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Pro- Fertility Effect of the Aqueous Leaf Extract of *Anogeissus leiocarpus* in Albino Wistar Rats

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Abstract

This study investigated the effect of the aqueous leaf extract of *Anogeissus leiocarpus* (AEAL) on the sperm count and motility of wistar rats. Twenty (20) adult male wistar rats were randomized into 4 groups of 5 rats each. Group 1 served as control and received 5ml/kg of 0.9% Normal saline, Groups 2, 3 and 4 received 100, 200 and 400 mg/kg AEAL respectively. Treatment was carried out p.o for 20 days, 24h after the last treatment, the rats were euthanized under chloroform anesthesia and the epididymis of rats was resected and the effects of the extract on sperm count, motility and epididymis were evaluated. The extract produced a dose- dependent significant ($P < 0.05$) increase in the mean sperm count and epididymal weight compared to the control group. A dose- dependent significant ($P < 0.05$) increase in mean fast sperm motility with a corresponding significant ($P < 0.05$) decrease in slow motile sperm cell was observed in the groups treated with AEAL compared to the control group. It was therefore concluded that the extract of *A. leiocarpus* possess pro-fertility effects.

Keywords: *Anogeissus leiocarpus*, Fertility, Aqueous, Wistar rats

1.0 Introduction

Sexual function greatly affects individual's quality of life, the normal male sexual response cycle consists of five phases: libido, erection, ejaculation, orgasm and detumescence subsequently. Any problem which affects satisfaction is considered sexual dysfunction (Abbas, 2016). Male sexual dysfunction is growing world widely. Sexual dysfunction has many etiological factors including various physical and psychological conditions (Malviya *et al.*, 2016). The male accounts for 30%–50% of entire infertility causes (Nejatbakhsh, 2016), 25% of them are unknown causes, without knowing the exact etiology, targeted management is not applicable, rising the use of empiric treatment in present conventional medicine without sufficient scientific evidence (Nejatbakhsh, 2016).

Recently the use of complementary and alternative medicines (CAMs) has increased in treating infertility (Weiss *et al.*, 2011). The long term use of these drugs however, is associated with a number of complications. It is therefore understandable why a lot of people resort to medicinal plants for the management of infertility. Although studies have reported the pro- fertility effect of many medicinal plants, to the best of our knowledge, no such study has been carried out on *A. leiocarpus*.

Anogeissus leiocarpus (DC) Guill and Perr family Combretaceae (Common name: Axlewood tree) has many applications in Nigeria. *A. leiocarpus* is used medically for the treatment of ascaricide, gonorrhoea, general body pain, blood clots, asthma, coughing and tuberculosis (Mann *et al.*, 2003). Information obtained from the Yorubas and South-Eastern people of Nigeria illustrate that the plant is also used as an antimicrobial agent against bacterial infections (Dweek, 1996). The leaves of the plant are used externally as a decoction in the eastern part of Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts and diabetic ulcers with good results. The powdered bark has also been mixed with 'green clay' and applied as an unusual face mask for serious blackheads

(Dweek, 1996). The infusion and decoctions are used as cough medicine, the pulped roots are applied to wounds and ulcers, the powdered bark is also rubbed to reduced tooth ache on gums, it is also used as vermifuges and the leaves decoction is used for washing and fumigation (Ibrahim *et al.*, 1997). This study was aimed at investigating the pro- fertility effect of the aqueous extract *A. leiocarpus* leaves.

2.0 Materials and Methods

2.1 Animals

Adult Male Wistar rats weighing 120–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean tap water and standard rodent feed.

2.2 Plant Collection and Identification

The leaves of *Anogeissus leiocarpus* were collected from a natural habitat in Abocho Area of Kogi State, Nigeria. The plants were identified and authenticated at Biological Science Department, Federal University, Lokoja, Kogi State.

