Neurochem Res (2017) 42:1219–1229
DOI 10.1007/s11064-016-2162-z

ORIGINAL PAPER

Quercetin Improves Neurobehavioral Performance Through Restoration of Brain Antioxidant Status and Acetylcholinesterase Activity in Manganese-Treated Rats

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Received: 30 October 2016 / Revised: 8 December 2016 / Accepted: 21 December 2016 / Published online: 31 January 2017

Abstract The present study investigated the neuroprotective mechanism of quercetin by assessing the biochemical and behavioral characteristics in rats sub-chronically treated with manganese alone at 15 mg/kg body weight or orally co-treated with quercetin at 10 and 20 mg/kg body weight for 45 consecutive days. Locomotor behavior was monitored using video-tracking software during a 10-min trial in a novel environment whereas the brain regions namely the hypothalamus, cerebrum and cerebellum of the rats were processed for biochemical analyses. Results indicated that co-treatment with quercetin significantly ($p < 0.05$) prevented manganese-induced locomotor and motor deficits specifically the decrease in total distance travelled, total body rotation, maximum speed, absolute turn angle as well as the increase in time of immobility and grooming. The improvement in the neurobehavioral performance of manganese-treated rats following quercetin co-treatment was confirmed by track and occupancy plot analyses. Moreover, quercetin assuaged manganese-induced decrease in antioxidant enzymes activities and the increase in acetylcholinesterase activity, hydrogen peroxide generation and lipid peroxidation levels in the hypothalamus, cerebrum and cerebellum of the rats. Taken together, quercetin mechanisms of ameliorating manganese-induced neurotoxicity is associated with restoration of acetylcholinesterase activity, augmentation of redox status and inhibition of lipid peroxidation in brain of rats.

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Keywords Quercetin · Manganese · Neurotoxicity · Acetylcholinesterase · Oxidative stress

Introduction

Manganese is a trace element and nutrient necessary for several cellular processes in both animals and humans. It is an element with janiform nature due to its' beneficial and potentially detrimental roles in biological systems. Low concentrations of manganese are required for normal growth, development and cellular homeostasis [1]. Manganese is an important cofactor in a variety of metalloenzymes, including antioxidant enzyme superoxide dismutase and those involved in neurotransmitter synthesis and metabolism [2–4]. Moreover, manganese is essential for normal immune function, brain function, adenosine triphosphate (ATP) regulation, reproduction and DNA repair [5, 6].

Although manganese is necessary at physiological concentration, excessive exposure to manganese is associated with severe central nervous system dysfunctions otherwise referred to as manganism [7, 8]. Manganese compounds are used in production of some fertilizers, pesticides and gasoline additive which are often released to the environment as combustion products from automobiles and industrial processes [9, 10]. Human exposure to manganese occurs through numerous routes including food, water, air and occupational factory. Interestingly, the absorption of manganese in humans is high and it is transported via the blood to the liver for metabolism and subsequently to the brain which is the principal target. Indeed, elevated manganese level reportedly affects several cellular processes through mechanisms including depletion of endogenous antioxidants, mitochondrial dysfunction, increased ROS production, calcium and

iron dyshomeostatis, altered acetylcholinesterase activity and dopaminergic dysregulation of neuronal activity [11, 12].

Cumulative evidence indicates that a healthy eating plan with appropriate intake of plant-based foods is essential in the prevention of diseases [13, 14]. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a natural flavonoid commonly found in fruits and vegetables including onions, berries, apples and red wine. The estimated dietary intake of quercetin ranges from 4 to 68 mg/day [15] and can increase to 200–500 mg/day in individuals consuming high quantities of fruits and vegetables rich in flavonoids. Moreover, quercetin is widely reported to have nutraceutical and pharmaceutical uses [16]. It is sold as a dietary supplement, with a recommended dosage of 1 g/day [17]. The numerous beneficial health effects of quercetin have been attributed to its antioxidant and anti-inflammatory properties [18, 19]. Previous in vitro studies on the neuroprotective effect of quercetin demonstrated that it increases the resistance of neurons to oxidative stress and excitotoxicity by modulating the mechanisms of cell death [20, 21]. Similarly, in vivo studies revealed that oral quercetin administration protected against PCBs-induced oxidative stress and apoptosis in hippocampus of adult rats [22], cell apoptosis in focal cerebral ischemia rat brain [23] and rotenone model of Parkinson's disease [24] and also ameliorated cognitive and emotional impairments in aged triple transgenic Alzheimer's disease (3xTg-AD) model mice [25]. However, there is no information in literature on the influence of quercetin on the neurotoxicity resulting from exposure to manganese.

The present study aimed at investigating the impact of quercetin on manganese-induced neurotoxicity by evaluating some locomotor activities, exploratory profiles and biochemical parameters in rats. A standard behavioral protocol for assessing novelty-associated behavioral stress responses [26, 27] was employed, using a video-tracking software (ANY-maze, Stoelting CO, USA). Furthermore, the hypothalamus plays a pivotal role in the synthesis of neurotransmitters and consequently in emotion control, the cerebellum is well-known to regulate equilibrium, posture, fine movement and motor learning whereas cerebrum is responsible for the control of voluntary movements and coordination of mental actions [28–30]. Thus, in order to understand the protective mechanism of quercetin against manganese-induced neurotoxicity, acetylcholinesterase (AChE) and antioxidant enzymes activities along with oxidative stress indices were analyzed in the hypothalamus, cerebrum and cerebellum of the treated rats.

Materials and Methods

Chemicals

Manganese chloride ($MnCl_2$), quercetin, epinephrine, glutathione, hydrogen peroxide, 5′,5′-dithio-bis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Animal Model

Sixty adult male Wistar rats (8 weeks old; 141 ± 2.5 g) obtained from the Faculty of Veterinary Medicine, University of Ibadan, Nigeria were used for this study. The animals were housed in plastic cages placed in a well-ventilated vivarium and subjected to natural photoperiod of 12-h light:12-h dark cycle. They were fed with rat chow and given drinking water ad libitum for a week before the commencement of the experiment. All the animals received humane care according to the conditions stated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health. The experimental protocols were performed after approval by the University of Ibadan Ethical Committee.

Experimental Design

The animals were randomly assigned to five groups of twelve rats each and were treated for 45 consecutive days as follows: Group I rats were orally administered corn oil alone at 2 mL/kg body weight and served as control. Group II rats were orally administered quercetin (Qt) alone at 20 mg/kg body weight whereas Group III rats were orally administered manganese (Mn) alone at a dose of 15 mg/kg. Group IV rats were co-administered with manganese and quercetin at 10 mg/kg ($Mn + Qt_{10}$) whereas Group V rats were co-administered with manganese and quercetin at 20 mg/kg ($Mn + Qt_{20}$).

