The protective effect of *Trigonella foenum-graecum* L. seeds extract in high fat diet-streptozotocin induced hyperglycemic mice

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**Keywords**: Anti-hyperglycemic, *Trigonella foenum-graecum*, Streptozotocin, free radical scavenging activity.

**ABSTRACT**

Diabetes mellitus is a chronic metabolic disorder which affects carbohydrate, protein, and fat metabolism. Associated with high morbidity and mortality, characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism. Hyperglycemia and hyperlipidemia result in development of oxidative stress, systemic and local inflammation which were critical factors for degradation of β-cells, insulin resistance and type II diabetes. Herbal medicine is one of the therapeutic strategies in the management of diabetes mellitus. It is considered to be less toxic and have fewer side effects than synthetic ones. The objective of this study was to investigate the possible protective effect of *Trigonella foenum-graecum* L. seeds (TFG) extract in High Fat Diet/Streptozotocin-induced toxicity in mice. The seeds of TFG were defatted by Soxhelt apparatus with n-hexane, then marc was macerated with ethanol to obtain total ethanol extract. Thirty-two male albino mice were fed high fat diet for two weeks plus single dose of streptozotocin 120mg/kg intraperitoneally to generate type II diabetes model with increased adiposity. The protective and hypoglycemic activity of seeds extract against toxicity and hyperglycemia induced was evaluated in mice. Glimepiride was used as a standard treatment. FBG, serum C-peptide, TC, Triglycerides, HDL, LDL levels were measured at the end of 28th day of experiment then the animal sacrificed and liver and pancreas sections were prepared and stained for histological evaluation. This study was resulted in that (TFG) seed extract cause; FBG, serum lipid profile in hyperglycemic mice significantly decrease, It also increase serum C-peptide, Glutathione and decrease Malondialdehyde levels significantly compared to untreated group; this was associated with improving histological features that impaired during STZ exposure. In conclusion, Defatted seeds extract has potential anti-hyperglycemic and hepatoprotective effect with free radical scavenging ability against STZ-induced toxicity.
1. INTRODUCTION

Currently, there is an increasing number of people suffering from diabetic diseases resulting from a sedentary lifestyle, the consumption of a high-caloric diet, obesity and others [1]. Diabetes is associated with hyperglycemia, characterized by a high blood glucose concentration, altered metabolism of lipids, carbohydrates and protein and increased risk of complication from various diseases [2]. This disorder can cause microvascular and macrovascular complications which increase the rate of morbidity and mortality [3]. Hyperglycemia and hyperlipidemia result in development of oxidative stress, systemic and local inflammation which were an essential factors for degradation of β-cells, insulin resistance and type II diabetes [4]. The main factors involved in the progression of (DM) are augmentation of lipid peroxidation, free radical production, impaired glutathione metabolism, alteration in antioxidant enzyme activity, and High glucose level can stimulate free radical
production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result state of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress [5]. Streptozotocin is an alkylating agent belongs to nitrosoureas drugs and it was a pancreatic beta-cell-specific cytotoxin, induced cytotoxicity in pancreatic cells was mediated by an increase in oxidative stress, alteration in cellular metabolism, and mitochondrial dysfunction[6]. Currently STZ is used mostly as an investigational Drug. For diabetes research in rodents models by inhibiting of β-cell O-GlcNAcase due to the diabetogenic effects[7]. Diverse oral hypoglycemic drugs used for the clinical treatment of diabetes have characteristic side effect profiles. Thus There is a worldwide development to return to natural resources which are culturally acceptable and economically feasible [8]. Trigonella foenum-graecum L. was one of the most ancient medicinal herbs, was now widely cultivated as spice, tea and herbal medicine possess various beneficial effects, [9]. like antiseptic, antimicrobial, bactericidal, anthelmintic, antioxidant, and effective as a cancer chemo preventive agent[10].

