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Effects of *Anthocleista Djalonensis* (Chev.) Methanol Root Bark Extract on Some Fertility Parameters in Male Rats

U. B. OKEKE[1ABCD], P. O. IGBINADUWA[1*ACEF], R. I. OZOLUA[2ACEF]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
[2]Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Anthocleista djalonensis* is a West African plant with several ethnomedicinal uses.
Objectives: This study evaluated the effect of *Anthocleista djalonensis* methanol root bark extract on fertility parameters in male rats.

Material and Methods: The rats were randomly allotted to four groups (n=5). Group A (control) received distilled water (10 ml/kg), groups B, C, D received oral doses of 100, 200 and 400 mg/kg/day of the extract respectively for 28 days. The mean body, testicular, and epididymal weights; sperm indices; and serum testosterone level were assessed.

Results: There were no significant changes on the body weights of the treated rats but their testicular and epididymal weights increased significantly ($P<0.05$) at the dose of 400 mg/kg in comparison with the control group. Sperm indices such as sperm count, motility, morphology and viability increased significantly ($P<0.05$) at the doses of 200 and 400 mg/kg/day when compared to the control. There was also a significant ($P<0.05$) increase in serum testosterone concentration at the dose of 400 mg/kg/day of the extract.

Conclusion: The results suggest that the methanol root bark extract of *A. djalonensis* improves fertility parameters in male rats thereby justifying its use in ethnomedicine.

Keywords: Sperm count, testosterone level, spermatogenesis, oxidative stress

INTRODUCTION

Infertility has been a source of worry to man from time immemorial. It has been estimated that 40-50% of infertility cases are due to the "male factors" (Kumar and Singh, 2015). Causes of male sterility are numerous, and include changes in the levels of certain hormone, untreated sexually transmitted diseases, sexual dysfunction, sperm DNA damage and varicocele (Mittal *et al.*, 2017). Seminal parameters such as volume, sperm count, sperm morphology, sperm vitality, and progressive motility have often been used for the evaluation of male fertility (Barratt, 2007; Kumar and Singh, 2015). Although approximately 40-50% of cases of male infertility are idiopathic (Australia Andrology, 2018), oxidative stress has been implicated as a root cause (Agarwal *et al*., 2014). Oxidative stress results from the action of excess oxidants or reactive oxygen species (ROS) against depleted antioxidant defence mechanisms in cells (Chandra, 2015). The quality and fertilizing capacity of sperm can be affected by excessive production of ROS in semen where they (excess ROS) override sperm or seminal antioxidant

defence mechanism causing oxidative stress (Sabeti *et al.,* 2016).

Most infertility management and supported reproductive procedures are expensive and with low success rates, ranging from 10 – 30% (Garceau *et al.,* 2002). Due to these, patients often lean on alternative therapies such as herbal medicines (Dabaja and Schlegel, 2014). The use of herbs in crude or polyherbal mixtures as fertility promoter in humans has witnessed a remarkable increase. Shortcomings such as low efficacy and untoward adverse effects related to conventional medicines have contributed to the surge in the demand and usage of herbal medicine (Umadeyi *et al.,* 2013; Ekor, 2014**).**

Anthocleista djalonensis (Longaniaceae) is a tall tree often armed with rigid spines, native to tropical West Africa. The leaves, roots and stem bark, are used in forklore medicine for treating different diseases which include male infertility (Edwin-Wosu, 2012). Decoction of the leaves and roots is used to treat general male infertility in south western Nigeria (Erabhor *et al.,* 2013; Anyanwu *et al.,* 2015). No scientific report exists on the effect of *A. djalonensis* root bark extract on male fertility. This study investigated the fertility enhancing property of methanol root extract of the plant on some fertility parameters in male Wistar rats.

METHODOLOGY

Collection and Identification of plant material

The roots of *A. djalonensis* were collected from a bush in Ikpoba Hill, Benin City, Nigeria in June 2017. The entire plant was authenticated by Dr. H.A Akinnibosun, a botanist at the Herbarium of Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where a voucher specimen (UBHA428) has been deposited.

Preparation of the extract

The roots were washed clean of sand after which the bark was peeled off from each, air-dried at room temperature and ground into fine powder. The powdered sample (1000 g) was extracted with 2.5 L of methanol by maceration for 72 h. The extract was filtered through cotton wool and Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator (yield = 14.91% w/w). The extract was then stored in a refrigerator at 4^{o}C until used.

Animals

Male albino rats weighing 150 - 200 g were obtained from Animal House, Igbinedion University, Okada, Nigeria. They were kept in wooden cages in the Animal House, Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were allowed to acclimatize for 14 days and had access to feeds and water *ad libitum.* The animals were exposed to natural conditions of temperature and lighting. Protocols for the experiments were in conformity with the ethical guidelines on the care and well-being of research animals (NIH, 2008). Ethical approval (reference: EC/FP/018/11) was obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City.

Phytochemical Analysis

Phytochemical screening of the methanol root extract of *A. djalonensis* was carried out using standard methods (Sofowora, 1993; Trease and Evans, 2002). The extract was screened for the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides.

