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Effects of Ethanolic Extract of *Cyperus esculentus* (Tiger Nut) Tubers on Blood Glucose Level and Lipid Profile in Alloxan-Induced Diabetes Mellitus in Male Wistar Rats

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ABSTRACT
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. The prevalence of Diabetes mellitus is expected to increase to 5.3% by 2030. This study aimed to determine the effects of ethanolic extract of *Cyperus esculentus* tubers on alloxan-induced diabetes mellitus and lipid profile in male Wistar rats. A total of 25 Wistar rats were used for the study. Diabetes was induced by injection of alloxan 100 mg/kg and rats with blood glucose levels 200 mg/kg and above were considered diabetic. The rats were randomly divided into five groups (n = 5), group 1, normal control, group II, negative (diabetic) control, and Group III and Group IV were diabetic rats treated with 400mg/Kg and 800mg/Kg body weight of the ethanolic extract of *Cyperus esculentus* tubers and group V were diabetic rats treated with 10mg/Kg body weight Metformin. All treatments lasted for 28 days and Blood glucose levels were checked at weeks 0, 1, 2, 3, and 4 respectively. At the end of the treatment period, the rats were sacrificed and the blood samples collected were used to determine serum insulin level and lipid profile. The result obtained revealed a statistically significant ($P<0.05$) decrease in blood glucose levels in groups III, IV, IV, and V as compared to the diabetic untreated group. The results of serum total cholesterol revealed a significant decrease in group I and group IV. However, high-density lipoprotein shows a significant decrease in group V as compared to the diabetic untreated group. There was also a statistically significant decrease ($P<0.05$) in low-density lipoprotein (LDL) levels in groups I, III, and IV when compared with the diabetic untreated group. There was also a statistically significant ($p < 0.05$) increase in serum insulin level in the normal control group and the group treated with 800mg/kg of extract. This study revealed the potential benefits of the ethanolic extract of *Cyperus esculentus* tubers in reducing blood glucose levels and its effects in reversing hyperlipidemia associated with diabetes mellitus.

Keywords:
Diabetes mellitus,
Cyperus esculentus,
Blood glucose,
Lipid profile.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both (Goyal, 2022). Chronic hyperglycemia in synergy with other metabolic aberrations in patients with diabetes mellitus can cause damage to various organ systems, leading to the development of disabling and life-threatening health complications. The most prominent of which are micro-vascular (retinopathy, nephropathy, and neuropathy) and macro-vascular complications leading to a 2-fold to 4-fold increased risk of cardiovascular diseases (Goyal, 2022).

According to the International Diabetes Federation (IDF), there were an estimated 463 million adults aged 20-79 years with diabetes mellitus worldwide in 2019, representing a global prevalence of 9.3% (IDF, 2023). This number is expected to increase to 578 million by 2030 and 700 million by 2045 if current trends continue (IDF, 2023). Also, the estimated prevalence of diabetes in the Sub-Saharan Africa region in 2019 was 4.1% (IDF, 2019). This prevalence is expected to increase to 5.3% by 2030 and 7.1% by 2045 if current trends continue (IDF, 2019). The IDF also reports that the region has a

particularly high proportion of undiagnosed diabetes cases, with approximately two-thirds of adults with diabetes remaining undiagnosed.

DM is broadly classified into three types by etiology and clinical presentation which are type 1 diabetes, type 2 diabetes, and gestational diabetes (GDM). Some other less common types of diabetes include monogenic diabetes and secondary diabetes (Sollis-Herrera, 2018).While medication and lifestyle changes are the primary treatments for diabetes, studies have shown that plants can also play a significant role in managing this condition (Kooti *et al.*, 2016; Jacob & Narendhirakannan, 2018; Kasole *et al.*, 2019).

