Trop J Nat Prod Res, April 2021; 5(4):744-748

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Influence of *Lophira lanceolata* Leaf Extract on Reproductive Indices and Hematological Parameters in Male Wistar Rats

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ARTICLE INFO

Article history:
Received 07 September 2020
Revised 11 January 2021
Accepted 10 April 2021
Published online 03 May 2021

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ABSTRACT

Lophira lanceolata is a medicinal plant used in folk medicine for the treatment of toothache, liver infections, cough, fever and heart pains. Currently, the patronage of medicinal plants as an alternative to synthetic drugs has increased. This present study aim to investigate the effects of *Lophira. lanceolata* leaf extract on reproductive indices, haematological parameters and serum biochemical parameter in male Wistar rats. Mature rats weighing 200-220g, n= 5 per group were administered *L. lanceolata* extract (100, 200 and 300 mg/kg body weight) by oral gavage for 21 days. The cauda epididymal sperm count, motility, morphology, livability and hematological parameters were assessed by standard procedures. The levels of testosterone, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined spectrophotometrically. Formalin-fixed, H&E stained tissues were examined using a light microscope. The results showed that administration of 100, 200 and 300 mg/kg body weight of *L. lanceolata* extract decreased the sperm count significantly ($p< 0.05$) by 27, 36 and 38% and the sperm motility by 50, 47 and 80% respectively. The percentage abnormality also increased by 10, 31 and 17% respectively. Similarly, histopathological changes were observed in the testes of rats orally administered *L.lanceolata* extract. However, there were no significant changes in the hematological indices and activities of AST, ALT and ALP compared to control. This study showed that *L. lanceolata* had adverse effects on male reproductive parameters but had no effects on hematological indices and liver enzyme activities. Therefore, the extract should be administered with caution.

***Keywords*:** *Lophira lanceolata*, Testes, Testosterone, Sperm count.

Introduction

Lophira lanceolata (LL), commonly known as iron wood, belongs to the family Ohanaceo. It is distributed in West and Central Africa including the northern states of Nigeria. The local name of the plant in Hausa is Namijinkande, Ikponhon in Yoruba, Okpopia in Igbo and Maganchi in Nupe. [1]The edible oil extracted from the plant is called '*meni oil*' and it has cosmetics and medicinal uses. [2] *L. lanceolata* is used for the treatment of toothache, liver infections, female sterility, cough, fever, heart pains, blood spitting, intercostals pain, stomach pain, dysmenorrhea, respiratory troubles, and to relieve the gripping of dysentery.[3]In Nigeria, it is used in the management of jaundice, measles and liver injury.[4, 5] Traditional knowledge of indigenous communities about plant diversity makes them major contributors to primary health care for majority of the world population. While some medicinal plants are effective in the management of certain ailments, a number of them may pose a potential threat to male reproductive organ.

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Citation: Nwaechefu OO, Arojojoye OA, Oyedeji TA, Ojo OO, Balogun OA, Kassim S. Influence of *Lophira lanceolata* Leaf Extract on Reproductive Indices and Hematological Parameters in Male Wistar Rats. Trop J Nat Prod Res. 2021; 5(4):744-748. doi.org/10.26538/tjnpr/v5i4.24

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Recent studies on *L. lanceolata* bark showed that it is a potent inhibitor of the development of Plasmodium sporogonic stages indicating that the plant contains antimalarial agents acting on transmissible stages in the human and mosquito host. [6] However, there is insufficient data on the effect of *L. lanceolata* extract on the male reproductive parameters. Therefore, the aim of this study is to investigate the effect of the aqueous extract of *L. lanceolata* leaves on reproductive indices haematological parameters and serum biochemical parameter in male Wistar rats.[3]

Materials and Methods

Plant collection and preparation of extracts

The leaves of *L. lanceolata* were obtained from a local vendor at Oje market in Ibadan, Oyo State, Nigeria in August, 2018. The leaves were identified and authenticated in the Department of Botany, University of Ibadan with Voucher specimen number UIH-22985. The leaves of *L. lanceolata* were air-dried, blended, soaked in water for 72 hours and filtered using a whatmann filter paper. The filtrate was concentrated using a rotary evaporator (Buchi Rotavapor-R coupled with Speedmac Edwards High Vacuum Pump, Thermo Circulator, product of Churchill Instrument Co. Ltd) under reduced pressure at 40^{o}C.

