BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 4 (2015) 307–313

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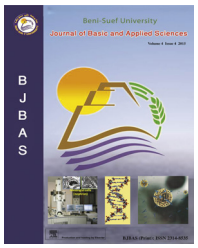

Full Length Article

Developed beverage from roselle calyx and selected fruits modulates β-cell function, improves insulin sensitivity, and attenuates hyperlipidaemia in diabetic rats

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ARTICLE INFO

Article history:
Received 29 May 2015
Accepted 20 November 2015
Available online 11 December 2015

Keywords:
Atherogenic indices
Antidyslipidaemic
Beta cell function
Hypoglycaemic
Insulin sensitivity

ABSTRACT

The aim of this study is to report the antidiabetic properties of a beverage developed from roselle calyx and selected fruits in male albino rats. The beverage was designed to contain 30% pawpaw (*Carica papaya* L.), 10% grapefruit (*Citrus paradisi*), 20% guava leaves (*Psidium guajava* L.) and 40% roselle calyx aqueous extracts. Four groups of five rats each were acclimatized on pelletized mouse chow for seven days, after which diabetes was induced by a single ip injection of alloxan in all groups except group 1, which served as control. Group 2 served as negative control while groups 3 and 4 were treated with the beverage at 2.5 and 5 ml/kg bw respectively. Food intake, body weight, and blood glucose levels were monitored. They were sacrificed by cervical dislocation after a 2 week treatment. Blood serum was analysed to evaluate insulin levels, β cell function, insulin resistance and lipid profile. Histological studies were carried out on pancreatic tissues. Treatment with both doses of the beverage led to a significant reduction ($p < 0.05$) in blood glucose, total cholesterol triglyceride, LDL and increased HDL levels. It also improved serum insulin levels, β cell function, reduced insulin resistance and restored pancreatic beta cells compared to the diabetic group. These antidiabetic properties may be as a consequence of modulation of the β-cell function, reduction of insulin resistance and preservation/restoration of β-cell integrity. However, treatment with the single dose showed signs of hyperinsulinaemia.

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http://dx.doi.org/10.1016/j.bjbas.2015.11.007

1. Introduction

The rising bane of diabetes especially in most developing countries has become a major global health issue (Erukainure et al., 2013). In 2010, 12.1 million people were estimated to be diabetic in Africa, and this is expected to rise to 23.9 million by 2030 (Sicree et al., 2009). Though it is mostly common among the elderly in most populations, prevalence rates have been predicted to significantly rise among the young and productive populations in the developing world (Gomes et al., 2006). Characterized by hyperglycaemia, diabetes is a metabolic disorder that affects carbohydrate, protein and lipid metabolism (Maritimi et al., 2003). This may be due to inadequate insulin release as seen in type 1 diabetes or insulin resistance resulting to type 2 diabetes (Meral et al., 2004). It increases the risk of several complications such as nephropathy, microangiopathy and retinopathy (Barar, 2000).

Increasing interests in complementary and alternative medicine have led to the development and search of novel therapy for diabetes (Yeh et al., 2002). Over the years, plants have been used in the treatment and management of diabetes (Gupta et al., 2005). This has been attributed to the presence of phytochemicals which are remedial against diabetes and other ailments.

Fruits have been well documented to be medicinal. This has been attributed to their antioxidant properties owing to the integrated action of oxygen radical scavengers such as β-carotene and ascorbic acid, calcium and dietary fibre (Bhardwaj and Pandey, 2011). Their daily consumption has been linked with reduced risk of cancer, cardiovascular disease, diabetes, stress and fatigue (Erukainure et al., 2012). Of particular interests are pawpaw (*Carica papaya* L.) and grape (*Citrus paradisi*) fruits. Their antidiabetic properties have been documented in several studies (Erukainure et al., 2012; Lans, 2006). Due to their perishable nature and poor storage facilities, the post-harvest shelf life of these fruits is very limited thereby leading to wastage (Bhardwaj and Pandey, 2011; Nwachukwu et al., 2010). Development of ready-to-serve beverage from blends of these fruits is a convenient option for their utilization and reduces post-harvest losses (Bhardwaj and Pandey, 2011). Blending juices with other medicinal plants such roselle calyx (*Hibiscus sabdariffa*) and guava leaves (*Psidium guajava* L.) improves the medicinal properties (Erukainure et al., 2012). The protective effect of these plants against diabetes has been reported. Guava leaves have been shown to inhibit increases in plasma glucose level in diabetic rats (Mazumdara et al., 2015). Aqueous extract of roselle calyx has been demonstrated as having beneficial effects on anti-oxidation and lipid lowering in experimental diabetic studies (Wang et al., 2011). A blend of the aforementioned fruits and medicinal plants to form a natural health drink suitable for the treatment and/or management of diabetes and its related complication has been reported (Erukainure et al., 2012; Okafor et al., 2010). Erukainure et al. (2012) reported the protective role of these blends against hyperglycaemia-induced oxidative stress in testicular tissues and sperm cells in rats.

