

Pre and Post-Treatment Effects: Estimation of Serum Testosterone and Lipid Peroxidation Levels on *Moringa olifera* Extract Induced Cadmium Exposed Rats

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ABSTRACT

Background: Cadmium (Cd), is a toxic metal which affects various organs including testis. It produces oxidative stress leading to male infertility. Moringa tree, is a natural plant with a great therapeutic value and hence it is found to be effective both in prevention and treatment of various conditions including reducing toxicity of hazardous materials. The aim of the present study was to examine the effects of Pre-and Post-treatment with *Moringa oleifera* leaf extract (MoE) on testis in cadmium exposed rats. **Materials and Methods:** The present study was conducted at the Department of Physiology, Kasturba Medical College (KMC), Mangalore, Manipal University (MU), Karnataka, India, between (2011-2013). This prospective study consisted a total of 30 rats. These were divided into 5 groups with group I being the control. Data were presented as mean \pm SD. student's *t* test was used as statistical tool, $p < 0.05$ considered statistically significant. Group IV and V were pre-and post-MoE treated groups respectively. Serum testosterone and tissue lipid peroxidation levels were estimated. **Results:** Treatment with MoE prior and after administration of cadmium, respectively showed an increase significantly in the testosterone levels and a decrease in the tissue lipid peroxidation as compared to the group treated with cadmium. However, the pre-treatment showed better results in combatting the toxic effects of cadmium. **Conclusion:** This study shows that *Moringa oleifera* leaf extract altered the testosterone and tissue lipid peroxidation levels. Also, pre-treatment showed better outcome.

Key words: Cadmium, MDA, Testosterone, Oxidative stress, *Moringa oleifera* extract.

INTRODUCTION

Cadmium has been found to produce various dysfunctions in our body, physiologically and biochemically. It can also lead to injury to the tissues.¹ Previous studies have shown that one of several mechanisms through which cadmium exerts its toxicity is by altering the antioxidant defence systems and increasing the production of cellular reactive oxygen species, like singlet oxygen, hydrogen peroxide and hydroxyl radicals.² This is usually followed by the development of oxidative stress in the target organs.^{3,4} Cadmium toxicity affects many organs adversely like, kidney, liver, lung, pancreas, prostate, ovaries, placenta etc. including testis.^{5,6} *Moringa oleifera* of Moringaceae family is one of the plant species that provide medicinal herbs which has been scientifically evaluated for its possible medicinal applications.⁷ Cadmium toxicity leads to alterations in the antioxidant defense system by fetching changes in the lipid peroxidation levels.⁸ *Moringa oleifera* is considered as a highly nutritious herb which has a lot of medicinal values and source of vitamins, minerals and other

phytochemicals with antioxidant property.⁷ Hence, extracts of this plant is indicated as an anti-cancer, anti-inflammatory and hepato-protective agent.^{9,10} Its anti-diabetic actions also has been demonstrated. Till recent times, there are ongoing scientific researches being carried on some of the reported medicinal properties of this plant.

Male infertility is one of the important problems nowadays and affects almost about 10% of couples in the society. Recently there are few studies reported on *Moringa oleifera* in improving the fertility rate. A study done by the Bureau of Plant Industry (BPI) revealed that the sperm count in men can be boosted by a steady diet of *Moringa* fruit.^{12,13} But till date there are no studies reported the comparative results *Moringa oleifera* prior and after the treatment on serum testosterone and tissue lipid peroxidation levels in cadmium exposed rats. So, the present study was designed to elucidate the effect of

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extract of *Moringa olifera* leaf on damage of testicles after the exposure of cadmium in wistar rats.

MATERIALS AND METHODS

The chemicals required for the biochemical assay were obtained from Durga Laboratories, India. Experiments were carried out after approval from the Institutional Animal Ethics Committee (IAEC).

Preparation of Extract

The leaves were collected and validated by a taxonomist. After drying the leaves at 37°C, powdered form of extract was prepared. 20 gm of powder was soaked in 100 ml of water and chloroform in a shaker for three days. It was further concentrated. Filtrate of this was concentrated to 10 % of its original. We concentrated the filtrate in rotary evaporator and dried in water bath.¹⁴

We procured male *Wistar Albino* rats (4 months old) from the institutional animal house. They were provided food and water *ad libitum*. Rats were maintained and procedures were conducted according to the institutional ethical committee guidelines.

