# Effects of *Moringa oleifera* on Total protein and lactate Dehydrogenase levels in testiculotoxic rats treated with cadmium chloride

**Ajilore BS and Atere TG**

**Original Article**

**ABSTRACT**

**Background:** The present study aimed at investigating the influence of *Moringa oleifera* (MO) on the levels of total protein and lactate dehydrogenase in cadmium-induced testicular toxicity.

**Methods:** Twenty-one adult male wistar rats were randomly divided into 3 groups, A, B and C (n=7) which were treated every day regularly for three weeks. Group A (Control) rats received 2ml per day 0.9% w/v Normal saline orally. Group B rats were treated with cadmium chloride 2.5 mg/kg bwt subcutaneously while group C rats were pre-treated orally with extract of MO 500 mg/kg bwt before treating with cadmium chloride 2.5 mg/kg bwt subcutaneously. Blood samples were collected prior sacrifice and sera used for estimations of total protein (TP) concentrations and lactate dehydrogenase (LDH) activities. Testes were harvested and homogenates used for estimation of TP concentrations and LDH activities. Weights of the testes were also recorded.

**Results:** The results showed significant ($p<0.05$) decrease in the weights of testes of rats treated with cadmium chloride when compared to control and pre-treated rats. There were significant ($p<0.05$) reductions in the levels of testicular TP and LDH activities in rats treated with cadmium chloride but sera TP concentrations and LDH activities were significantly ($p<0.05$) increased. However, pre-treatment with extract of MO restored TP concentrations and LDH activities to near normal values.

**Conclusion:** We concluded that leaves of *Moringa oleifera* possess potentials to restore deranged levels of LDH and TP observed in heavy metal-induced testicular toxicities.

**Keywords:** Testicular toxicity, cadmium, *Moringa oleifera*, total protein, lactate dehydrogenase

Corresponding Author: Dr. Ajilore, B.S (doctorajibam@yahoo.com)

Department of Biochemistry, College of Health Sciences, Osun State University, PMB 4494, Osogbo, Nigeria.

# L'effet de *Moringa oleifera* sur protéines totales et le lactate déshydrogénase niveaux dans Testiculotoxic les rats traités avec chlorure de Cadmium

**Ajilore B.S. & Atere T.G.**

**RÉSUMÉ**

**Antécédents:** La présente étude visant à déterminer l'influence de *Moringa oleifera* (MO) sur les niveaux de protéines totales et le lactate déshydrogénase de cadmium-induite toxicité testiculaire.

**Méthodes:** Vingt-et-un mâle adulte rats Wistar ont été aléatoirement répartis en 3 groupes (A, B et C (n= 7) qui ont été traités chaque jour régulièrement pendant trois semaines. Groupe A (Contrôle) rats reçu 2ml par jour 0,9 % w/v Normal salin oralement. Groupe B les rats ont été traités avec chlorure de cadmium 2,5 mg/kg par voie sous-cutanée du centenaire du traité relatif aux eaux limitrophes tandis que le groupe C des rats étaient pré-traitées oralement avec extrait de MO 500 mg/kg du centenaire du traité relatif aux eaux limitrophes avant de traiter avec chlorure de cadmium 2,5 mg/kg par voie sous-cutanée du centenaire du traité relatif aux eaux limitrophes. Échantillons de sang ont été prélevés avant sacrifice et de sérums utilisés pour les estimations de protéine totale (TP) les concentrations et le lactate déshydrogénase (LDH) activités. Les testicules ont été récoltés et les homogénats utilisées pour l'estimation des concentrations TP LDH et activités. Le poids des testicules ont également été enregistrées.

**Résultats:** Les résultats ont révélé une importante ($p<0.05$) diminution du poids des testicules chez les rats traités avec chlorure de cadmium lorsque comparé au contrôle et pré-traitées chez le rat. Il est significatif ($p<0.05$) réduction des niveaux des testicules TP et LDH activités chez des rats traités avec chlorure de cadmium mais sérums TP les concentrations et LDH activités étaient considérablement ($p<0.05$ ) augmenté. Toutefois, pré-traitement avec extrait de MO restauré TP les concentrations et LDH activités près de valeurs normales.

