Role of *Daucus carota* Ethanol Leaf Extract in Ameliorating Cadmium-Induced Oxidative Stress in Testes of Adult Wistar Rats

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ABSTRACT

Background and Objective. Cadmium remains the most pervasive and toxic pollutants which interferes with the endocrine function of the testes, thereby affecting testosterone synthesis, which is critical for maintaining male reproductive systems. This study investigated the role of *Daucus carota* ethanol leaf extract (DCELE) on cadmium induced toxicity in testicular cells of wistar rats.

Methods: Forty (40) adult male Wistar rats weighing 145-178kg were randomly divided into five groups (n= 8). Group A (control) received normal rat feed and water ad libitum. Group B received single dose of 8mg/kg body weight cadmium chloride ($CdCl_2$ only. Group C received 8mg/kg CdCl2 and a dose of 100mg/kg of DCELE. Group D received 8mg/kg body weight of $CdCl_2$ and 200mg/kg of DCELE. Group E received 8mg/kg body weight of $CdCl_2$ and 400mg/kg of DCELE twice daily. All treatments were administered through oral gavage for 28 days. Blood sample was collected using retro-orbital venous plexus puncture before sacrifice by cervical dislocation, for biochemical analysis

Results: Cadmium exposure significantly increased oxidative stress by increasing MDA and ROS while decreasing SOD, CAT and testosterone levels. Treatment with DCELE improved antioxidant enzyme activity and reduced lipid peroxidation, with DCELE co-treatment restoring SOD, CAT and testosterone level, reducing testicular oxidative stress and demonstrated improved testicular integrity. Results revealed that cadmium exposure when compared to control groups A is significant ($p < 0.05$).

Conclusion: Cadmium exposure significantly lowered antioxidant defense capacity and decreased circulating levels of testosterone. The DCELE could be a treatment strategy for individuals at risk of cadmium exposure, and an effective intervention for cadmium-induced toxicity in testicular tissue and potentially improving reproductive health

Key words: Cadmium, Ethanol, Extract, Oxidative, Rats

INTRODUCTION

Male infertility is an increasing global concern, with environmental toxicants such as cadmium (Cd) being major contributors as it interferes with the endocrine function of the testes, particularly affecting testosterone synthesis, which is critical for maintaining male reproductive health (El-Missiry *et al.*, 2019. Cadmium exerts its toxicological effects by inducing oxidative stress, disrupting endocrine functions, and impairing reproductive

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health. Specifically, it has been shown to target the hypothalamic-pituitary-testicular (HPT) axis, disrupting gonadotropin secretion and reducing testosterone levels, which are critical for spermatogenesis and male fertility (da Costa *et al*., 2021). It can disrupt cellular components such as lipids, proteins, and DNA, leading to apoptosis and necrosis in testicular cells (García-Gasca *et al*., 2021). It causes oxidative stress, which interferes with the synthesis of hormones and increases the production of reactive oxygen species (ROS) and a decrease in antioxidant defense mechanisms (Jones and Smith, 2019). Cadmium exposure results in a significant reduction in the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are crucial in neutralizing ROS (Patra *et al*.,2020). Acute administration of cadmium can induce testicular necrosis, hemorrhage and sterility in male rats (Chowdhury, 2009).

In response to the deleterious effects of cadmium, recent studies have explored the potential of natural antioxidants as therapeutic agents. This has necessitated the search for natural plant-based interventions with antioxidant, anti-inflammatory, and protective effects. *Daucus carota* (carrot), though well-studied for its root's nutritional value, has under-utilized leaves rich in potent bioactive compounds such as flavonoids, tannins, and phenolics. It may offer protective effect against oxidative damage. *Daucus carota* (carrot), particularly its leaves, has gained attention due to its rich composition of antioxidants, including β-carotene, polyphenols, and vitamins C and E (Khalid *et al*., 2021). Preliminary evidence suggests that ethanol extracts of carrot leaves exhibit antioxidant and cyto-protective effects. However, no study has comprehensively examined the protective effect of *Daucus carota* leaf extract against cadmium-induced reproductive toxicity. This notwithstanding, the role of *Daucus carota* leaf extract in mitigating cadmium-induced toxicity in testis is limited. Therefore, this significant gap in literature had to be addressed by this study.

METHODOLOGY

Materials: These were fresh *Daucus carota* leaves, forty wistar rats, normal saline, Whatmann filter paper, filter funnel, refrigerator, animal cages, metal wire, adhesive tape, heparinized test tubes, disposable syringes and needles, oral gavage, weighing scales, dissecting kits, laboratory coat and glass slides

Plant Collection/Identification and Animal Procurement: Fresh *Daucus carrot* leaves were obtained from Ogbe Awusa Market, Abakaliki, Ebonyi State of Nigeria. Identification and authentication of the leaves were done by a botanist in the Department of Science and Biotechnology in University of Nigeria Nsuka, Enugu State, Nigeria.

