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Neurotoxic effects of manganese chloride on the occipital cortex of adult wistar rats

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Abstract

Manganese (Mn) is the twelveth most abundant element on the earth. It is an essential trace metal that is required for a specific amount of enzymes required for normal cellular functions. Manganese deficiency and intoxication are associated with adverse metabolic and neuropsychiatric effects in humans. This study investigated the effect of manganese chloride on occipital cortex of adult Wistar rats.

Thirty-six (36) healthy adult Wistar rats of both sexes weighing between 120 – 200 g were separated into four groups; A, B, C and D. The Wistar rats were subjected to different doses of manganese chloride. The wistar rats in Group A were regarded as the Control which received only feed and distilled water daily for 29 days. Manganese chloride was administered at 10mg/kg, 20mg/kg and 30mg/kg orally to Group B, C and D respectively. The animals were sacrificed on day 30th of the treatment by cervical dislocation. The brain was harvested, weighed with a sensitive weighing balance, Part of the brain was removed and homogenized for biochemical analysis for MDA (Malondialdehyde), NO (Nitric Oxide) and SDH (Succinate dehydrogenase) and the remaining parts were fixed in 10% formol calcium and was processed for histological analysis using H & E staining method.

The result showed that the mean body weights of the Wistar rats reduced significantly ($P<0.05$) in group B , C and D manganese chloride treated group compared with Group A .The brain weights in group B, C and D shows insignificant increase ($P>0.05$) when compared with group A (control group).The biochemical analysis result showed significant increase ($P<0.05$) in MDA (Malondialdehyde), NO (Nitric Oxide) and SDH(Succinate dehydrogenase) in the brain of manganese chloride treated groups compared to Control. The histological analysis indicated that occipital cortex shows observable degenerative changes in the occipital cortex characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal and granule neurons that appear with fragmented cytoplasm and condensed nuclei in the manganese chloride treated compared with normal histoarchitecture of occipital cortex in the control.

The study concluded that Manganese Chloride treatment has adversely affected the occipital cortical tissue in the treated rats which may affect the occipital cortical functions.

Keywords: Manganese Chloride; Occipital Cortex; Degenerative changes; Neurons; oxidative stress

1. Introduction

Manganese (Mn) is an essential trace metal that is required for a specific amount of enzymes important for normal cellular functions [1] However, excess accumulation of Mn in the brain results in a neurological syndrome with cognitive, psychiatric and motor abnormalities [2, 3,]. Previous report has shown that excess exposure to Mn with the highest concentrations of Mn in the brain occur in the basal ganglia, specifically in the globus pallidus, caudate/putamen,

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and substantia nigra [4,5] These same studies have shown that Mn also accumulates in other brain structures within the cerebral cortex and in white matter [7,8]. The accumulation of Mn in the basal ganglia is likely to be responsible for a form of Parkinsonism with overlapping, but distinct clinical features with those seen in idiopathic Parkinson's disease (PD). Additionally, there has been a great deal of debate in the scientific literature regarding the possibility that Mn may have an etiological role in idiopathic PD or accelerate the expression of PD [6,7].

The occipital lobe is the smallest of the four lobes of the cerebral hemisphere. It is present posterior to the parietal and temporal lobes. Thus, it forms the caudal part of the brain. Relative to the skull, the lobe lies underneath the occipital bone. It rests on the tentorium cerebelli, which separates it from the cerebellum. The paired occipital lobes are separated from each other by a cerebral fissure. The posterior most part of the occipital lobe is known as the occipital pole. The occipital lobe is primarily responsible for visual processing. It contains the primary and association visual cortex [8].

2. Materials and Methods

Thirty six (36) adult wistar rats of both sexes weighing between 120-200g were divided into four groups (A, B, C, D) of Nine animals each.

1g of manganese chloride solute was dissolved in 200ml of distilled water thus forming 200mg/ml of manganese chloride solution.

