



# Russelioside B, a pregnane glycoside ameliorates hyperglycemia in streptozotocin induced diabetic rats by regulating key enzymes of glucose metabolism



Essam Abdel-Sattar <sup>a,\*</sup>, Shohda A. EL-Maraghy <sup>b</sup>, Riham Salah El-Dine <sup>a</sup>, Sherine M. Rizk <sup>b</sup>

<sup>a</sup> Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

<sup>b</sup> Biochemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

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## ABSTRACT

An alternative strategy to treat diabetes mellitus is the use of various natural agents possessing hypoglycemic effect. *Caralluma quadrangula* has been used in Saudi traditional medicine in cases of thirst and hunger and for the treatment of diabetes. The present study was designed to evaluate the improving effect of russelioside B, a pregnane glycoside isolated from *Caralluma quadrangula* on glucose metabolism in the liver of streptozotocin-induced diabetic rats. Diabetes mellitus was induced in rats by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight). Experimental rats were administered russelioside B at a dose of 50 mg/kg body weight once a day for 30 days. The results showed that RB improved the fasting serum glucose level, glycated hemoglobin percent, serum insulin level and lipid profile. A significant improvement was observed upon the administration of russelioside B on the activities of the key enzymes of carbohydrate metabolism (glucokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, and glycogen phosphorylase) in the liver of diabetic rats. Further, russelioside B administration to diabetic rats reverted gene expression of glucokinase, glucose-6-phosphatase, glycogen synthase and glycogen synthase kinase-3 $\beta$  to near normal levels. In conclusion, russelioside B possess antidiabetic and antihyperlipidemic effect in streptozotocin induced diabetic rats. Hence, administration of russelioside B may represent a potentially useful strategy for the management of diabetes.

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## 1. Introduction

Diabetes mellitus (DM) has emerged as a global epidemic, which is predicted to worsen in the coming decades, particularly in developing countries. Rapid urbanization, changes in nutrition, dietary patterns, sedentary habits, and obesity have all contributed to the progression of the epidemic in the Middle East. The current prevalence of diabetes in the Middle East and North Africa (MENA) region is estimated to be 9.2%, which is equivalent to 34.6 million people with diabetes. This figure is expected to double by 2035 to 67.9 million [1]. Nearly half of the people with type 2 diabetes mellitus (T2DM) remain undiagnosed, and in those who are diagnosed, glycemic control is suboptimal, which potentially increases

the risk of diabetes complications and results in poor health outcomes. As a global problem, no satisfactory therapeutic regimen can cure diabetes although most of them normalize blood glucose and fat levels, possess hypotensive activity, and improve microcirculation [2]. As reported previously, insulin injection and some oral antihyperglycemic agents, such as metformin and pioglitazone, display undesirable adverse effects [3].

Therefore, a search for new antidiabetic drugs is of high demand including alternative herbal medicine. The utility of natural products as sources of novel structures remains an ever evolving source for compounds of medicinal importance. The use of medicinal plants for the treatment various human diseases are well known and documented [4].

Several *Caralluma* species rich in pregnane glycosides showed antihyperglycemic activity of their crude extracts or their corresponding fractions [5–8]. *Caralluma quadrangula* extract has been used in Saudi traditional medicine in cases of thirst and hunger and for the treatment of diabetes [9]. In a previous study, we isolated in

\* Corresponding author.

E-mail addresses: [abdelsattar@yahoo.com](mailto:abdelsattar@yahoo.com), [essam.abdelsattar@pharma.cu.edu.eg](mailto:essam.abdelsattar@pharma.cu.edu.eg) (E. Abdel-Sattar).

a biologically-guided fractionation approach, four new acylated pregnane glycosides from the chloroform fraction of *Caralluma quadrangula* [10] and the isolated compounds were tested for their cytotoxic activity against breast cancer (MCF7) cell line. We have chosen for isolating one of its active principles, Russelioside B (RB), and tested its efficacy on glucose utilization pathways and on hepatic glucose production since both of them contribute significantly to plasma glucose levels. For this, the activities of key enzymes of carbohydrate metabolism (glucose utilization/production) are measured in streptozotocin-induced diabetic rats as there is no detailed study has been carried out on this aspect.

## 2. Materials and methods

### 2.1. Chemicals

Streptozotocin (STZ),  $\alpha$ -D-glucose, glucose-6-phosphate, 6-phosphogluconate, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP) and glycogen were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals were from pure analytical grade or from the highest purity available commercial grade.

