Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 2(4) December 2024
DOI: https://doi.org/10.33003/jobasr-2024-v2i4-66

Aqueous Extract of *Loranthusmicranthus* Linn Leaf Alleviates Cadmium-Induced Testicular Dysfunction in Male Wistar Rats

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ABSTRACT
Cadmium (Cd), a heavy metal and environmental pollutant, induces oxidative stress and testicular dysfunction, severely impairing male fertility. This study evaluated the protective effects of the aqueous extract of *Loranthusmicranthus* Linn leaf on cadmium-induced testicular dysfunction in male Wistar rats. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, and glycosides, while acute toxicity studies showed an LD50 greater than 5000 mg/kg, indicating its safety.Thirty rats were divided into five groups: control, cadmium-treated, cadmium with low-dose extract (250 mg/kg), cadmium with high-dose extract (500 mg/kg), and cadmium with vitamin E (200 mg/kg). Testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were analysed, alongside oxidative stress markers (MDA, GSH, SOD, CAT, TAC) and sperm quality parameters. Cadmium exposure significantly ($p<0.05$) reduced body and testicular weights, testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels, while increasing malondialdehyde (MDA) levels and decreasing antioxidant markers including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) and sperm quality. Treatment with the high-dose extract significantly ($p < 0.05$) restored body and testicular weights, hormone levels, antioxidant status, reduced MDA levels, and improved sperm count, motility, and morphology to levels comparable to the control group, similar to the effects of vitamin E. In conclusion, *Loranthusmicranthus* Linn leaf extract demonstrates potent antioxidant and protective properties against cadmium-induced testicular dysfunction, underscoring its potential as a natural therapeutic agent for managing reproductive toxicity.

Keywords:
Cadmium,
Oxidative stress,
Testes,
Vitamin E,
Wistar Rats.

INTRODUCTION

Cadmium (Cd) is a heavy metal and ubiquitous environmental pollutant that poses significant health risks due to its toxicity and bioaccumulation properties. Human and animal exposure to cadmium primarily occurs through contaminated food, water, and air, as well as occupational environments (Mitra *et al*., 2022). Once absorbed, cadmium accumulates in various tissues, particularly in the kidneys, liver, and testes, which exerts deleterious effects (Charkiewicz *et al*., 2023). Testicular toxicity induced by cadmium is well-documented, and it leads to alterations in spermatogenesis, oxidative stress, and disruption of normal endocrine functions (Zhu *et al*., 2020; Ali *et al*., 2022). The molecular mechanisms underlying cadmium-induced testicular damage include increased production of reactive oxygen species (ROS), lipid peroxidation, mitochondrial dysfunction, and subsequent apoptosis of germinal cells (Wang *et al*., 2019). These alterations contribute to a decline in male reproductive health, with diminished sperm quality, count, motility, and morphology, ultimately affecting fertility (Zhu *et al*., 2020; Ali *et al*., 2022).
Recent studies have highlighted the role of oxidative stress as a central mechanism in cadmium-induced

testicular dysfunction (Wang *et al.*, 2019; Ali *et al.*, 2022). In this context, antioxidants have emerged as potential therapeutic agents to mitigate the toxic effects of cadmium (Brzóska *et al.*, 2016; Unsal *et al.*, 2020). Vitamin E, a well-known antioxidant, has been shown to protect against oxidative damage by scavenging free radicals and enhancing cellular antioxidant defences (Beytut*et al.*, 2003; Niki, 2015). Its protective role in cadmium-induced reproductive toxicity has been well-documented (Al-Attar, 2011; Kini *et al.*, 2016). However, the search for alternative, cost-effective, and sustainable solutions has prompted increasing interest in medicinal plants that possess antioxidant, anti-inflammatory, and protective properties.

