Efficacy of saffron extract and fenugreek seeds supplementation on liver of streptozotocin induced diabetic rats

Doaa A. Ali, Hanaa Serag, Ahmed Abdeen and Radwa Refaat.
Zoology Department - Faculty of Science - Mansoura University, Mansoura, Egypt.
(Corresponding author email: doaasakr@mans.edu.eg)

Abstract

The present study aimed to investigate the possible hypoglycemic and hepatoprotective effects of saffron extract (100mg/kg b.wt) and fenugreek supplementation (1.4 g/day/kg b.wt) in STZ- induced diabetic rats at biochemical, histological, histochemical and ultrastructural levels. The results indicated that a single dose of STZ (50 mg /kg b wt, i.p) caused a significant increase in serum and liver total lipids, total cholesterol and triglyceride as well as serum low density and a very low density lipoprotein, accompanied by an increase in serum glucose level and oxidative stress marker, malondialdehyde and hydrogen peroxide level as well as serum aspartate and alanine aminotransferase activities. The results also reported a significant decrease in the high density lipoprotein, serum and liver total protein, serum albumin and globulin contents and A/G ratio, as well as a significant decrease in serum insulin level and the liver enzyme activities and the hepatic glutathione content. Histologically, STZ treated group showed degenerative changes in the pancreas and alteration in the structural integrity of the hepatocytes and their intracellular organelles. Histochemically, liver sections of STZ treated animals displayed marked decrease in the glycogen and total protein contents. On the other hand, the intake of saffron or fenugreek minimize the disturbance observed in the most tested parameters resulted from STZ administration and improve the liver structure and functions. Finally, it can be concluded that the intake of natural product such as saffron or fenugreek may be effective in reducing the diabetic liver injury induced by streptozotocin and the fenugreek supplementation showed more amelioration than saffron extract.

Keywords: Fenugreek, Histochemistry, Histopathology, Liver, Saffron, Streptozotocin, Ultrastructure

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder with numerous complications. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicted that about 366 million people are likely to be diabetic by the year 2030 (Oyedemi et al., 2011). Streptozotocin is mainly used to induce the experimental insulin-dependent diabetes which is usually accompanied with many clinical and metabolic disturbances (Montero et al., 2000). The liver is insulin-dependent tissue that plays a vital role in glucose and lipid homeostasis and is severely affected in diabetes disease as it recognized as major complications of diabetes (Sivajothi et al., 2007). Herbal medicines are being increasingly utilized in developed countries to treat a wide variety of clinical disease. Herbal remedies are considered to be effective and safe alternative in the management of treatment for diabetes mellitus and its complications (Heeba and Abd-Elghany, 2010).

Saffron (dried stigmas of the flowers of Crocus sativus L) is the world’s most expensive spice belongs to the Iridaceae family (Zargari, 1993). Saffron has been traditionally used as an antispasmodic, eupetic, gingival sedative and emmenagogue (Schmidt et al., 2007 and Kataria et al., 2011). Crocin, crocetin and Safranal are the major active constituents of saffron (Liakopoulou-Kyriakides and Kyriakidis, 2002). Previous studies have demonstrated that saffron and its active constituents have a wide variety of pharmacological effects such as antioxidant (Assimopoulou et al., 2005 and Chen et al., 2008). Antitumor (Chermahini et al., 2010), anti-genotoxic,
memory and learning enhancing (Abe and Saito, 2000 and Tamaddonfard et al., 2013), neuroprotective, analgesic and anti-inflammatory, anti-convulsant, antianxiety, antidepressant, anti-hypertensive (Razavi et al., 2013), and anti-hyperlipidemic effects (Srivastava et al., 2010, Bathaie and Mousavi, 2010 and Kataria et al., 2011). Also, crocetin has increased insulin sensitivity and ameliorated abnormalities related to insulin resistance such as impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension due to high-fructose diet and dexamethasone injection in rats (Xi et al., 2005;2007). Oxidative stress can cause insulin resistance and the long-term complications of diabetes; antioxidants may be very important in mitigating impaired insulin secretion and action in insulin resistance and prevent diabetes complications (Rahimi et al., 2005 and Evans, 2007).

Fenugreek (Trigonella foenum-graecum) is an annual herb belonging to family Leguminosae and is one of the oldest herbs known originating in the Mediterranean region and Asia (Azaizeh et al., 2006). Fenugreek seeds have been traditionally used in the treatment of gastrointestinal disorders, gout, wound healing, inflammation, hyperlipidemia, and diabetes (Miraldi et al., 2001; Basch et al., 2003 and WHO, 2004). Fenugreek have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant (Cowan, 1999; Amin et al., 2005; Abou El-Soud et al., 2007 and Kassaian et al., 2009). T. F. graecum seeds contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects (Billaud, 2001; Basch et al., 2003 and Puri et al., 2012).

The present study aimed to investigate the possible hypoglycemic and hepatoprotective properties of saffron extract and fenugreek supplementation in diabetic liver induced by STZ in rats at biochemical, histological, histochemical and ultrastructural levels.

