Journal of Infertility and Reproductive Biology

Journal of Infertility and Reproductive Biology, 2019, Volume 7, Issue 2, Pages: 11-16

Spermatogenic and Steroidogenesis Functions of Rat Testis following Exposure to *Alafia barteri* Leaf Extracts

Adelakun Sunday Aderemi[1*], Ogunlade Babatunde Samson[1], Aniah J. Akomaye[2], Ibiayo Ayooluwa Gabriel[3]

1 Department of Human Anatomy, School of Health and Health Technology, Federal University of Technology, Akure, Nigeria.
2 Department of Anatomy, College of Medicine, University of Abuja, Federal Capital Territory (FCT), Nigeria.
3 Department of Anatomy, College of Medicine, University of Lagos, Lagos, Nigeria

Received: 24/05/2018 Accepted: 12/06/2019 Published: 30/09/2019

Abstract

Background: In Africa and other continents herbal remedies have played essential roles in the treatment of all kinds of diseases since early times. Objectives: To study the impact of *Alafia barteri* on spermatogenesis and steroidogenesis in rats. Materials and Methods: Twenty (n=20) adult male wistar rats randomly assigned into four groups of five rats each consisting of a control which received only 2 ml/kg normal saline and treatment groups at the doses of 100, 300 and 500 mg/kg body weight daily for 28 days via gastric gavage. Parameter tested includes sperm quality, reproductive hormone, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and malondialdehyde (MDA) levels. Results: Sperm quality, total serum testosterone, SOD, CAT, and GPX levels were significantly increased in the *Alafia barteri* group in comparison to controls ($P < 0.05$). Also, rats in the *Alafia barteri* group showed a significant decreased in the level of plasma MDA ($P < 0.05$) in comparison to controls. There was a decreased in Follicle Stimulating Hormone (FSH) but no significant increase in the levels of leutinizing Hormone (LH). Conclusions: The administration of *Alafia barteri* extract significantly increased the sperm quality and possesses profertility properties, these profertility properties can be exploited in male fertility therapy.

Keywords: *Alafia barteri*, Catalas, Spermatogenesis, Superoxide Dismutase, Testosterone

1 Introduction

Infertility is one of the major health challenges in life, and approximately 30% of infertilities are due to a male factor [1,2]. Several conditions can interfere with spermatogenesis and reduce sperm quality and production [3]. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and production of normal sperm [4]. Several diseases, such as coronary heart diseases, diabetes mellitus and chronic liver diseases may interfere with the spermatogenesis process, and therefore sperm quality and quantity may be altered by these diseases [5]. Antioxidants are regarded as significant agents, which contribute to the overall health of the organism [6]. Poly-phenols, as dietary antioxidants, are associated with redox activities and have beneficial effects on health [7]. Oxidative stress is an important process that is involved in multiple conditions like infertility and inflammation [8]. Therefore, these diseases are controlled in people receiving antioxidant supplements [7]. Antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH), have a significant role in establishing a balance between reactive oxygen species (ROS) and antioxidant levels in serum [9]. The use of antioxidants may improve spermatozoa viability and longevity [9].

Alafia barteri is a high-climbing, scandent shrub with small, pure white or pink flowers [10]. It is used in ethnomedicine for the treatment of sickle cell anaemia, rheumatism, eye infections, febrifuges, as chew sticks and tooth ache [11]. The twining stem of *Alafia barteri* is used for the treatment of fever, inflammation and as binding materials for roots [12,13,14,15]. Antifungal properties of ethanol and water extracts of *Alafia barteri* leaves were reported [16]. In South-Western Nigeria (Lagos), *Alafia barteri* has been used for the treatment of malaria [17]. The decoction of root and leaves of the plant is also taken internally or applied externally to treat rheumatic pain, tooth ache and eye infections [18]. Preliminary phytochemical report on the stem extracts of *Alafia barteri* showed the presence of reducing sugars, steroids, flavonoids and anthraquinones [19]. To the best of our knowledge profertility potential of the plant has not been established. Current study thus focused on assessment of the effects of *Alafia barteri*, as an antioxidant source on sperm quality and hormone profile.

