Original article

Activity of *Raphia hookeri* root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan induced diabetic rats

Mbaka, GO.[1]*, Ogbonnia, SO.[2] and Banjo, AE.[3]

[1]Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi-Araba, Lagos, Nigeria
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria
*E-mail: mbaaka@yahoo.com

Abstract

Objective: To evaluate the effect of *Raphia hookeri* (RH) root extract on blood glucose, glycosylated haemoglobin (HbA_1C) and lipid profile on alloxan induced diabetic rats. **Methods**: Diabetic rats 5 per group received graded doses (50, 100 and 200 $mg.kg^{-1}$) of the extract or glibenclamide (10 $mg.kg^{-1}$) 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. Insulin assay was by Diagnostic Automation Kit while HbA_1C by standard protocol. In oral glucose tolerance test (OGTT), rats received the extract (1 $g.kg^{-1}$) or glibenclamide (0.01 $mg.kg^{-1}$) or vehicle and 30 minutes later received oral glucose load (1 $g.kg^{-1}$). Glucose was estimated at 30 minutes, 1, 2, 3 and 4 hours. The hypoglycaemic activity was assessed on normoglycaemic rat that received extract at 100, 250 and 500 $mg.kg^{-1}$ and estimated at 0, 4, 8 and 12 hours. **Results**: Treatment with RH root extract resulted in significant ($p < 0.05$) dose dependent decrease in fasting blood glucose level from day 3 to the end of experimental period compared to the vehicle group with the extract exhibiting stronger anti-diabetic activity than glibenclamide. RH caused hypoglycaemia after 4 hours with maximum decrease (54.7%) observed after 8 hours. The extract ameliorated dislipidaemia showing more marked activity than glibenclamide. RH extract exhibited insulin stimulatory effect by elevating the plasma insulin level of the diabetic treated animals. It also exerted effective decrease on plasma HbA_1C to a level comparable to normal. **Conclusion**: The RH roots extract attenuated hyperglycaemia by potentiating insulin release. It exhibited effective hypoglycaemic activity and ameliorated dislipidaemia.

Keywords: *Raphia hookeri*, anti-diabetes, beta cells, lipid profile, glycosylated haemoglobin.

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by the derangement of blood glucose homeostasis (MBAKA, ADEYEMI, ANUNOBI et al., 2008). The disease has remained topical due to its serious threat to public health. DM is evolving as a major health problem with its frequency known to have increased exponentially in the last few decades (ONAL, TIMUR, OKUTUEU et al., 2005). According to available statistics, nearly 10% of the world populations are afflicted by the ailment (VETRICHELVAN, JEGADEESAN and DEVI, 2002). It is a major degenerative disease in the world today afflicting many lives both in the developed and developing countries. The number of people living with the ailment is expected to multiply with major impact on the population of the developing countries due to increased rate of industrialization (GUPTA and PHATAK, 2003).

This clinical syndrome is traced to defects in insulin secretion and/or insulin action (KAHN, 1998) resulting in impaired metabolism in carbohydrates, fats, proteins and defects in electrolytes in the body with cumulative effect on the blood vessels and nerves (RAKESH, SANJAY, DEEP et al., 2008; AFOLAYAN and SUNMONU, 2010). It is aptly described as cardiovascular disease as many deaths associated with diabetes could be attributed to cardiovascular and vascular diseases including coronary arterial and peripheral vascular diseases. Diabetes therefore has been summarily defined as "[...] a state of premature cardiovascular death that is associated with chronic hyperglycaemia and also associated with blindness and renal failure [...]" (FISHER and SHAW, 2001). This definition highlighted the impact of the disease on the cardiovascular system and the need to tighten glucose control to attenuate cardiovascular disease risks factor.

Sustained hyperglycaemia is a key factor in the development and progression of diabetic complications. The major metabolic change caused by chronic hyperglycaemia is glycation of body proteins that more frequently leads to complications that affects among other things, nerves and arteries (SHARMA, 1993). Also, sustained hyperglycaemia in red blood cells is known to enhance irreversible production of glycosylation of haemoglobin (HbA_1C) which is now a marker in diabetic evaluation (AL-SHAMAONY, AL-KHAZRAJI and TWAIJI, 1994). The level of HbA_1C reveals integral blood glucose concentration over a period of time (ANITHA and CHANDRALEKHA, 2010).

Dislipideamia arising from diabetic complication is the major causative factor of atherosclerosis. In diabetes, there is serious alteration in the composition and concentration of lipids. The supervening increase in lipid perxidation causes the excess chylomicrons synthesized to accumulate within the vascular system which precipitates to the enrichment of very low lipoprotein (VLDL), low density lipoprotein (LDL) and triglycerides levels while a high density lipoprotein (HDL) particle decreases (KIM, KIM, LYU et al., 2009).

