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Methanolic leaf extract of *Pericopsis laxiflora* (benth. ex baker) meeuwen protects against cadmium-induced testicotoxicity in male sprague-dawley rats via antioxidant capabilities

Abdulrasaq Oyedeji[1]*, Christian Ukonu[1], Semiu Lawal[2], Luqmon Azeez[3]

[1]Department of Science Laboratory Technology, The Federal Polytechnic, Ilaro, Nigeria
[2]Department of Science Laboratory Technology, Ogun State Institute of Technology, Igbesa, Nigeria
[3]Department of Pure and Applied Chemistry, Osun State University, Nigeria

*Correspondence: olalekan.oyedeji@federalpolyilaro.edu.ng

ABSTRACT

Pericopsis laxiflora (Benth) Meeuwen is used in African folk medicine to mitigate numerous infections. This study evaluated the antioxidant, phytochemical and antimicrobial activities and protective potential of *P. laxiflora* methanol leaf extract against cadmium-induced testicotoxicity in male Sprague–Dawley rats. The methanol extract of *P. laxiflora* leaves (MLPL) obtained by cold maceration contained several phytochemicals, but no cardiac glycosides or terpenoids were found. It showed remarkable dose-dependent *in vitro* antioxidative activity. The extract had similar antibacterial effects as ciprofloxacin against *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, and *Pseudomonas aeruginosa*. However, it was more effective against *S. pneumoniae* than the other bacterial strains. Similarly, it exhibited antifungal activities comparable to Griseofulvin against *Candida albicans* and *Aspergillus spp*. The extract corrected the damage occasioned by intoxication with $CdCl_2$. It elevated testicular malonaldehyde like Quercetin. The increased NO and myeloperoxidase (MPO) activities induced by treating Sprague–Dawley rats with $CdCl_2$ were significantly reduced by the MLPL extract. The extract also attenuated the production of β-catenin and B-cell lymphoma 2 (BCL-2) protein in the testiculocytes of the rats and restored severe perivascular inflammation in liver cells. Through several mechanisms, MLPL showed protective effects against testis injury induced by cadmium chloride in Sprague–Dawley rats.

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Introduction

Medicinal plants have received greater attention because of their curative properties against various diseases. Sometimes, they serve as a complement or substitute for allopathic medical treatments because of people's orientation toward "green therapy" and using natural compounds from plants [1,2]. In tropical Africa, 80% of the population still uses medicinal plants to meet their primary healthcare needs because of poor economic performance, resulting in inequalities [3]. The perceived interest in plants for treatment is heightened by their efficiency, availability, relatively low cost, and fewer side effects [4]. The therapeutic effects of medicinal plants are primarily due to several compounds, such as polyphenols, flavonoids, and phenolic acids. These compounds have various beneficial effects on human health [4]. Because of the trust, acceptability, affordability, and accessibility of African medicinal plants as a source of health care, several African countries now cultivate medicinal plants on a large scale with facilities to manufacture herbal medicines [5].

In the realm of traditional African medicine, *Pericopsis laxiflora* is utilised to address a multitude of infections and various ailments such as abdominal discomfort, enteritis, peptic ulcers, cephalalgia, dermatophyte infections, envenomations, cardiac pain, visceral and pain gastritis [6]. In Guinea and Ghana, it serves as a therapeutic agent against colibacillosis, shigellosis, mycosis, and eczema, besides its application in treating malaria. Nigeria

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employs it as a traditional remedy for ulcers [7]. Research conducted by Doukourou *et al*. identified alkaloids, catechin, sterols, polyterpenes, polyphenols, tannins and flavonoids in the aqueous extracts of *P. laxiflora*, all of which exhibited considerable concentrations of electrolytes including potassium, calcium, iron, magnesium, sodium, and iron; however, the extract did not have a diuretic effect in Sprague-Dawley rats at the dosage administered [4].

Industrial wastewater is a complex mixture of trace metals and organic pollutants that are released into the environment and inevitably infiltrate aquatic sources, both surface and subterranean. Primarily among the metals released into the environment is cadmium. Cadmium is recognised for its capacity to disrupt the vascular integrity of the testis and inflict structural damage on the seminiferous tubules, Sertoli cells, and the blood-testis barrier. It impedes the development and functionality of Leydig cells and has been associated with the induction of Leydig cell tumours. Cadmium triggers reactive oxygen species (ROS) generation and induces DNA damage, thus epigenetically modifying somatic and germ cell functions, leading to male infertility. It also disrupts gonadal function, hormone regulation, and gametogenesis [8]. The incidence of infertility among couples has escalated by approximately 20% since 2019, with the highest prevalence observed in Western Sub-Saharan Africa. The declining global sperm count and persistent suboptimal semen quality are responsible for 40-50% of male infertility within this demography. One significant contributor to male infertility is the heightened exposure to environmental pollutants, including metals such as cadmium [9]. The use of plant-based materials as an alternative therapy for metal toxicity is emerging. They induce their therapeutic activities by heavy metal chelation, exerting anti-oxidation activities on the cells, and restoring normal physiological functions [10]. This study aimed to examine the phytochemical, antioxidant, and antimicrobial properties of *P. laxiflora* methanol leaf extract. Furthermore, it assessed the extract's ability to mitigate testicotoxicity caused by cadmium in male Sprague-Dawley rats. This will provide a low-cost intervention for infertility among the male population.