Experimental design and sample collection

Twenty male albino rats were randomly allotted into four groups (n=5). Group A served as the control and the rats were given distilled water (10 ml/kg/day, p.o.). Groups B, C and D were given oral doses of 100, 200, and 400 mg/kg/day of the extract respectively for 28 days. On the 29th day, the rats were sacrificed; blood samples were collected through cardiac puncture (under chloroform anaesthesia) into plain bottles for hormonal analysis. The testes and epididymes were dissected out, cleared of connective tissues and weighed.

Semen characteristics analysis

The left testes along with the epididymes were removed. The caudal epididymes were separated from the testes, blotted free of blood, and then placed in a pre-warmed Petri dish containing 1 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 a 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. Morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion (x100), and their percentages were calculated (WHO, 1999).

Estimation of serum levels of testosterone

The blood samples were allowed to clot before centrifuging at 10,000 rpm to obtain sera for the assay of testosterone using the Microplate Enzyme Immunoassay. The appropriate serum reference and specimen (0.01 ml) were pipetted into prepared microplate wells. Working Testosterone Enzyme Reagent (0.05 ml) was added to all the wells. Further preparations of the microplate was done using Testosterone biotin enzyme reagent and stop solution to prepare the well. The absorbance in each well was read at 450 nm wavelength in a microplate reader. The results were read within 30 min of adding the stop solution. The results were expressed in ng/ml (Monobind, 2012)

Statistical analysis and data presentation

Results were expressed as mean ± SEM (standard error of the mean). Data for the groups were compared using one-way analysis of variance with Kruskal-Wallis *post hoc* test. Differences between data were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of extract on body and reproductive organ weights

The effect of *A. djalonensis* methanol root extract on body weight and reproductive organ weight are shown in Table 1. There was no significant ($P > 0.05$) difference in body weight changes and reproductive organ weight of extract treated rats, although, a significant ($P < 0.05$) increase in testicular and epididymal weights in the group treated with 400 mg/kg/day of the extract was observed when compared to the control group. Studies by Choudhary and Steinberger (1975) have shown that androgens regulate the weight, size and secretory function of testes, epididymes and accessory organs. Alterations in the level of circulating androgen can affect structural and functional capacity of reproductive tissues with a resultant impact on spermatogenesis (Chinoy *et al*., 1982). Weight changes in reproductive organs can be used as a marker of altered circulating androgen levels, since the reproductive glands are extremely sensitive to androgen levels.

Table 1: Effect of *Anthocleista djalonensis* methanol root extract on body weight changes and reproductive organs weights

Treatment groups mg/kg	Body weight changes (g)	Organ weights (g)	
		Testes	Epididymes
Control	17.4± 4.9	1.14 ± 0.04	0.40 ± 0.03
100	26.8 ± 5.83	1.11 ± 0.02	0.44 ± 0.03
200	21.8 ± 10.74	1.21 ± 0.01	0.50 ± 0.02
400	9.4 ± 4.01	1.48 ± 0.02*	0.58 ± 0.03*

**P < 0.05 with respect to control. Values are mean ± SEM; n = 5*

Effect of extract on semen parameters

After 28 days treatment, the sperm count, percentage of progressive motility, viability, and morphology (normal-shaped sperm cells) increased significantly ($P < 0.05$) at the doses of 200 and 400 mg/kg/day of the extract when compared to the control as shown in Table 2. Conventional semen analysis is now used as the primary choice for fertility assessment and is frequently used to describe semen quality (sperm motility, morphology and viability) and concentration (sperm count) (Khatun *et al.,* 2018). The result of the present study suggests that the extract could improve fertility function of male rats as an increase in sperm quality and quantity were observed. This result might lend credence to the potential of the plant to enhance the fertilizing ability of sperm cells and its use as a fertility enhancer in traditional medicine.

Table 2: Effect of methanol root extract of *A. djalonensis* on sperm parameters in male rats

Treatment Groups (mg/kg)	% Progressive motility	% Morphology (Normal sperm)	% Abnormal sperm	% Viability	Sperm count
Control	65.8 ± 3.37	74.0 ± 5.97	26.0 ± 5.97	65.4 ± 6.15	63.2 ± 5.42
100	66.6 ± 6.44	67.6 ± 3.01	32.4 ± 3.01	68.8 ± 4.07	71.6 ± 2.42
200	70.4 ± 3.26	73.0 ± 4.15	27.0 ± 4.15	78.0 ± 3.74*	75.2 ± 3.19*
400	76.8 ± 3.43*	79.4 ± 2.33*	20.6 ± 2.87*	80.2 ± 3.12*	83.6 ± 4.13*

