

Molecular mechanisms of anti-hyperglycemic effects of *Costus speciosus* extract in streptozotocin-induced diabetic rats

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ABSTRACT

الأهداف: معرفة الطرق التي يعمل بها المستخلص الكحلي لجذور نبات القسطس كمضاد لارتفاع مستوى السكر بالدم في الفئران المصابة بالسكري. من خلال تقييم عملها على تخليق الأنسولين والتعبير الجيني للجينات المنظمة لمستوى سكر الدم في الفئران المصابة بالسكري باستخدام الستريبتوزوتوسين STZ.

الطريقة: أجريت هذه الدراسة في مختبر الكيمياء الحيوية، كلية الطب البيطري، جامعة الزقازيق، مصر بين يوليو وأغسطس 2013م. أجريت الدراسة على 60 من الفئران البيضاء بوزن 120 ± 20 غرام وأعمارها 6 أشهر والتي قسمت إلى 6 مجموعات (العدد=10). مجموعتان اشتملت على المجموعة المصابة بالسكري والمجموعة الضابطة غير المصابة بالسكري و 4 مجموعات عولجت بالستريبتوزوتوسين بجرعات بلغت 200، 400، 600 ملغ/كلغم من وزن الجسم و 600 ملغ/كلغم من وزن الجسم لعقار الجلبنكلوميد القياسي لمدة 4 أسابيع.

النتائج: أن مستخلص جذور الايثانول بجرعة 400ملغ/كلغم و 600ملغ/كلغم من وزن الجسم يؤدي إلى انخفاض مستوى السكر في الدم وزيادة مستوى الأنسولين و جلو كوكيناسي (GK)، وألدولاز، وكيناز البيروفات (PK)، ونازعة سكسينات (SDH)، ونشاط الجلبيكوجين سينسيز بالإضافة إلى ارتفاع مستوى تعبير الأنسولين، ومستقبلات الأنسولين (IRA) A، SDH، PK، GK، وبروتين ناقل الجلوكوز.

الخاتمة: أن المستخلص الكحلي لنبات القسطس يعمل كمضاد لارتفاع السكر في الجرذان المصابة بالسكري عن طريق تنشيط إنتاج الأنسولين من البنكرياس مع تحفيز حساسية الأنسجة له مما ينتج عنه زياده لدخول الجلوكوز بالأنسجة و ارتفاع مستوى أكسدته وتخزينه.

Objectives: To investigate the mechanisms of the anti-hyperglycemic effect of *Costus speciosus* (*C. speciosus*) root ethanolic extracts (CSREt) by assessing its action on insulin synthesis and glucose catabolic enzyme gene expression and activities in streptozotocin (STZ) diabetic rats.

Methods: This study was carried out at the Biochemical Laboratory, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt between July and August 2013. Sixty male albino rats (120 ± 20 g weight, and 6 months old) were used and divided into 6 groups (n=10). Two groups served as diabetic and nondiabetic controls. Four groups of STZ diabetic animals were given oral *C. speciosus* (CSREt) in doses of 200, 400, and 600 mg/kg body weight, and 600 µg/kg body weight of the standard drug glibenclamide for 4 weeks.

Results: The CSREt 400 and 600 mg/kg body weight induced a decrease in blood glucose and an increase in serum insulin level, glucokinase (GK), aldolase, pyruvate kinase (PK), succinate dehydrogenase (SDH), and glycogen synthase activities in addition to a higher expression level of insulin, insulin receptor A (IRA), GK, PK, SDH, and glucose transporting protein.

Conclusion: The *C. speciosus* has anti-hyperglycemic activity. It induces insulin secretion and release from cells, as well as stimulates the tissue's insulin sensitivity leading to an increase of the tissues' glucose uptake, storage, and oxidation.