Although treatment of diabetes with insulin and many oral hypoglycaemic agents has recorded huge successes, they were however, associated with some serious side effects like recurrent cases of hypoglyceamic coma and hepatorenal disturbances (SUBA, MURUGESAN, ARUNACHALAM et al., 2004). Moreover, these orthodox medications seem to be insufficient to prevent diabetic complication. These limitations to a large extent accounted for drift towards alternative therapies that include herbs/herbal formulations (LAWTON, AHMAD, HALLOWELL et al., 2005). The preferred choice of plants by many might not be unconnected with the historical successes recorded in the use of herbal product in traditional system of medicine in managing DM. Besides, herbal formulations were perceived to have fewer side effects and less toxic because of their rich natural source. Based on these and the support provided for its practice by the World Health Organization (WORLD..., 1980), several scientific investigations are being conducted with the view of identifying new active ingredient of natural source that would be more effective in the treatment of DM and diabetic complications.

Raphia hookeri (RH) commonly called raffia palm is a member of the family Palmaceae**.** The plant is commonly found in West Africa and in abundance particularly in South Eastern Nigeria, found in lowland and swamps where it grows in water up to 1 m deep. RH is an evergreen (palm), perennial plant usually single stemmed, but occasionally with 1-4 suckers and with very large leaves (AKPAN and USOH, 2004). Scientific research into the cultivation, management and uses of RH has received greater attention, particularly in Nigeria (NDON, 1985). The root extract of RH is used ethno botanically in the treatment and prevention of several diseases (AKPAN, AKPANYUNG and EDEM, 1996). The cool root extract is normally given to infant with stomach pain (AKPAN, AKPANYUNG and EDEM, 1996). There are claims by some herbalists that aqueous ethanol root extract of RH is effective in the treatment of DM and diabetic complications. The scientific evidence to support this claim is lacking. In view of this, the present study was undertaken to evaluate the effect of RH root extract on blood glucose, HbA_1C and lipid profile on alloxan induced diabetic rats.

2 Material and methods

2.1 Plant materials

The roots of *raphia hookeri* were obtained 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).

2.2 Preparation of the aqueous root extract of RH

The roots were washed and dried before being subjected to size reduction to a coarse powder with electric grinder. The root powder, 810 g, was extracted with 95% aqueous alcohol in three cycles using Soxhlet extractor. The crude extract was filtered with Whatman filter paper No. 4 and the filtrate concentrated *in vacuo* 30 °C to obtain 103 g dry residue (12.7% w/w) which was stored in an air tight bottle kept in a refrigerator at 4 °C till used.

2.3 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 hours 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 (INSTITUTE..., 1996).

2.4 Induction of diabetes

Rats were fasted for 18 hours and were induced with alloxan monohydrate, 150 $mg.kg^{-1}$ body weight (bwt), intra-peritoneally (ip) (MBAKA, ADEYEMI, ANUNOBI et al., 2008). Hyperglycaemia was confirmed when elevated blood glucose level was ≥250 $mg.dL^{-1}$ after 72 hours of injection (MBAKA, ADEYEMI, NORONHA et al., 2009).

2.5 Effect of the ethanol extract on oral glucose tolerance (OGTT)

The rats were fasted for 18 hours 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 $mL.kg^{-1}$ distilled water orally. Group two received 1 $g.kg^{-1}$ bwt of the ethanol root extract of RH diluted in water while group three received 0.01 $g.kg^{-1}$ bwt of glibenclamide by gavages. Thirty minutes after distilled water, aqueous extract or glibenclamide administration, the rats in the three groups were given oral glucose load at 1 $g.kg^{-1}$ bwt (PERFUMI, ARNOLD and TACCONI, 1991; MBAKA, ADEYEMI, NORONHA et al., 2009). Blood was collected from the animals at 30 minutes, 1, 2, 3 and 4hrs after the oral glucose load for the blood glucose estimation (MOSHI, UISO, MAHUNNAH et al., 1997; MBAKA, ADEYEMI, ANUNOBI et al., 2008).

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 $mg.kg^{-1}$ bwt respectively by gavages. Group IV received glibenclamide (10 $mg.kg^{-1}$ bwt), group V served as normal while group VI was diabetic control. Treatment was continued for 15 days. Blood was collected at days, 0, 3, 5, 7, 9, 11, 13 and 15 and analyzed for glucose by oxidase method (OLAJIDE, AWE and MAKINDE, 1999).

2.7 Evaluation of hypoglycaemic activity

Rats fasted for 18 hours 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 root extract dissolved in water at the concentration of 100, 250 and 500 mg.kg^{-1} respectively (SHARMA, DWIVEDI and SWARUP, 1997). The fourth group (IV), the control received distilled water (10 mL.kg^{-1}). Blood glucose level was determined at 0, 4, 8 and 12 hours later (MBAKA, ADEYEMI, NORONHA et al., 2009).

2.8 Lipid profile

Blood collected with heparinized tube was centrifuged within 5 minutes of collection at 4000 g for 10 minutes 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, NAJAFI, WONG et al., 2001). Low density lipoprotein-cholesterol (LDL-Chol) levels were calculated using Friedwald equation (CROOK, 2006).

2.9 Insulin and HbA_1C assay

The insulin level was determined using Diagnostic Automation insulin assay (Diagnostic Automation Inc. USA) as described by Clark and Hales (1994) while the HbA_1C assay was by standard protocol (CHANDALIA, SADIKOT, BHARGAVA et al., 1980).

