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Effect of *Raphia hookeri* Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. GOM designed the study, performed the statistical and histological analysis. He equally wrote the first draft of the manuscript and undertook the final editing of the paper. SOO wrote the protocol and part of the draft, KJO carried out most of the literature searches while PIA handled the tissue processing for histology. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To examine the effect of *Raphia hookeri* (RH) seed extract on blood glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats.
Materials and Methods: In the oral glucose tolerance test (OGTT), the animals received the extract (1 g/kg) or glibenclamide (0.01 mg/kg) or vehicle and 30 min later they received oral glucose load (1 g/kg). Glucose was estimated at 30min, 1, 2, 3 and 4 h. In hypoglycaemic study, the extract was administered at doses of 100, 250 and 500 mg/kg body weight (bwt) doses. In fasting blood glucose study (FBG), diabetic Wister rats, 5 per group, received graded doses (50, 100 and 200 mg/kg) of the extract or glibenclamide (10 mg/kg) or vehicle for 15 days. Blood was collected on days 0, 3, 5, 7, 9, 11, 13, 15 for glucose estimation. Lipid profile was analyzed using modified enzymatic procedure.

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Insulin assay was done by Diagnostic Automation Kit and HbA1C by standard protocol. The studies lasted for three weeks.

Results: The diabetic animals treated with the extract showed appreciable weight gain. In oral glucose tolerance test (OGTT), RH seed extract and glibenclamide treated rats blood glucose significantly ($P<0.05$) decreased in the peak values and the area under curve after 4 h of oral load with decreased values of 48.3±1.0 mg/dL (63.3%) and 62.0±0.8 mg/dL (51.6%) respectively. The hypoglycaemic activity at 250 and 500 mg/kg body weight (bwt) doses showed lowest plasma glycaemic decrease of 50.1% and 54.4% respectively after 8 h of oral administration. In FBG study, after 15 days of extract/glibenclamide treatment, the animals' blood glucose exacerbated by alloxan challenge returned to normal glycaemia with glycaemic decrease of 87.2±2.3 (79.3%); 57.0±1.7 (86.3%) and 55.0±0.3 mg/dL (87.1%) respectively while glibenclamide showed a maximum glycaemic decrease of 167.4±1.1 mg/dL (60.1%). The tissue morphology of the extract treated showed significant beta cells survivor. The extract ameliorated dislipidaemia and exerted significant ($p<0.05$) decrease in plasma HbA1C while marked increase in plasma insulin level occurred.

Conclusion: The extract effectively attenuated hyperglycaemia, caused marked decrease in HbAIC concentration and ameliorated dislipidaemia.

Keywords: Raphia hookeri; alloxan diabetes; beta cell; plasma HbA1C; lipid profile.

1. INTRODUCTION

The history of diabetes dates back to the ancient times and has remained a significant threat to life till date (Abebe and Ayehu, 1993; Mbaka et al., 2008). This debilitating disease is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives world over. It has emerged as the major cause of adult morbidity and mortality worldwide (Gulliford, 1994).

Diabetes mellitus (DM) is a disease of an endocrine pancreas caused by impaired metabolism of glucose, protein and lipids predisposing to hyperglycaemia (Pareek et al., 2009). It is probably the most common chronic disease that has proved difficult to manage despite advances made in clinical sciences to understand its causes and complications. The available drugs including insulin therapy and oral hypoglycaemic agents such as biguanids and sulfonylureas afforded effective glycaemic control but have failed to significantly alter the course of diabetic complications including rectinopathy, neuropathy, nephropathy, macro and micro-angiopathy (Akhtar and Ali, 1980; Grover et al., 2002; Dieye et al., 2007). Consequently, there has been a compelling need to explore the use of alternative therapy of plant source considered by many as best option because of the belief that they are safer and more effective in managing diabetic complications.

The chronic high level of blood glucose observed in diabetic condition could damage many body systems particularly blood vessels and nerves (Nagappa et al., 2003). DM has been aptly defined as "a genetically determined disorder of carbohydrate metabolism …with specific macrovascular complications and accelerated atherogenesis" (Rubin and Farber, 1988). The definition highlighted the serious cardiovascular risk posed by the disease. It has become more glaring that many deaths associated with diabetes are attributed to cardiovascular and vascular diseases including coronary arterial and peripheral vascular diseases. One of the major metabolic changes caused by chronic hyperglycaemia is the formation of advanced glycated end products (Al-Shamaony et al., 1994). The glycation of

body proteins in turn leads to complications affecting nerves and arteries (Sharma, 1993). Also, the level of glycosylated haemoglobin reveals integral blood glucose concentration over a period of time (Anitha and Chandralekha, 2010).

