Ameliorative properties of Iranian *Trigonella foenum-graecum* L. seeds and *Punica granatum* L. peel extracts in streptozotocin-induced experimental diabetic guinea pigs

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**ARTICLE INFO**

**Article history:**
Received 9 May 2016
Received in revised form 30 May, 2nd revised form 4 Jul, 3rd revised form 5 Jul 2016
Accepted 13 Oct 2016
Available online 5 Dec 2016

**Keywords:**
Diabetes
Iranian
*Trigonella foenum-graecum*
Seeds
*Punica granatum*
Peel
Streptozotocin

**ABSTRACT**

**Objective:** To assess the ameliorative properties of Iranian *Trigonella foenum-graecum* L. (*T. foenum-graecum*) seeds and *Punica granatum* L. (*P. granatum*) peel extracts against streptozotocin-induced diabetes in male guinea pigs.

**Methods:** Sixty guinea pigs were divided into six groups (10 guinea pigs per group). Group 1 consisted of normal animals. Groups 2 and 3 were treated with Iranian *T. foenum-graecum* seeds and *P. granatum* peel extract alone, respectively. Group 4 was treated with streptozotocin only; whereas Groups 5 and 6 receiving streptozotocin were treated with Iranian *T. foenum-graecum* seeds and *P. granatum* peel extract, respectively. All animals were treated for 30 days, and the body weight, blood and liver biochemical parameters were measured.

**Results:** Guinea pigs exposed to streptozotocin showed an alteration in body weight gain, fasting glucose level, kidney function parameters (blood urea nitrogen and creatinine) as well as decreased serum and hepatic total protein level. In addition, it increased the cholesterol and triglyceride level, while decreasing the hepatic glucose-6 phosphate dehydrogenase activity, glycogen, glutathione content and hepatic catalase activity. Oral treatment with *T. foenum-graecum* seeds and *P. granatum* peel extracts revealed significant protective properties with respect to body weight gain and other biochemical parameters studied.

**Conclusions:** The Iranian *T. foenum-graecum* seeds and *P. granatum* peel extracts are significantly potent in ameliorating diabetic condition induced by streptozotocin and improving various biochemical parameters in serum and liver of guinea pigs.

**1. Introduction**

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defects in insulin secretion and insulin action or both. The chronic hyperglycemia is concomitant with long-term destruction and dysfunction of various normal organs, chiefly the eyes, kidneys, nerves, heart, and blood vessels [1,2].

The incidence of diabetes is increasing all over the world due to shift in lifestyle, aging, and obesity due to physical dormancy. Unlike the West, where the elders are most affected, prevalence of diabetes in Asian countries is relatively high in young and middle-aged people [3]. The International Diabetes Federation has now classified Kingdom of Saudi Arabia to be among the top 10 countries worldwide with the highest incidence of diabetes in 2011 (16.2%) and the incidence is expected to be 20.8% in 2030 [4]. Additionally, the prevalence of some risk factors for type-2 diabetes mellitus in Kingdom of Saudi Arabia has also been assessed to be among the highest in the world [5].
Medicinal plants are considered as a source for drug discovery and are playing an essential role in drug development programs. Furthermore, numerous medicinal plants are rich in bioactive chemicals that are mostly free of undesirable effects and have powerful pharmacological actions.

Fenugreek (Trigonella foenum-graecum L. (T. foenum-graecum)) is an annual crop belonging to Fabaceae family and native to an area spreading from Iran to Northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. [6]. In different parts of the world, leaves, seeds and early seedlings of fenugreek are often used as curries, dyes, medicines and vegetable [7]. It has different active constituents such as flavonoids, alkaloids, vitamins and amino acids [8,9].

Punica granatum L. (Punicaceae) (P. granatum) is commonly known as pomegranate. It is considered to be an ancient and mystical plant. The term pomegranate is derived from the Latin words “pomum” (meaning apple) and “granatus” (meaning seeded) [10]. According to ancient Greek history, pomegranates are known as “the fruit of the dead” [11].

