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Article

Cyperus esculentus L. Protects Testis and Sperm Morphology of Hyperglycaemic Rats

Cyperus esculentus L. protege la morfología de los testículos y los espermatozoides de ratas hiperglicémicas

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SUMMARY

Cyperus esculentus L. (tiger nut) is a tuberous plant that promotes and protects reproductive functions, which are usually hampered in diabetics. The present study investigated the effect of *Cyperus esculentus* tuber extract (CETE) on testicular histology and sperm viability of alloxan-induced hyperglycaemic Wistar rats. Twenty-five adult male Wistar rats weighing 150-200g and grouped into five (n=5): Group 1, the control, administered tap water (20mL/kg), while groups 2-5 were administered a single intraperitoneal dose (120mg/kg b.w.) of alloxan, and each further received orally tap water (20mL/kg), CETE (100mg/kg), CETE (500 mg/kg) and metformin (500 mg/kg),

respectively for 21 days. The animals were sacrificed, their sperm collected for analysis, while the testes were harvested, and processed for histology. Results showed significantly increased ($p<0.05$) blood glucose and testosterone, and significantly decreased ($p<0.05$) sperm pH, motility, count, morphology and density, as well as disruptions and hypertrophy of the spermatogenic and Sertoli cells of the hyperglycaemic group. There were significant ($p<0.05$) blood glucose decline, while the sperm parameters and testicular weight improved with normal testicular histology in the 100 mg/kg CETE, 500 mg/kg CETE, and metformin-treated groups compared to the control and hyperglycaemic group. Treatment with CETE showed blood glucose amelioration and improved sperm quality, as well as testicular damage attenuation.

Keywords: *Cyperus esculentus*, Hyperglycaemia, Testes, Sperm quality

1. Background

Diabetes mellitus is a major challenge to healthcare, and one of the five leading causes of death in the world (Kumar *et al*., 2006). The prevalence of this disease condition for all age groups was estimated to be 171 million people (2.8%) in the year 2000, and estimated to reach 366 million (4.4%) by 2030 (Wild *et al*., 2004). Diabetes mellitus is classified into three subtypes; 1, 2 and gestational.

Gestational diabetes mellitus occurs only in pregnancy, which is also its predisposing factor. Type-2 diabetes arises either from the failure of insulin receptors to respond to insulin, relative insulin deficiency or both. In contrast, type-1 diabetes arises from destruction of beta (β) cells in the islets of Langerhans of the pancreas, leading to insulin insufficiency (Yoon and Jun, 2005). Thus, diabetes mellitus is generally characterized by hyperglycaemia, alongside impaired metabolic functions, with long-term organ damage and failure reported in chronic condition (Ceretta *et al.*, 2012; Lazio-de-la-Vega-Monroy and Fernandez-Mejia, 2013; Papatheodorou *et al*., 2016).

The testes are important reproductive organs in males severely affected by hyperglycaemia. Emerging evidence has shown that diabetes can cause male reproductive dysfunctions as reported in human and animal studies (Amaral *et al*., 2008; Mallidis *et al*., 2011; Ghlissi *et al*., 2012). Diabetes treatment involves classes of anti-diabetic drugs including the sulphonylurea; metformin and gliclazide being examples (Rojas and Gomes, 2013; Aldobeaban *et al.*, 2018; Ekong *et al*., 2022). However, this treatment may become complicated due to the adverse effects of diabetes on other body tissues.

To date, medicinal plants in various formulations are in use locally, to treat diabetes, although the scientific basis for these treatments is usually not considered (Brownlee, 2006; Salehi *et al.*, 2019; AlFaris *et al*., 2020). While researches are ongoing with these medicinal plants, they are postulated to offer a natural key towards unlocking diabetic complications (Babu *et al.*, 2006). Among the herbal remedies, the consumption of *Cyperus esculentus*, commonly called tiger nut (yellow nuts edge), is relatively popular in some societies as an antidiuretic agent (Gupta *et al*., 1971; Ghazanfar, 1994).

Cyperus esculentus Lativum (*C. esculentus*) is an underutilized tuber of the family, Cyperaceae, which produces spherical rhizomes from the base of the tuber. It grows freely and is consumed widely in Nigeria and other West African countries (Abaejoh *et al*., 2006). *C. esculentus* is reported to have anti-diabetic action (Onyenibe and Udogadi, 2019). On the other hand, animals treated with *C. esculentus* improved testicular functions, while protecting against lead acetate-induced testis histopathology (Al Essawe and Almashhadani, 2010; Udefa *et al*., 2020). The actions of *C. esculentus* may be attributed to its ability to reduce lipid peroxidation because of its antioxidant properties (Olabiyi, 2020). This study, therefore investigated the therapeutic effect of *C. esculentus* on the testes of alloxan-induced hyperglycaemia in adult male Wistar rats.