**Conclusion:** Nous avons conclu que feuilles de *Moringa oleifera* possèdent potentiels à restaurer deranged niveaux de LDH et TP observé en métal lourd-induite toxicités testiculaire.

**Mots-clés: T**oxicité testiculaire, cadmium, *Moringa oleifera*, protéines totales, lactate déshydrogénase

Corresponding Author: Dr. Ajilore, B.S (doctorajibam@yahoo.com)

Department of Biochemistry, College of Health Sciences, Osun State University, PMB 4494, Osogbo, Nigeria.

## INTRODUCTION

Cadmium is one of the most toxic industrial and environmental metals and possesses a continuing health hazard since it is rapidly distributed in tissues (1, 2). It is distributed widely in our environment and workplaces and is of great concern as an environmental and occupational toxicant, especially with increasing industrialization (3). Sources of cadmium include nickel-cadmium batteries, tobacco smoke, pigment plants, soldering activities and petroleum refining processes (4, 5). The exposure of humans to environmental contaminants that adversely affect the male reproductive function has been on the increase and has become a major concern to public health (6). Acute and chronic cadmium toxicity is associated with severe damage in testes in both humans and animals **(7).**

Cadmium exposure is strongly associated with reproductive toxicity in both animal and human populations culminating in infertility and cancers of the reproductive tissues (8, 9). The pathogenesis of testicular damage and spermiotoxicity following cadmium exposure is generally ascribed to oxidative damage (6, 10). Disruption of the blood vessels of the testis causing hemorrhage and edema has been recently suggested as mechanism responsible for cadmium-induced testicular toxicity (11). Cadmium also affects Leydig cells by promoting a reduction in steroidogenesis (12), and induces cell necrosis by production of reactive oxygen species (13).

*Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringacea (2). *Moringa* tree has gained popularity as a nutrition power plant that can feed the needy and save lives (14). The leaf is an excellent source of vitamin A, vitamin B, vitamin C, vitamin E, protein, calcium, potassium, selenium, zinc, specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids- lutein, alpha-carotene and beta-carotene, and xanthins (15). Extracts of various *Moringa* tissues have been used as anti-cancer (14), anti-trypanosomal (16), antimicrobial (17) and hepatoprotective and renoprotective agents (2).

In the present study, the effect of methanolic leaf extract of *Moringa oleifera* on lactate dehydrogenase (LDH) activity and total protein (TP) concentration as biomarkers of testicular toxicity was measured in male rats treated with cadmium chloride.

## MATERIALS AND METHODS

### *Plant Material and Extraction*

Fresh leaves of *Moringa oleifera* were collected from the National Centre of Genetic Resources and Biotechnology, Moor Plantation, Ibadan, Nigeria ($7^0$ 22' N; $3^0$ 50' E). The plant was identified at the Botany unit of NCGRB. The leaves of *Moringa olifera* were shade dried under laboratory conditions for three weeks and ground into fine powder by using an electrical mill. Three hundred and fifty gram (350g) of the dried powder was subjected to soxhlet extraction with 3.5 litres absolute methanol for 12 hours using modified method of Virdii *et al*. (18). The filtrate obtained was concentrated under reduced pressure and then lyophilized by freeze-drying.

### *Experimental Animals and Design*

Twenty-one (21) adult male wistar rats weighing between 200 g to 250 g were used for this study. The animals were kept in well ventilated wooden cages and had free access to standard rat pellets (Mosodun Feeds, Osogbo, Nigeria) and clean water.

The rats were randomly divided into 3 groups, A, B and C of 7 rats each and they were treated every day regularly for three weeks as follows:

Group A (Control) - Each rat in this group received 2ml per day 0.9% w/v Normal saline orally.

Group B (Cadmium only) – Each rat in this group received 2.5mg/kg body weight Cadmium chloride per day. Route of administration was subcutaneous.

Group C (Extract + Cadmium) – Each rat in this group was pre-treated with 500mg/kg body weight methanolic extract of *Moringa oleifera* orally one hour before subcutaneous 2.5mg/kg body weight Cadmium was given.

All the rats in the three groups were treated every day regularly for three weeks. Blood samples were collected by ocular puncture at the end of 3rd week of treatment and sera separated for biochemical assays. The rats were then sacrificed by cervical decapitation and testes were immediately harvested, cleared of adhering connective tissues, weighed and homogenized in freshly prepared 0.25M sucrose solution. The homogenate was further centrifuged at 6000 rpm for 5 minutes at $4^0$C using a refrigerated centrifuge (TGL-16G, BBran Scientific and Instrument Company, England) and supernatant obtained was used for biochemical assays.

