Effect of *Foeniculum vulgare* and Propolis on Liver in Alloxan Diabetic Rats

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**Abstract:** Background: *Foeniculum vulgare* (fennel) and propolis are high efficient antioxidant and antidiabetic agents. Propolis is produced by bees both includes phenolics and flavonoids which are the active ingredients. The aim of work is to study effects of *Foeniculum vulgare* and propolis on liver functions and liver tissues in diabetic rats. Materials and Methods: Eighty rats were divided into eight groups ten rats in each orally administered with propolis and fennel with different concentrations after induction of diabetes by alloxan then sacrificed and blood collected to do liver function tests on serum in addition to hematoxylen eosin liver sections were examined by microscope. Results: propolis and *foeniculum vulgare* decreases ALAT, ASAT and ALP; and increases albumin and total protein. In conclusion: fennel and propolis improve liver functions and liver tissue.

**Key words:** *Foeniculum vulgare* · Propolis · Liver · Alloxan · Diabetic Rats

**INTRODUCTION**

Niti et al. [1] reported that All around the world diabetes mellitus is the most common disease responsible for large number of deaths. Sugar level fluctuations may result to several serious complications like diabetic retinopathy (16.66%), neuropathy (24.66%), kidney problems (21.1%), foot ulcers (5.5%) and cardiovascular problems (23.6%).

Refaat et al. [2] reported that The most important reason for risk increase in diabetic patients is endothelial dysfunction and subclinical low grade systemic inflammation. Ischemia modified albumin (IMA) is produced as a result of serum albumin flowing through ischemic tissues and is a marker of oxidative stress and ischemia, as serum level of modified albumin rise in many diseases accompanied by ischemia.Wild fennels have radical scavenging activity [3]. The methanolic extract of *Foeniculum vulgare* has antioxidant activity through decreasing the malondialdehyde level. *Foeniculum vulgare* has wound healing effect and includes antibacterial peptides [4].

*Foeniculum vulgare* extract showed moderate activity and lipid peroxidation and the pure compounds present in *foeniculum vulgare* have higher antioxidant activity than crude extract [5]. The isolated compounds from *Foeniculum vulgare* are 3-caffeoylquinic acid, 4-caffeoylaquinic acid, ros-marinic acid, 1, 5-dicafeoylquinic acid, eriodictyol-7-rutinoside, kaempferol-3-o-glucoside, kaempferol-3-o-rutinoside and quercetin-3-o-galactoside [6].

*Foeniculum vulgare* Water and ethanol extracts have free radical scavenging, superoxide anion radical scavenging, effective reducing power, metal chelating activity and hydrogen peroxide scavenging activity [7].

Essential oil present in fennel has hepatoprotective activity in studying hepatoprotective activity of fennel on acute CCl, administration was found to be effective in decreasing levels of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and bilirubin. *Foeniculum vulgare* essential oil has hypoglycemic activity against streptozotocin induced diabetic rats [8]. *Foeniculum vulgare* includes natural antioxidants so it inhibits free radicals because it has high content of polyphenols and flavonoids [6, 9].

Propolis has different colors it is a resinous substance collected by *Apis mellifera* bees from plant sources [10]. Propolis is used to protect bees from insects and microorganisms it used as cement to seal cracks or open spaces in the hive, to immunify insect invaders [11].

Propolis has complex chemical composition collected from flower buds, sprouts, trees and other vegetal tissues. During collection bees mix the bees wax and the collected propolis with 13-glicoside enzyme found in their saliva,
hydrolyzing flavonoids glycosides into flavonoids a glycones. Propolis has many biological activities it effects on cartilage, dental pulp and bone regeneration, liver defense, immunological properties and antioxidant activity, antitoxic and immunomodulatory action. The flavonoids in propolis are quercetin, pectolinarigenin and faempferol in addition to acacetin and apigenin [12]. Propolis can remove free radicals from tissues and organs and allow of regeneration of ill organs and tissues by these flavonoids [11].