The major cause of vascular disease in diabetes has been identified as the alteration in lipid metabolism which is oxidative in nature (Baynes and Thrope, 1999). In such condition, there is an enhanced activity of the enzyme lipase responsible for lipolysis in the adipose tissue which consequently promotes the release of free fatty acids into the circulation (Agardh et al., 1999; Saravanan and Pari, 2005). The increase in free fatty acid in blood precipitates in the synthesis of cholesterol, phospholipids and triglycerides thereby increasing the risks of cardiovascular disease that include atherosclerosis (Sabu and Kuttan, 2002).

Raphia hookeri (RH) commonly known as Raffia palm is a member of Palmaceae family that grows in the eastern and western parts of Nigeria. It grows in fresh water swamps reaching a height of 9 m and possesses breathing roots thereby adapting it for life/support in water logged soils. The fruit is large, cone-shaped with a single hard nut having an outer layer of rhomboid-triangular and overlapping reddish brown scales. Between this outer layer of scales and the very hard seed is a yellow, mealy, oil-bearing mesocarp or pulp. RH is probably the most diversely useful plant in Nigeria as all it parts have various economic values. It is an important source of forest food species in southern Nigeria (Akachukwu, 2001). RH has equally shown to have beneficial therapeutic property as it is used in herbal medicine in the treatment of various illnesses. RH seed extract has been used ethno botanically and is claimed to have effective anti-diabetic activity. The fact that no scientific evidence to our knowledge exists to support the claim prompted the investigation. However, another consideration was the fact that the plant has been ascertained to be rich in triterpenes, flavonoids and saponins (Akpan and Usoh, 2004) which are known to be bioactive against diabetes (Loew and Kaszkin, 2002). This study therefore attempts to establish scientific basis for the use of RH seed in treating diabetes and diabetic complications.

2. MATERIALS AND METHODS

2.1 Plant Materials

The seed of *Raphia hookeri* were obtained in the month of November from swampy farm land at Ikorodu, Lagos State, Nigeria. They were authenticated by a taxonomist, Dr. O.A. Ugbogu, of the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen has been deposited in the herbarium (FHI/108941).

2.1.1 Preparation of the aqueous ethanol seed extract of RH

The fresh fruits of RH obtained from swampy farm land were spread in the sun for a week to enable for the softening and easy removal of the mesocarp. The seeds obtained were dried before being subjected to size reduction to a coarse powder with electric grinder. The seed powder, 1140 g, was extracted with 95% aqueous ethanol in three cycles using Soxhlet extractor. The crude extract was filtered with Whatman filter paper No. 4 and the filtrate concentrated in vacuo 30ºC to obtain 138 g residue weight (12.1% w/w). The residue which was in form of paste was stored in an air tight bottle kept in a refrigerator at 4ºC till used.

2.2 Animals

Wistar rats (150 ± 10 g) of either sex obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria, were kept under standard environmental condition of 12/12 hr light/dark cycle. They were housed in polypropylene cages (5 animals per cage), and were maintained on mouse chow (Livestock Feeds Nigeria Ltd), provided with water *ad libitum*. They were allowed to acclimatize for 9 days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

2.3 Induction of Diabetes

Rats were fasted for 18 h and were induced with alloxan monohydrate, 150 mg/kg body weight (bwt), intra-peritoneally (ip) (Mbaka et al., 2009). Hyperglycaemia was confirmed where elevated blood glucose level was ≥ 250 mg/dL after 72 h of injection (Mbaka et al., 2009).

2.4 Effect of the Aqueous Ethanol Extract on Oral Glucose Tolerance (OGTT)

The rats were fasted for 18 h and were randomized to three groups of five rats each. Blood was collected pre-treatment from each animal to determine their fasting blood glucose. The rats in group one received 2 ml/kg distilled water orally. Group two received 1 g/kg bwt of the 95% aqueous ethanol seed extract of RH diluted in water while group three received 0.01 g/kg bwt of glibenclamide by gavages. Thirty minutes after distilled water, aqueous ethanol extract or glibenclamide administration, the rats in the three groups were given oral glucose load at 1 g/kg bwt (Perfumi et al., 1991; Mbaka et al., 2009). Blood was collected from the animals at 30 min, 1, 2, 3 and 4 h after the oral glucose load for the blood glucose estimation (Moshi et al., 1997; Mbaka et al., 2008).

2.5 Hypoglycaemic Activity

Rats fasted for 18 h were randomly divided into four groups of 5 per group. The first three groups (I, II and III) were administered by gastric gavage (single dose) with the seed extract dissolved in water at the concentration of 100, 250 and 500 mg/kg respectively (Sharma et al., 1997). The fourth group (IV), the control received distilled water (10 ml/kg). Blood glucose level was determined at 0, 4, 8 and 12 h later (Mbaka et al., 2009).

