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PROTECTIVE AND DOSE-DEPENDENT EFFECTS OF *Cucumis melo* SEED OIL ON TESTICULAR FUNCTION AND OXIDATIVE STRESS IN TRICARBALLYLIC ACID – INDUCED TOXICITY IN MALE WISTAR RATS

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ABSTRACT

Cucumis melo seed oil (CMSO), rich in polyunsaturated fatty acids, tocopherols, and phenolic antioxidants, has demonstrated antioxidant and anti-inflammatory potential. This study evaluated the dose-dependent protective effects of CMSO on Tricarballylic acid (TCA)-induced testicular oxidative stress and functional impairment in male Wistar rats. Twenty-five male Wistar rats were randomly assigned into five groups (n = 5) and treated orally for 21 days: Control (normal saline), TCA only (200 mg/kg), and TCA (200 mg/kg) co-administered with CMSO at 100, 200, and 300 mg/kg. Body weight, oxidative stress markers (SOD, MDA), reproductive hormones (FSH, testosterone), sperm parameters, and testicular histology were assessed. TCA significantly decreased SOD activity (10.42±1.61 U/mg; p<0.05) and increased MDA (1.25±0.25 nmol/mg) compared to controls (18.02±3.00; 0.80±0.24). Co-administration of CMSO improved SOD activity in a dose-dependent pattern, with the highest level observed at 300 mg/kg (14.77±2.73; p<0.05). Testosterone suppression by TCA (3.22±0.05 ng/mL) was ameliorated by CMSO, especially at 200 mg/kg (4.02±0.45 ng/mL; p< 0.05). FSH levels showed mild dose-related increase. Sperm motility decreased markedly with TCA (35.26±13.35%) but improved only at 300 mg/kg CMSO (45.63±13.75%; p<0.05). Histology revealed focal degeneration, including tubular disorganization and germ-cell loss at medium and high CMSO doses. CMSO exerted dose-dependent biochemical protection against TCA-induced oxidative and hormonal disruptions, with the 300 mg/kg dose showing the greatest antioxidant effect. However, the persistence of histological alterations at higher doses suggests that biochemical improvements do not fully translate to structural recovery, highlighting the need for dose optimization and longer-duration studies to clarify therapeutic potential.

Keywords: *Cucumis melo* seed oil, Tricarballylic acid, Testicular toxicity, Oxidative stress, Sperm quality, Wistar rats

INTRODUCTION

Male infertility remains a significant global reproductive health challenge, contributing to nearly 30–50% of infertility cases among couples (Zegers-Hochschild *et al.*, 2009; Agarwal *et al.*, 2021). A growing body of evidence implicates oxidative stress as a major driver of male reproductive dysfunction. Excess reactive oxygen species (ROS) disrupt sperm membrane integrity, damage DNA, impair mitochondrial ATP-generating capacity, and ultimately reduce sperm quality and fertility potential (Agarwal *et al.*, 2020). The burden of oxidative stress–related infertility is particularly relevant in low- and middle-income regions, including parts of sub-Saharan Africa, where exposure to environmental toxicants and limited access to assisted reproductive care heighten vulnerability.

Experimental models of testicular oxidative damage frequently employ exogenous toxicants capable of disturbing redox homeostasis, including cadmium, bisphenol A, cisplatin, and heavy metals. In this context, tricarballylic acid (TCA) has emerged as a useful agent for inducing mitochondrial dysfunction and oxidative stress (Heidy *et al.*, 2017). Tricarballylic acid is a toxic exogenous tricarboxylic acid derivative produced by certain rumen microbes, and is known to inhibit mitochondrial aconitase, disrupt Krebs-cycle flux, and impair oxidative phosphorylation (Gumel *et al.*, 2019). Its interference with aconitase activity results in electron leakage from the electron transport chain, increased ROS generation, lipid peroxidation, and eventual apoptotic changes in susceptible tissues (Carvalho *et al.*, 2011). Although tricarballylic acid has primarily been studied in ruminants, emerging toxicological evidence suggests that its mitochondrial-disruptive properties can extend to other organs, including the testes, when administered experimentally (Maran & Priya, 2015). This mechanistic profile makes tricarballylic acid a relevant model for studying testicular oxidative stress, despite being less widely used than more established toxicants.

Given the centrality of oxidative imbalance in chemical-induced testicular injury, there is increasing interest in natural antioxidant compounds as potential protective agents. Plant-derived seed oils, rich in polyunsaturated fatty acids, tocopherols, phytosterols, and phenolic compounds, have demonstrated capacity to counter oxidative damage by attenuating lipid peroxidation, enhancing endogenous antioxidant enzymes, and modulating inflammatory pathways (Carvalho *et al.*, 2011; Zeb, 2016).

Cucumis melo (sweet melon), a widely cultivated fruit in tropical and subtropical regions including Nigeria, produces seeds that are frequently discarded despite their high nutritional and phytochemical value. *Cucumis melo* seed oil contains essential fatty acids, phenolics, tocopherols, and other bioactive constituents reported to possess antioxidant, anti-inflammatory, hypolipidemic, and metabolic-modulating properties (Chen & Kang, 2013; Górnaś *et al.*, 2014; Mallek-Ayadi *et al.*, 2018). These attributes suggest potential utility in mitigating oxidative stress in reproductive tissues; however, scientific evaluation of its effects on chemically induced testicular injury remains limited, especially in the context of toxins that impair mitochondrial function such as tricarballylic acid.

Considering the rising incidence of oxidative stress–mediated male infertility and the need for accessible, plant-based therapeutic options, investigating the protective potential of *Cucumis melo* seed oil is both timely and relevant. This study

therefore aims to evaluate the dose-dependent protective effects of *Cucumis melo* seed oil on testicular function and oxidative stress biomarkers in a tricarballylic acid–induced toxicity model in male Wistar rats.

