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The Phytochemical Composition and Some Biochemical Effects of Nigerian Tigernut (*Cyperus esculentus L.*) Tuber

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Abstract: The phytochemical composition of the tigernut tuber and the effect of the aqueous extract on some biochemical parameters such as blood glucose, serum protein, albumin and cholesterol, white blood cells, red blood cells, haemoglobin, erythrocyte sedimentation rate and packed cell volume were determined in rats administered different concentrations of the extract. From the result of the phytochemical analysis, the presence of alkaloids, cyanogenic glycosides, resins, tannins, sterols and saponins were observed in the raw tuber, however only alkaloids, sterols and resins were observed in the roasted tuber. Analysis of the antinutrient composition yielded oxalates (0.25±0.65 g/100 g), phytate (1.97±0.81 mg/100 g), saponins (0.88±0.02/100 g), tannins (9.50±0.46 mg/100 g) and cyanogenic glycosides (1.80±0.69 mg/100 g). Roasting numerically decreased the levels of the anti-nutritive factors analyzed. At the end of the treatment period, the mean weights of the animals increased. The blood glucose level decreased significantly in concentration dependent manner ($p<0.05$) and serum albumin level increased significantly in a concentration dependent manner ($p<0.05$) in the groups administered the different concentrations of the extract. There was no significant effect ($p>0.05$) on serum cholesterol and protein and on total and differential white blood cell, red blood cell, haemoglobin, packed cell volume and erythrocyte sedimentation rate. The results therefore indicate the absence of undesirable effect in the use of the tigernut tuber even in the raw form at least at the administered concentration and for the duration of feeding. The findings are of nutritional, health and industrial relevance since the tuber is currently being used as food in many homes in Nigeria.

Key words: *Cyperus esculentus*, tigernut, phytochemicals, biochemical effects

INTRODUCTION

The worsening food crisis and the consequent wide spread prevalence of malnutrition in developing and underdeveloped countries have resulted in high mortality and morbidity rates, especially among infants and children in low income groups (Enujuigba and Akanbi, 2005). The reliance on starchy roots and tubers and protein deficient cereals as main staples results in consumption of non-nutritious foods. The insufficient availability of nutrient rich diets and high cost of available ones have prompted an intense research into harnessing the potentials of the lesser known and underutilized crops, which are potentially valuable for human and animal foods to maintain a balance between population and agricultural productivity, particularly the tropical and subtropical areas of the world.

Cyperus esculentus (Tigernut) is an underutilized plant of the family *Cyperaceae*, which produces rhizomes from the base and tubers that are some what spherical (Cortes *et al*., 2005). The plant is not really a nut but a tuber first discovered some 4000 years ago (Lowe and Whitewell, 2000). It has other names like yellow nutsedge, chufa, flatsedge, rush nut, water grass, earth almond, northern nut grass and nut grass (Shilenko *et al*., 1979). *Cyperus esculentus* is known in Nigeria as aya in Hausa, ofio in Yoruba and akihausa in Ibo. *Cyperus esculentus* grows mainly in the middle belt and northern regions of Nigeria (Okafor *et al*., 2003), 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 fleshier body (Belewu and Abodurin, 2006). *Cyperus esculentus* can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called kuunu (Oladele and Aina, 2007).

Cyperus esculentus was reported as healthy and helps in preventing heart, thrombosis and activates blood circulation. It helps in preventing cancer, due to high content of soluble glucose. It was also found to assist in reducing the risk of colon cancer (Adejuyitan *et al*., 2009). The nut is rich in energy content (starch, fat, sugars and protein), mineral (phosphorus, potassium)

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and vitamins E and C (Belewu and Belewu, 2007). *Cyperus esculentus* is suitable for diabetic persons and also helps in loosing weight (Borges *et al*., 2008).

Food contains various compositions of nutrients and antinutrients and could have important or deleterious effects in the body when consumed. The composition of the nutrients and antinutrients, usually leads to side effects found in most plants which may lead to toxicity, hyperlipidaemia, excessive weight gain, hyperglycaemia, carotenemia, constipation, kidney stones, body odour, bad breath, allergies, diarrhoea, frequent urination and acne (Anonymous, 2009). In most of these side effects, the biochemical and haematological parameters are usually altered. For a food to be considered safe for human and animal health, its effect on these parameters need to be investigated to understand the nutritional potentials and safety of such foods with a view to determining their acceptability.

