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Original article

The Impact of Including *Moringa oleifera* Leaves to Lower Potassium Dichromate Toxicity on Rabbit Reproductive Outcomes Under Heat Stress

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ABSTRACT

Although chromium is a necessary element for healthy physiology, excessive amounts of it can be harmful to humans. Despite an increasing body of evidence about the effects of chromium exposure on human health, there is still a lack of agreement regarding semen quality. The incredibly lively and stimulating moringaoleifer (MO) is one of the first extensively dispersed species of the Moringaceae family. Due to its many health benefits, it has been highly respected since ancient times. Every portion of the tree has high alimentary properties, making it suitable for use in both commercial and nutritional contexts. This study's goal was to ascertain how feeding moringa oléifera leaves 'MO' to rabbits under heat stress could lessen the toxicity of potassium dichromate Cr'VI' and improve their ability to reproduce. The animals were divided into 4 groups: 400 mg 'MO'/kg bw, 5 mg Cr'VI'l/kg bw, 400 mg 'MO'/kg bw, and Cr'VI' (5 mg/kg bw) plus 'MO' (400 mg/kg bw). Results demonstrated that Cr'VI' treatment reduced ($P<0.05$) sperm motility index, sperm concentration, ejaculate volume, and semen beginning fructose content. There was a dose-dependent negative relationship between Cr'VI' and semen characteristics. Treatment with Cr'VI' led to a dose-dependent increase ($P<0.05$) in the number of aberrant and dead sperm. During treatment, treatment with, moringa oleifera 'MO' reduced the adverse effects of Cr'VI'. The outcomes showed that MO had a positive impact on mitigating the detrimental effects of Cr'VI' on male rabbit fertility and productivity. These results provide credence to the possibility of using moringa oleifera as a natural dietary supplement to improve reproductive health in stressed-out rabbits kept These findings support the use of moringa oleifera as a natural dietary supplement to enhance reproductive health in anxious rabbits housed in harsh conditions or near pollutants.

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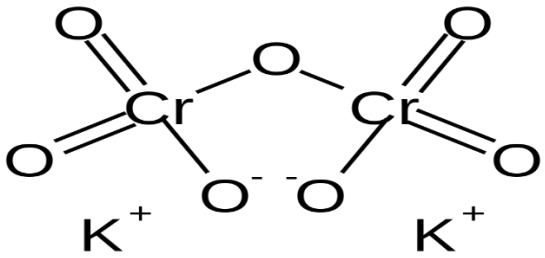
INTRODUCTION

Anthropology Because of its effects on both humans and the environment, anthropogenic exercise has become a crucial area of research [1]. Anthropogenic pollution has drastically altered the atmosphere, leading to a change in biodiversity [2]. This phenomenon results in the generation of wastes that are hazardous and needless. Heavy metals that are toxic, like hexavalent chromium (Fig,1), are produced by anthropogenic processes like electroplating, mining, wood

preservation, textile, dye, and stainless-steel manufacture, as well as tanning leather [3]. According to previous study [4], soil, water, and the air can all carry toxic chromium. Because of its mutagenicity, toxicity, and carcinogenicity, chromium is one of the main causes of acute disorders in humans [5]. According to the United States Environmental Protection Agency (USEPA), hexavalent chromium is unlikely to naturally degrade and presents a serious risk to human health and the environment [4]. It easily penetrates cells through the membrane's sulphate anion transport system and is then reduced to other lower oxidation states, which leads to an accumulation in many organs, a multitude of reactive oxygen species, or "ROS," and organ damage [6].



Figuer 1. Structure of potassium dichromate

are essential for halting the degeneration of other oxidizable products, like plastics, medications, and cosmetics. While there are other plant components with antioxidant action as well, polyphenols are the main culprits. Furthermore, studies on natural and synthetic antioxidants have revealed additional biological characteristics as anti-aging, anti-mutagenic, anti-carcinogenic, and anti-allergenic action [7]. Family *moringaceaeis, or Moringa* [8]. Although it may grow in poor soil, sandy, dry soil is ideal for it. Heat and sunlight are preferred by this plant [9]. It is a source of medicinal ingredients in addition to having high nutritional value components such protein, amino acids, carbs, minerals, vitamins, and organic acids [10].