Experimental Design

The present study was conducted at the Department of Physiology, Kasturba Medical College (KMC), Mangalore, Manipal University (MU), Karnataka, India. A total of 30 male *Wistar* rats were divided into five (5) groups as summarized in the Table below.⁴

Hormonal Assay

Testosterone was estimated in 2ml of blood which was collected from rats by cardiac puncture. Chemiluminescence Immuno Assay (CLIA) kit (Chen et al,1991) was used for the estimation of serum testosterone levels.¹⁵

Tissue lipid peroxidation (MDA) was analysed by using testis tissue homogenate. Malondialdehyde was estimated by the standard procedure described by Kartha and Krishnamurthy.¹⁶

Statistical Analysis

Data were presented as mean ±SD. Student's t test was used as statistical tool, p<0.05 considered statistically significant.

RESULTS

Results depicted in Figure 1, show prior and post treatment with aqueous *Moringa olifera* leaf extract on tissue lipid peroxidation level and serum testosterone level. A significant decrease in testosterone and increase lipid peroxidation (MDA) was observed (p < 0.0001) in cadmium treated group when compared to the control and pre-and post-treated *M. oleifera*

Table 1: Study Animals

GROUPS (n=6)	
I (Control)	Orally administered 0.9% saline
II (Experimental control)	Orally infused with <i>M. oleifera</i> extract, 100 mg/kg/bw for 10 days
III	Received a single oral dose of cadmium chloride (10 mg/kg/bw)
IV	Treated with <i>M. oleifera</i> leaf extract (100 mg/kg/bw) for 10 days, followed by cadmium chloride (10 mg/kg/bw) given orally for one day
V	Oral infusion of single dose of CdCl (10mg/kgbw) followed by <i>Moringa olifera</i> leaf extract (100mg/kgbw) 10 days

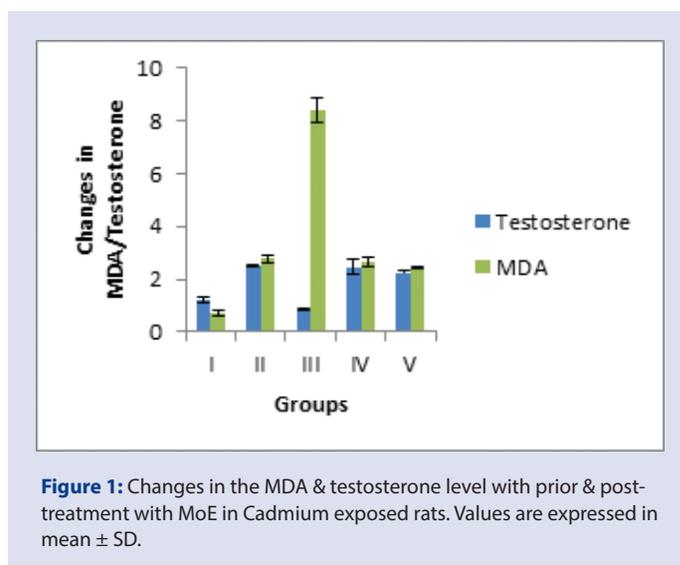


Figure 1: Changes in the MDA & testosterone level with prior & post-treatment with MoE in Cadmium exposed rats. Values are expressed in mean ± SD.

leaf extract (MoE). Highly significant decrease in lipid peroxidation of testis and significant increase in testosterone levels were observed when group IV and group V was compared to group III.

