Beneficial Effects of Ethanolic Seed Extract of Cyperus esculentus on Blood Glucose and Sperm Quality in Alloxan-induced Diabetic Rats

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Abstract

Background: An important complication of diabetes mellitus is the disturbance in the male reproductive system.

Aim: To determine the effects of ethanolic seed extract of C. esculentus on blood glucose and sperm quality in alloxan-induced diabetic rats.

Materials and Methods: Twenty-five (25) normal adult male wistar rats weighing between 150-200g were used. Group I Served as the positive control group and received distilled water only. Groups II-V were the test groups and were induced with diabetes. Group II was the negative diabetic control group with no treatment. Group III received 400mg/kg of C. esculentus. Group IV received 800mg/kg of C. esculentus while Group V received 150mg/kg of standard diabetic drug Metformin. Their fasting blood glucose levels were estimated. The animals were sacrificed and the semen sample was collected for sperm analysis.

Results: Significant (P<0.05) increase in blood glucose level in group II (108.00 ±3.52 mg/dL) and significant decrease in group IV (57.0 ±2.49 mg/dL) when compared with the control group I (74.60 ±1.63 mg/dL). No significant difference in blood glucose was observed in groups III (79.0 ±3.07 mg/dL) and V (77.0 ±2.55 mg/dL) when compared with the control group I. There was significant decrease (P<0.05) in sperm count in group II (3.80 ± 0.22 x10⁶/mL) and significant increase in group IV (7.68 ± 0.20 x10⁶/mL) when compared with the control group I (6.16 ± 0.26 x10⁶/mL). There was no significant difference (P>0.05) in sperm count in groups III (6.28 ± 0.19 x10⁶/mL) and V (5.60 ± 0.19 x10⁶/mL) when compared with control group I. The sperm motility and morphology significantly decreased in group II but significantly increased in group IV when compared with control group I. Groups III and V showed no significant difference in sperm motility and morphology when compared with the control.

Conclusion: C. esculentus seed extract may be beneficial in diabetes mellitus as well as its deleterious effect on sperm quality.

Key Words: Cyperus esculentus, Blood glucose, Diabetes, Sperm quality, Fertility

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1. Introduction

Diabetes mellitus is a major challenge in health care system around the world. It is considered as one of the five leading causes of death in the world (¹). The prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in 2000, and will be 4.4% by 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (²). Diabetes mellitus is characterized by hyperglycemic condition along with impaired metabolic functions. Two types of diabetes mellitus exist; the first type is characterized by insufficient production of insulin and in the other type, the target cells do not respond to insulin. The chronic hyperglycemia condition leads to long term damage and failure of target organs (³). Diabetes tends to damage cell membranes which results in elevated production of reactive oxygen species (ROS). The production of ROS appears to play a critical role in pathogenesis of diabetes mellitus (⁴).

An important complication of diabetes mellitus is the disturbance in the male reproductive system. Glucose metabolism is an important event in spermatogenesis. Diabetes mellitus induces molecular alterations which negatively affect sperm quality and function as well as fertility (⁵). Many studies in both human and animal studies have confirmed the deleterious effect of diabetes mellitus on sexual functions such as; semen parameters, nuclear DNA fragment and chromatin quality (⁶,⁷). The main goal of diabetes treatment is establishing of normal blood glucose and preventing or delaying its metabolic complications.
Tiger nut “Cyperus esculentus” is an underutilized tuber of family Cyperaceae, which produces rhizomes from the base of the tuber that is somewhat spherical (9). It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (9). In addition to being a food source, cyperus. esculentus tubers have several other purposes. For example, in Spain, they are used in the preparation of a milk-like beverage named “horchata” (10). Tiger nut has excellent nutritional qualities with a fat composition similar to olives (11). It is also gluten and cholesterol free (12). It is the richest food source of flavonoids (13) and also rich in water, fibers, alkaloids, digestible carbohydrates, saponins and fatty oils (glycerides). In addition, it also contains some elements, like phosphorus, potassium, calcium, copper, zinc, magnesium and manganese (14, 15). Among the Egyptian herbal remedies, consumption of tiger nut (cyperus esculentus) is relatively popular in some societies as an anti-diabetic agent (16, 17). It is said to be suitable for diabetic persons (18) as well as being a powerful aphrodisiac (19). A study carried out by Hassan (20) reported an appreciable hypoglycemia and hypolipidemia on streptozotocin-induced diabetic rats fed with tiger nut tubers. On the other hand, rats treated with cyperus esculentus relatively improved the induced histopathological changes in the testes of rats caused by effect of lead acetate (21). This improvement was attributed to the effect of the extract directly on spermatogenesis; by reducing lipid peroxidation and prevent, or decrease the formation of free radicals by acting as antioxidants. Also, a previous study by Al-Shaikh et al. (22) revealed protective effects of cyperus esculentus on testicular weight and spermatogenesis process in mice treated with lead acetate. They speculated that these effects could be due to either the antioxidant ability of C. esculentus or its positive influence on sex hormones. In addition, it has been claimed that treatment with cyperus esculentus methanolic extract improves sperm count and motility in male rats, which is associated with increased gonadotropins and testosterone serum levels (23)

