fenton reaction. Other important sources of ROS include the autoxidation of excitatory neurotransmitters, the enzymes xanthine oxidase, myleoperoxidase, cyclooxygenases, lipoxygenases, neutrophils and the microglia [19, 20]. These species are counter balanced by enzymatic antioxidants as glutathione peroxidase, superoxide dismutase and catalase, and non-enzymatic antioxidants as glutathione, beta-carotene, enzyme Q10, alpha-tocopherol, ascorbate, and uric acid [21]. Oxidative stress means that the oxidative balance inside the cell is tilled towards the oxidant side. Large amounts of reactive nitrogen (RNS) and oxygen species (ROS) and/or deficient cellular antioxidants cause increased oxidative stress. When the cell cannot tolerate the levels of ROS and RNS, the result is oxidative damage to proteins, nucleic acids, membrane lipids, and the mitochondria [19, 22]. Most neurodegenerative disorders occur due to increased neuro-inflammation and oxidative stress[23]. It is because of the high content of polyunsaturated fatty acids, the accumulation of the redox iron, the relatively low levels of antioxidants, that the brain tissue is vulnerable to oxidative stress [19,20, 24]. Studies also showed accumulation of iron and aluminum in the neuromelanin granules in patients with Parkinson's disease but not in controls [9] and aaluminum salts were found to enhance the iron-induced peroxidation of membrane lipids [25].

The present study aimed to investigate whether daily exposure to a low dose of $AlCl_3$ could enhance the neurobehavioral, motor and memory functioning and brain neurodegeneration caused by rotenone in mice. Rotenone is a pesticide of plant origin which has been shown to cause nigrostriatal neurodegeneration and is widely used in rodents to model human Parkinson's disease [26].

2. Materials and methods

2.1. Animals

Thirty two male Swiss albino mice (25-30 g), provided from the animal house of National Research Centre, were used. Mice were housed under temperature- and light-controlled conditions and allowed standard laboratory rodent chow and water ad libitum.

2.2. Chemicals and reagents

Rotenone and $AlCl_3$ were purchased from Sigma-Aldrich (St Louis, MO, USA). Rotenone was dissolved in dimethyl sulfoxide (DMSO). $AlCl_3$was dissolved in distilled water. Other chemicals and reagents were purchased from Sigma (USA).

2.3. Experimental design

Mice were divided in to four equal groups (8 mice/ group) randomly and received one of the following treatments:

Group 1: $AlCl_3$ at 2 mg/kg, daily by i.p injection

Group 2: Rotenone at 1.5 mg/kg, s.c., every other day for two weeks

Group 3: $AlCl_3$ at 2 mg/kg, i.p. daily + rotenone at 1.5 mg/kg, s.c., every other day

Group 4: Vehicle (saline + DMSO).

Preliminary experiments using higher dose of $AlCl_3$ (10 mg/kg) + rotenone resulted in death of all mice by the 5th rotenone injection.

Behavioral tests were done 24 hrs after last injection of rotenone, then mice were euthanized by cervical dislocation and their brains were quickly removed, washed and dissected on ice cold plate into two parts (cortex and rest of the brain), then stored at −80°C until further biochemical analyses were done.

2.4. Biochemical analysis

2.4.1. Determination of lipid peroxidation

Lipid peroxidation was determined by measuring thiobarbituric acid reactive species (TBARS). Its content in brain homogenates was measured as malondialdehyde (MDA) content according to colorimetric method of Ruiz-Larrea et al. with a peak absorbance at 532 nm using a Shimadzu spectrophotometer[27].

2.4.2. Determination of reduced glutathione

Ellman´s method was used in measuring reduced glutathione [28]. Ellman´s reagent DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] reacts with the free thiol group of GSH to form yellow color with a peak absorbance at 412 nm .

2.4.3. Determination of nitric oxide

Nitric oxide was determined using Griess reagent. Nitrate is converted to nitrite via nitrate reductase. The Griess reagent then reacts with nitrite to form a deep purple azo compound. The absorbance is measured at 540 nm using spectrophotometer [29].