Stock solution of quercetin (100 mg/mL) was prepared fresh every other day using corn oil as a vehicle. The doses of manganese and quercetin used in the present study were selected based on the pilot study in our laboratory and previously published data [31, 32].

Behavioral Experiments in a Novel Environment

The novel environment test was performed 24 h after the last treatment to assess the behavioral pattern of rats

according to standardize procedure [26]. Briefly, the rats were randomly selected and placed in the center of the apparatus (wooden box of 56 cm width × 56 cm length × 20 cm height) and allowed to freely explore the arena. The behavior of the rats was filmed during a 10-minute trial using a webcam (DNE webcam, Porto Alegre, Brazil) mounted above the apparatus and attached to a laptop. All experiments were conducted between 10:00 a.m. and 4:00 p.m. to maintain the same experimental conditions. The behavioral parameters were automatically computed at a rate of 30 frames per second using video-tracking software (ANY-maze, Stoelting CO, USA). Necessary caution was taken when transferring the rats from home cages to the novel environment to avoid stress associated with handling. All the rats were handled and tested using a standardized protocol (similar illumination, manipulation and time period in a day).

Evaluation of Neurobehavioral Parameters

Locomotor and exploratory activities were analyzed in the novel environment so as to reveal habituation to novelty stress. The locomotor pattern of the experimental rats were evaluated by behavioral endpoints including the total distance travelled, maximum speed, total time immobile, absolute turn angle, body rotation and grooming time. Analysis of the exploratory profile of the rats was performed using representative track and occupancy plots so as to evaluate the exploratory activity in the novel environment. The home base formation in the novel environment during a trial was defined as a place in the arena for which the experimental animal showed a preference in terms of occupancy, and as a starting and ending point of exploratory tours [33]. The home base formation of the animals was confirmed by both track and occupancy plots.

Sample Preparation for Biochemical Assays

Following the behavioral trial, the rats were sacrificed under light ether anesthesia. The cranium was opened and the brain were carefully excised and separated into hypothalamus, cerebrum and cerebellum [29]. The samples were homogenized in eight volumes of 100 mM potassium phosphate buffer (pH 7.4) and the resulting homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C and the supernatant obtained was subsequently used for the biochemical determinations. Protein concentration was assayed according to the method of Bradford [34].

Determination of Acetylcholinesterase Activity

Acetylcholinesterase activity was assayed in the hypothalamus, cerebrum and cerebellum according to the method of Ellman et al. [35]. The assay mixture was made up of 135 µL of distilled water, 20 µL of 100 mM potassium phosphate buffer (pH 7.4), 20 µL of 10 mM DTNB, 5 µL of diluted sample (1:10 v/v), and 20 µL of 8 mM acetylthiocholine as a substrate. The breakdown of acetylthiocholine iodide was analyzed for 5 min (30 s intervals) at 412 nm using a SpectraMax plate reader (Molecular Devices, CA, USA) and the results were expressed as µmol/min/mg protein.

Determination of Antioxidant and Oxidative Stress Indices in Hypothalamus, Cerebrum and Cerebellum

Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of autoxidation of epinephrine according to the method of Misra and Fridovich [36]. Briefly, 40 µL of the sample was added to 2.4 mL of 0.05 M carbonate buffer (pH 10.2) and the reaction started by the addition of 60 µL of freshly prepared 0.3 mM epinephrine. The increase in absorbance was analyzed for 150 s (30 s intervals) at 480 nm with a UV–visible spectrophotometer. Reaction mixture without the enzyme was used as blank. Value was expressed as nanomoles epinephrine oxidized/min/mg protein.

Catalase (CAT) activity was assayed by monitoring the disappearance of H_2O_2 according to established method [37]. Briefly, the reaction medium consisted of 1.8 mL of 50 mM phosphate buffer (pH 7.0), 180 µL of 300 mM H_2O_2 and 20 µL of sample (1:20 dilution). The reaction was analyzed for 2 min (15 s intervals) at 240 nm with a UV–visible spectrophotometer. Value was expressed as micromole H_2O_2 consumed/min/mg protein.

Hydrogen peroxide (H_2O_2) generation was assayed according to the method of Wolff [38]. Briefly, the ferrous oxidation with xylenol orange (FOX-1) reagent was prepared using 100 µ Mol/L xylenol orange, 250 µ Mol/L ammonium ferrous sulfate, 100 mmol/L sorbitol and 25 mmol/L H_2SO_4. The assay mixture consisting of 20 µL of the sample and 180 µL of FOX-1 reagent was vortexed and subsequently incubated at room temperature for 30 min. The absorbance was read at 560 nm using a SpectraMax plate reader (Molecular Devices, CA, USA) and the values extrapolated from H_2O_2 standard curve. The unit was expressed as the micromole H_2O_2 per mg protein.

Lipid peroxidation (LPO) was determined by measuring malondialdehyde (MDA), an end product of lipid peroxidation, according to the method described by Farombi et al. [39] with slight modification. Briefly, the reaction mixture consisted of 150 µL of 0.1 M of 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 mixture was heated at 90–95 °C for 20 min and after cooling to room temperature, they were centrifuged at 8000 × *g* for

10 min and the absorbance of the supernatant was measured at 532 nm using a SpectraMax plate reader (Molecular Devices, CA, USA). The level of MDA was calculated using the extinction coefficient (Σ) of 1.56×10^5 L/mol/cm and extent of LPO expressed as the μmole MDA formed per mg protein.

Histopathology

The brain samples were fixed in 10% neutral-buffered formalin and processed for histology according to established procedure [40]. Briefly, the fixed tissues were dehydrated using ascending concentrations of alcohol, cleared by xylene and embedded in paraffin wax. Subsequently, the tissues were cut into 4–5 μm sections using a microtome, fixed on the slides and stained with hematoxylin and eosin. The slides were viewed under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken using a Sony DSC-W 30 Cyber-shot (Sony, Tokyo, Japan) by pathologists who were blinded to the control and treatment groups.

Statistical Analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Bonferroni's post-hoc test to identify statistically significantly different groups using GRAPHPAD PRISM 5 software (Version 4; GraphPad Software, La Jolla, California, USA). Values of $p < 0.05$ were considered significant.