Forty (40) male wistar rats (145-178kg) were procured and maintained in metal cages, in the animal house of the Department of Anatomy, Ebonyi State University, Abakaliki. The animals were fed with standard rat feed daily and water ad libitum, and acclimatization for14 days, under 24 hours light/dark cycle at temperature of 24 ± 2^{0C}.

Cadmium procurement: $CdCl_2$ was purchased from Zayo-Sigma Chemicals Ltd, Jos, Nigeria; an agent and Partner of Sigma-Aldrich in Nigeria. Twenty (20) g of $CdCl_2$ was mixed with 100 milliliters of purified water and after which preserved in refrigerator for use.

Preparation of *Daucus carota* Ethanol Leaf Extract: The leaves were allowed to dry under shade for two weeks to prevent direct effects of sunlight on bioactive component of the leaves. After this, the leaves were grinded into a powdery form using a grinding machine. A 100g of the grind sample was macerated in 300ml ethanol, with intermittent agitation for 24 hours. The mixture was filtered with filter paper, and the extract was concentrated on a water bath. The remaining sample was macerated in 600ml ethanol with constant agitation for 72 hours. The extract was collected after 72hrs by filtration and concentrated using a water bath. The marc was re-soaked in 475ml ethanol for another 72 hours, filtered, and concentrated to obtain the DCELE in solid. Cadmium chloride (1800mg) was dissolved in 225ml of distilled water to obtain working solution from where various doses for *Daucus carota* leaf extract were calculated according to the weight of the animal.

Experimental Design: The rats were randomly placed in five (5) groups of eight (8) rats. They were subjected to different treatments as show below. These amounts were determined using an initial investigation indicating

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no signs of toxicity. Twenty (20) g of $CdCl_2$ was mixed with 100 milliliters of purified water and preserved in refrigerator for use. The $CdCl_2$ was dissolved in normal saline which was given orally at 8 mg/kg bwt dose.

- Group A (control) received normal feed and water ad libitum for 28 days
- Group B received 8mg/kg body weight of $CdCl_2$ only for 14days
- Group C received 8mg/kg body weight of $CdCl_2$ and100mg/kg of *Daucus carota* leaf extract for 14days
- Group D received 8mg/kg body weight of $CdCl_2$ and 200mg/kg *of Daucus carota* Leaf extract daily for 14days
- Group E received 8mg/kg body weight of $CdCl_2$ and 400mg/kg body weight of *Daucus carota* leaf extract for 28days.

Animal Sacrifice and Sample Collection: On the 28 day, at the conclusion of the experiment, and after the last weighing of the animals, the rats were sacrificed by cervical dislocation. Blood was collected using a 5ml syringe through venous orbital plexus puncture and immediately transferred into heparinized test tube. This was kept for 24 hours after which supernatant was collected in a test tube and centrifuged for 10minutes at 2500rpm. The plasma concentration of testosterone was determined using enzymatic kits and standard reagents according to the protocol of the manufacturer (NIADDK – NIH (USA)). The samples were homogenized and spinned in 10minutes, the resulting serum was used for the evaluation of Malondialdehyde(MDA), Catalase (CAT), Superoxide Dismutase (SOD) and reactive oxygen species(ROS) activity.

Evaluation of Oxidative Stress Markers

Estimation of Lipid Peroxidation (Malondialdehyde): The MDA level was determined according to the method reported by Gutteridge,1995. The absorbance was read in a UV/Vis spectrophotometer at 532 nm.

Lipid peroxidation in the tissue was estimated colorimetrically by thiobarbituric acid reactive substances(TBARS) using the method of Yagi (1976), the principle which is based on the formation of a TBA pink-colored chromogens as a result of the reaction between TBA and oxidation products that can be quantified by spectrophometry. It involved 0.1 ml of tissue in Tris HCl buffer, treated with 2 ml of BA-TCA-HCl reagent (thiobarbituric acid 0.37%, 15% TCA, 0.25 N HCl). The solution was placed in water bath for 15 minutes and cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptivity of malondialdehyde which was expressed as nmol/mg protein.

Thiobarbituric acid reactive substances (TBARS) method for detection of MDReaction of MDA with thiobarbituric acid at acidic pH at high temperatures with t

Assay of Catalase (CAT) Activity: Catalase activity was measured according the method of Aebi (1984). Tissue serum (0.1 ml) was pipetted into cuvette containing 1.9 ml of 50mM phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30% (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of the enzyme was expressed as units /mg protein

Assay of Superoxide Dismutase (SOD) Activity: Superoxide dismutase activity was measured according to the method of (Spitz and Oberley, 1989). Superoxide dismutase reduces superoxide to hydrogen peroxide. The reaction mixture contained 2.7 ml of 0.067M phosphate buffer, pH 10.2, 0.1 ml of 1.5mM NBT, 0.05 ml of 0.01M methionine and 0.1 ml of enzyme samples. The nitroblue tetrazolium (NBT) reduction served as an indicator of $O_2^{\bullet-}$ production. TheSOD competed with NBT for O_2, the percent inhibition of NBT reduction is a measure of the amount of SOD present at 560nm.

