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Experimental diabetes treated with trigonelline: effect on key enzymes related to diabetes and hypertension, β-cell and liver function

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Abstract Type 2 diabetes is quite diverse, including the improvement of insulin sensitivity by dipeptidylpeptidase-4 (DPP-4) inhibitor, α-glucosidase inhibitors, and the protection of β-cells islet. The aim of this study was to search the effect of trigonelline (Trig) on DPP-4, α-glucosidase and angiotensin converting enzyme (ACE) activities as well as β-cells architecture, and starch and glucose tolerance test. In surviving diabetic rats, the supplement of Trig potentially inhibited DPP-4 and α-glucosidase activities in both plasma and small intestine. The pancreas islet and less β-cells damage were observed after the administration of trig to diabetic rats. The increase of GLP-1 in surviving diabetic rats suppressed the increase of blood glucose level and improved results in the oral glucose and starch tolerance test. Trig also normalized key enzyme related to hypertension as ACE and improved the hemoglobin A1c and lipid profiles (plasma triglyceride, HDL-cholesterol, LDL-cholesterol, and total cholesterol), and liver indices toxicity. Therefore, these results revealed that Trig was successful in improving glycemic control, metabolic parameters, and liver function in diabetic rats. It is therefore suggested that Trig may be a potential agent for the treatment of type 2 diabetes.

Keywords Trigonelline · Diabetes · DPP-4 · α-Glucosidase · ACE · Pancreas · Rat

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Introduction

Diabetes mellitus is a major and growing public health problem throughout the world, with an estimated worldwide prevalence in 2008 more than 347 million people and is a heterogeneous disorder with varying prevalences among different ethnic groups and it is reported to constitute the 16th leading cause of global mortality [1, 2].

One of the therapeutic approaches used to decrease drug-treated diabetes is to retard the absorption of glucose through the inhibition of dipeptidyl peptidase 4 (DPP-4) which leads to increase in GLP-1 [3, 4]. A number of clinical data suggested that the GLP-1 pathway regulates blood glucose levels and has beneficial effects on beta cell proliferation and function, including insulin biosynthesis and secretion, in a glucose-dependent manner [5, 6]. Studies from diabetic animal models showed that GLP-1-based therapies increased β-cell neogenesis and reduced β cell apoptosis, and decreased α cell glucagon secretion [5–7]. Of special interest, alkaloids-containing herbs have been shown to display a wide range of attractive biological and pharmacologic activities, including antifungal, antibacterial, anti-inflammatory, and hypocholesterolemic actions [8–11]. More recently, the efficiency of fenugreek trigonelline ($C_7H_7O_2N$) as nutritional supplements and food additives has been widely established in the alleviation of a wide range of complications pertaining to various diseases, including hypercholesterolemia and obesity [12, 13]. Glimepiride, a second generation sulfonylurea agent, lowers blood glucose by stimulating the release of insulin from functioning pancreatic β cells (Fig. 1).

Trigonelline (Trig) is the major component of alkaloids fenugreek which has been used in the treatment of diabetes in traditional Chinese medicine [14, 15]. Previous studies in rats as in human have been shown that Trig reduces

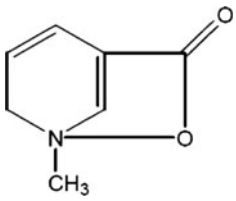

Fig. 1 Chemical structure of trigonelline

blood glucose concentrations in rats [16, 17] and in human [18, 19]. Moreover, the administration of Trig to surviving diabetic rats protects β-cells pancreas, increases insulin sensitivity index and insulin content [20], protects from stress oxidant, and stimulates antioxidant capacity in cell-free systems and human colon cell lines [21]. In addition, Trig regulates key enzymes of glucose and lipid metabolism as glucokinase, glucose-6-phosphatase, fatty acid synthase, carnitine palmitoyl transferase, glucokinase, and tumor necrosis factor-α production in diabetic and obese rats [22, 23]. However, few study reports the detailed beneficial effect of Trig on α-glucosidase, lipase, and DPP-4 activities. Accordingly, this study was undertaken to evaluate in the first time the effect of fenugreek Trig in the DPP-4, glycogen synthase, glycogen phosphorylase activities as well as pancreas β-cells architecture and insulin secretion of diabetic rats.

Materials and methods

Reagents

Trig hydrochloride (Trig, purity 99 %), Gly-pro-*p*-nitroanilide, alloxan, glucose, maltose, and starch were purchased from Sigma Chemicals (St. Louis, MO, USA). Total and glycosylated hemoglobin were assayed by kits from Biomaghred (Tunis, Tunisa).

Induction of diabetes

The assays of this study were conducted on adult male *Wistar* rats, weighing 179 ± 10 g, which were obtained from the local Central Pharmacy, Tunisia. All rats were kept in an environmentally controlled breeding room (temperature: 20 ± 2 °C, humidity: 60 ± 5 %, 12-h dark/light cycle) where they had free access to tap water. The animals fasted overnight before blood and tissue collection. Diabetes was induced in the rats by intraperitoneally administering of alloxan solution (150 mg/kg) [24]. Non-diabetic rats were intraperitoneally administered physiological saline and were kept in their cages for the next 24 h on 5 % glucose solution bottles to prevent hypoglycemia. 2 weeks later, the rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose levels of ≥2 g/L) were chosen for the subsequent experimental assays. The animals were handled in accordance with the guidelines set forth by the Tunisian Ethical Committee for the care and use of laboratory animals.

