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Protective Effect of Methanol Extract of Oxymitra Longipedicellata Leaf Against Cadmium Chloride-Induced Testicotoxicity in Male Rats

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Abstract- ***The aim and objective of this study is to evaluate the protective effect of methanol leaf extract of Oxymitra longipedicellata against Cadmium chloride-induced testiculotoxicity in male rats. Acute toxicity (LD_{50}) assessment revealed that the extract is relatively safe at lower doses, with toxic effects observed at higher concentrations (3000–5000 mg/kg). Forty-nine male Wistar rats were divided into seven groups and treated with cadmium chloride (2.5 mg/kg), extract (200 and 400 mg/kg), and quercetin (50 mg/kg) for 30 days. Cadmium exposure significantly ($P < 0.05$) reduced reproductive hormones (testosterone, FSH, LH), impaired sperm parameters, induced dyslipidemia, elevated liver enzymes, and disrupted antioxidant status, evidenced by decreased SOD, GST, and GSH levels alongside increased malondialdehyde (MDA). However, treatment with the extract significantly ameliorated these alterations in a dose-dependent manner. Notably, the 400 mg/kg dose restored hormonal balance, improved sperm quality, normalized lipid profile, reduced hepatotoxic markers, and enhanced antioxidant defense systems. The extract's effects were comparable to or slightly better than quercetin in some parameters. These findings suggest that Oxymitra longipedicellata methanol leaf extract exerts significant protective effects against cadmium-induced reproductive and oxidative damage, likely via its antioxidant and free radical scavenging properties. The study highlights its potential as a natural therapeutic agent for managing heavy metal-induced testiculotoxicity.***

Keywords: Cadmium toxicity; Oxymitra longipedicellata; Testiculotoxicity; Antioxidant; Reproductive hormones; Oxidative stress; Male fertility.

I. INTRODUCTION

The testes are highly vulnerable to cadmium toxicity due to their high metabolic activity and complex cellular organization. Experimental studies have shown that cadmium exposure results in severe structural and functional damage to testicular tissue, including degeneration of seminiferous tubules, disruption of the blood–testis barrier, and impairment of spermatogenesis (Iqbal et al., 2021; De Souza Predes et al., 2010). These pathological changes are often accompanied by reduced sperm count, motility, and viability, as well as decreased levels of reproductive hormones such as testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), ultimately leading to infertility (Nna et al., 2017).

Cadmium is a ubiquitous environmental pollutant with well-documented toxic effects on multiple organ systems, particularly the male reproductive system. Human exposure occurs through contaminated food, water, cigarette smoke, and occupational settings, leading to its accumulation in biological tissues due to its long biological half-life (Nna et al., 2017). Among its deleterious effects, cadmium chloride is known to induce testicular damage, characterized by impaired spermatogenesis, degeneration of seminiferous tubules, hormonal imbalance, and reduced fertility. The mechanism underlying cadmium-induced testiculotoxicity is largely attributed to oxidative stress, inflammation, and disruption of antioxidant defense systems, which collectively compromise cellular integrity and function in testicular tissues (Ali et al., 2022).

Medicinal plants are rich sources of bioactive phytochemicals, including flavonoids, phenolics, and alkaloids, which possess potent antioxidant, anti-inflammatory, and metal-chelating properties and also getting growing interest in their use for medicinal purpose in recent years. Methanol extraction has been widely employed to isolate these

compounds due to its high efficiency in extracting a broad spectrum of phytoconstituents (Nava-Solis et al., 2022). Previous studies have demonstrated that plant extracts such as Fragaria ananassa, Cyperus esculentus, and Pericopsis laxiflora can significantly ameliorate cadmium-induced testicular damage through antioxidant mechanisms (Nwangwa et al., 2025; Riberio et al., 2023; Elmallah et al., 2017). Similarly, quercetin, a well-known natural antioxidant, has shown strong protective effects against cadmium-induced reproductive toxicity (Ribeiro et al., 2023).