This paper aims to assess the anti-diabetic and/or hypoglycaemic effect of blends of pawpaw and grape fruits with aqueous extracts of guava leaves and roselle calyx in male albino rats.

2. Materials and methods

2.1. Plant materials

Unripe pawpaw fruits (Cg variety), grape fruits, and roselle calyx were purchased from Mushin market, Lagos Nigeria. Guava leaves were obtained from Ikorodu, Lagos, Nigeria. The fruits were washed and peeled, while the guava leaves and roselle calyx were sorted and washed. Production of beverage was carried out according to the method described by Okafor et al. (2010). The beverage was designed to contain 30% pawpaw (*C. papaya* L.), 10% grapefruit (*C. paradisi*), 20% guava leaves (*P. guajava* L.) and 40% roselle calyx aqueous extracts.

2.2. Animals

Twenty male albino rats of Wistar strain weighing about 150–200 g were used for the study. They were fed on standard rat pellet diet and allowed to adapt for one week. They were provided water ad libitum and maintained under standard laboratory conditions of natural photo period of 12-hr light–dark cycle. The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, Federal Institute of Industrial Research, Lagos, Nigeria.

2.3. Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 150 mg/kg of alloxan monohydrate in normal saline water in a volume of about 3 ml (Viswanathaswamy et al., 2011). After 72 hours, the diabetic rats (glucose level > 150 mg/dl) were separated and used for the study.

2.4. Experimental design

The rats were divided into four groups, each consisting of five animals.

Group 1 – consisted of normal rats and fed on pelletized mouse chows
Group 2 – consisted diabetic rats with no treatment administered
Group 3 – consisted of diabetic rats and treated with 2.5 ml/kg bw of the developed beverage
Group 4 – consisted of diabetic rats and treated with 5.00 ml/kg bw of the developed beverage

All rats were fed on pelletized mouse chows and were monitored daily for food and water intake, and body weight. The developed beverage was orally administered, groups 1 and 2 were orally administered with water. Blood glucose levels of the rats were monitored on a weekly basis with a glucometer. Treatment lasted for 14 days after induction. At the end of the treatment trials, the rats were fasted overnight and sacrificed by cervical dislocation.

The dose of the developed beverage and period of treatment were selected on the basis of previous studies by Erukainure et al. (2014).

Table 1 – Food intake, weight gain, and food efficiency ratio of experimental animals.

Parameters	Group 1	Group 2	Group 3	Group 4
Weight gain (g/day)	36.93 ± 5.02[c]	21.1 ± 2.13[c,d]	–4.18 ± 0.10[a,b,d]	21.66 ± 3.35[c]
Food consumed (g/day)	86.25 ± 10.02	75.00 ± 15.00	66.50 ± 9.08	77.75 ± 18.99
FER*	0.43 ± 0.18	0.28 ± 0.22	–0.06 ± 0.15	0.28 ± 0.20

Note: Values = mean + SD; n = 5.
[a] Statistically significant ($p < 0.05$) as compared with group 1.
[b] Statistically significant ($p < 0.05$) as compared with group 2.
[c] Statistically significant ($p < 0.05$) as compared with group 3.
[d] Statistically significant ($p < 0.05$) as compared with group 4.
* Food efficiency ratio = body weight gain/food intake.

2.5. *Serum preparation*

Blood was collected with a 5 ml syringe and needle by cardiac puncture. It was centrifuged at 3000 rpm for 10 min and the serum (supernatant) was analysed to evaluate serum insulin level by ELISA method using insulin kit (DRG International, Inc, USA) according to manufacturer's protocol.