Corresponding Author: Adelakun Sundayn Aderemi, Department of Human Anatomy, School of health and health Technology, Federal University of Technology, PMB 704, Akure. Nigeria, Telephone: 0813-335-4835, E-mail: saadelakun@futa.edu.ng.

2 Materials and methods

2.1 Plant material

The leaves of *Alafia barteri*, were collected from ipale forest, Irawo (7°25' N, 3°31'S), Oyo state Nigeria, in may,

2017. The plant sample was authenticated by professor Ogunkunle of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria and a voucher specimen deposited in the same unit for reference purpose.

2.2 Preparation of the plant extract

The leaves were thoroughly washed in sterile water and were air dried to a constant weight in the laboratory. The air-dried leaves were weighed using Gallenkamp (FA2406B, England) electronic weighing balance and were milled with automatic electrical Blender (model FS-323, China) to powdered form. The powdered plant sample (420 g) was extracted with 96% ethanol for 24h, at room temperature with constant stirring. This process was repeated twice for complete extraction. The extract was filtered through cheese cloth and then through Whatman #1 filter paper, the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C.

2.3 Animals and treatment

Male Wistar rats 8 weeks old, weighing 160± 180 g were obtained from the animal facility of Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were kept in polypropylene cages under room temperature (25°C), with 12 h light and 12 h dark cycle and were allowed to acclimatize for two weeks. The animals were fed with grower's mash (Farm support services Ltd, Ogbomoso, Nigeria) at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. They had access to water ad libitum. The animals were randomly assigned into four groups of five rats each consisting of (group A control) which received only 2 ml/kg normal saline and treatment groups (B,C,D) at the doses of 100, 300 and 500 mg/kg body weight of *Alafia barteri* extract daily for 28 days, respectively. Twelve hours after the administration of the last *Alafia barteri* dose, the rats were at the time of sacrifice first weighed, blood Samples were collected through ocular artery and centrifuged at 1,500 g/min at 4 °C for 10 min to obtain serum then animals were sacrificed under high ether anaesthesia. All experimental protocols followed the guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States [20].

2.4 Measurement of sperm parameters

The rats were anaesthetized with diethyl ether. A scrotal incision was made to exteriorize the testis and epididymides. The caudal epididymis was carefully removed, blotted free of blood and then placed in a prewarmed Petri dish containing 1.0 ml of physiological saline solution (maintained at 37°C). Several incisions were made on it to allow sperm swim out. Semen analysis was carried out immediately using the new improved Neubauer's haemocytometer counting chamber for determination of the concentration of spermatozoa. Sperm motility was also assessed immediately by counting both motile and immotile spermatozoa per unit area at the magnification of 40x. Sperm viability was assessed using eosin-nigrosin test. The percentages of unstained (live) and stained (dead) spermatozoa were calculated by counting 200 spermatozoa per sample. Morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion (100x) and their percentages were calculated.

2.5 Hormonal Analysis

Hormonal profile of the following endocrine markers (Testosterone TT, Follicule stimulating hormone FSH and Leutenizing hormone LH) were measured using commercially available immunoassay (ELISA) method (Randox Laboratories Ltd, Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Qt94QY). according to the manufacturer's instructions.

2.6 Malondialdehyde Concentration Measurement in Serum

Free radical damage was determined by specifically measuring malondialdehyde (MDA). The MDA, formed as an end-product of lipid peroxidation (LPO), was treated with thiobarbituric acid to generate a colored product measured at 532 nm (MDA detection kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7 Super Oxide Dismutase Activity Measurement in Serum

The activity of SOD was measured by following the method of Beyer and Fridovich [21].

2.8 Glutathione Peroxidase Activity Measurement in Serum

The GSH peroxidase activity (GPX) activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H2O2 in the presence of reduced GSH(10 mM), nicotinamide adenine dinucleotide phosphate (NADPH) (4 mM), and 1 U enzymatic activity of GSH reductase (GR) [22].

