MPRI-JAVS | INTERNATIONAL JOURNAL OF AGRICULTURAL AND VETERINARY SCIENCE
VOL. 02 NO. 1 OCT-2023 ISSN: 1115 – 831X

AMELIORATIVE EFFECT OF ETHANOL EXTRACT OF *PSIDIUM GUAJAVA* LEAVES ON MALE REPRODUCTIVE SYSTEM OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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Key word: Psidium guajava, antihyperglycemic, sperm motility, sperm count.

Abstract

Guava leaves have traditionally been used in the management of several diseases. This study was conducted to investigate the effect of an ethanolic extract derived from *Psidium guajava* on both blood glucose levels and the male reproductive system on streptozotocin-induced diabetic Wistar rats. HPLC analysis of the extract was also investigated. Various concentrations of guava leaves extracts (100 and 200 mg/kg) were administered orally to diabetic rats for 28 days daily. The extract's anti-diabetic effect was examined by measuring blood glucose for 28 days after administration of the extract. Sperm, testis, and epididymis samples were collected to determine sperm motility, sperm count, weight, and length of testis and epididymis. While testicular and epididymal tissues were collected for histopathological examination. Diabetes mellitus type 2 significantly increased the untreated group's blood glucose level, causing weight loss, decreased sperm motility, and sperm count, whereas *Psidium guajava* leaves extract significantly ($P<0.05$) decreased blood glucose levels. The sperm motility life span terminated at between 30 minutes in group treated with 200mg/kg of the extract which is a better lifespan compared to 10

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minutes recorded for the diabetic untreated group. The extract-treated groups show a significant (P<0.05) increase in sperm count, testicular weight, and epididymal length. The histology revealed that at a dosage of 100 mg/kg of the extract, there were testicular and epididymal damage, however, few lesions were observed in the group treated with 200mg/kg of the extract. These findings from this study showed that *Psidium guajava* has an ameliorative effect on the blood glucose level, sperm count, and reduced the histopathological effect on diabetic rats.

Introduction

Psidium guajava (PG) belonging to the family Myrtaceae is a large evergreen shrub or small tree that can reach a height of 15 m. it is native to and widely distributed in Mexico and Central America Indies. Today it is cultivated from Asia to Sub-Saharan Africa, having been introduced over the past 300 years in the United States (Kumar, *et al.,* 2021). *Psidium guajava* holds significance as a valuable food crop and medicinal plant with global popularity. It is renowned for its diverse therapeutic properties, which includes antispasmodic, antimicrobial, antioxidant, hypoglycemic, hepatoprotective, anti-allergic, anti-plasmodial, anti-cough, anti-nociceptive and anti-inflammatory activities. Additionally, it has been observed to increase platelet counts in patients grappling with dengue fever (Chechani, et al., 2023). studies on animal models have established the role of guava leave as a potent antitumor, anticancer, and cytotoxic agents (Yin, *et al.,* 2023).

In Nigeria, the main uses locally include treatment of diarrhea, typhoid fever, malaria, use as a chew stick, and treatment of certain skin conditions. Several research studies have been conducted to ascertain its anti-microbial, anti-malaria, anti-diarrhea, and its spasmolytic activity. Moreover, it has demonstrated effectiveness in managing both hypertension and diabetes (Babatola, et al, 2019). According to phytochemical analyses, anthocyanins, alkaloids, carotenoids essential oil, fatty acids, flavonoids, lectins, phenol, saponins, tannins, triterpenes, and vitamin C are revealed to be present in *Psidium guajava* leaves (Raj, et al., 2019). Diabetes is a major threat to global public health. Diabetes mellitus (DM) Type 2 is a complicated chronic metabolic disorder characterized by hyperglycemia, which is a result of defects in insulin secretion, action and inability of the tissue to utilize insulin, or both (Goyal, *et al.,* 2023). It is reported that between 2005 to 2013 the population of diabetic patients increased from 246 to 382 million with a significant prevalence among diverse ethnic groups and it is anticipated to rise to 592 million by 2035 (Shaw et al., 2010). Infertility is a prominent secondary consequence of diabetes due to histological damage to the

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epididymis and testes, and decreased spermatozoa motility (Jangir et al., 2014; Shi et al., 2017; Asafu-Adjei and Gittens, 2020). Most diabetes mellitus patients are diagnosed during their reproductive years, and it is expected that male fertility issues associated with DM will include a problem with sperm count, semen quality and impaired spermatogenesis invariably leading to infertility (Ding, et al.,2015; Maresch, *et al.,* 2018). Infertility being a significant health problem in the lives of a married couple worldwide where about 20-70% is due to male factor (Vander-Borght, and Wyns, 2018) is associated with diabetes complication and search for potent pharmacological compound which is cheap and available necessitated this current research work.

