

ANTI-DIABETIC POTENTIALS OF RUZU HERBAL BITTERS IN TYPE 1 DIABETES MELLITUS-INDUCED ALBINO RATS

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ABSTRACT

Diabetes mellitus, a metabolic disease characterized by hyperglycemia is an emerging epidemic of the 21st century. It is often managed or treated at a huge cost using conventional drugs. Some undesirable side effects are also associated with most of these conventional drugs. These overall problems led to the formulations of polyherbal medicines that include Ruzu herbal bitters (RHB), a product of Ruzu Natural Health Products and Services that are yet to be experimentally proven in the treatment of diabetes mellitus. Consequently, this study examined the anti-diabetic potentials of RHB in type 1 diabetes mellitus-induced experimental rats. Triple intraperitoneal injections of streptozotocin at 35 mg/kg, 45 mg/kg and 40 mg/kg body weight at three days interval were used to induce type 1 diabetes mellitus in male Albino rats of average weight 96.37 ± 11.42 g. The treatment groups were orally administered 15 mg/kg and 30 mg/kg of RHB for four weeks. The relative expressions of insulin, intestinal glucagon-like peptide 1 (GLP-1), and Glucose transporter 2 (GLUT-2) genes by quantitative polymerase chain reaction were subsequently determined. Results showed that there was significant difference ($p < 0.05$) between the initial and final blood glucose concentrations of animals administered 15 mg/kg and 30 mg/kg of RHB. Moreover, RHB at 30 mg/kg down-regulated insulin and GLP-I gene expressions, but up-regulated GLUT-2 gene expression. It is thus inferred that RHB demonstrated anti-diabetic potentials that could be a breakthrough in controlling the menace of diabetes mellitus, since it significantly reduced blood glucose concentrations and regulated insulin, GLP-1 and GLUT-2 gene expressions in diabetic rats.

Key-words: Anti-diabetic potentials; Diabetes; Hyperglycemia; Insulin; Ruzu herbal bitters

INTRODUCTION

The prevalence of diabetes mellitus (DM) is on a steady increase worldwide. It is identified as one of the main threats to human health in the 21st century (Datta *et al.*, 2013; Siddiqui *et al.*, 2013; Ezuruike and Prieto, 2014). According to Siddiqui *et al.* (2013), it was earlier considered as a mild disorder of the elderly people, but has now become a major cause of morbidity and mortality affecting children, youth and middle aged people. It is one of the five leading causes of death in the world, with type 2 diabetes mellitus (T2DM) occurring more frequently than type 1 diabetes mellitus (T1DM). However, type 1 diabetes

mellitus is more chronic and severe than type 2 diabetes mellitus (Ghasemi *et al.*, 2014). T1DM is an autoimmune endocrine metabolic disorder or disease in which the β -cells of the pancreas are destroyed and do not produce sufficient insulin, a hormone that stimulates blood glucose usage for energy production (Thivolet, 2002; Narendran *et al.*, 2005; Siddiqui *et al.*, 2013). Consequently, there is an imbalance in blood glucose levels between cells and blood (i.e. low blood glucose level (hypoglycemia) in cells and high blood glucose level (hyperglycemia) in blood) that could be detrimental to man.

Shaw *et al.* (2010) predicted that between 2010 and 2030, there would be 69% increase in number of adults with diabetes mellitus in developing countries and 20% increase in developed countries. International Diabetes Federation (IDF, 2017) revealed that 79% of adults with diabetes mellitus are living in low- and middle-income countries like Nigeria. Therefore, the disease is an epidemic one that requires quick intervention or remedy, especially in nations where there is upsurge in the incidence of this metabolic disorder. The danger diabetes mellitus poses and the need for quick intervention had led to the formulation of different polyherbal medicines for the treatment of this disease. Claims that these polyherbs possess anti-diabetic therapeutic potentials have resulted in a widespread patronage of the manufacturers or distributors for these products. The continuous usage of these polyherbs is further increased with the belief that polyherbal formulations reduced side effects compared with conventional or orthodox drugs (Parasuraman *et al.*, 2010).