2.3 Preparation of Extracts

The leaves of *Anogeissus leiocarpus* were shade-dried for five (5) days and pulverized using laboratory mortar and pestle. One thousand five hundred (1500) gram of the pulverized leaves was soaked in distilled water for 72- hours. The mixture was subsequently filtered using Whatmann filter paper (Size No1) and the extract was concentrated using free- dryer. The extract of *Anogeissus leiocarpus* was labelled as 'AEAL'.

2.4 Acute Toxicity Study

The oral median lethal dose (LD₅₀) of the extracts was determined in rats according to the method of Lorke (1983).

2.5 Experimental Design

Twenty (20) Male Wistar rats were used for the experiment. The rats were randomly divided into 4 groups of 5 animals each and treated as follows;

Group I: Control (5ml/kg of 0.9% Normal saline)

Group II: 100 mg/kg AEAL

Group III: 200 mg/kg AEAL

Group IV: 400 mg/kg AEAL

The treatment was done orally for 20 days, respectively. After 24 hours from the last treatment, the rats were euthanized using chloroform after which the rats were dissected and the epididymis was exposed by scrotal incisions and transferred into petri-dish. The weight of the epididymis was recorded for each rat.

2.6 Sperm analysis

The epididymis was crushed using a blunt forceps in a petri-dish and 1 ml of normal saline was added to semen and mixed thoroughly using a syringe to draw and release the mixture continuously (Verma *et al.*, 2002). The semen mixture was then sucked into a red blood cell pipette to the 0.5 mark, then normal saline was sucked up to the 101 mark. The normal saline in the stem of the pipette was discarded and the content of the bulb of the pipette was mixed thoroughly. A drop of the mixture was placed on the counting chamber which then spreads under the cover slip by capillary action. The counting chamber was then mounted on the slide stage of the microscope and viewed under x40 magnification. A grid system divides the counting chamber into five major squares each containing 16 smaller boxes. The count included all the sperm cells within the five major squares using the top and right or left and bottom system of counting as described by Verma *et al.* (2002) and Zaveneid and Polakoski (1977). The sperm count for a rat was calculated as $= n \times 1 \times 10^{-6} / \text{ml}$ of semen.

2.7 Sperm motility

A drop of the semen mixture was placed on a glass slide using 2 ml syringe, the preparation was placed on a microscope. Sperm motility was assessed as described by Sonmez *et al.* (2007). The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the caudal epididymis and data were expressed as percentages of fast motile, slow motile and non-motile spermatozoa. The percentage of motility was evaluated visually at x40 magnification.

2.8 Statistical Analysis

The results obtained were presented as mean \pm SEM. The data were analyzed using ANOVA and Turkey's post hoc test to determine the level of significance between the control and experimental groups. Values of $P < 0.05$ were considered to be of statistical significance.

