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EFFECTS OF ETHANOLIC EXTRACT OF *Psidium guajava* LEAF ON REPRODUCTIVE HORMONES IN MALE STREPTOZOTOCIN- INDUCED DIABETIC WISTAR RATS

[1]O. E. ADELEYE, [1]J. M. ENIKUOMEHIN, [1]E. S. AJIBOLA, [2]O. A. ADEKOYA, A. [3]I. ADELEYE, [4]T. SALIU, [2]O. T. ADENUBI

[1]Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria
*[2]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria
[3]Veterinary Teaching Hospital Medicine, Federal University of Agriculture, Abeokuta, Nigeria
[4]Nigeria natural Medicine Development Agency, Victoria Island, Lagos State, Nigeria
***Corresponding Author:** adekoyaoa@funaab.edu.ng **Tel:**+23

ABSTRACT

Diabetes mellitus (DM) is characterized by hyperglycaemia, inadequate production, and utilization of insulin, and by thirst, hunger, and loss of weight. Several diabetic studies in human and animal models have indicated that oxidative changes associated with DM can cause infertility. This necessitated investigation and comparison of the possible reproductive hormonal effects accompanied with the hypoglycemic effects of glibenclamide and quercetin (naturally-occurring flavonoid, strong antioxidant) present in *Psidium guajava* leaf extract (PGLE) on streptozotocin-induced diabetic male Wistar rats. Fifteen adult male Wistar rats (155 - 198 g) were divided into five (A-E) groups and treated daily for 14 days; Group A (125 mg/kg PGLE), B (250 mg/kg PGLE), C (5 mg/kg glibenclamide), D (Negative control) and E (Non-diabetic control). On day 14, blood samples were collected for haematology and serum. Testosterone, oestrogen, progesterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were analyzed using ELISA. Analysis of recorded data was performed by a One-way ANOVA and Tukey's test at $p \leq 0.05$. There was significant change in percentage weight gain (A: -7.02 ± 0.12, B: -5.02 ± 0.30, C: -4.33 ± 0.38%) and fasting blood glucose concentrations (A: 126.0 ± 1.16, B: 101.7 ± 1.45, C: 97.7 ± 1.45 g/dL) in PGLE groups. A significant increase in the FSH (A: 4.68 ± 0.07, B: 6.71 ± 0.07, C: 6.29 ± 0.09 ng/mL) and LH (A: 0.56 ± 0.005, B: 0.72 ± 0.004, C: 0.61 ± 0.009 ng/mL) levels of diabetic rats treated with PGLE was also observed. Although FSH and LH levels significantly increased, this could have resulted from stimulation of the hypothalamus which causes production of both hormones. This infers the potentials of *P. guajava* extracts as fertility booster, especially in male subjects with DM.

Key words: diabetes, gonadotropins, *Psidium guajava*, streptozotocin, testosterone

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INTRODUCTION

Diabetes mellitus (DM) is associated with damage, dysfunction and failure in organs and tissues, including the retina, kidney,

nerves, heart and blood vessels (He *et al.,* 2021). The International Diabetes Federation (IDF) estimates an overall prevalence of DM to be 366 million in 2011, and this is expected to rise to 552 million by 2030 (Whiting *et al.*, 2011). Diabetes mellitus has been known to be associated with chronic hyperglycemia (excess of glucose in the bloodstream), which is responsible for the several complications associated with the condition. Other factors complicating DM include heart disease, retinopathy, kidney disease, and neuropathy (Soumya and Srilatha, 2011).

The development of DM involves different pathogenic processes which range from abnormalities that are caused by insulin resistance, to autoimmune condition that results in the gradual destruction of the pancreatic beta-cells (American Diabetes Association, 2014). The functional deficiency caused by insulin lack in affected tissues or organs results in dysfunctions in protein, fat, and carbohydrate metabolism (American Diabetes Association, 2014). Diagnosis is based on testing glucose levels viz: fasting plasma glucose (FPG) or 2 hours plasma glucose (2 h PG) value, after a 75 g oral glucose tolerance test (OGTT) or recently, hemoglobin A1c (A1C) (threshold ≥ 6.5%) (American Diabetes Association, 2014).

