International Research Journal of Medicine and Biomedical Sciences Vol.5 (1),pp. 7-12, January 2020
Available online at http://www.journalissues.org/IRJMBS/
https://doi.org/10.15739/irjmbs.20.002

ISSN 2488-9032

Original Research Article

Assessment of neuroprotective effect of ascorbic acid against manganese dichloride induced cerebellar damage in female Wistar rats

Received 4 October, 2019 | Revised 29 November, 2019 | Accepted 9 December, 2019 | Published 14 January, 2020

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Manganese is an essential trace element that can elicit a variety of toxic responses upon prolonged exposure to elevated concentrations. The present study investigated the protective effects of ascorbic acid against cerebellar damage by manganese dichloride tetrahydrate in female Wistar rats. Thirty female Wistar rats weighing between 133 - 268g were divided into six groups lettered A to F. Group A served as the control group administered volumes of distilled water equal to those in treated groups. The test groups B, C, D, E and F were administered respectively with: ascorbic acid at 100mg/kg; manganese dichloride tetrahydrate at 148.4mg/kg; Manganese dichloride tetrahydrate at 445.2mg/kg; manganese dichloride tetrahydrate at 148.4mg/kg and ascorbic acid at 100mg/kg; and manganese dichloride tetrahydrate at 445.2mg/kg and ascorbic acid at 100mg/kg. The administration was by oral gavage for 18 days. The rats were euthanised by chloroform inhalation on the 19th day. The brain of each rat was removed, weighed and processed for H&E. The group administered with only ascorbic acid showed significant increase in body weight change, whereas the groups administered with $MnCl_2.4H_2O$ with or without ascorbic acid showed significant decrease in body weight. Organo-somatic index increased in the group administered with only ascorbic acid at 100mg/kg of body weight and significantly decreased in the group administered with low dose of $MnCl_2.4H_2O$ at 148.4 mg/kg and ascorbic acid at 100 mg/kg. Histological results revealed increase in Purkinje cells in group treated with ascorbic acid at 100 mg/kg of body weight, whereas the groups administered with $MnCl_2.4H_2O$ with or without ascorbic acid showed neurodegenerative changes. In conclusion, the administration of ascorbic acid did not completely reverse the effects of $MnCl_2.4H_2O$ as revealed in body weight and neurodegenerative changes in the cerebellum.

Keywords: Manganese dichloride tetrahydrate, ascorbic acid, cerebellum

INTRODUCTION

Manganese is one of the most abundant metal on earth. It is available in air, water, soil, and food, with food being the major source of manganese in the body. Its concentration in the environment is correlated with industrialization and traffic density (Joselow et al., 1978; USEPA 2003; Santamaria and Sulsky, 2010). Although it is a micronutrient, it is essential for many biological processes as it is a component of many important metalloproteinases, such as manganese superoxide dismutase, and pyruvate carboxylase (European-Commission 2011). Manganese homeostasis is tightly regulated within the body to avoid excess accumulation. Its availability is mainly regulated by

Table 1. Experimental Design

	Groups	Drug/Dosage	Duration
A	Control	Water and Feed alone	18 Days
B	Ascorbic acid	Ascorbic acid 100 mg/kg body weight	18 Days
C	Manganese dichloride (Low dose)	Manganese dichloride 148.4 mg/kg body weight	18 Days
D	Manganese dichloride (High dose)	Manganese dichloride 445.2 mg/kg body weight	18 Days
E	Manganese dichloride (Low dose) and Ascorbic Acid	Manganese dichloride 148.4 mg/kg body weight + Ascorbic acid 100 mg/kg	18 Days
F	Manganese dichloride (High dose) and Ascorbic Acid	Manganese dichloride 445.2 mg/kg body weight + Ascorbic acid 100 mg/kg	18 Days

its absorption through the digestive tract. Studies have shown that manganese chloride is the most aabsorbable chemical species of manganese (Keen et al., 2000; Ye et al., 2017; Chen et al., 2018).

High deposition of manganese is usually found in organs with high energy demands such as muscles, kidney and liver (USEPA 2003). Although found in mild concentrations in the brain, the brain is the most sensitive organ to manganese toxicity usually resulting in manganism (Francis and Forsyth, 1995; Crossgrove and Zheng, 2004). Manganism is associated with excess deposition of manganese in brain tissues, especially the substantia nigra, basal nucleus and cerebellum. The disease condition is characterised by reduced response speed, irritability, mood changes and compulsive behaviour. Protracted exposure to manganese have been reported to produce symptoms similar to idiopathic Parkinson's disease (Santamaria and Sulsky, 2010; Tuschl et al., 2013; Chen et al., 2015). In addition, children with learning disabilities including attention deficit disorders have been found to contain high levels of manganese in their hair compared to children with normal learning ability (Collipp et al., 1983; WHO 2011; Wessling-Resnick, 2019).