Ancient Egyptians considered the pomegranate as an indication of wealth and ambition. According to the Ebers Papyrus, one of the oldest medical writings from around 1500 BC, Egyptians used the pomegranate for the treatment of infection with tapeworm and other infections [12].

The most plentiful phytochemicals in pomegranate juice are polyphenols, including the hydrolyzable tannins called ellagitannins which are formed when ellagic acid and/or gallic acid binds with a carbohydrate, and also known as punicalagins. Pomegranate peel comprises as three times the total polyphenols as pomegranate seeds, as well as condensed tannins, catechins, galloallocatechins and prodelphinidins [9,13]. The higher phenolic content of the peel profits extracts for consumption in nutritional supplements [14].

Numerous studies were carried out to investigate the effects of natural products on diabetes induced by streptozotocin. The pomegranate has been considered beneficial to health because of its high antioxidant content [15].

The aim of the present work was to investigate the hypoglycemic effect of methanolic extracts of Iranian fenugreek seeds and pomegranate peel on streptozotocin-induced diabetic guinea pigs. The study also aimed to investigate the effects of these two potent edible natural products on various biochemical parameters in serum and liver, in turn, to assess their preventive and therapeutic effects.

2. Materials and methods

2.1. Chemicals

Streptozotocin was obtained from Sigma–Aldrich. All reagents used were obtained commercially and were of analytical grade.

2.2. Experimental animals

Male guinea pigs (490–550 g) were used. Animals were individually kept in stainless steel cages. Feed and water were provided ad libitum. The animals were fed with standard laboratory diet and allowed free access to water in an air-conditioned room with a 12 h light/12 h dark cycle. All experimental procedures involving animals were conducted in accordance to guidelines of the Ethics Committee of the Experimental Animal Care Society, College of Medicine, Ha'il University, and approved by Institutional Ethics Committee, College of Medicine, Ha'il University, Kingdom of Saudi Arabia.

2.3. Induction of diabetes mellitus

Experimental diabetes was induced in overnight fasted guinea pigs by single intraperitoneal injection of streptozotocin (150 mg/kg body weight, Sigma–Aldrich, Germany) dissolved in cold citrate buffer (0.05 mol/L, pH 4.5) [16]. Since streptozotocin is capable of inducing lethal hypoglycemia due to massive pancreatic insulin release, the guinea pigs were provided with 10% glucose solution after streptozotocin administration for the next 24 h to overcome streptozotocin-induced hypoglycemia. Animals with glucose levels between 180 and 210 mg/dL were considered to be diabetic and chosen for the experiments, while those with glucose levels outside this range were excluded.

2.4. Preparation of P. granatum peels extract

Pomegranate fruits were purchased from local market from Ha'il City. The peels were removed and dried in shade for 10 days. Methanolic extract of pomegranate peel was prepared based on the method of Hasona et al. [9]. P. granatum peel extract (500 mg/kg) was given orally to the animals in aqueous solution once per day [9].

2.5. Iranian T. foenum-graecum extract preparation

Seeds of Iranian fenugreek were obtained from local market from Ha'il City. The whole seeds were washed and crushed into fine powder using a grinding machine. Methanolic extract of fenugreek seeds was prepared based on the method of Hasona et al. [9]. Fenugreek seeds extract (500 mg/kg) was given orally to the animals in aqueous solution once per day [9].

2.6. Experimental design for animal study

The guinea pigs were randomly divided into six groups with 10 guinea pigs in each group. Group I served as normal control; Group II was treated with T. foenum-graecum extract orally for four weeks; Group III was treated with pomegranate peel extract orally for four weeks; Group IV was composed of streptozotocin-induced diabetic animals; Group V consisted of streptozotocin-induced diabetic guinea pigs which were treated with T. foenum-graecum extract in aqueous solution orally for four weeks; Group VI comprised streptozotocin-induced diabetic guinea pigs which were treated with pomegranate extract in aqueous solution orally for four weeks.

During the experimental period, body weight and blood glucose levels of all the guinea pigs were examined at regular intervals. At the end of the experimental period, the guinea pigs were fasted overnight, anesthetized, and sacrificed by cervical decapitation. The blood was collected with and without anticoagulant for plasma and serum separation, respectively.