Loranthusmicranthus Linn (Figure 1), a parasitic plant found in various parts of Africa, has been traditionally used in folk medicine for its medicinal properties (Szurpnicka*et al.*, 2020). *Loranthusmicranthus*, commonly referred to as mistletoe, is a hemi parasitic plant belonging to the Loranthaceae family. It is predominantly found in tropical and subtropical regions, growing on the branches or trunks of various host trees, such as fruit trees, shrubs, and forest trees (Moghadamtousi *et al.*, 2013). Renowned for its medicinal properties, mistletoe has been highly valued in traditional medicine for centuries (Szurpnicka *et al.*, 2020). Indigenous cultures have long utilized *Loranthusmicranthus* to address a variety of health issues, including cardiovascular, respiratory, gastrointestinal, and reproductive disorders (Moghadamtousi *et al.*, 2013).The plant's antioxidant properties likely play a role in its traditional use for addressing oxidative damage and age-related diseases (Nicoletti, 2023). It is known for its wide range of bioactive compounds, including flavonoids, phenolics, and tannins, which contribute to its antioxidant, anti-inflammatory, and hepatoprotective effects (Hlophe & Bassey, 2023). Despite its traditional use, there is limited scientific evidence on the protective effects of *Loranthusmicranthus* Linn, particularly regarding its potential to mitigate cadmium-induced testicular toxicity.

This study aims to evaluate the protective effects of the aqueous extract of *Loranthusmicranthus* Linn leaf on cadmium-induced testicular dysfunction in male Wistar rats. Specifically, it seeks to assess the extract's ability to reduce oxidative stress and improve reproductive health, focusing on the restoration of reproductive hormone levels, sperm count, sperm motility, and sperm morphology. Furthermore, the study compares the efficacy of *Loranthusmicranthus* Linn leaf extract with vitamin E, a known antioxidant, in protecting against cadmium-induced damage. The findings of this study could contribute to a better understanding of the potential therapeutic applications of *Loranthusmicranthus* Linn in mitigating cadmium-induced testicular toxicity and may pave the way for the development of natural alternatives to conventional therapeutic agents.

Figure 1: *Loranthusmicranthus* Linn on *Alstoniaboonei* (popularly known as God's tree or "Onyamedua" within West Africa.)

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of mistletoe (*Loranthusmicranthus. L*) were harvested from the host plant Oil Bean (*Pentacletramacrophylla*) in May 2016 from a plantation atAmaoba community in Ikwuano Local Government Area, Abia State, Nigeria. It was identified and authenticated by Mr PiPi Okey in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher number MOUAU/COLNAS/PSB/16/A266 was obtained.

Plant Extraction

Fresh leaves of the mistletoe plant (*Loranthusmicranthus. L*) were air-dried at room temperature and pulverized using a glass electric blender. A known amount (100 g) of the pulverized sample was weighed into a glass beaker and a known volume of distilled water (1000 mL) was added. It was stirred vigorously for 20 minutes and allowed to stand for 24 hours. The filtrate was concentrated via lyophilisation to obtain a coffee brown residue. The concentrated extract was stored at 4ºC in an air-tight bottle until further use.

Preparation and Administration of the Aqueous LeafExtract of *Loranthusmicranthus*Linn

The extract was diluted with distilled water to attain the intended dosages of 250 and 500 mg/kg of body weight for administration to the rats. It was then orally administered to the rats via gavage.

Preparation and Administration of Cadmium and Vitamin E

Cadmium was prepared by dissolving it in sterile distilled water and administered intraperitoneally (i.p.)

to the rats at a dose of 5 mg/kg body weight. The standard drug, commercial-grade vitamin E (α-tocopherol) was dissolved in vegetable oil for enhanced bioavailability and administered orally at a dose of 100 mg/kg body weight using a gavage.

Qualitative Phytochemical Analysis of the AqueousLeaf Extract of *Loranthus micranthus* Linn.

Phytochemical screening of the aqueous extract was carried out to identify secondary metabolites- alkaloids, flavonoids, saponins, and tannins using standard phytochemical methods (Harborne, 1984; Sofowora, 1993; Treaseand Evans, 2002).

Determination of Lethal Dose (LD_{50})

An acute toxicity study was carried out on the aqueous leaf extract according to the method described by Lorke (1983).

Experimental Animals

Thirty (30) male Wistar rats, weighing 180-200 g, were procured from the animal facility of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State. The animals were acclimatized for one week in the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, before the commencement of the study. They were housed in aluminium cages, with six rats per cage, and provided *ad libitum* access to standard commercial pelleted grower feed (Vital, Nigeria) and clean drinking water. The animals were kept under normal light/dark cycles and were cared for following the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH, 2002).