Reactive Oxygen Species

Horseradish Peroxidase (HRP) was used in conjunction with probes of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) to detect ROS in serum. The HRP-catalyzed oxidation of the probe produced a fluorescent signal that indicated the presence of ROS.

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Kit sinks were covered with an initial antibody against the desired antigen (ROS). By adding the desired antigen, followed by addition of a secondary antibody that bound to the antigen. The enzyme in Horseradish Peroxidase (HRP) was subsequently added and it stuck to the end of the secondary antibody. The chromogenic solutions containing the HRP enzyme substrate were then added. Oxygenated water (H_2O_2) was exposed to HRP and converted into hydroxide radicals. Subsequently, it was exposed to a reagent that oxidized and produced color. The color was studied with the ELISA Reader machine, the start-fax-2100 series manufactured by the United States, and was detected at 505 nm. The optical density specimen (O.D) and the sample concentration were determined based on the color. The more the color existed, the greater the light absorption was seen.

Statistical Analysis: Data obtained was expressed as mean ± SD. The level of homogeneity among the groups was tested using one-way Analysis of Variance (ANOVA). All groups were compared with each other for every parameter. The software for the analysis is graph pad prism version 9.0.1.5 The level of significance for all the analysis was set at ($p < 0.05$).

RESULTS

Cadmium-induced oxidative stress in testicular tissues results from the generation of reactive oxygen species (ROS) and a subsequent imbalance in antioxidant defenses. This oxidative stress leads to lipid peroxidation, which damages cell membranes and exacerbates testicular damage (Mori *et al.*, 2019). The increased ROS levels contribute to cellular dysfunction and loss of testicular integrity (Ali *et al.*, 2021). Lipid peroxidation products such as malondialdehyde (MDA) have been linked to cadmium toxicity and are used as biomarkers for oxidative stress (Yao *et al.*, 2022). Moreover, cadmium interferes with the endocrine function of the testes, particularly affecting testosterone synthesis, which is critical for maintaining male reproductive health (El-Missiry *et al.*, 2019)

Effects of Cadmium and *Daucus carota* leaf Extract on Oxidative Stress Markers

The Oxidative stress markers in this study is as presented in figure(1a) and (1b).

In figure 1a, levels of Malondialdehyde (MDA) in cadmium group B significantly increased compared to control group A ($p < 0.05$), and significantly decreased compared to treated groups (C, D and E) ($p < 0.05$). In figure (1b), the level of reactive oxygen species (ROS) in group B increased significantly compared to group A ($p < 0.05$). In contrast to treated groups (C, D and E), the levels of reactive oxygen species (ROS) maintained a steady decline compared to cadmium group B ($p < 0.05$).

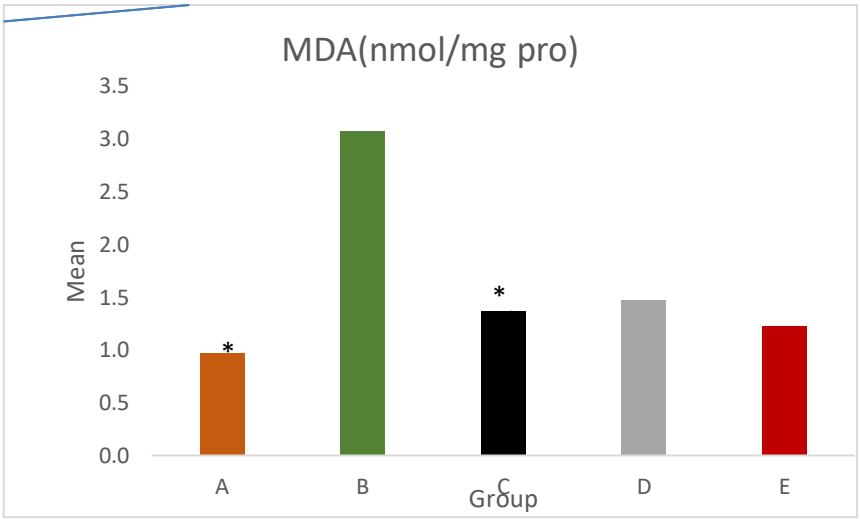

Fig. 1a: Effects of Cadmium and *Daucus carota* leaf extract on oxidative marker.

