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Full Length Research Article

Effect of Co-administration of Glibenclamide and Aqueous Calyx Extract of *Hibiscus Sabdariffa* on Oxidative Stress Markers in Streptozotocin-Induced Diabetic Rats

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Summary: The hyperglycaemia of diabetes mellitus (DM) induces oxidative stress which damages the tissues. Glibenclamide, an oral hypoglycaemic drug used in the treatment of DM has associated side effects. Natural products are considered safe in the treatment of chronic diseases. *Hibiscus sabdariffa* (HS) is a plant that has demonstrated antidiabetic activity. We aimed to determine the potential benefits of co-administration of HS and glibenclamide in ameliorating oxidative stress in streptozotocin (STZ)-induced diabetic rats. A total of 25 male albino Wistar rats were divided randomly into five groups: control (Non-DM), diabetic (DM), diabetic treated with 600µg/kg BW of glibenclamide (DM + GLIB), diabetic treated with 500mg/kg BW of HS (DM + HS), diabetic treated with both 600µg/kg BW of glibenclamide and 500mg/kg BW of HS (DM + GLIB + HS). The interventions were administered for a period of 28 days. The Non-DM rats were significantly heavier ($p<0.01$) compared to rats in the other treatment groups. Glibenclamide or HS alone and in combination, significantly lowered ($p < 0.001$) the final fasting blood glucose concentration of the rats in the respective treatment groups. HS and a combination of HS+ GLIB resulted in increased ($p<0.05$) serum activity of catalase, glutathione peroxidase and superoxide dismutase compared to the DM untreated rats. The serum level of malondialdehyde was significantly lowered ($p=0.000$) in rats that received a combination of HS + GLIB compared to the DM untreated rats. Co-administration of HS + GLIB showed beneficial regeneration of islet-cells in the pancreas. Co-administration of HS + GLIB appears to be more beneficial in the treatment of DM and associated oxidative stress than when given as single agents. Thus, a case for their incorporation as a combined therapy for DM should be considered.

Keywords: *Diabetes; Glibenclamide; Hibiscus sabdariffa; Streptozotocin; Rats*

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INTRODUCTION

The prevalence of diabetes mellitus (DM) is on the increase globally (Forouhi and Wareham, 2019), affecting about 451 million people in 2017 and estimated to reach 693 million by 2045 (Cho *et al*., 2018). The number of people living with DM and its sequelae in Nigeria as at 2017 was about 1.7 million (Cho *et al*., 2018). DM is characterized by defects in insulin secretion and/or insulin action leading to impairment of glucose, lipid and protein metabolism (Chandra *et al*., 2019). Consequently, hyperglycaemia results in the blood and other non-insulin dependent tissues such as the pancreas and brain (Piero *et al*., 2015; Glovaci *et al*., 2019).

Hyperglycaemia favours the development of oxidative stress via glucose autoxidation, glycation reactions with proteins and lipoproteins or when glucose enters the polyol pathway and gets converted to sorbitol (Oguntibeju, 2019). Hyperglycaemia, therefore, increases the concentration of free radicals in the body and overwhelm the capacity of the antioxidant enzymes to maintain them within normal physiological ranges and thus increasing oxidative stress (Tsuruta *et al*., 2010; Sharma and Kar, 2014). Oxidative stress has been implicated in several studies as an important player in the onset and advancement of DM (Oguntibeju, 2019; Badhwar *et al*., 2020).

Glibenclamide is one of the most frequently prescribed oral hypoglycaemic agents (Zhang *et al*., 2017) that stimulates insulin secretion from the existing β cells of the pancreas and reduces hepatic gluconeogenesis resulting in reduced blood glucose (Bolanle *et al*., 2018). However, the use of glibenclamide is limited due to prolonged hypoglycaemia (Heller and Novodvorsky, 2019), high secondary failure rate and other adverse events such as vomiting, weight gain, transient leucopoenia and purpura among others (Gopalakrishna *et al*., 2017).