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Diabetes mellitus (DM) may be defined as a state of chronic hyperglycemia caused by either relative, or absolute deficiency in the level of blood insulin.¹ A huge number of people in the world suffer from diabetes; the alarming increase in incidence of DM makes it a serious health problem.² The persistent hyperglycemia in diabetic patients is the inducer of DM complications, such as coronary artery disease, hyperlipidemia, cerebrovascular disease, blindness, renal failure, neurological complications, limb amputation, and premature death.³ The current available anti-diabetic drugs may have some limitations and side effects, therefore, the growing trend for DM treatment was directed to the use of natural agents, such as medicinal herbs.⁴ Medicinal plants have always been a part of human culture, and up to 80% of the world population relies on this system for some aspects of primary health care. A large number of folkloric plants are still in use as herbal therapies in the Kingdom of Saudi Arabia, among them is *Costus speciosus* (*C. speciosus* [Koenig]).⁵ The *C. speciosus* has a very wide distribution in India as a diabetic controller, usually diabetic patients eat one leaf daily to keep their blood glucose low.⁶ The Hexane extract of rhizome possess anti-hyperglycemic and hypo-lipidemic activity,⁷ reverses diabetes and its complications,⁸ improves hepatic antioxidant enzyme activity, affects neurotransmitters and monoamine oxidase activity,⁹ anti-inflammatory and antipyretic properties,¹⁰ and significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity.¹¹ The *C. speciosus* has a huge content of spirostanol glycosides, and furostanol glycoside 26-O-beta-glucosidase (F26G), which has hypoglycemic effects.⁵ The mechanism of the anti-hyperglycemic action of *C. speciosus* was not clearly studied, therefore the objective of this work was to investigate on a molecular level, the possible mechanisms of anti-hyperglycemic action of *C. speciosus* by monitoring its effects on the gene expression and activities of glycolytic enzymes in streptozotocin (STZ)-diabetic male rats.

Methods. This study was carried out at the Biochemical Laboratory, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt between July and August 2013. The *C. speciosus* roots (3 kg) were

obtained from Ragb's druggist company, Cairo, Egypt, and authenticated at the Plant Science Department, Suez Canal University, Ismailia, Egypt. The ethanolic extract of *C. speciosus* roots were prepared in 95% ethanol.⁸ The extractions continued until the solvent in the thimble becomes clear. After extraction, the extract was filtered, and the solvent was evaporated at room temperature. The dried residue was kept at 4°C.

Male albino Sprague-Dawley rats (n=60) weighing approximately 120±20, and 6 months old were used in this study. Rats were housed in polypropylene cages. They were allowed access to standard laboratory pellet diet and water *ad libitum*. The animals were housed in a light/dark cycle at an ambient temperature of 24±3°C. Feeding needles (18 gauge) were used for oral dosing. The experimental procedures were performed following the Egyptian guidelines for experimental animal care. The Veterinary Medicine Collage, Zagazig University Ethical Committee approved the current experiment. All experimental animals after acclimatization for 2 weeks before treatment were divided into 6 groups (n=10). The first group serves as normal control and received 5% Tween-80 (one ml/kg body weight [bwt]), and the rats in the other 5 groups were intraperitoneally (i.p.) injected with a single injection of STZ (65 mg/kg bwt) (Sigma-Aldrich Co., St. Louis, MO, USA) for the induction of diabetes. The second group was left as diabetic control. Other groups were treated with different doses such as 200mg/kg bwt of *C. speciosus* root ethanolic extracts (CSREt) in group 3, 400 mg/kg in group 4, 600 mg/kg in group 5, and group 6 was treated with 600 µg/kg bwt glibenclamide.⁸

The CSREt and glibenclamide were daily suspended in one mL of 2% Tween-80 and were administered to the animals daily for 4 weeks. The DM was induced by a single i.p. injection of STZ (65 mg/kg bwt) (Sigma-Aldrich Co., St. Louis, MO, USA). The STZ was freshly prepared in 0.01 M cold sodium citrate buffer (pH: 4.5) immediately before use.¹² The STZ-treated rats were administered with 5% weight/volume glucose for the next 24 hours to prevent hypoglycemia. After 72 hours, rats with fasting blood glucose more than 200 mg/dl were selected as diabetic rats. After 4 weeks of treatment, all rats were food deprived overnight, then sacrificed under anesthesia, and blood, liver, and pancreatic samples were collected. Blood samples were collected from the median canthus of the eye and the sera (approximately 800 µL) were separated by centrifugation, and stored at -20°C for biochemical investigation. Liver samples were obtained and prepared for further biochemical investigation. Other parts from the liver and pancreatic tissues were also frozen in on liquid nitrogen used for