2.6 Alloxan Induced Diabetic Rats

The diabetic animals were randomized to the following groups of 5 rats each: groups I, II and III received graded doses of the extract at 50, 100 and 200 mg/kg bwt respectively by gavages. The extract doses were determined based on the plant toxicity investigation earlier conducted which is outside the scope of this study. Group IV received glibenclamide (10mg/kg bwt); group V served as normal while group VI was diabetic control. Treatment was continued for 15 days. Blood was collected from the tail of the animals at days, 0, 3, 5, 7, 9, 11, 13 and 15 and analyzed for glucose by oxidase method (Olajide et al., 1999).

2.7 Lipid Profile

Blood collected with heparinized tube was centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which was analyzed for total cholesterol (TC), total triglyceride (TG) and high density lipoprotein-cholesterol (HDL-Chol) levels by modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). Low density lipoprotein-cholesterol (LDL-Chol) levels were calculated using Friedwald equation (Crook, 2006).

2.8 Insulin and HBA1C Assay

The insulin level was determined using Diagnostic Automation insulin assay (Diagnostic Automation Inc. USA) as described by Clark and Hales (Clark and Hales, 1994) while the HbA1C assay was by standard protocol (Chandalia et al., 1980).

2.9 Tissue Histology

The pancreatic tissue of animals from alloxan diabetic study was used for tissue histology. Tissue from the highest dose of the extract and glibenclamide treated, the negative and positive controls were fixed in Bouin's fluid for seven days before embedding in paraffin wax. The pancreatic tissue sectioned at 5 µm was stained with aldehyde fuchsin. Each section was examined under light microscope at high power magnification for structural changes and photomicrographs were taken.

2.10 Student's T-Test

All values were expressed as mean±standard error of mean and the statistical significance between treated and control groups were analyzed by means of Student's t-test. $P<0.05$ was considered significant.

3. RESULTS

3.1 Body Weight

The body weight changes of treated and untreated diabetic animals are indicated in Fig.1. The animals exhibited decrease in appetite and weight loss after alloxan induction. Treatment with the extract/glibenclamide resulted in significant ($p<0.01$) weight recovery after few days with improvement in appetite. In the untreated group however, progressive weight decrease occurred.

3.2 Effect of RH Extract on Oral Glucose Tolerance Test (OGTT)

Glucose tolerance was evaluated by OGTT (Fig. 2). Following oral glucose load in the untreated group, there was hyperglycaemia which reached a peak level 1h after the load. Although decrease in glycaemia occurred after 1 h of oral glucose load, blood sugar level however failed to return to baseline glycaemia after 4 h indicating glucose intolerance. In the extract and glibenclamide treated, significant ($P<0.05$) decrease in the peak values and the area under curve was observed after 4 h of oral load with decrease of 48.3±1.0 mg/dL (63.3%) and 62.0±0.8 mg/dL (51.6%) respectively. The extract showed more glucose tolerance compared to glibenclamide

