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EVALUATION OF THE EFFECTS OF *Gutenbergia nigritana* LEAVES EXTRACT ON CEREBELLUM OF ADULT MICE AND ITS IMPLICATION ON MANGANESE TOXICITY

Adekeye Adeshina Oloruntoba[1,3]*, Adumah Chizoba[1], Fafure Adedamola Adediran[1], Ajao Mayowa[1], Sabiu Saheed[2], Shallie Phillimon[3] and Adefule Adebayo Kehinde[3]

[1]Department of Anatomy, College of Medicine and Health Sciences, Afe babalola University, Ado Ekiti, Nigeria.

[2]Department of Biochemistry, School of Basic Medical Sciences, Kwara State University, Malete, Nigeria.

[3]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

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***Corresponding Author**
Adekeye Adeshina Oloruntoba
Department of Anatomy, College of Medicine and Health Sciences, Afe babalola University, Ado Ekiti, Nigeria.

ABSTRACT

Manganese is an important trace element in the biological system and its excessive exposure may lead to a neurological disorder known as manganism. Hence, checking the ameliorative effects of *Gutenbergia nigritana* leaves extract on the neuronal integrity of the cerebellum in motor deficit is of keen interest. Fifty adult male mice weighing between 25-35g were divided randomly into 5 groups. Manganese administration was for 7 days intraperitoneally at the dose of 40mg/kg body weight while *G. nigratana* extract was for 14 days and administered orally. Motor coordination was assessed in the animals using rotarod and parallel bar test. Animals sacrifice was done by humane killing followed by transcardiac perfusion fixation, the region of cerebellum were dissected out and fixed in 10% formal calcium for 72 hours, then processed for H&E stains for neuroarchitecture. Behaviourally, manganese treated group shows decreased motor activity on the rotarod, increased time of turn and time to edge during parallel bar test was noticed. $Mncl_2$ altered the integrity of purkinje neurons with appearance of indistinct cell bodies and scantily distributed granular cells. Manganese toxicity induces motor deficit from neurodegeneration but *Gutenbergia nigritana* extract revealed some ameliorative effect on the cells of the Cerebellar cortex.

KEYWORDS: Manganese, Intraperitoneally, Manganism, Neurodegeneration, Cerebellum.

1. INTRODUCTION

Manganese is an essential trace element that is required for body metabolism, bone formation and enzyme action in the body(Aschner *et al*., 2006). Despite its essentiality, excessive exposure to manganese for a long period of time is associated with adverse psychiatric, cognitive and motor disorders referred to as manganism (Pal *et al*.,1999; Olanow 2004; Perl and Olanow, 2007). Manganese (Mn) neurotoxicity can be caused by chronic inhalation of air containing Mn concentrations, parenteral nutrition, drinking water with high Mn concentration, eating manganese concentrated food (beans) for a very long time (Mergler, 1994; Pal *et al*.,1999; Rose *et al*., 1999; Bouchard *et al*., 2007; Iweala *et al*., 2014). A primary source for Mn intoxication is the occupational exposure in miners, welders, or workers in dry-cell battery factories (Aschner *et al*., 2007). The primary brain targets of Mn are the basal ganglia (BG), a group of related subcortical nuclei that include the caudate nucleus and putamen which together formed the striatum; the globus pallidus which comprises an external and internal segments (GPe and GPi, respectively) and the closely related subthalamic nucleus (STN) and substantia nigra (SN) *pars reticulata* (SNr) and *pars compacta* (SNc) (Parent,1996). These nuclei are components of circuits involving different areas of the cerebral cortex, thalamus and brainstem and are responsible for integrating and coordinating the information from various brain regions associated with motor and non-motor behaviors. In the brain, the striatum, globus pallidus (GP), SN, and STN have been reported as targets of Mn with the GP as the primary site for its accumulation and neurotoxicity (Yamada *et al*., 1986; Aschner*et al*., 2007). Manganism and true idiopathic Parkinson disease cause similar deficit within the central nervous system, they differ in the neurotransmitters upon which they act, manganese toxicity lies heavily on degeneration of GABAergic neurons in the Globus pallidus while Parkinson disease is more associated with the dopaminergic neurons in basal ganglia. However, PD and manganism share common mechanisms leading to dopaminergic (DAergic) neurodegeneration, mitochondrial dysfunction, aberrant signal transduction, oxidative stress and the activation of cell death pathways (Dobson *et al*., 2004; HaMai and Bondy, 2004; Latchoumycandane *et al*., 2005; Kitazawa *et al*., 2005). While the role of manganese on the striatum as been established, thus, little is known on its impact on the cerebellum. This research is designed to know the distribution of manganese in the cerebellum and if there is an improved motor coordination after been treated with gutenbergia