2. MATERIALS AND METHODS

Chemical: Streptozotocin (STZ) was purchased from Sigma–Aldrich, USA. Glimepiride was purchased from Sanofi-aventis, Cholesterol powder was purchased from BDH, England. Reagent kits for assay of lipid profile were purchased from Abbott diagnostic– USA, C-peptide diagnostic– ELSA assay kit was purchased from Kiel-wellsee Germany. Reagent ELSA-kits for determination of serum malondialdehyde (MDA), and reduced glutathione(GSH) were purchased from Shanghai- China. The work was done in accordance with the method prescribed in each diagnostic kit.

Methods
The herb included in the present study will be identified and authenticated by pharmacognacy department College of Pharmacy, Al-Mustansiriya University, Iraq) 

Trigonella foenum- graecum L. extraction

the fresh dried seeds were grounded into fine powder using an electrical grinder. The dried powder seeds(840 gm.) defatted by soxhelt apparatus with 1500 ml of n-hexane which spend about 5 hours. then it was sequentially extracted by adding 140 gm. in each of the six flasks, after that it was macerated with 80% ethanol, After that, the mixture filtered using whatman No.1 filter paper[11]. The filtrate was evaporated by rotary evaporator vacuum at 40°C until ethanol free extract was remained that contained the total constituents of active ingredients in TFG. Extract was placed in dark bottle and stored at 8°C for further use[12].

2.1 Experimental Animals
Thirty two apparently healthy, albino male mice, 2-3 months age, weight about 26-30g. were purchased from the department’s animal house- College of Medicine /AL Nahrain University. Before starting the study, The animals were acclimatized in standard
environmental conditions with fresh water and standard food (ad libitum) for a week before commencement of the experiment.

2.2 Hyperglycemia induction:
To generate a mouse model mimicking human type II diabetes with increased adiposity follow Xia et al., (2014) with small modification. STZ diabetogenic effect was induced in mice by a single intraperitoneal injection at 120mg/kg body weight (BW) dissolved in0.1 M sodium citrate buffer (pH 4.5). The STZ injection was administered after 2 weeks of HFD (30% fat, 70% standard chow) along with standard diet. immediately after a 16 h fasting period. Streptozotocin must be kept at an appropriate temperature prior to preparation. STZ should be used within 5 minutes of preparation and excess discarded; due to the potential to degrade[13].

Experimental Animals:
All experimental protocols were approved by the Ethics Committee of the College of Medicine /AL Nahrain University.
Group I (citrate buffer group): eight mice were injected with citrate buffer alone(I.P) after 2 weeks of HFD.
Group II: (Diabetic control group): eight mice were injected with STZ(I.P) 120mg/kg (BW) + HFD.
Group III (Glimepiride group): eight mice were injected with STZ(I.P) 120mg/kg (BW) + HFD, the animal were orally gavaged with 10 mg/kg(P.O) (BW) of glimepiride.
Group IV (Trigonella foenum-graecum group): eight mice were injected with STZ(I.P) 120mg/kg (BW) + HFD, the animal were orally gavage d with 100 mg/kg (BW) of Trigonella foenum-graecum seed extract.

blood glucose levels were measured in blood samples collected from tail vein puncture overnight fasting, using a glucose analyzer(ACCU-CHEK) [14].
Fasting blood glucose level 200mg/dl; mice were consider diabetic and selected for the study.
both citrate buffer and diabetic control mice received no treatment. The dose of Glimepiride was selected according to Fouad et al., (2013) [15]. All the control and experimental groups were then gavaged with DW and standard food at the last 4 week.

2.3 Blood collection:
At the end of experiment, all groups were subjected to blood collection under anesthesia by ether inhalation, the mice were fasted (water not restricted) overnight before blood collection. The blood was collected by the heart puncture. Blood sample was collected in a dry test tube and allowed to coagulate at room temperature for 30 min. The blood samples were separated by centrifugation at 3000RPM for 20 minute. at the end of 28th day of experiment then the animal sacrificed and liver and pancreas sections were prepared and stained for histological evaluation.

2.4 Histopathology of mouse pancreas and liver:
At the end of 28th day of experiment then the animal were sacrificed, liver and pancreas sections were prepared and stained for histological evaluation.
The isolated pancreas and hepatic tissues sections fixed in 10% neutral buffered formalin, dehydrated by passing through a graded series of alcohols, and embedded in paraffin blocks and 5 μm sections were prepared using a semi-automated rotary microtome. The sections were stained in hematoxylin and eosin (H-E) stains. The sections were mounted by distere phthalate xylene. Stains, sections were examined under light microscope [16] [17].