The concept of polyherbal formulation is well documented in ancient literature (Petchi *et al.*, 2014). Also, the use of herbal medicines alone or alongside prescribed (conventional) drugs for the management of diabetes mellitus is quite common in Nigeria (Ezuruike and Prieto, 2014). In fact, polyherbal formulations, which are combinations of two or more medicinal herbs or plants, are commonly used compared to single herb because of their perceived better and extended therapeutic potentials. However, little or no scientific data exist to support the anti-diabetic activities of most of these polyherbal medicines. In other words, the manufacturers' claims of anti-diabetic properties of these polyherbs have not been scientifically validated. Hence, this study was conducted to evaluate anti-diabetic potentials (therapeutic effects) of Ruzu herbal bitters in streptozotocin-induced type 1 diabetes mellitus Albino rat model.

MATERIALS AND METHODS

Drugs and Chemicals

Streptozotocin was obtained from Sigma Aldrich, USA, and metformin from Teva Pharmaceutical, Wales. All other chemicals used for the study were of analytical grade. All diagnostic kits were

procured from Lab-care diagnostics Ltd., India.

Polyherb

Ruzu herbal bitters (RHB), a product of Ruzu Natural Health Products and Services was purchased from a pharmaceutical store within Ondo State. It is made up of 3 main herbs namely: cluster pear or bush banana (*Uvaria chamae*), African crocus (*Curculigo pilosa*) and bitter apple or bitter cucumber (*Colocynthis citrullus*) according to the manufacturer. The polyherbal mixture is approved for use in Nigeria by National Agency for Food and Drug Administration and Control (NAFDAC). The polyherbal mixture was lyophilized, and then reconstituted to achieve the same unit (mg) with the standard drug (metformin). This makes it possible for us to use the same concentration/dosage unit (i.e. mg/kg) for both the polyherbal mixture and the standard drug.

Experimental Animals

Male Albino rats, average weight 96.37 ± 11.42 g were used for this study. The rats were obtained from the Animal Facility, University of Ibadan, Oyo State, and were acclimatized for two weeks before the commencement of the experiment. They were maintained in line with National Institutes of Health (2011) guide for the care and use of Laboratory animals. They were fed standard pelleted laboratory animal feed and water *ad libitum* at a room temperature of 22 ± 2 °C and $55 \pm 5\%$ relative humidity in a house where equal period (hour) of light and darkness was maintained (i.e. 12 h light/12 h darkness).

Induction of Type 1 Diabetes Mellitus (T1DM)

A modified method of Islam and Wilson (2012) was used to induce type 1 diabetes mellitus in rats. Successive triple intraperitoneal (i.p.) injection of streptozotocin dissolved in 0.1 M citrated buffer (pH 4.5) at (40 mg/kg, 45 mg/kg and 35 mg/kg of body weight) were given to the animals at three days interval after overnight fasting. The induction of type 1 diabetes mellitus was confirmed by the determination of high fasting blood glucose level with polydipsia (increased thirst) and polyuria (increased urination). Rats with fasting blood glucose level >200 mg/dl were selected for the

experiment.

Experimental Design

The streptozotocin-induced type 1 diabetic Albino rats were divided into four groups of five animals each in addition to the normal control group having the same number of animals. Group one, served as Normal Control (i.e. non-diabetic animals) - orally administered distilled water; group two served as Negative Control i.e. Diabetic Control (DC) - orally administered distilled water, while group three served as Positive Control i.e. Metformin (Met) - orally administered 15 mg/kg metformin, and group four and five served as Ruzu herbal bitters (RHB) treatment groups - orally administered RHB at 15 mg/kg and 30 mg/kg body weight respectively.

