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Moringa oleifera Ameliorates Histomorphological Changes Associated with Cuprizone Neurotoxicity in the Hippocampal *Cornu ammonis* (CA) 3 Region

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Summary: Cuprizone-induced neurotoxicity has severally been used to study demyelinating diseases like multiple sclerosis (MS), adversely affecting both the white and grey matters of the brain. Lesions have been observed in different regions of the brain including, corpus callosum, neocortex and the hippocampal formation. The current study explored the role of *Moringa oleifera* leaf extract in restoring the resultant histomorphological changes in cuprizone-induced hippocampal damage in Wistar rats. Twenty adult female Wistar rats with average weight of 163.74 ± 3.59 g were grouped into A: Control, administered with 1 ml of normal saline, B: received 0.4% cuprizone diet, C: received 1.875 mg/ml/day of *Moringa* extract, and D: received a combination of cuprizone and *Moringa* in similar doses. Administration was oral for 5 weeks. The weights of animals were assessed during treatment, and at the termination of experiment, the rats were euthanized and the brains were fixed in 4% paraformaldehyde. The tissue was processed for histological and histochemical examinations using the Haematoxylin and Eosin stain and cresyl fast violet stain to assess the general microarchitecture and neuronal cells respectively of hippocampal *cornu ammonis* (CA) 3 region. The body weight of cuprizone-treated rats was reduced and this was ameliorated significantly in animals that were co-administered with *Moringa*. Similarly, there were histological alterations in the CA3 region of the hippocampus with the presence of pyknotic pyramidal cells organized in clusters and CA3 cells with degenerative changes, but administration of *Moringa* led to a better organised and fairly intact histological appearance. Pharmaceutical development of *Moringa oleifera* into appropriate therapeutic formulations could offer some relief to patients of demyelinating conditions that have clinical features of neurological deficits.

Keywords*:* cuprizone, neurotoxicity, hippocampus, *Moringa*

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INTRODUCTION

Cuprizone intoxication has been used over time to study experimental demyelination and remyelination in the central nervous system (CNS) (Keegan *et al*., 2006). The copper chelator cuprizone causes a reproducible demyelination in corpus callosum within weeks of administration with spontaneous remyelination occurring after withdrawal (Matsushima *et al*., 2001).

The primary target of cuprizone toxicity is oligodendrocytes and various studies have demonstrated demyelination of hippocampal cortex in cuprizone model (Koutsoudaki *et al*., 2009; Kipp *et al*., 2011; Sun *et al*., 2016), with specific consequences on the behaviours and functions of the animal. The CA3 region of the hippocampus has more abundant internal connectivity compared with other regions of the hippocampus, and plays specific role in memory processes, susceptibility to seizures and neuro-degeneration (Cherubini and Miles, 2015). It is vulnerable to stress and seizure-induced damage (Belvindrah *et al*., 2014). Axonal fibres of CA3 pyramidal cells make excitatory contacts with neighboring excitatory and inhibitory neurons (Cherubini and Miles, 2015). Any injury therefore to this area of the hippocampus, though targeted at the myelin sheath, could affect the neuronal cells, especially pyramidal cells of the hippocampus, whose axons are myelinated by oligodendrocytes, with a resultant overall impairment in the function of the structure.

The cellular processes that occur during oligodendroglial damage involve other glial cells, such as microglia and astrocytes, which act as part of brain defense mechanism against the ongoing cellular injury occasioned by cuprizone cytotoxicity (Remington *et al*., 2007; Hibbits *et al*., 2012). Furthermore, evidence has emerged of damage to neuronal cells as well. A study by Hoffmann *et al*. (2008) demonstrated extensive neuronal degeneration in the hippocampus of cuprizone-treated mice. In addition, significant axonal loss has also been documented (Irvine and

Blakemore, 2008). According to Praet *et al*. (2014), most neurons are likely to survive acute cuprizone toxicity, but are however, more vulnerable to metabolic disturbance compared to oligodendrocytes (Praet *et al*., 2014).

The hippocampus is located in the medial temporal lobe. Its function is implicated in the processes of learning and memory,and is one of the structures of the CNS indispensable to long-term episodic memory (Bird and Burgess, 2008). Lesions in the hippocampus are associated with cognitive defects as seen in large proportions of multiple sclerosis patients (Rao *et al*., 1991). Despite the high functional impact, the knowledge about effective strategies for managing MS remains very low (Amato *et al*., 2006).

Traditionally, *Moringa oleifera* is used in many disease conditions throughout the world (mainly in Thai) (Rastogi *et al*., 2009). Many of its functions have been proven scientifically and these include antihypertensive, analgesic, anti-cancer, CNS depressant, antibiotics, anti-inflammatory, and antiepileptic properties (Faizi *et al*., 1994; Gupta and Mazumder, 1999). With a possibility of hippocampal damage following cuprizone intoxication, the current study explored the cytoprotective, anti-inflammatory, and anti-oxidative properties of *Moringa oleifera* leaf extract in restoring the resultant histomorphological changes in neuronal cells of the hippocampus.

