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RT-PCR ANALYSIS OF GENES EXPRESSION TO EVALUATE THE BIOMEDICAL IMPORTANCE OF MEDICAL-HERBAL EXTRACTS IN DIABETES TREATMENT

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Abstract

Fenugreek is an annual crop belonging to the legume family grown in most parts of the world. It is known to possess a number of medicinal properties, but a little phytochemical research has been carried out on the seeds of species; Trigonella hamosa Leguminosae. Previously we reported that Trigonella seeds extract are shown to have hypoglycemic effects on experimental diabetic rats. The aim of the current study is to understand the molecular mechanism of antidiabetic actions of Trigonella. Therefore, we examined the expressions levels of genes involved in glucose metabolism in liver of diabetic rats. Upon this basis, streptozotocin (STZ)-diabetic rats are used to assess hepatic glucokinase (GCK), insulin like growth factor-1 (IGF-1), and glucose transporters (GLUT-2) genes expressions after Trigonella administration by oral intragastric intubation for two months, using RT-PCR assay. The current findings demonstrated that Trigonella reduced significantly glucose level in diabetic rats, while significantly increases insulin serum level. GCK expression levels in hepatic tissue of diabetic-rats are found to be suppressed, while Trigonella treatment induces an increase in hepatic GCK activity. Diabetes due to STZ has a little effect on IGF-1 gene expression, while Trigonella administration elevates mRNA expression significantly. On the other hand, Trigonella reduce and normalize the

elevation of GLUT-2 gene expression, which increased due to STZ treatment. These results indicated that Trigonella are powerful antidiabetic agent, induce hypoglycemia by up-regulation of CGK and IGF-1 genes expression and induce an insulin mimetic activity.

Keywords: Genes Expression, RT-PCR, Diabetes mellitus, Liver, *Trigonella h.* Leguminosae

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease occurred due to either destruction of pancreatic β-cells or insulin resistance occurred as results of obesity and its related metabolic disorders (Hall, 2003; Havel, 2004). The two major types of DM; diabetes type 1 and diabetes type 2, are characterized by hyperglycemia, abnormal lipid and protein metabolism leading to many complications affecting human body systems (Mathur et al., 2011). Patient with diabetes for long term are at higher risk of heart disease, stroke, high blood pressure, blindness, kidney disease, nervous system disease, amputations, dental disease, and complications of pregnancy (Akinci et al., 2003). A disease of diabetes is remaining to be an expanding global health crisis, and expected to affect 300 million people by 2025 (Hjelm et al., 2003). In Kingdom of Saudi Arabia (KSA), diabetes has become more evident in the last two decades as a result of dramatic changes in the life style (Ammari, 2004). The prevalence of diabetes in KSA is now considered one of the highest in the world reaching as high as 23.7% of population (Alwakeel et al., 2009). Regrettably, DM is one of the five leading causes of death worldwide (Caliskan et al., 2006).

Patients suffer from diabetic prefer to use dietary modulators has antidiabetic activity to control blood glucose level. This tendency is because insulin cannot be used orally and its repeated injections have many undesirable adverse effects. In addition, most of hypoglycemic agents or drugs are not effective to decrease blood glucose levels in chronic diabetic patients (Cheng and Fantus, 2005). For these reasons, foods of medicinal value have been proved effective and thus are widely used as they combine two basic central factors: food and medication (Mohieldein et al., 2011; Oubre et al., 1997).

Annual coumarin-smelling herb Trigonella hamosa Leguminosae (Trigonella glabra Thumb) is one of the best known member of the herb fenugreek, grows as a weed in Egypt, the East Mediterranean region, and from KSA to Afghanistan and India (Boulos, 1999; Grower et al., 2002). We and others reported that fenugreek seeds can lower blood glucose and cholesterol in experimental diabetic animals (Khosla et al., 1995; Puri et al., 1995; Kumar et al., 2005; Puri et al., 2002; Salah-eldin et al., 2007). Also,

several studies included us, demonstrated that some plant extracts belonging to the fenugreek family decreased oxidative stress and enhanced the activities of several components of the endogenous antioxidant system (Annida et al., 2005; Anuradha and Ravikumar, 2001 ; Siddiqui et al., 2005; Salah-eldin, 2008).

The diabetogenic agent streptozotocin, used in this study, is selectively toxic to insulin-secreting β-cells of pancreatic islets (Strandelle et al., 1989), and it induced significant hyperglycemia accomplished by a decrease in insulin levels (Hussain, 2002; Sellamuthu et al., 2009). Liver is an insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis by regulating the interaction between the glucose utilization and production. Thus, a suitable antidiabetic agent should improve glucose-induced insulin secretion, hepatic glucose metabolism, and peripheral insulin sensitivity (Ferre et al., 1996). It has been demonstrated that, Trigonella exerts its protective effects in diabetes by decreasing morphological changes and preserving pancreatic ß-cell integrity (Salah-eldin et al., 2007), and by beneficially changing the hepatic antioxidants enzyme activities (Salah-eldin, 2008).