2 Materials and Methods

Streptozotocin (STZ) was purchased from Sigma Company (USA). It was applied as a single freshly prepared dose (50mg/kg body weight), dissolved in citrate buffer at pH 4.4, as previously recommended by Nandini et al. (2000).

Preparation of Saffron extract

An aqueous extract of saffron (Crocus sativus L) was prepared using (1g) dried stigmas powder of saffron, which were soaked in 100ml of double distilled water for one hour and homogenized. The homogenate was centrifuge at 2000 rpm for 10 min to remove the particles and supernatant was used for the experiment. The animals received a daily dose of saffron extract (100mg/kg b.wt) for 6 weeks according to Premkumar et al. (2003).

Fenugreek supplementation

Fenugreek seed (Trigonella foenum-graecum) powder was given in the diet in a dose (20 g/kg diet) for 6 weeks according to Mitra and Bhattacharya (2006).

Experimental design

The study was performed on male Wistar albino rats (Rattus rattus), weighing 120-140 g. Rats were housed in stainless steel cages at a well-ventilated animal house in a temperature-controlled room at 22–25°C, maintained under specific pathogen-free conditions on a 12hr light/dark cycle at the animal house lab., Faculty of Science, Mansoura University, Mansoura, Egypt. They were acclimated to laboratory conditions for 2 weeks prior to experiment; rats were permitted adequate standard diet and given water ad libitum. All experiments were carried out in accordance with the protocols approved by the Local Experimental Animal Ethics Committee. After the adaptation period, rats were randomly divided into six groups 10 rats each.

Group 1; The control group. Rats of this group were received a standard diet (SD) which composed of casein 15.0 %, starch, 67.0 %, corn oil 8.0%, salt mixture 4.0 %, vitamin mixture 1.0% and wood fiber 5.0 % for 6 weeks (Ulloa et al., 1988) and inter-peritoneal injected with a single dose of saline at PH 4.4 (50 mg /kg body weight) (Nandini et al., 2000). Group 2; Saffron treated group: Rats of this group were received SD with daily oral administration of an aqueous extract of saffron (100mg/ kg b.wt) for 6 weeks (Premkumar et al., 2003). Group 3; Fenugreek treated group: Rats of this group were fed diet supplemented with fenugreek powder daily for 6 weeks (Mitra and Bhattacharya, 2006). Group 4; Diabetic group: Rats were interperitoneally injected with a single dose of streptozocin (50 mg /kg b wt) after 4 week (Nandini et al., 2000). Group 5; Saffron+ Diabetic group: Rats of this group were received saffron as described in group 2 for 6 weeks, in the same time the animals were interperitoneally
injected with a single dose of streptozotocin (50 mg / kg bwt). **Group 6;** Fenugreek+ Diabetic group: Rats of this group were fed diet supplemented with fenugreek powder as described in group 3 for 6 weeks, and interperitoneally injected with a single dose of streptozotocin (50 mg / kg b wt).

**Blood sampling and biochemical investigations:**

At the end of the experimental period (6 weeks), all rats were fasted overnight and sacrificed under ether anesthesia. From each rat, a blood sample was collected into clean centrifuge tube and allowed to clot, then centrifuged at 3000 r.p.m for 10 minutes at 4°C for biochemical analysis. Immediately after collecting blood, the rats were dissected and three part of the liver from each rat were removed, the first one was accurately weighed and homogenized using homogenizer in a 10% fold volume of bidistilled water, the homogenate was kept frozen at –20°C until being analyzed, the second part of the liver and a part of the pancreas placed into 10% buffered neutral formalin for histological and histochemical studies, the third part of the liver fixed in 4F1G (pH 7.4), for transmission electron microscopic investigation.

**Serum glucose and insulin assay:**

Serum glucose level was determined using colorimetric kit according to the method of McCleary and Codd (1991), and serum insulin level was measured by enzyme linked immunosorbant assay (ELISA) kit according to the method of Flier et al (1976).

**Lipids profile assay:**

Total lipids level was estimated according to Frings et al (1972), total cholesterol, triglycerides level, and the level of serum HDL-C was estimated by colorimetric method of Young (1995), using HDL-C kit and the LDL-C and vLDL-C levels in serum were calculated according to equations described by Friedewald et al. (1972).

**Protein profile:**

Total protein content was estimated by colorimetric method according to the method of Henry (1964), total albumin was estimated in serum using diamond diagnostics kit according to Doumas et al. (1971) and globulin contents and A/G ratio were calculated.

**Liver functions:**

Serum and liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using colorimetric method according to the technique of Reitman and Frankel (1957). Malondialdehyde (MDA) content was determined according to the method of Ohkawa et al. (1979). Glutathione (GSH) content was determined according to the method described by Prins and Loose (1969). Hydrogen peroxide content was determined using colorimetric method of Aebi (1984).

**Histological examination**

The fixed liver and pancreas specimens were dehydrated in ascending series of ethyl alcohol and embedded in paraffin. Sections at 5μm thickness were stained according to the following histological stains: H&E (Weesner, 1968) and Masson’s Trichrome method (Masson, 1929) for collagen fibres.