Propolis modulated antioxidant enzymes and decrease lipid peroxidation processes in plasma, lungs, liver and brain of mice. Phenolic compounds present in propolis have hepatoprotective function [13 , 14]. Propolis ameliorates hepatotoxic effects of alcohol liver injury in mice [15]and also carbon tetrachloride induced liver damage in rats in addition to paracetamol induced liver damage in mice . The aim of work is to study effects of Foeniculum vulgare and propolis on liver functions and liver tissues in diabetic rats.

**MATERIALS AND METHODS**

All animal studies were transferred in accordance with criteria of the investigations and Ethics committee of the community laws governing the use of experimental animals.

**Experimental Animals:** The male albino rats (n=80) at average weight of (190 ± 10) at the beginning of the experiment. Obtained from the Egyptian holding company for biological product and Vaccines were used as experimental animals. The rats were transferred to the experimental environment one week prior to the initial of the experiment so as to ensure their environmental adaptation. The rats were transferred to the animal house in Zoology Department, Faculty of Science, Al-Azhar University; the rats were housed in regular designed cage and maintained in condition of good ventilation, normal temperature, and humidity range. Five rats were placed into each cage. Feed and water were provided *ad libitum* to the animals.

**Induction of Diabetes:** The animals were fasted overnight. Diabetes was induced by single intraperitoneal (i.p) injection of alloxan monohydrate (148mg/kg) in sterile normal saline (0.9%). The diabetic state was determined 72 hours after alloxan administration through the tail, using the one touch ultra-glucometer (Glucodocotor). Weekly record of blood glucose level was taken afterwards.

**Preparation:** Propolis bulks were cut into small pieces and mixed with deionized water and shacked at 95 c° for 2 hours according to therapeutic dose. Then cooled to room temperature and centrifuged at 1500 r.p.m for 5 minutes to obtain the supernatant [16]. This occurs in genetic engineering center Al-azhar University

**Representative Chemical Components in Propolis.**

**Foeniculum Vulgare:** Foeniculum vulgare seeds were collected from the local market in Egypt and identified by its morphological and microscopically characters.

**Preparation:** *Foeniculum vulgare* extracted by distilled water using soxhlet apparatus according to the Association of Official Analytical Chemists in physiology lab faculty of science al Azhar University.

**Experimental Design:** The patch of animals was distributed into eight groups as the following: Group 1 Control (C): negative control of normal rats, (n=10 Rats) rats of this group were neither treated nor injected by alloxan.

**Group 2 Diabetes Mellitus (DM):** positive control of alloxan injected rats, (n=10 Rats) rats of this group were injected by alloxan 148 mg/kg intraperitoneal.

**Group 3 Diabetes Mellitus+ 200 propolis (DM+200Pro):** Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 200 mg/ kg of propolis.

**Group 4 Diabetes Mellitus+ 400 propolis (DM+400Pro):** Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 400 mg/ kg of propolis.

**Group 5 Diabetes Mellitus+ 200 Foeniculum vulgare (DM+200FV):** Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 200 mg/ kg of *Foeniculum vulgare*.

**Group 6 Diabetes Mellitus+ 400 Foeniculum vulgare (DM+400FV):** Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with 400 mg/ kg of *Foeniculum vulgare*.

**Group 7 Diabetes Mellitus+200 propolis + 200 Foeniculum vulgare (DM+200Pro+200FV):** Rats of this
group were injected with alloxan 148 mg/kg intraperitoneal and treated with (200 mg/ kg of *Foeniculum vulgare*+ 200 mg/ kg of propolis).

**Group 8 Diabetes Mellitus+400 propolis + 400 *Foeniculum vulgare* (DM+400Pro+400FV):** Rats of this group were injected with alloxan 148 mg/kg intraperitoneal and treated with (400 mg/ kg of *Foeniculum vulgare*+ 400 mg/ kg of propolis).

