**IVERMECTIN-INDUCED REPRODUCTIVE TOXICITY IN RABBIT BUCKS AND THE PROTECTIVE ROLE OF *Moringa oleifera* LEAF EXTRACT AND VITAMIN C**

**Abstract**

This study investigated the adverse effects of ivermectin (IVM) on semen quality and hormonal levels in rabbit bucks and evaluated the protective role of *Moringa oleifera* leaf extract (MO) and vitamin C (VC). Twenty-five rabbit bucks were divided into five groups: control (normal saline), IVM-only (0.4 mg/kg weekly), IVM+MO (0.4 mg/kg IVM + 200 mg/kg MO), IVM+VC (0.4 mg/kg IVM + 200 mg/kg), and IVM+MO+VC (0.4 mg/kg IVM + 200 mg/kg MO + 200 mg/kg VC). Semen parameters (volume, sperm count, motility, morphology, livability) and serum hormones (testosterone, FSH, LH) were assessed after 8 weeks. Results revealed significant (*P*=.05) declines in semen quality and hormonal levels in the IVM group compared to controls, with improvements observed in supplemented groups. The IVM+MO+VC group showed the most notable recovery, nearing control values for sperm count (105.3 vs. 132.0 x10⁶/mL), motility (69.33% vs. 83.33%), and testosterone (4.33 vs. 5.07 ng/mL). The study concludes that IVM induces reproductive toxicity through oxidative stress and endocrine disruption, which MO and VC mitigate through antioxidant actions. Combined supplementation proved most effective, suggesting its potential as a protective strategy in breeding males. Recommendations include cautious IVM use in reproductive animals and adjunct antioxidant supplementation to preserve fertility.

**Keywords: Antioxidants; *Moringa oleifera*; Ivermectin; Reproductive toxicity; Vitamin C**

**1.0 INTRODUCTION**

Ivermectin (IVM) is a semisynthetic avermectin, a type of macrocyclic lactone, used as a broad-spectrum anthelmintic in both veterinary and human medicine (Pitterna *et al.*, 2009). IVM, 22, 23-dihydroavermectin B, consists of a natural avermectin mixture of B1a and B1b in a ratio of 80:20, produced from *Streptomyces avermitilis* (Zhang *et al.*, 2016). This antibiotic is widely recognized as an effective treatment for nematodes in animals and is the preferred medication for onchocerciasis, ascariasis, and enterobiasis in humans (Elzoghby *et al.*, 2015). IVM is predominantly effective as a single-dose treatment with a substantial safety margin, while certain instances require a repeated dosing schedule to eliminate the parasite (Foy *et al.*, 2019). Ivermetin has been shown to induce toxicity when used in high doses or over prolonged periods affecting various physiological systems in animals negatively (Abou El-Fetouh *et al.*, 2024). Khalil and Abu Samrah (2018) reported that the treatment of repeated doses of IVM induces cytogenotoxicity in bone marrow cells. IVM may permeate all bodily tissues, excluding the central nervous system (CNS), following any route of administration (Lankas *et al.*, 1989). In mammals, its action is mainly at GABA, a receptor in the CNS. At high concentrations, ivermectin promotes GABAergic inhibition by increasing GABA release and/or directly agonizing the GABA\_A chloride channel (Trailović & Nedeljković, 2011). This hyperpolarizes neurons and suppresses CNS activity. Clinically, overdoses cause progressive CNS depression (lethargy, ataxia, tremors), potentially leading to coma and death (Trailović & Nedeljković, 2011). IVM induces adverse effects ranging from moderate poisoning to central nervous system depression, as well as reprotoxicity, hepatotoxicity, and nephrotoxicity (Gonzalez *et al.*, 2012). One area of concern is its impact on the reproductive system, particularly in male animals. Semen quality is essential for the reproductive success and productivity of livestock (DeJarnette, 2005), and compromised semen characteristics due to drug toxicity can lead to fertility issues, reduced sperm motility, abnormal morphology, and decreased sperm count (Alp *et al.*, 2012).