**Histochemical investigation**

Total polysaccharides were detected using Periodic Acid Schiff’s (PAS) reaction (Pearse, 1985). Also, the total protein was detected using mercury-bromophenol blue stain (Mazia et al., 1953).

**Electron microscopic investigation**

Dissected liver samples were fixed in 4F1G in phosphate buffer (pH 7.2) at 4°C and post-fixed in 1% cold osmium tetroxide in phosphate buffer at pH 7.2 for three hours. The specimens were then dehydrated in graded ethanol and embedded in Epson-Araldite resin. Ultrathin sections were stained by uranyl acetate followed by lead citrate as described by Reynolds (1963) and examined on Joel Electron Microscope (JAPAN) operating at 60kV.

**Statistical analysis**

Data were analyzed statistically using Student’s t test using SPSS software and the data were presented as mean ± SEM. Differences with p ≤ 0.05 were considered significant.

3 **Results**

**Biochemical results**

The obtained data in the present study are represented in Tables 1, 2 and 3. Table(1) showed that a single intraperitoneal dose of streptozotocin (50 mg /kg b wt ) resulted in alterations in comparison with the control group including significant increases in serum and liver total lipids, total cholesterol and triglyceride as well as serum low density and a very low density lipoprotein but the decrement was reported in the high density lipoprotein. Meanwhile the intake of saffron or fenugreek ameliorated the above mentioned parameters. As shown in Table (2),
streptozotocin treated rats represented significant decrease in serum and liver total protein, serum albumin and globulin contents and A/G ratio accompanied by a significant decrease in serum insulin level in contrast to a significant increase in serum glucose level. However, supplementation of diabetic rats with saffron or fenugreek significantly improved the reduction in the above parameters, with the decrease in the serum glucose level; the improvement was more pronounced in the group administered with fenugreek more than that treated with saffron. Table (3) represented the liver malondialdehyde, hydrogen peroxide level and glutathione content, and serum and liver alanine aminotransferase and aspartate aminotransferase activities in different rat groups. Significant increase in oxidative stress parameters, MDA and H\textsubscript{2}O\textsubscript{2} as well as the serum AST and ALT enzyme activities with significant decrease in the antioxidant parameter, GSH content accompanied with the decrease in the AST and ALT activities in the liver of diabetic rat group were noticed when compared to the control group. On the other hand, the administration with saffron or fenugreek, improved both the serum and liver enzymatic activities and the oxidative and antioxidant parameters.