2.9 Catalase Activity Measurement in Serum

Serum CAT activity was determined according to the method of Beers and Sizer, as described by Arash [23], by measuring the decrease in absorbance at 240 nm due to the decomposition of H2O2 in a UV recording spectrophotometer. The reaction mixture (3 mL) contained 0.1 mL of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 mL of 30 mM H2O2 in phosphate buffer (pH 7.0). An extinction coefficient for H2O2 cm-1 was used for calculation. The specific activity of CAT was expressed as moles of H2 reduced per minute per mg protein, at 240 nm. An amount of40.0 M-1 cm-1 was used for calculation. The specific activity of CAT was expressed as moles of H2O2 reduced per minute per mg protein.

2.10 Data Presentation and Statistical Analysis

Data were expressed as Mean±SEM. Statistical differences between the groups were evaluated by one way ANOVA, followed by Dunnets comparison test to compare

between treated and control groups. Differences yielding p<0.05 were considered statistically significant. All statistical analysis of data was performed using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, California, USA).

3 Results

3.1 Body and reproductive organ weights of the male wistar rats

Table 1. Shown the absolute and relative body and organ weights of the control and *Alafia barteri* extract treated rats. Administration of the extract for 28 days significantly (P<0.05) increased the absolute and relative weights of the reproductive organs. There were also linear increases in the body weights of both the control and treatment groups.

3.2 Sperm parameters

Alafia barteri extract significantly (P<0.05) increased the sperm motility and sperm count of extract treated rats, however there was no significant difference in the percentages of abnormal sperm cells (morphology) in treatment groups in comparison with the control group after 28 days of administration. [Table 2].

3.3 Serum Testosterone, Follicule stimulating hormone and Leutenizing hormone

Administration of *Alafia barteri* extract for 28 days significantly (p<0.05) increased serum total testosterone in treated animals compared to controls. The results were 0.18±0.01, 0.23±0.03; 0.29±0.04 and 0.17±0.01 for the *Alafia barteri* extract group and controls respectively. The extract produced a non-significant decrease in the blood level, of Follicle Stimulating Hormone (FSH) of all the treated groups (0.14 ± 0.50, 0.12 ± 1.00, 0.11±2.01) when compared to the value of the control group (0.15±0.20). Also, there was a non-significant increase in the level of luteinizing hormone in the treated groups (0.12±0.03, 0.13±0.01, 0.11±0.03) when compared to the control (0.10±0.01) [Figure 1].

3.4 Malondialdehyde (MDA) and antioxidant enzymes

Administration of *Alafia barteri* extract for 28 days significantly decreased MDA concentration in the experimental group compared to controls (P<0.05), with 2.81±0.07, 2.43±0.13, 1.80±0.19 and 5.37±0.41, respectively. Also SOD concentration significantly increased in the experimental group compared to the control group (P<0.05), with the results of 1140±48.61, 1336.00±39.10, 1498.00±58.10and 922.60±36.89, respectively. The extract produced significant increased GPX concentration in the experimental group (139.00±1.33, 143.70±1.76, 150.80±2.66) compared to the control group (124.30±2.05) (P <0.05). Furthermore oral consumption of *Alafia barteri* extract for 28 days significantly (P <0.05) increased serum catalase activity in the experimental group (332.90±8.18, 346.60±6.57, 363.90±7.12) in comparison to the control group (303.30±9.61) [Table 3].

