Effects of methanolic leaf extract of *Momordica charantia* on testicular marker enzymes and gonadotrophic hormones in cadmium-induced gonadotoxic rats

Ajile Bamidele S1*, Babalola Olusegun O2, Ayannuga Olugbenga A3

**Abstract**

To investigate pre-treatment effects of *Momordica charantia* (MC) leaf against cadmium-induced gonad toxicity in male rats. Thirty male wistar rats weighing 200-300g were randomly divided into three groups (A, B and C) of ten rats each. Group A received normal saline orally. Group B was treated with 2.5mg/kg bwt cadmium subcutaneously while group C were pre-administered with 300mg/kg bwt MC extract orally before treating with subcutaneous cadmium 2.5 mg/kg bwt. Animals were treated every other day for six weeks. Serum testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were evaluated. Testis alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities were also evaluated. Histology of testis and accessory organs of reproduction was studied. The results showed significant (p<0.05) decrease in serum testosterone, LH, ALP, LDH and G-6-PDH levels in rats treated with cadmium. Histology showed varying degree of cellular degeneration in the testis and accessory organs of reproduction of rats treated with cadmium. However, pre-treatment with extract of MC improved testosterone, LH, ALP, LDH and G-6-PDH levels. We concluded that leaves of MC possess potentials to restore deranged levels of gonadotrophic hormones and testicular marker enzymes observed in heavy metal-induced gonads damage.

**Keywords:** *Momordica charantia*, cadmium chloride, gonadotoxicity, protective potentials

**Introduction**

Heavy metals including cadmium are found naturally in the earth but become concentrated as a result of human caused activities [1]. Cadmium can be found in mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilizers, paints and treated woods [2]. Exposure of humans to environmental contaminants that adversely affect male reproductive functions has been on the increase and has become a major concern to public health [3]. Cadmium causes reproductive toxicity in both animal and human populations which results in infertility and cancers of the affected reproductive organs [4, 5].

*Momordica charantia* (Bitter melon), the plant of study in this interest, is called Ejinrin weere among Yorubas of South-western, Nigeria, is used for a dazzling array of conditions among their people. Reported medicinal properties of the plant include antimicrobial, antihelminthic, anticancerous, antmutagenic, abortifacient, antidiabetic and rheumatism [6]. Some of the phytochemicals and compounds that have been isolated from bitter melon are: alkaloids, glycosides, phytosterols, saponins, phenolic compounds, flavanoids, proteins, few tannins, fats and fixed oils, resins, 5-hydroxytryptamines etc [7, 8].

The type of solvent has an important role in detecting plant compounds. Methanolic leaf extract was found to contain more potent antioxidant and high phenol compounds when compared with chloroformic leaf extract [9]. [10] reported that 300mg/kg bwt extract of *Momordica charantia* showed a significant increase in the levels of reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase, and also a significant decrease in the levels of thiobarbituric acid reactive substances and hydrogen peroxides in the liver and brain of rats treated with ammonium chloride. In the present study, the effects of methanolic leaf extract of *Momordica charantia* on the levels of male reproductive hormones and activities of testicular marker enzymes were measured in male rats treated with cadmium chloride.

**Materials and Methods**

**Collection and Identification of Plant**

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Fresh leaves of *Momordica charantia* (Bitter melon) were collected from a farm in Osu, Osun State, Nigeria. The plant was identified and authenticated at the IFE HERBARIUM, Department of Botany, Obafemi Awolowo University, Nigeria, where specimen copy was deposited. The Herbarium identification number was 16591.

**Solvent Extraction of *Momordica charantia***

The leaves of *Momordica charantia* were air-dried at room temperature for thirty days and ground into fine powder by using an electrical mill. The powder was kept in air-tight container until use. Three hundred and fifty gram of the dried powder was subjected to soxhlet extraction with 3.5 L of 70% (v/v) methanol for two consecutive days using modified method of [11]. Five hundred mls of warm water was added to the mixture to suspend the chlorophyll. The mixture was filtered to remove the suspended chlorophyll and filtrate was evaporated to dryness under reduced pressure using Buchi Rotary Evaporator.