The duration of treatment is for one month.

**Preparation of Tissue Homogenate:** After the animals have been sacrificed, livers were quickly excised, placed in chilled phosphate buffer solution (pH 7.4) at 4°C, One gram of liver is then taken to prepare 10% tissue homogenate using the same buffer solution utilizing tissue homogenizer, the homogenates were centrifuged in a cooling centrifuge with a temperature adjusted to + 4°C, at 4,000 p.m. for 10 min. The supernatants obtained were transferred into Eppendorf tubes, and preserved at-80°C in a deep freezer until used for analysis of antioxidant biomarker.

**Biochemical Parameters**

**Determination of Serum Alanine Aminotransferase (ALAT) Enzyme Activity (U/L):** Serum ALAT was determined according to the method of Bergmeyer, Horder& Rej [17] using kit from Elitech diagnostic Co. France. The kinetic determination of the alanine aminotransferase.

**Determination of Serum Aspartate Aminotransferase (ASAT) Enzyme Activity (U/L):** Serum ASAT was determined according to the method of Bergmeyer et al. [17] using kit from Elitech diagnostic Co. France. The kinetic determination of the alanine aminotransferase.

**Estimation of Serum Alkaline Phosphatase (ALP) Enzyme Activity (U/L):** Serum ALP was determined according to the method described by Bergmeyer et al. [18] using kit from Elitech diagnostic Co. France.

**Determination of Serum Total Protein Level (g/dl):** Serum total protein was determined according to the method described by Gornal et al.[19]using kit from Elitech diagnostic Co. France. 5- Determination of serum albumin level (g/dl):

Serum albumin was determined according to the method of Doumas, Watson& Biggs [20] using kit from Elitech diagnostic Co. France.

**Evaluation of Albumin Globulin (A/G) Ratio:** The A/G ratio is calculated using the formula, A/G ratio= (Albumin Level)/(Total Protein - Albumin).

**Determination of Total Serum Bilirubin Level (mg/dl):** Serum total bilirubin was determined according to the method of Dutt, Murphy and Thompson [21] using kit from Elitech diagnostic Co. France.

**RESULTS**

ASAT shows a significant decrease (p<0.05) in Control (Non Diabetic), Diabetic + treated with Propolis 200mg, Diabetic + treated with *F. vulgare* 200mg, Diabetic + treated with *F. vulgare* 400mg, Diabetic + treated with (*F. vulgare* 200mg+Propolis 200mg), when compared with Positive control (Diabetic) as shown in table (1) Where Mean and S.E was (247±20.81) in Positive control (Diabetic) and were (104.6±6.77) and (163.67±13.74) in Control (Non Diabetic) and Diabetic + treated with *F. vulgare* 200mg respectively.

ALAT shows a significant decrease (p<0.05) in Diabetic + Treated with Propolis 200mg, Diabetic + treated with Propolis 400mg, Diabetic + treated with *F. vulgare* 200mg, Diabetic + treated with (*F. vulgare* 200mg+Propolis 200mg), Diabetic + treated with (*F. vulgare* 400mg+Propolis 400mg) when compared with Positive control (Diabetic) as shown in table (1) Where Mean and S.E was (115.67±7.31) in Positive control (Diabetic) and were (61.33±20.43) and (25.33±7.35) in Diabetic + treated with *F. vulgare* 200mg and Diabetic + treated with (*F. vulgare* 400mg+Propolis 400mg) respectively.