2.10 Tissue histology

The pancreatic tissue from each group was fixed in Bouin's fluid for seven days before embedding in paraffin wax. The pancreatic tissue sectioned at 5 µm was stained with aldehyde fuschsin. Each section was examined under light microscope at high power magnification for structural changes and photomicrographs were taken.

2.11 Statistical analysis

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 Effect on weight of the animals

The body weight changes of diabetic animals treated and the untreated is indicated in Figure 1. The animals showed decrease in appetite and weight depreciation after alloxan induction. In the untreated group, progressive weight decrease occurred while in the extract/glibenclamide treated, there was weight appreciation after few days of treatment as well as showed increase in appetite.

3.2 Effect of RH extract on oral glucose tolerance test (OGTT)

Glucose tolerance was evaluated by OGTT (Figure 2). Following oral glucose load in the untreated group, hyperglycaemia occurred reaching a peak level 1hr after the glucose load. Decrease in glycaemia however occurred after 1hr but the blood sugar level failed to return to baseline glycaemia after 4 hours indicating glucose intolerance. In the extract/glibenclamide treated, significantly ($P < 0.05$) decrease in the peak values and the area under curve were observed with percentage decrease of 56.5 and 47.9 respectively compared to the untreated. The extract showed comparatively more effective glucose tolerance than the reference drug.

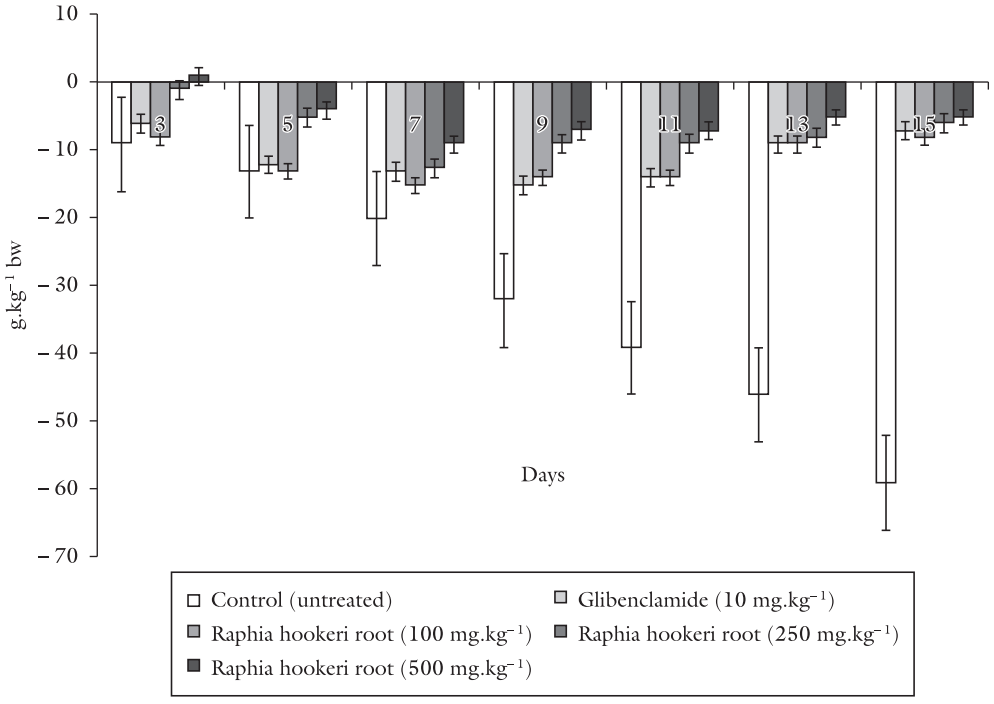

Figure 1. Weight difference in control and treated animals.

3.3 Effect of RH extract on fasting blood glucose

Alloxan diabetic animals showed (Figure 3) significant elevation of fasting blood glucose level (FBG). In the untreated (vehicle group), an increase in FBG level from day 0 was progressive all through the experimental period. 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 $mg.kg^{-1}$). At day 15 of treatment with the extract doses (50, 100 and 200 $mg.kg^{-1}$ bwt), FBG level which was exacerbated by alloxan returned back to base line with blood glucose levels of 87.2 ± 2.3 (79.3%); 57.0 ± 1.7 (86.3%) and 55.0 ± 0.3 $mg.dL^{-1}$ (87.1%) respectively. Treatment with glibenclamide showed a maximum glycaemic decrease of 167.4 ± 1.1 $mg.dL^{-1}$ (60.1%) indicating lower activity when compared to the extract doses.

