Ameliorative potential of Cortex Lycii on enzymes involved in carbohydrate metabolism in streptozotocin-nicotinamide induced diabetic rats

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Abstract: Cortex Lycii *(root back of Lycium chinense)* has is a famous traditional Chinese medicine which displays several pharmacological activities including antioxidant and antidiabetic properties. We investigated the effect of the ethyl acetate fraction (QCL) of Cortex Lycii on the enzymes involved in the metabolism of carbohydrate in diabetic rat models. Streptozotocin-nicotinamide (110 and 65mg/kg body weight, respectively) was used to induce diabetes. Diabetic rats were treated with QCL (100, 200 and 400 mg/kg) and glibenclamide (600 µg/kg) daily for six weeks. Upon the completion of treatment, fasting blood glucose (FBG), insulin, glycosylated haemoglobin (HbA1c), haemoglobin (Hb), hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase levels were measured by biochemical assays. Likewise, the body weight, food and water intake was monitored and measured. Diabetic rats displayed significant elevation in the blood glucose, glycosylated hemoglobin and a marked decrease in plasma insulin and hemoglobin. Furthermore, the levels of key enzymes including fructose-1,6-bisphosphatase, glucose-6-phosphatase phosphoenolpyruvate carboxykinase were significantly increased while the activity levels of hexokinase, glucose-6-phosphate dehydrogenase and glycogen were significantly down regulated in diabetic rats. However, treatment of diabetic rats with Cortex Lycii led to a significant reduction the FGB, food and water intake and an increase in the plasma insulin level. Treatment with Cortex Lycii also reversed the altered activity profiles of the key enzymes mentioned above in a dose dependent manner. Our results suggested that Cortex Lycii has a promising therapeutic option in the management of diabetic complications relating to glucose homeostasis and carbohydrate metabolism.

Keywords: Cortex lycii, diabetes mellitus, carbohydrate metabolism, glucose, streptozotocin, nicotinamide.

INTRODUCTION

Diabetes mellitus is a complex group of metabolic disorder that is associated with chronic hyperglycemia, abnormal carbohydrate, lipid and protein metabolism which occurs due to deficiency in insulin production or insulin resistance (Belvis *et al*., 2009; Akkati *et al*., 2011). The International Diabetes Federation has estimated by that about 400 million people have diabetes globally with this figure to increase to about 600 million by the year 2035 Federation (International Diabetes Federation, 2014). Diabetes is associated with several micro vascular and macro vascular complications and it is considered as a serious global health care challenge.

The continuous decline in the function of the β-cells of pancreas can lead to a progressive reduction in the secretion of insulin. As a result of this abnormality, there is an upset in the metabolism of carbohydrate, lipid and protein in diabetes. The liver has an active function to play in glucose regulation via glycolysis and gluconeogenesis and it is badly affected in diabetic condition (Baquer, 1999), leading to alterations in the functions of vital enzymes that are responsible for gluconeogenesis, glycolysis and glycogen metabolism. Furthermore, in diabetic situation, it has been observed that glucose-6-phosphatase and fructose-1,6-bisphosphatase activities is upregulated while that of hexokinase is reduced (Sivakumar and Subramanian, 2009; Nordlie *et al*., 1999).

Despite the enormous health challenges diabetes possess, there is still yet an effective treatment for the management and control of the complications associated with diabetes. At present the use of hypoglycemic agents such as biguanides α-glycosidase inhibitors, thiazolidenes and sulfonylureas are the available treatment options. However, these drugs have been associated with several side effects (Mallick *et al*., 2007; Park *et al*., 2013; Nathan *et al*., 2006). The World Health Organization has recommended the use of alternative therapeutic strategies which includes using natural products especially medicinal plants for the prevention and treatment of diabetes. As such several researches have focused in recent times on the use of natural medicines from plants and animals, as well as the active constituents obtained from these sources as possible therapeutic agents for the prevention and treatment of diabetes. This is due to their

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availability, safety and their effectiveness (Marles and Farnsworth, 1995; Kasetti *et al*., 2012).