Experimental procedure

A total of 50 rats (30 surviving diabetic rats and 20 control animals) were divided into four groups consisting of ten rats each.

Group 1 referred to surviving diabetic rats that were killed and named as diabetic rats at day 0 (D_0) (glycemia ≥2 g/L).

Group 2 designated the diabetic control rats that were named as diabetic rats after treatment (D_{30}).

Group 3 referred to Trig-treated diabetic rats at dose 100 mg/kg bw by gastric gavage route named (D_{30} + Trig).

Group 4 referred to glimepiride-treated diabetic rats at dose 5 mg/kg bw by gastric gavage route named (D_{30} + Gli).

Group 5 consisted of ten normal rats that were used as controls.

1 month after the administration of these fractions to the diabetic rats, the animals were killed by rapid decapitation and their trunk blood was collected. The serum was prepared by centrifugation (1,500×*g*, 20 min at 4 °C); the small intestine was removed, and all the samples were stored at −80 °C until further use.

At the end of the experimentation and for oral glucose tolerance test (OGTT), six rats from each group were received glucose 2 g/kg/bw by gastric gavage route. Blood samples were collected from tail at 5, 10, 20, 30, 60, and 90 min subsequent to received glucose (2 g/kg) and fasting glucose was measured.

Analytical methods

DPP-4 activity was determined by an enzymatic method using the H-gly-pro-7-amino-4-methylcoumarin substrate. The method is based on the ability of DPP-4 to degrade H-gly-pro-7-aminomethylcoumarin to 7-amino-4-methylcoumarin which is fluorescent. DPP-4 activity is expressed as milliunits of activity per milliliter plasma per minute of incubation with the substrate. The GOD, HDL, TC, TG, AST, ALT, PAL, total, and direct bilirubin were from Biomaghreb analyticals Tunis, Tunisia. The activities of maltase, lactase, and sucrase activities were obtained by measuring the amount of glucose released from various substrates [25]. The plasma and kidney serum ACE activity was measured using hippuryl-His-Leu as a synthetic substrate [26]. For histological studies, pieces of pancreas were fixed in a Bouin solution for 24 h, and then embedded

in paraffin. Sections of 5-μm thickness were stained with hematoxylin–eosin and examined under an Olympus CX41 light microscope.

Statistical analysis

Data are presented as mean ± SD. Determinations were performed from ten animals per group. The differences were examined using one-way analysis of variance and Fisher test (Stat View) and the significance value was accepted at $p < 0.05$.

Results

Effect of Trig on DPP-4 activity in diabetic rats

The findings indicated that compared to the control, there was a significant increase in the DPP-4 activity in both plasma and small intestine by 217 and 202 %, respectively, in diabetic rats. However, after the administration of Trig to surviving diabetic rats, a considerable reduction in plasmatic and intestinal DPP-4 activity by 45 and 43 %, respectively, was observed (Fig. 2).

Effect of Trig on glycogen synthase, glycogen phosphorylase activities, and liver glycogen content

Figure 3 illustrates the effect of Trig administration to surviving diabetic rats in terms of glycogen content, glycogen synthase, and glycogen phosphorylase activities in the liver of the control and experimental groups of rats. In fact, a considerable decrease in the glycogen synthase activity as well as in the glycogen content by 68 %, with a concomitant increase in the activity of glycogen phosphorylase, was noted in the livers of the untreated diabetic group of rats. However, compared to the control group, the administration of Trig to the diabetic rats restored the liver glycogen content and the activities of glycogen synthase and glycogen phosphorylase to near normal levels (Fig. 3).

Effect of Trig on activities of intestinal maltase, lactase and sucrase activities in diabetic rats

The findings indicated that compared to the control, there was a significant increase in the activities of three disaccharidases, namely maltase, lactase, and sucrase in the intestine of diabetic rats. However, after the administration of Trig to treated diabetic rats, a considerable reduction in intestinal maltase, lactase, and sucrase activities was observed, which provides sheer evidence to the gain contributions obtained by the administration of fenugreek saponins for the treatment of diabetes (Fig. 4).

