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EFFECT OF AQUEOUS CRUDE EXTRACT OF TIGER NUT (*CYPERUS ESCULENTUS*) ON PENILE ERECTION-RELATED BIOCHEMICAL PARAMETERS IN MALE *WISTAR* RATS

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ABSTRACT

There is an increase in the cases of erectile dysfunction and the remedies so far are not without side effects thus, this study is aimed at investigating the influence of aqueous crude extract of tiger nut (*Cyperus esculentus*) on some important biochemical indices involved in penile erection in male Wistar albino rats. Twenty-four adult male Wistar rats were divided into four (4) groups of five (5) rats each. One group (control) received distilled water, the other groups received 250, 500 and 1000 mg/kg of C. esculentus aqueous crude extract respectively for fourteen (14) days. Administration was done orally (once daily) with the aid of a gavage. At the end of the experimental period, blood samples were collected through the abdominal aorta into plain sample containers to obtain serum for biochemical analysis. Nitric oxide (NO), sex hormones, fasting blood glucose, lipid profile, serum liver enzymes (ALT and AST) and haematological parameters were assayed using standard methods. Body weight of the animals and organs (testis and kidney) were also monitored at intervals of seven (7) days after basal value had been taken. The results showed significant ($p<0.05$) reduction in body weight in the second week at 500 mg/kg in relation to basal values. Significant decrease and increase were observed in the weight of kidney and testes respectively in some of the groups when compared with the control group. Significant increase was observed in NO levels in the testes. Follicle stimulating hormone and testosterone increased in a non-dose dependent manner. With the exception of total cholesterol which was increased significantly in the group given 250 mg/kg, other assayed lipid panels were not significantly changed. The haematological indices also showed a non-dose dependent effect. Overall, the extract demonstrated a modulatory effect on biochemical markers relevant to erectile physiology, supporting its potential as a natural enhancer of male sexual function.

Keywords: Tiger nut, Testosterone, Testes, Nitric oxide, Kidney

INTRODUCTION

Penile erection is a physiological process where the smooth muscles within the penis's erectile tissues respond to specific chemical cues. The key chemical messenger for this process is nitric oxide (NO), which promotes muscle relaxation by increasing cellular cGMP levels. When a man consistently struggles to achieve or sustain an erection sufficient for sexual activity, it's known as erectile dysfunction (ED), a recognized clinical manifestation of male sexual dysfunction (NIH, 1996).

Currently, erectile dysfunction (ED) stands as a pervasive sexual health concern among men worldwide (Ariba *et al*., 2007). Data suggests that approximately 50% of men between 40 and 69 years old grapple with some form of ED (Rhoden *et al*., 2002). Within Nigeria, research indicates a high prevalence, with around 57.4% of men attending health centers reporting ED (Shaeer *et al*., 2003). The repercussions of ED extend beyond physical limitations; it can severely diminish a man's self-esteem because sexual performance is often viewed as integral to a fulfilling life. Such dysfunction can indeed disrupt various aspects of one's life (McCabe, 1997; Abolfotouh and Al Helali, 2001; Althof, 2002). Recent comprehensive analyses, like those found in *Sexual Medicine Reviews* (2024), further underscore the significant individual and public health burden of ED. Disorders of the endocrine system such as (diabetes mellitus, hypogonadism) and cardiovascular disorders (ischemic heart disease, peripheral vascular disease and hypertension) are part of the key factors of ED. A good candidate drug for erectile dysfunction should have the capacity to potentiate important biochemical indices involved in penile erection and being able to attenuate the predisposing factors to the risk factors of erectile dysfunction.

