ملخص البحث

التأثيرات الخاضعة لسكر الدم والمضادة للالتهابات لنبات البرسيم الحجازي في الفئران

أجريت هذه الدراسة لتحقيق الدور الوظيفي لبذور وبراعم البرسيم في السيطرة على مستويات السكر في الدم وعلاج الالتهابات في الفئران المصاب بهرم السكر. تم تقسيم (ن = 56) من ذكور الفئران البيضاء إلى مجموعتين رئيسيتين. المجموعة الأولى (ن = 8) تغذت على النظام الغذائي الأساسي خلال الفترة التجريبية كمجموعة ضابطة سالبة، والمجموعة الثانية (ن = 50) تم حقنها بمادة الألوكسان (120 ملغ/كجم من وزن الجسم) لإحداث مرض السكر وتم تقسيمها إلى خمسة مجموعات فرعية، ثم تم تغذيتهم على نظام غذائي مدعم بمستويين من بذور وبراعم البرسيم (5% و10%) لمدة أربعة أسابيع. في نهاية التجربة تم حساب نسبة الجلوكوز والأنسولين وإرنزيمات الكبد وجلوكوجين الكبد في سيرم الدم. كما تم حساب الهيموجلوبين السكري. وتم إصابة الفئران بالالتهابات عن طريق حقنها بالفوماليتين في مخالبها. حدث انخفاض في نسبة الجلوكوز وإرنزيمات الكبد والبيرويا والكيزاتين في سيرم الدم وأيضاً الهيموجلوبين السكري بشكل ملحوظ في المجموعات التي تغذت على الغذاء الأساسي المدعم بذور وبراعم البرسيم. كما أشارت النتائج إلى حدوث زيادة كبيرة في نسبة الإنسولين والجلوكوجين في السيرم بالمقارنة بالمجموعة المصابية بالسكر ولم يتم علاجها. أظهرت التحليل النسيجي لللكن والكلي تحت شكل خلايا الكبد والكليات الكلوية مع العلاج ببذور البرسيم وبراعمه. وكانت براهم البرسيم أكثر فاعلية من البذور في علاج السكر والالتهابات في الفئران المصابية. ولذلك، ينبغي تشجيع برامج التثقيف الغذائي لمعرفة الجمهور بقيمة بذور البرسيم وبراعمه ودوره في السيطرة على إرتفاع السكر في الدم وعلاج الالتهابات. وتشجيع اجراء البحوث في هذا المجال.

كلمات البحث:
بذور البرسيم الحجازي - براعم البرسيم الحجازي - النوع الثاني من مرض السكر - الالتهابات - الفئران
Abstract

The Hypoglycemic and Anti-inflammatory Effects of Alfalfa (*Medicago sativa L.*) Plant in Rats

This study was carried out to investigate the functional role of alfalfa seeds and its sprouts in controlling diabetes mellitus and treating inflammation in rats. Male albino rats (n= 56) were divided into two main groups. The first group (n=6) was fed on basal diet during the experimental period as -ve control, the second group (n=50) was injected with alloxan (120 mg/kg) for diabetes induction and was divided into five subgroups then fed on diets with alfalfa seeds and sprouts at two levels of intake (5% and 10%) for four weeks. Serum glucose, insulin, liver function enzymes serum liver glycogen and glycated hemoglobin were determined. Feeding of diabetic rats on diets supplemented with alfalfa seeds or sprouts decreased blood glucose levels significantly. Results showed that liver function enzymes and glycated hemoglobin levels were significantly increased. Also, insulin as well as glycogen level were increased significantly compared to diabetic untreated group. Alfalfa seeds and its sprouts helped in healing inflammation compared with the untreated diabetic ones. The histological studies showed improved in hepatocytes and renal tubules with alfalfa seeds and sprouts supplementation. Alfalfa sprouts were more effective in controlling diabetes mellitus and inflammation than alfalfa seeds. Therefore, nutrition education programs should be encouraged to inform the public about the hypoglycemic and anti-inflammatory effects of alfalfa seeds and sprouts. Further research is required.

**Keywords:** alfalfa seeds-alfalfa sprouts-diabetes mellitus- inflammation- rats.


WHO (2013): The burden of diabetes is increasing globally, particularly in developing countries.


