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Hormonal Protective Potential of Moringa oleifera Against Potassium Dichromate (Cr VI)-Induced Endocrine Disruption and Oxidative Stress in Male Rabbits

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ABSTRACT

Hexavalent chromium [Cr(VI)] is a well-documented environmental toxicant known to disrupt endocrine function and induce oxidative stress. This study investigated the protective role of *Moringa oleifera* (MO) against Cr(VI)-induced hormonal dysregulation and oxidative damage in male rabbits. Animals were divided into four groups: control, MO-treated, Cr(VI)-exposed, and MO+Cr(VI)-treated. Serum hormonal profiles and oxidative stress markers were analyzed. Results showed that Cr(VI) exposure significantly decreased testosterone (0.993 ng/ml), triiodothyronine (T_3, 1.396 ng/dl), and thyroxine (T_4, 2.934 µg/dl) levels ($p<0.05$), while elevating follicle-stimulating hormone (FSH, 0.869 mIU/ml) and luteinizing hormone (LH, 0.866 mIU/ml). Conversely, MO treatment markedly enhanced testosterone (2.764 ng/ml), T_3 (2.023 ng/dl), and T_4 (3.563 µg/dl) levels, while reducing LH (0.562 mIU/ml). Co-administration of MO with Cr(VI) restored testosterone (1.55 ng/ml), T_3 (1.568 ng/dl), and T4 (3.236 µg/dl) levels close to control values, with partial normalization of FSH and LH. Oxidative stress analysis revealed that Cr(VI) significantly elevated thiobarbituric acid-reactive substances (TBARS) in plasma (3.113 nmol/gT) and testes (27.3 nmol/gT), compared to controls (2.673 and 14.7 nmol/gT, respectively). MO administration lowered TBARS in plasma (2.462 nmol/gT) and testes (10.5 nmol/gT), while MO+Cr(VI) treatment markedly reduced lipid peroxidation relative to Cr(VI) alone. In conclusion, *Moringa oleifera* demonstrated a significant protective effect against Cr(VI)-induced endocrine disruption and oxidative stress by enhancing testosterone and thyroid hormone levels, modulating gonadotropins, and reducing lipid peroxidation. These findings highlight the therapeutic potential of MO as a natural antioxidant and hormonal regulator in mitigating heavy metal-induced reproductive toxicity.

Keywords: *Moringa oleifera, potassium dichromate, reproductive hormones, oxidative stress, rabbits.*

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1. INTRODUCTION

Hexavalent chromium [Cr(VI)], a widespread industrial and environmental pollutant, is recognized for its potent capacity to induce oxidative stress, genotoxicity, and endocrine-disrupting effects [1]. In animal models, Cr(VI) exposure has been shown to suppress steroidogenesis, reduce testosterone levels, and interfere with the hypothalamic pituitary gonadal axis (HPG), thereby impairing male reproductive function [2]. In particular, in vivo studies in rodents have demonstrated that Cr(VI) decreases the activities of key steroidogenic enzymes (e.g. 3β-HSD, CYP11A1), disrupts blood-testis barrier