### 2.2. Plant materials

The whole plant of *Caralluma quadrangula* (Forssk.) N.E.Br. (syn. *Stapelia quadrangula* Forssk.) was collected from Bani Salem, Abha–Al-Taif road, Saudi Arabia, in May 2010 and were dried in the shade. A specimen was deposited in the herbarium of College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (# CQ 1027).

### 2.3. Extraction and isolation

The air dried powdered aerial parts of *C. quadrangula* (480 g) were extracted with methanol (3 × 2 L) on cold using Ultra-turrax T50 homogenizer. The solvent was evaporated under reduced pressure to give 82 g of brown residue. Part of the residue (65 g) was suspended in distilled water (300 ml) and partitioned successively with chloroform (4 × 500 ml), and *n*-butanol (4 × 500 ml) to yield 8.8 and 35.8 g, respectively. Russelioside B (calogenin 20-*O*- $\beta$ -D-glucopyranosyl-3-*O*-[ $\beta$ -D-glucopyranosyl-(1 → 4)- $\beta$ -D-(3-*O*-methyl-6-deoxy)galactoside] was isolated from *n*-butanol fraction by chromatography on a Si gel column following procedure reported by Al-Yahya et al. [11]. The purity of compound RB was checked by melting point, superimposed IR, LC-ESI-MS [12] and by comparing its spectral data (<sup>1</sup>H- and <sup>13</sup>CNMR) with those reported in the literature [11].

### 2.4. Experimental animals

10–12 weeks male albino Wistar strain rats weighing 200–220 g were included in this study. All animals were housed in plastic cages with free access to drinking water and a pellet diet, under controlled conditions of humidity (50 ± 10%), light (12/12 h light/dark cycle) and temperature (25 ± 2 °C). At the end of the treatment, rats were fasted overnight and killed by cervical decapitation. The investigation complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The experimental protocol is conducted according to the guidelines of the ethical committee for animals experimentation at the faculty of pharmacy Cairo University.

### 2.5. Experimental design

Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of STZ (50 mg/kg body weight) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) [13,14]. STZ injected animals were allowed to drink 5% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. The animals were allowed to recover for three days before their blood glucose levels were tested using Accucheck Active glucose strips (Roche Diagnostics Polska Ltd., Warszawa, Poland) from blood samples collected via the tail vein. Rats were considered diabetic if their fasting blood glucose level was at or over 14 mmol/L. Animals that did not become diabetic were not used in the study.

The rats were assigned into the following groups:

**Group 1:** Six healthy rats have received both vehicles (0.1 M citrate buffer, pH 4.5, i.p.) once at the beginning of the experiment and daily oral dose of 0.5% aqueous solution of carboxy methyl cellulose (0.5% CMC) for 30 days and served as normal control group.

**Group 2:** Six diabetic rats were received a daily oral dose of 0.5% CMC for 30 days, served as a diabetic group.

**Group 3:** Six diabetic rats were received a daily oral dose of RB suspended in 0.5% CMC in a dose of 50 mg/kg body weight for 30 days, and served as a RB-treated group.

### 2.6. Sample collection

At the end of the experimental period, the rats were fasted overnight, sacrificed by cervical decapitation. The blood samples were collected and divided into two aliquots; one was processed for serum preparation used for assaying the levels of glucose, insulin, and lipid profile. The other was collected in heparinized tubes and used for the estimation of glycated hemoglobin percentage (HbA1c %). The liver tissue was dissected out, washed in ice-cold saline, and used to measure glycogen contents, glucose metabolism related enzymes and mRNA expression levels of glucokinase (GK), Glucose-6-phosphatase (G6Pase), glycogen synthase (GS) and glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ). Body weights of all the animals were recorded prior to the treatment and sacrifice.

### 2.7. Determination of LD<sub>50</sub>

LD<sub>50</sub> was determined according to the procedures developed by Karber (1931) [15]. Male Albino mice weighing 25–30 g and eight-week old were obtained from laboratory of National research center, Giza, Egypt. All the animals were maintained in a controlled environment condition of temperature (22 ± 3 °C) in alternative 12 highlight/dark cycles and were fed on standard diets with a regular supply of water and libitum. A minimum of 5 days acclimatization was allowed before the commencement of the study. The animals were fasted overnight prior dosing. A batch consisting of 10 male mice was administered with a single dose orally once with varied concentrations (500–5000 mg/kg bw) of the test compound dissolved in 10% tween 80 in distilled water. Similarly, control group I was administered 10% tween 80 in distilled water while control group II was administered distilled water only. All the animals (treated and control) were observed for 15 days. Observations were made at least once during the first 30 min and every 4 h in the first day and once every day for the next 14 days.