**P < 0.05 with respect to control. Values are mean ± SEM; n = 5.*

Serum testosterone concentration

Figure 1 shows that although serum testosterone levels increased with the dose of the extract, values were only significantly ($P < 0.05$) different at the dose of 400 mg/kg/day for the 28 days treatment. The effect of the extract in increased sperm concentration (Table 2) may be due to increased spermatogenesis resulting from high testosterone level. Testosterone is secreted by the Leydig cells under luteinizing hormone (LH) stimulation and is essential for promoting spermatogenesis (O'Donnell *et al.,* 2017**)**. LH and follicle stimulating hormone (FSH) are produced in the anterior pituitary gland and secreted episodically in response to the pulsatile release of gonadotrophin releasing hormone (GnRH) (Jayes, 1997). Studies have shown that testosterone deprivation in rodents has an effect in germ cells development to mature spermatids during stage VIII spermatogenesis in rats (Walker, 2011). Zirkin *et al.* (1989) have shown a dose-response relationship between the seminiferous tubule fluid testosterone concentration and the quantitative maintenance of advanced spermatogenic cells in rat testis. Singh *et al.* (1995) in a study showed that androgens (e.g. testosterone) can induce spermatogenesis in gonadotropin-deficient mice. Although spermatogenesis and sperm production is stimulated by testosterone, exogenous androgens use can influence the hypothalamic-pituitary-gonadal axis by exerting negative feedback in a dose- and duration-dependent fashion. This results in decreases in intra testicular testosterone (ITT), blunting of FSH production, and ultimately decrease or complete cessation of spermatogenesis, through similar mechanisms as endogenous testosterone (MacIndoe, 1997). In this present work, the extract was able to increase the serum testosterone level which indicates increase in androgen level in the treatment rats.

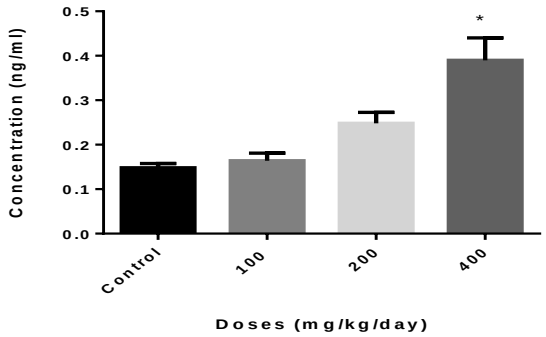

Fig. 1: Effect of oral 28-day methanol root extract of A. djalonensis on serum testosterone level in male rats. *P < 0.05 compared to control. Values are mean ± SEM; n = 5

Phytoconstituents

Preliminary phytochemical test of the methanol root extract of *A. djalonensis* revealed the presence of alkaloids, saponins, tannins, phenolics, flavonoids and terpenoids in the extract. Studies have shown that saponins could play an intermediary role in the production of androgens in stimulating the release of pituitary luteinizing hormone (LH) which would stimulate the production of testosterone by the Leydig cells (Gauthaman and Ganesan, 2008). Flavonoids have been associated in the maintenance of the synthesis of androgens by inhibiting 17β-estradiol aromatase, an enzyme involved in the conversion of testosterone to estrogen (Parandi *et al,* 2010). Alkaloids have been documented for both fertility, anti-fertility and aphrodisiac properties (Kumar *et al.*, 2010). Polyphenols have been shown to have positive effect on both male and female fertility due to their strong antioxidant properties (Seddiki *et al.*, 2017). Studies have shown that oxidative stress caused by reactive oxygen species (ROS) is the key and possible cause of idiopathic male infertility (Agarwal *et al.*, 2014). Excessive ROS production and depleted antioxidant defence mechanism of spermatozoa and seminal fluid causes a deleterious effect on spermatogenesis and the fertilizing capacity of

spermatozoa (Wagner *et al.*, 2018). Antioxidant phytochemicals can enhance male fertility through arrest of reactive oxygen species in spermatozoa, potentiation of spermatozoa and seminal fluid endogenous antioxidant system, or indirectly through the hypothalamic-pituitary gonadal axis. Studies by Igbinaduwa *et al* (2019) showed that *Anthocleista djalonensis* root extracts has high radical scavenging activities towards DPPH and hydrogen peroxide radicals. Findings by Muanya and Odukoya (2008) showed that *A. djalonensis* root extract in both raw and cooked fish homogenate inhibited lipid peroxidation, and used this as an index to assay aphrodisiac and fertility enhancing herbs. Murugesan *et al.* (2007) showed that antioxidants improved various processes of male reproductive function such as spermatogenesis and steroidogenesis. The presence of antioxidant phytochemicals in *A. djalonensis* root extract and its high antioxidant properties indicates that it could possess the capacity to scavenge excess reactive oxygen species (ROS) in spermatozoa responsible for oxidative damage of sperm cells. This could be responsible for the significant increase in sperm quality (Table 2) of rats in the extract treated groups.

CONCLUSION

The results of the study suggest that the methanol root extract of *A. djalonensis* may possess fertility-enhancing effects through improved sperm quantity, quality, and increased serum testosterone levels. This study lends credence to the use of the roots of the plant as a male fertility enhancer in ethnomedicine.

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*Address for correspondence: Patrick O. Igbinaduwa
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Benin,
Benin City. Nigeria
Telephone: +2348164848998
E-mails: Patrick.igbinaduwa@uniben.edu

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