

Ameliorative Effect of *Moringa oleifera* (drumstick) Leaf Extracts on Chromium-Induced Testicular Toxicity in Rat Testes

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ABSTRACT [ENGLISH/ANGLAIS]

Exposure to heavy metals has been known for its adverse effects on many body organs and Systems and thus their functions. Hence, this study was aimed to determine the ability of polyphenolic-rich *Moringa oleifera* Leaf Extract (MoE) in protecting rat testis against chromium-induced impairments based on some histopathological and biochemical clarification. Forty male Wistar rats were divided into four groups of ten animals each. The animals were administered either 150pp mg/kg potassium dichromate or 60 mg/kg body/weight of *Moringa Oleifera* extract (MoE) or the combination, for 100 days. The animals were sacrificed and testicular weight and volume and sperm parameters were determined. Chromium treated groups exhibited a significantly decrease in testis weight/volume, testis weight/body weight ratio, sperm parameters compared to the control and *Moringa oleifera* treated groups. Concurrent exposure to chromium and *Moringa Oleifera* extract significantly enhanced the sperm parameters compared to rats exposed to chromium alone. This showed that changes brought about by chromium toxicity were mildly averted by simultaneous treatment of *Moringa oleifera* leaf extract. This study concludes that chronic exposure to chromium produces marked testicular toxicity which can be prevented by concomitant administration of *Moringa oleifera* leaf extract.

Keywords: *Moringa oleifera*, chromium, toxicity, sperm parameters

RÉSUMÉ [FRANÇAIS/FRENCH]

L'exposition aux métaux lourds a été connu pour ses effets néfastes sur de nombreux organes et de Systèmes et donc leurs fonctions. Ainsi, cette étude visait à déterminer la capacité des polyphénoliques riches *Moringa oleifera* Leaf Extract (ME) dans la protection contre le testicule de rat induit des déficiences en chrome repose sur quelques éclaircissements histopathologiques et biochimiques. Quarante rats mâles Wistar ont été divisés en quatre groupes de dix animaux chacun. Les animaux ont été administrés soit bichromate de potassium 150pp mg / kg ou 60 mg / kg de poids corporel / poids de *Moringa oleifera* extrait (ME) ou la combinaison, pendant 100 jours. Les animaux ont été sacrifiés et le poids des testicules et le volume et les paramètres spermatiques ont été déterminés. Les groupes traités présentaient une chrome diminuer significativement dans le testicule poids / volume, rapport du poids des testicules poids / corps, les paramètres du sperme par rapport à la commande et de *Moringa oleifera* groupes traités. Exposition simultanée à du chrome et du *Moringa oleifera* extraire considérablement amélioré les paramètres du sperme par rapport à des rats exposés à du chrome seul. Cela a montré que les changements apportés par le chrome de toxicité ont été légèrement évitée par un traitement simultané de *Moringa oleifera* extrait de feuille. Cette étude conclut que l'exposition chronique au chrome produit une toxicité testiculaire marquée qui peut être prévenue par l'administration concomitante de *Moringa oleifera* extrait de feuille.

Mots-clés: *Moringa oleifera*, le chrome, toxicité, les paramètres du sperme

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INTRODUCTION

Metal induced toxicity is very well reported in literature. However, certain heavy metals like Cobalt, Copper, Iron, Manganese, Molybdenum, Selenium Zinc, manganese, and selenium occur in minute quantity known to be essential for metabolic functions and growth of the cells in the body [1, 2].

Abundant reports on the toxicity of certain heavy metal on human organs have been on the rise due to the pervasiveness of these metals in the environment. Arsenic, cadmium, lead, mercury, chromium and their inorganic compounds, are possibly the most potentially toxic metals in the environment. These metals have many industrial uses, which increase the possibility of human exposure resulting into various distinctive [3, 4, 5].

Chromium compounds have been reported to exert toxic effects on body tissues [6, 7, 8]. Two kinds of chromium compounds, chromium III and Chromium IV persist but with the latter being more toxic than the former because of its easy permeation through the cell by sulfate transport system [8].