Tiger nut (*Cyperus esculentus*), also known by names like chufa sedge, nut grass, or earth almond, is a resilient plant belonging to the sedge family. It grows year-round, reaching heights of up to 90 cm (Vilmorin, 1981). Each plant emerges as a single stem from a tuber, developing an intricate root system of interwoven underground stems, scales, small, strong, round tubers, and basal bulbs (Consejo *et al*., 2014). The underground stems are fleshy and thick, ranging from 0.3 to 1.9 cm in circumference, and come in yellow, brown, or black varieties (Ekeanyanwu *et al*., 2010). A single tiger nut plant can produce numerous tubers in one growing season (Rita, 2009). Remarkably, while the roots, foliage, basal bulbs, and rhizomes die off during cold seasons, the tubers can survive harsh weather and resprout the following season when soil temperatures reach around 6°C. These tubers can even remain viable and re-sprout several years later (Consejo *et al*., 2014). When tubers sprout, many rhizomes near the soil surface develop into basal bulbs. Tiger nuts are typically wind-pollinated (Miller, 2010).

In Nigeria, tiger nuts are commonly called "Aya" in Hausa, "Ofio" in the South-West, and "Aki awusa" in the South-East (Belewu & Abodurin, 2006). Three main varieties—yellow, brown, and black *Cyperus esculentus*—are cultivated there (Umerie *et al*., 1997). The yellow type is most preferred due to its fleshy texture, appealing appearance, and larger size, even though brown varieties are also common (Belewu & Abodurin, 2006). Tiger nuts are highly consumed by both humans and animals, prized for their rich nutrient and mineral content (Tunde-Akintunde & Oke, 2012). Notably, in Northern Nigeria, *Cyperus esculentus* is particularly popular, especially among men, due to its reputed aphrodisiac properties (Abireh *et al*., 2019). Few researches that have tried to provide scientific credence to this claim, generalized its

therapeutic potential targets with little or no information about the important biochemical indices involved in penile erection hence, the need for this study.

MATERIALS AND METHODS

Plant Collection and Identification

Five kilogram (5 Kg) of *C. esculentus* dry nuts was purchased from Wednesday Market in Dutsin-Ma, Katsina, Nigeria on 20th September, 2022, and was stored under room temperature before and after processing. *C. esculentus* was identified, authenticated and registered by a Botanist Dr. Daniel D. Musa (voucher number: HERB/FUDMA/PSB/00081) in the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, Nigeria.

Preparation of Plant Sample

The method of Osibemhe *et al*. (2020) was used for extraction with some slight modifications. The dry Tiger nuts were after purchase washed with clean water and the bad ones sorted out, sun dried before pulverizing to obtain fine powder using an electric blender. The powdered tiger nut (5 g) was macerated in 50 mL of water fresh daily, stirred vigorously, left unperturbed for 20 minutes at room temperature, and stirred at intervals. After 20 minutes, the dissolved tiger nut in water was administered to the rats without filtration. This was done daily for the period of administration. The crude extract was then preserved in an airtight container for daily use under room temperature.

Experimental Design

Twenty (20) adult male *Wistar* rats weighing between 110-150g were used for this study. Animals were housed in standard cages placed in well-ventilated housing condition. They were allowed to acclimatize for 7 days and were allowed access to food and drinking water *ad libitum*. Animals were properly cared for following the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health (NIH, 1992). They were randomly divided into four groups of five (5) rats each. Extract was administered as follows:

Group A served as control and were administered distilled water.

Group B (normal rats): received 250 mg/kg of *C. esculentus* aqueous crude extract.

Group C (normal rats): received 500 mg/kg of *C. esculentus* aqueous crude extract.

Group D (normal rats): received 1000 mg/kg of *C. esculentus* aqueous crude extract.

The choice of dosage was informed by reported safe dose of up to 2000mg/kg (Ekeanyanwu *et al*; 2010).The extract was administered orally once daily using a gavage for a period of 14 days. At the end of the administration, all the rats were sacrificed after an overnight fast under chloroform anesthesia. Blood samples were collected into a 5ml syringe through the abdominal aorta into plain sample containers to obtain serum for biochemical analysis.

Determination of Body Weight

Body weight was determined weekly after basal values using an analytical weight balance.

Determination Organ Weight

The reproductive organs (testes and kidney) were removed, cleaned free of fat, and weighed using an electronic weighing balance.