MATERIALS AND METHOD

Plant collection and Identification

Fresh leaves from guava tree were harvested at Gwagwalada, Abuja-Nigeria. It was identified by a taxonomist in the Department of Botany, University of Abuja, Nigeria. The leaves were air dried in a well-ventilated room free from dust.

Preparation of Crude Extracts

Crude extract was prepared using protocol by Abubakar and Haque 2020. Briefly, the dried leaves were pulverized into a fine powder using the industrial grinding machine. A total of 170 g of the ground leaves were placed in a 1000 mL beaker, 800 ml of 80% ethanol was added and thoroughly mixed, the solution was extracted using an orbital shaker for 72 h. The extract was obtained by filtering the solution using a Whatman No1 filter paper, it was evaporated to dryness and stored at 4 °C before use.

Experimental Animal

Twenty-five male Wistar rats weighing between 150 to 180 grams were purchased from Nile University, Abuja. They were kept in a metal cage and allowed to acclimatize at the Laboratory animal unit, University of Abuja for 2 weeks; they were provided standard pelletized rat feed and water *ad-libitum*. The animal experiments were done according to international guidelines for animal experimentation. The Institutional Animal Research Ethics Committee reviewed and certified the experimental protocol in conformity with the guidelines that follow national and international Laws and guidelines of the use of laboratory animal in Biomedical Research. The committee's directive was followed with the utmost caution.

Experimental Procedure

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The procedure by Furman, 2015 was used. Briefly, 20.15 g of citric acid and 7.36 g of sodium sulphate were weighed into 200 mL and 100 mL of distilled water, respectively. Then 50 mL of citric acid and 49 mL of sodium sulphate were mixed, and the Hanna checker® PH meter was used in adjusting the solution's pH to 4.5. Following the preparation of the citrated buffer solution, it was placed in the refrigerator to cool. Then, 250 mg of streptozotocin was dissolved in 25 mL of the citrated buffer solution, resulting in a concentration of 10 mg/mL. Each rat was dosed at a rate of 50 mg/kg of body weight. After 24 hours, the blood glucose level was measured using the Accu check glucometer®, and the glucose level ranges from 130 mg/dL to 543 mg/dL. Additionally, a 10% sucrose solution was prepared by dissolving 10 mg of sucrose powder in 100 mL of water and was provided to the rats as a water supplement for a period of 72 hours.

Experimental Grouping

There were five (5) animals in each group.
Group A: Non-diabetic rats and were administered distilled water only (No DM);
Group B: Diabetic rats and were administered distilled water only (DM);
Group C: Diabetic rats and were administered 100mg/kg of *Psidium guajava* leaves ethanolic extract orally (DM + 100mg/kg);
Group D: Diabetic rats and were administered 200 mg/kg of *Psidium guajava* leaves ethanolic extract orally (DM + 200mg/kg);
Group E: Diabetic rats and administered were treated with standard drug (Glibenclamide) at 5 mg/kg orally (DM + GLIB).

Determination of blood glucose

The measurement of blood glucose levels followed the method illustrated by (Furuhama,and Onodera in 1983). Blood from the tail vein was taken and the levels of blood glucose were determined. These measurements were taken at various time points, including before inducing diabetes (0 hours), as well as at 2-, 4-, and 6-hours post-induction of diabetes. Subsequently, blood glucose levels were monitored at 7, 14, 21, and 28 post induction of diabetes.

Determination of sperm motility

Mortimer, and Mortimer (2013) method was used to determine sperm motility. Feed was withdrawn from the animals' cages 24 h after the last treatment and the animals were asphyxiated using CO_2 followed. Using a non-precharged chamber, CO_2 is dispensed from a commercial cylinder with fixed pressure regulator and inline restrictor controlling gas flow within 30%-70% of the chamber volume per minute to

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comply with established Guidelines. CO_2 flow will be maintained for > 60 seconds following respiratory arrest (which may take up to 5 minutes), followed by bilateral thoracotomy to assure euthanasia. An incision was made on the scrotum and the epididymis was exteriorized. A needle is then used to puncture the caudal epididymis and the semen sample was taken unto a clean glass slide. One drop of warm normal saline was dropped on the semen and was covered with a cover slip, mounted under the microscope, and observed at ×40 magnification. Sperm motility was determined at an interval of 5 to 35 minutes. The percentage of motile sperm cells in a field relative to the number of non-motile sperm cells was used to describe sperm motility.