MATERIALS AND METHODS

Twenty adult female rats were obtained and housed in the Animal Facility of the Faculty of Basic Medical Sciences, University of Ilorin. The animals were acclimatized for two weeks.

Experimental Design

The animals were fed on standard rat feeds (except those that received cuprizone diet) and water *ad libitum*. They were divided into four groups. Group A (control): were given 1 ml of normal saline daily; Group B (Cuprizone group): were given 0.4% cuprizone diet; Group C (*Moringa* group): received 1.875 mg/ml *Moringa* leaf extract; while Group D (cuprizone +*Moringa*): received simultaneously 0.4% cuprizone and 1.875 mg/ml *Moringa* (Omotoso *et al*., 2018).

Animal Treatment

Cuprizone (bis-cyclohexanone oxaldihydrazone) was procured from Sigma-Aldrich Inc, Germany. It was prepared as 0.4% cuprizone mixed with standard rodent chow. For all treatment, route of administration was oral. The weights of the rats were taken twice a week. *Moringa oleifera* leaves were obtained and authenticated at the Department of Plant Biology, University of Ilorin. The ethanolic extract of the plant was carried out using the silica gel open column method at the Department of Chemistry, University of Ilorin, Nigeria. Out of all the fractionations obtained during the fractionation process, the MoF_6 fraction had the highest yield quantitatively; this formed the basis of its use in this study.

Termination of experiment

At the termination of experiment at the end of 5 weeks of administration, the rats were euthanised. The head was removed from the rest of the body and the cranium was dissected to remove the brain, which was weighed and fixed in 4% paraformaldehyde solution. The tissues were dehydrated through grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin and coronal sections of the cortex at 5 µ were obtained using rotary microtome. Tissue processing for histology and histochemistry was by the use of Haematoxylin and Eosin (for general architecture of the hippocampus) and cresyl fast violet stains (for demonstration of pyramidal neurons and Nissl bodies) respectively. The mounted sections were viewed with the aid of a light microscope.

Statistical analysis

The weights of the animals were analyzed using one-way analysis of variance (ANOVA). A Tukey post hoc test was used for group comparison when appropriate and a p value less than 0.05 was considered statistically significant.

RESULTS

Physical observation

The cuprizone group showed a decrease in body weight compared to control. A significant increase in body weight was observed in rats fed with *Moringa*. The rats in CPZ+MO group recorded a weight higher than CPZ group (Table 1).

Histological and histochemical observation

The histomorphological presentation of the *cornu ammonis* 3 (CA3) region of the hippocampus of rats in control group showed characteristically large pyramidal neurons with apical and basal dendrites projecting out of the large intensively stained soma. These pyramidal cells were laconically expressed and properly delineated in the CA3 region of *Moringa*-treated control animals (Figures 1 and 2). The pyramidal cells in the CA3 region of the hippocampus of rats treated with cuprizone showed pyknotic pyramidal cells organized in clusters and evidence suggestive of degeneration, including disintegration of neuronal cells, distorted cellular outline and irregular arrangement of cells (Figure 2). The cellular assortment of the CA3 region of the hippocampus alongside the polymorphic layer adjoining it on either side appeared distorted. Comparatively, the cellular assortment of pyramidal cells in the CA3 region of the rats that received a combined treatment of cuprizone and *Moringa oleifera* showed a better assortment and properly delineated cytoarchitectural manifestation

Table 1: Mean and standard error of initial weight, final weight and weight differences of experimental animals

Group	Final Weight (g)	Initial Weight (g)	Weight Difference (g)
Group A: Control	188.00 ±1.73	157.23± 1.65	30.77± 4.22
Group B: 0.4% CPZ	161.21± 2.41	168.93± 1.84	-7.72± 6.13*
Group C: 1.875 mg/ml *Moringa*	183.31 ±1.89	163.58 ±2.08	19.73± 6.44**
Group D: (CPZ+*Moringa*)	177.58 ±2.41	165.68± 1.97	11.9 ±7.95*

* and ** significant differences at p<0.05 relative to control and cuprizone groups respectively. CPZ=cuprizone.

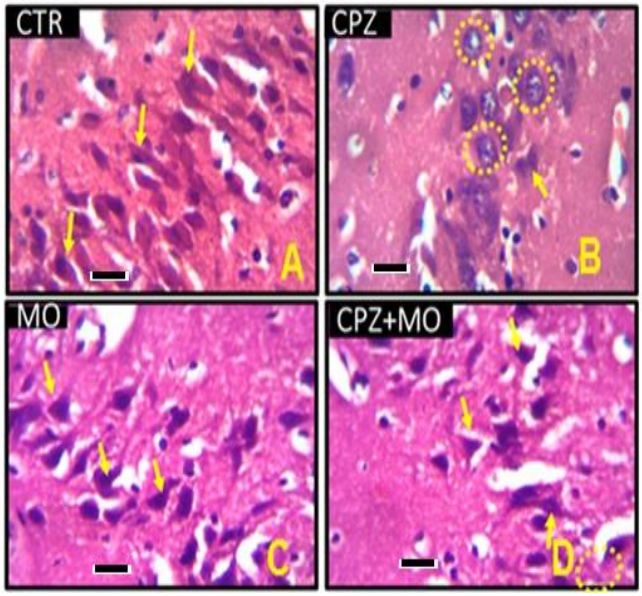

Figure 1: Representative photomicrograph of the *cornu ammonis* (CA3) region of the hippocampus of Wistar rats (H&E stain; Scale bar = 25µ).