Materials and methods

Chemicals and reagents

All the chemicals and reagents employed in this study were of scientific grade (Analar). Nitro blue tetrazolium (NBT), Griess reagent, 2,2–diphenyl–1–picrylhydrazyl (DPPH), Folin-Ciocalteu, 2,2–azinobis (3–ethylbenzothiazoline–6–sulfonic acid) (ABTS), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), nitric oxide (NO), butylated hydroxytoluene (BHT), potato dextrose agar (PDA), and catechin were purchased from Sigma Aldrich Chemical Company. The sodium salt of ethylenediaminetetraacetic acid (EDTA-Na), ascorbic acid, trichloroacetic acid (TCA), dimethyl sulphoxide (DMSO), ferric chloride ($FeCl_3$), saline phosphate buffer (SPB), thiobarbituric acid (TBA), dihydrate sodium nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$), cadmium chloride, ammonium sulphate, acetic acid, and potassium ferricyanide were of analytical grade and were purchased from Seglor Nigeria limited. All spectroscopic experiments were performed on a 752N UV-visible spectrophotometer.

Collecting plant material and preparing plant extracts

Fresh *P. laxiflora* leaves were collected from the plant garden of the Federal Polytechnic, Ilaro, in March 2023. They were air-dried, pulverised and extracted via cold maceration of 1000 g of the dried pulverised leaves in 2800 mL of 80% methanol for 72 hours at room temperature. The filtrate obtained was concentrated thereafter in a rotary evaporator and dried to a constant weight. This was kept at 20°C until further tests were performed.

Phytochemical Screening

The crude methanol extract of the leaf of *P. laxiflora* was screened for its phytochemical constituents using standard procedures [2].

In vitro screening of the leaf extract for its antioxidant activity

Free radical scavenging activity (DPPH): The free radical scavenging activity of methanolic extracts of *P. laxiflora* was measured by using the DPPH method of Abebe *et al*. [11]. The DPPH solution was prepared by dissolving 0.01 g of the DPPH in 100 mL of methanol, and a 3 mL portion was added to 1 mL of the graduated concentration (100–1500 µg/mL) of the test samples. The mixture was thereafter left to stand at room temperature (25°C) in a dark cupboard for 30 minutes, and the absorbance was read at 570 nm using methanol as the blank.

***Inhibition of lipid peroxidation (LPO)*:** Lipid peroxidation was determined as described by Al-Hakimi *et al*. [12]. A fresh cow liver sample obtained from the Polytechnic's animal farm was weighed and homogenised in a mortar with cold saline phosphate

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buffer (H_4NaO_5P, pH 7.4). Different concentrations of the plant extract (100–1500 µg/mL) were added to the liver homogenate. Lipid peroxidation occurred with the addition of 100 mL of the reaction mixture to a tube containing 1.5 mL of 0.67% thiobarbituric acid solution in 50% acetic acid. Subsequently, the resulting mixture was subjected to heating in a water bath maintained at a temperature of 80°C for 30 minutes. The absorbance of the resultant pink-hued complex was quantified at a wavelength of 535 nm using a spectrophotometer.

2, 2–azinobis (3–ethylbenzothiazoline–6–sulfonic acid (ABTS) scavenging activity: The evaluation of ABTS scavenging activity was conducted as per the protocol articulated by Ibitoye *et al*. [13]. An ABTS solution of three grams per litre (3 g/L) along with 132 g/mol NH_4SO_4 was allowed to equilibrate at ambient temperature for 12 hours in a dark environment before the application. The plant extract, prepared in various concentrations (100–500 µg/mL), was diluted with 0.6 mL of the ABTS and NH_4SO_4 mixture, followed by further dilution with 0.4 mL of ethanol. The absorbance readings were then taken at 745 nm.

Reducing power scavenging activity: The evaluation of reducing power activity was conducted utilising the approach delineated by Tijani *et al*. [14]. Specifically, 2 mL of the plant extract was added to 2.5 mL each of 1% potassium ferricyanide (10 mg/mL) and phosphate buffer (0.2M, pH 6.6), respectively. This resultant mixture was subjected to incubation at a temperature of 50°C for 20 minutes, subsequently followed by rapid cooling, the addition of 2.5 mL of 10% TCA, and centrifugation at 6500 rpm for 10 minutes. A 2.5 mL portion of the supernatant was subsequently diluted with a similar volume of distilled water, after which 0.5 mL of ferric chloride (0.1%) was incorporated and left for 10 minutes. The absorbance of the solution was determined spectrophotometrically at a wavelength of 700 nm.

Nitric oxide scavenging activity: The assessment of nitric oxide (NO) radical scavenging activity was performed under the protocol established by Deng *et al*. [15]. A 1.5 mM sodium nitroprusside solution in phosphate-buffered saline was combined with various concentrations of the plant extract (100 - 500 µg/ml) that had been dispersed in methanol and incubated at a temperature of 25°C for 5 hours. After the incubation, 1.5 mL of the resulting solution was extracted and diluted with 0.5 mL of Griess reagent. The absorbance of this mixture was then measured at a wavelength of 546 nm.

Superoxide scavenging activity (SO): The determination of superoxide scavenging activity was executed as per the method described by Debnath *et al*. [16]. An aliquot of 0.5 mL of the plant extract at varying concentrations (100 - 500 µg/mL) was prepared, to which 1 mL of DMSO was added, followed by 0.2 mL of NBT. The solutions were constituted in a 0.1M sodium phosphate buffer (pH 7.4). The absorbance of the solution was subsequently read at wavelengths of 540 and 560 nm.

