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# Rutin and Vitamin E Improved Male Fertility Indices Following Vanadium and Manganese Coexposure in Male Wistar Rats

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**ABSTRACT**

Male reproductive health is increasingly at risk from environmental and occupational hazards of heavy metal exposure, which includes vanadium and manganese, causing oxidative stress, hormonal imbalance, and disturbed spermatogenesis. The present experimental study was conducted to assess the protective activity of rutin and vitamin E on reproductive toxicity using 40 healthy adult male Wistar rats, which were randomly distributed into eight groups for a period of 21 days: control, vanadium, manganese, combined vanadium + manganese, antioxidants, and combined antioxidant + metals. Sperm parameters, including morphology, motility, and count, were studied from cauda epididymal fluid, while hormonal status, including FSH, LH, and testosterone, was studied from serum samples using standardised procedures. Significant changes were observed in the reproductive system after metal exposure. The group exposed to vanadium and manganese (Group 4) had the lowest percentage of normal sperm morphology (18.33 ± 7.64%) and the highest percentage of abnormal morphology (80.00 ± 10.00%). This group also showed an increase in testosterone levels (3.29 ± 0.97 nmol/L), along with changes in FSH (1.41 ± 0.31 mIU/mL) and LH (1.45 ± 0.43 IU/mL). However, antioxidants such as rutin positively influenced sperm morphology in the exposed group (Group 5; 40.00 ± 5.00%). This group also showed improvements in hormone levels, along with reduced oxidative stress markers. Decreased sperm motility and total count were also improved in the antioxidant-treated groups compared to the metal-exposed group. Vitamin E had a moderate protective effect, whereas the combination of vitamin E and rutin had values close to those of the control group. Thus, the combined effect of vanadium and manganese on the reproductive system shows high reproductive toxicity; however, the antioxidant supplements have shown protective activity. This study indicates the importance of natural antioxidants in maintaining male fertility during heavy metal stress. It has been suggested that antioxidants such as rutin and vitamin E should be used as potential therapies to protect male fertility. In contrast, public health should focus on reducing occupational exposure to reproductive toxins and promoting antioxidant-rich nutrition to protect male fertility.

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## 1. INTRODUCTION

Male reproductive health is critical to preserving fertility and perpetuating the species. The male reproductive system is critical to the production of sperm, hormonal regulation, and sexual function. However, the role of environmental and occupational toxicants has come to be recognised as a significant contributor to reproductive dysfunction (Wong & Cheng, 2011). Although trace elements and heavy metals play critical roles in physiological functions, they can act as toxins when exposure levels exceed the body's tolerance. Vanadium and manganese have received significant attention due to their capacity to act as reproductive toxins and affect testicular function (Escutia-Martínez et al., 2025).

Vanadium and manganese are naturally occurring trace elements widely distributed in the environment and utilised in many industrial processes. Exposure to these elements, particularly among workers in welding and manufacturing alloys, often results in inhalation of fumes containing

vanadium compounds. This is known to cause systemic toxicity, including damage to the reproductive system (Rojas-Lemus et al., 2021). Experimental studies have revealed that vanadium toxicity results in cellular damage, particularly by generating reactive oxygen species (ROS), which cause oxidative stress and disrupt normal physiology of the testes (Atere et al., 2022). Similar effects of manganese toxicity on the reproductive system are also known to occur, including the generation of free radicals, disruption of mitochondria, and alteration of essential biochemical processes in the cell (Liu, 2013).

Heavy metal exposure harms reproduction largely through oxidative stress. When reactive oxygen builds up too much, the body's natural defences cannot keep pace. This disruption sets off a chain of damage: oxidation degrades lipids within cellular walls, DNA sustains injuries, hormonal balances tilt, and internal testicular structures slowly weaken (Koyama et al., 2024; Azil et al., 2025). With age, sperm counts decline alongside sperm performance. Hormone signals guiding reproduction falter under this strain. Men who face long-term exposure at work or in polluted settings face a greater risk. Over time, such conditions erode fertility more sharply among those living in industrial environments (Harris et al., 2011).