Results

Quercetin Prevented Manganese-Induced Locomotor Deficits in Rats

Figures 1 and 2 depict the general locomotor performance of the experimental rats during a 10-minute trial in the novel environment. The body weight gain did not differ between the treated and control groups (data not shown). Similarly, administration of quercetin alone did not significantly altered the locomotor activities of the treated rats when compared with the control. However, the endpoint analyses showed that rats treated with manganese alone showed significant ($p < 0.05$) decrease in the total distance travelled, maximal speed, body rotation and turn angle whereas the total time immobile and grooming were significantly increased when compared with control. The percent decrease in the total distance travelled and maximum speed in manganese-treated rats were 60 and 52%, respectively. However, co-treatment with quercetin significantly reversed the locomotor performance when compared with

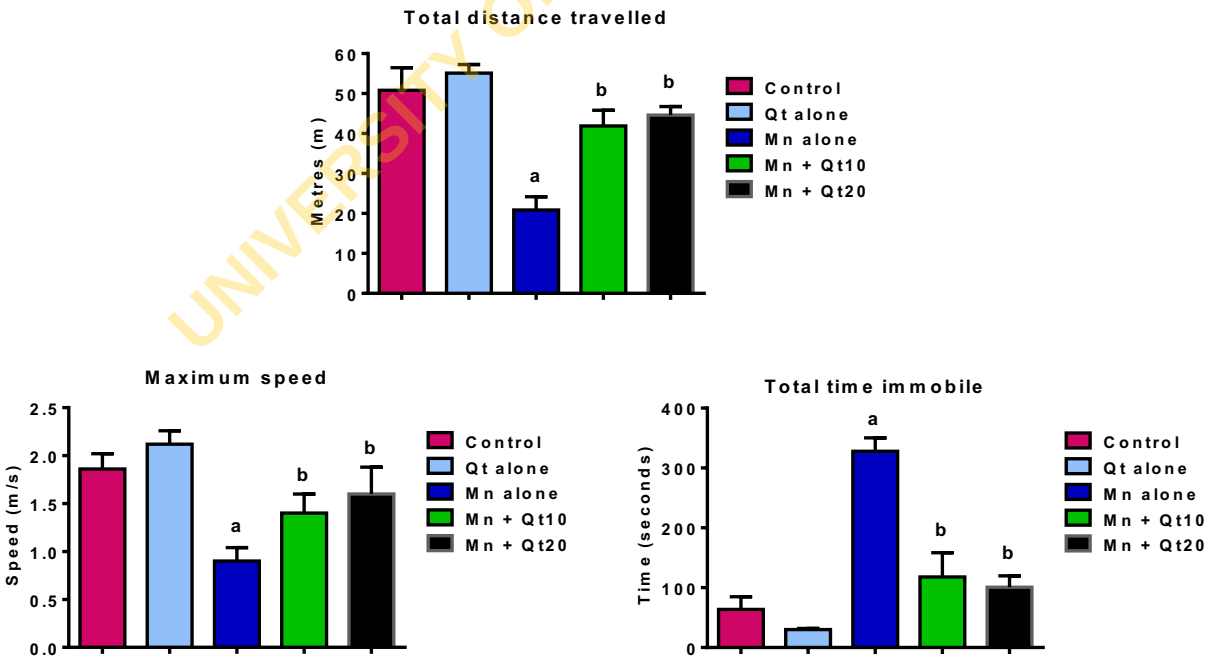

Fig. 1 Effects of quercetin on manganese-induced changes in the total distance travelled, maximum speed and time immobile during a 10-min trial. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight). The data are expressed as mean ± SD for 12 rats per group. *a* Values differ significantly from control ($p < 0.05$). *b* Values differ significantly from Mn alone ($p < 0.05$)

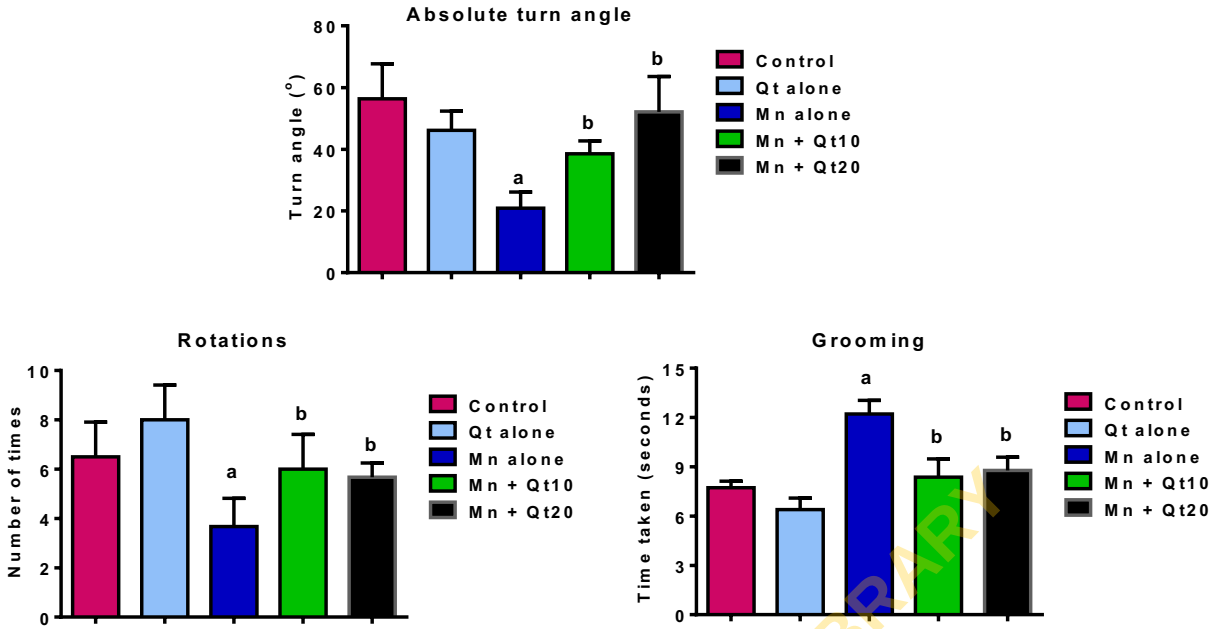

Fig. 2 Effects of quercetin on manganese-induced changes in the absolute turn angle, number of body rotations and time spent in grooming during a 10-min trial. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight). The data are expressed as mean ± SD for 12 rats per group. *a* Values differ significantly from control ($p < 0.05$). *b* Values differ significantly from Mn alone ($p < 0.05$)

rats treated with manganese alone. The percent increase in maximum speed was 56 and 101% whereas total distance travelled by the rats increased by 78 and 114% for $Mn + Qt_{10}$ and $Mn + Qt_{20}$, respectively. Moreover, quercetin administration significantly reversed manganese-induced decrease in body rotation and absolute turn angle in the rats. Administration of manganese alone significantly increased the total time immobile and grooming of the rats during the trial whereas quercetin co-administration significantly decreased the total time immobile and grooming when compared with rats treated with manganese alone.

