PROTECTIVE EFFECT OF MOMORDICA CHARANTIA EXTRACT ON LEAD-INDUCED MALE REPRODUCTIVE TOXICITY IN RATS

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ABSTRACT
Infertility is a reproductive health problem worldwide that affects many couples especially in developing countries. The present study aimed to investigate the protective effects of *Momordica charantia* extracts (MCE) on lead-induced male reproductive toxicity. Thirty male rats were randomly divided into five groups of six rats each. The rats were treated daily for 28 days. The phytochemical analyses showed that the plant possess polyphenols, flavonoids, alkaloids, saponins and tannins. The results obtained revealed that pretreatment of rats with MCE significantly (P<0.05) attenuated the decrease in body weight compared to untreated group. There was significant (P <0.05) increase in testicular MDA and significant (P <0.05) decrease in GSH levels in lead exposed rats compared to the control group. However, oral administration of MCE significantly reduced MDA Level and caused significant increase GSH level in a dose dependent manner when compared with untreated group. Similarly, lead exposure caused significant (P <0.05) decrease in sperm count, sperm motility, sperm viability and testosterone level compared to the control group. Oral administration of MCE caused significant (P <0.05) increase in these sperm parameters and testosterone when compared with the control group. Thus, based on the results obtained from the present study, it was found that MCE possess protective effect against lead induced male reproductive toxicity.

KEYWORD: Lead poison, sperm parameters, protective effect, testosterone, *Momordica charantia*. 
1. INTRODUCTION

Infertility is a reproductive health problem that affects many couples in the human population. Worldwide infertility is generally reported as occurring in 8-12% of all couples. Infertility is defined as one year of frequent, unprotected intercourse during which pregnancy has not occurred. Male factor was found to be responsible for the infertility in 42% of the subjects in Nigeria [1]. The most common aetiological factors responsible for male infertility were oligozoospermia and asthenozoospermia [2]. Lead (Pb) is ubiquitous and persistent heavy metals with no known biological function [3]. Lead poisoning, which can be traced to prehistoric time, is a broad spectrum of toxic effects in humans and animal systems especially urban cities due to its wide industrial activities and in the manufacture of batteries, toys and ammunitions [4]. Existing data suggest that lead exposure results in oxidative and has been reported to be the major mechanism of lead toxicity in most biological systems [5]. The deterioration of male reproductive health is one of the major manifestations of occupational and/or environmental exposure to lead [6]. It has been reported that acute or chronic exposure to lead poisoning are severe and almost all compartments of reproductive system are vulnerable targets [8]. Several reports also, suggest that workers exposed to lead suffered with oligospermia and asthenozoospermia [9], with altered sperm morphology [10]. It has also been reported that lead toxicity even extends to epididymis and results in altered sperm maturity [11]. Lead is also considered as endocrine disruptor which can modify hormonal metabolism by altering synthesis and/or breakdown of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) [12]. Thus it appears that, lead burden disturbs hormonal-mediated spermatogenesis and steroidogenesis of male reproduction [13]. Testosterone, the main male sex hormone, is formed and secreted by Leydig cells in testes in response to stimulation by of LH, plays a vital role in spermatogenesis and male fertility. It has been reported that exogenous administration of testosterone improves the spermatogenesis and normalization of maturation from round to elongated spermatids was a predominant action of testosterone in the restoration of spermatogenesis in rats [14]. Further, high doses of testosterone substantially promote spermatogenesis by maintaining or restoring testicular testosterone to about 40% of normal levels [15]. Tropical forest plant species have served as a source of medicines for people of the tropics for millennia. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids. *Momordica charantia* (bitter melon) belongs to the family of Cucurbitaceae which is a food as well as medicine. The plant has been reported to possess antimicrobial, antitumor, antidiabetic, antioxidative and anti-
inflammatory activities \(^{[16]}\). Despite the numerous use of, *Momordica charantia* in folk medicine and health food, little or no investigation had been reported regarding its *in-vivo* protective effects on lead induced male reproductive toxicity in rats. Therefore the present study was aimed to evaluate the anti-spermatogenic effect of *Momordica charantia* extract on lead-induced reproductive toxicity in male rats.