Moringa leaves have anti-inflammatory and antibacterial properties. Diarrhea and stomach ulcers are treated with leaf tea. Because moringa leaves are abundant in protein and fiber, they are a useful dietary supply for malnourished people. Leaves are used to treat ear, eye, and mucous membrane inflammation, fevers, and bronchitis. The leaves are said to be prescribed for anemia and used to treat scurvy skin disorders due to their high iron content. The leaves are the most nutrient-dense portion of the plant, containing protein, beta-carotene, vitamin K, vitamin C, provitamin A, and other important components [11]. Because *moringa oleifera's* (MO) phytochemical components have been shown to have antioxidant and antibacterial properties, (MO) is frequently utilized in a wide range of medical applications to treat conditions like cancer, inflammatory illnesses, digestive disorders, and asthma. Studies suggest that (MO) may be able to shield the body from oxidative stress [12], boost the immune system [13], exhibit antioxidant properties that can fight free radicals and the production of reactive oxygen species [14], and influence lipid metabolism in rats in a beneficial way [8]. Moreover, MO's strong antioxidants stop deterioration, extending the shelf life of goat meat products [15].

Previous studies have examined the use of MO as a feed supplement for rabbits [16] and chickens [17]. Moringa oleifera leaf also shows protective characteristics in spermatogonial cells, reducing the damage that cyclophosphamide injections in mice's cells cause [18]. It has been discovered that the hexane extract of MO enhances the functioning of the testis, epididymis, seminiferous tubule, and seminal vesicle in male mice [19]. Moreover, it was discovered by Barakat *et al.* [20] that 'MO', when combined with hormone supplementation, accelerated the maturation rate of sheep oocytes and suggested that it might work as a promoter to trigger the production of vital proteins and 'mRNA' expression for the maturational processes. Reproduction is an essential part of life and is essential to the survival of the human race. For livestock production to be successful, advanced reproductive technology is necessary [21], and food or nutrients have a significant impact on animal reproductive performance. Nutraceuticals are organic compounds made from plants that contain useful components that may improve animal reproduction [22]. The objective of this study was to evaluate the potential protective effects of Moringa oleifera (MO) leaves on the reproductive health of rabbits exposed to heat stress and potassium dichromate (Cr VI) toxicity. Specifically, the study aimed to determine whether feeding MO leaves could mitigate the adverse effects of Cr VI on semen quality and overall reproductive performance in rabbits.

METHODS

Tested compounds

The potassium dichromat (5 mg/ml) used in this work was obtained from the chemical department of the Faculty of Science, and the Moringa oleifera leaves were gathered from a home garden in Samno, Sabha, Libya. A masculine adult from New Zealand We used rabbits that weighed 2.008±49.21 kg at birth and were 6 months old.

Animals and treatments

The following methods were used to randomly divide the twenty mature male rabbits into four equal groups of five rabbits each. Group I: Every day for a period of twelve weeks in a row, rabbits were used as the control group. Group II: The rabbits received 'MO' treatment. Every day for a duration of 12 weeks, 400 mg/kg B.W. of 'MO' was given orally via gavage [23]. Group III: According to [24], the rabbits in this group were gavaged with a daily dose of 5 mg/kg B.W./day of Cr(VI). Group IV: The rabbits were simultaneously administered the MO daily at a dose of 400 mg/kg B.W./day, similar to group II, and gavaged with Cr(VI) daily for 12 weeks at a dose of 5 mg/kg B.W./day, similar to group III. The doses of MO and Cr(VI) were determined by taking the animal's body weight one week prior to dosing. The studied doses of MO and Cr(VI) were given daily for a duration of 12 weeks. Throughout the 12-week trial, each animal's body weight was recorded once a week. Every other week for the duration of the 12-week experiment, blood samples were drawn from each animal's ear vein.

Semen characteristics

Weekly semen collection was carried out during the 12-week trial period, yielding 60 ejaculates per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. A graded collection tube was used to measure each ejaculate's volume after the gel mass was removed. Using a mild eosin solution and an upgraded Neubauer hemocytometer slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg 11, and Germany), the concentration of sperm was determined [25]. Total sperm production is calculated by multiplying the volume of ejaculate semen by the concentration of semen. [26] states that as soon as the semen was extracted, the initial fructose content in seminal plasma was determined. To assess both dead and healthy spermatozoa, eosin and nigrosine blue staining were combined [27]. Using a light microscope with a moderate 10x magnification, the percentages of motile sperm were visually calculated. The total number of motile sperm was calculated by multiplying the fraction of motile sperm with the total amount of sperm produced. Reaction time was defined as the amount of time, measured in seconds, between testing a doe and the conclusion of the erection. The initial hydrogen ion concentration (pH) was determined using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany) as soon as feasible after collection. It was noted how much packed sperm (PSV) there was. The total functional sperm fraction (TFSF) was calculated by multiplying total sperm output (TSO), sperm motility (%), and normal morphology (%) [28].