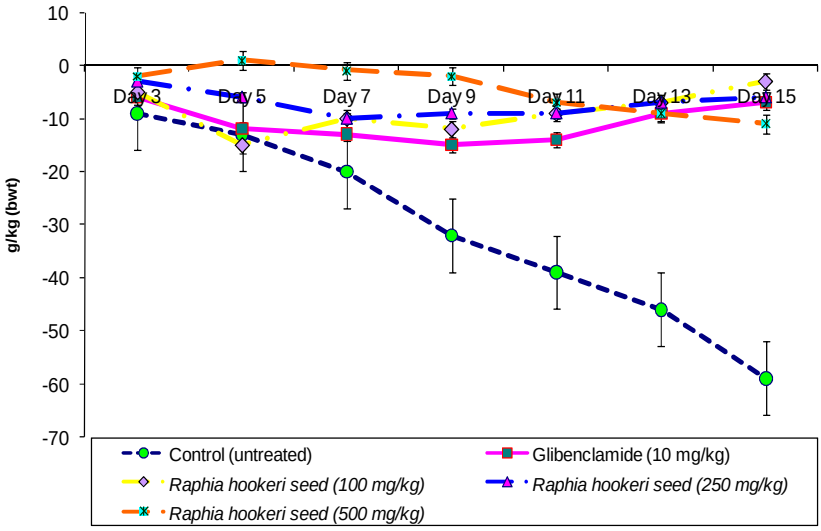


Fig. 1. Weight difference in control and treated animals

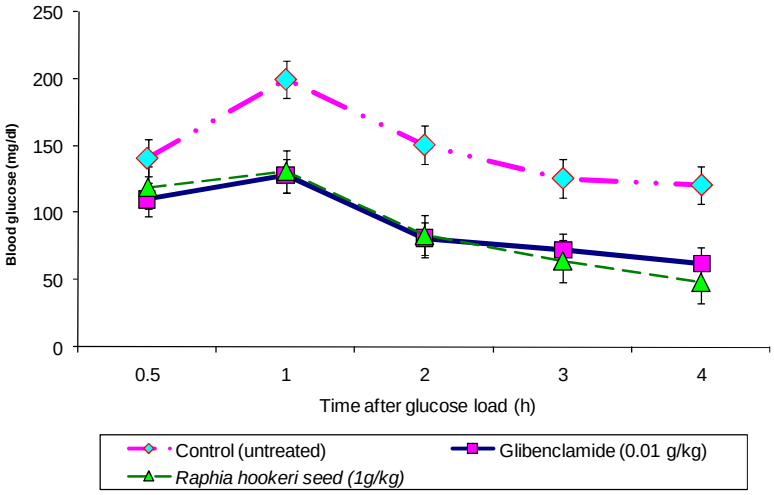


Fig. 2. The effect of seed extract on OGTT. Values represent mean ± n=5

3.3 Effect of RH Extract on Normoglycaemic Animals

The hypoglycaemic activity of the RH seed extract was evaluated at 4 h intervals. Treatment with 100 mg/kg bwt of the extract showed no significant change in glucose level in normoglycaemic animals (Fig. 3). But at 250 and 500 mg/kg bwt doses, significant ($P<0.05$) decrease occurred after 8 h of oral administration which was the lowest reduction with glucose levels of 26.2±0.5 (50.1%) and 31.0±0.2 mg/dL (54.4%) respectively. The effect produced after 12 h of extract administration was 38.0±1.3 (27.2%) and 37.1±0.5 mg/dL (45.8%) which shows comparatively less effect.

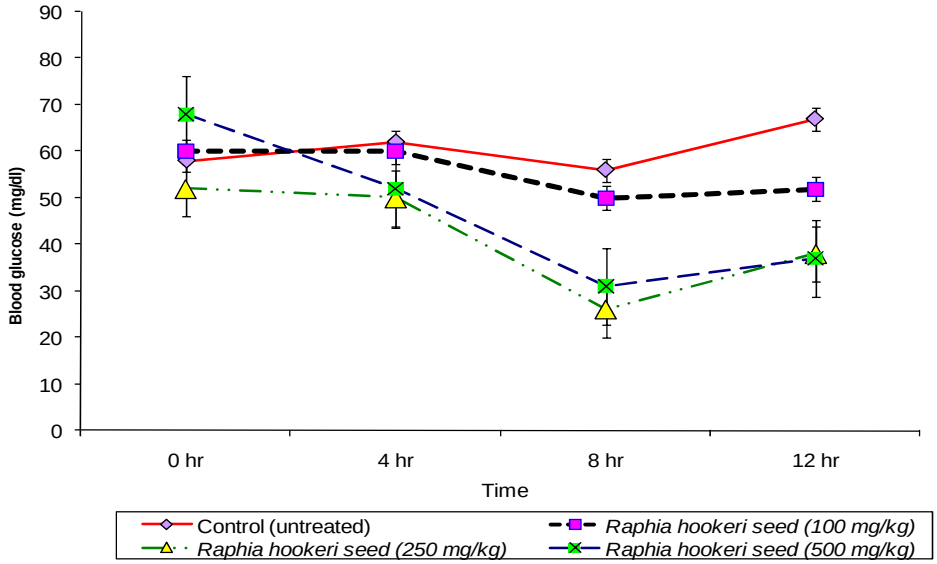


Fig. 3. Hypoglycaemic assessment of *Raphia hookeri* seed extract. Each bar represents Mean SEM (n=5)

3.4 Effect of RH Extract on Fasting Blood Glucose

The anti-diabetic property of RH seed extract was evaluated in alloxan diabetic rats. Significant elevation of fasting blood glucose (FBG) was established three days after alloxan challenge with glycaemic level > 250 mg/dL. In the untreated (vehicle group), FBG level increased progressively from day 0 (when diabetes was established) to the end of the experiment (Fig. 4).