2. Methods

Experimental Animal Handling

Twenty-five adult male Wistar rats of 150-200g in body weight obtained from Onos Animal Farm, Nnewi, Anambra State, were housed in the animal facility of the Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria. The rats were acclimatized for two weeks under a 12 h dark/light cycle. Ethical approval for the study was obtained from the Chukwuemeka Odumegwu Ojukwu University Ethical Committee, and the experiment complied with the guidelines of the United States National Research Council, for the care and use of animals for research (National Research Council, 2011).

Plant Materials and Extract Preparation

Fresh *C. esculentus* nuts (tiger nut) was obtained from a local market in Nnewi, Anambra State, Nigeria, and identified by a botanist at the Department of Biological Science of the Chukwuemeka Odumegwu Ojukwu University. Five kilograms of the nuts were cleaned and sun-dried for four weeks. The nuts were then milled to a fine powder using a manual engine grinder (Moelcorene, A.5 lander YCIA S.A). The powder was pulverized in five litres of 80% ethanol for 48 h. It was filtered with Whatman No.1 filter paper. 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.

Induction of Hyperglycaemia

Alloxan monohydrate (Sigma chemicals, St Louis, MO, USA.) was used to induce hyperglycaemia in normoglycaemic adult male Wistar rats after the two weeks of acclimatization. A concentration of 120 mg/kg body weight of alloxan monohydrate was given intraperitoneally to overnight fasted rats. After 24 h of administration, rats with glucose levels greater than 200 mg/dL were confirmed hyperglycaemic, and used for the study.

Experimental Design

Prior to induction of hyperglycaemia, the twenty-five rats were randomized into five groups of five rats each. Group 1 served as the control group and received by oral gavage, 20 mL/kg body weight of tap water only. Groups 2-5 were the experimental groups treated with a single dose of 120 mg/kg of alloxan monohydrate intraperitoneally. Group 2 served as the hyperglycaemic control (hyperglycaemic) and received in addition, tap water (20 mL/kg body weight) orally; groups 3-5 respectively received in addition, *C. esculentus* extract (*C. esculentus*, 100 mg/kg body weight), *C. esculentus* extract (*C. esculentus* 500 mg/kg body weight), and metformin (metformin, 500 mg/kg body weight) by oral gavages. The treatments were for 21 days.

Termination of the Experiment

On the 22nd day of the experiment, the animals were anaesthetized using 40 mg/kg ketamine hydrochloride. Blood was obtained through cardiac aspiration, and the serum testosterone level was analysed by enzyme-linked immunosorbent assay (ELISA) kit, while sperm were collected from extracted epididymis for sperm analysis using the diffusion method as described by Chapin *et al.* (1992). The testes were also harvested, weighed and fixed in Bouin's solution for further routine histological processing, and stained with haematoxylin and eosin protocol.

Sperm Analysis

Briefly, sperm were obtained by placing the epididymis in a Petri dish containing phosphate-buffered saline, and nicked in a few sites with a scalpel blade. The sperm were then allowed to diffuse into the saline for 15 min, and were incubated until adequately dispersed for analysis.

For sperm motility, a diluted suspension of the sample was placed on slides and mounted on the microscope. The motility was determined and the percentage of motile sperm, defined as the number of motile sperm/total number of sperm ×100, was calculated. `

In sperm morphology assessment, diluted suspensions of the sample on slides were mounted on a microscope and photomicrographs immediately obtained. The sperm morphology was then classified as normal or abnormal; there were classified as abnormal when i) normally shaped head separated from flagellum, ii) misshapen head separated from flagellum, iii) misshapen head with normal flagellum, iv) misshapen head with abnormal flagellum, v) degenerative flagella defect(s) with normal head, and vi) other flagella defect(s) with normal head.

For sperm count, a diluted suspension of the sample was loaded into a haemocytometer, mounted on the microscope and counted using Neubauer ruling. Each group's sperm were averaged, and the total sperm was calculated as mean count × dilution factor.

Statistical Analysis

All the data were tabulated and statistically analysed using GraphPad Prisms (version 6.0). One-way analysis of variance (ANOVA) followed by Bonferroni's post hoc tests were used for data comparison. $P < 0.05$ was regarded as statistically significant. Results were expressed as mean ± standard error of mean (SEM).