Oxymitra longipedicellata, a medicinal plant commonly found in tropical regions, has gained attention for its potential therapeutic properties. Traditionally used in herbal medicine, this plant is reported to possess various pharmacological activities, including antioxidant, anti-inflammatory, and cytoprotective effects. Oxymitra longipedicellata is a plant with emerging ethnopharmacological relevance, though its biological activities remain largely underexplored (Taiwo et al., 2013). Given the established antioxidant potential of plant-derived methanolic extracts, it is plausible that Oxymitra longipedicellata may possess bioactive compounds capable of mitigating oxidative stress and protecting against toxic insults such as cadmium exposure.

Therefore, this study was designed to investigate the protective effect of methanol leaf extract of Oxymitra longipedicellata against cadmium chloride-induced testiculotoxicity in male rats. The study focuses on evaluating reproductive hormones, sperm characteristics, lipid profile, liver function indices, and antioxidant defense parameters to elucidate the possible mechanisms underlying its protective action.

II. MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents employed in this study were of scientific grade (Analar) and were purchased from Seglor Nigeria limited. Randox kits such as Alanine amino transferase (ALT), Aspertate amino transferase (AST), Total cholesterol, Triglycerides, and so on, were from RANDOX registered agents in Lagos. Spectrophotometer (752N UV-visible) was employed for all spectroscopic experiments in this study.

Collecting plant material and preparing plant extracts

Fresh leaves of Oxymitra longipedicellata were collected from the botanical garden Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State. The leaves were air-dried, size reduced and extracted using cold maceration by soaking 5000mg of the dried leaves in two litres (2L) of 81% methanol for 72 hours at 770F. The mixture was filtered using No 1 wattman filter paper and filtrate was concentrated in a rotary evaporator then, dried to a constant weight.

Animals

All male Sprague-Dawley rats used in this study were aged four to five weeks and weighing between 160 and 200 grams and were procured from the Animal House of the Physiology Department at the University of Ibadan, Nigeria. The subjects were housed in well ventilated enclosures within the Departmental Animal House, maintained at a temperature range of 28 to 30 degrees Celsius, and subjected to regulated light cycles (12-hour light: dark). They received a diet of standard laboratory chow (Ladokun Feeds, Ibadan, Nigeria) along with water. All experimental procedures were conducted without the administration of anaesthesia, and the protocol adhered to the guidelines established by the National Institutes of Health (NIH).

Ethical approval:

Ethical approval was obtained from Ogun State Institute of Technology, Igbesa Ogun State. OGITECH institutional committee on the use and care of laboratory animals.

Determination of median lethal dose (LD50):

The LD50 test of the crude extract of methanol of Oxymitra longipedicellata leaf was determined, according to the method of Jaya et al. (2013), using 30 male wistar rats weighing 130g to 160g following the modified method of Jaya et al. (2013). The animals were randomly distributed into 5 treatment groups and a control group with each group containing five (5) animals. The control group given 0.3ml of corn oil while the treatment groups took a single oral dose of methanol extract of Oxymitra

longipedicellata (MLOL) at concentration of 200, 500, 1500, 3000 and 5000 mg/kg body weight respectively. The animals were closely observed in the first 4 hours and then hourly for the next 12 hours followed by 6 hourly interval for the next 2 weeks after the extract administration to observe for any death or display of any abnormal physiological, behavioral or neurological signs (Kwon et al., 2008). The median lethal dose (LD 50), therapeutic dose (TD50) was estimated for each group.

Induction of testicular damage in wistar rats

In this study, Cadmium chloride was used as toxicant which selectively damage the testes through possible mechanism of free radical generation within the testicular cells and consequently oxidative stress according the modified method of Oyedeji et al. (2025). Rats were intraperitoneally administered with cadmium chloride (CdCl2) (2.5mg/kg) dissolved in distilled water and were observed for the first 12hrs for any death or display of any abnormal physiological, behavioral or neurological signs.