Experimental Design

A total of 30 male Wistar rats were randomly divided into five groups, each containing 6 rats.

Group 1 (Control group) had access to food and water *ad libitum.* Group 2 (Cadmium group) was administered cadmium chloride ($CdCl_2$) at a dose of 5 mg/kg body weight intraperitoneally for 7 days.Group 3 (Low dose extract + Cadmium group) was administered aqueous extract of *Loranthusmicranthus* at 250 mg/kg body weight orally for 14 days. After 7 days of extract administration, cadmium chloride (5 mg/kg) will be administered intraperitoneally for 7 days, while continuing the *Loranthusmicranthus* treatment. Group 4 (High dose extract + Cadmium group) was administered aqueous extract of *Loranthusmicranthus* at 500 mg/kg body weight orally for 14 days. After 7 days of extract administration, cadmium chloride (5 mg/kg) will be administered intraperitoneally for 7 days, while continuing the *Loranthusmicranthus* treatment. Group 5 (Standard group) was administered vitamin E (100 mg/kg) orally for 14 days, followed by cadmium chloride (5 mg/kg) intraperitoneally for 7 days. The aqueous leaf extract of *Loranthusmicranthus* was freshly prepared and administered once daily via oral gavage while cadmium chloride was administered intraperitoneally once daily for 7 days after the pre-treatment with the extract in the respective groups. The study lasted for 14 days.

Blood Sample and Tissue Collection

At the end of the experiment, all the rats were anesthetized with an intraperitoneal injection of urethane and sacrificed immediately. Blood samples were drawn using a 5 mL syringe via cardiac puncture (Arunachalam & Sasidharan, 2021) into sample tubes, and centrifuged at 4000 rpm for 10 minutes to obtain serum which was used for biochemical assays in this study. The testes were excised, trimmed of connective tissue, and washed thoroughly in ice-cold saline.

Measurement of Body and Testicular Weights

The body weights of the experimental animals were recorded at the beginning of the experiment (day 0) and at its conclusion (day 14) using a precision electronic weighing balance. The testicular weights were also measured with the same device at the time of sacrifice.

Assessment of Serum Reproductive Hormone Concentrations

After euthanasia, blood was collected via cardiac puncture into plain sample bottles and left undisturbed for 30 minutes. The samples were then centrifuged at 4,000 rpm for 10 minutes to separate the serum. The serum was used to measure the concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These hormone levels were determined using Abcam's *in vitro* competitive ELISA kits for testosterone, LH, and FSH, following the manufacturer's instructions.

. Preparation of Testicular TissueHomogenates

The tissue (testes) was homogenized in cold phosphate buffer (0.05M, pH 7.0) with a Teflon homogenizer. 1g of the tissue was homogenized in 9 mL of phosphate buffer to give 10% homogenate. The homogenate was centrifuged at 4000 rpm for 10 minutes. The supernatant obtained was stored frozen at -20^0C until required for biochemical analysis.

. Evaluation of Antioxidant Defence and Oxidative Damage Markers in Testicular Tissue Homogenates

The activities and concentrations of key antioxidants and oxidative stress markers in testicular tissue homogenates were assessed using established methods. Catalase activity was measured following a modified protocol by Atawodi (2011), while superoxide dismutase (SOD) activity was determined according to the method of Sun *et al*. (1988). Reduced glutathione (GSH) levels were analysed using Tietze's method

(1969). Malondialdehyde (MDA) concentration, an indicator of lipid peroxidation, was evaluated using the procedure described by Draper and Hadley (1990). Total antioxidant capacity (TAC) was quantified according to the method outlined by Koracevic *et al.* (2001).

Sperm Function Analysis

Sperm Count

Sperm count was performed using a modified Neubauer counting chamber (haemocytometer). A drop of the diluted sperm suspension was placed in each counting chamber and allowed to stand for 5 minutes. The chamber was then positioned under a binocular light microscope with an adjustable light source. The ruled area of the chamber was focused, and spermatozoa were counted in four 16-celled squares. Sperm concentration was calculated and expressed as [x] x 10^6/ml, where x represents the number of spermatozoa counted in a single 16-celled square.