Phytochemical screening

Qualitative phytochemical screening of the aqueous extract of *L. lanceolata* leaves were carried out according to the method described by Sofowora. [7]

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Animal handling
The animals were housed in well ventilated cages and allowed to have 12 hr light/12 hr dark cycle ± 25^{o} and fed with commercial pellet diet (Vital feeds Ltd. Nigeria) and clean drinking water *ad libitum*. All experimental protocols were in line with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by Lead City University Ethical Research Board (LCU/ERB 0745).

Experimental design
Twenty Wistar rats weighing between 200-220 g were obtained from the Animal House, Lead City University, Ibadan, Nigeria. The animals were randomly divided into four groups of five animals per group. Animals in group 1 received normal saline while animals in groups 2, 3 and 4 received 100, 200 and 300 mg/kg body weight of *L. lanceolata* extract leaves for 21 days. After a twenty-four hour fast, all the rats were sacrificed by cervical dislocation and blood was collected by cardiac puncture for haematological analyses. The testes and epididymis were quickly removed and weighed. Samples from testes and epididymis were fixed with Bouin's solution.

Determination of relative testes and epididymis weight
Atrophy of the testes and epididymis were estimated by comparing the testes and epididymis weight to their respective body weight (relative weight).

Relative weight (mg/100 g body weight)

$$= \frac{Testes\ or\ Epidydymis\ weight\ (g)}{Total\ body\ weight\ (g)}\ x\ 100$$

Sperm motility assay
The right caudal epididymis was minced in a petri dish containing distilled water. After this, large tissues were removed by filtering through a nylon mesh in order to obtain a clear filtrate containing suspended sperm cell. Sperm suspension, 2-3 drops were put on a slide, and the diluents (buffered 2.9% sodium citrate solution) added and kept at 37^{o}C. This was added to the sperm suspension until the desired dilution was obtained. The motility of epididymis sperm cell was evaluated microscopically within 2-4 minutes after their isolation according to the method described by Zemjanis. [8] The results obtained was expressed in percentage.

Evaluation of epididymal sperm count
The sperm suspension obtained by mincing the caudal epididymis in distilled water was used for the sperm analysis. The sperm cells or spermatozoa were estimated by hemocytometer using the improved Neubauer (Deep 1/10 min, LABART, Germany) chamber as described by Pant and Srivasterva [9].

Sperm morphological abnormalities and percentage viability assay
Two drops of the sperm suspension was dispensed on a glass slide and was stained with Awa stain (0.6 g fast green and eosin negrosine, 0.2 g) dissolved in ethanol and distilled water, 1:2). Sperm morphology was examined using a light microscope at 100 X according to the method of Adedara and Farombi [10] The live / dead ratio of sperm cells were determined according to the methods of Wells and Awa [11]

Haematological studies
Blood was collected by cardiac puncture into EDTA bottles for haematological studies. For serum biochemical analysis, blood was collected in plain tubes and centrifuged at 3000 rpm for 15 minutes. The serum was dispensed into clean test tubes for biochemical analysis. The RBC, WBC *differentials* were estimated using the improved Neubauer counting chamber as described by Dacie and Lewis. [12] The Hb concentration was determined by the Cyanmethhaemoglobin method and the PCV was determined by the micro method, as described by Dacie and Lewis. [12]

Hormonal assay
Serum testosterone was assayed using the enzyme-linked immunosorbent assay ELISA kit (Immuno-Biological Laboratories Inc., Minneapolis, MN, USA).

Determination of liver enzyme activities
Assay for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were performed using commercially available kits adhering strictly to the manufacturer's instruction.

