Quercetin improves motor deficit in Manganese-induced Cerebellar toxicity in adult mice

Abstract

Introduction: Manganese (Mn) is an essential trace element needed for normal development and physiological processes in the body. However, chronic exposures or consumption of Mn can cause neurotoxicity in humans to affect balance and motor coordination. This present study is aimed at investigating the efficacy of quercetin on manganese-induced cerebellar damage of an adult mice.

Materials and methods: Forty (40) healthy BALB/c male mice weighing between 20-25g were used for the study. They were randomly grouped into five different groups; Group 1 received normal saline (C), Group 2 received 40mg/ kg per body weight of Manganese (Mn), Group 3 received 50mg/ kg per body weight of quercetin (Q), Group 4 served as an intervention group (POST) while Group 5 received both Manganese and quercetin concurrently (CONC). Behavioural, histological, immunohistochemical and biochemical analysis were carried after the study to validate our hypothesis that quercetin is neuroprotective.

Results: Results from behavioural analysis of motor coordination with parallel bar and suspension grip test revealed the effects of quercetin on manganese-induced damage on the cerebellum of adult mice. Manganese exposed mice exhibited increased latency of turn (LOT) when compared to the control group. There was a significant decrease in the LOT of Quercetin, concurrent and post treated groups when compared with manganese exposed group. However, Control (saline) group exhibited a decrease in time of turn compared to manganese exposed group. When using Grip strength test, manganese exposed mice exhibited decrease grip strength when compared to the control group. There was a significant increase in Quercetin grip strength when compared with manganese exposed group. However, Control (saline) group exhibited a significant increase in grip strength compared to manganese exposed group. Histological results also revealed that there was

no disruption neurons in the granular, molecular and purkinje layer with purkinje cells. Immunohistochemical results also showed that the normal control and quercetin-treated mice showed no expression of tumour necrosis factor alpha (TNF-α) when compared to the manganese-exposed mice with shrunken morphology. Markers of oxidative stress (SOD and MDA) was significant different in the experimental animals (manganese, post treatment and concurrent groups) when compared to the control and quercetin treated mice.

Conclusion: This study revealed that quercetin may have an antioxidant effects on the oxidative stress and neurodegeneration in the cerebellum thereby ameliorating the exhibited abnormal motor coordination caused by prolonged exposure to manganese.

Keywords; Parkinson disease, neurodegeneration, quercetin, manganese, oxidative stress

INTRODUCTION

Manganese (Mn) is a naturally occurring trace element essential for neuronal health associated with brain development (Takeda, 2003; Horning *et al.,* 2015) and this is expected to enter the body either by inhalation or ingestion. However, excessive accumulation in some brain areas such as substantia nigra (SN), the globus pallidus (GP) and the striatum has been recorded to produce neurotoxic effects that can lead to a neurodegenerative challenges resulting in a characteristic neurological disorder known as manganism akin to Parkinsonism (Lucchini, 1991; Rachel *et al.,*2004; Olanow, 2004) and this makes central nervous system especially the brain to be more susceptible to Mn toxicity leading to a certain morphological and neurobehavioural abnormalities involving motor control and movement (Racette *et al.*, 2001; Aschner and Aschner, 2005; Santamaria *et al.,* 2007; Burton and Guilarte, 2009). Patients suffering from manganism are known to exhibit a signature biphasic model of physical decline comprising an initial phase of

neurological deficits which are followed by motor deficits (Calne *et al.*, 1994; Olanow, 2004; Dobson *et al.*, 2004). Some researchers have also reported impaired fertility and libido alteration in workers afflicted with clinically identifiable symptoms of manganism associated to occupational exposure to Mn (Emara, 1971; Cherry, *et al.*, 2001; Neal and Guilarte, 2012) and suggested that impaired sexual function in man may be one of the earliest clinical manifestations of Mn toxicity.

The cerebellum is the largest part of the hindbrain made up of an outer cerebellar cortex containing arrays of nerve cell arranged within three layers and an inner medulla (Singh, 1998) to contribute to the preparatory activities of the motor neocortex (Ransdell *et al.*, 2018; Chabrol *et al.*, 2019).

Quercetin is one of the most abundant dietary polyphenolic compound commonly found in fruit and vegetables with potentially beneficial effects on health owing to its ability to reduce inflammation due to its antioxidant properties (Formica and Regelson, 1995; Davide *et al*., 2016) which might also work against degenerative brain disorder caused by Mn toxicity (Raman *et al*., 2019). This paper seeks to investigate the efficacy of quercetin as a supplemental therapy on manganese-induced damage in the cerebellar cortex of the adult mice.