Statistical analysis

Minitab software (version 17) was used for statistical analysis as necessary. Once a normal distribution for the data was identified and an appropriate $P < 0.05$ criterion was determined as critical, the statistical significance was ascertained using "ANOVA" analysis with the "Tukey" multiple comparison test.

RESULTS

According to Table 1, sperm motility index, total sperm output, ejaculate volume, sperm concentration, and semen starting fructose concentration were all significantly ($P<0.05$) reduced by Cr(VI) treatment. The number of aberrant and dead sperms rose ($P<0.05$) in a dose-dependent manner following Cr(VI) treatment, which had a detrimental influence on semen characteristics. The adverse effects of Cr(VI) during treatment were lessened by treatment with MO. The outcomes showed that MO had a positive impact on mitigating the detrimental effects of Cr(VI) l on male rabbit fertility and productivity. These results provide credence to the possibility of using *Moringa oleifera* as a natural dietary supplement to improve reproductive health in rabbits subjected to chemical and environmental stresses.

Table 1. The mean (±SE) of semen characteristics overall when male rabbits were treated with MO, Cr(VI), and/or their combination.

Items	'Con'	'MO'	'Cr(VI)'	'MO+Cr(VI)'
Reaction time (RT; sec.)	5.10±0.4333^{a}	3.88±0.234^{a}	5.62±0.453^{b}	4.97±0.207^{a}
Initial hydrogen ion concentration (pH)	8.00±0.028^{b}	7.31±0.004^{c}	8.28±0.061^{a}	8.03±0.010^{b}
Ejaculates volume (EV; ml)	0.65±0.021^{c}	0.90± 0.024^{b}	0.48±0.013^{d}	0.70±0.011^{a}
Sperm motility (SM; %)	57.1±0.9^{b}	66.4±1.8^{a}	47.6±1.5^{c}	58.6±0.9^{b}
Live sperm (LS; %)	69.8± 0.4^{b}	82.0±0.6^{a}	58.9±1.2^{c}	69.0±3.1^{b}
Dead sperm (DS; %)	33.9±1.02^{b}	24.5±0.89^{a}	42.3±0.41^{b}	33.1±0.82^{b}
Abnormal sperm (AbS; %)	23.8±0.7^{c}	17.9± 1.9^{a}	29.8±2.8^{b}	23.1±0.7^{a}
Normal sperm (NS; %)	79.8± 0.3^{b}	84.2±0.4^{a}	74.4± 0.7^{c}	79.6±0.8^{b}
Sperm concentration (SC; x10^{6}/ml)	296.8±4.6^{b}	330.4±4.5^{a}	229.2±4.2^{d}	270.5±3.9^{c}
Packed sperm volume (PSV; %)	16.65±0.22^{b}	19.16±1.73^{a}	13.53±0.09^{d}	15.52±0.21^{c}
Semen initial fructose (IF; mg/100 ml)	254.9±3.8^{a}	269.2±11.2^{a}	200.5±4.9^{c}	233.2±12.9^{b}