nigritana leave extract. Gutenbergia nigritana (Benth) of the family Asteraceae, a plant locally referred to as bush bitter leaf among the people of the Amassoma kingdom located in the southern-ijaw area of Bayelsa state in Nigeria is an erect branching herb about 1.3m in height, with wrinkled leaves and mauve to reddish purple florets in head (Augustine *et al*., 2013). The plant is an annual plant which can also be found in the western Nigeria and also known to grow in the tropical and subtropical regions of Central America, Eastern Brazil and West Africa. The leaf sap is traditionally used in Nigeria as a medicine for treatment of hypertension, asthma, and convulsion. It serves as one of the major ingredients required to prepare a decoction used in treatment of anemia and skin infections (Aluko, 2016).

2.0 MATERIALS AND METHODS

2.1 Reagents

Manganese (II) chloride ($Mncl_2.4H_2O$) tetrahydrate, Sodium hydrogen Phospahte (Na_2HPO_4), Potassium chloride (KCl), Potassium dihydrogen phosphate (KH_2PO_4) and all consumable used were purchased from Kermel laboratory (Colmar, France). The water used was glass-distilled and other reagents were of analytical grade.

2.2 Animals

Male BALB/c mice (8-10 weeks old) weighing 25-35g were procured from animal handling facility of the Department of Anatomy, University of Ibadan, Oyo state, Nigeria. The animals were housed and fed in the animal holding of Afe Babalola University, Ado Ekiti. The mice were allowed to acclimatize for a period of two weeks. All procedures involving animal handling in this study were carried out according to NIH guidelines, and were approved by the institutional Animal care and use committee, Afe Babalola University, Ado Ekiti, Nigeria.

2.3 Animal Treatment

Mice were divided into 5 groups (n=10). Group A were treated with manganese chloride ($Mncl_2$), Group B were treated with manganese first and later with *Gutenbergia nigritana* extract. Group C were treated with manganese and extract concurrently, group D were admistered with *Gutenbergia nigritana* extract alone, while group E received normal saline. The manganese and *gutenbergia nigritana* solution were freshly prepared and changed weekly. The Mn dose (40 mg/kg) and dosing route (intraperitoneal) used in this study for one week were model based on Jungmin, 2008 while the Gn dose (200mg/kg) and dosing route (orally) for two weeks were also due to a model based on a report by Aluko, 2016.

2.4 Assessment of Motor Function

Behavioural assessment were carried out at the end of administration to assess the motor coordination of mice, using a rotarod and parallel bar test (Jungmin and Kisok, 2008; Perona *et al.*, 2008). The animals were used to the behavioral apparatus before initiating the test. Both test were performed in the animal holding behavioural designated room of Afe Babalola University.

2.5 Collection and Preparation of Extract

Gutenbergia nigritana was collected during its blossoming stage in the month of May from a local farmland in Ikole-Ekiti, South-Western, Nigeria. The leaves were separated and dried under shade for five days and then pulverized into fine powder using an electric blender. A section of the fine powder was extracted in ethanol and the other in methanol for three days after which the extract was filtered. The filtrate was placed in a water bathe at a temperature of about 60^{o}c for a period of between 24-48 hours to dry. The dried extract was reconstituted in normal saline to give doses of 100 and 200mg/kg. Chemical tests were carried out using the ethanolic mode of extraction to identify the plant active constituents (saponin and tannin) which is in line with Kokate *et al.*, 2009.