Materials and Methods

Forty (40) healthy BALB/c male mice weighing about 22-30 g were used for this study. The mice were randomly assigned into 5 groups. They were housed in well ventilated cages, kept and maintained under laboratory condition of temperature, humidity and light. They were allowed to acclimatize for a period of two weeks and fed with broiler finisher pelletized meal and were also given water *ad libitum.* At the end of the acclimatization period, the mice were weighed and grouped into five different groups ; Group 1 was the control group and was given normal saline (C), Group 2 received 40mg per kg per body weight of Manganese only (Mn), intraperitoneally,

Group 3 received 50mg per kg per body weight of quercetin (Q), Group 4 received Manganese followed by quercetin as a post treatment group / Intervention (POST) and Group 5 received quercetin and Manganese concurrently (CONC). All experimental procedures were conducted in accordance with Afe Babalola University Ethical committee with protocol number AB/EC/19/10/51 in line with the National institute of Health Guide for care and use of Laboratory animals (NIH, 1985).

Behavioural study

The behavioural test was carried out in a closed area with proper illumination and sound control behavioural room in the animal house unit in Afe Babalola University. The animals were subjected to grip suspension and parallel bar test for motor coordination and locomotive activities.

Animal sacrifice

The mice that were set out for histological analysis were sacrificed by anaesthetized using ketamine followed by intracardiac perfusion fixation using sterile saline followed by 4% paraformaldehyde. The brain extracted from the mice were put in a specimen bottle containing 4% paraformaldehyde and the region of cerebellum was grossed for histological and immunohistochemical analysis. The remaining mice set out for biochemical analysis were sacrificed through cervical dislocation. The brain was harvested and kept in a specimen bottle and placed in a cooling chamber.

Biochemical analysis

The brain was carefully dissected, washed in ice cold saline, weighed and thereafter homogenized in phosphate buffer (pH 7.4) solution. The homogenate was centrifuged for 10 min at 5000x g to yield a pellet that was discarded and a low speed supernatant was kept for subsequent analysis.

The supernatant so obtained from the homogenized brain was used to assay for the markers of oxidative stress using Superoxide dismutase (SOD) and lipid peroxidation in form of Malondialdehyde (MDA) (Linsley *et al.,* 2005).

Statistical Analysis

Statistical analyses were done using one-way ANOVA (analysis of variance), differences between groups were evaluated using Newman-keuls for post hoc tests with the aid of GraphPad Prism V.5.0 (GraphPad Software, La Jolla California USA, www.graphpad.com). The outcomes of the statistical analysis were represented in graphs and bar charts with error bar representing the mean ± SEM (standard error of the mean). The significant level was set at $P < 0.05$.

RESULTS

Biochemical Assay

Lipid Peroxidation (MDA)

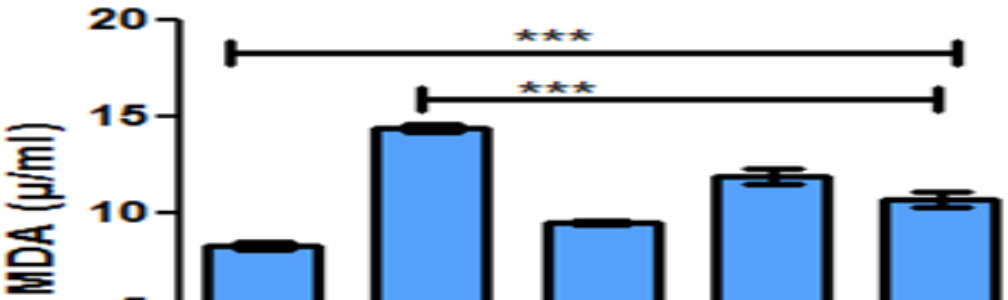

Figure 1: Graph representing the concentration of MDA (μ/ml) in the brain tissue. There is statistically significant difference in the control, Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); Concurrent (CONC); Malondialdehyde (MDA) ***$P < 0.001$.