Determination of Sperm count

Hu, et al. (2006) method was used to determine sperm count. The testicular and epididymal tissues were homogenized using a mortar and pestle, and they were subsequently mixed with 5 mL of standard saline solution. Following this homogenization, 0.5 mL of the resulting sample was introduced into 4.5 mL of Turks solution. The concentration of the spermatozoa and spermatid in the epididymis and testis were determined using hemocytometer respectively. A cover slip was placed on the hemocytometer and few drop of the homogenized solution were then added at the edge of the cover slip. With capillary action the solution spread across the surface of the hemocytometer after which it was viewed under the microscope at ×40 magnification. The total number of cell count is then multiplied by 250,000 to give the concentration of the cell in 10^6/ml.

Determination of the weight of testis, epididymis and length of epididymis

The testis and the epididymis were exteriorized and weighed using an analytical weighing balance and the length of the epididymis were measured using a ruler.

Determination Body Weight

The rats' weights were recorded using a weighing balance before and at 0, 7, 14, 21, and 28 days after inducing diabetes.

Histological Examination

The testicular and epididymal tissues were harvested and fixed in Bouin's fluid for 24 hours. The Bouin's fluid was then replaced with 10% neutral buffer formalin and tissue samples sent to Gariki General Hospital Abuja, Nigeria for processing. The processed tissues were examined under the microscope at ×40 magnification.

Statistical Analysis

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All generated data were presented as mean ± standard deviation (SD) and analyzed statistically by two-way analysis of variance (ANOVA). Analysis was done using GraphPad Instat® Prism 8.4.5 (2020) computer statistical software, with statistical significance defined as $p < 0.05$.

RESULT

Extraction yield

The ethanolic extract gave a yield of 14.1%. The dried extract was suspended in distill water to make two different concentrations of the ethanolic extract before administration into test animals.

Effect of EEPGL on body weight (gram)

The body weight is important in understanding the effect and outcome in a metabolic diseases state such as DM. Figure 1 showed a graph of the weekly body weight with a significant weekly reduction in the DM rats' weight throughout the experiment. Contrastingly, rats with No DM had increased body weight. On the other hand, treatment with extract at 100mg/kg and 200mg/kg experience reduced weight at the first week before improving then after which correlates with the reduction in their blood glucose level.

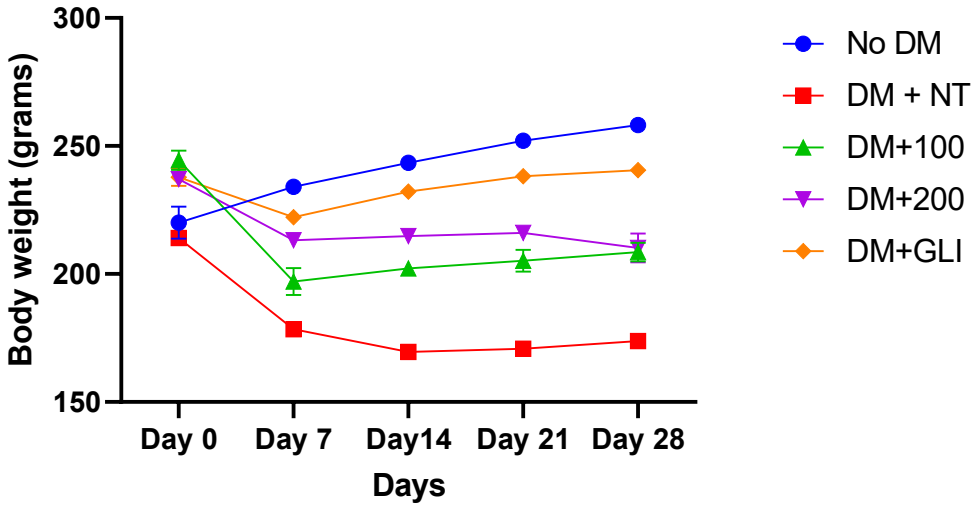

Figure 1: Graph showing the weekly body weight of Streptozotocin-Induced Diabetic Wistar Rats with significant reduction the body weight of diabetic rats.

Effect of EEPGL on weekly blood glucose level.

Concerning the weekly fated glucose level, blood glucose level above 200mg/dl is reported as diabetic. The control group (NO DM) had a glucose level below 200mg/dl throughout the experiment. In contrast, the diabetic rats without any

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treatment exhibited a slight reduction in blood glucose levels, although they remained above 200mg/dl. Diabetic rats treated with a 100mg/dl extract revealed ameliorative effect on their blood glucose levels, with the most notable improvement occurring during the third week of treatment. In contrast, diabetic rats treated with a 200mg/dl extract showed nearly identical outcomes to the group treated with 100mg/dl, achieving blood glucose levels below 200mg/dl by the third week. Remarkably, the standard drug, Glibenclamide at 5mg/kg, demonstrated a more significant enhancement in blood glucose levels during the second week.