Studies using both clinical and experimental models have demonstrated the benefits of antioxidants in ameliorating oxidative stress in tissues (Rivera-Barahona *et al*., 2017; Mehta *et al*., 2018). Compounds isolated from natural sources, such as plants are loaded with antioxidants that are more effective and inexpensive compared to conventional therapy in management of some diseases

(Mehta *et al*., 2018). Therefore, antioxidants or nutrients with high antioxidant capacity may offer additional health benefits with possibility of limiting the progression of DM and its associated complications (Fenercioglu *et al*., 2010). *Hibiscus sabdariffa* (HS) is a plant of the Malvaceae family (Zhen *et al*., 2016). It is used in preparation of local non-alcoholic cold or hot drinks. In Nigeria, the calyces of HS are processed into a beverage called "sobo" or "zobo" (Mojiminiyi *et al*., 2012). HS is loaded with antioxidants such as anthocyanins which are thought to be the primary way by which the plant exerts its biological activities (Nguyen *et al*., 2020). HS aqueous extract was shown to prevent hyperglycaemia, hyperlipidaemia and oxidative stress (Peng *et al*., 2011).

Considering the antioxidant property of HS and its previously demonstrated antihyperglycaemic effects, we hypothesised that a combination of HS and glibenclamide could be more effective in ameliorating oxidative stress than glibenclamide alone in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Study location: The study was conducted at the Animal house of the Usmanu Danfodiyo University Teaching Hospital complex, Sokoto and laboratories in the Department of Chemical Pathology, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto.

Ethical approval and compliance with ARRIVE guidelines: The protocols used in this study were approved by the Animal Research Ethics Committee of Usmanu Danfodiyo University, Sokoto, Nigeria and complied with international ethical guidelines and standards for the care and use of laboratory animals. The Animal Research: Reporting of *In vivo* Experiments (ARRIVE) guidelines have been used in writing up this manuscript.

Reagents: All reagents for the study were of good analytical grade. Accu check glucometer strips were from Roche, Germany. Biuret kit for protein estimation was from Prestige Diagnostics, UK. All the antioxidant enzymes (Glutathione peroxidase, Superoxide Dismutase and Catalase) and Malondialdehyde were assayed using the Laboratory kits from Cayman (USA).

Plant material: Dried calyces of HS were purchased from central market, Sokoto (coordinates: 13º05'N 05º15'E) and taken to Botany Department of Usmanu Danfodiyo University for authentication. A voucher number UDUH/ANS/0219 was collected and the plant specimen deposited at the herbarium of the same department of the institution.

Preparation of plant extract: The calyces were dried and pulverised with mortar and pestle. About 210 g of powder was extracted in 1400 ml of distilled water at 95ºC for two hours (Lin *et al*., 2007). The extracted powder was filtered (Whatman no. 1 filter paper) and concentrated at a temperature of 40ºC (Ibrahim *et al*., 2017) using hot air ovum (Hospibrand, USA). The residual powder extracts were recovered and preserved at 4ºc till use (Ali *et al*., 2003; Lin *et al*., 2007), and a yield of 55% was obtained.

Animals and Experimental Design: A total of 25 male adult Wistar albino rats aged 10-12 weeks (150- 200g) were purchased from the animal house, Usmanu Danfodiyo University, Sokoto. The rats were kept in standard cages in groups of five with 12-h light/dark cycles (lights on at 07.00 hours) at an ambient temperature. The rats had access to balanced and standard rats' pellets (Vital feeds, UAC, Jos, Nigeria) and plain tap water *ad libitum*.

The rats were allowed to acclimatize to the animal room conditions for five days prior to the induction of diabetes mellitus. All animal experimental protocols were conducted in compliance with animal care standards outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental design

The rats were randomly allotted to groups (n=5 per group) and treated as follows:

Group 1: Non- diabetic control rats, administered distilled water (0.5ml) orally

Group 2: Diabetic control rats administered distilled water (0.5ml) orally

Group 3: Diabetic rats, administered glibenclamide 600µg/kg body weight

Group 4: Diabetic rats, administered HS extract 500mg/kg body weight (Ibrahim *et al*., 2019).

Group 5: Diabetic rats, treated with HS (500mg/body weight) and glibenclamide 600µg/body weight (Erejuwa *et al*., 2010).