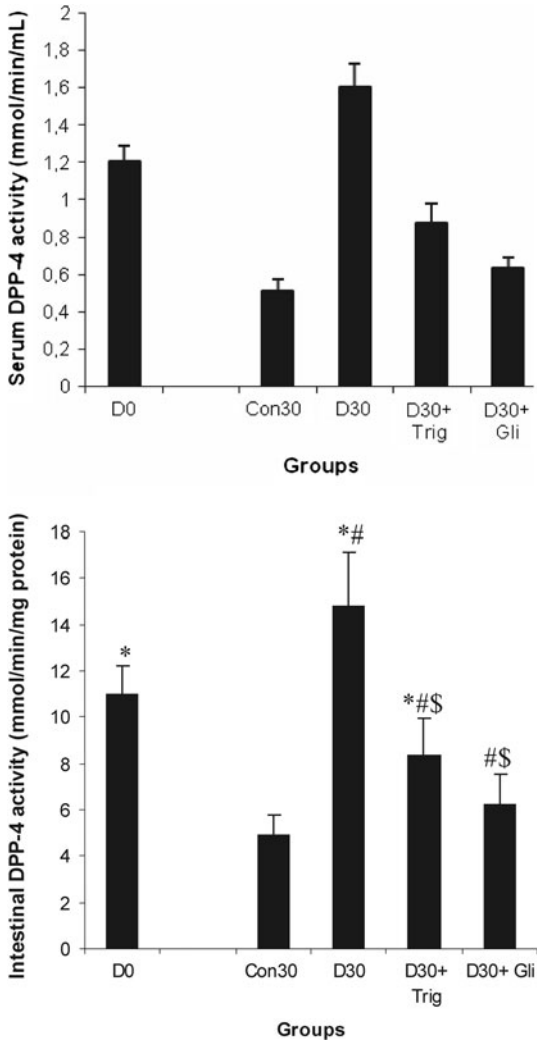

Fig. 2 Effect of the Trig on DPP-4 activity in serum and small intestine. Statistical analyses as given in table legend

Effect of Trig on blood glucose level and glucose tolerance test (OGTT) in diabetic rats

Figure 5 illustrates the effect of Trig administration to surviving diabetic rats in terms of serum glucose of the control and experimental groups of rats. In fact, a considerable increase in serum glucose level by 191 % was observed in diabetic rats as compared with those controls. However, compared to the control group, the administration of Trig to the diabetic rats restored the serum glucose, with the intent to assess the effect of orally administered Trig on systemic glucose homeostasis.

Figure 5 presents the blood glucose levels of normal, diabetic control, and Trig- and Gli-treated animals after oral administration of glucose (2 g/kg/mL). In diabetic animals, blood glucose levels reached a peak 1 h after glucose administration. Although the glucose levels started

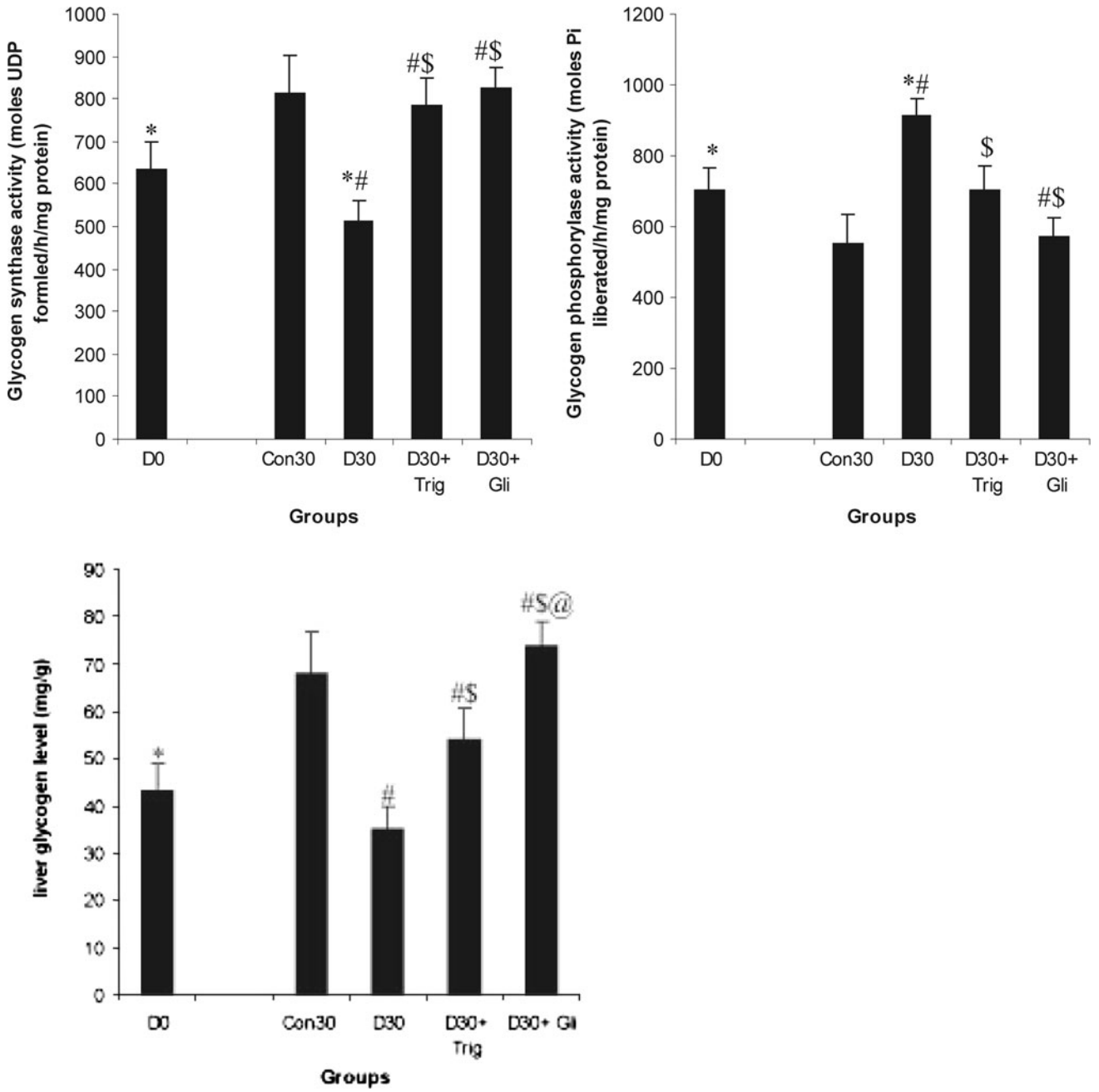

Fig. 3 Activities of glycogen synthase and glycogen phosphorylase, and level of hepatic glycogen content and blood glucose rates of control and experimental groups of rats. Statistical analyses as given in table legend

to decline, they continued to be high after 2 h. Trig-treated animals showed a significant decrease 1 and 2 h after oral glucose administration.