Testing for antimicrobial activity

Preparation, isolation and characterisation of the organism: Pure microbe cultures were obtained from Olabisi Onabanjo University's Department of Pharmaceutical Microbiology at the Faculty of Pharmacy in Sagamu, Nigeria. Bacterial isolates included both Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The experimental assessments also focused on *Candida albicans* and *Aspergillus* species because of their clinical and pharmacological significance. One gram of soil sample was weighed and added to 100 mL of distilled water in a sterile container. The container was sealed and vigorously stirred. A 1 mL portion of the resultant suspension was diluted aseptically. A 1-mL aliquot from a six-fold dilution was applied to PDA for fungi and bacteria. The plates were gently rotated to ensure uniform distribution of the inoculum and allowed to solidify under a laminar airflow hood. Each inoculated plate was incubated at a temperature of 37°C for 48 hours [17]. Following robust growth of the colonies, distinct colonies were sub-cultured onto fresh PDA and nutrient agar utilising the streak plate technique, and subsequently incubated at 37°C for an additional 72 hours. Each fungal colony was verified as the appropriate species via examination under a light microscope with the use of an oil immersion objective following staining with lactophenol cotton blue dye, whereas the bacterial colonies were subjected to Gram staining before microscopic observation. Pure isolates were subsequently transferred onto PDA and nutrient agar slants and stored at 4°C until required for further experimentation. The isolates were characterised based on their biochemical and cultural attributes.

Antimicrobial susceptibility testing: The agar well diffusion technique was used to evaluate the sensitivity of the microorganisms to the plant extract,

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as described by Jorgensen *et al*. [18]. Pure bacterial strains were grown in nutritious broth for 18 hours before usage. They were then subcultured onto Mueller-Hinton agar. One litre of distilled water was used to suspend 38 grams of the medium. The medium was dissolved by heating continuously and then boiling for 1 minute. The mixture was autoclaved at 121°C for 15 minutes, then cooled to room temperature. To create a uniform depth of 4 mm, sterile Petri dishes were filled with chilled Mueller-Hinton agar and set on a flat laboratory bench [13]. The Petri dish contents were left to harden at room temperature. The Mueller-Hinton agar was tested to maintain a final pH of 7.3±1 at 25°C and examined for the presence of inhibition zones. The extent of inhibition was quantified by measuring the inhibition zone diameter with a transparent ruler. The impact of the extract on bacterial and fungal organisms was assessed in comparison with that of the standard antibiotic ciprofloxacin, a broad-spectrum antibiotic effective against both bacteria and fungi, evaluated at a maximum concentration of 100 mg/mL.

Effect of methanol extract of P. laxiflora against cadmium chloride-induced testicotixicity

Animals: Forty-two (42) male Sprague-Dawley rats, aged four to five weeks and weighing between 160 and 200 grams, 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) [19].

Ethical approval

Ethical approval was obtained from the Federal Polytechnic, Ilaro institutional committee on the use and care of laboratory animals.

Experimental Design and Administration of the Extract

The Sprague-Dawley rats were randomly distributed into seven groups of six animals each and were allowed free access to feed and water for a week for acclimatisation before the commencement of the experiment. The grouping is shown in Table 1.

Table 1: Grouping of Experimental Sprague-Dawley rats

Groups	Rats Treatment
1	Control rats were administered 0.3 mL of olive oil per oral (p.o).
2	Intraperitoneally administered with $CdCl_2$(5 mg/kg) in distilled water.
3	Rats were intraperitoneally administered with $CdCl_2$(5mg/kg) in distilled water and MLPL at 100 mg/kg (p.o) dissolved in 0.3 mL of olive oil.
4	Intraperitoneally administered with CdCl2(5mg/kg) in distilled water and MLPL at 200 mg/kg (p.o) in 0.3 mL of olive oil.
5	Cadmium chloride (5mg/kg) in distilled water and Quercetin at 50 mg/kg dissolved in 0.3 mL of olive oil were administered orally.
6	Administered orally, MLPL was given at a dose of 200 mg/kg in 0.3 mL of olive oil.
7	Quercetin at 50 mg/kg in 0.3 mL of olive oil was administered orally.

The animals were pre-treated with MLPL for thirty (30) days, and $CdCl_2$ was administered to the animals in the last 3 days.

Preparation of samples: After receiving the final medication dose, the rats were starved overnight before being sacrificed via cervical dislocation. Blood was taken from the animals and stored in simple centrifuge tubes for two hours before being centrifuged to extract the serum. The animals' testes were extracted, washed in an ice-cold 1.15% KCl solution, dried, and weighed. The excised tissues were homogenised in 5 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 0.25 hours to generate the post-mitochondrial supernatant fraction (PMF), which was used to assess antioxidant indices. The tissues were also fixed in the Boin solution for histology and immunohistochemistry.

Assay methods: Total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) were estimated by using RANDOX kits, as described by Antar *et al*. [20]. Testicular nitric oxide level and myeloperoxidase activity were performed according to the method of Khan *et al*. [21]. Also, serum follicle-stimulating hormone, luteinising hormone, testosterone, sperm count, motility, live-to-death ratio, and sperm volume were analysed according to the method of Olaolu *et al*. [22].

Superoxide dismutase (SOD) was tested using Adaramoye *et al*.'s nitro blue tetrazolium reduction technique [23]. Catalase (CAT) and glutathione-S-transferase (GST) activity were measured per the method of Adebayo *et al*. [24]. In brief, GST activity was measured by measuring the rate of conjugate formation between glutathione (GSH) and 1-chloro-

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2,4-dinitrobenzene, whereas CAT activity was assessed by measuring the rate of hydrogen peroxide breakdown at 240 nm. The levels of reduced glutathione (GSH), hepatic glutathione peroxidase (GPx), and malonaldehyde were measured using the Adebayo *et al*. technique [24]. Reduced GSH levels were determined by measuring the rate of chromophore production in a reaction between DTNB and free sulfhydryl groups at 412 nm using the method of Baliyan *et al*. [25]. The extent of LPO was evaluated using the methods provided by Abeyrathne *et al*. and Candelaria *et al*. [26,27]. The cellular inflammatory response in the testes was assessed by calculating testicular myeloperoxidase activity and nitric oxide levels [28].