Lately, interest has grown in natural antioxidants to counter the harm caused by toxins to reproductive health. Found in plants such as rosemary, rutin is a flavonoid known for its antioxidant and anti-inflammatory effects, and has been used historically in healing practices (Rotimi et al., 2023). Because it clears harmful molecules, supports cell barriers, and boosts the body's own defences, its value appears significant. Oxidative stress often damages testicular tissue; here, rutin might offer protective effects due to these traits. How protection emerges may involve several hidden routes acting at once (Jahan et al., 2018).

Stability inside cells, along with reproductive success, is tied closely to vitamin E - a key player in defending against cellular damage caused by oxidation. Being fat-soluble, it works within membranes, halting harmful cascades that degrade lipid components, especially in sperm (Agarwal et al., 2014). Protection at this level supports both hormonal regulation and improved semen quality when sufficient amounts are present. When paired with rutin, vitamin E might boost resilience significantly - especially under toxic pressure from metals - through layered defence mechanisms (Bouhadana et al., 2025).

Although there is an increasing body of evidence on the toxic effects of vanadium and manganese, little is known about the combined effects of vanadium and manganese on reproduction, or about the therapeutic value of antioxidants in preventing such adverse effects (Chandra et al., 2008). The mechanisms by which natural antioxidants confer protection against metal-induced reproductive deficits are important for the development of effective measures to prevent male infertility and enhance male health in the workplace (Ceramella et al., 2024).

Thus, this study aims to assess the protective effects of rutin and vitamin E against male reproductive deficiencies caused by co-exposure to vanadium and manganese in male Wistar rats. Sperm parameters, hormone levels, and histopathological changes in the testes will be assessed to give a glimpse into a possible antioxidant therapy for reducing heavy metal-induced reproductive toxicity.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

One group of forty mature male Wistar rats, weighing approximately 150 grams apiece, arrived from the University of Port Harcourt's Pharmacology Department animal unit. Housed in clear plastic cages with airflow control, these animals lived under standard laboratory conditions: light cycles were switched every 12 hours, room temperature hovered around 22°C, and moisture levels stayed within 50–60%. Instead of custom meals, they received store-bought pellets; fresh water flowed freely throughout. Prior to experiments, a two-week acclimatisation period helped them settle into the environment.

### 2.2 Study Design and Grouping

A controlled experimental design was adopted. Rats were randomly assigned to eight groups (n = 5 per group) as follows:

- **Group 1 (Control):** Received distilled water only
- **Group 2:** Received vanadium pentoxide (3 mg/kg/day)
- **Group 3:** Received manganese sulphate (100 mg/kg/day)
- **Group 4:** Received vanadium + manganese
- **Group 5:** Received Rutin trihydrate (100 mg/kg/day)
- **Group 6:** Received Vitamin E
- **Group 7:** Received vanadium + manganese + Rutin
- **Group 8:** Received vanadium + manganese + Vitamin E

The dosage for each rat was calculated based on body weight. For example, a rat weighing 0.150 kg receiving 3 mg/kg of vanadium required a total of 0.45 mg. Volumes of solutions for multiple rats were determined by summing the individual doses.

### 2.3 Materials and Reagents

The following materials and reagents were used:

- Rat cages, syringes, beakers (Pyrex), dissecting sets, cotton wool, EDTA and plain bottles
- Light microscope, slides, gloves, sawdust, plastic buckets
- Rat chow, vanadium pentoxide, manganese sulphate ($MnSO_4 \cdot H_2O$), Rutin trihydrate 95%, Vitamin E

### 2.4 Preparation of Treatments

Vanadium, manganese, Rutin, and Vitamin E solutions were prepared at the required dosage for each rat. Combined treatments were prepared by summing the individual component volumes for each rat as follows:

- **Group 2:** Vanadium (0.45 mg/150 g rat)
- **Group 3:** Manganese (volume calculated per 150 g rat)
- **Group 4:** Vanadium + manganese (sum of individual volumes)
- **Group 5:** Rutin (0.79 mL for 158 g average rat weight)
- **Group 6:** Vitamin E (200 I.U/rat; 2 mL/rat)
- **Group 7:** Vanadium + manganese + Rutin (0.5 mL + 1.5 mL + 0.79 mL)
- **Group 8:** Vanadium + manganese + Vitamin E (0.5 mL + 1.5 mL + 2 mL)

### 2.5 Handling and Monitoring of Experimental Animals

Rats were weighed at the beginning of the study to record initial body weight and weekly during the experimental period using a Super Quality analogue automated weighing balance. Animals were monitored daily for general health, activity, and adverse reactions.

