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Lophira lanceolata protects testicular and spermatological damages induced by cisplatin in male Wistar rats

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Abstracts

Background: Chemotherapy is associated with male infertility. Cisplatin (CP), an antineoplastic agent has been successfully used for the treatment of diverse kinds of malignancies, however, the use of this effective agent could induce oxidative stress injury, nephrotoxicity, hepatotoxicity, and testicular damage. Combined CP chemotherapy with plant extracts can diminish the toxicity and enhance the antitumor efficacy of the drug. The objective of the study was to determine the protective effect *Lophira lanceolata* leaf extract (LLE) on CP-induced toxicity on male reproductive organs.

Methods: The study was carried out with 30 ($n = 30$) male Wistar rats (*Rattus norvegicus*). The rats were randomly assigned into 6 groups of 5 rats each. Rats in group 1 (Control) were administered distilled water per os. Rats in group 2 were administered 5 mg/kg of CP intraperitoneally (i.p). Rats in groups 3 and 4 were administered per os LLE at the doses of 200 and 400 mg/kg body weight and rats in groups 5 and 6 were administered 5 mg/kg body weight of CP + LLE at the doses of 200 and 400 mg/kg body weight respectively.

Results: The results showed a significant decrease in the sperm parameters in the group treated with CP alone when compared with the control and there in the sperm parameters in the groups administered CP + LLE. The body and organ weights of the rats were significantly ($p < 0.05$) decreased in the CP treated group relative to the control. However, there was an increase in the weight of the organs in the LLE pretreated groups. The photomicrographs showed degenerative changes in the testicular tissues of the rats administered CP alone whereas the group pretreated with the LLE showed amelioration induced by the CP. Our study revealed that CP treatment has deleterious effects on sperm parameters and testicular tissues and the accessory sex organs (Epididymis, prostate, seminal vesicles) of the rats. Oral administration of LLE at 200 and 400 mg/kg bodyweight for 26 days conferred protective effects against testicular damage induced by CP.

Conclusion: This study revealed that pretreatment with LLE protected against CP-induced testicular toxicity.

Keywords: Cisplatin, *Lophira lanceolata*, Spermatozoa, Reproductive organ, Toxicity

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Introduction

Chemotherapy involves the elimination of cancer cells or halting the growth of uncontrolled proliferative cells even at distant sites from the origin of the primary tumour by the use of chemical agents. Conversely, it does not discriminate between cancer and normal cells and destroys not only the rapid-growing cancer cells but also other rapidly dividing cells in the body [1]. Cisplatin, *cis*-43 diamminedichloroplatinum (CDDP), with the molecular formula *cis*-[Pt (NH₃)₂Cl₂] is an anti-neoplastic agent that has been efficaciously used for management of different types of cancers including bladder, lung, neck, head, and testicular cancers [2]. CP is a block of efficient platinum-derived alkylating agent that acts against proliferating and resting cells [3] by inducing DNA crosslinks and DNA double-strand breaks; both of these activities cause suppression in DNA transcription and replication, leading to apoptosis [4]. It is undoubtedly known that CP is a proven antineoplastic drug used to combat cancers including sarcomas, however, nephrotoxicity, hepatotoxicity, oxidative stress injury and testicular damage are amongst the utmost feared side-effects for patients embarking on cancer chemotherapy [5]. The testis is an organ with high proliferative capacity and it is involved in the transfer of genetic material from one generation to the other, thus adverse effects of chemotherapy on the testes can be very devastating [6]. Gonadotoxicity with deterioration in sperm parameters (motility, count, and viability), inhibition of spermatogenesis, testicular atrophy, and reduced fecundity as a sequel to CP administration have been proven in several studies [7]. There is relative spermotoxicity with the use of CP and almost all patients under chemotherapy show temporary or permanent azoospermia [8]. The mechanism underlying CP-induced testicular injury involves biochemical and physiological tissue disturbances consequential to the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [9] that enhance cellular damage and necrosis via lipid peroxidation of tissues, DNA lesions, and protein denaturation [10].