Antioxidants protect against the toxicity induced by drugs (Jomova *et al.*, 2024). *Moringa oleifera* and vitamin C are known to have antioxidant effects. *Moringa oleifera* is a plant mostly native to India and Africa, and it is extensively found and utilized in tropical and subtropical regions globally (Leone *et al.*, 2015). The leaves of moringa contain substantial quantities of protein, carotene, vitamins (A, B, C, and E), amino acids, minerals, and a diverse array of antioxidant chemicals, including polyphenols, flavonoids, proanthocyanidins, and flavanols (Khalafalla *et al.*, 2010). In rabbits, the oral administration or incorporation of *Moringa oleifera* leaf extract (MOLE) into semen extenders enhanced reproductive functions, including sperm motility, viability, and seminal antioxidant capacity (El-Seadawy *et al.*, 2017; Ajuogu *et al.*, 2019).

Vitamin C, or ascorbic acid, is a recognized natural antioxidant and a vital water-soluble vitamin necessary for several biological activities. It is linked to semen quality and fertility in animals (Fernandes *et al.*, 2011). This vitamin neutralizes free radicals, so mitigating the detrimental effects of Reactive Oxygen Species (ROS) on spermatogenesis and safeguarding sperm (Angulo *et al.*, 2011). The beneficial effects of ascorbic acid on stressors are deemed non-depressive, safer, and more feasible. It is inexpensive, readily accessible, non-toxic, easily administered, rapidly absorbed, lacks a withdrawal period, and has no significant effects at elevated dosages in vivo (Seifi *et al.*, 2010).

There is a growing interest in the use of natural supplements and antioxidants to mitigate drug-induced toxicity and protect reproductive health. However, limited research exists on the combined effects of *Moringa oleifera* leaf extract and vitamin C on the reproductive function of rabbit bucks exposed to ivermectin-induced toxicity. Hence, this study aims to fill this gap by investigating ivermectin induced reproductive toxicity in rabbit bucks and the protective role of *Moringa oleifera* leaf extract and vitamin C.

**2.0 MATERIAL AND METHODS**

**2.1 Experimental Animals and Management**

Twenty-five (25) apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) between 10 months and 1year old, weighing from 2.5 kg to 3.0 kg were used in this study. They were kept in hygienic, separate cages with a wire floor under standard circumstances. The rabbits were given feed and water *ad libitum*. The animals were maintained for two weeks to acclimatize prior to the commencement of the experiment. The experiment adheres to the guidelines set forth in the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH publication no. 83-23, revised 1996). The local ethics committee approved the study. The rabbits were weighed before and after the experiment.

## 2.2 Drug Acquisition and Preparation

Vitamin C (Em-Vit-C 100mg tablets) from Emzor® Pharmaceuticals Limited were purchased from reputable pharmaceutical shop within Wukari, Taraba State while ivermectin injectable 1% solution (InterChemie®) for veterinary use was purchased from Agrovet shop. The vitamin C tablets were initially made into a solution by dissolving them in distilled water before to administration to the rabbits.

**2.3 Plant Collection and Extraction**

Fresh *Moringa oleifera* leaves were sourced within Wukari, Taraba State, Nigeria. The leaves were allowed to air-dried to constant weight at room temperature and blender with Blender to powder. Two (2) kg of powdered *M. oleifera* leaves were extracted with ten (10) liters of methanol using a cold extraction technique. Four (4) grammes of the extract were diluted in 100 mL of distilled water to prepare a 4% (w/v) stock solution (Akorede et al., 2020).

**2.4 Experimental Design**

The rabbit bucks were randomly grouped into five groups which consisted of five rabbits each.

Group I received once weekly physiological saline (2 ml/kg body weight) subcutaneously.

Group II received subcutaneous administration of 0.4mg/kg body weight of ivermectin (which is the therapeutic dose) once weekly for 8 weeks.

Group III received 0.4mg/kg body weight of ivermectin, subcutaneously once weekly and oral administration of 200 mg/kg of *Moringa oleifera* leave extract for 8 weeks.

Group IV received 0.4mg/kg body weight of ivermectin, subcutaneously once weekly and oral administration of 200 mg/kg of Vitamin C for 8 weeks.

Group V received 0.4mg/kg body weight of ivermectin, subcutaneously once weekly and oral administration of 200 mg/kg of *Moringa oleifera* leave extract and 200 mg/kg of Vitamin C for 8 weeks.

**2.5 Semen Collection and Analysis**

Throughout the experimental period, the bucks were conditioned to ejaculate into an artificial vagina (AV) via a female teaser, as outlined by Munu *et al.* (2024). An improvised artificial vagina was constructed utilizing syringes, standard sample containers, and condoms. The AV was preheated in a hot water bath and held manually beneath the teaser, with the open end orientated caudally. As the buck initiates mounting, the artificial vagina (AV) was positioned more caudally to facilitate the male rabbit's penetration and ejaculation, which occurred swiftly after the penis entered the AV (Asibor *et al.*, 2022). The ejaculates were collected and maintained at a temperature of 37 °C using warm water.