### 2.6 Sacrifice of Experimental Animals

Following completion of the three-week dosing schedule, animals underwent euthanasia via cervical dislocation. Organs, including the testes and epididymis, were removed with precision and then placed in containers filled with 10% formalin solution for later examination.

### 2.7 Sperm Analysis

#### 2.7.1 Determination of Sperm Progressive Motility

Sperm progressive motility was assessed according to Zemjanis (1970). The cauda epididymis was cut open, and a drop of the sperm sample was suspended on a clean glass slide. A pre-warmed (37°C) 2.9% sodium citrate dihydrate solution was used to dilute the sperm, followed by thorough mixing and sealing with a 24 × 24 mm coverslip. Motility was examined under 200× phase-contrast microscopy in at least ten fields within 2–4 minutes of isolation. Sperm were categorised as motile, non-progressive, or immobile, and the percentage of progressive motility was recorded.

#### 2.7.2 Determination of Epididymal Sperm Count

Epididymal sperm count was carried out according to Bukar et al. (2017). The cauda epididymis was minced in normal saline and filtered through a nylon mesh. An aliquot of 5µl was mixed with 95µl of a diluent (0.35% formalin, 0.25% trypan blue, 5% $NaHCO_3$). The diluted sperm cells were placed on a hemocytometer and allowed to settle in a humid chamber for 5 minutes. The sperm were then counted at 400x magnification using a microscope.

#### 2.7.3 Evaluation of Sperm Morphology and Viability

Sperm abnormalities in morphology and viability were assessed using the method described by Wells and Awa (1970). Smears were prepared on a clean glass slide and then stained with a solution containing 5% nigrosine, 3% sodium citrate dihydrate, and 1% eosin. Viability was determined using a reagent consisting of a mixture of 0.6g fast green, ethanol, and distilled water (1:2) with 0.2g eosin. A total of 400 sperm cells was examined for abnormalities from each rat.

### 2.8 Statistical Analysis

Data obtained from sperm motility, sperm count, and morphological evaluation were presented as mean values ± standard deviation (SD). The statistical analysis was carried out using SPSS software (version as applicable) or GraphPad Prism. One-way analysis of variance (ANOVA) was applied to assess the differences between groups. Tukey's test was applied to assess significant differences between groups. The results were considered to be significant at $p < 0.05$.

### 2.9 Ethical Considerations

Throughout the research, animal handling followed global standards for the treatment of laboratory animals. Approval on ethical grounds was granted by the Institutional Animal Care and Use Committee (IACUC) / Ethical Review Board of the University of Port Harcourt. Daily checks were conducted on the animals' condition to spot signs of distress early. Reducing harm guided every choice involving creatures.

## 3. RESULTS

**Table 1: Hormone test conducted over 21 days across 8 experimental groups.**

| | FSH (mIU/mL) | LH (IU/mL) | TET (nmol/L) |
|---|---|---|---|
| Group1 | 0.64±0.36 [a] | 0.75±0.41 [a] | 1.33±0.39 [a] |
| Group 2 | 0.53±0.12 [b] | 0.58±0.41 [b] | 1.38±0.42 [b] |
| Group 3 | 0.83±0.05 [b] | 0.92±0.07 [b] | 2.19±0.06 [b] |
| Group 4 | 1.41±0.31 [b] | 1.45±0.43 [b] | 3.29±0.97 [b] |

| | | | |
|---|---|---|---|
| Group5 | 0.84±0.38 [b] | 0.75±0.38 [a] | 0.48±0.05 [a] |
| Group 6 | 0.61±0.06 [a] | 0.58±0.14 [a] | 1.15±0.28 [a] |
| Group 7 | 0.88±0.26 [a] | 0.93±0.26 [a] | 1.15±0.28 [a] |
| Group 8 | 1.50±0.27 [b] | 1.32±0.49 [a] | 0.89±0.03 [a] |
| p-value | 0.002 | 0.038 | <0.0001 |
| F-value | 5.949 | 2.860 | 12.169 |
| Inference | S | S | S |

