Journal of Advances in Medical and Pharmaceutical Sciences
8(4): 1-8, 2016, Article no.JAMPS.26576
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-fertility Effects of Ethanol Extract of *Salacia lehmbachii* Root Bark in Albino Rats

Grace A. Essiet[1*], Augustine D. Essien[1], Francis V. Udoh[1] and Akanimo Essiet[2]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.
[2]Department of Surgery, Division of Urology, Faculty of Medicine and Dentistry, University of Calabar, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GAE designed the study, wrote the protocol and the first draft of the manuscript. Author ADE managed the experimental process and did the literature searches. Author FVU carried out the statistical analysis of the study. Author AE edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/26576
Editor(s):
(1) Nissar Darmani, Professor of Pharmacology, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, California, USA.
Reviewers:
(1) Muhammad Asam Raza, University of Gujrat, Gujrat, Pakistan.
(2) Naglaa Gamil Shehab, Cairo University, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/15094

Original Research Article

Received 24th April 2016
Accepted 24th May 2016
Published 21st June 2016

ABSTRACT

Aim: With the reported impairment of male fertility by herbal remedies in the literature, ethanol extract of the root bark of *Salacia lehmbachii*, a commonly used remedy by locals for febrile illnesses, was studied to evaluate its effect on the fertilization potential of sperm cells in rats.
Methodology: Twenty four sexually mature male rats weighing 220-250 g and 24 virgin female rats weighing 150-180 g were used for this study. The male rats were randomly assigned 4 groups (n=6), labeled Control, A, B and C. Control rats received 2 mL of distilled water (vehicle) orally. Groups A, B and C received 250, 500 and 750 mg/kg body weight of ethanol extract of *Salacia lehmbachii* root bark (ESLR) respectively. Preparation of extract was by Soxhlet extraction of petroleum ether defatted plant residue using ethanol. Administration to the rats was orally using a cannula for 56 days. Natural ovulation was induced in the female rats after which they were paired one to one with the treated male rats and allowed overnight for mating. Vaginal smears from the

**Corresponding author: E-mail: geessiet@yahoo.com;*

female rats were examined for sperm cells the next morning as evidence of successful mating. Fertility indices were computed for the male rats. The rats were weighed, anaesthetized and the testes and left cauda epididymis harvested. Testicular weights and lengths were taken. Sperm from the left cauda epididymis was analyzed with an automated semen analyzer for counts while morphological characteristics were assessed with a microscope.
Results: There was a significant (P<.05) dose dependent decrease in testicular weights, sperm indices and fertilizing potential in treated rats. More primary and secondary sperm abnormalities were seen in treated rats and testicular cyto-architecture was altered at high doses.
Conclusion: At the doses used in this study, ESLR decreases fertility in male rats.

Keywords: Salacia lehmbachii; male fertility; ethanol extract; sperm indices; root bark.

1. INTRODUCTION

Fertility and conception are fundamental to the propagation of life. Conception usually follows sexual interaction between mature males and females. When conception fails to occur with up to 12 months of sexual activity without contraception, infertility is considered [1]. Infertility is a global problem and may be contributed to by either the male partner, the female partner or by both partners. It is a veritable cause of emotional and psychological distress [2,3]. Thirty percent (30%) of all cases of infertility has been documented as being attributable to the male factor [4]. Male fertility involves a process of spermatogenesis which should normally yield adequate quantities and quality of spermatozoa. There should also be a normal desire and ability to copulate [5]. These processes are controlled by male reproductive hormones. Spermatogenesis requires adequate numbers, quality and activity of Sertoli and Leydig cells in the testes as well as the supporting sustentacular cells [6]. Inadequacies in these cells states may result in abnormal sperm indices like low count, poor quality, or both. Abnormal sperm indices have been identified in about 90% cases of male infertility [7]. Although the causes of sperm abnormalities are unknown in 30- 40% cases, certain therapeutic agents including herbal remedies have been implicated as aetiological factors [8,9].

