Journal of Medical and Applied Biosciences
ISSN: 2277 - 0054
Volume 5, Number 2, 2013

Effect of Methanolic Extract of *Cyperus esculentus L.* (Tigernut) on Luteinizing Hormone, Follicle Stimulating Hormone, Testosterone, Sperm Count and Motility in Male Albino Wistar Rats

Agbai E.O[1]. and Nwanegwo C.O[2].

[1]*Department of Human Physiology, Madonna University Elele, Rivers State Nigeria.*
[2]*Department of Human Physiology, College of Medicine, Imo State University, Owerri Nigeria.*
E-mail: vivy4okey@yahoo.com

ABSTRACT

The effect of methanolic extract of *Cyperus esculentus L.* on follicle stimulating hormone, luteinizing hormone, testosterone and some sperm parameters in twenty four adult albino male wistar rats. After six weeks administration of extract of *Cyperus esculentus L.*, results showed statistically significant difference in LH levels at $P < 0.05$ between Group A (4.80 ± 1.04) compared with experimental Group B (5.70 ± 0.46), Group C (5.48 ± 0.27) and Group D (5.45 ± 0.37). FSH levels showed statistically significant difference at $P < 0.05$ between Group A (11.55 ± 0.46) compared Group D (15.20 ± 1.33). There was no statistically significant difference at $P > 0.05$ between Group A (11.55 ± 0.46) compared with Group B (11.30 ± 1.93) and Group C (11.08 ± 0.78). Testosterone levels showed statistically significant differences at $P < 0.05$ between Group A (3.30 ± 0.30) compared to Group B (3.75 ± 0.47), Group C (4.23 ± 0.34) and Group D (3.63 ± 0.63). The sperm count levels showed statistically significant differences at $P < 0.05$ between Group A (168.0 ± 4.54) compared to Group B (182 ± 4.94), Group C (174.25 ± 6.86) and Group D (176.0 ± 6.09). There was statistically significant difference at $P < 0.05$ between Group A (71.50 ± 1.55) compared to Group B (74.25 ± 1.54) and (76.25 ± 2.59). However, there was no statistically significant difference at $P > 0.05$ between Group A (71.50 ± 1.55) compared with Group C (68.50 ± 3.75). Results showed that methanolic extract of *Cyperus esculentus* significantly increased gonadotropins, testosterone and sperm parameters in a dose-dependent fashion.

Keywords: *Cyperus esculentus*, Tigernut, Follicle Stimulating Hormone, Luteinizing Hormone, Testosterone, Sperm Count, Sperm Motility.

INTRODUCTION

Cyperus esculentus is commonly known as earth almond, tiger nut, chufa, yellow nutsedge and zulu nuts. It is known in Nigeria as *aya* in Hausa, *ofio* in Yoruba and *akiausa* in Ibo where three varieties (black, brown and yellow) are cultivated

(Umerie *et al.,* 1997). Among these, only two varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive colour and fresher body (Belewu and Abodurin, 2006). Tigernut can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called tigernut milk (Oladele and Aina, 2007). *Cyperus esculentus* was reported to help in preventing heart, thrombosis and activates blood circulation, responsible for preventing and treating urinary tract and bacterial infection, assist in reducing the risk of colon cancer (Adejuyitan *et al.,* 2009), anti-diabetic, weight-losing effect (Borges *et al.,* 2008), and possesses anti-sickling property (Monago and Uwakwe, 2007). Research has indicated that *Cyperus esculentus* may play an important role in enhancement of fertility (Almsshadania and Al Essawe, 2010), thus we study the effect of methanolic extract of *Cyperus esculentus* on some reproductive hormones, sperm count and sperm motility in male albino wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty four newly weaned male albino wistar rats weighing 150-170g were obtained from the Animal house of the department of Veterinary Medicine, University of Nigeria. The rats were assigned into four groups (n = 6) and housed in a wire mesh cage on 12 hours light/ 12 hours dark cycle. They were acclimatized for two weeks and fed with normal rat chow (Growers Vital feeds, Nigeria) and tap water *ad libitum.* Animals care and treatment were conducted in conformity with the institutional guidelines that are in compliance with our institutional guide for the care and use of laboratory animals.