The distilled water, glibenclamide alone and glibenclamide with HS were administered once daily via oral gavage for four weeks post induction of diabetes. Fasting blood glucose of the rats was measured weekly.

Induction of Diabetes: DM was induced by intraperitoneal administration of Streptozotocin (STZ) (60 mg/kg body weight dissolved in 0.1M citrate buffer, pH 4.5) to rats following 12 hours fasting. Another group of rats were injected with citrate buffer alone without STZ. This group served as control. Three days after STZ injection, diabetes was established and measurement of blood glucose was made on blood obtained via a prick to the tail vein of the rats using lancets and a calibrated Accu- check glucometer (Roche, Germany). Rats with blood glucose concentrations of ≥12 mmol/L were considered diabetic (Mardiah *et al*., 2014).

Sample preparation: At the end of the treatment period, rats were fasted for 12hrs and anaesthetised using light ether soaked with cotton wool enclosed in plastic container. About 5ml of blood was collected through cardiac puncture into lithium heparin bottles for biochemical analysis.

Biochemical analysis

Estimation of serum glucose and protein concentration: Plasma glucose concentration was determined using a calibrated glucometer (Accu-check, Roche, Germany) that utilises the glucose oxidase method (Dickson *et al*., 2019). Plasma protein estimation was done using the biuret methods as described by Gornall *et al*. (1949).

Estimation of Lipid peroxidation: Lipid peroxidation assay was done using methods described by Niehaus and Samuelson (1968).

Estimation of antioxidant enzymes activities: Estimation of Glutathione peroxidase, Superoxide dismutase, and Catalase was done using Laboratory kits from Cayman (USA).

Histology of the pancreas: The pancreas was rapidly excised and fixed in 10% formalin. Histological analysis of the pancreas was done by using haematoxylin and eosin. The organ was brought out of fixative and examined macroscopically on a cutting bench. The pancreas was grossed and placed in a pre-labelled cassette. The tissues were dehydrated, cleared and impregnated using automatic tissue processor (Leica TPO102 model, China), after which they were embedded using embedding machine (Leica EG1 160 model, China). Section of the embedded tissue blocks were cut at 3µm using rotary microtome (Leica RM212 RT, China) and then floated out on labelled glass slides. The cut sections were allowed to dry on hot plates for 15 minutes and stained in haematoxylin and eosin stains. Stained sections were examined microscopically using x 10 and x 40 objective lenses. Photomicrographs of the pancreatic tissue sections of all the intervention rats were taken using an eye-piece-mounted camera and presented alongside with the control sections.

Statistical analysis

Data were analysed using the statistical software GraphPad Prism Version 7.0 (GraphPad Software Inc., San Diego, CA, USA) and expressed as means ± standard error of the mean. A one-way analysis of variance (ANOVA) was used to analyse the data, followed by a Tukey-Kramer *post hoc* test. The level of significance was set at $p \leq 0.05$.

RESULTS

Effect of HS aqueous calyx extract and glibenclamide on the initial and final body weights of streptozotocin-induced diabetic rats: The initial and final body weights of streptozotocin-induced diabetic rats that were treated with either an aqueous calyx extract of HS, glibenclamide or both are presented as Figure 1. Except for the DM vs DM + HS groups that were statistically different ($p = 0.023$, ANOVA), the initial body weights of the rats were similar ($p > 0.05$, ANOVA) across the other treatment groups. The final body weights of the rats in the Non-DM group were significantly higher than those of the untreated DM ($p = 0.000$, ANOVA), DM + GLIB + HS ($p = 0.000$, ANOVA) and DM + HS ($p = 0.011$, ANOVA) treatment groups. Diabetic rats treated with glibenclamide (DM + GLIB) were significantly heavier ($p = 0.010$, ANOVA) than their counterparts that were treated with both glibenclamide and HS.