The use of herbal preparations for the treatment of many endemic ailments is in common practice especially in developing countries. Often, such preparations are in form of plant extracts, some of which have been shown to adversely affect sperm parameters [10-14]. *Salacia lehmbachii* (Family, *Celastraceae*) is a medicinal plant about 3 meters in height, found commonly in the Southeastern states of Nigeria especially Bakassi forest reserves and parts of Southern Cameroun. It is used by the locals in these places for the treatment of malaria and other febrile illnesses. The median lethal dose (LD_{50}) of the aqueous root bark extract of the plant was found to be above 5000 mg/kg in albino rats and to contain alkaloids, glycosides, flavonoids, tannins, saponins and polyphenols [15]. The biological activities of this plant have been documented [15-18]. The antimalarial action of the plant has been scientifically established (Essien AD, University of Calabar, Calabar, Nigeria, unpublished results).The ethanol extract of the plant's root bark has been reported to have inhibitory effect on serum levels of male reproductive hormones [19]. The current study was designed to evaluate the effect of ethanol root bark extract of the plant on male fertility indices such as sperm parameters, success in mating and fertilizing potential of sperm in male albino rats.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The roots of *salacia lehmbachii* were sourced from Watt market, a local market in Calabar, capital of Cross River State, Nigeria. Authentication of the plant was undertaken by Botanists in the department of Botany of the University of Calabar and a Voucher Specimen with herbarium number 688 deposited therein.

2.2 Preparation of the Extract

The roots were washed with water to remove dirt and dried in an electric oven, thermostatically controlled at 40℃, for 12 hours. The bark was obtained by striking the dry roots on a hard surface and the pieces obtained pulverized into a coarse powder with a hand operated grain mill (Corona®, Columbia). The root bark powder was stored in an airtight container. Five hundred grammes of the root bark powder was Soxhlet extracted in a two-stage process beginning with petroleum ether (99.9%, Sigma Chemical

Limited, USA) at 65℃ as solvent for twelve hours to remove fats. The petroleum ether residue was dried, weighed and re-extracted with ethanol at 60℃ for 72 hours to give ethanol extract solution which was evaporated to dryness using a rotatory evaporator at a reduced temperature of 45℃ in-vacuo. The solid extract was weighed, put in a clean specimen bottle and preserved in a refrigerator, until required for the experiments.

2.3 Experimental Animals

Sexually mature male albino rats weighing between 220-250 g and twenty four virgin female rats within the weight range of 150-180 g were sourced from the animal house of the department of Pharmacology, University of Calabar and used for the study. They were housed in adequately ventilated plastic cages, each containing six properly identified rats. The animals were acclimatized for seven days to normal laboratory conditions (relative humidity: 50±5%, temperature: 28±2℃,12 hr of light-dark cycle and good ventillation) before the start of the experiment and maintained at the same conditions throughout the duration of experimentation. They were fed *ad libitum* with standard rat chow (Agro-Feeds, Calabar) and water (Water board, Calabar). The guidelines on Care and Use of Laboratory animals [20] were strictly observed. The experimental protocols were examined and approved by the appropriate ethics committee.

2.4 Animal Treatment

Twenty four male rats were randomly divided into four groups of six rats per group. The groups were labeled as Control, A, B and C. Control group rats were given 2mls of distilled water, the vehicle for the extract, while rats in groups A, B and C were treated with 250, 500 and 750 mg/kg body weight of the ethanol extract of *Salacia lehmbachii* root bark respectively. Administration which was orally using a gastric cannula was carried out daily for 56 days between the hours of 9 a.m. and 10 a.m. to mitigate the effect of the circadian rhythm. The female rats were not treated.

2.5 Evaluation of the Fertility Potential of the Male Rats

The method described by Yakubu and Afolayan [21] was adopted with slight modifications. At the end of the 56 day period, the control and treated male rats were caged individually. Ovulation in the female rats was induced by the natural method [22]. In this method, male and female rats are put in a single cage separated by wire gauze which allows enough closeness for sexual arousal but without the possibility of physical contact. The rats were allowed adequate food and water and kept in that state for four days. On the 5^{th} day, vaginal smears of the female rats were microscopically examined to ascertain if they were in the estrous phase. Each treated male rat was then paired with an ovulating female rat and mating was allowed to continue overnight. The female rats were observed the next morning for sperm plug in the vagina or sperm cells in the vaginal smears as evidence of successful mating. The following were used as fertility indices for the male rats:

i) Percentage mating success (number of mated rats **/** number of rats paired x 100)
ii) Percentage quantal frequency (number of pregnant rats/number of mated rats x 100)
iii) Percentage fertility success (number of pregnant rats **/** number of rats paired x 100)

2.6 Evaluation of Sperm Characteristics

At the end of experimentation period, animals were weighed, anaesthetized with chloroform vapour and sacrificed. The testes and epididymis were harvested and cleaned in between filter papers. The weight and axial measurements of each testis was obtained. Semen was collected from the epididymis as described by Oyeyemi and Ubiogoro [23] and the samples used to assess sperm parameters using an automated sperm analyzer ((Semelog, Semesco 150, Tokyo). Morphological characteristics of the sperm were evaluated with a light microscope (Olympus/3H, Japan).