Insulin resistance (IR) and β-cell (β) function were quantified using the homeostatic model assessment (HOMA) formula (Hermans and Lambert, 2002).

$$\text{HOMA-IR} = \frac{\text{Fasting blood glucose} \times \text{Fasting serum insulin}}{405} \quad (1)$$

$$\text{HOMA-}\beta = \frac{360 \times \text{Fasting insulin}}{\text{Fasting glucose} \times 63} \quad (2)$$

Insulin sensitivity (IS) was quantified using the quantitative insulin sensitivity check index (QUICKI) which is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose (Yokoyama et al., 2003).

$$\text{QUICKI} = \frac{1}{\log(\text{fasting insulin}) + \log(\text{fasting glucose})} \quad (3)$$

2.6. *Serum lipid profile and atherogenic indices*

Serum total cholesterol, triglyceride and high density lipoprotein (HDL) were measured by enzymatic colorimetric method using Randox kits. The concentration of very low density lipoprotein (vLDL) and low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedwald et al. (1972).

Atherogenic Index (AI) and Atherogenic Index of Plasma (AIP) were calculated as described by Takasaki (2005) and Onat et al. (2010) respectively. Cardiac Risk Ratio (CRR) and Atherogenic Coefficient (AC) were calculated according to the methods described by Ikewuchi and Ikewuchi (2009).

2.7. *Histopathology*

Pancreatic tissues from the sacrificed rats were sliced to a thickness of 3 mm and arranged in a tissue cassette with attached label. The tissues were processed using an automated tissue processor (Leitz 2005 model) according to manufacturer's protocol.

2.8. *Statistical analysis*

All samples were analysed in triplicate and repeated at least thrice for liability of data. Data were subjected to post-hoc test and statistical significance was established using one-way analysis of variance (ANOVA). Results were reported as mean + standard deviation. Significant difference was established at $P < 0.05$. Statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL).

3. Results

There was no significant difference in food consumed and food efficiency ratio (FER) in all experimental groups as depicted in Table 1. Significant reduced body weight was observed in rats treated with single dose.

Induction of diabetes led to significant increase ($p < 0.05$) in blood glucose level as shown in Fig. 1. Treatment with single and double dose of the beverage significantly ($p < 0.05$) reduced the blood glucose level.

Significant reduced ($p < 0.05$) serum insulin level was observed in the diabetic group as shown in Fig. 2. This was significantly increased on treatment with the beverage, with

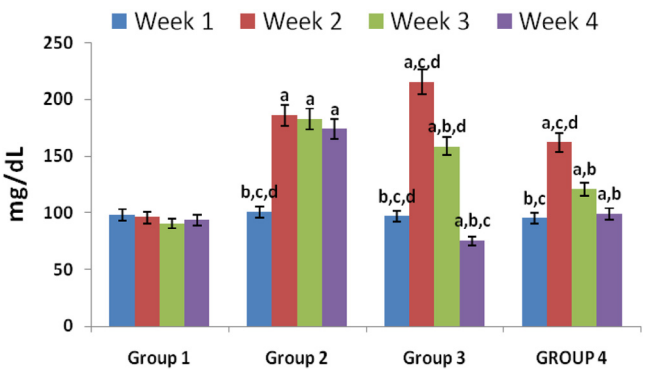

Fig. 1 – Fasting blood glucose level of experimental groups. Note: Values = mean ± SD; n = 5. a, statistically significant ($p < 0.05$) as compared with group 1; b, statistically significant ($p < 0.05$) as compared with group 2; c, statistically significant ($p < 0.05$) as compared with group 3; d, statistically significant ($p < 0.05$) as compared with group 4.

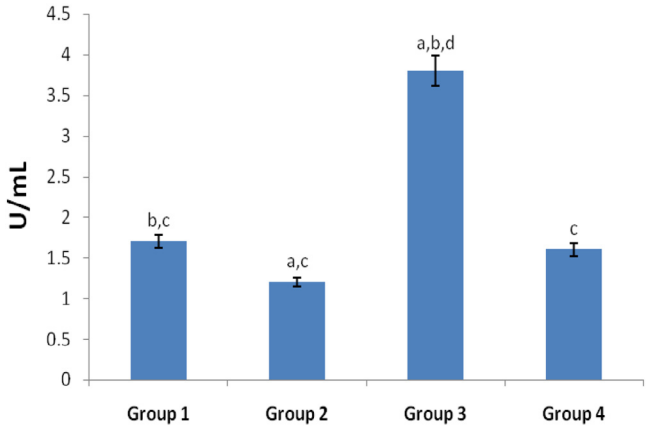

Fig. 2 – Fasting serum insulin level of experimental groups. Note: Values = mean ± SD; n = 5. a, statistically significant (p < 0.05) as compared with group 1; b, statistically significant (p < 0.05) as compared with group 2; c, statistically significant (p < 0.05) as compared with group 3; d, statistically significant (p < 0.05) as compared with group 4.

