Antidiabetic Activity of Vicia faba L. Vicine and its O-
Deglycosylation product, Divicine in Streptozotocin-
Induced Diabetic Rats

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ABSTRACT
This study was carried out to investigate the effect of Vicia faba Vicine and its aglucone, Divicine on control blood glucose, serum insulin, lipid profile level and protective effect against oxidative stress in streptozotocin induced diabetic rats. Oral administration of both Vicine and its aglucone Divicine at a concentration of 50mg/kg b.w daily for 30 days showed a significant decrease in fasting blood glucose, iron, transferrin, total iron binding capacity (TIBC), TG, TC, LDL-C, vLDL-C and atherogenic index as well as liver thiobarbituric acid reactive substances (TBARS). The treatment also resulted in a significant increase in plasma insulin, HDL-C, Ferritin, blood hemoglobin, HBA1c and glucose 6 phosphate dehydrogenase (G6PD) as well as blood and liver reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) of diabetic rats. The results clearly suggest that Vicine and Divicine may effectively normalize the impaired antioxidant status in streptozotocin induced diabetes. Also, the tested compounds exerted hypolipidemic and protective effects against lipid peroxidation by scavenging of free radicals by reducing the risk of diabetic complications. Finally, the effect was more pronounced in ethanolic Divicine compared to Vicine. Taken together, Vicine and Divicine have potential as preventive and therapeutic agents for oxidative stress in streptozotocin-induced diabetics and deserve clinical trial in the near future as an adjuvant therapy in diabetes.

Keywords: Antioxidant enzymes, Diabetes, Divicine, Fava beans (Vicia faba), Streptozotocin and Vicine

INTRODUCTION
Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated (Sandler et al., 2000 and Shewade et al., 2001). Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Baynes, 1991 and Ceriello, 2000). Diabetes is usually accompanied by increased production of reactive oxygen species (Baynes and Thorpe, 1999 and Young et al., 1995) or impaired antioxidant defenses (Halliwell and Gutteridge, 1990; Saxena et al., 1993 and McLennan et al., 1991). Antioxidants play an important role in protecting the human body against damage by reactive oxygen species. Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of streptozotocin (Coskun et al., 2005). Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001). Traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of Diabetes Mellitus. Medicinal plants are continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals. Traditional medicines provide better health coverage for 80% of the world population, especially in the developing countries (Mahalingam
and Krishnan, 2008). *Faba bean* (*Vicia faba*) (broad bean, horse bean) is an important member of the legume family with highly useful characteristics. A studies investigating the anti-diabetic and free radical scavenging effects of *Faba bean* showed a hypoglycemic and antioxidant response (Fatima and Kapoor, 2006 and Yang et al., 2006). The presence of *Vicine* or *Divicine* in faba beans seeds (Bjerg et al., 1985), may be is one of reasons of its antidiabetic activity.

![Vicine and Divicine](image)

On the other, the hypoglycemic effect of *Vicine* and anti-inflammatory property of *Vicine* and its aglucone *Divicine* isolated from *Faba bean* (*Vicia faba*) was reported (El Gengaihi et al., 1995 and Hussein, 2012). As an extension of our interested research program in the extraction and therapeutic evaluation of medicinal plants (Hussein, 2012 and Hussein and Hussein, 2013), we report herein, a facile route to explain the antidiabetic and antioxidant activities of *Vicine* and its aglucone *Divicine* isolated from *Faba beans* (*Vicia faba*) and their effect on lipid peroxides and enzymatic antioxidant in streptozotocin (STZ)-induced diabetic rats, in which may pave the way for possible therapeutic application.

**MATERIALS AND METHODS**

**Chemistry**

Melting points were determined on Gallen-kamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on shimadzu MR 470 infrared spectrophotometer using the KBr pellets. Microanalytical data (C, H, N) was determined at the Microanalytical Centre, Cairo University, Egypt. Mass spectra were run using HP Model MS-5988.

**Tested compounds**

1. *Vicine* pure crystalline sample was extracted from mature seeds of fava beans (*Vicia faba*) according to the procedure described by Arbid and Marquardt (1985).
2. *Divicine* pure crystalline sample was obtained by acid hydrolysis from *Vicine* according to the method described by Marquarott et al (1983).

