

# The Effect of Carbon Tetrachloride Extract of *Trigonella Foenum Graecum* Seeds on Glycogen Content of Liver in Streptozotocin-Induced Diabetic Rats

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**T**rigonella foenum graecum (fenugreek) is one of the best herbs known for its anti diabetic properties. This study was designed to compare the effects of the carbon tetrachloride extract of fenugreek with those of insulin on liver glycogen.

**Materials and Methods:** Diabetes was induced by streptozotocin in three groups of male Wistar rats. One group served as control (non-treated); in the second group NPH insulin was administered, and the last group received the carbon tetrachloride extract of fenugreek (4 g/kg) orally. Plasma glucose was measured before and after intervention. Water intake was measured daily and liver glycogen was determined at the end of the treatment.

**Results:** The results showed that fenugreek extract, like insulin, causes a significant decrease in plasma glucose and daily water intake ( $P < 0.05$ ). A significant increase was seen in liver glycogen of the groups treated with insulin ( $57 \pm 5$  mg/g of wet weight) and the extract ( $54 \pm 3$  mg/g of wet weight) compared to the control group ( $17 \pm 3$ ).

**Conclusions:** In conclusion, the results of this

study confirm the efficacy of the traditional use of fenugreek for diabetes treatment, and provide further insight into the formulation of carbon tetrachloride extract of fenugreek as an oral hypoglycemic agent in treatment of insulin dependent diabetic patients.

**Key Words:** *Trigonella foenum graecum* seeds, Diabetes, Carbon tetrachloride extract, Rat

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## Introduction

Diabetes is a common endocrine disorder, affecting more than 100 million people worldwide and the World Health Organization predicts this number will increase five-fold in the near future.<sup>1</sup> Although insulin is the most common conventional treatment for diabetes,<sup>2</sup> diet therapy approaches have demonstrated many advantages in developing countries.<sup>2,3</sup> Among herbs reported to possess anti-diabetic properties, *Trigonella foenum graecum* (fenugreek) is one of the best, in terms of efficacy and safety, history of traditional use, and results of research studies.<sup>4</sup> At present fenugreek is widely used as a sup-

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plementary treatment for diabetes.<sup>2</sup> Fenugreek causes dose-dependent reduction in blood sugar in normal and diabetic rats.<sup>5,6</sup> Human studies have shown that fenugreek causes reduction in blood glucose in type 1<sup>7,8</sup> and type 2<sup>7-9</sup> diabetes. Sauvaire et al. have reported that in rat and human pancreatic islet cells 4-hydroxy isoleucine as a component of fenugreek seeds, increased glucose-induced insulin secretion.<sup>10</sup> Fenugreek seeds contain Galactomannan which decrease blood sugar.<sup>11</sup>

Although the antihyperglycemic effect of fenugreek has been shown in animal and human studies, the exact mechanism(s) of the action remain to be elucidated.<sup>12</sup> The aim of this study was to determine the effect of carbon tetrachloride (CCl<sub>4</sub>) extract of fenugreek seeds on liver glycogen content in streptozotocin (STZ)-induced diabetic rats.

## Materials and Methods

**Materials:** STZ, NPH insulin, and ketamin hydrochloride, were purchased from the Upjohn (USA), Eli Lilly (Mexico), and Richter Gedeon Companies (Hungary) respectively. Carbon tetrachloride, glucose, trichloroacetic acid, sulfuric acid, and sodium chloride were obtained from Merck Company (Germany).

**Animals:** Male Wistar rats (150-230g) were obtained from the Razi Institute (Karaj, Iran). The animals were maintained in a 12h light/12h dark cycle at 22±3°C and 55% humidity with free access to water and food. Animals were handled in accordance with the criteria outlined in the guidelines for the care and use of laboratory animals.