Liver tissues were quickly excised, weighed and homogenized in a saline solution (0.9%), and centrifuged at 3000 r/min for 15 min. The supernatant was stored at −20 °C for measuring biochemical parameters related to oxidative stress.
2.7. Measurement of biochemical parameters

Serum glucose, blood urea nitrogen, creatinine, triglycerides and total cholesterol were measured using commercial kits purchased from United Diagnostics, Kingdom of Saudi Arabia. Total proteins were determined in serum and liver according to Biuret method using kits purchased from United Diagnostics, Kingdom of Saudi Arabia. For determination of liver glycogen, the frozen liver samples were prepared as per standard protocol [17].

The hepatic glucose-6-phosphate dehydrogenase activity was measured spectrophotometrically [18]. One unit of enzyme activity was defined as the quantity which catalyzes the reduction of 1 μmol/L of nicotinamide adenine dinucleotide phosphate per minute. The activity of this enzyme was recorded by using glucose-6-phosphate as a substrate and absorbance was measured at 340 nm.

The hepatic catalase activity was determined according to method of Cohen et al. [19]. The hepatic content of reduced glutathione (GSH) was assayed by the spectrophotometric technique according to Sedlack and Lindsay [20].

2.8. Statistical analysis

The SPSS version 18.0 for Windows (SPSS Inc., Chicago, USA) was used for the statistical analysis. Results were expressed as mean ± SE and differences were considered non-significant at P > 0.05, significant at P < 0.05, highly significant at P < 0.01 and very highly significant at P < 0.001.

3. Results

The body weight of diabetic control guinea pigs was reduced, whereas it was increased in other groups at the end of the experiment. Nondiabetic animals treated with T. foenum-graecum seeds and P. granatum peel extracts exhibited insignificant body weight gain as compared to normal ones. Oral administration of T. foenum-graecum seeds extract and P. granatum peel extract to diabetic animals was fairly beneficial since they elicited some weight gain (Table 1).

There was a highly significant reduction (P < 0.001) in the glucose level of diabetic guinea pigs treated with fenugreek seeds extract as well as pomegranate peel extract compared to the diabetic control. Also, streptozotocin-induced diabetic guinea pigs showed a significant decrease in serum total protein levels compared to normal control group (P < 0.001). While diabetic guinea pigs treated with fenugreek seeds (P < 0.01) or pomegranate peel extracts (P < 0.001) showed a significant increase in serum total protein levels compared with the diabetic control group (Table 2).

The study demonstrated significant (P < 0.001) decrease in serum total cholesterol and triglycerides levels after the continuous treatment with the tested materials as compared to diabetic untreated group which showed significant (P < 0.001) increase as compared to normal control (Table 2).

Serum creatinine and blood urea nitrogen levels were significantly increased in diabetic guinea pigs as compared to normal controls (P < 0.001). When diabetic guinea pigs were treated with T. foenum-graecum seeds extract and P. granatum peel extract, a significant normalization of these parameters was observed, when compared to untreated diabetic guinea pigs (Table 2).

The study showed significant (P < 0.001) decrease in liver glycogen content and hepatic total protein content in the diabetic control group as compared to normal control one. Concerning the diabetic animals treated with all the tested materials, there was significant (P < 0.001) increase in liver glycogen content and hepatic total protein content after the oral treatment of T. foenum-graecum seeds and P. granatum peel extracts (Table 3).

The activity of the hepatic glucose 6-phosphate dehydrogenase, catalase and hepatic GSH content were significantly decreased (P < 0.001) in the diabetic control group when compared to the normal control. On the other hand, the oral treatment with fenugreek seeds and pomegranate peel extract showed significant (P < 0.001) increase in the above mentioned parameters when compared to diabetic control animals (Table 3).

From above mentioned results, it was found that the pomegranate peel extract showed more promising and more effective results than fenugreek seeds extract in decreasing blood glucose level and potent antioxidant effect.