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.

Results and discussion

Plants can produce diverse chemical compounds essential for various biological processes and for safeguarding against assaults by predators like insects, fungi, and herbivorous mammals [29]. The MLPL, as shown in Table 2, contained alkaloids, flavonoids, anthraquinones, saponins and anthraquinone, while cardiac glycosides and terpenoids were absent. These phytochemicals are known to heighten sperm viability and also improve their morphology by free radical scavenging; hence, they are used as an alternative for the reversal of male infertility and associated conditions [30,31]. The MLPL, owing to its richness in phytochemicals like Bomba ceiba stem extracts, can ameliorate male fertility [32].

Table 2: Preliminary phytochemical screening of MLPL

Phytochemicals	MLPL
Alkaloids	++
Flavonoids	++
Anthraquinones	++
Cardiac glycosides	--
Saponins	++
Tannins	--
Phlobatanins	++
Terpenoids	--

Key to Table 1: ++ = Present; -- = Absent; MLPL = Methanol extract of P. laxiflora leaf

Methanol extract of *P. laxiflora* leaf showed remarkable antioxidant activities at graded concentrations relative to the standard, Catechin. The extract demonstrated the scavenging of free radicals in a concentration-dependent manner. Methanol extract of *P. laxiflora* leaf exhibited the scavenging abilities of DPPH and ABTS radicals dependent on the dose, showing activity comparable to the standard catechin, as indicated in Figure 1 and Table 3, respectively. The reduction capability of DPPH and ABTS radicals, which are absorbed at 517 and 745 nm, respectively, by MLPL is discussed by Onoja *et al*. (2021) [35]. It serves as a metric for its antioxidant activity. This suggests that the mechanism of action of *P. laxiflora* may involve either the inhibition or scavenging of DPPH and ABTS radicals, considering that both inhibitory and scavenging properties of antioxidants towards these radicals have been documented in a previous study by Tijani *et al*. [13]. The initiation of lipid peroxidation by ferrous sulphate occurs either through the formation of the ferryl-perferryl complex or via the hydroxyl (OH) radical in the Fenton reaction, as suggested by Ibitoye *et al*. [13].

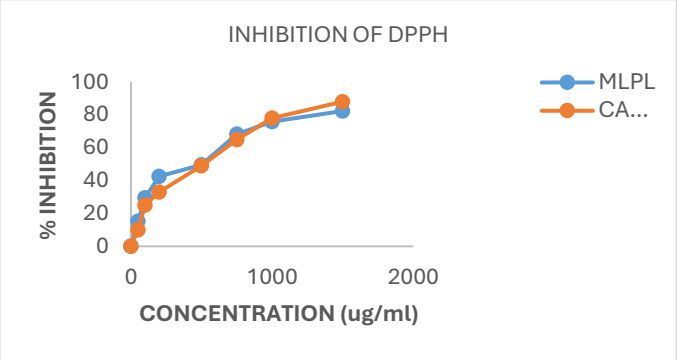

Figure 1: DPPH Scavenging activity of MLPL

The effectiveness of MLPL in decreasing the MDA level in liver phosphatidylcholine, superoxide and nitric oxide radicals in a dose-dependent manner indicates its strong antioxidant properties compared to catechin, and these are shown in Tables 3 and 4. The correlation between reducing power and antioxidant capacity (Figure 2) signifies the ability of an extract to provide electrons to radicals and reduce the oxidised intermediates of lipid peroxidation processes. The yellow hue of the test solution transformed into different shades of green and blue with increasing concentrations of MLPL, reflecting its reducing power. The presence of reducers in MLPL led to the conversion of the Fe^{3+}/ferricyanide complex utilised in this method, demonstrating the excellent reducing activity of MLPL in a dose-dependent manner relative to the reference catechin.

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Table 4: Antioxidant activity of MLPL measured as % inhibition of nitric oxide radicals and Superoxide radicals relative to catechin

Conc. (µg/mL)	% Inhibition of NO		% Inhibition of Superoxide Radical	
	Conc. (µg/mL)	Catechin	MLPL	Catechin
Control	0	0	0	0
100	-32.52±2.11	11.43±3.16*	19.45±2.16*	5.34±1.26*
200	-15.23±1.45	36.57±3.89*	28.34±2.86*	17.69±3.51*
500	-15.98±3.26	53.62±2.04*	41.43±5.36*	29.30±1.87*
750	26.57±3.14*	75.09±1.78*	47.61±2.54*	45.35±1.09*
1000	43.61±5.13*	84.22±3.84*	59.35±3.76*	67.49±0.46*
1500	67.11±3.86*	95.35±5.11*	76.37±4.11*	80.41±2.03*
IC_{50}^{++}	37.61*	19.81*	17.38*	21.57*

MDA–Malonaldehyde (Lipid peroxidation index), ABTS- 2,2-Azino-bis(3-ethylbenzo thiazoline-6-sulfonic acid radical, MLPL- Methanol extract of *P. laxiflora* leaf, IC50- Inhibitory concentration at 50 µg/mL * - represents statistical difference relative to controls at P < 0.05