Administration of the Manganese chloride solution was done orally using an oral metal cannula. Group A served as control group and received distilled water; group B, C and D received the Manganese Chloride solution doses of 10mg/kg,20mg/kg,30mg/kg body weight respectively for the period of 29 days The adult wistar rats were sacrificed on the 30th day of the treatment by cervical dislocation, the rats were dissected through an incision on the midline of the vertical surface of the head region and cranium was carefully removed, avoiding pressure on the underlying brain, the exposed brain was removed, quickly weighed using a sensitive analytical balance and fixed in 10% formol calcium, The gross morphology of the brain (occipital cortex) was assessed and then processed for light microscopy (histology).

2.1. Statistical Analysis

All data obtained were presented as mean± SEM. Statistical analysis of the data in this study was carried out approximately and tested for significance using the one way variance test (ANOVA). P values<0.05 were considered statistically significant. Statistical for the social science (SPSS) software version 11.0 was used for the statistical analysis.

2.2. Photomicrography

Digital photomicrography of the needed occipital cortical sections were obtained to show the morphological changes in the treated groups as compared to the control group. The photomicrography was taken at the Histology laboratory, Department of Anatomy, LAUTECH, Ogbomoso using a trinocular microscope with digital camera attached to one of the eyepieces

3. Results

Table 1 The mean ± S.E.M of the body weight of Wistar rats before and after administration

Groups	Initial Weight (g)	Final Weight (g)	Weight gain or loss
A	132.2 ± 7.03	151.4 ± 7.89	19.2
B	145.6 ± 3.77	134.6 ± 7.19	-11
C	142.2 ± 12.56	134.4 ± 6.85	-7.8
D	180.0 ± 5.77***	144.3 ± 6.04	-35.7

Significance: P >0.05, value greater than 0.05 were considered insignificant while values less than P<0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

Table 1 above shows that the body weights of adult wistar rats which started increasing from the group (A) which increased from mean value of 132.2 ± 7.03 at the initial stage of administration to 151.4 ± 7.89 at the final stage.

Group B which received a low doses of manganese chloride (10mg/kg) shows insignificant decreased in body weights of the adult wistar rats from mean value145.6 ± 3.77 at the beginning to 134.6 ± 7.19 at the final state as compared with the control (group A).

Group C which received a medium dose of manganese chloride (20mg/kg) shows insignificant decreased in body weights of the adult wistar rats from mean value in the initial stage 142.2 ± 12.56 to 134.4 ± 6.85 in the final state when compared with the control group (A).

Group D which received higher dose of manganese chloride (30 mg/kg) shows significant decreased in body weights of the adult wistar rats from mean value in the beginning 180.0 ± 5.77 to 144.3 ± 6.041 in the final state when compared with th econtrol group (A).

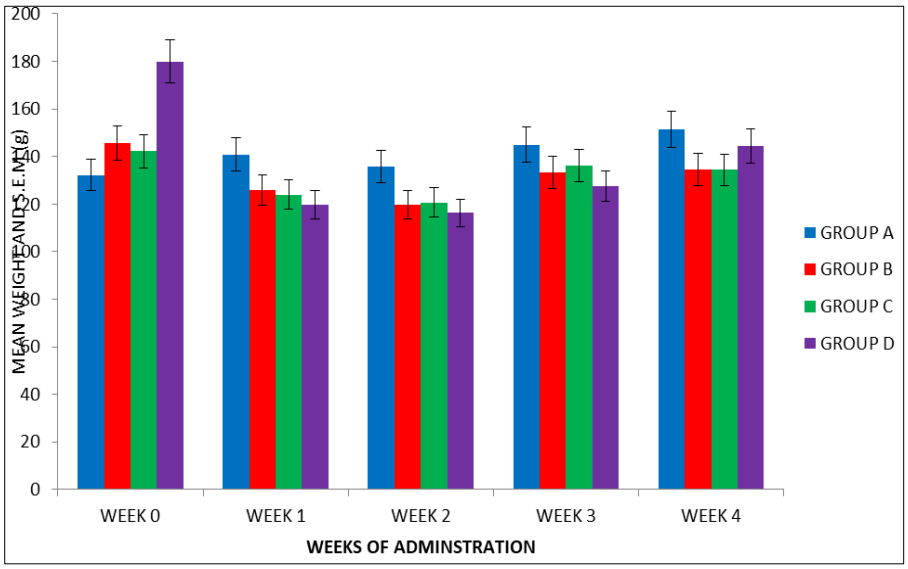


Figure 1 Histogram showing the body weights of the rats before and after administration

The graph above shows significant reduction ($p<0.05$)in the body weights of adult Wistar rats in group(A,B, C and D)during the administration of manganese chloride.