Gross evaluations of the ejaculates were conducted by analyzing the color, volume, and inspecting for overt signs of contamination, hemorrhage, or inflammatory changes. Samples from the different treatment groups were dropped on a pre-warmed glass slide and viewed at x100 magnification using light microscope. Individual motility was subjectively evaluated by counting the average number of the motile spermatozoa on the slide fields. The motility of the spermatozoa was quantified as a percentage (%). The sperm count was assessed via the Neubauer hemocytometer, as previously detailed by Atiq *et al.* (2011). For sperm morphology evaluation, the semen was smeared on a clean glass slide, dried and fixed with fixative (three volume of absolute methanol and one volume of glacial acetic acid), then it was stained with hematoxylin for 15 minutes and then washed, which was followed by staining with 1% eosin for 10 minutes and washed, then allowed to dry at room temperature and observed at x100 under a light microscope (Koziol & Armstrong 2022). The percentage of livability (%) was evaluated by mixing one drop of semen with 2-3 drops of warmed eosin-nigrosine stain on a heated slide, as outlined by Wells and Awa (1970). A thin smear was prepared and air-dried from the combination of semen and stain. The viable and non-viable sperm cells were counted separately, and the ratio of viable to non-viable sperm cells was determined.

**2.7 Determination of Hormonal Level**

A 5 ml blood sample was obtained in the morning from the marginal ear vein of each rabbit into plain test tubes, incubated for 60 minutes, and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Subsequently, serum was extracted from each test tube into sterile sample tubes, which were then utilized for the assessment of serum hormonal levels. Serum levels of FSH, LH, and testosterone were quantified utilizing Enzyme Linked Immunosorbent Assay (ELISA) kits sourced from Elabscience® (Texas, USA), in accordance with the manufacturer's guidelines included with the kits.

**2.8 Data Analysis**

The collected data were analyzed as mean ± standard error of the mean (SEM) and underwent one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test, utilizing GraphPad Prism version 9.0.1 (GraphPad Software, CA, USA). A P-value of .05 was deemed significant.

**3.0 RESULTS AND DISCUSSION**

**3.1 Result Presentation**

In Table 1, the control group consistently showed the most favorable semen parameters. Semen volume was significantly (*P=*.05) highest in the control (1.50 mL) and lowest in the IVM-only group (0.77 mL), demonstrating a negative effect of IVM on semen output. Supplementation with MO, VC, or both significantly (*P=*.05) improved semen volume relative to IVM alone. For sperm count, the control group had the highest count (132.0 x10⁶/mL), while IVM caused a drastic reduction (57.3 x10⁶/mL). Additions of MO, VC and a combination of MO+VC significantly (*P=*.05) increased sperm counts, with IVM+MO+VC yielding a significantly (*P=*.05) higher count (105.3 x10⁶/mL) than the other supplementations.

Sperm morphology and motility were likewise adversely affected by IVM (50.33% and 47.00%, respectively) and were significantly (*P=*.05) improved by the addition of supplements. Notably, the IVM+MO+VC group achieved morphology (82.33%) comparable to the control, suggesting a restorative effect of the combined treatment. Motility significantly (*P=*.05) improved from 47.00% in IVM alone to 69.33% in IVM+MO+VC which is lower the control (83.33%). Sperm livability also declined with IVM (48.00%) in comparison to the control and was significantly (*P=*.05) increased with supplementation, reaching 71.67% in the IVM+MO+VC group.

Table 1: Semen Characteristics of rabbit bucks exposed to Ivermectin, *Moringa oleifera* leaf extract and Vitamin C

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Control** | **IVM** | **IVM + MO** | **IVM + VC** | **IVM+MO+VC** |
| Semen Volume (mL) | 1.50±002a | 0.77 ±0.04c | 1.07±0.02b | 1.10±0.03b | 1.23±0.06b |
| Sperm count (x106/mL) | 132.0±4.8a | 57.3±3.6d | 83.7±3.2c | 92.67±2.1bc | 105.3±3.5b |
| Sperm Morphology (%) | 90.0±1.83a | 50.33±2.01c | 64.33±2.14b | 69.33±2.59b | 82.33±1.87a |
| Sperm Motility (%) | 83.33±.05a | 47.00±0.97e | 61.671.12d | 66.67±1.52c | 69.33±2.43b |
| % Sperm Livability (%) | 88.33±2.79a | 48.00±3.85c | 61.33±2.38b | 65.00±2.28b | 71.67±1.12b |

a,b,c,d,e = Means across rows with different superscripts are significantly different at p<0.05