Fasting blood glucose and weight of animals were monitored weekly during the course of the experiment for four weeks (i.e. 28 days). At the end of this period, the animals were fasted overnight and anaesthetized using chloroform, and fasting blood sugar level measured by drop of blood from tail vein of animal on ACCU CHEK Strip fixed with Glucometer (Mannheim, Germany) and the reading taken. Finally, key organs (pancreas and intestinal crypt) were collected for insulin, glucagon-like peptide 1 (GLP-1) and glucose transporter 2 (GLUT-2) gene expression analyses.

Blood Glucose Determination

Blood glucose levels of experimental animals were determined in accordance with Ghasemi *et al.* (2014) reported method.

RNA Isolation with RNA Snap Kit

RNA isolation for gene expression was carried out using Timao (1996) method with some modifications. Target tissues were excised and homogenized in Eppendorf tubes containing RNA isolation reagent (RNA Snap kits). Tissue homogenates were centrifuged for 30 min at

17000 rpm and the supernatant (RNA phase) was carefully aspirated into new sterile labelled tubes. Sodium acetate (3 M) and iso-amyl alcohol were added to precipitate RNA. The samples were then incubated at -7°C for an hour, centrifuged for 30 min at 17000 rpm and 70% ethanol added to recover pelleted RNA. Further centrifugation at 17000 rpm for 5 min was done and the tubes containing the samples (RNA) uncapped for ethanol to evaporate. The RNA was then air-dried and re-suspended into sterile tube containing nuclease-free water and total RNA concentration was determined by UV absorbance spectrophotometry (JENWAY 6305). Finally, all samples were diluted to the same concentration for RNA (mRNA) quantification.

cDNA Synthesis

Successfully isolated and quantified RNA was immediately converted to complementary DNA (cDNA). A 2 μl of super-enzyme (Reverse Transcriptase) containing oligo primer, dNTP's, reverse transcriptase, reverse transcriptase buffer and nuclease-free water were added to 20 μl of isolated RNA for cDNA synthesis. The sample was incubated at room temperature in a thermocycler running for 4 h at $65^{\circ}\text{C}/12\text{h}$.

Polymerase Chain Reaction (PCR) Amplifications

The reaction system was first optimized before amplification process began. Template (cDNA), nuclease-free water, forward and reverse primers, and master mix were used to carry out polymerase chain reaction (PCR) using multigen optimax PCR machine for complete enzymatic reaction. Amplification conditions are: 94°C pre-denaturation for 5 min, 94°C for 30 sec, annealing 55°C (T_m) for 30 sec and extension 72°C for 30 sec and then 5 min at 72°C for further and final extension in 30 cycles. Thereafter, amplicons were electrophoresed in 0.5% agarose gel.

Primers Used

Target Gene	Forward 5'-3'	Reverse 5'-3'
B-actin	ACACTTTCTACAATGAGCTGCG	ACCAGAGGCATACAGGACAAC
Insulin	GAGGCTCTGTACCGTGGTGTG	ACCTCCAGTGCCAAGGTTT
GLP-1	ACCGTTTACATCGTGGCTGG	CCCTGTGAATGGCGTTCTC
GLUT-2	CCTGGCGTCTTCAGAGAGTG	ACCGAGGAAGGAATCGGTTT

Statistical Analysis

Statistical analysis of data was performed using IBM SPSS Statistics, Version 21 Software. The results were expressed as mean \pm SEM, and statistical difference was evaluated using two-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test. Data were considered statistically significant at $p < 0.05$.

RESULTS

Table 1 showed that there was no significant ($p > 0.05$) difference in weights of animals between groups at week 0 (i.e. before treatment) and also at week 4 (after treatment) except, the normal control animals' weight that was significantly ($p < 0.05$) different from others at week 4. Also, there was no significant ($p > 0.05$) difference in animals' weights between the initial weights (week 0) and final weights (week 4) of experimental animals in all groups except in the normal control group.