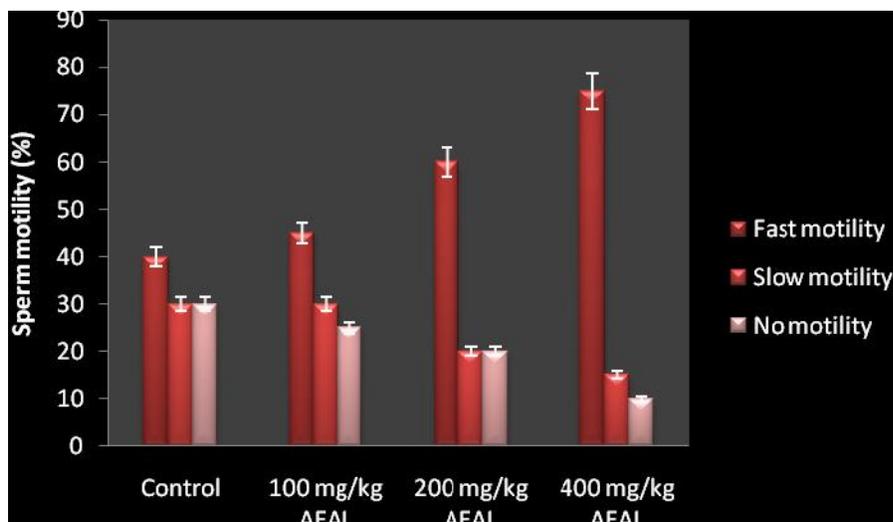
3.0 Results

The oral acute toxicity study did not reveal any mortality or signs of toxicity in rats up to a dose of 5000 mg/kg of aqueous leaf extract of *A. leiocarpus*. The oral LD₅₀ of the extract was then taken to be > 5000 mg/kg. Table 1 shows the effect of AEAL on the mean sperm count and epididymal weight of the rats. The mean sperm counts of the groups that received 200 and 400 mg/kg AEAL were significantly ($P < 0.05$) higher when compared to the control group. The mean epididymal weight of same groups were also significantly ($P < 0.05$) higher than the control group. There was also a significant ($P < 0.05$) increase in mean fast sperm motility with a corresponding significant ($P < 0.05$) decrease in slow motile sperm cell of the treatment groups compared to the control group (Figure 1).

Table 1: Effects of Aqueous Leaf Extract of *Anogeissus leiocarpus* on Sperm count and Epididymal Weight in Adult Male Wistar rats

Groups	Parameters	
	Sperm Count (million cells/ml)	Epididymal Weight (g)
Control (5ml/kg N/S)	40.22 ± 2.85	0.13 ± 0.02
100mg/kg AEAL	42.12 ± 2.66	0.15 ± 0.03
200mg/kg AEAL	50.28 ± 2.96 ^a	0.35 ± 0.09 ^a
400mg/kg AEAL	60.11 ± 3.11 ^b	0.43 ± 0.07 ^a

Data are presented as mean ± SD. (n=5). Means with different alphabets as superscript are significantly (P < 0.05) different

**Figure 1: Effects of Aqueous Leaf Extract of *Anogeissus leiocarpus* on Sperm Motility**

4.0 Discussion

Sexual activity is essential for procreation and general wellbeing as it bonds a relationship and has a calming effect. Sex involves the psychosocial and physiological activities to maintain a normal sexual function. Endocrine disorders or imbalance (Araujo *et al.*, 2000), life style patterns and substance abuse like smoking, alcohol consumption (Hammedeh *et al.*, 2010), psychological stress and neurological diseases, cardiovascular diseases (Adegite *et al.*, 2009), penile diseases and surgery and age affect sexual activity. A significant association had been found between impaired semen quality including sperm count, motility and morphology. This study investigated the effect of aqueous leaf extract of *A. leiocarpus* on sperm count and motility in wistar rats.

This study revealed that the extract significantly and dose-dependently increased the sperm count and sperm motility of rats. A significant increase in the epididymis was also observed with the extract and this could be due to increased androgen biosynthesis. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands and studies have shown that the level is positively correlated with the weight of testis, epididymis, seminal vesicle and prostate glands (Setty *et al.*, 1997). It is known that a major function of the epididymis is sperm maturation which leads to the acquisition of fertilizing ability and viability of spermatozoa. Therefore, improvement in the activities of the epididymis could have led to an increase in progressive motility of sperm in the experimental rats.

The increased sperm count and motility thereby shows that treatment with *A. leiocarpus* improves and enhances the fertilizing capacity of the Semen. These qualities were often used as a measure of sperm production, testicular function and/ or male fertility. Low sperm count and motility and high percentage abnormal spermatozoa level each have been associated with reduced fertility (Raji *et al* 2003).

5.0 Conclusion

Fertilization requires adequate and normal sperm count, morphology and motility to occur. With the observed effects of the extract on sperm count, sperm motility and epididymal weight, it is safe to say be that the extract of *Anogeissus leiocarpus* possess pro-fertility effect. This makes it a useful alternative for the management of male infertility.

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