2.7 Histological Examination of Rat Testes

Testes from sacrificed rats were fixed in 10% formalin and embedded in paraffin. Sections, 5 microns thick were prepared, stained with Hematoxylin and Eosin (H&E), examined under a light microscope (Olympus/3H, Japan) and photomicrographs obtained at magnifications 100 and 400.

2.8 Statistical Analysis

Data obtained from the study was processed using Statistical Package for Social Sciences (SPSS) version 20 and results presented by

descriptive statistics as means ± standard error of mean (SEM). Turkey's multiple comparison post hoc testing was carried out to compare the different study groups. Students t-test was used to compare significant differences between treated and control groups at discreet dose levels. Differences were considered significant at P values of $P<0.05$.

3. RESULTS

Treated rats produced significant ($P<0.05$) dose dependent reduction in the weights and lengths of testes compared to control (Table 1). Fertility indices namely mating success, quantal frequency and successful fertilization were significantly reduced ($P<0.05$) at high doses (500 mg/kg and 750 mg/kg) of the extract while low dose (250 mg/kg) produced no adverse effects (Table 1).

The extract significantly depressed sperm count ($P<0.05$) at all doses as shown in the comparison of the treated groups with control (Table 1). Sperm motility and morphology were also impaired at all doses but the impairment in each case was only significant ($P<0.05$) at the highest dose of 750 mg/kg (Fig. 1).

Morphological abnormalities of the sperm cells namely pin head, headless tail, tailless head, rudimentary tail, bent tail and bent mid-piece were also observed (Table 2). Compared to the control group pin head abnormality rate was significantly higher ($P<0.05$) in the treated group at high doses (500 and 750 mg/kg) of the extract. Increase headless tail abnormality rate was not statistically significant ($P>0.05$). However at high doses of the extract, (500 & 750 mg/kg), tailless head abnormality was significantly increased ($P<0.05$) in a dose dependent manner, while the percentage of cells with rudimentary tails was not significantly increased ($P>0.05$). Sperm cells with bent tail abnormality were also not significantly increased ($P>0.05$) in numbers while those with bent mid-piece abnormality showed a significant increase ($P<0.05$).

Testicular tissue sections of rats treated with 250 mg/kg of the extract showed no visible histological changes (Fig. 3), but at a higher dose (500 mg/kg), there were degenerating germ cells in several seminiferous tubules (Fig. 4) and at the highest dose (750 mg/kg), several degenerating seminiferous tubules (ST) and loss of interstitial space integrity were observed (Fig. 5).

Table 1. Effects of ethanol root bark extracts of *Salacia lehmbachii* on testicular weights and lengths; and fertility indices of male rats

Parameters	Control	Group A (250 mg/kg)	Group B (500 mg/kg)	Group C (750 mg/kg)
Testes wt (g)	1.10±0.55	1.00±0.81	0.96±0.74*	0.72±0.02*ab
Testes long. length (mm)	18.99±0.12	18.84±1.03	17.22±0.35*	16.67±0.29*ab
Testes trans. length (mm)	10.53±0.15	10.03±1.13	8.39±0.55*	7.29±0.29*ab
Mating success n (%)	6 of 6(100)	5 of 6(83.3)	4 of 6(66.7)*a	3 of 6(50.0)*ab
Quantal frequency n (%)	6 of 6(100)	5 of 5(100)	2 of 4(50.0)*a	1of 3(33.3)*ab
Fertility success n (%)	6 of 6(100)	5 of 6(83.3)	2 of 6(33.3.0)*a	1of 6(16.7)*ab
Sperm count (x106 cells/µL)	583.5±0.12	415.06±0.12*	386.19±0.12	301.12±0.12*ab
Percentage liveability	85.00±2.04	81.25±4.27	84.52±3.23	82.50±1.44