Table 2 The Initial and Final Body Weights of Wistar rats

Groups	Initial Weight (g)	Final Weight (g)	% Weight Gain or Loss
A	132.2 ± 7.03	151.4 ± 7.89	19.2
B	145.6 ± 3.77	134.6 ± 7.19	-11
C	142.2 ± 12.56	134.4 ± 6.85	-7.8
D	180.0 ± 5.77***	144.3 ± 6.04	-35.7

The above shows a decreased in final body weights of animals all group A, B, C and D. The initial and final weight in groups shows a significant reduction in the body weights of adult wistar rats during the administration of manganese chloride.

Above shows the body weight of adult wistar rats which started increasing from the group (A) increased from mean value of 132.2 ± 7.027 at the initial stage of administration to 151.4 ± 7.89 at the final stage by 19.2 % of weight gain.

Group B which received a low doses of manganese chloride (10mg/kg) shows insignificant decreased in body weights of the adult wistar rats from mean value145.6 ± 3.77 at the beginning to 134.6 ± 7.19 at the end decreases by -11% of weight loss as compared with the control (group A).

Group C which received a medium dose of manganese chloride (20mg/kg) shows insignificant decreased in body weights of the adult wistar rats from mean value in the initial stage 142.2 ± 12.56 to 134.4 ± 6.85 in the final state decreased by -7.8% weight loss when compared with the control group (A).

Group D which received higher dose of manganese chloride (30mg/kg) shows significant decreased in body weights of the adult wistar rats from mean value in the initial stage 180.0 ± 5.77 to 144.3 ± 6.04 in the final state decreased by -35.7% weight loss when compared with the control group (A).

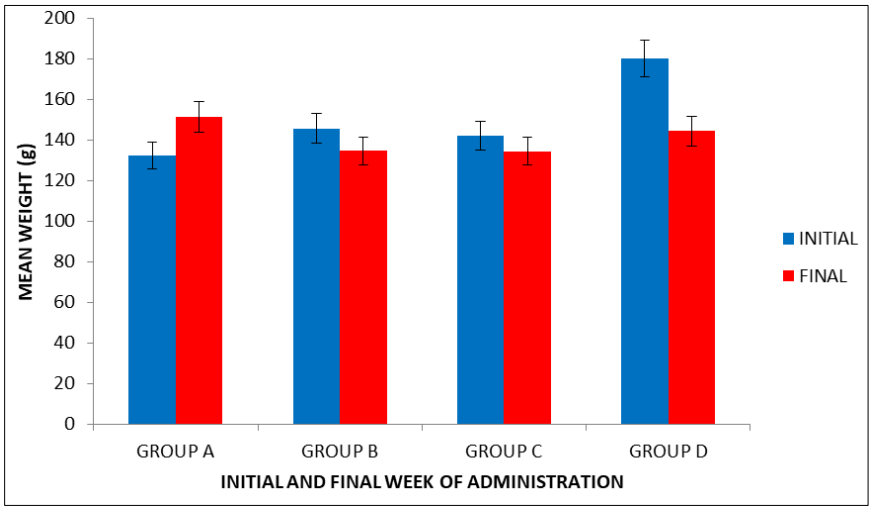


Figure 2 Histogram showing changes in initial and final body weights of adult Wistar Rats

The graph above shows significant reduction ($p<0.05$)in the initial and final body weights of adult wistar rats in group (B ,C and D)during the administration of manganese chloride compared to group A.