Diabetes treatment depends mainly on regulation of genes related to glucose and/or carbohydrate metabolism. Glucose must be first phosphorylated before being utilized by cells. This reaction is catalyzed by a family of enzymes called hexo-kinases, which are found in different organisms including humans (Cardenas et al., 1998). Mammalian glucokinase plays a key role in maintaining glucose homoeostasis (Matschinsky et al., 2006), and is the major glucose-phosphorylating enzyme expressed in hepatocytes and pancreatic β-cells. GCK is one of the essential factors for the glucose-stimulated insulin secretion (Bourbonais et al., 2012). Long-term regulation of hepatic GCK activity is controlled by its mRNA level. In the liver, expression of GCK is very closely dependent on the presence of insulin. Stimulation of transcription of genes encoding GCK, leads to a decreased glucose level (Celik and Erdogan, 2008).

Also, liver tissues might be the main source of circulating insulin like growth factor-1 (Pankov, 1999; Catanese et al., 1993). It was reported that, IGF-1 may probably involved in metabolic abnormality and complications associated with diabetes (Goya et al., 1999). IGF-1 is protein with high sequence similarity to insulin, widely present in mammalian tissues and has a number of bioactivities including regulation of metabolism and enhancement of growth and development of tissues (Pankov, 1999; Thrailkill, 2000; Cusi and DeFronzo, 2000; Zhuang et al., 1997). Moreover, glucose transport is the rate limiting step in carbohydrate metabolism (Maughana, 2009) which is facilitated by glucose transporters; GLUT-2 across the cell membrane (Anand et al., 2010). So, drugs facilitate GLUT-2 translocation and improve

insulin sensitivity together with carbohydrate and lipid metabolism could be beneficial for the treatment of diabetes (Kipmen-Korgun et al., 2009; Shepherd and Kahn, 1999). Therefore, the effect of Trigonella on the expression levels of GCK, IGF-1, and GLUT-2 genes were assessed in liver of diabetic rats to evaluate the molecular mechanism of action.

Recently there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants, because plant sources are usually considered to be safer, with fewer side effects than synthetic sources (Goldfrank et al., 1982; Sabu et al., 2002; Mentz and Schenkel, 1989). Trigonella effects on genes control carbohydrate have not been examined yet. Therefore an attempt has been made to investigate the molecular mechanism of Trigonella in lowering and control glucose levels in experimentally diabetic animals. Herein, the effect of Trigonella on the expression levels of GCK, IGF-1 and GLUT-2 genes were assessed in liver of STZ-induced diabetic rats using reverse transcription polymerase chain reaction (RT-PCR) assay.

Materials and methods:

1. Chemicals:

Streptozotocin (Streptozocin, STZ), agarose, ethidium bromide, chloroform, and isopropanol were purchased from Sigma Chemical Co, St. Louis, Mo, USA. Vehicles and related materials were from ADWIA pharmaceutical Co, Egypt. Oligo dT and primers were from Wako pure chemicals, Osaka, Japan. TriZol reagent was from Invitrogen, Carlsbad, CA. All other chemicals and reagents used will be of analytical grade.

2. Plant Material and Extract Preparation:

Seeds of *Trigonella hamosa* Leguminosae were chosen for the present study were collected and identified according to Boulos, (1999). Trigonella seeds are kindly obtained from the herbarium of the botany at faculty of science, Aswan University, Egypt. The active ethanolic fraction contains steroid saponins extract was prepared as previously describe by Salah-eldin et al. (2007).

3. Induction of Diabetes in Wistar Rats:

Wistar albino rats were purchase from Egyptian Co for Experimental Animals Import, Helwan, Egypt. Diabetes mellitus were induced in albino rats by a single intraperitoneal (i.p.) injection of STZ (66 mg/kg body weight) dissolved in 0.1 m. citrate buffer (pH 4.5) to overnight fasted rats (Hancu et al., 1998). After STZ injection, the rats will have free access to food and water and will be given 5% glucose solution to drink overnight to counter hypoglycemic shock. After three days, the fasting blood glucose

levels will be determined and rats showing fasting blood glucose more than 200 mg% will be considered diabetic and will be selected for the experimentation.

4. Experimental Design and Samples Collection:

A total of thirty six healthy male rats of 7 to 8 weeks old and weighing over 200 grams were used. All rats were kept for acclimatization for one week under laboratory condition. The rats were providing with food pelleted and tap water *ad libitum* and randomized into three groups. 1st group was normal non-diabetic control (Cont), and two diabetic experimental groups. The diabetic groups of rats were injected ip with STZ, and rats were divided into two group; the 2nd known as STZ diabetic (STZ-Diab) rats group received water only. The 3rd group treated with saponins aqueous extract at a dose of 45 mg/kg body weight known as diabetic rats treated with Trigonella (Diab+Trig).