Superoxide Dismutase

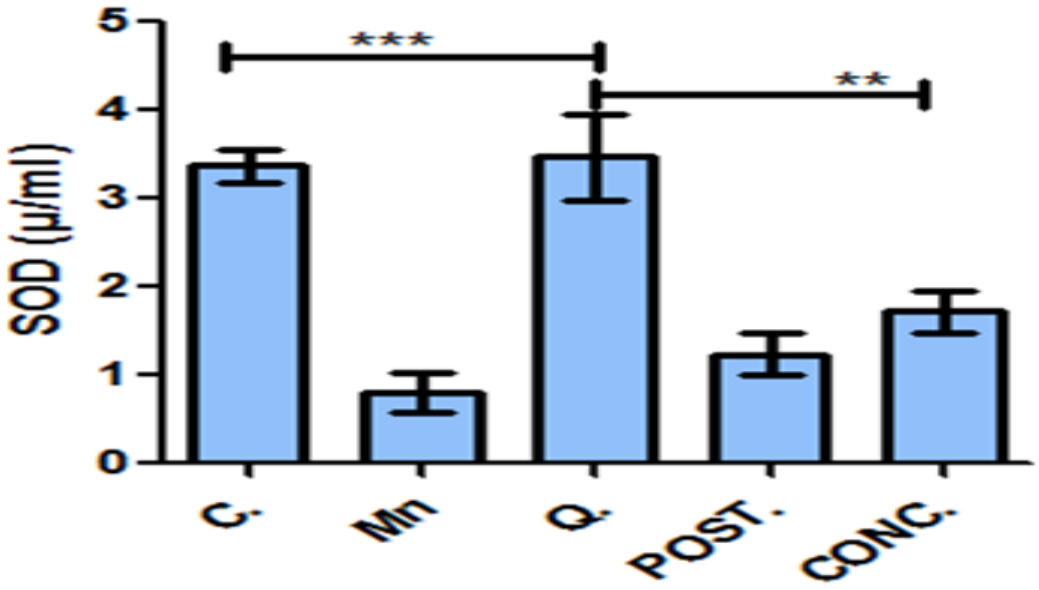

Figure 2: Graph representing the concentration of SOD (µ/ml) in the brain tissue. There is statistically significant difference in the control, Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); Concurrent (CONC); Superoxide Dismutase (SOD) ***P*<0.01, ***P<0.001.

BEHAVIORAL STUDIES

Motor coordination was assessed in the experimental animals using parallel bar and grip test.

Parallel bar test

In this test a significant increase in Latency of turn (LOT) scores were considered as abnormal motor coordination when the treatment groups were compared against control. Manganese exposed mice exhibited increased latency of turn (time to turn) and total time spent during the parallel bar test when compared to the control group. There was a significant decrease in the LOT of Quercetin, concurrent and post treated groups when compared with manganese exposed group.

However, Control (saline) group exhibited a decrease in time of turn compared to manganese exposed group (*p<0.05).

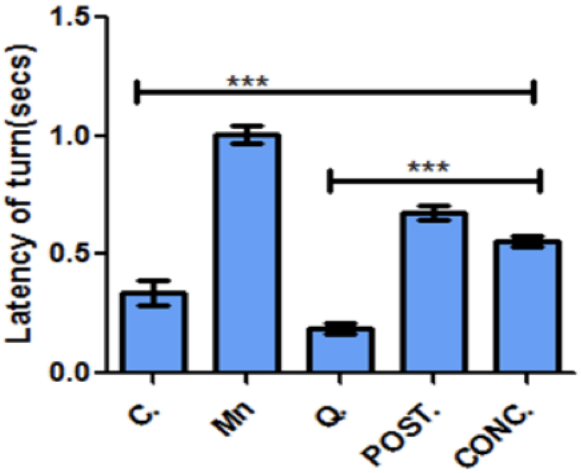

Figure 3: Graph showing latency of turn in Parallel bar test. Abnormal motor coordination were significantly reduced by the activity of quercetin.

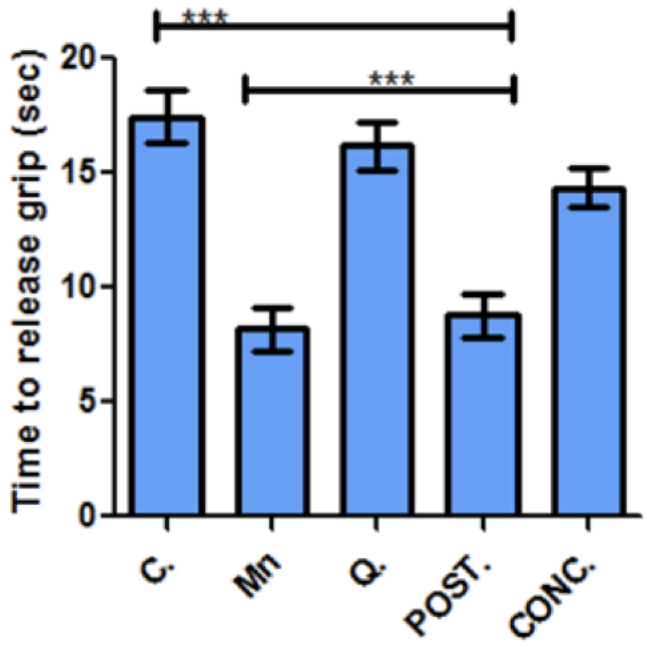

Figure 4: Graph showing the grip test of the experimental animals. There is statistically significant difference in the control group when compared with the Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); concurrent (CONC), ***$P < 0.001$.