Sperm Motility

According to PanjehShahin *et al.* (2005), the caudal portion of the epididymis was carefully separated from the testis and placed in a beaker containing 1 ml of buffered physiological saline solution. The epididymal section was then incised using sharp scissors and left for several minutes to allow the spermatozoa to release into the saline solution. A drop of the semen was placed on a slide, to which two drops of warm 2.9% sodium citrate were added. The slide was then covered with a cover slip and examined under a microscope at 400x magnification.

Sperm Morphology

Sperm morphology was assessed using a light microscope at 400x magnification. Caudal sperm from the original motility dilution was further diluted 1:20 in 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). A total of 500 spermatozoa from the sample were examined for morphological abnormalities (Ateşşahin *et al.*, 2006). Spermatozoa exhibiting a rudimentary tail, round head, or detached head were classified as morphologically abnormal.

Statistical Analysis

Data are presented as mean ± SEM and analysed using SPSS version 20 (IBM, USA). One-way ANOVA followed by Tukey's post-hoc test for multiple comparisons was used to assess statistical significance, with a P-value < 0.05 considered statistically significant.

RESULTS AND DISCUSSION

Results

Acute Toxicity Assessment of Aqueous Leaf Extract of *Loranthus micranthus* Linn.

The aqueous extract of *Loranthusmicranthus* Linn leaf, administered at doses up to 5000 mg/kg body weight in male Wistarrats, caused no mortality or observable behavioural changes. Based on this, the extract is considered safe at this dosage level, and its LD_{50} is estimated to be greater than 5000 mg/kg body weight.

Preliminary Phytochemical Screening of Aqueous *Loranthus micranthus* Linn Leaf Extract

The screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and glycosides in the extract. These bioactive compounds are known for their diverse pharmacological properties, including antioxidant, anti-inflammatory, and protective effects, which could contribute to the therapeutic potential of the extract, recovery, while the high-dose extract and vitamin E restored body weight to near-control values.

Table 1: Body Weight Changes in Response to Cadmium Exposure and Treatment with Aqueous *Loranthus micranthus* Linn Leaf Extract and Vitamin E

Groups/Treatment	Body weight (g) Day 0	Body weight (g) Day 14
Control	190.20 ± 5.1^a	210.50 ± 6.2^a
Cadmium (Cd) 5 mg/kg	188.50 ± 4.8^a	165.30 ± 5.0^c
Loranthusmicranthus (250 mg/kg) + Cd (5 mg/kg)	189.00 ± 4.9^a	190.20 ± 5.3^b
Loranthusmicranthus (500 mg/kg) + Cd (5 mg/kg)	190.30 ± 5.2^a	205.60 ± 5.8^a
Vitamin E (100 mg/kg) + Cd (5 mg/kg)	190.50 ± 5.0^a	208.40 ± 6.1^a

Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

Table 1 shows the body weights (days 0 and 14) across the different experimental groups. cadmium exposure caused a significant ($p < 0.05$) reduction in body weight on a day when compared to the control group. Administration of the low-dose extract showed partial recovery, while the high-dose extract and vitamin E restored body weight to near-control values.

Table 2: Protective Effects of Aqueous *Loranthus micranthus* Linn Leaf Extractand Vitamin E Against Cadmium-Induced Changes in Testicular Weight

Groups/Treatment	Left testicular weight (g)	Right testicular weight (g)
Control	1.80 ± 0.05^{a}	1.82 ± 0.04^{a}
Cadmium (Cd) 5 mg/kg	1.25 ± 0.03^{c}	1.27 ± 0.05^{c}
Loranthusmicranthus (250 mg/kg) + Cd (5 mg/kg)	1.55 ± 0.04^{b}	1.58 ± 0.04^{b}
Loranthusmicranthus (500 mg/kg) + Cd (5 mg/kg)	1.77 ± 0.06^{a}	1.80 ± 0.03^{a}
Vitamin E (100 mg/kg) + Cd (5 mg/kg)	1.79 ± 0.05^{a}	1.83 ± 0.05^{a}

Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

In Table 2, cadmium significantly reduced left and right testicular weights compared to the control group ($p < 0.05$), indicating its toxic effects. Co-administration of the extract at both 250 mg/kg and 500 mg/kg improved testicular weights compared to the cadmium group. However, the high-dose extract (500 mg/kg) restored testicular weights to values comparable to the control group ($p > 0.05$). Vitamin E (100 mg/kg) also showed a protective effect, with testicular weights similar to the control group ($p > 0.05$).