Effect of Trig on plasma ACE activity in diabetic rats

Figure 5 indicates that the plasma ACE activity in the diabetic rats underwent a potent increase in serum and kidney by 36 and 39 %, respectively, as compared to the nondiabetic rats. However, the administration of Trig to surviving diabetic rats restored back the activity of ACE in serum and kidney by 26 and 27 %, respectively (Fig. 6).

Effect of Trig on plasma hemoglobin and HbA1C

Figure 7 shows the HbA1C and hemoglobin levels of normal and experimental animals. Administration of Trig 50 mg/kg/day to diabetic rats significantly increased

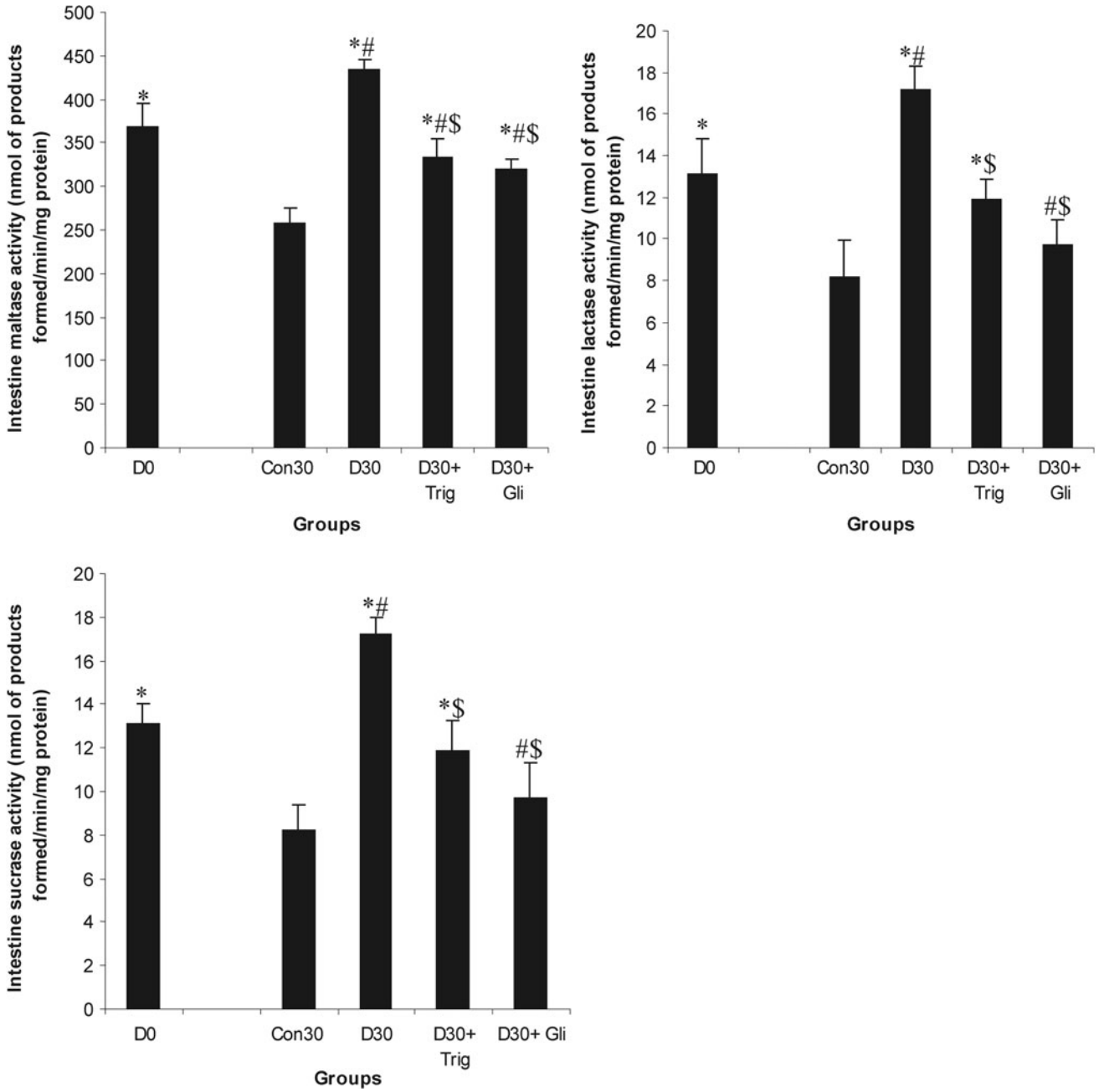

Fig. 4 Effect of Trig on lactase, maltase, and sucrase activities in the intestine of control and diabetic rats. Statistical analyses as given in table legend

hemoglobin levels and significantly decreased HbA1C levels.

Effect of Trig histological findings of pancreas tissues

The histopathological examination of the pancreatic tissues revealed that the pancreas of the control rats (Con) showed normal islets, whereas, those of diabetic rats showed a clear atrophy of β-cells (*D*). Conversely, a clear ameliorative action was observed in the pancreas of the diabetic rats after Trig administration (Trig, Gli) (Fig. 8).