Experimental Design and Administration of Extract

Forty-nine (49) male rats (Wistar strain) were randomly allotted into seven (7) groups of seven (7) animals each and allowed free access to feed and water for a period of a seven-day acclimatization period before the commencement of the experiment. The schedule of the extract and drug treatment is depicted in the table below

Grouping	Group name	Treatment
1	Positive Control	Received olive oil (per oral)
2	Negative Control ($CdCl_2$)	Received 2.5mg/kg of cadmium chloride ($CdCl_2$) by i.p
3	$CdCl_2$ + $MLOL_{200}$	Received 2.5mg/kg of $CdCl_2$ by i.p and 200mg/kg body weight of *Oxymitra longipedicellata* leaf's methanol extract (MLOL) orally
4	$CdCl_2$ + $MLOL_{400}$	Received 2.5mg/kg of $CdCl_2$ by i.p and 400mg/kg body weight of *Oxymitra longipedicellata* leaf's methanol extract (MLOL) orally
5	$CdCl_2$ + QUER	Received 2.5mg/kg of $CdCl_2$ by i.p and quercetin at 50mg/kg body weight orally
6	$MLOL_{400}$	Received 400mg/kg body weight of *Oxymitra longipedicellata* leaf's methanol extract (MLOL) orally
7	QUER	Received quercetin at 50mg/kg body weight orally

The animals were pre-treated with MELOL and rutin for thirty (30) days and CdCl2 will be administered to the animals in last 3 days. The route of administration of cadmium chloride was intraperitoneal (i.p) while the extract will be administered orally. The vehicle to be used will be olive oil.

Assay methods

Testicular and hepatic antioxidant assays which include glutathione-S transferase (GST) activity, Superoxide dismutase (SOD), reduced glutathione (GSH) level, nitric oxide level, glutathione peroxidase (GPx), malonaldehyde (lipid peroxidation and myeloperoxidase activity were all carried out following the method of Sundaram et al. (2021); Tijani et al. (2021); El-Demerdash et al. (2021). Furthermore, serum testosterone level, luteinizing hormone, follicle stimulating hormone and sperm analysis (sperm motility, count, sperm volume and sperm live-to-death ratio were analyzed in accordance to the method of Oyedeji et al. (2021); De Souza et al. (2010). Serum triglycerides (TG) level, Total cholesterol (TC) level, low- and high-density lipoprotein (HDL) were estimated by using RANDOX kits according to the method described by Nwangwa et al. (2025).

Statistical analysis

All values were expressed as the mean ± SD. Data were analysed using one-way ANOVA followed by the post hoc Duncan multiple range test for analysis of biochemical data using SPSS (10.0; SPSS Inc., Chicago, IL, USA) at a 0.95 confidence level.

III. RESULTS

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Table 1: Day 1 and 2 of Physical and Behavioral monitoring of rats on Lethal dose determination of methanol extract of Oxymitra longipedicellata leaf

S/N	Observation	DAY 1										DAY 2									
		Morning					Evening					Morning					Evening				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1.	Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2.	Hair	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3.	Mucus Membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4.	Skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5.	Nassal irritation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6.	Respiratory rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7.	Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	A	A
8.	Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9.	Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10.	Coma	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11.	Diarrhea	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	Y	Y
12.	Mortality	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	Total No of Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

N- Nil/ Normal; A- Abnormal; Y- Yes; 1- MLOL200; 2- MLOL500; 3- MLOL1500; 4- MLOL3000; 5- MLOL5000; MLOL- methanol extract of Oxymitra longipedicellata leaf

Table 2: Day 3 and 4 of Physical and Behavioral monitoring of rats on Lethal dose determination of methanol extract of Oxymitra longipedicellata leaf

S/N	Observations	DAY 3										DAY 4									
		Morning					Evening					Morning					Evening				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1.	Eyes	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2.	Hair	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3.	Mucus Membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4.	Skin	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5.	Nassal irritation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6.	Respiratory rate	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7.	Urination	N	N	N	A	A	N	N	N	A	A	N	N	N	A	A	N	N	N	A	A
8.	Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9.	Sleep	N	N	N	N	A	N	N	N	N	A	N	N	N	N	A	N	N	N	N	A
10.	Coma	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

11.	Diarrhea	N	N	N	N	Y	N	N	N	Y	Y		N	N	N	Y	Y	N	N	N	Y	Y
12.	Mortality/ Death	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	Y	Y
13	Total No of Death	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	1