Reference


Figure (1) Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on the Histology of Liver Tissues of Rats.
(A) Normal control diet       (B) positive control groups
(C) Basal diet supplemented with 5% alfalfa seeds powder.
(D) Basal diet supplemented with 10% alfalfa seeds powder.
(E) Basal diet supplemented with 5% alfalfa sprouts powder.
(F) Basal diet supplemented with 10% alfalfa sprouts powder.
(H and EX 200)
Table (6): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on Serum Liver Enzymes Concentration of Rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT u/l</th>
<th>AST u/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>26.58 ± 1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>48.43 ± 1.39&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (+)</td>
<td>51.87 ± 2.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.50 ± 5.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Seeds</td>
<td>44.77 ± 1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.70 ± 2.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Seeds</td>
<td>40.17 ± 1.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.92 ± 3.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Sprouts</td>
<td>32.28 ± 2.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>64.58 ± 3.49&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Sprouts</td>
<td>28.78 ± 1.48&lt;sup&gt;e&lt;/sup&gt;</td>
<td>52.12 ± 2.21&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values are expressed as means ± SD.

Means with different superscript letters in the column are significantly different at P ≤ 0.05.

Control (-) = Normal rats fed basal diet. Control (+) = Diabetic rats fed basal diet.

ALT : Alanine Aminotransferase. AST : Aspartate Aminotransferase

Table (7): Effect of Diet Supplementation with Alfalfa Seeds and Their Sprouts on the Paw's Thickness of Rats after Inflammation Induction.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time Zero Time</th>
<th>3 hours ± %</th>
<th>6 hours ± %</th>
<th>First day ± %</th>
<th>Second day ± %</th>
<th>Fourth day ± %</th>
<th>Sixth day ± %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+)</td>
<td>1.50 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.50 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>---</td>
<td>5.50 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.83 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.67 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Seeds</td>
<td>1.53 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.81 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13</td>
<td>4.83 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.08 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Seeds</td>
<td>1.57 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.85 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
<td>4.67 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.17 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Sprouts</td>
<td>1.50 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.17 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>4.33 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.17 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.83 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Sprouts</td>
<td>1.63 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33 ± 0.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39</td>
<td>3.83 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 0.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values are expressed as means ± SD.

Means with different superscript letters in the column are significantly different at P ≤ 0.05. Control (+) = Diabetic rats fed basal diet

% : Inhibition
Table (4): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on Feed Intake (FI), Body Weight Gain (BWG) and Feed Efficiency Ratio (FER) of Rats.

<table>
<thead>
<tr>
<th>Variables Groups</th>
<th>Feed Intake g/day</th>
<th>BWG g</th>
<th>FER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>15.12± 1.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>40.17± 2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.095 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (+)</td>
<td>20.17± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.00± 2.61&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.030 ± 0.005&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Seeds</td>
<td>17.75± 0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.33± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.043± 0.004&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Seeds</td>
<td>17.00± 0.63&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>23.67± 2.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.050± 0.006&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Sprouts</td>
<td>16.25± 1.08&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>26.42± 1.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.058± 0.004&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Sprouts</td>
<td>15.33± 0.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.00± 1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.065± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values are expressed as means ± SD. Means with different superscript letters in the column are significantly different at P≤ 0.05.
Control (-) = Normal rats fed basal diet. Control (+) = Diabetic rats fed basal diet.

Table (5): Effect of Diet Supplementation with Alfalfa Seeds and Its Sprouts on some glycemic parameters of Rats.

<table>
<thead>
<tr>
<th>Variables Groups</th>
<th>Insulin µ/ml</th>
<th>Glucose mg/dl</th>
<th>Glycogen ng/L</th>
<th>HbA&lt;sub&gt;1c&lt;/sub&gt;%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>13.94± 1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.17± 9.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>175.62± 2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.53 ± 0.94&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (+)</td>
<td>7.24± 0.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>235.83± 22.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.78± 4.91&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.58 ± 1.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Seeds</td>
<td>11.23± 1.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>151.00± 12.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110.00± 5.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.75 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Seeds</td>
<td>11.66± 1.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>149.33± 15.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117.85± 9.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.65 ± 1.04&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Alfalfa Sprouts</td>
<td>11.42± 1.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>140.00± 9.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120.02± 6.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.90± 1.64&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Alfalfa Sprouts</td>
<td>12.65± 1.41&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>121.50± 12.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>154.51± 2.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.98± 0.83&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values are expressed as means ± SD. HbA<sub>1c</sub>: Glycated hemoglobin. Means with different superscript letters in the column are significantly different at P≤ 0.05.
Control (-) = Normal rats fed basal diet. Control (+) = Diabetic rats fed basal diet.
Table (1): Chemical Composition of Alfalfa Seeds and Its Sprouts (Dry Weight %).

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Alfalfa Seeds</th>
<th>Alfalfa Sprouts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Protein</td>
<td>40.274</td>
<td>41.622</td>
</tr>
<tr>
<td>Fat</td>
<td>19.158</td>
<td>13.215</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>29.823</td>
<td>31.352</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>15.450</td>
<td>15.180</td>
</tr>
<tr>
<td>Moisture</td>
<td>07.245</td>
<td>10.273</td>
</tr>
<tr>
<td>Ash</td>
<td>03.498</td>
<td>03.536</td>
</tr>
</tbody>
</table>

Table (2): Active Flavonoid Compounds Content in Alfalfa seeds and Its sprouts (ppm).

