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Dietary supplementation of tiger nut alters biochemical parameters relevant to erectile function in L-NAME treated rats

Ayodeji A. Olabiyi[a,b,c], Fabiano B. Carvalho[c], Nathieli B. Bottari[c], Thauan F. Lopes[c], Pauline da Costa[c], Naiara Stefanelo[c], Vera M. Morsch[c], Afolabi A. Akindahunsi[a], Ganiyu Oboh[a,*], Maria Rosa Schetinger[c,*]

[a] Department of Biochemistry, Functional Foods and Nutraceuticals Unit, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria
[b] Department of Medical Biochemistry, Afe Babalola University, P.M.B. 5454, Ado-Ekiti, Nigeria
[c] Department of Biochemistry and Molecular Biology, Center of Natural and Exacts Sciences, Federal University of Santa Maria, Santa Maria, RS 97105-900, Brazil

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Adenosine (PubChemCID: 60961)
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Nitric oxide (PubChemCID: 145068)
Malondialdehyde (PubChemCID: 10964)
Vanadium chloride (PubChemCID: 62647)
Ammonium sulfate (PubChemCID: 6097028)
Sulfanilamide (PubChemCID: 5333)
Albumin (PubChemCID: 16132389)

ABSTRACT

Tiger nut tubers have been reportedly used for the treatment of erectile dysfunction (ED) in folk medicine without scientific basis. Hence, this study evaluated the effect of tiger nut on erectile dysfunction by assessing biochemical parameters relevant to ED in male rats by nitric oxide synthase (NOS) inhibitor, *N*ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) treatment. Rats were divided into five groups ($n = 10$) each: Control group; L-NAME plus basal diet; L-NAME plus Sildenafil citrate; diet supplemented processed tiger nut (20%) plus L-NAME;diet supplemented raw tiger nut (20%) plus L-NAME. L-NAME pre-treatment (40 mg/kg/day) lasted for 14 days. Arginase, acetycholinesterase (AChE) and adenosine deaminase (ADA) activities as well as nitric oxide levels (NO) in serum, brain and penile tissue were measured. L-NAME increased the activity of arginase, AChE and ADA and reduced NO levels. However, dietary supplementation with tiger nut caused a reduction on the activities of the above enzymes and up regulated nitric oxide levels when compared to the control group. The effect of tiger nut supplemented diet may be said to prevent alterations of the activities of the enzymes relevant in erectile function. Quercetin was revealed to be the most active component of tiger nut tuber by HPLC finger printing.

1. Introduction

Some benefits of tiger nut tubers have been documented for past decades without scientific validation. Recently, the usefulness has gained more attention due to the potential ability that was claimed in traditional medicine in the management of erectile dysfunction (ED). To this end, our research was directed to see if tiger nut tubers indeed have aphrodisiac properties. So far, from our research, we have been able to establish the ability of tiger nut tubers to enhance sexual behaviour, increase male sex hormone and showed antioxidant property as well as modulating key biochemical indices relevant to erectile function in normal adult male rats (Olabiyi et al., 2017; Olabiyi, Oboh, & Adefegha, 2016). In view of the published papers from our laboratory on the aphrodisiac potential of tiger nut tuber in normal adult male rats, we decided to further look at the effect of tiger nut tubers on relevant enzymes linked to erectile function in *N*ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) treated adult male rats since hypertension has been linked to ED. ED is defined as the consistent inability to obtain or maintain an erection for satisfactory sexual intercourse (Feldman, Goldstein, Hatzichristou, Krane, & Mckinlay, 1994). Basic science research on erectile physiology has been devoted to investigating the pathogenesis of ED and this has led to the conclusion that ED is predominantly a disease of vascular origin. The incidence of ED dramatically increases in men with diabetes mellitus, hypercholesterolemia, and cardiovascular disease. Endothelial dysfunction has been hypothesized to result in ED (Maas, Schwedhelm, Albsmeier, & Boger, 2002; Solomon, Man, & Jackson, 2003) and it is termed a phenotypical alteration of the endovascular lining of blood vessels characterized by

* Corresponding authors.
E-mail addresses: goboh2001@yahoo.com (G. Oboh), mariachitolina@gmail.com (M.R. Schetinger).

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pro-thrombotic, pro-inflammatory and pro-constrictive phenotype (Gokce, Keaney, & Vita, 1998). Endothelial function is readily measurable through multiple modalities as an established barometer of cardiovascular risk (Vita & Keaney, 2002). Measurement of endothelial function uses method designed to assess vasodilation of pharmacologic stimuli (for example, acetylcholine or bradykinin), mechanical stimuli (shear), or both. Furthermore, the vasodilator responses to these stimuli are primarily related to nitric oxide (NO) production capacity. Key research over several decades has identified NO as a central regulator of vascular endothelial function, with a loss of NO bioavailability identified as a central phenotypic characteristic of endothelial dysfunction (Ignarro, Edwards, Gruetter, et al., 1980). The formation of NO from its substrate L-arginine occurs in most tissues of the body, a reaction catalyzed by NO synthase (NOS). NO produced by endothelial nitric oxide synthase (eNOS) has been shown to be a vasodilator identical to the endothelium-derived relaxing factor produced in response to shear from increased blood flow in arteries (Ponting & Phillips, 1995). This dilates blood vessels by relaxing smooth muscle in their linings and activating guanylate cyclase (Burnett, Lowenstein, Bredt, Chang, & Snyder, 1992; Ignarro et al., 1990). Nevertheless, NOS and arginase compete for L-arginine as substrate (Giugliano et al., 2010), therefore inhibition of arginase is suggested to favour the bioavailability of NO. *N*ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) which is an NOS inhibitor has been utilized as experimental hypertension model both from other researchers (Biancardi, Bergamaschi, Lopes, & Campos, 2007; Lackland & Weber, 2015) and in our laboratory (Akinyemi et al., 2015; Cardoso et al., 2012) (Table 1).

