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Effect of Aqueous Extract of Tigernut (*Cyperus esculentus* L.) on Sperm Parameters and Testosterone Level of Male Albino Rats

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ABSTRACT

Tigernut is consumed by humans and livestock. It also has many medicinal uses. There is growing evidence of decline in human sperm count and quality. Hence, this study seeks to evaluate the effect of Aqueous Extract of Tigernut (AET) on testosterone level, weight of epididymes and testes, sperm count, semen pH, sperm motility, sperm viability and sperm head abnormalities in male rats as a model. The rats were divided into four groups of six rats each. The rats were treated with AET at 0.0, 0.6, 1.2 and 1.8 g kg⁻¹ b.wt. day⁻¹, respectively for nine weeks. After which, the rats were sacrificed and assessed for the parameters. The AET had a dose-dependent effect on all the parameters. In conclusion, AET has the capability of increasing the weights of the testes and epididymes, sperm count, sperm quality and testosterone level. Hence, AET could be used as a possible fertility booster and to attenuate sperm toxicity.

Key words: Tiger nut, sperm count, sperm quality, testosterone, sperm toxicity

INTRODUCTION

Tigernut (*Cyperus esculentus* L.) is cultivated for human consumption and as well as livestock feed. It is one of the underutilized and widely distributed plants in subtropical and tropical regions. In Nigeria, it is cultivated mainly in the Middle belt and Northern regions (Umerie and Enebeli, 1997; Okafor *et al.*, 2003). It also has many other common names such as chufa sedge, nut grass, yellow nutsedge, tigernut sedge, earth almond and Northern nutgrass (Shilenko *et al.*, 1979; Al-Shaikh *et al.*, 2013). Tiger nut is not really a nut but a tuber which belongs to the family Cyperaceae, with a slightly sweet and nutty flavor (Lowe and Whitewell, 2000; Al-Shaikh *et al.*, 2013).

Tigernut has been historically used in the cosmetic industry in the production of soap and oil (Al-Shaikh *et al.*, 2013) and presently in the production of the popular drink called “*kunu*”, it is also consumed as snack and delicacy, because of its rich milky taste (Amaal and Essraa, 2010) and rich sugar content (Kordyias, 1990; Umerie and Enebeli, 1997; Osagie and Eka, 1998; Pamplona-Roger, 2005). It is also rich in vitamin B₁, C and E and minerals such as calcium, magnesium and iron (Pamplona-Roger, 2005; Belewu and Belewu, 2007; Chukwuma *et al.*, 2010; Al-Shaikh *et al.*, 2013), as well as digestive enzymes such as catalase, peroxidase, lipase and among others (Pamplona-Roger, 2005). Medically, it has used in cases of flatulence, dysentery, debility, indigestion (Pamplona-Roger, 2005; Abano and Amoah, 2011), diarrhea (Pamplona-Roger, 2005; Al-Shaikh *et al.*, 2013), dyspepsia and colitis (Pamplona-Roger, 2005; Adejuyitan *et al.*, 2009). It is said to be suitable for diabetic persons (Borges *et al.*, 2008), as well as being a powerful aphrodisiac (Evans *et al.*, 1989).

There are growing evidences indicating a steady decline in human sperm count and quality (Auger *et al.*, 1975; Carlsen *et al.*, 1992; Fisch and Goluboff, 1996; Kaufmann *et al.*, 1998). Increased testicular weight, sperm concentration, sperm motility, sperm viability, progressive sperm motility and also the reduced percentage in sperm morphology abnormalities had been reported in rats and mice treated with tigernut (Amaal and Essraa, 2010; Al-Shaikh *et al.*, 2013), with potentials of attenuating sperm and reproductive toxicities.

In view of the afore mentioned, this study set out to evaluate the effect of Aqueous Extract of Tigernut (AET) on some sperm parameters, testosterone level and weight of testes and epididymes in albino rats as mammalian model; using short-term *in vivo* assays.

MATERIALS AND METHODS

Collection of plant material: Fresh tigernuts (*Cyperus esculentus* L.) were purchased from Bogobri Market, Calabar, Nigeria. The tiger nuts were identified and authenticated at the herbarium of the Department of Botany University of Calabar, Calabar, Nigeria. The tigernuts were screened and washed to remove sand and debris. They were dried and pulverized into fine powder.

Experimental animals: Twenty four healthy and sexually mature male albino rats of 12 weeks old weighing between 120-150 g were used in this study. The rats were obtained from the Experimental Animal Unit of Department of Genetics and Biotechnology, University of Calabar, Calabar. The rats were housed in conventional wire mesh cages under standard laboratory conditions. They were allowed free access to water and pellet feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of experimental animals and approved by the local Ethics Committee.

Experimental design and procedure: The twenty four male rats were randomly divided into four groups of six rats each. The animals were acclimatized for one week before the commencement of the study. The rats in the control and treatment groups were treated with 0.0, 0.6, 1.2 and 1.8 g of tigernut per kilogram body weight daily, respectively. The tigernut powder was soaked in distilled water overnight, filtered and administered in 2 mL aliquots daily through oral gavage. The treatment lasted for nine weeks.