**Animals**

Male albino rats weighing around 200 ± 10 gms were purchased from Faculty of Veterinary Medicine, Cairo University. All the rats were given a period of acclimatization for 15 days before starting the experiment. Animals were provided with diet and water *ad libitum* and were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21–25°C and humidity at roughly 56%. The animals were used accordingly to guidelines of the Committee on Care and use of Experimental Animal Resources of Faculty of Pharmacy October 6th University, Egypt.

**Induction of diabetes**

STZ-induced diabetes has been described as a useful experimental model to study the activity of hypoglycemic agents (Junod et al., 1969). After an overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 55mg/Kg b.w. The normal control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose range of above 250mg/dl) were considered as diabetic rats and were used for the further experiments. The change in the body weight experimental animals was observed throughout the treatment period.

**Experimental set up**

The animals were classified into six groups with eight animals in each. A suspended solution of 1.0g/ 100ml saline was prepared for intragastric intubation of rats.

- **Group I:** Normal control (was given physiological saline solution)
- **Group II:** Normal control treated with *Vicine* (50mg/kg b.w/day) suspended in saline, orally for 30 days (Hussein, 2012).
- **Group III:** Normal control treated with *Divicine* (50mg/kg b.w/day) suspended in saline, orally for 30 days (Hussein, 2012).
- **Group IV:** Diabetic control (STZ-induced diabetic rats, was given physiological saline solution) (Junod et al., 1969).
- **Group V:** Diabetic rats treated with *Vicine* (50mg/kg b.w./day) suspended in saline, orally for 30 days (Hussein, 2012).
- **Group VI:** Diabetic rats treated with *Divicine* (50mg/kg b.w./day) suspended in saline, orally for 30 days (Hussein, 2012).

After 30 days of treatment the fasted rats were sacrificed by cervical decapitation and the blood was collected using sodium fluoride as anticoagulant for determination of Hb, HbA1c, GSH, SOD, CAT, GPx, GST and G6PD. Plasma was collected for glucose, insulin, triacylglycerols, total cholesterol, HDL- c, LDL-c and VLDL-c determination. Serum was collected for iron, transferrin, ferritin and total iron binding capacity (TIBC) determination. The liver was dissected out, washed in ice-cold saline for GSH, SOD, CAT, GPx, GST, TBARS and total protein.
Biochemical Assays
Plasma glucose (Sasaki et al., 1972), insulin (Waldhauser et al., 1983), triacylglycerols (Matthews et al., 1985), total cholesterol (Fossati and Prencipe, 1982), LDL-cholesterol (Allain et al., 1974), LDL-cholesterol (Falholt et al., 1973) formula (LDL-cholesterol = total cholesterol – triacylglycerols/5 – HDL-cholesterol), VLDL-cholesterol concentration (Friedewald, 1973) formula (VLDL-cholesterol = triacylglycerols/5), the atherogenic index \[ \frac{\text{log (TG/ HDL-C)}}{2} \] was also calculated (Dobiasova and Frohlich, 2001), Serum iron (Ceretti and Ceriotti, 1980), transferrin (Hellsing, 1973), ferritin (Valberg, 1980) total iron bending capacity (TIBC) (piccardi and Nissen, 1972), blood Glutathione 6 phosphate dehydrogenase (G6PD) (Soood et al., 1981), blood and liver reduced glutathione (GSH) (Sedlak and Lindsay, 1968) and (Chanarin, 1989), respectively, superoxide dismutase (SOD) (Misra and Fridovich, 1979), catalase (CAT) (Tukahara et al., 1960), glutathione peroxidase (GPx) (Rotruck et al., 1973), glutathione-S-transferase (GST) (Habig et al., 1974), thiobarbaturic acid reactive substances (TBARs) (Uchiyama and Mihara, 1987), total- and glycated haemoglobin and liver protein (Ceritti and Ceriotti 1980), transferrin (Hellsing, 1973), haemoglobin by 35.45% and 19.20% (p<0.01), respectively, decrease insulin and total haemoglobin by 173.7% (p<0.01) and 62.34% (p<0.01), respectively, in diabetic control group compared to normal control rats. Administration of Vicine and Divicine tends to bring down the blood glucose by 24.9% (p<0.01) and 51.28% (p<0.01), and glycated haemoglobin by 19.09% (p<0.05) and 32.24% (p<0.01), respectively compared to untreated diabetic rats. Treatment of animals with Vicine significantly increase the level insulin and haemoglobin by 35.45% and 19.20% (p<0.05) compared to untreated diabetic rats. While, treatment of animals with Divicine significantly increase the level insulin and haemoglobin by 62.36% (p<0.01) and 28.86% (p<0.01) compared to untreated diabetic rats. The effect was more pronounced in case of

Statistical analysis
All the data were statistically evaluated with SPSS/13 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control. All the results were expressed as mean ± SD for eight animals in each group.