2.4.4. Determination of paraoxonase-1

Phenyl acetate was used to measure the activity of paraoxonase-1 enzyme. In this assay arylesterase hydrolyzes phenyl acetate forming phenol and the rate of hydrolysis is measured by monitoring the increase in the absorbance at 270 nm.The difference in absorbance within the first 60 sec is measured. Enzyme activity expressed as kU/l is calculated based on the molar extinction coefficient of 1310 M^{-1} cm^{-1} for phenol, pH 8.0 and 25°C [30].

2.4.5. Determination of butyrlcholinesterase

Butyrylcholinesterase (BChE) activity was measured using a colorimetric kit (Ben Biochemical Enterprise, Milan, Italy). The increase in absorbance at 405 nm is proportional to the activity of the cholinesterase in the brain homogenate.

2.5. Behavioral testing

2.5.1. Rearing activity

The cylinder test is used to assess spontaneous forelimb use. Mice were placed in a transparent Plexiglas cylinder for 6 min, the number of spontaneous rears made was measured for each animal [31].

2.5.2. Stair test

In Mice were placed at the bottom of a stair placed at an angle of 55° above the bench, and the time taken by the mouse to reach stair end was determined in seconds [32].

2.5.3. Wire hanging test

A steel rod (25 cm long, 0.2 cm in diameter), was used in three hanging trials for each mouse with a cut-off time of 3 min to evaluate their motor strength. The average hanging time for animal was recorded in seconds [33].

2.5.4. Water maze test

Morris water maze test was used to assess spatial working memory impairment [34]. The apparatus consisted of a glass tank (20 cm wide, 40 cm height and 70 cm in length) filled with colored water at 25 °C. The platform was hidden 1 cm below the surface of the water. The average time for each mouse to reach the hidden platform form three consecutive trials was calculated in seconds.

2.6. Histopathological studies

Three brains from each group were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at a thickness of 5 μm, and then stained with hematoxylin and eosin (H&E). Sections were examined using bright-field microscope (Optiphot 2; Nikon, Tokyo, Japan).

2.7. Statistical analyses

Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) was used for data analysis and post-hoc individual comparisons were performed with Duncan's multiple range test. The Statistical Package for Social Sciences (SPSS) software (SAS Institute, Cary, NC, USA) was used.

3. Results

3.1. Biochemical results

3.1.1. Lipid peroxidation

Both $AlCl_3$ and rotenone caused significant increase in malondialdehyde in the cerebral cortex and rest of brain tissue (subcortex). After treatment with $AlCl_3$, lipid peroxidation increased by 45.7% (25.71 ± 0.98 vs. 17.64 ± 0.81 nmol/g.tissue) and 44.3% (24.4 ± 1.6 vs. 16.91 ± 0.42 nmol/g.tissue) in cerebral cortex and subcortex, respectively. Meanwhile, rotenone-treated mice exhibited 57% (27.7 ± 1.3 vs. 17.64 ± 0.81 nmol/g.tissue) and 37.2% (23.2 ± 1.53 vs. 16.91 ± 0.42 nmol/g.tissue) increments in malondialdehyde in these brain regions. The combined treatment with $AlCl_3$ and rotenone did not result in increased brain malondialdehyde over that caused by only $AlCl_3$ or only rotenone. 3.1.2. Nitric oxide

3.1.2. Nitric oxide

Compared with the vehicle-treated control, the administration of either $AlCl_3$ or rotenone caused significant increase in nitric oxide by 57.7% and

43.6% in the cerebral cortex (27.6 ± 1.62 and 25.13 ± 1.18 vs. 17.5 ± 0.69 µmol/g. tissue)and by 47.7% and 44.4% in the subcortex (26.58 ± 0.49 and 26.0 ± 0.98 vs. 18.0 ± 0.33 µmol/g. tissue), respectively. The combination of $AlCl_3$ and rotenone caused 42.8% and 38% increments in nitric oxide in these brain regions, respectively.

3.1.3. Reduced glutathione

In $AlCl_3$-treated mice, the level of GSH in cerebral cortex and subcortex showed significant decrease by 27.5% (2.45 ± 0.03 vs. 3.38 ± 0.08 µmol/g. tissue) and 29.3% (2.58 ± 0.04 vs. 3.65 ± 0.17 µmol/g. tissue), respectively. Significant decreases in brain GSH by 33.4% (2.25 ± 0.09 vs. 3.38 ± 0.08 µmol/g. tissue) and 31.0% (2.52 ± 0.05 vs. 3.65 ± 0.17 µmol/g. tissue) were observed after rotenone only treatment. Meanwhile, the combined administration of both $AlCl_3$ and rotenone caused 28.7% and 33.4% decrease in GSH in these brain regions; values that were not significantly different from that caused by either toxicant alone.