Table 3: Protective Effects of Aqueous *Loranthus micranthus* Linn Leaf Extract and Vitamin E on Cadmium Chloride-Induced Hormonal Dysregulation in Male Rats

Groups/Treatment	Testosterone (ng/mL)	LH(mIU/mL)	FSH (mIU/mL)
Control	6.5 ± 0.4^{a}	3.2 ± 0.2^{a}	4.5 ± 0.3^{a}
Cadmium (Cd) 5 mg/kg	2.1 ± 0.3^{c}	1.0 ± 0.1^{c}	1.5 ± 0.2^{c}
Loranthusmicranthus (250 mg/kg) + Cd (5 mg/kg)	4.2 ± 0.5^{b}	2.1 ± 0.2^{b}	3.0 ± 0.4^{b}
Loranthusmicranthus (500 mg/kg) + Cd (5 mg/kg)	5.5 ± 0.6^{a}	3.0 ± 0.3^{a}	4.2 ± 0.3^{a}
Vitamin E (100 mg/kg) + Cd (5 mg/kg)	5.8 ± 0.5^{a}	3.2 ± 0.2^{a}	4.4 ± 0.4^{a}

Luteinizing hormone (LH), Follicle Stimulating Hormones (FSH). Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

The results in Table 3 indicate that cadmium ($CdCl_2$) exposure significantly ($p<0.05$) disrupts male reproductive hormone levels in Wistar rats, as evidenced by reduced testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels. Treatment with the extract (250 mg/kg and 500 mg/kg) and vitamin E (100 mg/kg) showed protective effects, restoring hormone levels toward control values. Testosterone, LH, and FSH levels in the treated groups significantly ($p<0.05$) improved compared to the cadmium-only group, with the higher dose of the extract and vitamin E achieving near-control levels. These findings highlight the potential of *Loranthus micranthus* and vitamin E to mitigate cadmium-induced endocrine dysfunction and reproductive toxicity.

Table 4: Protective Effects of Aqueous *Loranthus micranthus* Linn Leaf Extract and Vitamin E on Testicular Oxidative Stress Biomarkers in $CdCl_2$-Induced Toxicity

Groups/Treatment	MDA (µmole/mg testicular tissue)	GSH (U/mL)	SOD (µmole/g testicular tissue)	CAT (unit/g testicular tissue)	TAC (µmol/g testicular tissue)
Control	2.8 ± 0.3^{a}	5.9 ± 0.4^{a}	32.5 ± 1.8^{a}	60.5 ± 3.1^{a}	1.5 ± 0.1^{a}
Cadmium (Cd) 5 mg/kg	7.5 ± 0.6^{c}	2.2 ± 0.3^{c}	12.4 ± 1.2^{c}	22.1 ± 2.2^{c}	0.5 ± 0.1^{c}
Loranthusmicranthus (250 mg/kg) + Cd (5 mg/kg)	5.1 ± 0.4^{b}	4.1 ± 0.4^{b}	24.6 ± 1.5^{b}	44.8 ± 2.5^{b}	1.1 ± 0.2^{b}
Loranthusmicranthus (500 mg/kg) + Cd (5 mg/kg)	3.3 ± 0.4^{a}	5.5 ± 0.5^{a}	31.0 ± 2.0^{a}	58.4 ± 3.2^{a}	1.4 ± 0.2^{a}
Vitamin E (100 mg/kg) + Cd (5 mg/kg)	3.1 ± 0.3^{a}	5.6 ± 0.4^{a}	32.1 ± 1.9^{a}	59.1 ± 3.0^{a}	1.5 ± 0.1^{a}

Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Total Antioxidant Capacity (TAC). Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

The results in Table 4 demonstrate the protective effects of aqueous *Loranthusmicranthus* leaf extract and vitamin E against $CdCl_2$-induced oxidative stress and antioxidant depletion in testicular tissue. Cadmium exposure significantly ($p<0.05$) increased MDA levels and reduced GSH, SOD, CAT, and TAC levels when compared to the control, indicating oxidative damage and compromised antioxidant defence. Treatment with the extract (250 mg/kg and 500 mg/kg) and vitamin E (100 mg/kg) significantly ($p<0.05$) reduced MDA levels while restoring ($p<0.05$) GSH, SOD, CAT, and TAC levels compared to the cadmium treated group. The higher dose of the extract (500 mg/kg) and Vitamin E effectively restored these parameters to values comparable to the control group, highlighting their potent antioxidant and protective properties.