2.5. Statistical analysis: [18]

The statistical analysis was performed using The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA analysis) was used to significant compare between means of groups in this study, data were expressed as mean ±SEM, where P value <0.05 was consider to be significant.

3. RESULTS


Recovery of the extracts was calculated as yield (%) using the following equation: Yield (%) = [Wf / Wi] × 100, where (Wf) is the final weight of the crude extract powder and (Wi) is the initial weight of the raw material. The total percentage of crude extracts, extracted from (840gm) of Trigonella foenum-graecum have been mentioned in table 1.

3.2. Serum lipid profile:

From data presented in table2, it is observed that the administration of High Fat Diet / Streptozotocin -induced toxicity in mice (Group II), and cause significant increase in lipids profiles levels. Concurrent administration of ethanol extract of (TFG) at 100 mg/kg (BW), showed a significant reduction in the levels of serum TC, LDL, VLDL, as well as TG.

3.3. Fasting blood glucose and serum C-peptide:

FBG significant increase following induction, serum C-peptide also significantly decrease in mice (Group II), while there is a reduction in FBG and elevation in serum C-peptide level in groups treated with ethanol extract of (TFG) seed, glimepiride group respectively.

3.4. Antioxidant activities:

The serum MDA levels were significantly increased in induced(non-treated) group in comparison with (group I) injected citrate buffer only and significant reduction GSH level. Meanwhile, the ethanol extract of (TFG) seed and glimepiride treated group cause significant reduction in the MDA levels and significant elevation in GSH level.

Table1. Yield obtained from herb crude extract of Trigonella foenum-graecum seeds

<table>
<thead>
<tr>
<th>Extract</th>
<th>Weight (g)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trigonella foenum-graecum</td>
<td>74.92</td>
<td>8.92</td>
</tr>
</tbody>
</table>

Table2. Comparison between (citrate buffer) and induced diabetic (non-treated) group and induced treated with TFG seed extract in relation to different parameters.

Mean ±Error
### Table 3. Comparison between induced diabetic (non-treated) group and induced treated with TFG seed extract and induced treated with glimepiride in relation to different parameters.

<table>
<thead>
<tr>
<th>Group</th>
<th>(Citrate buffer)</th>
<th>(Induced)</th>
<th>Trigonella <em>foenum</em>– <em>graecum</em> extract</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-peptide (ng / ml)</td>
<td>1.15 ± 0.08 c</td>
<td>0.80 ± 0.03 d</td>
<td>1.91 ± 0.08ab</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>96.39 ± 0.01c</td>
<td>115.66 ± 0.01a</td>
<td>105.79 ± 0.01b</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>89.96 ± 0.009b</td>
<td>248.31 ± 11.75a</td>
<td>81.10 ± 0.95b</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.44 ± 0.12d</td>
<td>18.11 ± 0.009e</td>
<td>48.89 ± 0.02b</td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>37.60 ± 0.009b</td>
<td>245.62 ± 4.35a</td>
<td>35.90 ± 0.95b</td>
<td></td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>30.32 ± 0.01b</td>
<td>62.27 ±0.49a</td>
<td>16.08 ± 1.38c</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>115.25 ± 0.57b</td>
<td>305.06 ± 16.9a</td>
<td>109.25±2.11b</td>
<td></td>
</tr>
<tr>
<td>MDA (unit/ml)</td>
<td>25.77 ± 0.68bc</td>
<td>29.55 ± 0.50a</td>
<td>24.90 ± 0.47c</td>
<td></td>
</tr>
<tr>
<td>GSH (unit /ml)</td>
<td>48.54 ± 0.49c</td>
<td>45.40 ± 0.48d</td>
<td>49.55 ± 0.73bc</td>
<td></td>
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</table>

C-peptide level ¹, TC: total cholesterol ², TG: triglycerides ³, HDL: high density lipoprotein ⁴, LDL: low density lipoprotein ⁵, vLDL: very low density lipoprotein ⁶, blood glucose ⁷, MDA: Malondialdehyde ⁸, GSH: glutathione. ⁹