Levels of Malondialdehyde (MDA): Cadmium group significantly increased compared to control(*$p < 0.05$), and significantly decreased compared to treated groups(**$p < 0.05$).

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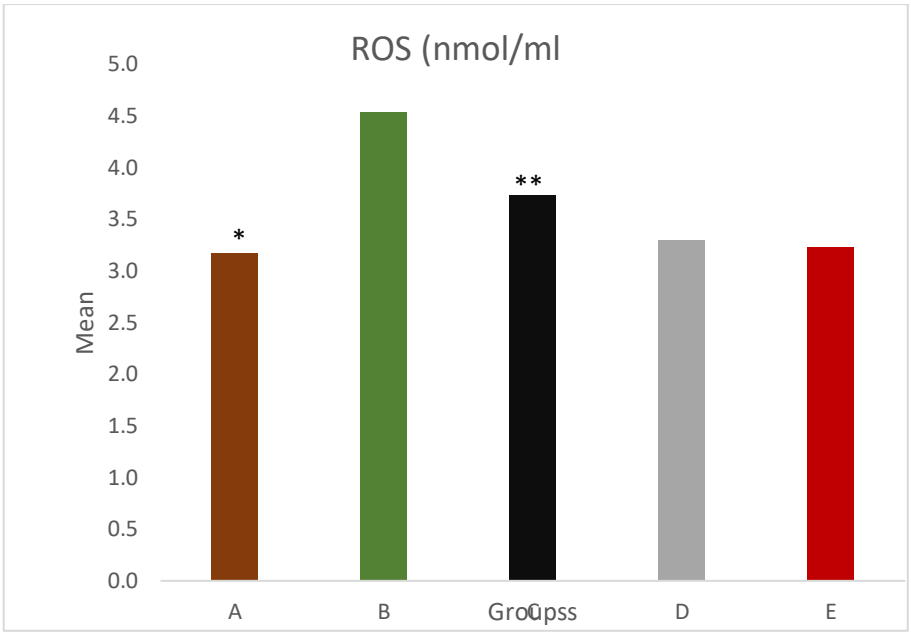

Fig 1b: Effects of Cadmium and *Daucus carota* Leaf Extract on Oxidative Marker

Levels of Reactive Oxygen Species (ROS). Cadmium group significantly increased compared to control (*p < 0.05), and significantly decreased compared to treated groups (**p < 0.05).

Effects of Cadmium and *Daucus carota* leaf Extract on Antioxidant Enzymes

The antioxidant enzymes in this study as presented in figure(2a) and (2b).

In figure 2a, levels of Superoxide Dismutase (SOD) in cadmium group B significantly decreased compared to control group A (p < 0.05) and significantly increased compared to treated groups C, D and E (p < 0.05). In figure (2b), the level of catalase (CAT) in group B decreased significantly compared to group A (p < 0.05). The treated groups (C, D and E), the levels of Catalase (CAT), progressively increased compared to group B (p < 0.05).

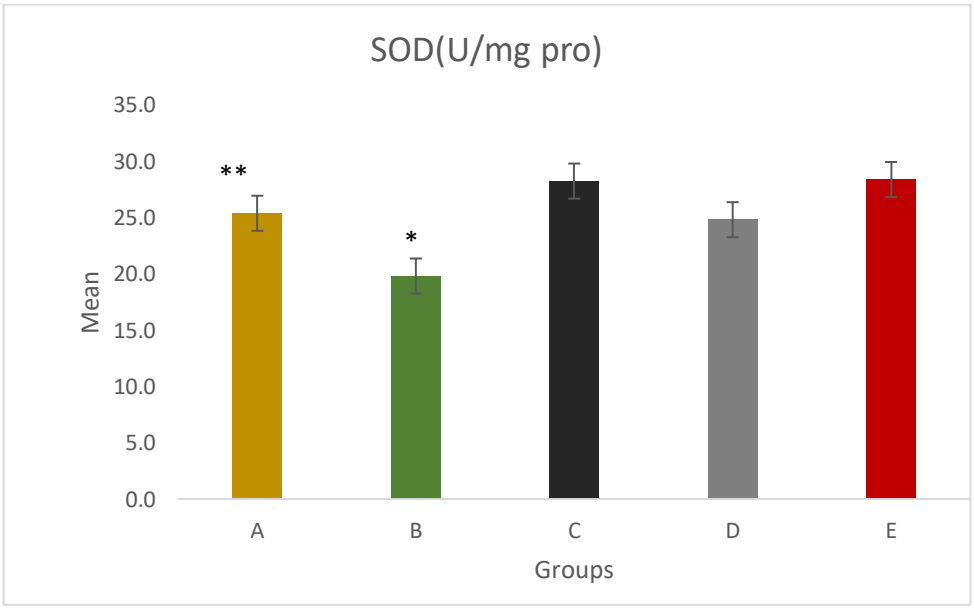

Fig 2a: Effects of Cadmium *and Daucus carota* Leaf Extract on antioxidant Enzyme

Levels of Superoxide Dismutase (SOD): Cadmium group significantly decreased compared to control ((**p < 0.05) and significantly increased compared to treated groups (*p < 0.05).