Key:

IVM= Ivermectin

IVM+MO= Ivermectin +*Moringa oleifera*

IVM+VC= Ivermectin + Vitamin C

IVM+MO+VC= Ivermectin +Folic Acid + Vitamin C

SEM=Standard Error of Mean

Table 2 presents the results of the hormonal levels of rabbit bucks exposed to ivermectin toxicity and subsequent supplementation with MO and VC. The control group had the highest concentrations of testosterone (5.07 ng/mL), FSH (4.80 ng/mL), and LH (4.77 ng/mL). Ivermectin (IVM) exposure significantly (*P=*.05) suppressed all three hormones, with testosterone at 2.17 ng/mL, FSH at 1.53 ng/mL and LH at 1.70 ng/mL. Supplementation with MO and/or VC significantly (*P=*.05) improved hormonal levels with the IVM+MO+VC group nearing control values—testosterone (4.33 ng/mL), FSH (4.33 ng/mL), and LH (4.30 ng/mL). The improvements suggest that these supplements mitigate the endocrine-disrupting effects of Ivermectin.

Table.2: Hormonal level of rabbit bucks exposed to Ivermectin, *Moringa oleifera* leaf extract and Vitamin C

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **Control** | **IVM** | **IVM + MO** | **IVM + VC** | **IVM+MO+VC** |
| Testosterone (ng/mL) | 5.07±0.14a | 2.17±0.11d | 3.50±0.13c | 3.80±0.05c | 4.33±0.11b |
| FSH (ng/mL) | 4.80±0.11a | 1.53±0.06d | 2.73±0.11c | 2.93±0.09c | 4.33±0.10b |
| LH (ng/mL) | 4.77±0.09a | 1.70±0.13e | 2.73±0.04d | 3.70±0.11c | 4.30±0.05b |

a,b,c,d = Means across rows with different superscripts are significantly different at p<0.05

Key:

IVM= Ivermectin

IVM+MO= Ivermectin +*Moringa oleifera*

IVM+VC= Ivermectin + Vitamin C

IVM+MO+VC= Ivermectin +Folic Acid + Vitamin C

SEM=Standard Error of Mean

**3.2 Discussion of Findings**

Ivermectin (IVM) exposure significantly impaired male rabbit fertility: ejaculate volume, sperm concentration, motility, viability and normal morphology all declined significantly, and serum testosterone, FSH and LH levels were likewise depressed. These findings concur with known avermectin reproductive toxicity. IVM readily crosses the blood–testis barrier and accumulates in germ cells, causing oxidative damage and apoptosis (Chavez-Varias *et al.*, 2024; Salman *et al.*, 2022). In males, avermectins induce testicular lesions that reduce sperm count and motility (Salman *et al.*, 202). The resulting germinal cell loss and Sertoli/Leydig cell damage explain lower sperm output and increased abnormalities. Ahmed *et al.* (2020) reported that IVM treatment provoked profound testicular cell apoptosis and marked infertility in rats, effects that were alleviated by antioxidant co-treatment (Ahmed *et al.*, 2020). In rabbits, as in other models, IVM triggered excessive reactive oxygen species (ROS), endoplasmic-reticulum stress and mitochondrial dysfunction within the seminiferous tubules (Chavez-Varias *et al.*, 2024). Such stressors activate unfolded protein responses and cell death in spermatogonia (Chavez-Varias *et al.*, 2024). Concurrently, neuroendocrine disruption occurred: IVM’s effects on GABAergic pathways and the hypothalamic–pituitary–gonadal (HPG) axis could suppress gonadotropin release and steroidogenesis (Salman *et al.*, 2022). In aggregate, these mechanisms account for the lower testosterone, FSH and LH measured in IVM-treated bucks. Similar endocrine impairment has been observed with other toxins: heavy metal or pesticide exposure in rabbits caused parallel drops in sex hormones and sperm quality (Yousef, 2005). Thus the IVM-induced alterations in seminal and hormonal parameters reflect a combination of direct testicular cytotoxicity and disturbed HPG signaling.