3. Results

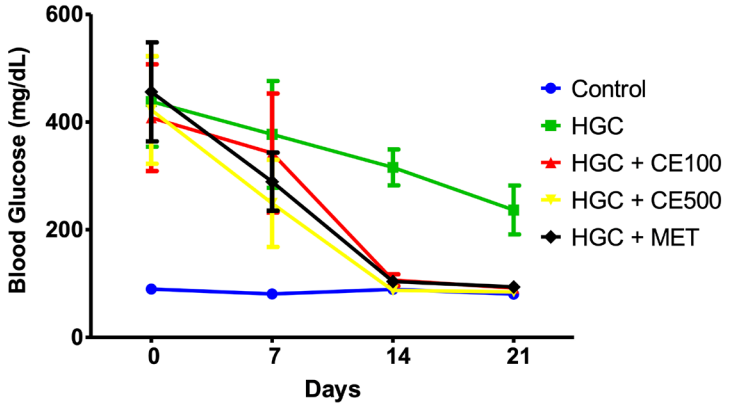
Blood Glucose Level

After induction of hyperglycaemia (Day 0), there was significantly increased ($p < 0.05$) blood glucose level of the hyperglycaemic, *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups compared to the control. By day 7 (seven days after alloxan induction), there was decreased blood glucose level of the hyperglycaemic, *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups, however, these levels were still significantly higher ($p < 0.05$) compared to the control. These test groups' glucose levels were not significantly different ($p > 0.05$) from each other (Figure 1).

By day 14, the blood glucose level of the hyperglycaemic group was significantly ($p < 0.05$) higher compared to the control, *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups. The blood glucose level of the *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups were not significantly ($p > 0.05$) different from the control (Figure 1).

By day 21, the blood glucose level of the hyperglycaemic group was significantly higher ($p<0.05$) compared with the control, *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups. There was however, no significant difference ($p > 0.05$) between the *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg, and metformin groups compared to the control (Figure 1).

Figure 1:
Effects of *C. esculentus* on Alloxan induced hyperglycaemia on blood glucose levels

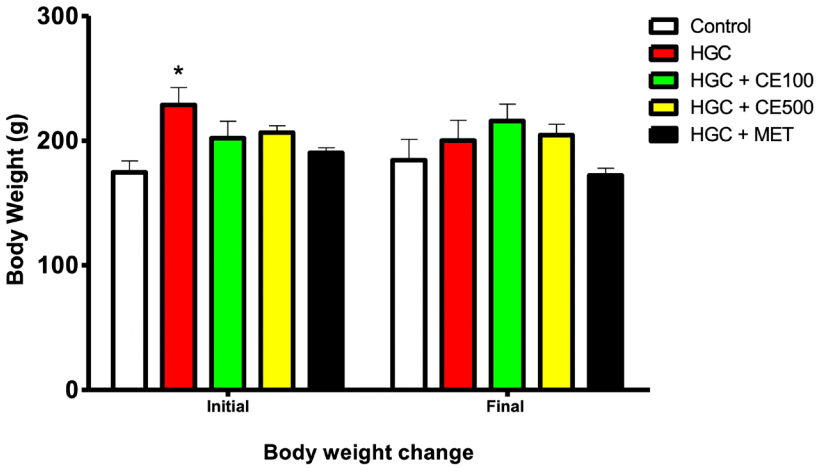


Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean, and significant at p = 0.0163, F-ratio = 3.936 (Initial body weight) and p < 0.05, F-ratio = 3.34, 2.11, 37.46 and 10.48 (days 7, 14 and 21, respectively); HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Body Weight

There was no significant difference (p > 0.05) in the initial body weight among the groups, except the hyperglycaemic group (p = 0.0163) compared with the control. There was no significant difference (p = 0.1700) in the final body weight among the groups compared with the control. However, the final body weights showed insignificant increase compared with the initial body weights, and except the hyperglycaemic, *C. esculentus* 500 mg/kg and metformin groups which declined insignificantly (p > 0.05) (Figure 2).

Figure 2:
Effects of *C. esculentus* on Alloxan induced hyperglycaemia on body weight

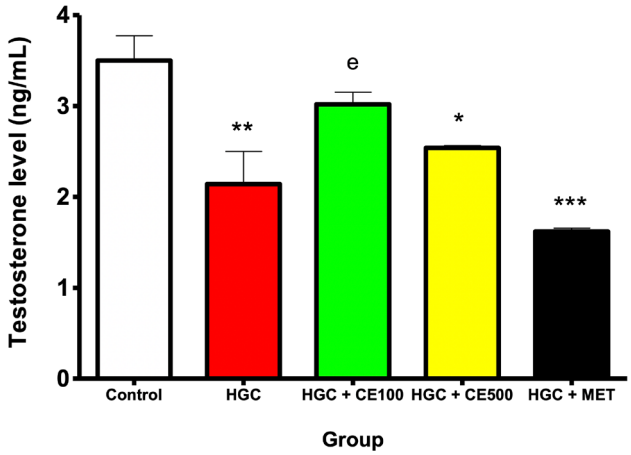


Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean, and * significantly different from control at p = 0.0163; F-ratio = 3.936 (Initial body weight) and p = 0.1700; F-ratio = 1.792 (Final body weight); HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Serum Testosterone Level

The serum testosterone level was significantly less ($p < 0.0001$) in the hyperglycaemic, *C. esculentus* 500 mg/kg and metformin groups compared with the control. The serum testosterone level of the *C. esculentus* 100 mg/kg group was not different ($p > 0.05$) compared with the control, but significantly ($p < 0.05$) higher than that of the metformin group (Figure 3).