The aim of the present study is to determine the phytochemical composition of the tuber and to ascertain if the tuber could have beneficial effect on biochemical parameters such as blood glucose, serum albumin, protein, cholesterol, red blood cell, haemoglobin, erythrocyte sedimentation rate, packed cell volume and total and differential white blood cell of the rats as our model for the research.

MATERIALS AND METHODS

Collection and preparation of tigernut tuber flour and the aqueous extract: Fresh tigernut tuber was purchased from a local market in Katsina, Katsina state, Nigeria. The tuber was identified and authenticated by Mr A. Ozougwu of Botany department, university of Nigeria, Nsukka, Enugu state. The tigernut tubers were cleaned, sorted and washed. The fresh tubers were dried in an oven (GallenKamp, England) at 37°C for one hour, milled separately using a laboratory electric mill (Retsch, 5657, GmbH, Germany) to pass through a 40-mesh sieve, packaged in glass jars and stored at 4°C in a refrigerator until analysis. A Quantity, 400 g of the fresh milled tubers was extracted by shaking it with 3 litres of n-hexane for one hour, three times to remove the oil. The defatted milled tubers were dried in a desiccator under vacuum. The water extract was obtained by stirring the dry defatted milled tubers with seven (7) litres of distilled water at room temperature (27±1°C) for twelve hours. The suspension was centrifuged at 3000 rpm for 10 min and the supernatant was filtered through white muslin cloth and then whatman filter paper No.1 under vacuum. The extract was concentrated using water bath at an optimum temperature of 65°C to avoid the denaturation of the bioactive compounds. The weight of the dry extract was determined. The different concentrations (500, 1000, 1500 and 2000 mg/kg) of the extract were prepared.

Table 1: The phytochemical composition of the tigernut tuber

Phytochemical	Raw	Roasted
Alkaloids	+++	+
Glycosides	-	-
Cyanogenic glycosides	+	-
Resins	+++	+++
Flavonoids	-	-
Cardiac glycosides	-	-
Tannins	+	-
Sterols	+++	+++
Saponins	+	-

+++ = Present in very high concentration, ++ = Present in moderately high concentration, + = Present in trace concentration, - = Not detected

Experimental animals: Adult male Wistar albino rats were purchased from the faculty of biological sciences animal house, University of Nigeria, Nsukka, Enugu state, Nigeria. The animals were about 12 weeks with average weight of 112.37±11.7 g. The animals were kept under standard conditions for 7 days with free access to water and food before starting the experiment. Albino mice, 20.50±4.27 g weights were used for the acute toxicity tests. The animals were housed in standard cages with food and water *ad libitum* at room temperature and provided with pelletized feed.

Experimental design: An acute toxicity study of the aqueous extract of tigernut was done by the method of Lorke (1983). Twenty five (25) male Wistar albino rats of 12 weeks were divided into five groups of five rats each of average weight were randomly assigned to five (5) cages labelled I, II, III, IV and V respectively and kept at room temperature (25°C). All the rats were allowed free access to water and feed *ad libitum* for a week to acclimatize them to laboratory conditions. After this period, the control animals (group I) were administered 0.2 ml of normal saline (0.9% NaCl) while groups II, III, IV and V were administered different concentrations of the extract. The extracts were administered for 30 days to the animals using the oral route by means of polythene cannula. The weights of the animals were taken before commencement of the feeding experiment and then later every six days interval. At the end of the 30 days, blood samples from each rat were collected through the orbital technique for analysis of haematological parameters like total and differential white blood cells, red blood cell, haemoglobin, packed cell volume, erythrocyte sedimentation rate and biochemical parameters like blood glucose, serum protein, albumin and cholesterol.

Phytochemical analysis: The phytochemical test for the presence and absence of saponins, alkaloids, flavonoids, cyanogenic glycosides, tannins, glycosides, and sterols were carried out according to the method described by Harbone (1984).

Antinutrient analysis: Percentage compositions of some antinutrients like oxalates, phytates, cyanogenic glycosides, saponins and tannins were determined by the method described by AOAC (1990). All determinations were done in triplicate determination.

Biochemical studies: Serum cholesterol was determined by the method of Meiatini *et al.* (1978), serum total protein by the method of Wooten (1964), blood glucose by the glucose oxidase method of Marks and Dawson (1965), serum albumin by the method of Doumas *et al.* (1971).