The rats were sacrificed under chloroform anaesthesia 24 h after the last treatment. Blood samples were collected from the rats through cardiac puncture for testosterone assay. The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for sperm count, semen pH, sperm motility, sperm viability and sperm head abnormality test. Data was collected on the following parameters: weight of testes and epididymes, semen pH, sperm motility, sperm viability, sperm count, sperm head abnormality test and testosterone level.

Weight of testes and epididymes: The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance.

Sperm parameters

Semen pH and sperm motility: Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponds to the pH and was read from the paper. Two drops of

sperm suspension was put on a microscope slide and cover slip was placed. The number of progressively motile sperm cells were counted and divided by the total number of spermatozoa counted under 40x lenses was expressed as a percentage (Ekaluo *et al.*, 2013a, b).

Epididymal sperm count: The epididymal sperm samples were obtained by macerating known weights of cauda epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80 µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed as million/mL of suspension (Ekaluo *et al.*, 2005).

Sperm viability test: The sperm viability test was conducted using “Eosin-Nigrosin one-step staining technique” (Bjorndahl *et al.*, 2003). A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five air-dried smears were prepared on glass slides for each sample. The slides were examined within 15 min for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

Sperm head abnormality test: A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.* (2009).

Testosterone assay: Blood samples collected through cardiac puncture were allowed to clot then centrifuged at 2500 rpm for 10 min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25°C to obtain the serum. Serum samples were assayed for level of testosterone using the Microwell (solid phase) enzyme linked immunosorbent assay (ELISA) technique utilizing the competitive binding principle; with analytical grade reagents from Syntron Bioresearch Inc., USA (Ekaluo *et al.*, 2010).

Statistical analysis: Data from epididymal sperm count, semen pH, sperm motility, sperm viability test, sperm head abnormality test, testosterone assay, weight of epididymes and testes were subjected to the analysis of variance (ANOVA) test, while observed differences in means were separated using Least Significant Difference (LSD) test.

RESULTS

Weight of testes and epididymes: The Aqueous Extract of Tigernut (AET) had an increasing effect on the weight of the testes and epididymes of the treated rats in a dose-dependent manner. Significant ($p < 0.05$) effects were observed in the weight of the testes of the rats treated with 1.2 and 1.8 g kg⁻¹ b.wt. when compared to the control as shown in Table 1.

Sperm parameters: Table 1 also shows the effect of AET on some sperm parameters. There was no significant ($p > 0.05$) effect of AET on the semen pH; although the semen pH was increased in a dose-dependent manner. The sperm motility was also increased in a dose-dependent manner from 75.11% in the control to 84.65% in 1.8 g kg⁻¹ b.wt. group. However, significant ($p < 0.05$) effect was

Table 1: Effect of aqueous extract of tigernut on sperm parameters, weight of testes and epididymes in rats

Parameters	Dose of tiger nut treatment (g kg ⁻¹ b.wt.)			
	0.0 (Control)	0.6	1.2	1.8
Sperm count (×10 ⁶ mL ⁻¹)	6.95±0.13 ^a	7.85±0.61 ^a	8.16±0.11 ^b	9.09±0.23 ^c
Sperm motility (%)	75.11±0.34 ^a	76.65±1.14 ^a	80.75±2.71 ^b	84.65±2.50 ^c
Semen pH	7.05±0.12 ^a	6.93±0.08 ^a	6.88±0.05 ^a	6.85±0.08 ^a
Sperm viability (%)	86.01±1.95 ^a	88.85±1.95 ^a	90.36±1.19 ^a	91.86±0.49 ^a
Sperm head abnormality (%)	4.26±0.09 ^a	4.50±0.10 ^a	5.34±0.29 ^a	5.53±0.18 ^a
Weight of testes (g)	1.16±0.02 ^a	1.28±0.02 ^a	1.51±0.05 ^b	1.60±0.04 ^c
Weight of epididymes (g)	0.30±0.05 ^a	0.35±0.02 ^a	0.38±0.01 ^a	0.38±0.03 ^a

^{abc}Values across the table with similar superscripts are not significantly different at 5% based on ANOVA

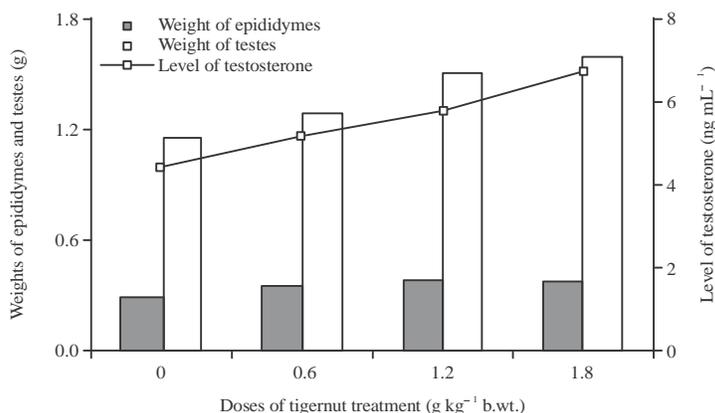


Fig. 1: Effect of aqueous extract of tigernut on testosterone level, weights of testes and epididymes in rats

