ANTIDIABETIC EFFECT OF COMPOUND (GII) FROM FENUGREEK (*TRIGONELLA FOENUM-GRAECUM L.*) SEED IN DIABETES MELLITUS

Greń, A., Formicki, G.

Institute of Biology, Pedagogical University of Cracow, Poland

ABSTRACT

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and its developed diabetic complications. These pathophysiological complications are often responsible for a decreased quality of life in diabetic. Hyperglycemia-induced increase in oxidative stress is implicated in diabetic complications. An anti-hyperglycemic compound named GII was purified from the water extract of the seeds of fenugreek (Trigonella foenum graecum TFG) and shown to be different from trigonelline and nicotinic acid isolated earlier from the same plant. Plants continuously produce an extraordinary variety of biologically active low-molecular-mass compounds. To evaluate antihyperglycemic and antioxidant role of TFG in male mice. The study was carried out on 28 selected male mice, average body weight 25-26g, bred in the constant light conditions LD 12:12 and fed standard diet with unlimited access to water. The mice were randomly divided into non-diabetic and diabetic groups. Diabetes was induced by a single intravenous injection of streptozotocin. Control animals received citrate buffer of the same volume instead. STZ-induced diabetic rats were included and retained for the experiments if their blood glucose was greater than 200 mg/dl. Animals were separated in four groups: [I] control (C; n=7); [II] diabetes (DM; n = 7); [III] control+trigonella foenum graecum (C+TFG; n=7); [IV] DM-treated with TFG (DM+TFG; n=7). TFG-100 mg/kg b.w. weight for 3 weeks, 1 week after diabetes induction. After completion of the experiment animals were anaesthetized and decapitated after last injection. The blood samples were collected from the carotid artery. The concentrations of glucose, cholesterol and triglycerides were estimated in the blood serum with STAMAR kits.

The present study infers that TFG leaf demonstrated remarkable antihyperglycemic activity in STZ-induced diabetic male mice. The potential antihyperglycemic action is plausibly due to its underlying antioxidant role.

Key words: diabetes, Trigonella foenum-graecum L., glucose, cholesterol, triglycerides, mice

INTRODUCTION

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and its developed diabetic complications. These pathophysiological complications are often responsible for a decreased quality of life in diabetic patients. Experimental evidence indicates that hyperglycemia induces a series of cellular events that increase the production of reactive oxygen species (ROS). The aim of the conservative treatment of diabetes type 1 is the metabolic control leading to the maintenance of the optimal values of blood glucose level and indicators of the lipid metabolism which represent parameters defining the so-called metabolic block. However, progressively wider knowledge concerning the pathogenesis of diabetes type 1 increases the spectrum of studies concerning the prevention and relieve of symptoms of the disease. Even though these drugs may be valuable in the management of diabetes mellitus, they have limitations due to undesirable adverse effects such as hypoglycemia, weight gain, secondary failure, and inability to arrest pancreas degeneration or diabetic complications which have been linked to oxidative stress. In view of the compelling evidence for a major role of oxidative stress in the development, progression, and complications of diabetes, antioxidants may serve as a potential therapy (Rains and Jain, 2011). Thus, an ideal therapy for diabetes mellitus would be a drug that not only possesses antihyperglycemic effect, but also enhances or protects the antioxidant defense system which is usually compromised. Various herbal extracts are known to possess antioxidant properties. Therefore, it seems that the preventive administration of the 75 plant compounds of antioxidative properties can efficiently protect an organism against pathological changes known as the metabolic block. In Poland as well as other parts of the world the management of diabetes and to overcome has complications. These plants are found to be effective and their low cost and minimal side effects have increased the interest of scientists to develop plant based drugs for managing diabetes. Fenugreek (*Trigonella foenum-graecum* L.-TFG) is an annual herb and a medicinal plant. The uses of the seeds and leaves of fenugreek are diverse. They are used as spices in food preparations to enhance or impart flavour. Fenugreek seeds are good sources of protein, fat, minerals and dietary fibre. Fenugreek seed extracts normalize the enhanced lipid peroxidation and relieve oxidative stress by providing antioxidants in diabetic mice (Tripathi and Chandra, 2002). TFG are a rich source of the polysaccharide galactomannan. They are also a source of saponins such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. Other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids such as choline and trigonelline. The well documented therapeutic uses of fenugreek are its hypoglycemic and hypolipidemic activity. Fenugreek (*Trigonella foenum-graecum* L. seed) is a food with traditional medicinal use in

diabetes (Puri et al., 2002). Beneficial effects have been demonstrated in diabetic animals and both insulin-dependent and non-insulin-dependent diabetic subjects (Al-Habori et al., 2001). Taking into account these factors, we decided to check influence of fenugreek on the chosen parameters indicating changes in an organism during diabetes mellitus type 1.