Haematological studies: The haemoglobin concentration was estimated using the cyanome-thaemoglobin photometric method. The packed cell volume was estimated using the micro-haematocrit centrifuge. The red blood cell and differential white blood cell was estimated using the improved Neubauer haemocytometer. Erythrocyte sedimentation rate was determined using the Westergren method (1957).

RESULTS AND DISCUSSION

The result of phytochemical screening shows that a higher content of alkaloids, sterols and resins than cyanogenic glycosides, saponins and tannins were detected in the raw Tigernut tuber. However, in the roasted Tigernut tuber, only alkaloids sterols and resins were detected and no other phytochemical assayed was detected. Alkaloids, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (Sofowora, 1993; Evans, 2005). Alkaloids are known for their toxicity, but not all alkaloids are toxic. They inhibit certain mammalian enzymic activities such as those of phophodiesterase, prolonging the action of cAMP. They also affect glucagons and thyroid stimulating hormones, while some forms have been reported to be carcinogenic (Okaka *et al.*, 1992). Some have been used either as an analgesic, antispasmodic, bactericidal agents (Frantisek, 1991). Saponins have been reported to be useful in reducing inflammation of upper respiratory passage and also chiefly as foaming and emulsifying agents and detergents (Frantisek, 1991). Tannins have astringent properties that hasten the healing of wounds and prevention of decay. Tannin compounds have antimicrobial activities and are responsible for preventing and treating urinary tract infections and other bacterial infections. The result of the determination of phytochemical test indicated that the tuber possess some biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al.*, 2006). They are usually present at low concentration in edible fruits, nuts, tubers and vegetables. Roasting reduced the amount of these phytochemicals in plant products (Piorrock *et al.*, 1984) as most of these phytochemicals are thermally unstable.

Analysis of the antinutrients composition of the raw tubers of *C. esculentus* showed that it contained 0.60±0.32 g/100 g oxalates, 2.40±0.40 mg/100 g phytates, 0.88±0.02 mg/100 g saponins, 9.62±0.29 g/100 g tannins and 1.08±0.69 mg/100 g cyanogenic glycosides. The roasted *C. esculentus* tuber contained 0.55±0.36 g/100 g oxalates, 1.06±0.24 mg/100 g phytate, 0.67±0.40 mg/100 g saponins, 7.10±0.35 g/100 g tannins and 0.86±0.44 mg/100 g cyanogenic glycosides. The levels of antinutrients analyzed were very low compared to those reported for nuts like the peanuts (Ejigui *et al.*, 2005). The presence of phytates in biological systems may chelate divalent metals like calcium, magnesium, or block the absorption of essential minerals in the intestinal tract (Dan, 2005) thus decreasing their bioavailability (Oberleas, 1973). Phytates chelate with mineral elements thereby having significant effects on the utilization of the minerals. They also react with basic residues of protein. Tannins and to some extent oxalates, binds to proteins thereby making them difficult to digest in the body. Oxalates can remove calcium in the form of calcium oxalate (Savage, 1993) in the blood and thus may result to kidney damage. Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-lumenal physicochemical interaction (Price *et al.*, 1987). They also exhibit structure dependent biological activity (Savage, 1993). The potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its consumption will produce a concentration of Hydrogen Cyanide (HCN) that is toxic to exposed humans. Cyanide causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. Cyanide also activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting tricarboxylic acid cycle (Akintonwa and Tunwashe, 1992). Odumodu (1992) and Okafor *et al.* (2003) had earlier reported low contents of these antinutrients in tigernut tuber flour compared with other local fruits, nuts, tubers and vegetables. Roasting numerically reduced the antinutrient composition of tigernut tuber flour.

Acute toxicity test are generally the first test conducted in any toxicity study. They provide data on the relative toxicity likely to arise from a single or brief exposure to any substance. Different plant extracts have been known to possess different levels of toxicity which majorly depends on the levels of antinutrients inherent in the plants (Sofowora, 1993). Preliminary investigations on the acute toxicity of the tuber extract of *C. esculentus* in mice showed that the aqueous extract of *C. esculentus* (tigernut) tuber was not toxic to mice at the administered concentrations.