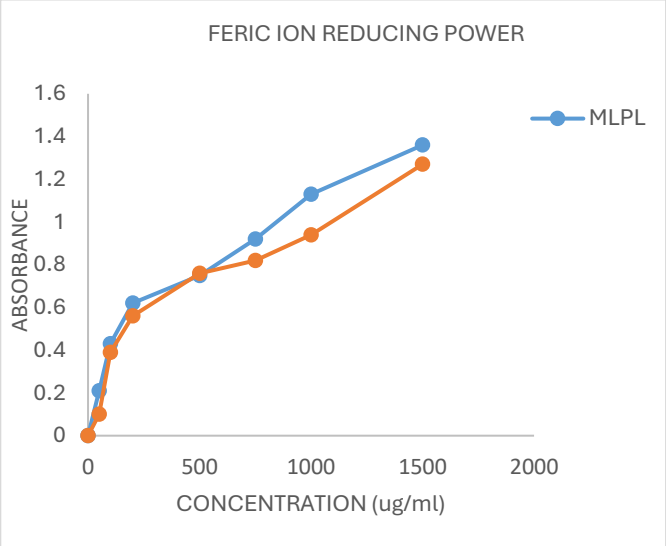

Figure 2: Ferric ion reducing potential of MLPL

The methanolic extract of *P. laxiflora* had antifungal activities comparable to griseofulvin against *C. albicans and A. spp.,* as shown in Table 5. A previous study had also shown the effectiveness of *P. laxiflora* stem bark against fungal strains, including *Trichophyton mentagrophytes* and *Trichophyton rubrum,* responsible for ringworm [7]. *Candida albicans* is known to have harmful effects on sperm function and fertilising ability. It was reported to cause decreased sperm motility and chromatin integrity, and sperm DNA fragmentation, ultimately causing decreased functional competence of the spermatozoa and increasing molecular apoptosis. Like *C. albicans, Aspergillus spp* is responsible for systemic mycoses in the male reproductive tract, causing urethritis, balanoposthitis, pseudotumor, orchitis and prostatitis [33,34].

Escherichia coli, S. aureus, and *S. pneumonae* are commonly associated with genitourinary tract infection, ultimately affecting the epididymis and testes. These conditions upset sperm quality, leading to infertility [34]. The methanolic extract of *P. laxiflora* showed activities against these pathogenic bacteria and could be used to reverse their pathogenicity

As observed in this study, intoxication with $CdCl_2$ caused a significant reduction in the serum level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, sperm count, volume, live-to-death ratio, and motility, testicular GSH, SOD and a significant elevation of testicular malonaldehyde. This finding is in line with Adaramoye and Lawal's (2013) [24] studies, where cadmium was shown to harm the testes of various mammals, consequently affecting sperm production. However, intervention with MLPL and Quercetin corrected the damage by bringing these parameters to normal

Table 5: Antifungal activities of MLPL

	Microorganisms	Zone of Inhibition (mm)	
		Control (Griseofulvin)	MLPL
100	*Candida albicans*	26.00±1.05	28.00±1.65
	Aspergillus spp	27.00±0.34	29.00±0.98
50	*C. albicans*	15.00 ± 2.49	20.00±1.15
	A. spp	18.00±0.82	23.00±1.88*
25	*C. albicans*	9.00±0.74	16.00±1.23*
	A. spp	11.00±0.96	18.00±1.35*
12.5	*C. albicans*	8.00±0.58	10.00±0.78*
	A. spp	8.00±0.36	9.00±0.65

MLPL- Methanol extract of *P. laxiflora* leaf, * - Statistically different relative to controls at P < 0.05

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Table 6: Antibacterial activities of MLPL

Concentration (mg/ml)	Microorganisms	Zone of Inhibition (mm)	
		Control (Ciprofloxacin)	MLPL
100	*S. aureus*	29.00 ± 1.32	25.00 ± 1.33
	S. pneumoniae	24.00 ± 0.78	29.00 ± 1.56
	E. coli	26.00 ± 1.67	23.00 ±1.09*
	P. aeruginosa	21.00 ± 0.83	21.00 ± 0.83
50	*S. aureus*	16.00 ± 2.20	17.00 ± 0.52
	S. pneumoniae	14.00 ± 0.93	19.00 ± 0.63
	E. coli	15.00 ± 1.21	15.00 ± 0.82
	P. aeruginosa	18.00 ± 0.74	16.00 ±0.47*
25	*S. aureus*	10.00 ± 0.47	10.00 ± 0.45
	S. pneumoniae	10.00 ± 0.72	8.00 ± 0.76*
	E. coli	8.00 ± 0.91	9.00 ± 0.12
	P. aeruginosa	9.00 ± 0.74	7.00 ± 0.32*
12.5	*S. aureus*	7.00 ± 0.65	5.00 ± 0.67*
	S. pneumoniae	8.00 ± 0.42	5.00 ± 0.71*
	E. coli	7.00 ± 0.81	4.00 ± 0.43*
	P. aeruginosa	8.00 ± 0.79	6.00 ± 0.26*

MLPL- Methanol extract of *P. laxiflora* leaf, * - Statistically different relative to controls at P < 0.05

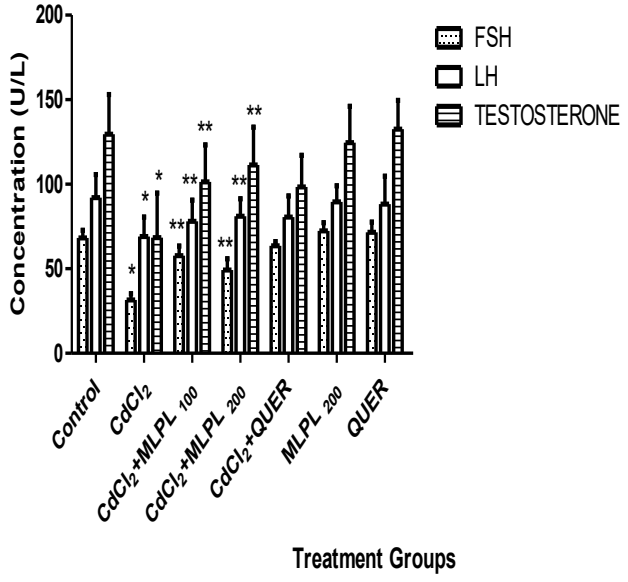

Figure 3: Effect of **MLPL** on the serum level of follicle-stimulating hormone, luteinising hormone, and testosterone in Sprague-Dawley rats intoxicated with cadmium chloride