N- Nil/ Normal; A- Abnormal; Y- Yes; 1- MLOL200; 2- MLOL500; 3- MLOL1500; 4- MLOL3000; 5- MLOL5000; MLOL- methanol extract of Oxymitra longipedicellata leaf

Table 3: Day 5 and 6 of Physical and Behavioral monitoring of rats on Lethal dose determination of methanol extract of Oxymitra longipedicellata leaf

S/N	Observations	DAY 5											DAY 6									
		Morning					Evening						Morning					Evening				
		1	2	3	4	5	1	2	3	4	5		1	2	3	4	5	1	2	3	4	5
1.	Eyes	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
2.	Hair	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
3.	Mucus Membrane	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
4.	Skin	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
5.	Nassal irritation	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
6.	Respiratory rate	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
7.	Urination	N	N	N	A	A	N	N	N	A	A		N	N	N	A	A	N	N	N	A	A
8.	Salivation	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
9.	Sleep	N	N	N	N	A	N	N	N	N	A		N	N	N	N	A	N	N	N	N	A
10.	Coma	N	N	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N	N	N	N
11.	Diarrhea	N	N	N	Y	Y	N	N	N	Y	Y		N	N	N	Y	Y	N	N	N	Y	Y
12.	Mortality/ Death	N	N	N	Y	Y	N	N	N	N	Y		N	N	N	N	N	N	N	N	Y	Y
13	Total No of Death	0	0	0	1	1	0	0	0	1	2		0	0	0	1	2	0	0	0	1	3

N- Nil/ Normal; A- Abnormal; Y- Yes; 1- MLOL200; 2- MLOL500; 3- MLOL1500; 4- MLOL3000; 5- MLOL5000; MLOL- methanol extract of Oxymitra longipedicellata leaf

Table 4: Impact of methanol extract of Oxymitra longipedicellata leaf on the serum level of Testosterone, Follicle Stimulating hormone (FSH), and Luteinizing hormone(LH) lipid profile of wistar rats intoxicated with cadmium chloride

Treatments	Testosterone (U/L)	FSH (U/L)	LH (U/L)
Control	32.51±3.15	22.30±1.05	38.27±2.43
$CdCl_2$	11.48±0.96[a]	9.45±0.55 [a]	21.60±1.52[a]
$CdCl_2$ + $MLOL_{200}$	24.76±1.65[b]	17.43±0.75 [b]	31.46±0.28[b]
$CdCl_2$ + $MLOL_{400}$	29.30±2.06 [b]	16.35±1.00 [b]	33.25±1.25 [b]
$CdCl_2$ + QUER	26.45±1.74 [b]	18.85±2.22 [b]	30.55±1.57 [b]
$MLOL_{400}$	31.46±1.30	16.90±1.57	41.16±2.15
QUER	26.60±2.05	24.35±2.11	35.54±1.95

MLOL- Methanol extract of Oxymitra longipedicellata leaf, CdCl2 - Cadmium Chloride, QUER- Quercetin, a - Significantly different from the control at P< 0.05, b - Significantly different from the CdCl2 group at P< 0.05

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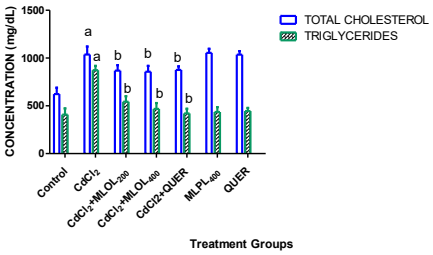
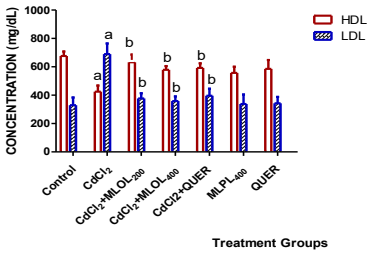


MLOL- Methanol extract of Oxymitra longipedicellata leaf, $CdCl_2$ - Cadmium Chloride,QUER- Quercetin, [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 1: Impact of methanol extract of Oxymitra longipedicellata leaf on the serum concentration of total cholesterol and triglycerides of wistar rats intoxicated with cadmium chloride