Table 5: Protective Effects of Aqueous *Loranthus micranthus* Linn Leaf Extract and Vitamin E on Cadmium Chloride-Induced Sperm Alterations in Male Rats

Groups/Treatment	Sperm Count (million/mL)	Sperm Motility (%)	Normal Sperm Morphology (%)
Control	72.5 ± 4.2^{a}	85.2 ± 3.2^{a}	90.3 ± 3.0^{a}
Cadmium (Cd) 5 mg/kg	25.3 ± 3.5^{c}	40.5 ± 4.1^{c}	45.1 ± 4.0^{c}
Loranthusmicranthus (250 mg/kg) + Cd (5 mg/kg)	50.2 ± 4.0^{b}	65.3 ± 3.8^{b}	70.4 ± 3.5^{b}
Loranthusmicranthus (500 mg/kg) + Cd (5 mg/kg)	69.8 ± 4.5^{a}	82.0 ± 4.2^{a}	88.2 ± 3.2^{a}
Vitamin E (100 mg/kg) + Cd (5 mg/kg)	71.0 ± 4.0^{a}	83.5 ± 3.5^{a}	89.5 ± 3.0^{a}

Values are represented as mean ± SEM (n=6). Means with different superscripts are significantly ($P < 0.05$) different while those with the same superscripts are not significantly different.

The results in Table 5 demonstrate the protective effects of aqueous *Loranthus micranthus* leaf extract and vitamin E against cadmium chloride ($CdCl_2$)-induced reproductive toxicity in male rats. Cadmium exposure significantly ($p<0.05$) impaired reproductive parameters, as evidenced by reductions in sperm count, motility, and normal morphology when compared to the control group. Co-treatment with the extract at both low and high doses (250 mg/kg and 500 mg/kg) mitigated these effects, with the high dose (500 mg/kg) restoring parameters to near-control levels. Similarly, vitamin E provided significant protection, yielding results comparable to

the control group. These findings indicate that *Loranthus micranthus*, particularly at higher doses, and vitamin E effectively counteract cadmium-induced oxidative damage, highlighting their potential as therapeutic agents for reproductive toxicity.

Discussion

This study investigated the detrimental effects of $CdCl_2$ on the male reproductive system, emphasizing its role in inducing oxidative stress, disrupting hormonal balance, and causing reductions in body weight, testicular weight, sperm count, motility, and morphology. Additionally, the study highlighted the protective effects of the aqueous extract of *Loranthusmicranthus* Linn leaf and vitamin E against these adverse outcomes.

The findings of this study indicate that the LD_{50} of the aqueous extract of *Loranthusmicranthus* Linn leaf exceeds 5000 mg/kg, suggesting that the extract exhibits low acute toxicity. The LD_{50} value represents the dose required to cause 50% mortality in a test population and serves as a critical index for classifying the toxicity of substances. According to toxicological guidelines established by the Organization for Economic Co-operation and Development (OECD, 2003), a value greater than 5000 mg/kg categorizes the substance as non-toxic or slightly toxic. This implies that the extract is relatively safe for therapeutic applications when administered at high doses without causing immediate or severe adverse effects. However, the absence of acute toxicity does not preclude the need for further research to evaluate chronic toxicity, bioaccumulation potential, and long-term effects. Additionally, studies are required to characterize the extract's pharmacological activities and elucidate its mechanisms of action at different dosages.

Phytochemical screening of the *Loranthusmicranthus* aqueous leaf extract revealed the presence of bioactive compounds, such as alkaloids, flavonoids, saponins, tannins, and glycosides (Ebhohon *et al*., 2023). These compounds are recognized for their antioxidant, anti-inflammatory, and protective properties, suggesting their potential role in mitigating cadmium-induced testicular injury. By neutralizing reactive oxygen species (ROS), these bioactive molecules may reduce oxidative damage, mitigate inflammation, and support tissue repair. Specifically, alkaloids and glycosides are known to stabilize cell membranes, promote detoxification, and facilitate regenerative processes (Macáková *et al*., 2019; Khan *et al*., 2022), positioning *Loranthusmicranthus* as a promising candidate for therapeutic interventions against cadmium-induced reproductive toxicity.These findings align with earlier research as reported by Onoja *et al*. in 2017 and Ebhohon *et al.* (2023).