Table (1): Effects of saffron extract and fenugreek seeds on Serum and liver lipid profile in streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Control</th>
<th>Saffron</th>
<th>Fenugreek</th>
<th>Diabetic</th>
<th>Saffron +Diabetic</th>
<th>Fenugreek + Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total lipids</strong></td>
<td>Serum (g/dl)</td>
<td>521±5.8</td>
<td>520±3.9</td>
<td>517±5.5</td>
<td>781±9.9*</td>
<td>613±7.2*</td>
<td>611±4.9*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-0.2</td>
<td>-0.8</td>
<td>50.2</td>
<td>17.6</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-21.6</td>
<td>-21.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver mg/g</td>
<td>65.3±3.1</td>
<td>65.8±2.7</td>
<td>64.0±3.2</td>
<td>88.1±3.5*</td>
<td>73.8±3.3*</td>
<td>71.6±2.3*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>0.8</td>
<td>-1.9</td>
<td>35.5</td>
<td>13.0</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-16.1</td>
<td>-18.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>Serum (g/dl)</td>
<td>92.5±3.0</td>
<td>80.7±3.1</td>
<td>89.4±3.4</td>
<td>131.5±2.6*</td>
<td>102.0±3.8*</td>
<td>110.1±4.1*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-12.7</td>
<td>-3.3</td>
<td>42.2</td>
<td>10.2</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-22.4</td>
<td>-16.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver mg/g</td>
<td>14.4±0.9</td>
<td>14.0±0.7</td>
<td>15.1±0.8</td>
<td>23.2±0.9*</td>
<td>18.6±0.5*</td>
<td>19.2±0.5*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-2.8</td>
<td>4.8</td>
<td>60.4</td>
<td>29.0</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-19.4</td>
<td>-16.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Triglyceride</strong></td>
<td>Serum (g/dl)</td>
<td>83.2±2.3</td>
<td>77.6±3.4</td>
<td>87.0±3.1</td>
<td>179.0±3.3*</td>
<td>105.6±2.6*</td>
<td>112.1±3.1*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-6.5</td>
<td>4.5</td>
<td>115.0</td>
<td>26.9</td>
<td>35.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-41.2</td>
<td>-37.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver mg/g</td>
<td>13.1±0.8</td>
<td>12.5±0.9</td>
<td>13.6±0.6</td>
<td>28.8±0.9*</td>
<td>15.8±0.9*</td>
<td>19.5±0.8*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-4.5</td>
<td>4.6</td>
<td>121.5</td>
<td>21.5</td>
<td>50.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-45.1</td>
<td>-32.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDL-c</strong></td>
<td>Serum (g/dl)</td>
<td>44.0±2.3</td>
<td>46.5±1.4</td>
<td>45.0±1.8</td>
<td>23.0±0.8*</td>
<td>30.1±1.2*</td>
<td>28.8±1.1*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>5.6</td>
<td>2.2</td>
<td>-48.0</td>
<td>-31.0</td>
<td>-34.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>30.4</td>
<td>25.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LDL-c</strong></td>
<td>Serum (g/dl)</td>
<td>33.5±0.7</td>
<td>30.1±0.8</td>
<td>32.3±0.9</td>
<td>71.5±1.9*</td>
<td>51.0±1.4*</td>
<td>60.7±1.7*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-10.1</td>
<td>-3.6</td>
<td>112.9</td>
<td>52.2</td>
<td>80.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-28.0</td>
<td>-15.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>vLDL-c</strong></td>
<td>Serum (g/dl)</td>
<td>16.2±0.6</td>
<td>15.2±0.7</td>
<td>15.5±0.5</td>
<td>35.7±0.8*</td>
<td>22.2±0.6*</td>
<td>24.4±0.6*</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>-6.1</td>
<td>-4.3</td>
<td>120.0</td>
<td>37.0</td>
<td>50.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>-37.8</td>
<td>-31.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ±SE (n=6 for each group).  
* Significant change at p≤0.05 comparing with control group,  
  ** Significant change at p≤0.05 comparing with diabetic group  
  % of change compared to control group &  ** % of change compared to diabetic group
Table (2): Effects of saffron extract and fenugreek seeds on serum and liver total proteins content, serum albumin, globulin, A/G ratio, glucose and insulin level in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th>Saffron</th>
<th>Fenugreek</th>
<th>Diabetic</th>
<th>Saffron + Diabetic</th>
<th>Fenugreek + Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (g/dl)</td>
<td>7.61±0.22</td>
<td>7.42±0.27</td>
<td>7.33±0.15</td>
<td>4.91±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89±0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.30±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>-2.4</td>
<td>-3.6</td>
<td>-35.5</td>
<td>-22.6</td>
<td>-17.2</td>
<td>28.3</td>
</tr>
<tr>
<td>Serum (mg/g wet)</td>
<td>1.40±0.06</td>
<td>1.33±0.06</td>
<td>1.31±0.07</td>
<td>0.48±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.88±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>-5.0</td>
<td>-7.1</td>
<td>-65.5</td>
<td>-42.0</td>
<td>-32.0</td>
<td></td>
</tr>
<tr>
<td>Total Protein (mg/g wet)</td>
<td>4.4±0.12</td>
<td>4.22±0.09</td>
<td>4.13±0.10</td>
<td>2.45±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.18±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.33±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>-4.1</td>
<td>-6.1</td>
<td>-44.3</td>
<td>-27.7</td>
<td>-25.0</td>
<td></td>
</tr>
<tr>
<td>Total albumin (mg/dl)</td>
<td>3.21±0.03</td>
<td>3.2±0.10</td>
<td>3.2±0.08</td>
<td>2.46±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.71±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.97±0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>-0.3</td>
<td>-0.3</td>
<td>-23.4</td>
<td>-15.6</td>
<td>-7.5</td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (mg/g wet)</td>
<td>1.37±0.02</td>
<td>1.31±0.03</td>
<td>1.29±0.01</td>
<td>0.99±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.12±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>-4.8</td>
<td>-5.8</td>
<td>-27.7</td>
<td>-14.6</td>
<td>-18.2</td>
<td></td>
</tr>
<tr>
<td>A / G ratio (mg/dl)</td>
<td>89.3±4.2</td>
<td>91.2±5.0</td>
<td>90.1±6.8</td>
<td>258.0±11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.2±7.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>143.3±6.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>2.1</td>
<td>0.7</td>
<td>189.0</td>
<td>82.0</td>
<td>60.7</td>
<td></td>
</tr>
<tr>
<td>Glucose level (µIU/ml)</td>
<td>3.4±0.58</td>
<td>3.5±0.14</td>
<td>3.24±0.19</td>
<td>1.03±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.96±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.1±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>**</td>
<td>2.9</td>
<td>-4.7</td>
<td>-69.7</td>
<td>-42.5</td>
<td>-38.2</td>
<td></td>
</tr>
<tr>
<td>Insulin level (µIU/ml)</td>
<td>90.0</td>
<td>103.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ±SE (n=6 for each group).

a  Significant change at p≤0.05 on comparing with control group
b  Significant change at p≤0.05 on comparing with diabetic group
*  % of change compared to control group & ** % of change compared to diabetic group

Histological observations.