Determination Fasting Blood Glucose (FBG)

Fasting blood glucose was determined weekly after basal values using glucometer.

Determination of Nitric Oxide (NO) Level

Nitric oxide was assayed by Griess method (Guevara *et al*., 1998). The Griess assay detects the red-pink colour produced by the reaction of Griess reagents with nitrites. Griess reagent was prepared fresh consisting of equal volume of sulfanilamide solution and *N*-(1-naphthyl) ehtylenediamine (NED) solution. The sulfanilamide solution was prepared by dissolving 1.5g of sulfanilamide in 100ml of 1M HCl/NED solution in 100ml distilled water. The reagent was protected from sunlight. Nitrite production was determined by mixing 1ml of the sample with 1ml of Griess reagent. After 10 minutes of incubation at room temperature, the absorbance was determined at 540nm. Nitric oxide concentrations were calculated from the sodium nitrite standard curve for serially diluted nitrite concentration.

Determination of Serum Testosterone Level, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH)

The hormones were assayed using the fully automated Finecare 3 Plus Immunoassay Analyser (WondFo) in triplicate following manufacturer's protocol.

Determination of Serum lipid profile (Triglyceride, Total cholesterol (TC), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL)

Lipid profile was estimated using Randox Diagnostic kits-United Kingdom.

Determination of Haematological Parameters

Haematological parameters (WBC, RBC and PLTS) were determined using automated analyser

Determination of Serum Liver Enzymes (ALT and AST)

Serum Liver Enzymes (ALT and AST) were determined using Biochemistry analyser and enzyme assay kits from Randox Laboratories Ltd. (United Kingdom).

Statistical Analysis

Data were subjected to analysis of compared mean. Results were presented as Mean ± standard deviation. One-way analysis of variance (ANOVA) was used for comparison of the means followed by Duncan post hoc test to compare the means using the Statistical Package for the Social Sciences (SPSS) version 20. Differences between means were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

The result in Table 1 shows the effect of the aqueous crude extract on body weight of male *Wistar* rats. A significant reduction in body weight was observed in group administered 500 mg/kg of the crude extract. This is in line with a previous study by Gambo and Da'u (2014), where rats administered methanolic extract of tiger nut displayed reduction in body weight.

Table 1: Effects of 14 days treatment with aqueous crude extract of *C. esculentus* (tiger nut) on body weight (g) of male *Wistar* rats

Group	Basal Weight (g)	Week 1 Weight (g)	Week 2 Weight (g)
A (control)	80.42±2.46^{a}	116.88±0.71^{b}	134.80±1.72^{c}
B (250mg/kg)	149.88±0.23^{a}	151.52±0.23^{a}	183.00±1.23^{b}
C (500mg/kg)	236.80±6.25^{b}	224.60±3.78^{ab}	221.28±3.78^{a}
D (1000mg/kg)	160.60±11.85^{a}	164.60±10.08^{a}	171.96±9.75^{a}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a row are not significantly different

The effect of aqueous crude extract of tiger nut on organ weight is shown in Table 2. There was a significant increase in the weight of testes in rats administered with tiger nut aqueous extract. The results agrees with the study of Amaal and Essraa (2010). The increase in testes weight can be linked to the presence of vitamin C in Tiger nut (Pamplona-Roger, 2005; Belewu and Belewu, 2007; Chukwuma *et al*., 2010) and its ability to protect against oxidative stress and alterations in the structure tissues of the testis (Karawya and El-Nahas, 2006; Nashwa and Venes, 2008; Fernandes *et al*., 2011; Al-Amoudi, 2012; Ekaluo *et al*., 2013). Furthermore, the weight gained by the testis may be attributed to enhanced sperm production (Kamtchouing *et al*., 2002; Nayernia *et al*., 2004; Amaal and Essraa, 2010; Ekaluo *et al*., 2013), and to improved production of the male sex hormones (Morakinyo *et al*., 2008). A significant difference in kidney weight was observed in group administered 250 mg/kg and 1000 mg/kg of the aqueous crude extract.