***Biochemical Estimations***

The total protein in the serum and testis homogenate was estimated using the method of Cheesbrough (19). Serum and testis homogenate lactate dehydrogenase (LDH) activity was estimated as described by Weisshaar *et al*. (20).

***Statistical Analysis***

Data, expressed as Mean ± SEM, were analyzed using analysis of variance (ANOVA of 1997 SAS Institute Package, version 9.1). Homogeneity of variance and squared deviations from group means was carried out by Levene's test, followed by Duncan's multiple range tests. Results were considered to be statistically significant when p values were less than 0.05 ($p<0.05$).

**RESULTS**

Table 1 showed the effect of cadmium, cadmium and extract of *Moringa oleifera* on the average weight of testes. There was significant ($p<0.05$) decrease in the weight of testes of rats that were treated with cadmium when compared to those in control group. Though, the weight of rats pre-treated with extract of *Moringa oleifera* was significantly ($p<0.05$) higher than those rats that were treated with cadmium alone.

Figures 1 and 2 showed the effects of cadmium, cadmium and extract of *Moringa oleifera* (MO) on total protein concentrations in the testes and sera of rats respectively. The protein level (82.46 g/l) in the testes of rats treated only with cadmium was significantly ($p<0.05$) reduced when compared to protein level (142.00 g/l) in control rats and protein level (126.12 g/l) in rats pre-treated with extract of *Moringa oleifera.* On the contrary, serum protein concentration (25.97 g/l) in rats treated only with cadmium was significantly ($p<0.05$) increased when compared to the levels in control rats (18.10 g/l) and in rats pre-treated with extract of MO (18.28 g/l).

Figures 3 and 4 showed the effects of cadmium, cadmium and extract of *Moringa oleifera* on lactate dehydrogenase activities in testes and sera of rats respectively. There was significant ($p<0.05$) reduction in the activities of testicular lactate dehydrogenase (LDH) in all the rats treated with cadmium chloride. LDH activity (1908.00 U/L) in rats treated only with cadmium was significantly ($p<0.05$) reduced when compared to the activities of the enzyme in testes of control rats (2714.00 U/L) and in rats that was pre-treated with extract of MO (2017.00 U/L). However, LDH activity in the sera of rats treated only with cadmium was significantly ($p<0.05$) higher than the activities of the enzyme observed in the sera of rats in other groups.

**DISCUSSION**

Cadmium exposure causes reduction in the weight of accessory organs of reproduction of male rats (22, 23). The significant decrease in the weights of testes observed in this study may be as a result of necrotic degeneration of testicular tissues (24).

The pathogenesis of testicular damage following cadmium exposure is generally ascribed to oxidative damage (6, 10) producing reactive oxygen species which attack essential cell constituents such as proteins, lipids and nucleic acids (25). The significant reduction in the testicular protein level and subsequent increase in the serum protein of the animals exposed to cadmium in our study was due to release of protein from testicular tissue into the circulation following oxidative damage and disruption of the blood

vessels of the testes.

The activity of lactate dehydrogenase (LDH) in testicular tissue is associated with the maturation of the germinal epithelial layer of the seminiferous tubules (26). LDH is found in sertoli and spermatogenic cells and plays an important role in testis energy production and biotransformation. Inhibition of LDH activity may lead to denaturalization of spermatogenic cells, therefore its activity is an important biochemical parameter in the evaluation of testicular function (27). In the present study, there was significant ($p<0.05$) reduction in the activity of testicular LDH and significant ($p<0.05$) increase in the level of the enzyme in serum of rats treated with cadmium. This could be due to release of the enzyme from testicular tissue into the circulation as a result of oxidative damage following cadmium chloride administration (23, 28).

*Moringa oleifera* leaf is highly nutritious and equally rich in antioxidants (14, 15, 29). The near normal protein concentrations and LDH activities observed in the testicular tissues and sera of rats used in this study may be as a result of numerous antioxidant properties of *Moringa oleifera*. One of the mechanisms by which *Moringa oleifera* leaf protects against chemical-induced toxicities is due to its ability to induce phase II detoxification pathway via promoting reduced glutathione (GSH) conjugation with toxic metabolites generated from CYP450 pathway (30).

