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**Effect of Aqueous Leaf Extract of *Ziziphus mucronata* on  
Sperm Count and Motility in Wistar Rats**

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**Abstract**

On an average, about 10% of all couples face difficulty in starting a family and this creates a feeling of great personal failure, particularly in regions where religious and socio-economic traditions have made it almost imperative for everyone to have children. A significant association had been found between infertility and impaired semen quality including sperm count, motility and morphology. In this study, we investigated the effect of the aqueous leaf extract of *Ziziphus mucronata* 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 200, 400 and 800 mg/kg of aqueous leaf extract of *Ziziphus mucronata* respectively. Treatment was done orally for 20 days, 24h after the last treatment, the rats were euthanized 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 compared to the control group. The mean epididymal weight of the treatment groups were also significantly ( $P < 0.05$ ) increased by the extract compared to the control group. The extract also dose- dependently produced 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. With the observed effects of the extract on sperm count, sperm motility and epididymal weight, it was concluded that the extract of *Ziziphus mucronata* possess pro-fertility effects. Hence its extract could be useful in the management of infertility in men.

**Keywords:** *Ziziphus mucronata*, Sperm count, Sperm motility, Wistar rats

## 1.0 Introduction

Infertility is a condition with psychological, economic and medical implications resulting in trauma and stress, particularly in a social set-up like ours, with a strong emphasis on childbearing. According to the International Committee for Monitoring Assisted Reproductive Technology, infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Zegers- Hochschild *et al.*, 2009). Infertility can be divided into primary infertility and secondary infertility. Primary infertility is the case, when the man has never impregnated a woman. Secondary infertility applies when the man has some time impregnated a woman, even if the women are not the partner in the present couple. The male infertility can be complete or partial termed as sub-fertility. It is possibly due to reduced number of spermatozoa (oligozoospermia), reduced sperm motility (asthenozoospermia), reduced sperm vitality (necrozoospermia), abnormal sperm morphology (teratozoospermia) or any combination of these. Majority of the cases of sub-fertility are caused by an intrinsic testicular disorder (Zegers- Hochschild *et al.*, 2009). Orthodox drugs exist for the management of infertility. 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 *Ziziphus mucronata*.

*Ziziphus mucronata* is a shrub or a small to medium-sized tree that is 10-20 m high with a spreading canopy. It is distributed throughout the summer rainfall areas of sub-Saharan Africa, extending from South Africa northwards to Ethiopia and Arabia (Orwa *et al.*, 2009). The plant is known as Buffalo thorn or cape thorn (English), Magaryar Kura (Hausa), Ekanase Adie (Yoruba), Gulam jabe (Fulani) and Umlahlabautu (Swahili) (Burkill, 1995). Other common species of the same genus include *Z. abyssinica*, *Z. jujube*, *Z. Mauritiana*, *Z. spina- Christi*, *Z. lotus*, *Z. vulgaris*, *Z. oxyphylla* (Burkill, 1995). In traditional medicine, a decoction or infusion of the glutinous roots is commonly administered orally as a painkiller for all sorts of pains as well as dysentery (Burkill, 1995) and in South Africa, a decoction of the root is used for lumbago and for scrofulous condition with swollen glands; the fruits are sometimes chewed to relieve toothache. The paste of the leaf is applied to glandular swelling. The Zulus take the powdered leaf and bark in water as an emetic agent for chest problem and other respiratory ailments. The poultice of the leaf can be applied to boils and other septic swellings of the skin and sometimes for urethral discharge, painful

conditions and urinary problems (Orwa *et al.*, 2009). Its leaf and root have also been used to treat diarrhoea, cough, sores, ear inflammation, asthma, syphilis, gonorrhoea, measles, and fever (Von Koenen, 2001).

## 2.0 Materials and Methods

### 2.1 Animals

Adult Male Wistar rats weighing 150–200g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

### 2.2 Plant Collection and Identification

The leaves of *Ziziphus mucronata* were collected from a natural habitat in Okpella Area of Edo State, Nigeria. The plants were identified and authenticated at Pharmacognosy Department, University of Nigeria, Nsukka.

### 2.3 Preparation of Extracts

The leaves of *Ziziphus mucronata* were shade- dried for five (5) days and pulverized using an electric blender. One thousand (1000) gram of the pulverized leaves was soaked in distilled water for 72- hours. The resulting mixture was filtered using Whatmann filter paper (Size No1) and the extract was concentrated using free- dryer. The extract of *Ziziphus mucronata* shall henceforth be referred to as AEZM.

### 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: 200 mg/kg AEZM
- Group III: 400 mg/kg AEZM
- Group IV: 800 mg/kg AEZM

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.