ALP shows a significant decrease (p<0.05) in Control (Non Diabetic), Diabetic + treated with Propolis 200mg, Diabetic + treated with Propolis 400mg, Diabetic + treated with *F. vulgare* 200mg, Diabetic + treated with (*F. vulgare* 200mg+Propolis 200mg), Diabetic + treated with (*F. vulgare* 400mg+Propolis 400mg) when compared with Positive control (Diabetic) as shown in table (1) Where Mean and S.E was (327±0.16) in Positive control (Diabetic) and were (121.8±0.05) and (175.25±0.18) in Control (Non Diabetic) and Diabetic + treated with *F. vulgare* 400mg respectively.
### Table 1: The means ± SE of ASAT, ALAT, ALP and Bilirubin concentration in induced-diabetic rats with alloxan and treated with Propolis and *F. vulgare* doses for one month

<table>
<thead>
<tr>
<th>Group</th>
<th>ASAT (U/L)</th>
<th>ALAT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-Diabetic)</td>
<td>Means: SE 104.6±6.77a</td>
<td>88±19.36a.c</td>
<td>121.8±0.05a</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>Positive control (Diabetic)</td>
<td>Means: SE 247±20.81c</td>
<td>115.67±7.31c</td>
<td>327±0.16b</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>Diabetic + treated with Propolis 200mg</td>
<td>Means: SE 130±19.02a,b</td>
<td>41.75±6.71b</td>
<td>135.25±0.04a</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Diabetic + treated with Propolis 400mg</td>
<td>Means: SE 194.33±42.75b,c</td>
<td>52±14.29a,b</td>
<td>172.33±0.13a</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>Diabetic + treated with <em>F. vulgare</em> 200mg</td>
<td>Means: SE 163.67±13.74a,b</td>
<td>61.33±20.43a,b</td>
<td>319±0.07b</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Diabetic + treated with <em>F. vulgare</em> 400mg</td>
<td>Means: SE 172±21.69b</td>
<td>52.5±6.65a,b</td>
<td>175.25±0.18a</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Diabetic + treated with (<em>F. vulgare</em> 200mg+Propolis 200mg)</td>
<td>Means: SE 160±14.43a,b</td>
<td>43.33±4.7b</td>
<td>141±0.01a</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>Diabetic + treated with(<em>F. vulgare</em> 400mg+ Propolis 400mg)</td>
<td>Means: SE 200.33±46.03b,c</td>
<td>25.33±7.35b</td>
<td>135.67±0.08a</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>F ratio</td>
<td>3.6</td>
<td>4.23</td>
<td>3.26</td>
<td>1.81</td>
</tr>
<tr>
<td>Probability</td>
<td>**</td>
<td>**</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Mean with dissimilar superscript letter are significantly different at (P<0.05). N.S = non-significant (p<0.001)=**

### Table 2: The means ± SE of total protein, albumin, globulin and Alb/glb ratio concentration in induced-diabetic rats with alloxan and treated with Propolis and *F. vulgare* doses for one month

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin</th>
<th>Alb/glb ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-Diabetic)</td>
<td>Means: SE 4.98±0.19a</td>
<td>2.66±0.08a</td>
<td>2.32±0.21</td>
<td>1.18±0.1</td>
</tr>
<tr>
<td>Positive control (Diabetic)</td>
<td>Means: SE 1.93±0.17c</td>
<td>0.76±0.08c</td>
<td>1.16±0.18</td>
<td>0.75±0.16</td>
</tr>
<tr>
<td>Diabetic + treated with Propolis 200mg</td>
<td>Means: SE 3.85±0.68a,b</td>
<td>1.92±0.38a,b,d</td>
<td>1.92±0.3</td>
<td>0.97±0.1</td>
</tr>
<tr>
<td>Diabetic + treated with Propolis 400mg</td>
<td>Means: SE 2.9±0.26b,c</td>
<td>1.6±0.26b,c,d</td>
<td>1.3±0.2</td>
<td>1.27±0.27</td>
</tr>
<tr>
<td>Diabetic + treated with <em>F. vulgare</em> 200mg</td>
<td>Means: SE 3.7±0.77a,b</td>
<td>1.9±0.57a,b,d</td>
<td>1.8±0.26</td>
<td>1.02±0.25</td>
</tr>
<tr>
<td>Diabetic + treated with <em>F. vulgare</em> 400mg</td>
<td>Means: SE 4±0.7a,b</td>
<td>2.25±0.35a,b</td>
<td>1.85±0.39</td>
<td>1.28±0.15</td>
</tr>
<tr>
<td>Diabetic + treated with (<em>F. vulgare</em> 200mg+Propolis 200mg)</td>
<td>Means: SE 3.3±0.28b,c</td>
<td>1.83±0.14a,b,d</td>
<td>1.5±0.17</td>
<td>1.24±0.12</td>
</tr>
<tr>
<td>Diabetic + treated with(<em>F. vulgare</em> 400mg+ Propolis 400mg)</td>
<td>Means: SE 2.73±0.94b,c</td>
<td>1.06±0.37c,d</td>
<td>1.66±0.59</td>
<td>0.69±0.16</td>
</tr>
<tr>
<td>F ratio</td>
<td>2.88</td>
<td>3.93</td>
<td>1.47</td>
<td>2.01</td>
</tr>
<tr>
<td>Probability</td>
<td>*</td>
<td>**</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Mean with dissimilar superscript letter are significantly different at (P<0.05), N.S = non-significant (p<0.01)=**(p<0.05)=**