MLPL- Methanol extract of *P. laxiflora* leaf, QUER- Quercetin, $CdCl_2$ - Cadmium chloride, FSH- Follicle Stimulating Hormone, LH- Luteinizing Hormone* - Significantly different from the control at P< 0.05, ** - Significantly different from the $CdCl_2$ group at P< 0.05

There was a significant reduction (P < 0.05) in the level of serum FSH (Fig. 3), LH (Fig. 3), testosterone (Fig. 3), sperm count (Fig. 5), motility (Fig. 4), volume (Fig. 5) and live-to-death ratio (Fig. 4) following the intoxication with cadmium chloride

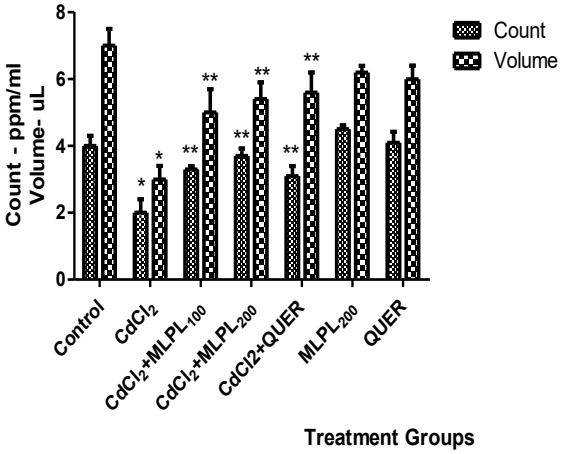

Figure 5: Effect of **MLPL** on the Sperm cells' count and sperm volume in Sprague-Dawley rats intoxicated with cadmium chloride

MLPL- Methanol extract of *P. laxiflora* leaf, QUER-Quercetin, $CdCl_2$ - Cadmium Chloride

* - Significantly different from the control at P< 0.05, ** - Significantly different from the $CdCl_2$ group at P< 0.05

specifically, by 74, 54, 69, 58, 66, 72 and 81%, respectively, relative to the control group. However, intervention with MLPL elevated these parameters by 54, 43, 59, 48, 63, 71 and 63% for 100 $mgkg^{-1}$ and 61, 58, 73, 69, 65, 73 and 78% for 200 $mgkg^{-1}$, respectively, when compared with the standard, Quercetin.

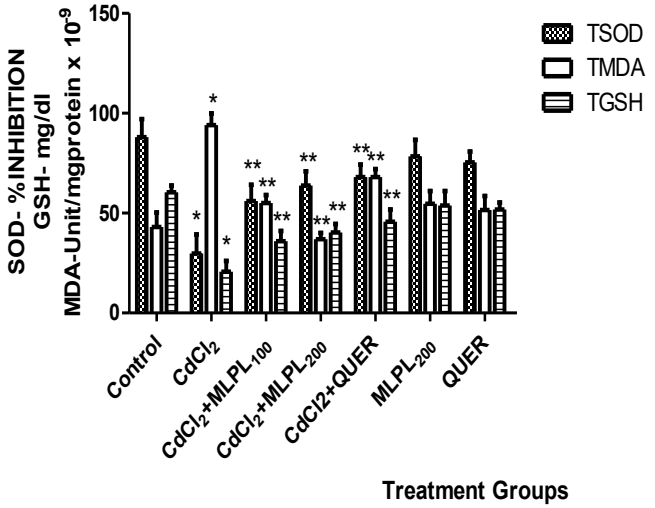

Figure 6: Effect of **MLPL** on the testicular activity of superoxide dismutase and the level of malonaldehyde in Sprague-Dawley rats intoxicated with cadmium chloride

MLPL- Methanol extract of *P. laxiflora* leaf, QUER-Quercetin, $CdCl_2$ - Cadmium chloride, TSOD -Testicular Superoxide Dismutase, TMDA- Testicular Malonaldehyde,* - Significantly different from the control at P< 0.05, ** - Significantly different from the $CdCl_2$ group at P< 0.05

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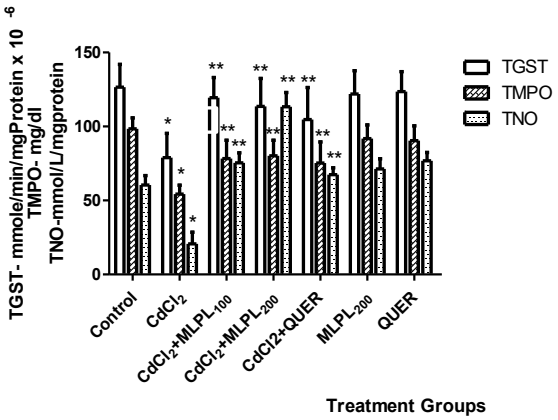

Figure 7: Effect of **MLPL** on the testicular activity of myeloperoxidase and glutathione-S-Transferase and level of nitric oxide in Sprague-Dawley rats intoxicated with cadmium chloride

MLPL- Methanol extract of *P. laxiflora* leaf, QUER- Quercetin, $CdCl_2$ - Cadmium Chloride, TMPO -Testicular Myeloperoxidase, TGST- Testicular Glutathione-S-Transferase, TNO- Testicular Nitric oxide, * - Significantly different from the control at P< 0.05, ** - Significantly different from the $CdCl_2$ group at P< 0.05

There was a significant reduction (P < 0.05) in the level of testicular SOD, GSH, GST, Myeloperoxidase, Nitric oxide and elevated testicular level of Malonaldehyde as shown in Figures 6 and 7 following the intoxication with cadmium chloride precisely by 34, 41, 37, 45, 32 and 57%, respectively relative to the control group. However, intervention with MLPL elevated these parameters by 65, 54, 45, 51, 68 and 49%, respectively, for 100 $mgkg^{-1}$ and 43, 46, 56, 32, 41 and 63% for 200 $mgkg^{-1}$, when compared with the standard, Quercetin.