3.4 Effect of RH extract on normoglycaemic animals

The hypoglycaemic effect of the RH extract was evaluated at 4 hours intervals. Treatment with 100 $mg.kg^{-1}$ bwt of the extract (Figure 4) showed no remarkable change in glucose level in normoglycaemic animals. However, at 250 and 500 $mg.kg^{-1}$ bwt doses, significant ($P < 0.05$) decrease occurred after 4 hours of oral administration representing

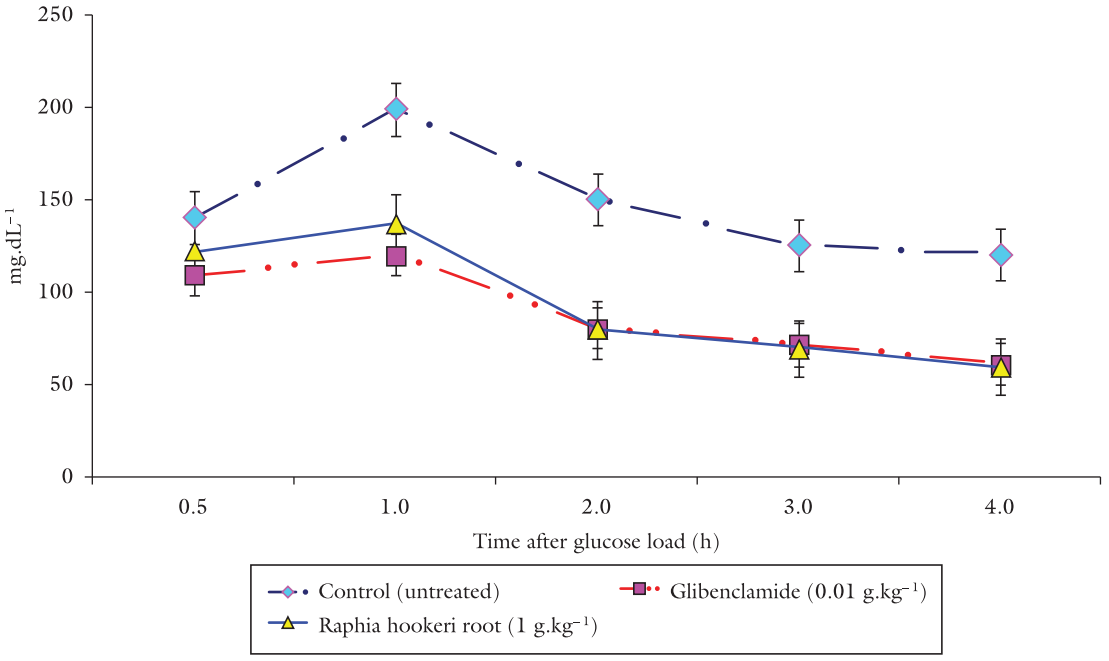

Figure 2. Effect of raphia hookeri root on OGTT. Values represent mean ± n = 5.

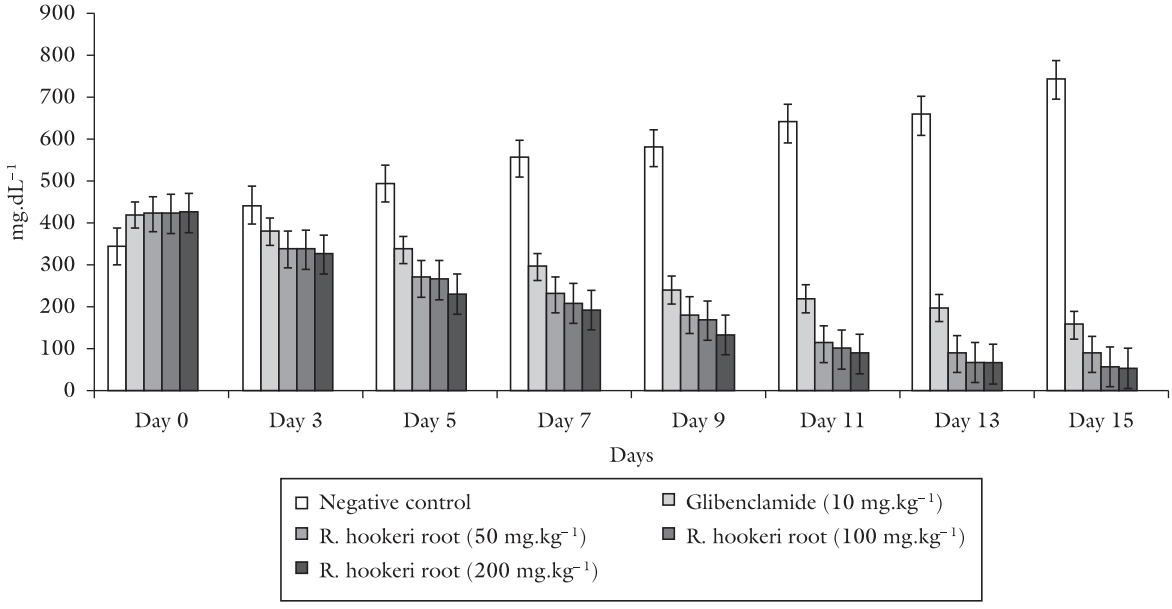

Figure 3. Plasma glucose level of rats treated with Raphia hookeri root extract. Each bar represente mean ± SEM (n = 5).

