Journal of Trace Elements in Medicine and Biology 53 (2019) 8–15

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Pharmacology

Rutin abrogates manganese—Induced striatal and hippocampal toxicity via inhibition of iron depletion, oxidative stress, inflammation and suppressing the NF-κB signaling pathway

Kpobari W. Nkpaa[a,*], Godspower I. Onyeso[b], Kale Z. Kponee[c]

[a] Environmental Toxicology Unit, Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Choba, Rivers State, Nigeria
[b] Department of Physiology, College of Medicine, Rivers State University, Port Harcourt, P.M.B. 5080, Rivers State, Nigeria
[c] Departments of Environmental Health and Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

ARTICLE INFO

Keywords:
Rutin
Manganese
Iron
Oxidative stress
Neuro-inflammation
NF-κB signaling

ABSTRACT

Excess exposure to Manganese (Mn) promotes oxidative stress and neuro-inflammation. Rutin (RUT) has been found to exhibit both anti-oxidative stress and anti-inflammatory properties. This study aimed to investigate the effects of RUT on Mn accumulation, endogenous iron (Fe) depletion, oxidative stress, inflammation and nuclear factor kappa B (NF-κB) signaling pathways in the hippocampus and striatum of Mn – induced rats. Rats were treated with 30 mg/kg Mn body weight alone or orally co-treated by gavage with RUT at 50 and at 100 mg/kg body weight for 35 consecutive days. Thereafter, we investigated Mn and endogenous Fe levels, acetylcholinesterase activity, oxidative stress markers, pro-inflammatory cytokines and nuclear factor kappa B (NF-κB) in the hippocampus and striatum of rats. The results indicate that Mn induced Mn – accumulation, Fe depletion, oxidative stress, inflammation and the activation of acetylcholinesterase activity and NF-κB signaling pathways in the hippocampus and striatum of the rats. However, RUT attenuated Fe depletion, oxidative stress and inflammation and suppressed acetylcholinesterase activity and NF-κB pathway via downstream regulations of tumor necrosis factor alpha, interleukin I beta and interleukin 6. Taken together, our present study demonstrates that RUT abrogates Mn – induced striatal and hippocampal toxicity via inhibition of Fe depletion, oxidative stress, inflammation and suppressing the NF-κB signaling pathways. Our results indicate that RUT may be of use as a neuroprotective agent against Mn – induced neuronal toxicity.

1. Introduction

Manganese (Mn) is ubiquitous in the environment and is an essential element required for many biological processes and systems in the human body [1]. The primary pathway of Mn exposure for humans and animals is via ingested food; secondary pathways are via drinking water and inhalation of Mn contaminated particulates in environmental and occupational settings. There have been public health concerns on the adverse health effects associated with elevated Mn exposure [1]. Occupational exposure to elevated Mn levels may lead to manganese poisoning, a toxic condition resulting from chronic exposure to Mn [2–4]. Epidemiological studies have suggested that workers exposed to Mn may develop adverse neurological effects that are sometimes progressive and irreversible [5]. Welders exposed to Mn has been shown to manifest working memory alteration associated with increased activity in the cingulate cortex and sensorimotor cortex due to subtle working memory deficits [6].

Mn induced dopaminergic neurodegeneration occurs via generation of reactive oxygen species (ROS) and dopamine activation [7]. Oxidative stress plays a key role in Mn – mediated toxicity especially on dopaminergic neurons, thereby causing morphological changes and reducing their viability [8,9]. Additionally, astrocytes are highly susceptible to Mn toxicity and have a high tendency to accumulate more Mn than neurons [7]. Elevated Mn exposure disrupts the neuronal activities of astrocytes which include ion buffering, neurotransmitter synthesis, nutrient delivery and response to toxin stimulation [10]. Mn exposure also induced a shift in the ratio of Fe (II)/(III) and leads to iron accumulation via translational repression of Amyloid Precursor Protein (APP) and H-Ferritin in rats' brain [11–13]. These disruptions can induce astrocyte apoptosis [14,15].

Several studies have indicated that iron (Fe) deficiency leads to increases in Mn concentrations in the brain [16]. Kelleher et al. [17] have reported that Fe deficiency anemia increases Mn absorption in the brain via the olfactory tract and leads to increased levels of divalent

* Corresponding author.
E-mail address: nkwilly@gmail.com (K.W. Nkpaa).

https://doi.org/10.1016/j.jtemb.2019.01.014
Received 7 September 2018; Received in revised form 22 December 2018; Accepted 23 January 2019

metal transporter-1 (DMT1); thus, Mn accumulation in the brain may be due to low Fe levels.

The interaction between Mn exposure and Fe deficiency is of great public health concern because of the ubiquity of Mn exposure. Previous studies have indicated that the populace in the Niger-Delta region of Nigeria are at increased risk of elevated Mn exposure via their drinking water and food [18,19]. Additionally, Fe deficiency is the most common nutritional deficiency worldwide, especially in children [20]. In Nigeria, the prevalence of Fe deficiency anaemia (IDA) among preschool children aged two and below is estimated at 69% [21] as against 26.7% reported in children in Malaysia [21]. Therefore, pharmacological agents that have neuroprotective properties capable of suppressing Fe depletion, oxidative stress, and neuro-inflammation in Mn exposed people may ease the public health burden associated with elevate Mn exposure.

Rutin, (RUT) also known as quercetin – O – rutinoside, is a citrus flavonoid suggested to have medicinal properties [22]. The primary source of RUT is citrus fruits such as lemons, buckwheat seeds, oranges, limes and grapefruits [22]. Animal studies have suggested that RUT has anti-cancer, antioxidant, and anti-inflammatory properties [23]. Bishnoi et al. [24] reported that RUT may have neuroprotective properties in animal model. The health benefits of RUT may be due to its ability to interact with important enzymes involved in antioxidant systems, signaling cascades involving cytokines or transcription factors [25,26]. Also, RUT has been reported to attenuate neurotoxicity via mechanisms involving enhancement of AChE activity, antioxidant status with concomitant inhibition of lipid peroxidation, neuro-inflammation and apoptosis in rats [27]. To the best of our knowledge, there have been no studies conducted on the association between exposure to RUT and Mn mediated neurotoxicity in animal model.