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molecular analysis. Blood glucose levels (mg/dl) were determined using glucose oxidase method by a kit that was obtained from SPINREACT, Girona, Spain. The levels of serum insulin were determined by Enzyme Linked Immunosorbent Assay kit specific for rat insulin (Cat. no. ezrmi-13kelisa, Billerica, MA, USA). One gram of each liver tissue samples were homogenized in 9 mL of the homogenizing buffer at pH 7.4 for preparation of tissue homogenate.¹³ This homogenate was used to assay the activities of the hepatic enzymes. The hepatic glucokinase (GK) activity was determined by measuring the glucose-6-phosphate (G6P) formed by GK by formation of nicotinamide-adenine dinucleotide phosphate in the presence of glucose-6-phosphate dehydrogenase enzyme.¹³ The activity of succinate dehydrogenase (SDH) was measured by monitoring the reduction of 2, 6-dichlorophenolindophenol at 600 nm.¹⁴ Hepatic aldolase activity was assayed by spectrophotometry at 340 nm using Aldolase Activity Colorimetric Assay kit (Biovision, Cat. no. K665-100, Milpitas, CA, USA), where one unit of aldolase activity is defined as the amount of enzyme that produced one μmol of NADH per minute at 25°C.¹⁵ Hepatic pyruvate kinase (PK) activity was determined in the liver homogenate with 50 mM glycylglycine, 15 mM ethylene diamine tetraacetic acid (EDTA), and 5 mM potassium phosphate. The total PK activity was determined in the supernatants as described.¹⁶ Glycogen synthase (GS) activity was measured in homogenates in the presence of 6.6 mM G6P, and the enzyme activity was expressed as mU/mg protein.¹⁷ Hepatic glycogen content was determined as previously described.¹⁸

Table 1 - Oligonucleotide primers sequence and the polymerase chain reaction (PCR) product size of the studied genes.

Gene	Oligonucleotide sequence	Size (bp)	Ref.
Insulin-1	f 5'-atggccctgtggatgcgctt-3'	331	19
	r 5'-tagttgcagtagttctccagc t-3'		
Insulin receptor A	f 5'-ttcattcaggaagaccttcga-3'	222	19
	r 5'-aggccagagatgacaagtgc-3'		
Glucokinase	f 5'-cacccaactgcgaatcacc-3'	162	19
	r 5'-catttgggggtgtggagtc-3'		
Glucose transporter 2	f 5'-ttagcaactgggtctgcaat-3'	243	19
	r 5'-tctctgaagacgccaggaat-3'		
Succinate dehydrogenase	f 5'-tggcttcacttctctgttg-3'	249	20
	r 5'-atcctcagttgtcctctt cca-3'		
Pyruvate kinase	f 5'-agagatggaggccttggggg-3'	300	21
	r 5'-tacgttgcttacctgctgtgr-3'		
Beta-actin	f 5'-tcactatcggcaatgtcgg-3'	260	22
	r gctcaggaggagcaatgatg-3'		

Amplified PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide in 1X Tris acetate ethylenediamine tetraacetic acid buffer (TAE) pH 8.3-8.5. The electrophoretic picture was visualized and analyzed by gel documentation system (Bio Doc Analyze, Biometra, Göttingen, Germany)

Hepatic homogenate protein concentration was measured using a Bio-Rad assay reagent.¹⁷ Total RNA was extracted from the liver and pancreatic tissues using RNeasy mini kit (Cat. No. 74104, Qiagen, China) following the manufacturer instructions. The amount of extracted RNA was quantified and qualified using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). The purity of RNA was checked and it ranged between 1.8 and 2.1 demonstrating the high quality of the RNA. First strand cDNA was produced using a specific kit that was supplied by Fermentas (Pittsburgh, PA, USA). The polymerase chain reaction (PCR) was carried out by (2x) PCR Master Mix (Fermentas Inc., Pittsburgh, PA, USA). A 2720 thermocycler (Applied Biosystems, Foster City, CA, USA) was used in performing the PCR reactions. We used 10 pmol/ μL of each forward and reverse primer for the measured genes. The housekeeping gene β -actin was used as a constitutive control for normalization. Primers were designed as previously published.¹⁹⁻²² All primers were provided by Sigma Aldrich (Chemie GmbH, Steinheim, Germany) as shown in Table 1. Amplified PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide in 1x Tris acetate EDTA buffer (TAE) (pH: 8.3-8.5) (Figure 1). The electrophoretic picture was