By elucidating the biochemical and histological responses of the testes to tricarballylic acid exposure and the modulatory effects of *Cucumis melo* seed oil, this research addresses an important knowledge gap and provides foundational evidence for the possible therapeutic application of *Cucumis melo* seed oil in managing oxidative stress–related testicular dysfunction.

MATERIALS AND METHODS

Ethical Consideration

All experimental procedures were carried out according to internationally accepted guidelines for the care and use of laboratory animals and were approved by the Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Baze University, Abuja.

Experimental Animals and Randomization

Twenty-five (25) adult male Wistar rats were obtained from the Animal House, Faculty of Basic Medical Sciences, Baze University Abuja. Animals were housed in standard ventilated plastic cages (30 × 20 cm), maintained under controlled environmental conditions (12 h light–dark cycle; temperature 22–25°C; relative humidity 50–60%), and provided standard rat chow and water *ad libitum*. Animals were acclimatized for 14 days. After acclimatization, rats were weighed and randomly assigned to groups using a simple randomization method (sealed-envelope technique) to minimize allocation bias.

Animal Cages

Five (5) well-ventilated plastic cages measuring 30 cm × 20 cm were obtained from the animal house and used to house the experimental animals throughout the study. Each cage was bedded with clean wood shavings and maintained under standard laboratory conditions. Environmental enrichment was provided in the form of nesting materials and cardboard tubes to promote natural exploratory behaviors and enhance animal welfare. The rats were allowed to acclimatize for one week prior to the commencement of the experiment and were fed standard rat chow with water available *ad libitum*.

Toxicant (Tricarballylic Acid)

The toxicant used in this study was tricarballylic acid (TCA), procured as potassium citrate–citric-acid syrup containing tricarballylic acid as the active component. The identity and concentration of tricarballylic acid were verified from the manufacturer's certificate of analysis. The compound is an exogenous mitochondrial toxin known to inhibit aconitase. The toxicant was stored at 4°C throughout the study.

Preparation and Extraction of *Cucumis melo* Seed Oil

Ripe *Cucumis melo* fruits were purchased from a local Abuja market. Seeds were manually removed, washed, and air-dried for seven (7) days. Dried seeds were milled into fine powder. Oil extraction was performed by maceration:

i. 50 g of seed powder were mixed with n-hexane at a 1:5 (w/v) ratio in amber bottles.
ii. The mixture was left to stand for 4 hours, centrifuged at 1,000 × g for 15 minutes, and filtered through Whatman No. 1 filter paper.
iii. Extraction was performed in duplicate.
iv. The solvent phase was removed using a rotary evaporator at 40°C, followed by nitrogen gas flushing to ensure complete removal of residual n-hexane.
v. Extracted oil was stored in amber vials at −20°C until use.

The oil yield (%) was calculated as:

$$\text{Yield} = \frac{Weight\ of\ Extracted\ Oil}{Weight\ of\ Dry\ Seed\ Powder} \times 100$$

Experimental Design

The twenty-five rats were randomly assigned into five groups (n = 5 per group):

Group I (Control): Normal saline (5 mL/kg, oral gavage)
Group II (TCA Only): Tricarballylic acid (200 mg/kg, oral gavage)
Group III (Low Dose): TCA (200 mg/kg) + seed oil (100 mg/kg)
Group IV (Medium Dose): TCA (200 mg/kg) + seed oil (200 mg/kg)
Group V (High Dose): TCA (200 mg/kg) + seed oil (300 mg/kg)

All treatments were administered orally via gavage once daily for 21 days.

Duration of Study

Acclimatization: 14 days
Treatment period: 21 days
Total duration: 35 days

Animal Sacrifice and Blood Collection

At the end of treatment, animals were fasted overnight, anesthetized with chloroform inhalation, and euthanized. Blood was collected by cardiac puncture using sterile syringes and placed into plain tubes. Samples were allowed to clot and centrifuged at 1,000 rpm for 10 minutes. Serum aliquots were stored at −20°C until analysis (avoiding repeated freeze–thaw cycles).

Organ Harvesting and Fixation

The testes and epididymides were excised, cleared of fat, and weighed. Tissues were fixed in 10% neutral buffered formalin (NBF) for histological processing. Protein concentration in tissue homogenates was estimated using the Lowry method (Maehre *et al.,* 2018).

Biochemical Assay

Superoxide Dismutase (SOD) Activity

SOD activity was assayed according to Winterbourn *et al.* (1975) as adapted by Rukmini *et al*. (2004). The reaction mixture consisted of phosphate buffer (0.067 M, pH 7.8), riboflavin, nitro-blue tetrazolium (NBT), methionine, and tissue homogenate. Absorbance was read at 560 nm. Activity was expressed as U/mg protein.

Lipid Peroxidation (Malondialdehyde – MDA)

MDA levels were estimated using the thiobarbituric acid reactive substances (TBARS) assay as described by Buege and Aust (1978). Absorbance was measured at 535 nm, and MDA concentration calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$, expressed as nmol/mg protein.

Hormonal Assays

Follicle-Stimulating Hormone (FSH)

Serum FSH was quantified using WHO-standardized enzyme immunoassay (EIA) kits (NIADDK–NIH, USA) according to the manufacturer's protocol.

Testosterone Assay
Plasma testosterone levels were determined via competitive enzyme immunoassay following the method of Tietz (1995). Absorbance was measured at 450 nm, and concentrations were extrapolated from standard curves.
Sperm Collection and Semen Analysis
The cauda epididymis was excised and minced in 1 mL of pre-warmed PBS (37°C) and incubated for 10 minutes for sperm dispersion.

Sperm Count
Ten microliters of diluted sperm suspension were loaded onto a Neubauer hemocytometer and counted at ×400 magnification. Results were expressed as million/mL.

Sperm Motility
A drop of sperm suspension was examined on a pre-warmed slide. Forward progressive motility was evaluated in five random fields and reported as a percentage of motile sperm based on WHO criteria (≥25 µm/s considered progressive).