12.9% and 29.7% decrease. Maximum decrease was established after 8 hours of the administration with plasma glucose levels of 37.2 ± 1.1 (40.3%) and 29.1 ± 0.3 (54.7%) respectively. The effect produced at 12 hours interval was less.

3.5 Effect of RH extract on lipid profile

The TC, TG and LDL-Chol were significantly ($p < 0.05$) higher in alloxan diabetic animals (Table 1) showing an increase of 286%, 339% and 220% respectively while HDL-Chol decrease by 51% compared to normal control animals. Treatment with RH extract at 50, 100 and 200 mg.kg^{-1} bwt doses and glibenclamide (10 mg.kg^{-1} bwt) resulted in significant ($p < 0.05$) decrease in plasma levels of TC {177.7 ± 3.1 (47%); 122.8 ± 4.1 (63%); 117.3 ± 4.3 (70%); 165.2 ± 1.4 (51%)}, TG {268.1 ± 3.6 (55%); 149.7 ± 2.1 (73%); 131.2 ± 3.8 (78%); 278.8 ± 3.9 (52%)} and LDL-Chol (199.4 ± 2.2 (38%); 127.4 ± 1.7 (60%); 112.1 ± 2.8 (68%); 175.4 ± 3.0 (43%)} compared to those of diabetic control rats with the extract treated exhibiting a dose dependent decrease. There was however significant recovery in HDL-Chol in the three extract doses and glibenclamide treated {27.1 ± 1.8 (40%); 34.4 ± 0.2 (78%); 34.3 ± 1.1 (92%); 30.1 ± 0.8 (57%)}.

3.6 Effect of the extract on insulin level

Figure 5 showed the plasma insulin levels in diabetic untreated and treated animals. In diabetic animals, there was marked decrease in plasma insulin level with significant ($p < 0.05$) increase in blood glucose level compared to normal. Administration of RH extract (50, 100 and 200 mg.kg^{-1} bwt) and glibenclamide (10 mg.kg^{-1}) to diabetic animals significantly ($p < 0.05$) increased the plasma insulin level compared to diabetic untreated with the extract treated showing dose dependent decrease.

3.7 Effect of RH on blood HbA_1C level

The summary of blood HbA_1C level is shown in Figure 6. The HbA_1C level was observed to be significantly increased in diabetic control animals when compared to normal. After treatment with RH extract and glibenclamide, there was significant ($p < 0.05$) decrease in plasma HbA_1C level compared to diabetic control in which the extract treated showed dose effect. The extracts at doses used exerted comparatively more effective decrease than glibenclamide.

3.8 Histopathology of pancreatic tissue

Aldehyde fuschsin stain was used to demonstrate the beta cells. In the normal control (Figure 7a), pancreatic tissue morphology showed intact beta cells which appeared more

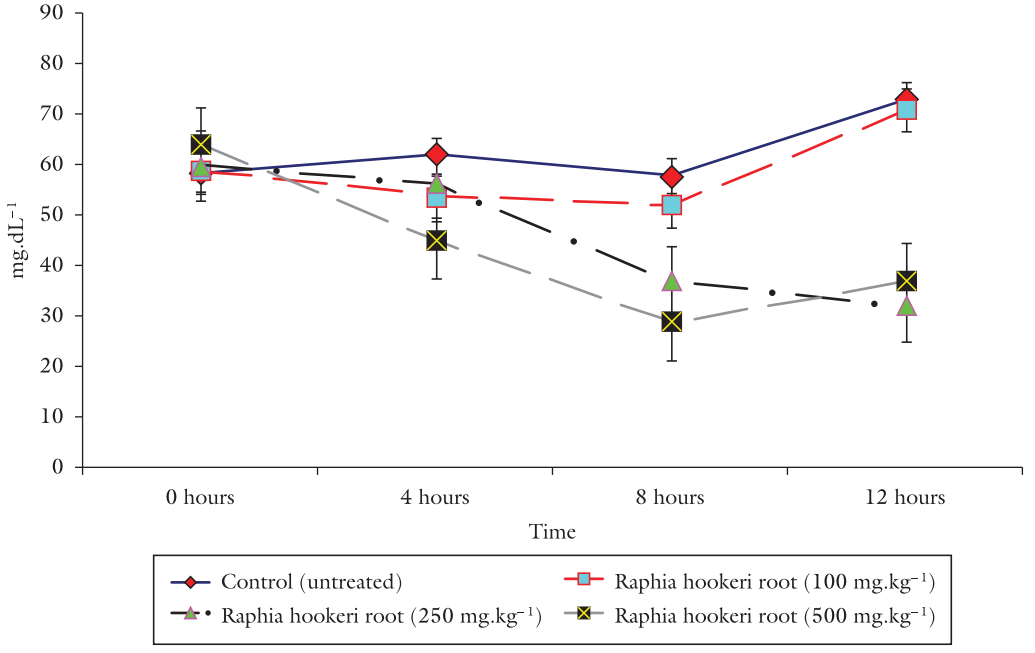

Figure 4. Hypoglycaemic assessment of raphia hookeri root extract. Each bar represent mean ± SEM (n = 5).