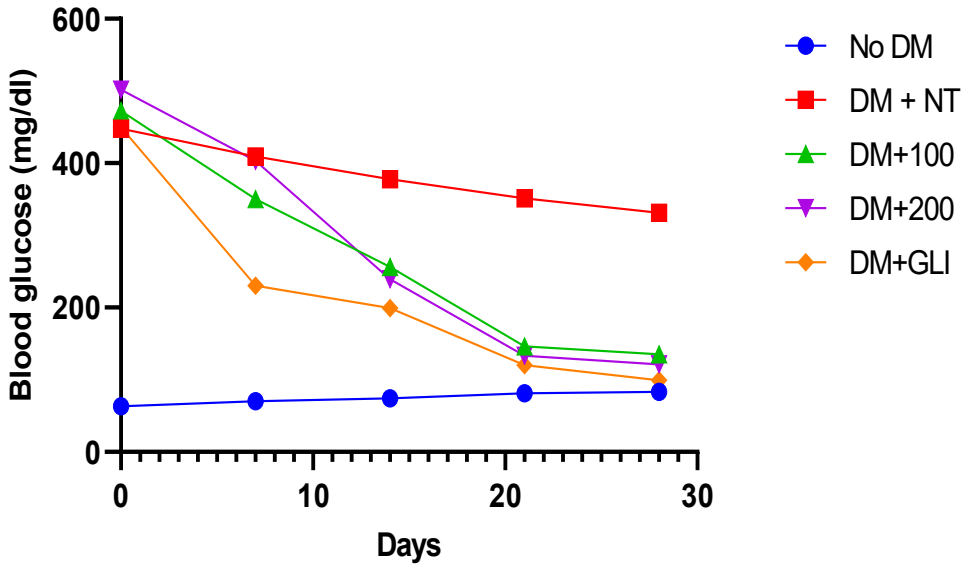

Figure 2: Graph showing weekly blood glucose level (mg/dL) of Streptozotocin-Induced Diabetic Wistar Rats.

Effect of EEPGL on sperm motility.

For effective fertilization to occur, sperm must be able to move to the site of fertilization from the vagina to the uterine horn. The sperm motility was graded in percentage with 5-minute intervals. The graph showed a rapid reduction in motility percentage for untreated diabetic rats, dropping from 98% to 25% within the first 5 minutes and reaching zero motility by the 15-minute mark. In contrast, rats treated with a 100mg/kg extract and those receiving a 200mg/kg extract exhibited a significant increase in motility percentages over time, with zero motility observed at the 30-minute mark.

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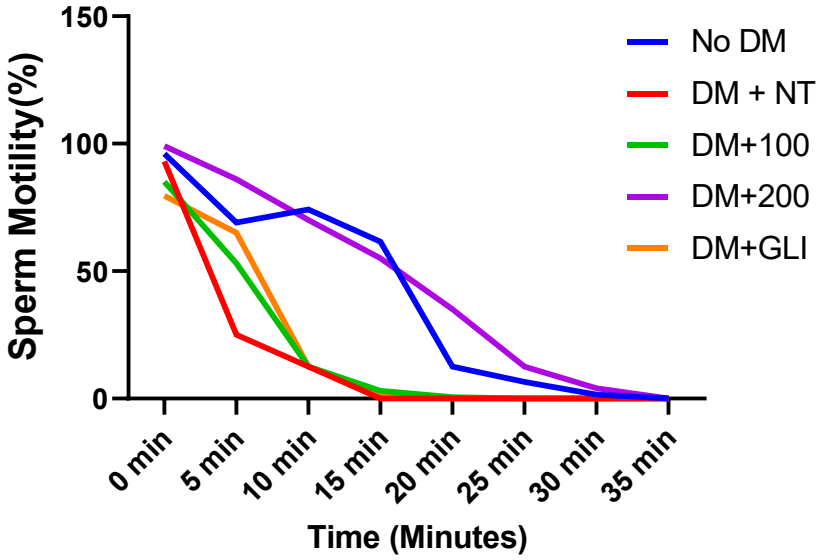

Figure 3: Graph showing the effect of Diabetes Mellitus on sperm motility in a Streptozotocin-Induced Diabetic Wistar Rats.