Tiger nut tuber "*Cyperus esculentus*" as considered by some authors originated from Africa and tropical Asia (Basine & Arslanoglu, 2020), while other authors consider that it is native to tropical and subtropical regions throughout the world (Asare *et al.*, 2020). It is a perennial crop cultivated extensively in Asia, East Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (Bazine & Arslanoglu, 2020; Asare *et al.*, 2020).

In Nigeria, Tiger nuts are known as 'Aya' by the Hausa and Tiv tribes, 'Ofio' by the Igbo tribe,'Ofio or Ijaa' by the Yoruba tribe and 'Aki Hausa' by the Idoma tribe (Onwukaeme *et al.*, 2015; Aina *et al.*, 2016; Aliyu *et al.*, 2017). Tiger nuts are commonly used in Nigerian cuisine. They can be consumed raw, roasted, or soaked in water to soften them before eating. They are used as a snack, added to beverages, and used as an ingredient in traditional dishes and desserts. They are also used as a thickening agent in soups and sauces (Adegbaju, 2019; Oguntona *et al.*, 2020).

Tiger nuts are a primary ingredient in the production of a popular Nigerian beverage called "kunun aya" or "tiger nut milk." The tubers are soaked, blended, and strained to extract a milky liquid that is sweet and nutty in flavor. They have been used in traditional Nigerian medicine for various purposes. They are believed to have aphrodisiac properties and are used to enhance fertility and as a natural remedy for digestive issues, constipation, asthma, and diarrhea (Ezekwe *et al.*, 2019; García-Mateos *et al.*, 2019).

Tiger nuts are also used as a component of animal feed in Nigeria. They are included in livestock diets to enhance growth and improve the nutritional value of the feed (Nworgu *et al.*, 2012). Tiger nut oil is used in Nigeria as a natural moisturizer for the skin and hair. It is also used in the production of soaps and lotions, as it is believed to have moisturizing and anti-aging properties (Adejumo, 2017). Tiger nuts tubers are used in the production of starch, flour, and oil, which have several industrial applications (Kwofie *et al.*, 2018).

Tiger nuts are rich in minerals such as phosphorus, potassium, calcium, magnesium, and iron. These minerals play important roles in various physiological processes inthe body, including bone health, muscle function, and nerve transmission. It's also rich in vitamins E and C, and a good quantity of vitamin B1 (Maduka & Ire, 2018; Iyuke *et al.*, 2019). Tiger nuts are a good source of carbohydrates, primarily in the form of starch. The carbohydrates in tiger nuts are slowly digested and can help maintain stable blood sugar levels (Iyuke *et al.*, 2019).

Tiger nuts are rich in phenolic compounds, including flavonoids, phenolic acids, and tannins. These compounds have been shown to possess antioxidant, anti-inflammatory, and anti-cancer properties (Sánchez-Machado *et al.*, 2017; Hanif *et al.*, 2020; García-Sánchez *et al.*, 2021). Tiger nuts contain a high-high-number of fatty acids, including oleic, linoleic, and palmitic acids. These properties make it an intriguing plant for investigating its potential effects in diabetes management. However, despite the growing interest in the therapeutic potential of *Cyperus esculentus*, the effects of its crude extract specifically in alloxan-induced diabetes mellitus in male Wistar rats remain relatively unexplored (Zhao *et al.*, 2018).

MATERIALS AND METHODS

Chemicals

Alloxan and ethanol were purchased from Zayo Sigma Chemicals. Tiger nut tubers were purchased from the local market in Masaka, Karu Area of Nasarawa State, Nigeria.

Experimental Animals and Management

A total of 25 male Wistar rats was used for this study. The rats were purchased and kept in cages in the Animal Care Unit, Bingham University Karu, where the study was carried out. The rats were allowed to acclimatize with the laboratory environment two weeks before the commencement of the experiment.

Plant Collection, identification and preparation

The tubers of *Cyperus esculentus* were obtained from the local market in Masak Karu Local Government Area of Nasarawa State, Nigeria and taken to the Department of Botany, Faculty of Life Sciences Ahmadu Bello University, Zaria and was identified and authenticated by Namadi Sunusi, a voucher number ABU900292 was given. The tubers were washed; air dried and grinded using pestle and mortar and 1850g of the powder was obtained.

