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QUERCETIN IMPROVES MOTOR DEFICIT IN MANGANESE-INDUCED CEREBELLAR TOXICITY IN ADULT MICE

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ABSTRACT

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. Quercetin is a polyphenolic flavonoid contained in some fruits and its association with the management of motor neurodegenerative disorders is yet to be fully understood. Hence, this is present study is aimed at investigating the efficacy of Quercetin on manganese-induced cerebellar damage of an adult mice. Forty (40) healthy BALB/c male mice weighing between 20-25grandomly selected into five group were used for the study. Behavioural, histological, immunohistochemical and biochemical analysis were carried to validate our hypothesis that Quercetin is neuroprotective. Manganese exposed mice exhibited increased latency of turn (LOT) when compared to the control group in parallel bar test. There was a significant decrease in the LOT in all groups that received Quercetin when compared with manganese exposed group. Also, when using grip strength test, manganese exposed mice exhibited decrease grip strength when compared to the control group and a significant increase in Quercetin grip strength when compared with manganese exposed group. Histological results also revealed that there were little or no disrupted neurons in the granular, molecular and purkinje layer of the cerebellum. 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 were significantly different in the experimental animals when compared to the control and Quercetin treated mice. This study therefore 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: Neurodegeneration, Quercetin, manganese, oxidative stress

INTRODUCTION

Manganese (Mn) is a naturally occurring trace element essential for neuronal healthassociated with brain developmentand this is expected to enter the body either by inhalation or ingestion (Horning et al. 2015, Adekeye et al. 2018a). The daily intakeof Mn has been set by the National Academy of Sciences (NAS) at 2.3 mg per day for men and 1.8 mg per day for women (Gutiérrez-Ravelo et al. 2020) but when the homeostatic regulation at the level of absorption or excretion of Mn is disrupted, elevated Mn concentrations in the blood circulation can cause neurotoxicity in humans. Notably, workers exposed to high airborne Mn levels are at elevated risk of developing a Parkinson's disease (PD)-like neurological disorder known as manganism (Cersosimo and Koller, 2006; Fafure et al. 2018). However, excessive accumulation in some brain areas such as substantia nigra (SN), the globus pallidus (GP) and the striatum has been recorded to produce neurotoxiceffects that can lead to a neurodegenerative challenges (Andruska and Racette, 2015; Fafure et al. 2018) 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 (Aschner and

Aschner, 2005; 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 (Dobson *et al.* 2004; Parmalee and Aschner, 2016). 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 (Neal and Guilarte, 2013) 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 hind brain made up of an outer cerebellar cortex containing arrays of nerve cell arranged within three layers and an inner medulla (Singh, 2006) 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 compounds commonly found in fruit and vegetables with potentially beneficial effects on health owing to its ability to reduceinflammation due to its antioxidant properties which might also work against degenerative brain disorder caused by Mn toxicity (Spagnuolo et al. 2018; Kang et al. 2020). 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 4received 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). Motor coordination and locomotive activities in the experimental animals were estimated by parallel bar and grip suspension test as previously described by Adekeye et al. (2018b).

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 (Adekeye et al. 2020). The brain extracted from the mice wereput in a specimen bottle containing 4% paraformaldehyde and the region of cerebellum was grossed for histologicaland immunohistochemical analysis. The remaining mice set out for biochemical analysis were sacrificed through cervical dislocation (Adekeye et al. 2018b). 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 (pH7.4) 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)with an established procedure used by Sharif et al. (2019).

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 Graph Pad Prism V.5.0 (Graph Pad 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 revealed a statistically significant decrease in Malondialdehyde (MDA) activities of the Quercetin treated animal when compared to manganese and other treatment groups (***p<0.001). The level of oxidative stress with respect to MDA was high with Manganese but Quercetin treatment was considerably lower when compared to the control group.