RESULTS AND DISCUSSION
Table 1 shows the specific physicochemical properties of Vicine and Divicine. Melting point of Vicine and Divicine are 239–241 and 201–205, respectively.

Table 1. Physico-chemical properties and molecular formulae of Vicine and Divicine

<table>
<thead>
<tr>
<th>Compd.</th>
<th>M.P. [°C]</th>
<th>Yield (%)</th>
<th>Mol. Formula (Mol. Wt.)</th>
<th>Elemental analyses</th>
<th>Calcd./Found [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Divicine</td>
<td>201-203</td>
<td>93</td>
<td>C₂₃H₂₅N₂O₁₂ (456)</td>
<td>C 43.80 H 4.23 N 39.43</td>
<td>43.30 4.02 39.74</td>
</tr>
</tbody>
</table>

Table 2 shows the changes in blood glucose, insulin, total- and glycated haemoglobin levels in normal control and experimental groups of rats. There was a significant increase in blood glucose and glycated haemoglobin by 173.7% (p<0.01) and 62.2% (p<0.01), respectively, decrease insulin and total haemoglobin by 62.34% (p<0.01) and 32.56% (p<0.01), respectively, in diabetic control group compared to normal control rats. Administration of Vicine and Divicine tends to bring down the blood glucose by 24.9% (p<0.01) and 51.28% (p<0.01), and glycated haemoglobin by 19.09% (p<0.05) and 32.24% (p<0.01), respectively compared to untreated diabetic rats. Treatment of animals with Vicine significantly increase the level insulin and haemoglobin by 35.45% and 19.20% (p<0.05) compared to untreated diabetic rats. While, treatment of animals with Divicine significantly increase the level insulin and haemoglobin by 62.36% (p<0.01) and 28.86% (p<0.01) compared to untreated diabetic rats. The effect was more pronounced in case of
simultaneous administration of Divicine- treatment compared to administration of Vicine-treatment.

Table 2. Effect of Vicine and Divicine on plasma glucose, insulin, blood total- and glycosylated haemoglobin in control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (μU/ml)</th>
<th>Haemoglobin (g/dl)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>99.33±6.71</td>
<td>54.76±6.05</td>
<td>13.51±1.66</td>
<td>7.36±0.16</td>
</tr>
<tr>
<td>Control + Vicine</td>
<td>102.67±11.29</td>
<td>43.99±5.35</td>
<td>12.82±2.07</td>
<td>6.84±0.09</td>
</tr>
<tr>
<td>Control + Divicine</td>
<td>83.83±6.70</td>
<td>50.10±7.855</td>
<td>12.44±2.13</td>
<td>7.22±0.11</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>272.21±42.7**</td>
<td>20.62±2.47**</td>
<td>9.11±0.77**</td>
<td>11.94±0.25**</td>
</tr>
<tr>
<td>Diabetic + Vicine</td>
<td>204.17±14.07**</td>
<td>27.93±2.02**</td>
<td>10.86±0.84*</td>
<td>9.66±0.08**</td>
</tr>
<tr>
<td>Diabetic + Divicine</td>
<td>132.50±12.84**</td>
<td>33.49±1.49**</td>
<td>11.74±2.08**</td>
<td>8.09±0.17**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *p<0.05 and **p<0.01. Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.