Quercetin Ameliorated Manganese-Induced Reduction in Exploratory Profile in Rats

Figure 3 depicts the representative track plots of the walking traces and the occupancy plots of control rats and those treated with manganese alone or in combination with quercetin within the apparatus. The control rats demonstrated a usual behavioral profile by walking around the novel apparatus whereas the densities of the track and occupancy plots of rats exposed to quercetin alone seems to be higher than control. There were marked reduction in the density of track plots of manganese-treated rats, thus corroborating the decrease in locomotor activity. Similarly, the occupancy plot analyses of manganese-treated rats revealed a marked decrease in exploration with longer time spent in a particular region of the arena when compared with control. However, as shown by the increase in the densities of track and occupancy plots, quercetin co-administration significantly reversed manganese-induced decrease in exploration and home base formation by the rats.

Quercetin Modulated Manganese-Induced Increase in Acetylcholinesterase Activity in Hypothalamus, Cerebrum and Cerebellum of Rats

Figure 4 depicts the modulatory effect of quercetin on AChE activity in different brain regions of rats treated with manganese. Administration of manganese caused a significant increase in AChE activity in the hypothalamus, cerebrum and cerebellum of the treated rats. The percent increases in the AChE activity were 101, 50 and 59% in the hypothalamus, cerebrum and cerebellum, respectively in manganese-treated rats. However, co-administration with quercetin significantly modulated the effect of manganese evidenced by the decrease in AChE activity in the brain regions of the treated rats. The percent reduction in AChE activity in the hypothalamus was 38 and 59%; cerebrum was 42 and 50% and cerebellum was 33 and 38% for $Mn + Qt_{10}$ and $Mn + Qt_{20}$, respectively, when compared with rats treated with manganese alone.

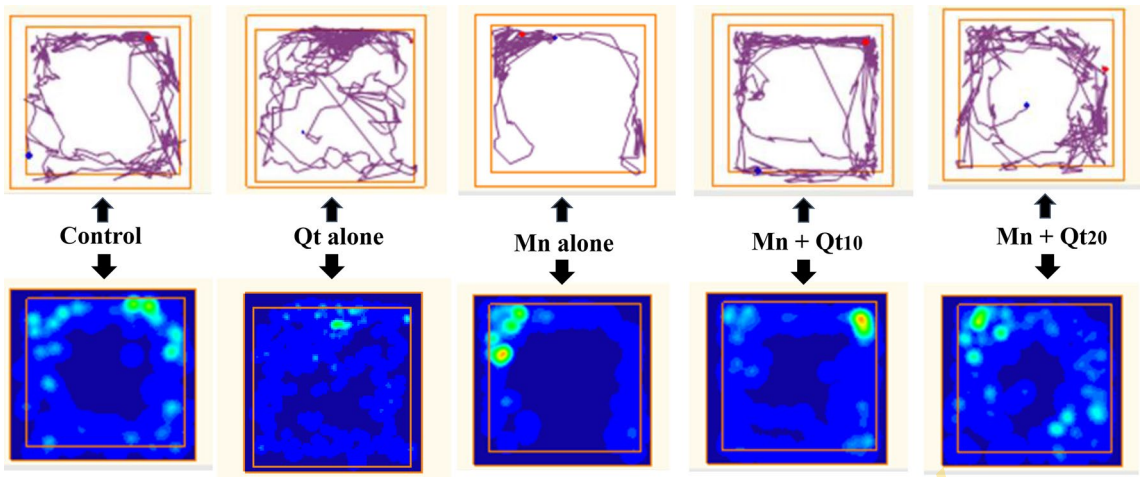

Fig. 3 Effects of quercetin on exploratory profiles in manganese-treated rats represented by track and occupancy plots during a 10-min trial. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight).The representative track plots (*upper panel*) showing the path travelled by rats in the novel environment. The *light green* spots in the occupancy plot (*lower panel*) denotes home base formation, the regions of frequent immobile episodes. The data were analyzed using video-tracking software (ANY-maze, Stoelting CO, USA). (Color figure online)

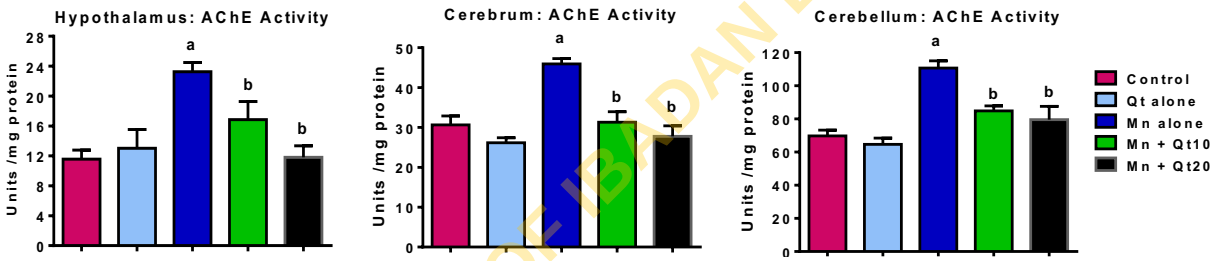

Fig. 4 Effects of quercetin on acetylcholinesterase activity in the hypothalamus, cerebrum and cerebellum of manganese-treated rats. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight).The data are expressed as mean ± SD for 12 rats per group. *a* Values differ significantly from control ($p < 0.05$). *b* Values differ significantly from Mn alone ($p < 0.05$)

Quercetin Prevented Manganese-Induced Decrease in Antioxidant Enzymes Activities in Hypothalamus, Cerebrum and Cerebellum of Rats

Figure 5 depicts the effects of quercetin on the antioxidant status in hypothalamus, cerebrum and cerebellum of manganese-treated rats. When compared with the control, administration of quercetin alone did not cause any treatment-related effects on SOD and CAT activities in the rats. Administration of manganese alone significantly decreased SOD and CAT activities in hypothalamus, cerebrum and cerebellum in the treated rats when compared to the control. Activity of SOD decreased by 35, 34 and 27% whereas CAT activity decreased by 48, 64 and 50% in the hypothalamus, cerebrum and cerebellum, respectively in manganese-treated rats. However, co-administration of quercetin significantly increased the SOD and CAT activities when compared with rats treated with manganese alone. The percent increase in SOD activity in the hypothalamus was 40 and 55%; cerebrum was 33 and 23% and cerebellum was 19 and 27% whereas CAT activity increased in the hypothalamus by 46 and 69%; cerebrum by 121 and 129% and cerebellum by 59 and 91%, respectively for $Mn + Qt_{10}$ and $Mn + Qt_{20}$, respectively when compared with rats treated with manganese alone.