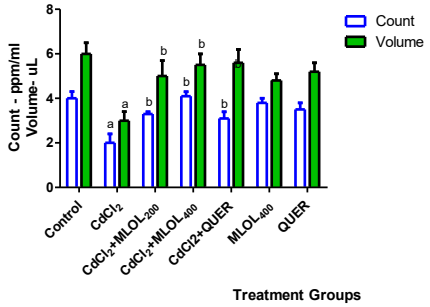
MLOL- Methanol extract of Oxymitra longipedicellata leaf, $CdCl_2$ - Cadmium Chloride, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, QUER- Quercetin, [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 2: Impact of methanol extract of Oxymitra longipedicellata leaf on the serum concentration of HDL and LDL of wistar rats intoxicated with cadmium chloride

Table 5 Impact of methanol extract of Oxymitra longipedicellata leaf on the serum activities of AST, ALT, GGT and Bilirubin in wistar rats intoxicated with cadmium chloride

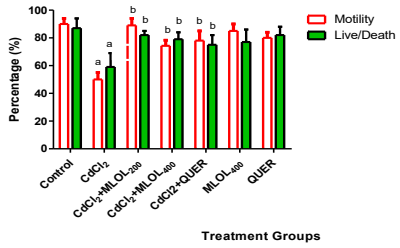
Treatments	AST	ALT	GGT	BILIRUBIN
Control	132.46±12.13	206.43±16.76	401.78±22.47	72.65±5.57
$CdCl_2$	235.76±10.84[a]	364.82±12.26 [a]	686.57±25.42 [a]	125.43±7.29 [a]
$CdCl_2$ + $MLOL_{200}$	142.35±12.43[b]	215.54±14.50 [b]	471.87±35.17 [b]	83.75±8.23 [b]
$CdCl_2$ + $MLOL_{400}$	158.71±15.10 [b]	246.38±10.28 [b]	426.27±31.67 [b]	75.12±8.85 [b]
$CdCl_2$ + QUER	147.92±9.82 [b]	222.8±16.11 [b]	497.36±29.26 [b]	83.96±8.47 [b]
$MLOL_{400}$	126.16±9.10	218.39±5.23	438.18±27.42	93.29±10.34
QUER	119.63±11.41	246.82±13.85	448.48±30.38	98.23±7.94

MLOL- methanol extract of Oxymitra longipedicellata leaf, QUER- Quercetin, CdCl2 - Cadmium Chloride, ALT-Alaninie Amino Transferases, AST- Aspartate Amino Transferase, GGT- Gamma Glutamyl Transferase, a - Significantly different from the control at P< 0.05, b - Significantly different from the CdCl2 group at P< 0.05



MLOL- Methanol extract of Oxymitra longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 3: Effect of methanol extract of Oxymitra longipedicellata leaf on the Sperm count and volume Ratio in wistar rats intoxicated with cadmium chloride



MLOL- Methanol extract of Oxymitra longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 4 Effect of methanol extract of Oxymitra longipedicellata leaf on the Sperm cells' motility and Live/Death Ratio in wistar rats intoxicated with cadmium chloride

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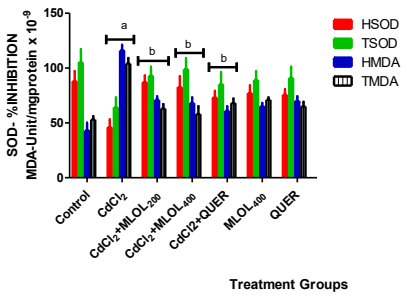
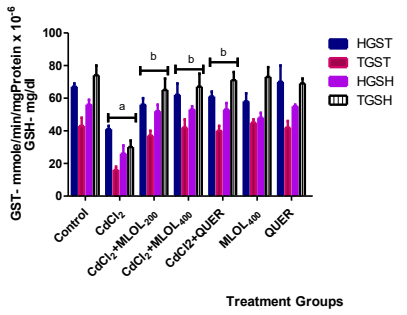