The hippocampus is responsible for the integration of spatial learning and memory information. Moreover, the striatum is one of the nuclei in the subcortical basal ganglia of the forebrain. It receives dopaminergic and glutamatergic inputs and serves as the primary input to the rest of the basal ganglion nuclei. It also responsible for the coordination of multiple aspect of motor/locomotive function, cognition, action planning, decision making, stimulus response, reinforcement and learning [28]. Any alteration in the hippocampus and striatum may lead to impaired spatial learning/memory and neurobehavioral deficits. Therefore, this study aims to investigate the neuroprotective effects of RUT on Mn – induced neurotoxicity in the hippocampus and striatum of rats via mechanisms involving the inhibition of endogenous Fe depletion, AChE activity, antioxidant status with concomitant inhibition of lipid peroxidation, neuro-inflammation and NF-κB signaling pathways.

2. Methods

2.1. Chemicals

Analytical grade of manganese chloride (as $MnCl_2{\cdot}4H_2O$, ≥99.9%) and rutin hydrate (Quercetin-3-rutinoside hydrate), epinephrine, hydrogen peroxide, 1-chloro-2,4-dinitrobenzene (CDNB), 5′,5′-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), glutathione and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Interleukin 1 beta (IL - 1β), tumor necrosis factor alpha (TNF – α), interleukin 6 (IL – 6) and nuclear factor kappa B (NF-κB) Rat ELISA Kit were purchased from Elabscience Biotechnology Company, (Beijing, China) and ABCAM (UK). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

2.2. Animal model

Sixty adults male Wistar rats weighing between 140–160 g (8 weeks old) were purchased from the Department of Biochemistry, Faculty of Science, University of Port Harcourt and used in the study. The rats were housed in standard polypropylene cages with a 12-h light/dark cycle in a well-ventilated rats house and fed on rat pellets and water ad libitum in their home cages for a period of 2 weeks before the commencement of the experiment. The experimental protocol and animal care were carried out according to the requirements outlined in the Guide for the Care and Use of Laboratory Animals approved by the National Academy of Science (NAS) and published by the National Institute of Health.

2.3. Experimental design

The experimental design comprised of seventy-five animals of five groups of fifteen rats each and were treated for 35 consecutive days as follows:

Group I (Control): Rats were orally treated with normal drinking water alone for 35 consecutive days.

Group II (RUT alone): Rats were orally treated with RUT alone at 100 mg/kg body weight for 35 consecutive days.

Group III (Mn alone): Rats were orally treated with Mn alone at a dose of 30 mg/kg body weight for 35 consecutive days.

Group IV (Mn + RUT_{50}): Rats were orally co-treated with Mn at 30 mg/kg body weight and RUT at 50 mg/kg body weight for 35 consecutive days.

Group V (Mn + RUT_{100}): Rats were orally co-treated with Mn at 30 mg/kg body weight and RUT at 100 mg/kg body weight for 35 consecutive days.

The doses of Mn (30 mg/kg) and RUT (50 and 100 mg/kg) used in the present study were chosen based on the results from a pilot study conducted in our laboratory and previously published data [27]. The Mn and Fe levels in the food pellets and RUT were examined as shown in Table 1 and certified Mn and Fe free before administration to the experimental animals.

2.4. Tissues preparation

After 24 h of treatment, the final body weights of the experimental rats were taken before they were sacrificed under light ether anesthesia. Thereafter, the striatum and hippocampus were carefully dissected from 60 rats (i.e. 12 rats from each group). Thereafter, the brain regions from each group were weighed and homogenized with the aid of a Teflon homogenizer in eight volumes of 50 mM Tris−HCl buffer (pH 7.4) containing 1.15% potassium chloride. The resulting homogenate was centrifuged at 12,000 × *g* for 15 min at 4 °C to obtain the post-mitochondrial fraction and the supernatant obtained was subsequently used for the biochemical and molecular determinations. Protein concentration was determined at 595 nm according to the method of Bradford [29].

2.5. Analysis of Mn and Fe by atomic absorption spectrometer (AAS)

The hippocampus and striatum of 3 rats from each group were digested according to the method of Mello et al. [30] and Nkpaa [19]. The brain regions were oven – dried at 120 °C. Thereafter, they were transferred into a beaker and concentrated HNO_3 was added and heated

Table 1
Mn and Fe content of the food pellet and rutin administered on the experimental animal.

Samples	Metal content	
	Mn (mg/kg)	Fe (mg/kg)
Food pellet	0.18 ± 0.05	0.65 ± 0.08
Rutin	BDL	BDL

The data are expressed as mean ± S.D. for 5 replicates of the samples. BDL = below detection limit of Mn and Fe.