Effect of HS aqueous calyx extract and glibenclamide on the baseline and final fasting blood glucose concentration of streptozotocin-induced diabetic rats: Table 1 shows the mean baseline (FBG1) and final (FBG 2) fasting blood glucose concentration of streptozotocin-induced diabetic rats that were treated with either an aqueous calyx extract of HS, glibenclamide or both. The initial post-induction fasting blood glucose concentration of non-diabetic rats was significantly lower ($p < 0.001$, ANOVA) than that of the rats in the other treatment groups. Administration of glibenclamide alone and HS alone and in combination significantly lowered ($p < 0.001$, ANOVA) the final fasting blood glucose concentration of the rats in the other treatment groups.

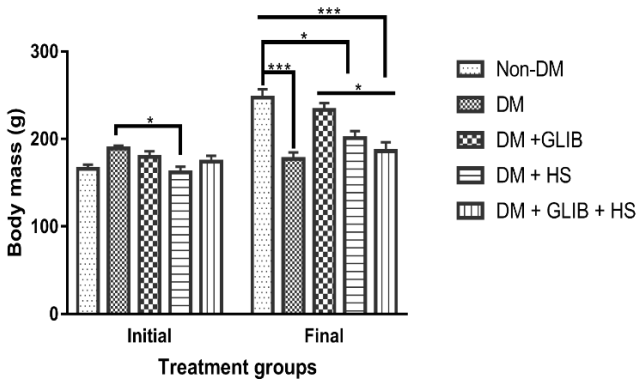

Figure 1:

Initial and final body weights of streptozotocin-induced diabetic rats

* = means are significantly different at $p < 0.05$.

*** = means are significantly different at $p < 0.001$.

Initial= initial body mass, Final= final body mass, Non-DM= administered with distilled water only, DM= administered streptozotocin 60mg/kg body weight, DM + GLIB= administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight, DM + HS= administered streptozotocin 60mg/kg body weight + HS 500mg/kg body weight, DM + GLIB + HS =administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight + HS 500mg/kg body weight. Data expressed as mean ±SEM, n= 5 per group.

Table 1:

Initial and final fasting blood glucose concentration of streptozotocin-induced diabetic rats

Treatment groups	FBG1 (mmol/L)	FBG2 (mmol/L)
Non-DM	4.32 ± 0.32^{a}	4.68 ± 0.24^{a}
DM	27.14 ± 2.99^{b}	24.00 ± 2.08^{c}
DM + GLIB	19.70 ± 2.48^{b}	8.70 ± 1.14^{b}
DM + HS	22.10 ± 3.74^{b}	9.38 ± 0.95^{b}
DM + GLIB + HS	25.60 ± 2.29^{b}	6.58 ± 0.72^{ab}

a,b,c = means with different superscripts are significantly different at $p \leq 0.005$ across columns. FBG1= baseline fasting blood glucose concentration, FBG2= final fasting blood glucose concentration. Non-DM= administered with distilled water only, DM= administered streptozotocin 60mg/kg body weight, DM + GLIB= administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight, DM + HS= administered streptozotocin 60mg/kg body weight + HS 500mg/kg body weight, DM + GLIB + HS =administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight + HS 500mg/kg body weight. Data expressed as mean ±SEM, n= 5 per group

Effects of HS aqueous calyx extract and glibenclamide on serum activities of catalase, glutathione peroxidase and superoxide dismutase: The serum activities of catalase, glutathione peroxidase, superoxide dismutase in streptozotocin induced diabetic rats that were treated with either an aqueous calyx extract of HS, glibenclamide or both are presented in Table 2.

The serum activity of catalase of Non-DM rats was significantly higher compared to the DM (p = 0.000, ANOVA) and DM + GLIB (p=0.006, ANOVA) respectively. However, the activity of catalase in DM + HS (p=0.021, ANOVA) and DM + GLIB + HS (p=0.001, ANOVA) were significantly higher compared to DM untreated rats.

Similarly, the serum activity of glutathione peroxidase was significantly higher in Non-DM compared to other treatment groups; DM (p=0.000, ANOVA), DM+ GLIB (p=0.000, ANOVA), DM+ HS (p=0.000), but the DM+ GLIB +HS group was significantly higher (p=0.011, ANOVA) compared to DM, DM +G:LIB and DM + HS
The serum activity of Superoxide dismutase of Non-DM was significantly higher when compared to DM (p=0.000, ANOVA) and DM + GLIB (p=0.000, ANOVA). However, the activity of superoxide dismutase was significantly higher in DM + GLIB + HS (p= 0.006, ANOVA) compared to DM.