Histology
Fresh portions of testes was fixed in Bouins' fluid and then dehydrated, with ethanol. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Sections (4–5 µM) were stained with conventional histological stains. The tissues were viewed under a light microscope and the photomicrograph taken. Stained sections of control and rats orally administered *L. lanceolata* extract were examined for alterations in the architecture, portal triads and hepatocytes.

Statistics
The data derived from the study was analyzed with one way analysis of variance (ANOVA) and Turkey post-hoc test using SPSS 16 package. A probability value of $p < 0.05$ was considered statistically significant.

Results and Discussion

No pathological changes were observed in testes section of the normal control rats as normal spermatogenic epithelium was revealed (Figure 1 A). However, rats administered 100 mg/kg showed disruption of the seminiferous tubule outlines depleted spermatogenic epithelium (Figure 1B). Figure 1C showed that treatment with 200 mg/kg of the extract caused necrosis of the seminiferous tubules, pyknosis of the nuclei and general disorganization of the histo-architecture of the testis. Testes section of rats orally administered 300 mg/kg of the extract showed depleted spermatogenic epithelium.
Medicinal plants have shown usefulness in the treatment of many disease conditions, however, some of them are capable of producing wide range of undesirable effects that affect some physiological parameters.[13, 14] This study examined the effects of oral administration of the *L. lanceolata* extract on reproductive indices, hematological indices, biochemical parameters and after twenty one days.
The weight of testes decreased following oral administration of the extract in the groups orally administered *L. lanceolata* extract compared with the control, atrophy of the testes was observed at highest concentration as shown in table 2. It is well known that the weight and size of organ is closely related to the secretory function. It is expected that this reduction of weight of testes would affect the reproductive function and parameters. Our result support previous data which showed that acute fluid loss, proteolysis and lipolysis are responsible for the decrease in organ weight in the rats orally administered *L. lanceolata* extract.[15, 16] Phytochemical screening of extract showed the presence of alkaloids, cardenolides, flavonoids, polyphenols, saponin and polyphenols (Table 1) *explaining* its' usefulness as therapeutic agents against malaria, jaundice, measles and liver injury.
Spermatogenesis is a complex process by which undifferentiated germ cells undergoes multiplication and maturation to form functional haploid spermatozoa. Endocrine regulation by testosterone and the architecture of the Sertoli cells and seminiferous tubules also forms an important decisive factor in spermatogenesis. From our result sperm count and motility decreased significantly ($p<0.05$) by 27, 36, 38 and 50, 47, 80% respectively when administered 100, 200 and 300mg/kg L. *lanceolata* extract compared with control. The percentage abnormality also increased by 10, 31, and 17% at the respective doses (Table 3). The decrease in sperm viability (live/dead) agreed with reduction in the progressive sperm motility because immobile sperms were considered dead as they took up the Eosin/Nigrosinstain when the smear was examined.
Our findings showed that while increased sperm abnormality was observed in groups orally administered *L. lanceolata* extract, the livability increased while motility and count were decreased.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Similarly, pathological features showed depleted spermatogenic epithelium, necrosis of the seminiferous tubules, and general disorganization of the histo-architecture of the testis culminating in reduced spermatogenesis. This gives credence to the previous study of Auta and Hassan.[17] Several plants have been reported to adversely affect reproductive function, despite their therapeutic effects, these include *Barleriaprionitis*, *Dendrophthoefalcata*, among others.[18-20] Testosterone is a male hormone that has significant impact on spermatogenesis.[21] Testosterone production is directly dependent on the concentration (or activity) of luteinizing hormone (LH), in the milieu secreted by the anterior pituitary gland.[22] Testosterone has profound influence on germ cell development and differentiation. It exerts a negative feedback action on LH secretion, and also on FSH (at higher concentration) acting on hypothalamic-pituitary axis.[23]

Table 1: *Phytochemical composition of L. lanceolata* extract

Phytochemical component	RESULT
Alkaloids	+
Cardenolides	+
Anthraquinone	-
Saponin	+
Tannins	+
Flavonoids	+
Polyphenols	+

+ = positive; - = negative

Table 2: Effect of aqueous extract of *L. lanceolata* extract on the weight of organs in Wistar rats