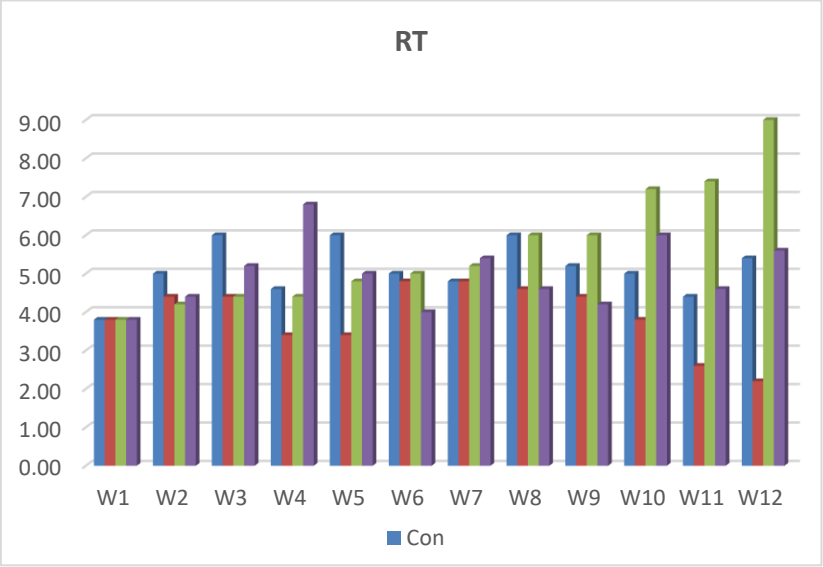


Figure 2. Variation in response time when male rabbits are treated with 'MO', Cr (VI), or both of them together.

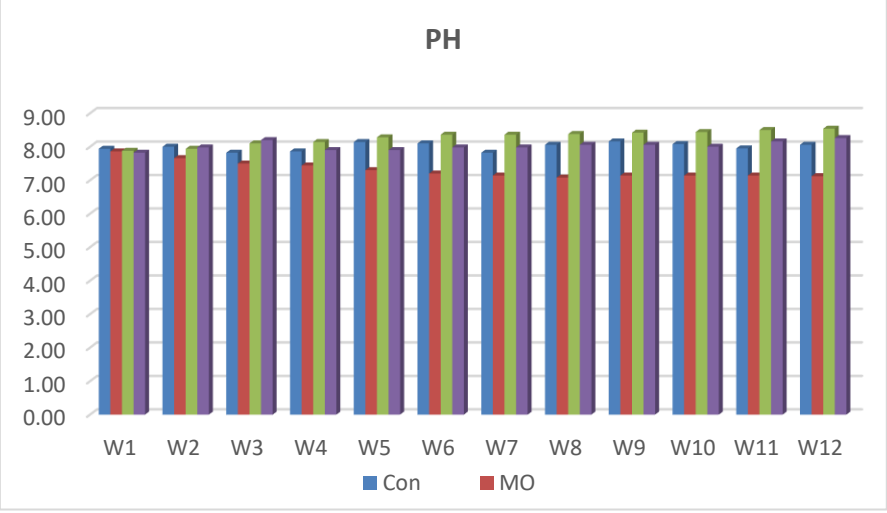


Figure 3. Variation in the starting concentration of hydrogen ions when male rabbits are treated with 'MO', Cr (VI), or both of them together.

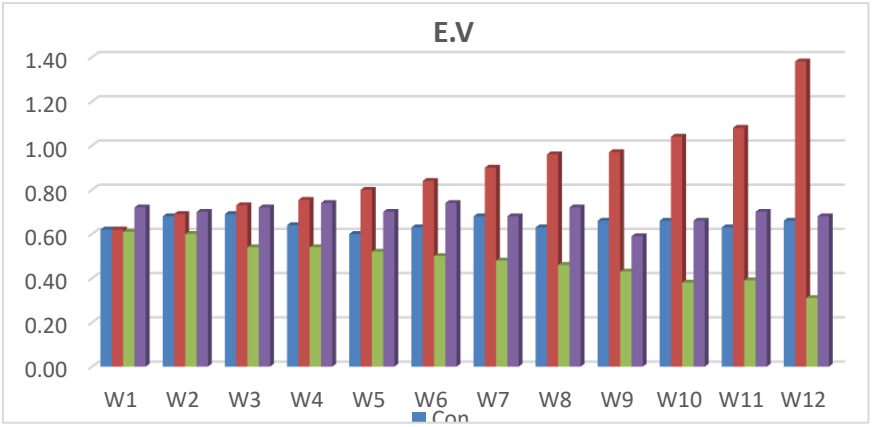


Figure 4. Variation in ejaculate volume in male rabbits treated with 'MO', Cr (VI), or both of them together.

