Effects of the *Trigonella foenum-graecum* L. seed extract and chromium picolinate supplementation in streptozotocin induced diabetes in rats

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Our objective was to determine the effects of the *Trigonella foenum-graecum* seed extract and chromium picolinate in streptozotocin (STZ) induced diabetes in rats. We used 60 female Sprague Dawley rats aged between 2-3 months, who were divided into 6 groups: control (C); vehicle received physiological saline solution intraperitoneally (i.p.) + physiological saline solution orally, diabetes control group (DC); 50 mg/kg STZ i.p. + physiological saline solution orally, *T. foenum-graecum* (TFG); 50 mg/kg STZ i.p. + 150 mg/kg TFG seed extract orally, chromium picolinate (Crpic); 50 mg/kg STZ i.p. + 30 µg/kg Chromium picolinate orally, *T. foenum-graecum* + chromium picolinate (TFG+Crpic); 50 mg/kg STZ i.p. + received 150 mg/kg TFG + 30 µg/kg Crpic orally, insulin (I); 50 mg/kg STZ i.p. + 1 IU insulin subcutaneously. The treatment lasted for 21 days. On the 14th and 21st days, we found a decrease in the FBG of animals treated with insulin, TFG, TFG and Crpic combined (all p < 0.001), and Crpic (p < 0.01) compared with the diabetes control group. Moreover, there was a significant decrease in the plasma triglyceride and very low density lipoprotein (VLDL) levels after a treatment with Crpic, I, TFG, and TFG+Crpic compared with diabetes control group (p < 0.001). Administration of TFG+Crpic caused a significant increase in plasma high density lipoprotein (HDL) levels (p < 0.05) compared with diabetes control group. There was a significant decrease in the plasma tumor necrosis factor alpha (TNF-α) level after a treatment with Crpic (p < 0.05). The TFG, TFG and Crpic combined, and insulin treatment significantly increased the insulin-positive β cells compared with diabetes control group (p < 0.001). These results show that the TFG extract may have an insulinotropic effect on the β cells of the islets of Langerhans, or may prevent the damage of the pancreatic β cells. Crpic did not stimulate insulin secretion from the β cells in the Langerhans islets. We concluded that Crpic may exert its antihyperglycemic effects by facilitating the interaction between insulin and its receptor. It is recommended that the TFG seed extract and Crpic supplements may help in alleviating or reducing the hyperglycemia-related chronic complications of diabetes.

**Keywords:** Diabetes mellitus, *Trigonella foenum-graecum*, Chromium picolinate, Proinflammatory cytokines, Lipid profile

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Oxidative stress is significantly increased in patients with type 1 or type 2 diabetes and in diabetic animal models, because prolonged hyperglycemia increases the generation of cytotoxic free radicals via various metabolic pathways (nonenzymatic and oxidative glycosylation, increased activity of protein kinase C, and increased activity of the sorbitol and hexosamine pathways)1,2. There is no therapy for type 1 diabetes, and the applied treatments cause complications and are expensive. Therefore, plants with a possible antidiabetic effect, such as *Trigonella foenum-graecum* L. (TFG, fenugreek), *Momordica charantia* L., *Ganoderma lucidum*, and *Mirabilis jalapa* L., are of great interest3. These medicinal plants are widely used in folk medicine4,5.

TFG is an annual plant that belongs to the *Leguminosae* family. TFG seeds have antihyperglycemic, antihyperlipidemic, antioxidant, and diuretic effects6,7. The most important phytochemicals isolated from TFG are saponins, trigonelline, alkaloids, phosphate, potassium, proteins (4-hydroxyisoleucine), choline, vitamin C, betacarotene, nicotinic acid, and folic acid8,9. Mohammed *et al.*10 showed that the GLUT4 content of the skeletal muscle cell membranes in diabetic rats is decreased; however, it was restored upon TFG treatment. In addition, TFG treatment prevented lipid peroxidation and increased the level of antioxidants in various tissues11,12.

Chromium is an essential trace mineral that plays an important role in the regulation of lipid and blood glucose levels13-15. Chromium chloride, chromium
nicotinate, and chromium picolinate are commonly used formulations of the trivalent chromium\textsuperscript{16}. Crpic may have a glucose-regulating activity through its enhancing action on insulin, and may increase the phosphorylation of insulin receptor substrate 1 and the glucose transporter GLUT4\textsuperscript{17,18}. Recent studies showed that chromium may increase the cellular antioxidant activity in rats\textsuperscript{19,20}. Increased chromium mobilization and utilization intensified the glucose metabolism, and stimulated cortisol secretion from the cortex of the kidney\textsuperscript{21}.