Table 3 shows the changes in plasma TG, TC, HDL-C, LDL-C, vLDL-C and atherogenic index levels in normal control and experimental groups of rats. There was a significant increase in plasma TG, TC, LDL-C, vLDL-C and atherogenic index levels by 157.7% (p<0.01), 123.83% (p<0.01), 268.2% (p<0.01), 156.99% (p<0.01) and 110.28% (p<0.01) respectively, and decrease HDL-C by 13.68% (p<0.05) in diabetic control group compared to normal control rats. Treatment of animals with Vicine significantly decrease the level TG, TC, LDL-C, vLDL-C and atherogenic index levels by 30.04% (p<0.01), 27.69% (p<0.01), 38.43% (p<0.01), 30.10 (p<0.01) and 23.33% (p<0.01) and increase HDL-C by 14.12% (p<0.05) compared to untreated diabetic rats. While, treatment of animals with Divicine significantly decrease the level TG, TC, LDL-C, vLDL-C and atherogenic index levels by 66.26% (p<0.01), 35.39% (p<0.01), 36.00% (p<0.01), 66.31(p<0.01) and 60.0% (p<0.01) and increase HDL-C by 16.30% (p<0.05) compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of Divicine- treatment compared to administration of Vicine-treatment.

Table 3. Effect of Vicine and Divicine on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), vLDL-cholesterol (vLDL-C) and atherogenic index in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>vLDL-C (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>117.50±5.76</td>
<td>103.50±4.80</td>
<td>43.83±4.53</td>
<td>36.17±6.1</td>
<td>23.6±4.3</td>
<td>0.42±0.006</td>
</tr>
<tr>
<td>Control + Vicine</td>
<td>112.67±6.52</td>
<td>110.00±6.47</td>
<td>47.17±6.92</td>
<td>40.50±4.90</td>
<td>22.33±3.6</td>
<td>0.378±0.009</td>
</tr>
<tr>
<td>Control + Divicine</td>
<td>108.00±4.62</td>
<td>102.33±4.47</td>
<td>44.33±4.77</td>
<td>36.45±5.22</td>
<td>21.6±4.7</td>
<td>0.386±0.006</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>302.83±7.51**</td>
<td>231.67±8.54**</td>
<td>37.83±9.74*</td>
<td>133.19±8.77*</td>
<td>60.56±5.0**</td>
<td>0.90±0.017**</td>
</tr>
<tr>
<td>Diabetic + Vicine</td>
<td>211.67±8.87*</td>
<td>167.50±13.12*</td>
<td>43.13±3.54*</td>
<td>82.00±5.33**</td>
<td>42.33±7.2**</td>
<td>0.69±0.019**</td>
</tr>
<tr>
<td>Diabetic + Divicine</td>
<td>102.17±6.02**</td>
<td>149.67±5.00**</td>
<td>44.00±4.23*</td>
<td>85.24±6.9**</td>
<td>20.4±3.4**</td>
<td>0.36±0.017**</td>
</tr>
</tbody>
</table>

Values represent the mean ± SE (n=8). Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.

LDL-C (mg/dl) = TC-HDL-C-TG/5. vLDL-C (mg/dl) = [Triglycerides/5]. Atherogenic index = log(TG/HDL-C)

- *Significantly different from control group at p<0.05.
- **Significantly different from control group at p<0.01.