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Alfalfa Seeds (ppm)</th>
<th>Alfalfa Sprouts (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Romarinic</td>
<td>5.38</td>
<td>10.27</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.39</td>
<td>36.79</td>
</tr>
<tr>
<td>Narenginin</td>
<td>0.15</td>
<td>8.53</td>
</tr>
<tr>
<td>Kampferol</td>
<td>0.36</td>
<td>3.44</td>
</tr>
<tr>
<td>Luteolin</td>
<td>33.07</td>
<td>57.86</td>
</tr>
<tr>
<td>Rutin</td>
<td>78.43</td>
<td>20.70</td>
</tr>
</tbody>
</table>

Table (3): Active Phenolic Compounds Content in Alfalfa Seeds and Its Sprouts (ppm).

<table>
<thead>
<tr>
<th>Phenolic Compounds</th>
<th>Alfalfa seeds (ppm)</th>
<th>Alfalfa sprouts (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringic</td>
<td>1582.19</td>
<td>45351.08</td>
</tr>
<tr>
<td>Gallic</td>
<td>10.80</td>
<td>9.40</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>33.42</td>
<td>233.06</td>
</tr>
<tr>
<td>Catechein</td>
<td>107.32</td>
<td>839.47</td>
</tr>
<tr>
<td>Chlorogenic</td>
<td>82.07</td>
<td>471.75</td>
</tr>
<tr>
<td>Epicatechen</td>
<td>10.72</td>
<td>59.88</td>
</tr>
<tr>
<td>Caffeic</td>
<td>1.24</td>
<td>16.82</td>
</tr>
<tr>
<td>P-Coumaric</td>
<td>7.14</td>
<td>5.24</td>
</tr>
<tr>
<td>Ferulic</td>
<td>7.05</td>
<td>39.97</td>
</tr>
<tr>
<td>Iso-ferulic</td>
<td>4.32</td>
<td>13.26</td>
</tr>
<tr>
<td>Ellagic</td>
<td>59.06</td>
<td>10.05</td>
</tr>
</tbody>
</table>
(Navarro et al., 1993) which gives an indication on the hepatotoxic effect of alloxan. The present results showed that treatment of diabetic rats by alfalfa seeds and sprouts significantly lowered ALT and AST concentrations in diabetic animals compared with untreated diabetic ones. The ability of alfalfa seeds (15% of diet) to cause significant reduction in serum ALT and AST were confirmed by Al sadek, (2013) in hyperlipedemic rats. These improvements may be related to the effect of some constituents such as phytochemicals in alfalfa seeds and sprouts which function as antioxidant and in turn may improve the function of hepatocytes. The sprouts however showed more effective decrease in ALT and AST may be due to its high content in flavonoids such as quercetin and phenolic compounds such as ferulic acid compared to alfalfa seeds. each compound had significant effect in decreasing ALT and AST (El Gohary et al., 2013, AbouZaid et al., 2014).

Formalin-induced paw edema is also one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthritis (Joseph et al., 2009). Different phenolic acid compounds, such as gallic acid, vanillic acid, caffeic acid, coumaric acid, and epicatechin as antioxidants, have several biological activities to reduce lipid peroxyl radicals, inhibit lipid peroxidation, and protect our cells against oxidative damage (Birošová et al., 2007, Penarrieta et al., 2008). Alfalfa seeds and its sprouts helped in inflammation healing compared with +ve control. This improvement may be due to the presence of active phenolic compounds such as ferulic acid and flavonoid compounds such as naringenin and quercetin. The alfalfa sprouts however showed the highest improvement in inflammation healing compared with +ve control. This improvement may be due to that it contains a larger proportion of the active compounds. Flavonoids have been reported to have high anti-inflammatory properties (Igwe and Okwu, 2013). The present results was in the same line with (Hong et al., 2009b) that component of alfalfa sprout ethyl acetate extract exerted anti-inflammatory effect. Also, Ramos et al., (2012) reported that the oral administration of the red clover dry extract inhibition of rat paw edema. Several studies revealed that ferulic acid (Srinivasan et al., 2007), catechins (Elseweidy et al., 2008), naringenin (Annadurai et al., 2013) and p-coumaric acid (Yoon et al., 2013) have antioxidant and anti-inflammatory effects.