### 2.8. Biochemical analysis

Fasting serum glucose (FSG) was assayed enzymatically

(Stanbio, San Antonio, TX, USA). HbA1c % was measured by high performance liquid chromatography (HPLC) using hemoglobin testing kits (Bio-Rad D-10, France). Serum insulin level was determined by ELISA (DRG International, New Jersey, USA) according to the manufacturers' instructions. The concentrations of serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and triglyceride (TG) were measured by using kits supplied by Stanbio Laboratory (Boerne, Texas, USA) at the end of the experiment. Atherogenic index (AI) was calculated from the data. The atherogenic index (AI) was calculated according to the following formula:

$$AI = TC/HDL - C$$

### 2.9. Measurement of carbohydrate metabolic enzymes

GK activity was estimated by recording the increase in the optical density at 340 nm due to the reduction of NADP in the presence of an excess of glucose-6-phosphate dehydrogenase (G6PDH) [16]. Glycogen phosphorylase was estimated according to Hers and Van Hoof (1966) in which, glycogen phosphorylase acts on the terminal glucose-1-phosphate in glycogen molecule, splitting it into glucose and inorganic phosphate [17]. The liberated inorganic phosphate is then estimated colorimetrically at 680 nm. G6Pase activity was estimated in liver homogenate after incubation with glucose-6-phosphate at 37 °C for 15 min, the reaction was stopped by addition of 10% trichloroacetic acid. The activity was estimated by measuring the amount of inorganic phosphate released [18] and G6PDH activities were assayed in the liver according to the methods of Glock and McLean (1953), by recording the rate of reduction of NADP at 340 nm [19]. The activity measured at pH 7.6 with 6-phosphogluconate as substrate was that of 6-phosphogluconate dehydrogenase while the activity measured at the same pH but using 6-phosphogluconate and glucose-6-phosphate as substrates were referred to the summation of 6-phosphogluconate dehydrogenase and G6PDH. The G6PDH was taken as the difference between the two activities. Glycogen content was determined by dehydration of glycogen solution by heating with concentrated sulphuric acid, resulting in the development of pink color, the intensity of which is directly proportional to the glycogen concentration [20]. Protein content was measured by the method of Lowry et al. (1951) [21].

### 2.10. RNA extraction and real-time PCR analysis

The mRNA expression levels of GK, G6Pase, GS and GSK-3 $\beta$  were examined by quantitative real time-polymerase chain reaction (qRT-PCR). Total RNA extraction from the liver was done using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The purity and concentration were determined spectrophotometrically at the optical density of 260 and 280 nm before use. The optical density ratio at 260/280 nm ranged from 1.7 to 2.0. The isolated total RNA was reverse-transcribed into complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions and all products were stored at -20 °C. The expression of target genes were analyzed by qPCR using the SYBR Green PCR Master MIX (Applied Biosystems, California, USA) with the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) and relative quantification software (Applied Biosystems, Foster City, CA). The sequences of the primers used are listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the house-keeping gene. The thermal cycle protocol consisting of initial denaturation at

95 °C for 10 min followed by 40 cycles with 30 s denaturation at 95 °C and 30 s annealing/extension at 60 °C. As a relative quantification, fold changes were calculated following the  $2^{-\Delta\Delta Ct}$  method. For each sample, the Ct value of target gene mRNA was normalized to the GAPDH endogenous control as  $\Delta Ct$  ( $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{GAPDH}}$ ). The fold change of the target gene mRNA in the experimental sample relative to control sample was determined by  $2^{-\Delta\Delta Ct}$ , where  $\Delta\Delta Ct = \Delta Ct_{\text{Experimental}} - \Delta Ct_{\text{Control}}$  [22].

### 2.11. Statistical analysis

All the values were expressed as mean  $\pm$  SEM of 6 rats from each group and statistically evaluated by one-way analysis of variance followed by Tukey-Kramer's multiple comparisons tests using Statistical Package for the Social Sciences (SPSS) Version 16.0 (SPSS, Cary, NC, USA). A value of  $P < 0.05$  was considered statistically significant.

## 3. Results

RB was first isolated by Al-Yahya et al. (2000) from *Caralluma russeliana* in a high yield (~3% of dry powder) [11] and further isolated from *Caralluma tuberculata* (unpublished data) and quantified by LC-MS [12].

