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Spermatogenesis and Steroidogenesis Functions of Rat Testis Following Exposure to *Alafia barteri* Leaf Extracts

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Abstract

In Africa and other continents, herbal remedies have played essential roles in the treatment of all kinds of diseases since early times. This study is focused on the impact of *Alafia barteri* (*A. barteri***)** leaf extract on spermatogenesis and steroidogenesis in rats. Twenty adult male wistar rats randomly were chosen and assigned into four groups of five (n=5) rats each, consisting of a control which received only 2 mL/kg of normal saline and the treatment groups receiving the doses of 100, 300 and 500 mg/kg of body weight daily for twenty-eight days via gastric gavage. The parameters tested included sperm quality, reproductive hormones, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and malondialdehyde (MDA) levels. Sperm quality, total serum testosterone, SOD, CAT, and GPX levels were significantly increased in the *A. barteri* groups in comparison to controls ($P < 0.05$). Also, rats in the *A. barteri* groups showed a significant decrease in the level of plasma MDA ($P < 0.05$) in comparison to controls. There was a decrease in Follicle Stimulating Hormone (FSH), but no significant increase in the levels of luteinizing Hormone (LH). The administration of *A. barteri* extract significantly increased the sperm quality and improved the profertility properties; these profertility properties can be exploited in male fertility therapy.

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

1. Introduction

Infertility is one of the major health challenges in life. Approximately 30 % of infertility can be attributed to male factors (Carlsen *et al*., 1992; Isidori *et al*., 2006). Several conditions can interfere with spermatogenesis and reduce sperm production and quality (Arash *et al.*, 2009). Among these factors include drug treatment, chemotherapy, toxins, air pollution, and insufficient vitamins' intake which may also have harmful effects on spermatogenesis and the production of normal sperm (Mosher and Pratt, 1991). Several diseases, such as coronary heart diseases, diabetes mellitus, and chronic liver diseases may (also) interfere with the spermatogenesis process, and therefore sperm quality and quantity may be altered by these diseases (Shalaby and Mouneir, 2010).

Antioxidants are significant agents, which can contribute to the overall health of the organism (Saliha *et al.,* 2014). For example, Polyphenols are dietary antioxidants associated with redox activities and have quite beneficial effects on health (Scalbert *et al.,* 2005). Oxidative stress is a critical process that can (be) involved in multiple conditions such as infertility and inflammation (Dragsted, 2003). However, infertility and inflammation can be controlled with antioxidant supplements (Scalbert *et al.,* 2005). 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 (Khaki *et al.*,2009). Therefore, the use of antioxidants may improve spermatozoa viability and longevity (Khaki *et al.*, 2009).

Alafia barteri is a high-climbing, scandent shrub with small, pure white or pink flowers (Irvine, 1961). It is used in ethnomedicine for the treatment of sickle cell anaemia, rheumatism, eye infections, febrifuges, toothache, in addition to being used as chew sticks (Adekunle and Okoli, 2002). The twining stem of *A. barteri* is used for the treatment of fever, inflammation, and the roots are used in the manufacture of binding materials (Burkill, 1985; Daziel, 1937; Irvine, 1961; Nadkarni, 1976; Leeuwenberg, 1997). Antifungal properties of ethanol and water extracts of *A. barteri* leaves were reported (Adekunle and Okoli, 2002). In South-Western Nigeria (Lagos), *A. barteri* has been used for the treatment of malaria (Olowokudejo *et al.,* 2008). The decoction of the root and leaves of this plant can be taken internally or applied externally to treat rheumatic pain, toothache, and eye infections (Odugbemi, 2008). A preliminary phytochemical report on the stem extracts of *A. barteri* showed the capability of reducing sugars, and the presence of steroids, flavonoids and anthraquinones (Hamid and Aiyelaagbe, 2011). To the best of our knowledge, the spermatogenesis and

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steroidogenesis functions of *A. barteri* have not been established. This study is aimed at investigating the impact of *Alafia barteri* leaf extract on spermatogenesis and steroidogenesis in rats.

2. Materials and Methods

2.1. Plant Material

The leaves of *A.barteri*, were collected from Ipale forest, Irawo (7°25' N, 3°31'S), Oyo state Nigeria, in May, 2017. The plant samples were authenticated by Prof. Ogunkunle of the Department of Pure and Applied Biology at Ladoke Akintola University of Technology, Ogbomoso in Nigeria and a voucher a specimen was deposited at the same Department for reference purposes.

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 obtain a powdered form. The powdered plant sample (420 g) was extracted with 96 % ethanol for twenty-four hours, at room temperature with constant stirring. This process was repeated twice for a complete extraction. The extract was filtered using a cheese cloth and then using 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 aged eight weeks old, weighing 160± 180 g were obtained from the animal facility of the Department of Anatomy at Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were kept in polypropylene cages under room temperature (25°C), in a cycle of twelve hours of light and twelve hours of dark, 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 of normal saline and the treatment groups (B, C, D) receiving the doses of 100, 300 and 500 mg/kg body weight of the *A. barteri* extract daily for twenty-eight days, respectively. Twelve hours after the administration of the last *A. barteri* dose, the rats were at the time of sacrifice. They were first weighed, blood samples were collected through ocular artery and centrifuged at 1,500 g/min at 4 °C for ten minutes to obtain serum. The animals were then 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 (NIH, 1985).

