

Ethanolic extract of *Moringa oleifera* leaves alleviates cuprizone-induced nephrotoxicity in rats

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Abstract

Some medicinal properties have been ascribed to various parts of *Moringa oleifera* (MO), including its therapeutic potentials in gastrointestinal and hepatorenal disorders. In this study, we investigated the potentials of ethanolic leaf extract of *Moringa oleifera* in ameliorating cuprizone-induced nephrotoxicity. Twenty adult female albino rats were grouped into 4: Group A, the Control received 1 ml normal saline; Group B received 0.4% cuprizone (CPZ); Group C was given 1.875 mg/ml *Moringa oleifera* ethanolic leaf extract; and Group D was co-administered with CPZ and MO ethanolic leaf extract at same doses for 5 weeks. Thereafter, the animals were euthanized and intracardial blood samples were collected to assay serum creatinine and uric acid, while the kidneys were fixed in 4% paraformaldehyde for histopathological evaluation. CPZ-treated rats demonstrated significant weight loss and elevated serum creatinine and uric acid levels. The histological framework was characterised by distorted structural arrangement, cellular degeneration and loss of renal corpuscles. These changes were appreciably amended in animals treated with a combination of CPZ and MO ethanolic leaf extract. These findings suggest that MO may relieve renal complications associated with demyelinating diseases.

Keywords: *Moringa*, Cuprizone, Multiple Sclerosis, Renal complications

Introduction

Cuprizone (bis-cyclo- hexanone oxaldihydrazone) is a copper chelator that induces consistent demyelination by causing death of oligodendrocytes [1]. Following withdrawal of the drug, spontaneous remyelination occurs as early as 4 days after [2], underscoring the importance of the cuprizone model to study the pathology of demyelination and the physiology of remyelination [3].

Overtime, the brain has been considered the main target organ implicated in cuprizone toxicity. Nevertheless, few works have reported that cuprizone has variable effects on other organs of the body like kidneys, liver and the heart [4].

Part of the mechanism of action of cuprizone is the formation of megamitochondria, and organs like kidneys, liver and the heart have been studied for the presence of megamitochondria. Hoppel and Tandler [5] showed that cuprizone inhibits mitochondrial-coupled oxidative phosphorylation in the liver in *in vivo* and not *in vitro* studies, while *in vivo* studies of mitochondria of cardiac origin had no effect on oxidative phosphorylation.

Katarai *et al.* [6] considered the kidney as an important site of cuprizone-induced injury. The kidney is an important organ because of its role in getting rid of harmful materials and excretion of drugs and body waste products. It is essential for maintaining the internal environment which allows maximum cellular function as well as removes toxic substances that are generated by metabolism [7].

Moringa oleifera (MO) commonly known as drumstick or horseradish tree is a small to medium-sized deciduous tree native to northern India, Pakistan and Nepal [8] and widely distributed in the tropics, West Africa and Central America as well as the Caribbean [9]. The different parts of the tree including the leaves, roots, seeds and fruits have proven health benefits, and they have been said to possess anti-inflammatory, antioxidant, antitumor, antipyretic, and antihypertensive properties [10]. Both human and animal studies have documented the benefits of *Moringa oleifera* in kidney diseases and its role in improving renal physiology, including the ability of the herbal preparations to decrease the blood levels of urea and creatinine, decrease proteinuria and slow down the progression

of chronic renal disease [11 - 15]. Due to conflicting reports of the effect of CPZ on the kidneys and renal functions, it has become imperative to access the nephrotoxic effect of cuprizone and determine if *Moringa oleifera* could ameliorate such effects if and when present.

Materials and Methods

Approval to use animals was obtained from the Ethical Review Committee of the Faculty of Basic Medical Sciences, University of Ilorin and the study was carried out in line with the rules and guidelines set by the committee. Cuprizone was purchased from Sigma-Aldrich, Germany.

Ethanollic Extraction of *Moringa oleifera* Leaves

Fresh *Moringa oleifera* leaves weighing about 1.5 kg were obtained from in Ilorin, Kwara State and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, where a voucher specimen with a voucher number UILH/001/1008 was deposited. The extraction process was done at the Chemistry Laboratory, University of Ilorin. Fresh MO leaves were air-dried for about two weeks and kept away from sunlight to avoid thermal degradation. After this, the leaves were pulverized to allow adequate solvent penetration into them. The pulverized leaves were divided into two portions; 500 g and 789 g. Column chromatography was employ to fractionate the extract. The process of separation of *Moringa* concentrate and ethanol using rotary evaporation was done. The *Moringa* concentrate was then passed through column chromatography for fractionation in a silica gel open column. Out of all the fractionations obtained, the MoF₆ fraction was used for this study to access any possible role in alleviating potential renal damage associated with cuprizone.