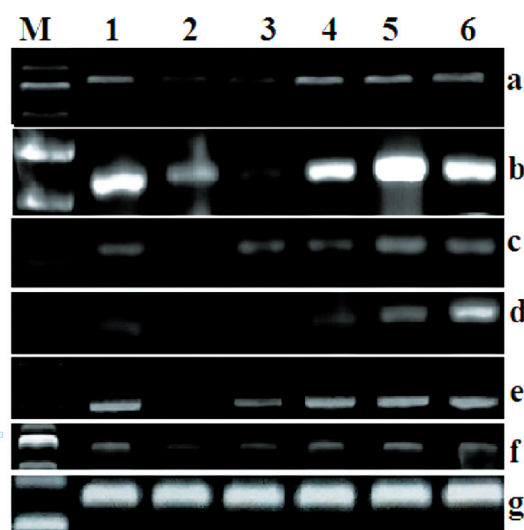


Figure 1 - Agarose gel electrophoresis picture of insulin (a), insulin receptor A (b), glucokinase (c), succinate dehydrogenase (d), pyruvate kinase (e), glucose transporter 2 (f), and beta-actin (g) genes expression. M - 100pb DNA marker, 1 - control group, 2 - diabetic group, 3 - diabetic and CSREt (200 mg/kg bwt), 4 - diabetic and CSREt (400 mg/kg bwt), 5 - diabetic and CSREt (600 mg/kg bwt), 6 - diabetic and glibenclamide (600 $\mu\text{g}/\text{kg}$ bwt). bwt - body weight

visualized and analyzed by gel documentation system (Bio Doc Analyze, Biometra, Göttingen, Germany).

The statistical analysis of the data was carried out in Predictive Analytics SoftWare Statistics for Windows version 18 (IBM SPSS Statistics, Endicott, New York, USA). One-way analysis of variance was used to determine the statistical differences between groups followed by Duncan's multiple range test to analyze the inter-grouping homogeneity. Data were presented as mean \pm standard deviation. $P < 0.05$ was considered statistically significant.²³

Results. The STZ induced diabetic group had high blood glucose and low serum insulin levels, hepatic GK, SDH, aldolase, PK, and GS activities, and hepatic glycogen content compared with control rats. While animals administered with CSREt showed a decrease in blood glucose levels and induced the activities of hepatic GK, SDH, aldolase, PK, and GS, as well as increase the levels of serum insulin and hepatic glycogen when compared with the diabetic rats. The CSREt action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt, and less effect with a low dose 200 mg/kg bwt in comparison with the

standard drug glibenclamide (Table 2). The STZ induced diabetic rats had significant decrease in the levels of pancreatic insulin, hepatic insulin receptor A (IRA), GK, SDH, PK, and glucose transporter 2 (GLUT2) relative gene expression compared with control rats. While animals administered with CSREt showed an increase in the levels of pancreatic insulin, hepatic IRA, GK, SDH, PK, and GLUT2 relative gene expression when compared with the diabetic rats. The CSREt action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt, and less effect with the low dose 200 mg/kg bwt in comparison with the standard drug glibenclamide (Table 3).

Discussion. In the present study, we examined the mechanism of the anti-hyperglycemic effect of *C. speciosus* root ethanolic extract on STZ-induced diabetic rats. Our hypothesis is *C. speciosus* induces insulin production and tissues' insulin sensitivity, leading to an increase in the tissues glucose uptake, storage, and oxidation. In this study, we demonstrated the anti-hyperglycemic action of CSREt in STZ-induced diabetic rats. It decreased the blood glucose level in

Table 2 - Effect of *Costus speciosus* extract on blood glucose, insulin levels, and glucose catabolism enzyme activities in streptozotocin-diabetic rats.

Parameters	Control group	Diabetic group	Diabetic + extract (200 mg/kg bwt)	Diabetic + extract (400 mg/kg bwt)	Diabetic + extract (600 mg/kg bwt)	Diabetic + glibenclamide (600 μ g/kg bwt)
Glucose (mg/dl)	80.5 \pm 14.3 ^c	365 \pm 28.17 ^a	297.5 \pm 34.8 ^b	160.5 \pm 30.9 ^c	118.5 \pm 35.12 ^{cd}	99.4 \pm 21.23 ^{de}
Insulin (μ U/ml)	14.4 \pm 1.52 ^a	6.6 \pm 0.86 ^d	6.8 \pm 0.79 ^d	9.3 \pm 0.92 ^c	10.2 \pm 1.02 ^c	12.6 \pm 0.98 ^b
GK	1.21 \pm 0.02 ^a	0.16 \pm 0.008 ^d	0.21 \pm 0.01 ^c	0.98 \pm 0.09 ^b	1.03 \pm 0.05 ^b	1.18 \pm 0.05 ^a
SDH	14.85 \pm 0.82 ^a	9.13 \pm 0.49 ^d	9.3 \pm 0.76 ^d	11.4 \pm 1.34 ^c	13.15 \pm 1.03 ^b	14.82 \pm 1.1 ^a
Aldolase	13.4 \pm 1.33 ^a	7.05 \pm 0.89 ^c	6.98 \pm 0.92 ^c	11.6 \pm 0.92 ^b	13.4 \pm 1.07 ^a	13.1 \pm 1.05 ^a
PK	60.4 \pm 3.12 ^a	39.5 \pm 1.89 ^d	37.5 \pm 2.14 ^d	51.5 \pm 3.11 ^c	55.5 \pm 4.12 ^{bc}	59.5 \pm 2.35 ^{ab}
Glycogen	23.4 \pm 1.25 ^a	8.2 \pm 0.98 ^c	11.3 \pm 1.6 ^d	17.4 \pm 1.34 ^c	20.15 \pm 1.03 ^b	21.34 \pm 1.3 ^b
GS	3.7 \pm 0.08 ^a	1.98 \pm 0.1 ^d	2.01 \pm 0.06 ^d	2.8 \pm 0.17 ^c	3.2 \pm 0.22 ^b	3.8 \pm 0.32 ^b