Table 3 The mean ± S.E.M of BRAIN weight of adult wistar rat after administration of manganese chloride

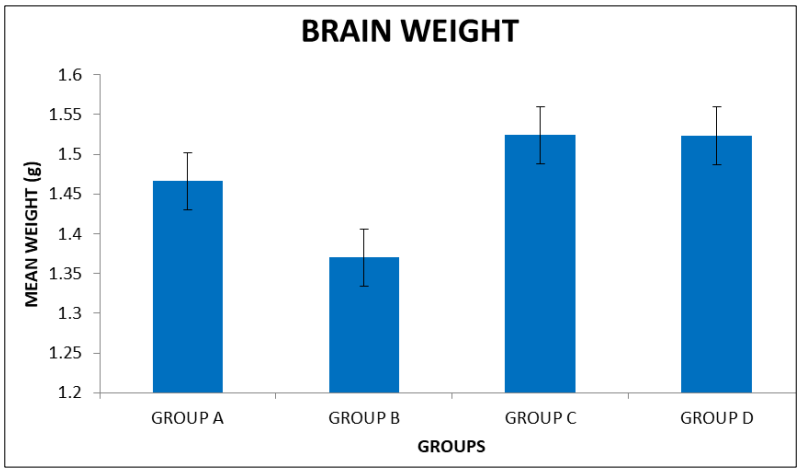
Groups	Brain weight(mean±S.E.M)	Relative brain weight %
A	1.460 ± 0.04	0.98
B	1.367 ± 0.09	1.02
C	1.524 ± 0.05	1.13
D	1.523 ± 0.04	1.05

Above shows the brain weight of adult wistar rats with mean value of 1.46± 0.04 and relative brain weight of 0.98%

Group B which received a low doses of manganese chloride (10mg/kg) shows insignificant increase in brain weights of the adult wistar rats from mean value1.367 ± 0.09 of relative brain weight of 1.02% as compared with the control (group A).

Group C which received a medium dose of manganese chloride (20 mg/kg) shows insignificant increased in brain weights of the adult wistar rats mean value of 1.524 ± 0.05 with relative brain weight of 1.13% when compared with the control group (A).

Group D which received higher dose of manganese chloride (30 mg/kg) shows insignificant increased in brain weights of the adult wistar rats of mean value of 1.523 ± 0.04 with relative brain weight% when compared with the control group (A).



Significance: P < 0.05, value greater than 0.05 are considered insignificant while values less than 0.05 are considered significant (*). Values are expressed as mean ± Standard error of mean.

Figure 3 Histogram showing the mean± SEM weights of BRAIN of Adult Wistar Rats

4. Biochemical Evaluation

Table 4 The effect of manganese chloride on the levels of MDA, NO and SDH in the brain

GROUPS	MDA (nmol/gtissue)	NO (µmol/g)	SDH(µmol/g tissue)
A	20.66± 0.10	3.97 ± 0.26	2.32 ± 0.43
B	26.43 ± 1.87**	5.59±0.40**	3.12 ± 0.47
C	24.66±0.92**	5.51± 0.46*	3.56 ± 0.29*
D	31.39±1.80***	6.36±0.45***	4.32± 0.30**

Significance: P < 0.05, values greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as mean ± Standard error of mean.

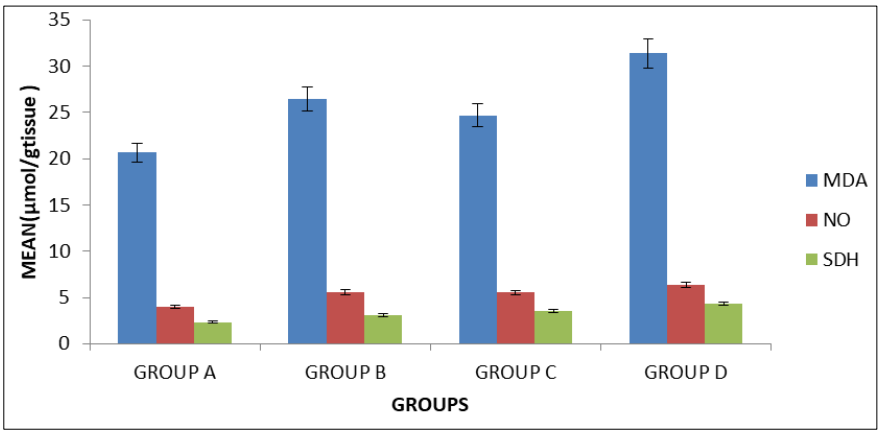


Figure 4 Histogram showing the changes in level of MDA, NO, SDH

- MDA: Malondialdehyde.
- SDH: Succinatedialdehyde.
- NO: Nitic Oxide.