Histological examination of the pancreas:
Pancreatic tissue of the control group showed normal pancreatic structure. The pale stained islets of langerhans "the endocrine component" were scattered throughout the exocrine component with well define boundary. The islets are made up of irregular cords or masses of polygonal epithelial cells (Fig 1A). Pancreatic sections of animals treated with either saffron or fenugreek showed normal pancreatic architecture with no observable pathological finding in the cells of islets of langerhans (Figs 1B&1C). On the other hand, pancreas section of STZ-treated group showed degenerative changes in islets of langerhans, homogenization of the center and apparent relative reduction in the size and number of islets. Some of the islet cells showed vacuolated cytoplasm with nuclear changes as karyolysis, disappearing of nucleus as well as residue of destructed cells was observed (Fig 1D). In other foci, irregular outline of the islet and an increased in reticent septa were noticed (Fig 1E). However, histological examination of pancreatic tissue of saffron or fenugreek + STZ induced diabetic rat showed improvement in the islet structure and restoration of normal pancreatic architecture (Figs 1F&1G).
Table (3): Effects of saffron extract and fenugreek seeds on liver malondialdehyde content, hydrogen peroxide level, glutathione content, serum and liver alanine aminotransferase and aspartate aminotransferase in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Saffron</th>
<th>Fenugreek</th>
<th>Diabetic</th>
<th>Saffron + Diabetic</th>
<th>Fenugreek + Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver MDA</td>
<td>84.7±2.80</td>
<td>79.8±2.10</td>
<td>89.0±2.90</td>
<td>219.5±4.70</td>
<td>155.0±3.50</td>
<td>169.0±2.80</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>-5.7</td>
<td>5.1</td>
<td>159.0</td>
<td>82.9</td>
<td>99.2</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>29.4</td>
<td>23.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver H2O2</td>
<td>0.15±0.01</td>
<td>0.15±0.01</td>
<td>0.14±0.01</td>
<td>0.48±0.04</td>
<td>0.30±0.02</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>0.0</td>
<td>-6.6</td>
<td>220.0</td>
<td>100.0</td>
<td>113.3</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>-37.5</td>
<td>-33.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver GSH</td>
<td>3.5±0.07</td>
<td>3.4±0.08</td>
<td>3.6±0.09</td>
<td>2.7±0.02</td>
<td>3.0±0.04</td>
<td>3.1±0.07</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>-2.9</td>
<td>2.9</td>
<td>-22.9</td>
<td>-14.3</td>
<td>-11.4</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>11.1</td>
<td>14.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT Serum</td>
<td>33.2±0.9</td>
<td>31.3±1.2</td>
<td>34.6±1.1</td>
<td>68.3±2.6</td>
<td>52.0±1.6</td>
<td>44.4±1.5</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>-5.7</td>
<td>4.2</td>
<td>105.7</td>
<td>56.6</td>
<td>33.7</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>-23.6</td>
<td>-34.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST Serum</td>
<td>74.5±0.7</td>
<td>77.1±1.6</td>
<td>73.6±0.8</td>
<td>50.8±1.2</td>
<td>63.5±1.0</td>
<td>58.4±1.3</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>3.4</td>
<td>-1.2</td>
<td>-31.9</td>
<td>-14.8</td>
<td>-21.6</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>25.0</td>
<td>15.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT Liver</td>
<td>60.1±1.0</td>
<td>54.4±1.3</td>
<td>59.2±1.5</td>
<td>80.7±2.2</td>
<td>68.0±1.3</td>
<td>70.2±1.1</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>-9.5</td>
<td>-1.5</td>
<td>34.2</td>
<td>13.3</td>
<td>16.6</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>15.0</td>
<td>12.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST Liver</td>
<td>139.9±1.9</td>
<td>141.7±0.9</td>
<td>137.3±1.5</td>
<td>77.8±0.7</td>
<td>96.3±1.2</td>
<td>93.2±2.0</td>
</tr>
<tr>
<td>**</td>
<td>*</td>
<td>1.8</td>
<td>-1.9</td>
<td>44.4</td>
<td>31.11</td>
<td>33.3</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>23.8</td>
<td>19.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ±SE (n=6 for each group).

a Significant change at p≤0.05 on comparing with control group
b Significant change at p≤0.05 on comparing with diabetic group
* % of change compared to control group & ** % of change compared to diabetic group

**Histological examination of the liver:** The liver section of the control group showed the hepatocytes arranged in hepatic cords forming a network around the central vein (Fig 2A). Liver sections of fenugreek and saffron treated groups exhibited normal architecture of the liver (Figs 2B & 2C). While liver sections of STZ–treated group displayed remarkable degenerative changes pronounced in loss of the normal liver architecture (Fig 2F) and dilation and inflammation in central and portal veins (Fig 2D). Also, in other foci, the blood sinusoids between the hepatic cells were dilated (Fig 2E). The hepatocytes appeared to be suffering from ballooning degeneration with marked cytoplasmic vacuolation fatty infiltration were observed with pyknotic and karyolitic nuclei (Figs 2D & 2F). On the other hand, liver sections of Saffron + STZ treated group exhibited some degree of improvement with minor histological changes versus the control animals pronounced in reduction in fat accumulation, less blood sinusoids dilations and less number of necrotic cells were observed. However, focal necrotic area still observed around the central vein (Fig 2G). Also, liver sections of Fenugreek + STZ treated group revealed a marked degree of recovery. The hepatic tissue showed a marked reduction in fat accumulation, absence of leucocytic infiltration, less blood sinusoids dilations and few necrotic cells (Fig 2H).
Collagen Fibers

Liver sections of control and saffron and fenugreek treated animals showed negligible amount of collagen fibers around the central vein (Figs 3A-3C). Liver section of STZ-induced diabetic animals illustrated an increase in the amount of collagen fibers around the central vein (Fig 3D). Meanwhile, liver sections of animals treated with saffron + STZ showed negligible amount of collagen fibers around the central vein (Fig 3E). Also, examination of liver sections of animals treated with fenugreek + STZ showed small amount of collagen fibers around the central vein compared to that of STZ-induced diabetic animals (Fig 3F).