Table 1: Experimental Animals' Weight Data

Weeks	Treatment Groups				
	Normal Control	Diabetic Control	Metformin (15mg/kg b.wt)	Ruzu (15mg/kg b.wt)	Ruzu (30mg/kg b.wt)
					(g)
Week 0	99.28 \pm 8.97 ^a	88.67 \pm 6.14 ^a	94.41 \pm 4.68 ^a	92.32 \pm 9.57 ^a	95.67 \pm 7.61 ^a
Week 1	124.23 \pm 9.03 ^b	89.20 \pm 7.06 ^a	100.93 \pm 3.85 ^a	95.30 \pm 10.36 ^a	96.07 \pm 5.72 ^a
Week 2	135.97 \pm 8.26 ^b	89.69 \pm 7.75 ^a	98.76 \pm 8.21 ^a	96.14 \pm 11.15 ^a	97.39 \pm 5.96 ^a
Week 3	145.85 \pm 12.95 ^b	86.35 \pm 6.03 ^a	97.29 \pm 10.07 ^a	94.08 \pm 10.83 ^a	93.04 \pm 2.54 ^a
Week 4	148.30 \pm 10.16 ^b	83.76 \pm 6.19 ^a	106.39 \pm 14.32 ^a	86.13 \pm 10.96 ^a	95.47 \pm 2.71 ^a

Note: Comparison is both within groups (down column) and across groups (across column). Mean \pm SEM values with the same alphabet superscript are not significantly different from each other at ($p < 0.05$) using Duncan's New Multiple Range Test (DNMRT). Normal control (non-diabetic animals); Diabetic control (diabetic animals).

Table 2: Experimental Animals' Blood Glucose Concentrations

Weeks	Treatment Groups				
	Normal Control	Diabetic Control	Metformin (15mg/kg b.wt)	Ruzu (15mg/kg b.wt)	Ruzu (30mg/kg b.wt)
					(mg/dl)
0	72.00 \pm 5.25 ^a	330.20 \pm 26.42 ^b	342.40 \pm 21.89 ^b	335.80 \pm 20.60 ^b	340.75 \pm 33.87 ^b
1	74.80 \pm 3.97 ^a	404.20 \pm 52.47 ^b	374.20 \pm 38.03 ^b	351.00 \pm 44.13 ^b	380.75 \pm 23.32 ^b
2	68.40 \pm 2.66 ^a	240.00 \pm 44.83 ^b	385.25 \pm 38.45 ^b	338.00 \pm 48.26 ^b	235.50 \pm 79.02 ^b
3	72.75 \pm 4.27 ^a	196.60 \pm 55.17 ^a	195.33 \pm 57.71 ^a	189.40 \pm 25.87 ^a	193.33 \pm 62.83 ^a
4	71.75 \pm 2.25 ^a	249.80 \pm 62.32 ^a	291.50 \pm 10.50 ^a	281.75 \pm 76.70 ^a	197.67 \pm 85.86 ^a

Note: Comparison is both within groups (down column) and across groups (across column). Mean \pm SEM values with the same alphabet superscript are not significantly different from each other at ($p < 0.05$) using Duncan's New Multiple Range Test (DNMRT). Normal control (non-diabetic animals); Diabetic control (diabetic animals).

Table 2 revealed that baseline blood glucose levels of streptozotocin-induced type 1 diabetes mellitus animals in the four diabetic groups were significantly ($p < 0.05$) higher than that of the normal control group before treatments, but three to four weeks after treatments, there was no significant ($p > 0.05$) difference between the different experimental groups. Although, there were significant ($p < 0.05$) reductions in blood glucose levels of diabetic animals in all the four groups (diabetic control inclusive) three to four weeks after treatments in comparison with baseline values, 30 mg/kg RHB brought about more reduction in blood glucose levels (i.e. 340.75 \pm 33.87 to 193.33 \pm 62.83 mg/dl) than the other treatment groups, and it is the only diabetic treatment group where blood glucose levels of experimental animals is arguably and fairly constant between week three to four after treatment just like the normal control group.