Many natural products of plant origin safeguard against drug-induced toxicity [11]. Herbal medicine is gaining importance day-by-day in the management of several disorders, however, to date, just minimal information exists on medicinal plants that are described to relieve CP-induced reproductive side effects as a result of chemotherapy and new approaches to improve tolerance and reduce sequelae of cancer chemotherapy are urgently needed.

Iron wood (*Lophira lanceolata*) is a tree that is socioeconomically and culturally valuable to inhabitants of Africa and it is a traditional medicine recipe that has been used to treat several disorders. Earlier

scientific reports on the leaf extract of this plant show that it possesses antibacterial [12] and antiplasmodial [13] activities. Decoctions of fresh/dried young leaves can treat pain caused by intestinal worms, dysentery, and diarrhoea for children. Women take the decoction of roots against menstrual pain, intestinal disorders, and malaria [14, 15] reported that its flowers are effective for treating several ailments including sexual weakness, epilepsies, yellow fever, and several others. Although, *Lophira lanceolata* is believed to have aphrodisiac properties and has been used in the management of sexual dysfunction, however, to the best of our knowledge, its ameliorative or protective effect against CP-induced reproductive toxicity has not been investigated. We therefore aimed at evaluating the protective effects of LL in CP treated male Wistar rats. To achieve this, we evaluated spermatogenesis, organo-somatic indices (OSI) of the testes, seminal vesicles, prostate and epididymis along with the histopathological alterations in the testis of the experimental rat models.

Materials and method

The drug used for the induction of testicular and spermatological damages

The CP used was obtained from Kwality pharmaceuticals Pvt. Ltd. Nag Kalan, Majitha Road, Amritsar- India.

Plant collection and preparation

Fresh leaves of *Lophira lanceolata* were collected from within the premises of the Federal University of Agriculture, Makurdi, Benue State, Nigeria, and identified by a botanist. A voucher specimen (UAM/FH/0328) has been deposited in the Herbarium of the College of Forestry, Federal University of Agriculture, Makurdi. The leaves were collected and washed under running tap water and air-dried under room temperature for two (2) weeks. The dried leaves were pulverized with a kitchen electric grinding machine and stored in an airtight container. About 200 g of smooth powdered leaves of *Lophira lanceolata* were placed in a conical flask containing 4000 ml of distilled water. This was kept at room temperature for 48 h with frequent agitation every 2 h. Filtration was done with a Whatman™ no. 1 filter at the end of the period. The filtrate was concentrated by evaporating at 45 °C using a water bath. The concentrated extract was weighed to determine the yield and stored in the refrigerator until needed.

Experimental animal management and treatment

Adult male Wistar strain albino rats (150-230 g) were obtained from Animal house, College of medicine,

Benue State University, Makurdi. The rats were kept in standard cages in a well-ventilated laboratory animal house under controlled temperature ($24 \pm 27^\circ\text{C}$) and photoperiod (12 L,12D).and were fed with Growers Mash[®] produced by Grand Cereals and Oil Mills Ltd. Bukuru, Jos. The rats were acclimatized for 14 days to the best laboratory conditions before the experiment were provided with a balanced diet, water was provided ad libitum. The experimental protocol was by the guidelines on the care and wellbeing of research animals [16] and was approved by the Departmental Ethics Committee with an assigned number (VPB/01/03/2019). The rats were handled according to the standard protocols for the use of laboratory animals for experiments.

Thirty (30) male albino rats weighing 150-230 g were recruited in this study. The rats were randomly assigned to 6 groups of five rats in each group. Group 1 rats received distilled water only orally for 26 days. Groups 2 rats were given distilled water for 20 days orally and on day 21 the rats were treated with CP at a dose of 5 mg/kg intraperitoneally (i.p). Groups III and IV were administered doses of LLE 200 and 400 mg/kg body weight (bwt) respectively for 26 days. Groups V and VI were administered doses LLE at 200 and 400 mg/kg body weight respectively for 26 days and the same rats were treated with CP 5 mg/kg on the 21st day as in group II. The body weights of the experimental rats were recorded weekly.