2.6 Biochemical Analysis

Biochemical analysis was done to determine the catalase and superoxide dismutase activity (SOD) and to account for the total protein. Brain excised were fixed in 30% sucrose and later homogenize for further processing. Catalase and SOD activity were obtained in fraction of the cerebellum following the method by Sinha, Misra and Fridovich respectively (Martins *et al.*, 2012). Total protein determination Aliquots from the homogenates were separated for protein measurements that were assessed according to Bradford. Results for each of the biochemical assays were corrected for protein content in the samples (Martins *et al.*, 2012).

2.7 Statistical Analysis

Data obtained from activities of Oxidative stress markers and motor coordination performance of the treated group were compared with those of controls using one way analysis of variance (ANOVA). All results are presented as mean ± SEM and are considered statistically significant at $p<0.05$.

3.0 RESULTS

3.1 Neurobehevioural assessment on motor coordination

After the 3rd week of Mn exposure intraperitoneally, From the figure one (1) that revealed locomotive activities with the use of rotarod test, control mice show improved locomotor skills by staying on the accelerating rotating rod for longer period of time (sec). Mn treated group (20 or 40mg/kg) showed significant reduction when compared with control group as their motor activity decreased after 3 weeks of administration (*p<0.05). The post treatment group ($Mncl_2$/extract) revealed a statistical significant differences when compared with the control group in terms of improved motor activities. On the other hand, there was no significant difference between G.*nigritana* treated group and control group. Also from the figure two (2), There was a statistically significant difference in the level of latency of turn (LOT) when the control group was compared with the treated groups. Manganese group (20 and 40mg/kg) showed very significant increase when compared with the control group. (***p<0.001, ***p<0.001). It can also be seen that the post treatment group both at low and high doses revealed a statistically significant difference when compared with the control group. (**p<0.01, ***p<0.001).

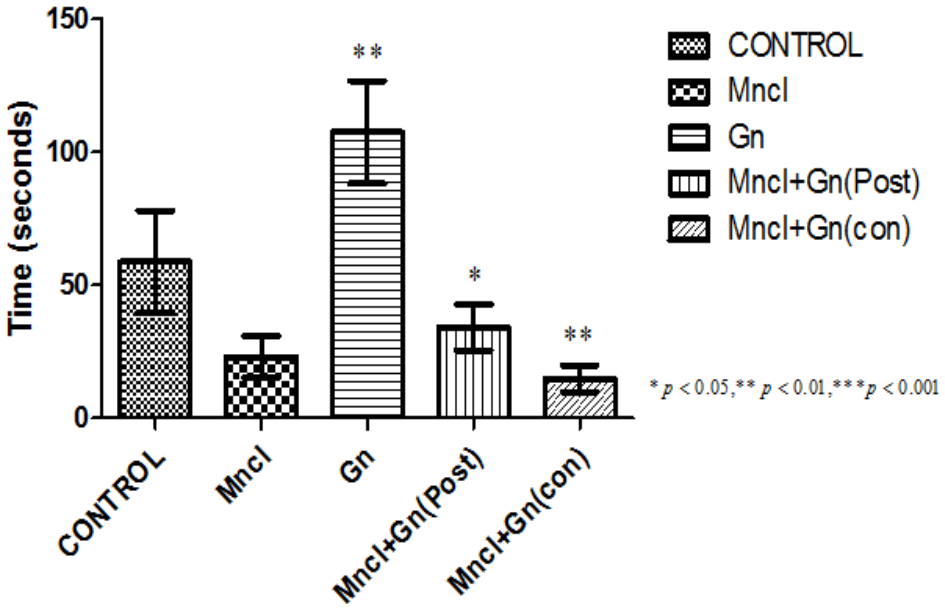

Figure 1: Bar chart showing latency of fall (LOF) of the experimental animals.