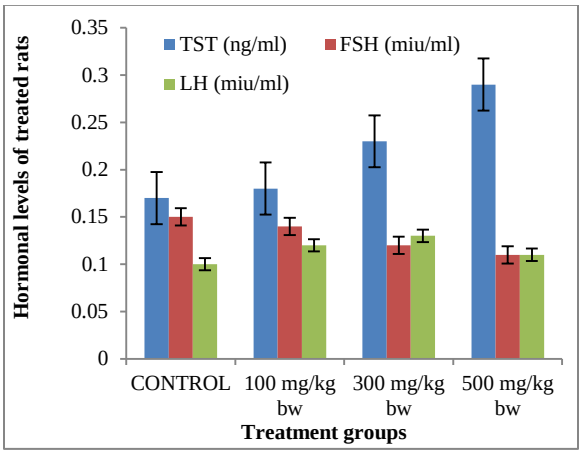

Figure 1: Effect of *Alafia barteri* extract on serum Testosterone, Follicule stimulating hormone and Leutenizing hormone of male wistar rats. Values are expressed as Mean ± S.E.M, n=5 in each group, * represent significant dissimilarly from the control group at p < 0.05. One-Way ANOVA. TST: Testosterone, FSH: Follicle stimulating hormone, LH: Leutinizing hormone, Miu: Milli international unit, ng: Nanogramme. bw: body weight. A: 2 ml/kg normal saline, B: 100 mg/kg bw *Alafia barteri*, C: 300 mg/kg bw *Alafia barteri*, D: 500 mg/kg bw *Alafia barteri*

4 Discussion

Herbal remedies have played important roles since early times in the treatment of all kinds of diseases in Africa and other continents, owing to the challenges confronting the appropriate delivery of official health care to millions of people in urban, remote and rural communities [24]. Herbal remedies constitute a strong component of traditional, complementary and alternative medicine [19]. In many countries, plants are used in the treatment of diseases due to presences of antioxidants, which is a characteristic phenomenon in traditional medicine [25]. Antioxidants neutralize the oxidation process by biding to free radicals, chelating catalytic metals and acting as oxygen scavengers [26].

The Impact of *Alafia barteri* leaves ethanolic extract on spermatogenic and steroidogenesis functions of rats testis have not been studied. In this study, we investigated some of the effects of the leaf extract of *Alafia barteri* on spermatogenesis in order to elucidate some of the possible implications that could occur following its consumption. The effect of extract of *Alafia barteri* in this study shown that *Alafia barteri* has a potential to increase mean body weights and weights of reproductive organs [Table 1]. Androgens regulate the weight, size and secretory function of testes, epididymes and accessory organs [27]. This is also constant with the findings of Shittu *et al*., [28] that increased cellular activities are key factor to be considered in the evaluation of organ weights.

Table 1: Effect of *Alafia barteri* extract on body and reproductive organ weights of male wistar rats

Parameters	Groups			
	A (2 ml/kg) control	B(100mg/kg bw)	C(300mg/kg bw)	D(500mg/kgbw)
Initial Body Weight (g)	166.70±70	169.00±1.72	167.20±1.71	167.50±1.11
Final Body Weight (g))	190.60±3.64	193.50±3.40	188.60±3.42	191.50±3.52
Testes Absolute weight (g)	1.88±0.08	2.18±0.08*	2.37±0.09*	2.50±0.03*
Epididymis Absolute weight (g)	0.36±0.02	0.46±0.02*	0.48±0.01*	0.57±0.01*
Ventral prostate Absolute weight (g)	0.28±0.08	0.37±0.04*	0.42±0.01*	0.48±0.01*
Seminal vesicle Absolute weight (g)	0.46±0.01	0.53±0.01*	0.60±0.01*	0.62±0.02*
Vas deferens Absolute weight (g)	0.13±0.01	0.12±0.01	0.14±0.01	0.15±0.02*

Values are expressed as Mean ± S.E.M, n=5 in each group, * represent significant dissimilarly from the control group at $p < 0.05$. One-Way ANOVA. bw: body weight

A: 2 ml/kg normal saline, B: 100 mg/kg bw *Alafia barteri*, C: 300 mg/kg bw *Alafia barteri*, D: 500 mg/kg bw *Alafia barteri*

Table 2: Effect of *Alafia barteri* extract on sperm characteristics of male wistar rats.

Groups	Parameters				
	Morphology (%)				
	Sperm motility (%)	Sperm count ($x10^6$/ml)	Viability (%)	Normal	Abnormal
A (2 ml/kg)control	64.60±2.67	56.13±13	57.87±0.71	75.50±1.09	24.50±1.00
B (100mg/kg bw)	68.40±2.48	65.78±1.44*	58.49±0.84	74.80±1.02	25.20±1.02
C (300mg/kg bw)	73.52±2.53	71.21±1.92*	62.01±0.83*	74.20±1.63	25.80±1.63
D (500mg/kgbw)	77.61±2.49*	75.16±2.50*	65.07±0.97*	78.25±1.10	21.75±1.10