**Experimental Animals**

Thirty Wistar adult male rats with an average weight range of 200 g to 300 g were used for this study. The animals were acclimatized for 2 weeks at the Medical Animal House of the College of Health Sciences, Obafemi Awolowo University, Nigeria where they had free access to standard rat pellets and clean water.

**Treatment of Experimental Animals**

Thirty Wistar male rats weighing 200-300 g were randomly divided into 3 groups (A, B and C) of 10 rats each. A was the control group and received oral 45 mg/kg body weight 0.9% w/v Normal saline. Group B was treated with subcutaneous 2.5 mg/kg body weight cadmium and group C was pre-treated with oral 300 mg/kg body weight methanolic extract of *Momordica charantia* one hour before treating with subcutaneous 2.5 mg/kg body weight cadmium. All the animals in the three groups were treated every other day for the duration of six weeks. The weight of the body of each rat was recorded at 0, 3 and 6 weeks post-treatment in the three groups.

**Sacrificing of the Experimental Animals**

Five rats from each group were sacrificed after 3 and 6 weeks of treatment. Blood samples were collected by ocular puncture at the two time intervals before sacrificing the animals and the sera separated for the following hormone assays: Testosterone, Leutinizing hormone (LH) and Follicular stimulating hormone (FSH).

At the two time intervals mentioned above, the testes were isolated from each rat, washed 3 times with potassium phosphate buffer (0.1 mol/L, pH 7.2), weighed and used to determine activity of the following testicular marker enzymes: Lactate dehydrogenase (LDH), Acid phosphatase (ACP), Alkaline phosphatase (ALP), and Glucose-6-phosphate dehydrogenase. Histological studies of the testes and other accessory organs of reproduction (seminal vesicle, epididymis and prostate gland) were also carried out.

**Biochemical Studies**

**Preparation of Blood Serum**

Fresh blood (5-10 mL) sample was collected at two time intervals mentioned above by ocular puncture from each rat into a clean plain labeled tube, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes in a Gallenkhamp table centrifuge at room temperature. The clear serum was separated and kept at -200C till assay.

**Preparation of Testis Homogenate**

The testes isolated from each rat at the two time interval were washed with potassium phosphate buffer and weighed. 0.5 g of testis was cut into small pieces and homogenized in 5 mL potassium phosphate buffer (testis/total buffer is 1:10). The supernatant obtained after the homogenate was centrifuged at 6000 rpm for 5 minutes at 40C was used as an enzyme extract for determination of enzyme activities.

**Estimation of Biochemical Parameters**

**Assay of Serum Testosterone**

Serum testosterone was estimated as described by [12] using Randox kit. The assay is based on competitive binding technique.

**Assay of Serum Luteinizing Hormone (LH)**

Serum leutinizing hormone was estimated as described by Kosasa [13] using Randox kit. The assay is based on competitive binding technique.

**Assay of Follicle Stimulating Hormone (FSH)**

Serum follicle stimulating hormone was estimated as described by [14] using Randox kit. The assay is based on competitive binding technique.
Assay of Testis Homogenate Alkaline phosphatase (ALP) and Total Acid phosphatase (ACP) Activities

The testis homogenate alkaline phosphatase and total acid phosphatase activities were estimated as described by [15] using Fortress kit.

Assay of Testis Homogenate Lactate dehydrogenase (LDH) Activity

The testis homogenate lactate dehydrogenase (LDH) activity was estimated as described by [16] using Randox kit.

Assay of Testis Homogenate Glucose-6-phosphate dehydrogenase (G-6-PDH) Activity

The testis homogenate glucose-6-phosphate dehydrogenase activity was estimated as described by [17] using Randox kit.

Histological Studies

At the 3rd and 6th weeks post-treatment, testis, epididymis, seminal vesicle and prostate tissues were harvested from the sacrificed rats. Organs were quickly cleaned, and immediately fixed in 10% formalin. The tissues were transferred into an automatic processor where they went through a process of dehydration. The tissues were then cleared in xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised, hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined with a Leica DM750 microscope interfaced with Leica ICC 50 camera. Micrographs were obtained at different magnifications and archived for comparison.