MATERIAL AND METHODS

The study was carried out on 28 selected male mice, average body weight 25-26 g, bred in the constant light conditions LD 12:12 and fed standard diet with unlimited access to water. The mice were randomly divided into non-diabetic and diabetic groups. Diabetes was induced by a single intravenous injection of streptozotocin (75 mg/kg, STZ, Sigma-Aldrich Co., USA). STZ was freshly prepared by dissolving it in citrate buffer (pH 4.5, Sigma-Aldrich Co., USA). Control mice received citrate buffer of the same volume instead. STZ-induced diabetic mice were included and retained for the experiments if their blood glucose was greater than 200 mg/dl. Blood glucose was measured by using a glucometer (ACCU-CHEK, ADVANTAGE, Roche Diagnostics, Germany). Animals were separated into four groups: [I] control (C; n=7); [II] diabetes (DM; n = 7); [III] control+trigonella foenum (C+TFG-100 mg/kg b.w.; n=7); [IV] DM-treated with TFG (DM+TFG; n=7). TFG was administered in a dose of 100 mg/kg body weight once a day for 3 weeks, one week after diabetes induction. The extract was given orally by gastric intubation. After completion of the experiment animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery. The concentrations of glucose, cholesterol and triglycerides were estimated in the blood serum with STAMAR kits. All the experiments were performed with the acceptance (No. 36/2010) of the Local Ethical Committee, Cracow.

Biochemical analysis

The concentrations of glucose, cholesterol and triglycerides were estimated in the blood serum with STAMAR kits. Glucose estimation was based on the enzymatic reaction of glucose with oxygen catalyzed by glucose oxidase. The reaction leads to the formation of D-gluconate and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with phenol and 4-aminoantipyrine producing chinone dye and water. We measured the intensity spectrophotometrically at the wavelength of 500 nm. The estimation of cholesterol was based on the reaction of cholesteryl ester with water. The reaction was catalyzed by cholesterol esterase. As a result of this reaction cholesterol and fatty acids are produced. At the second stage, cholesterol is oxygenised by cholesterol oxydase producing 4-cholestenon-3

and hydrogen peroxide. Hydrogen peroxide reacts with the participation of peroxidase with phenol and 4-aminoantipyrine. As a result, chinone dye and water are produced. The intensity of the color was estimated spectrophotometrically at the wavelength of 500 nm.

The enzymatic estimation of triglycerides involves three stages. First, lipase hydrolyses triglycerides to glycerol and fatty acids. Then, glycerol, at the presence of glycerol kinase and ATP, is phophorylised to 3-P-glycerol. 3-P-glycerol oxidase (GPO) catalyses the formation of hydrogen peroxide which reacts with p-chlorophenol and 4-aminoantipyrine (4-AA) producing a coloured complex. The intensity of the colour is directly proportional to the concentration of triglycerides. All the spectrophotometric measurements were performed using Marcel 330 spectrophotometer.

Statistical analysis

The statistical analysis of the results was carried out with Statistica program version 8.

The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analysed using one-way ANOVA followed by post hoc analysis with Tukey's test.

RESULTS

During the experiments, a statistically significant increase in the concentration of glucose, cholesterol and triglycerides in the animal model of diabetes type 1 (induced by the administration of streptozotocin-STZ). However, the administration of fenugreek (*Trigonella foenum-graecum L.*-TFG) seed extract caused a decrease in the concentration of the studied substances in all experimental groups. It was also observed that simultaneous administration of TFG seed extract limits the negative effects of streptozotocin on the concentration of glucose, cholesterol and triglycerides.