Cortex Lycii is the root bark of *Lycium chinense*, a famous traditional Chinese medicinal herb widely used as both medicine as well as a functional food (Ministry of Health of RP China, 2002). It is used as part of the ingredient in soup, congee, and herbal tea. Traditionally Cortex Lycii is used as a heat cleansing agent, for the treatment of cough, pneumonia, hematemesis, night sweats, inflammations and diabetes. Phytochemical studies indicated the presence of several secondary metabolites in Cortex Lycii, including alkaloids, flavonoids, phenolic acids and coumarins (Potterat, 2010; Yao *et al*., 2011). Previous pharmacological studies on the antidiabetic properties of plants in the genus *Lycium* have been documented. For instance, Ye *et al* reported that Cortex Lycii Radicis extracts (the root back of *Lycium barbarum*) could improve the metabolism of lipids as well as insulin resistance in obese diabetic rats (Ye *et al*., 2008). Similarly, Gao *et al* reported the hypoglycemic properties of Cortex Lycii Radicis in diabetic mice models (Gao *et al*., 2007). However, to the best of our knowledge there has been no report on the effect of Cortex Lycii on key enzymes involved in carbohydrate metabolism in experimental diabetes. Hence, the aim of this study is to evaluate the effect of Cortex Lycii on carbohydrate metabolic enzymes in streptozotocin-nicotinamide induced diabetic rat

MATERIALS AND METHODS

Chemical and reagent

Streptozotocin, glibenclamide and nicotinamide were obtained from Sigma-Aldrich (St. Louis, MO, USA), α-Glucosidase powder was obtained from MP Biomedical (France). All other chemicals and reagents used were of analytical grade.

Extraction of root back of Lycium chinense

The plant specimen was obtained from Dongta, Jiangsu, China in August 2015. It was identified by one of the authors (Assoc. Prof. Hongxia Chen). A reference sample (LC/201503001) was kept at the School of Pharmacy, Jiangsu University. The plant material (3kg) was dried, powered and extracted with 90% ethanol (3×5 L) under reflux. The crude ethanol extract was concentrated, suspended in water and partitioned using n-hexane, ethyl acetate and 1-butanol. The ethyl acetate fraction (QCL) was dried under vacuum pressure and subsequently used for the experiment.

Identification of chemical constituents in QCL

The ethyl acetate crude extract (QCL, 20.0g) of the root bark of *Lycium chinense* was subjected to silica gel column chromatography eluting with CH_2Cl_2/CH_3OH (100:1 to 2:1) to afford six main fractions (Fa-Ff). Fractions Fa (158.9mg) and Fb (900mg) were purified on silica column chromatography and gel filtration chromatography (Sephadex LH-20) to afford compounds 1-4. Compounds 5-7 were obtained from fractions Fc (821.7mg) using Sephadex LH-20 and reverse phase HPLC, respectively. Fraction Fd (1.5g) was purified on silica chromatography eluting with (CH_2Cl_2/CH_3OH, 3:1 to 0:1), Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1) and RP-HPLC (CH_3OH/H_2O (34:66) to obtain compounds 8-10. Fraction Fe (1.3g) was purified on repeated column chromatography (CH_2Cl_2/CH_3OH, 8:2 to 2:1) and RP-HPLC (CH_3OH /H_2O, 25:75) to give compounds 11-15. The structural elucidation of all compounds was carried out using IR, MS and NMR spectral analyses and comparison with previous literature value.

Animals

36 male Sprague Dawley rats (150-200g) were acquired from animal house facility in the university. The animals were accommodated in polypropylene cages in an air conditioned room (24±3.0˚C and 40-50% humidity, a 12h light and 12h dark cycle) and allowed to have access to food and water *ad libitium*. The rats were acclimatized to the conditions for two weeks prior to the experiment. All protocols used during the experimental procedures were in accordance with the guidelines for the Care and Use of Laboratory Animals of the National Institute of Health (NIH, No 85-23, 1996) and the ethical committee of the Animal Use at Jiangsu University.

Induction of type 2 diabetes

The rats were fasted for 18 hours and 110mg/kg nicotinamide was given intraperitoneally. After 15min, freshly prepared streptozotocin (65mg/kg) in 0.1M citrate buffer (pH 4.5) was administered to the rats (Masiello *et al*., 1998). Control rats were similarly injected with vehicles only. After 72h of administration, fasting blood glucose (FBG) of the rats were measured to confirm diabetes using an Accu-Chek glucometer. Rats with FBG level greater than 250mg/dl were considered diabetic and included in further experiment.

Experimental design

Rats were randomly allotted into seven groups of six rats per group.

