Evaluation of clinical activity and safety of Daflon 500 mg in type 2 diabetic female patients

Sherine Maher Rizk a, Nagwa Ali Sabri b,*

Received 15 November 2008; accepted 10 May 2009
Available online 7 August 2009

KEYWORDS
Diabetes mellitus; Daflon®; Cardiovascular disease risk

Abstract Background: The incidence of cardiovascular disease in patients with type 2 diabetes mellitus is approximately twice as high as in the non-diabetic population.

Aim: To investigate the hypoglycemic and hypocholesterolemic effects of Daflon® 500 mg (DF) administration together with its tolerability and efficacy in reducing the cardiovascular metabolic risk factors in female patients with type 2 diabetes.

Methods: In a well-adapted controlled single-blinded randomized parallel design the tolerability and the efficacy of Daflon® (500 mg) either alone or with oral hypoglycemic, twice daily for 45 days, was studied in 36 female patients with type 2 diabetes.

Results: None of the patients in the studied groups were reported to have any adverse events throughout the treatment period (45 days), liver and kidney function tests were within normal limits and there was no significant difference between the pre-treatment (day 0) and post-treatment (day 45) values. Female patients receiving Daflon® either alone or with oral hypoglycemic showed significant decrease in serum glucose; fructosamine; total cholesterol; LDL–cholesterol; triglycerides; malondialdehydes (as index of lipid peroxidation) and C-reactive protein (CRP) levels along with increase in the levels of nitric oxide and blood glutathione.

Conclusion: This study has shown that Daflon® (500 mg, twice daily for 45 days) is helpful in reducing glucose level and the risk of cardiovascular disease in type 2 diabetic patients.

Recommendation: Further clinical trials are essential for strengthening the evidence base on the role of this drug in the cardiovascular risk in diabetic patients.

© 2009 King Saud University. All rights reserved.
prospective studies have shown an association between the degree of hyperglycemia and increased risk of microvascular complications, myocardial infarction, stroke, macrovascular mortality and all cause mortality (Stratton et al., 2000). The presence of plasma lipid abnormalities, which include reduction in high density lipoprotein cholesterol (HDL–C), an alteration in the structure and concentration of the low density lipoprotein cholesterol (LDL–C) and increased triglycerides is associated with a particular high risk of cardiovascular complication (Krauss, 2004). In addition, several experimental, epidemiologic and clinical studies support the notation that oxidative stress plays a significant role in type 2 diabetes mellitus and in the development of cardiovascular diseases (Jacob and Burri, 1996). Reactive oxygen species (ROS), which convert Ox-LDL into highly oxidized LDL, which is, in turn, taken up by macrophages to form foam cells (Channon, 2002). Foam cells combine with leukocytes to become the fatty streak, which is converted to fibrous plaque that will protrude into the arterial lumen, calcified, yielding a fibrous cap that surrounds a lipid-rich core. In acute coronary syndromes (e.g., myocardial infarction), when fibrous plaques rupture, the formation and release of thrombi may ultimately occlude vessels (Madamanchi et al., 2005). It is also reported that ROS production was significantly higher in unstable versus stable angiogenesis, which suggests that ROS might also modulate plaque stability (Channon, 2002).

Impairment in the bioavailability of nitric oxide (NO) results in compromised vasodilatation with subsequent increase the cardiovascular risk (Endemann and Schiffrin, 2004). As such, glucose; lipid and oxidative stress lowering therapy with increased NO (nitrite/nitrate) level may help to reduce cardiovascular risk in these patients.

In our study, we measure serum C-reactive protein (CRP) as a marker for increased risk of cardiovascular disease. CRP is released in response to inflammatory markers present within atherosclerotic plaques. Several studies have shown a correlation between elevated levels of CRP and cardiovascular events in patients with coronary heart disease, independent of serum lipid concentrations and may be a stronger predictor of cardiovascular disease than LDL cholesterol (Ridker et al., 2004). Previous reports stated that CRP is one of the strongest independent predictors of future cardiovascular events both in patients with CVD and in healthy subjects (Le Mellédo et al., 2004). The American Heart Association recommends obtaining CRP levels in patients at intermediate risk of a cardiovascular event. In these patients an elevated CRP (> 3 mg/L) is considered to confer high risk (Pearson et al., 2003).