3.1.4. Paraoxonase-1

In cerebral cortex, mice treated with $AlCl_3$ or rotenone showed significant inhibition of PON-1 activity by 76% and 54.9%, respectively (2.75 ± 0.14 and 5.1 ± 0.39 vs. vehicle control value of 11.3 ± 0.76 kU/l). The combination of $AlCl_3$ and rotenone resulted in 87.2% decrease in PON-1 activity as compared to the vehicle-treated group. In the subcortex, both $AlCl_3$ and rotenone significantly inhibited PON-1 activity by 48.2% and 41.5%, respectively. In addition, the combination of $AlCl_3$ and rotenone caused 57% inhibition of PON-1 activity compared to the vehicle control value.

3.1.5. Butyrylcholinesterase

BChE in cerebral cortex decreased by 36.2% after treatment with rotenone (90.0 ± 5.4 vs. 141.0 ± 6.5 U/l). The administration of $AlCl_3$ or $AlCl_3$/rotenone caused significant increase in BChE by 125.5% and 48.9% compared to vehicle control (318 ± 15.9 and 210 ± 14.7 vs. 141.0 ± 6.5 U/l) (Fig. 3).

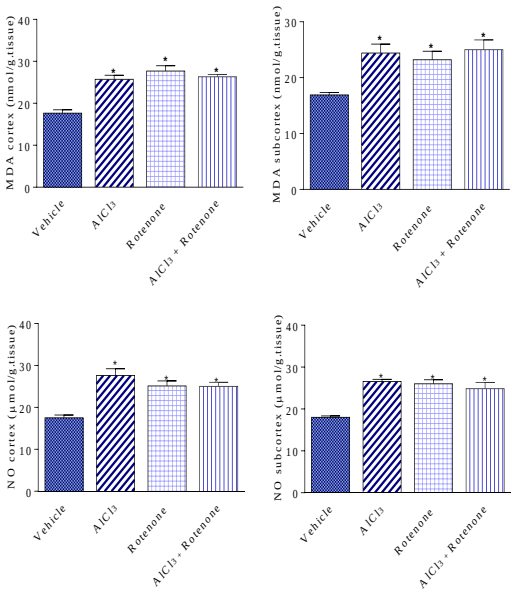

Figure 1. Effect of treatment with AlCl3 , rotenone or both on malondialdehyde (MDA) and nitric oxide (NO) in mice brain. *: p< 0.05 vs. vehicle.

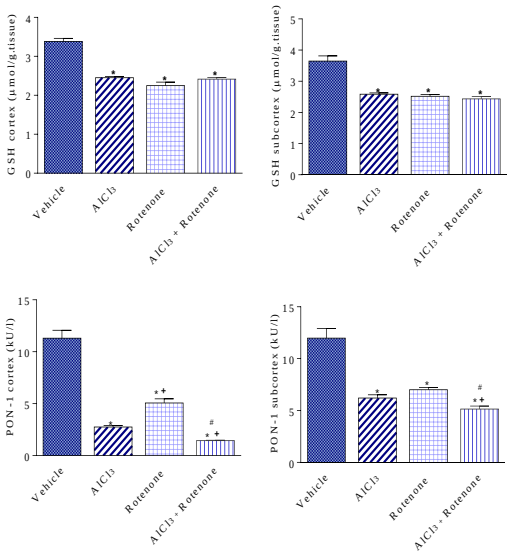

Figure 2. Reduced glutathione (GSH) levels and paraoxonase-1 activities in mice brain after treatment with AlCl3, rotenone or both. *: p< 0.05 vs. vehicle. +: p<0.05 vs. AlCl3. #: p< 0.05 vs. rotenone.

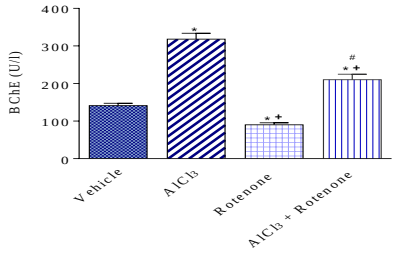

Figure 3. Butyrylcholinesterase (BChE) activity in mice treated with AlCl3, rotenone or both. *: p< 0.05 vs. vehicle. +: p<0.05 vs. AlCl3. #: p< 0.05 vs. rotenone.