Supplementation with *Moringa oleifera* leaf extract (MO) and/or vitamin C significantly mitigated IVM’s deleterious effects. Bucks receiving MO and/or vitamin C showed substantial recovery of semen volume, sperm count, motility, viability and normal morphology, and near-normal hormone levels. This protective outcome is in agreement with numerous studies on these supplements. Dietary *Moringa* is rich in antioxidants and has been shown to improve male reproductive performance. El-Kashef (2022) reported that male rabbits fed *Moringa* leaves exhibited increased semen quality and higher serum testosterone, LH and FSH which aligns with the findings of this study. Similarly, Jeje *et al.* (2022) demonstrated that *Moringa* leaf extract restored sperm counts, motility and testosterone levels in rats with drug-induced testicular injury. Vitamin C’s effects were also evident. Ascorbate supplementation is known to boost semen quality and protect against toxin damage. In rabbits challenged with a reproductive toxicant, ascorbic acid significantly raised ejaculate volume, sperm concentration, motility and percentage of live normal sperm (Raji *et al.*, 2023). In this study, vitamin C co-treatment reversed many IVM-induced declines in sperm viability and motion. The combination of MO and vitamin C provided complementary antioxidant defenses that preserved sperm integrity and steroidogenic function under IVM stress.

The divergent outcomes with and without MO/VC reflect their impact on oxidative stress pathways. Ivermectin’s reproductive toxicity appears largely mediated by redox imbalance. *In vitro* work on germ cells shows that IVM triggers ROS generation, calcium dysregulation and protein aggregation, inducing endoplasmic-reticulum (ER) stress and apoptosis (Chavez-Varias *et al.*, 2024). Excessive ROS can damage sperm membranes and DNA, impairing motility and morphology. In rabbits, elevated oxidative stress likely accounted for the poorer semen quality after IVM. In contrast, *Moringa* and vitamin C exert strong antioxidative actions. *Moringa* leaves contain high levels of vitamin C, vitamin E, carotenoids, flavonoids and phenolic acids, which act as powerful radical scavengers (El-Kashef, 2022). These phytochemicals raise seminal antioxidant capacity and upregulate enzymes like superoxide dismutase and glutathione peroxidase (Mohlala *et al.*, 2023). As a result, lipid peroxidation is reduced and germ cell membranes are stabilized. Indeed, *Moringa* supplementation has been shown to increase testicular glutathione peroxidase and maintain seminiferous tubule integrity (Mohlala *et al.*, 2023). Vitamin C, which accounts for much of the antioxidant pool in semen, likewise neutralizes oxidants and regenerates other antioxidants (Panah *et al.*, 2023). By bolstering antioxidant defenses, these supplements likely prevented IVM-induced Leydig cell apoptosis and preserved steroidogenic capacity. *Moringa* leaf extract has even been reported to upregulate key steroidogenesis genes (e.g. StAR, CYP11A1) and increase testicular testosterone in rats (Mohlala *et al.*, 2023). The combined action of MO and vitamin C reduced ER and mitochondrial stress in germ and Leydig cells, maintaining spermatogenesis and hormone output.

**4.0 Conclusion**

This study demonstrated that prolong administration of therapeutic dose of ivermectin (IVM) induced significant reproductive toxicity in male rabbits, as evidenced by marked reductions in semen quality and reproductive hormone levels. The deleterious effects of IVM include diminished semen volume, sperm count, motility, morphology, and viability, suppressed testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations. These impairments are consistent with oxidative stress-mediated testicular damage and endocrine disruption. However, supplementation with Moringa oleifera leaf extract and vitamin C, individually and in combination, significantly mitigated these effects. The combination treatment of MO and VC proved most effective, restoring reproductive parameters to near-control levels. These findings revealed the protective potential of natural antioxidants in combating drug-induced reproductive toxicity and show Moringa oleifera and vitamin C as agents for preserving male fertility in veterinary practice. Based on the findings of this study, it is recommended that Veterinarians should exercise caution in prolong administration of ivermectin to breeding male rabbits and other livestock species, especially during active reproductive periods and that supplementation with *Moringa oleifera* leaf extract and vitamin C is recommended alongside ivermectin treatment to mitigate its reproductive toxicity.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**Ethical Approval**

All authors declare adherence to "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) and relevant national legislation where appropriate. All experiments have been reviewed and approved by the relevant ethics committee.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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