Figure 3:
Effects of *C. esculentus* on Alloxan induced hyperglycaemia on testosterone level



Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean, and *,**,*** significantly different from control at $p < 0.05$, 0.01, 0.001, respectively; [e] significantly different from HGC + CE 100 at $p < 0.05$; F-ratio = 12.03; HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Sperm pH

There was significantly decreased ($p < 0.05$) sperm pH of the hyperglycaemic group compared with the control, *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg and metformin groups. There was, however no difference in the sperm pH among the *C. esculentus* 100 mg/kg, *C. esculentus* 500 mg/kg and metformin groups compared to the control (Table 1).

Table 1:
Effects of *Cyperus esculentus* on Alloxan induced hyperglycaemia on sperm pH

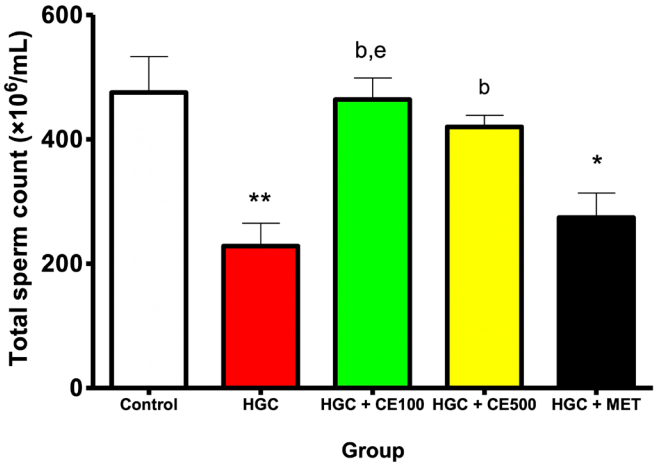
Group	Sperm pH (F-ratio = 5.75)
Control	5.66 ± 0.33
Hyperglycaemic	5.00 ± 0.00*
C. esculentus 100 mg/kg	6.00 ± 0.00[b]
C. esculentus 500 mg/kg	6.33 ± 0.33[b]
Metformin	5.50 ± 0.00 [b]

Data presented as mean ± standard error of mean, and values were considered significant at $p<0.05$; * significantly different from control; [b] significantly different from hyperglycaemic group

Total Sperm Count

The total sperm count was significantly less ($p = 0.0004$) in the hyperglycaemic, *C. esculentus* 500 mg/kg, and metformin groups compared to the control. However, the total sperm count was not significantly ($p = 0.05$) different in the *C. esculentus* 100 mg/kg group compared to the control, but significantly higher ($p < 0.05$) than the hyperglycaemic and metformin groups (Figure 4).

Figure 4:
Effects of *C. esculentus* on Alloxan induced hyperglycaemia on the total sperm count



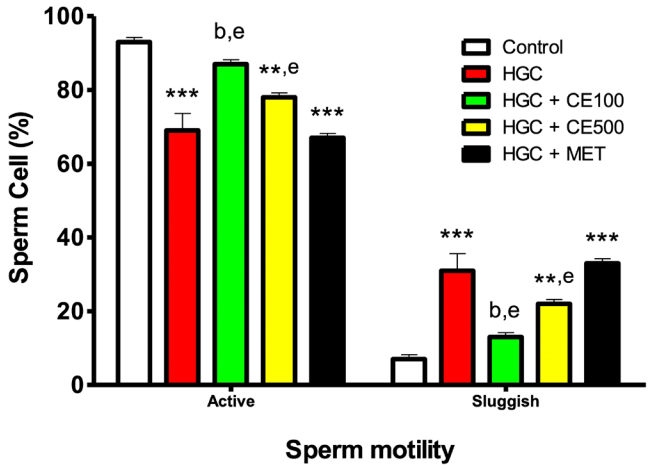
Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean, and *,** significantly different from control at p<005, 0.01, respectively; [b] significantly different from HGC at $p < 0.05$; [e] significantly different from HGC + MET at $p < 0.05$; F-ratio = 8.300; HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Sperm Motility

Active sperms were significantly less ($p = 0.0001$) in the hyperglycaemic, *C. esculentus* 500 mg/kg, and metformin groups compared to the control. However, the *C. esculentus* 100 mg/kg group active sperms were not different ($p > 0.05$) from the control, but significantly higher than the hyperglycaemic and metformin groups. The *C. esculentus* 500 mg/kg group active sperms were also significantly higher than the metformin group (Figure 5).