Values are expressed as Mean ± S.E.M, n=5 in each group, * represent significant dissimilarly from the control group at $p < 0.05$. One-Way ANOVA. bw: body weight

A: 2 ml/kg normal saline, B: 100 mg/kg bw *Alafia barteri*, C: 300 mg/kg bw *Alafia barteri*, D: 500 mg/kg bw *Alafia barteri*

Table 3: Effect of *Alafia barteri* extract on malondialdehyde (MDA) and antioxidant enzymes

Parameters	Groups			
	A (2 ml/kg normal saline)control	B (100 mg/kg bwt Ab)	C (300mg/kg bwt Ab)	D (500 mg/kg bwt Ab)
Malondialdehyde	5.37±0.41	2.81±0.07*	2.43±0.13*	1.80±0.19*
Super oxide dismutase, u/g Hb	922.60±36.89	1140±48.61*	1336.00±39.10*	1498.00±58.10
Glutathione peroxidase, u/mg Hb	124.30±2.05	139.00±1.33*	143.70±1.76*	150.80±2.66*
Catalase, u/mg Hb	303.30±9.61	332.90±8.18*	346.60±6.57*	363.90±7.12*

Values are expressed as Mean ± S.E.M, n=5 in each group, * represent significant dissimilarly from the control group at $p < 0.05$. One-Way ANOVA. bwt: body weight, Ab: ***Alafia barteri***

A: 2 ml/kg normal saline, B: 100 mg/kg bw *Alafia barteri*, C: 300 mg/kg bw *Alafia barteri*, D: 500 mg/kg bw *Alafia barteri*

Result from current study revealed impacts of *Alafia barteri* on spermatogenesis in a dose dependent manner. Evidenced in our study, was an improved sperm concentration, motility, percentage normal and abnormal morphology sperm of the groups of animal treated with Alafia *barteri* extract for duration of 28 days in comparison to the control group rats. This shows that administration of *Alafia barteri* extract successfully increases the sperm qualities. It has been reported that *Alafia barteri* rich in antioxidant constituents such as total polyphenols, flavonoids, tannins, alkaloids, saponnins, vitamin E, vitamin C and vitamin A [29]. Therefore, it is plausible deducing that this rich antioxidant constituent of *Alafia barteri* boosted the testicular non-enzymatic and enzymatic antioxidants to effectively scavenge the free radicals preventing lipid peroxidation. The consequence is hereby reflected in the increased sperm count and sperm motility. This finding is in consonance with the reports by Rodrigues *et al*. [30] and Bansal and Bilaspuri [31] . Also Herbal antioxidants eliminate and suppress ROS formation, and the reduction of ROS is a crucial factor in the production of sperm cells and optimization of the fertility rate [32, 33]. The administration of *Alafia barteri* in this study could increases glucose metabolism leading to the production of pyruvate which is known to be the preferred substrate essential for the activity and survival of sperm cells [34, 35]. Although pathophysiology of male infertility has always been unclear, evidence shows that antioxidative changes are probably responsible for the abnormal spermatozoa function and fertilization capacity [36]. Seminal plasma, which is a rich source of antioxidants, protects sperm against oxidative stress by enzymes with powerful

antioxidant activity such as CAT, SOD and GPX. According to previous studies, a decreased level of antioxidants in seminal plasma of infertile men is correlated with the elevated level of MDA, which results in important LPO [23]. GPX is an important antioxidant, which protects the epididymis and the ejaculated spermatozoa [37]. This study showed that administration of *Alafia barteri* extract as an antioxidant increased SOD, GPX, and CAT, leading to the elimination of ROS. Therefore, sperm cells are protected from oxidation. In the same vein GPX, CAT and SOD could also increase serum antioxidant levels in male rats. It has the potential to restore fertility and normal spermatogenesis, and to improve hormonal level and sperm quality while in meantime decreasing the MDA level.