Histological Analysis

Histological observation made at magnification of x800 with the aid of OPTO-EDU Image view research microscope to understand the histoarchitectural integrity of cerebellar cortex (H& E stain). Normal distribution of cells was observed with three defined layers in control and quercetin group but there was a remarkable neuronal disruption of the purkinje cells due to neurotoxic effects due to neurotoxic effect of the manganese (Figure 5).

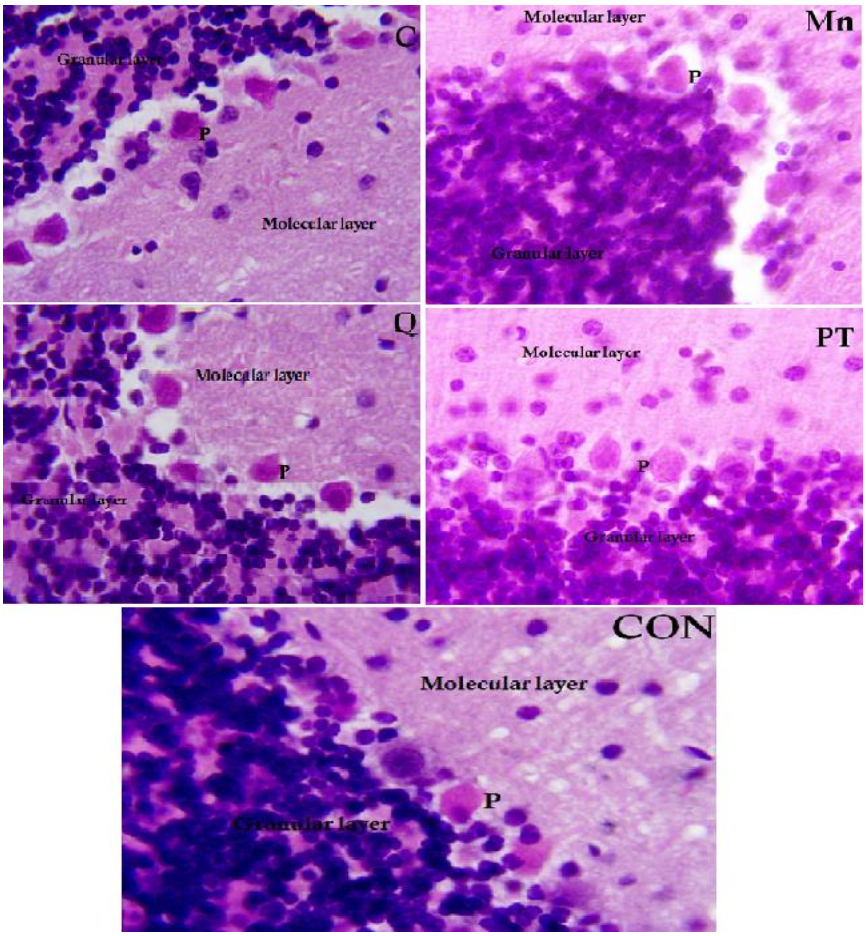

Figure 5: Photomicrograph of the cerebellar section of the manganese-induced mice with quercetin. Stained with H&E. Mg x800.

IMMUNOHISTOCHEMISTRY

Immunohistochemical studies on Cerebellar section evaluating the ameliorative roles of Quercetin following manganese chloride induced neurotoxicity.

TNF-α

From the slide below, control and quercetin group reviewed no expression of tumour necrosis factor alpha; expression of TNF-alpha and shrunken morphology was pronounced in the $Mncl_2$ group when compared with the control; Post treatment group revealed little expression of tumour necrosis factor alpha when compared to the $Mncl_2$, while concurrent group revealed little or no expression of TNF-alpha.