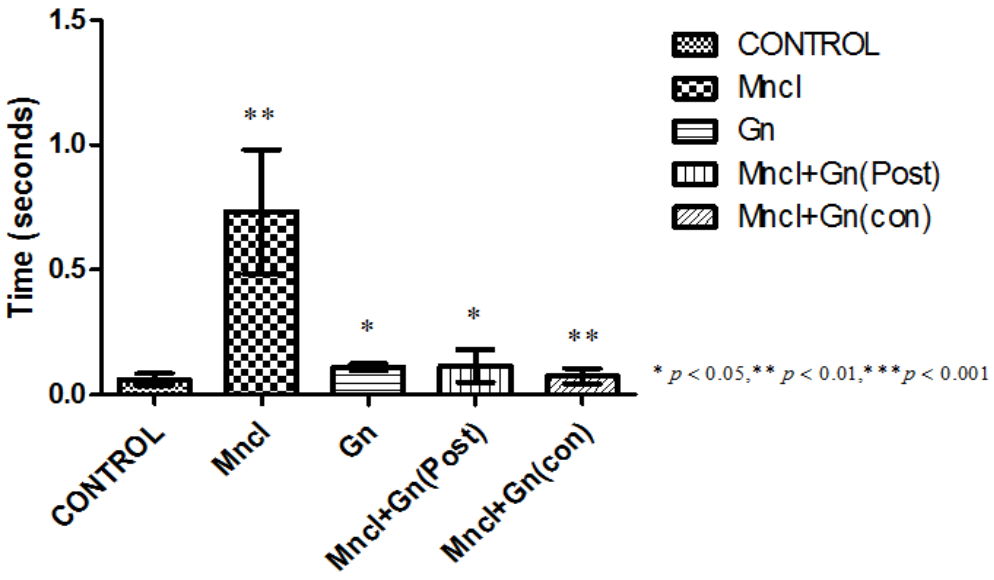

Figure 2: Bar chart showing latency of turn (LOT) of the experimental animals.

3.2 Relative Organ Weight

From the figure three (3), there was a statistically significant difference in the level of relative organ weight when the control group was compared with the treated groups (*p<0.05). Manganese group showed a well remarkable reduction both at low and high dose when compared with the control group. (*p<0.05). It can also be seen that the aqueous leave extract of Gutenbergia *nigritana* extract at high dose revealed a statistically significant difference when compared with the control group. Relative organ weight can be calculated by brain weight/body weight multiply by 100.

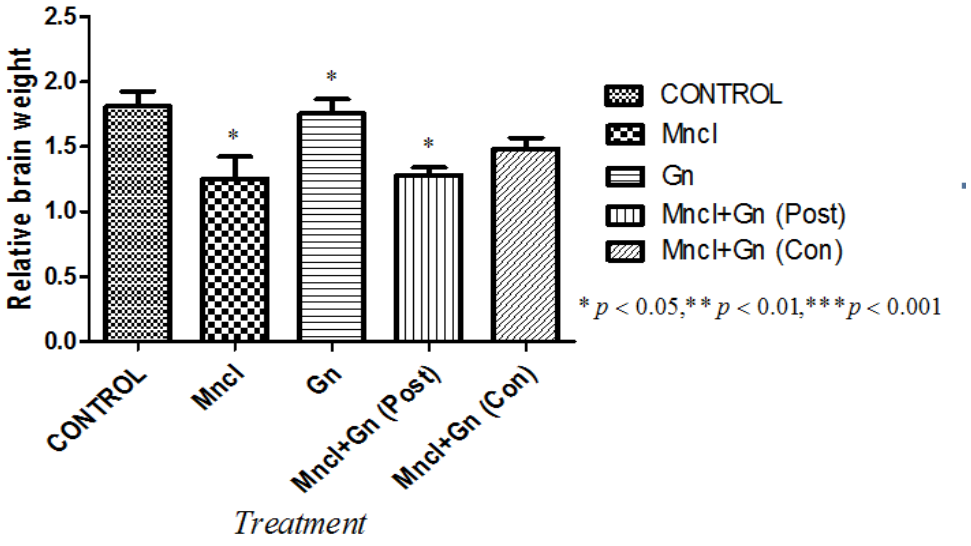

Figure 3: Bar chart showing the Relative brain weight of the experimental animals.