Collection and preparation of tissues

About 24 h after the last treatments, the rats were anesthetized with pentobarbitone sodium (PS) at a dose of 60 mg/kg i.p. The testes and accessory organs of each rat were collected and weighed using a Mettler meter. Epididymal sperm was collected by cutting one epididymis into small pieces in 5 ml of physiological saline at room temperature. The testes were preserved in 10% formalin for histopathological analysis.

Sperm parameters

Determination of Epididymal sperm motility A sperm motility test was carried out using the method described by the World Health Organization [17]. The individual motility was determined by the formula;

$$\text{Motility (individual) (\%)} = \frac{\text{Number of motile sperm}}{\text{Total no. of sperm (motile + immotile)}} \times 100$$

Determination of Epididymal sperm viability Sperm viability test was determined by the method described by the [17, 18].

The sperm viability was calculated as given below

$$\text{Viable (\%)} = \frac{\text{number of viable sperm}}{\text{total no. of sperm (viable + non - viable)}} \times 100$$

Determination of sperm concentration Sperm concentration of each sample was determined using the improved Neubauer haemocytometer after dilution in 0.9% normal-saline [19].

Determination of acrosome integrity The sperm acrosome integrity was determined by the method described by [20].

Acrosome integrity was determined by placing a drop (100 μL) of sperm sample on a clean, grease-free slide and mixed with a single drop of Giemsa stain. The spermatozoa were allowed to interact with the stain for at least 2 min and then a smear was prepared. The prepared smear was air-dried and examined under oil immersion objective (100 \times magnification) to determine the percentage of spermatozoa with intact acrosomes. The spermatozoa that pick the Eosin-Nigrosine stain means Acrosome integrity is compromised or dead. The spermatozoa with intact Acrosome integrity does not pick the stain. The mean results were expressed as percent intact acrosomes.

Determination of sperm morphology Sperm morphology was determined by the method described by [17].

The sperm cells were scored as follows:

Normal morphology: sperms with normal head and tail. Abnormal morphology: sperm cells with isolated heads – misshapen head or not; head misshapen head with abnormal tail and fused sperm. The percentage of abnormal forms were evaluated; Normal semen has fewer than 30% abnormal forms [21].

Histological analyses of the testicular tissues

Histopathological examination was carried out according to the method described by [22]. The testicular tissues were fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The stained-glass slides were viewed under a light microscope at $\times 100$.

Statistical analysis

Results are expressed as mean \pm standard error of mean (Mean \pm SEM). The data were analyzed by one-way analysis of variance (ANOVA). All statistical analysis was carried out using Graph Pad Prism Version 6.1 for windows.

Results

Protective effects of LLE on the bodyweight of Wistar rat treated with CP

Table 1 shows the initial body weight, final body weight, and weight gain of the experimental rats.

Accordingly, the loss in body weight of the rats was greater in the groups administered CP and CP+ LLE) than in the control group. There was a significant ($p < 0.05$) decrease in body weight in the group treated with CP only when compared with the controls, however, there was an increase in the body weights in the groups co-administered CP + LLE in a dose-dependent manner.

Effects of LLE on the sperm parameters in albino rats

Treatment of rats with LLE alone at graded doses did not affect the parameters studied as compared to the control value. Administration of CP alone caused a significant decrease in sperm motility, sperm concentration, and a significant increase in the percentage of abnormal sperm and acrosomal integrity (dead) when compared to the control group. Pretreatment of the rats with LLE for 21 days before CP treatment significantly showed an increase in the percentage motility (Table 2).