**Diabetes induction:** To induce diabetes, STZ (90 mg/kg i.v.) was administered in 3 divided doses for 3 consecutive days. Ten days after STZ injection, animals with plasma glucose concentrations higher than 250 mg/dl, were considered diabetic.<sup>13</sup> Diabetic rats then divided into three groups of 10 rats each. One group of rats received no treatment, the second group were treated with NPH insulin (10 U/kg) for 3 days,<sup>14</sup> and the

third group received the carbon tetrachloride (CCl<sub>4</sub>) extract of fenugreek seeds (4g/kg) orally twice daily for 3 days. At the end of the third day, animals were anesthetized with 50 mg/kg ketamin hydrochloride; the abdomen was opened and the liver was removed. Plasma was separated immediately and plasma glucose was measured on the same day, but liver samples were kept at -80 ° C (5-12 days) for glycogen measurement. Daily water intake was measured throughout the three days.

**Extraction by carbontetrachloride:** Fenugreek seeds were purchased from a local provider and verified by the faculty of pharmacy (Division of Botany) Isfahan, Iran. Extraction was carried out by the maceration method. Fenugreek seeds were powdered and CCl<sub>4</sub> (2ml/g) was then added in three steps. About one third of total CCl<sub>4</sub> was added to the powder and kept for 3 days, solvent was removed and, then about one fourth of CCl<sub>4</sub> was added for 2 days. Finally, after removing the solvent, the remainder of CCl<sub>4</sub> was added. The resulting solutions were combined and filtered through Wathman paper (number 1). The volume of the extract was reduced as much as possible, and then the extract was kept at 30°C to remove remaining CCl<sub>4</sub>. The amount administered orally to the animals was calculated based on the original weight of the seeds used for extraction, e.g. 4g/kg means the administered extract contains the extracted substance from 4 g of the seeds.

**Plasma Glucose measurement:** Plasma glucose concentration was determined using the orthotoluidine method.<sup>15</sup> Interassay and intraassay coefficient of variations for high and low controls were 4.5; 6.2 and 5.5; 7.4 percent, respectively.

**Liver Glycogen determination:** Liver glycogen content was determined by the Kemp micromethod with minor modifications;<sup>16</sup> in this method, glycogen is hydrolyzed, and glucose is released in the vicinity of hot sulfuric acid producing 5-hydroxymethyl furfural with light red color. Intensity of the color is proportional to glucose concentra-

tion.<sup>16,17</sup> In brief, 75 mg of liver was homogenized in 5ml of trichloroacetate acid (TCA) for 2 minutes; then the tubes containing the homogenate were kept in at 100°C for 15 min; after that the tubes were immersed in cold water and evaporated volume was replaced with 10% TCA. Tubes were centrifuged at 1000 g for 5 min and 250 µl of supernatant was added to 750 µl of 10% TCA, plus 3 ml of sulfuric acid and after mixing the tubes were again placed in 100°C for 6.5 min. Later than tubes were immersed in cold water and the absorbance of the resulting light red color was read with spectrophotometer at 520 nm against glucose standards. The level of liver glycogen was calculated based on mg per gram of liver wet weight.<sup>17</sup>

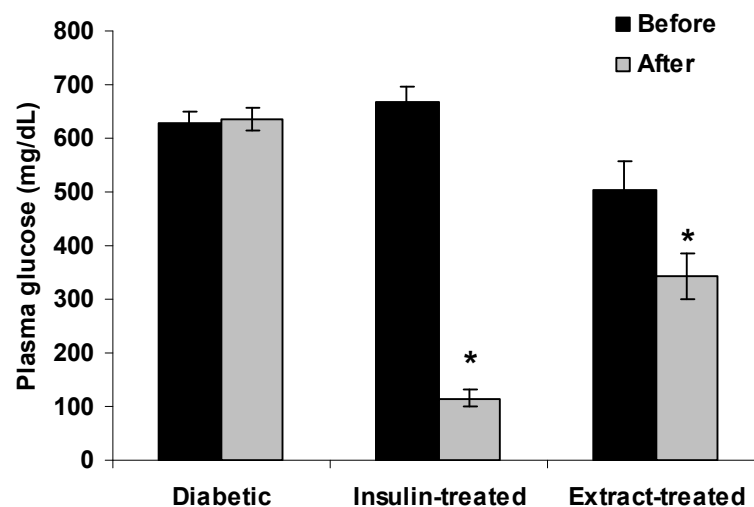
**Statistical Analysis:** Data analyses were done with SPSS program (version 11.5). Results were expressed as mean±SEM. Paired t-test was used for comparison between plasmagluose before and after treatment. Water intake and liver glycogen contents were compared using the one way analysis of variance (ANOVA), and Tukey's HSD used as post hoc. P-values less than 0.05 were considered significant.