Sperm Morphology
Sperm smears were stained using Eosin–Nigrosin. Two hundred spermatozoa per sample were evaluated under ×40 magnification. Results were expressed as the percentage of morphologically normal sperm.

Histological Processing and Scoring
Fixed testes were processed using standard paraffin-embedding. Sections (4 µm) were stained with hematoxylin and eosin (H&E). Histological evaluation included seminiferous tubule integrity, germ-cell layering, interstitial cell morphology, and evidence of degeneration.
A semi-quantitative scoring method (Johnsen score) was used by two independent blinded observers.

Statistical Analysis
Data were analyzed using IBM SPSS Version 23. Results were expressed as Mean±SEM. Intergroup comparisons were performed using one-way ANOVA followed by LSD post hoc testing. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION
Body Weight Changes
The results in Figure 1 show the effects of Tricarballylic acid (TCA) and *Cucumis melo* Seed Oil (CMSO) on the body weight of rats. All groups experienced weight gain over the experimental period, but the extent varied significantly. Group I (Control) and Group II (TCA only) showed comparable weight gains (229.37 g and 238.90 g respectively), suggesting that TCA alone did not adversely affect body weight. Notably, Group III (TCA + 100 mg/kg CMSO) had the highest weight gain (269.77 g), indicating a potential synergistic or nutritive effect of low-dose CMSO in combination with TCA. Conversely, Group IV (TCA + 200 mg/kg CMSO) showed the lowest and statistically significant reduction in weight gain (152.80 g), with final body weight significantly lower than both the control and TCA-only groups (*$P < 0.05$).
This suggests a possible dose-dependent adverse or suppressive effect of CMSO at 200 mg/kg. Group V (TCA + 300 mg/kg CMSO) showed a restoration of weight gain (228.70 g) to near-control levels, implying a reversal of the negative impact seen at the 200 mg/kg dose.
These results imply that, while CMSO at 100 mg/kg enhances weight gain when combined with TCA, the 200 mg/kg dose impairs it significantly.

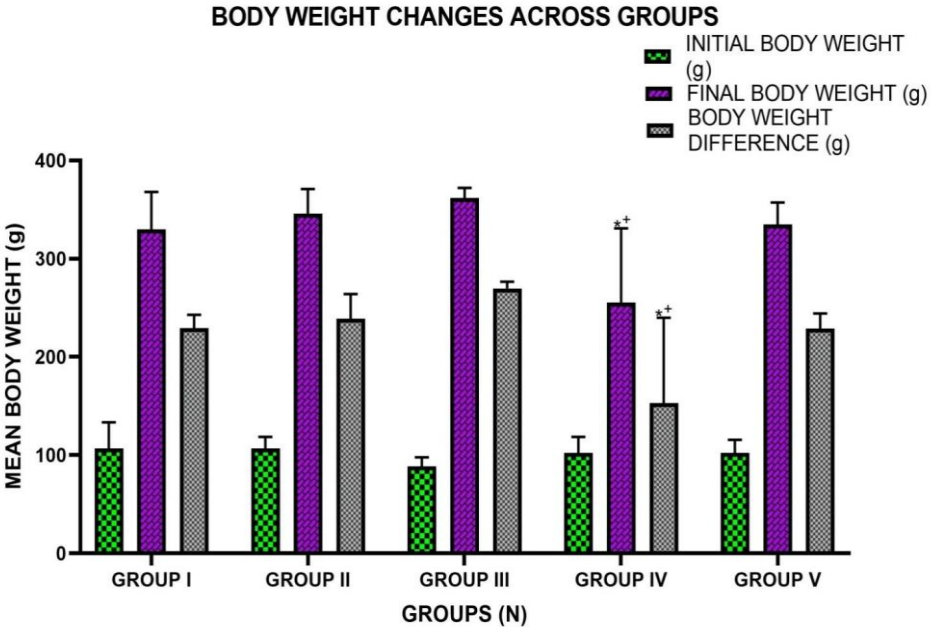


Figure 1: Body Weight Changes across Groups
*+ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

Oxidative Stress Markers
Figure 2 shows the oxidative stress markers; Superoxide Dismutase (SOD) activity and Malondialdehyde (MDA) levels across the experimental groups. Group I (control) showed the highest SOD activity (18.02±3.00 U/mg protein) and the lowest MDA concentration (0.80±0.24 nmol/mg protein). Administration of 200 mg/kg TCA in Group II significantly reduced SOD activity (10.42±1.61 U/mg protein) and increased MDA levels (1.25±0.25 nmol/mg protein), demonstrating enhanced oxidative stress and cellular damage compared to control. Groups III - V, which received TCA combined with varying doses of CMSO, showed partial

restoration of SOD activity and reduced MDA levels relative to Group II. Notably, Group V (TCA + 300 mg/kg MSO) showed a significantly higher SOD level (14.77±2.73 U/mg protein) compared to TCA-only treated Group II, suggesting a dose-dependent antioxidant effect of CMSO. However, MDA levels remained elevated in all treated groups compared to control, indicating persistent but attenuated lipid peroxidation.

These results suggest that TCA induces oxidative stress by impairing antioxidant defenses and promoting lipid peroxidation. CMSO co-administration mitigates these effects by enhancing antioxidant enzyme activity, with the highest dose showing the most protective effect.