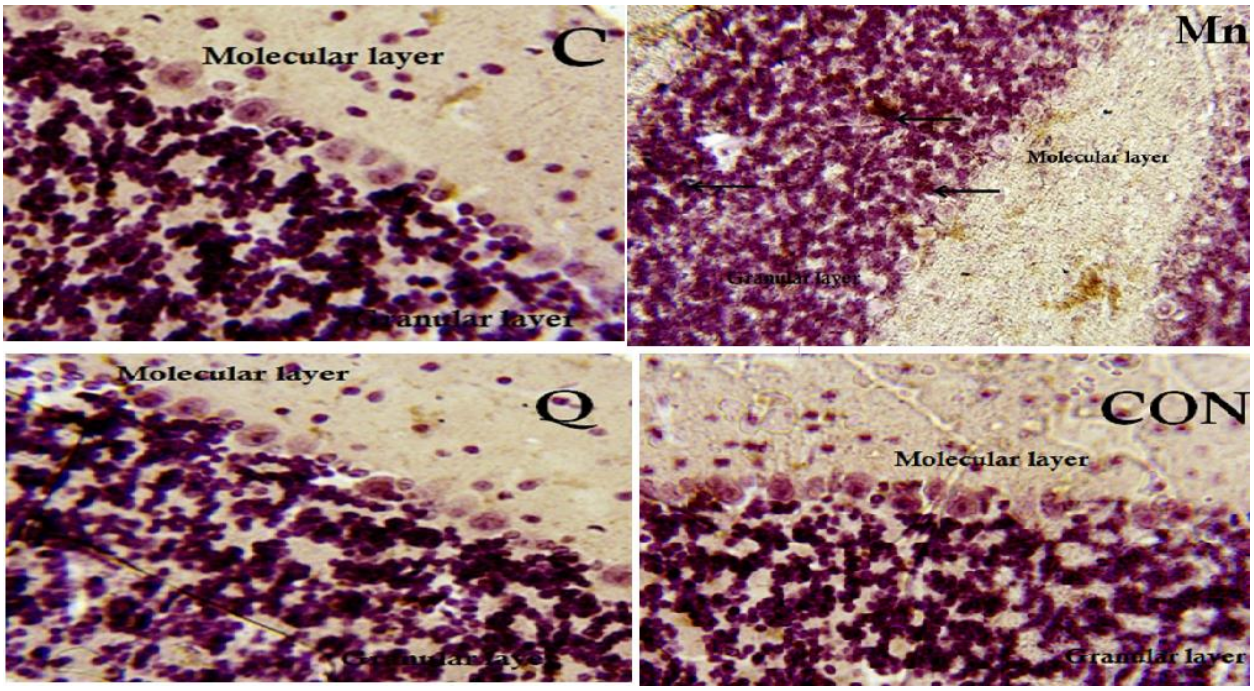

Figure 6: Photomicrograph showing the cerebellum TNF-α positive cell of mice exposed to

manganese chloride and quercetin. The apoptotic cells were characterized by dark brown precipitate, brown-stained nuclei and shrunken morphology. Mag. X800

DISCUSSION

The study was carried out to investigate the effect of quercetin on manganese induced damage in the cerebellum. Manganese is essential in neuronal health but its excess accumulation has been known to be implicated in the brain as a neurotoxin affecting motor neocortex which a characteristic neurological disorder known Manganism. Quercetin is a flavonoid naturally found in fruits and vegetables with antioxidant, anti-inflammatory and neuroprotective properties (Muhammed *et al.*, 2016). The results obtained from the morphological changes in the brain and body weights clearly showed significant difference when manganese treated mice were compared with controls with no eye colour changes. The manganese treated mice showed a significant reduction of body weight which was not experience in all groups that received quercetin and was corroborated by worked done by Pappas et al., (1997). Reduced brain and cerebellar weights in the manganese treated mice might be due to pathologic changes as a result of neurotoxic effects of manganese leading to loss of cellular architectural design as well as vacuolations of the brain section in mice treated with Mn that could be responsible for cerebellar dysfunction. Exposure of some Chilean miners to manganese concentrations in air has been confirmed by research done by Ansola et al., 1994 to have resulted in neurological disorders. Accumulation of Manganese often lead to changes in neuronal degeneration and brain biochemistry (Tarale et al., 2016; Taylor et al., 2019).

In the biochemical assay, Malonaldehyde (MDA) test was done to evaluate the level of lipids peroxidation as a marker of oxidative stress in cellular activities. Lipid peroxidation in biological membranes is considered as one of the major mechanisms of cell injuries in aerobic or working

organisms, subjected to oxidative stress. the chain reaction of lipids peroxidation increases the number of free radicals in the cells which lead to further peroxidation (Ooh and Rocha, 2007).

It was also observed from our biochemical results that a significant differences in the experimented animals in the treatment groups, (manganese, quercetin, post treatment and concurrent groups when compared to the normal control animals and a very statistically significant difference showed changes in the markers of oxidative stress (MDA and SOD) from figure 1and 2 indicating that quercetin attenuate manganese-induced cerebellar toxicity in mice via inhibition of oxidative stress which supports work done by Abubakar et al., (2019).