- 1st Group: 12 rats, normal non-diabetic control (Cont).
- 2nd Group: 12 rats, STZ diabetic (STZ-Diab).
- 3rd Group: 12 diabetic rats, treated with Trigonella (Diab+Trig).

Trigonella treatments were continued for two months by gastric tube, on a daily basis. Six rats were sacrificed by decapitation from each group after the experimental period of one and two months.

5. Blood Glucose and Insulin Levels Estimation:

Blood was collected for the biochemical analyses after decapitation. Bio Merieux Co. (Marcy-L-Eto-L charobonnieres Les Bains, France) kits were used for colorimetric determination of plasma glucose; it was determined enzymatically according to Cooper and Daniel, (1970). Insulin levels were determined by radio immunoassay kits according to Goetz et al., (1963). Livers tissues were collected from all groups, washed by normal saline solution, dried by towel, flash frozen in liquid nitrogen and subsequently frozen at –70ºC until the time of RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) analysis.

6. Total RNA Isolation and RT-PCR Assay for Selected Genes in Liver:

The Primers of GCK (Mahmoodi et al., 2013), IGF-1 (Hwang et al., 2009), and GLUT-2 (Soliman et al., 2013) genes were taken from literature. β-actin (Hwang et al., 2009) gene was used as an internal standard (housekeeping gene). The primers were checked for their Tm values, hairpin loops, dimers, cross-dimers and number of repeats and runs using Net Primer (Oligoanalyzer 3.1). Frozen liver samples of each group were thawed and used for RNA extraction. Homogenization of 200 mg of frozen tissue samples was carried after addition of 1 mL TriZol (Invitrogen, Carlsbad,

CA) using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). One milliliter of the tissue homogenate was transferred to a microfuge tube and total RNA was extracted via chloroform extraction followed by nucleic acid precipitation with isopropanol. The pellet was washed with 75% ethanol and resuspended in molecular biology grade water. Nucleic acid concentration was determined by optical density at 260 nm (Smart-Spec; Bio-Rad Laboratories, Hercules, CA) and RNA integrity was evaluated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA). The purity of RNA at 260/280 OD ratio and RNA integrity was evaluated and only a high purity samples (OD 260/280 >1.8) were subjected to further manipulation.

For cDNA synthesis, total RNA (1 μg) was denatured at 72°C for 5 min and then reverse transcribed using 100 units of Moloney Murine Leukemia virus reverse transcriptase (Gibco), 50 pmol of oligo (dT) primer and 20 nmol of dNTPs in a total volume of 10 μL at 37°C for 1 h. After heating at 94°C for 5 min, PCR amplification was performed with 2.5 units Taq DNA polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl2 and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 50 μL. The RT-PCR conditions were as follows: cycling for 35-40 cycles, each cycle consisting of denaturation for 30 sec at 94ºC followed by annealing for 30 sec at 52-55ºC (according to gene of interest as described in Table 1) and extension for 1 min at 72ºC. The template concentration and the cycle number were optimized to ensure linearity of response and to avoid saturation of the reaction. The PCR products were resolved and electrophoresed in 1.5% agarose gels, stained with ethidium bromide and visualized under UV lamp. The bands were identified based on the product size and documented using a Gel documentation system (Gel DocTMXR System, Bio-Rad) and the prints were scanned. The scanned images were quantified densitometrically with the aid of NIH image program (http//rsb.info.nih.gov/nih-imag/). The results were normalized to the levels obtained for the β-actin gene by taking a ratio of the value obtained for the gene of interest to that of β-actin and then relative to the control.

Table (1): PCR Conditions, Primer Sequences and Expected Product Size for the Genes Amplified.

Genes	(cDNA)		Primer Sequences	RT-PCR Product size	Annealing Temp/Time	Cycles Number
Glucokinase	GCK	Forward Reverse	5'-ACTGACTATCCGGCTACATG-3' 5'-GATTCCTGCTTGAATAGTGC-3'	199 bp	55°C /45 sec	35 Cycles
Glucose Transporter 2	GLUT-2	Forward Reverse	5'-AAGGATCAAAGCCATGTTGG-3' 5'-GGAGACCTTCTGCTCAGTGG-3'	330 bp	55°C /1 min	40 Cycles
Insulin like Growth Factor 1	IGF-1	Forward Reverse	5'-CTG GGT GTC CAA ATG TAA CT-3' 5'-GTA TCT TTA TTG GAG GTG CG-3'	170 bp	52°C /45 sec	35 Cycles
ß-actin	ß-actin	Forward Reverse	5'-AGC CAT GTA CGT AGC CAT CC-3' 5'-CTC TCA GCT GTG GTG GTG AA-3'	230 bp	55°C /1 min	40 Cycles

PCR cycle of respective genes are shown above, while temperature and time of Denaturation and Elongation steps of each PCR cycle are 94°C, 30 sec and 72°C, 60 sec, respectively.