3.3. HISTOLOGICAL RESULTS

Heamatoxylin and Eosin Stain

Third week of Mn (20 and 40mg/kg) exposures compared to the control mice shows obliterated and scanty distributed purkinje cells with densely packed granular cells of the cerebellum.Post treated groups shows obliterated purkinje cells, dark and densely packed granular cells. While G.*nigritana* treated group shows evenly distributed purkinje cells with densly packed granular cells.

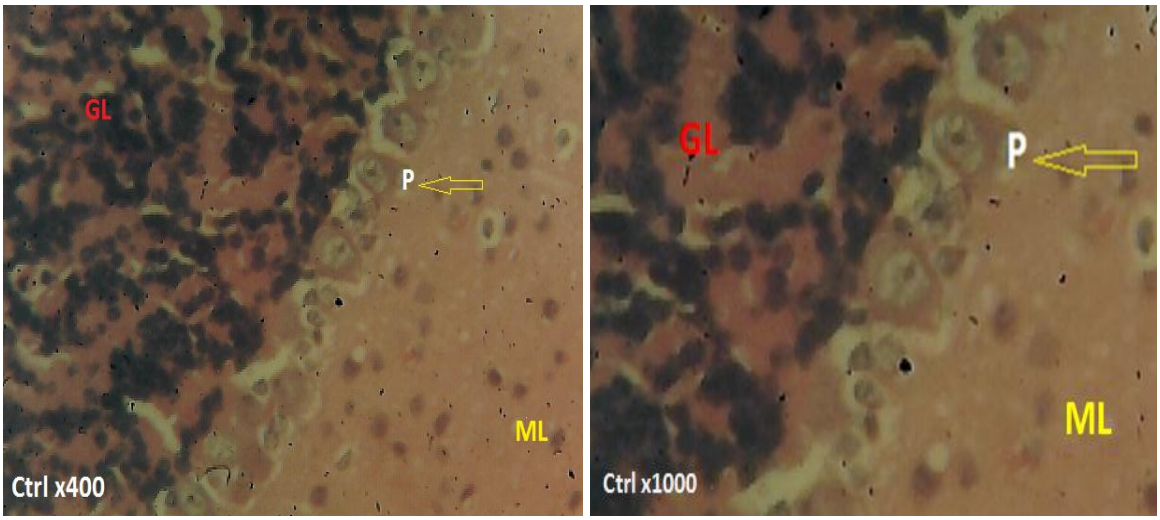

Figure 4: Photomicrograph of cerebellar section of the control. H&E x400 & x1000, showing the GL(granular layer), ML(molecular layer) and P(purkinje).