Statistical Analysis

All data obtained were subjected to statistical analysis using One-way analysis of variance (ANOVA) of 1997 SAS Institute Package (version 9.1) and the results were expressed as Mean ± SEM. Results were considered to be statistically significant when p values were less than 0.05.

Ethical Consideration

The study was performed with the approval of Institutional Ethical Committee on the use of animals for research work.

Results

Table 1 showed the effect of cadmium and extract of Momordica charantia on the hormones involved in spermatogenesis. There was no significant difference in serum testosterone and luteinizing hormone (LH) levels in all the treatment groups at the third week post-treatment. However, testosterone and LH levels in rats treated with cadmium alone exhibited a significant decrease (p<0.05) at the sixth week post treatment compared to what observed in rats in control and those pre-treated with extract of MC where there were significant (p<0.05) increase in the levels of the hormones. However, there was increase (though not significant) in the levels of follicle stimulating hormone (FSH) in all the treatment groups at the two intervals previously mentioned.

Table 1: Effect of Cadmium and Extract of Momordica charantia (MC) on Serum Testosterone (nmol/L), Luteinizing hormone (mIU/mL), and Follicle stimulating hormone (mIU/mL) Levels of Male Rats.

<table>
<thead>
<tr>
<th>Serum Hormone</th>
<th>Duration of Treatment (Weeks)</th>
<th>Control</th>
<th>Cadmium only</th>
<th>Extract of MC + Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>3</td>
<td>5.00±1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.36±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.16±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>22.33±7.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.15±1.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Luteinizing Hormone</td>
<td>3</td>
<td>2.82±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.90±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.55±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30±0.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.20±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone</td>
<td>3</td>
<td>3.22±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.34±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.75±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). Means of the same parameter at the same treatment interval with different Duncan superscripts along the row are statistically significant at p<0.05.
Table 2 showed the effect of cadmium and extract of *Momordica charantia* on the activities of alkaline phosphatase (ALP), total acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) at third and sixth week post treatment intervals. ALP level was significantly (p<0.05) reduced in rats challenged with cadmium when compared to rats in control group at the end of three weeks of treatment. Though the enzyme level was also reduced significantly (p<0.05) in rats pre-treated with extract of MC but significantly (p<0.05) higher than that of the rats treated with cadmium alone at the same treatment interval. Similarly, ALP activity was significantly (p<0.05) reduced in rats treated with cadmium alone and in those pre-treated with extract of MC at sixth week post-treatment. However, there was no significant (p<0.05) difference in the activity of ACP in all the treatment groups at the two-time treatment intervals previously mentioned.

LDH activities were significantly (p<0.05) reduced in rats treated with cadmium alone and in those pre-treated with extract of MC at third week post-treatment while the enzyme level in rats treated with cadmium alone was significantly (p<0.05) higher than that of rats pre-treated with extract of MC. Similarly, LDH activity was significantly (p<0.05) depressed in rats treated with cadmium alone and in those pre-treated with extract of MC at the end of six weeks of treatment but the LDH level in rats pre-treated with extract of MC was significantly (p<0.05) higher than that of rats treated with cadmium alone at sixth week post-treatment. Also, G-6-PDH activity was significantly (p<0.05) reduced in rats challenged with cadmium at the two-time treatment intervals but the enzyme level was significantly (p<0.05) higher in rats pre-treated with extract of MC at the two-time treatment intervals.

### Table 2: Effect of Cadmium and Extract of *Momordica charantia* (MC) on Testicular Homogenate Alkaline Phosphatase (U/l), Total Acid Phosphatase (U/l), Lactate Dehydrogenase (U/l), and Glucose-6-phosphate Dehydrogenase (U/l) Levels of Male Rats.

<table>
<thead>
<tr>
<th>Testicular Enzyme</th>
<th>Duration of Treatment (wks)</th>
<th>Control</th>
<th>Cadmium only</th>
<th>Extract of MC + Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>3</td>
<td>270.07±34.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.28±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.70±27.23&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>276.00±24.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.82±10.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.48±6.95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>3</td>
<td>13.52±4.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.53±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.27±5.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10.28±3.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.43±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>3</td>
<td>683.43±179.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>394.77±61.44&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>352.81±112.12&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>534.44±167.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140.33±20.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202.22±90.14&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose-6-phosphate Dehydrogenase</td>
<td>3</td>
<td>874.90±302.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>490.30±231.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>733.57±297.46&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>538.40±359.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235.53±70.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>260.79±50.24&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). Means of the same parameter at the same treatment interval with different Duncan superscripts along the row are statistically significant at p<0.05.