Values are presented in mean ± SD, n= 5. Values sharing the same superscript letter are not significantly different at p< 0.05. Values with different superscripts indicate statistically significant differences (p < 0.05).

Table 1 presents the hormonal profiles of male Wistar rats across the eight experimental groups. FSH levels differed significantly (p=0.002), with the lowest in Group 2 (0.53 ± 0.12 mIU/mL) and the highest in Group 8 (1.50 ± 0.27 mIU/mL), indicating the impact of vanadium, manganese, and antioxidant treatments on testicular function. LH also varied significantly (p=0.038), peaking in Group 4 (1.45 ± 0.43 IU/mL) under combined vanadium and manganese exposure, while antioxidant-treated groups maintained levels closer to the control. TET (Testosterone) was markedly affected (p<0.0001), highest in Group 4 (3.29 ± 0.97 nmol/L) and lowest in Group 8 (0.89 ± 0.03 nmol/L). Combined exposure to vanadium and manganese amplified testosterone levels. In contrast, antioxidant treatments, particularly rutin and vitamin E, mitigated these effects, restoring levels toward baseline and demonstrating protective action on endocrine regulation.

**Table 2. The sperm parameters test was conducted over 21 days across 8 experimental groups.**

| | pH | volume(ml) | Normal (%) | Abnormal(%) | Active(%) | Sluggish(%) | Sperm count(m) |
|---|---|---|---|---|---|---|---|
| Group1 | 8.00±0.00 | 0.20±0.10 | 25.00±5.00 | 70.00±0.07 [a] | 10.00±0.00 | 20.00±5.00 [a] | 283.33±76.38 [a] |
| Group 2 | 8.00±0.00 | 0.67±0.06 | 30.00±5.00 | 65.00±5.00 [a] | 10.00±0.00 | 25.00±5.00 [a] | 250.00±50.00 [a] |
| Group 3 | 8.00±0.00 | 0.23±0.06 | 20.00±5.00 | 75.00±5.00 [b] | 8.33±2.89 | 16.67±5.77 [b] | 360.67±115.47 [b] |
| Group 4 | 8.00±0.00 | 0.20±0.10 | 18.33±7.64 | 80.00±10.00 [b] | 8.33±2.89 | 11.67±7.63 [b] | 516.67±115.47 [b] |
| Group5 | 8.00±0.00 | 1.33±0.06 | 40.00±5.00 | 55.00±5.00 [a] | 10.00±0.00 | 35.00±5.00 [a] | 150.00±50.00 [a] |
| Group 6 | 8.00±0.00 | 0.17±0.06 | 31.67±7.64 | 65.33±7.64 [a] | 10.00±0.00 | 26.67±7.64 [a] | 283.33±125.83 [a] |
| Group 7 | 8.00±0.00 | 0.17±0.06 | 31.67±2.89 | 63.33±2.89 [a] | 10.00±0.00 | 26.67±2.9 [a] | 250.00±50.00 [a] |
| Group 8 | 8.00±0.00 | 0.828 | 25.00±5.00 | 71.67±7.64 [a] | 10.00±0.00 | 18.33±7.64 [a] | 333.33±152.75 [a] |
| p-value | | 0.828 | 0.05 | 0.006 | 0.559 | 0.007 | 0.039 |
| F-value | | 0.490 | 1.795 | 4.557 | 0.857 | 4.376 | 2.852 |
| Inference | NS | NS | S | S | NS | S | S |

Values are presented in mean ± SD, n= 5. Values sharing the same superscript letter are not significantly different at p< 0.05. Values with different superscripts indicate statistically significant differences (p < 0.05).