In view of the aforementioned, since the main goal of diabetes treatment is to establish normal blood glucose and prevent or delay its metabolic complications; this study is therefore designed to determine the effects of ethanolic seed extract of cyperus esculentus on the blood glucose and sperm quality of alloxan induced-diabetic rats. A standard anti-diabetic drug metformin was also used to compare the observed effects. To our knowledge, no study has been done on this so far.

II. Materials and Methods

Plant materials and Extract Preparation
Fresh nuts of Cyperus esculentus (Tiger nuts) were obtained from a local market, in Nnewi, Anambra state Nigeria during the month of July, 2018. It was identified by a taxonomist in Botany Department of Nnamdi Azikiwe University Awka, Anambra State, Nigeria. Large quantities of the nuts were washed and air dried for 4 weeks. Thereafter, the tubers were milled to fine powder using manual engine grinder (Modelcorene, A.5 lander YCIA S.A). The milled sample was soaked in 5L of 80% Ethanol for 48 hours. Thereafter, it was filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated under reduced pressure in a vacuum at 45°C using a rotary evaporator (Searl Instruments Ltd. England) into a colloid form and stored at 4°C until use.

Experimental Animals
A total of twenty-five (25) normal adult male wistar rats weighing between 150-200g were used for this study. They were housed in the animal house of Human Physiology Department, Nnamdi Azikiwe University, Nnewi. The animals were acclimatized for a period of two weeks before the commencement of the experiment. They were kept in standard cages where they were subjected to an intensive nutritional program and were allowed free access to water and feed ad libitum throughout the period of the experiment. The rats were kept in plastic cages and they were given distilled water throughout the experiment. The animal room was well ventilated with a temperature range of 25°C-27°C under day / light 12-12 hour photoperiodicity. All procedures used in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of Animals (24).

Induction of Diabetes
Alloxan was used to induce diabetes mellitus in normoglycemic adult male wistar rats. A concentration of 150mg/kg body weight was given intraperitoneally to overnight fasted rats. After 24 hours of Alloxan administration, rats with glucose levels > 200 mg/dL were confirmed diabetic and were used for the study.

Experimental Design
After the induction of diabetes, the animals were randomized into 5 groups of 5 rats each. Group I served as the positive control group and received distilled water only. Groups II- V were the test groups and were induced with diabetes. Group II was the negative diabetic control group with no treatment. Group III was
diabetic and received 400mg/kg of *Cyperus esculentus*. Group IV was diabetic and received 800mg/kg of *Cyperus esculentus* while Group V was diabetic and received 150mg/kg of standard diabetic drug Metformin. The extracts were administered orally in the early hours of each day (within 8am to 10am) throughout the period of 21 days of extract administration.

**Blood Glucose Level Determination and Sample Collection**

At the end of the administration, their blood glucose levels were estimated after an overnight fast according to Iyare and Obaji (25) using glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostic, USA). This was done by tail puncture. A drop of blood was collected into the marked point of the strip inserted into the glucometer. Afterwards, the animals were sacrificed by cervical dislocation (26) and the semen sample was collected from the caudal epididymis by dissecting it. Semen analysis was done by using Monical Cheesbrough procedure (27).

Semen of 1ml was added to 20ml of diluted sodium bicarbonate- formalin and well mixed. Using the Pasteur pipette, the Neubauer ruled chamber was filled with the well mixed diluted semen and left for about 5 minutes. Then it was viewed through the microscope to count the sperm in the fluid. A drop of semen was placed on the slide. Normal saline of one drop was added to the semen and then covered with a cover slide. Using 40x objective, the normal and abnormal spermatozoa was examined and 10x objective was used for confirmation. One hundred spermatozoa were counted and the percentages showing normal and abnormal morphology were estimated. Also the percentage that was motile was estimated.