Pro-inflammatory cytokines such as TNF-\(\alpha\), IL-1 and IL-6 closely related to improved insulin resistance\textsuperscript{22}, leading to cytotoxic effects on pancreatic beta cells\textsuperscript{23}, and impaired insulin mediated glucose uptake\textsuperscript{24,25}. The aim of the present study was to examine the effect of Crpic and TFG administration, both together and individually, on hyperglycemia, hyperlipidemia, the level of proinflammatory cytokines, and percentage of insulin-containing pancreatic \(\beta\) cells.

Methodology

Ethic statement

This study was conducted under the approval of the Local Ethics Committee for Animal Experiments at Firat University (2012/104). We used 60 female Sprague Dawley rats aged between 2-3 months, who were divided into 6 groups (10 rats each) for the experiments. The animals were maintained under standard conditions (temperatures (23±2 \(^\circ\)C), humidity (55±5 %) and 12 hrs light: 12 hrs dark cycle). They were fed with balanced and standard commercial pelleted diet for rats by \textit{ad libitum} throughout the experiment. Rat food composition; water: maximum 12%, crude protein: minimum 24%, crude cellulose: maximum 7%, crude ash: maximum 8%, hydrochloric acid insoluble ash: maximum 2%, Sodium chloride: maximum 1%, calcium: minimum-maximum 1-2.8%, phosphorus: minimum 0.9%, sodium: minimum-maximum 0.5-0.7%.

Experimental design

Type 1 diabetes was induced with a single intraperitoneal (ip.) injection of streptozotocin (STZ, 55 mg/kg, Sigma-Aldrich, S0130) in groups DC, TFG, Crpic, TFG+Crpic, and I. Animals in group C were injected with physiological saline solution. Control (C); vehicle received physiological saline solution intraperitoneally (i.p.) + physiological saline solution orally, diabetes control group (DC); this group received a single dose 50 mg/kg STZ i.p. (50 ml citric acid + 40 ml disodium hydrogen phosphate buffer pH 4.5) + physiological saline solution orally, \textit{T. foenum-graecum} (TFG); this group received a single dose 50 mg/kg STZ i.p + 150 mg/kg TFG seed extract orally, chromium picolinate (Crpic); this group received a single dose 50 mg/kg STZ i.p + 30 \(\mu\)g/kg Chromium picolinate orally, \textit{T. foenum-graecum} + chromium picolinate (TFG+Crpic); this group received a single dose 50 mg/kg STZ i.p. + received 150 mg/kg TFG + 30 \(\mu\)g/kg Crpic orally, insulin (I); this group received a single dose 50 mg/kg STZ i.p. + 1 IU insulin subcutaneously. Rats were defined as diabetic if their FBG level was >200 mg/kg 72 hrs after STZ administration. The treatment lasted for 21 days.

Preparation of the \textit{T. foenum-graecum} seed extract

TFG seeds were purchased at a local herbal market. Thousand gm of the TGF seeds were powdered finely and dissolved in a mixture of ethyl alcohol and water (2:8) with a ratio of 1:5 (seed weight: solvent volume) according to Hamza \textit{et al.}\textsuperscript{26}. The mixture was kept at room temperature in a shaking water bath for 2 days. It was filtered through filter paper, and the excess solvents were evaporated under pressure at 50 \(^\circ\)C. Nine gm of the extract were obtained from 250 ml maceration.

Preparation of the homogenate and biochemical analysis

At the end of the experiment, blood samples were collected from region of a femoralis bifurcation and liver, pancreas samples were excised under an anesthesia induced with 0.4 ml/kg pentobarbital sodium. The blood samples were then centrifuged at 3000 rpm for 10 min at 4 \(^\circ\)C and the plasma samples were stored at −20 \(^\circ\)C until use. The liver samples were homogenized in phosphate buffer saline (PBS 1:9) at room temperature using a homogenizer. The homogenate was centrifuged at 10,000 gm for 5 min at 4 \(^\circ\)C, and the supernatants were assayed. The levels of TNF-\(\alpha\) and IL-1\(\alpha\) were determined with ELISA kits (Invitrogen and Hangzhou Eastbiopharm, respectively). The level of glycosylated hemoglobin (HbA\textsubscript{1c}) and the lipid profile were measured with test kits (Ceragem and Abbott Diagnostics, respectively).

Histopathological evaluations

Pancreas samples from the control and experimental groups were fixed in 10% formalin, and
processed according to the routine procedure. We cut 4-µm-thick paraffin sections, stained them with hematoxylin-eosin (HE), and evaluated the results using a light microscope.