** (P<0.01). Means having with the different letters in same column differed significantly.
VLDL (mg/dl)\textsuperscript{VI}

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<td>(Glimepiride)</td>
<td>29.38 ± 1.04 \textsuperscript{a}</td>
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<td>30.95 ± 0.82 \textsuperscript{a}</td>
<td>29.76 ± 0.91 \textsuperscript{a}</td>
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<td>30.98 ± 1.50 \textsuperscript{a}</td>
<td>30.80 ± 1.44 \textsuperscript{ab}</td>
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Glucose (mg/dl)\textsuperscript{VII}

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MDA (unit/ml)\textsuperscript{VIII}

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GSH (unit/ml)\textsuperscript{IX}

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C-peptide level \textsuperscript{I}, TC: total cholesterol \textsuperscript{II}, TG: triglycerides \textsuperscript{III}, HDL: high density lipoprotein \textsuperscript{IV}, LDL: low density lipoprotein \textsuperscript{V}, VLDL: very low density lipoprotein \textsuperscript{VI}, blood glucose \textsuperscript{VII}, MDA: Malondialdehyde \textsuperscript{VIII}, GSH: glutathione \textsuperscript{IX}

** (P<0.01). Means having with the different letters in same column differed significantly.

Table 4. Mean±Error body weights of study groups.

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* (P<0.05), NS: Non-Significant. Means having with the different letters in same column differed significantly

3.5 Histopathology examinations

1. Liver histopathology
Image: 1. (A & B) Section of Mice liver for (citrate buffer group) showing mild and focal microvesicular steatosis (fatty changes) (red arrow) of hepatocyte of the liver H&E. (20X)

Image: 2. (A & B) Section of Mice liver tissue for (STZ group) showing marked and diffused microvesicular steatosis (fatty changes) (red arrow) and hypertrophy of the hepatocyte (green arrow), dilated and congested vein and sinusoid with mild periportal inflammation H&E. (20X)
Image: 3. (A &B) Section of Mice liver tissue for (glimepiride group) showing restoration of the normal hepatocyte architecture with disappearance of the mild hepatocyte steatosis (red arrow) and decrease in the periportal inflammation H&E. (20X)

Image: 4. (A &B) Section of Mice liver tissue for (Trigonella foenum- graecum group) showing restoration of the normal hepatocyte architecture with disappearance of hepatocyte steatosis (red arrow), with no portal or decrease interface inflammation H&E. (20X)
2. Pancreas Histology

Image 5. Section of Mice pancreatic tissue section for (citrate buffer group) showing normal pancreatic islets (red arrow), and normal pancreatic acini (green arrow) H&E. (20X)

Image 6. Section of Mice pancreatic tissue for (STZ group) showing shrinkage and reduction in overall number of pancreatic islet (red arrow), and size of acini (green arrow), and abnormal architecture H&E. (20X)
Image: 7. Section of Mice pancreatic tissue section for (Glimepiride group) showing improvement in the structure of the pancreatic islet (red arrow), and acini (green arrow) H&E. (20X)

Image: 8. Section of mice pancreatic tissue section for (Trigonella foenum-graecum group) showing improvement in the architecture of the pancreatic islet (red arrow), and normal in the size, round in shape of acini (green arrow) H&E. (20X)
4. DISCUSSION

Trigonella foenum-graecum L. was one of the most ancient medicinal herbs[9]. It has been shown to have a variety of biological activities like in the treatment of menstrual disorders, including premenstrual symptoms and spasmodic dysmenorrhea, for certain menopausal conditions, for insufficient lactation. The total amount of FG seeds used in this study were 840 gm., n-hexane was used for defatted FG oil from the seeds[20] in order to exclude the surplus fatty components or lipids that interfere with both extraction process by adhering on the flask and biologic effect inside the body[21].