Effect of Trig on liver function and metabolism

Table 1 illustrates that the activities of AST, ALT, PAL, and the level of total and direct bilirubin in the plasma of the diabetic rats witnessed a significant increase when compared to nondiabetic rats. Moreover, a significant increase of TG,

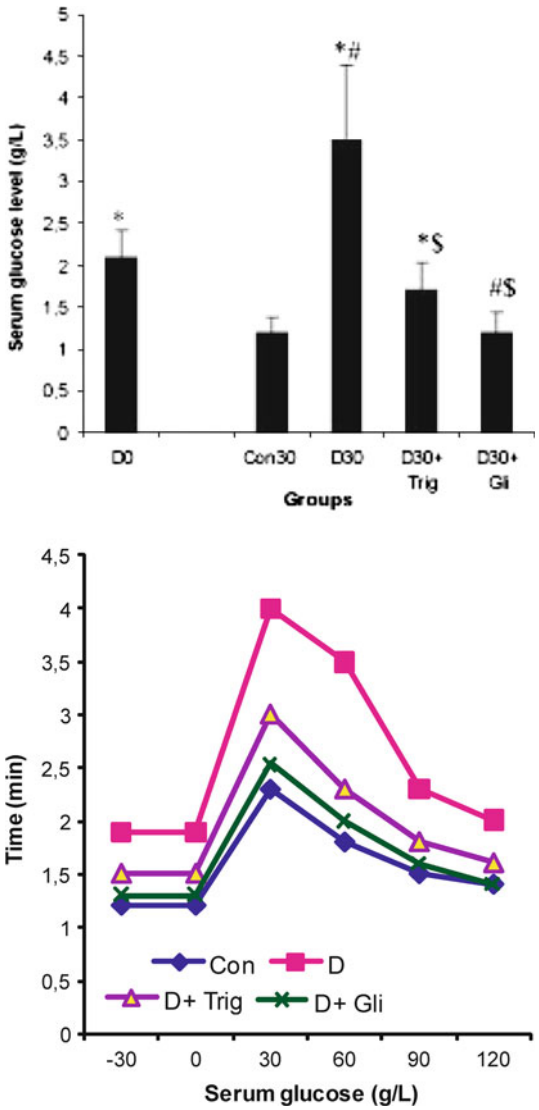

Fig. 5 Oral glucose and starch tolerance test on control and experimental groups of rats

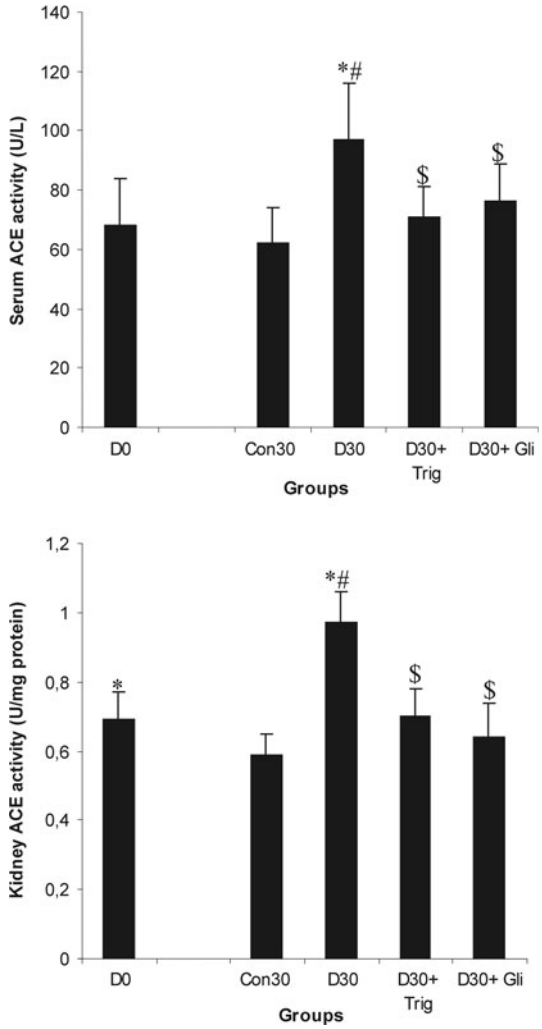

Fig. 6 Effect of Trig on serum and kidneys ACE activity of control and diabetic rats. Statistical analyses as given in table legend

LDL-C, TC, and decrease in HDL-C was noted in plasma of diabetic rats. However, the supplement of Trig was found to bring about a potent decrease in terms of the three indices of liver toxicity and normalized lipid profile as near to normal rats. Findings from further histological analysis were found to confirm the positive effect of Trig in liver tissues. As shown in liver of diabetic rats, fatty cysts, indicated by arrow, appeared in the hepatic tissues of diabetic rats as compared to normal rats. However, the administration of Trig and Gli to surviving diabetic rats protects liver tissues (Trig, Gli) (Fig. 9).