In animals from the control group, the concentration of glucose was 5.02±0.07 mmol/l. The highest and statistically significant increase (p=0.001) of 86% was observed after the administration of streptozotocin (9.34±0.07 mmol/l). However, after the injection of TFG a decrease of 16% (4.21±0.14 mmol/l) in the concentration of glucose in comparison with the control values was found (p=0.001). Moreover, it was observed that combined administration of TFG and STZ caused an increase of 42% (7.14±0.07 mmol/l) in the concentration of glucose in comparison with the control animals (p=0.001). On the other hand, the average concentration of glucose in animals from this experimental group was lower in comparison with the values for the experimental group treated with STZ exclusively (p=0.001) (Tab.1, Fig.1).

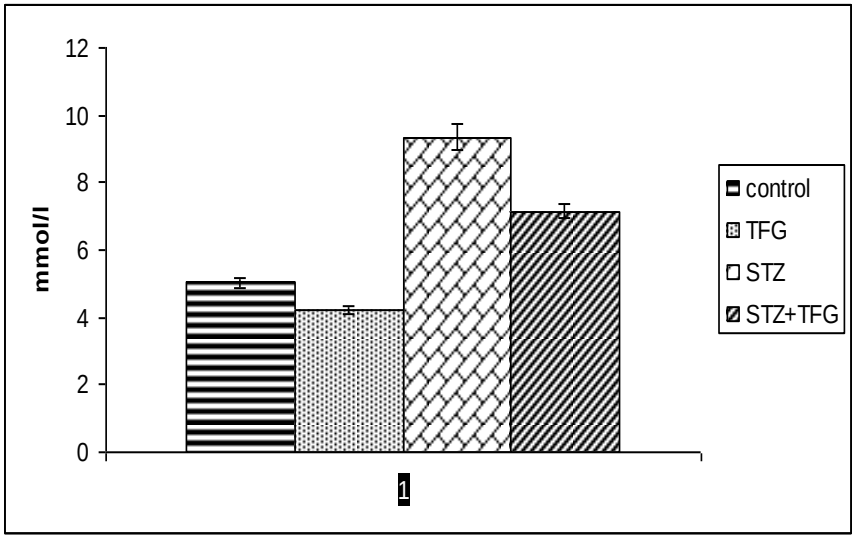
Administration of STZ caused a statistically significant increase in cholesterol concentration by 40% (6.95±0.29 mmol/l) in comparison with the values for the control group (4.97±0.21 mmol/l) (p=0.001). The injection of TFG decreased the concentration of cholesterol by 7% (4.62±0.07 mmol/l) in comparison with the control (p=0.05). It was found that the combined administration of TFG and STZ caused an increase in the concentration of cholesterol by 5% (5.24±0.14 mmol/l) in comparison with the control values (p=0.01). On the other hand, the increase was lower comparing to animals treated with STZ exclusively (p=0.001) (Tab.1, Fig.2).

Similar tendencies were noted in the triglyceride concentrations. The highest increase (p=0.001) in the concentration of triglycerides was observed after the administration of STZ by 50% (3.43±0.21 mmol/l), and somewhat lower and insignificant increase (p=0.001) after the combined administration of TFG and STZ (2.72±0.14 mmol/l) – 19% in comparison with the control values (2.29±0.33 mmol/l). However, after the administration of TFG a significant (p=0.001) decrease of 27% (1.68±0.07 mmol/l) was observed in comparison with the concentration of triglycerides in the control animals (Tab.1, Fig.3).

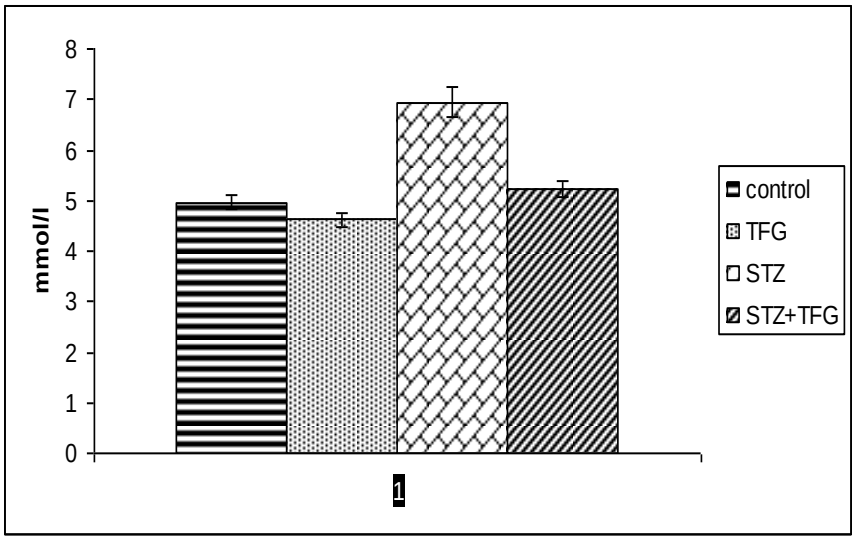
Table 1. Concentrations of glucose, cholesterol and triglycerides in blood serum (mmol/l±SD) **after administration of streptozotocin** (STZ) **and *Trigonella foenum-graecum L.*** (TFG)