Table 2:
Serum activities of catalase, glutathione peroxidase and superoxide dismutase

Treatment groups	CAT (nmol/mg)	GPx (nmol/mg)	SOD (nmol/mg)
Non-DM	0.55 ± 0.02^{a}	1.5 ± 0.12^{a}	8.26 ± 0.23^{a}
DM	0.19 ± 0.03^{b}	0.60 ± 0.03^{b}	3.19 ± 0.14^{be}
DM + GLIB	0.32 ± 0.06^{bc}	0.78 ± 0.06^{bd}	3.97 ± 0.21^{bc}
DM + HS	0.39 ± 0.04^{ac}	0.98 ± 0.03^{cd}	4.40 ± 0.86^{cd}
DM + GLIB + HS	0.46 ± 0.02^{ac}	1.15 ± 0.07^{cd}	4.03 ± 1.96^{bc}

a,b,c,d,e= means with different superscripts are statistically different at $p \leq 0.05$.
CAT= catalase, GPx= glutathione peroxidase, SOD= superoxide dismutase. Non-DM= administered with distilled water only, DM= administered streptozotocin 60mg/kg body weight, DM + GLIB= administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight, DM + HS= administered streptozotocin 60mg/kg body weight + HS 500mg/kg body weight, DM + GLIB + HS =administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight + HS 500mg/kg body weight. Data expressed as mean ±SEM, n= 5 per group.

Effects of combination of HS aqueous calyx extract and glibenclamide on serum level of malondialdehyde: The serum levels of malondialdehyde in streptozotocin induced diabetic rats that were treated with either an aqueous calyx extract of HS, glibenclamide or both are presented in Figure 2.

The serum activity of malondialdehyde in Non-DM was significantly lower when compared with the DM (p= 0.000, ANOVA) and DM + GLIB (p=0.000, ANOVA). However, the malondialdehyde activity was significantly lower in DM + HS (p=0.014, ANOVA) and DM + GLIB +HS (p= 0.000, ANOVA) when compared with the DM.

Effects of HS aqueous calyx extract and glibenclamide on the pancreatic histology of streptozotocin induced diabetic rats: Figure 3 is a micrograph of pancreatic histology showing various degree of endocrine gland (beta cells) destruction on different group of diabetic rats induced by streptozotocin. The **slide A** is a Non-DM group, which displays normal pancreatic histology with intact endocrine and exocrine glands. **Slide B** represents the DM group which was administered (60mg/kg body weight) of streptozotocin. The histology shows destruction of islet cells as a result of fibrosis of the endocrine gland**. Slide C** was administered streptozotocin also shows destruction of islet cells while **slide D** and **E** show signs of destroyed and regenerating islet cells

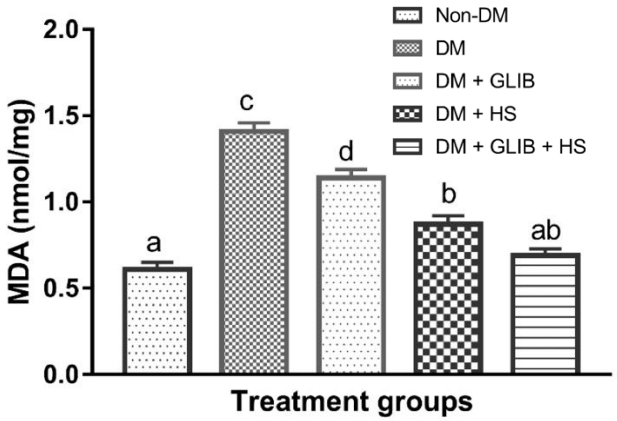

Figure 2:
Serum levels of malondialdehyde (MDA)
a, b, c, d = means various levels of malondialdehyde across the rats group induced with streptozotocin treated with either aqueous extract of HS, glibenclamide or both, MDA = malondialdehyde. Non-DM= administered with distilled water only, DM= administered streptozotocin 60mg/kg body weight, DM + GLIB= administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight, DM + HS= administered streptozotocin 60mg/kg body weight + HS 500mg/kg body weight, DM + GLIB + HS =administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight + HS 500mg/kg body weight. Data expressed as mean ±SEM, n= 5 per group.