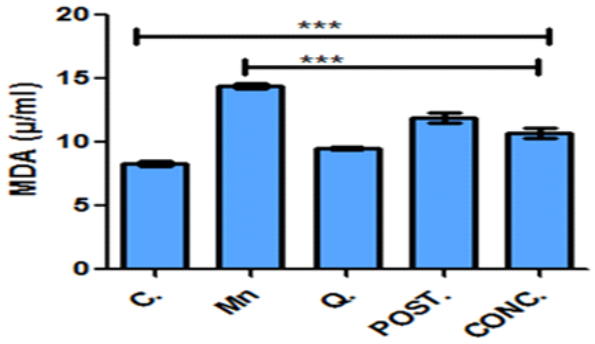

Figure 1: Graph representing the concentration of MDA (µ/ml) in the brain tissue. There is statistically significant difference in the control, Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); Concurrent (CONC); Malondialdehyde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

Superoxide Dismutase

There was a statistically significant increase in SOD level of the quercetin treated animals and control group when compared to Manganese and other experimental groups (**P<0.01, ***P<0.001). quercetin reduces the level of oxidative stress by increasing the activities of SOD but excess manganese causes an increase the oxidative stress by reduction of the SOD as shown in Figure 2.

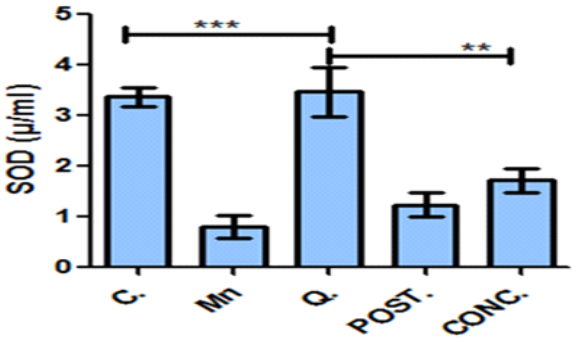

Figure 2: Graph representing the concentration of SOD (µ/ml) in the brain tissue. There is statistically significant difference in the control, Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); Concurrent (CONC); Superoxide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

Behavioral Studies

Results of motor coordination assessed in the experimental animals using parallel bar and grip suspension test were shown in the figure 3 and 4.

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 (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. 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).

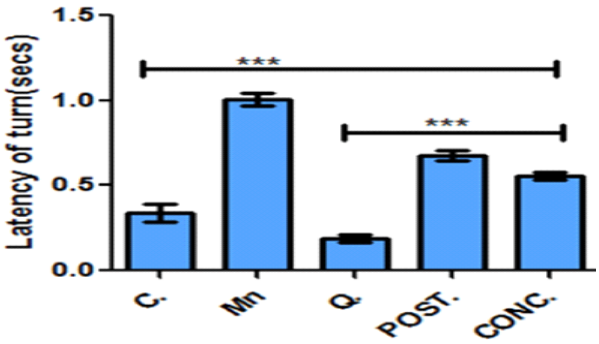

Figure 3: Graph showing latency of turn in Parallel bar test. Abnormal motor coordination were significantly reduced by the activity of quercetin.

Grip Suspension test

A significant decrease in release grip period was considered as abnormal motor coordination when the treatment groups were compared against control (Fafure et al. 2018). Manganese exposed mice exhibited decreased grip strength when compared to the control group. There was a significant increase in grip strength of the quercetin, concurrent and post-treated groups when compared with manganese exposed group (Figure 4). The control group exhibited a significant increase in grip strength when compared to manganese exposed group (***P<0.001)

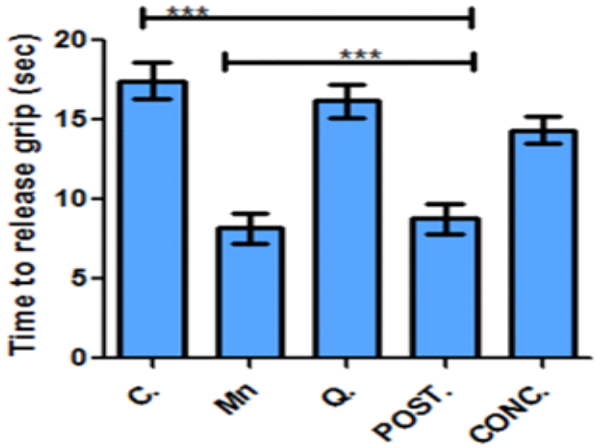

Figure 4: Graph showing the grip test of the experimental animals. There is statistically significant increase in the control group when compared with the Manganese, Quercetin, intervention and concurrent treated groups. Control (C); Manganese (Mn); Quercetin (Q); Intervention (POST); concurrent (CONC),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

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 wasobserved 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).