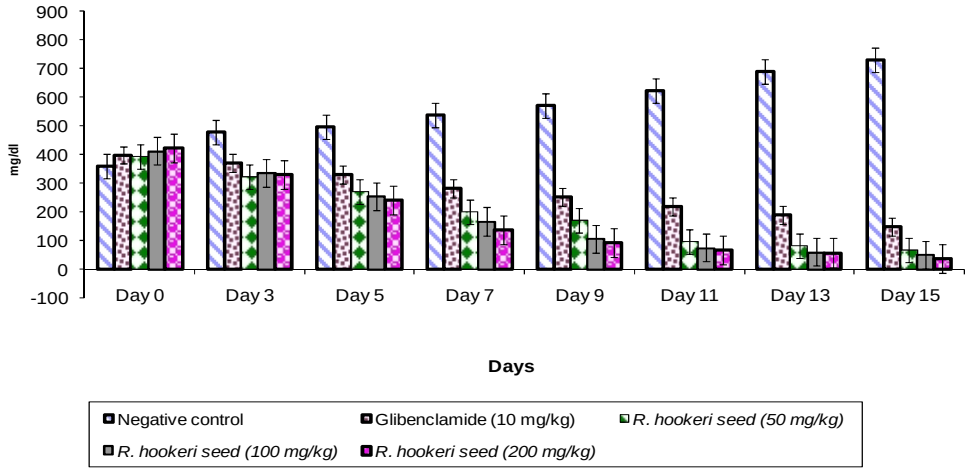


Fig. 4. Plasma glucose level of rats treated with *Raphia hookeri* seed extract

Treatment with RH extract resulted in significant ($p<0.05$) dose dependent decrease in FBG level from day 3 compared to the vehicle group. Similarly, decrease in FBG level occurred in glibenclamide treated (10 mg/kg). At the end of 15 days of extract treatment (50, 100 and 200 mg/kg), FBG level of the animals which was exacerbated by alloxan challenge returned to normal glycaemia with blood glucose level of 87.2±2.3 (79.3%); 57.0±1.7 (86.3%) and 55.0±0.3 mg/dL (87.1%) respectively. Treatment with glibenclamide showed a maximum glycaemic decrease of 167.4±1.1 mg/dL (60.1%) indicating that the reference drug exhibited lower activity compared to the extract.

3.5 Effect of RH Extract on Lipid Profile

There was significant increase ($p<0.05$) in TC, TG and LDL-Cholesterol in alloxan diabetic animals while HDL-Cholesterol decreased markedly (Table 1). In diabetic animals treated with different concentrations of RH extract (50, 100 and 200 mg/kg bwt), significant ($p<0.05$) decrease occurred in plasma levels of TC {148.6±0.3 (60.8%); 134.4±2.2 (75.2%); 107.2±3.3 (81.8%)}, TG {228.0±3.9 (52.8%); 180.8±4.5 (63.1%); 138.1±2.2 (71.0%)} and LDL cholesterol {175.5±2.2 (52.6%); 160.4±4.2 (60.5%), 118.1±3.0 (70.5%)}. These parameters also showed significant decrease in glibenclamide treated with TC decreasing to 165.2±1.4 (43.1%), TG 278.8±4.9 (40.4%), LDL 175.4±3.0 (50.7%). There was however, significant recovery in HDL cholesterol in both the extract and glibenclamide treated.

Table 1. Biochemical analysis showing the lipid profile

	Dose mg.kg^{-1}	**TOTAL CHOL mg.100ml^{-1}**	**HDL mg.100ml^{-1}**	**LDL mg.100ml^{-1}**	**TG mg.100ml^{-1}**
Normal		97.7±1.4	38.7±0.5	125.5±4.1	141.1±2.8
Diabetic		348.4±4.2	18.3±0.4	304.1±6.1	598.7±5.7
Glibenclamide	10	165.2±1.4*	30.1±0.8*	175.4±3.0*	278.8±4.9*
R. hookeri seed	50	148.6±0.3*	31.0±1.1*	175.5±2.2*	228.0±3.9*
R. hookeri seed	100	134.4±2.2*	33.1±0.5*	160.4±4.2*	180.8±4.5*
R. hookeri seed	200	107.2±3.3*	33.8±1.2*	118.1±3.0*	138.1±2.2*

*Values are Mean±SEM; n=5,*p<0.05 compared to control (Student's t-test)*

3.6 Effect of the Extract on Insulin Level

Fig. 5 demonstrated the plasma insulin levels in untreated and treated diabetic animals. In diabetic animals, there was considerable decrease in plasma insulin level with significant ($p<0.05$) increase in blood glucose level compared to normal. The administration of RH extract (50, 100 and 200 mg/kg bwt) and glibenclamide (10 mg/kg) resulted in significant ($p<0.05$) increase in plasma insulin levels compared to diabetic untreated with the extract treated showing dose dependent decrease.