Experimental Animals

Twenty (20) adult female albino rats with average weight of 150±3 g were sheltered in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin. The rats were allowed to acclimatize for two weeks, and fed on standard pelletized growers mash and water. The nutrient content of the feed was as stated: 10% crude fibre, 15% crude protein, 7% Fat, 1% calcium, 0.35% phosphorus and 2,550 kcal/kg of metabolized energy.

The rats were randomly grouped into 4:

- i. Group A (Control) received 1 ml of Normal saline for five(5) weeks.
- ii. Group B (Cuprizone) received 0.4% CPZ for five (5) weeks.
- iii. Group C (*Moringa*) received 1.875 mg/ml *Moringa* leaf extract for five (5) weeks.
- iv. Group D (Cuprizone and *Moringa*) received 0.4% CPZ and 1.875 mg/ml *Moringa* leaf extract for five (5) weeks.

Animal Sacrifice and Tissue Collection

The rats were euthanized a 24 hr after the last administration. Blood samples were obtained for biochemical analysis and the kidneys were also excised, decapsulated, weighed and fixed in 4% buffered paraformaldehyde for histological evaluation.

Biochemical Analysis

Biochemical analysis was carried out to assay the levels of serum creatinine and uric acid. The results obtained were subjected to statistical analysis using the version 16.0 of the Statistical Package for Social Sciences (SPSS) software. Data were presented as mean $\pm$ SEM. Values were considered to be significant at $p < 0.05$ and $p < 0.01$. The results obtained from the SPSS software was used to plot graphs using the 10.0 version of the graph pad application.

Results

Changes in Weight of Experimental Animals

The changes in the weight of experimental animals were compared across the groups (Figure 1). Animals in cuprizone group showed a significant decrease ($p < 0.05$). in weight compared to the control The loss of weight experienced by animals in cuprizone group was also significant ($p < 0.01$) when compared to the *Moringa* group. Animals that received *Moringa* only had higher weight increase compared to the control ($p > 0.05$) and cuprizone group ($p \leq 0.01$).

Comparing the kidney weight of animals in the other experimental groups with the control, no significant difference was observed ($p>0.05$) (Figure 2). The relative kidney weight was compared across the groups, no statistically significant difference was found between the treated groups and the control ($p>0.05$) (Figure 3).

Biochemical Analysis

Figures 4 and 5 show the mean and the standard deviation of creatinine and uric acid levels in all the treatment groups. The levels of creatinine increased significantly ($p<0.01$) in the cuprizone treated group compared to the control group. This increase was also significant ($p<0.01$) compared to the *Moringa* treated group. A significant increase ($p<0.05$) was also observed in the group treated with both cuprizone and *Moringa* compared to the *Moringa* group.

An increase in the level of serum uric acid was observed in the group treated with cuprizone compared to the control, although this increase was not statistically significant ($p>0.05$). However, the increase was statistically significant ($p<0.05$) in the group treated with cuprizone and *Moringa*.

Histological Observations

The renal parenchyma of rats in the Control appeared normal, and no significant alterations were observed in the renal tissue of animals exposed to *Moringa* compared to the Control. However, the kidney architecture of rats that were fed on cuprizone diet showed marked derangement from the normal, with obvious irregular outline, distorted cellular arrangement, cellular degeneration and disintegration and loss of renal corpuscles. The severity of the degree of changes was lower in the cuprizone + *Moringa* group, compared with the cuprizone group (Figures 6 and 7).

Discussion

The kidneys are important organs necessary for normal functioning of the urinary system and regulation of homeostasis [16]. Cuprizone is a toxic substance that has been used extensively to study the pathogenesis of de- and re-myelination in the central nervous system [3,7, 18], but not many works have been conducted on its effects on organs of the body outside the nervous system. *Moringa oleifera* used in this study was associated

with increase in body weights exceeding those of Control rats. Although cuprizone treatment was associated with loss of weight, just as previously reported [19, 20], co-treatment with *Moringa oleifera* significantly abolished cuprizone-induced weight loss [21]. Patients with multiple sclerosis have been noted to present with impaired renal functions [22] and they are said to be prone to acute injury rather than a chronic renal pathology [23]. The occurrence of acute renal failure in these patients is related to impaired bladder function with resultant recurrent urinary tract infection and development of kidney stones [24, 25] and females appear to be more affected by these renal complications [26].