Preparation of Extracts

Fresh nuts of *Cyperus esculentus* were obtained from a local market at Ogbete market in Enugu State. The seeds were screened to remove the bad nuts and were washed, sundried and grounded into fine powder with the aid of a corona Japan made grinding machine and also with a mortar and pestle. About 50g of the powdered form of the seeds were macerated in 250ml of methanol, stirred vigorously and left undisturbed for 48 hours after which it was sieved using white handkerchief. The filtrate was gotten and poured in a beaker and was concentrated to dryness in an electric oven (Gallenkamp^{R}) carefully regulated at 65^{0}C and left until all

the methanol evaporated and only the extract (oil) remains in the beaker.

Administration of Extracts

Group A served as a Control fed with normal rat chow for six weeks. The experimental Group B was fed with normal rat chow plus oral administration of 200mg/kg of methanolic extract of *Cyperus esculentus* per body weight for six weeks. The experimental Group C was fed with normal rat chow plus oral administration of 400mg/kg *Cola nitida* and 200mg/kg of *Cyperus esculentus* per body weight for six weeks. The experimental Group D was fed with normal rat chow plus oral administration of 600mg/kg of *Cyperus esculentus* per body weight for six weeks.

Sample Collection

At the end of six weeks of experiment, the animals were anaethesized in a chloroform chamber and the blood samples were obtained through cardiac puncture. Blood samples drawn were put in a well labeled non-heparinized EDTA sample tube which was allowed to stand for 3 hours in ice water and later centrifuged at 7000g for 10 minutes. The serum was then collected and stored in a refrigerator for 24 hours before hormonal assay.

HORMONAL ASSAY

Testosterone

Step 1: Reaction of Antiserum with Serum Testosterone: Testosterone occurs largely bound to corticosteroid binding protein and albumin. In this stage a blocking agent which binds to the serum binding protein but not to the antibody is used to displace testosterone from serum binding proteins thus making it available for antibody binding.

The test tubes to be used were first labeled appropriately, 4 test tubes for plasma of test animals and 4 test tubes for plasma of control animals. 50µl of serum, 100µl of testosterone EIA blocking reagents, 100µl of testosterone EIA antiserum and 100µl testosterone EIA separation reagent were pipette into all 8 tubes covered and briefly vortexed mixed. After which the test tubes were incubated in a water bath for 2 hours. The racks of the test tubes were then placed on the base of the magnetic separators and left for 5-10 minutes; this was to allow all magnetic particles sediment with all tubes still in contact with the magnetic base. The separator was inverted over a sink to decant the supernatant liquid from the test tubes.

To separate the hormones bound to the magnetic particles from the

other components of the plasma rack. Tubes were removed from the magnetic base and 100µl of diluted testosterone EIA was buffered added to the test tube. The tubes were briefly vortexed mixed, placed on the magnetic separator for 5-10 minutes and the supernatant liquid decanted. This is a wash step.

Step 2: Reaction of Antiserum with Enzyme Labeled Testosterone: 700µl of diluted testosterone EIA enzyme label was pipette into test tubes. Test tubes were covered and briefly vortexed mixed, then placed in water bath for 15 minutes. After which racks of tubes was placed on magnetic separator for 5-10 minutes and the supernatant liquid decanted. The content of the tubes were then washed twice following the same wash procedure above but using testosterone EIA wash buffer 2.

Colour Development Step: The same procedure but tubes are incubated in water for 1 hour. This was done using seroenzyme 1 machine.

Measurement of Optical Density: This machine determines the optical density of the samples from programmed standards at 500nm and 492nm.

FSH and LH

100µl of plasma from test animals were pipette into each of the 4 test tubes and the test tubes were labeled A, B, W and X. The same procedure was repeated for plasma from control animals and the test tubes labeled C, D, Y and Z. Tubes A-D used to test for FSH while tubes W-Z were used for LH. 100µl of FSH EIA magnetic antibody and 100µl of LH EIA magnetic antibody was added to tubes A-D and W-Z respectively. The tubes were then covered with aluminum film and briefly water mixed. After mixing, the tubes were transferred to the water bath. Tubes A-D was allowed to incubate for 15 minutes and tubes W-Z for 30 minutes. The temperature of the water bath was 37^0C. To separate the hormone bound to magnetic particles from other components of plasma, the samples were washed. 500µl of diluted FSH/LH EIA wash buffer were briefly water mixed. The rack of tubes were placed on a magnetic separator and allowed for 5 minutes. After which the supernatant liquid was decanted by inverting the rack and separator.