Table 4. Effect of Vicine and Divicine on serum iron, transferrin, ferritin and total iron bending capacity (TIBC) in control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Iron (mg/dl)</th>
<th>Ferritin (mg/dl)</th>
<th>Transferrin (mg/dl)</th>
<th>TIBC (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>161.35±9.12</td>
<td>29.45±2.44</td>
<td>215.30±8.89</td>
<td>457.33±19.10</td>
</tr>
<tr>
<td>Normal Control + Vicine</td>
<td>126.79±8.70</td>
<td>27.70±3.26</td>
<td>223.42±10.28</td>
<td>469.72±18.80</td>
</tr>
<tr>
<td>Normal Control + Divicine</td>
<td>107.89±7.92</td>
<td>27.64±3.20</td>
<td>211.92±9.37</td>
<td>474.11±17.00</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>70.15±5.09**</td>
<td>12.03±4.09**</td>
<td>244.68±7.48*</td>
<td>508.25±25.85*</td>
</tr>
<tr>
<td>Diabetic + Vicine</td>
<td>100.72±9.70**</td>
<td>16.09±2.67**</td>
<td>172.40±13.25*</td>
<td>364.78±16.52**</td>
</tr>
<tr>
<td>Diabetic + Divicine</td>
<td>106.67±6.49**</td>
<td>18.75±2.48**</td>
<td>148.61±10.44**</td>
<td>417.99±15.46*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *p<0.05 and **p<0.01. Diabetic control rats were compared with normal control rats. Experimental groups were compared with the diabetic control rats.
Table 4 shows the changes in plasma iron, ferritin, transferrin and total iron binding capacity (TIBC) levels in normal control and experimental groups of rats. There was a significant decrease in plasma iron and ferritin by 56.52% (p<0.01) and 59.15% (p<0.01), respectively, and increase transferrin and total iron binding capacity (TIBC) by 13.64% (p<0.05) and 11.13% (p<0.05), respectively, in diabetic control group compared to normal control rats. Treatment of animals with Vicine significantly increase the level iron and ferritin by 37.7% (p<0.01) and 33.47% (p<0.01), respectively, and decrease transferrin and total iron binding capacity (TIBC) by 19.92% (p<0.05) and 28.22% (p<0.01), respectively, compared to untreated diabetic rats. While, treatment of animals with Divicine significantly increase the level iron and ferritin by 52.06% (p<0.01) and 55.86% (p<0.01), respectively, and decrease transferrin and total iron binding capacity (TIBC) by 39.26% (p<0.01) and 17.75% (p<0.05), respectively, compared to untreated diabetic rats. The effect was more pronounced in case of simultaneous administration of Divicine- treatment compared to administration of Vicine-treatment.

Table 5. Level of reduced glutathione (GSH) and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and thiorubarbic acid reactive substances (TBARs) in liver of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µg/g Hb)</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>GST</th>
<th>(G6P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>107.33 ± 6.34</td>
<td>25.68 ± 2.35</td>
<td>33.50 ± 3.06</td>
<td>118.16 ± 3.78</td>
<td>13.89 ± 1.89</td>
<td>21.2 ± 2.73</td>
</tr>
<tr>
<td>Normal + Vicine</td>
<td>75.56 ± 5.38</td>
<td>23.09 ± 1.34</td>
<td>28.75 ± 2.31</td>
<td>110.85 ± 5.04</td>
<td>11.49 ± 1.35</td>
<td>19.89 ± 3.27</td>
</tr>
<tr>
<td>Normal + Divicine</td>
<td>97.37 ± 4.09</td>
<td>22.36 ± 2.60</td>
<td>29.61 ± 3.07</td>
<td>100.01 ± 4.90</td>
<td>10.66 ± 1.52</td>
<td>20.73 ± 2.86</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>52.06 ± 4.20**</td>
<td>16.67 ± 2.80</td>
<td>20.80 ± 2.63**</td>
<td>62.79 ± 2.87**</td>
<td>5.44 ± 0.97**</td>
<td>10.48 ± 3.19**</td>
</tr>
<tr>
<td>Diabetic + Vicine</td>
<td>62.20 ± 5.88**</td>
<td>15.68 ± 2.22**</td>
<td>22.12 ± 5.57**</td>
<td>83.33 ± 2.86**</td>
<td>6.96 ± 0.97**</td>
<td>14.55 ± 3.71**</td>
</tr>
<tr>
<td>Diabetic + Divicine</td>
<td>80.64 ± 3.56**</td>
<td>18.40 ± 2.67**</td>
<td>27.56 ± 2.94**</td>
<td>97.54 ± 2.21**</td>
<td>7.27 ± 0.84**</td>
<td>16.43 ± 4.00**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *p<0.05 & **p<0.01; Control rats compared to administration of Vicine-treatment.