Quercetin Inhibited Manganese-Induced Oxidative Stress in Hypothalamus, Cerebrum and Cerebellum of Rats

Figure 6 depicts the effects of quercetin on the biomarkers of oxidative stress determined in the hypothalamus, cerebrum and cerebellum of the rats. There was no significant difference between the measured oxidative stress biomarkers, namely H_2O_2 generation and MDA (an index of LPO) in the control and rats treated with quercetin alone.

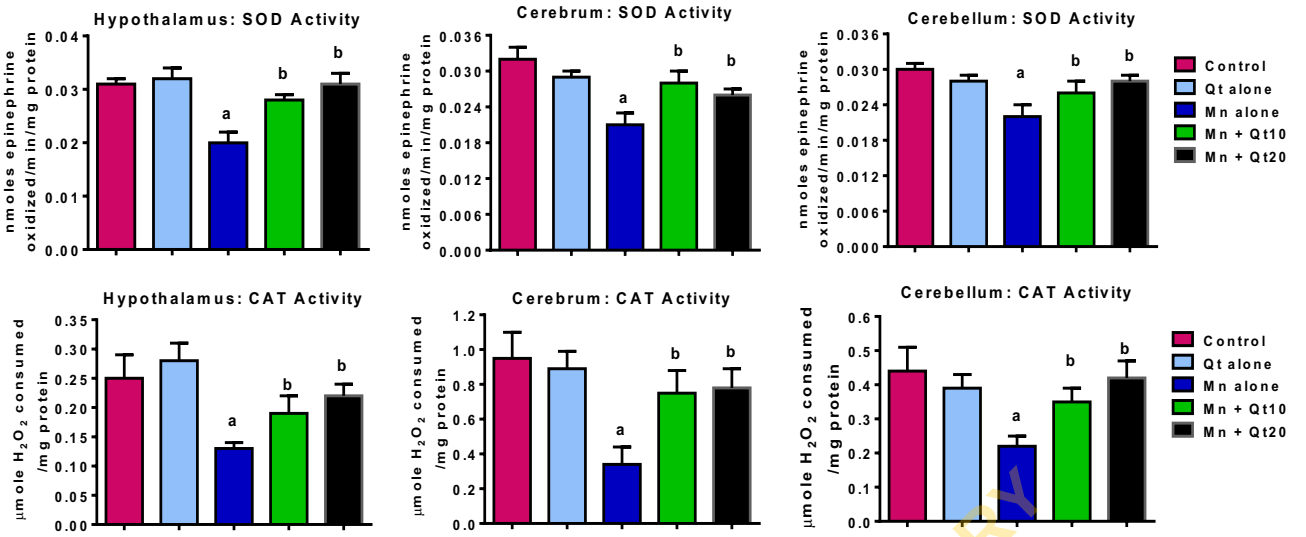

Fig. 5 Effects of quercetin on superoxide dismutase (SOD) and catalase (CAT) activities in the hypothalamus, cerebrum and cerebellum of manganese-treated rats. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight). The data are expressed as mean ± SD for 12 rats per group. *a* Values differ significantly from control ($p < 0.05$). *b* Values differ significantly from Mn alone ($p < 0.05$)

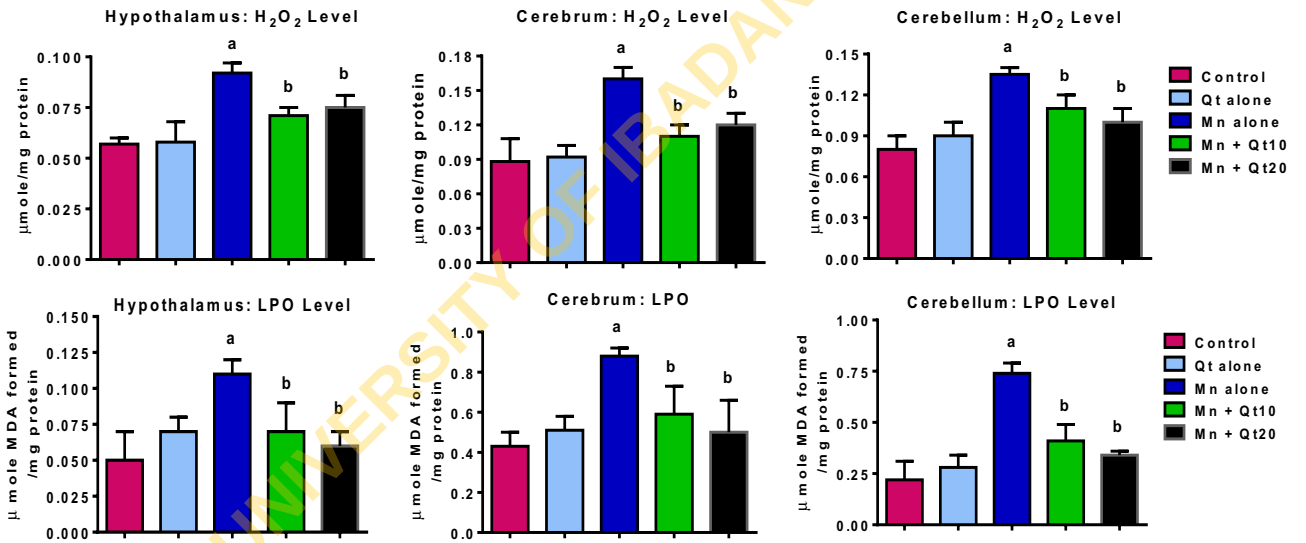

Fig. 6 Effects of quercetin on hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO) levels in the hypothalamus, cerebrum and cerebellum of manganese-treated rats. Manganese, Mn; Quercetin, Qt; Mn alone (15 mg/kg body weight); Qt alone (20 mg/kg body weight); Qt_{10} (10 mg/kg body weight); Qt_{20} (20 mg/kg body weight). The data are expressed as mean ± SD for 12 rats per group. *a* Values differ significantly from control ($p < 0.05$). *b* Values differ significantly from Mn alone ($p < 0.05$)

However, administration of manganese alone resulted in significant elevation in the levels of H_2O_2 generation and LPO in the hypothalamus, cerebrum and cerebellum compared with the control group. The level of H_2O_2 increased by 61, 17 and 25% whereas LPO increased by 120, 105 and 236% in the hypothalamus, cerebrum and cerebellum, respectively in manganese-treated rats. However, co-administration of quercetin significantly decreased the H_2O_2 and LPO levels when compared with rats treated with manganese alone. Rats co-administered with $Mn + Qt_{10}$ showed 23, 31 and 19% reduction in H_2O_2 level whereas they showed 36, 33 and 45% reduction in LPO level in the hypothalamus, cerebrum and cerebellum, respectively when compared with rats treated with manganese alone. Furthermore, rats co-administered with $Mn + Qt_{20}$ showed 18, 25 and 26% reduction in H_2O_2 level whereas

they showed 45%, 43% and 54% reduction in LPO level in the hypothalamus, cerebrum and cerebellum, respectively when compared with rats treated with manganese alone.