The kidney maintains the blood creatinine and uric acid in a normal range. Creatinine and uric acid have been found to be indicators of kidney function. Elevated creatinine and uric acid levels signify impaired kidney function or kidney disease [27].

Serum uric acid is commonly elevated in subjects with kidney disease [28]. Several studies have documented that an elevated serum uric acid level independently predicts the development of kidney pathologies [27, 29].

Observations from the current study further strengthen the view of the presence of renal pathology in individuals with multiple sclerosis [30, 31]. Cuprizone toxicity markedly raised the levels of creatinine and uric acid. Elevated uric acid level has been implicated in development of chronic kidney diseases [27]. On the other hand, exposure to *Moringa oleifera* lowered creatinine and uric acid levels in experimental animals when compared with the Control, and this may suggest a beneficial role in favour of renal functions. This was also reflected in the animals co-administered with cuprizone and *Moringa oleifera*, where it was observed that *Moringa oleifera* was able to reduce the high levels of creatinine and uric acid in the serum to levels close to the Control.

The distorted cellular layout and cytoarchitecture of the kidneys observed in the cuprizone treated rats and the marked diffuse glomerular degeneration and enlargement of Bowman's space when compared to the control group may be as a result of the cytotoxic effects of cuprizone on the microanatomy of the kidney with consequent derangement in functions that could be detrimental to the health status of the animals. The histopathological alteration in the kidney of animals treated with CPZ

which is followed by elevated uric acid level is consistent with previous findings that suggested that elevated serum uric acid level is associated with both the presence of intrarenal arteriolar lesion [28, 29].

The microarchitectural re-organisation perceived in the kidney of rats co-administered with cuprizone and *Moringa oleifera* may be part of the attempts of *Moringa oleifera* ethanolic leaf extract to repair possible structural damage associated with cuprizone ingestion. This development also corroborates the biochemical changes observed in the serum levels of creatinine and uric acid as earlier mentioned. This could positively affect the physiological functions of the kidney and improve glomerular functions.

The proposed mechanism of *Moringa oleifera* to reduce nephrotoxicity might be due to its antioxidant properties [32]. Antioxidant potential of this herb has been reported in many studies [33-35]. The various parts of *Moringa oleifera* plant (especially the seeds and leaves) are documented to contain many active constituents as glucosinolates, isothiocyanates, thiocarbamates and various flavonoids which contributed to the antioxidant activity [36]. Flavonoids in MO have been reported to inhibit chemical-induced nephrotoxicity due to its potent antioxidant activity [37].

Conclusion

In conclusion, cuprizone consumption elevates serum creatinine and uric acid levels and disrupts renal architecture, while co-administration of the MoF₆ fraction of ethanolic extract of *Moringa oleifera* leaves ameliorated these effects. Hence, ethanolic extract of *Moringa oleifera* leaves may offer some beneficial effects to patients of multiple sclerosis or other related demyelinating conditions in managing associated renal involvement or complications.

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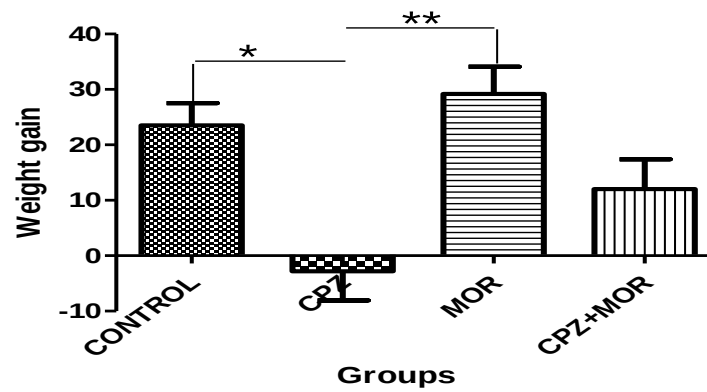


Figure 1: Body weight changes of the control, cuprizone (CPZ), *Moringa* (MOR) and cuprizone+*Moringa* (CPZ-MOR) groups, with statistically significant difference between groups at $p < 0.05^*$ and $p < 0.01^{**}$.