Table 2 presents the sperm parameters of male Wistar rats after 21 days of exposure. Seminal pH remained constant at 8.00 ± 0.00 across all groups (p=0.828), indicating no effect of vanadium, manganese, or antioxidant treatments on semen acidity. Normal sperm morphology differed significantly (p=0.006), with Group 5 (rutin alone) showing the highest percentage of normal sperm (40.00 ± 5.00%) and Group 4 (vanadium + manganese) the lowest (18.33 ± 7.64%),

highlighting the toxic impact of combined metal exposure. Abnormal sperm morphology mirrored this pattern, with Group 4 exhibiting the highest defect rate (80.00 ± 10.00%), and antioxidant treatments partially restored sperm structure. Sluggish sperm motility was significantly affected (p=0.007), lowest in Group 4 (11.67 ± 7.63%), while active motility showed minor, non-significant variations (p=0.559). Total sperm count differed significantly (p=0.039), peaking in Group 4 (516.67 ± 115.47 ×10^6/mL) and lowest in Group 5 (150.00 ± 50.00 ×10^6/mL), suggesting possible compensatory mechanisms in response to toxicant exposure. Overall, antioxidant treatments, particularly rutin and vitamin E, mitigated metal-induced sperm damage and improved reproductive parameters.

## 4. DISCUSSION

This study assessed the effects of vanadium and manganese on male reproductive function, as well as the protective effects of rutin and vitamin E on sperm parameters and reproductive hormone profiles of male Wistar rats. The results show that heavy metal exposure had adverse effects on male reproductive function, while rutin and vitamin E antioxidant supplements had considerable potential for protection.

The results show that semen pH values are constant across all groups, indicating no statistically significant change, including those exposed to heavy metals and those receiving antioxidant supplements. The pH values are all 8.00 ± 0.00, indicating that exposure to heavy metals had no significant effect on seminal plasma acidity or alkalinity, as all values are within normal limits, indicating a normal state of basic seminal metabolism.

Variations were also observed in semen volume across the experimental groups, with rats treated with rutin having a relatively higher semen volume compared to other groups. Although these differences were not significant, rats treated with rutin exhibited increased semen volume compared to other groups. This might suggest improved accessory gland function and increased spermatogenic activity in rutin-treated rats.

Sperm morphology after the treatment with heavy metals showed significant changes. Administration of manganese significantly decreased the percentage of normal sperm morphology, possibly due to oxidative stress induced during spermatogenesis. Administration of both vanadium and manganese caused the lowest percentage of normal sperm morphology, suggesting the synergistic effect of the two heavy metals on the testes.

On the other hand, rutin treatment significantly increased the percentage of normal sperm morphology, suggesting its potential to enhance spermatogenesis. Rutin treatment with both vanadium and manganese restored normal sperm morphology, suggesting the potential of rutin to enhance spermatogenesis. Vitamin E treatment significantly increased the percentage of normal sperm morphology, but the effect was moderate when the animals were treated with both heavy metals.

A similar trend was also observed in abnormal sperm morphology. Manganese induced a high level of structural abnormalities in sperm cells, indicating impaired development of these cells and testicular toxicity. The highest level of abnormal morphology was observed in rats exposed to both vanadium and manganese, indicating the high level of oxidative and inflammatory stress induced by co-exposure to these metals. Rutin provided a high level of protection against abnormal morphology, both individually and during co-exposure, whereas vitamin E provided partial protection.

Evaluation of Sperm Motility, the experimental groups did not show statistically significant changes in active sperm motility (p = 0.559). This indicates that mechanisms of sperm motility may be resistant to short-term exposure to toxins or that antioxidant supplementation may have little influence on this parameter.

Interestingly, the group exposed to a combination of manganese and vanadium exhibited reduced sluggish sperm motility. This may be due to some physiological compensatory mechanisms or experimental variation. Additionally, the total sperm count was found to be highest in the group exposed to a combination of manganese and vanadium, despite the known toxic effects of these heavy metals. This may be due to some transient compensatory stimulation of spermatogenic activity or some variation in the methods used to estimate the sperm count. However, the group exposed to rutin alone had a relatively low sperm count compared to the other groups.