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3.2. Motor and behavioral results

3.2.1. Rearing behavior

In both $AlCl_3$-, and rotenone-treated mice, the rearing behavior using one arm significantly decreased 28.6% and 42.9% while rearing using two arms significantly decreased by 39.3% and 60.8% compared with the vehicle-treated group. Mice given $AlCl_3$/rotenone showed no movements (Fig. 4A &B).

3.2.2. Stair test

The time spent in ascending the stair significantly increased by 50.1% and 397.1% in mice treated with $AlCl_3$ or rotenone compared to the vehicle control value. The combination of $AlCl_3$ and rotenone significantly increased the time spent on ascending the stair to 549%. Values are 10.45 ± 1.0, 34.6 ± 2.1, 45.2 ± 2.7 and 6.96 ± 0.8 sec for $AlCl_3$, rotenone, $AlCl_3$/rotenone, and vehicle, respectively (Fig. 4C).

3.2.3. Wire hanging test

Compared with the vehicle control group, injections of $AlCl_3$ or rotenone significantly decreased the time the mouse spent in the wire-hanging test by 33.9% and 51.1%, respectively (13.48 ± 1.1 and 9.97 ± 0.46 vs. 20.4 ± 0.71 sec) whilst mice given the combination of $AlCl_3$ and rotenone showed 63.5% decrease (7.45 ± 0.59 vs. 20.4 ± 0.71 sec) (Fig. 4D).

3.2.4. Water maze test

The latency to find the plate form in the WMZ test significantly increased by treatment with $AlCl_3$ or rotenone compared to the vehicle control group. Mice treated with $AlCl_3$ /rotenone showed marked increase in latency (Fig. 5).

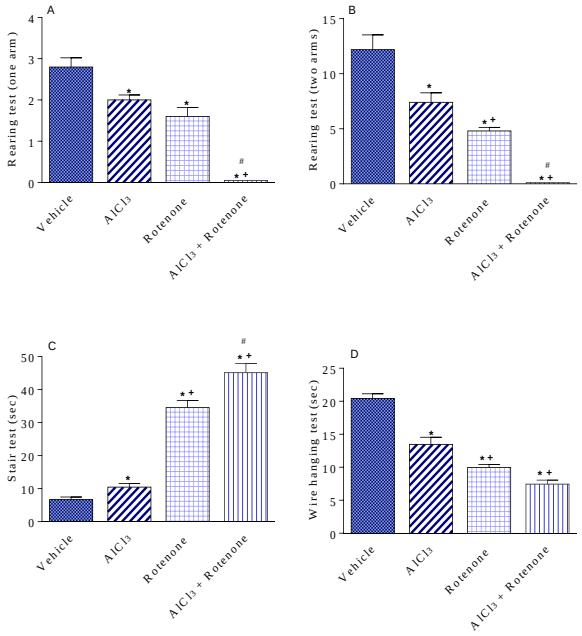

Figure 4. Rearing behavior, stair and wire hanging tests in mice after with AlCl3, rotenone or both. *: p< 0.05 vs. vehicle. +: p<0.05 vs. AlCl3. #: p< 0.05 vs. rotenone.

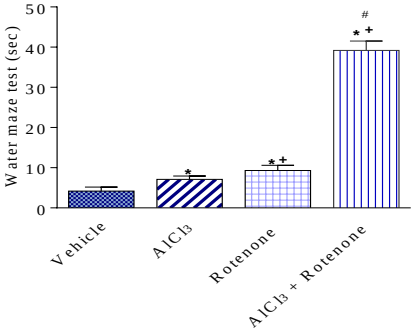

Figure 5. Effect of AlCl3, rotenone or both on the latency to find a submerged platform in the Morris water maze test in mice. *: p< 0.05 vs. vehicle. +: p<0.05 vs. AlCl3. #: p< 0.05 vs rotenone.