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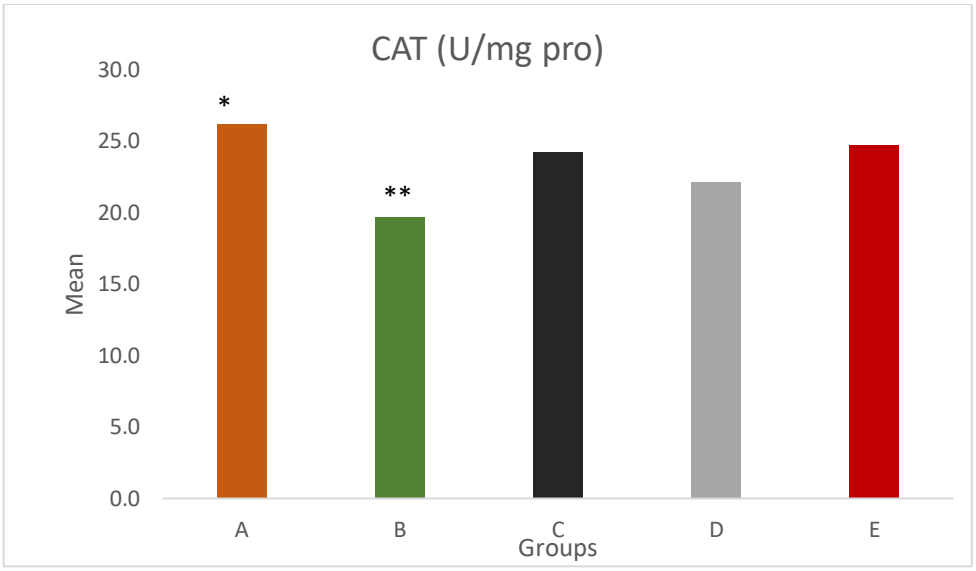

Fig 2b: Effects of Cadmium and *Daucus carota* Leaf Extract on Antioxidant Enzyme

Levels of catalase (CAT): Cadmium group significantly decreased compared to control ((**p < 0.05) and significantly increased compared to treated groups (*p < 0.05).

Effects of Cadmium on Animal Percentage Weight Gain

The body weight analysis is presented in figure 2c. The control group (A) exhibited a weight gain (14.3%), while cadmium exposure in group B led to a significant weight drop when compared to group A (p< 0.05), indicating toxicity-related metabolic effects. Group C, treated with DCLE, had a weight increase, significantly different from group B. Similarly, groups D and E showed no statistically significant difference (p>0.05).