Adenosine, like NO, is also a potent vasodilator and has long been implicated in the regulation of erectile function (Andersson, 2001) as well. However, adenosine is deaminated to inosine by the action of adenosine deaminase (ADA); hence, inhibition of this enzyme has been found to improve smooth muscle relaxation (Fredholm, IJzermen, Jacobson, Klotz, & Linden, 2001). Acetylcholinesterase (AChE), found primarily on red blood cell membranes, neuromuscular junctions, and neural synapses exists in multiple molecular forms (Wang & Tang, 2005) function in the hydrolysis of neurotransmitter-acetylcholine to choline and acetic acid. Decrease of acetycholinesterase activity makes acetylcholine available from nonadrenergic and noncholinergic nerve endings to stimulate the production of NO in the endothelial cell and its importance in male erectile function have been suggested. The brain controls all sexual functions, from perceiving arousal to initiating and controlling the psychological, hormonal, nerve, and blood flow changes that leads to the relaxation of the smooth muscle. Hippocampus and hypothalamus have been regarded as important integration centers for sexual behaviour and penile erection (Sachs & Meisel, 1988). Oxidative stress has also been implicated as a result of imbalance between free radicals and antioxidants in a number of degenerative disease conditions due to the formation of reactive oxygen species (ROS) and thiobarbituric reactive substance (TBARS).

Tiger nut tuber is a member of the grass family Cyperaceae and has many other names like Zulu nut, yellow nut grass, ground almond, edible rush and rush nut (Eteshola & Oraedu, 1996). It is cultivated both for animal and human consumption and could be eaten raw, roasted, grated, baked or used for ice cream and beverage making (Belewu & Abodunrin, 2006). Tiger nut had been reported to contain arginine (Martínez-Valls, 2003) which is a precursor of nitric oxide, able to reduce colon cancer risk (Adejuyitan, Otunola, Akande, Bolarinwa, & Oladokun, 2009) and serve as a source of milk "horchata" (Rubert, Sebastiá, Soriano, Soler, & Mañes, 2011). Also, reports have been made of the tuber to be one of the natural remedy-based treatments for inflammatory diseases such as atherosclerosis and heart attack (Martínez-Valls, 2003; Salem, Zommara, & Imaizumi, 2005) thereby improving blood circulation (Chukwuma, Obioma, & Cristopher, 2010). Enhancement of sexual behaviour, male sex hormone and antioxidant property as well as the modulatory effect of tiger nut supplementation on key biochemical indices relevant to erectile function in normal adult male rats has also been reported from our laboratory (Olabiyi et al., 2016, 2017). Of all the importance attributed to tiger nut tubers, its effect on relevant enzymes linked with L-NAME treated rats has not been reported. Therefore, this study sought to investigate the effect of dietary supplementation of tiger nut tubers (raw and processed) on the relevant biomolecules involved in erectile function in L-NAME treated rats.

Table 1
Diet formulation for basal and supplemented diets for the control and test groups.

Groups					
Component	I	II	III	IV	V
Skimmed milk	37.5	37.5	37.5	33.1	33.1
Oil	10.0	10.0	10.0	10.0	10.0
Vitamin mix.	4.0	4.0	4.0	4.0	4.0
Corn Starch	48.5	48.5	48.5	32.9	32.9
PRO				20.0	
RAW					20.0
Total (g)	100	100	100	100	100

Note: PRO: Processed tiger nut, RAW: Raw tiger nut,
Skimmed milk - 32% protein. One gram of vitamin mixture 3200 IU vitamin A, 600 IU. Vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 1 mg vitamin B2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin H2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 Mg manganese, 0.08 mg selenium, 12.4 mg zinc, and 0.5 mg antioxidant.

2. Materials and methods

2.1. Chemicals and reagents

L-arginine, adenosine, acetylthiocholine iodide, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and Coomassie brilliant blue G were procured from Sigma-Aldrich, Inc., (St Louis, MO, USA). N-(l-naphthyl) ethylenediamine dihydrochloride, Tris–HCl buffer, bovine serum albumin and vanadium III chloride (VCl_3) were from Reagen (Colombo, Paraná (PR), Brazil). Thiobarbituric acid (TBA) and trichloroacetic acid (TCA) are sourced from BDH Chemicals Ltd., (Poole, England). All other chemicals and reagents used were of the highest purity, while the water was glass distilled.

2.2. Plant material and sample preparation

Tiger nut tubers (*Cyperus esculentus* L.) were obtained from the local market in Akure, Nigeria. The tubers were thoroughly washed under running water tap to remove contaminants and the quantities obtained were divided into two portions. The first portion of the tubers was roasted in an electric oven for 30 min at 120 °C (categorized as processed; PRO) while the other portion was oven dried at 45 °C (categorized as raw (RAW) to a constant weight. The two samples were pulverized, defatted in cold *n*-Hexane and kept in an air-tight container prior to analysis. Furthermore, proximate analyses of the samples were carried out (data not published) to determine the nutritional content of the tuber which was used in the diet formulation. Voucher specimen of *C. esculentus* L. has been deposited in Ekiti State University, Ado Ekiti Herbarium.

2.3. Preparation of extracts

The nuts were extracted twice for 24 h in 10 ml of 70% ethanol and 30% distilled water per gram milled material on a mechanical shaker and then filtered using Whatman filter paper. The first and second extracts were combined using rotary evaporator (Heidolph Laborota 4000