Table 6. Level of reduced glutathione (GSH) and activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and thiorubarbic acid reactive substances (TBARs) in liver of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µg/g protein)</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>GST</th>
<th>(TBARs) µM/mg protein</th>
<th>MDA/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>227.16 ± 5.22</td>
<td>125.27 ± 4.06</td>
<td>22.36 ± 1.46</td>
<td>37.25 ± 2.15</td>
<td>42.85 ± 2.75</td>
<td>3.12 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Normal + Vicine</td>
<td>251.55 ± 8.18</td>
<td>110.36 ± 7.11</td>
<td>20.52 ± 1.73</td>
<td>35.04 ± 3.23</td>
<td>38.90 ± 5.08</td>
<td>3.23 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Normal + Divicine</td>
<td>232.64 ± 7.93</td>
<td>115.58 ± 6.38</td>
<td>19.44 ± 2.16</td>
<td>36.68 ± 2.55</td>
<td>39.54 ± 4.11</td>
<td>3.05 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>205.44 ± 6.35**</td>
<td>76.59 ± 3.59**</td>
<td>13.25 ± 0.98**</td>
<td>21.40 ± 2.37**</td>
<td>27.61 ± 2.07**</td>
<td>8.37 ± 0.07**</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Vicine</td>
<td>215.16 ± 11.48*</td>
<td>95.41 ± 5.37</td>
<td>17.60 ± 3.33</td>
<td>30.94 ± 4.54</td>
<td>3.86 ± 3.55</td>
<td>5.64 ± 0.09</td>
<td></td>
</tr>
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<td>Diabetic + Divicine</td>
<td>230.64 ± 9.55**</td>
<td>108.40 ± 4.65</td>
<td>19.37 ± 1.26**</td>
<td>34.07 ± 3.77**</td>
<td>37.49 ± 3.49**</td>
<td>4.57 ± 0.08**</td>
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Values are given as mean ± SD for groups of eight animals each. Values are statistically significant at *p<0.05 & **p<0.01; Control rats compared to administration of Vicine-treatment. The effect was more pronounced in case of simultaneous administration of Divicine-treatment compared to administration of Vicine-treatment.
Figure 1. Effect of vicine and divicine on the histopathology of liver in STZ-treated rats. (a, b and c) normal control group & vicine and divicine treatment group showing normal appearance of liver histology. Diabetic control group showing proliferation of bile ductule, lymphocytic infiltration and pyknotic nuclei (d). Diabetic rats continuously treated with vicine and divicine show normal hepatic architecture except low degree of lymphocytic infiltration around central veins (e &f).

Histopathology Examination
Histopathological examination of liver sections of the normal control groups showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. The hepatocytes are polygonal cells with well preserved cytoplasm, nucleus with prominent nuclei (figure a). Likewise, in Vicine or Divicine -treated animals, liver sections showed normal hepatic architecture (figure 1b&c). On the other hand, in the hepatotoxic positive STZ-treated control group, histological examination showed showing proliferation of bile ductules and lymphocytic infiltration and pyknotic nuclei (figure 1d). Histopathological examination also showed good recovery of STZ-induced proliferation by vicine and divicine as compared to STZ-treated group (figure 1 e&f). Vicine or Divicine -treatment at 50mg/kg b.w showed remarkable histological normal hepatic architecture except low degree of lymphocytic infiltration around central veins to those of the STZ- treated group. They showed nearly ordinary patterns with an increase normal hepatocytes parenchyma and a reduced development of fibrous septa and lymphocyte infiltration.