Table 4 revealed increase in level of MDA in the treated group when compared with the contol,it increase significantly (p<0.05) from 20.66± 0.10 to 26.43 ± 1.87 in group B, 24.66±0.92 in group C and 31.39±1.80 in group D.

The level of NO increased significantly ($p<0.05$) in the treated groups compared with the control, it increased from 3.97 ± 0.26 to 5.59 ±0.40 in group B,5.51± 0.46 in group C, 6.36 ±0.45 in group D.

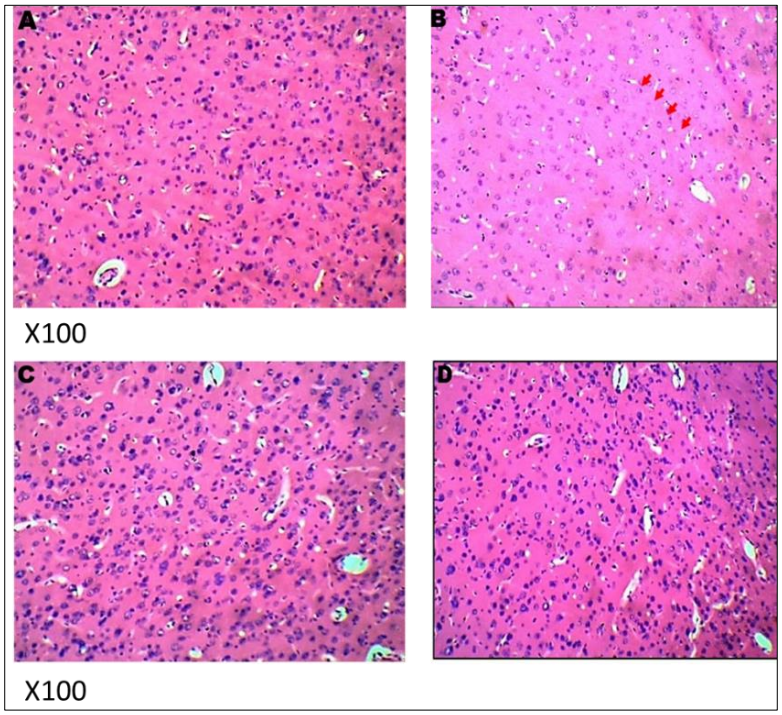


Figure 5 A-D Occipital cortex of group A [control] and B, C, and D treated with 0.3, 0.6, 0.8 mg/kg manganese chloride respectively for 29 days

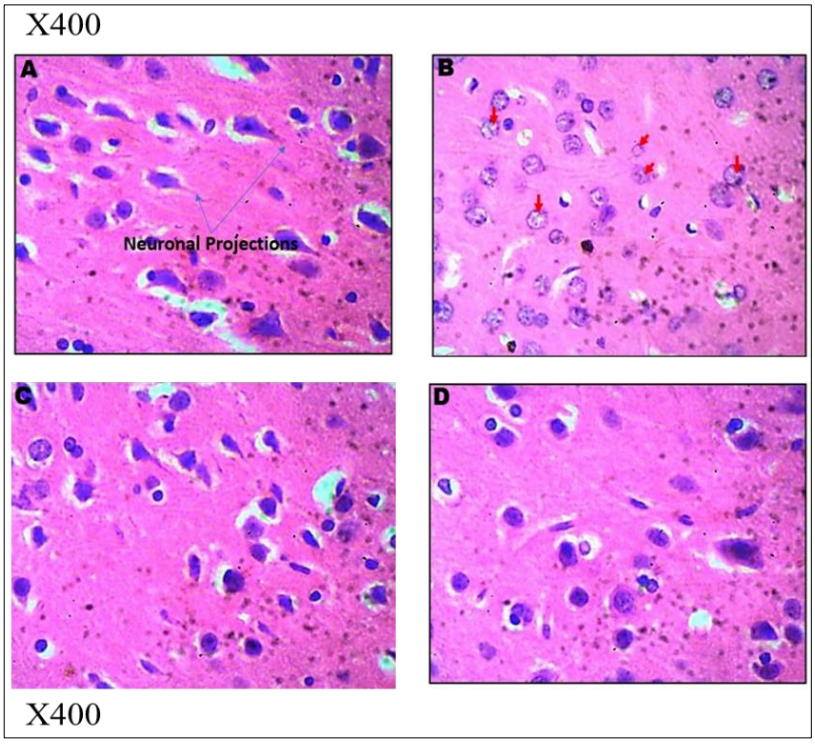


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Figure 6 A-D Occipital cortex of group A [control] and B, C, and D treated with 0.3, 0.6, 0.8 mg/kg manganese chloride respectively for 29 days