Table 7: Effect of MLPL on the serum lipid profile of Sprague Dawley rats intoxicated with cadmium chloride

Treatments	Total Cholesterol	Triglycerides	HDL	LDL
Control	643.34±43.23	636.87±36.48	401.78±22.47	356.34±31.57
$CdCl_2$	1403.67±100.45	949.71±51.19	245.56±56.34	1242.66±47.44
$CdCl_2$ + $MLPL_{100}$	/965.45±87.17	711.35±62.37	576.21±44.68	405.38±27.49
$CdCl_2$ + $MLPL_{200}$	923.98±95.23	561.57±71.45	547.82±41.32	465.22±41.78
$CdCl_2$ + QUER	994.89±92.65	682.38±54.79	454.03±57.85	581.39±21.54
$MLPL_{200}$	781.30±68.64	498.26±75.87	564.91±20.54	301.82±19.43
QUER	713.03±83.56	578.31±83.98	504.67±39.40	269.64±27.88

MLPL- Methanol extract of *P. laxiflora* leaf, QUER- Quercetin, $CdCl_2$ - Cadmium chloride , HDL- High-Density Lipoprotein, LDL- Low-Density Lipoprotein, * - Significantly different from the control at P< 0.05, ** - Significantly different from the $CdCl_2$ group at P< 0.05

A significant elevation (P < 0.05) of the level of serum cholesterol, triglycerides, LDL and lowered HDL were noticed when the animals were challenged with cadmium chloride, precisely by 54, 43, 68 and 61%, respectively, relative to the control group. These results are presented in Table 7 and Figure 7, respectively. However, supplementation with MLPL lowered these parameters by 76, 67, and 59%, respectively, for 100 mg kg-1 and 72, 69, 82, and 54% for 200 mg kg-1, respectively, when compared with the standard, Quercetin. Nitric oxide (NO), known as a signalling molecule, plays a pivotal role in the pathogenesis of inflammation by inducing an anti-inflammatory response under normal physiological conditions. Conversely, NO can act as a pro-inflammatory mediator, triggering inflammation through excessive production in abnormal states. It is synthesised and released into endothelial cells with the assistance of NOSs, which convert arginine into citrulline, thereby generating NO [37]. Myeloperoxidase (MPO), a heme-containing peroxidase, is primarily produced by polymorphonuclear neutrophils. This enzyme is released into the extracellular fluid following oxidative stress and various inflammatory responses. Therefore, the controlled release of MPO at the site of infection is essential for its effectiveness, as unregulated release can exacerbate inflammation and lead to tissue damage, even in the absence of inflammation. Numerous studies have linked MPO-derived oxidants to various types of tissue injuries and the pathogenesis of major chronic diseases such as liver diseases, diabetes, cardiovascular diseases, and cancer.

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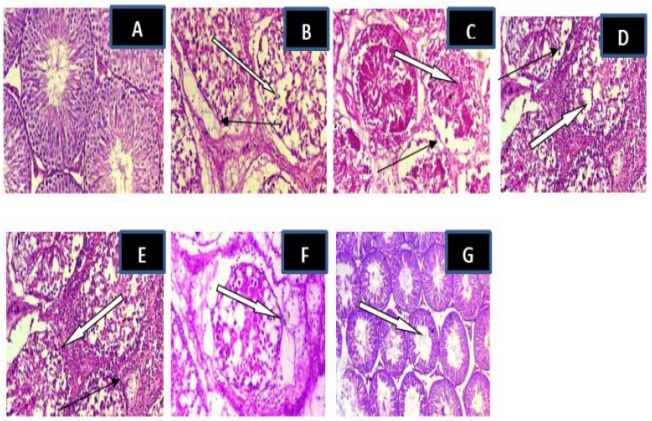

Figure 8: Effect of methanol extract of *P. laxiflora* leaf on the testicular histology of Sprague Dawley rats intoxicated with cadmium chloride

A(Control) - testicular section showing normal seminiferous tubules and other germ cells, as well as Sertoli cells

B ($CdCl_2$) - testicular section showing atrophic seminiferous tubules with severely degenerated germ cells and vacuolations

C ($CdCl_2$ +MLPL100) - testicular section showing atrophic seminiferous tubules with mildly degenerated germ cells as well as multiple vacuolations

D ($CdCl_2$ +MLPL200)- testicular section showing atrophic seminiferous tubules with mildly degenerated germ cells as well as multiple vacuolations

E ($CdCl_2$ +QUER) - testicular section showing atrophic seminiferous tubules with mildly degenerated germ cells as well as Sertoli cells

F (MLPL200)- testicular section showing normal seminiferous tubules and other germ cells as well as Sertoli cells

G (QUER)- testicular section showing normal seminiferous tubules and other germ cells as well as Sertoli cells

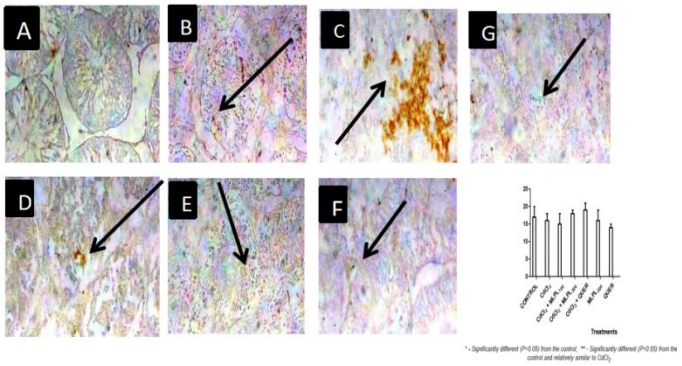

Figure 9: Effect of methanol extract of *P. laxiflora* leaf on the testicular Vimetin of Sprague-Dawley rats intoxicated with cadmium chloride