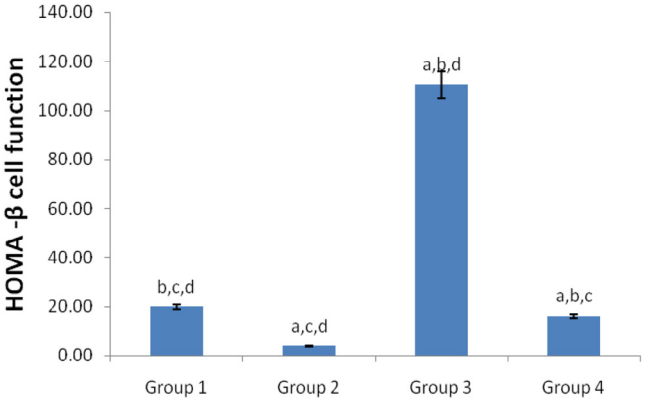

Fig. 4 – HOMA-β cells (β cell function) of experimental groups. Note: Values = mean ± SD; n = 5. a, statistically significant (p < 0.05) as compared with group 1; b, statistically significant (p < 0.05) as compared with group 2; c, statistically significant (p < 0.05) as compared with group 3; d, statistically significant (p < 0.05) as compared with group 4.

rats treated on single dose showing a rather higher level as compared to the normal (control) group.

Effect of the beverage on insulin resistance and sensitivity is shown in Fig. 3. Significant increase in insulin resistance was observed in rats treated with single dose of the beverage; this was followed by the untreated diabetic rats, while rats treated with double dose had the lowest value. Reduced insulin sensitivity was observed in rats on single dose treatment, this was followed by the untreated, while the double dose treated had the highest value.

Induction of diabetes led to significant reduction in β-cell function as observed in the untreated diabetic rats (Fig. 4). This was significantly reversed (p < 0.05) on treatment with the beverage at both doses.

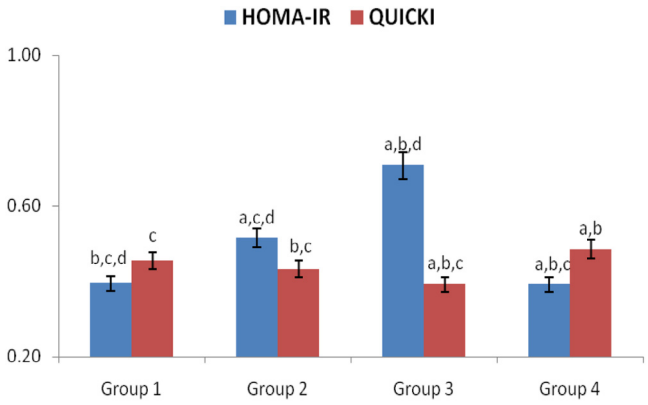

Fig. 3 – HOMA-IR and insulin sensitivity (QUIKI) of experimental groups. Note: Values = mean ± SD; n = 5. a, statistically significant (p < 0.05) as compared with group 1; b, statistically significant (p < 0.05) as compared with group 2; c, statistically significant (p < 0.05) as compared with group 3; d, statistically significant (p < 0.05) as compared with group 4.

Induction of diabetes led to a significant increase in serum cholesterol, triglyceride, vLDL and LDL levels and a reduced HDL level as depicted in Fig. 5. This was significantly reduced on treatment with the beverage.

Significant alteration in atherogenic indices was observed in the experimental groups as shown in Fig. 6. The untreated group displayed significantly higher (p < 0.05) levels. These values were significantly lower (p < 0.05) in the treated groups.