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight</th>
<th>Body weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Group I</td>
<td>506.37 ± 7.33</td>
<td>574.00 ± 11.75</td>
</tr>
<tr>
<td>Group II</td>
<td>524.37 ± 18.15</td>
<td>585.00 ± 13.63</td>
</tr>
<tr>
<td>Group III</td>
<td>544.63 ± 25.29</td>
<td>600.12 ± 23.57</td>
</tr>
<tr>
<td>Group IV</td>
<td>516.75 ± 21.95</td>
<td>466.13 ± 23.94</td>
</tr>
<tr>
<td>Group V</td>
<td>510.25 ± 9.29</td>
<td>524.12 ± 8.70</td>
</tr>
<tr>
<td>Group VI</td>
<td>130.40 ± 1.06</td>
<td>5.45 ± 0.13</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. **: P < 0.01, ***: P < 0.001 compared to initial body weight; ***: P < 0.001 as compared to normal control group; ***: P < 0.001 as compared to diabetic control group.

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting serum glucose level (mg/dL)</th>
<th>Serum total protein level (g/dL)</th>
<th>Serum total cholesterol level (mmol/L)</th>
<th>Serum triglyceride level (mmol/L)</th>
<th>Serum blood urea nitrogen level (mmol/L)</th>
<th>Serum creatinine level (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>72.90 ± 1.75</td>
<td>6.75 ± 0.14</td>
<td>0.85 ± 0.10</td>
<td>0.71 ± 0.01</td>
<td>7.68 ± 0.26</td>
<td>35.11 ± 0.72</td>
</tr>
<tr>
<td>Group II</td>
<td>70.20 ± 1.85</td>
<td>6.89 ± 0.12</td>
<td>0.69 ± 0.01</td>
<td>0.68 ± 0.02</td>
<td>7.39 ± 0.20</td>
<td>34.50 ± 1.05</td>
</tr>
<tr>
<td>Group III</td>
<td>65.70 ± 1.65</td>
<td>7.09 ± 0.22</td>
<td>0.61 ± 0.01</td>
<td>0.66 ± 0.02</td>
<td>6.22 ± 0.13</td>
<td>33.86 ± 0.98</td>
</tr>
<tr>
<td>Group IV</td>
<td>195.40 ± 1.28***</td>
<td>4.10 ± 0.19***</td>
<td>1.57 ± 0.02***</td>
<td>1.39 ± 0.01***</td>
<td>14.57 ± 0.42***</td>
<td>71.43 ± 0.99***</td>
</tr>
<tr>
<td>Group V</td>
<td>139.20 ± 1.25***</td>
<td>5.17 ± 0.12***</td>
<td>0.97 ± 0.02***</td>
<td>1.07 ± 0.01***</td>
<td>12.78 ± 0.22***</td>
<td>53.16 ± 0.87***</td>
</tr>
<tr>
<td>Group VI</td>
<td>130.40 ± 1.06***</td>
<td>5.45 ± 0.13***</td>
<td>0.81 ± 0.02***</td>
<td>0.82 ± 0.02***</td>
<td>10.75 ± 0.26***</td>
<td>46.36 ± 1.04***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. **: P < 0.05, ***: P < 0.01, ****: P < 0.001 as compared to normal control group; **: P < 0.05, ***: P < 0.01, ****: P < 0.001 as compared to diabetic control group.
The effect of fenugreek and pomegranate extract on liver biochemical parameters in guinea pigs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic glycogen content (µg/mg of liver)</th>
<th>Hepatic total protein content (mg/g of tissue)</th>
<th>Hepatic glucose-6-phosphate dehydrogenase activity (IU/mg of tissue)</th>
<th>Hepatic catalase activity (IU/g protein)</th>
<th>Hepatic GSH content (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>89.35 ± 2.47</td>
<td>103.95 ± 1.83</td>
<td>120.32 ± 2.19</td>
<td>6.98 ± 0.04</td>
<td>14.45 ± 0.29</td>
</tr>
<tr>
<td>Group II</td>
<td>88.20 ± 2.06</td>
<td>110.39 ± 0.82**</td>
<td>121.78 ± 2.73</td>
<td>7.48 ± 0.05***</td>
<td>17.09 ± 0.35***</td>
</tr>
<tr>
<td>Group III</td>
<td>91.75 ± 1.81</td>
<td>108.19 ± 2.18</td>
<td>130.94 ± 2.36</td>
<td>7.66 ± 0.02***</td>
<td>21.00 ± 0.43***</td>
</tr>
<tr>
<td>Group IV</td>
<td>23.00 ± 1.50***</td>
<td>76.00 ± 1.69***</td>
<td>52.22 ± 2.45***</td>
<td>4.22 ± 0.03***</td>
<td>8.80 ± 0.24***</td>
</tr>
<tr>
<td>Group V</td>
<td>33.81 ± 1.38***</td>
<td>89.25 ± 2.18***</td>
<td>72.46 ± 2.10***</td>
<td>5.39 ± 0.04***</td>
<td>11.44 ± 0.23***</td>
</tr>
<tr>
<td>Group VI</td>
<td>53.87 ± 1.56***</td>
<td>92.37 ± 1.35***</td>
<td>70.56 ± 1.23***</td>
<td>6.11 ± 0.02***</td>
<td>14.22 ± 0.22***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. **: P < 0.01, ***: P < 0.001 as compared to normal control group; +++: P < 0.001 as compared to diabetic control group.