Histopathological slide of the testis of Wistar rats treated with LLE and CP (Table 3)

Summary of the slides

- A: Histopathological slide of the testes in the normal control group (group 1) showing normal seminiferous tubule and normal germinal epithelium. X100 magnification
- B: Histopathological slides of the testes of rats in the Cisplatin treated group (group 2) show atrophic and degenerative seminiferous tubules and lots of degenerated spermatozoa. X100 magnification
- C: Histopathological slides of the testes of rats in LLE 200mg/kg treated group (group 3) showing normal

seminiferous tubule and normal germinal epithelium

- D: Histopathological slides of the testes of rats in LLE 400mg/kg treated group (group 4) showing congested blood vessels, normal seminiferous tubules, and normal spermatozoa. X100 magnification
- E: Histopathological slides of the testes of rats treated with LLE at 200mg/kg + CP (group 5) showing restored seminiferous tubules towards normality. X100 magnification
- F: Histopathological slides of the testes of rats treated with LLE at 400mg/kg + CP (group 6) showing restored degenerative changes in the seminiferous tubules towards normality. X100 magnification.

Discussion

One of the mammalian tissues reported to be the most susceptible to CP toxicity is the germ cells. CP treatment has been reported to induce oxidative stress with damaging consequences on the testes and DNA resulting in infertility and hence, there is a need, therefore, to avert such side effects to preserve the testicular tissues of patients undergoing CP treatment [7]. The results of the present study suggested that CP treatment is very deleterious to the male reproductive organ and LLE showed a cytoprotective function in the rat's testicular tissues. These data are confirmed by our observation of the increased reproductive organ weights, improved spermatological parameters, and histopathological examination.

The results of the present study showed that the treatment with CP causes a reduced body weight gain process [23] in their previous work reported that the two most serious health problems in patient chemotherapy, especially with CP are depressed appetite and consequent weight loss may be due to reduced feed efficiency. Conversely, an increase in body weight was

Table 1 Protective effect of oral administration of LLE for 26 days on body weight of Male Wistar rats treated with CP

Groups	Initial body weights (g)	weight at 2 nd week	weight at 3 rd week	final body weight (g)	Weight gain
Dist Water	158.6±6.14	172.8±12.08	179.0±12.81	195.0±10.37	37
CP	170.8±4.36	181.6±3.92	191.0±5.20	184.8±7.10	13
LLE 200mg	171.8±4.41 ^b	186.0±7.15 ^b	190.8±5.12 ^b	195.4±5.27 ^b	24
LLE 400mg	174.6±2.69 ^b	186.6±2.44 ^b	195.0±2.61	198.6±6.21	24
CIS+LLE 200mg)	190.4±5.19 ^{ab}	198.8±4.88 ^b	206.4±7.20	209.0±7.38	19
CIS+LLE 400mg)	181.0±3.26 ^b	193.6±2.58 ^b	200.4±3.53	201.4±3.49 ^b	20

Note: The values are presented as Means ± SEM, (n = 5) ^a Significant Differences as compared with normal control group $p < 0.05$ ^b Significant Differences as compared with the negative control group at $p < 0.05$

Table 2 Protective effect of oral administration of LLE for 26 days on the weight of the relative organs in Wistar male rats treated with CP

Groups	Testes (left)	Testes (right)	Sem.v (left)	Sem.v (right)	Epid. (left)	Epid. (right)	Prostate
Dist water	0.63±0.01	0.62±0.01	0.18±0.01	0.16±0.01	0.23±0.01	0.22±0.01	0.06±0.002
CP	0.31±0.03	0.30±0.03	0.04±0.002	0.03±0.002	0.15±0.01	0.14±0.008	0.04±0.005
(LLE200m)	0.61±0.02 ^b	0.60±0.02 ^b	0.06±0.005 ^a	0.05±0.005 ^a	0.24±0.01 ^b	0.23±0.001 ^b	0.06±0.006
(LLE 400mg)	0.60±0.01 ^b	0.59±0.01 ^b	0.10±0.02	0.09±0.02	0.21±0.009 ^b	0.20±0.007 ^b	0.06±0.009
(CP+ LLE 200mg)	0.49±0.03 ^{ab}	0.47±0.03 ^{ab}	0.10±0.02	0.09±0.02	0.20±0.008	0.19±0.009	0.06±0.003
(CP+ LLE 400mg)	0.49±0.02 ^{ab}	0.48±0.02 ^{ab}	0.16±0.03 ^b	0.14±0.03 ^b	0.15±0.01 ^a	0.14±0.01 ^a	0.08±0.007 ^b