## Results

**Effect of fenugreek extract on plasma glucose:** In the insulin treated group the blood glucose was 669±28, and 115±16 mg/dl, before and after treatment respectively ( $p<0.05$ ). In the extract treated group, plasma glucose was reduced compared to before treatment (344±43 vs. 504±54 mg/dl). In diabetic rats, that received no treatment, before and after plasma glucose was not significantly different (629±22 vs. 635±22 mg/dl) (Fig. 1).

**Effect of fenugreek extract on water intake:** Daily water intake in diabetic group was 146±5 ml/day, which was significantly higher compared to the insulin (28±3) or extract (25±4) treated animals ( $p<0.05$ ) (Fig. 2).

**Effect of fenugreek extract on liver glycogen:** Fenugreek extract significantly increased liver glycogen content. Liver glycogen levels in the non-treated, insulin treated and extracts treated animals were 17±3, 57±5, and 54±3 mg per gram of wet weight respectively ( $p<0.05$ ) (Fig. 3).



**Fig. 1.** Effect of fenugreek extract (4 kg/kg twice a day for 3 days) and insulin (NPH insulin 10 U/kg for 3 days) on blood glucose in diabetic rats. Samples for blood glucose obtained before and after intervention. The number of experiment (n) is 10 in each group \*:  $P<0.05$  compared to before intervention.

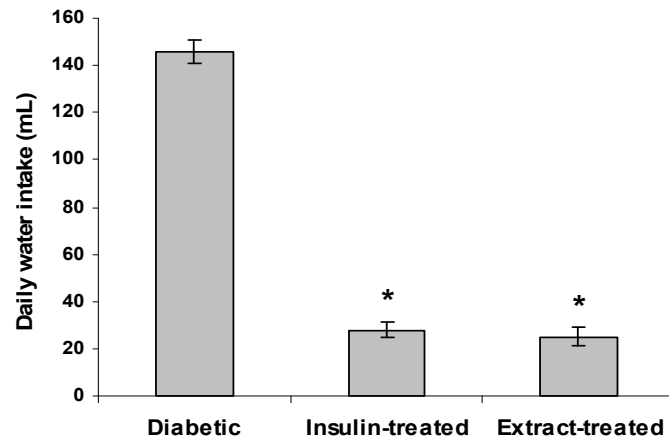


Fig. 2. Effect of fenugreek extract (4kg/kg twice a day for 3 days) and insulin (NPH insulin 10 U/kg for 3 days) on daily water intake in diabetic rats. The number of experiment (n) is 10 in each group  
\* P<0.05 compared to before intervention

