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Cyperus Esculentus L. (Tigernut) Increases Estrogen Level in Female Albino Wistar Rats

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ABSTRACT

The effect of methanolic extract of *Cyperus esculentus L.* on follicle stimulating hormone, luteinizing hormone, prolactin and estrogen in twenty four albino wistar rats and in four control rats. After six weeks administration of extract of *Cyperus esculentus L.*, results showed no statistically significant difference in LH levels at $P > 0.05$ between Group A (5.23 ± 0.92) compared with experimental Group B (5.55 ± 0.29), Group C (5.33 ± 0.74) and Group D (6.10 ± 0.15). FSH levels showed statistically significant difference at $P < 0.05$ between Group A (12.20 ± 0.94) compared to Group B (11.30 ± 1.93) and Group C (12.18 ± 1.24). There was no statistically significant difference at $P > 0.05$ between Group A (12.20 ± 0.94) compared with Group D (14.18 ± 2.00). Prolactin levels showed no statistically significant differences at $P > 0.05$ between Group A (1.83 ± 0.38) compared with Group B (2.03 ± 0.05), Group C (1.88 ± 0.11) and Group D (2.25 ± 0.21). The estrogen levels showed statistically significant differences at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group B (19.85 ± 2.17). There was also statistically significant difference in estrogen levels at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group C (24.03 ± 4.31). There was a statistically significant difference at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group D (31.40 ± 4.19). Our finding revealed that methanolic extract of *Cyperus esculentus* significantly increased serum estrogen level as the dose concentration of extract increased, but did not statistically increase follicle stimulating hormone, luteinizing hormone and prolactin levels.

Keywords: *Cyperus esculentus,* Tiger nut, Follicle Stimulating Hormone, luteinizing hormone, Prolactin, Estrogen.

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 Igbo 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 assist in reducing the risk of colon cancer (Adejuyitan *et al.,* 2009), and suitable for diabetic persons and also helps in losing weight (Borges *et al.,* 2008), and possesses anti-sickling property (Monago and Uwakwe, 2007). It has been hypothesized that *Cyperus esculentus* has an effect on the reproductive functions thus; this present study investigates the effect of methanolic extract of *Cyperus esculentus* on some reproductive hormones in female albino wistar rats.

Materials and Methods

Experimental Animals

Twenty four newly weaned female albino wistar rats weighing 140-160g 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 the Guide for the Care and Use of Laboratory Animals (United State National Research Council, 1996).

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

Estrogen, prolactin, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured using Enzyme Linked Immuno sorbent Assay (ELISA). This assay is based on the high specificity of antibodies to bind molecules which in this case hormones. The antibody is tagged with an enzyme. Since the enzyme labeled with antibody reacts/binds with the hormone, the amount of the hormone present can be obtained by introducing a substrate for the enzyme that forms a colored product and the optical density (OD) of the sample is measured. The intensity of colour is proportional to the concentration of the bound hormone.

Tests for LH and FSH

Test tubes A-D were used for LH while tubes W-Z were used for FSH. Step 1:100µl of plasma from test animals were pipette into the tubes A-D and W-Z. The same procedures were repeated for animals from control group. 100µl of LH EIA magnetic antibody were added to test tubes A-D, and 100µl of FSH EIA magnetic antibody were added to tubes W-Z. The tubes were covered with aluminum foils and briefly vortex mixed. After mixing, the tubes were transferred to a water bath. Tubes A-D was allowed to incubate for 30 minutes, while tubes W-Z were allowed to incubate for 15 minutes. The temperature of the water bath was kept at 37^0C. To separate the hormones bound to magnetic

particles from other components of plasma, the plasma samples were washed. 500µl of dilute LH/FSH EIA wash buffer was added to the test tubes A-D and W-Z respectively and briefly vortex mixed. The rack of the tubes were placed on the magnetic separator and allowed to stand for 5 minutes after which the supernatant liquid was decanted by inventing the rack and separator.
The rack was removed from the magnetic separator and 300µl of dilute LH EIA labeled antibody was added to test tubes A-D and 250µl of dilute FSH EIA labeled antibody was added to test tubes W-Z. All the test tubes were covered and vortex mixed. After mixing, the tubes were transferred to the water bath. Tubes A-D was allowed to incubate for 2 hours and test tubes W-Z were allowed to incubate for 1 hour. All the tubes were then washed twice as described above. The tubes were washed twice to ensure that all unbound labeled antibody was removed.

Blood was centrifuged and plasma collected. Standard and internal QC samples were reconstituted as directed in full protocols. Magnetic antibody enzyme labeled antibody and substrate solution were prepared as directed in the full protocols. Buffer was also diluted in full protocols.