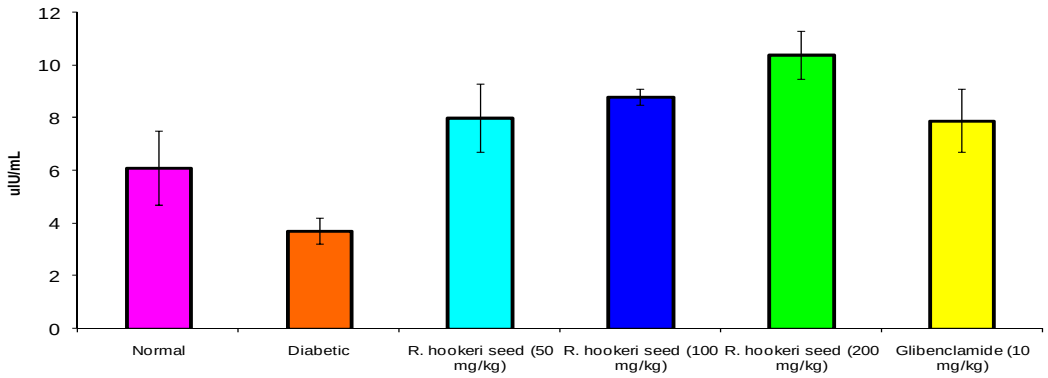


Fig. 5. Plasma insulin level post treatment. Values represent mean ± n=5

3.7 Effect of Rh on Blood HBA$_1$C Level

Fig. 6 showed the summary of blood HbA$_1$C level. The level of HbA$_1$C was observed to be significantly increased in diabetic animals compared to normal. The administration of RH extract/glibenclamide resulted in significant ($p<0.05$) decrease in plasma HbA$_1$C level compared to diabetic untreated with the extract doses exhibiting more effective decrease than glibenclamide.

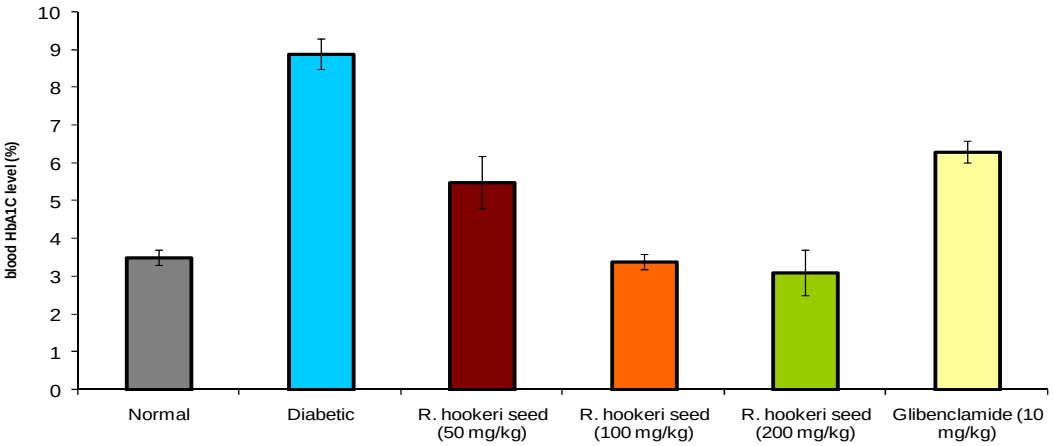


Fig. 6. Plasma HbA1C level post treatment. Values represent mean ± n=5

3.8 Histopathology of Pancreatic Tissue

Gomori aldehyde fuchsin stain was used to demonstrate the pancreatic beta cells. The normal pancreatic tissue morphology showed intact beta cells which appeared more numerous than the poorly expressed alpha cells (Fig. 7A). The islet formation was demarcated from the surrounding pancreatic acini by thin fibrous tissue capsule and within it was spotted blood vessels. The photomicrograph of the extract treated rats showed cellular lesion with majority of the beta cells showing normal appearance (Fig. 7B). In the glibenclamide treated rats (Fig. 7C), there was more extensive beta cells necrosis with spots of survivor cells. The photomicrograph of diabetic untreated rats (Fig. 7D) showed more extensive necrotic changes with islet mass forming a shrunken amorphous eosinophilia.