integrity, and increases apoptosis of Leydig cells, culminating in reduced serum testosterone and compromised spermatogenesis [3]. Concurrently, Cr(VI) also impacts thyroid hormone homeostasis. Disruption of triiodothyronine (T_3) and thyroxine (T_4) levels has been observed in animals exposed to heavy metals, mediated via increased reactive oxygen species (ROS) production, lipid peroxidation, and altered expression of thyroid enzyme systems. These disturbances in thyroid hormones may further exacerbate metabolic and reproductive dysfunction [4]. Oxidative stress is now established as a major mechanism by which Cr(VI) exerts its endocrine and reproductive toxicity. Excess generation of free radicals overwhelms endogenous antioxidant defenses, resulting in lipid peroxidation, cell membrane damage, and hormonal dysregulation. Biomarkers such as thiobarbituric acid reactive substances (TBARS) rise significantly in response to Cr(VI) exposure, correlating with declines in testosterone and alterations in gonadotropins (FSH, LH) [5]. *Moringa oleifera* (MO) is a medicinal and nutritional plant rich in phytochemicals such as flavonoids, polyphenols, vitamins C and E, and carotenoids. Several reports have shown that MO leaf or seed extracts possess strong antioxidant, anti-inflammatory, and hormone-modulating properties [6]. For example, MO supplementation in male animal models improves reproductive hormone levels, enhances sperm quality, and suppresses ROS, thereby offering protective effects against oxidative damage. Among female models, MO extracts have restored normal levels of FSH, LH, estrogen, and protected ovarian structure under chemically-induced [7]. Despite these promising data, the capacity of MO to mitigate the specific endocrine disruption caused by Cr(VI) particularly in terms of testosterone, thyroid hormones (T_3, T_4), FSH, and LH has not been fully elucidated in male rabbits, a species of relevance for toxicology and reproductive biology [8]. Accordingly, the present study aims to evaluate the hormonal protective potential of Moringa oleifera against Cr(VI)-induced endocrine disruption and oxidative stress in male rabbits, by assessing serum levels of testosterone, thyroid hormones and gonadotropins, together with oxidative stress biomarkers [9]. This work seeks to clarify whether MO can restore hormonal balance and reduce oxidative damage in the context of heavy-metal toxicity, thereby contributing to potential therapeutic and preventive strategies.

2. MATERIALS AND METHODS

Potassium dichromate ($K_2Cr_2O_7$; 5 mg/ml) was obtained from the Chemistry Department, Faculty of Science, Sabha, Libya. Fresh leaves of *Moringa oleifera* were collected from a home garden in Samno, Sabha, Libya. Twenty mature male New Zealand White rabbits (7 months old; 2008.03 ± 49.21 g) were individually housed and fed a standard pelleted diet containing 15.8% crude protein, 11.3% crude fiber, 3.7% ether extract, and supplemented with vitamins and minerals[10]. Rabbits were randomly assigned to four groups (n=5 each): Control: received corn oil daily by gavage for 12 weeks. MO: received *M. oleifera* (400 mg/kg B.W/day) in corn oil for 12 weeks. Cr(VI): received $K_2Cr_2O_7$ (5 mg/kg B.W/day) for 12 weeks. MO+Cr(VI): received $K_2Cr_2O_7$ (5 mg/kg B.W/day) and *M. oleifera* (400 mg/kg B.W/day) concurrently for 12 weeks. Blood samples were collected biweekly from the ear vein in the morning before feeding. Plasma and serum were separated by centrifugation (860 × g, 20 min) and stored at –80°C. Hormonal concentrations of testosterone, estradiol, progesterone, FSH, LH, T_3, and T_4 were measured using commercial Coat-A-Count RIA kits (DSL, Texas and Los Angeles, USA). Plasma and testicular thiobarbituric acid-reactive substances (TBARS) were quantified as described by [11]. Statistical Analysis: Data were analyzed using ANOVA followed by Tukey's multiple comparison test (GraphPad Prism 8; Minitab v17). Results are expressed as mean ± SE, with $P < 0.05$ considered statistically significant.