The significant weight loss observed in the cadmium-exposed group aligns with previous studies, which suggest that cadmium induces oxidative damage, disrupts hormonal regulation, and causes metabolic imbalances, leading to weight reduction (Nna *et al*., 2017; Abdel-Wahab *et al*., 2021; Onoja *et al*., 2021; Elish *et al*., 2022). However, treatment with *Loranthusmicranthus* and vitamin E showed recovery, indicating their protective effects likely due to their antioxidant properties, which counteract the oxidative stress caused by cadmium (Ebhohon *et al.*, 2023). Notably, the high-dose *Loranthusmicranthus* extract (500 mg/kg) offered protection similar to Vitamin E, highlighting its potential as a natural therapeutic agent against cadmium-induced toxicity. The findings of this study align with those reported by Alkhedaide *et al*. (2016), Abdel-Wahab *et al.* (2021), Onoja *et al*. (2021), and Elish *et al*. (2022). These results emphasize the importance of antioxidants in mitigating the detrimental effects of heavy metals on body weight and overall health (Marini *et al*., 2022). In addition, the significant reduction in testicular weight in the cadmium-only group suggests testicular toxicity, likely resulting from oxidative damage, hormonal imbalances, and apoptosis in testicular cells (Abdel-Wahab *et al*., 2021). The restoration of testicular weight observed in the *Loranthusmicranthus* and vitamin E-treated groups further supports their protective role, with the high-dose treatments showing the most significant improvements. These findings suggest that both *Loranthusmicranthus* and vitamin E are effective in mitigating cadmium-induced testicular toxicity, underscoring the role of antioxidants in combating oxidative stress and preserving reproductive health (Kaltsas, 2023).

Cadmium is a well-documented reproductive toxicant that induces oxidative stress in testicular cells, disrupting the hypothalamic-pituitary-gonadal (HPG) axis (Lafuente, 2013) and Leydig cell function (Wu *et al*., 2017), leading to decreased testosterone synthesis. This disruption stems from oxidative damage to the testes and impaired gonadotropin secretion due to cadmium-induced injury to the hypothalamus or pituitary gland (Ali *et al*., 2022). In this study, cadmium administration significantly reduced levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, reflecting dysfunction within the HPG axis.The results of this study are consistent with those documented by Alkhedaide *et al*. (2016), Nna *et al*., (2017), Abdel-Wahab *et al*. (2021), Onoja *et al*. (2021), and Elish *et al*. (2022).

Co-administration of the varying doses of the extract (250 mg/kg and 500 mg/kg) with cadmium significantly improved serum levels of testosterone, LH, and FSH, with the higher dose nearly restoring these parameters to control levels. The outcomes of this study are in agreement with the findings reported by Alkhedaide *et al*. (2016), Nna *et al*. (2017), Abdel-Wahab *et al*. (2021), Onoja*et al*. (2021), and Elish *et*

al. (2022).These effects are attributed to the antioxidant properties of the phytochemicals in the extract, particularly flavonoids and alkaloids, which likely reduced oxidative stress, restored mitochondrial function, and enhancedsteroidogenic enzyme activity (Ebhohon *et al*., 2023). Additionally, the extract may protect the hypothalamus and pituitary gland from oxidative damage, thereby maintaining gonadotropin secretion. Similarly, in this study, vitamin E (100 mg/kg) demonstrated comparable efficacy in restoring hormone levels. As a potent antioxidant, vitamin E scavenges free radicals, prevents lipid peroxidation, and preserves the integrity of Leydig cells, supporting steroidogenesis and normal reproductive function (Chen *et al*., 2005; Soleimani Mehranjani & Taefi, 2012).The combined results suggest that *Loranthusmicranthus* and vitamin E mitigate cadmium-induced reproductive toxicity by countering oxidative stress, preserving testicular structure and function, and restoring hormonal balance.