Histological examination of the experimental rat pancreas is shown in Fig. 7. Reduced concentration and hypocellularity of islets of Langerhans was observed in the untreated diabetic group (Fig. 7b). Treatment with single dose of the beverage revealed a higher concentration of normocellular islets of Langerhans (Fig. 7c). Treatment on double dose revealed normo-cellular islets of Langerhans but not as concentrated as that of group 3 (Fig. 7d). All experimental groups showed normal exocrine pancreas.

4. Discussion

Diabetes has been described as a looming epidemic and a major threat to global public health, with the biggest impact on adults of working age in developing countries (WHO and IDF, 2004; Saini, 2010). It is characterised mainly by hyperglycaemia, insulin resistance, dyslipidaemia and β cell failure (Vijayaraghavan, 2010). This study reports the therapeutic potentials of beverage produced from roselle calyx and selected fruit blends on diabetic rats.

The importance of weight loss in diabetes particularly type 2 has been reported (Pi-Sunyer, 2005). However, in this study, the rats were not obese making the observed weight loss in the single dose treated group a serious concern.

Increased blood glucose has been recognized as the primary defining characteristics of diabetes. Also known as hyperglycaemia, it occurs as a result of low insulin levels and/

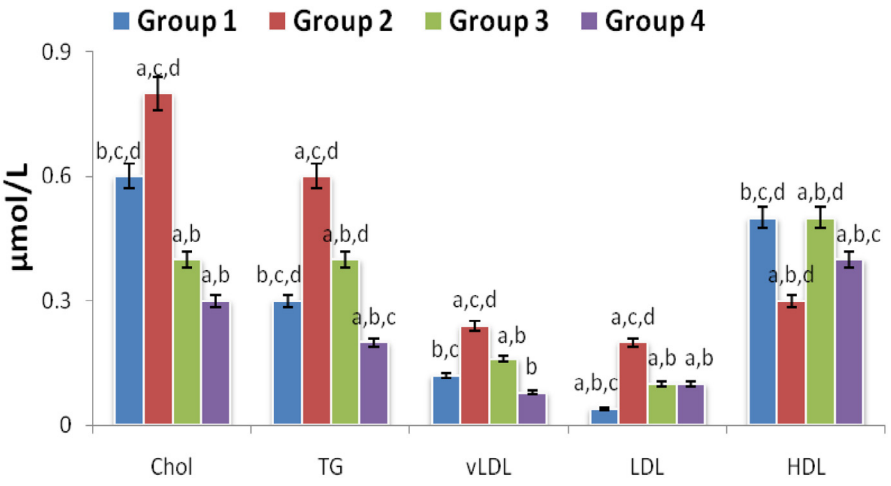

Fig. 5 – Lipid profile of experimental groups. Note: Values = mean ± SD; n = 5. a, statistically significant ($p < 0.05$) as compared with group 1; b, statistically significant ($p < 0.05$) as compared with group 2; c, statistically significant ($p < 0.05$) as compared with group 3; d, statistically significant ($p < 0.05$) as compared with group 4.

or by resistance to insulin at the cellular level (type 2 diabetes) (Sommerfield et al., 2004). Both doses of treatment led to significant ($p < 0.05$) reduction in blood glucose levels, indicating a hypoglycaemic effect. This could be attributed to the reported phytochemical and mineral properties of the beverage (Okafor et al., 2010). The observed hypoglycaemic property could also be attributed to the synergetic effect of the reported medicinal properties of the fruits (Bhardwaj and Pandey, 2011).

The observed increase in serum insulin level by both doses indicates insulin release by the pancreatic beta cells. This release corroborates with the hypoglycaemic activity of the beverage. However, the serum insulin level of the single-dose treated group was rather high which may suggest an occurrence of hyperinsulinaemia owing to insulin resistance. This is further portrayed by the high HOMA-IR and reduced QUIKI values which indicate low insulin sensitivity. As a response to reduced insulin sensitivity, insulin secretion is theoretically expected to increase (Dixon et al., 2003). Insulin resistance is the core metabolic abnormality in type 2 diabetes and has been associated with most diabetic complications (Monzillo and Hamdy, 2003). The increased beta cell function also correlated with the observed increased insulin level of the treated groups, indicating reversibility of beta cell dysfunction with hypoglycaemic activity.