MLOL- Methanol extract of Oxymitra longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride, HSOD -Hepatic Superoxide Dismutase, TSOD -Testicular Superoxide Dismutase, HMDA- Hepatic Maalonaldehyde, TMDA- Testicular Maalonaldehyde [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 5: Effect of methanol extract of Oxymitra longipedicellata leaf on the hepatic and testicucular activity of Superoxide dismutase and level of Malonaldehyde in wistar rats intoxicated with cadmium chloride



MLOL- Methanol extract of Oxymitra longipedicellata leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride, HGST- Hepatic Glutathion-S-Transferase, TGST- Testicular Glutathion-S-Transferase, HGSH- Hepatic Reduced Glutathione, TGSH- Testicular Reduced Glutathione, [a] - Significantly different from the control at P< 0.05, [b] - Significantly different from the $CdCl_2$ group at P< 0.05

Figure 6: Effect of methanol extract of Oxymitra longipedicellata leaf on the Hepatic and testicucular activity of Glutathion-S-Transferase and level of Reduced Glutathione in wistar rats intoxicated with cadmium chloride

IV. DISCUSSION

The findings from this acute toxicity study (LD50) demonstrate that the methanol extract of Oxymitra longipedicellata leaf (MLOL) exhibits a clear dose- and time-dependent toxicological profile in Wistar rats (Table 1-3). During the initial 48 hours (Days 1 and 2), all treated groups, including those receiving the highest doses (3000 and 5000 mg/kg), showed no mortality and no significant alterations in physiological or behavioral parameters, indicating an absence of immediate acute toxicity. However, from Day 2 onward, mild abnormalities such as altered urination and the onset of diarrhea began to emerge, particularly at higher doses. By Days 3 and 4 (Table 2), these effects became more evident, with consistent occurrences of abnormal urination, diarrhea, and slight disturbances in sleep among rats administered 3000 and 5000 mg/kg, alongside the first recorded deaths at the highest dose. This toxic effect progressed further on Days 5 and 6 (Table 3), with increased mortality observed in both 3000 and 5000 mg/kg groups, accompanied by persistent diarrhea and behavioral changes, whereas the lower dose groups (200–1500 mg/kg) remained largely normal throughout the observation period. Notably, there were no significant changes in critical parameters such as respiratory rate, salivation, coma, or mucous membrane condition across all groups, suggesting that the extract's toxicity may be more localized or specific rather than causing immediate systemic failure. Overall, these results indicate that MLOL is relatively safe at lower doses but produces significant toxic effects at higher concentrations, with the median lethal dose (LD_{50}) likely situated between 3000 and 5000 mg/kg, reflecting a moderate safety margin for the extract.

Cadmium chloride ($CdCl_2$) is widely used as an experimental model for studying male reproductive toxicity due to its potent accumulation in testicular tissue and long biological half-life. Evidence shows that cadmium exposure induces severe structural and functional damage to the testes, particularly targeting Sertoli cells, Leydig cells, and the blood–testis barrier (BTB). Disruption of these components leads to impaired spermatogenesis, reduced testosterone synthesis, and consequent infertility (Oyedeji et al., 2025). Mechanistically, cadmium chloride–induced testiculotoxicity is strongly associated with oxidative stress, characterized by increased lipid peroxidation and depletion of endogenous antioxidant systems such as superoxide dismutase, catalase, and glutathione (Ali et al., 2022). This oxidative imbalance promotes cellular damage, vascular disruption, and degeneration of seminiferous tubules, ultimately resulting in decreased sperm count, motility, and viability (Oyedeji et al., 2025; Iqbal et al., 2021).

Furthermore, cadmium chloride triggers apoptotic and molecular signaling pathways that exacerbate

testicular injury. Studies demonstrate that $CdCl_2$ exposure upregulates pro-apoptotic proteins (e.g., Bax) while downregulating anti-apoptotic proteins (e.g., Bcl-2), leading to activation of caspases and germ cell apoptosis. Additional mechanisms include endocrine disruption via suppression of steroidogenic enzymes and testosterone production, as well as interference with calcium signaling and nitric oxide pathways that are essential for spermatogenesis (Oyedeji et al., 2025; Nna et al., 2017). Cadmium also alters gene expression and induces epigenetic modifications, further impairing germ cell development and fertility potential (Iqbal et al., 2021, Elmallah et al., 2017). Collectively, these findings establish cadmium chloride as a potent testiculotoxic agent acting through oxidative stress, apoptosis, hormonal imbalance, and structural disruption of testicular architecture