3.3. Histopathological results

3.3.1. Cortex and striatum

Heamatoxylin and eosin stained sections of the cerebral cortex and striatum from control group showed the normal histological structure of neuron with demarcated nuclei (Figs.6A & 7 A). The brain of the group treated with $AlCl_3$showed that most neuron cells were nearly as in the control animals, while few cells were more darkly stained and vacuolated (Figs. 6B & 7B). In the rotenone only group, the most consistent observations were severe degenerative changes, vacuolation, shrunken cytoplasm, and extensively dark pyknotic and apoptotic nuclei in neurons of the cortex and striatum brain tissues. Also, congestion was observed in the blood vessels, focal gliosis of cerebral cortex and the haemorrhage was

noticed only in the meninges (Figs.6C & 7C). The previously mentioned degenerative changes were also observed in the group that received rotenone and $AlCl_3$ but more or less than the rotenone only group (Figs. 6D& 7D).

3.3.2. Hippocampus

The heamatoxylin and eosin staining revealed that hippocampal pyramidal cells in the control group were arranged neatly and tightly cells were round and intact with nuclei stained clear (Fig. 8A). The $AlCl_3$ treated group showed more or less histological picture as compared to the control group with few pyknotic nuclei (Fig. 8B). There was obvious tissue damage for the rotenone group as indicated by the decreased number of hippocampal pyramidal cells, There were damaged cells, as revealed by shrinkage, by the stain, the lack of a clear nuclear membrane, and the nucleolus had disappeared (Fig. 8C). The pyramidal cells were more or less affected in the group that received rotenone and $AlCl_3$; had deeply stained nuclei and became irregular in shape (Fig. 8D).

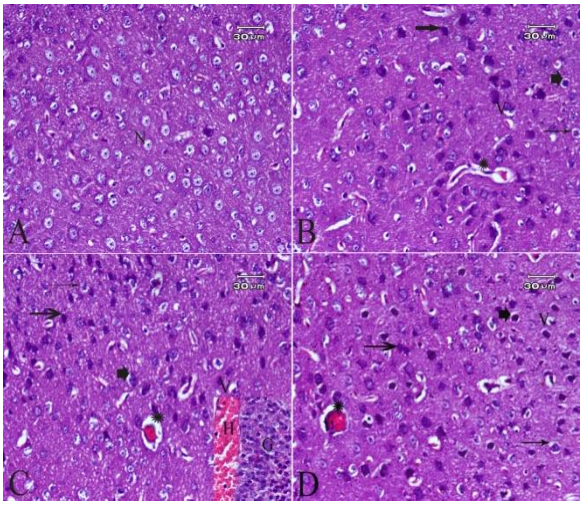

Figure 6. Representative photomicrographs of cerebral cortex sections from: (A) Vehicle-treated group showing normal neurons cells (N). (B) AlCl3 group showing few pyknotic and apoptotic nuclei (arrow). (C) Rotenone group showing marked neuronal cell degeneration (thin arrow), vacuolation (V), with dilated blood vessels (*), pyknotic (arrow) and apoptotic cells (arrowhead). In situ haemorrhage (H) was noticed only in the meninges and focal gliosis (G). (D) Rotenone and AlCl3 showing more or less neuronal cell degeneration (thin arrow), vacuolation (V), with dilated blood vessels(*), pyknotic (arrow) and apoptotic cells (arrowhead) (H & E X 400).

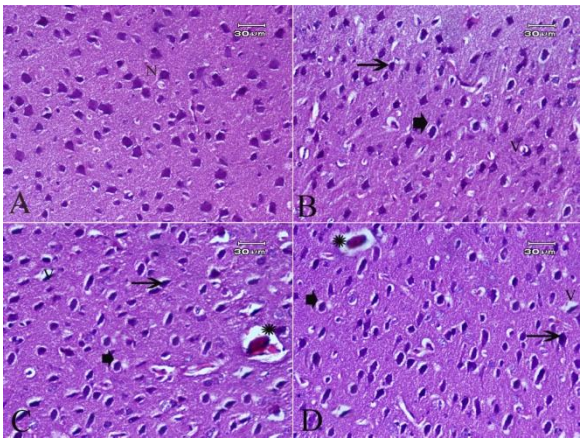

Figure 7. Representative photomicrographs of the striatum sections from: (A) Vehicle-treated group showing normal neurons cells (N). (B) AlCl3 group showing few pyknotic and apoptotic nuclei (arrow). (C) Rotenone group showing marked neuronal cell degeneration (thin arrow), vacuolation (V), with dilated blood vessels (*), pyknotic (arrow) and apoptotic cells (arrowhead). (D) Rotenone and AlCl3 showing more or less neuronal cell degeneration (thin arrow), vacuolation (V), with dilated blood vessels(*), pyknotic (arrow) and apoptotic cells (arrowhead) (H & E X 400).