Quercetin Ameliorated Manganese-Induced Histopathological Changes in Brain of Rats

Figure 7 depicts the histopathological changes observed with the light microscope in the cerebellar and cerebral sections from the experimental rats. The cerebellum and cerebrum of rats from control (Fig. 7a1, b1) and quercetin alone (Fig. 7a2, b2) groups appeared structurally and functionally normal. However, there were obvious pathological features in the cerebellum (Fig. 7a3) and cerebrum (Fig. 7b3) sections in rats treated with manganese alone. The results showed marked neuronal degeneration and congestion of the macro and micro-circulation (black arrow) of the cerebellum whereas cerebrum showed focal area of vacuolation with marked haemorrhagic lesion and edema (green notched arrow). The sections from rats co-administered with quercetin at 10 mg/kg (Fig. 7a4, b4) showed normal cerebellum whereas the cerebrum showed mild haemorrhage (yellow notched arrow). The cerebellum and cerebrum sections of rats co-administered with quercetin at 20 mg/kg (Fig. 7a5, b5) appeared normal and comparable with control.

Discussion

The modulation of toxicant-induced neurotoxicity possibly by naturally occurring phytochemicals represents a major contemporary interest. The results of the present study obviously demonstrated, for the first time, the efficacy of quercetin in ameliorating neurotoxicity due to excessive exposure to manganese. The neuroprotective effects of quercetin were evidenced by reversal of manganese-induced locomotor deficits, enhancement of AChE activity, increase in oxidative stress and inhibition of antioxidant enzymes activities in rats. The modulation of these biochemical parameters and brain histopathology in manganese-treated rats by quercetin is related to the improvement in the neurobehavioral performance of the treated rats.

In the present study, rats treated with manganese exhibited significant locomotor impairment as evidenced by the decrease in speed and distance moved along with increased time of immobility in the novel environment. Moreover, administration of manganese caused alteration in the motor posture patterns evidenced by marked reduction in the body rotation and turn angle. The decrease in locomotor activity observed in manganese-treated rats is consistent with previous studies [41, 42]. However, the reversal of manganese-induced locomotor and motor deficits in rats co-treated with quercetin clearly demonstrated the protective role of quercetin in manganese neurotoxicity. Grooming in animals is an act of cleaning the outer body surface. The translational value of rodent self-grooming as an index of disturbed motor functions and survival behavioral patterns in models of brain disorders is a well acceptable concept [43]. The increase in grooming time observed in rats treated with manganese alone in the present study is consistent with previous study [44]. The ability of quercetin to prevent manganese-induced increase in time spent grooming in the rats in the present investigation further suggests the modulatory role of quercetin in manganese mediated disturbance of motor function in the treated rats.

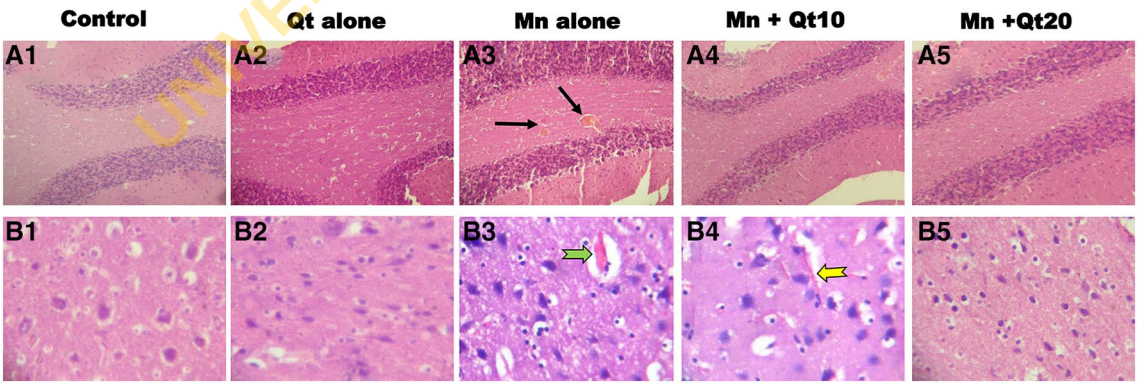

Fig. 7 Representative photomicrographs of cerebellar and cerebral sections from the experimental rats. The cerebellum and cerebrum of rats from control (**a1, b1**) and quercetin alone (**a2, b2**) groups showing normal morphology. The cerebellum of rats treated with manganese alone (**a3**) showing marked neuronal degeneration and congestion of the macro and micro-circulation (*black arrow*). The cerebrum of rats with manganese alone alone (**b3**) showing focal area of vacuolation with marked haemorrhagic lesion and edema (*green notched arrow*). The cerebellum and cerebrum of rats co-treated with quercetin at 10 mg/kg (**a4, b4**) showing normal cerebellum whereas the cerebrum showed mild haemorrhage (*yellow notched arrow*). The cerebellum and cerebrum of rats co-treated with quercetin at 20 mg/kg (**a5, b5**) appeared structurally normal and similar to control. Original magnification: ×250. (Color figure online)

Moreover, the adverse effect of manganese exposure on the exploratory activity of the rats was pronounced in comparison with control. The decrease in the exploration during the trial was confirmed by track and occupancy plots. Exploratory activity represent a major way by which animals gather information about their spatial environment [45]. An organism have a habit of establishing a safe place or home-base where it spends more time in and frequently returns to when exploring a novel environment [33, 46]. The densities of the track and occupancy plots of rats exposed to quercetin alone appears to be higher than control, thus signifying that quercetin alone changed the behavior of the rats. The reversal in the manganese-induced decrease in exploration and home base formation as evidenced by the increase in the densities of track and occupancy plots in rats co-treated with quercetin suggests the protective role of quercetin in manganese-induced disorganization of spatial behavior in the rats.