The results presented in Table 4 indicate that administration of cadmium chloride ($CdCl_2$) caused a significant ($P < 0.05$) reduction in serum levels of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) when compared to the control group, confirming its deleterious effect on the reproductive endocrine system. However, treatment with the methanol extract of Oxymitra longipedicellata leaf (MLOL) at both 200 and 400 mg/kg significantly ($P < 0.05$) ameliorated these reductions, as evidenced by the marked increase in hormone levels relative to the $CdCl_2$-only group. Notably, the higher dose (400 mg/kg) showed a more pronounced restorative effect, bringing testosterone and LH levels closer to normal control values, suggesting a dose-dependent protective activity. Similarly, the standard antioxidant, quercetin, also significantly improved hormone levels, though the effects of MLOL, particularly at 400 mg/kg, were comparable or slightly superior in some parameters.

The result presented in Figure 1 and 2 shows the effect of cadmium chloride ($CdCl_2$) intoxication and treatment with methanol extract of Oxymitra longipedicellata leaf (MLOL) and quercetin on serum lipid profile, specifically total cholesterol and triglycerides, in Wistar rats. Exposure to $CdCl_2$ caused a marked and significant increase (a, $P < 0.05$) in both total cholesterol (fig. 1), triglyceride (fig. 1) and low density lipoprotein (LDL) levels (fig. 2) and reduction in high density lipoprotein (HDL) level (fig. 2) compared to the control group. This elevation indicates that cadmium toxicity disrupts lipid metabolism, leading to hyperlipidemia. Such alterations may be attributed to oxidative stress–induced damage to hepatic tissues, which impairs lipid regulation, enhances lipid synthesis, and/or reduces lipid clearance. The rise in triglycerides further suggests increased mobilization of fatty acids and possible impairment in lipoprotein lipase activity. However, co-administration of MLOL (200 and 400 mg/kg) significantly reduced (b, $P < 0.05$) total cholesterol, triglyceride and LDL levels and elevated level of HDL when compared to the $CdCl_2$ group. The reduction was more pronounced at the higher dose (400 mg/kg), indicating a dose-dependent hypolipidemic effect of the extract. Similarly, quercetin treatment also significantly lowered lipid levels, demonstrating comparable efficacy.

The results from Figures 3 and 4 demonstrate that cadmium chloride ($CdCl_2$) exposure significantly ($P < 0.05$) impaired sperm quality in Wistar rats, as evidenced by marked reductions in sperm motility, live/dead ratio, sperm count, and semen volume compared to the control group. This confirms the well-established reproductive toxicity of cadmium, likely mediated through oxidative stress and testicular damage. However, co-administration of the methanol extract of Oxymitra longipedicellata leaf (MLOL) at both 200 and 400 mg/kg significantly ($P < 0.05$) improved all sperm parameters relative to the $CdCl_2$-only group, indicating a protective and restorative effect. Notably, the higher dose (400 mg/kg) exhibited a more pronounced improvement, with values approaching those of the control group, suggesting a dose-dependent response. Similarly, treatment with quercetin (QUER), a known antioxidant, also significantly ameliorated cadmium-induced damage, although the effects of MLOL, particularly at 400 mg/kg, were comparable or slightly superior in some parameters. Furthermore, administration of MLOL or QUER alone maintained normal or near-normal sperm characteristics, indicating no adverse effects on reproductive function. Overall, these findings suggest that MLOL possesses potent protective effects against cadmium-induced reproductive toxicity, likely through antioxidant mechanisms that preserve sperm integrity, viability, and function.