100µl of plasma from test animals were introduced into each of 4 test tubes and test tubes were labeled A, B, W, and X. The same procedures were repeated for plasma from control animals and test tubes labeled C, D, Y, and Z. The tubes were removed from the magnetic separator. 500µl of substrate solution was added to test tube plus one empty tube that served as the substrate blank tube. The tubes were covered and vortex mixed. After mixing, the tubes were placed inside a water bath. Tubes A-D were left for 1 hour and tubes W-Z were left for 30 minutes, after which 1 ml of diluted stop buffer was added to all 9 separator for a minimum of 10 minutes. Measurement of optical density was done using serono serozyme 1 machine which determines the OD of samples from programmed standard of 500nm and 492nm.

Test for Prolactin

The test tubes were labeled appropriately, 4 tubes for plasma from test animals and 4 tubes for control animals. 150µl of plasma and 200µl of enzyme conjugate reagent were dispensed into each of the four tubes and gently mixed for 10 minutes and incubated at room temperature for 45 minutes. The incubation mixture was removed and rinsed with distilled water five times. The tubes were struck onto absorbent paper to remove all residual water droplets. 100µl of TMB reagent was added into each test tube and gently mixed for 10 seconds,

and incubated at room temperature n the dark for 20 minutes. The reaction was stopped by adding 100µl of stop solution to each and gently mixed for 30 minutes. The OD was red at 450nm with a microtitre plate reader within 15 minutes.

Test for Estrogen

The test tubes were labeled appropriately; 4 tubes for plasma from test animals and 4 tubes for control animals. 150µl of plasma and 200µl of estradiol antibody were introduced into all the test tubes. The tubes were covered, briefly vortex mixed and incubated in water at 37°C for 2 minutes. 200µl of enzyme labeled estradiol was introduced into all the test tubes and the test tubes were covered and briefly vortex mixed, then incubated in water bath for 20 minutes. 200µl of separation reagent was added to the test tubes. The test tubes were covered, vortex mixed and incubated in water bath for 15 minutes. The tubes were then placed in the magnetic separator for 5 minutes and supernatant liquid was decanted. To remove the unbounded enzyme label, the contents of the tubes were washed twice using the wash step described in the procedure for LH and LH assay. Estradiol EIA was buffer used.

Statistical Analysis

The results obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 18.0 for windows and expressed as mean ± SEM. Analysis of Variance (ANOVA) with Tukey test for multiple comparisons was used to analyze LH, FSH, prolactin and estrogen data. Statistical significance was defined as $P < 0.05$.

Results

Effect of 200mg/kg, 400mg/kg and 600mg/kg Methanolic Extract of *Cyperus esculentus* Extract on Plasma LH and FSH Concentration

In the results in figure 1, LH levels showed no statistically significant difference at $P > 0.05$ between Group A (5.23 ± 0.92) compared with experimental Group B (5.55 ± 0.29), Group C (5.33 ± 0.74) and Group D (6.10 ± 0.15). Conversely, FSH levels showed statistically significant difference at $P < 0.05$ between Group A (12.20 ± 0.94) compared to Group B (11.30 ± 1.93) and Group C (12.18 ± 1.24). There was no statistically significant difference at $P > 0.05$ between Group A (12.20 ± 0.94) compared with Group D (14.18 ± 2.00).

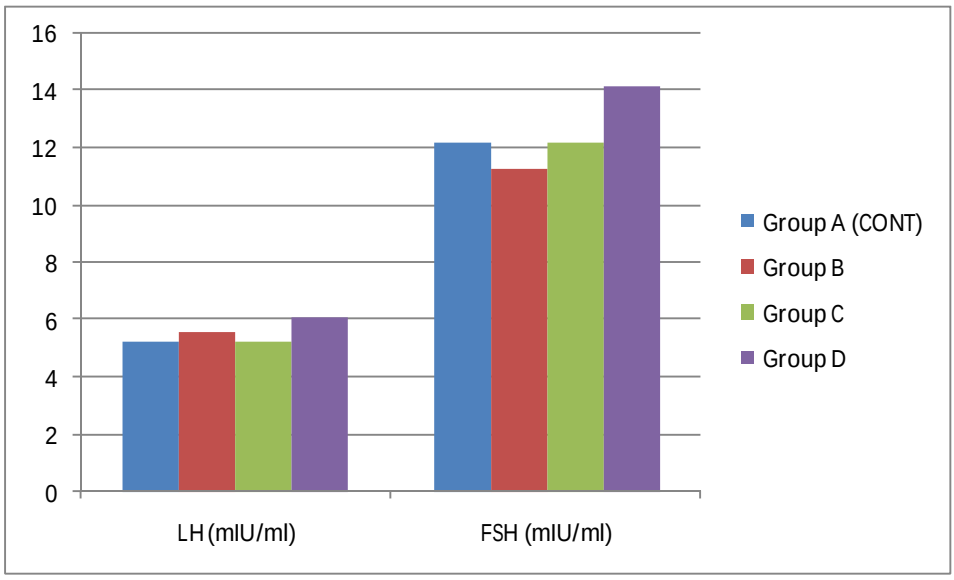

Figure 1: Effect of 200mg/kg, 400mg/kg and 600mg/kg Methanolic Extract of *Cyperus esculentus* on LH and FSH Level in Female Albino Wistar Rats

Effect of 200mg/kg, 400mg/kg and 600mg/kg Methanolic Extract of *Cyperus esculentus* Extract on Plasma Prolactin and Estrogen Concentration

In the results in figure 2, prolactin levels showed no statistically significant differences at $P > 0.05$ between Group A (1.83 ± 0.38) compared with Group B (2.03 ± 0.05), Group C (1.88 ± 0.11) and Group D (2.25 ± 0.21).