DISCUSSION

The study was designed to investigate the effects of HS and/or glibenclamide on STZ-induced diabetes and its complications in a rat model. Upon induction of diabetes using STZ, the fasting blood glucose (FBG1) levels of the rats were all significantly elevated compared to the untreated rats. Streptozotocin is a specific cytotoxic drug that destroys the pancreatic β-cells, there by denying secretion and regulatory action of insulin (Al-Nahdi *et al*., 2019). The diabetic effect results in production of reactive oxygen species (ROS). Excessive ROS attacks protein, lipids, cellular membrane; and organs like pancreas, kidney and liver (Bathina *et al*., 2016).

Hypoglycaemic drugs are either too expensive or have undesirable side effects including haematological, neurological (e.g. coma) and disturbances of liver and kidney functions (Grieb, 2016). Controlling diabetes without any side effects is still a growing challenge in the health sector. Thus, the quest for effective, safer and affordable antidiabetic natural products like plant extracts (Saad *et al*., 2015).

In the current study, the untreated diabetic rats had significantly lower final body weight compared to their non-diabetic counterparts (control). The administration of HS and/or GLIB on the other hand improved significantly the body weights of the diabetic rats.

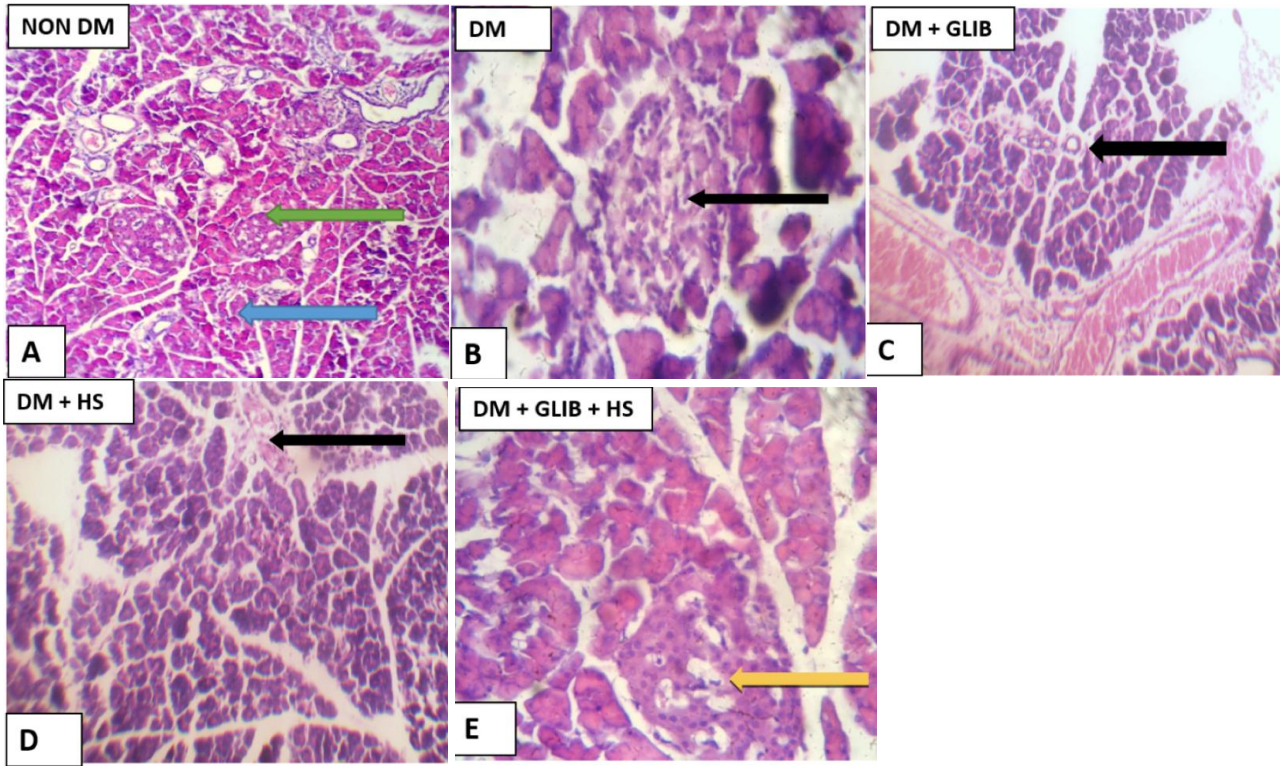

Figure 3:
Micrographs of pancreas histology (H and E staining, x 400) of streptozotocin-induced diabetic rats.
The green arrow points to normal endocrine gland, the blue arrow points to normal exocrine gland, the black arrow points to destroyed endocrine gland and yellow arrow shows regenerating islet cells. A= Non-DM; administered with distilled water only, B= DM; administered streptozotocin 60mg/kg body weight, C=DM + GLIB; administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight, D= DM + HS; administered streptozotocin 60mg/kg body weight + HS 500mg/kg body weight, E= DM + GLIB + HS; administered streptozotocin 60mg/kg body weight + glibenclamide 600µg/kg body weight + HS 500mg/kg body weight. tissue section = 3-5µm.

The significant reduction in body weight of the diabetic rats was probably as a result of impaired glucose metabolism due to absent or insufficient insulin. In diabetes, lack of insulin activates sensitive lipase which acts on adipose tissues, causing its break down and thus leading to rapid weight loss as observed in DM untreated rats (Wu and Yan, 2015).