Gonadotropin-releasing hormone (GnRH) release is stimulated in the hypothalamus by insulin (INS), which also enhances the activity of HPGA. (Sahu et al., 2017). Additionally, in vitro INS stimulates gonadotropin (GN) secretion triggered by luteinizing hormone-releasing hormone (LHRH). Insufficient secretion of INS in DM patients can damage the HPGA (He et al., 2021). The secretion of INS is necessary for the anterior pituitary cells to properly utilize glucose and abnormalities in how the body utilizes glucose that are caused by INS deficiency, and these abnormalities lower GN secretion and pituitary protein production. The release of GnRH is disrupted by hyperglycemia, which lowers the secretion of GN and prolactin. This decreases the secretion of testosterone (T) from Leydig cells, which ultimately results in abnormalities in spermatogenesis (He et al., 2021). Because of the DM-mediated damage of Sertoli cells or Leydig cells, the secretion of T is decreased, resulting in an increase in the levels of FSH and LH through the negative feedback effect of HPGA. Hyperandrogenemia and insulin resistance are the two most significant pathogenic alterations in reproductive function brought on by HPGA sexual endocrine disease. (He et al., 2021).

The pathogenesis of male infertility and sexual dysfunction caused by abnormal HPGA may be related to the abnormal INS signaling and the dysregulation of kisspeptin expression in hypothalamus (Liu & Tang, 2017).

Streptozotocin (STZ; N-nitro derivative of glucosamine) occurs naturally, and it is a broad-spectrum antibiotic which destroys the β- cells of the islets of Langerhans in the pancreas, responsible for the production of insulin in the body of mammals (Szkudelski, 2001; Damasceno *et al*., 2014; Kintoko *et al*., 2014). Using STZ to induce DM in rats is a remarkably simple procedure that requires little or no expertise. Injecting 60 mg/kg of STZ via the intravenous (IV) or intraperitoneal (IP) routes, results in the degeneration of the β cells, and the effects are noticed clinically within two days post injection (Furman, 2021).

There are various plants that have been discovered to serve as drugs for various diseases like DM (Patel *et al.*, 2012). *Psidium guajava* Linn. (Family Myrtaceae) fruit, known as guava, grows in the tropics, and can be eaten raw. It is also sold as beverages, syrups and jams. It is extraordinarily rich in antioxidants like vitamin C and polyphenolic compounds (Thaipong and Boonprakob, 2005). All plant parts (roots, fruits, leaves, stem, barks, seeds, and flowers) of the plant are prescribed in folkloric medicine to manage respiratory and gastrointestinal tract (GIT) ailments, diabetes, and cancer (Shadia *et al.,* 2018; Naseer *et al.,* 2018). *P. guajava* leaves have anti-inflammatory, antibiotic, analgesic, hepatoprotective and antioxidant activities (Gutiérrez *et al.,* 2008). It contains quercetin, which is its most pharmaco-active composition (Adeleye *et al.*, 2020).

MATERIALS AND METHODS

Animals

Fifteen adult male Wistar rats (155 – 198 g) were obtained from the Teaching and Research Animal House, University of Ibadan, Nigeria. They were housed in well-ventilated standard rat cages in the Experimental Animal Unit of the College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. They were kept in 12-hour light/12-hour darkness conditions, fed conventional rat diet, and always had access to water, unless otherwise stated. When conducting this study, the rules, regulations and institutional policies set forth by FUNAAB's committee on animal care ethics and usage were strictly adhered to. Approval number (FUNAAB/COLVET/CREC/2021/05/16) was given.

Plant material

The Department of Pure and Applied Botany, College of Biosciences, FUNAAB confirmed the *P. guajava* leaves that were collected from a farm in Abeokuta, Ogun State. The leaves were dried for four weeks at room temperature. The coarse particles were soaked in 97% ethanol for three days in a room after the dry leaves were reduced into small particles. The filtrate was evaporated at 60^0C using a vacuum rotary evaporator after the mixture was filtered using Whatman's filter paper. The moist dark residue of the *P. guajava* leaves extract (PGLE) was allowed to dry in vacuo, before being stored in the refrigerator (4^0C) until needed. One gram of residue was dissolved in 20 mL distilled water to make the stock solution, which had a concentration of 50 mg/mL.