Extraction

The grinded tubers were macerated in ethanol (70%) at a ratio of 5ml/g of the powder with continuous stirring for three days at 4^{o}C. The extract was filtered and lyophilized to obtain a semi solid powder about (275g), which was stored at 4^{o}C. 200g of the extract was then dissolved in

water 5ml/g of the extract then partitioned with Ethyl acetate 5ml/g of the extract. A separation funnel was used to separate the ethyl acetate fraction from the aqueous then N-butanol 5ml/g of the extract was also added to the aqueous stirred and allowed for few hours then separated to get the N-butanol fraction of the extract leaving the aqueous fraction. The three fractions; ethyl acetate, n-butanol and aqueous fractions were then evaporated to dryness in an oven at 37 ^{0}C and a semi solid fraction was obtained which forms the ethyl acetate, n-butanol and aqueous fractions of *Cyperus esculentus*. The residue obtained was kept in a sealed container at 4 ^{0}C in a refrigerator until used (Tanko *et al*., 2014).

Induction of Diabetes Mellitus

Diabetes mellitus was induced after fasting the rats for 16 hours, the animals were intra-peritoneally injected with alloxan (100mg/kg body weight) dissolved in ice cold 0.1 M sodium citrate buffer which was followed by oral administration of 2-3 ml sucrose solution 10% (w/v) for one day (Burcelin *et al*., 1995). Animals were fasted overnight and one drop blood sample was obtained by pricking the lateral tail vein using a sterile surgical scissors and immediately the blood glucose level was determined 72 hours after the induction of diabetes with alloxan. Fasting blood glucose levels were determined by using the glucose oxidase method (Trinder, 1969) with FINEST TOUCH Glucometer and results obtained were reported as mg/dl (Rheney and Kirk, 2000). Animals with blood glucose level above 200 mg/dl were considered diabetic (Kim *et al*., 2008).

Experimental Design

The rats were divided into five (5) groups of five rats as follows:

Group 1: This served as an experimental control (non diabetic) group with a total number of five Wistar rats (n = 5). The rats in this group were fed with animal feed and water for four (4) weeks.

Group 2: This served as the diabetic negative control group, with a total number of five Wistar rats (n = 5). The rats in this group were diabetic and fed with animal feed and water for four (4) weeks.

Group 3: A total number of five Wistar rats (n = 5). The rats in this group were diabetic and treated with400mg/kg body weight ethanolic extract of *Cyperus esculentus* for four weeks.

Group 4: A total number of five Wistar rats (n = 5). The rats in this group were diabetic and treated with 800mg/kg body weight ethanolic extract of *Cyperus esculentus* for four weeks.

Group 5: A total number of five Wistar rats (n = 5). The rats in this group were diabetic and treated with metformin 10mg/kg body weight for four weeks

Blood sample collection and serum preparation

At the end of the four weeks treatment period, the Wistar rats were anesthetized and euthanized and blood samples were collected from the animals through cardiac puncture. About 5mL of blood was collected into specimen bottles and allowed to clot and separate by centrifugation at 3,000 g for 10 minutes using the Bench Centrifuge. The supernatant obtained was used for the determination of Insulin

Determination of blood glucose level

Animals were fasted overnight and one drop blood sample was obtained by pricking the lateral tail vein using a sterile surgical scissors and immediately the blood glucose level was determined. Fasting blood glucose levels was determined by using the glucose oxidase method (Trinder, 1969) with fine touch Glucometer and results was reported as mg/dl (Rheney and Kirk, 2000). Animals with blood glucose level above 200 mg/dl were considered to be diabetic (Kim *et al*., 2008).