Antioxidants, flavonoids and vitamins in *Alafia barteri* extract support sperm morphology, sperm survival and sperm function which could be regarded as a regular supply of additional nutrients to the treated rats over the control groups. The improved sperm quality in treatment group suggested that *Alafia barteri* extract could generate simulative influence on hypothalamus. Although, in our study there was decreased in the blood level of follicle stimulating hormone, increased in the level of serum testosterone and luteinizing hormone of treated group rats indicates the positive impact of *Alafia barteri* extract in rats, this accord with previous study that antioxidant improves steroidogenesis by enhancing the primary effect of leydig cell endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis [38]. Moreover spermatogenesis and male fertility depends on presence of testosterone in testis. Reduction of serum testosterone presumably associated with changes in body composition, muscle strength, diminished energy, and sexual function. Therefore, androgens and partial androgens are important for the maintenance of spermatogenesis and male fertility [39], especially in various disease which exacerbate the decline in testosterone.

5 Conclusion

This investigation confirmed that oral administration of the leaf extract of *Alafia barteri* has no toxic and disruptive interference on spermatogenesis and steroidogenesis in wistar rats. To the best of our knowledge, this is the first study reporting the effect of *Alafia barteri* on semen and hormonal profile in wistar rats. The oral administration of extract of *Alafia barteri* possesses profertility. These profertility properties can be exploited in male fertility therapy.

Conflicts of interest

The authors declare no conflicts of interest.

References

[1] Carlsen E, Giwercman A, Keiding N, et al. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992;305: 609-613.

[2] Isidori AM, Pozza C, Gianfrilli D, et al. Medical treatment to improve sperm quality. J. Reprod Biomed. 2006;12:704-714.

[3] Arash K, Fathiazad F, Mohammad N, et al. The effects of Ginger on spermatogenesis and sperm parameters of rat . International Journal of Reproductive BioMedicine. 2009;7:7-12

[4] Mosher WD, Pratt WF. Fecundity and infertility in the United States: Incidence and trends. J. Fertil. Steril. 1991;56: 192-193.

[5] Shalaby MA, Mouneir SM. Effect of zingiber officinale roots and cinnamon zeylanicum bark on fertility of male diabetic rats. Glob Veter. 2010; 6: 341–7.

[6] Saliha R, Syed TR, Faizal A, et al,. The Role of Vitamin E in Human Health and Some Diseases. Sultan Qaboos Univ Med J. 2014;14: 157–165.

[7] Scalbert A, Manach C, Morand C, et al. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr. 2005;45: 287–306.

[8] Dragsted LO. Antioxidant actions of polyphenols in humans. Int J Vitam Nutr Res. 2003;73:112–9.

[9] Khaki A, Fathiazad F, Nouri M, et al,. Evaluation of androgenic activity of allium cepa on spermatogenesis in the rat. Folia Morphol (Warsz). 2009;68: 45–51.

[10] Irvine FR. Woody Plants of Ghana. With special references to their uses London; Oxford University Press. 1961

[11] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotech. 2005;4: 685-688.

[12] Burkill HM. The useful plants of West Tropical Africa. 2nd Ed. Vol.1. Families, A-D. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 1985; Pp. 960.

[13] Daziel JM. The useful plants of West Tropical Africa. Crown Agents for Overseas Governments and Administrations, London, United Kingdom. 1937; Pp. 612.

[14] Nadkarni AK, Nadkarni KM. India Materia Medica. With ayurvedic, Unani-tibbi, Saddha, allopathic, homeopathic, naturopathic and home remedies, appendices and indexes. Revised, enlarged and reprinted 3rd Ed. vol. 1&2. Bombay: Popular Prakashan. 1976

[15] Leeuwenberg AJM. Series of revisions of Apocynaceae 43. *Alafia Thouars*. Kew Bulletin. 1997;52: 769-839.

[16] Adekunle AA, Okoli SO. Antifungal activity of the crude extracts of Alafia bacteri Oliv.(apocynaceae) and chasmanthera depeddeers. Hochst. (menisermaceae). Hamdard medicus. 45: 52-56.

[17] Olowokudejo JD, Kadiri AB, Travil VA. An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. Ethnobot. Leaflets. 2008;12, 851–856.