Results of the histological examination of the liver of hyperglycemic rats fed on diet supplemented with alfalfa seeds and its sprouts revealed a dose dependent reduction of degenerative changes caused by alloxan. The histological findings are affirmed by the observed improvements in AST and ALT. The curative effect of alfalfa seeds and their sprouts may be related to the presence of certain constituents such as flavonoids and phenolic compounds.
Another possible mechanism by which alfalfa seeds and sprouts supplementation may bring their hypoglycemic action is attributed to their fiber content. In this context, Moharib and El-Batran, (2008), Robert et al., (2012) reported that fiber supplementation for type 2 diabetes mellitus can reduce fasting blood glucose and HbA1c in rats and human respectively. Possible mechanisms for metabolic improvements with dietary fiber include delay of glucose absorption from intestine, increase in pancreatic extraction of insulin and increased insulin sensitivity at the cellular level (Vuksan et al., 2009).

Liver glycogen content was significantly reduced in alloxan induced diabetic rats as compared to -ve control group. Daisy and Rajathi, (2009) reported that the decrease in glycogen content of liver and skeletal muscle observed in diabetic rats is probably due to lack of insulin in the diabetic state. In the present study, alfalfa seeds and sprouts supplementation restored the depressed glycogen levels in diabetic rats compared to untreated diabetic group possibly by increasing the level of insulin as a result of the presences of phytochemicals which may enhance insulin secretion or have insulin like effects. Alfalfa sprouts however, showed the highest improvement in liver glycogen content may be due to that it contain a larger proportion of the active compounds. Malini et al., (2011) stated that oral administration of ellagic acid to diabetic rats significantly increased liver and muscle glycogen content by stimulating the remnant β-cells to release insulin. In accordance with the present results, Kumar et al., (2005) reported that in the diabetic group, HbA1C level increased significantly suggesting glycosylation of hemoglobin in the presence of hyperglycaemia. In the alfalfa seeds and sprouts supplemented diabetic groups, marked decreases in HbA1C concentration were observed when compared to that of diabetic untreated animals indicating decrease in blood glucose level and recovery to hemoglobin. The sprouts however, showed more effective decrease, confirming its anti-diabetic potency may be due to that alfalfa sprouts contain active compound such as syringic and chlorogenic acid in higher amounts than in alfalfa seeds. This effect seems to be a result of the noticed reduction of blood glucose level and the improvement in insulin secretion. Active constituents found in alfalfa seeds and sprouts may play a role in improving HbA1C concentration of diabetic treated rats. Several studies concluded that administration of ellagic acid (Malini et al., 2011), chlorogenic acid (Nishi et al., 2013), syringic acid (Srinivasan et al., 2014) and p-coumaric acid (Amalan and vijayakumar, 2015) significantly reduced levels of HbA1c in diabetic treated rats.

The activities of liver enzymes (AST and ALT) increased significantly in diabetic rats were in agreement with previous findings reported by Hamden et al., (2008). The increased activities of serum AST and ALT may be due to the leakage of these enzymes from the liver cytosol into the blood stream.
alfalfa seeds and sprouts treated diabetic groups, showed significant improvement in body weight gain and moderation in food intake compared to positive control group. This may be related to the protein content of alfalfa seeds and sprouts which may has protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis and may also be due to proper glycemic control. In addition, the presence of some active phenolic compounds such as p-coumaric, protocatechuic acid and ferulic acid in alfalfa seeds and sprouts may have indirect effect on body weight gain and feed intake through their effects on controlling serum glucose and improving serum insulin. Compared to diabetic rats fed on diet supplemented with alfalfa seeds, diabetic rats fed on diet supplemented with alfalfa sprouts showed better improvements in body weight gain and feed intake perhaps due to that alfalfa sprouts contain higher levels of many active compounds than alfalfa seeds. Several studies reported that protocatechuic acid (Harini and Pugalendi, 2010), ferulic acid (Choi et al., 2011), p-coumaric acid (Amalan and vijayakumar, 2015) had the ability to increase body weight of the diabetic rats.

The results of the present study indicated that alfalfa seeds and its sprouts significantly reduced fasting serum glucose level and significantly improve serum insulin concentration in diabetic rats. In accordance with the present results, Winiarska et al., (2007) found that Medicago sativa L. extract modulate post-challenge carbohydrate metabolism in type 2 diabetes animal model by enhancing insulin secretion. On the same line, Baxiet et al., (2010) reported that Medicago sativa L. leaf extract was found to be very effective in lowering diabetic hyperglycaemia in four weeks by about 42%, while Qiu et al., (2012) reported that red clover extract treatment attenuated hyperglycemia in type 2 diabetic animals.