Diabetes mellitus is caused by an absolute or relative lack of insulin that, among other consequences, leads to an increase in plasma glucose concentration. Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (Baynes and Thorpe, 1997). Many plant extracts and plant products have been shown to have significant antioxidant activity (Anjali and Manoj, 1995) and useful in treatment of several ill fated diseases including diabetes (Scartezzini and Speroni, 2002). Also, Johnston
Mohammadi and Naik, 2005, reported that aglycones and nonglycosylated polyphenols inhibited glucose uptake whereas glycosides and phenolic acids were ineffective. These data suggest that aglycones inhibit facilitated glucose uptake whereas glycosides inhibit the active transport of glucose (Johnston et al., 2005). The present study was conducted to evaluate the beneficial effects of Vicine and Divicine on antioxidant status in STZ-induced diabetic rats. This study has investigated a possible method to isolate the active polyphenolic compound; Vicine from Fava beans (Vicia faba) Arbid and Marquardt (1985) and by hydrolysing it, its aglucone Divicine was obtained Marquarott et al. (1983). Structure elucidation of Vicine and Divicine were established based on both elemental analysis and spectral data. Infrared spectrum of Vicine indicated the presence of (NH₂), (OH), (CH-aliphatic), (C = N), (C-O-C) and (C-O-C) groups. Mass spectrum of Vicine revealed a molecular ion peak at m/z 304 (100%). The value of this peak in mass spectroscopy instrumental equals the elemental analysis data which equals the calculated molecular weight of Vicine. Infrared spectrum of Divicine exhibited bands of (NH₂), (CH-aliphatic), (C = N), (C-O-C) and (C-O-C). Mass spectrum of Divicine revealed a molecular ion peak and a base peak at m/z 143 (M⁺+H, 100%). The value of this peak in mass spectroscopy instrumental equals the elemental analysis data which equals the calculated molecular weight of Divicine. Also, mass spectrum and elemental analysis of Divicine indicated the breakdown of glycosidic bond in Vicine by hydrolysis and lost one glucose moiety. The preliminary studies conducted by this work revealed the non-toxic nature of Vicine and /or Divicine on normal rats. Although no literature data have given any insight into the possible toxicological potentials of Vicine and its aglucone Divicine, the dose of 50mg/kg b.w. chosen for treating animals did not cause obvious signals of toxicity. Acute treatment of the normal control group rats with saline alone did not produce any significant change (p > 0.05) in the blood glucose, haemoglobin and plasma insulin concentrations of either the fasted normal or the fasted STZ-treated diabetic rats. However, Vicine and Divicine, produced significant reductions (p < 0.01) in the blood glucose levels of fasted STZ-treated diabetic rats (table 2). In our study, elevated blood glucose level and decrease insulin level were observed in STZ-induced diabetic rats and it may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. Oral administration of Vicine and Divicine at 50 mg/kg to the diabetic rats significantly reduced blood glucose level compared to diabetic control rats. Also, the decreased insulin levels were noticed in diabetic rats compared to control rats which directly support and represent STZ-mediated beta cell destruction or damage. In diabetic rats, treatment of Vicine and Divicine increased the insulin level compared to diabetic control rats. Hence, the hypoglycemic activity of Vicine and Divicine may be due to its protective action against STZ-mediated damage to the pancreas beta cells and also, possibly because of regeneration of damage beta cell or increased insulin release or secretion. HbA1c is the product of non-enzymatic reaction between glucose and free amino group of Hb (glycosylation) (Mohammadi and Naik, 2008). It is marker of evaluation of long term glycemic control in diabetic patients and predicts risks for the development and/or presence of diabetic complication (Tembhurne and Sakarkar, 2010). Our study results showed that increased level of HbA1c and decreased Hb level were observed in diabetic rats compared to normal control rats which indicate the occurrence of glycosylation in diabetic rats due to hyperglycemia. Administration of Vicine and Divicine to the diabetic rats significantly reduced HbA1c level and increased Hb level compared to diabetic control rats. This action represents that Vicine and Divicine has ability to prevent the development of diabetes associated complications. The present results confirmed with the results of Fatima and Kapoor, 2006, who reported that Vicine showed a significant hypoglycemic effect in STZ-diabetic rats.

Hyperglycemia is an important contributor for the cardiovascular diseases risk. Both type 1&2 diabetes are independent risk factors for (CHD) (Grundy et al., 1999). The vascular diseases occurred in diabetes due to disturbance in lipoprotein metabolism which causes acceleration of atherosclerosis (Maser et al., 1991). In diabetic condition, increased level of TC, TG and reduced level of HDL-c along with altered composition of LDL-c particles were commonly reported (Howard et al., 2000). In the present study, administration of STZ showed alteration of normal lipid profiles such as increase of TC, TG, LDL-c and VLDL-c levels as well as decreased HDL level compared to normal control rats (table 3). These altered lipid profiles were reversed to near normal level after treatment of Vicine and Divicine in STZ-induced diabetic rats. This lipid lowering action may be due to proper stabilization of glucose level and increase in insulin level after administration of Vicine and Divicine which may normalize the disturbed lipid metabolism in diabetic rats, therefore hypolipidemic after Vicine and Divicine administration, support its ability to prevent the CHD diseases associated with diabetes.