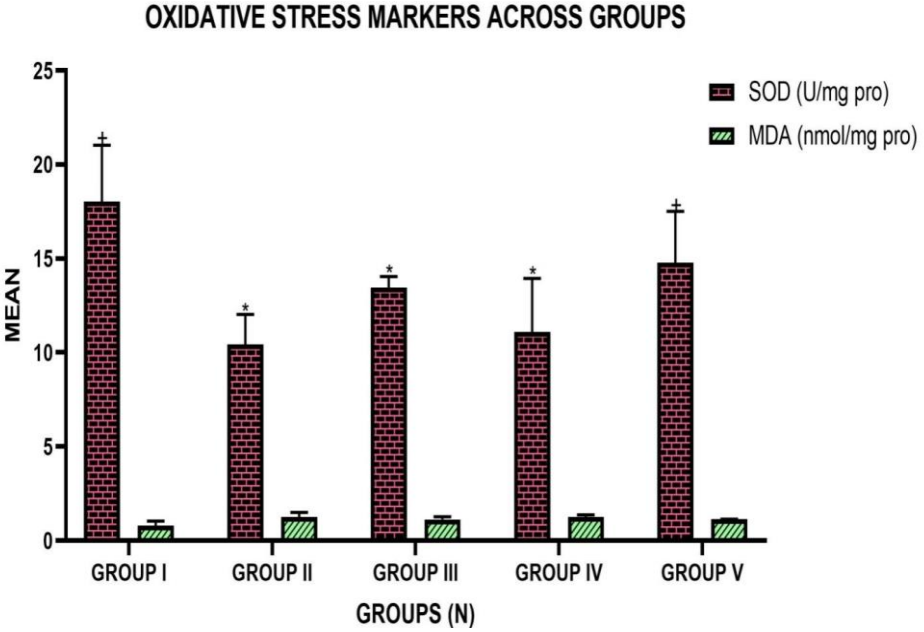


Figure 2: Oxidative Stress Markers across Groups
*+ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

Reproductive Hormone Levels

Results in Figure 3 show the reproductive hormone levels, specifically Follicle Stimulating Hormone (FSH) and Testosterone. The control group (Group I) treated with normal saline exhibited the highest mean levels of FSH (3.22±0.91 ng/ml) and Testosterone (5.50±0.46 ng/ml). Group II, treated with 200 mg/kg TCA alone, showed a significant reduction ($P<0.05$) in both FSH (1.27±0.15 ng/ml) and Testosterone (3.22±0.05 ng/ml), indicating the suppressive effect of TCA on reproductive hormones.

Co-administration of CMSO with TCA in Groups III, IV, and V resulted in a partial but statistically significant increase ($P<0.05$) in hormone levels compared to Group II. Notably, Group IV (200 mg/kg TCA + 200 mg/kg CMSO) demonstrated the highest improvement among treated groups, with FSH at 1.55±0.12 ng/ml and Testosterone at 4.02±0.45 ng/ml, the latter showing significant difference ($P<0.05$) compared to both the control and TCA-only groups. This suggests a dose-dependent ameliorative effect of CMSO on TCA-induced reproductive hormone suppression.

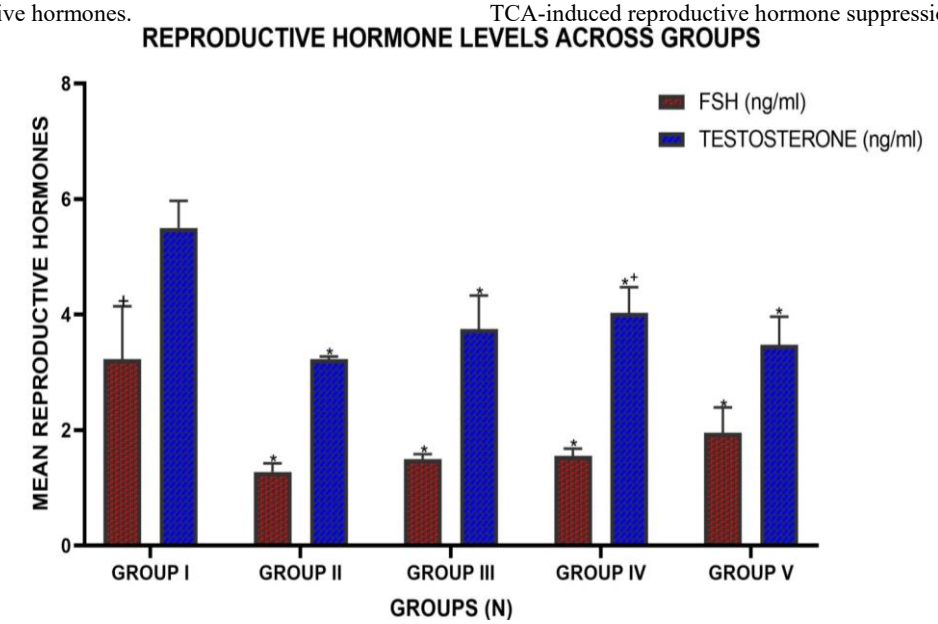


Figure 3: Reproductive Hormones Levels across Groups
*+ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

Sperm Count

The results in Figure 4 depict the sperm count across experimental groups. The control group (Group I) had the highest mean sperm count (30.60±7.67 x10⁶/ml), significantly higher than all treatment groups. TCA alone (Group II) markedly reduced sperm count (17.86±2.02 x10⁶/ml), indicating its deleterious effect. Co-treatment with CMSO (Groups III–V) did not significantly improve sperm count, with the lowest value in Group V (14.66±8.07 x10⁶/ml). This suggests that CMSO, at the tested doses, did not effectively reverse TCA-induced sperm count reduction.