Table 1. Biochemical analysis showing the lipid profile.

	Dose mg.kg^{-1}	T CHOL mg.dL^{-1}	HDL mg.dL^{-1}	LDL mg.dL^{-1}	TG mg.dL^{-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 ± 3.9*
R. hookeri root	50	177.7 ± 3.1*	27.1 ± 1.8*	199.4 ± 2.2*	268.1 ± 3.6*
R. hookeri root	100	122.8 ± 4.1*	34.4 ± 0.2*	127.4 ± 1.7*	149.7 ± 2.1*
R. hookeri root	200	117.3 ± 4.3*	34.3 ± 1.1*	112.1 ± 2.8*	131.2 ± 3.8*

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

numerous than the poorly expressed alpha cells. Thin fibrous tissue capsule demarcated the islet formation from the surrounding pancreatic acini and within the islet was spotted blood vessel. The photomicrograph of the extract treated (Figure 7b) showed mild cellular lesion with predominant beta cells survivor. In the glibenclamide treated (Figure 7c), there was more extensive beta cells necrosis with spots of survivor cells. The photomicrograph of diabetic control (Figure 7d) showed more extensive necrotic changes with islet mass forming a shrunken amorphous eosinophilia.

4 Discussion

Different parts of RH are used to achieve herbal remedies which include metabolic disorders by the rural populace in Nigeria particularly in South Eastern part where it is found in abundance. DM is known to be associated with polydipsia, polphagia, polyuria, hyperglycaemia and weight loss (BRAGANCA, 1996). In this study, chemical parameters like plasma blood glucose level and lipid profile were examined in alloxan induced diabetic rats treated with RH root extract/glibenclamide. Treatment with the extract/glibenclamide recorded weight increase. Weight loss is usually a major feature in diabetes characterized by muscle wasting (SWANSTON-FLAT, DAY, BAILEY et al., 1990) and loss of tissue protein (CHATTERJEA and SHINDA, 2002). The observed weight gain therefore, could be as a result of improvement in the condition of the pancreas by the extract by ameliorating the activity of the diabetogenic agents and controlling muscle wasting.

Effective clearance of excess glucose in the blood to maintain glucose homeostasis is crucial to decreasing the risk of developing macro- vascular complications (ATTELE, ZHOU, XIE et al., 2002). The study showed that the extract/glibenclamide exerted anti-hyperglycaemic activity

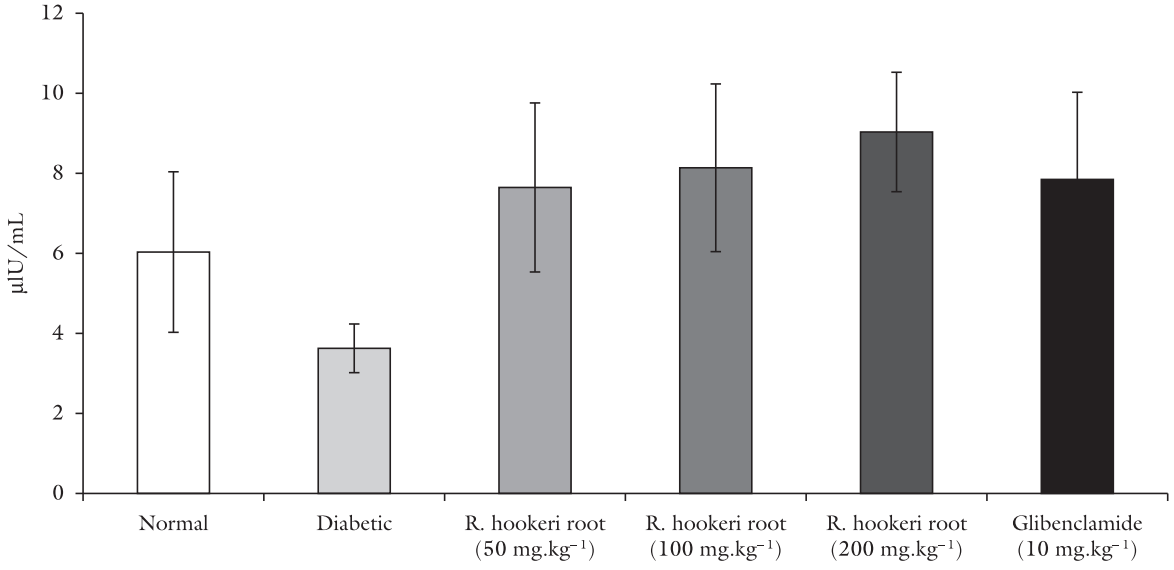

Figure 5. Plasma insulin level post treatment. Values represent mean ± n = 5.