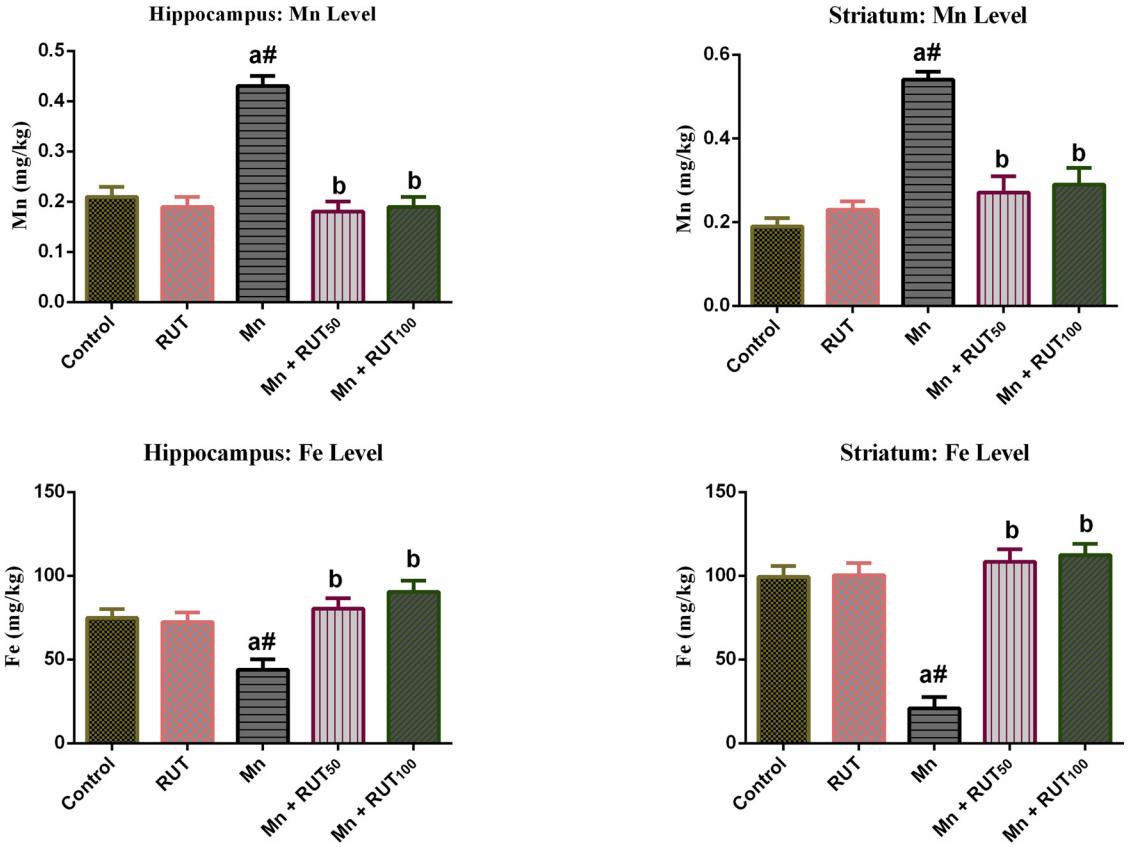

Fig. 1. Effects of Rutin on bio-metals (Mn and Fe) levels (mg/kg) in the hippocampus and striatum (A–D) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 3 rats per group. a: Mn differs significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differs significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

to boiling. After digestion (as indicated by a clear light solution), the contents were then transferred into a test tube, cooled and diluted. A part of the solution was then used for the Mn and Fe analysis with the aid of atomic absorption spectrophotometer (AAS) (GBC Avanta, PM AAS, A6600 Australia). Values less than 0.001 mg/kg were below detectable limit. The hippocampal and striatal samples were analyzed in triplicate and the variation in coefficient was usually less than 10%. Concentrations of Mn and Fe were expressed as milligram per gram dry weight in hippocampus and striatum.

2.6. Biochemical assays

Acetylcholinesterase activity was determined using acetylthiocholine iodide as substrate according to the method of Ellman et al. [31]. In brief, the assay mixture consisted of 110 µL of distilled water, 20 µL of 100 mM potassium phosphate buffer (pH 7.4), 20 µL of 10 mM DTNB, 30 µL of diluted sample, and 20 µL of 8 mM acetylthiocholine as a substrate. The degradation of acetylthiocholine iodide was analyzed for 5 min (30 s intervals) at 412 nm spectrophometrically and the results were expressed as µmol/min/mg protein.

Superoxide dismutase (SOD) was assayed by measuring the inhibition of autoxidation of epinephrine at pH 10.2 (30 °C) according to the method of Misra and Fridovich [32]. In brief, 50 µL of the sample was added to 2.4 mL of 0.05 M carbonate buffer (pH 10.2) and the reaction was initiated by the addition of 60 µL of 0.3 M of freshly prepared epinephrine. The increase in absorbance at 480 nm was monitored for 150 s at 30 s intervals with the aid of UV–vis spectrophotometer. Reaction mixture without the enzyme was used as the blank. SOD values were expressed as nanomoles epinephrine oxidized/min/mg protein.

Catalase (CAT) activity was assayed using hydrogen peroxide as substrate according to the method of Clairborne [33]. In brief, the assay medium, containing 1.8 mL of 50 mM phosphate buffer at pH 7.0, 180 µL of 19 mM of H_2O_2 and 20 µL sample, was allowed to run for 3 min at 30 s intervals with absorbance measured at 570 nm with a UV–vis spectrophotometer. CAT value was expressed as micromole H_2O_2 consumed/min/mg protein.

Glutathione peroxidase (GPx) activity was assayed according to the method of Rutruck et al. [34]. In brief, the reaction mixture consisted 100 µL of 10 mM sodium azide, 200 µL of 4.0 mM GSH, 500 µL potassium phosphate buffer, 100 µL of 2.5 mM H_2O_2, and 50 µL of striatum sample. The total volume was made up to 2.0 mL with distilled water. The reaction mixture was incubated for 3 min at 37 °C. Subsequently, the reaction was terminated by adding 500 µL of 10% TCA. The residual glutathione level was determined by centrifuging the reaction mixture. The supernatant was thereafter removed and to this, 2.0 mL of 0.3 M dipotassium hydrogen orthophosphate solution and 500 µL of DTNB reagent were added. The formed color was read at 412 nm. GPx activity was expressed as unit's/mg protein.

Lipid peroxidation (LPO) was determined by measuring malondialdehyde (MDA), an end – product of lipid peroxidation, according to a modified method described by Farombi et al [35] with slight modification. In brief, the reaction mixture consisted of 150 µL of 0.1 M phosphate buffer, 50 µL of sample, 100 µL of 10% TCA and 100 µL of 0.75% 2 – thiobarbituric acid (TBA) in 0.1 mol/L HCl. The reaction mixture was heated at 90–95 °C for 30 min. After cooling at room temperature, they were centrifuged at 8000 × *g* for 10 min and the absorbance of the supernatant was read against distilled water at 532 nm. The level of MDA was calculated using the extinction coefficient of 1.56×10^5 L/mol/cm and was expressed as the micromole MDA formed/mg protein.

2.7. Enzyme – linked immunosorbent assay

The concentrations of interleukin 1 beta (IL - 1β), interleukin 6 (IL – 6), tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB) were determined using commercially available ELISA kits (Elabscience Biotechnology Company, Beijing, China and ABCAM, UK) with the aid of DNM 9602 Microplate Reader (China) in accordance with the procedure described in the assay manual.

2.8. Statistical analysis

Statistical analyses were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups (e.g., Mn alone vs. control, Mn alone vs. Mn + $RUT_{50\ or\ 100}$, RUT alone vs. Mn alone and Mn + $RUT_{50\ or\ 100}$ vs. control) followed by Bonferroni's post-hoc test using GRAPHPAD PRISM 6 software (Version 5; GraphPad Software, La Jolla, California, USA). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of RUT on bio-metals levels in the hippocampus and striatum of Mn treated rats

Fig. 1(A–D) shows the Mn and Fe levels assayed in the hippocampus and striatum of control and rats exposed to Mn alone or in combination with varying doses of RUT. Mn-treated rats had a significant ($p < 0.0001$) increase in Mn levels in the hippocampus and striatum when compared to control rats. Endogenous Fe levels in the hippocampus and striatum were significantly ($p < 0.0001$) decreased when compared to control rats. The exposure of Mn alone significantly increased Mn by 105% and 184% whereas Fe decreased by 41% and 79% in the hippocampus and striatum, respectively. However, co – treatment with RUT (i.e. Mn + RUT_{50} and Mn + RUT_{100}) significantly decreased Mn levels and increased Fe levels in the hippocampus and striatum, respectively.