Diabetes Induction

After an overnight fast, STZ (Sigma-Aldrich, USA) was administered IP in a single dose of 60 mg/kg body weight, diluted in 0.4 ml of freshly made citrate buffer (pH 4.5) to induce DM. A drop of blood was drawn from each rat's tail vein 48 hours after STZ administration, and the blood glucose level was determined using a digital glucometer (Accu-chek© Advantage, Roche Diagnostics, Germany). Diabetic rats were defined as those with blood glucose levels ≥ 350 mg/dl and were included in the research.

Experimental Procedure

The fifteen rats were randomly assigned to five groups of three rats each, and groups A through D received IP doses of 60 mg/kg STZ to produce the diabetic state. The following was applied to each group; Group A (DT125) and B (DT250) received 125 and 250 mg/kg PGLE respectively, Group C (DTG) was given a standard antidiabetic agent (glibenclamide), and Group D (DNT) were diabetic but not treated. Group E (CTR) animals were not diabetic and re-

ceived no treatment. At the end of the experiment (14 days), five mls of blood was collected from the rats, full blood count and serum analysis for sex hormonal levels was done.

Hormonal assay

Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone levels were determined by analyzing the plasma using ELISA (Enzyme-linked immunosorbent assay) kit (Inteco™ UK) according to the manufacturer's protocols.

Fasting blood glucose determination

The rats were fasted of feed for 18 hours before their blood samples were taken, but they were given water *ad libitum*. An Accu-Chek® glucometer was used to analyze the blood glucose concentration via the glucose oxidase method (Nagappa et al., 2003). Briefly, a drop of blood was placed on the test strip and strip inserted into the glucometer after it was turned on. The result was viewed from the screen and recorded. This method involves the use of enzymes. The test strips are loaded with enzymes which react with the glucose in the blood. There is a color change due to the reaction which the meter measures and translates into the concentration of glucose in mg/dL. Blood glucose values for each rat were repeated thrice and the average reading was used. This was conducted thrice within a week for the two-week period of the research.

Data analysis

Data were gathered, collated, and presented in the proper statistical data format, with mean ± standard error of means (SEM) being used to express the results. Analysis of variance (ANOVA) was used to analyze the data, and then Tukey's test was used to compare means. The Statistical Package for the Social Science (SPSS) software (version 16.0; SPSS Inc., USA) was used for all analysis and P values less than 0.05 were significant following the guidelines as recommended by Feldman *et al.* (2003).

RESULTS

Blood glucose levels from the 15 rats used for the research were recorded and analyzed (Figure 1). The weights of the rats were also recorded and analysed (Figure 2). The blood serum and the various sex hormonal levels were examined and analysed using the ELISA method (Figures 3 – 7).

Effect of administration of Psidium guajava leaf extract on fasting blood glucose of control and test rats

There was significant decrease (P=0.017) in all test groups (DT125: 126.0 ± 1.16 g/dL, DT250: 101.7 ± 1.45 g/dL and DTG: 97.7 ± 1.45 g/dL) when compared with DNT (350.7± 2.96 g/dL). There was no significant difference when the test groups were compared to each other (Figure 1).