Control	TFG	STZ	STZ+TFG
	Glucose		
5.02±0.07	4.21±0.14*	9.34±0.07*	7.14±0.07*
	Cholesterol		
4.97±0.21	4.62±0.07**	6.95±0.29*	5.24±0.14***
	Triglycerides		
2.29±0.33	1.68±0.07*	3.43±0.21*	2.72±0.14*

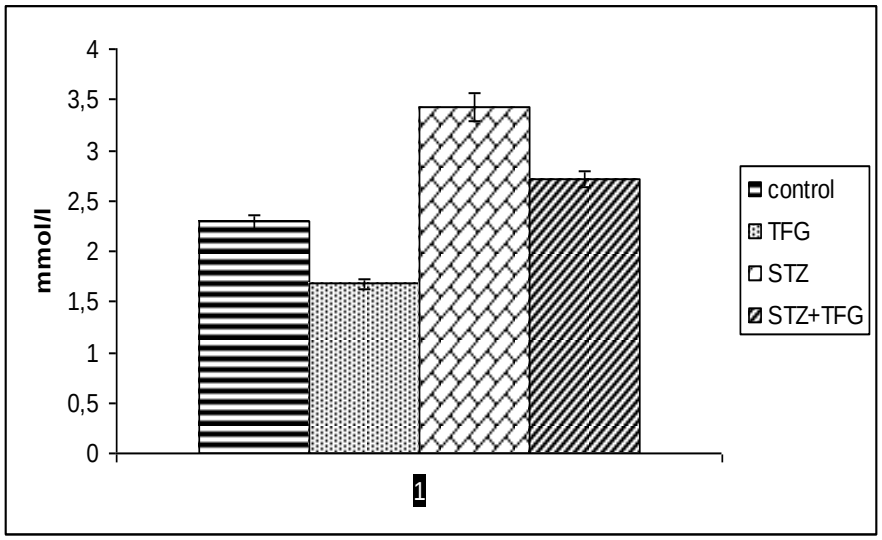
*differences statistically significant compared to the control group at p=0.001
**differences statistically significant compared to the control group at p=0.05
***differences statistically significant compared to the control group at p=0.01



Ryc.1. Concentrations of glucose in blood serum (mmol/l±SD) **after administration of streptozotocin** (STZ**) and *Trigonella foenum-graecum L.*** (TFG)



Ryc.2. Concentrations of cholesterol in blood serum (mmol/l±SD) **after administration of streptozotocin** (STZ) **and *Trigonella foenum-graecum L.*** (TFG)



Ryc.3. Concentrations of triglycerides in blood serum (mmol/l±SD**) after administration of streptozotocin** (STZ) **and *Trigonella foenum-graecum L.*** (TFG)

DISCUSSION

Fenugreek has a long history of medical uses in Ayurvedic and Chinese medicine, and has been used for numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health. Preliminary animal and human trials suggest possible hypoglycemic and antihyperlipidemic properties of oral fenugreek seed powder (Basch et al., 2003).