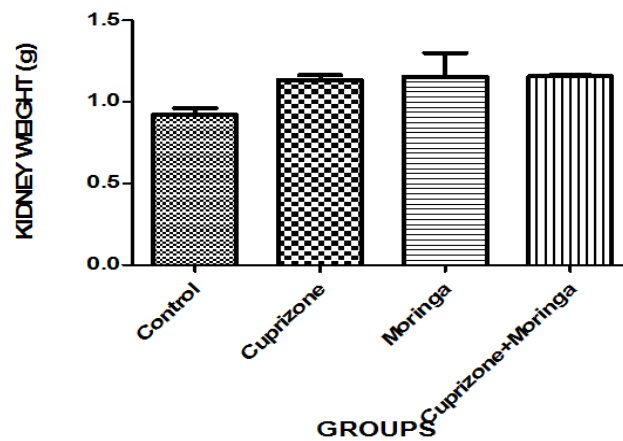


Figure 2: Organ (Kidney) weights of the control and treated animals, showing no statistically significant difference between groups

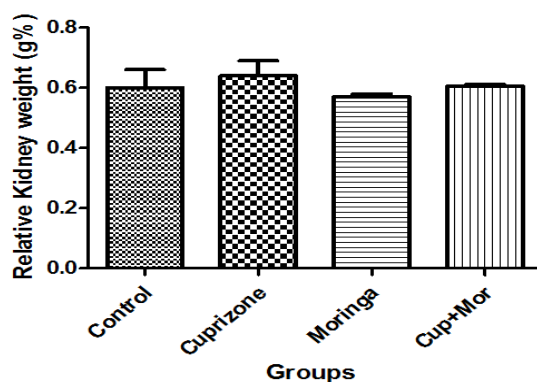


Figure 3: The relative kidney weights of the control, cuprizone, *Moringa* and cuprizone+*Moringa* (CPZ-MOR) groups of treated animals, showing no statistically significant difference between groups.

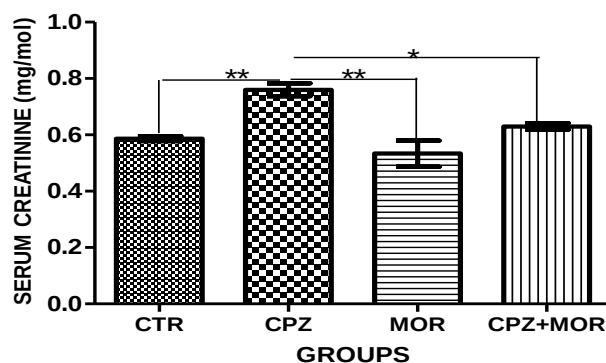


Figure 4: Changes in the levels of serum creatinine of the control (CTR), and animals treated with cuprizone (CPZ), *Moringa* (MOR) and cuprizone plus *Moringa* (CPZ-MOR), showing with statistically significant difference between groups at $p < 0.05^*$ and $p < 0.01^{**}$ compared with control.

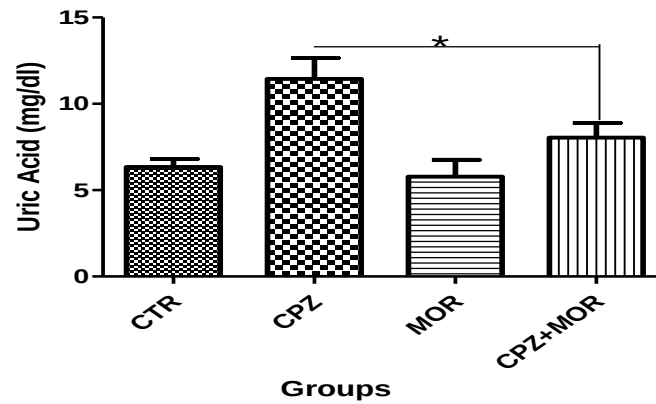


Figure 5: Changes in the levels of serum uric acid of the control (CTR), and animals treated with cuprizone (CPZ), *Moringa* (MOR) and cuprizone plus *Moringa* (CPZ-MOR), showing with statistically significant difference between groups at $p < 0.05^*$, compared with control.