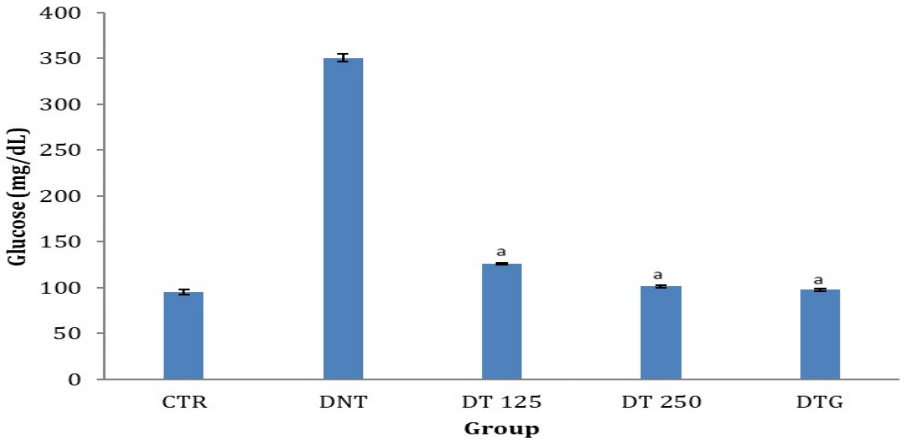

Figure 1: Effect of PGLE administration on fasting blood glucose concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, $^{a}p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

Effect of administration of Psidium guajava leaf extract on relative weight gain of control and test rats

There was general weight loss in all groups except the control group (Figure 2). However, there was significant increase (P=0.017) in all test groups (DT125: -7.02 ± 0.12%, DT250: -5.02 ± 0.30% and DTG: -4.33 ± 0.38%) when compared with DNT (-10.33 ± 0.78%). There was no significant difference when the test groups were compared to each other (Fig. 2).

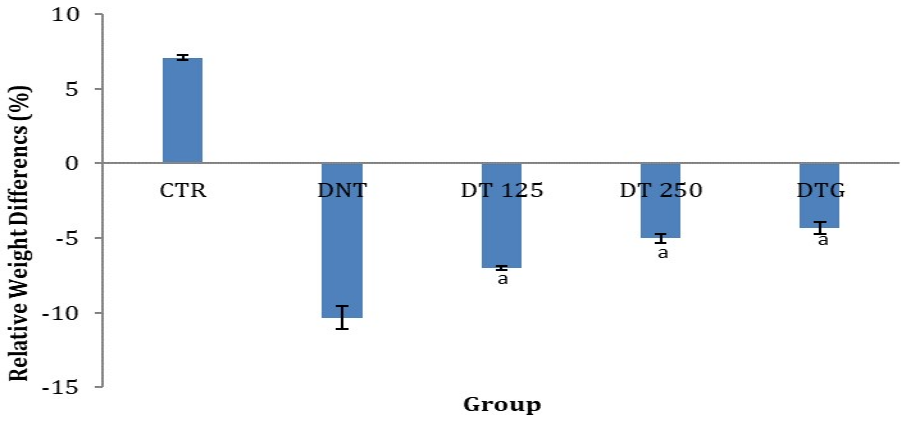

Figure 2: Effect of PGLE administration on relative weight gain of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, $^{a}p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg/kg glibenclamide

Effect of administration of Psidium guajava leaf extract on oestrogen concentrations of control and test rats

There was significant decrease (P=0.023) in all test groups (DT125: 10.39 ± 0.18 pg/mL, DT250: 5.18 ± 0.13 pg/mL and DTG: 4.09 ± 0.12 pg/mL) when compared with DNT (13.8 ± 0.27 pg/mL). There was significant increase in DT125 compared with DT250 and DTG, but there was no difference between DT250 and DTG (Fig. 3).

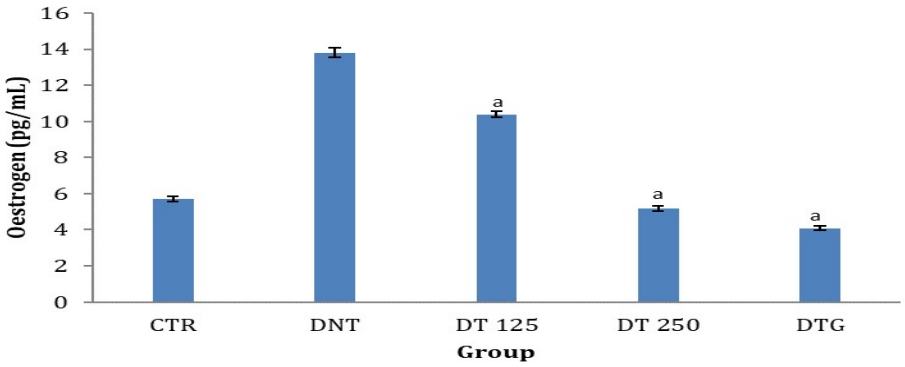

Figure 3: Effect of PGLE administration on oestrogen concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, [a]$p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