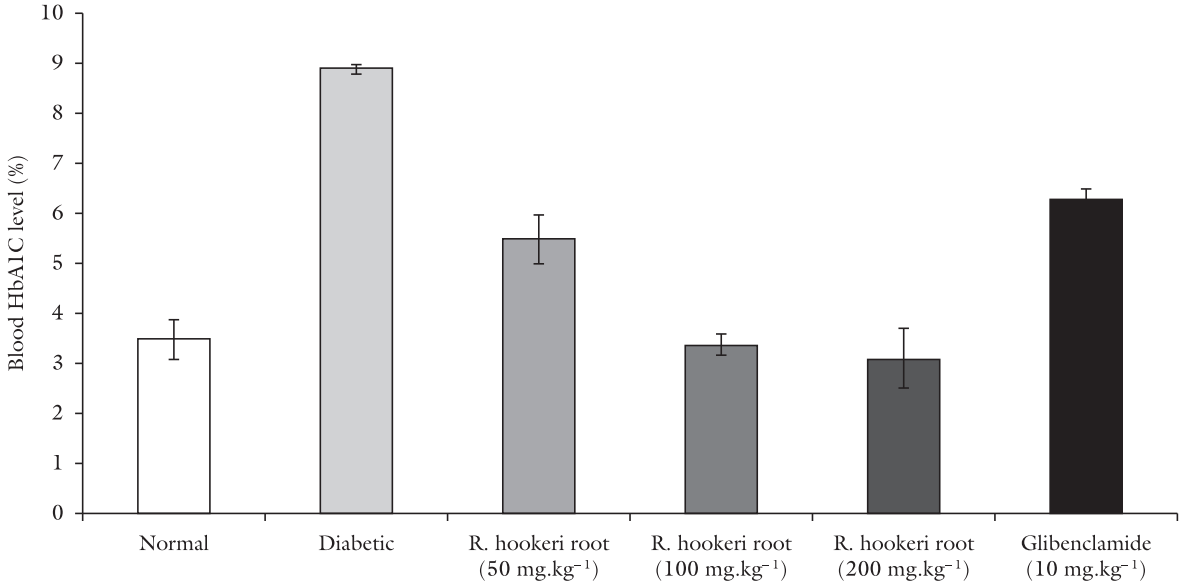

Figure 6. Plasma HbA^1C level post treatment. Values represent mean ± n = 5.