Labeled Antibody Reaction

The rack was removed from magnetic separator and 250µl of diluted FSH EIA labeled antibody

was added to tubes A-D and 300µl of diluted LH EIA labeled antibody was added to tubes W-Z. All test tubes were then covered and vortex mixed. After mixing, the tubes were transferred to the water bath. Tubes A-D were left to incubate for 60 minutes and tubes W-Z for 2 hours, after which all tubes were then washed twice as described above. The tubes were washed twice to ensure that all unbound labeled antibody were removed.

Colour Development Step

The tubes were removed from the magnetic separator. 500µl of substrate was pipette into all test tubes plus one empty tube that served as the substrate blank tube. The tubes were covered and vortex mixed. After mixing, the tubes placed into a water bath. Tubes A-D were left for 30 minutes and tubes W-Z for 1 hour, after which 1ml of diluted stop buffer was added to all nine tubes and the test tubes were briefly vortex mixed. The tubes were then placed in the magnetic separator for a minimum of 10 minutes.

Sperm Analysis

The peritoneal cavity was opened with an incision running to the thoracic region and down to the lower abdominal region. The testis was carefully removed, washed in normal saline solution (0.9%) and caudaepididymis was stored in normal saline (0.9%) at 37^0C until sperm count and motility were carried out.

Epididymis Sperm Count

Epididermal spermatozoa were collected by cutting the caudal region of the epididymis into small piece of 5ml of Ringer's solution at 37ºC. Assessment of sperm count was performed according to Freud and Carol (1965). Briefly both caudal epididymis from each rat were placed in 2ml of normal saline pre-warmed to 37ºC, small cuts were made into two caudal epididymis, where the spermatozoa were obtained and suspended in a saline solution. 200µl of the suspension was transferred to both chambers of neubauer hemocytometer using a pastuer pipette by touching the edge with a cover slip and allowing each chamber to be filled by capillary suction.

Sperm Motility

The semen is mixed very well, then a drop is put on a slide (this procedure is called wet mount or net preparation) then viewed with microscope. It is recorded in percentage. There are actively motile cells, moderately motile and sluggishly motile cell. The actively and sluggishly motile cells were checked and recorded in percent. The moderately motile was also

recorded in percent. The non motile cells are also derived (Cheesbrough *et al.,* 2002).

RESULTS

Results showed a statistically significant difference in serum LH level between Group A (4.80 ± 1.04 mIU/ml) compared to groups B, C, and D at $P < 0.05$ [Group B (5.70 ± 0.46), Group C (5.48 ± 0.27), and Group D (5.45 ± 0.37) mIU/ml].

There was statistically significant difference in serum FSH level between Group A (11.55 ± 0.46 mIU/ml) compared to Group D (15.20 ± 1.33 mIU/ml) at $P < 0.05$. There was no statistically significant difference between CONT (2.914±0.2017pg/ml) compared with Group B (11.30 ± 1.93 mIU) and Group C (11.08 ± 0.78 mIU/ml) at $P > 0.05$.

Results showed statistically significant difference in serum testosterone level between Group A (3.30 ± 0.30 mIU/ml) compared to Group B (2.66±0.49mIu/ml) and Group C (4.23 ± 0.34 mIU/ml) at $P < 0.05$. There was no statistically significant difference between Group A (3.30 ± 0.30 mIu/ml) compared with Group D (3.63 ± 0.63 mIU/ml) at $P > 0.05$.

There was statistically significant difference in sperm count between Group A (168.00 ± 4.54 x 10^6/ml) compared to other groups [Group B (182.50 ± 4.94x10^6/ml), Group C (174.25 ± 6.86x10^6/ml) and Group D (176.00 ± 6.09x10^6/ml)] at $P < 0.05$.

However, there was statistically significant difference in sperm motility between Group A (71.50 ± 1.55) compared to Group B (74.25 ± 1.54) and Group D (76.25 ± 2.59). There was no statistically significant difference between Group A (71.50 ± 1.55) compared with Group C (68.50 ± 3.75) at $P > 0.05$.