Effect of administration of Psidium guajava leaf extract on progesterone concentrations of control and test rats

There was significant decrease (P=0.035) in all test groups (DT125: 10.19 ± 0.08 ng/mL, DT250: 15.14 ± 0.14 ng/mL and DTG: 14.75 ± 0.09 ng/mL) when compared with DNT (7.43 ± 0.09 ng/mL). There was significant decrease in DT125 compared with DT250 and DTG, but there was no difference between DT250 and DTG (Fig. 4).

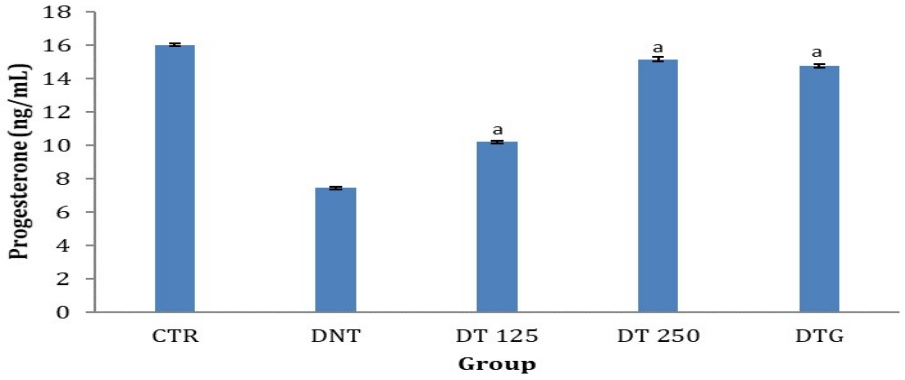

Figure 4: Effect of PGLE administration on progesterone concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, [a]$p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

Effect of administration of Psidium guajava leaf extract on testosterone concentrations of control and test rats

There was significant decrease (P=0.041) in all test groups (DT125: 3.47 ± 0.07 ng/mL, DT250: 4.74 ± 0.25 ng/mL and DTG: 4.24 ± 0.04 ng/mL) when compared with DNT (1.35 ± 0.17 ng/mL). There was no significant difference when the test groups were compared to each other (Fig. 5).

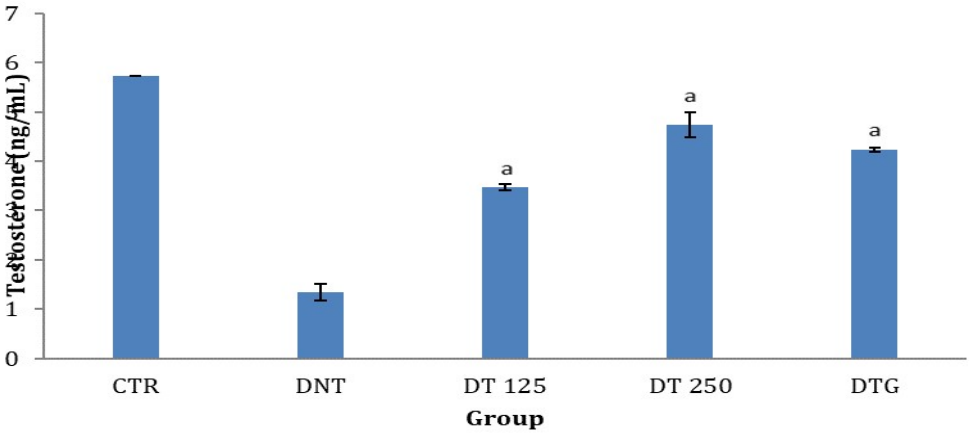

Figure 5: Effect of PGLE administration on testosterone concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, $^{a}p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

Effect of administration of Psidium guajava leaf extract on follicle stimulating hormone concentrations of control and test rats

There was significant increase (P=0.034) in all test groups (DT125: 4.68 ± 0.07 ng/mL, DT250: 6.71 ± 0.07 ng/mL and DTG: 6.29 ± 0.09 ng/mL) when compared with DNT (4.80 ± 0.08 ng/mL). There was significant increase in DT125 compared with DT250 and DTG, but there was no difference between DT250 and DTG (Fig. 6).