Results from behavioural test for motor coordination of the animals with parallel bar and Grip strength tests also support the efficacy of the quercetin in mice exposed to Manganese. There was a significant increase in Latency of turn (LOT) scores in Parallel bar test and these were considered as an abnormal motor coordination of the manganese treated mice compared with normal control and quercetin treated mice (Figure 3). Manganese exposed mice exhibited increased latency of turn (time to turn) and total time spent during the parallel bar test when compared to the control group. A significant decrease in the LOT of Quercetin, concurrent and post treated mice observed when compared with manganese exposed mice only. When using Grip strength test, a significant decrease in time to release grip were considered as abnormal motor coordination when the treatment groups were compared against control. Manganese exposed mice exhibited decrease grip strength when compared to the control group. There was a significant increase in Quercetin grip strength, concurrent and post treated groups when compared with manganese exposed group (Figure 4). However, control group exhibited a significant increase in grip strength when compared to manganese treated mice and this revealed efficacy of quercetin to promote behavioural deficiency caused by the Manganese (Chakraborty *et al.,* 2014).

Histological analysis showed that the cerebellar cortex of the groups with quercetin revealed a well-defined 3 layers with normal distribution of the neuronal cells but there was a disruption in the layers and cells organization with manganese treated mice with clustered and denseness in orientation when compared to the control group (Figure 5) and this was supported by VanBogaert and Dallemagne (1945) who worked on atrophy of the cerebellar cortex on monkey (Schilling et al., 2019; Qi et al., 2017). The efficacy of quercetin was more pronounced in the co-treated animals with protective effect on the neuronal density and neuronal branching morphogenesis **(**Bournival et al., 2012; Ghosh et al., 2013; Chakraborty et al., 2014; Ay et al., 2017**).** According to the research done Entaz *et al*., (2017), administration of quercetin in exposed mice with intoxicants shows improvement of histological alterations in terms of alignment and number of the purkinje cells within the 3 layers of the cerebellar cortex (Fafure *et al.,* 2018).

Immunohistochemistry results revealed that the level of apoptosis occur in the granular and purkinje cell layers of the cerebellar cortex in all the experimental animals but it was more pronounced in the Manganese treated mice only and reduced drastically with the administration of quercetin when compared with normal control mice. DNA fragmentation is said to be the hallmark of apoptosis according to Iglesias-Guimarais et al., (2003), Therefore, tunnel assay was carried out to detect DNA fragmentation and level of apoptosis in this study. This Immunohistochemistry results showed that the normal control and quercetin treated mice little or no expression of tumour necrosis factor alpha while in manganese treated mice revealed a remarkable expression of TNF-alpha and shrunken morphology of the cerebellar cells as seen in Figure 5 and 6.

Conclusion: This study revealed that quercetin may have an antioxidant effects on the oxidative stress and neurodegeneration in the cerebellum thereby ameliorating the exhibited abnormal motor coordination caused by prolonged exposure to manganese.

REFERENCES

1) Adebayo EA, Ishola OR, Taiwo OS, Majolagbe ON, Adekeye BT (2009). Evaluations of the methanolic extract of Ficus exas- perata stem, bark, leaf and root for phytochemical analysis and antimicrobial activities. Afr. J. Plant Sci. 3 (12): 283-287.

2) Aschner JL, Aschner M (2005). Nutritional QW aspect of manganese homeostasis. *Molecular Aspect of Medicine.* 26:4-5

3) Calne DB, Chu NS, Huang CC, Lu CS, Olanow W *(1994).* Manganism and idiopathic Parkinsonism: similarities and differences. *Neurology ;44(9):1583-6*

4) Callan AC, Hinwood AL (2011). Mechanisms of lead and manganese neurotoxicity. *Rev Environmental Health;* 26:13.

5) Carlsson CM, Mhyre TR, Boyz JT, Hamill RW, Maguire-Zeis KA (2015). Parkinson'sdisease. *National center for Biotechnological Information* 65:389-455.

6) Carroll W, Bhidayasiri R (2009). Parkinsonism *"International Neurology*" *"A Clinical Approach. Blackwell Publishing Ltd"* 24-10.

7) Cherry N, Labreche F, Collins J, Tulandi T (2001). Occupational exposure to solvents and male infertility. Occup Environ Med. 58(10): 635–640. doi: 10.1136/oem.58.10.635

8) Christine CW, Aminoff MJ (2004). Clinical differentiation of parkinsonian syndromes: prognostic and therapeutic relevance. *Am J Me*d 117(6):412-419.