### 3.1. Determination of LD<sub>50</sub>

The investigated RB was found to be non-toxic up to a maximum soluble dose (LD<sub>50</sub> = 5.0 g/kg body wt) when tested for its acute toxicity in mice.

### 3.2. Effect of RB on body weight, FSG, HbA1c and insulin

Results represented in Table 2 showed that body weight was significantly decreased in diabetic rats in comparison with normal control rats. Meanwhile, administration of RB failed to ameliorate this fall in body weight. Diabetic rats showed a significant increase in FSG and HbA1c%, a matter which reflected an overt hyperglycemia in this group, whereas serum insulin level was significantly reduced as compared with normal control rats. On administration of RB to diabetic rats, a significant decrease in FSG (55.8%) and HbA1c% (29.6%) and a significant increase in insulin levels (40.8%) were observed by the end of the experimental period.

### 3.3. Lipid profile and atherogenic index

Our observation provides further support to the growing body of evidence showing that STZ-induced diabetes can also induce anomaly of serum TC, TG, HDL-C, and AI. Table 3 showed that the levels of serum TC, TG and the AI were significantly increased by 28%, 27% and 56%, respectively, whereas, HDL-C significantly decreased in diabetic rats reaching to 83% of the values obtained in normal control. RB administration showed a significant decrease in the levels of serum TG, and AI with a significant increase in the level of HDL-C after 30 days treatment when compared with the diabetic group.

### 3.4. Hepatic glycogen content and glycogen metabolizing enzymes

Diabetic animals showed marked decline in liver glycogen and GS gene expression, roughly by about 42% and 38% of control values, respectively. STZ induced an increase in glycogen phosphorylase and expression of GSK-3 $\beta$  were amounting to approximately 1.3 and 2.8 folds, respectively compared to their control counterparts (Fig. 1). Conversely, oral treatment with RB to diabetic

**Table 1**  
The oligonucleotide primers sequence of studied genes and the house keeping gene.

Accession no.	Gene name	Forward primer	Reverse primer
L41631	GK	5'-TCCACCTCCAGAGATTAG-3'	5'-AGGAAGAGGAAGAGGATAG-3'
U00445	Glc-6-Pase	5'-GCATTGCCAGGAAGAGAAAG-3'	5'-AACTGAAGCCGTTAGACATAG-3'
NM_030678.3	GS	5'-ACGGGCTGAATGTGAAGAAG-3'	5'-GAAGTCCAGGTGCCATAAA-3'
NM032080.1	GSK-3 $\beta$	5'-TGGTCCGAGGAGAGCCCAATGT-3'	5'-TGCCTTGGTGGAGTTCCGGG-3'
NM_008084	GAPDH	5'- AACAGCAACTCCACTCTTC-3'	5'- TGGGTGCAGCGAAGCTTTAT-3'

**Table 2**  
Effect of russelioside B administration on changes in the body weight, FSG, HbA1c and insulin levels.

	Normal	Diabetic	Diabetic + russelioside B
Body weight (g) (day 0)	210.33 $\pm$ 7.39	214.67 $\pm$ 8.07	211.67 $\pm$ 8.29
Body weight (g) (day 30)	237.83 $\pm$ 6.35	188.83 $\pm$ 8.59 <sup>a*</sup>	183.50 $\pm$ 10.68 <sup>a*</sup>
Body weight gain (g)	27.50 $\pm$ 4.33	-25.83 $\pm$ 3.40 <sup>a*</sup>	-23.17 $\pm$ 0.65 <sup>a*</sup>
FSG (mmol/L) (day 0)	4.66 $\pm$ 0.34	18.37 $\pm$ 0.98 <sup>a*</sup>	18.85 $\pm$ 1.01 <sup>a*</sup>
FSG (mmol/L) (day 30)	4.85 $\pm$ 0.24	19.42 $\pm$ 1.41 <sup>a*</sup>	8.57 $\pm$ 0.83 <sup>ab*</sup>
HbA1c%	3.46 $\pm$ 0.27	8.41 $\pm$ 0.51 <sup>a*</sup>	5.92 $\pm$ 0.32 <sup>a*b*</sup>
Insulin ( $\mu$ g/l)	0.99 $\pm$ 0.07	0.45 $\pm$ 0.04 <sup>a*</sup>	0.76 $\pm$ 0.06 <sup>ab*</sup>

Data are expressed as mean  $\pm$  SEM for six animals in each group. FSG, fasting serum glucose and HbA1c%, glycated hemoglobin percent.