## CONCLUSION

We concluded that cadmium induces testicular toxicity, and *Moringa oleifera* leaf has appreciable potentials to restore deranged lactate dehydrogenase and total protein levels seen in heavy metal-induced testicular damage.

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**Conflict of Interest**

The authors declare no conflict of interest.

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**Table 1: Effects of Cadmium, Cadmium and Extract of *Moringa oleifera* (MO) on Average weight (g) of Testes**

| Treatment Group | Average Weight of Testis (g) |
|---|---|
| Control | 0.90±0.07 [a] |
| Cadmium only | 0.42±0.03 [b] |
| Extract of MO + Cadmium | 0.50±0.06 [ba] |

Values are expressed as mean ± SEM (n=7). Means with different Duncan superscripts are statistically significanty different at $p<0.05$.

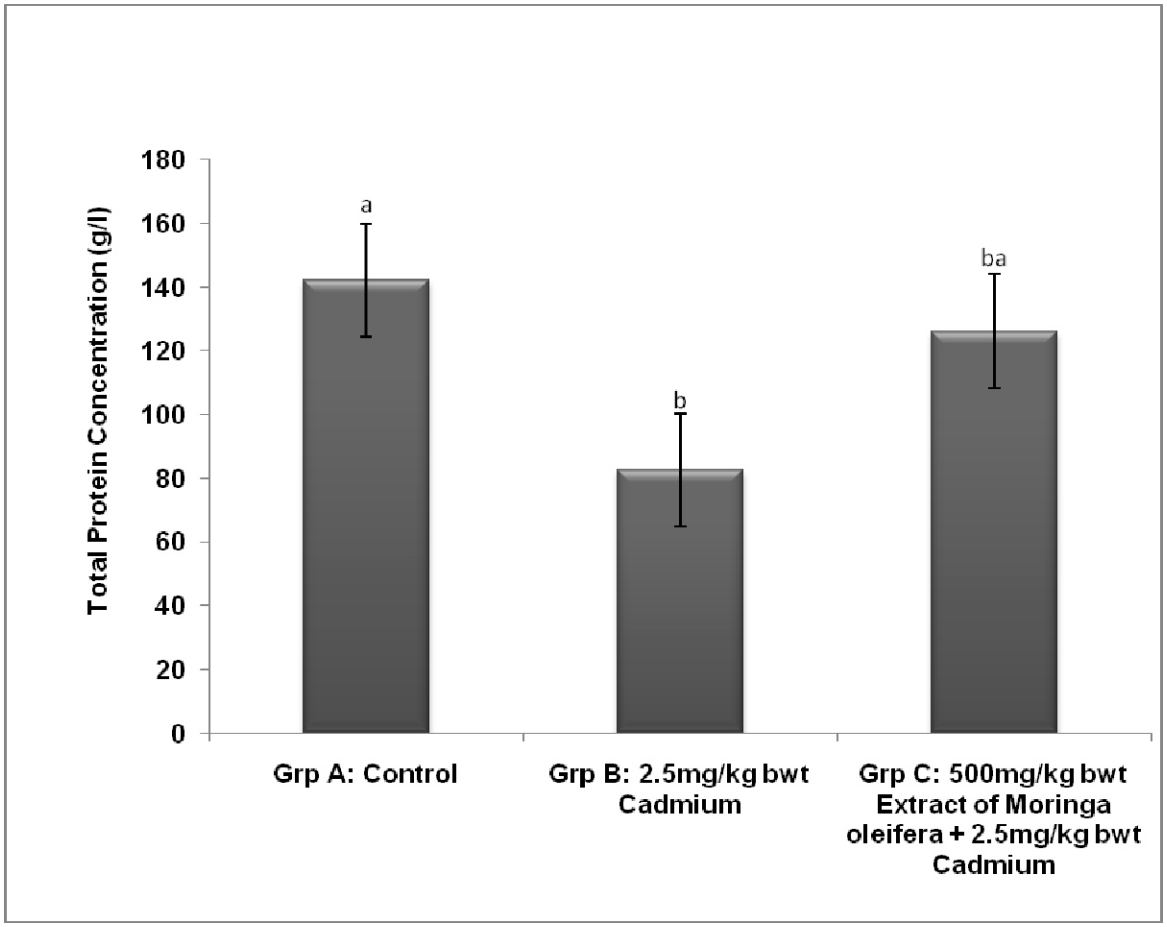


**Figure 1: Effects of Cadmium, Cadmium and Extract of *Moringa oleifera* on Total Protein Concentration (g/l) in Testes of Rats**

Values are expressed as mean ± SEM (n=7). Means with different Duncan superscripts are statistically significantly differently different at $p<0.05$.