Determination of Serum Insulin

Insulin ELISA is based on solid phase sandwich ELISA method. The samples and conjugate reagent (anti-Insulin biotin & hormone receptor protein (HRP)) were added to the wells coated with Streptavidin. Insulin in the serum binds to the matched pair antibodies, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate were washed off, through a washing step. Upon addition of the substrate, the intensity of color was proportional to the concentration of Insulin in the samples. A standard curve was prepared by relating the color intensity to the concentration of Insulin (Beischer, 1983).

Determination of Lipid Profile

Serum levels of lipid profile; total cholesterol, HDL-cholesterol, LDL and triglycerides were determined according to the methods of Frings et al. (1972), Allian et al. (1974), Burstein et al. (1970) and Fossati and Prencipe (1982), respectively.

Data Analysis

Data obtained from the study was analyzed using One Way Analysis of Variance (ANOVA), a statistical package SPSS version 25 was used and the results obtained were presented as Mean ± Standard error of mean and appropriate post hoc test was used to determine the level of significance and p values ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Table 1: Blood Glucose Levels (mg/dL)

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Control	114.60±4.80[a]	92.80±5.82[a]	116.20±5.08[a]	97.40±6.05[a]	109.0±6.79[a]
D cntl	321.75±93.28	481.50±73.03	249.0±35.26	275.50±64.96	317.0±91.30
D+400mg/Kg ECE	408.0±49.10	303.67±96.15	210.67±61.67	220.67±85.10	116.33±13.17[b]
D+800mg/Kg ECE	403.50±59.38	317.50±59.16	127.25±23.67[b]	114.25±23.77[b]	113.0±10.98[b]
D+10mg/Kg Met.	486.75±94.02	495.25±44.32	122.25±12.45[b]	116.50±31.0[b]	94.75±8.63[b]

D = Diabetic, ECE = ethanolic extract of *Cyperus esculentus*, Met. = Metformin, [a] and [b] = statistical significance at $p < 0.05$.

Table 2: Serum Insulin Levels

Groups	**Insulin** (μIU/ml)
Control	117.95±41.41[b]
Diabetic control	29.99±7.67
Diabetic+ 400mg/Kg extract	49.38±11.67
Diabetic+800mg/Kg extract	102.91±31.79[a]
Diabetic+10mg/Kg Metformin	48.43±13.18

[a] and [b] = statistical significance at $p < 0.05$

Table 3: Serum Lipid Profiles

GROUPS	**TC (**mmol/l)	**TG (**mmol/l)	**HDL (**mmol/l)	**LDL (**mmol/l)
Control	1.73±0.08[a]	1.33±0.08	0.66±0.02	0.80±0.60[a]
D cntl	2.26±0.07	1.31±0.10	0.67±0.02	1.33±0.03
D+400mg/Kg ECE	2.00±0.24	1.78±0.33	0.72±0.04	0.92±0.14[b]
D+800mg/Kg ECE	1.73±0.12[b]	1.35±0.11	0.70±0.02	0.75±0.10[b]
D+10mg/Kg Met.	2.16±0.28	1.40±0.17	0.69±0.02[b]	1.19±0.22

D = Diabetic, ECE = ethanolic extract of *Cyperus esculentus*, Met. = Metformin, TC = Total cholesterol, TG = Triglyceride, HDL = High-density lipoprotein, LDL = Low-density lipoprotein [a] and [b] = statistical significance at $p < 0.05$

Discussion

Tiger nut tuber contains active ingredients such as sterols, alkaloids, tannins, saponins, resins, flavonoids, and vitamins E and C (Marchyshyn *et al*., 2021). The phytochemicals in tiger nuts are exceptional and can be used in the production of drugs and therapeutic diets (Ihenetu *et al*., 2021). Tiger nut contains 62 % flavonoid compounds, 23 % phenolic acids and their derivatives, and 15 % phenylethanoid glycosides (Mayer, 2019). The concentration of saponin, tannin, phytate, oxalate, hydrogen cyanide, and hemagglutinin reduce after fermentation (Ji and Gi, 2018). Several studies have revealed the therapeutic potential of *Cyperus esculentus* in lowering blood glucose levels and anti-oxidant properties.