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 was then placed in a pre-warmed 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 x40. 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. The morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion (100x) and their percentages were calculated. (WHO, 1999)

2.5. Hormonal Analysis

Hormonal profile of the following endocrine markers (Testosterone TT, Follicle stimulating hormone FSH, and Luteinizing hormone LH) were measured using commercially available immunoassay (ELISA) method (Randox Laboratories Ltd, Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Qt94QY) in accordance with 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 (1987).

2.8. Glutathione Peroxidase Activity Measurement in Serum

The GSH peroxidase and GPX activity were quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H_2O_2 in the presence of reduced GSH (10 mM), nicotinamide adenine dinucleotide phosphate (NADPH) (4 mM), and 1 U enzymatic activity of GSH reductase (GR) (Yoshikawa *et al.*,1993).

2.9. Catalase Activity Measurement in Serum

Serum CAT activity was determined according to the method of Beers and Sizer, as described by Arash, 2015, by measuring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 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 H_2O_2 in phosphate buffer (pH 7.0). An extinction coefficient for H_2O_2 cm-1 was used for calculation. The specific activity of CAT was expressed as moles of H_2 reduced per minute per mg protein, at 240 nm. An amount of 40.0 M-1 cm-1 was used for calculation. The specific activity of CAT was expressed as moles of H_2O_2 reduced per minute per mg protein.

2.10. Statistical Analysis

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

At the end of the experiment (twenty-eight days), there was an increase in the relative body weight of the *A. barteri* extract-treated groups when compared with the control groups. The absolute weight of Testes, Epididymis, Ventral prostate, Seminal vesicle and Vas deferens of the *A. barteri* extract-treated rats, significantly ($p<0.05$) increased in comparison to the control groups (Table 1).

3.2. Sperm Parameters

The *A. barteri* extract significantly ($p<0.05$) increased the sperm motility and sperm count (68.40±2.48, 73.52±2.53, 77.61±2.49) and (65.78±1.44, 71.21±1.92, 75.16±2.50) in the extract- treated rats when compared with the control (64.60±2.67) and (56.13±13). However, there was no significant difference in the percentages of abnormal sperm cells (morphology) in the treated groups (25.20±1.02, 25.80±1.63, 21.75±1.10) in comparison to the control groups (24.50±1.00) after twenty-eight days of administration (Table 2).

3.3. Serum Testosterone, Follicle Stimulating Hormone and Lutenizing Hormone

The administration of *A. barteri* extract for twenty-eight days significantly ($p<0.05$) increased the serum total testosterone in the 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 *A. barteri* extract group and controls respectively. The extract produced a non-significant decrease in the blood level, of Follicle Stimulating Hormone (FSH) of the all 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

The administration of *A. barteri* extract for twenty-eight days significantly decreased MDA concentration in the experimental group compared to the 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±58.10 and 922.60±36.89, respectively. The extract produced a 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, the oral consumption of *A. barteri* extract for twenty-eight 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).

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/kg bw)
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 *A. barteri*, C: 300 mg/kg bw *A. barteri*, D: 500 mg/kg bw *A. 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 (2mL/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/kg bw)	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*

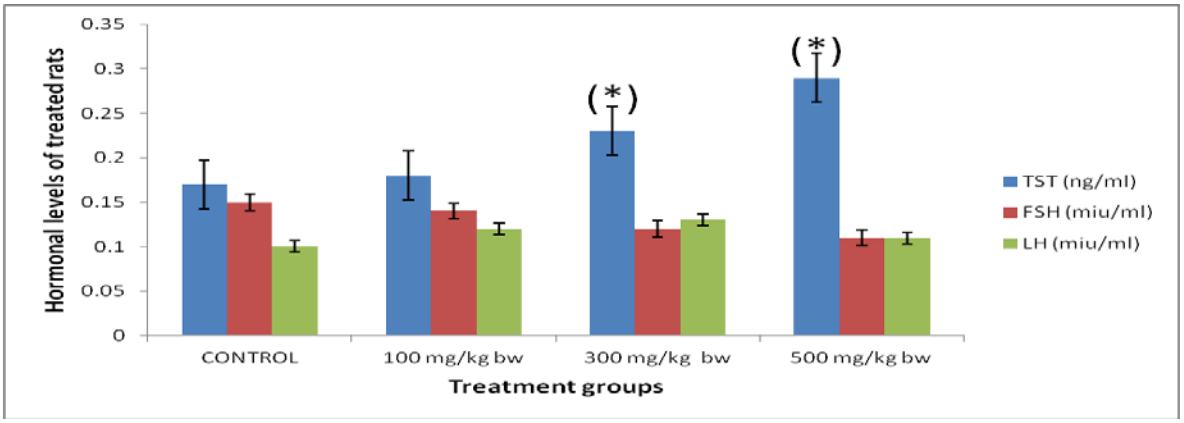

Figure 1: Effect of *Alafia barteri* extract on serum Testosterone, Follicule stimulating hormone and Luteinizing hormone of male wistar rats. Values are expressed as Mean ± S.E.M, n=5 in each group, * represents significant dissimilarly from the control group at $p < 0.05$. One-Way ANOVA. TST: Testosterone,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

4. Discussion

Medicinal plants are used in the treatment of several diseases attributed to the presence of antioxidants. (Virgili *et al.*, 2001).