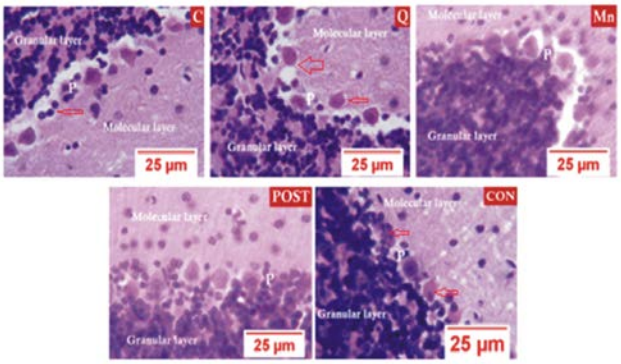

Figure 5: Photomicrograph of the cerebellar section of the manganese-induced mice with quercetin. Stained with H&E. Mg x800.

Immunohistochemistry:

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

TNF-α : From the figure 6 below, control and quercetin group revealed little or no expression of tumour necrosis factor alpha indicated with **dark brown precipitate**; expression of TNF-alpha and shrunken morphology was more pronounced in the $Mncl_2$ group (Mn) when compared with the control which indicates a well marked neurodegeneration; Post treatment group also 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.

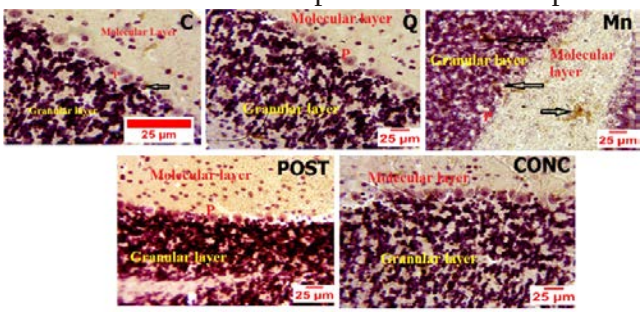

Figure 6: Photomicrograph showing the cerebellum TNF-α positive cell of mice exposed to manganese chloride and Quercetin. The apoptotic cells were characterized by dark brown precipitate, brown-stained nuclei and shrunken morphology. Mag. X800

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 hasknown to be implicated in the brain as a neurotoxin affecting motor neocortex which a characteristic neurological disorder known Manganism (Ye et al. 2017; Fafure et al. 2018, Adekeye et al. 2018a). Quercetin is a flavonoid naturally found in fruits and vegetables with antioxidant, anti-inflammatory and neuroprotective properties that may be therapeutic in neuronal injury (Costa et al. 2016; Khan et al. 2019). 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 reduction of body weight and cumulative feed intake which was not experienced in all groups that received quercetinand this was corroborated by worked done by Pei et al. (2020). Reduced brain and cerebellar weights in the manganese treated mice might be due to pathologic changes as a result of neurotoxic effects of manganese (Neal and Guilarte, 2013) leading to loss of neuronal degeneration and cellular architectural design as well as vacuolations of the brain section (figure 5) and this might contribute to cerebellar dysfunction and motor deficit which is in accordance with previous research done by Tarale et al. (2016) and 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 (Gaschler and Stockwell, 2017). It was also observed from our biochemical results that a significant increase in the experimental animals when compared to the normal control animals and a very statistically significant increase in the markers of oxidative stress (MDA and SOD) from figure 1 and 2 indicates 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 testand 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 miceobserved 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 Schilling et al. (2020). The efficacy of Quercetin was more pronounced in the co-treated animals with protective effect on the neuronal density and neuronal branching morphogenesis (Ghosh et al. 2013; Chakraborty et al. 2014; Ay et al. 2017). The histological results obtained in this research confirmed that Quercetin showed improved histological integrity in terms of alignment and density of the neuronal cells within the layers of

the cerebellar cortex which is in accordance with research done by Bahar et al. (2017).

Immunohistochemistry results from figure 6 further revealed the level of apoptosis that occur in the granular, purkinje and molecular cell layers of the cerebellar cortex in all the experimental animals but it was more pronounced in the Manganese treated mice group only and reduced significantly 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 micehad little or no expression of tumour necrosis factor alpha whilemanganese treated mice revealed a remarkable expression of TNF-alpha and shrunken morphology of the cerebellar cells as seen in Figure 6 that contributed to the motor deficit experience in the experiment and this was much in line with previous work done by Comella and Yuste (2013).

CONCLUSION

This study therefore clearly 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.

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