*Legend: Values are in percentages * = significantly different from control at P<0.05, a= significantly different from value for 250 mg/kg at P<0.05 b= significantly different from value for 500 mg/kg at P<0.05*

Table 2. Sperm cell abnormalities in the treated and control rats

Sperm abnormality (%)	Control	Group A (250 mg/kg)	Group B (500 mg/kg)	Group C (750 mg/kg)
Pin head	6.75±0.29	7.10±0.41	8.05±0.48*a	9.25±0.25*ab
Headless tail	1.75±0.48	1.85±0.31	1.88±0.63	1.87±0.25
Tailless head	3.15±0.33	3.75±0.21	5.00±0.24*a	6.23±0.31*ab
Rudimentary tail	1.25±0.11	1.34±0.10	1.42±0.08	1.40±0.09
Bent tail	4.50±0.32	6.01±0.38*	6.55±0.41*	7.40±0.40*a
Bent mid-piece	5.05±0.63	6.12±0.58*	7.10±0.50*a	7.35±0.48*a

*Legend: Values are in percentages * = significantly different from control at P<0.05 a= significantly different from value for 250 mg/kg at P<0.05 b= significantly different from value for 500 mg/kg at P<0.05*

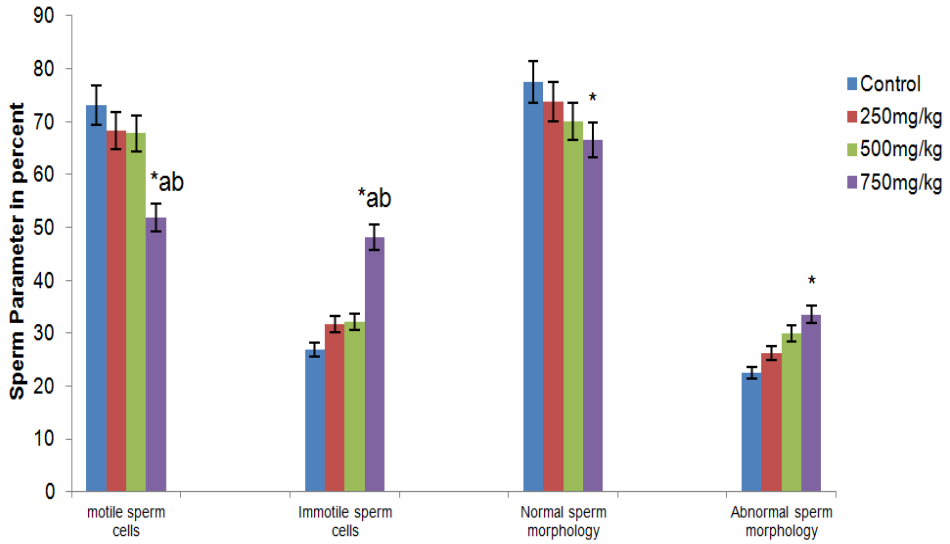
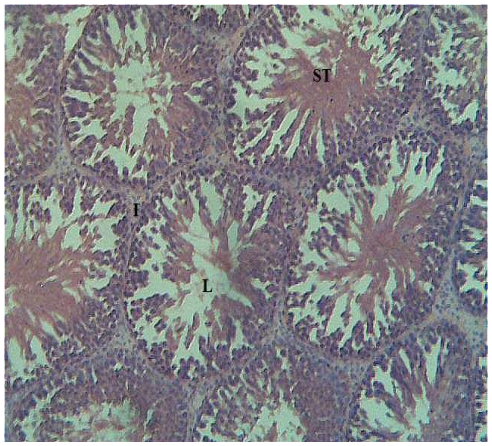


Fig. 1. Effects of ethanol extract of *Salacia lehmbachii root bark on sperm motility* and *morphology*

*Values are expressed as mean ± SEM. n = 6. * = significant different from control at p<0.05 a = significant different from value for 250 mg/kg ESL at p<0.05 b= significant different from value for 500 mg/kg ESL at p<0.05*



Magnification x 100. H&E stain

Fig. 2. Photomicrograph of testicular tissue section of rats treated with distilled water (Control) for 56 days