2. MATERIALS AND METHODS

2.1 Chemicals, Reagents and Drugs

Lead acetate (purchased from Merck India Ltd Mumbai, India), testosterone assay kits (purchased from Randox Laboratories Ltd, United Kingdom) Trichloro acetic acid (TCA), thiobarbituric acid (TBA), reduced glutathione (GSH), pyrophosphate, ethylene diamine tetra acet acid disodium salt (EDTA) 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), \( \beta \)-nicotinamide adenine dinucleotide hydrogen (NADH) were obtained from Sigma Chemical (St. Louis, MO, USA). Disodium hydrogen phosphate (\( \text{Na}_2\text{HPO}_4 \)), dihydrogen potassium phosphate anhydrous (\( \text{KH}_2\text{PO}_4 \)) (purchased from Merck India Ltd Mumbai, India). All other chemicals and reagents were of analytical grade.

2.2 Plant Sample Collection and Identification

The leaves of *Momordica charantia* Linn were collected in polythene bags from Damba Village Gusau, Zamfara State, Nigeria. The samples were identified and authenticated by Mr. U. S. Gallah of the herbarium unit, Department of Biological science, Ahmadu Bello University Zaria Nigeria, and a voucher number was given as VN/552/2014.

2.3 Plant Extract Preparation

The leaves of *Momordica charantia* Linn were cleaned, washed with tap water and dried under shade. The dried leaves were broken into small pieces using pestle and mortar and then pulverized using electric blender into fine powder. Five hundred grams (500g) of fine powder were weighed and soaked into two liters of distilled water. The mixture was shaken regularly at interval of 2 hours and kept at room temperature for 24 hours. After 24 hours, the homogenate was filtered using muslin cloth and the filtrate obtained were re-filtered using Whatman No. 1 filter paper, the filtrate obtained was evaporated to dryness using water bath set at 45ºC.
2.4 Phytochemical Analyses

Quantitative phytochemical analyses of stem bark extract of *M. charantia* were carried out according to the following methods: Tannins [17], saponins and alkaloids [18] and flavonoids [19].

2.5 Acute Toxicity Study

The median lethal dose (LD50) of aqueous leaves extract of *Momordica charantia* was carried out according to the method of described by [20]. The method involved two phases of which nine rats were grouped into three groups of three rats each. They received single dose of 10, 100 and 1000 mg/kg body weights of the extracts respectively. In the second phase also nine rats were grouped into three groups of three rats each and they received 1600, 2900 and 5000 mg/kg body weights. The rats were observed daily for any signs of toxicity including death for twenty four hours.

2.6 Experimental Animals

A total of thirty apparently healthy male Wister albino rats of three months old, weighing between 180-200g were purchased from the animal house, Department of Biological Science, Bayero University Kano, Nigeria. The animals were kept in clean plastic cages at 25 ±3.0°C under 12 hr light and dark cycles and were, allowed free access of water and standard pellet diets *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment. The experiments complied with the guidelines of our institution’s animal ethics committee which was established in accordance with the internationally accepted principles for laboratory animal use and care.

2.7 Experimental Design and Treatment

Thirty male rats weighing 180-200 g were randomly divided into five groups of six rats each. The rats were treated daily for 28 days as follows:

**Group 1:** Normal control: Were given orally distilled water only

**Group 2:** Positive group: Were given orally lead acetate 740 mg/Kg bwt only

**Group 3:** Standard group: Were given orally 50 mg/kg bwt proviron + lead acetate 740 mg/Kg bwt

**Group 4:** Were given orally 250 mg/Kg bwt *Momordica charantia* extract + lead acetate 740 mg/Kg bwt

**Group 5:** Were given orally 500 mg/Kg bwt *Momordica charantia* extract + lead acetate 740 mg/Kg bwt.
2.8 Evaluation of Body and Weights
The initial and final body weights of each experimental rat in all the groups were recorded.

2.9 Tissue and blood sample preparation
At the end of 28 days treatment period, the rats were fasted overnight and decapitated; blood was collected from the heart of the animals placed into two test tubes one without anticoagulant for serum preparation and the other one with hepanized tube and stored at -20ºC until needed. Serum was used estimation of testosterone level and whole blood was used for determination Pb level. Thereafter, all testis tissue of each animal was homogenized in phosphate buffer (pH 7.4) to give a 20 % w/v homogenate. This homogenate was centrifuged at 3300 g for 15 min at 4 ºC and the supernatant was stored at -70 ºC until analysis. The clear supernatant was used for the estimation of serum testosterone levels, MDA, GSH and total protein.