4. Discussion

This study highlights the comparative effects of the crude methanolic extract of Iranian fenugreek seeds and pomegranate peel in streptozotocin-induced diabetic male guinea pigs.

Intraperitoneal injection of streptozotocin induces diabetes by selectively abolishing the pancreatic β cells [16]. In the present study, streptozotocin-induced diabetic guinea pigs were chosen for the experiment. Streptozotocin-induced diabetes is characterized by a significant reduction in body weight due to increased catabolism of tissue proteins and unavailability of carbohydrate as energy source as a consequence of lacking insulin secretion, leading to significant decrease in the body weight of diabetic guinea pigs, which was detected in the current work, and this is in concordance with the findings of Salwe et al. [21]. The significant improvement in the body weight detected in diabetic guinea pigs administrated with extracts indicates the favorable properties of the extract in controlling muscle protein synthesis.

The present study revealed significant elevation of serum glucose level after four weeks of single intraperitoneal streptozotocin injection in the diabetic control group when compared to normal control group. These results are in accordance with the finding of several researchers using streptozotocin-induced diabetic animals [15,22].

In the present study, the treatment with Iranian fenugreek seeds and pomegranate peel extracts given to streptozotocin-induced diabetic guinea pigs showed a marked hypoglycemic effect when compared with the diabetic non-treated group. In spite of showing hypoglycemic activity, the diabetic treated groups remained diabetic.

The hypoglycemic effect of the tested extracts may be due to the active principles present in these extracts such as polyphenols and flavonoids [23] which possess the properties of regenerating pancreatic β cell, increasing insulin secretion, enhancing glucose uptake by adipose or muscle tissues, inhibiting glucose absorption from intestine and glucose production from the liver and resolving the problem of insulin deficiency.

Our results are similar to the finding of Aboonabi et al. [15] who reported that the treatment with aqueous extract of pomegranate peel has hypoglycemic effects on diabetic rats induced by streptozotocin.

Diabetes mellitus impairs the ability of the liver in glycogen production. The glycogen metabolism is regulated in vivo by the enzymes glycogen synthase and glycogen phosphorylase. The reduced glycogen stored in the diabetic guinea pigs is probably due to lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthetase systems [24]. The significant decline in the glycogen level was observed in the diabetic control group after streptozotocin administration. Oral treatment with fenugreek seeds or pomegranate peel extracts to diabetic guinea pigs restored the level of glycogen, indicating the improved glucose homeostasis, and this is in accordance with the findings of other studies [25-27].