Invariably, sluggish sperms were significantly more ($p = 0.0001$) in the hyperglycaemic, *C. esculentus* 500 mg/kg, and metformin groups compared to the control. However, the *C. esculentus* 100 mg/kg group sluggish sperms were not different ($p > 0.05$) from the control, but significantly less than the hyperglycaemic and metformin groups. The *C. esculentus* 500 mg/kg group sluggish sperms were also significantly less than the metformin group (Figure 5).

Figure 5:
Effects of *C. esculentus* on Alloxan induced hyperglycaemia on sperm motility



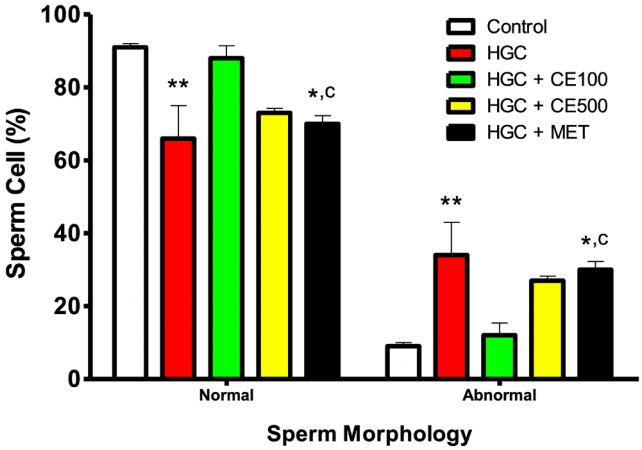
Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean, and **,*** significantly different from control at $p < 0.001$, 0.001, respectively; [b] significantly different from HGC at $p < 0.05$; [e] significantly different from HGC + MET at $p < 0.05$; F-ratio = 23.37; HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Sperm Morphology

The percentage of sperm with normal morphology was significantly less ($p = 0.0019$) in the hyperglycaemic and metformin groups compared to the control. However, the percentage of normal sperm morphology was not different ($p > 0.05$) in the *C. esculentus* 100 mg/kg group compared to the control, *C. esculentus* 500 mg/kg, hyperglycaemic and metformin groups (Figure 6).

Invariably, the abnormal sperm morphology was significantly ($p = 0.0019$) higher in the hyperglycaemic and metformin groups compared to the control. However, the abnormal sperm morphology was not different ($p > 0.05$) in the *C. esculentus* 100 mg/kg group compared to the control and *C. esculentus* 500 mg/kg groups (Figure 6).

Figure 6:
Effects of *Cyperus* esculentus on Alloxan induced hyperglycaemia on sperm morphology

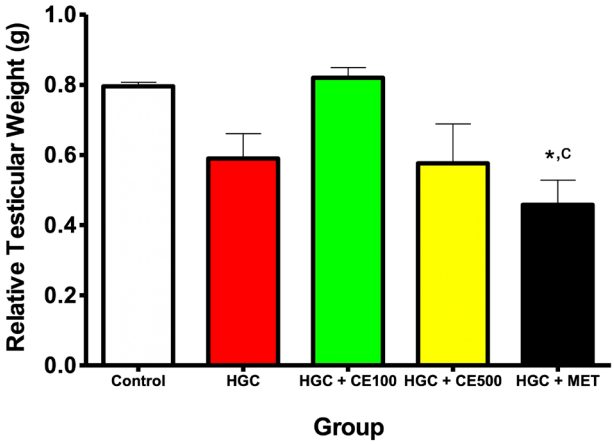


Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean. *,** significantly different from control at p = 0.05, 0.001, respectively; [c] significantly different from HGC + CE 100 at p < 0.05; F-ratio = 6.265; HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

Relative Testicular Weight

There was no significant difference (p > 0.05) in the relative testicular weight in the hyperglycaemic as well as the *C. esculentus* 100 and 500 mg/kg groups compared to the control. However, there was significantly less (p = 0.0055) relative testicular weight in the metformin group compared with control and the *C. esculentus* 100 mg/kg groups (Figure 7).