Previous studies found similar observation of weight loss in diabetic animal models (Videla *et al*., 2009; Saad *et al*., 2015; Nafizah *et al*., 2017). The improved weight gain may be attributed to the anti-diabetic effect of both HS and glibenclamide, through improvement in glucose utilisation and limiting the breakdown of adipose tissue in rats (Adisakwattana *et al*., 2012). It is important to mention that the administration of GLIB alone to diabetic rats was more effective in improving the body weight of the rats than that of HS alone or HS and glibenclamide combined. Normal weight is an indicator of good diabetic control and glibenclamide is known to cause weight gain (Kasolo *et al*., 2019). Thus, with regards to body weight, glibenclamide may be more beneficial than HS in diabetic rats.

Following the induction of diabetes, the fasting blood glucose concentrations of the rats were significantly elevated compared to the untreated control rats. However, when the rats were treated with either an aqueous extract of HS, glibenclamide or a combination of both HS and glibenclamide, the blood glucose concentration reduced to levels almost similar to that of the control rats. A combination of HS and glibenclamide was more effective in lowering the blood glucose concentration compared to when they were administered independently. HS has been shown to possess anti-diabetic properties in previous studies. For example, when HS extract was administered to diabetic rats at 200mg/kg body weight, it led to a drastic reduction in serum glucose of rats (Peng *et al*., 2011). HS has also been shown to inhibit the enzyme pancreatic α- amylase leading to a slowing down of digestion of carbohydrates to more absorbable forms of monosaccharides (Adisakwattana *et al*., 2012). Huang *et al*. (2009) have also previously demonstrated that an HS extract suppressed the high glucose stimulated cell proliferation and migration in a dose dependent manner. Additionally, HS contains phytochemicals such as, saponins, glycosides and flavonoids which are believed to have hypoglycaemic effects, thus reducing hyperglycaemia (Idris *et al*., 2012).
In this interventional study, the activities of the antioxidant enzymes assayed (catalase, superoxide dismutase and glutathione peroxidase) were significantly reduced in DM untreated rats. However, treatment with HS and/ or glibenclamide improved the activities of the enzymes in diabetic rats. The findings in this study corroborate those of other studies that also found reduced serum activities of antioxidant enzymes in diabetes (Szkudelski, 2001; Sepici-Dincel *et al*., 2007; Singh *et al*., 2017).