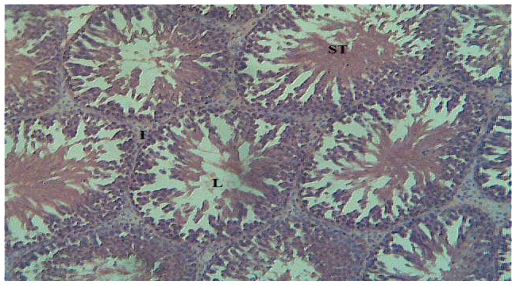
Normal testicular tissue section showing several seminiferous tubules (ST) with lumen (L) and interstitial space (I) containing the interstitial cells. The seminiferous epithelium shows germ cells at different stages of development

4. DISCUSSION

The evaluation of certain andrological parameters is important in the determination of fertility in males [24-26]. When the percentage of sperm cell abnormalities in semen reaches a critical level (≤ 10%), male infertility may result [27]. The weight of the testis is a known sensitive end point used for assessment of the direct effect of a compound on testicular cells. Alterations in testicular cell activity may affect the metabolic and secretory functions of the gland [28]. In this study, testicular sizes obtained by measurements of the longitudinal and transverse dimensions and weights of the testes were significantly reduced (*P*<.05) in rats treated with high doses (500, 750 mg/kg) of extract compared to the control group. The alterations in testicular size may explain the observed changes in sperm indices. To affect testicular size, much of the extract would have gained entry into testicular cells to affect its functions. This is in spite of the blood-testis barrier known to restrict entry of xenobiotics into testicular tissue [29], it has been established that a healthy testicular and epididymal microenvironment is necessary for proper spermatogenesis [30] and any disturbance to this environment manifests as alterations in sperm characteristics. The observed depression in sperm parameters (total sperm count, motility and morphology) in the extract-treated rats may thus have been from disturbance of epididymal microenvironment.

Sperm count depicts fairly accurately, the efficiency of spermatogenesis as it shows the picture at all stages of sperm production, and has a high correlation with fertility [31]. Findings of

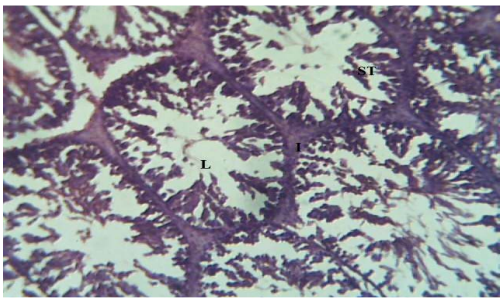
significant decrease (*P*<.05) in sperm count in treated rats compared to control may be from inhibited spermatogenesis. Although low sperm count may result from direct cytotoxic action of a compound on sperm cells, this is unlikely to be the case in this study since the live/dead ratio of sperm cells in treated rats were not significantly (*P*>.05) different from those of the control.



Magnification x 100. H&E stain

Fig. 3. Photomicrograph of testicular tissue section of rat treated with 250 mg/kg body weight of ethanol extract of *Salacia lehmbachii* for 56 days

Photomicrograph of testicular tissue section showing several seminiferous tubules (ST) with lumen (L) and interstitial space containing the interstitial cells. There is no visible lesion

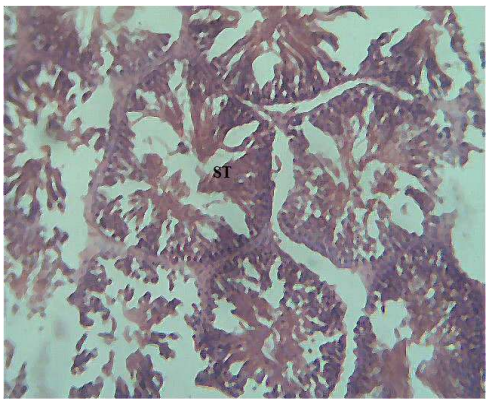


Magnification x 100. H&E stain

Fig. 4. Photomicrograph of testicular tissue section of rat treated with 500 mg/kg body weight of ethanol extract of *Salacia lehmbachii* for 56 days

Photomicrograph of testicular tissue section showing several seminiferous tubules (ST) containing degenerating germ cells. The interstitial space (I) contains the interstitial cells

Sperm morphology is equally important in male fertility because a spermatozoon defective in size and shape may not undergo capitation to enable it reach and penetrate an ovum [32,33]. Sperm motility and morphology in treated rats were significantly (P<.05) depressed in this study. Disturbance of epididymal microenvironment mentioned above may also have hindered normal maturation of the sperm cells resulting in the observed defects in shape and form rendering the sperm cells non-motile. Histologically it may be inferred that the extract, at high doses (500, 750 mg/kg), has a deleterious effect on the seminiferous tubules of rats because of the finding of germ cells with various degrees of degenerative change in the testicles of treated rats.