Figure 7:
Effects of *C. esculentus* on alloxan induced hyperglycaemia on relative testicular weight



Data was analysed using ANOVA followed by post hoc Bonferroni multiple comparison test. Data presented as mean ± standard error of mean. * significantly different from control at p < 0.05; [c] significantly different from HGC + CE 100 at p < 0.05; F-ratio = 5.065; HGC - Hyperglycaemic; CE100 - *C. esculentus* 100 mg/kg; CE500 - *C. esculentus* 500 mg/kg; MET - Metformin

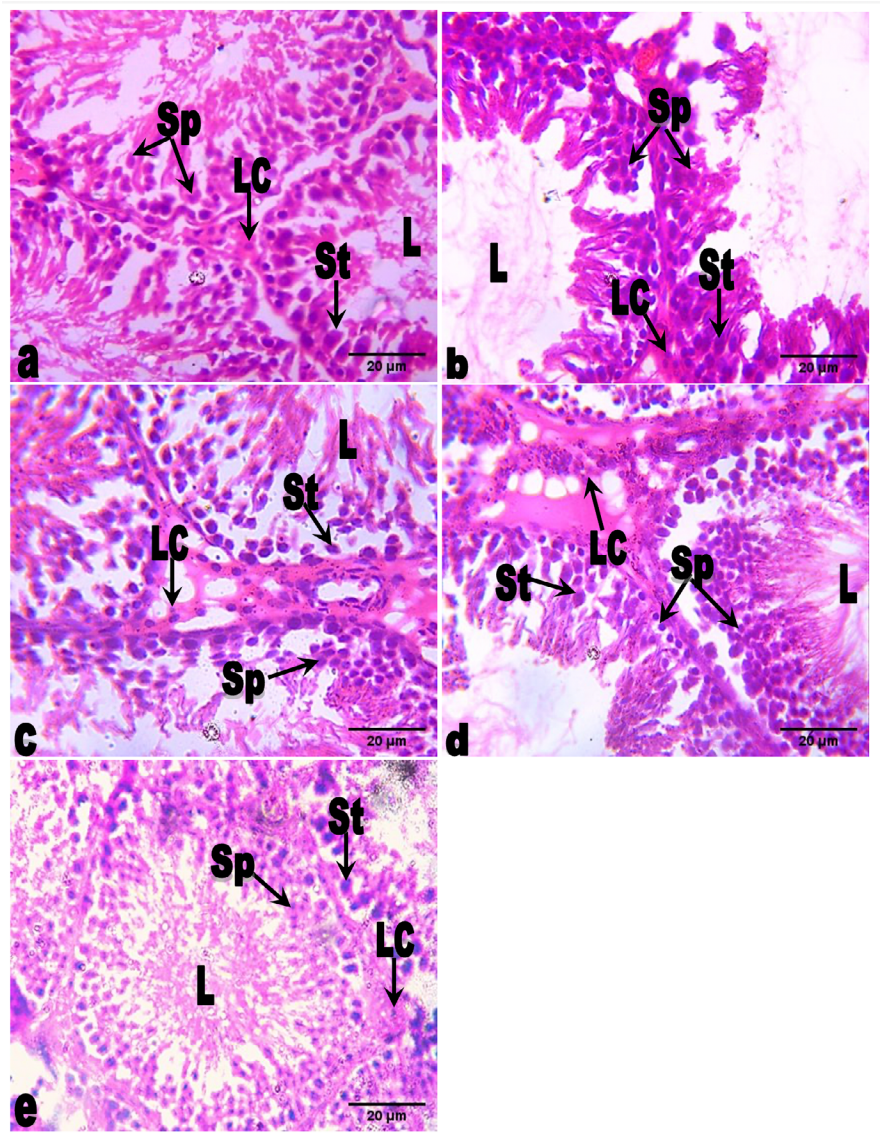
Histology of the Testis

The testes of the control group showed normal morphology: The seminiferous tubules lined by a basement membrane of single layer cuboidal cells; within filled with stratified spermatogenic epithelium, and Sertoli cells having ovoid nuclei. The interstitial cells of Leydig traverse between the seminiferous tubules, with prominent nuclei (Figure 8a).

The testes of the hyperglycaemic group showed disruption of the spermatogenic cells: The seminiferous tubules were devoid of these cells, while the Sertoli cells appeared slightly atrophied compared with the control. The Leydig cells appeared less pronounced in the interstitial area (Figure 8b). The testes of the *C. esculentus* 100 mg/kg group showed mild disruption of the spermatogenic cells, although some of the Sertoli cells appeared slightly atrophied compared with the control (Figure 8c).

The testes of the *C. esculentus* 500 mg/kg group showed slightly improved morphology of the spermatogenic and Sertoli cells compared with the control (Figure 8d). The testes of the metformin group showed slightly atrophied spermatogenic and Sertoli cells, with less prominent Leydig cells compared with the normal control (Figure 8e).