On the other hand *Moringa oleifera* (MoE) tree also known as drumstick tree is a rapid growing deciduous shrub or small tree of about 13m tall and 35 cm in diameter with an umbrella-shaped open cap [9]. It has also been reported [10] that, *Moringa oleifera* oil and micronutrients contain antitumor, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters. *Moringa oleifera* (MoE) contains specific plant pigments with demonstrated powerful antioxidative ability such as vitamins C, E, A, caffeoylquinic acids, carotenoids - lutein, alpha-carotene and beta carotene, kaempferol, quercetin, rutin [11, 12, 13].

In view of the fact that chromium IV has been shown to induce testicular toxicity, the effect being through oxidative stress and the antioxidant properties of phytochemical constituents of MoE influence oxidative stress in tissue of rats, we therefore aimed at investigating the protective efficacy of MoE on chromium induced testicular derangement in rat.

MATERIALS AND METHODS

Animals

Forty male Wistar rats (*Rattus norvegicus*), six weeks old, weighing about 190-200g were used as experimental animals in this study. They were obtained from the Animal Facility of Department of Anatomy, College of Medicine University of Ilorin, Nigeria. The animals were kept in the research section of the animal house of the Department of Anatomy University of Ilorin, Nigeria and allowed to acclimatize over a period of two weeks. The chosen animals were housed in stainless steel cages at standard atmospheric temperature (25±5 °C) and had a 12/12- hour light-dark cycle (light on at 6.00-18.00h). They were fed (rat chow) and allowed access to water ad libitum.

Chemicals

Potassium dichromate ($K_2Cr_2O_7$) was obtained from the department of Chemistry, University of Lagos, Nigeria. As described by Radike et al. [14], it was dissolved in deionised water at a concentration of 150 ppm chromium.

Moringa oleifera and the Aqueous Extraction Procedure

Samples of *Moringa oleifera* leaves were collected from a plantation in Nasarawa state, Nigeria on 15th of July, 2010. The leaves were authenticated by Dr A.B Kadiri in the herbarium of the department of Botany, University of Lagos, Lagos state, Nigeria and a voucher specimen number DCN 87 was referenced. The leaves were carefully washed in sterilized water and the water was then sapped from the leaves.

Animal Grouping and Treatment

The rats were at random divided into four experimental groups: A (Control), B (MOE treated), C (Chromium treated) and D (Chromium + MOE treated), with each group containing 10 animals. The MOE treated, chromium treated and Chromium + MOE treated groups received oral administration of potassium dichromate dissolved in deionised water at a concentration of 150ppm chromium and oral administration of 50 mg/kg of MOE daily [14,15]. The control groups were administered (orally) distilled water at 2.5 ml/kg b.w all through the experiment [15]. All treatments were for eight weeks.

Acute Oral Toxicity Study of *Moringa oleifera* Leaves Extract

As described earlier by Saalu et al. [15], the acute oral toxicity study for *Moringa oleifera* leaves extract was carried out using the Organization for Economic Cooperation and Development (OECD) (2000) Guidance Document on Humane End points that should condense the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure. Six animals were weighed and independently identified. The first animal was given the test dose – *Moringa oleifera* leaves extract 2000 mg per kg body weight. The second and third animals were in tandem dosed and the fourth and fifth animals successively dosed. The results were evaluated as follows, S (which denotes survival) and X (which denotes death). The animals were observed individually at least once during the first 40 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 5 hours), and daily thereafter for a total period of 14 days. All observations were systematically recorded with individual records maintained for each animal.

Animal Sacrifice, Organ Weight and Volume Evaluation

The rats were at the time of sacrifice first weighed and then cervical dislocation was carried out. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs. The testis were detached and cleared free of the surrounding tissue. The testis was weighed with an electronic analytical and precision balance, while the testis volumes were measured using immersion method.

Sperm Characteristics

As described earlier by Saalu et al. [16,17], Sperm motility, concentration and progressive motility were determined by removing the cauda part of epididymis and placing it in a beaker containing 1 mL physiological saline solution after which each section was quickly incised with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the saline solution. Semen drops were placed on the slide and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined under the microscope using X40 objective for sperm motility. Sperm count was done under the microscope using enhanced Neubauer haemocytometer.