3.2. Effects of RUT on acetylcholinesterase activity in the hippocampus and striatum of Mn-treated rats

Fig. 2(A and B) shows the effect of RUT on acetylcholinesterase activity in the hippocampus and striatum of Mn – treated rats. The experimental rats treated with Mn alone showed a significant ($p < 0.0001$) increase in acetylcholinesterase activities in the hippocampus and striatum when compared with controls. However, co-treatment with RUT (i.e. Mn + RUT_{50} and Mn + RUT_{100}) significantly ($p < 0.0001$) inhibited acetylcholinesterase activities when compared with Mn alone.

3.3. Effects of RUT on oxidative stress indices in hippocampus and striatum of Mn-treated rats

Fig. 3(A–D) and Fig. 4(A and B) shows the effect of RUT on oxidative stress indices in the hippocampus and striatum of Mn – treated rats. The experimental rats treated with Mn alone showed significant ($p < 0.0001$) decrease in SOD, CAT, GPx activities in the hippocampus and striatum when compared with controls. However, co-treatment with RUT significantly ($p < 0.0001$) prevented decreases in SOD, CAT, and GPx activity in the hippocampus and striatum when compared with Mn alone. Additionally, treatment of experimental rats with Mn alone significantly ($p < 0.0001$) increased LPO levels in the hippocampus and striatum when compared with control rats. However, co-treatment with RUT (i.e. Mn + RUT_{50} and Mn + RUT_{100}) significantly ($p < 0.0001$) prevented the increased LPO levels when compared with the Mn alone group as shown in Fig. 4(C and D).

3.4. Effects of RUT on pro-inflammatory cytokines in the hippocampus and striatum of Mn-treated rats

Fig. 5(A–D) and Fig. 6(A and B) depicts the effects of RUT on pro-inflammatory cytokines namely TNF – α, IL - 1β and IL – 6 in the hippocampus and striatum of Mn – treated rats. The rats treated with Mn showed a significant ($p < 0.0001$) increases in TNF – α, IL - 1β and IL – 6 levels in the hippocampus and striatum when compared with the control. However, rats that were co-treatment with RUT (i.e. Mn + RUT_{50} and Mn + RUT_{100}) demonstrated significant ($p < 0.0001$) inhibition of Mn – induced increases in pro-inflammatory cytokines in the rat brain regions when compared with the Mn alone group.

3.5. Effects of RUT on NF-κB in the hippocampus and striatum of Mn-treated rats

Fig. 6(C and D) depicts shows the levels of NF-κB in the hippocampus and striatum of Mn – treated rats. The rats treated with Mn alone showed significant ($p < 0.0001$) increases in NF-κB in the hippocampus and striatum when compared with the controls. However, co-treatment with RUT (i.e. Mn + RUT_{50} and Mn + RUT_{100}) significantly ($p < 0.0001$) prevented the increase in NF-κB when compared with the Mn alone group.

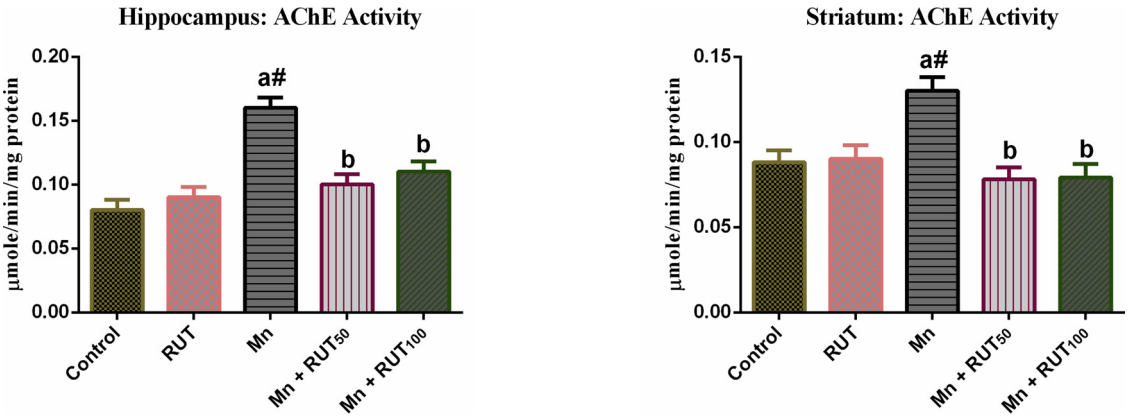

Fig. 2. Effects of Rutin on acetylcholinesterase (AChE) activity (µmole/min/mg protein) in the hippocampus and striatum (A and B) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 6 rats per group. a: Mn differs significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differs significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