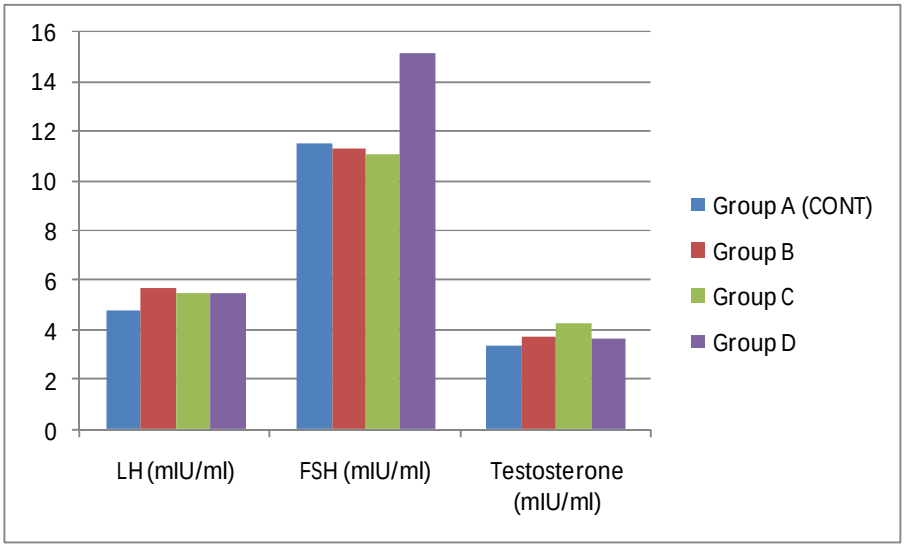

Figure 1: Effect of Methanolic Extract of *Cyperus esculentus* on LH, FSH and Testosterone in Male Albino Wistar Rats

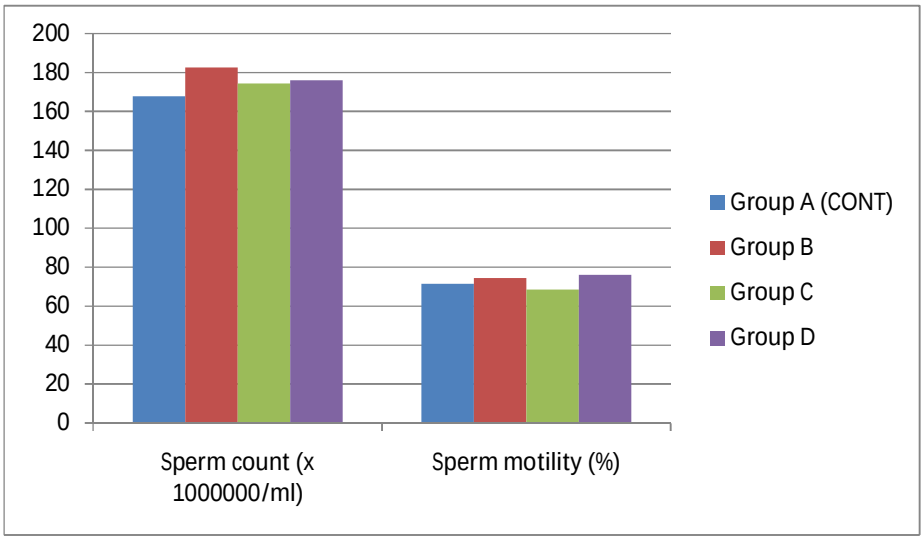

Figure 2: Effect of Methanolic Extract of *Cyperus esculentus* on Sperm Count and Sperm Motility in Male Albino Wistar Rats

DISCUSSION

In this study, the effect of *Cyperus esculentus* on gonadotropins, testosterone and sperm count and motility was studied in adult male albino wistar rats. The plasma testosterone level in figure 1 showed significant increase at reduced dose concentration of the extract. Testosterone is aromatized to estradiol and/or converted to dihydrotestosterone by aromatase

and reductase enzymes present within the epididymis (Shittu *et al.,* 2007). The presence of some phytochemical contents such as apigenin is an effective inhibitor of aromatase enzyme that converts androgen to oestrogen (Jeong *et al.,* 1999). *Cyperus esculentus* may have increased serum level of testosterone at a low dose by inhibiting the aromatase enzyme; this is evidenced at the same dose concentration (400mg/kg) that increased testosterone level also caused an increase in the sperm count and motility as shown in figure 2. Leydig cells secrete testosterone by the stimulatory effect of LH (Udoh *et al.,* 2005c). In this present study, the serum level of LH was increased also in a dose-dependent manner indicating that the methanolic extract of C*yperus esculentus* indicating that this increase in the serum level of testosterone could probably be due the increase of LH. Thus, the increase in the serum testosterone level could possibly due to a decreased activity of aromatase enzyme or as a result of hormonal interplay at the level of the hypothalamic-pituitary-testicular axis.