The Impact of *Alafia barteri* (*A. barteri*) Oliv., Apocynaceae leaves extract on spermatogenesis and steroidogenesis functions of rats' testis have not been studied. The current study investigated the effects of the leaf extract of *A. barteri* on spermatogenesis and steroidogenesis in order to elucidate its possible implications that could occur following its following consumption. The effect of the extract of *A. barteri* shown in this study revealed a potential increase in mean body weights and weights of reproductive organs (Table 1). Previous observation revealed that androgens regulate the weight, size and secretory function of testes, epididymes and accessory organs (Choudhary and Steinberger, 1975). In addition, This finding is consistent with the findings of Shittu *et al*., (2007) which showed that increased cellular activities are key factors to be considered in the evaluation of organ weights.

The results of the current study reveal the impact of *A. barteri* on spermatogenesis in a dose- dependent manner. Results also shows improved sperm concentration, motility, percentage normal and abnormal morphology sperm of the groups of animal treated with *A. barteri* extract for the duration of twenty-eight days in comparison to the control group rats. This showed that the administration of *A. barteri* extract successfully increased sperm qualities. It has been reported that *A. barteri* is rich in antioxidant constituents such as total polyphenols, flavonoids, tannins, alkaloids, saponnins, vitamin E, vitamin C and vitamin A (Lasisi *et al.*, 2016). Therefore, it is plausible to deduce that the rich antioxidant constituent of *A. barteri* boosted the testicular non-enzymatic and enzymatic antioxidants to effectively scavenge the free

radicals preventing lipid peroxidation as reflected in the increased sperm count and sperm motility. This finding is consistent with the reports of Rodrigues *et al*.,2005 and Bansal and Bilaspuri, 2011. Furthermore, Herbal antioxidants eliminate and suppress ROS formation; the reduction of ROS is a crucial factor in the production of sperm cells and optimization of the fertility rate (Khaki *et al.*, 2010; Henkel, 2005). The administration of *A. barteri* in this study has increased 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 (Dua and Vaidya, 1996; Egbunike *et al*., l986). 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 (Akinloye *et al.*,2005). Seminal plasma, which is a rich source of antioxidants, protects sperm against oxidative stress by several enzymes with a 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 (Arash, 2015). GPX is an important antioxidant, which protects the epididymis and the ejaculated spermatozoa (Sunde, 1984). This study showed that the administration of *A. barteri* extract as an antioxidant increased SOD, GPX, and CAT, leading to the elimination of ROS thereby protecting sperm cells from oxidation. In addition, *A. barteri* has the potential to restore fertility and normal spermatogenesis, and to improve hormonal levels and sperm quality with concomitantly decreasing the MDA level.

It could be inferred in this present study that antioxidants such as flavonoids and vitamins present in *A. barteri* extract supported sperm morphology, sperm survival, and sperm function which could be regarded as a regular supply of additional nutrients to the treated rats in comparison with the control groups. The improved sperm quality in the treated groups suggests that the *A. barteri* extract can generate simulative influence on hypothalamus. Although the current study shows that there was a decrease in the blood level of follicle-stimulating hormone, the increase in the level of serum testosterone and luteinizing hormone of the treated rat groups indicates the positive impact of *A. barteri* extract in rats. This finding is consistent with a previous study which revealed that antioxidants improve steroidogenesis by enhancing the primary effect of the leydig cell endocrine function in addition to increasing the circulatory testosterone production and the stimulation of spermatogenesis (Prasad and Rajalakshnmi, 1989). Moreover, spermatogenesis and male fertility depend on the presence of testosterone in testis. The reduction of serum testosterone is presumably associated with changes in the body composition, muscle strength, diminished energy, and sexual function. Therefore, androgens and partial androgens are important for the maintenance of spermatogenesis and male fertility (Vahdani and Khaki, 2014), especially in various diseases which exacerbate the decline in testosterone.

5. Conclusion

This investigate on confirmed that the oral administration of the leaf extract of *A. barteri* has no toxic or disruptive interference with spermatogenesis and steroidogenesis in wistar rats. The present study revealed that the oral administration of the *A. barteri* extract possesses profertility properties. These profertility properties can be exploited really well in male fertility therapy.

Conflicts of interest

The authors declare no conflicts of interest.

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