Magnification x 100. H&E stain

Fig. 5. Photomicrograph of testicular tissue section of rat treated with 750 mg/kg body weight of ethanol extract of *Salacia lehmbachii*

Photomicrograph of testicular tissue sections showing several degenerating seminiferous tubules (ST) and loss of interstitial space (I) integrity

Fertility in a male rat is determined by sperm parameters, sexual desire and copulating abililty. All all of these are controlled by reproductive hormonal activity [5]. The inhibitory effect of the extract on sperm indices has already been discussed above. The observed decrease in mating success rates, quantal frequency and fertility success rates in rats treated with high doses (500, 750 mg/kg) of the extract suggest that sexual desire and copulation were depressed during the period of experimentation. The depressive effect of the extract on serum levels of male reproductive hormones in rat models had been shown in an earlier study as mentioned before. The antifertility effect of the extract may thus be attributed to its depressant effect on male sex hormones which control all aspects of male fertility. Other authors have reported similar detrimental effects on male rat fertility following treatments with medicinal plants

like *Carica* papaya [34], *Achilea mellefolium* flowers [35], *Hibiscus sabdariffa* [10] *and Quassia amara* [36].

5. CONCLUSION

Findings from this work has shown clearly that exposure of rats to the ethanol extract of *Salacia lehmbachii* root bark for 56 days causes dose dependent decrease in sperm parameters, fertility indices as well as degeneration of seminiferous tubules in treated rat testes. The above indicates that frequent usage of this extract may impair fertility in the male. There is therefore the need to be cautious when using the extract for treatment of ailments in man.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that guidelines on Care and Use of Laboratory animals in research were followed. The experimental protocol was approved by the appropriate ethical committee.

ACKNOWLEDGEMENTS

The authors acknowledge Mr. Ekerette Ekere of Department of Hematology, University of Calabar teaching hospital for technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bashir A, Tahir M, Samee W, Munir B. Effect of tribulus terrestris on testicular development of immature albino rats. Biomedica. 2009;25:63-68.
2. Cui W. Mother or nothing: The agony of infertility. Bull World Health Organ. 2010; 88:881–882.
3. Chachamovich JR, Chachamovich E, Ezer H, Fleck MP, Knauth D. Investigating quality of life and health-related quality of life in infertility: A systematic review. J Psychosom Obstet Gynaecol. 2010;31: 101–110.
4. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992;305:609-613.
5. Oyeyem M, Olukole S, Esan O. Sperm morphological studies of West African dwarf bucks treated with pumpkin plant (*Cucurbita pepo*). Int J Morphol. 2008; 26:121-126.
6. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D, Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. J Steroid Biochem Mol Biol. 2008;109(3-5):323-330.
7. Isidori AM, Pozza C, Gianfrilli D, Isidori A. Medical treatment to improve sperm quality. J Reprod Biomed. 2006;12:704-714.
8. Mosher WD, Pratt WF. Fecundity and infertility in the United States: Incidence and trends. J Fertil Steril. 1991;56:192-193.
9. Steven RL, David HB, MZ, Barry W, Hussein A, Brian C, Harry F, Patricia B. Abnormal sperm morphology is highly predictive of pregnancy outcome during controlled ovarian hyperstimulation and intrauterine insemination. J Assisted Reprod Genet. 1996;13(7):569-572.
10. Arabi OH, Kahil MA, Ibrahim NA. The effects of aqueous extract of *Hibiscus sabdariffa* on spermatogenesis and sperm parameters of mice. Int J Cur Res Rev. 2014;6(10):51-56.
11. Venma PK, Sharma A, Mathur A, et al. Effects of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. Asian J Androl. 2002;4(1):43-47.
12. Verma OP, Joshi BC, Kumar S, Chaherjee SN. Antifertility effect of malvaviscuss conzattii greenm flower extract (SC) on male albino mice. Indian J Exp Biol. 1980; 18:561-4.
13. Adhikary P, Banerji J, Chowdhury D. Antifertility effect of piper linn. extract on ovary and testis of albino rats. Indian J Exp Biol. 1989;27:868-70.
14. Parandin R, Sadeghipour HR, Haeri RSA. Evaluation of antifertility effect and recovery of the seed oil constituents of Iranian species of *Melia azadrach L.* in Male Rats. J Rep Contracep. 2008;9(3): 161-166.
15. Takem LP, Lawal BAS, Udia PM. Analgesic and acute anti-inflammatory activities of aqueous root extract of *Salacia lehmbachii.* Br J Pharm Res. 2013;4(18): 2172-2181.
16. Essien AD, Takem LP, Udia PM. Role of *Salacia lehmbachii* extract in renal