The hypoglycemic effects alfalfa seeds and sprouts may be attributed to the constituents found in the two forms alfalfa plant. From the phytochemical analysis, it was found that both alfalfa seeds and sprouts, in lower extent for most, contain flavonoids such as (quercetin, naringenin and rutin) and phenolic compounds(such as syringic, catechein, chlorogenic, p-coumaric, ferulic, gallic, ellagic, protocatechuic, hesperidin, and caffeic). Compared to diabetic rats fed on diet supplemented with alfalfa seeds, and sprouts showed better improvements in serum insulin and glucose may be due to that alfalfa sprouts contain active compound such as syringic, catechein, chlorogenic and protocatechuic higher amounts than in alfalfa seeds. On the light of this, several studies concluded that quercetin (Narenjkar et al., 2011), ellagic acid and rutin (Oyedemi et al., 2011) and naringenin (Tsai et al., 2012) caused significant decrease in the serum glucose.

Phenolic compounds have been found to be beneficial in controlling diabetes and many other diseases as evident from earlier studies (Vasco et al., 2008). The benefits towards many of these conditions come in part through the antioxidant characteristic of phenols (Zinca and Vizireanu, 2013). Both supplements showed presence of phenolic compounds. Several studies revealed the anti hyper glycemic effects of syringic acid (Srinivasan et al., 2014)
mm, while rats fed on diet supplemented with 5% alfalfa sprouts exhibit reduction paw’s thickness by 24.18%, while rats fed on diet supplemented with 10% alfalfa sprouts exhibit reduction paw’s thickness by 39.45%.

After 6 hours of inflammation induction, rats fed on diet supplemented with 5% alfalfa seeds exhibit reduction paw’s thickness by 12.18%, while rats fed on diet supplemented with 10% alfalfa seeds exhibit reduction paw’s thickness by 15.09% with mean value of 4.67±0.29 mm. Rats fed as diet supplemented with 5% alfalfa sprouts exhibit reduction paw’s thickness by 21.27% with mean value of 4.33±0.58 mm, while rats fed on diet supplemented with 10% alfalfa sprouts exhibit reduction paw’s thickness by 30.36%.

Similarly, results of percent inflammation inhibition and paw’s thickness after one, four and six days of inflammation induction revealed that all treatments were able to reduce the incidence of inflammation especially 10% alfalfa sprouts.

Concerning histological results, microscopically, liver of -ve control rats fed on normal diet revealed normal histological structure as shown in Fig.1. Liver of rat from +ve control group showed obvious changes such as congestion of hyperplastic bile duct, and congested hepato-portal blood vessel. Liver of the diabetic group fed on diet supplemented with 5% alfalfa seeds revealed moderate changes in the liver tissue including dilated blood sinusoids with disorganized hepatic cords. Also, the diabetic group fed on diet supplemented with 10% alfalfa seeds revealed moderate changes in the liver tissue including vacuolated enlarged hepatocytes with narrowed blood sinusoids.

On the other hand, diabetic group fed on diet supplemented with 5% alfalfa sprouts indicated mild liver changes including vacuolated enlarged hepatocytes. Interestingly, microscopic examination of liver tissue of diabetic group fed on diet supplemented with 10% alfalfa sprouts showed apparently healthy hepatic parenchyma.

Discussion

Regarding bioactive compounds found in alfalfa seeds and sprouts, Kundan et al., (2011) reported that extract phytochemical screening of Medicago sativa L. extract showed the presence of flavonoids. This study was undertaken to evaluate the anti-diabetic activity of alfalfa seeds and their sprouts and its usefulness in managing inflammation.

The present results were in agreement with Gupta et al., (2012), and Mbaka et al., (2012) who reported that, diabetic rats showed reduction in weight gain compared with non diabetic rats. Liang et al., (2013) reported that when insulin is deficient and the cells cannot metabolize glucose for energy, the cells compensate by increasing their metabolism of proteins and fats. However,
improvement was observed in the group receiving 5% alfalfa seeds while the greatest improvement was observed in the group receiving 10% alfalfa sprouts.

The level of HbA1C was significantly increased in diabetic rats compared to normal rats representing 13.58 ± 1.48% and 5.53 ± 0.94%, respectively (Table 5). The administration of alfalfa seeds at 5% and 10% to the diet of diabetic rats resulted in significant (p≤0.05) decrease in HbA1C levels compared to the diabetic untreated positive control group representing 11.75 ± 0.91% and 10.65 ± 1.04% vs. 13.58 ± 1.48%, respectively. On the contrary, the administration of alfalfa sprouts at the same levels to the diet of diabetic rats resulted in significant decrease in HbA1C level compared to the diabetic untreated positive control group representing 9.90 ± 1.64% and 7.98 ± 0.83% vs. 13.58 ± 1.48%, respectively. It can be concluded that 10% of alfalfa sprout is the most effective level in the diet which give the best results in glycogen and glycated haemoglobin.