Table 3 showed the effect of cadmium and extract of *Momordica charantia* on the weights of the genital organs. The weights of testes, epididymis, seminal vesicles and prostate glands were reduced significantly (p<0.05) in all the rats treated with cadmium at the end of third and sixth week treatment intervals.

### Table 3: Effect of Cadmium and Extract of *Momordica charantia* (MC) on the Weight (g) of Genital Organs of Male Rats Relative to Body Weight

<table>
<thead>
<tr>
<th>Weight of Genital Organ</th>
<th>Duration of Treatment (wks)</th>
<th>Control</th>
<th>Cadmium only</th>
<th>Extract of MC + Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Testis</td>
<td>3</td>
<td>1.07±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.10±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Left Testis</td>
<td>3</td>
<td>1.02±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.49±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.08±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epididymis</td>
<td>3</td>
<td>0.21±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.22±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>3</td>
<td>0.87±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.06±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65±0.01&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>0.33±0.08&lt;sup&gt;ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prostate Gland</td>
<td>3</td>
<td>0.33±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.34±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22±0.02&lt;sup&gt;ba&lt;/sup&gt;</td>
<td>0.12±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). Means of the same parameter at the same treatment interval with different Duncan superscripts along the row are statistically significant at p<0.05.
Table 4 showed effect of cadmium and extract of *Momordica charantia* on body weights of rats at zero, third and sixth week post-treatment intervals. There was significant (p<0.05) difference between the weights before commencement of treatment. The weights of rats that were treated with cadmium alone and those that were pre-treated with extract of MC were significantly (p<0.05) higher than the weights of the rats in control group at zero week. There was significant (p<0.05) weight loss in rats treated with cadmium alone and those that were pretreated with extract of MC, whereas, there was significant (p<0.05) increase in the weights of rats in control group at the third and sixth week post-treatments. The percentage change in weight at the end of 3rd week post-treatment was 4.65%, 17.57% and 18.75% for control, cadmium, and cadmium with extract of MC rats respectively. The percentage change in weight at sixth week post-treatment was 8.14%, 34.12% and 29.23% for control, cadmium, and cadmium with extract of MC rats respectively. The rate of weight gain in control rats at three week post-treatment was 3.33 g/wk. The rate of weight loss in rats treated with cadmium alone was 17.33 g/wk while the rate of weight loss in rats pre-treated with extract of MC was 17.00 g/wk at three week post treatment. The rate of weight gain in control rats at six week post-treatment was 2.92 g/wk. The rate of weight loss in rats pre-treated with extract of MC was 13.25 g/wk.

**Table 4: Effect of Cadmium and Extract of *Momordica charantia* (MC) on Average Body Weight (g)**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Control</th>
<th>Cadmium</th>
<th>Cadmium and Extract of MC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks</strong></td>
<td><strong>% of change</strong></td>
<td><strong>% of change</strong></td>
<td><strong>% of change</strong></td>
</tr>
<tr>
<td>0</td>
<td>215.00±4.77c</td>
<td>296.00±8.84a</td>
<td>272.00±15.55a</td>
</tr>
<tr>
<td>3</td>
<td>225.00±4.26b</td>
<td>244.00±6.53a</td>
<td>221.00±11.20b</td>
</tr>
<tr>
<td>% of change</td>
<td>+4.65</td>
<td>-17.57</td>
<td>-18.75</td>
</tr>
<tr>
<td>6</td>
<td>232.50±2.50a</td>
<td>195.00±6.45b</td>
<td>192.50±11.09b</td>
</tr>
<tr>
<td>% of change</td>
<td>+8.14</td>
<td>-34.12</td>
<td>-29.23</td>
</tr>
</tbody>
</table>