<sup>a, a\*</sup> Significant different from normal at  $P < 0.05, 0.01$ , respectively.

<sup>b\*</sup> Significant different from normal at  $P < 0.01$ .

**Table 3**  
Effect of russelioside B administration on changes in serum lipid profile in STZ-induced diabetic rats.

	Normal	Diabetic	Diabetic + russelioside B
TC (mmol/L)	1.91 $\pm$ 0.11	2.45 $\pm$ 0.09 <sup>a</sup>	2.05 $\pm$ 0.13
TG (mmol/L)	0.88 $\pm$ 0.05	1.12 $\pm$ 0.06 <sup>a*</sup>	0.92 $\pm$ 0.03 <sup>b</sup>
HDL-C (mmol/L)	0.79 $\pm$ 0.04	0.66 $\pm$ 0.02 <sup>a</sup>	0.82 $\pm$ 0.04 <sup>b*</sup>
AI (Unit)	2.42 $\pm$ 0.14	3.77 $\pm$ 0.18 <sup>a*</sup>	2.53 $\pm$ 0.22 <sup>b*</sup>

Data are expressed as mean  $\pm$  SEM for six animals in each group. TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein- cholesterol and AI, atherogenic index.

<sup>a, a\*</sup> Significant different from normal at  $P < 0.05, 0.01$ , respectively.

<sup>b, b\*</sup> Significant different from normal at  $P < 0.05, 0.01$ , respectively.

rats partially recovered the level of glycogen, the activity of glycogen phosphorylase and GS and GSK-3 $\beta$  mRNA expression levels to near normal control rats.

### 3.5. Hepatic key enzymes of carbohydrate metabolism

Fig. 2 portrays the changes in the activities of carbohydrate metabolizing enzymes in the liver of control and experimental rats. The activities of GK, and G6PDH were significantly decreased by 60% and 31%, respectively, whereas the activity of G6Pase was significantly increased by 95% in diabetic rats when compared to normal control rats. Upon treatment of diabetic rats with RB, it normalized the activity of G6PDH with partial recovery in the activities of GK and G6Pase activities.