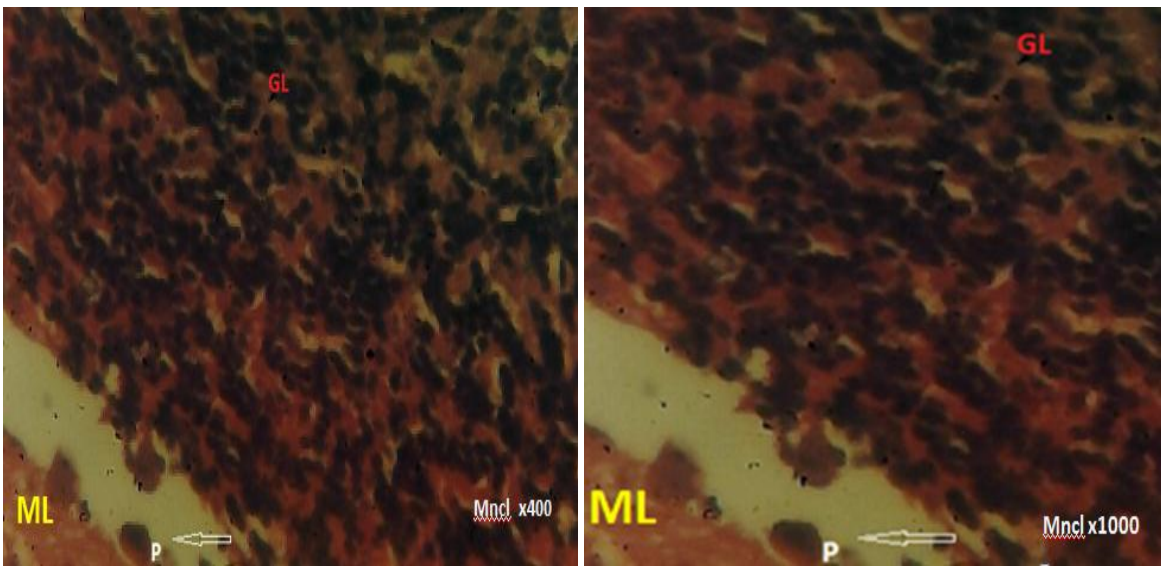

Figure 5: Photomicrograph of cerebellar section of the Mn treated group. H&E x400 & x1000, showing the GL(granular layer), ML(molecular layer) and P(purkinje).

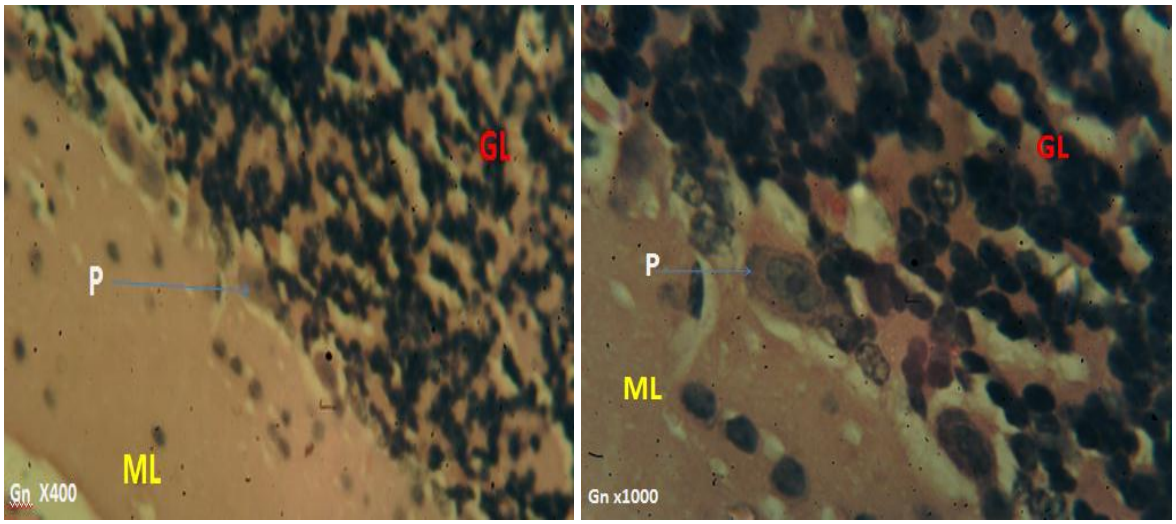

Figure 6: Photomicrograph of cerebellar section of the Gn treated group. H&E x400 & x1000, showing the GL(granular layer), ML(molecular layer) and P(purkinje).