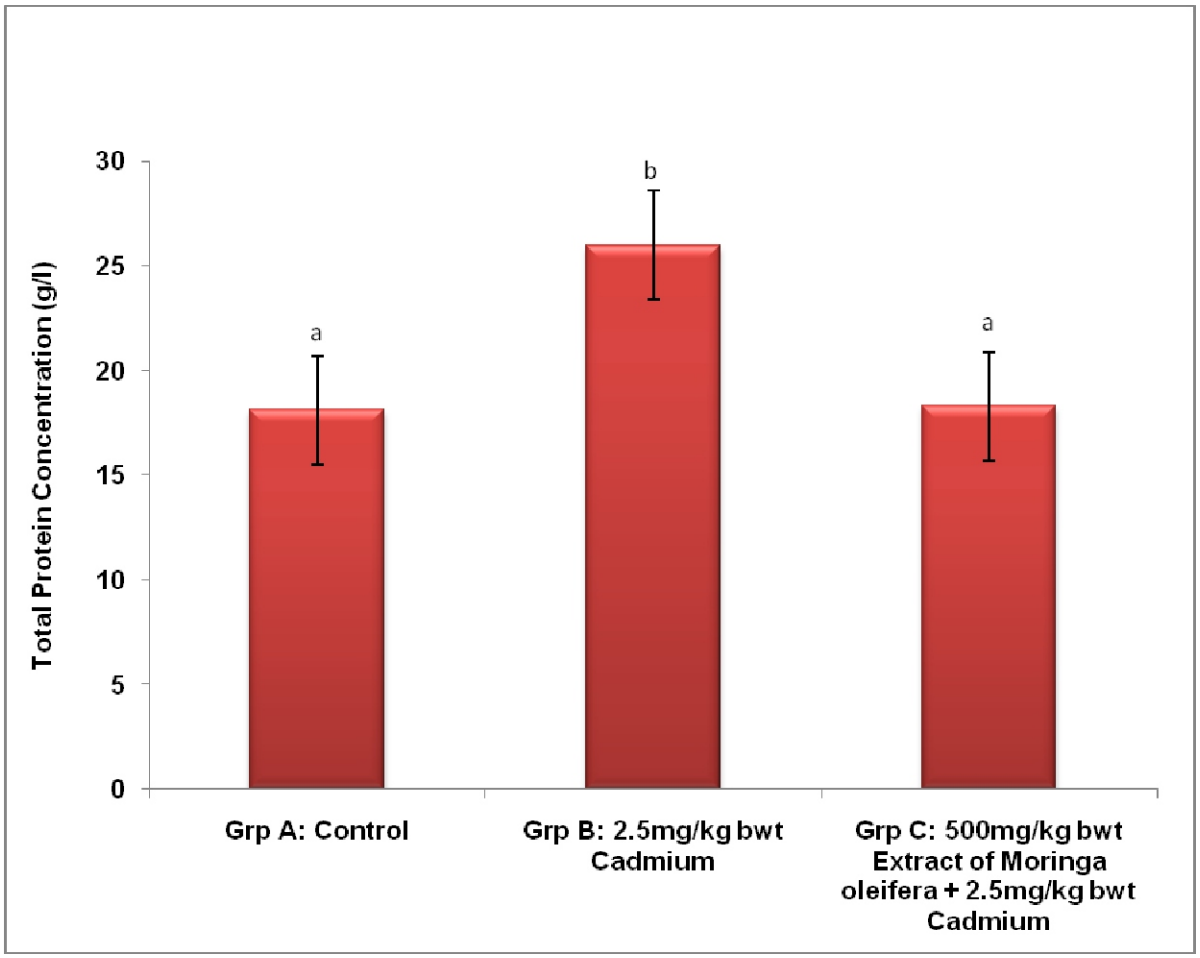


**Figure 2:Effects of Cadmium, Cadmium and Extract of *Moringa oleifera* on Total Protein Concentration (g/l) in Sera of Rats**

Values are expressed as mean ± SEM (n =7). Means with different Duncan superscripts are statistically significantly different at $p<0.05$.