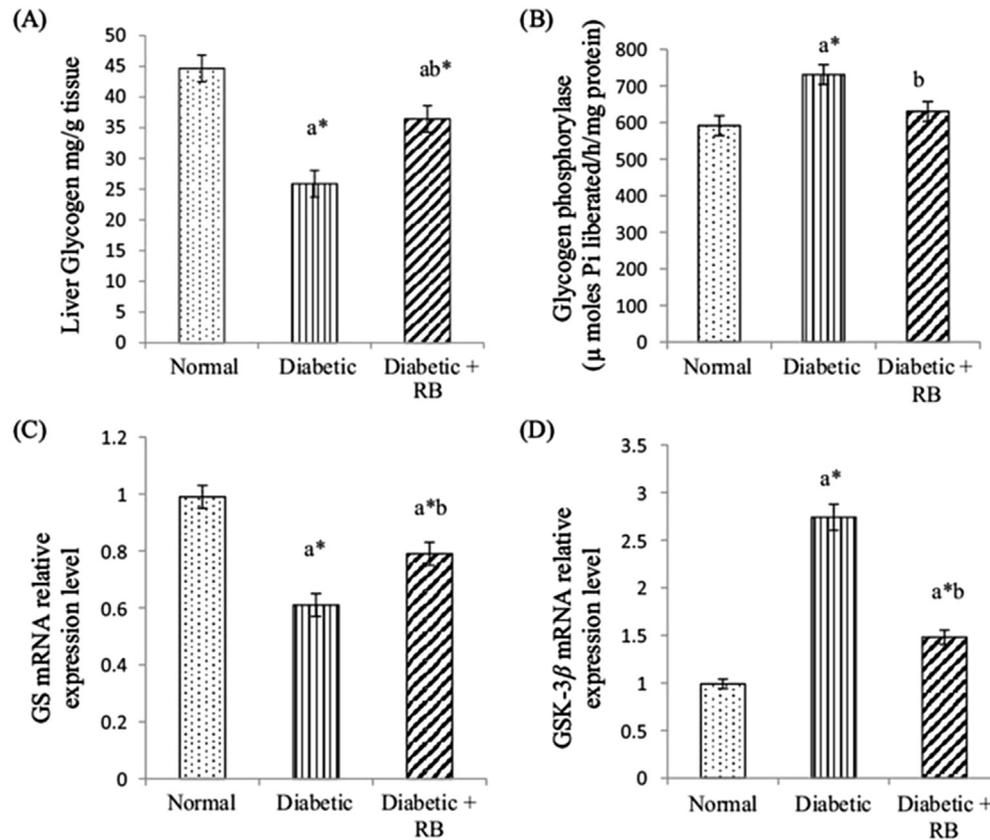
## 4. Discussion

The objective of this study is to investigate the potential anti-hyperglycemic effect of RB (Fig. 3) as well as to study some of the metabolic pathways related to glucose metabolism. Administration of RB to diabetic rats decreased the elevated FSG level which is an essential trigger for the liver to revert its normal homeostasis during experimental diabetes. Furthermore, RB supplement significantly lowered the HbA1c level, a useful parameter in the monitoring of long-term blood glucose with a concomitant increase in serum insulin level. The hypoglycemic action of RB may have two mechanisms of actions. RB may reduce the blood glucose level by

intestinal glucose absorption, or/and by stimulation of insulin release from the pancreas. Yoshikawa (2002) proposed that steroidal glycosides partially stay on the surface of the small intestine and lower glucose absorption, thus controls the rise in blood sugar levels [23]. On the other hand, the assimilated portion of the pregnane glycoside is metabolized via the production of progesterone [24]. Progesterone have a significant role in the control of hyperglycemia at least in the animal where the pancreatic  $\beta$ -cells are dysfunctional, through the production of insulin due to the progesterone receptor activated nitric oxide (NO) synthesis in the liver cells [25]. Thus, RB effect on glucose solidly confirmed its antihyperglycemic activity. Furthermore, pregnane glycoside was found to down regulate the production of corticosteroid, one of insulin antagonists, in human adrenocortical cells [26].

In the present study, STZ- induced diabetic rats showed signs of weight loss, polyuria, polydipsia, and polyphagia. Decrease in body weight of diabetic rats is due to catabolism of fats and proteins. Due to insulin deficiency, the protein content is decreased in muscular tissue by proteolysis [27]. Moreover, there is no significant difference in the final body weight and body weight gain trend between the diabetic control rats and RB-treated diabetic rats. The failure of RB to restore the decreased body weight may be due to the appetite suppressant effect of pregnane glycoside [28,29].

The STZ-induced diabetic rats exhibited obvious changes of lipid metabolism as evidenced from significant elevations of serum TC and TG, as well as AI, and reduced HDL-C levels. The high concentration of serum lipids in the diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots since insulin inhibits the hormone sensitive lipase. The marked hyperlipemia that characterized the diabetic state may, therefore, be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [30]. The present study demonstrates that RB was able to reduce both TG and TC to significant levels which are very encouraging. It has additionally shown a significant increase in the levels of HDL- C. The decline in TG and TC level after administration of RB could be due to the increase in insulin level promoted by RB. It was found that RB inhibits cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton-induced hyperlipidemia [31]. The reduction of serum TG following treatment with RB might also facilitate glucose oxidation



**Fig. 1.** Effect of pregnane glycoside administration on changes in liver A: glycogen content; B: glycogen phosphorylase activity; C: and mRNA expression level of glycogen synthase (GS) and D: glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) of STZ-induced diabetic rats. Data are expressed as mean  $\pm$  SE for six animals in each group. <sup>a</sup>, <sup>a\*</sup>Significant different from normal at  $P < 0.05$ ,  $0.01$ , respectively. <sup>b</sup>, <sup>b\*</sup> Significant different from normal at  $P < 0.05$ ,  $0.01$ , respectively.