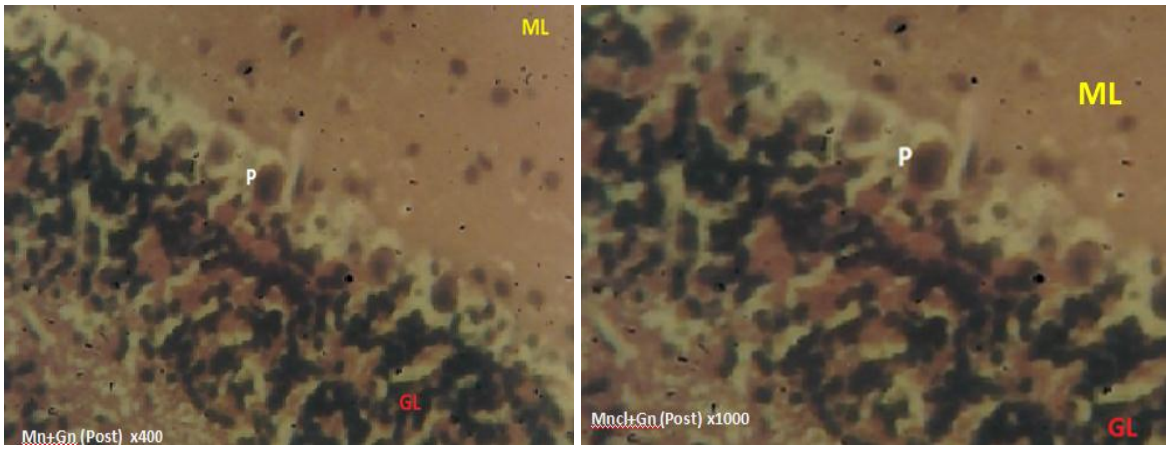

Figure 7: Photomicrograph of cerebellar section of the Post treated group. H&E x400 & x1000, showing the GL(granular layer), ML(molecular layer) and P(purkinje).

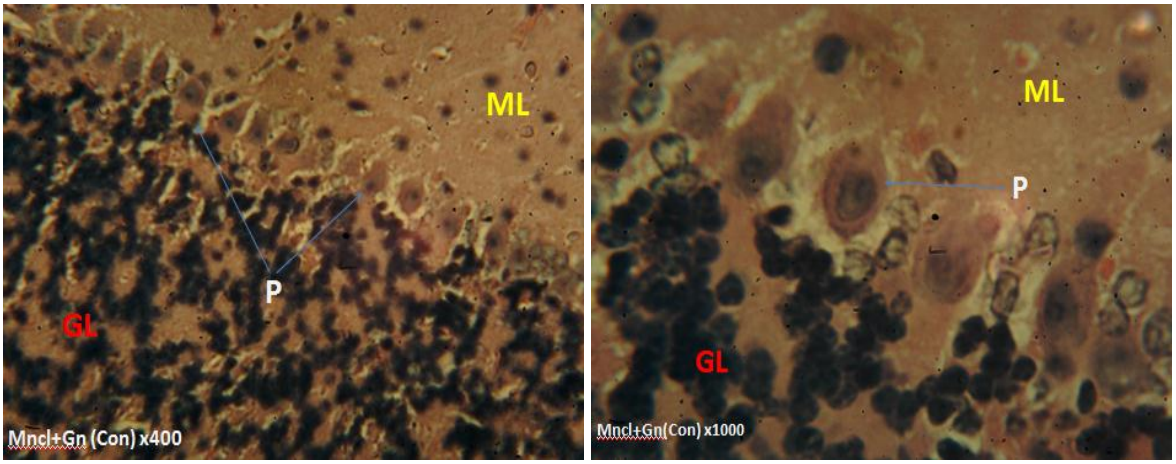

Figure 8: Photomicrograph of cerebellar section of the Concurrent treated group. H&E x400 & x1000, showing the GL(granular layer), ML(molecular layer) and P(purkinje).