Estimation of Plasma Levels of Testosterone

As earlier described by Saalu et al. [16], Plasma testosterone concentrations were estimated using the Enzyme Immunology Assay kits (Immunometrics, London U.K) according to manufacturer's protocol. Plasma samples were collected and stored at -20°C until assayed. The EIA kits contain controlled testosterone EIA substrate reagents and EIA quality control samples. A quality control sample was run for the hormone at the beginning and at the end of the assay variation. The EIA kit used had a sensitivity level of 0.3nmol/L (0.1 ng/mL). The intra and inter assay variations were 11.00% and 10.10%, respectively.

Assay of Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity was measured by the method described by Saalu et al. [15]. The reaction mixture contained 2.0ml of 0.4M Tris- HCL buffer, pH 7.0, 0.01 ml of 10mM sodium azide, 0.2ml of enzyme, 0.2ml of 10mM glutathione and 0.5ml of 0.2mM. H_2O_2 . The contents were incubated at 37°C for 10 minutes followed by termination of the reaction by the addition of 0.4ml 10%

(v/v) TCA, centrifuged at 5000 rpm for 5 minutes. The absorbance of the product read at 430nm and expressed as nmol/mg protein.

Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation in the testicular tissue was estimated calorimetrically by thiobarbituric acid reactive substance TBARS method [18]. A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagent (thiobarbituric acid 0.37% TCA) and placed in water bath for 15 min, cooled. The absorbance of clear supernatant was measured against reference blank at 535nm. Concentration was calculated using the molar absorptivity of malondialdehyde which is $1.56 \times 10^5 \text{M}^{-1}$ and expressed as nmol/mg protein.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA), and are generally presented as mean \pm SEM. Statistical significance between each group pairs was based on t – test.

RESULTS

Acute Oral Toxicity Studies

There were no deaths of mice dosed at 2000mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD50 was calculated to be greater than 3000mg/kg body weight /orally.

Gross Anatomical Parameters

Body Weight Changes

Table 2 shows that rats in Chromium treated and Chromium/MoE treated groups lost body weight compared to rats in the control and MoE treated groups. Though the weight loss in chromium treated group was more significant ($p < 0.005$) compared to chromium/MoE treated group ($p < 0.05$).

Changes in Testis Weights and Testis Volume

Table 2 also shows that testis weight, testis volume and testis weight/body weight ratios of the rats in chromium treated groups were significantly lower ($p < 0.005$) than rats treated with chromium/MoE ($p < 0.05$). There was no significant change in the testis weight, testis volume, testis

weight/body weight ratio in the control and MoE treated groups.

Testosterone Level

Table 3 showed a significant ($p < 0.005$) decrease in the testosterone (TT) level of the chromium treated group when compared to the control and MoE treated groups. Following co-treatment with MoE, the level of TT increase significantly ($p < 0.05$) when compared to the chromium-alone treated groups.

Testicular Oxidative Stress

As shown in table 3, the activities of testicular enzyme-glutathione peroxidase (GPx) in chromium treated groups decreased significantly ($p < 0.005$) when compared to the control and MoE treated groups. Following co-

administration with MoE, the activity of GPx increased significantly ($p < 0.05$) in the chromium/MoE treated groups compared to the chromium-alone treated groups but as shown in table 3, the changes in the activities of GPx in the chromium/MoE treated groups was not significant when compared to the control and MoE treated groups.

The testicular content of Malondialdehyde (MDA) in the chromium-alone treated groups was significantly elevated ($p < 0.005$) when compared to the control and MoE treated groups. There was a significant ($p < 0.05$) decrease in the MDA level of the chromium/MoE treated groups when compared to chromium-alone treated groups. However, the changes were not significant when compared to the control and MoE treated groups (Table 3).