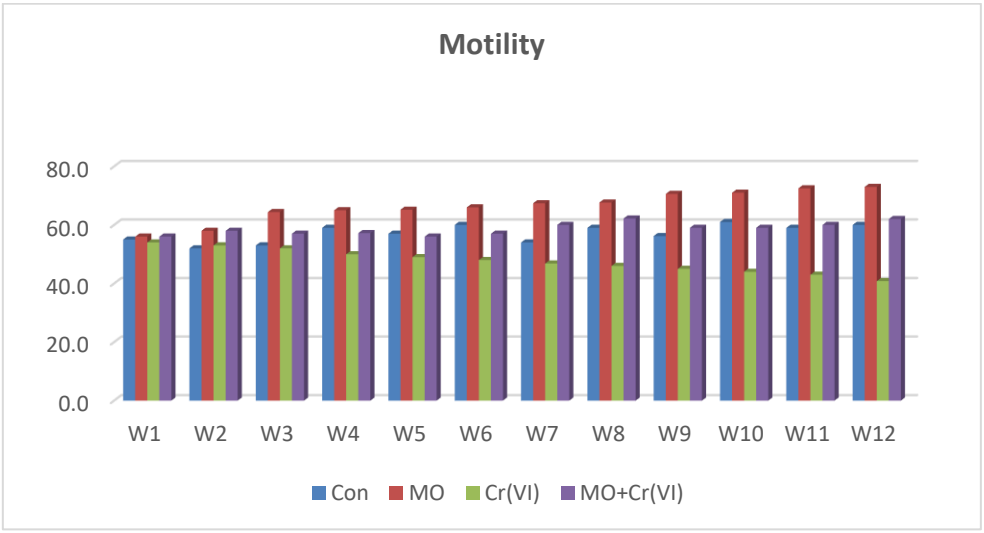


Figure 5. Modification in sperm motility in male rabbits treated with 'MO', Cr (VI), or both combined.

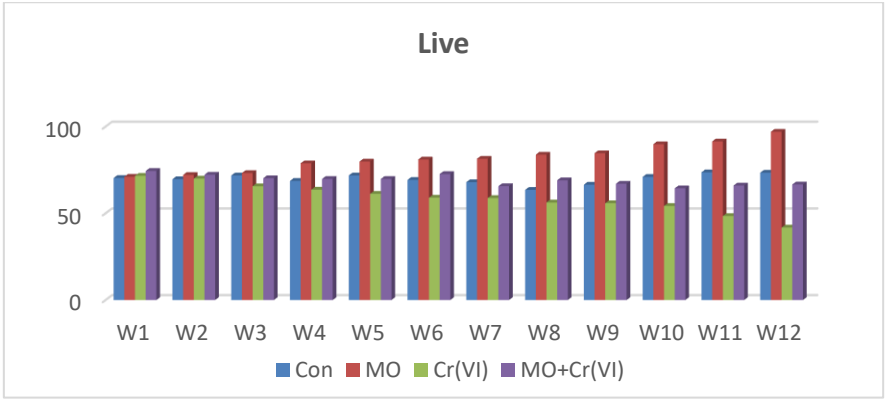


Figure 6. Modification in live sperm when treating male rabbits with 'MO', Cr (VI), or both combined.

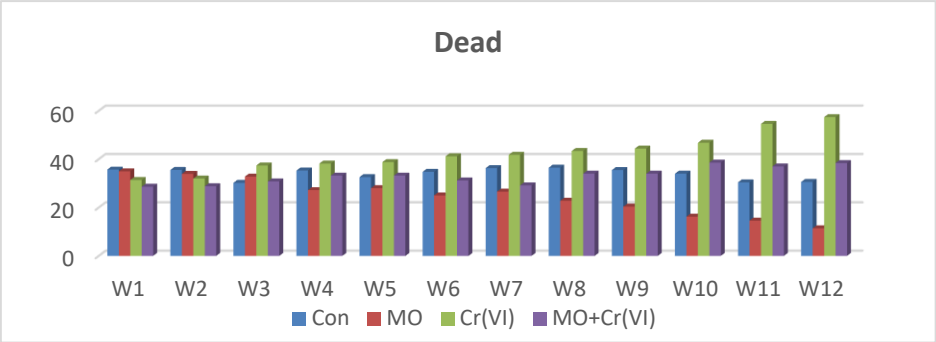


Figure 7. Modification in dead sperm when treating male rabbits with 'MO', Cr (VI), or both combined.