Groups 1- Normal control rats (NC)
Group 2- Normal rats + 400 mg/kg QCL (N + QCL)
Group 3- Diabetic control (DC)
Group 4 Diabetic+100mg/kg of QCL (D+QCL100 mg/kg)
Group 5- Diabetic+200mg/kg QCL (D + QCL 200 mg/kg)
Group 6- Diabetic+400mg/kg QCL (D + QCL 400 mg/kg)
Group 7- Diabetic + 250 mg/kg Glibenclamide (D + Gli 600 µg/kg)

The extract and drug were administered by gastric probe once daily for six weeks. The body weight, fasting blood glucose, food and water consumption of all rats in each group were monitored throughout the experimental period. After six weeks the rats were sacrificed and blood samples were obtained in tubes with potassium oxalate

and sodium fluoride (3:1) for the determination of insulin levels in the plasma. The whole blood sample was used for the estimation HbA1c and Hb levels. Samples of the liver and kidney tissues were dissever, washed with PBS, homogenized using 0.1M Tris-HCl buffer (pH 7.4) and centrifuged at 4000g for 10min. The supernatant collected was used for further enzyme analysis.

Biochemical assays

The level of plasma insulin was determined based on the method of Trinder 1969 (Trinder, 1969) with the aid of a commercially available reagent and ELISA kit (Boehringer Ingelheim, Mannheim, Germany). Hb and HbA1c assay were determined using the cyanmethemoglobin method (Drabkin and Austin, 1932).

Carbohydrate metabolic enzymes assay

Hexokinase activity was estimated based on the previously described protocol of Brandstrup *et al*. (Brandstrup *et al*., 1957). Glucose-6-phosphate dehydrogenase activity was measured using the method described by Ells and Kirkman (Ellis and Kirkman, 1961). Phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase activities was measured using the protocol of Pogson and Smith with modifications based on the method of Kin *et al*. and Gancedo and Gancedo, respectively (Pogson and Smith, 1975; Kin *et al*., 2004; Gancedo and Gancedo, 1971). Furthermore, glucose-6-phosphatase activity was assayed using the method of Koide and Oda (Koide and Oda, 1959).

Determination of glycogen and glycogen metabolic enzymes

Glycogen was extracted and determined using Morales *et al*. protocol (Morales *et al*., 1973).

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined based on previous literature (Yuan *et al*. 2012). In brief, 50µL of sample, 50µL of 0.1Mm of phosphate buffer (pH 6.9) and 100µL of α-glucosidase solution (pH 6.9, 0.2 U/mL, in 0.1M phosphate buffer) were added in a 96-well plates (25°C for 15min). After incubation, 50µL of 5mM *p*-nitrophenyl-α-$_D$-glucopyranoside (pNPG) solution in 0.1M phosphate buffer (pH 6.9) was added to each well, and the absorbance was measured at 405 nm on a micro-plate reader. The inhibitory activity of the compounds was expressed as the concentration of sample that caused 50% inhibition of the enzyme activity (IC_{50}). Acarbose was used as positive control. The percentage inhibition was calculated based on the following equation:

$$\text{Inhibition percentages}\,(\%) = \frac{AC\ (AS - Ab)}{AC} \times 100$$

Where A_C = absorbance of control
A_S = absorbance of sample
A_b = absorbance of blank (without pNPG solution).

STATISTICAL ANALYSIS

All data were presented as mean ± SD. Statistical analysis was performed using one-way analysis of Variance (ANOVA) followed by Post hoc analysis using Tukey test for the determination of differences among means. $P<0.05$ were considered statistically significant.

RESULTS

Effect of QCL on body weight, food and water intake

Table 1 indicates the effect of QCL on food intake, body weight as well as water intake. In the diabetic rats, it was observed that food and water consumption was significantly increased in comparison to untreated normal control rats. Similarly, there was a marked decline in the weight of the diabetic control rats as compared to normal rats. However, QCL significantly attenuated the loss of body weight, food and water consumption observed in the diabetic rat models in a dose dependent manner ($p< 0.05$).