In the present study, a decreased total iron and serum ferritin with a significant increase in serum transferrin and total iron binding capacity (TIBC) in diabetic rats control group (P<0.01) (table 4). In type 2 diabetes with insulin deficiency, the rate of iron deposition in body organs was increased (Jiang et al., 2004) leading to decrease the serum iron level. Body iron stores, have been implicated in the development of oxidative stress and conversely, decreased iron stores improve insulin sensitivity and insulin secretion control group (Jiang et al., 2004 and Fernandez-Real et al., 2005). However, the oral administration of Vicine and Divicine to the diabetic group of rats significantly reverted back iron, ferritin, transferrin and total iron binding capacity (TIBC) levels to near normal values which show the anti-lipid peroxidative property of Vicine and Divicine in Type 2 experimental diabetes. The mentioned
structural conditions may be found in a Divicine molecule which, in the in vitro systems efficiently scavenges hydroxyl radical (OH•), superoxide radical (LOO•), superoxide anion radical (O₂−•), singlet oxygen (¹O₂), and nitrogen oxide (NO•). But the mentioned structural conditions not allowed to Vicine molecule, so it have lower chelating potency less than its aglycone Divicine (Scheme 1).

Scheme 1. Chelating effect of Divicine

Literature reports outline that iron catalyzed oxidative stress mediates apoptosis of pancreatic islets with a resultant decrease in insulin secretory capacity (Cooksey et al., 2004). The beneficial effect of iron chelators on endothelial dysfunction suggests the role of iron in vascular disease. Iron chelation therapy may present a novel way to interrupt the cycle of catalytic iron-induced oxidative stress and tissue injury and consequent release of catalytic iron in diabetes and to prevent diabetes mellitus –related complications (Swaminathan et al., 2007).

STZ utilize low affinity glucose transporter 2 in the plasma membrane and its selectivity accumulation in pancreatic beta cells and also it damage other organs which can express this transporter, particularly kidney and liver (Lenzen, 2008). Also, a number of study revealed that oxidative stress plays a major role in the development, progression of diabetes and its related complications (Pham-Huy et al., 2008; Oyedemi and Afolayan, 2011; Sajeesh et al., 2011 and Kumar et al., 2011 ). In diabetic state, free radical generation via increased glycolysis, auto-oxidation of glucose and non-enzymatic protein glycation (Sharma et al., 2010). Moreover, drastic reduction of in vivo antioxidant enzymes level in various tissues was reported in diabetic condition (Sharma et al., 2010). In our study, decreased levels of blood and liver SOD, CAT, GPx, GST and GSH as well as increased liver level of TBARs were observed in STZ-induced rats (table 5&6). The reduction of above enzymes directly reflect the oxidative stress in diabetic rats and these enzyme level changes may be due to generation of free radical by auto-oxidation of glucose, glycosylation in hyperglycemic condition as well as STZ mediated generation of ROS by its NO donor property to the intracellular molecules. In the present study, increased SOD, CAT, GPx, GST and GSH as well as reduced TBARs level were noticed in diabetic rats after administration of Vicine and Divicine. The above action represented that antioxidant property of Vicine and Divicine in diabetic condition and hence Vicine and Divicine possesses a potential to reduce or prevent the diabetic micro- and macrovascular complication.

In the present study, the histological findings prove that Vicine or Divicine affected the recovery of liver structure in STZ-induced liver proliferation. Indeed, there was show normal appearance of hepatocytes around C.V (Central vein) in most of hepatic lobules in rats treated with vicine and divicine-groups compared to control diabetic-group. Histological studies confirmed the hepatoprotective effect of the tested compounds. Diabetic rat liver sections showed fatty degeneration of hepatocytes. Vicine or Divicine-treatment (50mg/kg) almost normalized these effects in the histoarchitecture of liver. Furthermore, the severe fatty changes in the livers of diabetic rats caused by STZ were treated in the STZ- treatment groups. Therefore, from this study the Vicine and Divicine could be a hepatoprotective against STZ induced liver damage in rats.

Our data confirm that Vicine and Divicine possesses blood glucose lowering action in diabetic condition. Moreover it has hypolipidemic and antioxidant activities in diabetic state; therefore it has an ability to prevent diabetic complications. Anthyperglycaemic, hypolipidemic and antioxidant Effect of Vicine and its aglucone Divicine in streptozotocin-induced diabetic rats has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. In conclusion, the present study showed that oral administration of Vicine and Divicine increases insulin sensitivity and reduces metabolic complications along with oxidative stress in diabetic rats. Further studies are essential to establish the role of Vicine and Divicine in controlling type 2 diabetes and its complications.

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