Table 2: The antinutrient composition of the tigernut tuber

	Components				
Sample	Oxalates (g/100 g)	Phytate (mg/100 g)	Saponin (g/100 g)	Tannins (mg/100 g)	Cyanogenic glycosides (mg/100 g)
Raw	0.60±0.32	2.40±0.40	0.88±0.02	9.62±0.29	1.08±0.69
Roasted	0.55±0.36	1.06±0.24	0.67±0.40	7.10±0.35	0.86±0.44

Values are mean±standard deviation of triplicate determination

Table 3: The biochemical parameters of the animals at the end of experimental period

	Groups				
Parameters	Group I NS	Group II 500 mg/kg	Group III 1000 mg/kg	Group IV 1500 mg/kg	Group V 2000 mg/kg
Blood glucose (g/dl)	71.5±4.04	60.25±3.40*	56.75±2.50*	54.00±3.46*	48.50±4.66*
Serum protein (g/dl)	6.92±0.27	7.43±0.63	7.39±0.45	7.16±0.61	7.19±0.35
Serum albumin (g/dl)	3.35±0.48	3.14±0.72	4.08±0.29*	4.18±0.31*	3.93±0.30*
Serum cholesterol (mg/dl)	88.10±15.12	86.49±17.65	91.35±3.24	75.94±18.89	79.91±8.79

Values are mean±standard deviation of quintuplicate determination, *Means significant different ($p<0.05$) compared to the control.
N = 5, NS = Normal Saline

The result of the effect of administration of the various concentrations (500, 1000, 1500 and 2000 mg/kg) of *C. esculentus* tuber extract on biochemical parameters such as blood glucose, serum protein, albumin and cholesterol are presented in Table 3. The result showed that there was significant increase ($p<0.05$) in serum albumin and a significant decrease ($p<0.05$) in blood glucose, but there was no significant effect ($p>0.05$) on serum protein and cholesterol. Since total serum proteins and albumin are generally influenced by total protein intake (Onifade and Tewe, 1993), the results obtained indicate nutritional adequacy of the dietary and the extract proteins. Abnormal serum albumin usually indicates an alteration of normal systemic protein utilization (Apata, 1990). Awosanya *et al.* (1999) have demonstrated the dependence of blood protein on the quality and quantity of protein source. The reported low level of phytate in the tuber could also have led to the increased absorption of protein from the rat diet. Phytate acts as a chelator, forming proteins and mineral bioavailability (Davies and Gathlin, 1991). Since glucose level was significantly ($p<0.05$) lowered and cholesterol levels were not affected abnormally, possibilities of anorexia, diabetes, liver dysfunction and mal-absorption of fat, which are the symptoms of abnormal glucose and cholesterol levels in blood (Bush, 1991) are ruled out. The glucose lowering potentials of the extract may be ascribed to modifications in glucose uptake in the intestine. It is well known that soluble fibres generally increase transit time through the gut, slow emptying of the stomach and slow glucose absorption (Swaminathan, 2002). *Cyperus esculentus* tubers have high dietary fibre content (Umerie and Enebeli, 1997), so they may play a major role in lowering blood glucose level. This observation supports an earlier hypothesis that the tuber may be important for diabetics and those seeking to reduce weight (Kordyias, 1990).

The result of the effect of administration of the various concentrations (500, 1000, 1500 and 2000 mg/kg) of *C. esculentus* (tigernut) aqueous tuber extract on haematological parameters such as red blood cells, total and differential white blood cells, haemoglobin, packed cell volume and erythrocyte sedimentation rate is presented in Table 4. The result show that there was no significant effect ($p>0.05$) on these haematological parameters. The results obtained for all treatment groups indicate nutritional adequacy of the tuber extract and the rat diet since they did not indicate malabsorption or under nutrition (Church *et al.*, 1984). These observations were related to the composition of the tuber extract and health status of the animals since none of the animals died as a result of any diseases. Hackbath *et al.* (1983) had earlier recorded a strong influence of food components on haematological traits, packed cell volume and haemoglobin concentration being very strong indicators of nutritional status of animals. It is well known that various antinutritional substances and xenobiotics can cause haemolysis, nutrients malabsorption and abnormal haemopoesis which could arise from liver damage (Chubb, 1982), antinutrient analysis of the tigernut tuber shows that it has low concentration of these antinutrients. The result of the total and differential white blood cell count indicate that the animals were healthy because decrease in number of white blood cells is an indication of allergic conditions, anaphylactic shock and certain parasitism while elevated value indicate to the existence of a recent infection, usually with bacteria (Ahamefule *et al.*, 2008). The mean body weight change in rats after every six days following administration of 500, 1000, 1500 and 2000 mg/kg body weight extract of *C. esculentus* tuber extract are presented in Table 5. A general increase in physical activities, food and water intake were observed for all the animals during the feeding experiment. There was initial increase in weight which was sustained. The increased weight could be due to increased feed and water intake observed all through the experimental period. The increase in weight of the animals suggests that they increasingly accumulated calories from the normal rat diet and from the nutrient rich extracts.