**Immunohistochemistry**

Tissue sections from the pancreas were immunostained for insulin/proinsuline using the avidin–biotin–peroxidase complex (ABC) technique. Serial 4-µm-thick sections were dewaxed in xylene, and rehydrated with decreasing series of alcohol. Sections were incubated in 3% hydrogen peroxide for 30 min to block the endogenous peroxidase activity. Sections were then washed with Tris-buffered saline (TBS) for 5 min, and were incubated in citrate buffer saline in a microwave oven for 20 min for antigen retrieval. After a 5 min wash with TBS, the sections were incubated in streptavidin peroxidase complex (1:300, Dako) for 60 min at room temperature. The signal was developed using 3-amino-9-ethylcarbazole (AEC) as chromogen, and Mayer’s hematoxylin was used to counterstain the sections. The primary antibody was omitted from the negative control used to counterstain the sections. The primary antibody was used to counterstain the sections. The primary antibody was used to counterstain the sections.

After further washes in TBS, all sections were incubated in streptavidin peroxidase complex (1:200, Dako) in TBS for 60 min at room temperature. The sections were incubated in biotinylated rabbit anti-mouse IgG (1:200, Scientific Pierce) overnight at 4°C. Sections were washed with TBS thrice for 5 min each, and were incubated in 5% normal rabbit serum in TBS followed by incubation in avidin–biotin–peroxidase complex (ABC) technique

Results

The effects of TFG, Crpic, TFG + Crpic, and I administration on the levels of FBG, HbA₁c, and on the percentage of insulin-positive β cells are shown in Table 1. In the DC group, we found a significant increase in the FBG level compared with that in the C group (p < 0.001). We found a decrease in the FBG levels in animals treated with TFG, I, TFG + Crpic (all p < 0.001), and Crpic (p < 0.01) on the 14th and 21st days compared with that in the DC group. In the DC group, we found a significant increase in the HbA₁c levels compared with that in the C group (p < 0.001), but no statistical difference was found between the diabetic groups (p > 0.05). Histopathologically, no significant difference was observed in the HE stained pancreas tissue among the groups. Immunohistochemistry revealed a prominent positive staining for insulin/proinsulin in the β cells of the pancreatic islets, which was localized in the cytoplasm of beta cells. In the DC group, the percentage of immunopositive cells decreased significantly compared with that in group C (p < 0.001). In addition, immunostaining was visible in the limited areas of the cytoplasm of β cells in the DC and Crpic groups. However, in the TFG, TFG + Crpic, and I groups, the number of insulin-positive β cells increased significantly compared with that in the DC group (p < 0.001) (Fig.1).

**Table 1—Effect of TFG extract and Crpic on fasting blood glucose, HbA₁c, levels and Insulin‘ beta cells, mean ± SD of 10 animals in each group**

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>DC</th>
<th>TFG</th>
<th>Crpic</th>
<th>TFG+Crpic</th>
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<tr>
<td>GLUCOSE (mg/dl)</td>
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<tr>
<td>0th</td>
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<td>105.20±2.35</td>
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<td>104.00±2.75</td>
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<td>86.50±7.39</td>
<td>83.40±3.92</td>
<td>357.88±36.42</td>
<td>381.83±41.15</td>
<td>371.29±31.6</td>
<td>379.57±45.64</td>
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<tr>
<td>7th</td>
<td>80.20±9.13</td>
<td>83.11±36.87</td>
<td>369.75±24.56</td>
<td>376.67±47.50</td>
<td>367.00±32.60</td>
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<td>14th</td>
<td>100.60±4.72</td>
<td>477.75±39.63</td>
<td>351.57±44.22</td>
<td>411.71±37.45</td>
<td>366.20±39.39</td>
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<tr>
<td>21st</td>
<td>102.88±3.83</td>
<td>517.44±28.14</td>
<td>336.67±49.70</td>
<td>453.40±30.68</td>
<td>374.00±29.39</td>
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<td>HbA₁c (%)</td>
<td>4.4±0.48</td>
<td>5.76±0.39</td>
<td>5.29±0.36</td>
<td>5.54±0.31</td>
<td>5.46±0.21</td>
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<tr>
<td>Insulin‘ beta cells</td>
<td>38.00±10.07</td>
<td>4.85±1.8</td>
<td>17.46±5.4</td>
<td>10.92±5.6</td>
<td>16.64±4.1</td>
<td>18.92±4.8</td>
</tr>
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</table>

*p < 0.05, **p < 0.01, ***p < 0.001 as compared with control with diabetes, #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with control.

**Statistical analysis**

One-way ANOVA was used to determine whether there are significant differences between the groups. Duncan’s multiple range test was used to detect significant differences pairwise between the groups. A value of p < 0.05 was considered as significant. All statistical tests were performed with SPSS 18.