**Statistical Analysis**

All data were tabulated and statistically analyzed using SPSS version 20.0. Results were expressed as Mean ± standard deviation (M ± SD). One way analysis of variance (ANOVA) followed by Bonferroni’s Post hoc test were used for data comparison. P < 0.05 was taken as statistically significant.

**III. Results**

**Effect of Ethanolic seed Extract of *Cyperus esculentus* on Blood Glucose Level of Alloxan Induced Diabetic Male Wistar Rats**

Table 1 shows a statistically significant (P<0.05) increase in blood glucose level in group II (108.00±3.52 mg/dL) when compared with the control group I (74.60±1.63 mg/dL). No significant difference in blood glucose was observed in groups III (79.0±3.07 mg/dL) and V (77.0±2.55 mg/dL) when compared with the control group I. Group IV (57.0±2.49 mg/dL) however, showed significant decrease in blood glucose level when compared with the control group I.

**Effect of Ethanolic Seed Extract of *Cyperus esculentus* on Sperm count of Alloxan- Induced Diabetic Male Wistar Rats**

The result (table 2) shows a significant decrease (P<0.05) in sperm count in group II (3.80 ± 0.22 x10^6/mL) when compared with the control group I (6.16 ± 0.26 x10^6/mL). There was no significant difference (P>0.05) in sperm count in groups III (6.28 ± 0.19 x10^6/mL) and V (5.60 ± 0.19 x10^6/mL) when compared with control group I. However, group IV (7.68 ± 0.20 x10^6/mL) showed significant increase (P<0.05) in sperm count when compared with control group I.

**Effect of Ethanolic Seed Extract of *Cyperus esculentus* on sperm motility in alloxan- induced diabetic rats**

Figure 1 shows statistically significant decrease in actively motile sperm in group II when compared with control group I. Groups III and V showed no significant difference, while group IV showed significant increase in actively motile sperm when compared with the control group I.

**Effect of Ethanolic Seed Extract of *Cyperus esculentus* on sperm morphology in alloxan- induced diabetic male wistar rats**

Figure 2 shows a significant decrease in normal sperm and increase in abnormal sperm in group II when compared with the control group I. Groups III and V showed no significant difference in sperm morphology whereas group IV showed significant increase in normal sperm and decrease in abnormal sperm when compared with the control group I.
Table 1: Effect of Ethanolic Seed Extract of *Cyperus esculentus* on Blood Glucose Level in Alloxan Induced Diabetic Male Wistar Rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO OF RATS (n)</th>
<th>TREATMENT</th>
<th>MEAN ± SEM (mg/dl)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>Normal Positive Control</td>
<td>74.60 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>Diabetics Negative Control</td>
<td>108.00 ± 3.52*</td>
<td>0.00</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>Diabetes + 400mg/kg of <em>Cyperus esculentus</em></td>
<td>79.02 ± 3.07</td>
<td>1.00</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>Diabetes + 800mg/kg of <em>Cyperus esculentus</em></td>
<td>57.0 ± 2.49*</td>
<td>0.00</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>Diabetes + 150mg/kg Metformin</td>
<td>77.0 ± 2.55</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 2: Effect of Ethanolic Extract of *Cyperus esculentus* on Sperm count in Alloxan Induced Diabetic Male Wistar Rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO OF RATS (n)</th>
<th>TREATMENT</th>
<th>MEAN ± SEM (X10^6/ml)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>Normal Control</td>
<td>6.16 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>Diabetics Negative control</td>
<td>3.80 ± 0.22*</td>
<td>0.00</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>Diabetes + 400mg/kg of <em>Cyperus esculentus</em></td>
<td>6.28 ± 0.19</td>
<td>1.00</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>Diabetes + 800mg/kg of <em>Cyperus esculentus</em></td>
<td>7.68 ± 0.20*</td>
<td>0.04</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>Diabetes + 150mg/kg Metformin</td>
<td>5.60 ± 0.19</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Figure 1: Effect of Ethanolic Seed Extract of *Cyperus esculentus* on sperm motility in alloxan- induced diabetic rats

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IV. Discussion

The result of the present study showed an increase in blood glucose in group II which was induced with diabetes without any treatment. Diabetes mellitus is characterized majorly by increased blood glucose level among other characteristics. 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 \cite{28, 29}. The non significant difference in blood glucose observed in groups III and V as well as the significant decrease observed in group IV showed that the extract possesses the potential of reducing or eliminating diabetes mellitus. This can be attributed to the extract having high content of Arginine, which liberates the hormone that produces insulin \cite{30}. Recent study by Sabiu et al \cite{31} has also revealed the ability of the seed extract of *Cyperus esculentus* to inhibit the activities of α-glucosidase and α-amylase. As a result of this, the rate of starch hydrolysis to free glucose is either effectively reduced or abolished. The study also suggested a higher affinity of the enzyme for *Cyperus esculentus* than the substrate, thereby strategically modulating further carbohydrate hydrolysis.