Histochemical observations.

Total polysaccharides: Total polysaccharides in the hepatocytes of control animals appeared in the form of deeply stained reddish granules as shown by their strong PAS positive reaction (Fig 4A). Both Saffron and fenugreek-treated animals displayed no difference...
Fig (4): Liver histochemical demonstration for total polysaccharides of STZ and/or saffron and fenugreek -treated rats. Liver section of control rats (A) showing normal distribution of total polysaccharides with the glycogen flight phenomenon (→), liver sections of saffron (B) and fenugreek (C) -treated rats showing strong PAS positive reaction with normal distribution of total polysaccharides, liver section of STZ treated rats (D) displaying a marked decrease in total polysaccharides, liver section of saffron+ STZ -treated rats (E) demonstrating a slight decrease in total polysaccharides in the hepatocytes, liver section of fenugreek + STZ (F) -treated rats illustrating restoration of total polysaccharides content in the hepatocytes (PAS reaction, scale bar = 50 µm)

in their polysaccharides contents in comparison with control group (Figs 4B&4C). In liver section of STZ-induced diabetic animals, the polysaccharides contents has been diminished in the hepatocytes and most of the cells appeared with cytoplasmic vacuolization (Fig 4D). Examination of liver sections of animals treated either with saffron+STZ or fenugreek + STZ showed improvement in polysaccharides content when compared with STZ-induced diabetic animals (Figs 4E&4F).

**Total protein:** The proteins in liver cells of a control rats appeared in the form of small bluish irregular particles against a weakly to moderately stained ground cytoplasm (Fig 5A). Normal distribution and contents of the proteins materials were observed in the liver tissues of either saffron or fenugreek treated groups (Figs 5B&5C). Liver section of STZ-induced diabetic animals revealed cytoplasmic vacuolization in a large number of hepatocytes and the proteins remnants were less reactive with bromophenol blue than before (Fig 5D). Liver sections of saffron + STZ treated animals showed reduction in the proteins contents and cytoplasmic vacuolization in the hepatocytes near or around the central vein. However, in other foci, restoration of proteins materials was observed in other hepatocytes (Fig 5E). Examination of liver sections of fenugreek + STZ -treated animals revealed normal content and distribution of proteins materials all over the hepatocytes as in the control group (Fig 5F).

**Ultrastructural observations.**

In electron microscope preparations, the hepatic cells from the control group showed normal rounded nucleus with electron-Lucent euchromatin and scattered areas of heterochromatin (Fig 6A). The cytoplasm showed a granular appearance there were numerous rounded and elongated mitochondrial profiles with electron-dense matrix. Also, there were profiles of rough endoplasmic reticulum between the mitochondria (Figs 6A&6B). The bile canaliculi were wide channels formed by opposing hepatic cells.
Fig (6): Electron micrographs of liver section of control rats (A,B&C), saffron (D,E &F) and fenugreek (G,H&I) treated rats showing normal hepatocytes, bile canaliculi and blood sinusoids. Abbreviations; nucleus (N), mitochondria (M), rough endoplasmic reticulum (RER), space of Disse (DI), endothelial cells (EN), Zonula occludens (ZO), Zonula adherens (ZA), bile canaliculi (BC), microvilli (MV), lipid droplets (LD), Kupffer cells (KC) and red blood cells (RBCs).

Microvilli of the hepatic cell were found to project into the lumen of the bile canaliculus, and junctional complex secured the attachment of the hepatic cells around these canaliculi (Fig 6B). The plasma membrane of the hepatic cells facing the blood sinusoids was formed of microvilli which was called space of Disse. The hepatic sinusoids were lined by an extremely thin walled discontinuous layer of endothelial and Kupffer cells. The endothelial cells were extremely thin with an electron-lucent cytoplasm. A large Kupffer cells were macrophage lining the sinusoids (Fig 6C).

The structure of the hepatic cells, bile canaliculi and blood sinusoids of both saffron and fenugreek treated rats were found to be quite normal as those of the control animals (Figs 6D-6I).