7. Statistical analysis:

Results obtained are expressed as means ± standard error (X ± SE). To determine differences among the treated groups, data were statistically analyzed using a computerized one-way ANOVA followed a by t-test, according to Sendecor and Cochron, (1982). Differences between means were considered significant with a value of $P \leq 0.05$.

Results

It is clear from the previous study that the harmful effects of chronic diabetes appeared after 20 days and reached a maximum after 30 days of experiment (Salah-eldin et al., 2007). Our further study was carried out on how Trigonella supplementation of the diet countered the harmful effects of chronic diabetes, by improving antioxidant defense and oxidative damage, after one month of treatment (Salah-eldin, 2008). Since insulin was decreased in diabetic-rats and Trigonella administration normalized blood sugar level, we investigate the molecular mechanism of Trigonella action as a hypoglycemic agent by analysis the expressions levels of GCK, IGF-1 and GLUT-2 genes in normal control non-diabetic, diabetic, and diabetic-treated rats using RT-PCR assay.

1. Glucose and Insulin Levels Recovered by Trigonella Treatment:

It is well established that STZ causes destruction of pancreatic -cells, leading to a marked reduction in serum insulin level and hyperglycemia. Figures 1 and 2 showed the variation in the levels of blood glucose and insulin, respectively during experiments. The level of glucose was significantly ($P \leq 0.05$) elevated in STZ-diabetic rats (STZ-Diab) after four days of treatment and persisted throughout the experiment. After one and two months of STZ injection, rats showed markedly elevation in blood

glucose levels, and the values recorded reached 633.3 mg% and 566.2 mg%, respectively (Figure 1). We found a sign of recovery in glucose levels after treatments by Trigonella. In diabetic-treated group (Diab+Trig), Trigonella induce a significantly ($P \leq 0.05$) lowering in glucose levels to 394.3 mg% and 142.1 mg% after one and two months of experiments, respectively.

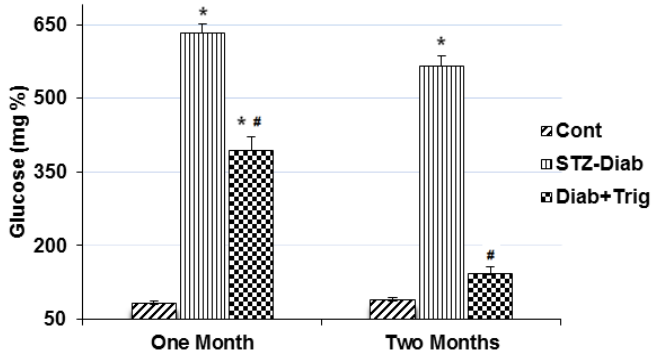

Figure (1): Variations in the glucose levels in non-diabetic control (Cont) rats, streptozotocin-diabetic (STZ-Diab) rats, and diabetic-rats received oral Trigonella extract (Diab+Trig) after one and two months of experiments (n=6). (*) Values are significant different ($P \leq 0.05$) compared to control. (#) Values are significant different ($P \leq 0.05$) compared to diabetic group control.

A severe reduction in serum insulin levels due to STZ treatment in STZ-Diab group, from 7.8 mIU/ml to 3.9 mIU/ml after one month, and from 8.1 mIU/ml to 4.5 mIU/ml after the second month of experiments (Figure 2). A significant ($P \leq 0.05$) recovery of insulin levels was recorded to 5.4 mIU/ml and 7.1 mIU/ml in diabetic-treated group with Trigonella after the first and second month, respectively. The present study clarified that Trigonella increases insulin levels by elevating insulin synthesis and secretion.

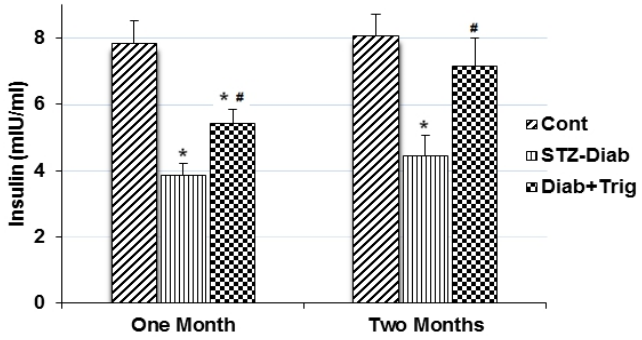

Figure (2): Variations in the insulin levels in non-diabetic control (Cont) rats, streptozotocin-diabetic (STZ-Diab) rats, and diabetic-rats received oral Trigonella extract (Diab+Trig) after one and two months of experiments (n=6). (*) Values are significant different ($P \leq 0.05$) compared to control. (#) Values are significant different ($P \leq 0.05$) compared to diabetic group control.