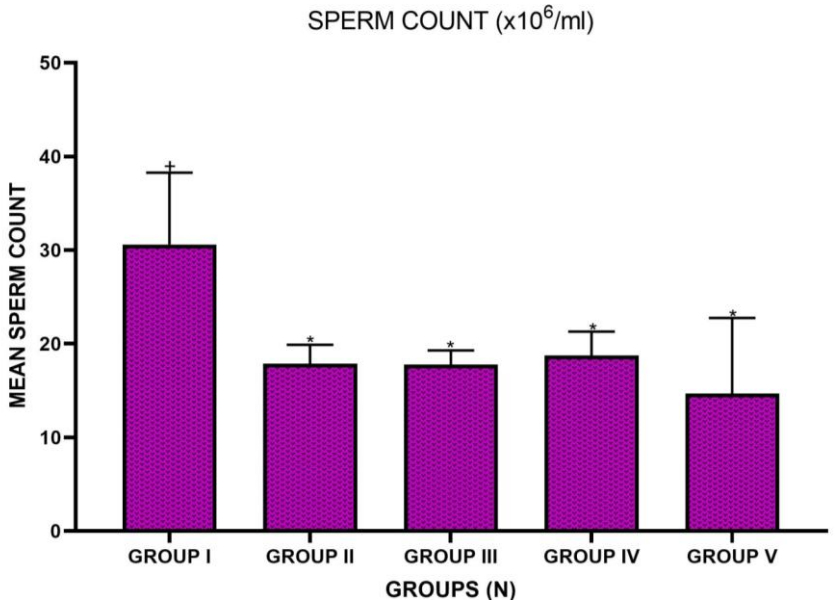


Figure 4: Sperm Count across Groups

*⁺ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

Sperm Morphology

Sperm morphology remained relatively stable across all groups as shown in Figure 5. Group II (TCA) had a slightly higher mean morphology (65.26±9.10%) than the control (58.86±21.66%), while CMSO-treated groups (III–V) showed variable values with no clear improvement. These results indicate that TCA and CMSO did not significantly distort or improve normal sperm morphology.

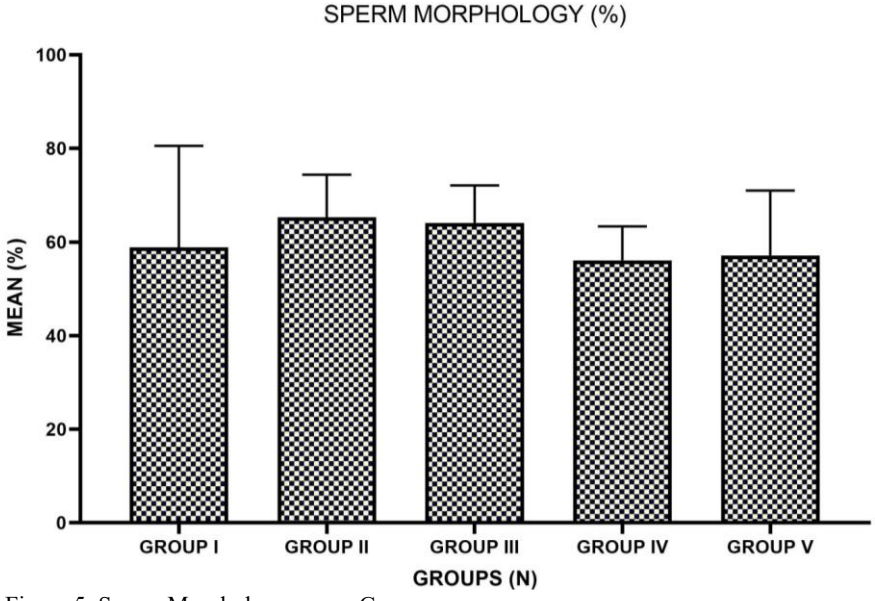


Figure 5: Sperm Morphology across Groups

*⁺ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

Sperm Motility

Figure 6 shows the mean sperm motility across groups. TCA reduced sperm motility compared to the control (35.26±13.35% vs. 44.40±6.06%). CMSO at 100 mg/kg and 200 mg/kg (Groups III and IV) further reduced motility (28.06±6.76%, 30.10±6.29%), while 300 mg/kg CMSO (Group V) restored motility to control level (45.63±13.75%). This suggests a possible dose-dependent protective effect of CMSO on sperm motility at higher doses.

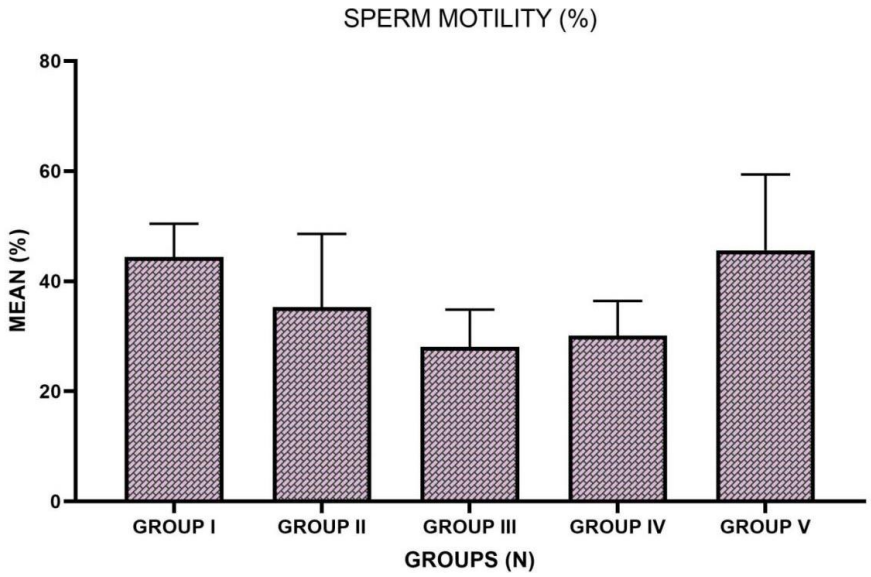
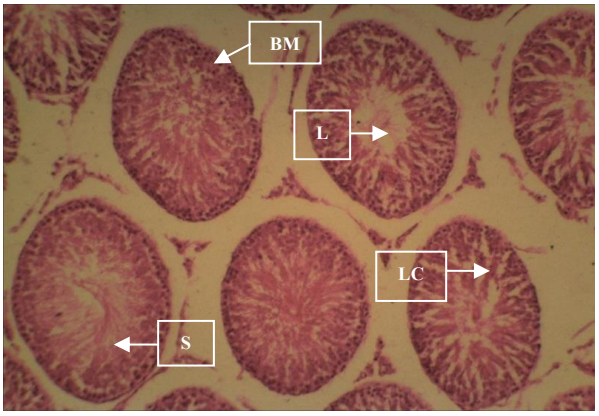