Table 2: Effects of 14 days' Treatment with Aqueous Crude Extract of C. Esculentus (tiger nut) on Weight (g) of Testis and Kidney of Male Wistar Rats

Group	Testis Weight (g)	Kidney Weight (g)
A (control)	1.86±0.19^{a}	1.54±0.11^{c}
B (250mg/kg)	2.24±0.15^{b}	1.26±0.02^{ab}
C (500mg/kg)	2.74±0.16^{b}	1.31±0.10^{bc}
D (1000mg/kg)	2.36±0.13^{b}	1.04±0.07^{a}

Values are means ± SEM (n=5). Values with the same superscript within a column are not significantly different

The limited gain in body weight and the male sex organ by the test groups were compared with the control as shown in Table 3. The result showed no significant difference in the relative organ weight of the testes. However, there was a significant decrease in relative organ weight of kidney in the test group when compared to the control. The results of the relative organ weight of the testes obtained in this study is in agreement with those of Allouh *et al*. (2015) where no significant difference in organ to body weight ratio between control and treated rats was observed.

Table 3: Effects of 14 days Treatment with Aqueous Crude Extract of C. Esculentus (Tiger nut) on Organ to Weight Ratio (%) of Male Wistar Albino Rats

Group	Testis	Kidney
A (control)	1.38±0.14^{a}	1.14±0.09^{b}
B (250mg/kg)	1.32±0.08^{a}	0.69±0.02^{a}
C (500mg/kg)	1.23±0.07^{a}	0.59±0.04^{a}
D (1000mg/kg)	1.40±0.15^{a}	0.61±0.03^{a}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different

Table 4 shows the effect of tiger nut extract on fasting blood glucose level. The result showed that there was a significant reduction in fasting blood glucose in group administered 250 mg/kg and 500 mg/kg of the aqueous extract in week 2. Reduction in fasting blood glucose may be due to the presence of some amino acids and fibre in tiger nut. Several studies have suggested that the high fiber and arginine found in tiger nut has the ability to stimulate insulin which will in turn mop up excess blood sugar and stop the detrimental effect of diabetes (Wu and Meininger, 2000; Oladele and Aina, 2007). Sánchez *et al*. (2012) and Gambo, (2014) observed that higher amounts of tiger nut aqueous crude extract had significantly lower blood glucose levels compared to those of the control group. However, our study only showed significantly low blood glucose level in group administered 250 mg/kg and 500 mg/kg of the aqueous extract in week 2. The highest dose group (1000 mg/kg) did not show significant reduction in blood glucose level.

Table 4: Effects of 14 days' Treatment with Aqueous Crude Extract of C. Esculentus (tiger nut) on Fasting Blood Glucose (mmol/L) of Male Wistar Albino Rats

Group	Basal (mmol/L)	Week 1 (mmol/L)	Week 2 (mmol/L)
A (control)	7.74±2045^{a}	7.76±0.36^{a}	8.00±0.59^{a}
B (250mg/kg)	6.10±0.29^{a}	7.60±0.46^{b}	5.28±0.35^{a}
C (500mg/kg)	6.18±0.15^{a}	6.68±0.19^{b}	5.80±0.13^{a}
D (1000mg/kg)	6.98±0.24^{a}	7.72±0.19^{a}	7.06±0.23^{a}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a row are not significantly different.

Table 5 shows the effect of tiger nut extract on nitric oxide level. There was a significant increase in the level of nitric oxide in testes of group treated with 250 mg/kg of the aqueous extract as compared to the control. However, no significant difference was observed in nitric oxide from kidneys when the treated group was compared to the control. The result in this study correspond with a previous report by Amalia *et al*. (2008) where they observed a significant increase in NO level in male *Wistar* rats treated with *Kukai* bulbs extracts.