These data indicated that rats in STZ-Diab group did not grow normally, which would be expected, because their insulin levels were very low and glucose levels were too high. The decrement in plasma insulin level in STZ-Diab rats may be due to destruction of β-cells. Meanwhile, Trigonella administration in Diab+Trig group increased insulin levels significantly near to control levels. The results of insulin were reflected on glucose levels, which were higher in diabetic rats but normalized after two months of Trigonella administered in diabetic rats.

2. RT-PCR Analysis for Genes Modulate Carbohydrate Metabolism in Hepatic Tissue:

To investigate the alteration in carbohydrate metabolism by Trigonella, mRNA from liver tissue was subjected for RT-PCR analysis. The expressions levels of GCK, IGF-1 and GLUT-2 genes were assessed in normal control albino rats (Cont), STZ-diabetic (STZ-Diab) rats, and diabetic rats treated with Trigonella (Diab+Trig) groups.

2.1. GCK Gene Expression in Liver after Trigonella Administration:

The mRNA levels of glucokinase in rat's liver were analysis. The expression level of the GCK gene in the normal control group (Cont) was considered as reference to calculate and normalize the expression in the other groups. Comparing with control rats, diabetes was found to suppress GCK gene expression slightly in liver tissue in STZ-Diab group (Figure 3). Trigonella treatment in Diab+Trig group elevated hepatic GCK gene expression when compared with the control and diabetic groups after one and two months of treatments. RT-PCR results showed that, Trigonella administration in diabetic rats induced a significant ($P \leq 0.05$) increase in the gene expression after one and two months to 141.1 % and 169.2%, respectively.

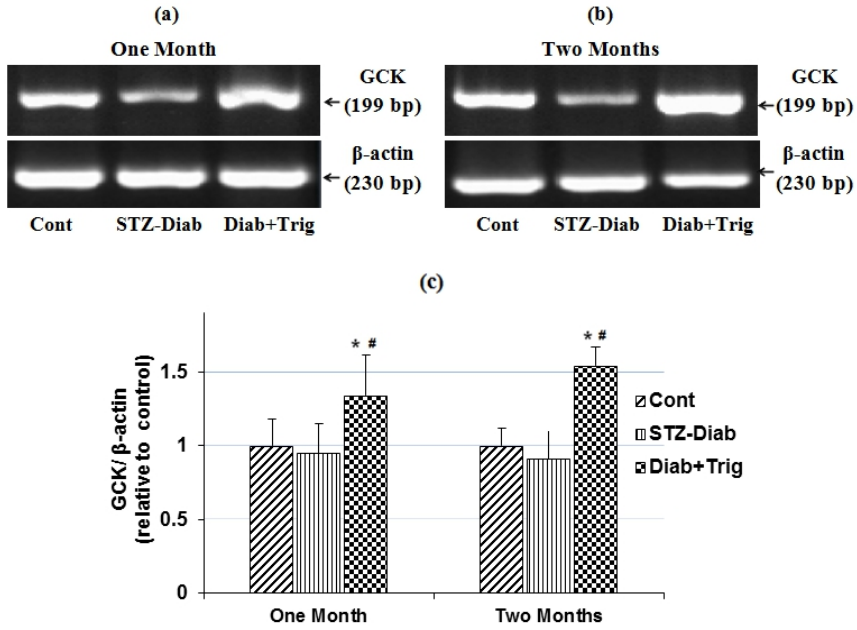

Figure (3): RT-PCR analysis of GCK expression in liver tissues. Data of control non-diabetic (Cont), experimentally diabetic (STZ-Diab), and Trigonella-treated diabetic rats (Diab+Trig) are shown after one and two months. RNA was extracted and reverse transcribed (1 μg) and then RT-PCR analysis was carried out for GCK gene. The densitometric analysis of the expresses bands after one month (a) and two months (b) of treatments. Data were normalized with that of β-actin and then calculated as relative to the control group (c). (*) Values are significant different ($P \leq 0.05$) compared to control. (#)Values are significant different ($P \leq 0.05$) compared to diabetic group control.

2.2. IGF-1 Gene Expression in Liver after Trigonella Administration:

Herein, data shown that insulin levels sharply decreased in diabetic rats, and then increased near to non-diabetic control after Trigonella treatment. In this study, a change in insulin like growth factor 1 expression was non-significantly increased in diabetic as shown in Figure 4 compare to control non-diabetic rats. After Trigonella administration for two months only, IGF-1 mRNA expression is significantly ($P \leq 0.05$) increased. The over expression of this gene was recorded when diabetic rats treated with Trigonella in Diab+Trig group compare to normal control and diabetic-untreated rats.