9) Cosgrove KP (2007). Evolving knowledge of sex difference in brain structure, function, chemistry. *Biol psychiastry*. 62; 847

10) Chabrol FP, Blot A, Mrsic-Flogel TD (2019). Cerebellar Contribution to Preparatory Activity in Motor Neocortex. Neuron. 103(3): 506–519.e4. doi: 10.1016/j.neuron.2019.05.022

11) Cotzias GC, Miller ST, Papavasiliou PS, Tang LC (1976). Interactions between manganese and brain dopamine. *Med. Clin. North Am.* 729–738.

12) Dobson AW, Erikson KM, Aschner M (2004). Manganese neurotoxicity. *Ann NY Acad Sci 1012:115-28.*

13) Barmore (2019). Movement Disorders Fellow at the University of Florida. *a Parkinson's Foundation Centre of Excellence 224-18*

14) Avila DS, Aschner M(2016). Encyclopedia of Food and Health, *Modern Pharmacology Publication* . 117-151 35

15) Davidsson L, Cederblad A, Lonnerdal B, Sandstrom B (1991). The effect of individual dietary components on manganese absorption in humans. *Am. J. Clin. Nutr 54* .1065–1070

16) Davis CD, Zech L, Greger JL (1993). Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses, *Proc. Soc. Exp. Biol. Med 202,* 103–108

17) Dorman DC, Struve MF, James RA, McManus BE, Marshall MW, Wong BA,(2001). Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. *Toxicology Science*. 242–251.

18) Neal P, Guilarte TR (2012). Mechanisms of Heavy Metal Neurotoxicity: Lead and Manganese. J Drug Metab Toxicol S5DOI: 10.4172/2157-7609.S5-002

19) Formica JV, Regelson W (1995). Review of the biology of quercetin and related bioflavonoid. *Food and Chemical Toxicology.* ***33*** *(12):1061–80*

20) Guilarte TR, Burton NC, McGlothan JL, Verina T, Zhou Y, Alexander M, (2008). Impairment of nigrostriatal dopamine neurotransmission by manganese is mediated by pre-synaptic mechanism(s): implications to manganese induced parkinsonism. *J Neurochem* 107:1236–1247.

21) Andruska KM, Racette AB (2015). Neuromythology of Manganism. *CurrEpidemiol.* 143-148.

22) Horning KY, Caito SW, Tipps KG, Bowman AB, Aschner M (2019). **Manganese** Is Essential for Neuronal Health. Annu Rev Nutr. 2015; 35: 71–108. doi: 10.1146/annurev-nutr-071714-034419

23) Justesen U, Knuthsen P (2001). Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. *Food Chemistry. **73** (2): 245–50.*

24) Lindsay MB (2018). Central nervous system and sensory motor pathway "*Anatomy and physiology Textbook* 250-275

*25) M*itchell AE, Hong YJ, Koh E, Barrett DM, Bryant DE, Denison RF, Kaffka S (2007). "Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes*". Journal of Agricultural and Food Chemistry. **55** (15): 6154–9.*

26) Luk EE, Culotta VC (2001). Manganese superoxide dismutase in Saccharomyces cerevisiae acquires its metal co-factor through a pathway involving the Nramp metal transporter,''. *J Biol Chem. ;276(50):47556–62.*

27) Malecki EA, Devenyi AG, Beard JL, Connor JR (1999) 'Existing and emerging mechanisms for transport of iron and manganese to the brain. *J Neurosci Res.*56(2):113–22.

28) Mims MP, Guan Y, Pospisilova D, Priwitzerova M, Indrak K, Ponka P, Divoky V, Prchal JT (2005) "Identification of a human mutation of DMT1 in a patient with microcytic anemia and iron overload. Blood.*;105(3):1337–42.*

29) Mitchell AE, Hong YJ, Koh E, Barrett DM, Bryant DE, Denison RF, Kaffka S (2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes*". Journal of Agricultural and Food Chemistry. **55** (15): 6154–9.*

30) Goldstein JM, Seidman LJ, Horton NJ, Makris N, Kennedy DN, Caviness VS, Faraone SV, Tsuang MT(2001). Normal Sexual Dimorphism of the Adult Human Brain Assessed by In Vivo Magnetic Resonance Imaging. *Cerebral Cortex*,11(6)490–497 https://doi.org/10.1093/cercor/11.6.490

31) Neal AP, Guilarte TR (2013) "Mechanisms of lead and manganese neurotoxicity*". Toxicol Res (Camb)*. 2013;2(2):99-114.

32) Nevo Y. (2007). Directed mutagenesis investigation of coupling properties of metal ion transport *by DCT1*. *Biochim Biophys Acta*;1778(1):334–41.

33) Olanow CW (2004). "Manganese-induced parkinsonism and Parkinson's disease. *Ann N Y Academy Sci. 1012:209–23.*

34) Orgad S, Nelson H, Segal D, Nelson N (1998). Metal ions suppress the abnormal taste behavior of the Drosophila mutant malvolio. *J Exp Biol.; 201*(Pt 1):115–20.