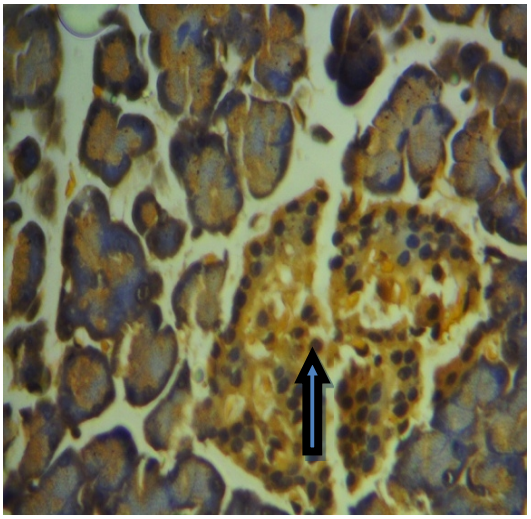


Fig. 7a. showed normal Islet cells. The black arrow indicated the deeply stained beta cells (X 400).

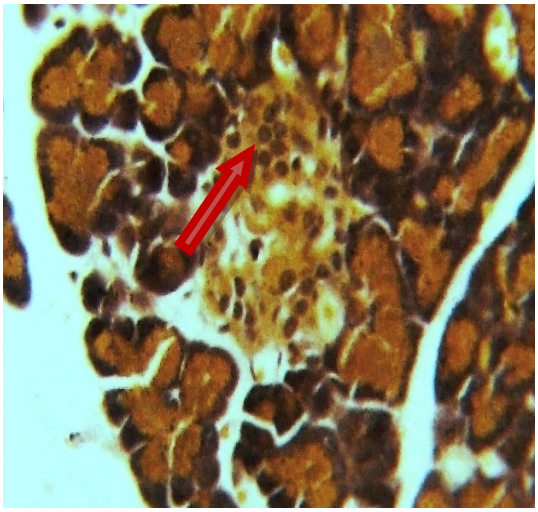


Fig. 7b. showed the pancreatic tissue post-treated with RH seed extract. The red arrow point on the cluster of dark spotted beta cells (X 400).

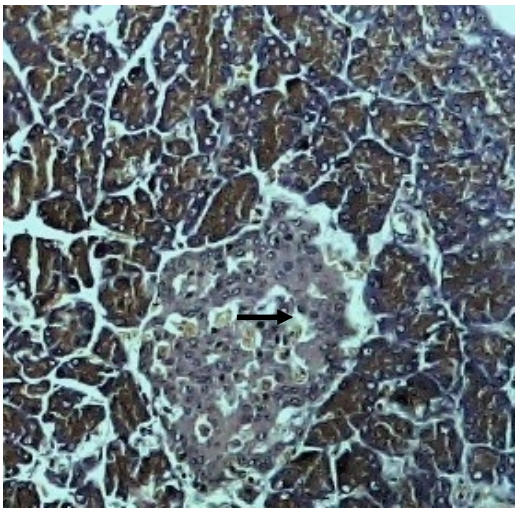


Fig. 7c. indicated pancreatic tissue post-treated with glibenclamide. Arrowed is the area of necrotic beta

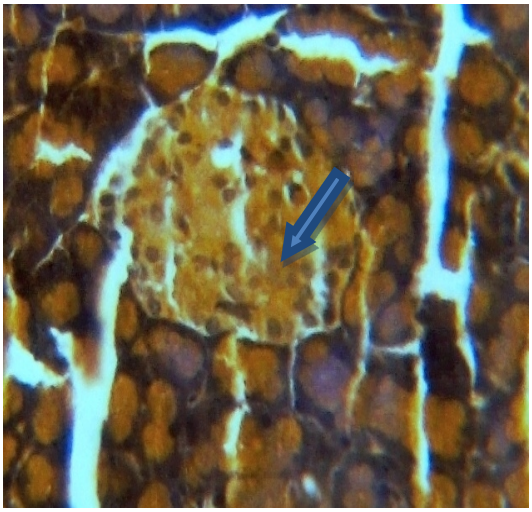


Fig. 7d. Diabetic control with arrow pointing to area of amorphous mass of eosinophilia (X 400).

4. DISCUSSION

This study was undertaken to evaluate the anti-diabetic activity of RH seed extract and its usefulness in managing diabetic complications. In this study, there was depreciation in body

weight in the alloxan diabetic animals. However, in RH seed extract treatment, weight recovery occurred overtime which might be as a result of reversal in tissue degeneration. A body weight change is an important characteristic of diabetic condition. It constitutes one of the complications of DM caused by muscle wasting (Swanston-Flat et al., 1990) and loss of tissue protein which is utilized by cells as alternative source of energy (Chatterjea and Shinde, 2002; Pareek et al., 2009).