Organ Weight (g)	Control	100 mg/kg	200 mg/kg	300mg/kg
Epididymis	0.30 ± 0.01	0.38 ± 0.01	0.33 ± 0.01	0.39 ± 0.01
Testes	2.46 ± 0.02	2.14 ± 0.09	2.04 ± 0.06	1.78 ± 0.04

Values are expressed as mean ± standard error of mean (n = 5). The significant difference between groups was analyzed using ANOVA

Table 3: The effects of *L. lanceolata* extract on reproductive indices in Wistar rats

Reproductive indices (%)	Control	100 mg/kg	200 mg/kg	300 mg/kg
Sperm Abnormality	10.66 ± 0.46	11.72 ± 0.23*	13.99 ± 1.25*	12.56 ± 0.94*
Livability	95.20 ± 1.27	66.60 ± 2.98*	91.60 ± 3.09*	66.00 ± 7.16*
Sperm volume	5.16 ± 0.02	5.16 ± 0.02	5.18 ± 0.02	5.16 ± 0.02
Sperm motility	84.00 ± 2.48	42.00 ± 1.34*	44.00 ± 2.48*	15.00 ± 2.48*
Sperm count	128.60 ± 6.00	93.40 ± 7.42*	81.40 ± 3.98*	79.60 ± 5.44*

Values are expressed as mean ± standard error of mean (n = 5). The statistical significance of difference between groups where analyzed using ANOVA. *= Statistical difference ($p > 0.05$) compared with control.

Table 4: Effect of *L. lanceolata* extract on serum testosterone level in Wistar rats

Hormonal Profile	Control	100 mg/kg	200 mg/kg	300 mg/kg
Testosterone	0.035 ± 0.009	0.037 ± 0.014	0.036 ± 0.013	0.032 ± 0.010

Data are presented as mean ± standard error of mean (n = 5)

Table 5: Effects of *L. lanceolata* extract on hematological parameters in Wistar rats

Hematological indices	Control	100 mg/kg	200 mg/kg	300 mg/kg
PCV (%)	37 ± 0.70	38 ± 0.72	38 ± 1.04	37 ± 1.88
Hemoglobin (g/dl)	11 ± 0.13	12 ± 0.33	12 ± 0.5	11 ± 0.34
Red blood cell (%)	6 ± 0.24	6 ± 0.13	6 ± 0.31	6 ± 0.71
MCV (FL)	60 ± 1.62	59 ± 0.75	62 ± 1.04	61 ± 1.00
MCH (pg)	18 ± 0.73	18 ± 0.42	19 ± 0.40	19 ± 0.7
MCHC (%)	31 ± 0.63	31 ± 0.35	31 ± 0.37	31 ± 0.57
Neutrophils (%)	29 ± 2.32	23 ± 3.45	24 ± 2.32	30 ± 3.81
Monocytes (%)	2.40 ± 0.25	2.40 ± 0.64	2.60 ± 0.25	2 ± 0.62
Eosinophils (%)	1 ± 0.35	2 ± 0.50	2 ± 0.50	1 ± 0.80
Lymphocyte (%)	67.20 ± 2.97	71.80 ± 2.54	70.40 ± 3.54	66.80 ± 3.50

Values are expressed as mean ± standard error of mean (n = 5) the statistical significance of difference between groups were analyzes using ANOVA.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Effects of *L. lanceolata* extract on serum biochemical parameters in Wistar rats

Groups	Treatments	AST	ALT	ALP
I	Control	14.3 ± 0.9	12.5 ± 0.4	13.8 ± 1.0
II	100mg /kg	12.0 ± 0.8	13.3 ± 0.2	14.7 ± 0.7
III	200mg /kg	13.0 ± 0.6	14.3 ± 0.6	18.4 ± 0.6
IV	300mg /kg	15.0 ± 2.0	12.5 ± 0.6	18.4 ± 0.5