In most cases, the presence of high concentrations of manganese is not detected until symptoms are expressed (Francis and Forsyth 1995; Howe et al., 2004; Santamaria and Sulsky, 2010). This raises the need for protection against manganese toxicity in environments prone to manganese pollution like many industrialized cities. Although the mechanisms by which manganese damages the brain tissues are not clearly understood, oxidative stress have been identified as one of such mechanisms (Kontur and Fechter 1988; Avila et al., 2013; Peres et al., 2016; Chen et al., 2018).

Ascorbic acid, also known as vitamin C is a pharmaceutical drug consisting of two inter convertible compounds L-ascorbic acid and L-dehydroascorbic acid. It is a potent free radical scavenger in the extracellular fluid, protecting cells against oxidative damage by its ability to reduce potentially damaging relative oxygen species. Ascorbic acid in itself is of low toxicity and has only minor adverse effects such as diarrhoea, nausea and other digestive disturbances, due to osmotic withdrawal of water from the intestinal contents by the unabsorbed ascorbic acid residues. Amounts higher than 2000mg daily have been estimated to result in severe side effects (ODS-NIH 2019; Wax, 2019). It is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent. Several studies have reported the neuroprotective function of ascorbic acid in association with its antioxidant property (Animoku et al., 2015; Ali et al., 2018; Animoku et al., 2018). Therefore, this study was aimed at investigating the protective effect of ascorbic acid against the potential toxicity of manganese to the cerebellum.

MATERIALS AND METHODS

This study was carried out in the animal facility of Basic Medical Sciences, University of Uyo, Nigeria. Ethical approval for the study was obtained from the Animal Ethical Committee of Faculty of Basic Medical Sciences, University of Uyo, Nigeria. All Histological preparations were carried out at the histology laboratory of the Department of Anatomy, University of Uyo Nigeria.

A total of 30 female Wistar rats weighing between 133 to 268g were used for the study. Five animals were housed in a cage with rubber cylinders for environmental enhancement. The rats were given food and water ad libitum. All animal experiments were performed in accordance with the National Institute of Health Guide for the use and care of laboratory animals (2011).

The rats were divided at random into six groups of five each. Group A was control and B to F were treatment groups as illustrated in Table 1. Dosage of manganese dichloride tetrahydrate ($MnCl_2.4H_2O$) was calculated using the LD_{50} for $MnCl_2.4H_2O$ of 1484 mg/kg at 10 % and 30 % for low and high dose respectively, which is related to its environmental concentration in the soil (Holbrook et al., 1975; Pinsino et al., 2012).

Drug Administration

Manganese dichloride tetrahydrate (MnCl2.4H2O) was used for the study. White tablets of ascorbic acid produced

Table 2. Effects of Manganese dichloride and Ascorbic acid on Body Weight Change

	Groups Drug/Dosage	D0	D6	D12	D18
A	Water and Feed alone	156±6.14	178±4.47	183±4.88	186±5.44
B	Ascorbic acid 100 mg/kg body weight	156±6.14	160±5.24	159±6.61	163±6.65
C	Manganese dichloride 148.4 mg/kg body weight	217±4.10	221±3.48	228±1.20	230±3.21
D	Manganese dichloride 445.2 mg/kg body weight	181±2.36	181±2.42	186±2.95	186±2.90
E	Manganese dichloride148.4mg/kg body weight and Ascorbic acid 100 mg/kg	252±7.44	249±6.66	242±6.25	241±5.40
F	Manganese dichloride 445.2 mg/kg body weight and Ascorbic acid 100 mg/kg	198±3.52	197±2.0	194±2.39	197±1.67

Values are expressed as Mean ± SEM, n=5; D0= Initial weight, D6= weight at day 6 of administration, D12= weight at day 12 of administration, D18= Final weight

by Emzor Nigerian ltd. were crushed into powdered form and used for the study. Each dose was diluted in 1ml of water and given by oral gavage following administration of $MnCl_2.4H_2O$ daily for 18 days.

Sample Collection

After 18 days administration, the animals were weighed and euthanized by chloroform inhalation on the 19th day. The cerebellum of each rat was excised and weighed. They were then rinsed in normal saline to clear all bloodstains before fixing in 10 % buffered formalin for tissue processing.