(A): Enlarged section of normal liver structure in adult albino rat showing hepatic polygonal cells (HC) with round nucleus, hepatocytes arranged in cords (H), normal central vein (CV) and blood sinusoids (BS). (H&E x400).
Enlarged section in liver of adult diabetic albino rat showed dilated blood vessels (DBV), irregular hepatic cords, multi necrotic cells (N), pyknotic nuclei(P), karyolysis (K), karyorrhexis (Kr) and more distribution of Kupffer cells (KC). (H & E X400).

Enlarged section in liver of adult diabetic albino rat treated with 200 mg of *Foeniculum vulgare* + 200 mg of propolis showed hepatocytes moderately arranged in cords (H) represented by pyknotic nuclei (P) and necrotic cells (N). (H & E X400).

Enlarged section in liver of adult diabetic albino rat treated with 400 mg of *Foeniculum vulgare* + 400 mg of propolis showed normal structure hepatocytes like as control, normal central vein (CV) with normal blood sinusoids (BS), some pyknotic nuclei (P) and karyolysis (K). (H & E X400).

Total protein shows a significant increase (p<0.05) in Control (Non Diabetic), Diabetic + treated with Propolis 200mg and Diabetic + treated with *F. vulgare* 400mg when compared with Positive control (Diabetic) as shown in table (2) Where Mean and S.E was (1.93±0.17) in Positive control (Diabetic) and were (4.98±0.19) and (3.8±0.68) in Control (Non Diabetic) and Diabetic + treated with Propolis 200mg respectively.

Albumin shows a significant increase (p<0.05) in Control (Non Diabetic), Diabetic + treated with Propolis 200mg, Diabetic + treated with *F. vulgare* 200mg, Diabetic + treated with *F. vulgare* 400mg and Diabetic + treated with (F. vulgare 200mg+Propolis 200mg) when compared with Positive control (Diabetic) as shown in table (2) Where Mean and S.E was (0.76±0.08) in Positive control (Diabetic) and were (2.66±0.08) and (1.83±0.14) in Control (Non Diabetic) and Diabetic + treated with (F. vulgare 200mg+Propolis 200mg) respectively.

Serum activities of ALAT and ASAT are indicators of hepatotoxicity and are also used as biomarkers for early acute hepatic damage [28]. Increased levels of these enzymes indicate cellular infiltration and disturbance in the functioning of the hepatic cell membranes. Serum levels of ASAT and ALAT were significantly elevated in the diabetic untreated group when compared to the normal untreated group, signifying hepatotoxicity and acute hepatic damage [27].