3. RESULTS

Table 1 shows the mean ± SE values of plasma testosterone, triiodothyronine (T3), thyroxine (T4), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in male rabbits across the four experimental groups. Testosterone levels were highest in the MO group (2.764 ± 0.228 ng/ml) and lowest in the Cr(VI) group (0.993 ± 0.118 ng/ml), with the MO+Cr(VI) group showing intermediate levels (1.55 ± 0.098 ng/ml) comparable to the control (1.487 ± 0.045 ng/ml). T3 levels followed a similar pattern, with the MO group showing the highest value (2.023 ± 0.075 ng/dl) and the Cr(VI) group the lowest (1.396 ± 0.070 ng/dl). The MO+Cr(VI) group (1.568 ± 0.053 ng/dl) was intermediate between control (1.687 ± 0.041 ng/dl) and Cr(VI) groups. T4 levels were highest in the MO group (3.563 ± 0.117 µg/dl), lowest in the Cr(VI) group (2.934 ± 0.093 µg/dl), and intermediate in the MO+Cr(VI) group (3.236 ± 0.059 µg/dl), relative to control (3.172 ± 0.043 µg/dl). FSH levels were lowest in the MO group (0.736 ± 0.021 m/IU/ml), highest in the Cr(VI) group (0.869 ± 0.025 m/IU/ml), and intermediate in the MO+Cr(VI) group (0.805 ± 0.023 m/IU/ml), compared with control (0.787 ± 0.018 m/IU/ml). LH levels were lowest in the MO group (0.562 ± 0.038 m/IU/ml), highest in the Cr(VI) group (0.866 ± 0.013 m/IU/ml), and intermediate in the MO+Cr(VI) group (0.819 ± 0.015 m/IU/ml), relative to control (0.813 ± 0.012 m/IU/ml).

Table 1.Average of blood plasma testosterone (ng/ml), triiodothyronine (T_3; ng/dl), thyroxine (T_4; ug/dl), follicle stimulating hormone (FSH; m/IU/ml) and luteinizing hormone (LH; m/IU/ml) of male rabbits treated with MO, Potassium dichromate ($K_2Cr_2O_7$) and/or their combination (means ± SE).

Animal	*Testosterone*	*Triiodothyronine*	*Thyroxine (T4;*	*Follicle stimulating*	*Luteinizing*

Groups	*(ng/ml)*	*(T_3; ng/dl)*	*ug/dl)*	*hormone (FSH;m/IU/ml)*	*hormone (LH; m/IU/ml)*
Control (Mean±SE)	1.487±0.045^{b}	1.687±0.041^{b}	3.172±0.043^{ab}	0.787±0.018$^{a\,a}$	0.813±0.012^{a}
MO (Mean±SE)	2.764±0.228^{a}	2.023±0.075^{a}	3.563±0.117^{a}	0.736±0.021^{a}	0.562±0.038^{a}
'Cr(VI)' (Mean±SE)	0.993±0.118^{c}	1.396±0.070^{c}	2.934±0.093^{b}	0.869±0.025^{b}	0.866±0.013^{b}
'MO+Cr(VI) (Mean±SE)	1.55±0.098^{b}	1.568±0.053^{b}	3.236±0.059^{b}	0.805±0.023^{ab}	0.819±0.015^{ab}

Data are expressed as mean ± SE of 5 rabbit. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same letters mean that there is no significant difference ($p>0.05$).

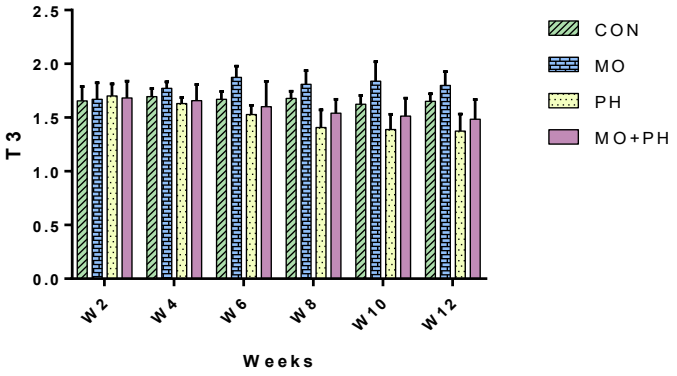

Figure 1. Changes in triiodothyronine (T_3; ng/dl) during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