The level of SDH increased significantly ($p<0.05$) in the treated groups compared with the control. It reduced from 2.32 ± 0.43 to 3.12 ± 0.47 in group B, 3.56 ± 0.29 in group C, and 4.32± 0.30 in group D.

Normal histological features of the occipital cortex are observed in groups A this is characterized by a normal large pyramidal as well as granule neurons and oligodendrite. Treated With 10mg/ Kg Of Manganese chloride For 30 Days histological features of the occipital cortex are observed in group B treatment caused conspicuous degenerative changes in the cortex that was characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal and granule neurons that appear with fragmented cytoplasm and condensed nuclei. Wide Perineural spaces can be seen surrounding degenerating neurons, axons and dendrites are scarcely appreciable around neurons in this group, neuronal population appear scarcely appreciable in this group (red arrow Group C treatment showed mild similarity with group B treatment (red arrow).group D shows the pyramidal cells are characterized with long axonsthat extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears destorted, with degraded nuclear and cytoplasmic content, both pyramidal and satellite cells appear darkly stained with signs of diffused content with distinct layering.

5. Discussion

This study investigated the effects of manganese chloride on the occipital cortex of an adult Wistar rats. In this present study, the result obtained from the body weights (fig. 4.2) Groups B and C shows insignificant decrease ($p>0.05$) when compared to control. However there was a significant decrease ($p <0.05$) in group D when compared to control, The loss in body weight of the wistar rats in this study may be due to anorexia (loss of appetite) which has been reported to be induced by heavy metal ingestion, in relation to the previous work [9] .

Another possible explanation for loss of body weights may be due to the decrease muscle mass and cachexia due to the oxidative stress induced by manganese chloride as obtained in the previous report has induced by manganese chloride as obtained in the previous report has indicated that heavy metal toxicity is associated with oxidative stress [10], Similarly, weight loss according to previous studies documented is associated with muscle wasting leading to low body weights as compared with the previous work done [11].

Manganese chloride has a predilection to accumulate within the brain mitochondria resulting in free radicals' formation and neural cell death [12]. Biologically, the balance between oxidants' production and endogenous antioxidant defense mechanisms is warranted and any disruption for this balance results in oxidative stress damage [13]. Manganese chloride -treated group exhibited a decreased final body weight compared to the control group .according to previous work [14].

The result obtained in Table (4.3) Shows an insignificant increase in the relative brain weights which has been shown in the previous study that rats treated with manganese chloride shown in the previous study that rats treated with manganese chloride shows an insignificant increase ($p > 0.05$) in their brain weights compared to group A (control) although not dosage dependent, However , it has been reported a significant increase in the brain manganese level in rats [15] .Moreover, other investigators have documented a negative effect of manganese exposure on brain weights when the manganese chloride treated rats were compared with control, after treatment and this was in agreement with findings that rats treated with manganese chloride had a significant decrease in the brain weight compared to control [16] .

Accumulation of Mn in brain is associated with a relatively long half-life and a sluggish elimination rate [17]. Based on this fact, our findings showed that brain Mn levels were markedly elevated in $MnCl_{2-}$ treated rats which has been similarly reported [18]. The results of biomedical parameters investigated shows significant increase ($P<0.05$)in level of MDA (malondialdehyde) in group B, C and D manganese treated groups as compared with group A, MDA which is known to be the final product of lipid perioxidation. The result of this study is consistent with the reported increase in the level of lipid peroxidation which is probably because of the acceleration of manganese oxidation or its reaction with dopamine, thus an increase in the occurrence of oxidative stress in tissues of treated rats. Mn exposure induces lipid peroxidation MDA as obtained in various region of the brain.