Effect of QCL on FBG, plasma insulin, HbA1c and Hb levels

The effect of QCL on FGB, plasma insulin, HbA1c and Hb in all the rat models are shown in table 2. FBG in the diabetic control rats was notably increased while the plasma insulin level was observed to be markedly decreased as compared to the untreated control group. Likewise, in the diabetic control rats, there was a marked increase in blood HbA1c and a corresponding reduction in the Hb levels. However, in diabetic rats treated with QCL and glibenclamide, a significantly attenuation of the altered levels of glucose and HbA1c. Furthermore, the reduced plasma insulin and Hb levels observed in the diabetic control rats were significantly restored in QCL and glibenclamide treated groups in a concentration dependent manner.

Effect of QCL on gluconeogenesis parameters

As highlighted in table 3, it was observed that the hepatic and renal activities of gluconeogenesis parameters (glucose-6-phosphatase, fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase) in the diabetic rats were markedly upregulated in comparison to the rats in the control group. However, treatment with QCL and glibenclamide significantly attenuated the altered activities in the diabetic rat groups.

Effect of QCL on hexokinase and glucose-6-phosphate dehydrogenase activities

The level of the activities of hexokinase and glucose-6-phosphate dehydrogenase in the untreated diabetic rat group were significantly reduced to about 1.5 and 2.1 fold respectively when compared to the control rats. In diabetic groups treated with QCL and glibenclamide, it was observed that the activities of these enzymes were up regulated in a concentration dependent manner (table 4).

Table 1: Effect of QCL on body weight, food and water intake in normal and diabetic rats

Treated groups	Body weight (g)		Food intake (g/rat)	
	Initial	Final	Initial	Final
NC	195.10 ± 14.39	240.14 ± 14.17	14.48 ± 1.18	16.49 ± 1.05
N + QCL (400 mg/kg)	198.21 ± 13.98	235.62 ± 14.31	15.26 ± 1.40	18.03 ± 1.16
DC	196.31 ± 15.12	134.89 ± 15.98##	58.83 ± 4.16	75.06 ± 4.48##
D + QCL (100 mg/kg)	193.68 ± 14.11	189.89 ± 15.10**	46.59 ± 1.28	39.10 ± 3.25**
D + QCL (200 mg/kg)	195.22 ± 13.83	206.97 ± 14.21**	37.12 ± 1.09	30.13 ± 2.17**
D + QCL (400 mg/kg)	199.05 ± 16.03	220.09 ± 15.86**	32.69 ± 1.19	24.74 ± 1.67**
D + Gli (600µg/kg)	194.85 ± 14.27	229.43 ± 14.85**	30.09 ± 1.26	22.42 ± 1.37**

Treated groups	Water intake (ml/rat)	
	Initial	Final
NC	84.65 ± 6.21	83.87 ± 6.17
N + QCL (400 mg/kg)	88.03 ± 6.34	86.72 ± 6.09
DC	168.02 ± 11.68	184.66 ± 13.22##
D + QCL (100 mg/kg)	132.44 ± 10.11	120.36 ± 10.07**
D + QCL (200 mg/kg)	125.51 ± 9.10	108.11 ± 8.69**
D + QCL (400 mg/kg)	114.16 ± 8.32	96.03 ± 7.45**
D + Gli (600 µg/kg)	109.21 ± 7.98	93.66 ± 7.98**

Table 2: Effects of QCL on blood glucose, HbA1c, Hb and plasma insulin levels in normal and diabetic rats

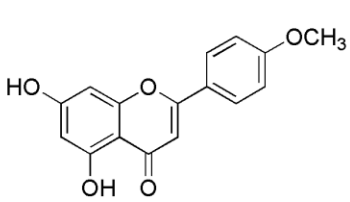
Treated groups	Blood glucose (mg/dl)	Insulin (ng/ml)	HbA1c (%)	Hb (g/dl)
NC	84.45 ± 2.45	3.34 ± 0.17	6.02 ± 0.38	14.22 ± 1.05
N + QCL (400 mg/kg)	85.10 ± 3.01	3.21 ± 0.21	5.92 ± 0.40	14.10 ± 1.45
DC	301.14 ± 8.99##	0.69 ± 0.08##	13.74 ± 1.16##	7.42 ± 0.73##
D + QCL (100 mg/kg)	214.10 ± 6.20**	1.37 ± 0.11**	9.73 ± 0.68**	8.98 ± 0.88**
D + QCL (200 mg/kg)	172.05 ± 4.01**	2.01 ± 0.09**	8.41 ± 0.32**	10.23 ± 1.17**
D + QCL (400 mg/kg)	101.54 ± 2.11**	2.98 ± 0.14**	7.32 ± 0.29**	12.74 ± 1.13**
D + Gli (600 µg/kg)	109.11 ± 2.71**	2.83 ± 0.16**	6.89 ± 0.33**	12.98 ± 1.57**