Effect of EEPGL on Organ somatic index of the epididymis and testis

Although not significant, the epididymal weight reduced in the DM but not treated group with relative increase observed in the 100mg/kg, 200mg/kg and the glibenclamide treated. A similar trend was noticed in the testes' weight, with no apparent alterations in the length of the epididymis.

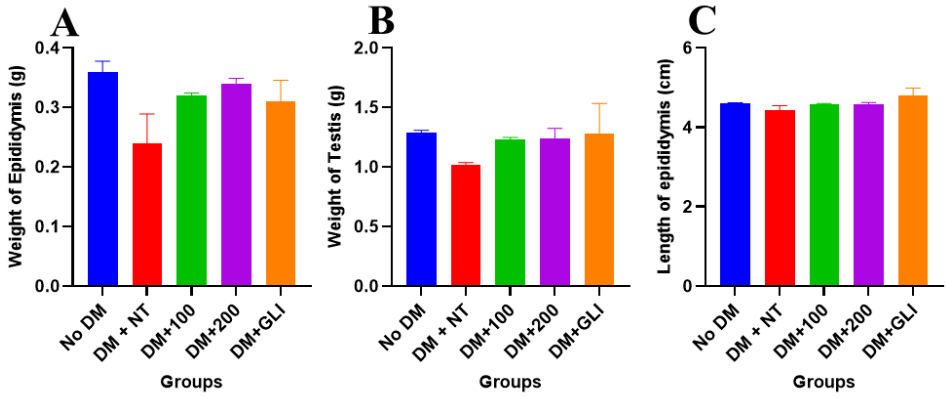

Figure 4: Showing the Organ somatic index of Streptozotocin-Induced Diabetic Wistar Rats. A represent the weight of the epididymis, B represent the weight of the testes and C denotes the graph of length of epididymis.

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Effect of EEPGL on sperm count

Concerning the gonadal sperm count, there was a significant (p≤0.001) reduction in the sperm count of diabetic rats with no treatment compared to the non-diabetic rats. However, the treatment with 100mg/kg of extract significantly increased the sperm count with same effect as the glibenclamide treated diabetic rat. On the other hand, 200mg/kg of extract increased more significantly the sperm count than the NO DM, DM+100 and DM+GLI group.

However, the extra gonadal sperm count also revealed almost the same result with significant (p≤0.001) reduction in the DM+NT group nd with ameliorative effect of extract on the extragonadal sperm count.

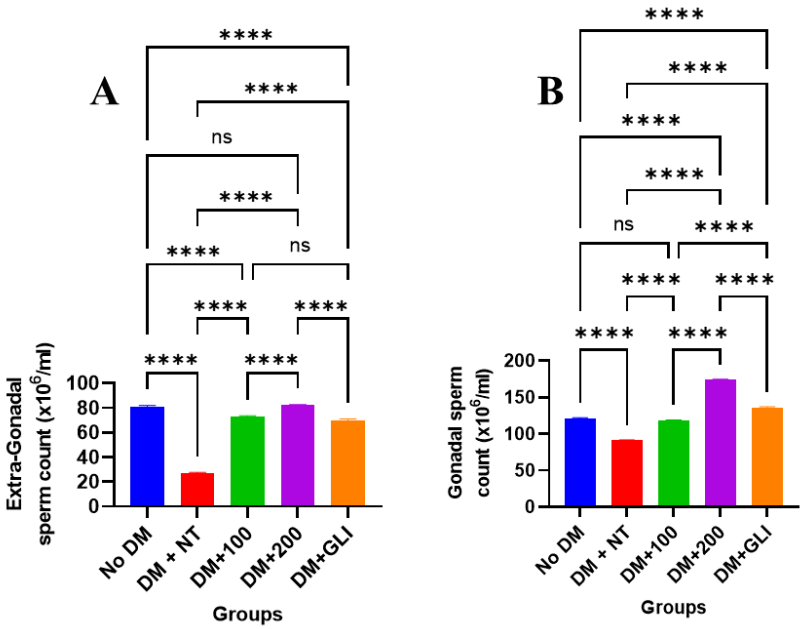

Figure 5. Showing result of A, Extragonadal sperm count and B, the gonadal sperm count in Streptozotocin-Induced Diabetic Wistar Rats. ns- Not significant, ****-(p≤0.0001).

Effect of EEPGL on histology of the testis

The diabetic non-treated group's testicular histology revealed loss of interstitial connective tissue and spermatogonia necrosis; the group treated with Glibenclamide at a dose of 5 mg/kg exhibited loss of interstitial connective tissue, spermatogonia necrosis, and interstitial hemorrhage; the group treated with 100 mg/kg of the extract exhibited loss of interstitial connective tissue and spermatogonia necrosis; however, the 200 mg/kg extract-treated group displayed partial restoration of the connective tissue along with spermatid necrosis. The testicular tissue's natural architecture was visible in the histology of the non-diabetic group.