Table 5: Effects of 14 days' Treatment with Aqueous Crude Extract of C. Esculentus (Tiger nut) on Nitric Oxide (NO) of Male Wistar Rats

Group	Testis	Kidney
A (control)	0.40 ± 0.87^{a}	0.41 ± 0.05^{a}
B (250mg/kg)	0.64 ± 0.89^{b}	0.35 ± 0.01^{a}
C (500mg/kg)	0.43 ± 0.38^{ab}	0.40 ± 0.05^{a}
D (1000mg/kg)	0.29 ± 0.54^{a}	0.42 ± 0.11^{a}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different

Administration of tiger nut aqueous extract resulted in a significant decrease in the levels of serum testosterone in group administered 250 mg/kg of the extract (Table 6). This finding is in contrast to those of Allouh *et al*. (2015) who reported a significant increase in serum testosterone levels in the test groups. In addition, there was no significant increase in LH levels in this study. The result obtained is comparable to those of Abireh *et al***.** (2019).

Table 6: Effects of 14 days' Treatment with Aqueous Crude Extract of C. Esculentus (tiger nut) on Male Sex Hormones of Male Wistar Rats

Group	FSH (IU/L)	LH (IU/L)	Testosterone (ng/dl)
A (control)	2.30 ± 0.22^{a}	1.44 ± 0.16^{a}	3.46 ± 0.26^{b}
B (250mg/kg)	9.64 ± 1.66^{b}	1.42 ± 0.25^{a}	0.82 ± 0.27^{a}
C (500mg/kg)	2.20 ± 0.42^{a}	1.54 ± 0.30^{a}	3.56 ± 0.28^{b}
D (1000mg/kg)	2.20 ± 0.46^{a}	1.64 ± 0.23^{a}	3.44 ± 0.34^{b}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different. FSH-Follicle Stimulating Hormones, LH- Luteinizing Hormone

A significant increase in total cholesterol (TC) was observed in group treated with 250 mg/kg of the extract (Table 7). Increase in serum cholesterol concentration has been associated with increase in aphrodisiac activity since cholesterol has been linked to production of steroid hormones (Erhabor and Idu, 2017). Furthermore, the result showed no significant changes in TG, HDL and LDL as compared to the control. The non-significant differences observed suggests that tiger nut was able to maintain lipid profile homeostasis in the animals which is advantageous given influence high amount of lipid in the blood on erection.

Table 7: Effects of 14 Days' Treatment with Aqueous Crude Extract of C. Esculentus (tiger nut) on Lipid Profile of Male Wistar Rats

GROUP	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A (control)	100.60 ± 3.14^{a}	88.40 ± 5.84^{a}	26.20 ± 2.24^{a}	92.08 ± 2.16^{a}
B (250mg/kg)	114.20 ± 4.84^{b}	98.00 ± 7.04^{a}	27.80 ± 2.85^{a}	106.00 ± 3.24^{a}
C (500mg/kg)	102.80 ± 2.63^{ab}	94.00 ± 4.39^{a}	27.40 ± 1.72^{a}	94.20 ± 2.52^{a}
D (1000mg/kg)	101.80 ± 4.82^{ab}	86.60 ± 6.70^{a}	28.40 ± 2.84^{a}	90.72 ± 3.61^{a}

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different. TC-Total Cholesterol, TG-Triglyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein.

The results of the haematological indices in Table 8 shows that the administration of tiger nut to rats at different doses caused significant changes in most of the parameters at 250 and 500 mg/kg body weight. The increased RBC could be as a result of the abundance of iron in tiger nut (Addy and Eteshola, 1984; Jeong *et al*., 2000; Shaker *et al*., 2009).