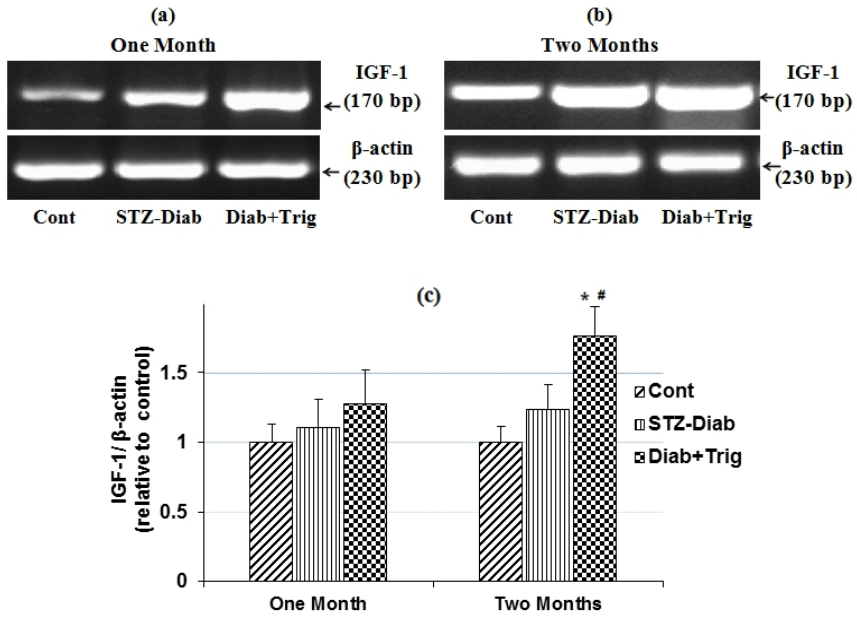

Figure (4): RT-PCR analysis of IGF-1expression in liver tissues. Data of control non-diabetic (Cont), experimentally diabetic (STZ-Diab), and Trigonella-treated diabetic rats (Diab+Trig) are shown after one and two months. RNA was extracted and reverse transcribed (1 μg) and then RT-PCR analysis was carried out for IGF-1 gene. The densitometric analysis of the expresses bands after one month (a) and two months (b) of treatments. Data were normalized with that of β-actin and then calculated as relative to the control group (c). (*) Values are significant different ($P \leq 0.05$) compared to control. (#)Values are significant different ($P \leq 0.05$) compared to diabetic group control.

2.3. GLUT-2 Gene Expression in Liver after Trigonella Administration:

Trigonella administration normalized the increase in glucose in diabetic rats, and decreased insulin level. So, we tested the physiological importance of insulin dependent glucose transporter 2_translocation. STZ administration in STZ-Diab group was found to increase GLUT-2 gene expression non-significantly ($P \leq 0.05$) in liver compared to control rats after one and two months of treatments (Figure 5). In Diab+Trig group, Trigonella reduce hepatic GLUT-2 gene expression when compared with the diabetic group. As shown in Figure 5, diabetic rats showed an increase in GLUT-2 mRNA expression and that increase was inhibited and become close to control after Trigonella administration in a way to regulate the excessive hepatic glucose utilization.