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VOL. 02 NO. 1 OCT-2023 ISSN: 1115 – 831X

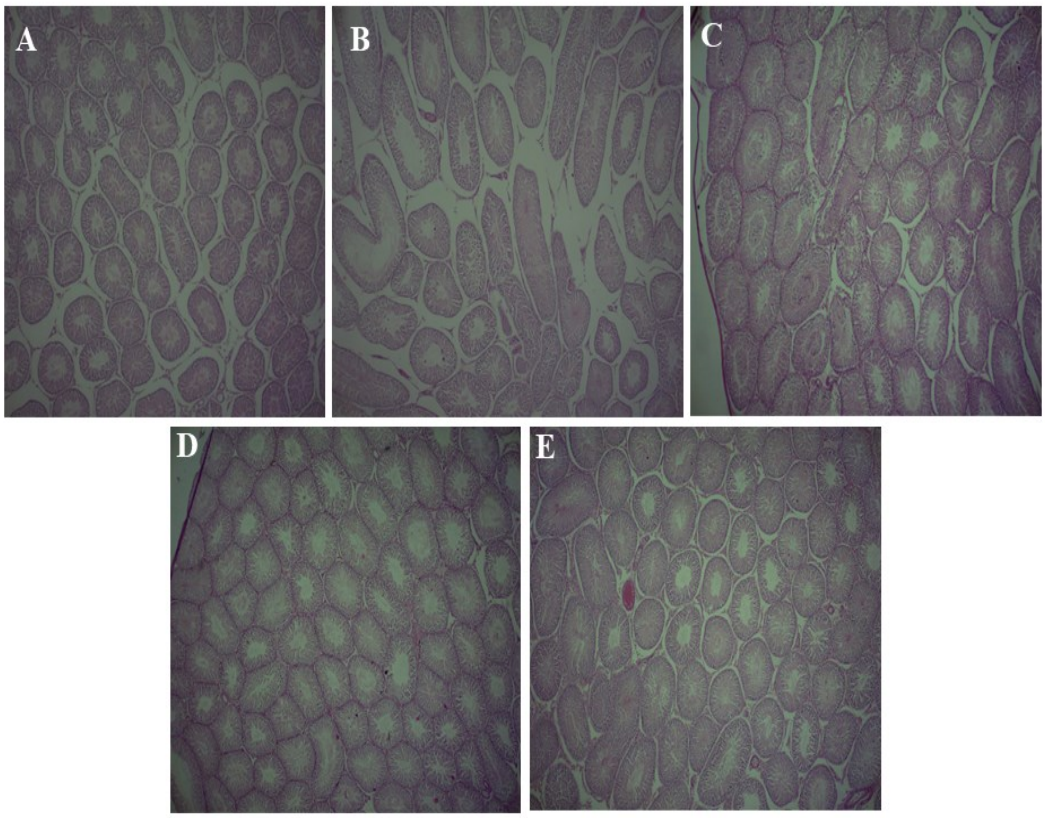

Figure 6: light microscopic examination of testicular tissue (stained hematoxylin stain) obtained from various experimental groups. (A) Non-diabetic group showing normal architecture of the testicular tissue; (B) Diabetic non-treated group showed pathological changes in the interstitial connective tissue and necrosis of the spermatogonia; (C) Diabetic group treated with Glibenclamide (standard drug) shows necrosis of the spermatogonia and infertile hemorrhage; (D) Diabetic group treated with 100 mg/kg showing loss of interstitial connective tissue and necrosis of the Spermatogonia; (E) Diabetic treated with 200mg/kg showing partial restoration of the connective tissue with necrosis of the spermatids.

Effect of EEPGL on histology of the Epididymis

The histology of the epididymis show vacuolation of the epithelial lining for the diabetic non-treated group; the group treated with the standard drug Glibenclamide at 5 mg/kg showed shrinkage of the epithelium; the group treated with 100 mg/kg of the extract show total disorganization of the epididymis and loss of sperm cells; however, the 200 mg/kg extract treated group shows increase interstitial space and vacuolation in the epithelial lining. While the non-diabetic group show the normal architecture of the epididymal tissue.