Acetylcholinestarase (AChE) is a vital enzyme for cholinergic neurotransmission and its regulatory role in many neurobehavioral processes is well established [47]. The results of the present study showed that AChE activity was markedly increased in hypothalamus, cerebrum and cerebellum of rats exposed to manganese. This finding is in agreement with previous observations where excessive exposure to manganese reportedly increased brain AChE activity in rat [48, 49]. The increase in AChE activity in rats treated manganese could result in decreased acetylcholine levels in the synaptic cleft. The diminution in this important neurotransmitter and neuromodulator consequently reduces cholinergic neurotransmission efficiency and impairs locomotor and exploratory activities in the rats. However, the attenuation of manganese effect on AChE activity in rats co-treated with quercetin might boost neurotransmission and consequently increase motor function, locomotion, and exploration as observed in the manganese-treated rats.

Advances in the understanding of manganese neurotoxicity have implicated oxidative stress as a major mechanism [50, 51]. However, the antioxidant defense systems represent one of the major survival mechanisms of cells during exposure to environmental contaminants including metals. Antioxidant enzymes including SOD and CAT are actively involved in the defense against oxidative cell injury owing to their ability to mop up free radicals [52]. The decrease in SOD and CAT activities in the hypothalamus, cerebrum and cerebellum of manganese-treated rats in the present study suggests enzyme inhibition and inability to scavenge free radicals in the brain of the rats. The decrease in these antioxidant enzymes reported herein are consistent with the previous studies [53, 54]. Moreover, elevated levels of H_2O_2 and MDA observed in the three regions of the brain during this study indicates a state of oxidative stress subsequent to the reduction in antioxidant defenses and accumulation of ROS in manganese exposed rats. However, quercetin mediated reversal of the manganese-induced oxidative stress were evidenced by increased SOD and CAT activities with concomitant reduction in H_2O_2 and LPO levels in the brain. These findings therefore support the historic antioxidant properties of quercetin [16, 19, 55].

The histological lesions in brain of rats treated with manganese alone in the present study were characterized by marked neuronal degeneration and congestion of the macro and micro-circulation of the cerebellum while focal area of vacuolation with marked haemorrhagic lesion and edema were observed in the cerebrum of the rats. These histopathological findings may be related to the induction of oxidative damage in the brain of the treated rats. However, quercetin significantly ameliorated manganese-induced brain lesions in the treated rats. The ability of quercetin to maintain structurally and functionally active brain somewhat similar to the control corroborates the biochemical data on its protective effects against manganese neurotoxicity in the rats.

Taken together, the neuroprotective influence of quercetin on manganese-induced neurotoxicity as evidenced by improvement in the neurobehavioral performance is attributed to its ability to maintain AChE and antioxidant enzymes activities with concomitant suppression of oxidative insult. Quercetin may thus represent an important antioxidant to be considered for ameliorating manganese neurotoxicity in the future.

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

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Erikson KM, Syversen T, Aschner J, Aschner M (2005) Interactions between excessive manganese-exposure and dietary iron-deficiency in neurodegeneration. Environ Toxicol Pharmacol 19:415–421.
2. Takeda A (2003) Manganese action in brain function. Brain Res Rev 41: 79–87.
3. Golub MS, Hogrefe CE, Germann SL, Tran TT, Beard JL, Crinella FM, Lonnerdal B (2005) Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, sow formula or soy formula with added manganese. Neurotoxicol Teratol 27:615–627
4. Aschner M, Guilarte TR, Schneider JS, Zheng W (2007) Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol 221:131–147