Values are mean ± standard error of mean, n = 5

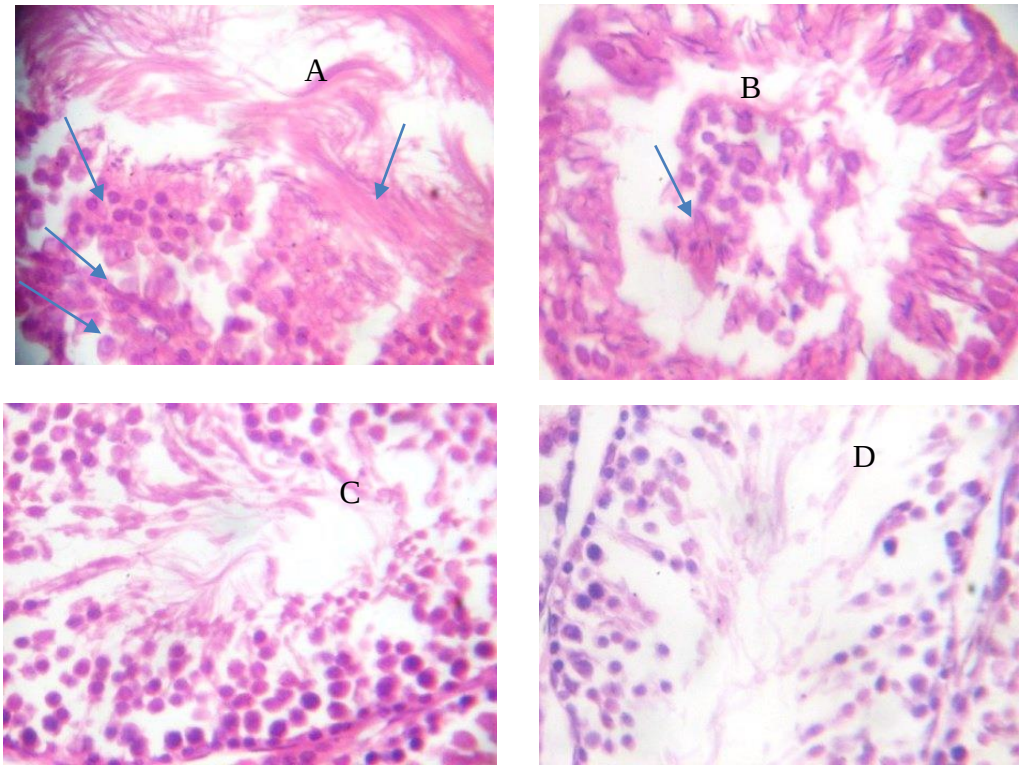

Figure 1: Effects of *L. lanceolata* extract on testicular structure in Wistar rats. Representative photomicrographs showing the testicular structures of rats at 400X magnification in experimental groups. **(A)** Control; **(B)** 100 mg/kg **(C)** 200 mg/kg **(D)** 300 mg/kg H&E Stain X400

The testosterone level has been used scientifically as a reproductive parameter. Our result showed that administration of the extract did not have significant effect on testosterone levels.

The results in Table 5 showed the assessment of hematological parameters the result suggest that extract was not toxic to the blood parameters as the levels of Hb, PCV, RBC, MCHC, WBC, MCV, MCH, neutrophils, monocytes, and eosinophils were not significantly affected relative to control. This revealed safety of the plant on the hematological parameters studied. Our current haematological data support the earlier reports on *Maerua crassifolia*.[24] Similarly, ALT, AST, and ALP activities (table 6) were not significantly elevated in the serum of rats orally administered *L. lanceolata* extract relative to control. Furthermore, the hepatocellular biomarkers showed that ALT, AST, and ALP activities were not affected by the administration of extract in rats, showing there was no hepatotoxicity. Liver cell damage releases transaminases into blood stream, where they can be measured they are therefore the index of liver injury.[25] Our result are consistent with the findings of Mensah, 2019.[26]

Conclusion

This study showed that administration of *L. lanceolata* extract altered reproductive parameters but had no effects on hematological indices and liver enzyme activities. Therefore, the extract should be administered with caution.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Acknowledgements

The authors acknowledge the laboratory staff of Department of Biochemistry, Lead City University, Ibadan.

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