Figure 1 showed that RHB (30 mg/kg) and metformin (15 mg/kg) down regulated pancreatic insulin gene expression of experimental animals in comparison with other treatment groups, normal control group inclusive. Furthermore, figure 2 revealed that RHB (15 mg/kg and 30 mg/kg) and metformin (15 mg/kg) up-regulated intestinal crypt glucose transporter 2 (GLUT-2)

gene expression in experimental animals in comparison with normal control and diabetic control groups. Additionally, figure 3 showed down regulation of intestinal crypt glucagon-like peptide 1 (GLP-1) gene expression of animals administered RHB (30 mg/kg) than the other treatment groups except diabetic control group.

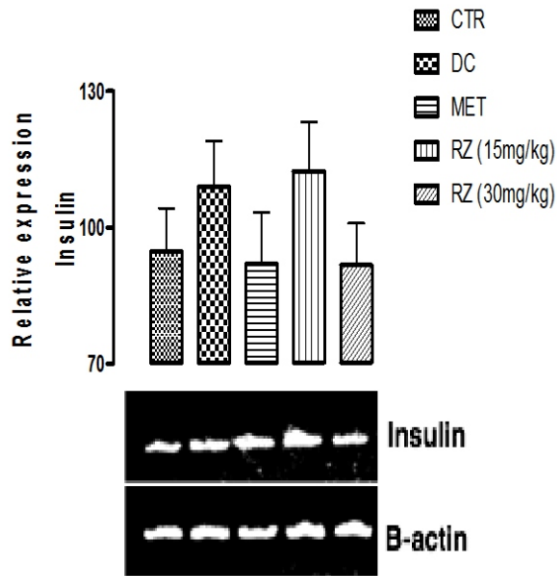


Figure 1: Pancreatic Insulin Gene Expression of Experimental Animals after Treatments

Key: CTR – Normal control; DC – Diabetic control; MET – Metformin; RZ – Ruzu herbal bitters; B-actin (beta actin). The down-regulation of insulin gene at 30 mg/kg RHB signifies that the polyherb at this dosage potentiated or mediated the hypoglycemic effect of the polyherb seen in table 2, because insulin is expressed in relation to blood glucose concentration.

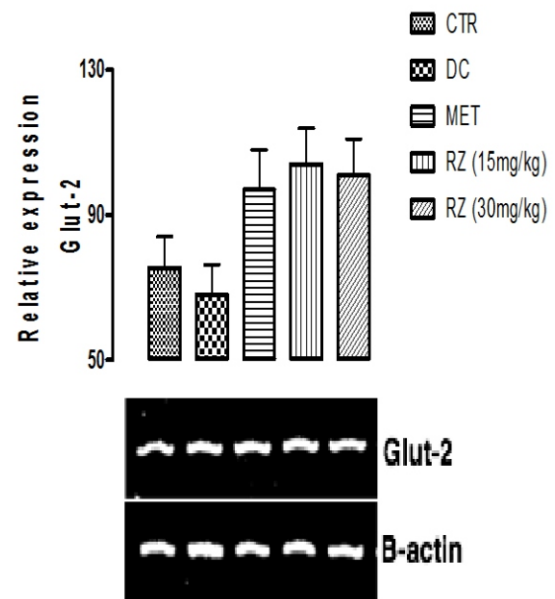


Figure 2: Intestinal Crypt Glucose Transporter 2 Gene Expression of Experimental Animals

Key: CTR – Normal control; DC – Diabetic control; MET – Metformin; RZ – Ruzu herbal bitters; Glut-2 (Glucose transporter 2); B-actin (beta actin).

The up-regulation of Glut 2 in RHB-treated animals signifies that RHB stimulated expression of Glut 2 in glucose concentration-dependent manner in order to facilitate glucose transportation into the cells for effective use by the body cells thus, reducing the concentration in the blood.