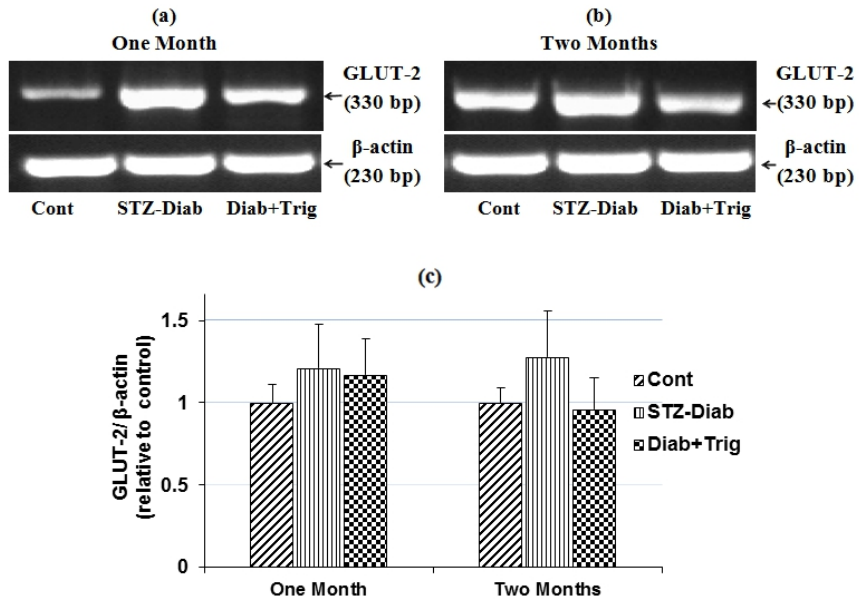

Figure (5): RT-PCR analysis of GLUT-2 expression in liver tissues. Data of control non-diabetic (Cont), experimentally diabetic (STZ-Diab), and Trigonella-treated diabetic rats (Diab+Trig) are shown after one and two months. RNA was extracted and reverse transcribed (1 µg) and then RT-PCR analysis was carried out for GLUT-2 gene. The densitometric analysis of the expresses bands after one month (a) and two months (b) of treatments. Data were normalized with that of β-actin and then calculated as relative to the control group (c). (*) Values are significant different ($P \leq 0.05$) compared to control. (#)Values are significant different ($P \leq 0.05$) compared to diabetic group control.

Discussion

Diabetes is a chronic metabolic disorder that affects approximately 3% of population worldwide (Kim et al., 2009). Many sufferers from diabetes become aware that they are diabetic, only when they develop one of its life-threatening complications. Knowledge and awareness about DM, its risk factors, complications and management are important aspects for better control and good quality of life (Wild et al., 2004; Ángeles-Llerenas et al., 2005). Usage of oral hypoglycemic drugs to treat diabetes has several limitations, such as adverse effects and high rates of secondary failure (Kim et al., 2009). Those adverse effects forced the diabetic patients to use herbal medication that has a similar degree of efficiency without side effects. Keeping in mind, the sustained reductions in hyperglycemia will decrease the risk of developing microvascular diseases and reduce diabetes complications (Gaster and Hirsch, 1998).

Changes in gene expression are an important component of the pathogenesis of diabetes (Yechoor et al., 2002; Sreekumar et al., 2002). In recent years, numerous plant extracts and plant formulations have been shown to regulate the expression of genes in metabolic pathways of different

diabetic animal models (Xu et al., 2009; Kim et al., 2009; Kwon et al., 2005). Also, multiple transcription factors are involved in the regulation of carbohydrate metabolism either directly or through interaction with insulin, and a large number of insulin-regulated genes have been identified in liver tissue (O'Brien and Granner, 1996). Our previous studies have reported the hypoglycemic effect and antihyperlipidemic potency of Trigonella in STZ-induce diabetes in rats (Salah-eldin et al., 2007; Salah-eldin, 2008), while the mode of action of this plant is not reported yet.

Present study revealed that, injection of STZ at dose of 66 mg/kg body/weight induced significant hyperglycemia in albino rats. This dose caused damage to □ -cells of the islets of Langerhans and emergence of clinical diabetes within four days as results of autoimmune process (Weiss, 1982), and STZ treatment induced significant hyperglycemia (Hussain, 2002; Sellamuthu et al., 2009). STZ causes destruction of□ -cells of pancreas resulting in marked decrease in insulin levels, it could be predicted that glycogen levels in tissues (muscle and liver) decrease as the influx of glucose in liver is inhibited in the absence of insulin and recovers on insulin treatment (Vats et al., 2004). Hyperglycemia may be attributed to enhancement of gluconeogenesis as a result of absence of insulin (Yao et al., 2006).

Hepatic glucokinase is a key enzyme in glucose homeostasis, and it is a potential target for treatment strategies of diabetes. It was reported that glucokinase enzyme activity was decreased by more than 90% in the liver diabetic rats (Zhang et al., 2009). So, in the current study, we evaluate the antidiabetic mechanism(s) of Trigonella, on the key enzyme of carbohydrate metabolism at mRNA level in livers of diabetic rats using RT-PCR. In this study, GCK expression levels in the livers of diabetic rats were slightly suppressed, while Trigonella-treated diabetic group showed an increased. The increased activity of hepatic GCK in the Trigonella treated group may cause an increase in glycolysis and utilization of glucose for energy production. Run in agreement with our data, it has been reported that GCK-knockout mice have mild hyperglycemia (Postic et al., 1999), whilst rats over expressing GCK in the liver have reduced blood glucose (Ferre et al., 1996).

Current results demonstrate that Trigonella reduce significantly glucose level in diabetic rats. The decrease in the blood glucose concentration in Trigonella treated diabetic rats may be associated with enhancement of GCK mRNA expression in the liver, thus increasing the level of glycolysis. In addition, blood insulin concentrations were increased in Trigonella-treated groups compared with the diabetic untreated group. This suggests that insulin may be enhances transcription of GCK gene in hepatocytes (Iynedjian et al., 1989; Matschinsky et al., 1993), which employ

a different promoter than that employed by -cells (Shelton et al., 1992). This agrees with the finding that hepatic glycolytic enzyme activity is controlled primarily at the transcription level by insulin (Friedman et al., 1997). High insulin levels have been shown to inhibit hepatic glucose production by means of stimulation of GCK gene transcription (Celika et al., 2009). In this study, the changes in GCK could be partly attributed to the insulin level because plasma insulin level was elevated in Trigonella treated rats than the diabetic one and become near to control group. These data are in agreement with finding about other medical plants like, Caffeic acid phenethyl ester (CAPE) treatment (Celika et al., 2009), Persian Shallot treatment (Mahmoodi et al., 2013), and Cinnamon extract treatment (Soliman et al., 2013)