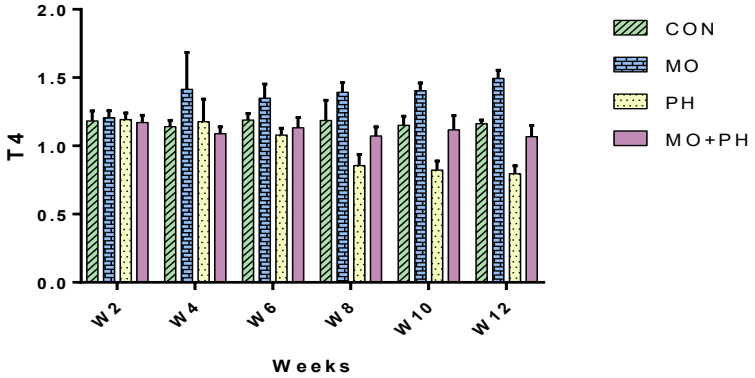

Figure 2.Changes in thyroxine (T_4; ug/dl) during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

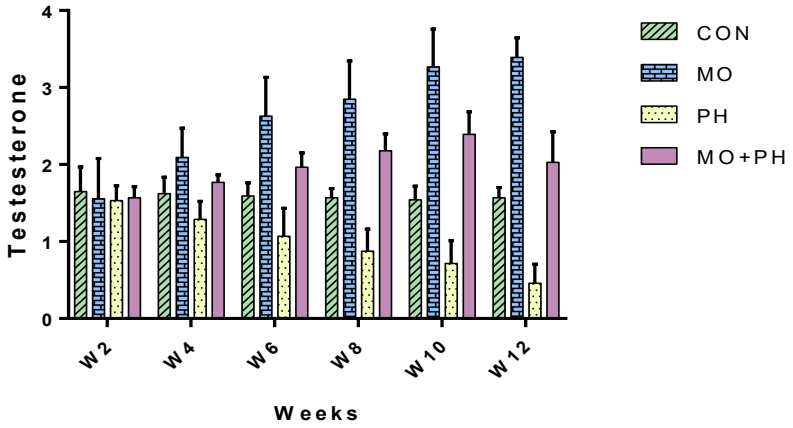

Figure 1.Changes in testosterone (ng/ml) during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

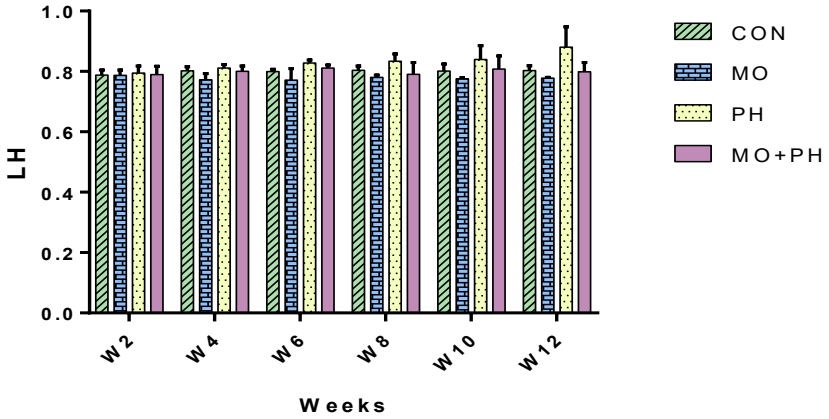

Figure 4. Changes in luteinizing hormone (LH; m/IU/ml) during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

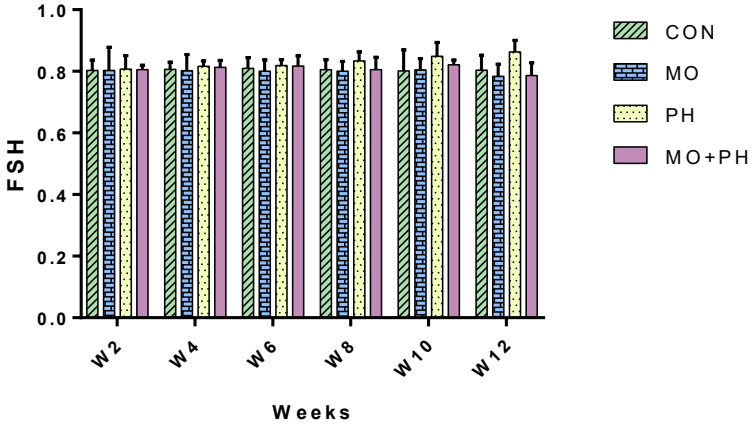

Figure 5.Changes in follicle stimulating hormone (FSH; m/IU/ml) during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