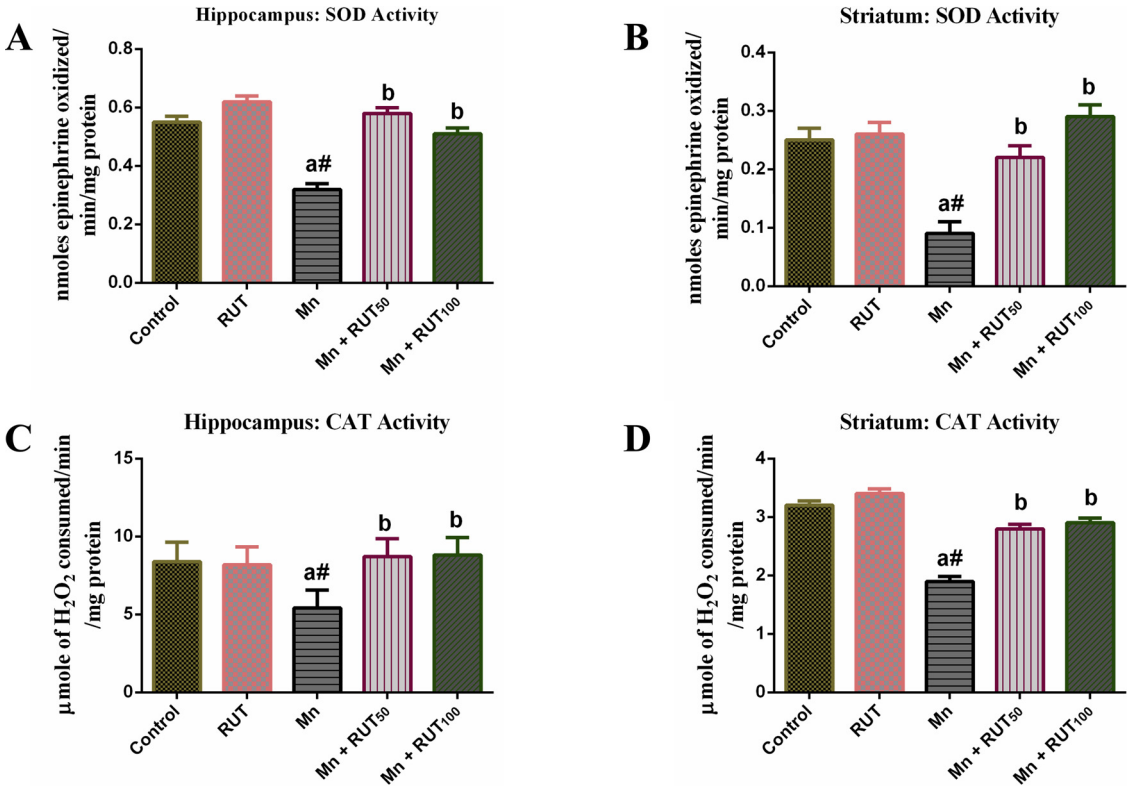

Fig. 3. Effects of rutin on SOD and CAT activities in the hippocampus and striatum (A–D) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 6 rats per group. a: Mn differ significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differ significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

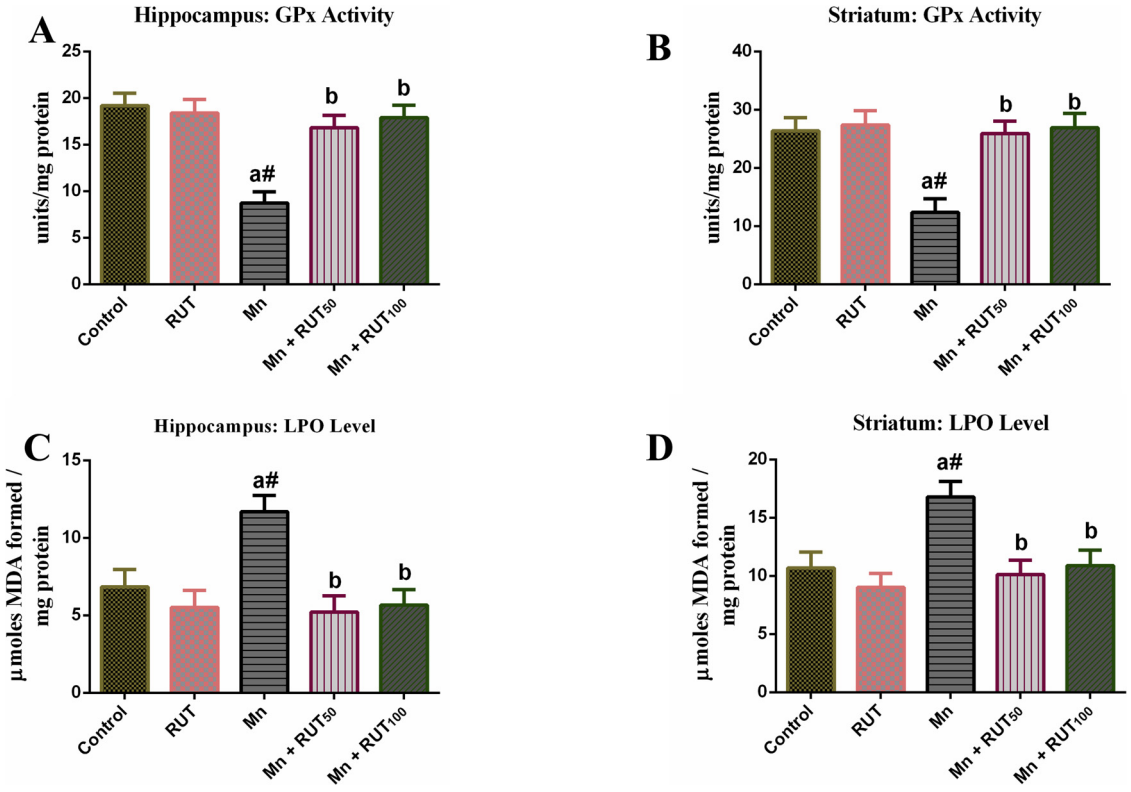

Fig. 4. Effects of rutin on GPx activity and LPO level in the hippocampus and striatum (A–D) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 6 rats per group. a: Mn differ significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differ significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