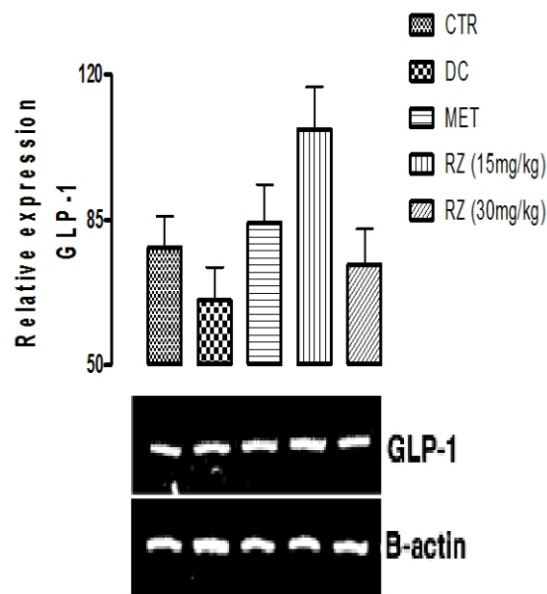


Figure 3: Intestinal Crypt Glucagon-like Peptide 1 Gene Expression of Experimental Animals

Key: CTR – Normal control; DC – Diabetic control; MET - Metformin; RZ – Ruzu herbal bitters; GLP-1 (Glucagon-like peptide 1); B-actin (beta actin).

The up-regulation of GLP-1 at 15 mg/kg RHB and converse down-regulation at 30 mg/kg signifies that RHB regulated GLP-1 expression in glucose concentration-dependent manner in RHB-treated animals. It was also expressed in relation to blood glucose concentration.

DISCUSSIONS AND CONCLUSION

Streptozotocin (STZ), a β -cytotoxin induces 'chemical diabetes' in a wide variety of animal species including rats (Akbarzadeh *et al.*, 2007) by selectively damaging the insulin-secreting β -cells of the pancreas (Ghasemi *et al.*, 2014). This leads to decrease in insulin levels, a condition termed hypoinsulinaemia (Rodrigues and McNeill, 1986; Siddiqui *et al.*, 2013) as a result of the destruction of the islet of Langerhans of β -cells of the pancreas.

The non-significant reduction in body weights (reductions and/or unchanged body weight) in streptozotocin-induced type 1 diabetes mellitus experimental animals compared to significant increase in body weights of normal control animals three to four weeks after treatments, suggests the effectiveness of streptozotocin in damaging DNA, or/and destroying structural proteins and lipids in the β -cells-containing tissues like pancreas, kidney, liver, intestines etc. through the synergistic action of both nitric oxide (NO) and reactive oxygen species (ROS) free radicals (Szkudelski, 2001), which in turn affect the animals' body weights. But, the almost constant

body weights of diabetic animals administered 30 mg/kg of RHB compared to slight decrease in weights of diabetic control animals suggests the ability of the polyherb at this dose to aid the recovery of animals from the devastating effects of streptozotocin and maintain body weights to prevent underweight or overweight. Overweight can result into obesity, a likely side effect when oral hypoglycemic agents such as sulfonylureas, thiazolidinediones etc are used for type 1 diabetes mellitus treatment. According to Lorenzati *et al.* (2010), all sulfonylureas and thiazolidinediones are associated with weight gain and in some cases underweight, and metformin, a biguanide (another class of synthetic hypoglycemic agents/drugs) used in this study is not an exception. The weight maintenance ability of RHB at 30 mg/kg in experimental animals may be due to its ability to prevent further destruction of the pancreatic β -cells initiated or caused by STZ in the course of inducing T1DM, while at the same time stimulating gradual regeneration of affected β -cells that in turn improve insulin levels, stabilize blood glucose levels, increase serum protein levels and inhibit muscle wasting (Choudhari *et al.*, 2017). According to Casteneda (2002), uncontrolled diabetes mellitus is associated with

severe muscle wasting, while according to Gillespie (2006) decrease in body weights in diabetes is due to impairment of insulin action in the conversion of glucose into glycogen. The observed body weights of experimental animals in this study are consistent with previous report of Kumar *et al.* (2012) that reported reductions in body weights of diabetic control rats. The body weights results are also in agreement with the findings of Choudhari *et al.* (2017) who reported that Ojamin (OJ) polyherb and metformin improved diabetic rats' weights.