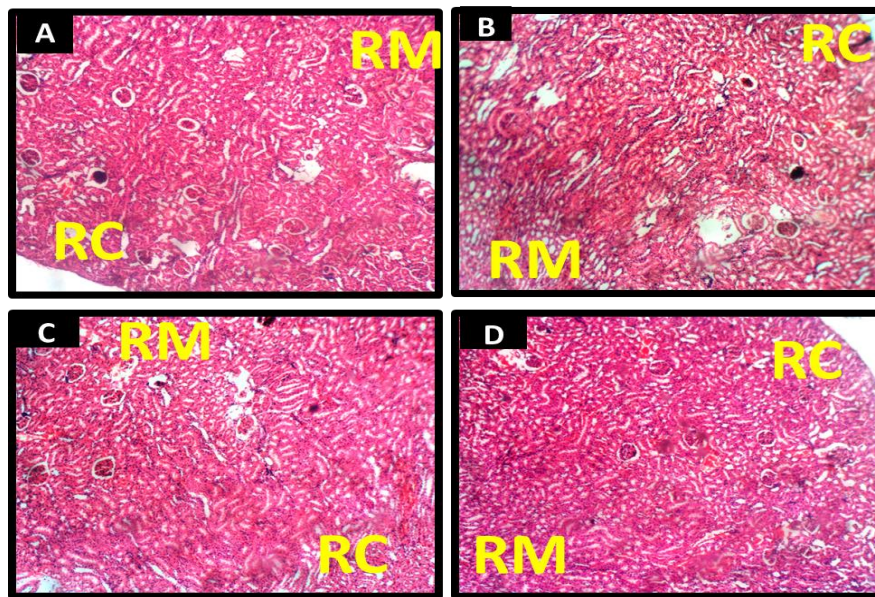


Figure 6: Representative photomicrographs of a panoramic view of the kidney revealing the renal cortex (RC) and renal medulla (RM). A= control, B = cuprizone, C = *Moringa*, D = cuprizone and *Moringa*. Haematoxylin and Eosin x40

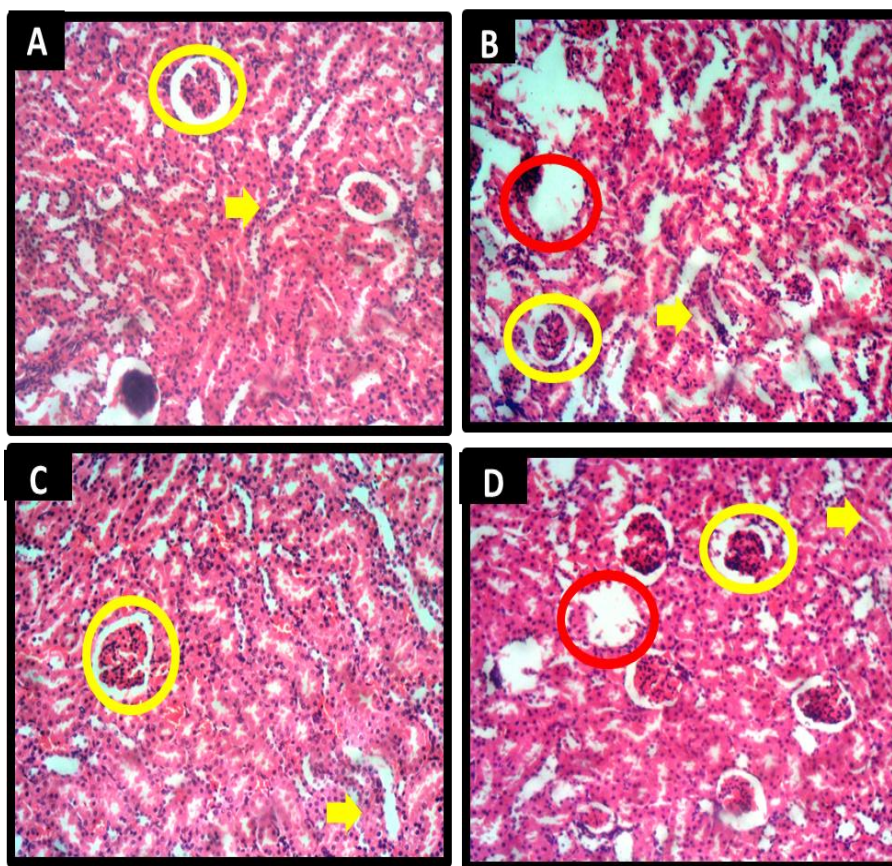


Figure 7: Representative photomicrographs of kidney showing disruptive and degenerating changes in B and distortions of the normal architectural pattern. A and C showed apparently normal renal corpuscle (yellow circle) and a well arranged cytoarchitecture (yellow arrow), while B and D presented with loss of renal corpuscle cells (red circle). A= control, B = cuprizone, C = *Moringa*, D = cuprizone and *Moringa*. Haematoxylin and Eosin; x100