Table 4: The red blood cell count, total and differential white blood cell count haemoglobin concentration, erythrocyte sedimentation rate and packed cell volume of the animals at the end of experimental period

Haematological indices	Group I NS	Group II 500 mg/kg	Group III 1000 mg/kg	Group IV 1500 mg/kg	Group V 2000 mg/kg
RBC ($x10^6$/µL)	8.50±0.19	8.74±0.58	8.63±0.67	8.54±1.55	8.67±0.15
Hb (g/dl)	17.25±1.28	16.94±1.29	16.99±0.95	17.71±1.00	17.91±0.63
PCV (%)	44.37±2.56	46.00±1.08	45.63±4.23	45.00±0.00	44.13±1.32
ESR (mmHr)	0.76±0.12	0.73±0.07	0.82±0.10	0.70±0.55	0.69±0.07
tWBC ($x10^3$/µL)	13.96±2.64	13.51±1.82	13.57±2.72	16.61±2.72	14.53±1.33
Neutr ($x10^3$/µL)	2.78±0.82	2.52±0.46	1.59±0.44	2.77±1.00	3.18±1.24
Lymph ($x10^3$/µL)	10.69±1.88	10.73±1.50	11.62±2.61	13.38±2.62	11.03±1.42
Eosin ($x10^3$/µL)	0.06±0.07	0.07±0.08	0.11±0.13	0.09±0.10	0.00±0.00
Mono ($x10^3$/µL)	0.39±0.20	0.24±0.15	0.24±0.18	0.33±0.13	0.18±0.67
Baso ($x10^3$/µL)	0.03±0.06	0.03±0.07	0.00±0.00	0.40±0.80	0.12±0.15

Values are mean±standard deviation of quintuplicate determination, *Means significant different ($p<0.05$) compared to the control.
N = 5, NS = Normal Saline. RBC = Red Blood Cell, Hb = Haemoglobin, PCV = Packed Cell Volume, ESR = Erythrocyte Sedimentation Rate, tWBC = total White Blood Cell, Neutr = Neutrophil, Lymph = Lymphocyte, Eosin = Eosinophil, Mono = Monocytes, Baso = Basophils

Table 5: The mean body weight of rat administered aqueous tuber extract of tigernut

Periods	Group I NS	Group II 500 mg/kg	Group III 1000 mg/kg	Group IV 1500 mg/kg	Group V 2000 mg/kg
0 day	113.25±15.09	113.50±6.62	114.74±12.20	111.24±9.62	110.47±5.83
6^{th} day	138.05±8.00	115.20±9.97	139.00±16.02	121.50±17.65	134.40±13.00
12^{th} day	147.30±11.47	130.32±9.35	142.94±15.35	125.38±17.26	139.34±12.42
18^{th} day	157.07±8.60	141.90±8.20	149.40±14.57	133.90±17.92	152.14±14.02
24^{th} day	160.15±9.47	143.80±9.30	158.10±15.06	141.02±18.45	159.14±15.40
30^{th} day	174.95±7.61	149.92±10.45	166.48±15.87	148.36±19.06	171.28±11.53

Values are mean±standard deviation of quintuplicate. N = 5, NS = Normal Saline

Although the animals used in this study were fed with normal rat diet, the tigernut tuber extract might have allowed proper absorption of the nutrients which have allowed proper utilization of the nutrients. Low level of active/toxic principles may have stimulated appetite and increased feed utilization resulting in increased weight gain. The tuber of *C. esculentus* is used in making a refreshing beverage called kuunu in Nigeria which is consumed mostly in the Northern region of Nigeria (Belewa and Abodurin, 2008). There have not been any reported cases of toxicity in humans.

The present study confirms the tigernut tuber contains important nutrients and some essential macro and micro nutrient necessary for good human and animal health. Roasting the tuber as a processing step reduced the antinutrients composition. But unlike several other underutilized crops, it does not produce any undesirable effects even when consumed raw. The findings indicate that the tigernut tuber which is popularly eaten raw is rich in important food properties when compared with other crops has no negative effect, at least in rats and considering the economic situation in Nigeria and the near zero economic value of this tuber, its cultivation and consumption should be encouraged.

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