Table 8: Effects of 14 Days' Treatment with Aqueous Crude Extract of C. Esculentus (Tiger nut) on Hematological Parameters of Male Wistar Rats

Group Parameters	A (control)	B (250mg/kg)	C (500mg/kg)	D (1000mg/kg)
WBC (10^3/µL)	6.36±0.66^a	11.92±1.05^b	6.74±1.01^a	7.20±0.55^a
LYM (%)	87.92±1.65^c	66.58±5.86^a	80.74±2.46^{bc}	75.48±2.53^{ab}
MON (%)	4.52±0.95^a	11.40±1.14^{bc}	6.50±1.35^{ab}	12.74±2.60^c
NEU (%)	4.14±0.29^a	10.26±1.55^c	7.80±0.98^{bc}	6.00±1.23^{ab}
EOS (%)	0.26±0.05^{ab}	0.38±0.12^b	0.28±0.05^{ab}	0.15±0.05a
BAS (%)	3.14±0.38^a	7.04±1.50^b	4.68±0.49^{ab}	5.66±0.89^{ab}
RBC (10^6/µL)	5.48±0.51^a	7.31±0.19^b	6.90±0.44^b	6.55±0.89^b
HGB (g/dL)	12.58±0.33^a	13.72±0.18^a	13.70±0.81a	3.02±0.12^a
HCT (%)	34.50±2.20^a	41.47±0.61^b	43.36±1.15^b	37.84±0.33^a
MCV (fL)	59.20±1.13^a	57.72±2.03^a	58.46±0.47^a	57.84±0.95^a
MCH (pg)	21.96±1.88^a	19.30±0.67^a	19.86±0.33^a	19.88±0.30^a
MCHC (g/dL)	37.36±2.61^a	33.26±0.21^a	33.96±0.30^a	34.40±0.39^a
RDWC (%)	14.82±1.01^a	16.64±0.50^a	16.30±0.27^a	16.06±0.29^a
RDWS (%)	32.46±1.60^a	32.48±2.06^a	34.80±0.80^a	34.26±1.33^a
PLT (10^3/µL)	465.20±112.88^a	566.40±82.33^a	575.00±42.69^a	446.20±59.22^a
MPV (fL)	4.98±2.04^a	7.70±0.08^a	6.16±1.54^a	8.06±0.12^a
PCT (fL)	0.00±0.00^a	0.43±0.61^b	0.00±0.00^a	0.00±0.00^a
PDW (%)	0.00±0.00^a	14.44±0.65^b	0.00±0.00^a	0.00±0.00^a
PLCR (fL)	0.00±0.00^a	11.22±0.78^b	0.00±0.00^a	0.00±0.00^a

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different

WBC -White Blood Cell, LYM- Lymphocyte, MON-Monocyte, NEU-Neutrophil, EOS -Eosinophil, BAS-Basophil, RBC-Red Blood Cell, HGB-Hemoglobin, HCT-Hematocrit, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration, RDWC, Red Blood Cell Volume Distribution C-width, RDWS-Red Blood Cell Volume Distribution S-width, PLT, Platelet, MPV, Mean Platelet Volume, PCT-Plateletcrit, PDW-Platelet Volume Distribution Width, PLCR ---- Platelet Large Cell Ratio

Table 9 showed no significant increase in serum liver enzymes. The result indicate that the administered doses are not deleterious to the liver. Also, tiger nuts have hepatoprotective properties as posited by Mehta *et al*. (1999). This could be one of the functions of unsaturated fatty acids on the walls of the liver that keep the liver integrity and the permeability of the walls of the liver in place (Owu *et al.,* 1998).

Table 9: Effects of 14 Days Treatment with Aqueous Crude Extract of C. Esculentus (Tiger nut) on Liver Enzymes (AST & ALT) of Male Wistar Albino Rats

Group	AST (U/L)	ALT (U/L)
A (control)	17.40±1.17^a	7.80±0.97^a
B (250mg/kg)	16.20±0.80^a	6.60±0.75^a
C (500mg/kg)	16.20±1.02^a	7.00±0.80^a
D (1000mg/kg)	18.00±1.00^a	8.40±0.90^a

Values are means ± SEM (n=5). Values with the same superscript alphabet within a column are not significantly different

CONCLUSION

In conclusion, the results presented indicated that tiger nut positively affected nitric oxide (NO) levels, a vital component in smooth muscle relaxation. We also observed that these doses effectively balanced most of the predisposing factors for erectile dysfunction. Consequently, *C. esculentus* shows significant potential as a means to enhance erections and improve conditions related to erectile dysfunction.

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