Under physiological conditions, free radicals generated are scavenged by antioxidant enzymes like SOD, CAT and GPx, thus, preventing the development of oxidative stress (Saad *et al*., 2015). But in diabetes and its complications,

there is an increased generation of superoxide, hydrogen peroxide anion and lipid peroxide radicals (Gaya *et al*., 2009; Guardiola and Mach, 2014). Thus, causing reduction of antioxidant enzymes due to their increased utilisation to combat the oxidative stress.

CAT is an antioxidant that breaks down hydrogen peroxide (H_2O_2) into H_2O and O_2. The reduced activity of CAT observed in the diabetic rats might be a consequence of elevated superoxide radical (O^{2-}) since increased O^{2-} is known to inactivate catalase enzyme (Kono and Fridovich, 1983; Erejuwa *et al*., 2010).

GPx is an antioxidant enzyme involved in the detoxification of hydrogen and lipid peroxides and acts as a peroxynitrite reductase (Yun *et al*., 2019). The activity of GPx in the plasma that was initially reduced in the diabetic rats was improved with treatment with HS and/ or glibenclamide. However, the activity was more improved in the rats that were administered with both HS and glibenclamide. Generally, antioxidants protect tissues against oxidative damage (Costantini, 2019). Several studies have reported on the antioxidant potentials of HS (Olaleye and Rocha, 2008; Mossalam *et al*., 2011; Villasante *et al*., 2019). HS is thought to exert this antioxidant activity by scavenging free radicals and reactive oxygen species, inhibition of xanthine oxidase activity and prevention of cell damage via lipid peroxidation (Farombi and Fakoya, 2005; Shalgum *et al*., 2019).

SOD metabolizes O_2^- to H_2O_2 (Chung, 2017). Since the non-diabetic rats had normal SOD activity, the reduced SOD activity in the serum of diabetic rats might indicate high levels of O_2^- being generated as a result of chronic hyperglycaemia. Reduced SOD in the serum of STZ induced diabetic rats has also been reported by Qazi and Molvi (2018).

In our study, the levels of lipid peroxidation as indicated by malondialdehyde (MDA) in untreated diabetic rats was significantly higher than the other treatment groups. However, though treatment with both HS alone and glibenclamide alone reduced the level of peroxidation, the combination of HS and glibenclamide together was more beneficial in this regard. This finding indicates that GLIB alone might not offer protection against lipid peroxidative damage but requires multi-therapy approach for effective results. HS extracts have previously been reported to inhibit the formation of malondialdehyde (Usoh *et al*., 2005) and formation of thiobarbituric reactive substances (Hirunpanich *et al*., 2005). Therefore, HS may potentially be useful in alleviating the lipid peroxidation associated with diabetes.

In the current study, untreated diabetes resulted in fibrosis around the endocrine gland on the very few glands which signify cell injury as a result of cytotoxic effects of administered STZ. STZ is a glucose analogue that is selectively accumulated in pancreatic beta-cells via a GLUT 2 glucose transporter in the plasma membrane (Wu and Yan, 2015).

The pancreatic tissues of the rats treated with HS alone and those treated with a combined HS and glibenclamide showed signs of regeneration of islet cells probably as a result of antioxidant property of aqueous extract of HS. Hyperglycaemia is associated with pancreatic β-cell damage due to its toxic effects (Erejuwa *et al*., 2010). Interestingly, a recent study has demonstrated that treatment with HS in a type 1 diabetes rodent model improved the volume of the pancreatic islets and the numerical density of the β-cells depleted by STZ (Adeyemi and Adewole, 2019). Glibenclamide has also been shown to protect pancreatic cells damage through antioxidant mechanisms especially when it is administered in combination with natural polyphenols (Erejuwa *et al*., 2010). It is therefore safe to assert that HS and glibenclamide when combined may produce a more portent protection in the pancreatic cells against the oxidative damage effects of diabetes.

In conclusion, co-administration of glibenclamide and HS aqueous extract may be more beneficial in alleviating the oxidative stress generated in STZ-induced diabetes mellitus in a rodent model than when they are administered as single agents. Thus, complimentary therapy with glibenclamide and HS should be considered in the management of diabetes.

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