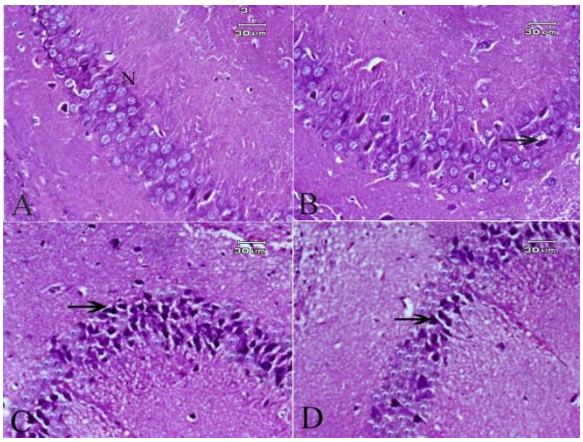

Figure 8. Representative photomicrographs of the hippocampal sections from: (A) Vehicle-treated group showing normal neurons cells (N). (B) AlCl3 group showing few pyknotic nuclei (arrow). (C) Rotenone group showing marked neuronal cell degeneration with pyknotic nuclei (arrow). (D) Rotenone and AlCl3 showing showing marked neuronal cell degeneration with pyknotic nuclei (arrow) (H & E X 400).

4. Discussion

In this study, the effect of daily exposure to a low dose of $AlCl_3$ and/or rotenone on brain oxidative stress, brain neurodegeneration and motor and memory functioning in mice was examined. The findings in the present study can be summarized as follows: the systemic administration of either $AlCl_3$ or rotenone resulted in (i) brain oxidative stress indicated by the rise in the level of the lipid peroxidation end

product malondialdehyde [35, 36, 37] and by the decrease in the antioxidant and free radical scavenger reduced glutathione [38, 39, 40, 41] ; (ii) increased brain nitric oxide; (iii) inhibition of PON-1 activity; (iv) decreased exploratory behavior in the cylinder test; (v) impaired motor strength and coordination in the wire hanging and stair tests; (vi) impaired performance in the WMZ test; (vii) neurodegneration. The two toxicants however, exerted differing effects on BChE activity in cerebral cortex being decreased following rotenone but increased after $AlCl_3$ or $AlCl_3$/rotenone. The combined treatment with $AlCl_3$/rotenone resulted in worse performance on motor and behavioral testing compared with that caused by either toxicant alone.

Several studies have indicated increased oxidative stress biomarkers and depletion of cellular antioxidants eg., reduced glutathione content, total antioxidant capacity, and catalase and superoxide dismutase activities in the brain of rodents treated with rotenone [42,43,44,45]. The pesticide is a highly lipophilic and readily crosses blood brain barrier [42]. Rotenone inhibits the mitochondrial complex I resulting in increased generation of superoxide ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2)[46]. There is also evidence for microglia activation causing an increase in $O_2^{\cdot-}$ and hypochlorous acid [47]. Rotenone causes increased intracellular reactive oxygen species and oxidative stress is considered an important mechanism underling the neurotoxicity of rotenone because the extent of neurodegeneration induced by the toxicant could be attenuated by antioxidants [48, 49, 50]. Studies also showed that rats treated with $AlCl_3$ exhibited significant increases in brain lipid peroxidation and depletion of reduced glutathione, indicative of increased oxidative stress [51,52]. Rats treated with the metal also exhibited significant decrease in brain superoxide dismutase and catalase activities [51,52]. Aluminum however, has a fixed oxidation number, and thus cannot participate in redox reactions. It is suggested that aluminum impairs iron homeostasis, displace iron from binding sites and results in iron-initiated free radical-induced tissue damage [25,53]. In support of this notion the finding of an increase in iron content within the glia cells surrounding the neuritic plaques [54] and the presence of aluminum in amyloid fibers in the cores of senile plaques in Alzheimer's disease [55] as well as the accumulation of iron and aluminum in the neuromelanin granules in Parkinson's disease [9].