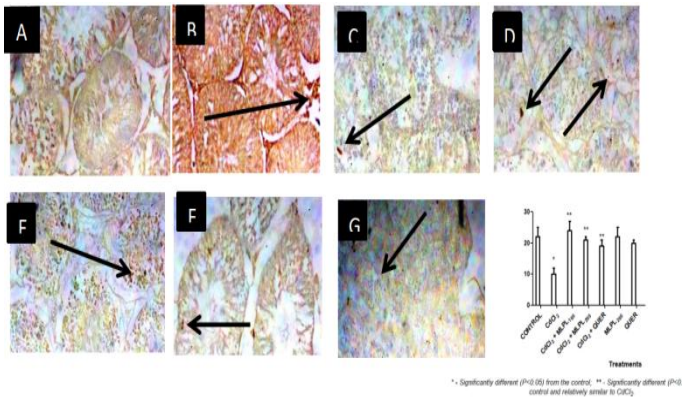

Figure 10: Effect of methanol extract of *P. laxiflora* leaf on the testicular BAX of Sprague Dawley rats intoxicated with cadmium chloride.

Consequently, an elevated level of MPO activity serves as a valuable diagnostic tool for assessing inflammatory and oxidative stress biomarkers in these conditions [21].

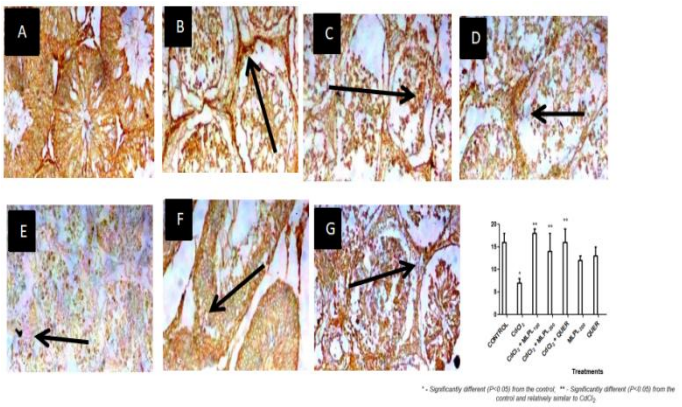

Figure 11: Effect of methanol extract of *P. laxiflora* leaf on the testicular BCL-2 of Sprague Dawley rats intoxicated with cadmium chloride

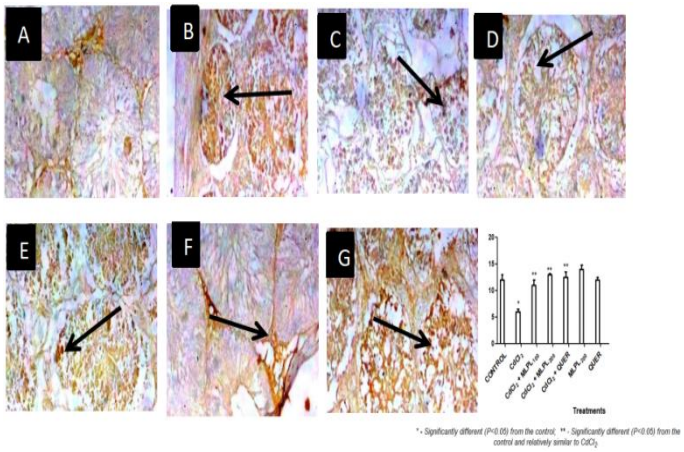

Figure 12: Effect of methanol extract of *P. laxiflora* leaf on the testicular Beta-Catenin of Sprague Dawley rats intoxicated with cadmium chloride

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As demonstrated in the present investigation, and shown in Figures 11 and 12, a notable increase in NO levels and MPO activity was observed in rats treated with $CdCl_2$ compared to the control group, indicating inflammation because of the toxic effects of $CdCl_2$ on the testes. Methanol extract from *P. laxiflora* leaf, along with Quercetin, exhibited promising anti-inflammatory properties by significantly reducing NO and MPO levels. Furthermore, strong induction of anti-apoptotic proteins, BCl2 and β-catenin in the testiculocytes of rats intoxicated with $CdCl_2$ was halted in rats treated with MLPL and Quercetin, thus suggesting the fact that $CdCl_2$-induced testicotoxicity may be because to repressed apoptosis. Histopathological investigation of testis slices showed poor architecture with severe peri-vascular infiltration of inflammatory cells in $CdCl_2$-treated rats, whilst treatment with MLPL and Quercetin restored normalcy in the architecture of the liver with mild infiltration of cells.

Conclusions

Methanol extract of *P. laxiflora* leaf (MLPL) has been found to possess excellent antioxidant, antibacterial and antifungal activity in this study. Furthermore, cadmium chloride caused testicular damage in rats through some mechanisms, which include impairment of the oxidant/antioxidant balance, pro-inflammatory enzymes and inhibition of apoptosis. Still, MLPL decreased these debilitating effects by elevating the activities of antioxidant enzymes and up-regulating the expression of anti-inflammatory and apoptotic proteins. This activity is comparable to the standard Quercetin. The dose of MLPL used relative to quercetin conserves the testes from injury, and protective functions may be because of the number of phytochemicals present in the extract.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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