Induction of diabetes caused significant elevation in both alanine amino transferase (ALT) and aspartate amino transferase (AST) values in serum of positive control group compared to the negative control group representing 51.87 ± 2.26u/l vs. 26.58 ± 1.29u/l, respectively for ALT concentration and 95.50 ± 5.41 u/l vs. 48.43± 1.39 u/l for AST levels, respectively (Table 6). The concentration of ALT significantly decreased in the diabetic groups supplemented with alfalfa seeds or its sprouts at 5% and 10% levels of intake compared with the positive control group. The highest reduction in ALT concentration was observed when rats were fed diet supplemented with 10% alfalfa sprouts followed by 5% alfalfa sprouts.

The addition of the tested two levels of alfalfa seeds and its sprouts caused significant reduction in AST concentrations compared to the positive control group (Table 6). AST values were 82.70 ± 2.09 u/l and 67.92 ± 3.71 u/l for 5% and 10% alfalfa seeds groups, respectively, while they were 64.58 ± 3.49 u/l and 52.12 ± 2.21u/l for 5% and 10% alfalfa sprouts groups, respectively. The most pronounce improvement in AST activities was observed in the group of diabetic rats supplemented with 10% alfalfa sprouts in the diet. No significant difference was observed between this group and AST value of negative control group representing 52.12 ± 2.21u/l vs. 48.43 ± 1.39u/l, respectively.

Diet supplemented with the two levels of alfalfa seeds as well as sprouts significantly decreased the paw’s thickness of experimental rats after induction of pedal inflammation by formalin compared by the diabetic group. After 3 hours of inflammation induction, rats fed diet supplemented with 5% alfalfa seeds exhibit reduction paw’s thickness by 12.54% with mean value of 4.81 ± 0.29mm, while rats fed on diet supplemented with 10% alfalfa seeds exhibit reduction paw’s thickness by 11.81% with mean value of 4.85 ± 0.32
Feed efficiency ratio (FER) of negative control group was significantly higher than that of positive control group ($P \leq 0.05$) with a mean value of $0.095 \pm 0.008$ and $0.030 \pm 0.005$, respectively. Diabetic rats fed on diets supplemented with alfalfa seeds and its sprouts at the two levels of intake showed significant increase in FER with mean values of $0.043 \pm 0.004$, $0.050 \pm 0.006$, $0.058 \pm 0.004$ and $0.065 \pm 0.003$, respectively compared to the positive control group.

The lowest improvement in FER was observed in the diabetic group received 5% alfalfa seeds, while the greatest improvement was observed in the diabetic group received 10% alfalfa sprouts compared with the positive control group.

Serum insulin concentration of normal rats fed on basal diet (negative control group) was $13.94 \pm 1.80 \mu$/ml, while it was $7.24 \pm 0.69 \mu$/ml for diabetic rats fed on basal diet (positive control group) as shown in Table (5). Serum insulin concentration was decreased significantly by inducing diabetes. When diabetic rats were fed on diet supplemented with 5 and 10% alfalfa seeds, serum insulin concentrations were significantly improved with mean values of $11.23 \pm 1.57 \mu$/ml and $11.66 \pm 1.78 \mu$/ml, respectively compared to the positive control group. The same trend was observed in serum insulin concentrations of rats had diets supplemented with 5% and 10% alfalfa sprout. Diet supplementation with 10% alfalfa sprout had the best serum insulin concentration among all tested groups compared with the negative control group.

Positive control group showed significant increase in serum glucose level representing $235.83 \pm 22.72 \text{mg/dl}$ compared with negative control ($92.17 \pm 9.13 \text{mg/dl}$) (Table 5). Fasting blood glucose concentration in diabetic rats had fallen down following the administration of alfalfa seeds and its sprouts in the diet at the two levels of intake compared with the positive control group representing $151.00 \pm 12.43 \text{mg/dl}$, $149.33 \pm 15.58 \text{mg/dl}$, $140.00 \pm 9.55 \text{mg/dl}$ and $121.50 \pm 12.72 \text{mg/dl}$ for 5%, 10% seeds and 5% and 10% alfalfa sprouts, respectively. Interestingly, the results revealed that the most pronounced reduction in fasting blood glucose level was observed when rats were fed on diet containing 10% alfalfa sprouts.

Positive control diabetic group showed significant lower serum glycogen concentration than that of the negative normal control group $175.62 \pm 2.60 \text{ng/L}$ vs. $65.78 \pm 4.91 \text{ng/L}$, respectively (Table 5). When rats were fed on alfalfa seeds or sprouts at any level of intake, their serum glycogen concentrations were increased significantly compared with positive control group. Results showed that serum glycogen levels in the diabetes treated groups fed on diets supplemented with 5% or 10% alfalfa seeds and 5% or 10% alfalfa sprouts were $110.00 \pm 5.50 \text{ng/L}$, $117.85 \pm 9.88 \text{ng/L}$, $120.02 \pm 6.34 \text{ng/L}$ and $154.51 \pm 2.85 \text{ng/L}$, respectively. The lowest significant glycogen
Results

The chemical analysis of alfalfa seeds and its sprouts (Table 1) revealed that protein contents were 40.274% vs. 41.622%, concentrations reached fat were 19.158% vs. 13.215%, while carbohydrate were 29.823% vs. 31.352%, crude fiber 15.450% vs. 15.180%, moisture 07.245% vs. 10.273% and ash 03.498% vs. 03.536%, respectively.