Furthermore Recently, there has been growing interest in the role played by lipid peroxidation in metal toxicity, with numerous studies undertaken using malondialdehyde (MDA) as biomarker of oxidative stress [20,21] A growing body of evidence has indicated that trace metals play important roles in a number of biological processes by activating or inhibiting enzymatic reactions, by competing with other elements and metalloproteins for binding sites, by affecting the permeability of cell membranes, or through other mechanisms [22,23]. Previous research shows the effects that the

doses of Manganese chloride have on the induction of lipid peroxidation in the various regions of the brains of the treated rats. After injection with Manganese chloride (50 mg /kg), we observed that the degree of lipid peroxidation increased significantly in the frontal cortex, corpus callosum, hippocampus, hypothalamus, medulla, and cerebellum, and at 100 mg/kg, the levels in the frontal cortex, corpus callosum, striatum, hypothalamus, medulla, and cerebellum had all increased significantly. At 25 mg/kg, a significant increase of the lipid peroxidation level was found only in the frontal cortex, corpus callosum, and cerebellum [24].

During a previous study, the peroxidase activity was found significantly ($p<0.05$) higher in the fish liver and brain under all metal exposure treatments as compared to the control group. Manganese-exposed fish showed higher peroxidase activity due to the production of reactive oxygen species as compared to the control fish in which peroxidase activity remained lesser due to the balanced production of ROS and optimum peroxidase activity. [25]

The level of NO (Nitric Oxide) in group B, C and D that receives 10mg/kg, 20mg/kg and 40mg/kg of manganese chloride also increased significantly ($P< 0.05$) as compared and highly detrimental neurotoxic effects by the excess production of many factors such as inducible nitric oxide synthase (iNOS), tumor necrosis factor a (TNF-a), and interleukin-Ib (IL-Ib) [26,27].

NO, in the previous study demonstrated that excessive Mn accumulated largely in brain of cocks exposed to Manganese and significantly affected the concentrations of select microelements and was associated with significant increase in NO and iNOS activity, which is related to the cerebral damage process [28]..

The activity of SDH (Succinate dehydrogenase) enzyme in group B, C and D that received 10mg/kg,20mg/kg and 40mg/kg increased significantly ($P< 0.05$) as compared with group A, however significant decrease in SDH (mitochondrial complex II) was observed as a result of increased administration of manganese chloride dosage.

The result obtained from the histological analysis shown observable degenerative changes, clustered satellite cells that appeared with fragmented cytoplasm and condensed nuclei. Wide perineural spaces can be seen surrounding degenerating neurons, axon and dendrite are scarcely appreciable around neurons, axons and dendrite are scarcely appreciable around neurons, neuronal population appeared scarcely appreciable in this group, this histological changes may be due to overexposure of accumulation of manganese which indicated that mitochondria, nucleous and synaptosomes of neurons and astrocyte of globus pallidus are described as primary sites of Mn accumulation and toxicity in the brain [29].

Histopathology of the related metal rats shows that, Cerebral Cortex of Aluminium chloride (1000mg/L) treated rats for 40 days showed vacuolation (prominent in the molecular layer. The outer pyramidal layer showed shrunken pyramidal cells with vacuoles contained condensed or partially degenerated neurons, karyorhexus and karyolysis of the nuclei. Hyaline necrosis also detected. Deep cerebral cortex layer showed area of cytoplasmic and nuclear vacuolation. As well as, edema with tissue necrosis, gliosis and vascular congestion are clearly observed in the white matter. Congo red stained tissue sections revealed focal extracellular amyloid deposition in cerebral cortex [30],

6. Conclusion

In conclusion, findings of this study concluded that long term exposure to manganese chloride could be a risk factor in cellular damage and neurodegeneration in wistar rats.

Recommendation

Based on the result of this study, I strongly recommend that the general public should avoid Manganese exposures in a variety of environmental settings, nutritional sources, contaminated foods, infant formulas, water, soil, and air with natural or man-made contaminations. Cumulative evidence on Mn toxicities and the vast public interest in this metal speak volumes of its public health importance, calling for a thorough understanding of its risk, the mechanism of its harm, some forms of effective clinical interventions, and any applicable strategy for prevention of present neurological and neuropsychiatric disorders. However further research should be done to compliment the findings of this research.

7. Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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