Figure 8:
Photomicrographs of sections of the testes showing the effect of *Cyperus esculentus* on alloxan induced hyperglycaemia in rats (H & E, ×400):



The control group testis shows normal appearance of the spermatogenic (Sp), Sertoli (St) and interstitial cells of Leydig (LC).

a. The hyperglycaemic group testis shows disruption of the spermatogenic cells (Sp), with atrophy of the Sertoli cells (St).

b. The *C. esculentus* 100 mg/kg group testis shows mild disruption of the spermatogenic cells (Sp), with some of the Sertoli cells (St) appearing hypertrophied.

c. The *C. esculentus* 500 mg/kg group testis shows normal appearance of the spermatogenic (Sp) and Sertoli (St) cells.

d. The metformin group testis shows slight atrophy of the spermatogenic (Sp) and Sertoli (St) cells.

L – lumen.

4. Discussion

This study investigated the effect of *Cyperus esculentus* tuber extract on testicular histology and sperm cells viability of alloxan-induced hyperglycaemic Wistar rats. In the present study, alloxan administration induced hyperglycaemia with associated increased serum glucose, indicating a deficient insulin secretion, probably due to β-cell destruction, which is reported with alloxan (Yarmolenko *et al.*, 2020). The high blood glucose declined after treatment with either *C. esculentus* or metformin, indicating their actions in mitigating hyperglycaemia. Metformin is known to reduce blood glucose (Aldobeaban *et al.*, 2018), however, the action of *C. esculentus* may be due to its active metabolites, which as in other plants are responsible for the numerous potentials. The present result corroborates a previous report of *C. esculentus* oil reducing glucose level of type 2 diabetic Wistar rats (Onyenibe and Udogadi, 2019). At concentration of 100 mg/kg the effect of the extract corresponded with that of the standard drug metformin, but with increased concentration to 500 mg/kg, further decline in blood glucose was observed.

There was no significant difference in the final body weights among the test groups compared with the control, although there were insignificantly increased compared with the initial body weights, except in the hyperglycaemic, *C. esculentus* 500 mg/kg and metformin groups which declined insignificantly. Hyperglycaemia is characterized by continuous loss of muscle mass (Hirata *et al.*, 2019), which may have been a reason for the decline in body weight in the present study, although not reversed with subsequent *C. esculentus* 500 mg/kg and metformin treatments.

Deteriorative effects of hyperglycaemia on the male sexual and reproductive functions such as decreased libido and impotence, testicular structural changes and dysfunction have been reported (Naziroglu, 2003; Amaral *et al.*, 2008). The testosterone levels were significantly less in the hyperglycaemic, *C. esculentus* 500 mg/kg and metformin groups compared with the control. Testosterone level decreases in diabetes, whose hallmark is hyperglycaemia, as well as with metformin treatment (Kim *et al.*, 2014; Cai *et al.*, 2021), which the present study aligns with. However, the testosterone level of the *C. esculentus* 100 mg/kg group was not different compared with the control, supporting its protective action on the male androgen as previously reported (Udefa *et al.*, 2020).

Growing evidence indicates that oxidative stress is increased in diabetes due to the production of reactive oxygen species (ROS) and decreased efficiency of antioxidant defences (Amaral *et al.*, 2008; Ceretta *et al.*, 2012; Lazio-de-la-Vega-Monroy and Fernandez-Mejia, 2013). ROS and free radicals have adverse effects on sperm motility and fertility, thus oxidative damage of lipids and DNA of spermatozoa is associated with declining motility and diminished fertility of human sperm (Naziroglu, 2003). There were decreased sperm pH, motility, sperm count and normal sperm cell density in the hyperglycaemia group compared with the control, indicating the adverse effects of hyperglycaemia. Hyperglycaemia is reported to affect the male sexual and reproductive functions negatively (Naziroglu, 2003; Amaral *et al.*, 2008), which may be the case in the present study. The observed negative effects in sperm quality is in line with a study by Mallidis *et al.* (2011), who reported that diabetes mellitus, with hyperglycaemia, induces molecular alterations that negatively affect sperm quality and function, as well as fertility. Studies using alloxan-induced type 1 diabetes animal models have also yielded similar result (Hafez, 2010; Ghlissi *et al.*, 2012).

Treatment with C. *esculentus* extract at 100 and 500 mg/kg showed improved sperm quality such as increased sperm count, motility and morphology, indicating a mitigation of the adverse sperm effects compared with the hyperglycaemic and control groups. These results are in agreement with a study by Al Essawe and Almashhadani (2010), who reported increased sperm count and quality in mice treated with ethanol seed extract of *C. esculentus*. These effects could be due to either the antioxidant ability

of *C. esculentus* or its positive influence on sex hormones (Agbai and Nwanegwo, 2013). The protective role of *C. esculentus* against oxidative stress could also be responsible for this effect. Treatment with metformin also showed a mitigation of the adverse sperm effects compared with the hyperglycaemic and control groups. Metformin reverses hyperglycaemia, which may have also reversed the associated adverse sperm effects, as in the present study.