This study evaluates the antidiabetic property of *Cyperus esculentus* on blood glucose. The results obtained from the studies revealed a significant ($p < 0.05$) decrease in blood glucose levels in all the rats treated with 400 and 800 mg/kg body weight of ethanolic extract of *Cyperus esculentus* tubers as compared to the alloxan-induced diabetic untreated rats. The antidiabetic property of *Cyperus esculentus* may be attributed to the presence of several phytochemicals present in the plant. Among the phytochemicals present are flavonoids and studies revealed that flavonoids have multiple positive health effects on metabolic disorders, such as cardiovascular disease, cancer, obesity, and diabetes (Middleton *et al*., 2000). They also serve as antioxidants that modulate oxidative stress in the body by neutralizing the effect of nitrogen and oxygen species, thus preventing the disease (Kawser *et al*., 2016). The antidiabetic activity of flavonoids supports the regulation of carbohydrate digestion, insulin signaling, insulin secretion, glucose uptake, and adipose deposition (Vinayagam & Xu, 2015). They target multiple molecules that are involved in the regulation of several pathways, like improving β-cell proliferation, promoting insulin secretion, reducing apoptosis, and improving hyperglycemia by regulating glucose metabolism in the liver (Graf *et al*., 2005). A US study on 200,000 women and men evaluated the association between dietary intake of flavonoid subclasses and type 2 diabetes, confirming that a higher consumption of anthocyanins lowers the risk of diabetes (Wedick *et al*., 2012). It is hypothesized that the majority of flavonoids bioactivity occurs due to their hydroxyl group, α, and β ketones (Barone *et al*., 2009).

The results from the serum insulin showed that there was a significant ($p < 0.05$) increase in serum insulin in the diabetic rats treated with ethanolic extract and ethyl acetate fraction of *cyperus esculentus* tubers in alloxan-induced diabetic Wistar rats.

The result of insulin level showed that there was a statistically significant ($p < 0.05$) increase in serum insulin level in the group treated with 800 mg/kg body weight ethanolic extract of *cyperus esculentus* tubers. The alloxan used in this study has the ability to cause diabetes in rats by damaging the insulin-secreting cells of the pancreas leading to hyperglycemia (Lenzen, 2008; Ewenighi *et al.*, 2015). The results for serum insulin also agree with the study of Hassan (2007) on the effect of dietary supplementation with tiger nut tubers on streptozotocin-induced diabetic rats which revealed an increase in serum insulin after dietary supplementation with *Cyperus esculentus* tubers.

The lipid profile obtained in this study showed a significant ($p < 0.05$) decrease in total cholesterol and low-density lipoprotein (LDL) in the groups treated with ethanolic extract of *Cyperus esculentus* and metformin as compared to the diabetic non-treated group. This finding is in line with the study of Tanko *et al* (2017) on the effects of Rutin on lipid and liver function enzymes in alloxan-induced hyperglycemic rats, the results revealed a significant decrease in the lipid profile of the treated groups compared to the diabetic untreated group. The decrease in low-density lipoprotein observed in the ethanolic extract-treated group reveals that *Cyperus esculentus* tubers offer strong protection against hyperlipidemia.

CONCLUSION

This study reaffirmed the negative effects of diabetes mellitus on blood glucose levels, lipid profiles, and serum insulin production and secretion in the pancreas. The study also shows that ethanolic extract of *Cyperus esculentus* (Tiger nuts) tubers in higher doses (i.e 800mg/Kg body weight) have a more beneficial effect than lower doses (400mg/Kg body weight) in the prevention and management of diabetes mellitus through the presence of the phytochemical components in the extract which contribute to and make up the anti-oxidant and anti-diabetic activities of the extract. It has been shown to have similar effects as the known anti-diabetic drug, Metformin.

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