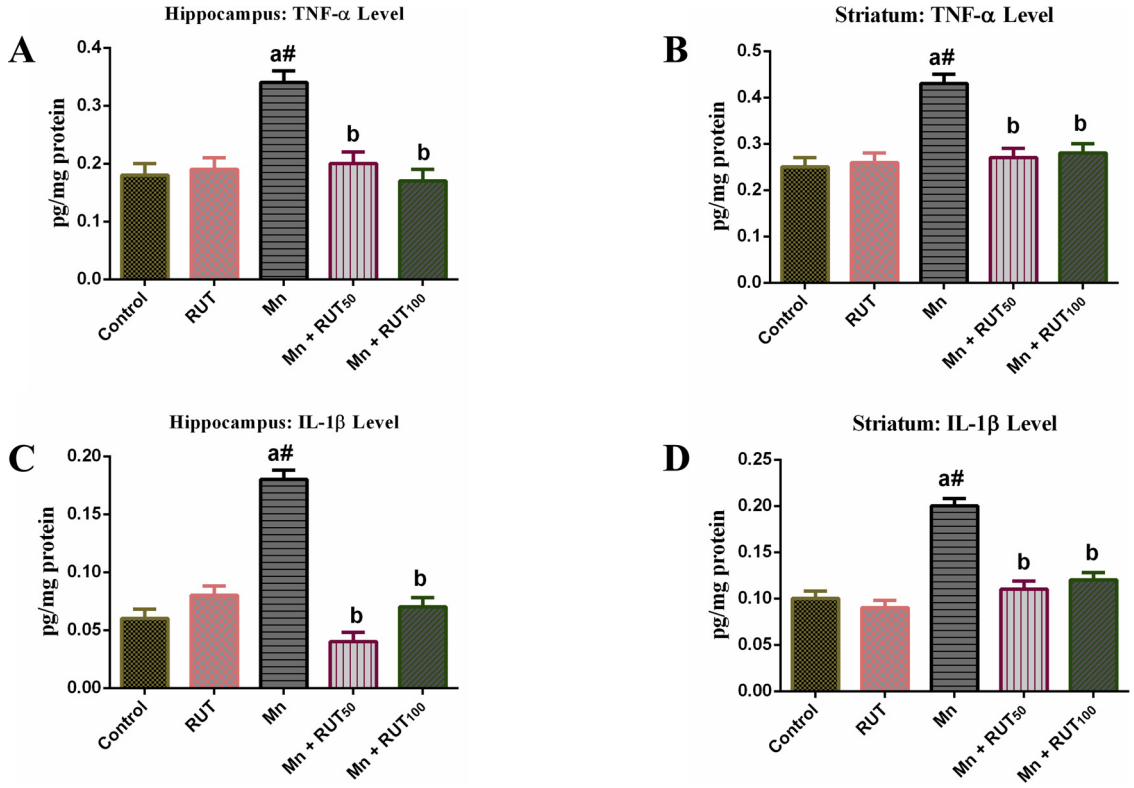

Fig. 5. Effects of rutin on TNF - α and IL - 1β levels in the hippocampus and striatum (A–D) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 6 rats per group. a: Mn differ significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differ significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

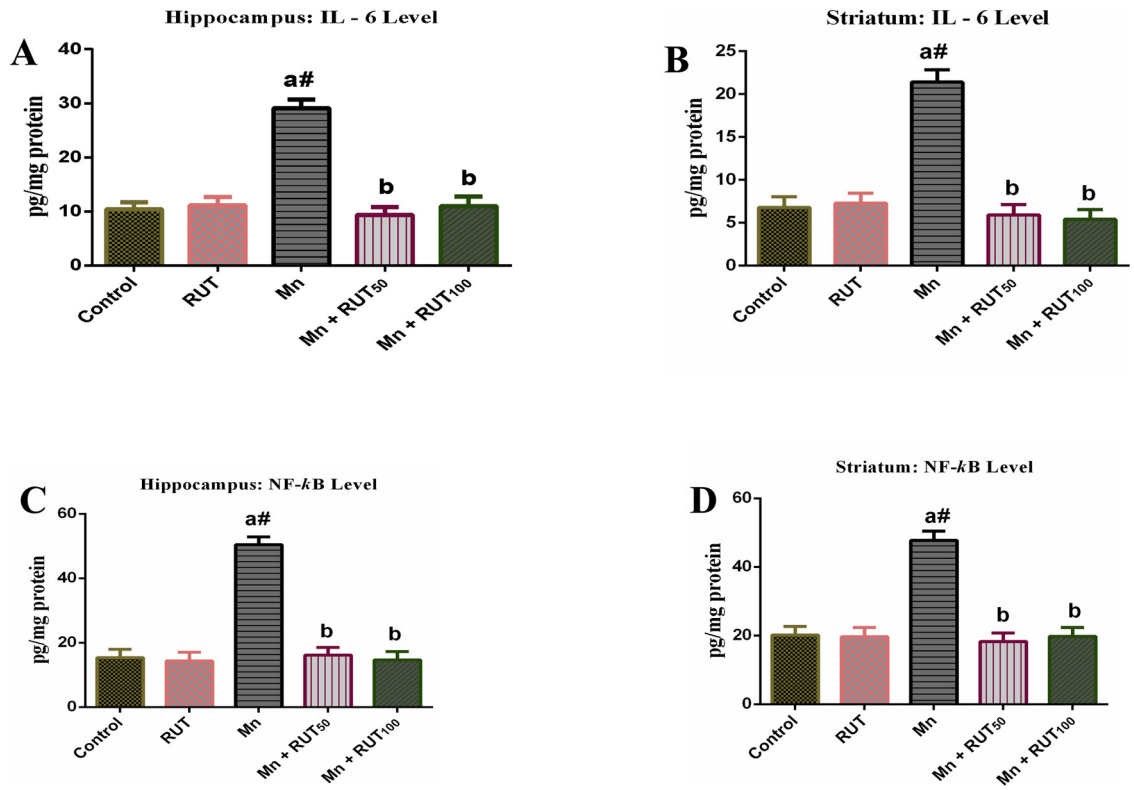

Fig. 6. Effects of rutin on IL – 6 and NF-κB level in the hippocampus and striatum (A–D) of Mn – treated rats. Mn (30 mg/kg body weight); Rutin; RUT_{50} (50 mg/kg body weight); RUT_{100} (100 mg/kg body weight). The data are expressed as mean ± S.D. for 6 rats per group. a: Mn differ significantly from control ($p < 0.05$). b: Mn + $RUT_{50\ or\ 100}$ differ significantly from Mn alone. #: RUT alone differ significantly from Mn alone.

4. Discussion

It is evident from the present study that Mn induced decreases in endogenous Fe levels in the hippocampus and striatum after 35 days of exposure. Mn and Fe are competitive inhibitors since they are both divalent positives ions and usually compete in binding to the blood carrier proteins and pass via the blood-brain-barrier [36]. Studies have also shown that long-term exposure to Mn significantly reduced Fe in human and animal models [37]. The accumulation of Mn in the hippocampus and striatum may have been a result of Fe deficiency in these brain regions. This is consistent with other studies which reported that Fe deficiency results in increased Mn concentrations in the brain [12]. The interaction between Mn neurotoxicity and Fe deficiency may have profound implications such as oxidative stress and neuro-inflammation in these brain regions.

Acetylcholinesterase is responsible for cholinergic neuro-transmission termination by rapid acetylcholine hydrolysis [38]. This neurotransmitter acetylcholine has been long associated with learning and memory in previous reports [38]. Elimination of vesicular acetylcholine transporter as a result of decreased in acetylcholine in the hippocampus and striatum may led to deficits in long-term potentiation that may impair spatial memory and locomotion [38]. In the present study, there was a consistent increase in acetylcholinesterase activity in the hippocampus and striatum of rats exposed to Mn alone. This increase may decrease acetylcholine levels in the synaptic cleft, thereby, resulting in impairment of spatial learning, memory and locomotion. However, co-treatment with RUT significantly prevented the increase in acetylcholinesterase activity in these brain regions. This result indicates that RUT may abrogate neurobehavioral deficits in Mn – induced neurotoxicity attributed to acetylcholinesterase inhibition.