Electron microscopic examination of the liver tissue of STZ - diabetic rats showed major alteration in the structural integrity of the intracellular organelles with disintegrals or dissolution of the cytoplasm. Also, numerous lipid droplets of variable size were observed within the hepatocytes (Figs 7A&7C). The rough endoplasmic reticulum was ill-
Fig (7): Electron micrograph of liver section of STZ treated rats (A& B & C) showing the presence of the nucleus (N) with two nucleoli (Nu), mitochondria (M) with electron-lucent matrix, lipid droplets (LD) and disintegration of the cytoplasm (arrow heads) with ill-defined RER, dilated bile canaliculi (BC), broken and detached endothelial cells (EN), RBCs infiltration between the hepatocytes and distention of the blood sinuosoids with proteinaceous material. Liver section of both saffron+ STZ treated rats (D&E&F) and fenugreek + STZ treated rats (G&H&I) displaying an improvement in the hepatocytes, bile canaliculi and blood sinuosoids structure. Abbreviations; nucleus (N), electron-dense mitochondria (M), rough endoplasmic reticulum (RER), narrow space of Disse (arrow heads), endothelial cells (EN), Zonula occludents (ZO), Zonula adherens (ZA), bile canaliculi (BC), microvilli (MV), lipid droplets (LD), Kupffer cells (KC) and red blood cells (RBCs).

defined as compared to that of the control group and some of them showed detachment of ribosomes in the cytoplasm (Fig 7B). Altered mitochondria with electron-lucent matrix and intercristal swelling were also noticed (Fig 7B). Dilated bile canaliculi with a plenty of pleomorphic microvilli were observed. However, the blood sinuosoids appeared broken with detachment of the endothelial cells and infiltration of RBCs between the hepatocytes (Fig 7C).

In Saffron+ STZ treated diabetic group, there was an apparent decrease in hepatocytes degeneration and the nucleus appeared normal (Fig 7D). Profiles of rough endoplasmic reticulum around the electron-dense mitochondria were observed. However some of the mitochondria in the group still suffer from intercristae swelling. Also, an obvious decrease in the lipid droplets in the cytoplasm was noticed (Figs 7D &7E). The bile canaliculi with aplenty of pleomorphic microvilli were observed (Fig 7E). On the other hand the blood sinuosoids with Kupffer and endothelial cells and narrow space of Disse was noticed (Fig 7F).
Preadministration of fenugreek to STZ treated diabetic rats restored most of the liver ultrastructure changes. The liver tissue of this group showed rounded nucleus with normal distribution of chromatin materials and prominent nucleolus. Also, profiles of normal mitochondria and cisternae of RER were observed in the cytoplasm (Fig 7G). Moreover the bile canaliculi and the blood sinusoids seemed to be normal with respect to that of the control group (Figs 7H&7I).

4 Discussion

Diabetes mellitus is metabolic disorder occurs due to human genetically susceptibility, resulting from defect in insulin secretion, insulin action or both (Kumar et al., 2013). Many traditional herbs are being used by diabetic patients to control the disease (Yahaya, 2009). Nonetheless very few studies are preformed to investigate the efficacy of these herbs clinically. There have been growing interests in the application of natural components as anti diabetic agents (Srinath et al., 2011).

In the present study, the STZ-treated rats showed a significantly increase of plasma glucose level and decrease of plasma insulin level compared to that of control animals. Also, pancreas section of STZ-treated group showed degenerative changes in islets of langerhans, homogenization of the center and apparent relative reduction of the size and number of islets. STZ is a cytotoxic compound obtained from the soil microbes Streptomyces achoromogenes. STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood glucose in animals (Kumar et al., 2012; 2013). In this study, animals treated with Saffron+ STZ or fenugreek+ STZ remarkably attenuated the change in blood sugar, serum insulin levels and improved the pancreatic islet cells structure. Little and Sacks (2009) and Kianbakht and Hajighaee (2011) found that saffron, crocin and safranal showed antihyperglycemic and antihypoinsulinemic effects on diabetic rats. The hypoglycemic effect of saffron extract and its constituents may be due to its inhibitory effects on oxygen free radical, MDA levels and intracellular ca concentration in endothelial cell and activating superoxide dismutase (Xiang et al., 2006). Also its inhibitory effect on pancreatic lipase may be another mechanism which may act by reducing the absorption of fat and cholesterol (Sheng et al., 2006). Meanwhile the hypocholesterolemic effect of T. Foenum greacum seeds could be attributed to saponin content of the seeds or interference with cholesterol biosynthesis in the liver (Petit et al., 1995; Abd elaziz, 2011 and Rajarajeswari et al., 2012). The amino acid 4 hydroxyisoleucine present in fenugreek seeds may also decrease the plasma triglyceride level (Sharma, 1986). Moreover, the lipids lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone T4 (Mitra et al., 1995 and Abd elaziz, 2011).

Biochemical and histochemical results in the present study of STZ induced diabetic rats showed significant decrease in the total protein and albumin content. This decrease can be attributed to increase protein catabolism and the low serum albumin levels.
of the diabetic animals could be attributed to impaired renal disturbance or impaired liver function for these rats (Iroaganachi et al., 2015). The pre-administration of diabetic rats either with saffron extract or fenugreek showed significant improvement in the total protein and albumin contents when compared with diabetic rats. These results may be due to their antidiabetic role which improved pancreatic β cells and increased serum insulin level which has anabolic effect in protein (Abou El-Soud et al., 2007; Arasteh et al., 2010 and Elgazar et al., 2013).