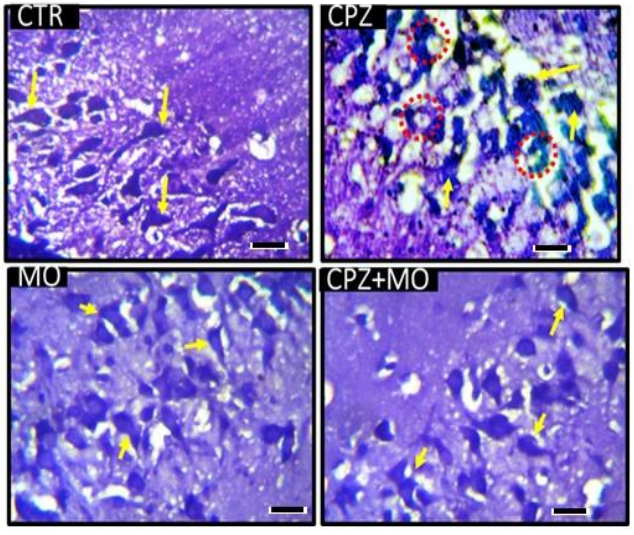

Figure 2: Representative photomicrograph of the *cornu ammonis* (CA3) region of the hippocampus of Wistar rats showing well demonstrated pyramidal cells in the Control, (CPZ) but marked architectural breakdown in the CPZ group (cresyl fast violet stain; Scale bar = 25µ)). CTR = control, CPZ = cuprizone, MO = *Moringa oleifera*, CPZ+MO = cuprizone plus *Moringa oleifera.*

when compared to that of the cuprizone-treated animals.

Histochemical demonstration of the *cornu ammonis* also showed well-preserved pyramidal neurons in the control group which were equally positive for Nissl bodies. The *cornu ammonis* of animals exposed to cuprizone diet had less positivity for Nissl staining, which was ameliorated following the use of *Moringa oleifera* (Figure 2).

DISCUSSION

Cuprizone administration is usually associated with weight loss (Franco-Pons *et al*., 2007; Hoffman *et al*. 2008; Benetti *et al*., 2010). However, according to the study by Benetti *et al*. (2010), mice fed on cuprizone diet lost weight up till the 5th week of exposure, but there was weight gain after this period. Similarly, in the current study, a reasonable level of weight loss was observed in the rats exposed to cuprizone (however, this study did not exceed 5 weeks). When co-administered with cuprizone, *Moringa* led to an appreciable weight gain, though not as high as the weight of rats treated with *Moringa* only.

Moringa oleifera has nutritional benefits and can support growth, as proteins are the most abundant nutrients in the leaf of *Moringa* (Brilhante *et al*., 2017). Although the weight increase in the current study was not up to that of the control, weight increase associated with *Moringa oleifera* leaf extract was able to ameliorate the weight loss associated with cuprizone diet.

In the current work, the micro-architecture of the *cornu ammonis* 3 of the hippocampus suffered some degree of distortion and disintegration in animals that received cuprizone diet, with the evidence of distorted cellular arrangement and degenerated cellular structures. Nissl bodies are rough endoplasmic reticulum present in the soma of neurons and are the site for neuronal protein synthesis (Fedorenko and Uzdensky, 2010). Cuprizone treatment in the current study led to depletion in Nissl bodies store, as revealed by the poor Nissl staining. However, co-administration of *Moringa* with cuprizone resulted in a more positive Nissl staining. This structural observation is corroborated by the work of Praet *et al*. (2014), where cuprizone treatment caused adenosine triphosphate (ATP) shortage and oxidative stress which led to the disruption of the proper functioning of the

endoplasmic reticulum, with a consequent impairment in protein synthesis.

This study further underscores the cytoprotective role of *Moringa oleifera*, as previously documented, in preserving the cellular architecture against toxic insults (Gupta and Mazumder, 1999; Rastogi *et al*., 2009).

Aside having a toxic effect on oligodendrocytes, our work revealed that cuprizone alters the morphology and integrity of pyramidal neurons of the hippocampus. However, co-administration with *Moringa* brought about a better cellular assortment and properly delineated cytoarchitecture of the hippocampal CA3 region. This intervention with *Moringa oleifera leaf extract* could help preserve the integrity of the hippocampus and the axonal internal connectivity vital for restoring and maintaining the neuronal functions of the hippocampus in individuals suffering from dymyelinating conditions of the central nervous system.

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