Table 2 presents the mean ± SE values of thiobarbituric acid-reactive substances (TBARS) in plasma and testes homogenates of male rabbits across the experimental groups. Plasma TBARS levels were highest in the Cr(VI) group (3.113 ± 0.087 nmol/gT) and lowest in the MO group (2.462 ± 0.051 nmol/gT). The MO+Cr(VI) group (2.601 ± 0.038 nmol/gT) showed intermediate values, compared with control (2.673 ± 0.025 nmol/gT). Testes TBARS levels were highest in the Cr(VI) group (27.3 ± 2.64 nmol/gT) and lowest in the MO group (10.5 ± 0.21 nmol/gT). The MO+Cr(VI) group (15.28 ± 1.64 nmol/gT) was intermediate, relative to control (14.7 ± 1.50 nmol/gT). Figures 6 and 7 illustrate the changes in plasma and testes TBARS, respectively, during the 12-week treatment period across the different experimental groups.

Table 2. Average of testes homogenates thiobarbituric acid-reactive substances (TBARS;nmol/gT) in male rabbits treated with MO, ptasium dichromate Cr (VI) and/or their combination (means ± SE).

Animal Groups	*Thiobarbituric acid-reactive substances (TBARS; nmol/gT)*	*Thiobarbituric acid-reactive substancesTestes (TBARS; nmol/gT)*
Control (Mean±SE)	2.673 ± 0.025[a]	14.7 ± 1.50[b]
MO (Mean±SE)	2.462 ± 0.051[b]	10.5 ± 0.21[b]
***'Cr(VI)'* (Mean±SE)**	3.113 ± 0.087[a]	27.3 ± 2.64[a]
***'MO+Cr(VI)'* (Mean±SE)**	2.601 ± 0.038[b]	15.28 ± 1.64[b]

Data are expressed as mean ± SE of 5 rabbit. Within each row, means with different superscript (a, b, c or d) were significantly different at $p<0.05$. Where means superscripts with the same letters mean that there is no significant difference ($p>0.05$).

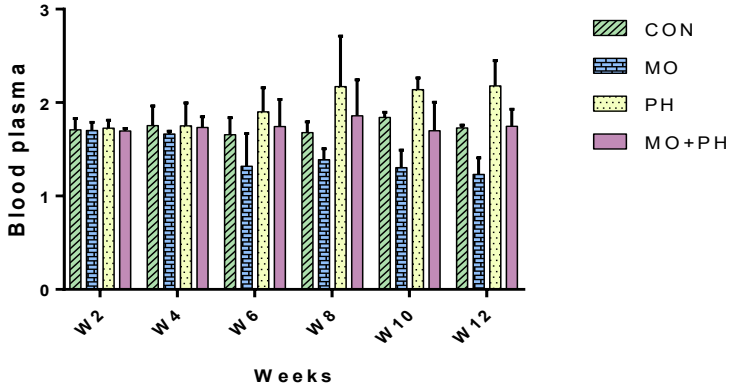

Figure 6.Changes in thiobarbituric acid-reactive substances (TBARS) in plasma during treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