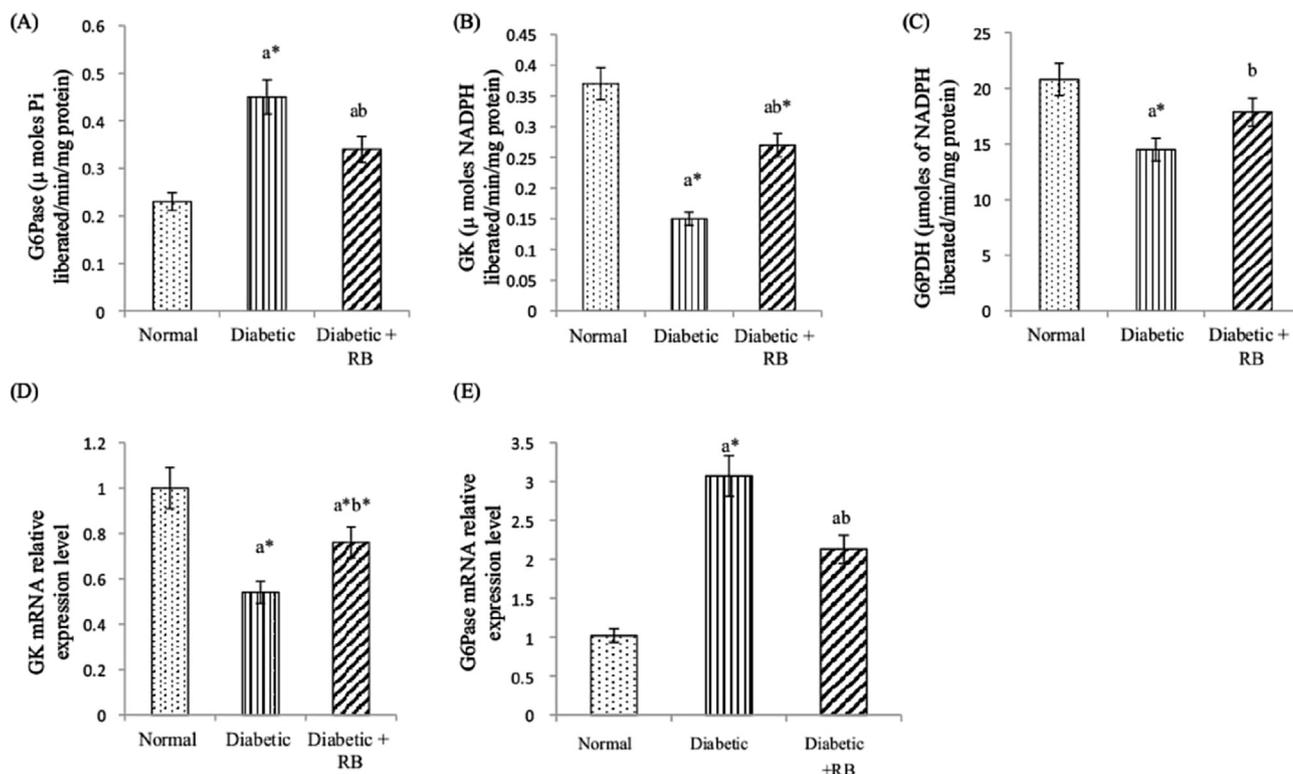
and utilization and subsequently, lead to the reduction of hyperglycemia.

Glycogen is the primary intracellular form in which glucose is stored and its levels in various tissues, particularly the liver, are a direct indication of insulin activity as insulin enhances intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase [32]. In the present study, liver glycogen content was markedly decreased in STZ-induced diabetic rats as well as the expression of GS enzyme with a concomitant increase in the activity of glycogen phosphorylase and GSK-3 $\beta$  expression. Since STZ causes selective destruction of pancreatic  $\beta$ -cells resulting in an apparent decline in insulin levels which is responsible for the decreased glycogen levels in liver, as it depends on insulin for entry of glucose [33]. During diabetic conditions, the glycogen levels, GS activity and the response to insulin signaling are diminished and glycogen phosphorylase activity is significantly increased [34]. RB glycoside administration to diabetic rats regulated the activity of glycogen metabolizing enzymes by stimulating the remnant  $\beta$ -cells to secrete more insulin thereby normalized the altered glycogen content in the liver. Activation of GSK-3 $\beta$  leads to the reduction in glycogen synthesis. Hence, we studied the effect of RB in modulating this insulin signaling molecule. Increased expression of GSK-3 $\beta$  seen in diabetic animals was decreased in RB-treated animals. The decrease in the mRNA expression of GSK-3 $\beta$  in our study is correlated with an increase in hepatic glycogen content.

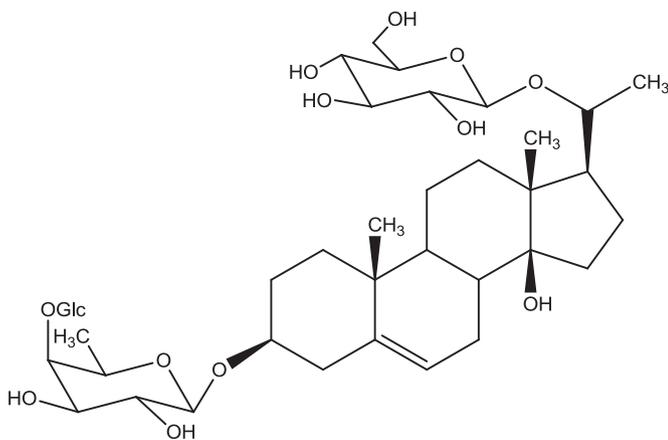
In the current study, detailed examination of enzymes involved in hepatic glucose metabolism demonstrated a significant decrease in GK activity and its expression in the liver of diabetic rats. GK