Table 3: Effect of QCL on hepatic and renal activities of fructose-1,6-bisphosphatase, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in normal and diabetic rats

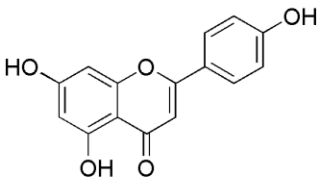
Treated groups	Fructose 1,6-bisphosphatase (mmol of Pi liberated/min/ mg protein)		Glucose-6-phosphatase (mmol of Pi liberated/min/ mg protein)	
	Liver	Kidney	Liver	Kidney
NC	0.19 ± 0.02	0.17 ± 0.01	0.36 ± 0.03	0.80 ± 0.06
N + QCL (400 mg/kg)	0.18 ± 0.01	0.17 ± 0.02	0.37 ± 0.03	0.79 ± 0.076
DC	0.39 ± 0.03##	0.35 ± 0.03##	0.68 ± 0.06##	1.42 ± 0.13##
D + QCL (100 mg/kg)	0.32 ± 0.02**	0.31 ± 0.01**	0.59 ± 0.05**	1.26 ± 0.08**
D + QCL (200 mg/kg)	0.27 ± 0.02**	0.26 ± 0.02**	0.51 ± 0.04**	1.03 ± 0.07**
D + QCL (400 mg/kg)	0.23 ± 0.02**	0.24 ± 0.02**	0.44 ± 0.04**	0.89 ± 0.07**
D + Gli (600 µg/kg)	0.21 ± 0.01**	0.22 ± 0.01**	0.42 ± 0.03**	0.85 ± 0.07**

Treated groups	Phosphoenolpyruvate carboxykinase (nmol/min/mg protein)	
	Liver	Kidney
NC	1.95 ± 0.13	0.50 ± 0.04
N + QCL (400 mg/kg)	1.98 ± 0.14	0.51 ± 0.04
DC	3.42 ± 0.26##	0.93 ± 0.08##
D + QCL (100 mg/kg)	2.81 ± 0.23**	0.75 ± 0.06**
D + QCL (200 mg/kg)	2.59 ± 0.19**	0.67 ± 0.06**
D + QCL (400 mg/kg)	2.28 ± 0.17**	0.60 ± 0.05**
D + Gli (600 µg/kg)	2.25 ± 0.16**	0.57 ± 0.04**

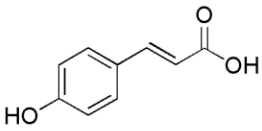
Data are expressed as means ± SD (n = 6) and analyzed by ANOVA followed by Tukey test. ##$p < 0.05$ vs. normal control group. **$p < 0.05$ vs. diabetic control group.