Figures 1 and 2 showed the histomorphological changes of the testis, epididymis, seminal vesicle and prostate glands at the 3rd and 6th weeks post-treatment respectively. Figure 1 showed depletion of the cellular components of the seminiferous tubules such as the spermatogonia, spermatocytes and spermatids in the experimental groups. There was associated disruption of the seminiferous tubule architecture. In addition, there was a disruption of the interstitial space with depletion/regeneration of the Leydig cells. Remnants of the spermatids were also noted. Normal epididymis with distinct cellular boundaries, microvilli and well-formed connective tissue were noted in EA and EC while distortion of the connective tissue and absence of luminal microvilli were noted in EB. While well laid out epithelium with cuboidal to columnar epithelial cells and lamina propria were noted in SA and SB, epithelial cells of SC were clumped together with poor cellular boundary. Focal epithelial thickening were noted in the experimental group prostate (PB, PC). Figure 2 showed cellular depletion/regeneration in the experimental group seminiferous tubules alongside the interstitial space (TB, TC). However, few spermatogonia were noted in TC. Luminal microvilli were noted in the epididymis of the EA and EC with well-marked connective tissue. EB however showed features of pseudo-stratification of epithelial cells with a slight disruption of the cellular arrangement and poor cellular boundaries (EB). While the surrounding smooth muscle appears normal in the control and experimental seminal vesicle, clumping and disorganization of epithelial cells as well as disruption and thinning of lamina propria were noted in SC. There is also epithelial discontinuity and marked thinning of the surrounding smooth muscle. The prostate showed well laid out characteristic cuboidal to columnar epithelium in PA and PB, epithelial thinning with focal thickening were noted in PC.
Figure 1: Photomicrographs of Groups A, B and C (A, B, C respectively) testis (T), Epididymis (E), Seminar vesicle (S) and Prostate gland (P) after 3 weeks of treatment showing normal seminiferous tubule with spermatogonia (red arrow), spermatocyte (yellow arrow), spermatid (white arrow) and Leydig cell (L) in TA while disruption and degeneration of seminiferous and interstitial cell were noted in TB and TC with remnant of spermatid (green arrow). Epididymis with normal cellular and connective tissue histoarchitecture (EA), microvilli (brown arrow) were noted in EA and EC. Disruption of cells was noted in EB alongside disorganization of connective tissue. The epithelium (black arrow), and smooth muscle (orange arrow) appear normal in SA and SB, while cellular clumping and thinning of smooth muscle were noted in SC. PA showed normal histoarchitecture of the prostate gland while focal (white arrow) thickening of epithelium was noted in PB and PC. Stain H&E. Mag X40 (PA, PB, PC, SB), X100 (EA, EB, EC, SA, SC), X400 (TA, TB, TC).
Figure 2: Photomicrographs of Groups A, B and C (A, B, C respectively) testis (T), Epididymis (E), Seminar vesicle (S) and Prostate gland (P) after 6 weeks of treatment showing normal seminiferous tubule with spermatogonia (red arrow), spermatocyte (yellow arrow), spermatid (white arrow) in TA while degeneration of seminiferous and interstitial cell were noted in TB and TC with remnant of spermatid (green arrow). Few spermatogonia (red arrow) were noted in TC. Epididymis with normal cellular and connective tissue histoarchitecture (EA), microvilli (brown arrow) were noted in EA and EC. Cellular and connective tissue degeneration were noted in EB. The epithelium (black arrow), and smooth muscle (orange arrow) appear normal in SA and SB, while cellular disruption was noted in SC. PA and PB showed normal
Discussion

The current study was designed to investigate the possible protective effect of methanolic extract of the leaf of *Momordica charantia* (MC) on cadmium-induced gonad damage in male rats. The complete male gonad development is dependent on the balanced endocrine interplay of hypothalamo-pituitary-gonadal axis. LH and FSH are released from pituitary gland. FSH binds with receptors in the sertoli cells and stimulates spermatogenesis while LH stimulates the production of testosterone in Leydig cells of testis [18]. The significant (p<0.05) reduction in sera levels of testosterone and luteinizing hormone following subcutaneous administration of cadmium to male rats may be as a result of cadmium-induced oxidative damage of the gonads as previously reported [2, 19]. The elevation of FSH levels (though not significant) in all the rats treated with cadmium at the two-time post-treatment intervals may be due to feed back mechanism of testosterone. Cadmium exposure through puberty of male rats results in decreased circulating levels of LH and testosterone and increased FSH [20]. Pre-treatment with extract of *Momordica charantia* significantly (p<0.05) raised testosterone level at sixth week post-treatment. This was as a result of significant (p<0.05) increase in LH level to near normal in rats in this group.