Figure 6: Sperm Motility across Groups
*$^{+}$ = statistically significant difference at $P<0.05$ compared to groups I and II respectively

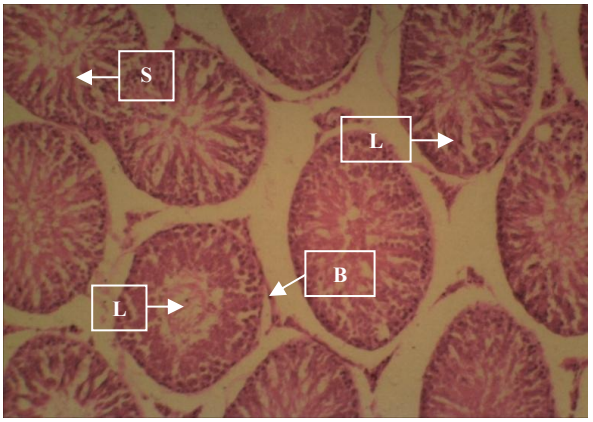
Histological Profile

Histological assessment of testicular tissue from Group I - III revealed a typical testicular histology, characterized by abundant spermatozoa arranged in radiating patterns towards the luminal region of the seminiferous tubules, alongside intact Leydig cells. Additionally, retention of spermatids within the tubules was noted.

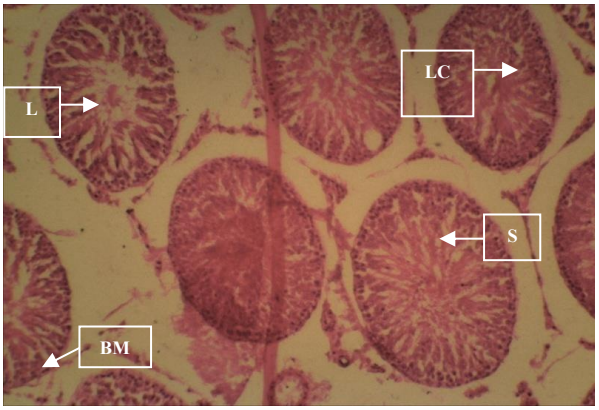
Conversely, groups IV - V exhibited varying degrees of testicular morphology abnormalities. Seminiferous tubules in these groups displayed features such as spermatid retention, tubular atrophy, and diffuse disorganization of germ cells. Furthermore, degenerative changes were evident in the testicular architecture, marked by a conspicuous absence of interstitial space and evidence of necrosis. Microscopic examination revealed the presence of maturing spermatogenic cells within the seminiferous tubules, accompanied by disruptions such as ruptured nuclear membranes and fragmentation of nuclei (karyorrhexis). Additionally, spermatogonia cells with densely stained nuclei were observed alongside normal sperm cells. Although most seminiferous tubules exhibited normal characteristics with radiating sperm, some basement membranes appeared thickened and hyalinized.



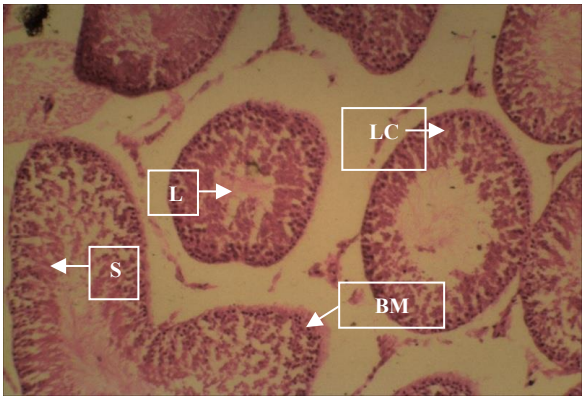
Group I: Testicular Section from Group I showing intact Basement Membrane (BM), Lumen (L), Spermatogonia (S), & Leydig Cells (LC) (H&E x10)



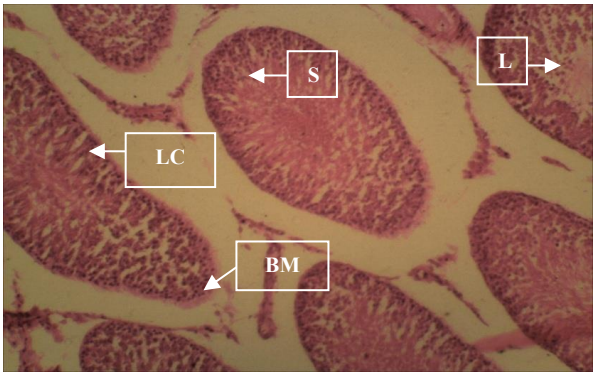
Group II: Testicular Section from Group II showing intact Basement Membrane (BM), Lumen (L), Spermatogonia (S), & Leydig Cells (LC) (H&E x10)



Group III: Testicular Section from Group III showing intact Basement Membrane (BM), Lumen (L), Spermatogonia (S), & Leydig Cells (LC) (H&E x10)



Group IV: Testicular Section from Group IV showing diffuse Basement Membrane (BM), mildly atrophied Lumen (L), disorganized Spermatogonia (S), & Leydig Cells (LC), with cellular debris (H&E x10)



Group V: Testicular Section from Group V showing diffuse Basement Membrane (BM), mildly atrophied Lumen (L), disorganized Spermatogonia (S), & Leydig Cells (LC), with cellular debris (H&E x10)

Discussion

This study examined the effects of *Cucumis melo* seed oil on Tricarballylic acid–induced alterations in body weight, oxidative stress markers, reproductive hormones, sperm parameters, and testicular histology in male Wistar rats. Tricarballylic acid, the toxic agent used in this study is a mycotoxin-associated acid known to impair energy metabolism and induce oxidative stress.