### 2.5.1 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 =  $n \times 1 \times 10^{-6}$ /ml of semen.

### 2.5.2 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.

### 2.6 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 statistically significance.

### 3.0 Results

**Table 1** shows the observations from the acute toxicity study of AEZM in rats. There were no signs of toxicity such as physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects. More so, there was no mortality up to a dose of 5000 mg/kg of aqueous extract of *Ziziphus mucronata*. The oral LD<sub>50</sub> of each of the extract was then taken to be > 5000 mg/kg. **Table 2** shows the effect of AEZM on the mean sperm count and epididymal weight of the rats. The mean sperm counts of the groups that received 400 and 800 mg/kg AEZM 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. As presented in **Figure 1**, there was 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. Also there was significant ( $P < 0.05$ ) decrease in immotile sperm cells in the treatment groups compared to the control group.

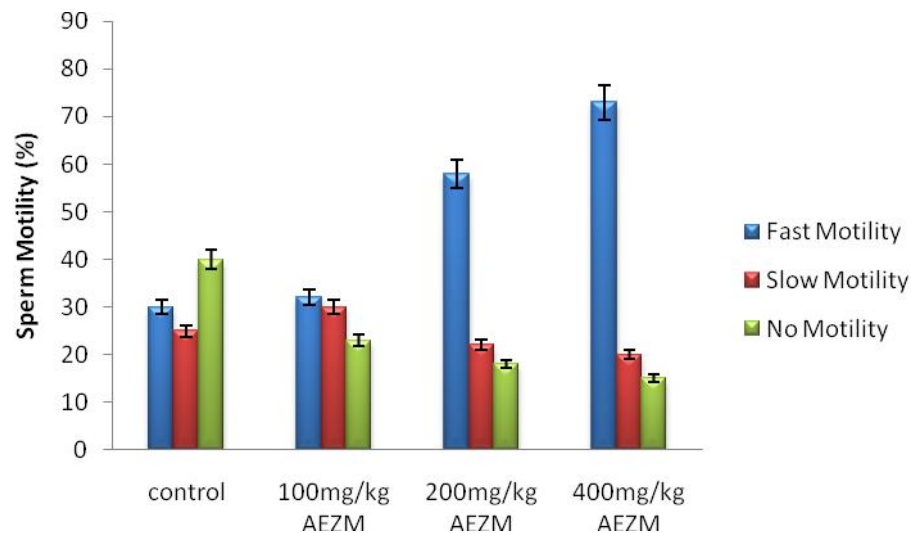
**Table 1: Observations from the Acute Toxicity Study of the Aqueous Leaf Extract of *Ziziphus mucronata* in Rats**

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	AEZM (10)	0/3	-
	2	AEZM (100)	0/3	-
	3	AEZM (1000)	0/3	-
II	1	AEZM (1600)	0/1	-
	2	AEZM (2900)	0/1	-
	3	AEZM (5000)	0/1	-

**Table 2: Effects of Aqueous Leaf Extract of *Ziziphus mucronata* on Sperm count and Epididymal Weight in Adult Male Wistar rats**

Groups	Parameters	
	Sperm Count (million cells/ml)	Epididymal Weight (g)
Control	35.12 $\pm$ 1.34	0.15 $\pm$ 0.04
200mg/kg AEZM	37.18 $\pm$ 2.22	0.18 $\pm$ 0.02
400mg/kg AEZM	48.29 $\pm$ 2.37 <sup>a</sup>	0.29 $\pm$ 0.06 <sup>a</sup>
800mg/kg AEZM	58.30 $\pm$ 2.85 <sup>b</sup>	0.28 $\pm$ 0.09 <sup>a</sup>

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



**Figure 1: Effects of Aqueous Leaf Extract of *Ziziphus mucronata* on Sperm Motility**

#### 4.0 Discussion

Infertility has become an ominous problem. On an average, about 10% of all couples face difficulty in starting a family and this creates a feeling of great personal failure, particularly in regions where religious and socio-economic traditions have made it almost imperative for everyone to have children. 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 *Ziziphus mucronata* on sperm count and motility in wistar rats.

The results of the study suggested that *Ziziphus mucronata* have a beneficial effect on male reproductive functions in rats. This is confirmed by our observation on the increased sperm counts and motility produced by the administration of 200-800mg/kg of the plant extract. The significant increase in the epididymis 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 *Z. mucronata* 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

With the observed effects of the extract on sperm count, sperm motility and epididymal weight, it can be concluded that the extract of *Ziziphus mucronata* possess pro-fertility effect. Hence its extract could be useful in the management of infertility in men.

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