Note: The values are presented as Means ± SEM (n = 5)^a Significant Differences as compared with normal control group $p < 0.05$ ^b Significant Differences as compared with the negative control group at $p < 0.05$

observed in the groups co-administrated CP and LLE, and this may suggest that LLE administration improved the appetite of the rats thereby abrogating the anorexic effect induced by CP treatment. To our knowledge, this is the first report of LLE on the prevention of cisplatin-induced weight loss. In this study, the relative reproductive organ weights of male rats exposed to CP was significantly ($p < 0.05$) reduced when compared to control group, the decline in the reproductive organ weight was mitigated by co-administered of CP and LLE at 200 mg/kg and 400 mg/kg in group 5 and 6 respectively. The therapeutic amelioration could be attributed to the Phytochemicals present in the LLE that abrogated the effect of the CP on the reproductive organs. Our present findings on CP induced testicular impairment also endorsed the earlier findings of [24] who reported that a notably reduced testes and accessory sex organs weight in rats administered CP. Also, the prior work of [25] has confirmed that CP caused a decline in the body, testicular, epididymis, and seminal vesicle weights in experimental rats. There are reports of biochemical and testicular tissue alterations such as in germinal epithelium and long-term failure of spermatogenesis due to its alkylation property following CP treatments [26].

The epididymis is an essential structural part of the testis conferring fecundity capacity on the spermatozoa

and the development of rapid progressive motility. It is understood that certain anomalies may originate from this critical region [27]. Cisplatin treatment alone significantly ($p < 0.05$) resulted in caudal epididymal sperm morphological abnormalities when compared with the control. This result is consistent with [7] who established that the toxicity effects caused by CP treatment include a decrease in sperm count, alteration in sperm motility, and an increase in abnormal sperm ratios. This is in agreement with previous reports on platinum compounds in humans [28] and animal [29, 30] also reported that the percentage of normal sperm cells was decreased in mice treated with a single dose of CP.

Furthermore, in experimental animals, acute exposure to chemotherapeutic agents like CP has revealed an increase in the occurrence of germ-cell apoptosis. It can also lead to decreased reproductive organ weights, azoospermia, and degenerated spermatogenic cells [31, 32]. In the current study, the fertilizing ability of the CP treated group (group 2) was significantly reduced. The reduction in fertility of the rats could be due to high percentages of deformed/abnormal sperm cells (headless, bent sperm, and tailless sperm) as compared to the control. However, the spermatozoa recovered from the cauda epididymis of the rats in groups 5 and 6, co-administered CP and LLE at 200 and 400 mg/kg

Table 3 Protective effects of oral administration of LLE for 26 days on sperm parameters of CP treated male Wistar rats

Groups	% Sperm motility	% Sperm viability	Sperm conc.	Acrosome integrity (live)	Acrosome Integrity (dead)	Sperm Morp. (normal)	Sperm morp. (abn)
Dist water	66.0 ± 2.45	65.2±2.24	5.40±0.18	69.18±1.29	31.27±1.67	65.25±3.97	34.75±3.97
CP	40.0 ± 4.47	40.0±4.47	3.95±0.20	22.46±2.78	77.54±2.78	28.60±0.82	71.40±0.82
LLE 200mg	88.6 ± 1.86 ^{ab}	88.6±1.86 ^{ab}	9.65±0.15 ^{ab}	77.74±4.63 ^b	22.29±4.63 ^b	80.93±4.45 ^{ab}	19.06±4.45 ^{ab}
LLE 400mg	81.25±2.39 ^{ab}	81.25±2.39 ^{ab}	7.44±0.40 ^{ab}	70.03±0.66 ^b	29.98±0.66 ^b	73.98±1.92 ^b	26.02±1.92 ^b
(CIS+LLE 200mg)	73.0 ± 3.00 ^b	73.0±3.00 ^b	7.70±0.29 ^{ab}	71.39±0.76 ^b	28.61±0.76 ^b	71.29±0.48 ^b	28.70±0.48 ^b
(CIS+LLE 400mg)	80.24±3.15 ^{ab}	80.25±3.15 ^{ab}	8.44±0.39 ^{ab}	74.65±1.21 ^b	25.35±1.21 ^b	79.48±1.11 ^{ab}	20.52±1.11 ^{ab}