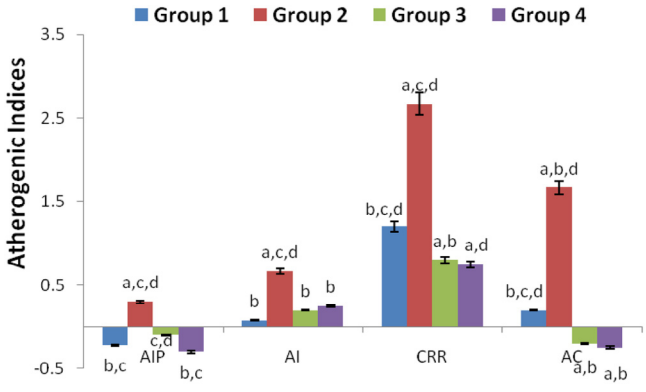

Fig. 6 – Atherogenic indices of experimental groups. Values = mean ± SD; n = 5. a, statistically significant ($p < 0.05$) as compared with group 1; b, statistically significant ($p < 0.05$) as compared with group 2; c, statistically significant ($p < 0.05$) as compared with group 3; d, statistically significant ($p < 0.05$) as compared with group 4.

Dyslipidaemia has been associated with the early stage of diabetes (Adiels et al., 2008). It is characterized by elevated triglyceride levels, low high-density lipoprotein cholesterol (HDL) levels, and a dominance of low-density lipoprotein (LDL) particles (Erukainure et al., 2014). These were observed in the untreated diabetic group. However, treatment on both doses led to an anti-dyslipidaemic effect. Raised levels of free fatty acids, cytosolic triglycerides have been associated with insulin resistance and suggested to affect beta cell function in diabetics (Bakker et al., 2000; Dixon et al., 2003). Therefore, the improved insulin sensitivity and beta cell function could provide a possible explanation for the reversibility (Dixon et al., 2003).

An atherogenic lipid triad of high serum levels of triglycerides, HDL and preponderance of small, dense LDL particles has been associated with atherogenesis in diabetes (Nesto, 2005). It is aggravated by insulin resistance and/or metabolic syndrome. Higher levels of atherogenic index (AI), atherogenic index of plasma (AIP), cardiac risk ratio (CRR) and atherogenic coefficient (AC) observed in the untreated diabetic group indicate an occurrence of atherogenesis. This can be attributed to the reduced serum insulin level and increased insulin resistance. Atherogenic indices are powerful indicators of the risk of cardiovascular disease (CVD); the higher the value, the higher the risk of developing the disease and vice versa (Ikewuchi and Ikewuchi, 2009). The reduced levels in the treated group could thus be attributed to the increased serum insulin level and improved insulin sensitivity. Thus, indicating the protective potential of the beverage against diabetic-induced CVD.

Histopathological studies revealed reduction in the concentration and hypocellularity of islets of Langerhans in the