The results in Table 5 reveal that cadmium chloride ($CdCl_2$) exposure caused a significant ($P < 0.05$) elevation in serum liver biomarkers—AST, ALT, GGT, and bilirubin—compared to the control group, indicating pronounced hepatocellular injury and impaired liver function. However, co-treatment with the methanol extract of Oxymitra longipedicellata leaf (MLOL) at both 200 and 400 mg/kg significantly ($P < 0.05$) reduced these elevated enzyme levels relative to the $CdCl_2$-only group, demonstrating a protective effect against cadmium-induced hepatotoxicity. The 400 mg/kg dose of MLOL showed a more substantial improvement, particularly in reducing GGT and bilirubin levels closer to normal values, suggesting a dose-dependent hepatoprotective activity. Similarly, quercetin (QUER), used as a standard antioxidant, also significantly ameliorated the toxic effects of $CdCl_2$, although the effects of MLOL, especially at the higher dose, were comparable in restoring liver enzyme activities. Furthermore, administration of MLOL or QUER alone did not significantly elevate liver enzymes compared to the control, indicating their relative safety and lack of hepatotoxic effects.

As depicted in Figure 5, exposure to $CdCl_2$ markedly disrupted antioxidant balance, as evidenced by a significant reduction in hepatic and testicular superoxide dismutase (HSOD and TSOD) activities, alongside a pronounced increase in malondialdehyde (HMDA and TMDA) levels. This indicates enhanced lipid peroxidation and oxidative damage in both liver and testicular tissues. However, treatment with MLOL (200 and 400 mg/kg) and quercetin significantly restored SOD activity and reduced MDA levels compared to the $CdCl_2$ group (b, $P < 0.05$). The higher dose of MLOL (400 mg/kg) showed greater efficacy, suggesting a dose-dependent antioxidant effect. The reduction in MDA levels implies that MLOL mitigates lipid peroxidation, likely due to its phytochemical constituents with free radical scavenging properties. Quercetin, a known antioxidant, showed comparable protective effects, further validating the oxidative stress–ameliorating potential of the plant extract.

In Figure 6, a similar trend is observed in glutathione-related parameters. $CdCl_2$ exposure significantly decreased hepatic and testicular glutathione-S-transferase (HGST, TGST) activities and reduced glutathione (HGSH, TGSH) levels, indicating depletion of endogenous antioxidant defenses. This depletion compromises the detoxification of reactive oxygen species and electrophilic metabolites. Treatment with MLOL and quercetin significantly elevated GST activity and GSH levels compared to the $CdCl_2$ group (b, $P < 0.05$), demonstrating restoration of antioxidant capacity. Again, the higher dose of MLOL exhibited a more pronounced effect, suggesting improved enhancement of cellular redox status. Overall, these findings suggest that $CdCl_2$ induces severe oxidative stress by impairing enzymatic (SOD, GST) and non-enzymatic (GSH) antioxidant systems while increasing lipid peroxidation (MDA). The administration of MLOL effectively counteracts these effects, likely through its antioxidant phytochemicals that enhance endogenous defense mechanisms and scavenge free radicals. The comparable efficacy with quercetin further supports the potential of Oxymitra longipedicellata as a natural therapeutic agent against cadmium-induced hepatotoxicity and testicular toxicity.

V. CONCLUSION

Cadmium chloride exposure resulted in profound reproductive, biochemical, and oxidative alterations, including suppression of reproductive hormones (testosterone, FSH, and LH), impairment of sperm parameters, dyslipidemia, hepatotoxicity, and increased oxidative stress markers. However, administration of methanol extract of Oxymitra longipedicellata (MLOL) significantly ameliorated these deleterious effects in a dose-dependent manner. The extract restored hormonal balance, improved sperm quality (motility, count, and viability), normalized lipid profile, and reduced elevated liver enzymes. Furthermore, MLOL enhanced antioxidant defense systems by increasing superoxide dismutase (SOD), glutathione (GSH), and glutathione-S-transferase (GST) levels while reducing malondialdehyde (MDA), thereby mitigating lipid peroxidation and oxidative damage.

The protective efficacy of MLOL, particularly at 400 mg/kg, was comparable to or slightly better than standard antioxidants such as quercetin in some

parameters. These findings suggest that the protective mechanism of MLOL is largely mediated through its antioxidant and free radical scavenging properties, which help preserve testicular structure and function. In the overall, this study establishes Oxymitra longipedicellata as a promising natural therapeutic agent for managing cadmium-induced reproductive toxicity and associated oxidative stress.

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