Discussion

One of the therapeutic approaches used for the decrease of postprandial hyperglycemia and hyperlipidemia is the stimulation of GLP-1 by inhibiting DPP-4. Accordingly, recent research seems to have granted special interest for the search of effective natural DPP-4 inhibitors. In the same vein, alkaloids isolated from plant sources have attracted a great deal of attention in the biomedical arena particularly for their broad spectrum of therapeutic properties and relatively low toxicity [27]. In this study, we report that administration of Trig to surviving diabetic rats restored β-cells mass and architecture in response to glucose stimulation. These results match previous findings that demonstrate administration of fenugreek alkaloids to diabetic rats had lower blood glucose and this was accompanied by an increase in plasma insulin concentration [20, 28, 29]. Moreover, it has reported that administration of fenugreek alkaloids to diabetic rats increased the number of β-cells, the islets appeared more organized and less vacuolated. The hypoglycemic effect of fenugreek alkaloids may be due to the regeneration of β cells number. Puri et al. [29] reported that hypoglycemic effect of

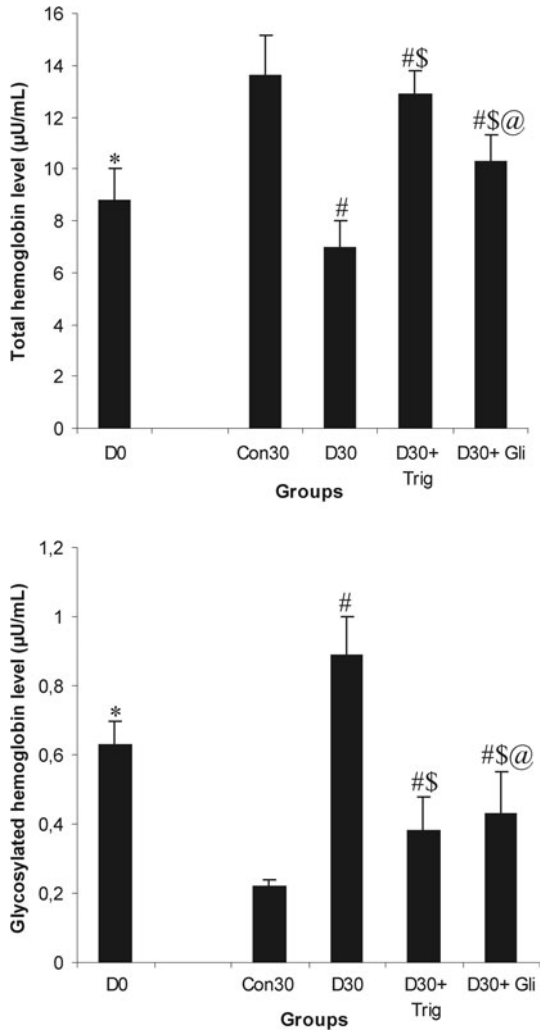

Fig. 7 Plasma hemoglobin and glycosylated hemoglobin levels in normal and diabetic rats. Statistical analyses as given in table legend

fenugreek may be mediated through stimulating insulin synthesis and/or increasing secretion β pancreatic cells of langerhans [30–33].

This data demonstrate that administration of a Trig significantly inhibited DPP-4 activity in both plasma and small intestine and further enhanced the concentration of active GLP-1. In fact, the augmentation of GLP-1 action is now widely used for the treatment of type 2 diabetes. Indeed, GLP-1 not only acts as an incretin to lower blood glucose via stimulation of insulin secretion from islet β-cells but also exerts actions independent of insulin secretion, including inhibition of gastric emptying and acid secretion, reduction in food ingestion and glucagon secretion, and stimulation of β-cell proliferation.

Moreover, the findings of this experimental study showed that, when compared to their nondiabetic counterparts, the diabetic rats underwent a significant increase in maltase, sucrase, and lactase activities in agreement with previous study [34]. Interestingly, the findings indicate that the administration of Trig to surviving diabetic rats significantly decreased the intestinal maltase, lactase, and sucrase activities in intestine of diabetic rats. The reduction of intestinal disaccharidase activities lowers the conversion of oligosaccharides and disaccharides into absorbable monosaccharide. It leads to a hypothesis that inhibition of intestinal disaccharidases limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, and therefore inhibitors of disaccharidase may be useful in the treatment of diabetes [34].

The ACE plays a dominant role in the regulation of the water electrolyte balance and blood pressure. Activation of this system has been considered to be a main cause of renovascular hypertension. The importance of ACE inhibitors in the chronic treatment of various cardiovascular diseases such as hypertension, congestive heart failure, myocardial infarction, diabetic nephropathy, or renal dysfunction is now well established. This study showed that diabetes was associated with increase of plasma ACE activity, and this activity was decreased significantly with the addition of Trig which stands in agreement with findings recently reported in the literature, where it has been reported that administration of Trig to rats is associated with decrease in diastolic and mean arterial pression [33].

In diabetes, the glycation and subsequent browning (glycoxidation) reactions are enhanced by elevated glucose levels and there is some evidence that glycation itself may induce the formation of oxygen-derived free radicals [34]. HbA1C levels are monitored as a reliable index of glycemic control in diabetes [35]. In this study, HbA1C levels followed a similar pattern, i.e., they were elevated in diabetic rats and the extent of this increase was directly proportional to fasting blood glucose levels. Total hemoglobin decreased in the diabetic group, possibly due to the increased formation of HbA1C. This result was well correlated with an earlier report of decreased hemoglobin levels in experimentally diabetic rats [36]. The increase in total hemoglobin and decrease in HbA1C levels in animals receiving Trig may have been due to the decreased blood glucose levels.