Nitric oxide is synthesized from L-arginine by the action of the enzyme nitric oxide synthase (NOS). The constitutive endothelial (eNOS) and neuronal (nNOS) isoforms of the enzyme are the source for the physiologically relevant low concentrations of nitric oxide. In contrast, higher amounts of nitric oxide generated for relatively longer lime are produced by the inducible isoform (iNOS) expressed on activated astrocytes and microglia following their stimulation by ROS, pro-inflammatory cytokines and bacterial lipopolysaccharide [56]. High levels of nitric oxide have been implicated in the development of neurodegeneration in Parkinson's and Alzheimer's diseases [57]. This increase in nitric oxide is attributed to the action of iNOS. Nitric oxide causes neurotoxicity owing to the formation of reactive nitrogen species eg., peroxynitrite anions (ONOO–) generated by the reaction of nitric oxide and superoxide, which readily causes nitrosylation and nitrotyrosination of proteins [58]. Previous studies indicated increased expression of iNOS together with increased nitric oxide content in rat brain following treatment with $AlCl_3$ [54] or rotenone [43,60]. Authors demonstrated that treatment with specific inhibitors of iNOS or nNOS prevents neurodegenertion caused by $AlCl_3$ [61,62] or rotenone in rats [63,64] with decrease in oxidative stress, thereby, indicating a role for nitric oxide-mediated toxicity as an important mechanism underlying neuronal injury caused by these toxicants.

In the present study, however, combined treatment with rotenone and $AlCl_3$ did not result in higher level of oxidative stress compared with that caused by either agent alone, suggesting the presence of an interaction between the two toxicants. In their study, Mèndez-Aèlvarez et al. [65] showed that in the rat brain mitochondrial preparations, $^{\bullet}OH$ production and the increase in lipid peroxidation (TBARS production) by 6-hydroxydopamine autoxidation was significantly reduced in presence of Al^{+3} . Since ascorbic acid increased $^{\bullet}OH$ production but reduced TBARS production, the decrease in lipid peroxidation by Al^{+3} was attributed to a reduction of negative charge density due to binding of Al^{+3} to lipid membranes. Moreover, in vivo, the presence of Al^{+3} resulted in significantly lower dopaminergic denervation (tyrosine hydroxylase immunoreactivity) in rats receiving intrastriatal injections of the nigrostriatal toxin 6-hydroxydopamine.

Our findings indicate that treatment with $AlCl_3$ and/or rotenone was associated with marked decrease in brain PON-1 activity which is consistent with previously published data [38,43, 45, 49, 51, 59, 66]. We also observed an additive effect for the two toxicants in inhibiting PON-1 activity in the cerebral cortex. Paraoxonase-1 belongs to a group of detoxifying enzymes comprising three isoforms: PON-1, PON-2 and PON-3 [67]. In recent years, PON-1 has become a focus of interest in view of the evidence linking the enzyme with the development of neurodegeneration in Parkinson's and Alzheimer's diseases; (i) PON-1 hydrolyzes the toxic metabolites of several organophosphate insecticides [67,38,39] and exposure to these compounds have been suggested to account for an increase in the risk for developing idiopathic Parkinson's disease [68,39]; (ii) genetic variation in enzyme activity and hence its catalytic

efficiency in hydrolyzing some organophosphate insecticides was also found to determine the individual's susceptibility to these agents [69] and thus the susceptibility for developing Parkinson's disease in subjects exposed to these compounds [70]; the activity of PON-1 was also decreased in Alzheimer's disease and other dementia patients, thereby, suggesting a role for the enzyme in the neurodegeneraion [71,72]. Paraoxonase-1 also possesses antioxidant and antiinflammatory activities [73] and their decrease would render the neuronal cells more vulnerable to oxidative stress and inflammatory events, with ensuing neurodegeneration.