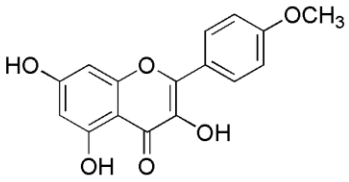
1. Acacetin



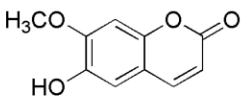
2. Apigenin



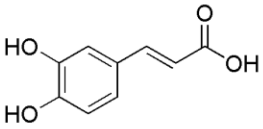
3. *p*-Coumaric acid



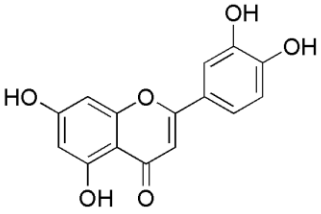
4. Kaempferide



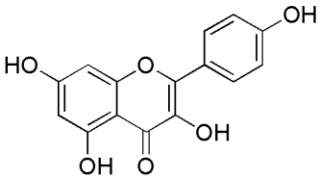
5. Isoscopoletin



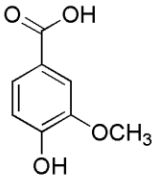
6. Caffeic acid



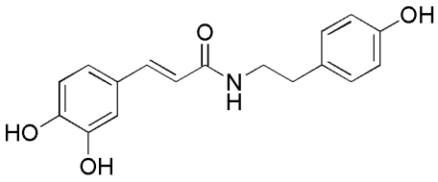
7. Luteolin



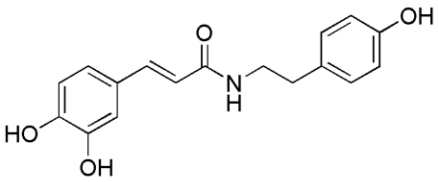
8. Kaemferol



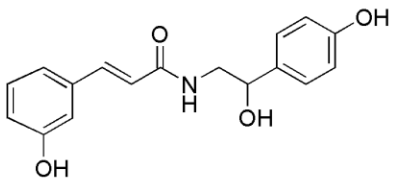
9. Vanillic acid



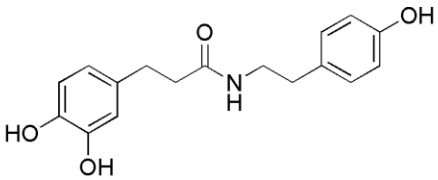
10. Trans-N-caffeoyltyramine



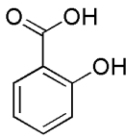
11. Cis-N-caffeoyltyramine



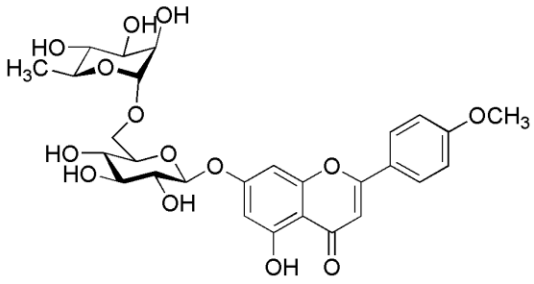
12. Trans-N-feruloyloctopamine



13. Dihydro-N-caffeoyltyramine



14. Gentisic acid



15. Linarin

Fig. 1: Chemical structures of isolated compounds from QCL

Table 4: Effect of QCL on hepatic hexokinase and glucose-6-phosphate dehydrogenase levels in normal and diabetic rats

Treated groups	Hexokinase (μmol of glucoses min/g protein) phosphorylated/	Glucose-6-phosphate dehydrogenase ($\times 10^{-4}$ ml U/mg protein)
NC	157.12 ± 12.03	4.77 ± 0.39
N + QCL (400 mg/kg)	155.26 ± 11.86	4.74 ± 0.34
DC	104.01 ± 8.82##	2.30 ± 0.15##
D + QCL (100 mg/kg)	120.62 ± 9.08**	2.64 ± 0.19**
D + QCL (200 mg/kg)	132.10 ± 9.47**	3.14 ± 0.23**
D + QCL (400 mg/kg)	143.72 ± 10.99**	3.64 ± 0.28**
D + Gli (600 μg/kg)	146.97 ± 11.21**	3.97 ± 0.30**

Table 5: Effects of QCL on liver and muscle tissues glycogen content in normal and diabetic rats

Treated groups	Liver glycogen content (mg/g tissue)	Muscle glycogen content (mg/g tissue)
NC	64.08 ± 4.33	8.04 ± 0.58
N + QCL (400 mg/kg)	63.19 ± 4.65	7.89 ± 0.62
DC	20.70 ± 0.98##	3.56 ± 0.31##
D + QCL (100 mg/kg)	33.84 ± 2.18**	4.40 ± 0.39**
D + QCL (200 mg/kg)	40.69 ± 3.95**	5.31 ± 0.41**
D + QCL (400 mg/kg)	47.10 ± 4.48**	6.10 ± 0.47**
D + Gli (600 μg/kg)	56.32 ± 4.10**	6.62 ± 0.50**

Data are expressed as means ± SD (n = 6) and analyzed by ANOVA followed by Tukey test. ##$p < 0.05$ vs. normal control group. **$p < 0.05$ vs. diabetic control group**.**

Table 6: α-glucosidase inhibitory activity of some selected compounds

Treated groups	α-glucosidase inhibition (IC_{50} μM)
trans-*N*-caffeoyltyramine	4.74 ± 0.28
cis-*N*-caffeoyltyramine	7.08 ± 0.76
trans-*N*-feruloyloctopamine	5.58 ± 0.17
dihydro-*N*-caffeoylytramine	5.03 ± 0.14
Linarin	45.03 ± 9.21
Kaempferide	8.11 ± 0.11
Acarbose	193.11 ± 12.68

Effects of QCL on glycogen content

The results obtained indicated that there was a marked decrease in the levels of glycogen in the untreated diabetic group as against the normal control rats. Howbeit the oral administration of QCL and glibenclamide to the experimental diabetic groups significantly prevented the reduction in the levels of tissue glycogen in comparison to the untreated diabetic control group (table 5).