Alfalfa sprouts had higher flavonoids compounds than alfalfa seeds (Table 2). Flavonoid compounds found in alfalfa seeds and their sprouts include for example romarinic, quercetin, narenginin, kampferol, luteolin and rutin. The highest concentration of flavonoid compounds was found in alfalfa seeds as rutin with mean value of 78.43 ppm, which was decreased in the sprouts (20.70 ppm). However, luteolin content was found in alfalfa seeds with concentration of 33.07 ppm, which was increased in alfalfa sprouts with value of 57.86 ppm.

Alfalfa sprouts are higher than alfalfa seeds in most of the active phenolic compounds (Table 3). Phenolic compounds found in alfalfa seeds and its sprouts include for example syringic (1582.19 vs. 45351.08 ppm), gallic (10.80 vs. 9.40 ppm), protocatechuic (33.42 vs. 233.06 ppm), catechin (107.32 vs. 839.47 ppm), epicatechen (59.88 vs. 10.72 ppm), caffeic (16.82 vs. 1.24 ppm), p-coumaric (5.24 vs. 7.14 ppm), ferulic (39.97 vs. 7.05 ppm), iso-ferulic (13.26 vs. 4.32 ppm) and ellagic (10.05 vs. 59.06 ppm), respectively.

Syringic compounds had the highest concentration in both alfalfa seeds and their sprouts. Followed by catechin compound. The third concentration was chlorogenic in both seeds and sprouts.

The mean value of feed intake (FI) of the negative control rats was 15.12±1.05 g/day but when rats were become diabetic (positive control), their FI was increased significantly with a mean value of 20.17±0.93 g/day (Table 4). Diabetic rats fed diet supplemented with 5% and 10% alfalfa seeds showed significant decrease in FI compared to the positive control rats. The most pronounced improvement in FI was observed in the group of diabetic rats supplemented with 5% and 10% alfalfa sprouts as no significant difference was observed between them and that of negative control group.

Regarding body weight gain (BWG) in Table 4, positive control group showed significant loss in body weight compared to the negative control group. BWG values of diabetic groups supplemented with 5% and 10% alfalfa seeds and its sprouts were significantly increased compared favorably to that of positive control group in the following magnitude of increasing order 21.33±1.21 g > 23.67±2.16 g > 26.42±1.36 g > 28.00±1.67g vs. 17.00±2.61g, respectively. Alfalfa sprout supplementation at 5 and 10% induced better BWG compared to 5 and 10% alfalfa seeds supplementation.
Induction of Inflammation:
At the last week of the feeding trial, three rats were selected from each of the five diabeticsubgroup then 0.1 ml of formalin (4%) was injected in the left hind paw of ether anaesthetized rats in order to induce inflammation (Northover and Subramanian, 1962). The paw’s thickness was measured just before and 3 h, 6 h, 1 day, 2 days, 4 days, and 6 days after formalin injection using skin caliber. The anti-inflammatory effect of alfalfa seeds powder and its sprouts was assessed by calculating percent change in the thickness of rats’ paws compared with the positive control group at the same time.

Blood Samples and Tissue Collection:
At the end of the feeding trial, rats were fasted over-night, lightly anaesthetized by diethyl ether. Blood samples were collected from hepatic portal vein into dry clean centrifuge tubes. Blood samples were left to clot at room temperature, and then centrifuged for 15 minutes at 3000r.p.m. Sera were transferred into dry clean eppendorf tubes and kept frozen at -20°C till biochemical analysis. Livers were removed immediately after sacrificing, excised, rinsed, blotted dry with tissue paper, and kept in formalin solution (10%) for histological examination.

Biological Evaluation and Biochemical Analysis:
Feed intake (FI) was recorded every day throughout the experimental period. Body weight gain (BWG) was determined according to Chapman et al., (1959). Fasting serum insulin and glucose were determined according to Temple et al., (1992) and Trinder (1969), respectively. Serum glycogen was determined according to Mendel et al., (1954) while glycatedhaemoglobin (HbA1c) was determined according to Trivelli et al., (1971). Serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) levels were analyzed according to Reitman and Frankel, (1975).

Histopathological Examination:
Specimens from liver were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6μm in thickness were cut out, deparaffinized and stained with Haematoxylin and Eosin (H&E) for examination under the light microscope (Bancroft et al., 1994).