Testicular marker enzymes are sensitive biochemical endpoints and may be used as a marker reflecting cadmium toxicity to the reproductive system [21]. The results of this study showed significant decrease (p<0.05) in the activity of alkaline phosphatase, ALP in the testes of all rats exposed to cadmium which was marked in rats treated with cadmium alone. Decrease in the activity of acid phosphatase, ACP (though not significant) in all rats challenged with cadmium was also observed. This supports previous studies that cadmium exposure results in gonads damage with decreased activities of ALP and ACP [22]. The decreased activities of testicular ALP and ACP observed may be as a result of decreased testosterone levels in rats treated with cadmium and this is a reflection of testicular degeneration [23]. 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 [5, 24]. Glucose-6-phosphate dehydrogenase, G-6-PDH is present in sertoli, leydig and spermatogenic cells, and it is associated with the functions of these cells. The significant (p<0.05) reductions in the activities of LDH and G-6-PDH in testes of all rats exposed to cadmium could be due to imbalance production of these enzymes following oxidative damage by cadmium. However, *Momordica charantia* significantly (p<0.05) increased the activities of ALP, LDH and G-6-PDH in the testes of rats pre-treated with the extract possibly by counteracting the oxidative activity of cadmium.

Cadmium exposure led to significant (p< 0.05) weight loss in rats, though, both the percentage and the rate of weight loss was more in the rats treated with cadmium alone. This result supports previous work where the same dose of 2.5 mg/kg bwt cadmium was administered to rats five times a week for the period of four weeks and resulted to significant decrease in body weights of male rats [22]. Similarly, the treatment of rats in this study with cadmium resulted in significant decrease in weights of testes, epididymis, seminal vesicles and prostate glands. The reduction in the weights of the accessory organs of reproduction of male rats treated with cadmium is as a result of the necrosis of seminiferous tubules.

Histological study of the testis showed well arranged seminiferous tubules with distinct interstitial in control rats at the two-time treatment intervals. The testicular tissue appears normal with full complement of spermatogenic cell line (spermatogonia, spermatocytes and spermatids). There was varying degree of cellular degeneration within the seminiferous tubules and interstitial in rats challenged with cadmium which was marked in rats treated with cadmium alone. There was complete destruction of germ cells and seminiferous tubules in rats treated with cadmium alone. This damage was characterized by necrosis and destruction of nucleus. In rats pre-treated with methanolic extract of MC, few spermatogonia with well-arranged remnant of spermatids were observed. Cadmium induces testicular necrosis despite the fact that very little cadmium accumulates in the testis. A study showed that a single low dose of cadmium exposure causes spermatogenesis failure [25]. Cadmium induced testicular damage causes vascular necrosis and increased vascular permeability [26]. The histological study of the epididymis showed distorted epithelium, absence of microvilli, distorted basal cells and clumping of the nucleoli in rats treated with cadmium alone. However, in rats pre-treated with extract of MC the microvilli were preserved. Cellular disorganization and distorted lamina propria were observed in the seminal vesicles of rats treated with cadmium. There was varying degree of cellular disorganization and loss of characteristic pseudostratified epithelia in the prostate of rats treated with cadmium. The cellular degeneration and disorganization observed in the histomorphological study was also due to decreased in the activities of the testicular marker enzymes previously described following cadmium administration. The activity of ALP is related to the mitosis of spermatogenic cells [21], LDH activity in testicular tissue is associated with the maturation of the germinal epithelial layer of the seminiferous tubules [27] and G-6-PDH is associated with the function of leydid cells.

Conclusion

In conclusion, the results inferred that cadmium induces toxicity of the gonads, and that leaves of MC at the dose investigated possess potentials to restore deranged levels of gonadotrophic hormones and testicular marker enzymes observed in heavy metal-induced gonads damage.
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References