Organo-Somatic Index

The organo-somatic index was calculated using the formula (Ebong et al., 2008).

$$\frac{\text{Weight of Organ}}{\text{Weight of animal}} \times \frac{100}{1}$$

Tissue processing

After fixation, the cerebella were cut transversely to remove 5-6mm of tissue from their inferior pole for histological processing. The cut sections were put through series of tissue processing procedures for hematoxylin and eosin (H&E) staining as described in Bancroft and colleagues (2013).

Statistical Analysis

All data were expressed as Mean ± Standard Error of Mean (SEM). One way analysis of variance (ANOVA) was carried out to evaluate the significant difference between means of different groups with 95% confidence limit. Least significant differences (LSD) were used to determine significant results. Differences between groups were considered statistically significant at $p<0.05$.

RESULT

Body Weight

The body weight of groups A, C, and D showed a trend towards weight gain in the animals, while groups D and F showed trend towards weight loss in the animals (Table 2).

Organo-Somatic Indices

The mean brain-weight result showed no significant difference in any group, the organosomatic index for the brain indicated significant increase in group B and a significant decrease in group E.(Table 3 and 4).

Histological Observations

The photomicrograph of the cerebellum of group A showed normal histoarchitecture and cellular layers of the cerebellum (Figure 1), while numerous Purkinje cells and very active granule cells as indicated with their deep stains, were observed for group B. There was necrosis of cells evident in vacuolations in molecular and Purkinje cell layers (around Bregmann cells) in group C. The vacuolations within the molecular and Purkinje cell layers were enormous in group D with degeneration of pyramidal cells, and evenly stained granule cells (Figure 1). Similar degenerations of the cerebellum were observed for groups E and F, which were given combined administration of Ascorbic acid and manganese chloride at low and high dose respectively.

DISCUSSION

The cerebellum plays an important role in motor control. It is also involved in some cognitive functions such as regulating fear and pleasure responses (Wolfe and Liu 2007). Although it does not initiate movement, it is important in movement coordination, precision and accurate timing. It integrates input from sensory systems of

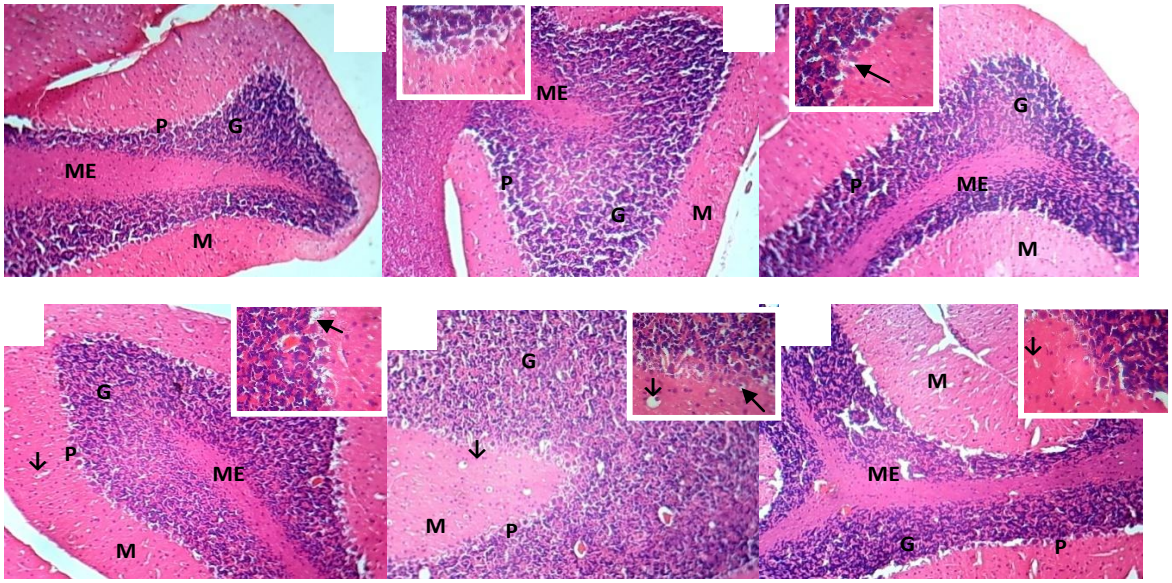

Figure 1: Photomicrographs of the cerebellum of experimental rats. A: cerebellum from control group. B: cerebellum from group B treated with ascorbic acid. C: cerebellum from group C treated with low dose of. D: cerebellum from group D treated with high dose of MnCl2. E: cerebellum from group E treated with low dose of MnCl2 and ascorbic acid. F: cerebellum from group F treated with high dose of MnCl2 and ascorbic acid. M-Molecular Layer, P-Purkinje Cell Layer, G-Granular Layer, ME-Inner Medullar Layer, single arrows - Vacuolation around Bregmann Cell, ↓↓-Vacuolation in Molecular Layer. (H&E; x100; insert x400)

the spinal cord and other parts of the brain to fine tune motor activity. Damage to the cerebellum produces disorders in fine movement, equilibrium, posture and motor learning (Fine et al., 2002). It is one of the areas of the brain affected by excess deposition of manganese through oxidative stress reaction.