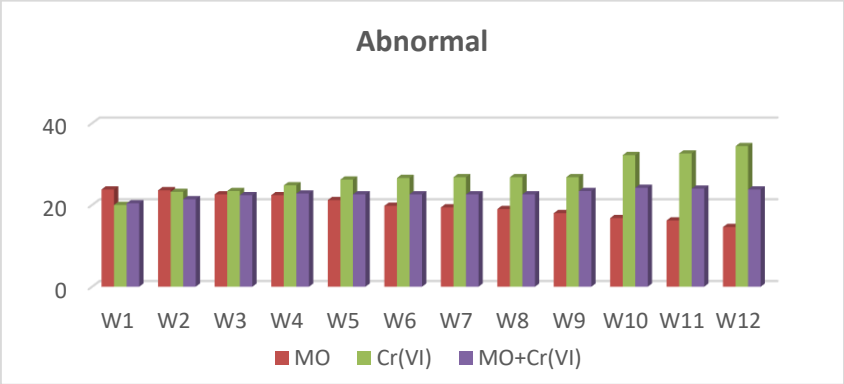


Figure 8. When male rabbits are treated with 'MO', Cr (VI), or both at once, abnormal sperm undergo a change.

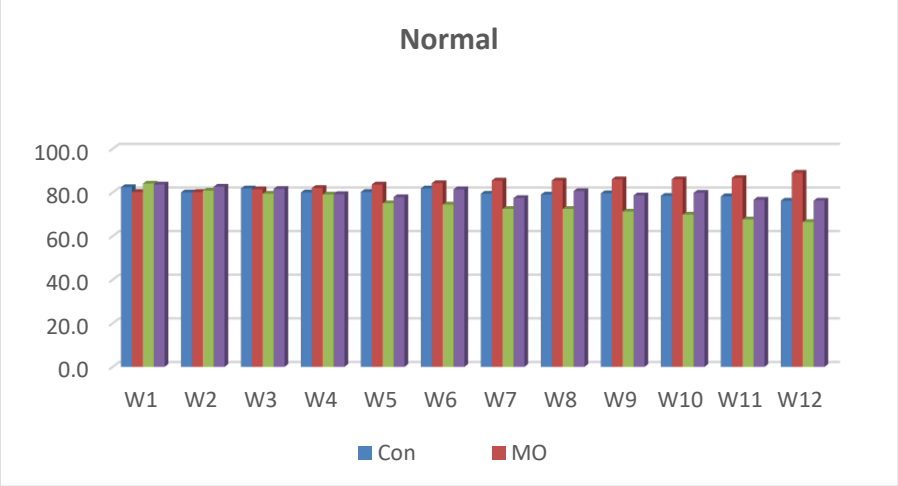


Figure 9. Modification in Normal Sperm' when treating male rabbits with Cr (VI), 'MO', or both at once.

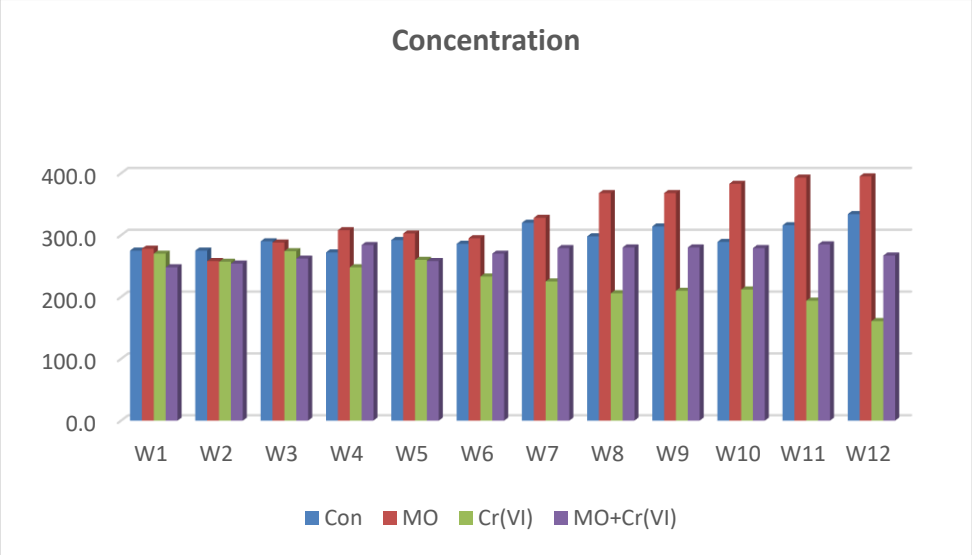


Figure 10. Shift in sperm concentration in male rabbits treated with Cr (VI), 'MO', or both at the same time.