plays a key role in the first step of hepatic glucose metabolism. Hepatocytes are able to respond to a fluctuation in blood glucose level by parallel changes in the rate of glucose phosphorylation. The key enzyme in the regulation of that process is GK. The regulation of GK activity has been strongly correlated with corresponding changes of mRNA and with rates of transcription of GK gene. In diabetic animals, where glycolysis is inoperative and gluconeogenesis is increased, glucokinase mRNA is very low [35] and decrease in its expression, could be also attributable to insulin hyposecretion [36]. RB administration to that group of rats showed an increase in GK activity as well as its expression in compared to diabetic rats. This result could be attributed to the profound increase in insulin concentration in such group of rats. Therefore, whether the normalization of GK in diabetic animals is due to direct effect of RB and/or to the impact of insulin on GK needs to be further clarified.

The hepatic gluconeogenic enzyme, G6Pase, is an important enzyme in the last step of gluconeogenesis and glycogenolysis where it catalyzes the hydrolysis of glucose-6-phosphate to glucose. Glucose is transported out of the liver to increase blood glucose concentration. Physiologically, insulin slows down hepatic glucose production by reducing G6Pase activity [37]. In the present study, the hepatic gluconeogenic enzyme, G6Pase activity was elevated significantly in diabetic rats. This may be due to the increased synthesis of the enzymes as we observed an increase in the expression of it in hepatic tissue. The increased G6Pase activity, in turn, led to a further increase in hepatic glucose production and aggravation in the glucose metabolic imbalance [38]. Administration of RB significantly decreased the activity and expression of G6Pase enzyme in diabetic rats. The level of plasma insulin was



**Fig. 2.** Effect of pregnane glycoside administration on changes in liver A: glucose-6-phosphatase (G6Pase); B: glukokinase (GK); C: glucose-6-phosphate dehydrogenase (G6PDH) activities and mRNA expression level of D: glukokinase (GK) and E: G6Pase of STZ-induced diabetic rats. Data are expressed as mean  $\pm$  SE for six animals in each group. <sup>a</sup>, <sup>a\*</sup>Significant different from normal at  $P < 0.05$ ,  $0.01$ , respectively. <sup>b</sup>, <sup>b\*</sup> Significant different from normal at  $P < 0.05$ ,  $0.01$ , respectively.



**Fig. 3.** Structure of russelioside B.

found to increase significantly in diabetic rats treated with RB which may be a leading cause for the significant reduction in the level of the gluconeogenic enzyme. The reduction in the activity of this gluconeogenic enzyme can result in the decreased concentration of glucose in the blood.

Decreased activity of G6PDH enzyme, the rate-limiting step of the pentoses monophosphate shunt pathway, was observed in diabetic rats which are in agreement with other studies [39]. Xu et al. (2005), suggested that both posttranslational modification (phosphorylation) and decreased expression of G6PDH enzyme play a role in diabetes. Diabetic rats treated with RB showed a significant increase of liver G6PDH activity, via increased secretion of insulin, which might increase the influx of glucose into the

pentose monophosphate shunt and this resulted in a decrease in FSG level [40].

## 5. Conclusion

To the best of our knowledge, this is the first study on the antidiabetic activity of RB glycoside in an animal model. Since, RB exhibited anti-hyperglycemic and anti-hyperlipidemic potential, which acts by improving insulin secretion and the alterations in the carbohydrate and lipid metabolism, we can conclude that RB isolated from aerial parts of *Caralluma quadrangula* represent as a good natural product candidate could be used in the management of diabetes mellitus. Further deeper mechanistic study is needed in a future study to elucidate the interaction between RB and the receptors of the specific genes coding for glycolytic enzymes, in addition to the investigation of other minor related compounds. Similar to *Caralluma fimbriata* extract, a useful nutraceutical used as an anti-obesity/appetite-suppressant [41], the alcohol extract or RB from *Caralluma quadrangula* could represent another example to be developed as support or in treatment of diabetes mellitus after deeper future studies.

## Competing interests

The authors declare that there is no conflicts interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.cbi.2016.03.033>.

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