This study also showed both a significant decrease in glycogen synthase activity and a remarkable increase of glycogen phosphorylase activity, which stands in agreement with findings recently reported in the literature. Together, the decrease in the activity in glycogen synthase and the increase in liver glycogen phosphorylase led to a considerable decrease in liver glycogen content and increase in blood glucose, which is also in agreement with previously reported data [35]. However, the administration of Trig alkaloid to surviving diabetic rats regulated the glycogen contents in the liver by mobilizing key enzymes that stimulated glycogen synthesis through increasing

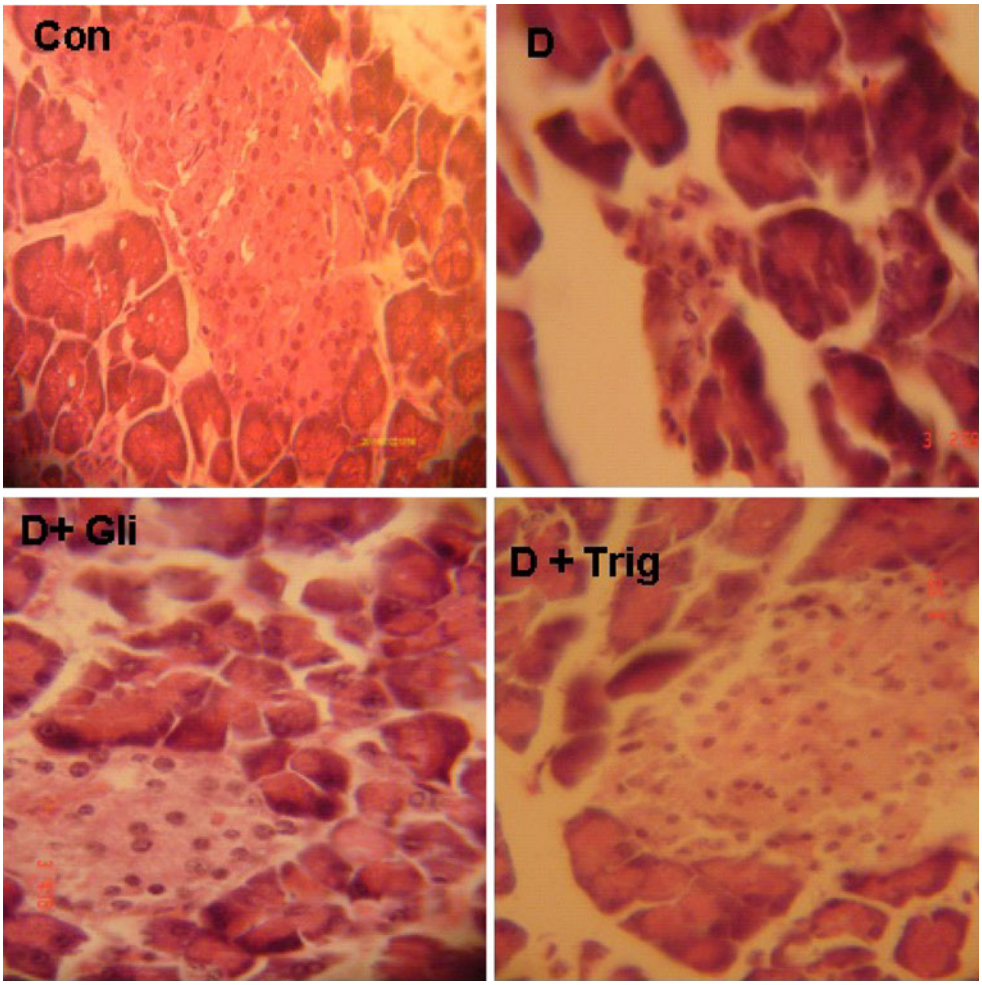

Fig. 8 Effect of the trig on the histological changes of rats' pancreas by HE staining (×100). Normal control rats showed normal β-cells. Severe injury in β-cells in the pancreas of male rats given alloxan for 1 month. In diabetic rats treated with Gli: an ameliorative actions were observed compared to diabetic rats after 4 weeks. Pancreatic β-cells showing protective effect in diabetic rats treated with Trig

Table 1 Effect of the Trig on liver indices and lipid profile in serum

	Control	D_0	D_{30}	D_{30} + GII	Diab + Gli
Liver dysfunction indices					
AST	48 ± 12	58 ± 16*	89 ± 17*$^{\#}$	51.6 ± 18$^{\$}$	69 ± 11$^{\$}$
ALT	41 ± 5.2	451 ± 5.2	66 ± 9*$^{\#}$	49 ± 7$^{\$}$	54 ± 11$^{\$}$
Total bilirubin	1.25 ± 0.31	1.6 ± 0.3	2.69 ± 0.4*$^{\#}$.	1.74 ± 0.58$^{\$}$	1.81 ± 0.49$^{\$}$
Direct bilirubin	0.51 ± 0.06	0.59 ± 0.05	0.83 ± 0.09*$^{\#}$	0.61 ± 0.07$^{\$}$	0.60 ± 0.04$^{\$}$
PAL	128 ± 22	1.98 ± 23*	272 ± 31*$^{\#}$	169 ± 18*$^{\$}$	179 ± 21*$^{\$}$
Lipid profile					
T-Ch	1.72 ± 0.21	1.89 ± 0.23	2.13 ± 0.2$^{*\#1}$	1.80 ± 0.18$^{\$}$	1.85 ± 0.22$^{\$}$
HDL-Ch	0.93 ± 0.1	0.76 ± 0.05	0.45 ± 0.08*$^{\#}$	0.94 ± 0.1$^{\#\$}$	0.81 ± 0.08$^{\$}$
LDL-Ch	0.79 ± 0.12	1.04 ± 0.12	1.44 ± 0.13*	0.86 ± 0.14$^{\$}$	1.04 ± 0.21$^{\$}$
TG	0.74 ± 0.15	0.88 ± 0.19	1.31 ± 0.13*$^{\#}$	0.83 ± 0.21$^{\$}$	1.14 ± 0.11$^{\$}$