Statistical Analysis:
Data were statistically analyzed using SPSS, PC statistical software (version 16). Results were expressed as mean ± SD. Differences among groups were analyzed by analysis of variance (ANOVA) using Duncan’s test. A p value of ≤ 0.05 was considered statistically significant according to Armitage and Berry, (1987).
2. Methods:

Preparation of alfalfa dried seeds and their sprouts:

Alfalfa seeds were cleaned, washed, air dried and well grinded to get the fine powder. In order to obtain the alfalfa sprouts, alfalfa seeds were cleaned, washed, soaked overnight then water was poured out. The seeds were let to sit for five days and were rinsed twice a day with cold water and were exposed to the sun for 15 minutes after the fifth day. Finely grounded into the powder. Fresh alfalfa sprouts were freeze-dried at biology department, animal research institute, Giza, Egypt using Freeze-Dryer Lyophilizer (Heidolph) at -50ºC. Fresh alfalfa sprouts(100 gm )yielded 20 g freeze dried powder.

Experimental Animals and diet:

Fifty six adult male albino rats of Sprague-Dawley strain weighting (160-170g) were used. Rats were accessed to the experimental diets and water *ad-libitum*. All rats were fed on the basal diet one week prior to the starting of the experiment as acclimatization period. The basal diet (AIN-93G) was formulated according to Reeves *et al.*, (1993). Salt mixture was prepared according to Hegested *et al.*, (1941) and vitamin mixture was prepared according to Campbell (1963).

Induction of Diabetes Mellitus:

Diabetes was induced by inter-peritoneal injection with freshly prepared alloxan monohydrate (120 mg / body weight of rat ) dissolved in 0.1 M sodium citrate buffer (pH 3.0). After a period of 72 h, rats with fasting blood glucose >150 mg/ dl were considered to be diabetic ( Venkatesh *et al.*, 2008 , Neeli *et al.*, 2007 ).

Experimental Design:

After acclimatization period, rats were divided into two main groups. The first main group (n=6) was fed on the basal diet during the experimental period (four weeks) and kept as a negative control (-ve). The rest of the animals (n = 50) were injected with alloxan for diabetes induction and were divided into five subgroups (10 rats each) fed on the following diet schema for four weeks:

**Subgroup (1):** Diabetic rats were fed on basal diet (positive control).

**Subgroup (2):** Diabetic rats were fed on basal diet supplemented with 5% alfalfa seeds powder.

**Subgroup (3):** Diabetic rats were fed on basal diet supplemented with 10% alfalfa seeds powder.

**Subgroup (4):** Diabetic rats were fed on basal diet supplemented with 5% freeze dried alfalfa sprouts.

**Subgroup (5):** Diabetic rats were fed on basal diet supplemented with 10% freeze dried alfalfa sprouts.
The Hypoglycemic and Anti-inflammatory Effects of Alfalfa (Medicago sativa L.) Plant in Rats

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Introduction

Diabetes mellitus, is one of the most common endocrine metabolic disorders has caused significant morbidity and mortality (Patel et al., 2012). The number of diabetic patients in 2014 was 347 million worldwide, and the expected number in 2030 is 552 million, which represents an increase of more than 50% (ADA, 2014). More than 80% of people with diabetes live in low- and middle-income countries (WHO, 2014). Untreated, diabetes can lead to cardiovascular disease, blindness and kidney failure (WHO, 2012). The causes are complex, but are in large part due to rapid increases in overweight, obesity and physical inactivity (WHO, 2013).

Alfalfa (Medicago sativa L.) is a genus plant distributed widely throughout the world. The common name of alfalfa is Lucerne and it is called the "father of all plants" (Caunii et al., 2012). Although alfalfa is very important and well known as a livestock forage, in some parts of the world these leaves are eaten raw or cooked as a vegetable in human diet. Seeds and sprouts are a favorite salad ingredient and has been indicated to be helpful in lowering cholesterol levels in animal and human studies (Colodny et al., 2001) and reduce inflammation (Hong et al., 2009a). As a result of alfalfa medicinal functions, it has become very important lately. The plant has been reported to have antioxidant effects (Kundan and Anupam, 2011). So, this study was conducted to explore the functional role of alfalfa seeds and its sprouts in controlling blood glucose levels and treating inflammation in diabetic rats.

Materials and Methods

1. Materials:
Alloxan monohydrate, casein, cellulose, vitamin mixture, mineral mixture and formalin were obtained from El-Gomhoria Company, Cairo, Egypt. Alfalfa seeds (Medicago sativa L.) were obtained from agriculture research center, Giza, Egypt. Rats were obtained from Helwan Station, Cairo, Egypt. Kits for biochemical analysis were obtained from Alkane company, Cairo, Egypt.