The extract exhibited effective glycaemic control by decreasing the peak blood glucose concentration and the area under OGTT curve (Fig. 2). The return to baseline glycaemia after 4 hours in OGTT indicated an enhanced glucose utilization triggered by insulin production from the beta cells. It was also evident from the result that RH seed extract effectively lowered FBG level in alloxan induced diabetic animal. The decrease was dose dependent with extract treatment exhibiting comparably higher activity than the reference drug. The factor responsible for the anti-diabetic effect was not explored but it must have been due to the activity of terpenoids, flavonoids, alkaloids and phenolic compounds present in the plant (Akpan and Usoh, 2004). These compounds have been established to have anti-diabetic effect (Jung et al., 2006). The mode of action was possibly by insulin stimulatory effect on the beta since significant increase in plasma insulin level was observed when alloxan diabetic animals were treated with RH seed extract. This explains the efficacy of extract in reducing blood glucose level as well as improving glucose tolerance in diabetic cases. Plants potentiating insulin stimulatory effects have been reported (Latha and Pari, 2004; Mbaka et al., 2009; Bera et al., 2010). Interestingly, the blood glucose level in the extract treated Wister rats decreased to basal glycaemia after a rise above 400 mg/dL, the occurrence suggested a quantitative change in the residual beta cells by differentiating and proliferating after damaging effect by the diabetogenic agent. The photomicrograph of pancreatic tissue of animals treated with the extract showed cellular density that was comparable to normal which supported the assumption that beta cells restoration might have occurred after alloxan damage. However, further investigation is required. In glibenclamide treated, there were cellular abnormalities coupled with significant depletion in beta cells population. The RH seed extract, at 250 and 500 mg/kg bw respectively exerted significant hypoglycaemic effect after 8 h of oral administration on normoglycaemic rats. The ability of the extract to enhance insulin release from the beta cells may have equally accounted for the hypoglycaemic effect in normoglycaemic rats.

A rise in glucose level in alloxan diabetes usually occurs with corresponding increase in plasma lipids which represents a risk of coronary heart disease (Prince et al., 1999). The abnormal high concentration of serum lipids is more often due to increase in the mobilization of free fatty acids from the peripheral fat deposits which occur when there is a lack in insulin that inhibits the hormone sensitive lipase production (Udayakumar et al., 2009). In this study, treatment with RH seed extract led to the levels of the blood lipids to near normal. The extract exhibited hypocholesterolaemic and hypotriglyceridaemic activity as well as significant decrease in LDL-cholesterol. The decrease in plasma triglyceride level was vital since is a predictor of coronary heart disease (CHD) such as atherosclerosis (Saravanan and Pari, 2005; Anitha and Chandralekha, 2010). The decrease in LDL-cholesterol was however indicative of decrease in phospholipids level known to be present in cell membrane and make up vast majority of the surface lipoprotein forming lipid bilayer which is key to LDL-cholesterol formation (Cohn and Roth, 1996). Although it had been established that many factors are responsible for accelerated atherosclerosis observed in diabetes, lipoproprotein abnormalities have been incriminated as key contributor (Anitha and Chandralekha, 2010). It is because LDL-cholesterol is the major transporter of low molecules cholesterol in the body known as “bad cholesterol”. On the other hand, there was increase in HDL-cholesterol which

helps to lower plasma cholesterol level. The extract therefore has shown to ameliorate dislipidaemia and consequently exhibited promising potential in minimizing cardiovascular risk factor.

The rate of formation of HbA_{IC} has been observed to be proportional to blood glucose level (Anitha and Chandralekha, 2010). In essence it reveals the integral blood glucose concentration over a period of time. The assessment of HbA_{IC} level is increasingly becoming popular as an important means of determining the efficacy of blood glucose clearance by anti-diabetic agents. It is considered a reliable index in glycaemic control (Koenig et al., 1978). In the diabetic group, HbA_{IC} level increased significantly suggesting glycosylation of Hb in the presence of hyperglycaemia. Glycosylated Hb shows reduced affinity to oxygen (Bunn et al., 1997), a process that aid free radical release. In the RH extract/glibenclamide treated, marked decrease in HbA_{IC} concentration was observed when compared to that of diabetic animals indicating decrease in blood glucose level and recovery to Hb. The extract however showed more effective decrease, confirming its anti-diabetic potency. A number of medicinal plants have been reported to reduce HbA_{IC} formation (Latha and Pari, 2004; Bera et al., 2010).

5. CONCLUSION

Plants afford effective glycaemic control which made their uses more compelling in managing diabetic cases. In this investigation, RH seed extract effectively attenuated hyperglycaemia and minimized the susceptibility of oxygen free radicals release by preventing HbA_{IC} formation. It also showed a beneficial effect on cardiovascular risk factors by ameliorated dislipidaemia. The plant has therefore exhibited promising potentials that if exploited could be of value in the management of diabetes mellitus

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of our Institution”

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.”

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Authors wish to thank Prince Musibau Sikiru, Ogun State, Nigeria, who assisted with the plant collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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