Values are given as mean ± SD for group of ten animals each. Values are statistically presented as follows: *$p < 0.05$ significant differences compared to controls; $^{\#}p < 0.05$ significant differences compared to diabetic at day 0; $^{\$}p < 0.05$ significant differences compared to diabetic rats at day 30; $^{@}p < 0.05$ significant differences compared to diabetic rats treated with trig

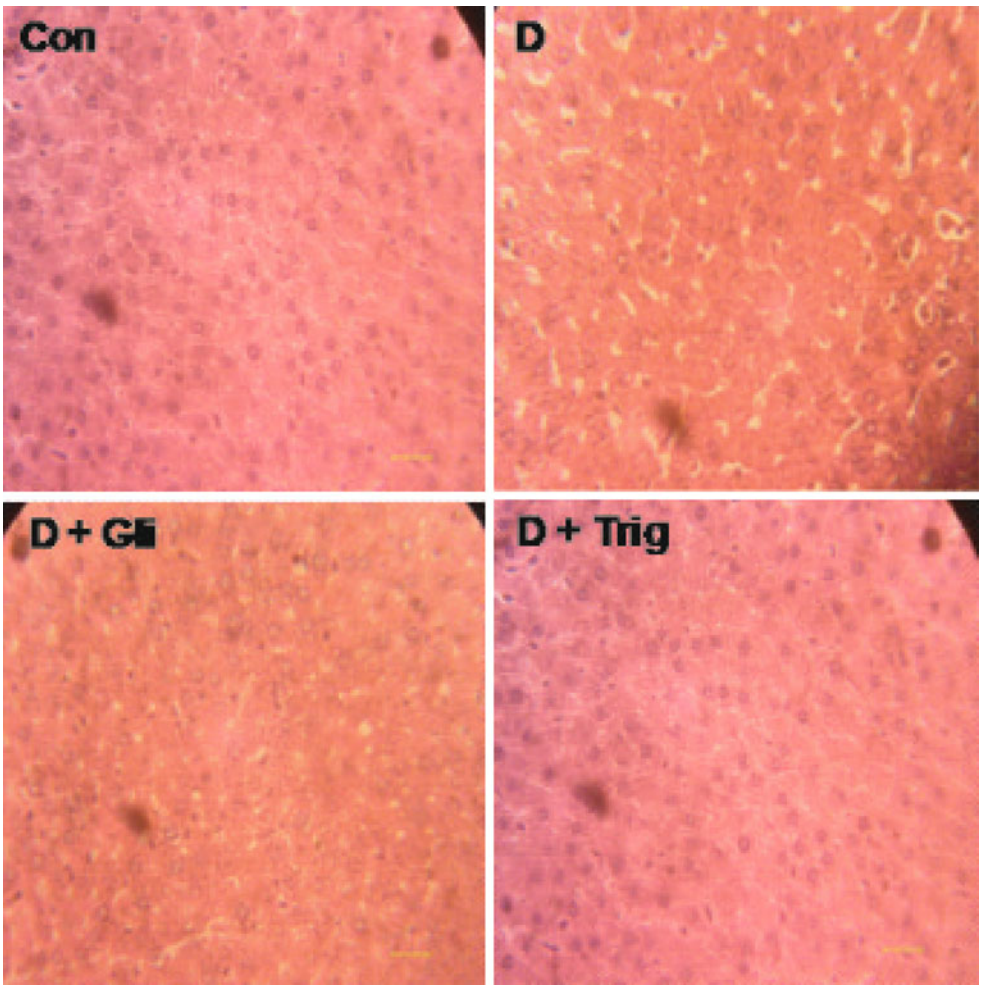

Fig. 9 Histopathological studies of liver in the control and experimental groups of rats. Section of the liver from control rat; diabetic rat at day 30 showing fatty cysts apparition in liver tissues; in diabetic rats treated with Gli: a few ameliorative actions were observed compared to diabetic rats. Diabetic rat treated with Trig, a protective action was shown

glycogen synthase and inhibiting glycogen phosphorylase which lead to modulation of blood glucose and liver glycogen rates.

This study also showed that the administration of Trig to surviving diabetic rats potentially improves this index, reflected by an increase in insulin sensitivity in various bodies and the amelioration of the ability of pancreatic β-cells to secrete insulin [34].

In conclusion, results from this study demonstrate that Trig alkaloid exhibited promising therapeutic effects on postprandial hyperglycemia. Accordingly, Trig can exploit new pathways for the treatment and prevention of diabetes and related diseases, and can be considered as potential therapeutic agents for the treatment of diabetes.

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