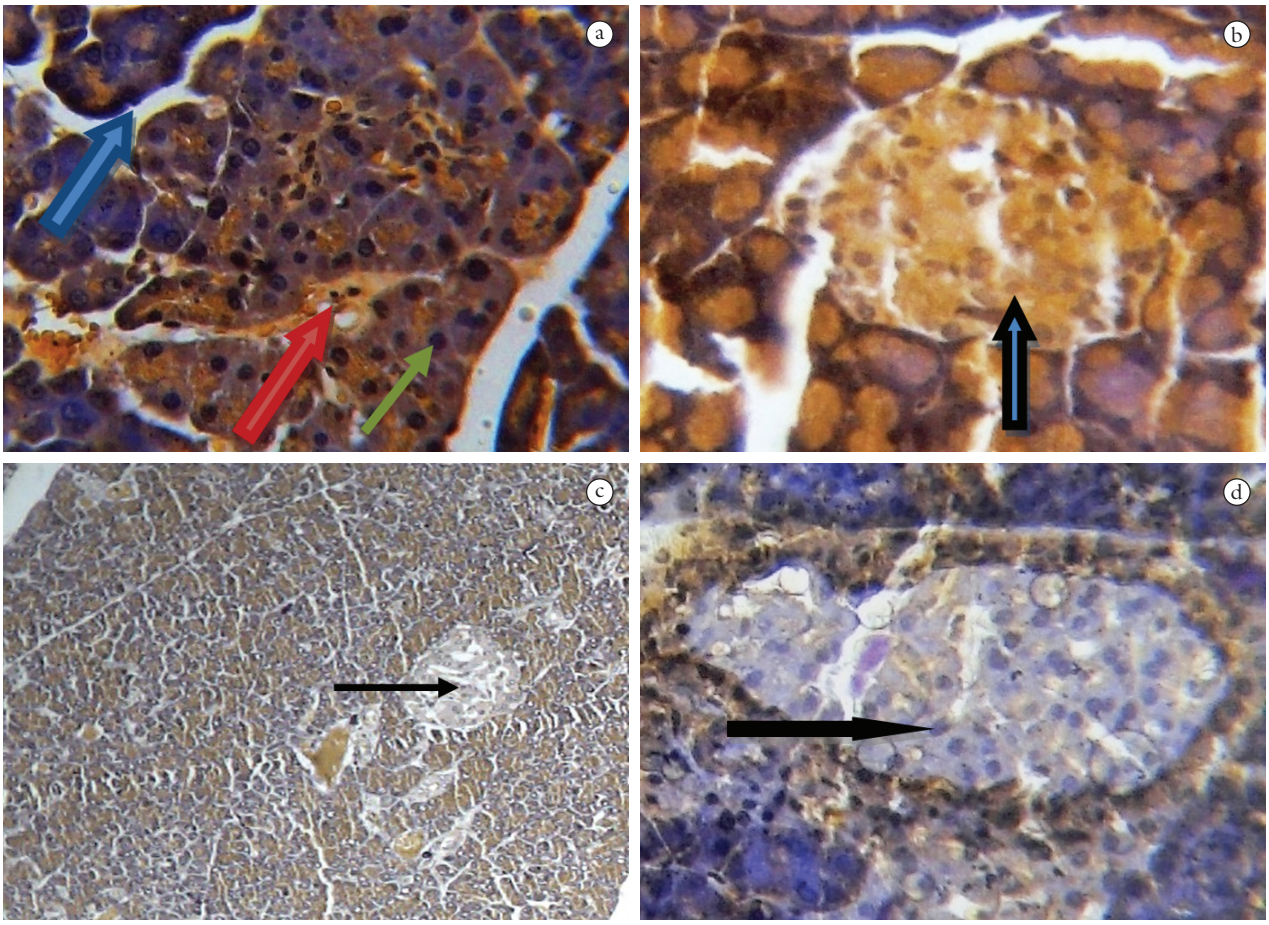

Figure 7. a) Normal Islet cells, green arrow point to beta cell, red to blood vessels and blue at the islet capsule (×400). b) Pancreatic tissue post-treatment with glibenclamide. Arrowed is the area of necrotic beta cells (×400). c) Diabetic control, arrow pointing to necrotic amorphous islet mass (×400). d) Pancreatic tissue post-treatment with RH extract. The beta cells (arrowed) are deeply stained (×400).

by decreasing the peak blood glucose concentration and the area under OGTT curve with the extract exhibiting comparably more activity than the reference drug after 4 hours of oral glucose load. In FBG study, it was apparent RH extract enhanced glucose utilization since it significantly lowered the blood glucose level in alloxan diabetic rats. The mode of action was not explored. However, the photomicrograph of pancreatic tissue showed more numerous beta cells compared to treatment with the reference drug which suggested that the extract may have effected quantitative change by initiating differentiation and proliferation of beta cells after alloxan damage. There was also strong indication from insulin assay that the extract caused insulin stimulatory effect which altogether was responsible for the decrease of blood glucose to a base line level. Reports that some plants used for treating diabetic cases can effect beta cells regeneration have been put forward (NAGAPPA, THAKURDESAI, VENKAT RAO et al., 2003; KIM, KIM, LYU et al., 2009). The potentiation of insulin release from the beta cells may have equally accounted for the hypoglycaemic activity of the extract. Many anti-diabetic plants have been shown to possess hypoglycaemic activity (VALCHEVA-KUZMANOVA, KUZMANOV, TANCHEVA et al., 2007; UDAYAKUMAR, KASTHURIRENGAN, MARIASHIBU et al., 2009; MBAKA, ADEYEMI, NORONHA et al., 2009; PAREEK, SHARMA, KHAIJA et al., 2009). The use of oral hypoglycaemic agents such as biguanides and sulfonylureas (RANG and DALE, 1991) offered great relief to diabetic patients. The success recorded necessitated intensified search for safer and more effective hypoglycaemic agents.

Treatment with RH extract/glibenclamide in alloxan diabetic rats resulted in significant decrease in plasma HbA1C level with the extract treatment exhibiting more effective decrease. This implied that RH extract effectively lowered plasma glucose level leading to recovery in haemoglobin content. The assessment of glycosylated haemoglobin level has proven a useful tool in ascertaining if blood glucose level is adequately controlled in the course of treatment since the rate of formation of HbA1C has been observed to be proportional to blood glucose level (ANITHA and CHANRALEKHA, 2010). Studies have reported decrease in haemoglobin level following glycosylation in hyperglycaemic condition (VENKATESWARAN and PARI, 2002; LATHA and PARI, 2004).

In alloxan induced diabetes, increase in blood glycaemia is usually accompanied by elevated level in plasma TC, TG and LDL which is a significant threat to cardiovascular risk factors like coronary heart disease (PRINCE, MENON and

GUNASEKARAN, 1999). Insulin, a potent inhibitor of lipolysis when lacking or drastically reduced as a result of beta cells damage, would lead to increase in mobilization of free fatty acids from the peripheral fat deposit by the activity of hormone sensitive lipase (UDAYAKUMAR, KASTHURIRENGAN, MARIASHIBU et al., 2009). This condition more often accentuates the risk of cardiovascular diseases. In this study, the levels of the lipid profile were brought to near normal following treatment with RH extract. It was probable that there was an inhibition of the activity of hormone sensitive lipase in adipose tissue thereby suppressing fatty acid release. Besides, insulin may have activated lipoprotein lipase to hydrolyze excess TG and LDL in the blood stream hence preventing the progression of coronary heart disease. The result equally showed that the HDL-cholesterol appreciated significantly in the RH treated which has a beneficial effect on the cardiovascular system.

5 Conclusion

This study showed that *raphia hookeri* root extract attenuated hyperglycaemia leading to recovery of haemoglobin content by possibly potentiating insulin release. It exhibited effective hypoglycaemic activity and ameliorated dislipidaemia. The above findings showed the plant root to have promising value in the development of potent phyto-medicine for treating DM and have therefore justified its folk use in the management of DM and diabetic complications.

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