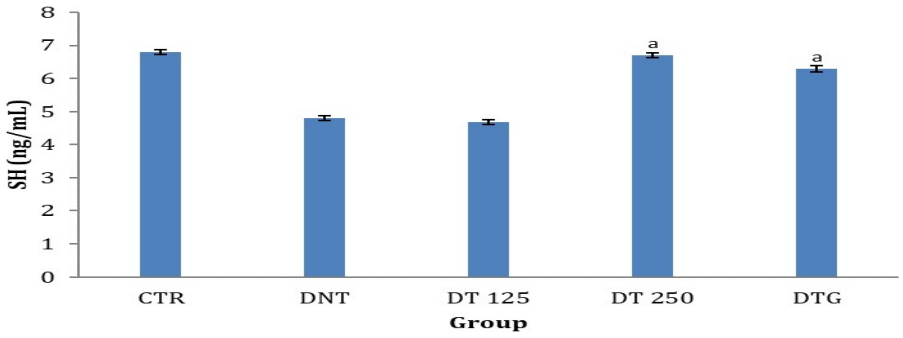

Figure 6: Effect of PGLE administration on follicle stimulating hormone concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, $^{a}p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

Effect of administration of Psidium guajava leaf extract on luteinizing hormone concentrations of control and test rats

There was significant decrease (P=0.013) in all test groups (DT125: 0.56 ± 0.005 ng/mL, DT250: 0.72 ± 0.004 ng/mL and DTG: 0.61 ± 0.009 ng/mL) when compared with DNT (0.47 ± 0.005 ng/mL). There was no significant difference when the test groups were compared to each other (Fig. 7).

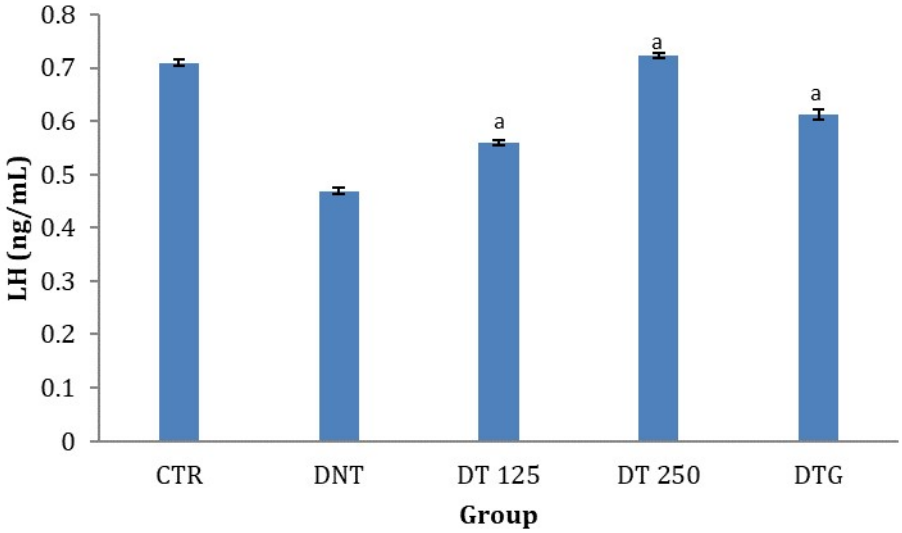

Figure 7: Effect of PGLE administration on Luteinizing hormone concentration of streptozotocin-induced diabetic rats. Columns stand for mean ± SEM, n = 3, [a]$p < 0.05$ = significant difference from DNT. CTR = non-diabetic control; DNT = diabetic untreated; DT 125 = 125 mg/kg PGLE; DT 250 = 250 mg/kg PGLE; DTG = 5 mg /kg glibenclamide