210. Determination of whole blood lead level (BLL)
Lead content of whole blood was measured using an atomic absorption spectrophotometer (Shimadzu 680A, with graphite furnace, Shimadzu, Japan) and expressed as micrograms per deciliter (μg/dl) according to the method described by [21].

2.11 Evaluation of serum testosterone level
Serum level of testosterone was determined by enzyme linked immunosorbant assay (ELISA) using a commercial kits (Randox Laboratories Ltd, United Kingdom). The assay was done strictly according to the procedure given along with the kit. The serum level of testosterone was expressed as ng/mL.

2.12 Evaluation of sperm parameters of Testicular Functions
The epipidymal sperm count, motility and viability were evaluated by the following method of [22]. Briefly, epididymal spermatozoa were obtained by mincing the epididymis with anatomical scissors in 5 ml of Ham’s F12 medium and incubated at 32ºC for 2 minutes. An aliquot of this solution was placed in Neubauer haemocytometer and motile sperms were counted by using light microscope at x 400 magnification. Non-motile sperm numbers were first determined, followed by counting total sperm. Sperm motility was expressed as a percent of motile sperm from the total sperm counted.
2.13 Assay of lipid peroxidation (Malondialdehyde)
Lipid peroxidation in the testicular tissue was measured colorimetrically by thiobarbituric acid reactive substance (TBARS) method described by [23]. Concentration was estimated using the molar absorptive of malondialdehyde, which is \(1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}\) and it was expressed as nmol mg\(^{-1}\) protein.

2.14 Assay of testicular reduced glutathione (GSH) activity
Reduced GSH in the testicular tissue was measured according to the method described by [24]. The absorbance was read at 412nm, it was expressed as nmol-1 protein.

2.15 Statistical Analysis
The Data was analyzed and presented as the mean ± standard deviation using GraphPad Prism 5.01. Analysis of variance (ANOVA) and Turkey’s Post hoc test was employed to test the significance of difference between the groups and P < 0.05 was considered as statistical significant.

3. RESULTS AND DISCUSSION
3.1 Acute Toxicity
The present study was aimed at evaluating the protective role of Momordica caharnatia extract (MCE) against lead-induced male reproductive damage. The result of acute toxicity study indicated that, the LD50 of aqueous leaves of Momordica charantia extract (MCE) was greater than 5000 mg/kg (Data not showed). The extract did not exhibit any sign of toxicity and no animal death was recorded throughout the period of this study. This is an indication that, the leaves of MCE was relatively safe.

3.2 Phytochemical analysis and effect of MCE on rat body weight
The result of phytochemical studies presented in (Figure 1a) indicated that the amount of metabolites detected. For instance, polyphenolics were the highest concentration of 0.98g/100g whereas tannins were having the least concentration of 0.26g/100g amongst of all the phytochemicals analyzed. The presence secondary metabolites such as flavonoids, polyphenols and alkaloids contribute to the medicinal property to the plant. The effect of oral administration of lead on animals final body weight revealed that, lead poisoning caused significant (P < 0.05) decrease in body weight when compared to control group. However, pretreatment of proviron (standard) and MCE to animals prior to lead exposure attenuated the
decrease in body weight in dose dependent manner (Figure 1 b). The results of a decreased in body weight obtained in our investigation was in agreement with the study conducted by [25].

Figure 1: Phytochemical analyses (a), the effect of MCE on body weight of lead exposed rats (b). Values are Mean ± SD (n=6). Column with different superscripts are statistically different compared to control at P<0.05