35) Post JE (1996). Manganese oxide minerals: crystal structures and economic and environmental significance. *Proc Natl Acad Sci*; 96(7):3447–54

36) Ransdell JL, Nerbonne JM (2018). Voltage-Gated Sodium Currents in Cerebellar Purkinje Neurons: Functional and Molecular Diversity. Cell Mol Life Sci. 75(19): 3495–3505. doi: 10.1007/s00018-018-2868-y

29) Slimestad R, Fossen T, Vågen IM (2007)."Onions: a source of unique dietary flavanoid". *Journal of Agricultural and Food Chemistry*. ***55*** *(25): 10067–*

30) Takeda A(2003). Manganese action in brain function. *Brain Res Brain Res Rev*. 2003;41(1):79-87.

31) Winkel-Shirley B (2001). "Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology"*Plant Physiology*. ***126*** *(2): 485–93.*

32) Iglesias-Guimarais V, Gil-Guiñon E, Sánchez-Osuna M, Casanelles E, García-Belinchón M, Comella JX, Yuste VJ (2013). Chromatin Collapse during Caspase-dependent Apoptotic Cell Death Requires DNA Fragmentation Factor, 40-kDa Subunit-/Caspase-activated Deoxyribonuclease-mediated 3′-OH Single-strand **DNA** Breaks. *J Biol Chem.* 288(13): 9200–9215. doi: 10.1074/jbc.M112.411371

33) Taylor CA, Hutchens S, Liu C, Jursa T, Shawlot W, Aschner M, Smith DR, Mukhopadhyay S (2019). SLC30A10 transporter in the digestive system regulates brain manganese under basal conditions while brain SLC30A10 protects against neurotoxicity. *J Biol Chem.* 294(6): 1860–1876. doi: 10.1074/jbc.RA118.005628

34) Tarale P, Chakrabarti T, Sivanesan S, Naoghare P, Bafana A, Krishnamurthi K (2016). Potential Role of Epigenetic Mechanism in Manganese Induced Neurotoxicity. *Biomed Res Int.* doi: 10.1155/2016/2548792

35) Bournival J, Plouffe M, Renaud J, Provencher C, Martinoli M (2012). Quercetin and Sesamin Protect Dopaminergic Cells from MPP^+-Induced Neuroinflammation in a Microglial (N9)-**Neuronal** (PC12) Coculture System . *Oxid Med Cell Longev*. doi: 10.1155/2012/921941

36) Ghosh A, Sarkar S, Mandal AK, Das N (2013). Neuroprotective Role of Nanoencapsulated Quercetin in Combating Ischemia-Reperfusion Induced Neuronal Damage in Young and Aged Rats. *PLoS One*. 8(4): e57735. doi: 10.1371/journal.pone.0057735

37) Chakraborty J, Singh R, Dutta D, Naskar A, Rajamma U, Mohanakumar KP (2014). Quercetin Improves Behavioral Deficiencies, Restores Astrocytes and Microglia, and Reduces Serotonin Metabolism in 3-Nitropropionic Acid-Induced Rat Model of Huntington's Disease. *CNS Neurosci Ther.* 20(1): 10–19. doi: 10.1111/cns.12189

38) Ay M, Luo J, Langley M, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2017). Molecular Mechanisms Underlying Protective Effects of Quercetin Against Mitochondrial Dysfunction and Progressive Dopaminergic Neurodegeneration in Cell Culture and

MitoPark Transgenic Mouse Models of Parkinson's Disease. *J Neurochem.* 141(5): 766–782. doi: 10.1111/jnc.14033

39) Schilling K, Gao Y, Christian M, Janve V, Stepniewska I, Landman BA, Anderson AW (2020). A Web-Based Atlas Combining MRI and Histology of the Squirrel Monkey Brain. *Neuroinformatics.* 17(1): 131–145. doi: 10.1007/s12021-018-9391-z

40) Ye Q, Park JE, Gugnani K, Betharia S, Pino-Figueroa A, Kim J (2017). Influence of iron metabolism on manganese transport and toxicity. *Metallomics.* 9(8): 1028–1046. doi: 10.1039/c7mt00079k

41) Abubakar K, Mailafiya MM, Danmaigoro A, Chiroma SM, Abdul Rahim EB, Md Zuki Abu Bakar (2019). Curcumin Attenuates Lead-Induced Cerebellar Toxicity in Rats via Chelating Activity and Inhibition of Oxidative Stress. *Biomolecules*. 9(9): 453 doi: 10.3390/biom9090453