seen in 1.2 and 1.8 g kg⁻¹ b.wt. treatment groups when compared with the control group. The AET had no significant ($p>0.05$) effect on sperm viability, but the sperm viability was increased in a dose-dependent manner from 86.01-91.86% as in sperm viability. The sperm count was increased in a dose-dependent manner by AET in all the treatment groups from 6.95×10^6 mL⁻¹ in control group to 9.09×10^6 mL⁻¹ in 1.8 g kg⁻¹ b.wt. group and significant ($p<0.05$) effects were seen in 1.2 and 1.8 g kg⁻¹ b.wt. treatment groups when compared with the control group. There was no significant ($p>0.05$) effect of AET on sperm head abnormality; however there were slight increases in a dose-dependent manner from 4.26% in control group to 5.53% in 1.8 g kg⁻¹ b.wt. treatment group.

Testosterone assay: The aqueous extract of tigernut had significant ($p<0.05$) increasing effect on the level of testosterone in all the treatment groups when compared with the control group. The testosterone levels were increased in a dose-dependent manner from 4.45 ng mL⁻¹ in the control group to 6.75 ng mL⁻¹ in the 1.8 g kg⁻¹ b.wt. treatment group. Figure 1 shows that AET had a dose-dependent proportional effect on testosterone level, weights of testes and epididymes in treated rats.

DISCUSSION

The effect of AET on the increasing weight of testes and epididymes agrees with the report of Amaal and Essraa (2010). This could be attributed to its rich vitamin (Pamplona-Roger, 2005; Belewu and Belewu, 2007; Chukwuma *et al.*, 2010) and the protective role of particularly

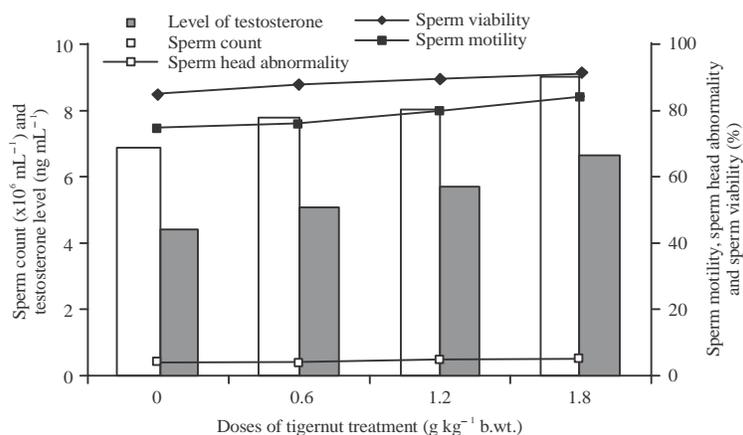


Fig. 2: Effect of aqueous extract of tigernut on testosterone level and sperm parameters in rats

vitamin C against oxidative stress and morphological changes of the testicular tissues (Karawya and El-Nahas, 2006; Nashwa and Venes, 2008; Fernandes *et al.*, 2011; Al-Amoudi, 2012; Ekaluo *et al.*, 2013a, b) and also indicated by corresponding increase in testicular and epididymal weights; as well as the protective role of AET against oxidative stress and morphological changes is also an indication of its potentials in attenuating sperm and reproductive toxicities (Ekaluo *et al.*, 2013a, b).

Increase in testicular and epididymal weights have been observed to be an indication of higher sperm production (Kamtchouing *et al.*, 2002; Nayernia *et al.*, 2004; Amaal and Essraa, 2010; Ekaluo *et al.*, 2013a, b), which could be due to increased androgen biosynthesis as evidenced by a significant increase in testosterone level (Morakinyo *et al.*, 2008) in AET treated rats (Fig. 1). The increase in testosterone level suggests a possible androgenic property of AET (Kamtchouing *et al.*, 2002; Morakinyo *et al.*, 2008). Figure 2 shows the concomitant dose-dependent improvement in sperm count and sperm quality (semen pH, sperm motility, sperm viability and sperm head abnormality) which could also be attributed to increase in testosterone's stimulation of the spermatogenic cells to undergo successful spermatogenesis, sperm maturation in the epididymes and the secretory activity of the accessory sex glands (Greenspan and Stawler, 1997).

In general, damage to the sperm cell is said to occur either by physiological, cytotoxic or genetic mechanism. Exposure to the chemicals could produce pituitary hypothalamic or sex hormonal effects, which in turn could affect spermatogenesis or exposure could cause abnormalities in seminal fluid resulting in functional or structural impairment of sperm. It may also arise as a consequence of naturally occurring level of mistakes in the spermatozoon differentiating process during spermatogenesis (Bakare *et al.*, 2005; Ekaluo *et al.*, 2005).

CONCLUSION

In conclusion, this study shows that Aqueous Extract of Tigernut (AET) has antioxidant and androgenic properties with the capability of increasing the weights of the testes and epididymes, sperm count, sperm quality and testosterone level. Hence, AET could be used as a possible fertility booster and in attenuating sperm and reproductive toxicities.

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