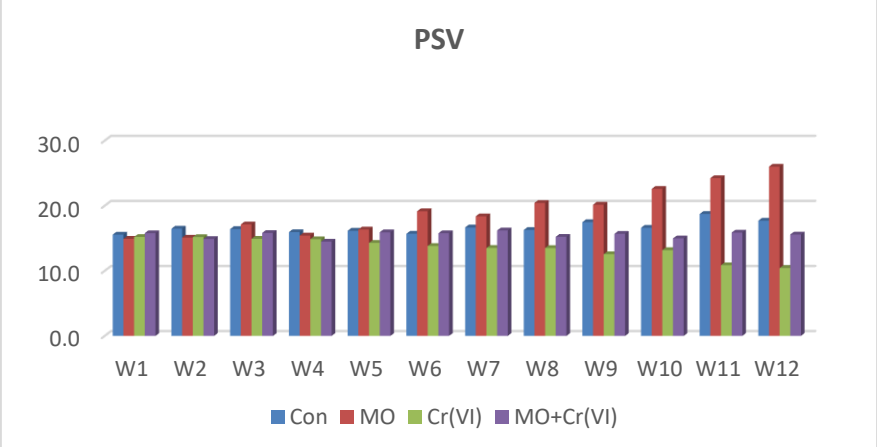


Figure 11. Variation in packed sperm volume in male rabbits treated with 'MO', Cr (VI), or both at the same time.

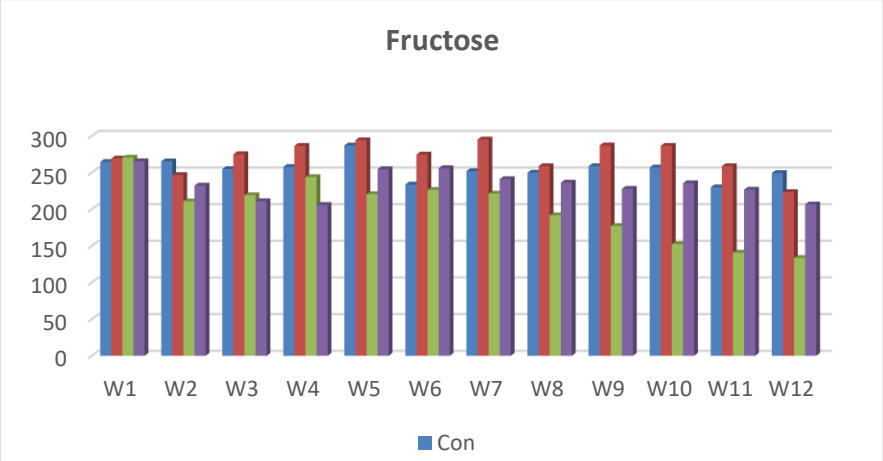


Figure 12. Modification in the early fructose content of semen in male rabbits treated with 'MO', Cr (VI), or both at the same time.

DISCUSSION

By lowering the toxicity of potassium dichromate (Cr(VI)), the current study aimed to evaluate the effect of dietary additions of Moringa oleifera (MO) leaves on male rabbits' capacity to breed under heat stress conditions. The results demonstrated that adding MO leaves as a supplement significantly lessened the detrimental effects of Cr(VI) on several reproductive indices. The Cr(VI) treatment significantly decreased sperm concentration and ejaculate volume in comparison to the control group. These results are consistent with earlier research showing that heavy metals, such as chromium, can harm reproductive organs and cause oxidative stress, which can affect reproductive capabilities [29]. Nevertheless, these indicators improved when MO leaves were added to the diet, indicating that MO may have a protective impact. According to [30], the antioxidants and phytochemicals in MO may mitigate the oxidative damage brought on by Cr(VI), hence increasing sperm production. Cr(VI) exposure also had a negative impact on sperm motility and viability, as seen by a marked decline in the motility index and an increase in the quantity of aberrant and dead sperm. These outcomes are in line with research showing that Cr(VI) can impair sperm cells' ability to function and maintain their structural integrity [31]. However, the group that received MO leaf treatment showed better sperm motility and viability, perhaps as a result of MO's antioxidant qualities, which guard against cellular damage [32].