The observed non-significant difference in blood glucose across all groups, including the diabetic control group three to four weeks after treatments; may be a signal of spontaneous recovery of rats from devastating effect of streptozotocin at low dosage/concentration. Junod *et al.* (1969) discovered some decades ago that a dose of 35 mg/kg of STZ resulted in spontaneous recovery of 25% diabetic rats from diabetic state. Streptozotocin rapidly undergoes metabolic degradation in rat liver and has a half-life of 6.9 min (Battell *et al.*, 1999) or 15 min (Srinivasan and Ramarao, 2007). Islam and Wilson (2012) also found that a single dose of 25 mg/kg or < 35 mg/kg of streptozotocin in rats produces no major effect, but they observed a stable diabetes state when 55 mg/kg to 65 mg/kg body weight of streptozotocin were used to induce diabetes. However, these high dosages/concentrations were avoided in this study due to high mortality rate associated with them. So, as observed in this study, using low doses of streptozotocin to induce diabetes mellitus in Albino rats seems to allow gradual recovery with time (signs of spontaneous recovery) since there was reduction in blood glucose levels of diabetic control animals although higher reduction was observed in 30 mg/kg RHB treated animals.

The observed significant reductions in blood glucose levels (i.e. 335.80 ± 20.60 mg/dl to 281.75 ± 76.70 mg/dl and 340.75 ± 33.87 mg/dl to 197.67 ± 85.86 mg/dl) of diabetic animals administered RHB (15 mg/kg and 30 mg/kg respectively) at the end of four weeks of treatment indicate the polyherb's anti-diabetic potentials at these therapeutic doses. This anti-diabetic

potential/activity might have involved one or more of its compounds/constituents that worked synergistically at this dose to induce hypoglycemic effect (decrease in blood glucose levels) on the rats (Sy *et al.*, 2005). The more pronounced decrease in blood glucose at 30 mg/kg of RHB further implies that RHB may possess yet-to-be discovered constituent(s) that could stimulate β -cell regeneration and secrete insulin in the pancreas, stimulate binding of insulin to its cognate receptor, and stimulate GLUT-2 to facilitate the entrance of glucose into the various cells/tissues (muscle, liver etc.) from the blood. This could be the possible mechanism that led to the observed decrease in blood glucose levels of treated animals. The constituent could as well have acted as antioxidant to scavenge free radicals in diabetic experimental rats that enhanced the observed RHB hyperglycemia-inhibiting activities (Choudhari *et al.*, 2017).

Furthermore, the improved glycemic effect of RHB at both 15 mg/kg and 30 mg/kg in comparison with that of metformin (15 mg/kg) as shown in table 2 is an indication that this polyherbal mixture is more effective than conventional hypoglycemic drugs (meglitinides, thiazolidinediones, α -glucosidase inhibitors, incretin mimetics (agonists), sulfonylureas and dipeptidyl peptidase 4 (DPP-4) inhibitors). The observed results affirm the statement of Lorenzati *et al.* (2010) that "metformin does not directly stimulate insulin secretion, but rather increases its action to mediate glucose utilization in peripheral tissues like muscle and liver, particularly after meals", and hence could not perform optimally in the treatment of type 1 diabetes mellitus, a diseased condition where insulin-producing cells are destroyed.