Central cholinergic pathways are fundamental to cognitive functioning and memory [74]. In Alzheimer's disease, there is selective degeneration of the basal forebrain cholinergic complex, loss of acetylcholine and reduced choline acetyltransferase activity [12,13]. Consequently, the use of centrally acting centrally acting cholinesterase inhibitors is the therapeutic strategy employed for enhancing the residual cholinergic neurotransmission in these patients [75]. These drugs inhibit acetylcholinesterase (AChE) and, to a varying degree, butyrylcholinesterase (BChE), a second cholinesterase in brain [76, 77]. Both AChE and BchE inactivate the neurotransmitter acetylcholine (ACh) and thus ameliorate the cholinergic deficit in Alzheimer's disease. Inhibition of brain BChE was shown to elevate brain ACh and enhances cognition [78]. In this study and consistent with previous reports [51, 52, 66], treatment with $AlCl_3$ caused significant increase in BChE activity. Rats given $AlCl_3$ (10 mg/kg, i.p.) for 6 weeks exhibited significantly higher AChE and BChE activities in the cerebral cortex compared to control animals [51, 59, 66]. Increased AChE activity in cerebral cortex and hippocampus was also reported in mice following $AlCl_3$ at 10 mg/kg for 90 days [79], while $AlCl_3$ at 50 mg/kg for 8 weeks increased both AChE and BChE activities in mouse brain [52]. In contrast to the effect of $AlCl_3$, rotenone was shown to inhibit cortical AChE and BChE activities [45, 80]. The loss of dopaminergic tone in nigrostriatal pathway and the consequent dopaminergic/cholinergic imbalance that occurs in PD is responsible for the motor manifestations of the disease. Anticholinergic drugs are therefore used in early Parkinson's disease for symptom control, particularly the associated resting tremor [81]. Inhibition of AChE activity is thus likely to worsen the motor features of the disease. Our results showed that BChE activity in cerebral cortex decreased by rotenone but increased after $AlCl_3$. In rats co-administered $AlCl_3$/rotenone, BChE activity was significantly increased compared with the vehicle group.

This study also examined the neurobehavioral and motor performance following treatment with $AlCl_3$, rotenone or both. The administration of either $AlCl_3$, rotenone decreased exploratory behavior in the cylinder test, impaired motor strength and coordination in the wire hanging and stair tests and impaired performance in the WMZ test. Rearing is an exploratory behavior and also provides a possibility to investigate the spatial and motor behavior and thus brain function [82]. Rearing is also a measure of anxiety-like behavior [83]. Studies reported an attenuating effect of rotenone on rearing behavior and motor coordination [37,38, 39, 84,85]. In their study, Cannon et al. [85] found that the effect of rotenone on rearing behavior in rats reached significance after the 6^{th} day of rotenone injection with the deficit being reversible by the dopaminergic agonist apomorphine, indicating the presence of residual dopaminergic tone. In this study, the rearing behavior also decreased to less degree by treatment with $AlCl_3$. Other researchers reported decreased vertical activity in mice given 1 mg/kg aluminum lactate/g diet [86]. Fewer entries into the centre of the open field were also recorded in mice receiving 0.3 mg/kg aluminum hydroxide [87]. In the present study, after combined treatment of $AlCl_3$/rotenone, mice ceased to explore their environment, suggesting an additive neurotoxicity. Other behavioral measures including grip strength, skilled reaching, memory and spatial navigation revealed significant impairments after treatment with $AlCl_3$ or rotenone in accordance with other studies [38,39,40,43,49,52,59]. Nevertheless, the combined administration of $AlCl_3$/rotenone resulted in severe impairment in motor and memory functioning, compared with that caused by either agent alone. These observations indicated greater neurotoxicity from the $AlCl_3$/rotenone combination. Changes in brain ACh induced by $AlCl_3$ and/or rotenone might have accounted at least in part for the neurobehavioral and motor impairments observed in mice treated with these toxicants.

5. Conclusions

In summary, the present study indicates the involvement of oxidative stress in the neurotoxicity caused by either $AlCl_3$ or rotenone and shows for the first time that the motor, behavioral and memory deficits induced by rotenone in mice were increased by the concomitant administration of a low dose of $AlCl_3$, suggesting that increased intake of this metal is likely to have adverse effects on the motor symptomatology

and possibly the response to treatment in subjects with idiopathic Parkinson's disease.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

This works was not supported by research **grants.**

8. Authors' contributions

Omar M. E. Abdel-Salam: Study conception and design and data analysis; Marwa El-Sayed El-Shamarka: preparation of drugs, performed experiments, behavioral tests, biochemical assays and data analysis; Enyat Omara: histopathological studies and their interpretation; Omar M. E. Abdel-Salam, Marwa El-Sayed El-Shamarka, Enyat Omara: manuscript preparation, revision and final approval of the version to be published.

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