The estrogen levels showed statistically significant differences at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group B (19.85 ± 2.17). There was also statistically significant difference in estrogen levels at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group C (24.03 ± 4.31). There was a statistically significant difference at $P < 0.05$ between Group A (16.83 ± 1.48) compared to Group D (31.40 ± 4.19).

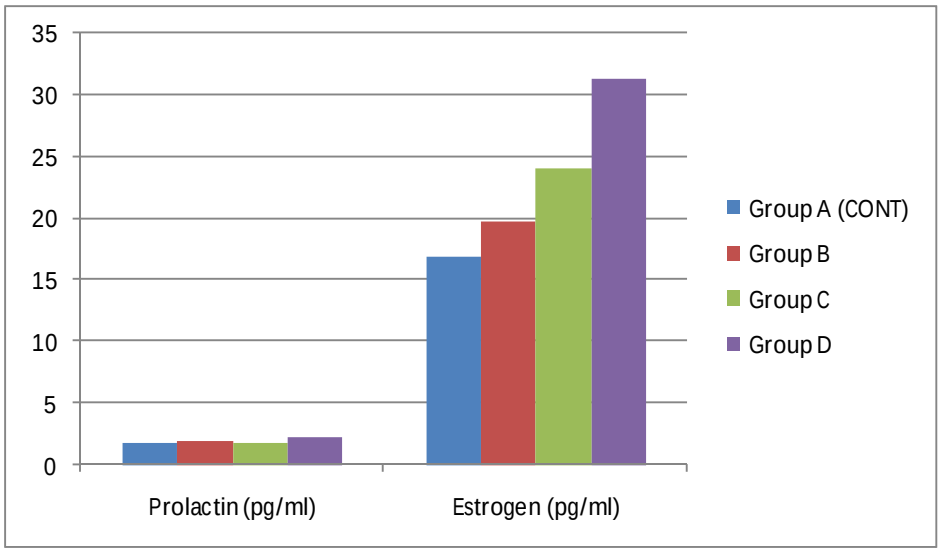

Figure 2: Effect of 200mg/kg, 400mg/kg and 600mg/kg Methanolic Extract of *Cyperus esculentus* on Prolactin and Estrogen Level in Female Albino Rats

Discussion

This present study is aimed at investigating the effect of methanolic extract of *Cyperus esculentus* on gonadotropins, prolactin and estrogen. Ekeanyanwu *et al.,* (2010) showed that *Cyperus esculentus L.* contains phytochemical contents such as sterol, resins and alkaloids. Alkaloids and flavonoids have been shown to reduce plasma concentration of LH, estradiol and FSH (Lauritzen *et al.,* 1997; Browning *et al.,* 1998; Bianco *et al.,* 2006). Udoh *et al.,* (2009) also reported that increased dose of alkaloid extract significantly decreased serum levels of FSH, LH and testosterone. These reports could explain our findings in figure 1 showing a slight increase in the LH and FSH levels (albeit not statistically significant) as the dose concentration of extract is increased.

Plant sterols have shown to possess estrogenic effect (Malini and Vanithakumari, 1993). Sex steroids are a type of sex hormone. they include androgens, estrogens and progestagens, which affect sex differences and support reproduction (Callewaert *et al.,* 2010) and phytosterols can be used as precursors to almost all kinds steroid hormones including sex hormones, anabolic steroids and even adrenocortical hormones (Aparicio and Martin, 2008; Doukyu, 2009). Results in figure 2 showed that the level of estrogen was significantly increased in a dose-dependent manner, and this increase could be as a result of high content of sterol in *Cyperus esculentus*. Plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones (Brinker, 1997). Thus, the high level of

estrogen observed in this present study could explain the small increase in LH and FSH respectively.

There was no statistically significance increase in prolactin level as shown in figure 2. High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and gonadotropin-releasing hormone (Fitzgerald and Dinan, 2008). From our findings, *Cyperus esculentus* may not have an effect on prolactin synthesis, and could be independent on the gonadotropin level.

This present study, therefore, suggests that methanolic extract of *Cyperus esculentus* increased the serum level of estrogen in a dose-dependent manner and this estrogenic property could be useful in menopause and other related cases of hypoestrogen.

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