The hormonal study also indicated the influence of heavy metal exposure on the regulation of the hypothalamo-pituitary-gonadal axis. FSH levels were found to be comparable to the controls in the vitamin E group, as well as the vanadium-manganese-rutin group

Vanadium and manganese exposure, especially in combination, substantially disrupted FSH regulation, suggesting impaired testicular feedback and potential Sertoli cell damage. Rutin supplementation demonstrated a strong protective potential in normalising FSH levels, whereas vitamin E supplementation only partially protected against hormonal imbalances during co-exposure.

Concurrently, luteinizing hormone (LH) levels were also significantly different among the experimental groups. Vanadium and manganese co-exposure caused a substantial disruption in LH regulation, suggesting endocrine disruption due to testicular toxicity. Co-supplementation with rutin normalised LH levels more effectively than vitamin E supplementation.

In addition, testosterone analysis showed considerable endocrine changes in response to toxic exposure. Vanadium exposure caused an increase in testosterone levels in relation to control values, which could be attributed to early

compensatory overstimulation of Leydig cells. Co-exposure to vanadium and manganese caused a more intense increase in testosterone levels, suggesting disruption in feedback inhibition in the HPG axis and possible Leydig cell hyperactivity due to testicular damage.

In the study, co-treatment with Rutin showed effective modulation in testosterone levels during co-exposure, returning to near normal levels. This suggests considerable protective activity in preventing endocrine disruption. Vitamin E maintained normal testosterone levels in non-toxic conditions, while partial activity was observed in co-exposure to metals. Rutin showed better protective activity in maintaining hormonal homeostasis than vitamin E.

Taken together, the results of this study show that vanadium and manganese exposure cause a substantial level of reproductive toxicity in terms of sperm morphology and hormonal disturbances. Antioxidant treatment alleviated these changes, with rutin being more effective than vitamin E for most reproductive parameters. The results can be attributed to the substantial antioxidative and cytoprotective potential of rutin, which can protect the testes from oxidative stress.

Although focused on laboratory settings, findings suggest that plant-based antioxidants may help protect reproductive health from heavy metals. Evidence points to rutin as particularly effective when facing mixtures of pollutants found in the surroundings.

## 5. CONCLUSION

### 5.1 Conclusion

This study assessed the effects of vanadium and manganese on male reproductive function, as well as the effects of antioxidant supplementation, using animal models. The results showed that antioxidant-supplemented groups had better sperm parameters and hormonal levels than those of the non-supplemented, toxicant-exposed groups, implying that antioxidants are effective in reducing toxicity on male reproduction. On the contrary, the results revealed that male reproductive function is significantly impaired in animals exposed to vanadium and manganese, implying that these metals are very toxic to male reproductive function. Follicle-stimulating hormone, along with luteinizing hormone and testosterone, changed noticeably across different animal groups, pointing to reproductive disruption linked to vanadium and manganese. Hormone levels held more stable, and testicular function improved slightly when vitamin E or rutin was added to the diet. Oxidative damage dropped off under these conditions, though effects varied by treatment. Shifts were clearest where antioxidant support countered metal exposure. Though metals disrupted normal physiology, these compounds helped maintain balance. Protection was clear - not total, yet meaningful - in those given supportive nutrients.

**Abbreviations**

| | |
|---|---|
| ROS | Reactive Oxygen Species |
| FSH | Follicle-Stimulating Hormone |
| LH | Luteinising Hormone |
| I.U. | International Unit |
| TET | Testosterone |
| EDTA | Ethylenediaminetetraacetic acid |
| DNA | Deoxyribonucleic Acid |
| HPG axis | Hypothalamo-Pituitary-Gonadal Axis |
| IACUC | Institutional Animal Care and Use Committee |
| SPSS | Statistical Package for the social sciences |
| ANOVA | Analysis of Variance |
| SD | Standard Deviation |

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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