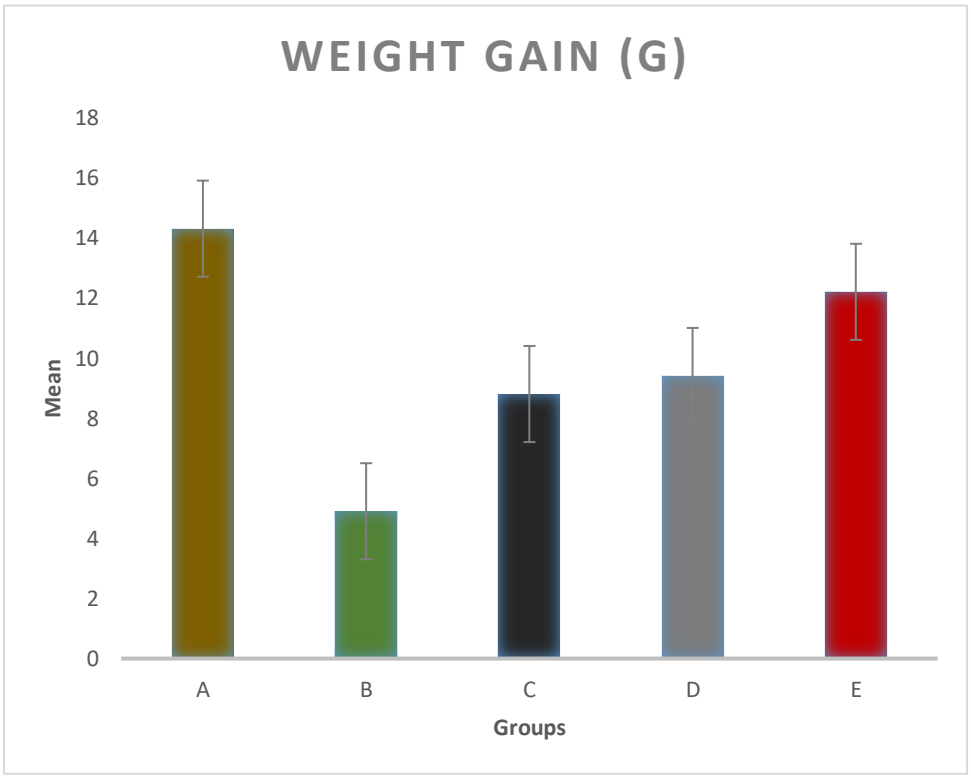

Figure 2c: Effects of cadmium on animal percentage weight gain following treatment with *Daucus carota* leaf extract. Percentage body weight gain in control, Cadmium group significantly decreased compared to control (**p < 0.05) and significant increased compared to treated groups (*p < 0.05).

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Effects of *D. carota* Leaf Extract on Testosterone Level Following Cadmium Exposure

The control group recorded the highest serum testosterone levels, while cadmium exposure significantly reduced testosterone levels ($p < 0.05$), indicating cadmium's detrimental effect on testosterone synthesis. In contrast *Daucus carota* leaf extract treated groups improved testosterone levels compared to cadmium group ($p < 0.05$). see Fig 2d.

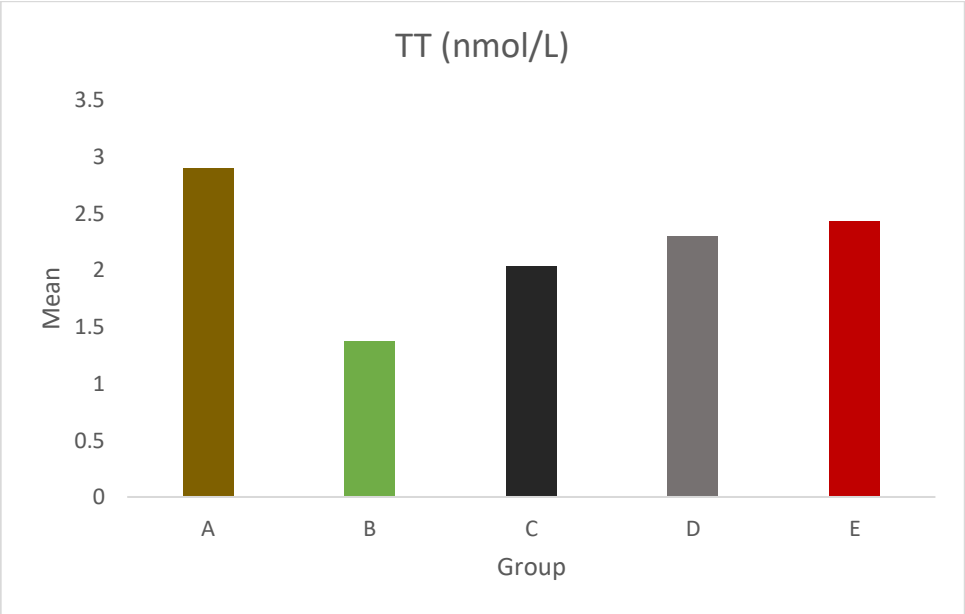

Figure 2d: Effects of *D. carota* Leaf Extract on Testosterone level following Cadmium Exposure. Levels of testosterone (TT): Cadmium group significantly decreased compared to control ((**$p < 0.05$) and significantly increased compared to treated groups (*$p < 0.05$).

DISCUSSION

Biochemical Analysis

The biochemical analysis in this study evaluated oxidative stress markers and antioxidant activity in the testes of cadmium exposed adult wistar rats and the ameliorative effects of *Daucus carota* leaf extract. The elevated levels of MDA and ROS resulted in decreased antioxidant enzyme markers Superoxide Dismutase(SOD) and Catalase (CAT) (figure 2a and 2b). Treatment with *Daucus carota* leaf extract showed decline in oxidative stress markers and enhanced antioxidant enzyme activity. Findings show that exposure to cadmium result to elevated oxidative stress, orchestrated by increased levels of Malondialdehyde (MDA). In the control group, cadmium exposure significantly elevated MDA levels (figure1a), confirming cadmium's pro-oxidant effects and its role in promoting lipid peroxidation. This is in line with a previous study that Cd decreases antioxidant activity and increased MDA content of the mice testis, hence, inducing oxidative stress damage in the testis (Yao et al.,2022). Reactive oxygen species and lipid peroxidation in cadmium induced oxidative stress in tissue has been reported by Song et al. (2023). The *Daucus carota* leaf extract treated groups significantly reduced MDA levels indicating nature-based antioxidant role to humiliate lipid peroxidation in testis of rats by scavenging free radicals and reducing lipid peroxidation (Oboh et al., 2022).