4. DISCUSSION

Manganese (Mn) is an important trace element responsible for human health, being absolutely necessary for development of brain, metabolism and the antioxidant system (Xin *et al*., 2015; Jungmin and Kisok, 2008). Nevertheless, excessive exposure or intake may lead to a condition known as manganism, a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian-like symptoms (Prashant *et al.*, 2016; Ebany *et al.*, 2013; Marcelo *et al.*, 2012; Brad *et al.*, 2011; Micheal *et al.*, 2009) Hence, Mn has a paradoxal effect in animals, High Mn exposure may result to neurodenegeration and astrogliosis and is characterized by a complex behavioral syndrome including motor deficit (Marta and Michael, 2013; Julie *et al.*, 2009; Yuko *et al.*, 2009; Ebany *et al.*, 2013; Krishna *et al.*, 2014). In this study, no morphological changes were observed in the skin/fur and eyes colour of the treated animals when compared to the control. Usually people addicted to drugs do not often show changes in the skin or eye colour when used for a short term. During the course of administration as it has been pointed out in figure 3 that explain about relative organ weight, there was an increase in average body weight of the group treated with only manganese chloride and a slight decrease in body weight in the gutenbergia extract group but there was no significant difference between the treated groups when compared with the control. According to figure 1 and 2, Behavioral changes was observed in the group treated with Manganese, Gutenbergia nigritana (Gn) extract, and Mn+Gn group when compared with the control group. The treated group with manganese chloride and Mn+Gn showed slow movement when compared to the control group. Animals in Gn extract group were more active and faster than the other treated groups which is similar to animals in the control group. The cerebellum plays a vital role in motor coordination and control of fine movement. From the studies carried out according to Figure 4 which is the control group revealed a distinct preservation of the three layers of the cerebellum cortex which include outer molecular layer followed by middle purkinje layer containing purkinje cells (PCs) and innermost granular layer as shown at x400 and x1000 magnification. The granular layer of all the group showed normal morphology and the purkinje cells were numerous in the control group (Fig 4-8) but there was degeneration of purkinje cell in the $MnCl_2$ (Fig 5) which may be due to release of Reactive oxygen species (ROS), increase pyknotic nuclei and marked of karryohexis. Figure 7 also slightly showed that there was reduced pyknotic nuclei and nuclear fragmentation which can invariably caused reduction of degeneration of purkinje cell in $MnCl_2$+Gn extract group due to antioxidant properties of the gutenbergia nigritana extract. Figure 6 and 8 revealed preservation of the the three layers of the cerebellar cortex and

purkinje cells are well marked which evidence of reduction of oxidative stress, neuroinflammation and absence of karryohexis. Converging evidence indicates that PCs of the cerebellar cortex are an essential component of the neural machinery necessary for controlling movements and keeping them finely tuned (Mauk *et al.*, 2000; Thach, 1992). Purkinje cells are large neurons with many branching extensions that is found in the cortex of the cerebellum of the brain and plays a fundamental role in controlling motor movement by releasing GABA-neurotransmitter which exerts inhibitory actions on certain neurons which reduces the transmission of nerve impulses. These inhibitory functions enable purkinje cells to regulate motor movement, which may explain the inability of the animals to perform fine motor movement. The neurobehavioural analysis from the rotarod and parallel bar test results revealed that Gutenbergia extract is very potent in ameliorating motor deficit when compared with the control group in terms of latency of fall and turning reduction.

5. CONCLUSION

The significant and elicited neuroprotective potential of extract of *Gutenbergia nigritana* leaves may be ascribed to its antioxidative properties. This was achieved by the extract via release and induction of ROS scavenging enzymes which subsequently stalled auto-oxidation of membrane-bound lipids and neuronal damage. In conclusion, administration of excessive manganese lead to increase pyknotic nuclei, increase gliosis, neuroinflammation that causes decrease in population of viable neurons in the cerebellar cortex especially at the purkinje layers which were later recovered or reduced to minimal levels by the leave extract by virture of its antioxidant activities. However it can be drawn from these findings that *Gutenbergia nigritana* extract may have some ameliorating effect which improve motor deficit caused by the activities of manganese neurotoxicity.

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Conflict of interest

The authors declared no conflict of interest.

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