The present study demonstrates that Mn treatment is associated significant decreases in SOD, CAT and GPx activities and increased LPO levels in the hippocampus and striatum. This may be as a result of the high polyunsaturated fatty acid content of the brain which renders it highly sensitive to stress-induced neurodegeneration via ROS generation which may lead to oxidative damage and lipid peroxidation [39] in the tissues. Several reports have indicated that oxidative stress induced by free radicals is a major pathophysiological process in secondary neuronal damage [39]. However, co – treatment with RUT significantly prevented oxidative damage via improving the antioxidant status along with concomitant inhibition of LPO in the hippocampus and striatum; therefore, indicating the antioxidant and anti-lipid peroxidative properties of RUT in Mn – induced neurotoxicity.

The brain is highly susceptible to inflammation which may induce neurobehavioral deficits and functional alteration of the brain [23]. Neuro-inflammation has been reported to be an important pathophysiological process involved in secondary neuronal damage [40]. However, the underlying molecular mechanism of Mn – mediated neuro-inflammation is not fully understood. In the present study, we investigated the role of NF-κB in Mn – induce neuro-inflammation. NF-κB signaling pathway plays a major role in inflammation. Under normal biological conditions, NF-κB is bound to IκBα, its inhibitory protein in the cytosol. Activation of IκBα results in its phosphorylation and degradation, thereby resulting in the phosphorylation and translocation of NF-κB into the nucleus from the cytosol. Nucleus bound NF-κB activates the transcription of its target genes such as TNF-α, IL-1β, and IL-6 [41]. The present study shows that Mn induced the activation of NF-κB signaling pathways in the hippocampus and striatum which regulates genes involved in inflammation. However, RUT seems to effectively abrogates the activation of NF-κB signaling pathways and may have been responsible for the decrease in the pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) observed in the hippocampus and striatum. Our results therefore show the anti – inflammatory properties of RUT in Mn – induced neuro-inflammation.

5. Conclusion

Taken together, our present study demonstrated that RUT seem to abrogates Mn – induced striatal and hippocampal toxicity via inhibition of iron depletion, acetylcholinesterase activity, oxidative stress and neuro-inflammation and suppression of the NF-κB signaling pathways. These results indicate that RUT may be used as neuroprotective agents against Mn – induced neuronal toxicity.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This research was done without specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

[1] X. Ge, F. Wang, Y. Zhong, Y. Lv, C. Jiang, Y. Zhou, D. Li, B. Xi, C. Su, H. Cheng, Y. Ma, F. Xiong, Y. Shen, Y. Zou, Manganese in blood cells as an exposure biomarker in manganese-exposed worker's healthy cohort, J. Trace Elem. Med. Biol. 45 (2018) 41–47.

[2] M. Aschner, K.M. Erikson, E. Herrero Hernandez, R. Tjalkens, Manganese and its role in Parkinson's disease: from transport to neuropathology, Neuromolecular Med. 11 (4) (2009) 252–266.

[3] K. Neth, M. Lucio, A. Walker, B. Kanawati, J. Zorn, P. Schmitt-Kopplin, B. Michalke, Diverse serum manganese species affect brain metabolites depending on exposure conditions, Chem. Res. Toxicol. 28 (7) (2015) 1434–1442.

[4] K. Neth, M. Lucio, A. Walker, J. Zorn, P. Schmitt-Kopplin, B. Michalke, Changes in brain metallome/metabolome pattern due to a single i.v. injection of manganese in rats, PLoS One 10 (9) (2015) e0138270.

[5] W. Laohaudomchok, X. Lin, R.F. Herrick, S.C. Fang, J.M. Cavallari, R. Shrairman, A. Landau, D.C. Christiani, M.G. Weisskopf, Neuropsychological effects of low-level manganese exposure in welders, Neurotoxicology 32 (2) (2011) 171–179.

[6] Y. Chang, J.J. Lee, J.H. Seo, H.J. Song, J.H. Kim, S.J. Bae, J.H. Ahn, S.J. Park, K.S. Jeong, Y.J. Kwon, S.H. Kim, Y. Kim, Altered working memory process in the manganese exposed brain, NeuroImage 53 (2010) 1279–1285.

[7] A. Benedetto, C. Au, D.S. Avila, D. Milatovic, M. Aschner, Extracellular dopamine potentiates Mn-induced oxidative stress, lifespan reduction, and dopaminergic neurodegeneration in a BLI- 3-dependent manner in *Caenorhabditis elegans*, PLoS Genet. 6 (8) (2010) 1001084.

[8] T.R. Guilarte, Manganese neurotoxicity: new perspectives from behavioral, neuroimaging, and neuropathological studies in humans and non-human primates, Front. Aging Neurosci. 5 (2013) 23.

[9] M. Farina, D.S. Avila, J.B. da Rocha, M. Aschner, Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury, Neurochem. Int. 62 (2013) 575–594.

[10] M.K. Chen, J.S. Lee, J.L. McGlothan, E. Furukawa, R.J. Adams, M. Alexander, D.F. Wong, T.R. Guilarte, Acute manganese administration alters dopamine transporter levels in the non-human primate striatum, Neurotoxicology 27 (2) (2006) 229–236.

[11] B. Michalke, K. Fernsebner, New insights into manganese toxicity and speciation, J. Trace Elem. Med. Biol. 28 (2) (2014) 106–116.

[12] K. Fernsebner, J. Zorn, B. Kanawati, A. Walker, B. Michalke, Manganese leads to an increase in markers of oxidative stress as well as to a shift in the ratio of Fe (II)/(III) in rat brain tissue, Metallomics 6 (4) (2014) 921–931.

[13] V. Venkataramani, T. Doeppner, D. Wilkommen, C.M. Cahill, G. Ye, Y. Liu, A. Southon, A. Aron, H.Y. Au-Yeung, X. Huang, D. Lahiri, F. Wang, A.I. Bush, G.G. Wulf, P. Strohel, B. Michalke, J.T. Rogers, Manganese causes neurotoxic iron accumulation via translational repression of amyloid precursor protein and H-Ferritin, J. Neurochem. 147 (6) (2018) 831–848.