Table 1: This table shows the results of Acute Toxicity Test for *Moringa oleifera* leaves Extract (Up and Down Procedure) in Rats

Test serial number	Animal Identity	Dose of MoE mg/kg	Short term result (48 hours)	Long term results (14 days)
1	REP	2000	S	S
2	LEP	2000	S	S
3	TC	2000	S	S
4	RLT	2000	S	S
5	LLT	2000	S	S
6	I	2000	S	S

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RLT = Right leg tagged; LLT = Left leg tagged, I = Intact rat

Table 2: This table shows the changes in gross anatomical parameters of control and treated Wistar rats

Treatment groups	Initial body weight (g)	Final body weight (g)	Body weight Diff (g)	Testis weight (g)	Testis volume (ml)	Testis weight /Body weight ratio
Control	210.5±7.4	210±6.0	1.4	1.35±2.4	1.33±2.5	0.006
MoE	200±3.5	200±4.5	1	1.30±4.3	1.31±3.3	0.006
Chromium	225.5±2.8	197.5±1.3	28**	0.40±0.2**	0.41 ± 0.3**	0.002**
Chromium/MoE	215.5±6.5	210.7±4.8	4.8**	0.98±0.4*	1.0 ± 0.2*	0.005*

Values are expressed as mean ± SD for n=10; * $p < 0.05$, ** $p < 0.005$ significantly dissimilar from control.

Table 3: This table shows the serum testosterone (TT), testicular antioxidative enzyme (GPx) and testicular MDA

Treatment groups	TT (ng/mL)	GPx (nmol/mg protein)	MDA (nmol/mg protein)
Control	2.02±0.7	0.85±0.14	0.90±0.16
MoE	2.15±2.1	0.74±0.04	0.85±0.3
Chromium	1.44±0.3**	0.35±0.05**	2.51±0.05**
Chromium/MoE	1.98±3.1*	0.69±0.16*	1.44±0.15*

Values are expressed as mean±SD for n=10; * $p < 0.05$, ** $p < 0.005$ significantly dissimilar from control.

Sperm Parameters

Morphology

As shown in Table 3, sperm morphology of the control, MoE and chromium/MoE treated groups were normal compared to chromium treated group which showed a significant ($p < 0.005$) decline in the number of sperms that are morphologically normal (increase in morphologically abnormal sperm).

Concentration

Chromium-alone exposed group showed a significant ($p < 0.01$) decline in sperm concentration (oligospermia) compared to both the control and MoE treated groups (Table 3).The Chromium/MoE treated group showed a serene oligospermia; With their sperm concentration being significantly lower ($p < 0.05$) than both the control and MoE treated groups.

Motility

As shown in table 3, there was a significant decrease ($p < 0.05$) in sperm motility of both the chromium and chromium/MoE treated groups as compared to the control and MoE treated groups. However there was no significant difference in sperm motility of the chromium

and chromium/MoE treated groups when compared to each other.

Progressivity

Spermatozoa in chromium and chromium/MoE treated groups showed a slothful progressive movement compared to the rapid progressiveness showed in the control and MoE treated groups (Table 4).

DISCUSSION

Reproductive toxicity from heavy metal exposure in males is one of the areas of concern in toxicology today and our result in this present study showed that exposure to chromium (150ppm chromium/orally for 100 days) in rat caused stern testicular toxicity resulting in the obstruction of spermatogenesis and steroidogenesis, evidenced by the reduction in the testicular weight/body weight ratio, sperm concentration, testosterone level and increase in the activities of GPx and testicular content of MDA of the chromium-alone treated group. Our result is in conformity with the report by Roy et al. [4], which showed that sodium chromate in rats caused shrinking of nuclear size of testicular cells and decline in cell population of spermatogenic cells.

Table 4: This table shows the sperm parameters

Treatment Groups	Sperm count($\times 10^6$ /ml)	Sperm motility%	Sperm progresivity		Sperm morphology %Normal
Control	120.3±3.4	73.6±1.5	a1	71.6±2.1	6.7±3.3
MoE	127±5.7	70.2±0.7	a1	75.2±1.3	7.3±4.1
Chromium	48.6±2.2**	30.4±1.3*	b1	50.1±2.2**	21.2±7.1**
Chromium/MoE	98.3±5.7*	56.3±1.5*	b1	70.1±3.1	6.0±1.1

A sperm is alleged to be morphologically abnormal if the head is detached or undeveloped tail. Values are expressed as mean±SD for n=10 in each group
* $p < 0.05$, ** $p < 0.005$ significantly different from control. a1= Rapid linear motility; b1= Non or slow linear motility

Numerous reports have shown that testicular toxicity of chromium results to male subfecundity, spermatogenic and steroidogenic impairment [8, 19, 20].