The ability of RHB to stimulate and regulate insulin gene expression when blood glucose level was high could be the reason for its high insulin gene expression (up-regulation) in experimental animals administered 15 mg/kg RHB when animals were in hyperglycemic condition, but low insulin gene expression (down-regulation) when blood glucose level was reduced as seen in animals administered 30 mg/kg of RHB from table 2 and figure 1. In a normal pancreatic β -cells, increase

in blood glucose concentration triggers insulin secretion but inhibits its release when the concentration is low (Thorens and Mueckler, 2010). Therefore, since at 15 mg/kg RHB where fasting blood glucose of experimental animals was high (281.75 ± 76.70 mg/dl), insulin gene expression was also high, but became low (i.e. decreased) at 30 mg/kg when fasting blood glucose level was reduced (197.67 ± 85.86 mg/dl), it is opined that RHB should possess health benefiting effect on insulin hormone that enabled it to regulate insulin gene expression of the animals in glucose concentration-dependent manner. It is also thought that the polyherb have regenerating effect on β -cells of the pancreas (i.e. ability of β -cells to overcome or recover from negative effect of streptozotocin to become functional to regulate insulin gene expression in glucose concentration-dependent manner as observed in table 2 and figure 1) that made experimental animals to up-regulate pancreatic insulin gene when blood glucose concentrations were high but conversely when blood glucose concentrations were low. According to Marty *et al.* (2007), glucose is the principal regulator of insulin secretion in intact pancreatic β -cells, and modulates the expression of insulin gene in β -cells of the endocrine pancreas. The finding on insulin gene expression from this study is in tandem with the position of Thorens and Mueckler (2010). Antoine *et al.* (1997) posited that “glucose is an important regulator of gene transcription in most prokaryotic and eukaryotic species”.

Additionally, the up-regulation in pancreatic insulin gene expression at 15 mg/kg RHB should in turn have caused the up-regulation in intestinal glucose transporter 2 (GLUT-2) gene expressions in animals observed in this study in order to facilitate the entrance of glucose from the blood into cells and peripheral tissues, and enhanced its clearance from the blood. According to Tobin *et al.* (2008), insulin has been reported to induce the internalization of apically expressed GLUT-2 in order to enhance glucose absorption, a process that is impaired in insulin resistance or insulin destruction. GLUT-2 activity is essential for both glucose secretion and keeping intracellular glucose-6-phosphate (Glu-6-P) concentration low

and could be needed to allow normal regulation for insulin secretion by glucose in β cells (Petchi *et al.*, 2014).

The gene expression of intestinal glucagon-like peptide 1 (GLP-1) as expected was up-regulated at 15 mg/kg RHB because of high blood glucose levels so as to augment insulin action, but was subsequently down-regulated at 30 mg/kg RHB (figure 3) when blood glucose levels had been reduced (table 2). This suggests that the polyherb could also have acted like GLP-1 agonist to stimulate GLP-1 gene expression and activity when blood glucose was high and a likely antagonist when blood glucose was low. Hence, RHB possesses the ability to decrease blood glucose levels in a glucose-dependent manner by enhanced insulin secretion and GLP-1 activities that led to the observed pronounced hypoglycemic effects. GLP-1, an incretin hormone, has insulinotropic effects and is associated with numerous regulatory and protective effects (Drucker and Nauck, 2006). Thus, for GLP-1 to have been well regulated in glucose-dependent manner suggests that: RHB might possess ability to inhibit DPP-4 action (an enzyme that degrades GLP-1 in one or two minute(s) after its production (Demuth *et al.*, 2005)), which in turn enhanced insulinotropic effects of GLP-1 that led to the observed decrease in blood glucose levels of animals.

Conclusively, the observed results in this study unequivocally established the anti-diabetic effects of RHB especially at 30 mg/kg therapeutic as it reduced elevated blood glucose concentrations and as well regulated insulin, GLP-1 and GLUT-2 gene expressions in glucose-dependent manner in studied animals.

Conflict of Interest: None

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