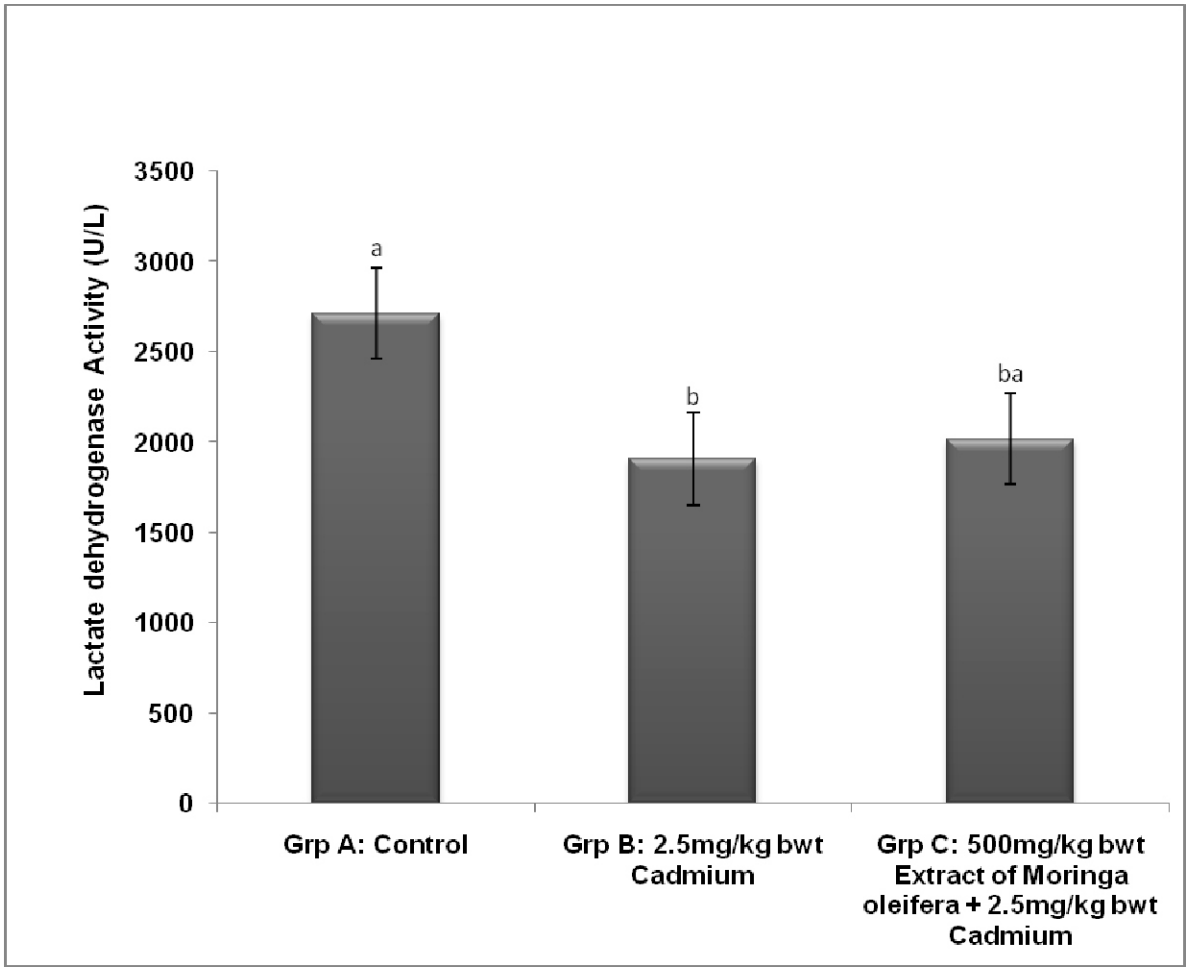


**Figure 3:Effects of Cadmium, Cadmium and Extract of *Moringa oleifera* on Lactate dehydrogenase Activity (U/L) in Testes of Rats**

Values are expressed as mean ± SEM (n=7). Means with different Duncan superscripts are statistically significantly different at $p<0.05$.