The protective effect of ascorbic acid against oxidative stress in brain tissues has been investigated and confirmed in several toxicity studies (Oularbi et al., 2016; Ali et al., 2018; Animoku et al., 2018). In this study, ascorbic acid was found to confer considerable protection against manganese cerebellar-toxicity.

No significant difference was found in body weight and brain weight between the groups. In these rats, the weight lost from tissue damage may have been cancelled out by the weight contributed by manganese deposition in the brain as have been established in many studies of manganese toxicity (Avila et al., 2013; Tuschl et al., 2013; Chen et al., 2015).

Organo-somatic index is a significant biomarker of toxicity (Reddy 2009; Ariwariokuma et al., 2011).

In this study, organo-somatic indices revealed that the group administered only ascorbic acid at 100mg/kg of body weight showed significant increase in brain somatic index, while the group administered with low dose of $MnCl_2.4H_2O$ at 148.4mg/kg and ascorbic acid at 100mg/kg of body weight showed significant decrease in brain somatic index indicating some level of toxicity by manganese. This result is similar to that of Reddy et al. (2009) who reported a decrease in brain-somatic index for fluoride induced neural toxicity in rats. The insignificant difference for other $MnCl_2.4H_2O$ treatment groups may be due to the accumulation of the heavy metal in the brain contributing to the weight of the brain.

Photomicrographs of H&E sections from group B administered with only ascorbic acid showed no degeneration or loss in Purkinje cells indicating none-toxic effect of ascorbic acid. There was degeneration and necrosis of cells in the cerebellum of rats administered with $MnCl_2.4H_2O$ with or without ascorbic acid compared with control group, which showed normal histology of the cerebellum. This is in agreement with Chandra and Shukla (1978) who reported changes in brain chemistry and neuronal degeneration in mice and rats due to excess exposure to manganese.

In addition, Fonnum and Lock (2000) had identified purkinje and granule cells as important targets in the cerebellum for toxic substances. This is further supported in this study by the remarkable reduction of Purkinje cell population in all groups administered with manganese chloride with or without Ascorbic acid. These findings indicate severe toxicity of manganese on the cerebellum similar to what have been reported for other substances resulting in the degeneration and loss of Purkinje cells in the cerebellar cortex of Wistar rats (Ajibade et al., 2008).

However, comparison between manganese-administered groups showed improvements in the groups with ascorbic acid co-administration. This suggests partial protection of

Table 3. Effects of Manganese dichloride and Ascorbic acid on Brain Weight Change

Groups (n = 5)	Average brain weight (g)
Group A	1.84 ± 0.14
Group B	1.83 ± 0.09
Group C	1.94 ± 0.11
Group D	1.67 ± 0.57
Group E	1.97 ± 0.36
Group F	1.86 ± 0.24

No significant difference observed (mean ± SD)

Table 4. Organo-Somatic Index

Groups (n = 5)	Average Brain somatic index
Group A	0.98 ± 0.08
Group B	1.13 ± 0.06*
Group C	0.84 ± 0.07
Group D	1.07 ± 0.06
Group E	0.81 ± 0.03*
Group F	0.97 ± 0.05

*values significantly different from corresponding control ($p < 0.05$) (mean ± SD)

the cerebellum by ascorbic acid against manganese toxicity. The inability of ascorbic acid to completely protect the cerebellum against manganese toxicity may be due to manganese overload from cumulative deposition in the cerebellum or damage to the cerebellar cells through other mechanisms than oxidative stress.

Conclusion

This study showed that increase exposure to manganese dichloride tetrahydrate above required quantity in the body could cause adverse effects such as decreased weight loss and neurodegenerative changes in the histoarchitecture of the cerebellum. This degeneration has been clearly illustrated in the loss of Purkinje cells in the cerebellar tissue. It has been found that co-administration does not fully protect against this damage, which may suggest mechanisms of manganese toxicity to the cerebellum other than oxidative stress. Further studies may consider manganese deposition in the cerebellum from co-administration with ascorbic acid. Understanding the different mechanisms of manganese toxicity to the cerebellum, and the brain at large, is required for development of effective protective measures against this damage.

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