Several workers have reported that STZ-induced diabetes mellitus and association between specific diabetic complications and disturbances in various tissues, such as diabetic nephropathy and cardiovascular diseases, but only limited data is available on the possible association between diabetic complications and liver function (Arkkila et al., 2001; Zafar et al., 2009). Liver is one of the most important organs that maintain blood glucose levels within normal limits thus enhancement of blood sugar yield to imbalance of oxidation-reduction reactions in hepatocytes (Waer and Helmy, 2012). The present study showed that the aminotransferases (AST and ALT) levels were significantly increased in the liver of STZ-treated animals. Elevated activities of serum aminotransferases are a common sign of liver diseases and are observed more frequently among people with diabetes than in general population (Arkkila et al., 2001).

Oxidation stress plays a key role in the pathogenesis of chronic diseases such as diabetes and diabetic complications (Liu et al., 2012 and Tamaddonfard et al., 2013). In this study there is significant decrease in GSH content and significant increase in MDA content and \( \text{H}_2\text{O}_2 \) level in the liver tissues of diabetic rats. The data of the present study also revealed that pretreatment of diabetic rats either with saffron extract or fenugreek markedly improves the antioxidant status of liver tissue as GSH level significantly increased and MDA level and \( \text{H}_2\text{O}_2 \) level markedly decreased. Assimopoulou et al. (2005); Kanakis et al. (2007) and Chen et al. (2008) and Tamaddonfard et al. (2013) reported that saffron and its active constituent's crocin and crocetin are effective in quenching free radicals and have antioxidant properties. On the other hand, other studies revealed the antioxidant activity of fenugreek seeds and ought this activity partly to the presence of flavonoids and polyphenols (Jayadev et al., 2001 and Annida and Stanely, 2005).

In the present histological and ultrastructural study, STZ-treated animals displayed alteration in the structural integrity of the hepatocytes and their intracellular organelles. The hepatocytes showed marked cytoplasmic vacuolation with pyknotic and karyolitic nuclei. Numerous lipid droplets of variable size, ill-defined rough endoplasmic reticulum and altered mitochondria were observed within the hepatocytes. Moreover, the blood sinusoid appeared broken with detachment of the endothelial cells and infiltration of RBCs between the hepatocytes. Slater (1984) reported that the endoplasmic reticulum is particularly liable to the free radical attack, not only because it is considered as a site of radical production but also due to the enrichment of its membrane with polyunsaturated fatty acids which are susceptible to free radical attack. Such results are in agreement with Damjanov (2000) who stated that, the damage of RER leads to a decrease in protein synthesis as observed in the present biochemical and histochemical findings. In addition, the numerous lipid droplets observed in the cytoplasm of the hepatocytes of the present study may be attributed to impaired protein synthesis as a result of RER damage, and accordingly inhibition of hepatic lipoprotein manufacture which leads to release of the lipids and its accumulation in the liver hepatocytes (Ganong, 1995; Ohno et al., 2000 and Zafar et al., 2009). Also, Powell et al. (2007) and Khalil et al. (2010) declared that diabetes is one of the metabolic causes of steatosis. Moreover, liver sections of STZ-induced diabetic animals illustrated an increase in the amount of collagen fibers around the central vein. Sricharoenvej et al. (2012) and Madhankumar (2016) attributed this finding to the activation of hepatic stellate cells. The accumulation of numerous collagen fibers may lead to more advanced and severe liver injury (Iredale, 2003 and Friedman, 2008). Also, oxidation stress in particular lipid peroxidation induces collagen synthesis (Muriel and Moreno, 2004) as demonstrated in the present study. In diabetes, hyperglycemia causes increased oxygen free radicals from glucose oxidation and protein glycosylation (Tesfamariam, 1994 and Al Shaikh, 2010). Therefore, the dramatic histological and ultrastructural alternation observed in the present study may be attributed to increased oxidation stress.

The present study showed the administration with saffron extract or fenugreek to STZ-treated animals resulted in amelioration in serum and liver ALT and AST activities and improved most of the abnormalities in the liver histological and ultrastructure when compared with diabetic group. The protective effects of saffron extract and fenugreek
may be related to their active constituents; antioxidant properties and its effect in reducing blood glucose level and radical scavenging role (Cam et al., 2003; Mohammad et al., 2011; Elgazar et al., 2013 and Das, 2014).

Histochemically, the current work showed weak PAS positive reaction in liver sections of STZ-treated animals indicating marked decrease in the glycogen content in the hepatocytes and this result was confirmed biochemically, corroborating Welt et al. (2004), Khalaf and Abdel Gabbar (2008) and Onaolapo and Onaolapo (2012) who suggest that in the diabetic individuals, the synthesis and the action of glycogenolytic and glycogenic enzymes would be impaired, which would decrease or prevent the synthesis of glycogen or increase in its breakdown in the liver. However, liver sections of fenugreek + STZ treated animals showed an increase in the polysaccharides content and this may be attributed to the decreased activity of gluconeogenic enzymes (Abd elaziz, 2011).

In conclusion, the present study showed that STZ-induced diabetes impaired liver structure and function and confirmed that saffron extract and fenugreek supplementation were able to attenuate these effects through its hypoglycemic and antioxidant properties. Therefore, the present study provides a scientific rationale for the use of saffron extract and fenugreek supplementation as antidiabetic and hepatoprotective agents.

5 References


Chermahini, H.S.; Abd Majid, F. A.; Sarmidi, M.