Furthermore, other components of the extract such as; roundine, morphine, p-cymol, tubocurarine and digoxigenein have been implicated either as ROS scavengers or hypoglycemic agents \cite{32}.

At concentration of 400mg/kg (group III), the effect of the extract seems the same as that of the standard drug (group V). But with increased concentration of 800mg/kg (group IV) further reduction of blood glucose was observed.

The observed negative effects in sperm quality such as decreased sperm count, decrease in actively motile sperm and increase in abnormal sperm in group II is in line with a study by \cite{5} who reported that diabetes mellitus induces molecular alterations which negatively affect sperm quality and function as well as fertility. Also, several studies using STZ-induced type 1 diabetes animal model have shown decreased daily sperm production, sperm count and motility \cite{33, 34}, and increased percentage of spermatozoa with abnormal morphology \cite{35}. Studies using alloxan-induced type 1 diabetes animal models \cite{36, 37} have also yielded similar result. Sperm cells from men with type 1 diabetes have also been observed to have structural defects with nuclear and mitochondrial DNA fragmentation, reduced motility, and decreased zona pellucida binding \cite{6, 38, 39}.

Spermatozoa need specific carriers, known as glucose transporters (GLUTs) to mediate the glucose uptake from the surrounding medium into the cell \cite{40}. Diabetes has been shown to be associated with a depletion of GLUTs \cite{41}. Therefore, diabetic individuals are known to possess an inability to transport glucose, which supports an association of this disease with disruptions in sperm metabolism and consequently sub fertility or even infertility.
Conversely, the groups that received the seed extract of *Cyperus esculentus* produced positive effects in sperm quality with group IV showing increased sperm count, increase in actively motile sperm and decrease in abnormal sperm. However, the standard drug group (group V) showed similar effect with group III. This result is in agreement with a study by Ekalu et al. [22] who reported increase in sperm count and quality in rats treated with aqueous extract of *C. esculentus*. These effects could be due to either the antioxidant ability of *C. esculentus* or its positive influence on sex hormones [23]. The protective role of *C. esculentus* against oxidative stress could also be responsible for this effect. Furthermore, a study by Mohammed et al. [24] revealed the presence of considerable amount of several components such as quercetin, vitamins E and C, and the mineral zinc in tiger nut these could also contribute to the beneficial effect of *C. esculentus* observed in the present study.

Quercetin is a dietary flavonoid that exhibits strong antioxidant activity. A previous study by Khaki et al. [25] revealed significant increase in sperm count, percentages of viability and motility, and total serum testosterone in diabetic rats treated with quercetin. Taepongisorat et al. [26] reported that subcutaneous injections of quercetin over a period of 1 week significantly increased testis weight and improved sperm quality in rats.

Vitamin C has long been established as an agent to play a crucial role in the differentiation process of the spermatogonial cells to sperm [27]. Vitamin E has strong antioxidant properties and inhibits the lipid peroxidation created by the free hydroxyl and superoxide radicals. It protects the cell membrane of sperm cell from damages of reactive oxygen species. In vivo studies, supplementation of vitamin E was found to be effective in reduced number and motility of sperms caused by reactive oxygen species [28].

Zinc has many important functions in spermatozoa physiology, including effects on lipid flexibility and sperm membrane stabilization [29].

V. Conclusion

The present study has revealed that *C. esculentus* seed extract may be beneficial in the treatment of diabetes mellitus as well as its deleterious effect on sperm quality, thereby improving fertility. The effect is related to the presence of phytochemical constituents, vitamins and minerals contained in the seed extract. It has been shown to have similar or even better effect as the known diabetic drug metformin. This may therefore be used in place of the conventional drugs for the treatment of diabetes. Nevertheless, further studies need to be carried out on effects of this extract on some vital organs.

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References


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