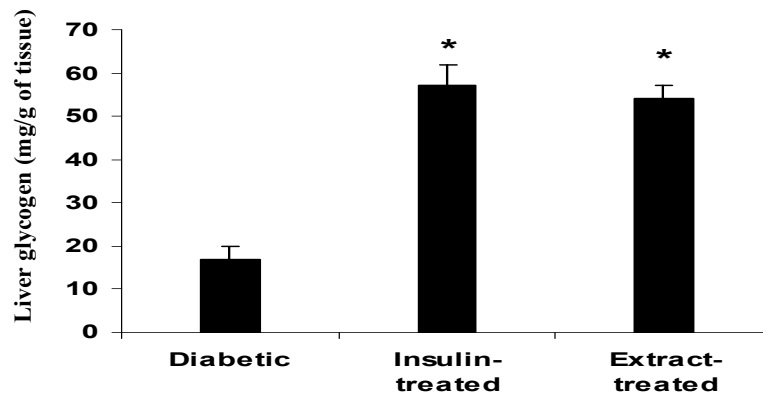


Fig 3. Effect of fenugreek extract (4kg/kg twice a day for 3 days) and insulin (NPH insulin 10 U/kg for 3 days) on liver glycogen content in diabetic rats. The number of experiment (n) is 10 in each group  
\* P<0.05 compared to before intervention

## Discussion

The results of this study showed that the CCl<sub>4</sub> extract of fenugreek causes significant decrease in plasma glucose and increased glycogen content of the liver in STZ-induced diabetic rats. This hypoglycemic effect is in accordance with the findings of others.<sup>9,18,19</sup> In this study, the CCl<sub>4</sub> extract of the seed was used because Zaree et al.<sup>20</sup> have reported that

compared to other extracts, CCl<sub>4</sub> extract of fenugreek has stronger glucose decrement activity. In the present study, the carbon tetrachloride extract of fenugreek returned liver glycogen content to the normal levels in diabetic rats, an effect comparable to insulin. Ruby et al.<sup>21</sup> have reported that following exercise, the 4-hydroxy isoluecine, present in fenugreek seeds, increases the rate of glyco-

gen synthesis in skeletal muscle. Other studies have shown that fenugreek seeds<sup>22</sup> and leaves<sup>23</sup> prevent liver glycogen depletion in STZ-induced diabetic rats, although levels do not fully normalize as happens with insulin.<sup>23</sup> In this study, the CCl<sub>4</sub> fenugreek extract had effects similar to those of insulin on the glycogen content of the liver. Probably one of the factors that can explain this small discrepancy is the type of the extract.

Another result of this study is the lowering of daily water intake by CCl<sub>4</sub> extract of fenugreek. Considering the remarkable hypoglycemic effect of the extract used in this study, decrease in water consumption is expected. Sharma et al.<sup>24</sup> have showed that fenugreek seeds decrease polydipsia and polyuria in type 2 diabetic patients. Also it has been shown that fenugreek seeds decrease urine glucose up to 54 percent in type 1 diabetic patients.<sup>25</sup>

Various mechanisms such as high fiber content, high viscosity, inhibition of intestinal glycosidase activity,<sup>6</sup> inhibition of glucagon release, increase in sensitivity to insulin,<sup>4</sup> and potentiation of insulin action<sup>3</sup> are suggested as being responsible for antihyperglycemic effect of fenugreek. In addition, some ingredients of fenugreek such as hydroxyisoleucine, trigonelline, and comarin<sup>4</sup> also have antihyperglycemic effects. Type of extraction

used in this study suggests that the effective compound(s) may not have the polar character and the fiber content is not high; it is therefore possible that some factor other than fiber content is effective. On the other hand, type of the animal model (STZ-induced) used in this study is similar to type 1 diabetes in which insulin secretion is either absent or very low;<sup>12</sup> therefore with high probability the CCl<sub>4</sub> extract of the fenugreek seeds would not exert its effect through insulin release and it probably contains substance(s) with insulin-like activity.

In conclusion, the results of this study show that CCl<sub>4</sub> extract of fenugreek seeds, when administered orally, reduce plasma glucose and water intake and increase liver glycogen in STZ-induced diabetic rats, effects that are comparable with those of insulin. The results of this study suggest that the seeds of *Trigonella foenum graecum* contain substance(s) which could be used orally by type 1 diabetic patients to control the disease. Of course, possible side effects need to be tested first.

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