Effect of MCE on blood lead (Pb) and total protein level
The effect of MCE pretreatment on blood lead levels (BLL) was investigated. The results obtained revealed that administration of lead to the animals caused a significant (P < 0.05) increase in blood lead level (BLL) in lead treated groups when compared with the normal control group. However, pretreatment of animals with both MCE and proviron (Standard) prior to lead poisoning, lowered the BLL level significantly (P < 0.05) when compared with lead-non-treated group in a dose-dependent relation compared to the control group. The reduction of blood lead level in rats treated with MCE and proviron may be due to its chelating property. The chelating agents may form an insoluble complex with lead to remove it from lead-burdened tissues [26]. Similarly, administration of lead caused a significant (P <0.0%) decrease in total protein in all the treated groups compared to the control group as shown in (Figure 2). However, pretreatment of rats with MCE caused a significant (P <0.05) increase in total protein level when compared with the lead toxin group.
Effect of MCE on MDA and GSH level
The effect of lead administration on the Malondialdehyde (MDA) and reduced gluathathione (GSH) level was investigated. The results showed in (Figure 3a & b) shows that MDA concentration was significantly (P <0.05) increased in the testes of rats after treatment with lead when compared with the control group whereas, pretreatment of animals with both proviron (Standard) and MCE significantly (P <0.05) lowered the concentration of MDA when compared with the lead exposed group in a dose-dependent manner. Oral administration of lead to the animals caused a significant decrease in reduced glutathione (GSH) level when compared with the control group. However, pretreatment of rats with MCE and proviron (standard drug), resulted in significant (P <0.05) increase in GSH level compared to the lead toxic group. Existing data suggest that lead exposure results in oxidative stress this has been reported to be the major mechanism of lead toxicity in most biological systems [27]. That the testicular injury observed following treatment of rats with lead acetate was brought about due to oxidative stress is evident from a marked increase in the level of lipid peroxidation products (MDA) and also of reduced GSH level of hepatic tissue. These two parameters are considered as the primary biomarkers of oxidative stress. The results obtained from present study showed significant increased lipid peroxidation and depletion of GSH which indicate an increased in ROS generation which can cause damage to sperm and other cytoplasmic membrane structures through peroxidation of lipids, proteins and nucleotides, thereby altering sperm motility. The stimulation of lipid per-oxidation observed.
in the current study as a result of lead exposure could be due to the formation of free radicals through an exhaustion of antioxidants as shown by \[28\].

![Figure 3: Effect of MCE on MDA and GSH level. Values are Mean ± SD (n=6). Columns with different superscripts are statistically different compared to control at P<0.05.](image)

**Effect of MCE on Sperm parameters and testosterone level**

The effect of lead on epididymal sperm count, sperm motility and viability was evaluated. The result obtained shown in (Figure 4) revealed that, oral administration of lead to male rats caused a significant (P < 0.05) decrease in sperm count, sperm motility and viability when compared with the control group. On the other hand, pretreatment of male rats with *Mormodica charantia* extract (MCE) caused a statistically significant (P < 0.05) increase in sperm count, sperm motility and viability in a dose-dependent relation when compared with the lead toxin group (positive control). The effect of lead on the testosterone level was evaluated and the results shown in (Figure 4b) indicated that, oral administration of lead to the rats caused a significant (P < 0.05) decrease in serum levels of testosterone compared to the control group. However, pretreatment of MCE to the animals prior to lead administration resulted in a significant (P < 0.05) increase in testosterone level compared to lead toxin group (Non-MCE treated group). That is MCE restored the testosterone level near the level observed in the control group in a non-dose dependent manner. Many studies on male animal reproductive system have documented lead as a toxicant for reproductive functions \[29\]. The possible explanation is that lead may have a direct negative effect on sperm and testicular histological structure. Moreover, the decrease in sperm motility and quality can be due to indirect effects of lead, like increase of ROS (reactive oxygen substances) generation in sperm cells. By causing lipid oxidation, ROS alter the integrity and the fluidity of cellular membrane structures which is essential for sperm motility, structural integrity, and ultimately
for sperm viability [30]. The results obtained revealed that, sperm count, sperm motility and sperm viability were significantly decreased in lead exposed rats. The results are in agreement with earlier reports obtained by [31], who reported that lead reduces significantly epididymal and testicular sperm counts including daily sperm production. Similarly, lead-treated group in our study showed significant decrease in serum testosterone level compared to control group. These results were in accordance with a study conducted by [31].

![Figure 4: Effect of MCE on Sperm parameters and testosterone level. Columns are Mean ± SD (n=6). Values with different superscripts are statistically different compared to control at P<0.05.]

CONCLUSION
The results of the present study found that, *Momordica charantia* extract (MCE) possess protective effect against lead-induced reproductive toxicity in male rats. The effect observed demonstrated dose-dependent response and was comparable to standard drug (proviron). The presence of certain secondary metabolites such as polyphenols, flavonoids and alkaloids in this plant could be responsible for the observed activity.
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REFERENCE