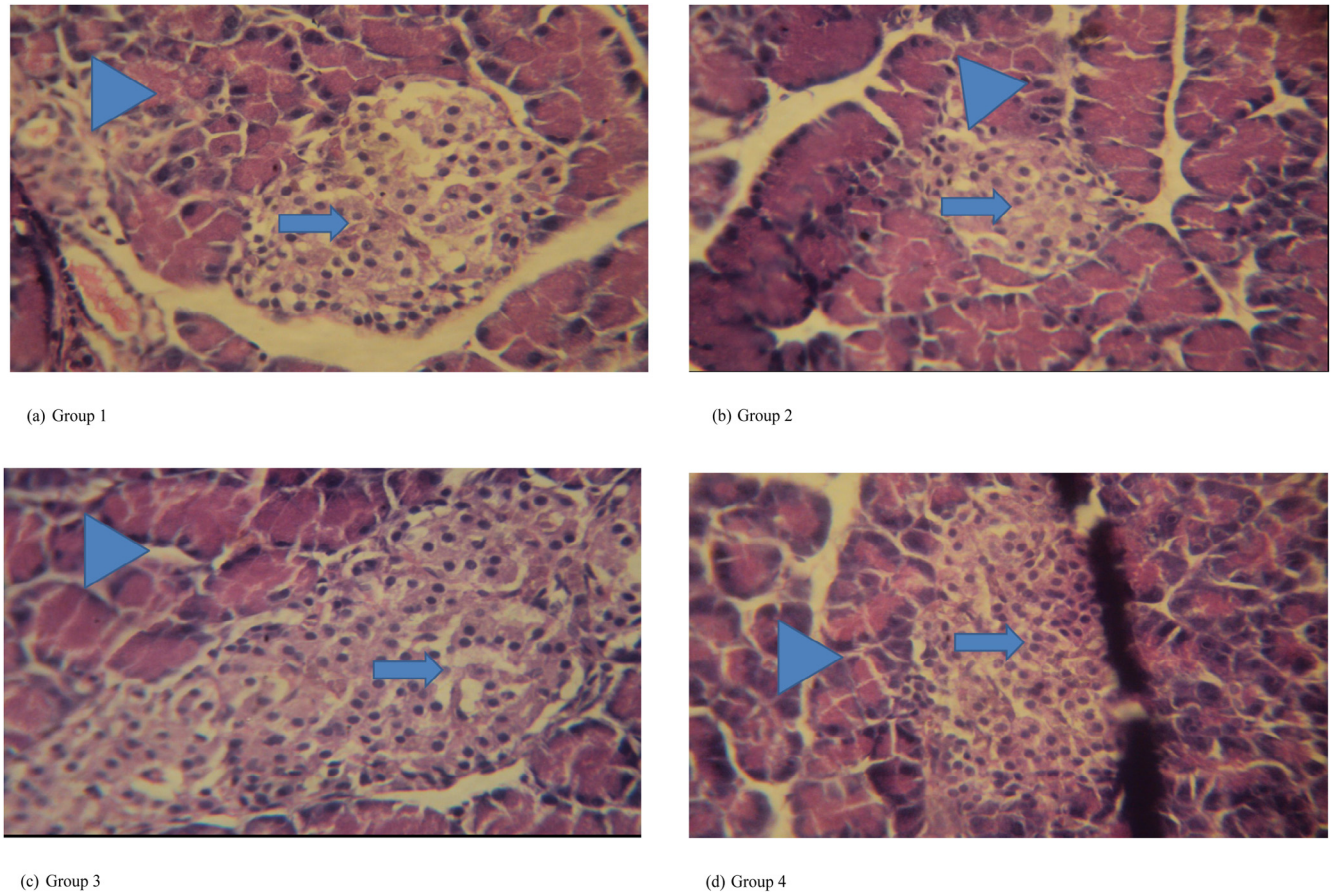

Fig. 7 – Histological examinations of experimental rat pancreas (haematoxylin and eosin stains ×400). (a) Normal control rat pancreas showing patches of abundant β-cells. (b) Pancreas of untreated (diabetic) groups showing reduced number of pancreatic β-cells. (c) Pancreatic tissues of treatment group administered single dose of the beverage showing pancreatic β-cells almost similar to that of control. (d) Pancreatic tissues of the treatment group administered double dose showing increased number of pancreatic β-cells. Legend: The arrow head refers to islets of Langerhans, while the triangle indicates the exocrine portion.

untreated diabetic group indicating diabetes. However, the pancreatic beta cells were not totally destroyed indicating an early stage of diabetes. Impairment of insulin secretion and beta cell function has been linked to shrinkage of the pancreatic β cells as evident in Figs. 2 and 5. Insulin resistance then sets in. These features greatly affect the long term control of blood glucose leading to diabetic complications (Kaku, 2010). Treatment with both doses led to restoration of shrunk beta-cells which corroborates with the reduced blood glucose level, insulin resistance and improved beta-cell function.

5. Conclusion

Treatment with the developed beverage led to reduced blood glucose, more or less appropriate serum insulin, and increased high-density-lipoprotein cholesterol levels in diabetic rats, thus revealing an antidiabetic potential. This may be as a consequence of modulation of the β-cell function, reduction of insulin resistance and preservation/restoration of β-cell integrity. However, only treatment at 5.00 ml/kg bw had conclusive beneficial effects. The beverage can thus be used as an adjunct drink in the treatment and management of diabetes.

Acknowledgment

The authors are sincerely grateful to the management of the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria for funding of this project. The authors report no conflict of interest.

Authors' contributions

OO, OBO, AO and GNE conceived the project. All authors except YLS and GNE were involved in the production of the beverage. OLE, OO, OBO, and GNE designed the experiment. All authors except OBO, GNE and OVO were involved in the animal trials and analysis. OLE and OCO wrote the manuscript. All authors read and approved the manuscript.

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