The ROS (figure1b) significantly increased in cadmium group, confirming testicular oxidative stress caused by cadmium exposure thereby releasing free radicals. In a previous study, Cd inhibits the electron transfer chain and induces ROS. The homeostasis of ROS is maintained by the production of ROS and the antioxidant system (Wang et al., 2004). Following the treatment with *Daucus carota* leaf extract, ROS level declined, indicating the ability of nature-based antioxidant to scavenge free radicals. This finding is consistent with previous report by Sen et al. (2004), indicting antioxidants protection in rat testes from cadmium induced reactive oxygen species.

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In figure 2a, SOD decreased significantly in group B (cadmium group) compared to the control group A. This level of reduction suggests that cadmium exposure enhanced oxidative stress by reducing antioxidant defense activity. Previous studies have shown that this decrease in SOD highlights the vulnerability of testicular cells to oxidative stress when exposed to cadmium, potentially leading to cellular damage (Liang *et al.*, 2021). In contrast, groups C, D and E which received *Daucus carota* leaf extract post-cadmium exposure exhibited significantly increased SOD activity compared to Group B. In this study, DCLE as an antioxidant, stabilized the cell membranes and restored SOD levels to counteract oxidative damage. This aligns with previous studies which deposited that *Daucus carota* ethanol leaf can enhance antioxidant capacity against cadmium induced oxidative damage in rat testis (Jahan *et al*., 2014).

Similarly, in figure2b, CAT activity decreased significantly in group B (cadmium group), indicating severe oxidative stress. This is in line with a previous study by Zhang et al. (2023), which showed cadmium-induced rat testicular dysfunction and its mechanism of chronic stress. In the control group, CAT activity was high. This reduction in CAT activity showed that cadmium impairs the cellular antioxidant response, allowing hydrogen peroxide to accumulate and increase testicular oxidative stress. Our finding is consistent with a study by Zhu *et al*. (2020), which revealed the toxicological effects of cadmium exposure on mammalian testis. In treated groups, *Daucus carota* leaf extract, administration showed an improvement in CAT activity when compared to group B. The recovery though not fully restored when compared to group A, reflects the antioxidant's capacity to improve the body's oxidative defense mechanisms. This is in line with the result of another study which revealed that, when enzymatic activity required to detoxify hydrogen peroxide is impaired, oxidative stress and potential cellular damage remains inevitable (Saeed *et al*., 2022). The reduction in these enzymes suggest that cadmium exposure suppressed the testicular antioxidant mechanisms, leading to oxidative stress and testicular damage (Zhang et al., 2023; Zhu et al., 2020). Treatment in all the groups with *Daucus carota* leaf extract restored SOD and CAT levels, indicating its antioxidant response as therapeutic agents.

Percentage body Weight gain (PBWG)

In figure 2c, the control group (A) exhibited a high weight gain. Cadmium exposure in group B led to a significant weight drop when compared to group A, indicating toxicity-related metabolic effects. Groups C, D and E treated with DCLE, showed a weight increase, with significant different from group B. No significant difference was recorded among treated groups. This result is in line with the result of (Biswas *et al*., 1965), which documented that administration of cadmium chloride lowered testicular weight gain than the controls, with significant decrease in epididymal sperm count and motility, abnormal spermatozoa and serum testosterone levels.

Serum testosterone level

In figure 2d, the control group recorded the highest serum testosterone levels. Cadmium exposure significantly reduced testosterone levels, indicating cadmium's detrimental effect on testosterone synthesis (Huang et al., 2022). In contrast *Daucus carota* leaf extract co-administration improved testosterone levels, with the group treated with 100 mg/kg of the extract showing a moderate increase compared to cadmium-alone group. This recovery demonstrates *Daucus carota* leaf extract's potential to counteract cadmium-induced hormonal impairment (Gong et al., 2023). Testosterone is a vital reproductive hormone, and its levels serve as biomarkers of testicular function (Ahmed *et al.*, 2021)

CONCLUSION

The results of this study provide significant insights into the effects of cadmium-induced oxidative stress on testicular function in male Wistar rats and the potential protective role of *Daucus carota* leaf extract on cadmium-induced toxicity. Cadmium is known to induce oxidative stress, which can compromise cellular and enzymatic defenses, ultimately affecting reproductive health and function.