Our end result also showed that concurrent treatment with *Moringa oleifera* (50 mg/kg/orally for 100 days) slightly prevented the toxicity brought about by chromium exposure and this is evidenced by an enhancement in the testicular morphology and an improved sperm concentration, motility and plasma testosterone coupled with a decrease in the activity level of GPx and Testicular MDA in chromium/MoE treated group.

Metal induced toxicity is very well reported in literature and one of the main mechanisms behind heavy metal toxicity has been ascribed to oxidative stress [6, 22, 23]. Evaluation of lipid peroxidative activities of antioxidative enzymes such as GPx in tissues has always been used as a biomarker for tissue damage [15, 19]. Hence, increase in the activities of GPx and testicular level of MDA from this present study proved that chromium caused oxidative and testicular injury. A growing amount of study has provided abundant evidence which has established the fact that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules [23]. One of the best

indications supporting this hypothesis is provided by the wide spectrum of nucleobase products typical for the oxygen attack on DNA in cultured cells and animals [24].

Diverse reports in the past few decades have shown that chromium intoxicates the testis by generating reactive radicals, which results in cellular damage like diminution of enzyme activities, damage to lipid bilayer and DNA [4,19], amounting to amplified oxidative stress damages in the sperm membranes, proteins and DNA. This may perhaps explain the reduced testis weight/body weight ratio, sperm concentration, motility and plasma testosterone with complementary increase in abnormal sperm rates as seen in the chromium-alone treated group. Simultaneous treatment with *Moringa oleifera* leaf extract, which has been severally shown to contain bioflavonoid and other effective antioxidants [15, 27, 28] resulted in a notable amelioration of the unbalanced sperm parameters of the testis as seen in the chromium/MoE treated group. *Moringa oleifera* leaf extract pretreatment has been shown to shield testes from a variety of toxic substances [19]. Siddhuraju and Becker [12] and Saalu et al. [15] reported that *Moringa oleifera* contains fundamental antioxidant and phenolic compounds that help in protecting the testis against morphologic, spermatogenic and oxidative changes brought about by toxic materials and certain antineoplastic agents.

Knowing that toxic actions of metals are oxidative in nature, which has been shown by Antilla [7], Stohs and Bagchi [19], and Leonard et al. [23]; it is indicative that *Moringa oleifera* was able to attenuate the toxicity of chromium due to its antioxidative potential. This is in agreement with studies by Saalu et al. [15], Bonde [25], and Faizi et al. [26] which have shown the antioxidative properties of *Moringa oleifera* and its ability to elevate a variety of antioxidant enzymes and testicular biomarkers. This could provide a rationalization for the finding in our study why the experimental group treated with chromium/MoE showed enhanced sperm parameters and decrease in activities of GPx and Testicular MDA. In this regard, our results are in accordance with the report [18] which proved that the sperm cytoplasm contained very low concentrations of free radical-sweeping enzymes and as a result; an increase in the antioxidant enzyme system levels by *Moringa oleifera* treatment can favor reproductive potentials. From this present study, we could say that the toxic effects of chromium on rat testis can be prevented by *Moringa oleifera* leaf extract.

Though further studies to clarify the clear-cut mechanism and actual concentration of *Moringa oleifera* leaf extract required for such protection, are suggested.

CONCLUSION

The increased oxidative stress resulting from chromium intoxication in testicular tissue might be accountable, at least in part, for the histopathological changes evidenced in our study. However, *Moringa oleifera* leaf extract had a protective effect against the toxicity which was evidenced by improvement in sperm parameters of rats treated with chromium and *Moringa oleifera* leaf extract.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors

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