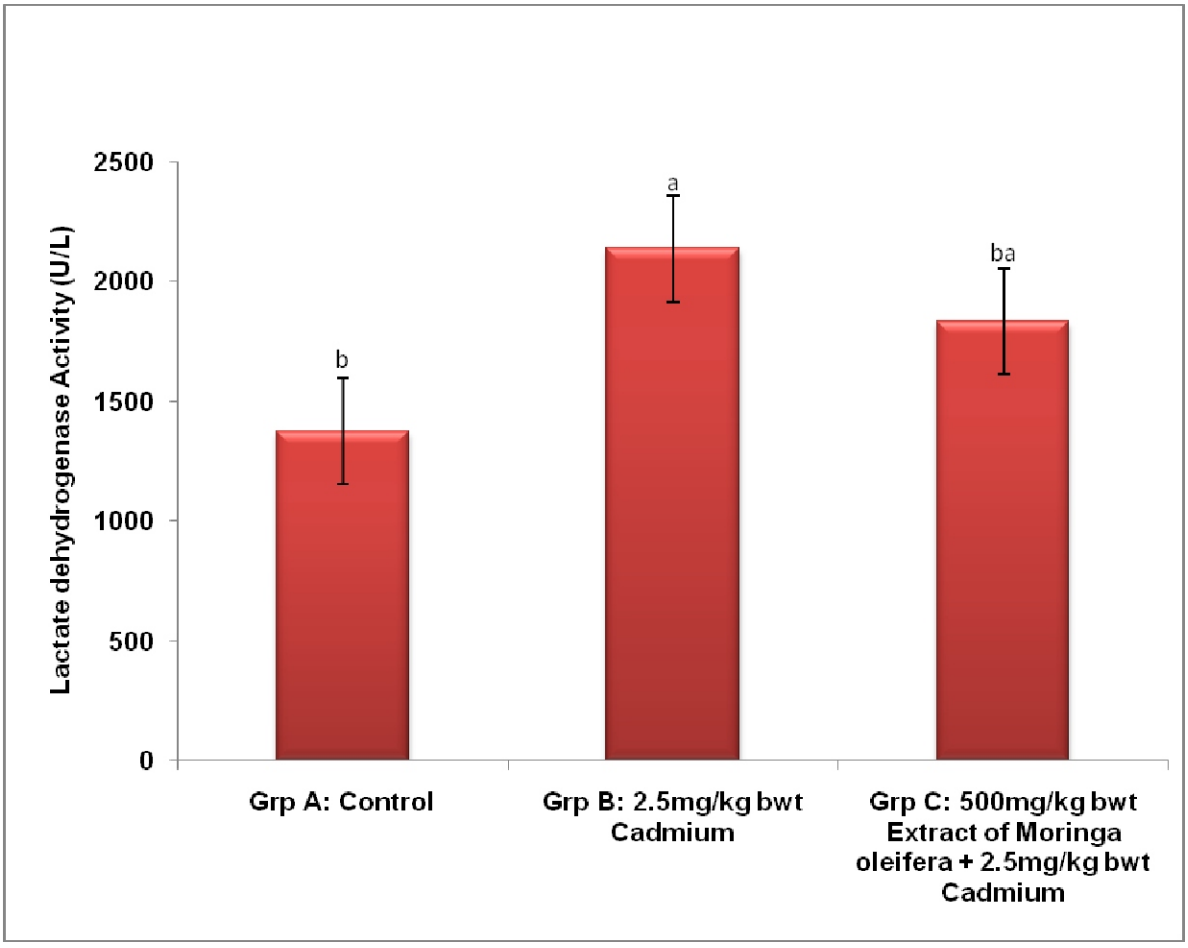


**Figure 4: Effects of Cadmium, Cadmium and Extract of *Moringa oleifera* on Lactate dehydrogenase Activity (U/L) in Sera of Rats**

Values are expressed as mean ± SEM (n=7). Means with different Duncan superscripts are statistically significantly different at $p<0.05$.