Body weight outcomes showed that Tricarballylic acid alone did not markedly reduce weight gain relative to the control group, suggesting limited systemic metabolic toxicity at the administered dose. The increase in body weight in the 100 mg/kg CMSO group may indicate a nutritive influence of CMSO, consistent with reports describing its rich essential fatty acid and bioactive composition (Adeyemi *et al.*, 2021; Ogunleye *et al.*, 2022). In contrast, reduced weight gain at 200 mg/kg CMSO suggests that this intermediate dose may have introduced physiological stress or reduced metabolic efficiency. The near-control values observed at 300 mg/kg CMSO indicate that CMSO did not exert overt systemic toxicity at the higher dose.

The reduction in SOD activity and elevation of MDA following Tricarballylic acid exposure aligns with earlier findings that this compound impairs redox homeostasis and promotes mitochondrial reactive oxygen species generation (Almeida *et al.*, 2020). Co-administration of CMSO produced partial increases in SOD and modest reductions in MDA, suggesting that CMSO may attenuate oxidative damage. These observations are consistent with documented antioxidant properties of *Cucumis melo* seed oil attributed to its tocopherols, phenolics, and unsaturated fatty acids (Bello *et al.*, 2021; Chen *et al.*, 2023). However, MDA levels remained above control values in all CMSO-treated groups, indicating that lipid peroxidation was only partly mitigated rather than fully corrected. Such partial responses parallel findings from classical testicular toxicants such as cadmium, bisphenol A (BPA), and cisplatin, where antioxidants frequently reduce but do not normalize oxidative indices (Almeida *et al.*, 2020; Nwosu *et al*., 2020).

Tricarballylic acid significantly decreased FSH and testosterone levels, supportive of endocrine disruption involving both hypothalamic–pituitary signaling and Leydig cell steroidogenesis, as previously described in reproductive toxicant models (El-Boshy *et al.*, 2022). CMSO administration produced dose-related improvements, with the 300 mg/kg dose showing the most consistent enhancement of testosterone levels, aligning with antioxidant-supported restoration of steroidogenic function reported in earlier studies (Nwosu *et al.*, 2020; Ibrahim *et al.*, 2023; Zhang *et al.*, 2024). These patterns are consistent with oxidative disruption models, where reduction of oxidative load is associated with improved hormone biosynthesis (El-Boshy *et al.*, 2022).

Despite these hormonal improvements, sperm count remained significantly reduced across all CMSO-treated groups. This is not unexpected because full spermatogenic turnover in rats requires approximately 48–52 days (Patel *et al.*, 2022), and the duration of exposure in this study may not have permitted complete recovery of sperm output. Similar delayed or limited

recovery has been reported in chemically induced testicular injury models, even with antioxidant supplementation (Musa *et al.*, 2021; Patel *et al.*, 2022). The lack of significant change in sperm morphology suggests a degree of structural resilience of spermatozoa despite biochemical and endocrine disruptions. Sperm motility showed improvement only at the highest CMSO dose, which reflects partial stabilization of sperm membrane integrity under reduced oxidative stress (Olawuyi *et al.*, 2023).

Histopathological analysis provided structural evidence supporting the biochemical findings. While the control and low-dose groups exhibited largely preserved testicular architecture, moderate to high CMSO doses in combination with Tricarballylic acid produced varying degrees of tubular distortion, spermatogenic disruption, and degenerative changes, including nuclear fragmentation and basement membrane thickening. These abnormalities are similar to those observed in established testicular toxicant models such as cadmium or cisplatin, where oxidative and endocrine mechanisms converge to disrupt germ cell organization (Abdullahi *et al.*, 2021; Kumar *et al.*, 2022; Olawuyi *et al.*, 2023). Although CMSO demonstrated some mitigating influence biochemically, the histological findings indicate that CMSO did not prevent Tricarballylic acid–induced tissue damage and at some doses might not adequately counteract underlying toxic processes.

Overall, the findings suggest that *Cucumis melo* seed oil may attenuate, but does not fully reverse, Tricarballylic acid–induced oxidative stress, endocrine suppression, and testicular injury. The dose-dependent patterns across biochemical, hormonal, sperm, and histological endpoints indicate potential partial benefits of CMSO, particularly at higher doses for antioxidant and endocrine parameters. However, the incomplete recovery across outcomes underscores the need for further mechanistic studies, longer-duration experiments that encompass full spermatogenic cycles, and comparisons with standard antioxidant interventions used in testicular toxicology.

CONCLUSION

This study demonstrates that tricarballylic acid, a mycotoxin-associated metabolic disruptor, induces oxidative stress, hormonal suppression, impaired sperm quality, and testicular structural damage in male Wistar rats. Co-administration of *Cucumis melo* seed oil produced partial and dose-dependent mitigation of some biochemical and endocrine disturbances, characterized by partial biochemical improvement but limited functional recovery and structural preservation. These findings highlight the need for longer-duration studies covering a full spermatogenic cycle, dose-optimization studies, and mechanistic investigations.

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REFERENCES

Abdullahi, A. M., Ibrahim, A. S., & Musa, A. I. (2021). Dose-dependent effects of natural oils on testicular oxidative stress and histopathology in rats. *Journal of Experimental Biology and Agricultural Sciences*, 9(5), 712–720.

Adeyemi, O. S., Akinyemi, A. I., & Bello, O. M. (2021). Nutritional and antioxidant potentials of melon seed oil in experimental models. *Food Science and Nutrition*, 9(4), 1970–1980.

Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., & Shah, R. (2020). Male oxidative stress infertility (MOSI): Proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *World Journal of Men's Health, 38*(3), 296–312.

Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2021). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology, 19*(1), 1-15.

Almeida, F. L., Da Silva, F. M., & Souza, C. M. (2020). Tricarboxylic acid cycle disruption and oxidative stress in rat models of toxicity. *Toxicology Reports*, 7, 1234–1242.