Note: The values are presented as Means ± SEM, (n = 5)^a Significant Differences as compared with normal control group $p < 0.05$ ^b Significant Differences as compared with the negative control group at $p < 0.05$

respectively have high percentages of normal sperm with head, body, and tail intact that can naturally ascend the female reproductive tract to the site of fertilization and also to achieve fertilization [33]. Though descriptions for the recorded abnormalities could be conjectural, however, the aberrations could have resulted from the abnormal chromosome, a slight modification in testicular DNA, or errors during the process of spermatogenesis [34]. The data from the present study shows that the treatment of rats with LLE has the potential to improve the reproductive fitness of rats. Interestingly, treatment with LLE alone at the dose of 200 mg/kg and 400 mg/kg increased sperm count respectively. A corresponding increase in spermatozoa motility was also observed especially in the group treated with LLE at 200 mg/kg. These sperms were capable of forwarding movement needed for fertilization with no defects in flagella substructures.

The reproductive toxicity of CP was further substantiated by the histological analysis of the testes. Testicular sections of control (group) revealed copious seminiferous tubules that were regularly spaced with all cells of the spermatogenic series and interstitial spaces that were essentially normal (slide A). Conversely, rats exposed to CP (group 2) showed disruption of the normal architecture of the testes, e.g., testicular atrophy, degenerative changes in the seminiferous tubules, presence of spermatozoa (Headless and tailless spermatozoa, bent spermatozoa) and degenerative changes in spermatogonia cell lining the seminiferous tubules, associated with incomplete spermatogenesis (slide B). The results of this study are consistent with the findings of [35] in a similar study in which Wistar rats that were exposed to endosulfan, an organochlorine pesticide developed testicular atrophies [36] in another but akin study also established that the exposure of Wistar rats to chlorpyrifos for 30 days at various dosage levels caused degeneration in seminiferous tubule having decreased number of spermatogenic elements in exposed rats.

Histological sections of LLE alone (200 and 400 mg/kg) did not show any deleterious changes in seminiferous tubular architecture and interstitium (slide C and D)

There was a remarkable tissue restoration in the photomicrographs of rats in the groups that were co-administered CP + LLE at 200 mg/kg and 400 mg/kg respectively. The photomicrographs showed the appearance of normal seminiferous tubules and interstitial spaces occupied by the Leydig cells. Most of the seminiferous tubules were close together with regular outlines and narrow interstitium (Slide E and F).

Conclusion

This result may suggest that LLE exhibited a protective effect on the testicular tissues and could favour normal spermatogenesis in the seminiferous tubules of the testes of the rats which may be attributed to the phytochemicals it contains. This strengthens the medicinal use of this herb in managing reproductive related problems.

Abbreviations

LLE: *Lophira lanceolata* leaf extract; LL: *Lophira lanceolata*; CP: Cisplatin; CDDP: Cisplatin, *cis*-diamminedichloroplatinum; ip: Intraperitoneally; PS: Pentobarbitone sodium; Sem V: Seminal Vesicle; Epid: Epididymis; abn: Abnormal; Conc: Concentration; Morph: Morphology; SEM: Standard error of mean; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; n: Number of animals; no: Number; ANOVA: One-way analysis of variance

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Authors' contributions

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The protocols of this study were approved by the Animal Welfare and Ethics Committee of the College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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