In present study, it was essential for us to develop a suitable animal model of hyperglycemia, insulin resistance, and mitochondrial dysfunction, resembling the characteristics seen in human type II diabetes mellitus. High-fat diet was one of unhealthy lifestyle that can cause abnormal cholesterol levels[22]. Also, [23] Rui-Li et al (2006) reported that high fat diet (HFD) induced abnormal increases in serum concentrations of total cholesterol, lipid peroxidation, TG, and LDL-cholesterol, and a decrease in HDL-cholesterol concentration in addition to decreased lipoprotein lipase activity, accompanied by a depressed antioxidant defense system. Single high-dose injection of STZ could induce hypoinsulinemia, hyperglycemia, and mitochondrial dysfunction,[24] In addition, obesity, insulin resistance, which cause further augmentation of hyperglycemia, which mimicking human type II that induced by HFD[25]. Therefore, HFD/STZ-induced diabetic model was a useful animal model for screening hypoglycemic drugs and studying the corresponding mechanisms; which agree with Li et al. (2014) induction protocol and support present results. The results showed that HFD/STZ diabetic mice had a significant reduction in BW, and an obvious increase in blood glucose, serum TG, and serum TC levels in induced (non-treated) group which were rescued to near normal with the treatment of (TFG) seed ethanol extract and glimepiride. Hyperglycemia and hyperlipidemia result in development of oxidative stress, systemic and local inflammation which were an essential factors for degradation of β-cells, insulin resistance and resembling type II diabetes[4] Oxidative stress in STZ induced diabetic animals is due to glucose auto-oxidation, protein glycation, formation of advanced glycation products that generates free radicals[26] which play a main role in beta cell destruction linked to STZ, and these result agree with Ahangarpour et al. (2014) demonstrated that hyperglycemia-induced destruction of pancreatic beta cells could be a consequence of the oxidative stress; therefore, antioxidant compounds possess protective effect against the damage induced by hyperglycemia. Malondialdehyde (MDA), which was a product of lipid peroxidation or reaction of oxygen with unsaturated lipids[27] were highly significant upsurge in induced (hyperglycemic) mice (Table 3.2). The elevated levels of MDA in induced (hyperglycemic) mice suggest increased lipid peroxidation in fat deposits that could be released and have detrimental effects on hepatocytes and other hepatic cells. In hepatocytes, ROS and lipid peroxidation products further impair the respiratory chain, either directly or indirectly through oxidative damage to the mitochondrial genome. These features, in turn, lead to the generation of extra ROS, and a vicious cycle ensues. Finally, ROS and
lipid peroxidation products also activate stellate cells, thus resulting in fibrosis [28] Besides, the results were supported by histological examination which showed degenerative changes in the liver and reticular changes of islets as evidence of fibrosis on pancreas; image 2&6.

Glutathione is an intracellular hydrophilic antioxidant[29] . It was the most vital endogenous defense system against oxidative stress in body and it plays a role in maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function [30] . In this study, the serum lipid profile and MDA and fasting blood glucose levels was found to be declined with ethanolic extracts of *Trigonella foenum-graecum* , *Artemisia vulgaris* and their combinations in comparison with induced (non- treated) group; Table 3.3. The reason behind that may owing to the presence of polyphenols; which possess the ability to scavenge reactive oxygen species[31].

Normal architecture in the liver histology, no steatosis, no inflammation, and no triaditis or necrosis was observed indicate that *Trigonella foenum-graecum* processes good antioxidant activity.

The histopathological study of diabetic treated group specified increased volume density of islets and increased proportion of beta cells, in the diabetics that received the extracts, which may be a sign of regeneration; Image:4.

Signs of regeneration of β -cells, potentiation of insulin secretion from surviving β cells of the islets of Langerhans and decrease of blood glucose level that have been reported following consumption of some plant extracts.

The results of present study showed appositive correlation between hypoglycemic effect and free radical scavenging activity in TFG ethanol extract.

5. CONCLUSION

Findings suggest Defatted seeds extract treatment has potential anti-hyperglycemic and hepatoprotective effect with free radical scavenging ability against STZ-induced toxicity. In addition to therapeutic protective effect against diabetes by decreasing oxidative stress and preserving pancreatic β-cell integrity. Consequently TFG may be clinically useful for protecting β cell against oxidative stress.

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