function. Int J Pharm Pharm Sci. 2014;2 (1):23-28.
17. Takem LP, Lawal BAS, Udoh FV, Abe NP. Anti-abortificient activities of aqueous root extract of *Salacia lehmbachii* in wistar rats. J Pharm Pharm Sci. 2014;1(3):195-199.
18. Okechi WN, Lawal BAS, Wokota NP, Hassan J. Evaluation of *in-vivo* and *in-vitro* antioxidant activities of methanol extract of *Salacia lehmbachii* loes leaf. European J Med Plants. 2015;8(4):214-223.
19. Essiet GA, Essien AD, Akuodor GC, Udoh FV, Essiet A. Effect of ethanol extract root bark of *Salacia lehmbachii* loes on male reproductive hormones in albino rats. European J Med Plants; 2016. "In press".
20. Organization for Economic Co-operation and Development (OECD). Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. AIWC Bulletin. 2000; 11(1-2):1-27.
21. Yakubu MT, Afolayan AJ. Reproductive toxicologic evaluations of bulbine natalensis baker stem extract in albino rats. Theriogenology. 2009;72:322–332.
22. Udoh FV, Udo PB. Hepatotoxicity of the NeOH-extract of *Carica papaya* (pawpaw) seeds in wister rats. Pharm Biol. 2005; 43(4):349-352.
23. Oyeyemi MO, Ubiogoro O. Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of large white boar in Nigeria. Int J Morphol. 2005;23(3):235-239.
24. Saba AB, Oridupa OA, Oyeyemi MO, Osanyigbe OD. Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of lagenaria breviflora roberts. Afr J Biotech. 2009;8(7):1170-1175.
25. Garner DL, Hafez ESE. Spermatozoa and seminal plasma. In: Hafez ESE, (eds.) Reproduction in farm animals (6th ed.) Lea and Febiger, Philadelphia, USA. 1993;165-187.
26. Zemjanis R. Collection and evaluation of semen. In: Diagnostic and Therapeutic Techniques in Animal Reproduction. William and Wilkins company, Baltimore, USA. 1977;242.
27. Creasy DM. Evaluation of testicular toxicology: A synopsis and discussion of the recommendations proposed by the society of toxicologic pathology. Birth Defects Result Part B. Dev Reprod Tox. 2003;68:408-415.
28. Dua AA, Vaidya SR. Sperm motility and morphology as changing parameters linked to Sperm count variations. J Postgrad. 1996; 42:93–96.
29. Craft I, Bennett V, Nicholson N. Fertilizing ability of testicular spermatozoa. Lancet. 1993;342:864–866.
30. Meistrich ML, Finch M, da Cunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. Cancer Res. 1982; 42:122-131.
31. Noarkes DE, Parkinson TJ, England GCW, Arthur GH. Normal reproduction in male animals. In: Arthur's veterinary reproduction and obstetrics (8th edn.). Saunders Publishers, Edinburgh. 2004: 673-694.
32. Thomas MJ, Thomas JA. Toxic responses of the reproductive system. In: Casarett & doull's toxicology- the basic science of poisons (6th ed). McGraw-Hill Medical Publishing Division, New York. 2001;677-681.
33. Chinoy NJ, Padman P. Anti-fertility investigation on the benzene extract of *Carica papaya* seeds in male albino rats. J. Med. Aromatic Plant Sci. 1996;19(2): 422-426.
34. Parandin R, Ghorbani R. Effects of alcoholic extract of *Achilea mellefolium* flowers on fertility parameters of male rats. Int J Pharm Tech Res. 2010;2(4):2492-2496.
35. Parveen S, Das S, Kundra CP, Pereira BM. A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats. Reprod Toxicol. 2003;17(1):45-50.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15094