Regarding to the results of ASAT, it shows a significant decrease (p<0.05) in Control (Non Diabetic), Diabetic + treated with Proposes 200mg, Diabetic + treated with *F. vulgare* 200mg, Diabetic + treated with *F. vulgare* 400mg, Diabetic + treated with (F. vulgare 200mg+Propolis 200mg), when compared with Positive control (Diabetic) this is may be due to elevated transaminase activities. This results agreement with Babatunde et al. [30].

Serum activities of ALAT and ASAT are indicators of hepatotoxicity and are also used as biomarkers for early acute hepatic damage [25]. Increased levels of these enzymes indicate cellular infiltration and disturbance in the functioning of the hepatic cell membranes [26]. Serum levels of ASAT and ALAT were significantly elevated in the diabetic rats when compared to the normal rats, signifying hepatotoxicity and acute hepatic damage [27].

Serum activities of ALAT and ASAT are indicators of hepatotoxicity and are also used as biomarkers for early acute hepatic damage [28]. Increased levels of these enzymes indicate cellular infiltration and disturbance in the functioning of the hepatic cell membranes [26]. Serum levels of ASAT and ALAT were significantly elevated in the diabetic rats when compared to the normal rats, signifying hepatotoxicity and acute hepatic damage [27].
Regarding to the results of total protein, it shows a significant increase (p<0.05) in Control (Non Diabetic), Diabetic+ treated with Propolis 200mg and Diabetic + treated with F. vulgare 400mg when compared with Positive control (Diabetic) this is may be due to free radicals that caused liver cells damage and this leading to decrease in protein synthesis in diabetic group. This results Agreements with Juan Carlos Romero and Strick [31]. Kumar et al. [32], Oršoliæ et al. [33] and Hassan [34].

Regarding to the results of albumin, it shows a significant increase (p<0.05) in Control (Non Diabetic), Diabetic + treated with Propolis 200mg, Diabetic + treated with F. vulgare 200mg, Diabetic + treated with F. vulgare 400mg and Diabetic + treated with (F. vulgare 200mg + Propolis 200mg) when compared with Positive control (Diabetic) may be due to free radical that caused liver cells damage and this leads to decrease in albumin synthesis in diabetic group.

Thakran et al. [35] found that in liver and kidney of alloxan-induced diabetic rats histopathological studies showed liver degenerative and early nephropathic changes.

The present study suggested that propolis and Foeniculum vulgare have ameliorative effects on the liver and kidney of alloxan-induced diabetic rats as in Figure A, C and D.

The liver is an organ of prime importance and plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bio regulation of fats, carbohydrates, amino acids and proteins. A number of pharmacological and chemical agents act as hepatotoxins and produce a variety of liver ailments [36]. The present study demonstrated that histopathological observation of the liver sections of alloxan-induced diabetic rats showed dilated blood vessels, irregular hepatic cords, multi necrotic cells, pyknotic nuclei, karyolysis, karyorrhexis and more distribution of Kupffer cells. The most prominent change was the presence of smaller or larger vacuole-like spaces in the hepatocyte cytoplasm. This was probably a result of increase quantity of fat within cells due to impaired metabolism of fatty acids as in figure (B). Another characteristic of damaged cells indicating necrosis. This results are in agreement with Ragavan and Krishnakumari [37] and Oršolie et al. [33] which found periportal Vacuolization with focal necrosis in the rat liver treated with single intraperitoneal injections of alloxan in a dose of 120 mg /kg body weight, while Khalil et al. [38] with a higher dose of 150 mg/ kg found disorganization of the hepatic cords and vacuolized hepatocytes with picnotic nuclei. Patel et al. [39] stated that Antioxidants have been reported to prevent oxidative damage caused by free radicals.

CONCLUSION

Propolis and Foeniculum Vulgare:

- Decrease alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase
- Increases albumin and total protein
- Improve liver functions and liver tissue

Recommendation: We recommend with using fennel and propolis to improve liver functions and liver tissue in diabetic rats.

REFERENCES