α-glucosidase inhibitory activity

As shown in table 6, all tested compounds showed significant α-glucosidase inhibitory. Trans-*N*-caffeoyltyramine and dihydro-*N*-caffeoylytramine displayed the highest inhibitory activity at an IC_{50} value of 4.74 and 5.03 μM, respectively.

DISCUSSION

Lycium species are used in TCM as treatment for inflammatory disorders, night sweat and diabetes. The different species of *Lycium* vary in their bioactive constituents as well as their medicinal effects. Several pharmacological and medicinal properties of *Lycium* species have been extensively explored, however the effects of the root back of *Lycium chinense* on carbohydrate metabolic enzymes in type II diabetes is yet to be explored. In this study, we induced diabetes in experimental rats using streptozotocin-nicotinamide and the ethyl acetate extracts of the root bark of *Lycium chinense* was administered to the diabetic rats.

Diabetes mellitus is a lifelong severe metabolic disorder affecting a large population globally. It is characterized by insulin resistance which eventually progresses to a drastic reduction in the secretion of insulin. Streptozotocin is frequently used to induce diabetes in animal models (Kim *et al*., 2003; Matteucci *et al*., 2008). It displays cytotoxic effects by damaging the pancreatic β-cells which is responsible for insulin secretion thereby leading to a reduced biosynthesis and secretion of insulin (Riad *et al*.,

2007; Szkudelski, 2001; Wilson and Leiter, 1990) leading to a state of hyperglycemia, a hallmark of diabetes mellitus (Saini, 2010). Furthermore, streptozotocin cytotoxicity has also been linked to the ROS generation leading to oxidative insult which damages the β-cells through DNA fragmentation (Lekshmi *et al*., 2015). It is believed that substances which display antioxidant/free radical scavenging activities can possibly promote the rejuvenation of pancreatic β-cells as well as exert protective effects streptozotocin-induced pancreatic islets destruction (Coskun *et al*., 2005).

Nicotinamide possesses antidiabetic effects and it is administered before streptozotocin injection in order to partially protect the pancreatic β-cells from complete damage as well as partially preventing the decline in the secretion of insulin (Szkudelski, 2012). During diabetes mellitus, there is a drastic fall in insulin production leading to a state of hyperglycemia. Insulin is responsible for the regulation of the activities of some vital enzymes, thus the insufficient production of insulin leads to altered glucose and carbohydrate metabolism (Sellamuthu *et al*., 2012). It was observed in this study that there was a significant reduction in plasma insulin and a corresponding increase in the glucose level of diabetic rats. The oral administration of QCL to the experimental diabetic rats led to a decline in the blood glucose and an increase in insulin levels in treated rats, revealing its anti-hyperglycemic properties.

Polyphagia, polydipsia, polyuria and a drastic reduction of body weight were clearly noticed in the diabetic rat which is synonymous to hyperglycemia (Balamurugan *et al*., 2011). The excessive weight loss indicates muscle wasting and breakdown of structural proteins as a result of loss of energy source (Salahuddin and Jalalpure, 2010; Sundaram *et al*., 2014; Sharma *et al*., 2011). The results obtained from our study showed that there was a decline in the body weight which was markedly increased after the administration of QCL and glibenclamide. Furthermore, there was a significantly decreased in the food and water consumption of the experimental animals treated with QCL.