Ethical Approval

Ethical approval was sought for and obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki. The ethical code of this study is MPC/2024/05//001

REFERENCES

1. Aebi H, Lester P (1984). Catalase in vitro. In: Packer L, editor. Methods in Enzymology. Vol. 105. Academic Press; New York:. pp. 121–126.
2. Ahmed, M., Zhang, W., Li, T., and Liu, Y. (2021). Impact of environmental contaminants on reproductive hormones: Insights from recent studies. Environmental Toxicology and gPharmacology, 75, 103326.
3. Biswas NM, Deb C.(1965). Testicular degeneration in rats during hypervitaminosis A. Endokrinologie. ;49:64–69.
4. Chowdhury A R (2009) Recent advances in heavy metals inducedeffects on male reproductive function-A retrospective. AlAmeen J. Med. Sci. 2, 37-42.
5. El-Missiry, M. A., Othman, A. I., and Amer, M. A. (2019). L-carnitine protects against cadmium-induced reproductive toxicity in rats: A role of oxidative stress and apoptosis. Reproductive Toxicology, 87, 89-97.
6. García-Gasca, T., Armienta, M. A., and Delgado-Lamas, G. (2021). Antioxidant responses in rats exposed to cadmium and the protective role of silymarin. Journal of Environmental Science and Health, Part A, 56(5), 536-544.
7. Gong, Z., Zhao, W., Li, F., and Wu, H. (2023). Disruption of follicle-stimulating hormone levels due to heavy metal exposure: Mechanisms and mitigation. Toxicology Reports, 10, 99–106.
8. Gutteridge, J. M. (1995). Lipit peroxidation and antioxidants as biomarkers of tissue damage, Clin. Chem;41(12):1819– 1828.
9. Huang, Y., Zhang, X., and Wang, L. (2022). Oxidative stress and reproductive toxicity: The role of cadmium. Reproductive Toxicology, 59(2), 45-52.
10. Jahan, S., Zahra, A., Irum,U, Iftikhar,N., Ullah ,H.(2014). Protective effects of different antioxidants against cadmium induced oxidative damage in rat testis and prostate tissues. Syst. Biol. Reprod. Med., 60, pp. 199-205,
11. Jones, P., Smith, R., and Patel, M. (2017). Industrial sources of cadmium pollution on Environmental Science and Technology, 52(7), 342-355.
12. Khalid, S., et al. (2021). Phytochemical and antioxidant profile of Daucus carota leaves. Journal of Medicinal Plants Research, 15(4), 89–96.
13. Liang, X., Li, D., and Zhang, X. (2021). Assessment of cadmium-induced oxidative stress and protective role of antioxidants. Toxicological Sciences, 181(1), 115–126.
14. Oboh, G., Olasehinde, T. A., and Ademosun, A. O. (2022). Cadmium-induced oxidative stress and the protective role of antioxidants: A biochemical analysis. Biological Trace Element Research, 200(1), 315-324.
15. Patra, D. K., Archarya, S., Pradhan, C., Patra, H. K. (2020). Poaceae plants as potential phytoremediators of heavy metals and eco-restoration in contaminated mining sites. Environmental Technology and Innovation, 21: 101293, https://doi.org/10.1016/j.eti. 2020.101293
16. Saeed, M., Ali, A., and Khan, M. (2022). Cadmium-induced oxidative stress and the protective role of antioxidants. Journal of Toxicology and Environmental Health, 86(4), 123-135.
17. Sen Gupta, R., Sen Gupta, E., Dhakal, B. K., Thakur, A. R., and Ahnn, J. (2004). Vitamin C and vitamin E protect the rat testes from cadmium- induced reactive oxygen species. Mol. Cells 17, 132–139.
18. Song, J., Wang, H., and Liu, Y. (2023). Reactive oxygen species and lipid peroxidation in cadmium-induced oxidative stress. Antioxidants, 12(3), 678.
19. Spitz, D. R., Oberley, L. W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. Annal Biochem, 179(1): 8-18 doi: 10.1016/0003-2697(89)90192-9
20. da Costa, C. S., Oliviera, T. F., Freitas-Lima, L. C., Padilha, A., Krause, M., Carneiro, M. T. W. D., Salgado, B. S., Graceli, J. B.(2021). Subacute cadmium exposure disrupts the hypothalamic-pituitary-

gonadal axis, leading to polycystic ovarian syndrome and premature ovarian failure features in female rats. Environ Pollut. 269:116154 doi: 10.1016/j.envpol.2020.116154.

21. Wang, Y., Fang, J., Leonard, S.S., Rao, K.M(2004). Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic. Biol. Med., 36, 1434–1443
22. Yagi, K.(1976). A simple fluorometric assay of lipoperioxide in blood plasma. Biochem Med. 15(2): 212-216. doi: 10.1016/0006-2944(76)90049-1
23. Yao, C., Zhang, H., and Liu, S. (2022). Role of lipid peroxidation in cadmium toxicity: Biomarkers and mechanisms. Toxicology Reports, 9, 203-211.
24. Zhang, X., Wu, J., and Yang, L. (2023). Apoptotic pathways involved in cadmium-induced testicular damage. Toxicology Letters, 366, 134-143.
25. Zhu, Q., Li, X., Ge, R.,(2020). Toxicological Effects of Cadmium on Mammalian Testis Front. Genetics, 11 (2020), p. 527,