Cr(VI) therapy decreased the initial fructose concentration in semen, a measure of seminal vesicle activity. According to[33] , the toxic effects of Cr(VI) on accessory sex glands and seminal vesicles may be the cause of this decrease. On the other hand, the addition of MO leaves preserved normal fructose levels, presumably by guarding against oxidative damage to the seminal vesicles and guaranteeing spermatozoa had enough energy [34]. Exposure to Cr(VI) considerably reduced overall reproductive performance as measured by total sperm production and functional sperm fraction. This is consistent with studies demonstrating decreased fertility and reproductive health as a result of chromium toxicity [35]. These metrics increased when MO leaves were added, confirming the theory that MO can promote reproductive health under stressful situations.

The preventive properties of MO are probably attributed to its capacity to lower oxidative stress and enhance antioxidant defense systems [36]. Exposure to Cr(VI) considerably reduced overall reproductive performance as measured by total sperm production and functional sperm fraction. This is consistent with studies demonstrating decreased fertility and reproductive health as a result of chromium toxicity [35]. These metrics increased when MO leaves were added, confirming the theory that MO can promote reproductive health under stressful situations. The preventive properties of MO are probably attributed to its capacity to lower oxidative stress and enhance antioxidant defense systems [36]. Heat stress increases oxidative stress and interferes with endocrine functioning, exacerbating the deleterious effects of Cr(VI) on reproductive health [37].

The ability of MO as a dietary supplement to lessen the combined effects of environmental pollutants and heat stress is highlighted by the protective effects of MO leaves during heat stress. According to Sinha *et al.* [38], MO's antioxidative and anti-inflammatory qualities are essential for preserving reproductive function in such unfavorable circumstances.

CONCLUSION

The findings of this study suggest that supplementing male rabbits' diets with Moringa oleifera leaves might significantly lessen the reproductive toxicity that potassium dichromate produces, particularly in situations where the rabbits are under heat stress. This preventive effect of 'MO' is most likely due to its strong antioxidant content, which helps to reduce oxidative stress and improve overall reproductive health. Further studies should examine the specific mechanisms by which 'MO' produces these protective effects and evaluate the viability of 'MO' in different animal models and environmental contexts.

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تأثير إضافة أوراق المورينجا أوليفيرا في خفض سمية ثنائي كرومات البوتاسيوم على النتائج الإنجابية للأرانب تحت الإجهاد الحراري

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المستخلص
هدفت هذه الدراسة إلى تقييم تأثير إضافة أوراق المورينجا إلى النظام الغذائي في تخفيف سمية كرومات البوتاسيوم على الأداء التناسلي للأرانب الذكور تحت ظروف الإجهاد الحراري. أظهرت النتائج أن علاج كرومات البوتاسيوم له تأثيرات ضارة على العديد من المعايير التناسلية، بما في ذلك حجم السائل المنوي، وتركيز الحيوانات المنوية وتركيز الفركتوز في السائل المنوي. كانت هذه التأثيرات السلبية تعتمد على الجرعة.ومع ذلك، أظهرت الأرانب التي تلقت أوراق المورينجا تحسناً ملحوظاً في هذه المعايير التناسلية مقارنةً بالأرانب التي تعرضت فقط لعلاج كرومات البوتاسيوم .يشير ذلك إلى أن أوراق المورينجا يمكن أن تكون لها دور وقائي بفضل محتواها الغني من مضادات الأكسدة والفيتامينات التي تعمل على تقليل الأضرار الناتجة عن الإجهاد التأكسدي الذي يسببه كرومات البوتاسيوم.كما أظهرت الدراسة أن الإجهاد الحراري يعزز من التأثيرات السلبية لعلاج كرومات البوتاسيوم على الصحة التناسلية، وأن إضافة أوراق المورينجا يمكن أن تخفف من هذه التأثيرات بشكل كبير.تشير هذه النتائج إلى أن أوراق المورينجا يمكن أن تكون مكملًا غذائيًا طبيعيًا مفيدًا لتحسين الصحة التناسلية للأرانب المعرضة للإجهاد البيئي والكيميائي، خاصةً في البيئات ذات درجات الحرارة المرتفعة.
الكلمات المفتاحية: ثاني كرومات البوتاسيوم , السائل المنوي, ارانب, المورينجا اوليفيرا