HbA1c is a reliable marker index for monitoring the level of glucose and a useful tool in the therapeutic control and management of diabetes (Kasetti *et al*., 2010; Subash Babu *et al*., 2007). Hyperglycemia in diabetes, leads to an upsurge in the reaction of non-enzymatic glycosylation of Hb resulting in HbA1c. Previous reports have indicated that the level of HbA1c directly corresponds to the blood glucose level (Calisti and Tognetti, 2005). Complications such as retinopathy, nephropathy, neuropathy which are associated with diabetes are associated with high levels of HbA1c (Anand *et al*., 2010). Results from this study showed that there was an increase in the level of HbA1c in untreated diabetic rats, indicating the process of glycosylation as a result of hyperglycemia. The administration of QCL and glibenclamide significantly increased the level of Hb and reduced HbA1c levels in the treated diabetic rats. It is assumed that there was amelioration in the secretion of insulin. Thus suggesting that Cortex Lycii might have the ability to alleviate the onset and progression of diabetic complications.

The liver is a vital organ in the body which is involved in several metabolic processes. It plays an active function in glucose homeostasis via gluconeogenesis or glycogenolysis (Berg *et al*., 2001). Increased hepatic gluconeogenesis has been implicated in the pathological processes leading to type 2 diabetes. Previous report from Berg *et al*. reported that there was an increase in the activities of gluconeogenic enzymes both hepatic and peripheral tissues of diabetic rats (Soty *et al*., 2012). Fructose-1,6-bisphosphatase is responsible for the conversion of fructose-1,6-bisphosphate to fructose 6-phosphate in gluconeogenesis, while pyruvate carboxylase is an enzyme that is responsible for catalyzing the conversion of oxaloacetate to phosphoenolpyruvate. Glucose-6-phosphatase is involved in catalyzing the reaction involving the hydrolysis of glucose-6-phosphate, giving rise to glucose and phosphate group, the last enzymatic reaction that completes the last step in gluconeogenesis and glycogenolysis, which is important in the regulation of the release of glucose into the blood (Chandramohan *et al*., 2015; Mohamad *et al*., 2004). Insulin is responsible for curbing the activities of these enzymes in normal body conditions. However, in diabetic state, the activities of these enzymes are upregulated in the liver and kidney (Kang *et al*., 2008). Our study indicated the increased activities of these enzymes in the untreated diabetic rats which may be as a result of insufficient insulin, thus increasing the production of glucose. However, QCL treatment down regulated the activities of these enzymes in the experimental diabetic rats.

Hexokinase, is an enzyme that has an important role it plays in glucose utilization and it is responsible for catalyzing the phosphorylation of glucose to form glucose-6-phosphate by transferring phosphate group from ATP to the hydroxyl group on position 6 in glucose. There was a decrease in the activity of hexokinase in the diabetic rats as a result of inadequate insulin secretion. QCL treatment to experimental diabetic animals augmented hexokinase activity in the liver thus enhancing glucose metabolism and homeostasis.

Glycogen is a multiple branched polysaccharide that is produced by glycogen synthase and it serves as the intracellular energy storage form of glucose in the liver. Two important enzymes; glycogen synthase and glycogen phosphorylase are responsible for glycogen metabolism and the determination of glycogen is considered as a

critical parameter in the assessment of antidiabetic potentials (Zhanga *et al*., 2009). Glycogen levels have been reported to be reduced in diabetic situations due to the absence or insulin deficiency and the enhanced activity of glycogen phosphorylase (Xie *et al*., 2014). The oral administrations of QCL to streptozotocin-induced diabetic rats significantly increased the glycogen content.

Phytochemicals constituents of Cortex Lycii such as flavonoids, phenolic acids, phenolic amides, and coumarins have been reported (Pandey and Rizvi, 2009). The *in vitro* α-glucosidase inhibitory activity of the six major isolated compounds (trans-*N*-caffeoyltyramine, cis-*N*-caffeoyltyramine, trans-*N*-feruloyloctopamin, dihydro-*N*-caffeoylytramine, linarin and kaempferide) was determined. All the tested compounds showed significant inhibitory activity at an $IC5_0$ value of 4.74, 7.08, 5.58, 5.03, 45.03 and 8.11μM, respectively which was significantly lower than the value for acarbose (193.11 μM). Flavonoids and phenolic amides have been proven to be potent antidiabetic agents (Li *et al*., 2011). Fifteen known compounds (fig. 1) were identified with the aid of spectroscopic data analysis and comparison with previous report in literatures.

CONCLUSION

In conclusion, our investigation indicated that the administration of QCL to diabetic experimental rats could decrease blood glucose level, restore insulin secretion and glucose homeostasis through modulatory action on the activities of key enzymes involved in glucose utilization and production.

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