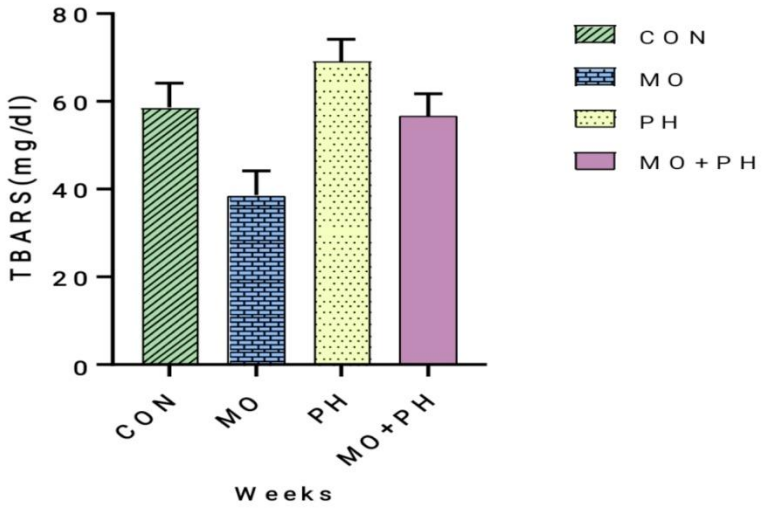

Figure 7.Changes in thiobarbituric acid-reactive substances (TBARS) in testesduring treatment of male rabbits with MO , ptasium dichromate Cr (VI) and/or their combination.

4. DISCUSSION

The present study demonstrated significant alterations in plasma sex and thyroid hormones, as well as lipid peroxidation markers, in male rabbits treated with Moringa oleifera (MO), potassium dichromate (Cr VI), and their combination. Testosterone levels were markedly elevated in the MO-treated group compared with control, whereas Cr(VI) exposure resulted in a pronounced reduction. The intermediate values observed in the MO+Cr(VI) group suggest a mitigating effect of MO against Cr(VI)-induced testosterone suppression. These findings are consistent with previous studies reporting the androgen-enhancing effects of Moringa leaf extracts in male rodents, potentially through antioxidant and steroidogenic pathways [12]. Thyroid hormones, T_3 and T_4, showed a similar pattern, with MO treatment resulting in higher plasma concentrations, Cr(VI) causing significant reductions, and the MO+Cr(VI) group showing partial recovery toward control levels. This indicates that MO may counteract the thyroid-disruptive effects of Cr(VI), in agreement with studies highlighting Moringa's protective effects on thyroid function under oxidative stress conditions [13,14]. The gonadotropins, FSH and LH, displayed an inverse response relative to testosterone and thyroid hormones. Cr(VI) exposure significantly increased FSH and LH levels, while MO treatment reduced these hormones below control values, and the combination group exhibited intermediate levels. These results suggest that MO may modulate hypothalamic-pituitary-gonadal axis activity and partially normalize Cr(VI)-induced hormonal imbalance. Similar modulatory effects of plant-derived antioxidants on FSH and LH have been documented in rodent models under heavy metal stress[15]. Oxidative stress evaluation via TBARS in plasma and testes homogenates revealed that Cr(VI) significantly increased lipid peroxidation, whereas MO treatment significantly lowered TBARS levels in both matrices. The MO+Cr(VI) group showed intermediate TBARS levels, closer to control, highlighting the antioxidant potential of MO. These findings align with prior reports demonstrating that Moringa possesses strong free radical-scavenging activity, which can protect against heavy metal-induced oxidative damage in reproductive organs [16]. Collectively, these results indicate that Moringa oleifera exhibits a protective role against Cr(VI)-induced endocrine disruption and oxidative stress in male rabbits, likely through a combination of antioxidant activity and modulation of steroidogenic and thyroid pathways. The partial normalization of hormone levels and reduction of TBARS in the MO+Cr(VI) group underscore the therapeutic potential of MO as a protective dietary supplement in environments contaminated with heavy metals.

5. CONCLUSION

This study demonstrates that potassium dichromate (Cr VI) induces marked endocrine disruption and oxidative stress in male rabbits. Supplementation with *Moringa oleifera* significantly improved hormonal balance, reduced lipid peroxidation, and restored physiological parameters toward normal. These findings suggest that *Moringa oleifera* may serve as a natural protective agent against heavy metal-induced reproductive and endocrine toxicity.

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