[18] Odugbemi T. A text book of medicinal plants from Nigeria. Lagos: University of Lagos Press. 2008; p.545.

[19] Hamid AA, Aiyelaagbe OO. Preliminary phytochemical, antibacteria and antifungal properties of Alafia barteri Stem grown in Nigeria. Eur. J. Med. Plants. 2011; 2:26–32.

[20] NIH . Guide for the use of laboratory animals DHHS, PHS. NIH Publication No 85-23, rev. ed. NIH. 1985

[21] Beyer WJ, Fridovich . Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem. 1987; 161:559–66.

[22] Yoshikawa T, Naito Y, Kishi A, et al. Role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut. 1993;34:732–7.

[23] Arash K. Effect of *Cinnamomum zeylanicumon* on Spermatogenesis. Iran Red Crescent Med J. 2015; 17:18668

[24] Badole SL, Zanwar AA, Ghule AE, et al. Analgesic and anti-inflammatory activity of alcoholic extract of stem bark of *Pongamia pinnata* (L.) Pierre. Biomed. Aging Pathol. 2012;2:19-23.

[25] Virgili F, Scaccini C, Packer L, et al. Antioxidants in Food. Cardiovascular disease and nutritional phenolics. Cambridge: Woodhead Publishing Ltd. 2001; pp. 87–99.
[26] Kelen M , Tepe B. Screening of antioxidative properties and total phenolic compounds of various extracts of three different seed of grape varieties (Vitis vinifera L.) from Turkish flora. Pak J Biol Sci. 2007;10:403–8.
[27] Choudhary A, Steinberger E. Effect of 5α-Reduced Androgens on Sex Accessory Organs. Initiation and Maintenance of Spermatogenesis in the Rat. Biol. Reprod. 1975;12: 609-617.
[28] Shittu LAJ, Bankole MA, Oguntola JA, et al. Sesame leaves intake improve and increase epididymal spermatocytes reserve in adult male Sprague Dawley rat. Scientific Research and Essay. 2007;8: 319-324.
[29] Lasisi AA, Olayiwola MA, Balogun SA, et al. Phytochemical composition, cytotoxicity and in vitro antiplasmodial activity of fractions from *Alafia barteri* olive (Hook F. Icon)-Apocynaceae. Journal of Saudi Chemical Society . 2016;20: 2–6
[30] Rodrigues HG, Diniz YS, Faine LA, et al. Antoxidant effect of Saponin: potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis, Int. J. food Sci. Nutr. 2005;56 ; 79–85.
[31] Bansal AK , Bilaspuri GS.Impacts of oxidative stress and antioxidants on semen functions, Vet. Med. Int. http://dx.doi.org/10.4061/2011/686137, Article ID 686137. 2011; p.7
[32] Khaki A, Fathiazad F, Nouri M, et al. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytother Res. 2010;24:1285–91.
[33] Henkel R. The impact of oxidants on sperm function. Andrologia. 2005;37:205–6.
[34] Dua AA,Vaidya SR. Sperm motility and morphology as changing parameters linked to sperm count varintions, J. Postgraduate Med. 1996;42: 93-96.
[35] Egbunike GN, Branscheid W, Plisterer J et al. Changes in porcine sperm lactate dehydrogenase isoenzymes during sperm maturation. Andrologia. 1986; 18: 108-113.
[36] Akinloye O, Arowojolu AO, Shittu OB, et al. Selenium status of idiopathic infertile Nigerian males. Biol Trace Elem Res. 2005;104:9–18.
[37] Sunde RA. The biochemistry of seleno proteins. J Am Oil Chem Soc. 1984;61:1891–900.
[38] Prasad MRN; Rajalakshnmi M. Spermatogenesis and Accessory Gland Secretions. In: Textbook of Biochemistry and Human Biology, Talwar GP, Srivastava LM and Moudgil LM (Eds.), 2nd Edn. Prentice-Hall of India Pvt. Ltd., New Delhi, India. 1989; pp: 883.
[39] Vahdani V ,Khaki A. Effect of Allium cepa Seed Extract on Serum Testosterone in Rats. Crescent J Med Biol Sci. 2014;3:110-112.