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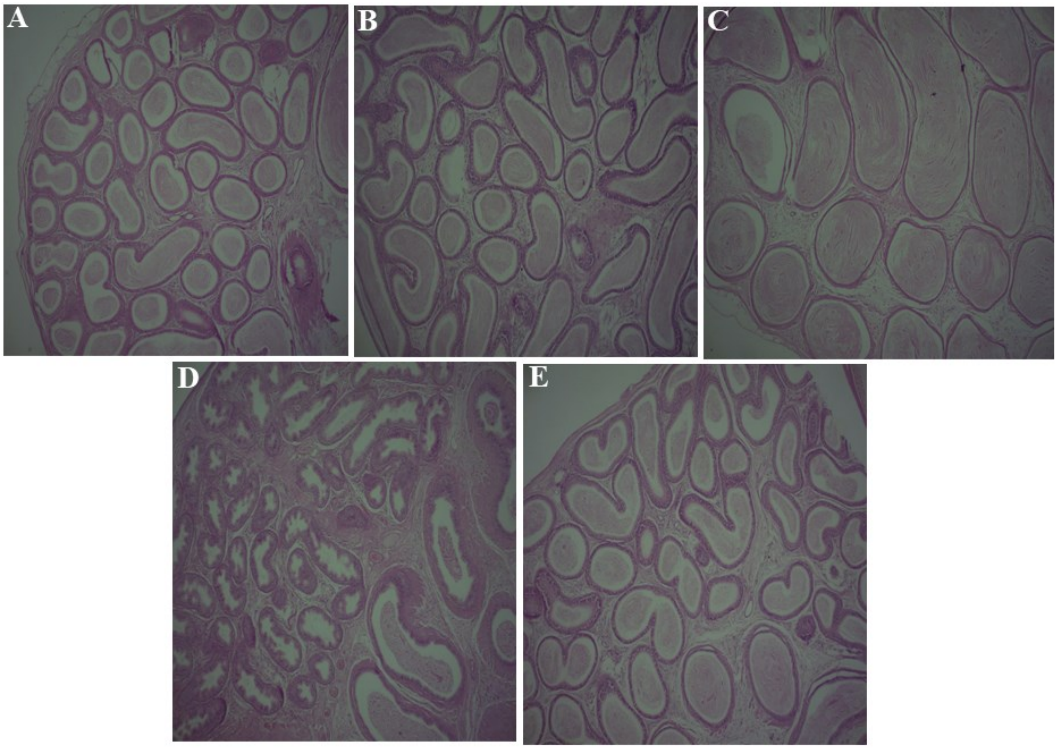

Figure 7: Microscopic examination of the epididymis (stained with hematoxylin) obtained from various experimental groups. (A) non-diabetic group showing normal architecture of the epididymal tissue; (B) diabetic non-treated showing vacuolation of the epithelial lining; (C) The group treated with Glibenclamide showing shrinkage of the epithelium. (D) The untreated diabetic group showing total disorganization of the epididymis and loss of sperm cells; (E) Group treated with 200 mg/kg showing increase interstitial space and vacuolation in the epithelial lining.

DISCUSSION

The observed weight reduction effect of the extract may be attributed to the presence of flavonoids like quercetin, isoflavones, and catechins. Kefayati et al. (2017) reported that these plant metabolites can contribute to weight reduction and serve as a supplement for fruit and vegetable intake, potentially aiding in the treatment of obesity.

The study shows a significant reduction in body weight of rats which aligns with the findings of Shabbir et al. (2020). Shabbir and colleagues reported that groups treated with guava pulp juice exhibited notably lower body weights compared to the control group (diabetic untreated). Their study also highlighted the effectiveness of *P. guajava* seed in reducing body weight, glycaemia, cholesterol, and triglyceride levels while

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significantly increasing the level of HDL-c in Wistar rats compared to the control group.

Psidium guajava has long history of traditional use in managing various infectious and metabolic diseases. The administration of EEPGL led to a significant reduction in blood glucose level among diabetic rats induced by streptozotocin. Several studies have shown that oral administration of *Psidium guajava* extracts has consistently produced a significant lowering effect on blood glucose levels in hyperglycemic rats induced by alloxan or streptozotocin, as demonstrated in previous research (Kameswara et al., 2001; Vinayagam et al., 2018; Kumari et al., 2016). The observed hypoglycemic effect of the *Psidium guajava* extract in the experiment could be attributed to its phytochemical constituents, particularly the presence of a flavonoid called 3,5-diaminobenzoic acid, as determined through chromatographic analysis. The peak and Rf are 97% similar with the standard. It's worth noting that benzoic acid and benzoate, are traditionally recognized as food preservatives. It has also been revealed in recent research to possess antioxidant, antidiabetic, and anti-obesity properties (Jaradat et al., 2022). Guava leaf extracts have exhibited a wide range of physicochemical, pharmacological, and biological attributes, encompassing anti-inflammatory, antidiabetic, antioxidant, antitumor, and immunomodulatory activities (Kumar, *et al.,* 2016Brian). Moreover, Sarian et al. (2017) have reported that flavonoids are linked to significant improvements in the function of pancreatic islet β-cells and in the morphology of hepatocytes in diabetic mice. Furthermore, the consequences of diabetes on male reproductive function can be multifaceted, potentially impacting the endocrine system, spermatogenesis, and even causing difficulties in erection and ejaculation, which may ultimately lead to infertility (He et al., 2021). Notably, diabetes has been associated with adverse effects on semen quality, affecting parameters such as sperm volume, sperm count, sperm morphology, total sperm motility, and sperm DNA fragmentation, as discussed in studies by Lotti and Maggi (2023) and Ding *et al.* (2015).