Testicular effects showed no difference in testicular weight, except in the metformin group. However, there were disruptions and atrophy of the spermatogenic and Sertoli cells of the hyperglycaemic group, indicating adverse actions of arising from alloxan-induced hyperglycaemia. Alloxan-induced hyperglycaemia is reported with advers effects on other body tissues (Lucchesi *et al.*, 2015; Yarmolenko *et al.*, 2020), which may be the case in the testis of the present study. Similarly, hyperglycaemia induced by other chemicals are reported to cause adverse testicular changes (Naziroglu, 2003; Amaral *et al.*, 2009), indicating that hyperglycaemia may have been the reason for the adverse presentation of the testis.

The testes of the 100 and 500 mg/kg *C. esculentus* groups showed mild disruption to normal spermatogenic and Sertoli cells, compared with the control and hyperglycaemic group. This may indicate a reversal of the adverse hyperglycaemic effect on the testes by *C. esculentus. C. esculentus* administration is reported with ameliorative effects, either in normal or hyperglycaemic states in animal models (Al Essawe and Almashhadani, 2010; Agbai and Nwanegwo, 2013; Onyenibe and Udogadi, 2019). These may have been the case in the positive outcome of the present study

Treatment with metformin showed a mild adverse testicular effect compared with the hyperglycaemia and control groups, indicating its mitigating ability.

5. Conclusion

This study showed that alloxan-induced hyperglycaemia resulted in decreased sperm pH, motility, count, and density, as well as adverse testicular histology. Treatments with 100 and 500 mg/kg *Cyperus esculentus* normalized blood glucose level, improved sperm viability and testicular histology. Metformin also normalized blood glucose level, but its ameliorative action on sperm viability and testicular histology was not as good. It is therefore inferred that treatment with *Cyperus esculentus* tuber extract may be a better treatment of hyperglycaemia and its associated adverse testicular condition.

List of Abbreviations

CETE - *Cyperus esculentus* tuber extract

ELISA - enzyme-linked immunosorbent assay

SEM - standard error of mean

6. Declaration of Interests

The authors declare that there is no conflict of interests that could prejudice the impartiality of the research reported.

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8. Contribution by Authors

AAE conceived and designed the research, interpreted the data, drafted the initial manuscript.

ICN collected and assembled data

CAI critically revised the article for important intellectual content

COE collected and assembled data

MBE Analysed and interpreted data, critically revised the article for important intellectual content

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RESUMEN

Cyperus esculentus L. es una planta tuberosa que promueve y protege las funciones reproductivas, que generalmente se ven afectadas en los diabéticos. El presente estudio investigó el efecto del extracto de tubérculo de *Cyperus esculentus* (CETE) sobre la histología testicular y la viabilidad de los espermatozoides de ratas wistar con hiperglicemia inducida por alloxan. Veinticinco ratas Wistar macho adultas que pesaban 150-200 g y se agruparon en cinco (n = 5): el grupo 1, el control, administró agua del grifo (20ml / kg), mientras que los grupos 2-5 se les administró una dosis intraperitoneal única (120 mg / kg p.v.) de alloxan, y agua del grifo por vía oral (20ml/kg), CETE (100 mg/kg), CETE (500 mg/kg) y metformina (500 mg/kg), respectivamente durante 21 días. Los animales fueron sacrificados, su esperma recolectada para su análisis, mientras que los testículos fueron retirados y procesados para histología. Los resultados mostraron un aumento significativo ($p<0,05$) de la glucosa en sangre y la testosterona, y una disminución significativa ($p<0,05$) del pH, la motilidad, el recuento, la morfología y la densidad de los espermatozoides, así como interrupciones e hipertrofia de las células espermatogénicas y sertoli del grupo hiperglucémico. Hubo una disminución significativa ($p<0,05$) de la glucosa en sangre, mientras que los parámetros espermáticos y el peso testicular mejoraron con la histología testicular normal en los grupos de 100 mg / kg de CETE, 500 mg / kg de CETE y tratados con metformina en comparación con el grupo de control e hiperglucémico. El tratamiento con CETE mostró una mejora de la glucosa en sangre y una mejora de la calidad de los espermatozoides, así como atenuación del daño testicular.

Palabras clave: *Cyperus esculentus*, Hiperglicemia, Testículos, Calidad del esperma