Alkaloids in plants have been reported to cause an increase in the serum level of FSH in male rats (Udoh *et al.,* 2009). FSH stimulates the Sertoli cells to convert spermatids to sperm; the increase in FSH observed in figure 1 is dependent on increase dose concentration of *Cyperus esculentus*. This increase in FSH was reflected by an increase in sperm motility and a slight increase in the sperm count. In the result, 400mg/kg of *Cyperus esculentus* decreased the FSH, sperm count and sperm motility but increased the LH and testosterone. 200mg/kg of *Cyperus esculentus* significantly increased LH, sperm count, testosterone and FSH although 600mg/kg significantly increased FSH and sperm motility. Increase in LH and FSH in male improve spermatogenesis and fertility (Almsshadania and Al Essawe 2010). In this view, it is credible to suggest that *Cyperus esculentus* increased LH and FSH levels and enhances spermatogenesis and fertility in male albino wistar rats. Therefore, this present study concludes that oral administration of *Cyperus esculentus* improve reproductive functions in adult male albino rats by altering the plasma levels of gonadotropins, testosterone and sperm functions in a dose-dependent manner.

REFERENCES

Adejuyitan J.A., Otunola E.T., Akande E.A., Bolarinwa I.F. and Oladokun F.M. (2009). Some Physicochemical Properties of Flour Obtained from Fermentation of Tigernut (*Cyperus esculentus*) Sourced from a Market in Ogbomosho, Nigeria. *Afr. J. Food Sci.,* 3:51-55.

Almashhadania A.M. and Al Essawe M.A. (2010). The Effect of *Cyperus esculentus* on Sperm Function Parameters in Prepubertal Mice as a Good Model for Human. *Journal of Baghdad for Science* 7:389-393.

Belewu M.A. and Abodurin A.O. (2006). Preparation of Kuunu from Unexploited Rich Food Source: Tigernut (*Cyperus esculentus L*). *Pak. J. Nutr.,* 7:109-111.

Cheesebrough M. (2002). District Laboratory Practice in Tropical Countries (Second Edition Part 2); Chapter 7:15.

Freund M., and Carol B. (1964). Factors Affecting Haemocytometer Counts of Sperm Concentration in Human Semen. *J. Reprod. Fertil.,* 8: 149-155.

Jeong H.J., Shin Y.G., Kim I.H., and Pessuto J.M. (1999). Inhibition of Aromatase Activity by Flavonoids. *Arch. Pharm. Res.* 22: 309-312

Monago C.C. and Uwakwe A.A. (2007). Proximate Composition and In-vitro Anti-sickling Property of Nigerian *Cyperus esculentus* (Tigernut Sedge). *Trees for Life Journal* Vol. 4:2.

Oladele A.K. and Aina J.O. (2007). Chemical Composition and Functional Properties of Flour Produced from Two Varieties of Tigernut (*Cyperus esculentus*). *Afr. J. Biotechnol.* 6:2473-2476.

Shittu .A.J., Shittu R.K., Ogundipe O., Tayo A., and Osinubi A.A. (2007). Hypoglycemic and Improved Testicular Parameters in *Sesamum radiatum* Treated Normo-glycaemic Adult Male Sprague Dawley Rats. *African Journal of Biotechnology*, Vol. 8 (12), Pp. 2878-2886.

Udoh F.V., Udoh P.B. and Umoh E.L. (2005c). Activity of Alkaloid Extract of *Carica papaya* Seeds on Reproductive Function in Male Wistar Rats. *Pharmaceutical Biol*., 43(6):563-567.

Udoh P.P., Udoh F.V., Umoren E.R., James U.W., Okeke C.P. and Agwu B. (2009). Effect of Caricapryl-99 Seed Alkaloid Extract on the Serum Levels of Sex Hormones and Pituitary Gonadotropins in Male Albino Rats. *Niger. J. Physiol. Sci.* 24(1):13-15.

Umerie S. C., Okafor P. E. and Uka A. S. (1997). Evaluation of Tubers and Oil of *Cyperus esculentus*. *Biresour. Technol.,* 61:171-173.

Reference to this paper should be made as follows: Agbai E.O. and Nwanegwo C.O. (2013), Effect of Methanolic Extract of *Cyperus esculentus L.* (Tigernut) on Luteinizing Hormone, Follicle Stimulating Hormone, Testosterone, Sperm Count and Motility in Male Albino Wistar Rats. *J. of Medical and Applied Biosciences,* Vol. 5, No. 2, Pp. 52 – 61.