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ATILA & YÜCE: EFFECTS OF TFG SEED EXTRACT & CRPIC SUPPLEMENTS ON DIABETIC RATS
The levels of plasma TNF-\(\alpha\), liver TNF-\(\alpha\), and IL-1\(\alpha\) were significantly higher in the DC group compared with that in the C group (\(p < 0.05\), \(p < 0.001\), and \(p < 0.01\), respectively). In addition, there was a significant decrease in the plasma TNF-\(\alpha\) level after a treatment with Crpic (\(p < 0.05\)). Similarly, the level of TNF-\(\alpha\) in the liver was significantly reduced in group I compared with that in the DC group (\(p < 0.001\)) (Table 2).

The plasma total cholesterol and LDL-C levels were not significantly different in the experimental groups (\(p > 0.05\)). On the other hand, plasma triglyceride and VLDL-C levels were significantly increased in the DC group compared with that in the C group (\(p < 0.001\)); however, there was a significant decrease in these levels in the TFG, Crpic, TFG + Crpic, and I groups compared with that in the DC group (\(p < 0.001\)). In addition, there was a significant increase in the plasma HDL-C level in the TFG + Crpic group (\(p < 0.05\)) (Table 3).

**Discussion**

There is currently no treatment for type 1 diabetes. Therefore, traditional plants and essential trace minerals are investigated for their antihyperglycemic, antioxidant, and antihyperlipidemic effects to treat diabetes.

The present results showed that FBG levels were decreased in the TFG, TFG + Crpic, Crpic groups 14 and 21 days after diabetes induction. The TFG and TFG + Crpic treatments were more effective than the Crpic treatment (Table 1). Previous studies found similar effects on fasting glucose levels in diabetic rats, rabbits, dogs and patients with type 1 or type 2 diabetes. The extract may be mediated by 4-hydroxyisoleucine and trigonelline. 4-hydroxyisoleucine may have an important role in cell death prevention pancreatic \(\beta\) cells, and may stimulate insulin secretion from the \(\beta\) cells in the Langerhans islets. Crpic did not stimulate insulin secretion from the \(\beta\) cells in the Langerhans islets. It was suggested that the antihyperglycemic effect of Crpic may facilitate the interaction between insulin and its receptor. Crpic administered at therapeutic doses improved glucose tolerance, but has no hepatotoxic or nephrotoxic effects.

Pro-inflammatory cytokines such as IL-1, TNF-\(\alpha\), TNF-\(\beta\), and IFN, may be cytotoxic to \(\beta\) cells, as they can increase the levels of nitric oxide and free radicals in the \(\beta\) cells. Few studies investigated the effect of Crpic and TFG administration on the cytokine levels in diabetes. Our results showed that diabetic rats have increased TNF-\(\alpha\) and IL1-\(\alpha\) cytokine levels. The Crpic treatment significantly decreased the plasma TNF-\(\alpha\) levels compared with that in the DC group (Table 2). This study showed that IL-1\(\alpha\) and TNF-\(\alpha\) cytokines may have important roles in the pathogenesis of type 1 diabetes mellitus. Jain et al. showed a significant increase in TNF-\(\alpha\) and IL-6 levels in diabetic rats, and this increase was prevented by chromium picolinate and chromium niacinate treatment.

The present study showed that plasma triglyceride and VLDL-C levels were increased in the DC group; however, there was a significant decrease in the TFG.
Significance of study

*T. foenum-graecum* L. seeds have numerous medicinal properties such as their insulinotropic, antihyperlipidemic, antioxidant, and diuretic effects. They can restore β cells in the islets of Langerhans. TFG is one of the oldest traditionally used plants in many Asian and African countries. Similar to chromium supplementation, may facilitate the interaction between insulin and its receptor. This study showed that TFG extract may have an insulinotropic effect on the β cells of the islets of Langerhans, or may prevent the damage of the pancreatic β cells but wasn’t determined the insulinotropic effect of Crpic. Therefore, it should be expanded to investigate of plant species used in traditional medicine. Moreover, we believe that our study is a step to determining the mechanisms of action of chromium picolinate and *T. foenum-graecum* L. seed extract that have important role in the regulation of lipid and blood glucose levels.

Conclusion

In summary, the experiments showed that the administration of TFG extract and Crpic, both together and individually, is beneficial for hyperglycemia and hyperlipidemia in type 1 diabetic rats. TFG extract may have an insulinotropic effect on the β cells of the islets of Langerhans, or may prevent the damage of the pancreatic β cells. Crpic did not stimulate insulin secretion from the β cells in the Langerhans islets. We concluded that Crpic may exert its antihyperglycemic effects by facilitating the interaction between insulin and its receptor. These results show that TFG extract and Crpic supplements may help to alleviate the hyperglycemia-related chronic complications of diabetes.

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