The results of this study clearly indicate that the effect of fenugreek (*Trigonella foenum graecum* L.-TFG) is equivalent to the reference oral hypoglycemic drug. TFG significantly reduced hyperglycemia, hypercholesterolemia and hypertriglyceridemia. The constituents of fenugreek might have lowered glucose levels either by promoting insulin secretion or by increasing insulin receptor sensitivity (Madar et al., 1988). Similar results were obtained by Puri et al (2002). The treatment produced significant attenuation of the glucose tolerance curve and improvement in the glucose induced insulin response, suggesting that the hypoglycemic effect may be mediated through stimulating insulin synthesis and/or secretion from the beta pancreatic cells of Langerhans (Puri et al., 2002). Annida et al. (2004) studied the effect of fenugreek leaves. The fenugreek leaves were supplemented in the diet daily to diabetic rats for 45 days, and food intake was recorded daily. Their results show that blood glucose and tissue lipids were elevated in STZ-induced diabetic rats. Supplementation of fenugreek leaves lowered the lipid profile in STZ-induced diabetic rats (Annida et al. 2004).

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Regulation of the major metabolic

pathways of fat, carbohydrate, and protein is of critical importance to organism functions and is achieved by several hormones. Insulin, a pancreatic hormone, is essential in the regulation of carbohydrate, lipid, and protein metabolism. It acts to preserve and to create energy reserves in the body by inhibiting catabolic processes such as lipolysis, gluconeogenesis, proteolysis, and glycogenolysis. The soluble dietary fibre (SDF) fraction of *Trigonella foenum graecum* (TFG-sdf) has previously been shown to reduce postprandial elevation in blood glucose level of type 2 model diabetic rats by delaying the digestion of sucrose (Borida et al., 1997; Madar et al., 1988).

Oxidative stress (OS), a feature of diabetes mellitus is defined as an increase in the steady-state levels of reactive oxygen species (ROS) and may occur as a result of increased free radical generation and/or decreased anti-oxidant defense mechanisms. Increasing evidence indicates that hyperglycemia is the initiating cause of the tissue damage in DM, either through repeated acute changes in cellular glucose metabolism, or through long-term accumulation of glycated biomolecules and advanced glycation end products (AGEs). AGEs are formed by the Maillard process, a non-enzymatic reaction between ketone group of the glucose molecule or aldehydes and the amino groups of proteins that contributes to the aging of proteins and to the pathological complications of diabetes mellitus. Fenugreek seed showed an encouraging antioxidant property and can be valuable candidate in the treatment of the reversal of the complications of diabetes (Kaviarasan et al., 2004; Genet et al., 2002; Tripathi and Chandra, 2002; Anuradha and Ravikumar, 2001).

Treatment of diabetic mice with aqueous extracts of TFG for 3 weeks after establishment of hyperglycemia resulted in significant reduction of glucose, cholesterol and triglycerides levels in blood serum which might be due to enhanced peripheral glucose utilization or these plant extracts potentiate the insulin effect by rejuvenation of damaged pancreatic β cell (Jelodar et al., 2005). Hypoinsulinemia due to streptozotocin induced diabetes leads to several biochemical alterations including lipid peroxidation. Increased plasma total lipid, triglycerides and total cholesterol are common in diabetics. This may be due to increased activity of lipases (sensitive to insulin) which results in increased lipolysis. Adjunct use of fenugreek seeds improves glycemic control and decreases insulin resistance in mild type-2 diabetic patients. There is also a favourable effect on hypertriglyceridemia (Gupta et al., 2001).

The therapy of TFG could lower the blood glucose level and ameliorate clinical symptoms in the treatment of diabetes mellitus type 1 and the therapy was relatively safe. It seems that *Trigonella foenum graecum* extract may be used for several purposes in diabetics

such as lowering of blood glucose levels or delaying complications (atherosclerosis, nephropathy, neuropathy and gastroparesis etc). This anti-infective properties may be an added benefit as diabetic are known to be more susceptible to infections. Further investigations are necessary to find out the active components present in *Trigonella foenum graecum* extract. Future studies should also take into consideration the mechanisms of action of active components of *Trigonella foenum graecum* and therapeutic potential of the plant in the treatment of diabetes and related complications. Our results lead to the conclusion that this novel fenugreek (*Trigonella foenum graecum* L.) preparation corrects metabolic alterations in diabetes by exhibiting insulin-like properties and has a potential for clinical applications.

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Contact address

Agnieszka Greń, PhD., Department of Biochemistry and Animal Physiology,, Pedagogical University of Cracow, ul. Podbrzezie 3, 31-054 Cracow, E-mail: agrenagren@gmail.com
Fax: +48126626682