5. Erikson KM, Aschner M (2003) Manganese neurotoxicity and glutamate–GABA interaction. Neurochem Int 43:475–480
6. Aschner JL, Aschner M (2005) Nutritional aspect of manganese homeostasis. Mol Asp Med 26:353–362.
7. Bowman AB, Kwakye GF, Herrero Herna´ndez E, Aschner M (2011) Role of manganese in neurodegenerative diseases. J Trace Elem Med Biol 25:191–203
8. Chen P, Chakraborty S, Mukhopadhyay S, Lee E, Paoliello MM, Bowman AB, Aschner M (2015) Manganese homeostasis in the nervous system. J Neurochem 134:601–610
9. US EPA (2003) Health Effects Support Document for Manganese. U.S. Environmental Protection Agency, Office of Water. EPA. EPA-822-R-03-003. Washington, D.C.
10. Dobson AW, Erikson KM, Aschner M (2004) Manganese neurotoxicity. Ann N Y Acad Sci 1012:115–128
11. Horning KJ, Caito SW, Tipps KG, Bowman AB, Aschner M (2015) Manganese is essential for neuronal health. Annu Rev Nutr 35:71–108
12. Chen P, Miah MR, Aschner M (2016) Metals and neurodegeneration [version 1; referees: 3 approved] F1000Research, 5(F1000 Faculty Rev):366
13. Willett WC (2002) Balancing life-style and genomics research for disease prevention. Science 296:695–698
14. Liu RH (2013) Health-promoting components of fruits and vegetables in the diet. Adv Nutr 4: 384S–392 S
15. Chen C, Zhou J, Ji C (2010) Quercetin: a potential drug to reverse multidrug resistance. Life Sci 87:333–338
16. Boots AW, Haenen GRMM, Bast A (2008) Health effects of quercetin: from antioxidant to nutraceutical. Eur J Pharmacol 585:325–337
17. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM, Lines TC (2007) A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 45:2179–2205
18. Garcia-Mediavilla V, Crespo I, Collado PS, Esteller A, Sanchez-Campos S, Tunon MJ, Gonzalez-Gallego J (2007) The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and downregulation of the nuclear factor kappaB pathway in Chang Liver cells. Eur J Pharmacol 557:221–229
19. Barcelos GRM, Grotto D, Serpeloni JM, Angeli JPF, Rocha BA, Souza VVO, Vicentini JT, Emanuelli T, Bastos JK, Antunes LMG, Knasmuller S, Barbosa F Jr (2011) Protective properties of quercetin against DNA damage and oxidative stress induced by methylmercury in rats. Arch Toxicol 85:1151–1157
20. Jiménez-Aliaga K, Bermejo-Bescós P, Benedí J, Martín-Aragón S (2011) Quercetin and rutin exhibit antiamyloidogenic and fibril disaggregating effects in vitro and potent antioxidant activity in APPswe cells. Life Sci 89:939–945
21. Choi SM, Kim BC, Cho YH, Choi KH, Chang J, Park MS, Kim MK, Cho KH, Kim JK (2014) Effects of flavonoid compounds on beta-amyloid-peptide-induced neuronal death in cultured mouse cortical neurons. Chonnam Med J 50:45–51
22. Selvakumar K, Bavithra S, Suganthi M, Benson CS, Elumalai P, Arunkumar R, Krishnamoorthy G, Venkataraman P, Arunakaran J (2012) Protective role of quercetin on PCBs-induced oxidative stress and apoptosis in hippocampus of adult rats. Neurochem Res 37:708–721
23. Yao RQ, Qi DS, YU HL, Liu J, Yang LH, Wu XX (2012) Quercetin attenuates cell apoptosis in focal cerebral ischemia rat brain via activation of BDNF-TrkB-PI3K/Akt signaling pathway. Neurochem Res 37:2777–2786
24. Karuppagounder SS, Madathil SK, Pandey M, Haobam R, Rajamma U, Mohanakumar KP (2013) Quercetin up-regulates mitochondrial complex-I activity to protect against programmed cell death in rotenone model of Parkinson's disease in rats. Neuroscience 236:136–148
25. Sabogal-Guáqueta AM, Muñoz-Manco JI, Ramírez-Pineda J, Lamprea-Rodriguez M, Osorio E, Cardona-Gómez GP (2015) The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 93: 134–145
26. Adedara IA, Rosemberg DB, Souza DO, Kamdem JP, Farombi EO, Aschner M, Rocha JBT (2015) Biochemical and behavioral deficits in lobster cockroach Nauphoeta cinerea model of methylmercury exposure. Toxicol Res 4: 442–451
27. Adedara IA, Rosemberg DB, de Souza D, Farombi EO, Aschner M, Souza DO, Rocha JBT (2016) Neurobehavioral and biochemical changes in Nauphoeta cinerea following dietary exposure to chlorpyrifos. Pest Biochem Physiol 130: 22–30
28. Gonçalves JF, Fiorenza AM, Spanevello RM, Mazzanti CM, Bochi GV, Antes FG, Stefanello N, Rubin MA, Dressler VL, Morsch VM, Schetinger MR (2010) N-acetylcysteine prevents memory deficits, the decrease in acetylcholinesterase activity and oxidative stress in rats exposed to cadmium. Chem Biol Interact 186:53–60
29. Igado OO, Olopade JO, Adesida A, Aina OO, Farombi EO (2012) Morphological and biochemical investigation into the possible neuroprotective effects of kolaviron (*Garcinia kola* bioflavonoid) on the brains of rats exposed to vanadium. Drug Chem Toxicol 35:371–380
30. Adedara IA, Abolaji AO, Idris UF, Olabiyi BF, Onibiyo EM, Ojuade TD, Farombi EO (2017) Neuroprotective influence of taurine on fluoride-induced biochemical and behavioral deficits in rats. Chem Biol Interact 261:1–10
31. Ponnapakkam TP, Bailey KS, Graves KA, Iszard MB (2003) Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. Reprod Toxicol 17:547–551
32. Nabavi SF, Nabavi SM, Latifi AM, Mirzaei M, Habtemariam S, Moghaddam AH (2012) Mitigating role of quercetin against sodium fluoride-induced oxidative stress in the rat brain. Pharm Biol 50:1380–1383
33. Mintz M, Russig H, Lacroix L, Feldon J (2005) Sharing of the home base: a social test in rats. Behav Pharmacol 16: 227–236
34. Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254
35. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95
36. Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
37. Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105:121–126
38. Wolff SP (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. Methods Enzymol 233:182–189
39. Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO (2000) Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a Garcinia kola seed extract. Food Chem Toxicol 38:535–541
40. Bancroft JD, Gamble M (2008). Theory and practice of histology techniques, 6th edn. Churchill Livingstone Elsevier, London, pp 83–134.
41. Dodd CA, Ward DL, Klein BG (2005) Basal Ganglia accumulation and motor assessment following manganese chloride exposure in the C57BL/6 mouse. Int J Toxicol 24:389–397
42. Moreno JA, Yeomans EC, Streifel KM, Brattin BL, Taylor RJ, Tjalkens RB (2009) Age-dependent susceptibility to manganese-induced neurological dysfunction. Toxicol Sci 112:394–404

43. Tartaglione AM, Armida M, Potenza RL, Pezzola A, Popoli P, Calamandrei G (2016) Aberrant self-grooming as early marker of motor dysfunction in a rat model of Huntington's disease. Behav Brain Res 313:53–57
44. Schneider JS, Decamp E, Koser AJ, Fritz S, Gonczi H, Syversen T, Guilarte TR (2006) Effects of chronic manganese exposure on cognitive and motor functioning in non-human primates. Brain Res 1118:222–231
45. Gorny JH, Gorny B, Wallace DG, Whishaw IQ (2002) Fimbria-fornix lesions disrupt the dead reckoning (homing) component of exploratory behavior in mice. Learning Memory 9:387–394
46. Eilam G, Golani I (1989) Home base behaviour of rats (Rattus norvegicus) exploring a novel environment. Behav Brain Res 34:199–211
47. Ballard CG, Greig NH, Guillozet-Bongaarts AL, Enz A, Darvesh S (2005) Cholinesterases: roles in the brain during health and disease. Curr Alzheimer Res 2:307–318
48. Chtourou Y, Fetoui H, Garoui EM, Zeghal N (2012) Improvement of cerebellum redox states and cholinergic functions contribute to the beneficial effects of silymarin against manganese-induced neurotoxicity. Neurochem Res 37:469–479
49. Lebda MA, El-Newwshy MS, El-Sayed YS (2012) Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene. Neurotoxicology 33:98–104
50. Milatovic D, Zaja-Milatovic S, Gupta RC, Yu Y, Aschner M (2009) Oxidative damage and neurodegeneration in manganese-induced neurotoxicity. Toxicol Appl Pharmacol 240:219–225
51. Martinez-Finley EJ, Gavin CE, Aschner M, Gunter TE (2013) Manganese neurotoxicity and the role of reactive oxygen species. Free Radic Biol Med 62:65–75
52. Ebokaiwe AP, Adedara IA, Owoeye O, Farombi EO (2013) Neurotoxicity of Nigerian bonny light crude oil in rats. Drug Chem Toxicol 36:187–195
53. Chtourou Y, Fetoui H, Garoui EM, Boudawara T, Zeghal N (2012) Improvement of cerebellum redox states and cholinergic functions contribute to the beneficial effects of silymarin against manganese-induced neurotoxicity. Neurochem Res 37:468–479
54. Latronico T, Brana MT, Merra E, Anna Fasano A, Di Bari G, Casalino E, Liuzzi GM (2013) Impact of manganese neurotoxicity on MMP-9 production and superoxide dismutase activity in rat primary astrocytes. Effect of resveratrol and therapeutical implications for the treatment of CNS diseases. Toxicol Sci 135:218–228
55. Lee JC, Kim J, Park JK, Chung GH, Jang YS (2003) The antioxidant, rather than prooxidant, activities of quercetin on normal cells: quercetin protects mouse thymocytes from glucose oxidase-mediated apoptosis. Exp Cell Res 291:386–397