DISCUSSION

Effect of heavy metal exposure on testes (biology of reproduction) has become one of the major concern for the society as it is directly related to infertility.^{17,18,19} Recently, anti-diabetic actions of *Moringa olifera* have also been reported.²⁰ Cadmium is a toxic metal, implicated in oxidative stress^{10,11} and embryonic tissue.^{21,22} Oxidative stress arises from a variance between the production of oxidants and physiological antioxidant capability.²³ Lipid peroxidation is a measure of oxidative stress. Hence in the present experiment we measured tissue lipid peroxidation and serum testosterone levels.²³ Our results showed significant increase in the testicular MDA of the cadmium alone treated group (oral 10mg/kg/bw, single dose). Testosterone levels were found to be decreased significantly in these rats. These findings indicate testicular toxicity. Our previous study reports on Pre-treatment of *Moringa olifera* in rats also showed the similar results on cadmium induced rats.²⁴ Imbalance of steroidogenic activity observed in this study are in accordance with the previous reports.^{25,26} Increased MDA level suggests that CdCl₃ triggered testicular oxidative damage by enhancing the production of reactive oxygen species.²⁷ Treatment with *M. oleifera* (pre and post 100 mg/kg oral for 10 days) showed decreased toxic effect caused due to exposure of cadmium, which is evidenced by an increase in the serum testosterone level coupled with decrease in the tissue MDA levels. However, the pretreated group showed a better effect in reducing the toxicity than the treated group after exposure to cadmium *M. oleifera* leaf extract (proved of having bio flavonoid and other effective antioxidants) administration prior to cadmium exposure and post to cadmium exposure caused in significant improvement of toxicity in the testis.^{9,10} Studies have also reported that pre-treatment with *M. oleifera* leaf extract (containing fundamental antioxidant and phenolic compounds) protects the testes against various lethal substances and the oxidative damage caused by them.²⁶ Our study suggest that *Moringa oleifera* extract could diminish cadmium induced toxicity which owe to their antioxidant property. This may explain the fact that, the experimental group treated cadmium and *M. oleifera* leaf extract (MoE) showed an increase in testosterone concentration and subsequent reduc-

tion in testicular MDA. Studies have shown antioxidant activities of *M. oleifera* leaves.²⁸ Active components of this plant scavenge free radicals. It offers protection against oxidative damage on cellular macro molecules. Free radical scavengers may include polyphenols, flavonoids, and phenolic compounds.²⁹ *Moringa oleifera* could enhance the antioxidant system present in the epididymis which in turn preserved and facilitated the process of spermatogenesis. Apparently, it may have reversed concentrations of gonadotropins (FSH and LH) and ultimately testosterone.³⁰ Therefore the present study suggests that *M. oleifera* leaf extract (MoE) can be used as preventive and convalescent agent to attenuate the deleterious effects of cadmium on the testis

CONCLUSION

Plant extract used in the present study offers a cheap alternative therapeutic potential in treating cadmium induced testicular damage and infertility.

Limitation of the Study

Further studies are warranted in isolating the active components responsible for the reversal of cadmium induced decrease in sex hormone and testicular damage.

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CONFLICT OF INTEREST

No conflict of interest are declared.

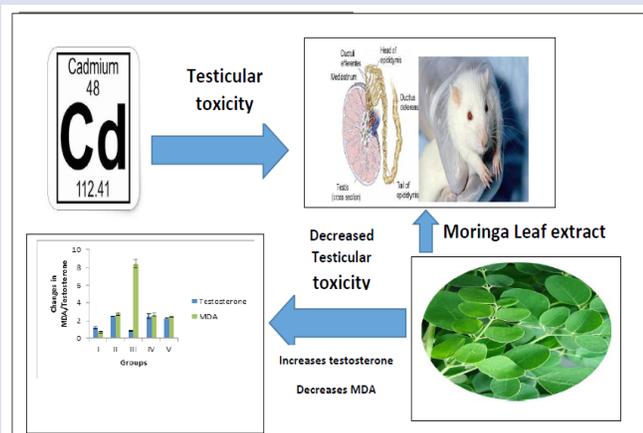
ABBREVIATION USED

MOE: *Moringa olifera* extract; **FSH:** Follicular Stimulating Hormone; **LH:** Leutinizing Hormone.

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GRAPHICAL ABSTRACT



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SUMMARY

- Cadmium (Cd) exposure produces testicular toxicity. The pre & post treatment with aqueous leaf extract of *Moringa olifera* (MoE) showed a significant reduction in the testicular toxicity with pre-treatment response being better than post treatment. The decrease in the toxicity might be due to presence of antioxidants.

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