Bello, T. A., Olawuyi, O. T., & Olufunmilayo, A. E. (2021). Antioxidant activity of Cucumis melo seed oil in mitigating oxidative stress in vivo. *Biomedicine & Pharmacotherapy*, 137, 111311.

Bosky, N. (2022). Testicular anatomy and physiology. *International Journal of Reproductive Medicine*, 2022, Article ID 9938574.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, *52*, 302–310.

Carvalho, L. M. J., Machado, B. A. S., Ferreira, I. M. P. L. V. O., & Faria, J. A. F. (2011). Fruit seeds and their oil as potential functional food ingredients: A review. *Food Science and Technology, 44*(3), 587–593.

Chen, J., Wang, L., & Li, H. (2023). Phytochemical composition and antioxidative properties of melon seed oil: Implications for reproductive health. *Phytotherapy Research*, 37(1), 80–89.

Chen, Y., & Kang, J. (2013). Hypoglycemic effects of melon seed extract in type 2 diabetic rats. *Phytotherapy Research, 27*(6), 886–891.

Drake, R. L., Vogl, A. W., & Mitchell, A. W. M. (2015). *Gray's Anatomy for Students* (3rd ed.). Elsevier.

El-Boshy, M. E., Abdel-Daim, M. M., & El-Sayed, Y. S. (2022). Impact of toxicants on reproductive hormones and testicular function: A review. *Environmental Toxicology and Pharmacology*, 89, 103820.

Górnaś, P., Rudzińska, M., & Segliņa, D. (2014). Evaluation of antioxidant capacity and fatty acid composition of fruit seeds oils. *Food Chemistry, 162*, 87–93.

Gumel, M., Sharif, N. M., & Usman, A. (2019). Citric acid: Properties, production, and applications. *Food Science and Biotechnology, 28*(5), 1359–1373.

Heidy, T. M., Mohamed, A. H., & Fatma, M. A. (2017). Production of organic acids by submerged fermentation: A review. *Biotechnology Reports, 15*, 76-85.

Ibrahim, R. M., Mahmoud, M. E., & El-Far, A. H. (2023). Protective effects of natural antioxidants on testicular toxicity induced by environmental pollutants. *Environmental Science and Pollution Research*, 30(11), 30020–30033.

Jin, Y., Zhang, Z., & Liu, X. (2024). Hormetic responses to phytochemicals: A review of dose-dependent effects on metabolism and oxidative stress. *Phytochemistry Reviews*, 23(1), 1–19.

Kumar, S., Singh, R., & Verma, A. (2022). High-dose effects of plant oils on testicular morphology and function in rodent models. *Journal of Toxicological Sciences*, 47(3), 189–198.

Kumar, V., Abbas, A. K., & Aster, J. C. (2013). *Robbins Basic Pathology* (9th ed.). Elsevier Saunders.

Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I.-J. (2018). Protein Determination—Method Matters. *Foods*, *7*(1), 5. https://doi.org/10.3390/foods7010005

Mallek-Ayadi, S., Ksouri, R., Trabelsi, N., & Hamdaoui, G. (2018). Nutritional and antioxidant potential of melon (Cucumis melo L.) seeds. *Industrial Crops and Products, 113*, 137–144.

Maran, J. P., & Priya, K. A. (2015). Impact of tricarboxylic acid cycle intermediates on male fertility: A review. *Journal of Reproductive Toxicology, 54*, 1-9.

Musa, A. I., Abdullahi, A. M., & Ibrahim, A. S. (2021). Effects of antioxidants on sperm parameters in chemically induced testicular toxicity. *Reproductive Toxicology*, 100, 27–35.

Nwosu, E. C., Okonkwo, C. U., & Obi, A. I. (2020). Role of antioxidants in ameliorating endocrine disruption in male reproductive toxicity. *Andrologia*, 52(9), e13600.

Nyam, K. L., Wong, N. K., & Shah, N. M. (2009). Physicochemical properties and antioxidant activity of melon seed oil. *Journal of the American Oil Chemists' Society, 86*(8), 823–829.

Ogunleye, T., Olaniyan, O., & Ajayi, O. (2022). Bioactive compounds in melon seed oil and their metabolic effects. *Nutrition and Metabolism*, 19(1), 55.

Olawuyi, O. T., Bello, T. A., & Adeyemi, O. S. (2023). Dose-dependent effects of melon seed oil on sperm motility and testicular oxidative stress in rats. *Andrology Reports*, 5(2), 110–119.

Patel, R., Desai, P., & Mehta, K. (2022). Antioxidant therapy for male infertility: A review of recent evidence. *Journal of Assisted Reproduction and Genetics*, 39(7), 1565–1576.

Rukmini, C., Raghuram, T. C., & Vijayaraghavan, K. (2004). *Manual of laboratory techniques*. New Delhi: National Institute of Nutrition, Indian Council of Medical Research.

Smith, J. A., Roberts, L. T., & Chen, W. (2023). Biphasic effects of plant-based compounds on metabolism: A review of hormesis in natural products. *Journal of Nutritional Biochemistry*, 112, 108860. https://doi.org/10.1016/j.jnutbio.2022.108860

Winterbourn, C. C., Hawkins, R. E., Brian, M., & Carrell, R. W. (1975). The estimation of red cell superoxide dismutase activity. *Journal of Laboratory and Clinical Medicine*, *85*(2), 337–341.

Zeb, A. (2016). Phenolic content and antioxidant activity of melon seed oil. *Journal of Food Science and Technology, 53*(2), 1024–1031.

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., De Mouzon, J., & Sokol, R. (2009). The International Committee for Monitoring Assisted Reproductive Technology (ICMART) glossary on infertility and fertility care. *Fertility and Sterility, 92*(5), 1520-1524.

Zhang, Y., Li, H., & Wang, X. (2024). Antioxidant supplementation and restoration of testosterone synthesis in rodent models of testicular toxicity. *Endocrine Connections*, 13(1), e210658.

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