To our knowledge, we report for the first time that Trigonella supplementation causes an increase in GCK mRNA expression in liver of diabetic rats. Current results is consistent with previous studies that showed GCK mRNA expression increase in Naringin (Jung et al., 2004), 1-Deoxynojirimycin (Li et al., 2011) and epigallocatechin gallate, a main polyphenolic constituent of green tea (Nakagawa et al., 2002) treated rats. In liver, an increase in GCK activity leads to enhanced glycolysis and hepatic glucose uptake (Grimsby et al., 2003). The increase in insulin concentrations in diabetic rats supplemented with Trigonella could either be caused by direct stimulation of insulin secretion in response to feeding or by a protective effect of Trigonella on the pancreas. The results of our study are in line with recently published data, which suggest that other plants preserves and protects the pancreas by its strong antioxidative capacity (Jung et al., 2004; Song et al., 2003). This would ultimately lead to enhanced pancreatic function and improved insulin secretion in response to feeding. The lipid peroxidation may attribute to the hypoinsulinemia caused by STZ progressive deterioration of normal pancreatic β-cell function. This hypoinsulinemia induced an increase in the activity of fatty acyl Co-A oxidase that initiate the β-oxidation of fatty acids resulting in lipid peroxidation (Oberley , 1988; Baynes and Thorpe, 1996). In the previous study, administration of Trigonella extract significantly decreased lipid peroxide biomarkers; Thiobarbituric acid reactive substances (TBARS) in liver of diabetic rats. These results are underlined by the significant increase in hepatic activities and expressions of antioxidant enzymes in diabetic rats treated with Trigonella (Salah-eldin, 2008).

IGF-1 is a single polypeptide with 70 amino acids, widely expressed in mammal tissues and it has similar structures and functions like those of insulin (Pankov, 1999; Thrailkill, 2000). The liver is the main source of circulating IGF-1 (Sjogren et al., 2002), thus hepatic tissues were used for the expression study. IGF-1 induced its effect via binding to specific

receptors on target cells. Insulin regulates IGF-1 expression either directly or indirectly by increasing the number of growth hormone hepatic receptors. Because of IGF-1 induced glucose taken and improved the insulin sensitivity (Scharf et al., 2000; Reinmuth et al., 2002), increased its expression augmented this effect in diabetic rats treated by Trigonella. Therefore, the increase of gene expression of IGF-1 by Trigonella administration can be considered a new mechanism of antidiabetic effect of this herbal medical plant. In the present study, hepatic IGF-1 gene expression was not significantly changed in STZ-diabetic rats compared to the control non-diabetic one. On contrary, previous findings reported by Goya et al. (1999) and Li et al. (2004) indicating a down regulation of IGF-1 in diabetic rats after acute or chronic administration of diabetic-induce agent; Alloxan. IGFs are part of a complex system that cells use to communicate with their physiologic environment, since at high concentrations is capable of activating the insulin receptor, it can also complement for the effects of insulin (Scarth, 2006). As discussed above, the antihyperglycemic effect of Trigonella was observed via up-regulation of IGF-1 gene expression in diabetic rats.

GLUT-2 is a transmembrane carrier protein that enables passive glucose movement across cell membranes. It is the principal transporter for transfer of glucose between liver and blood and for renal glucose reabsorption (Freitas et al., 2005). In this study, the normalization of GLUT-2 expression after Trigonella administration may be a reflect pathway to reverse the glucose uptake in liver cells while Trigonella administration increases IGF-1 expression to induce insulin like effects.

In summary, there has been a growing interest in hypoglycemic agents from natural products of Saponins found in *Trigonella hamosa* L. In current study Trigonella significantly reduced glucose level, while gently increases insulin serum level. These findings suggest that, Trigonella could restore the damaged pancreas and stimulate the secretion of pancreatic insulin. Mean-time, Trigonella has probably ability to accelerate the hepatic glucose metabolism may be via regulating the expression of the functional genes of GCK, IGF-1 and GLUT-2 as documented by RT-PCR studies. In fact, this antihyperglycemic action of Trigonella is likely to be associated with a marked enhancement of the GCK and IGF-1 mRNA expressions in liver.

Conclusion

We can conclude that Trigonella extract induce beneficial effects in diabetic-rat models, by affect the genes related to carbohydrate metabolism via upregulation of GCK and IGF-1 expressions in a way to control the

metabolic biohazards accompanied diabetes. Moreover, Trigonella is a good herbal medication with insulin mimetic activity.

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