Cadmium exposure significantly increased malondialdehyde (MDA) levels in testicular tissue, indicating heightened lipid peroxidation and oxidative stress (Haouem & El Hani, 2013). Treatment with *Loranthusmicranthus* (250 mg/kg and 500 mg/kg) and vitamin E (100 mg/kg) significantly reduced MDA levels, with the higher dose of *Loranthusmicranthus* restoring MDA to near-control levels. This reduction highlights the potent antioxidant properties of the extract, which neutralizes ROS and protects cellular membranes from oxidative damage. Additionally, cadmium exposure depleted glutathione (GSH), a critical antioxidant involved in redox homeostasis (Lushchak, 2012). Both *Loranthusmicranthus* and vitamin E treatments restored GSH levels, with the higher dose of *Loranthusmicranthus* showing exceptional efficacy, reinforcing its role in enhancing endogenous antioxidant defences.Thefindings of this study align with those reported by Alkhedaide *et al*. (2016), Nna *et al*. (2017), Abdel-Wahab *et al*. (2021), Onoja *et al*. (2021), and Elish *et al*. (2022).

The activities of key antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), were also assessed. Cadmium significantly reduced SOD and CAT activities, indicating impaired detoxification of superoxide radicals and hydrogen peroxide. Treatment with *Loranthusmicranthus* and vitamin E significantly restored these enzyme activities, with the higher dose of *Loranthusmicranthus* achieving near-complete restoration to control values. Enhanced activities of SOD and CAT underscore the ability of these treatments to bolster the antioxidant defence system and mitigate oxidative damage. The results of this study are consistent with those documented by Alkhedaide *et al*. (2016), Nna *et al*. (2017), Abdel-Wahab *et al*. (2021), Onoja *et al*. (2021), and Elish *et al*. (2022).

Total antioxidant capacity (TAC), a comprehensive measure of antioxidant potential (Silvestrini *et al*., 2023), was significantly reduced by cadmium exposure. Both *Loranthusmicranthus* and vitamin E significantly improved TAC, suggesting that their protective effects involve the upregulation of antioxidant enzymes and the replenishment of non-enzymatic antioxidants like GSH. These findings establish *Loranthusmicranthus* and vitamin E as potent agents against cadmium-induced oxidative stress and reproductive damage.The findings of this study are in line with those reported by Alkhedaide *et al*. (2016), Abdel-Wahab *et al*. (2021), and Elish *et al*. (2022).

Cadmium exposure significantly impairs male fertility by reducing epididymal sperm count, motility, and morphology through mechanisms involving oxidative stress, testicular inflammation, mitochondrial dysfunction, and cellular damage. These disruptions compromise spermatogenesis and overall sperm quality (Dutta *et al*., 2021). However, *Loranthusmicranthus* leaf extract, particularly at higher doses, effectively mitigates these effects, restoring sperm parameters to near-normal levels. The extract's protective efficacy is comparable to that of vitamin E, a well-known antioxidant, highlighting its potential as a natural therapeutic alternative for managing cadmium-induced reproductive toxicity. The results of this study are in agreement with the findings of Nna *et al*. (2017), Abdel-Wahab *et al*. (2021), and Elish *et al*. (2022). The phytochemical composition of *Loranthusmicranthus*, rich in flavonoidsandother antioxidants, likely underpins its protective effects by neutralizing reactive oxygen species (ROS), enhancing endogenous antioxidant enzyme activities (e.g., superoxide dismutase and catalase), and preventing oxidative damage and apoptosis in testicular cells (Ebhohon *et al*., 2023). These findings position *Loranthusmicranthus* as a promising natural remedy for preventing or reversing cadmium-induced testicular injury and male infertility, offering a plant-based alternative to synthetic antioxidants.

CONCLUSION

The present study demonstrates the protective effects of *Loranthusmicranthus* and vitamin E against cadmium-induced oxidative stress and testicular damage. Their efficacy in restoring antioxidant defences, mitigating lipid peroxidation, and improving reproductive parameters positions them as promising therapeutic agents. Future research should explore their molecular mechanisms, long-term safety, and efficacy in chronic toxicity models to optimize their use in managing reproductive toxicity.

Conflict of Interest: The authors affirm that there are no conflicts of interest related to the publication of this manuscript.

Funding: This research did not receive any funding.

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