[14] V. Evren, M. Apaydin, A. Khalilnezhad, O. Erbas, D. Taskiran, Protective effect of edaravone against manganese-induced toxicity in cultured rat astrocytes, Environ. Toxicol. Pharmacol. 40 (2) (2005) 563–567.

[15] Y. Deng, D. Xu, B. Xu, Z. Xu, Y. Tian, W. Feng, W. Liu, H. Yang, G0/G1 phase arrest and apoptosis induced by manganese chloride on cultured rat astrocytes and protective effects of riluzole, Biol. Trace Elem. Res. 144 (1–3) (2011) 832–842.

[16] K.M. Erikson, Z.K. Shihabi, J.L. Aschner, M. Aschner, Manganese accumulates in iron deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations, Biol. Trace Elem. Res. 87 (2002) 143–156.

[17] T. Kelleher, E. Ryan, S. Barrett, M. Sweeney, V. Byrnes, C. O'Keane, Increased DMT1 but not IREG1 or HFE mRNA following iron depletion therapy in hereditary haemochromatosis, Gut 53 (2004) 1174–1179.

[18] K.W. Nkpaa, B.A. Amadi, M.O. Wegwu, Hazardous metals levels in groundwater from Gokana, Rivers State, Nigeria: non-cancer and cancer health risk assessment, Hum. Ecol. Risk Assess. 24 (1) (2018) 214–224.

[19] K.W. Nkpaa, K.C. Patrick-Iwuanyanwu, M.O. Wegwu, E.B. Essien, Health risk assessment of hazardous metals for population via consumption of seafood from

Ogoniland, Rivers State, Nigeria; a case study of Kaa, B-Dere, and Bodo city, Environ. Monit. Assess. 188 (2016) 9.
[20] Y.A. Seo, Y. Li, M. Wessling-Resnick, Iron depletion increases manganese uptake and potentiates apoptosis through ER stress, Neurotoxicology 38 (2013) 67–73.
[21] O.S. Akodu, E.A. Disu, O.F. Njokanma, O.A. Kehinde, Iron deficiency anaemia among apparently healthy pre-school children in Lagos, Nigeria, Afr. Health Sci. 16 (March1) (2016) 61–68.
[22] N. Kamalakkannan, S. Mainzen, P. Prince, The antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats, Basic Clin. Pharmacol. Toxicol. 98 (2006) 97–103.
[23] S. Nafees, S. Rashid, N. Ali, S.K. Hasan, S. Sultana, Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NF-kB/MAPK pathway, Chem. Biol. Interact. 231 (2015) 98–107.
[24] M. Bishnoi, K. Chopra, S.K. Kulkarni, Protective effect of rutin, a polyphenolic flavonoid against haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes, Fundam. Clin. Pharmacol. 21 (2007) 521–529.
[25] S.O. Abarikwu, C.A. Otuechere, M. Ekor, K. Monwuba, D. Osobu, Rutin ameliorates cyclophosphamide-induced reproductive toxicity in male rats, Toxicol. Int. 19 (2012) 207–214.
[26] N. Gupta, R.S. Chauhan, J.K. Pradhan, Rutin: A Bioactive Flavonoid, Handbook of Medicinal Plants and Their Bioactive Compounds, (2014), pp. 51–57.
[27] K.W. Nkpaaa, G.I. Onyeso, Rutin attenuates neurobehavioral deficits, oxidative stress, neuro-inflammation and apoptosis in fluoride treated rats, Neurosci. Lett. 682 (2018) 92–99.
[28] K.W. Nkpaa, B.A. Amadi, I.O. Awogbindin, A.O. Abolaji, I.A. Adedara, M.O. Wegwu, E.O. Farombi, Ethanol exacerbates manganese – induced neurobehavioral deficits, striatal oxidative stress and apoptosis *via* regulation of p53, caspase-3 and Bax/Bcl-2 ratio-dependent pathway in rat striatum, Biol. Trace Elem. Res. (2018), https://doi.org/10.1007/s12011-018-1587-4.
[29] M.M. Bradford, Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[30] C.F. Mello, C.K. Kraemer, A. Filippin, V.M. Morsch, A.L.S. Rodrigues, A.F. Martins, M.A. Rubin, Effect of lead acetate on neurobehavioral development of rats, Braz. J. Med. Biol. Res. 31 (1998) 943–950.
[31] G.L. Ellman, K.D. Courtney, V. Andres Jr, R.M. Feather-Stone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95.
[32] H.P. Misra, I. Fridovich, The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 (1972) 3170–3175.
[33] A. Clairborne, Catalase activity, in: A.R. Greewald (Ed.), Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, FL, 1995, p. 2 37–242.
[34] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (1973) 588–590.
[35] E.O. Farombi, J.G. Tahnteng, A.O. Agboola, J.O. Nwankwo, G.O. Emerole, Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron–a *Garcinia kola* seed extract, Food Chem. Toxicol. 38 (2000) 535–541.
[36] A. Nastiti, K. Oginawati, M. Santoso, Manganese exposure on welders in small-scale mild steel manual metal arc welding industry, J. Appl. Sci. Environ. Sanit. 5 (3) (2010) 227–238.
[37] A. Bahrami, A. Jafari, H. Asilian, M. Taghizadeh, A. Arjmand, H. Akbari, Biomarkers of occupational manganese toxicity, Biosci. Biotechnol. Res. Asia 12 (3) (2015) 2147–2156.
[38] J. Massoulie, The origin of the molecular diversity and functional anchoring of cholinesterases, Neurosignals 11 (2002) 130–143.
[39] P.L. Croxson, P.G. Browning, D. Gaffan, M.G. Baxter, Acetylcholine facilitates recovery of episodic memory after brain damage, J. Neurosci. 32 (40) (2012) 3787–13795.
[40] C. Yang, H. Ling, M. Zhang, Z. Yang, X. Wang, F. Zeng, C. Wang, J. Feng, Oxidative stress mediates chemical hypoxia induced injury and inflammation by activating NF-κb-COX-2 pathway in HaCaT cells, Mol. Cells 31 (2011) 531–538.
[41] Y. Yao, Y.-Y. Yang, M.-W. Zhou, N. Liu, H.-Y. Xing, X.-X. Liu, F. Li, Ketogenic diet attenuates oxidative stress and inflammation after spinal cord injury by activating Nrf2 and suppressing the NF-_B signaling pathways, Neurosci. Lett. 683 (2018) 13–18.