Glucose-6-phosphate dehydrogenase is an enzyme that catalyzes the first step in the hexose monophosphate pathway of glucose metabolism, and produces ribose which is incorporated into nucleotides and reduced form of nicotinamide adenine dinucleotide phosphate, the major cytoplasmic reducing compound. Our result has shown a significant decrease in activity of hepatic glucose-6-phosphate dehydrogenase in the diabetic control group as compared to normal one. Our findings are consistent with those of other researches [28,29] which showed that hepatic glucose-6-phosphate dehydrogenase activity was decreased after streptozotocin injection.

Fenugreek seeds or pomegranate peel extract administration to diabetic guinea pigs showed elevated hepatic glucose-6-phosphate dehydrogenase activity when compared to diabetic control guinea pigs, indicating that increased amounts of reduced form of nicotinamide adenine dinucleotide phosphate are required for the detoxification process. Also, the increased activity of glucose-6-phosphate dehydrogenase indicates an improvement in glucose utilization by pentose phosphate pathway.

The defect in insulin action/secretion leads to defective amino acid/protein metabolism. The defective amino acid/protein metabolism could be attributed to enhanced muscle proteolysis and reduced protein synthesis. In the present work, administration of fenugreek seeds or pomegranate peel extracts to diabetic guinea pigs significantly inhibited proteolysis due to insulin deficiency and improved total protein level; these findings are in accordance with those of other studies [27,30].

The blood urea nitrogen was elevated with the simultaneous decline in blood total protein levels in streptozotocin-induced diabetic guinea pigs as a consequence of increased breakdown of blood and tissue proteins due to negative nitrogen balance. Furthermore, the concentration of glucose in diabetic state causes severe imbalance between protein metabolism and negative nitrogen balance. This, in turn, explains the elevated blood urea nitrogen and creatinine levels in diabetic guinea pigs treated with streptozotocin only.

The improvement in the blood urea nitrogen and creatinine levels in diabetic animals treated with fenugreek seeds or pomegranate peel extracts indicates renal protective nature of these extracts. Our results are in accordance with the finding of Pradeepa et al. [25] who reported that *Pithecellobium dulce* fruit...
extract possessed significant antidiabetic activity and reverted urea and creatinine levels to almost normal levels.

Streptozotocin induced a highly significant elevation of total cholesterol and triglycerides levels in the serum of diabetic untreated group as compared to normal one. Hypertriglyceridermia and hypercholesterolemia are major factors involved in the progress of atherosclerosis and coronary heart disease which are the secondary worries of diabetes [31]. Absence of insulin is accompanying with an elevation in cholesterol levels due to the enhanced mobilization of fats from the adipose tissue to the plasma [32].

In the present study, the treatment with fenugreek seeds or pomegranate peel extracts reduced serum levels of total cholesterol and triglycerides in diabetic treated groups. This may be due to increased cholesterol excretion (in feces) and decreased cholesterol absorption. Our findings are consistent with those of other researchers [9,33].

L-γ-Glutamylcysteinylglycine (GSH) is a tripeptide which plays a significant role in detoxification of reactive oxygen species, conjugation, elimination of lethal particles and regulation of inflammatory cytokine cascade. Diminution of GSH in tissues leads to a deficiency of the cellular protection against reactive oxygen species and causes peroxidative damage. Hence there are studies demonstrating the role of the GSH in cellular functions, such as detoxification of endogenous and exogenous compounds [34].

In this study, a marked decrease in the hepatic GSH content and the activity of hepatic catalase in untreated diabetic guinea pigs were observed. The decrease results from the accumulation of lipid peroxides and increased oxidative stress in diabetic guinea pigs. Treatment of guinea pigs with extracts of fenugreek seeds or pomegranate peel enhanced the streptozotocin-induced decline in the GSH content as well as the activity of catalase. The results, therefore, provide support to the antioxidant effects of fenugreek seeds or pomegranate peel.

In the current study, the administration of the methanolic extract of pomegranate peel and fenugreek seeds showed significant hypoglycemic and antioxidant activity in streptozotocin-induced diabetes. However, the pomegranate peel extract was proved to be more potent and more effective than fenugreek seeds extract.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to College of medicine, Hail University, KSA for supporting and providing all facilities to carry out this work.

References