Data are expressed as means \pm SD. Different lowercase letters in the same row indicate significant differences ($p < 0.05$).
GK - glucokinase (U/g liver tissue), SDH - succinate dehydrogenase (nmol.mg protein⁻¹min⁻¹), aldolase (U.mg protein⁻¹), PK - pyruvate kinase (mU.mg protein⁻¹), hepatic glycogen (μ g.gm Wet.Wt⁻¹), GS - glycogen synthase (mU/mg protein), bwt - body weight

Table 3 - Effect of *Costus speciosus* extract on the relative gene expression (relative to beta-actin gene expression) of insulin-1, insulin receptor A, glucokinase, succinate dehydrogenase, pyruvate kinase, and glucose transporter 2 in streptozotocin-diabetic rats.

Gene	Control	Diabetic	Diabetic + CSREt (200 mg/kg bwt)	Diabetic + CSREt (400 mg/kg bwt)	Diabetic + CSREt (600 mg/kg bwt)	Diabetic + glibenclamide (600 μ g/kg bwt)
Insulin	139.6 \pm 14.3 ^a	57.5 \pm 8 ^d	62 \pm 11 ^d	108.5 \pm 15.9 ^c	121.7 \pm 25.12 ^b	136.2 \pm 21.23 ^a
IRA	157.1 \pm 19.2 ^a	68 \pm 7 ^c	73.7 \pm 9 ^c	125.7 \pm 18 ^b	133.5 \pm 15 ^b	152.1 \pm 23 ^a
GK	145.9 \pm 23 ^a	92.7 \pm 12 ^d	97 \pm 10 ^d	125.1 \pm 16 ^c	137 \pm 20 ^b	141.9 \pm 16 ^a
SDH	138 \pm 9 ^a	74 \pm 5 ^d	79.1 \pm 8 ^d	105.8 \pm 13 ^c	125 \pm 17 ^b	131.4 \pm 21 ^a
PK	93 \pm 6 ^a	56.2 \pm 8 ^d	60.2 \pm 6 ^d	69.1 \pm 4 ^c	80.3 \pm 6 ^b	91.2 \pm 15 ^a
GLUT2	147.5 \pm 22 ^a	65.3 \pm 6 ^d	70.6 \pm 8 ^d	116.7 \pm 17 ^c	133.2 \pm 20 ^b	143.2 \pm 23 ^a

Data are expressed as means \pm SD. Means in the same row with different letters (a, b, c, d and e) are statistical significant at $p < 0.05$. IRA - insulin receptor A, GK - glucokinase, SDH - succinate dehydrogenase, PK - pyruvate kinase, GLUT2 - glucose transporter 2, bwt - body weight, CSREt - *Costus speciosus* root ethanolic extracts