The diabetic untreated group, which received only distilled water, experienced a significant increase in glucose levels and this has been reported to negatively affect spermatogenesis because elevated glucose levels cause derangement in functions of Sertoli cells that play significant role in the control of spermatogenesis which ultimately results to male infertility (Banday, *et al.,* 2020). The hyperglycemia which may be seen in the diabetic group may significantly impairs male fertility, thus affecting most especially quality of sperm, the group treated with 200 mg/kg recorded sperm motility up to 30 min which is comparable to the non-diabetic group. This outcome illustrates that the extract has an ameliorative effect which lowers the distortions in concentration of insulin arising from high blood glucose which affect

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the functiions of Sertoli cells and spermatogenesis (Ajiboye, *et al.,* 2017). Additionally, the compound identified in the extract possesses antioxidant properties that may inhibit lipid peroxidation induced by reactive oxygen species (ROS), which attack the sperm plasmalemma. Spermatozoa can be destroyed by peroxidation, because major percentage of their cytoplasm is taken away at the final stage of spermatogenesis, this leaves them exposed at this stage when they need endogenous antioxidants system protecting them from ROS (Kim, and Parthasarathy 1998). Furthermore, the testes contain an abundance of polyunsaturated fatty acids, similar to the composition of the sperm cell's plasma membrane, rendering them susceptible to the peroxidation process as well.

Sperm count, motility, viability, and morphology are major indicators of fertility in the male subject and are the model fingerprints in spermatogenesis and maturation of epididymis within the testis. Reduced sperm count, motility and elevated abnormal sperm cells are all related and connected with reduced fertility rate (Tanga, *et al.,* 2021). The decline in sperm count and motility, along with a decrease in the weight of the testes and epididymis within the diabetic group administered distilled water, indicates that it predisposes to decreased fertility, aligning with findings from previous research (Dcunha, et al., 2022). The ethanolic extract exhibited a significant effect on the sperm motility which supported the report of Adeleye *et al.,* (2020) who reported that EEPGL simultaneously increase sperm motility at 100 and 200 mg/kg, also ethanolic extract shows a significant effect on the sperm count which is accordance with the earlier report by Adeleye *et al.,* (2020) who report an increase in sperm count at 100, 200 mg/kg of EEPGL and group treated with Glibenclamide at 5 mg/kg in streptozotocin-induced diabetic rats. There was significant improvement on the relative testis and epididymis weight in rats treated with extract.

The 200 mg/kg of the extract demonstrated an enhancement in the histology of the tissue with necrosis of the spermatids which is an advantage over other groups with necrosis of the spermatogonia. The 200 mg/kg oral extract administration of EEPGL ameliorates the damage in the formation of spermatogonia to development of spermatids. These findings align with Adeleye et al.'s (2020) report, which also highlighted significant improvement in testicular cell architecture with a 200 mg/kg dosage of ethanolic extract from *Psidium guajava.* The observed reduction in blood glucose levels, coupled with the improvements in sperm motility, viability, and sperm count in this study, suggests that EEPGL may be a good medicinal plant for treating male infertility induced by diabetes mellitus.

CONCLUSION

The ethanol extract of Psidium guajava has blood glucose lowering effect on streptozotocin diabetes induced rat at 100 and 200 mg/kg wish justify its traditional

use in the treatment of diabetes. A compound identified in the extract, 3,5, para benzoic acid has been previously tested as an antioxidant, anti-obese and antidiabetic compound. Diabetic mellitus causes male infertility though reduction of sperm viability, motility and sperm count, the extract causes increase sperm count and improve sperm motility better than Glibenclamide. The epididymis and testicular architecture destroyed in diabetic group was restored in the treated group.

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