DISCUSSION

This study revealed that PGLE caused a dose-dependent reduction in blood glucose values. This finding is in line with Gutiérrez *et al.* (2008) who showed that phytochemicals in *P. guajava* have antispasmodic, antimicrobial, antioxidant, hepatoprotection, antiallergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflamatory and antinociceptive activities. Adeleye et al. (2020) had previously reported that *Allium cepa*(Alliaceae family) peel extracts showed a similar response as PGLE in STZ-induced diabetic male Wistar rats, supporting the findings of this research and, *Petroselinum Crispum* (Parsley) (Cheraghi *et al.*, 2021). Oral supplementation of arachidonic acid, a polyunsaturated fatty acid completely prevented hyperglycemia and enhanced insulin sensitivity by suppressing IL-6 and TNF-a production and restoring antioxidant status to normal in STZ-induced diabetic Wistar rats like findings in this research (Gundala *et al.*, 2018).

Diabetes mellitus, is recognised to be characterised by multi-systemic pathologies due to hyperglycemia caused by the cells' inability to take in glucose (Kaveeshwar and Cornwall,

2014). Reactive oxygen species (ROS) are created when glucose is auto oxidized, and when their production exceeds the body's antioxidant system's capacity to scavenge them, and severe neuropathy, macro- and microvascular dysfunction result (Bajaj and Khan, 2012). This causes the body to physiologically choose other methods of producing glucose (glycogenolysis and lipolysis), which ultimately leads to weight loss. This was demonstrated in this research in both the PGLE-treated and untreated diabetic groups. However, the glibenclamide-treated diabetic rats gained weight. Sulfonylureas (glibenclamide) are most commonly prescribed as an antidiabetic agent but have challenges with causing weight gain. Uncontrolled weight gain could alter the serum lipid profile of the diabetic patient (Balsells *et al*., 2015).

The effect of *P. guajava* leaves on reproductive hormones showed a decrease in the oestrogen levels, an increase in progesterone, testosterone, FSH and LH levels. Physiologically, in male animals, secretion of oestrogen is reduced. However, in the diabetic state, the untreated group had an increased level (Uboh *et al*., 2010). This may suggest that in diabetic animals, the level of oestrogen is increased before the commencement of treatment, thereby affecting the sexual potency of such male animals. Once the treatment is started, the oestrogen levels are seen to decrease.

Findings of Ferdinand *et al.* (2014), noted that *P. guajava* leaves caused an increased in testosterone hormone production, suggesting an effect on steroidogenesis in Leydig cells of the testes. Also, increased levels of FSH and LH in this study shows that in diabetic untreated rats, production of FSH and LH is reduced prior to treatment. Once treatment is commenced, the hypothalamus is stimulated to cause more production of both hormones.

CONCLUSION

This research looked at *P. guajava*'s impact on a few reproductive hormones in STZ-induced diabetic Wistar rats. Since the extract returned the lowered hormonal values in test animals to normal, ethanol extract of *P. guajava* leaves could be used in the treatment of infertility. This uptake reduces the generation of ROS, restoring normal levels of reproductive hormones. It would be necessary to standardise the dosage and evaluate the mechanism by which *P. guajava* produces this effect before the extract could be utilised by diabetic patients.

Significance statement

DM is reaching epidemic proportions globally. In a STZ-induced type 2 diabetes mellitus animal model, it was shown that oral supplementation of *P. guajava* leaves extract can encourage quick glucose uptake by liver and other peripheral tissues with a small reduction of intestinal glucose absorption completely preventing hyperglycemia, reducing the generation of ROS, and restoring normal levels of reproductive hormones. These results suggest that *P. guajava* leaves extract may function as an anti-diabetic agent.

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Nil

Conflict of interest

All authors do not have any conflict of interest to declare.

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