male STZ-induced diabetic rats particularly with the high dose (600 mg/kg bwt), as well as the standard drug glibenclamide. The anti-hyperglycemic effect of CSREt was dose dependent. Bavarva and Narasimhacharya⁸ recorded the anti-hyperglycemic action of CSREt for the first time in alloxan-induced diabetic rats. The *C. speciosus* crude extracts decreased the serum glucose levels in diabetic rats.²⁴ The anti-hyperglycemic effect of CSREt might be exerted by increasing insulin synthesis, and release by the beta cells of the islet of Langerhans and inducing the sensitivity of cell receptors to insulin. This appears through the induction of expression of the insulin gene in pancreatic cells and IRA in hepatic cells, and increasing the serum insulin levels consequently increased glucose uptake through induction of GLUT2 gene expression. The hypoglycemic effect of eremanthin (active substance of *C. speciosus*) was exerted through potentiation of insulin synthesis and release from the existing beta cells, as well as increasing the tissues sensitivity of insulin to glucose uptake.²⁵ The STZ induces a selective destruction of pancreatic β -cells leading to poor glucose utilization inducing hyperglycemia, but leaving many of the surviving beta cells, which can be regenerated.²⁴ Such regeneration is enhanced by the administration of CSREt, and results in stimulating insulin release through increasing the level of gene expression, and so increasing its level in the blood, which can improve glucose metabolism. Insulin receptors are expressed with different ranges in all tissues that are sensitive to insulin.²⁶ This enforces our results, which showed high hepatic IRA gene expression levels in the groups that were administered high doses of CSREt.

Hepatic glucose utilization was induced possibly due to the induction of gene expression of the GLUT2 gene. The latter is a membrane bound glucose transporter present mainly in the liver, and not dependent on insulin. It has a high glucose Michaelis constant (K_m) and so the transporting of glucose into hepatic tissue is unlimited.¹⁹ Glucose that is transported to the liver is either oxidized, or stored as glycogen. The CSREt improved the activity and gene expression of hepatic glucose catabolic enzymes GK, aldolase, PK, and SDH in harmony by increasing the level of gene expression of insulin gene and serum insulin levels. The GK activity and gene expression was increased in the CSREt treated rats when compared with diabetic non-treated rats. The gene expression of GK is correlated with enzyme activity. The GK enzyme catalyzes the first hepatic glycolysis reaction, it has a low blood glucose affinity

with high K_m . So it has a high sensitivity to blood glucose level change.¹⁹ The GK activity and expression levels are decreased in diabetic patients.²⁷ The GK is a strong diabetic therapy marker because it enhances the hepatic glucose uptake and insulin secretion from pancreatic tissue.²⁸ Aldolase is a bi-functional enzyme in both glycolysis and gluconeogenesis; it is closely related to PFK-1 (phospho-fructokinase-1) in its action as it cleaves its product (F1, 6 bisphosphate) into glyceraldehyde-3-phosphate and dihydroxy acetone phosphate. Its level was reported lower in the diabetic models,²⁹ the increase in its activity after CSREt treatment may refer to improvement of glucose oxidation. The PK is a glycolytic enzyme playing a central role in hepatic glucose and lipid metabolism. It is regulated by phosphorylation, allosteric modification, hormones, and nutrients. Excess glucose utilization by tissue induces glycolysis, L-Pyruvate kinase (L-PK) activity, glycogenesis, de novo-synthesis of fatty acids, and lipid storage. The L-PK gene transcription is induced by insulin stimulated glucose metabolism.²¹ Treatment with 400 mg/kg bwt of CSREt causes an increase in the serum insulin level by 40.9% and 600 mg/kg bwt by 54.5%. The elevation in the serum insulin could result in activation of L-PK gene expression, and the enzyme activity that presented in our results, which refer to increase in the glycolytic pathway by treatment with 400 and 600 mg/kg bwt of CSREt, whereas treatment with 200 mg/kg bwt failed to produce this effect. The SDH is an oxidative mitochondrial enzyme that controls transcription of metabolism-related genes in mitochondria, and promotes glucose and lipid metabolism. The SDH mRNA expression levels were reduced in diabetic animals.³⁰

Our study showed that treatment with 400 and 600 mg/kg bwt of CSREt can increase the SDH mRNA levels and activity that improves the oxidative status in diabetic rats.¹⁰ Glycogenesis is another pathway for glucose utilization in the liver that is directly affected by insulin; the decrease in insulin level in diabetic rats results in the decrease of GS activity, and so decrease in liver glycogen content.³¹ Such decrease in the activity was reversed by CSREt treatment with doses of 400 and 600 mg/kg bwt by the correction of insulin level in the blood. The hypoglycemic action of CSREt was accompanied by the activation of GS, and increases the hepatic glycogen content.³²

The STZ induces a selective destruction of pancreatic β -cells, which may be regenerated giving false results. Further future studies are needed on genetically

engineered/knock out genes for diabetes in rat models. The anti-hyperglycemic effect of *C. speciosus* needs to be examined in diabetic humans.

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