Daflon® (micronized purified flavonoid fraction of *Rutaecae aurantiae*, consisting of 90% diosmin and 10% hesperidin) has been used in clinical practice to treat a variety of lymphoedemas, such as post haemorrhoids (Cospite, 1994), post-phlebitic syndrome, and idiopathic cyclic oedema syndrome (Pecking, 1995), radical mastectomy oedema (Pecking et al., 1997), chronic venous insufficiency (Le Deyehat et al., 1997) and varicose ulcers (Guilhou et al., 1997).

Finally, the aim of this study is to evaluate the hypoglycemic and hypcholesterolemic effects of Daflon® 500 mg (DF) in type 2 diabetic female patients. To date, no clinical trials have been conducted to assess the suitability of this drug in decreasing the risk of cardiovascular in type 2 diabetes. In this study, we are presenting our observations on the tolerability and efficacy of Daflon® (500 mg, twice a day for 45 days) in reducing cardiovascular metabolic risk factors in patients with type 2 diabetes.

## 2. Experimental and methods

### 2.1. Patients and methods

#### 2.1.1. Chemicals

All chemicals used in this study were of analytically pure grade from Sigma–Aldrich (GERMANY) or from purest grade available.

Daflon® 500 mg tablet was kindly supplied by Servier, France.

#### 2.1.2. Study design and treatment regimen

Thirty-six female patients of type 2 diabetes, between the ages of 40.00–69.00 years (mean of 50.67 ± 3.01), who had signed a written informed consent of the study, were admitted to National Institute of Diabetes in Egypt, for a period of four days and randomly allocated into two drug groups:

- Group A received Daflon® (500 mg) + oral hypoglycemic twice a day for 45 days, and
- Group B received Daflon® (500 mg) twice a day for 45 days.

In addition, 18 female normal volunteers were participated in this study, and served as normal control between the ages of 36.00–47.00 years (mean of 41.667 ± 5.129).

The appropriate drug regimen was administered to the patients twice daily (morning and evening doses) from day 1 to day 3 (three days excluding day 0, i.e. day of admission) of hospitalization, under the direct supervision of the medical team. Patients were discharged on the morning of day 4 with a pack of the appropriate drug capsules (for the next 7 days: to complete the treatment up to day 10). The patients were educated and instructed by the physician and the researchers to comply with the dosage schedule. Thereafter, patients were requested to attend weekly to the institute to allow measurement of blood glucose level and to receive the drugs needed for the next week.

All patients were clinically monitored for any adverse reactions (such as abdominal pain, nausea, vomiting, chest pain, diarrhea, fever, headache, myalgia and chills) at 8 h intervals for first 24 h and thereafter every 24 h for further two days (until the end of day 3). All systemic reactions, if any, were recorded in a pre-designed form. Laboratory investigations on biochemistry parameters (blood urea, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AST) were assessed on day 0, (pre-treatment), and on completion of treatment (day 45).

#### 2.1.3. Patients’ data collection and follow-up

The following information would be collected; demographic data including; age, occupation, medication history including; past and current medication (prescribed and OTC) drug allergies and adverse effects, disease history and its complications (if present), family history, social habits and subjective parameters. Patients were given the Daflon® (DF) tablets with administration instructions, following-up was performed through weekly meeting with those patients in the national institute of diabetes, besides their telephones and home address were registered for more following-up procedures to ensure
their compliance and adherence to the DF tablets 500 mg dosage regimen (twice daily) and to document any subjective improvement or deterioration.

(i) Inclusion criteria

(1) Female type 2 diabetic patients.
(2) Disease history not more than 8 years.
(3) Patients who comply with the regular visits to the national institute of diabetes.
(4) Patients with moderate hyperglycemia.

(ii) Exclusion criteria

(1) Type 1 diabetic patients.
(2) Disease history more than 8 years.
(3) Patients not comply with the regular visits to the national institute of diabetes.
(4) Patients with severe hyperglycemia.
(5) Patients suffering from acute or chronic renal diseases.
(6) Patients with known liver disease(s).
(7) Patients with coronary artery diseases.
(8) Patients with a history of hyper- and/or hypo-glycemic coma.
(9) Patients with severe diabetic complications.
(10) Illiterate patients.

2.1.4. Patients' evaluation and biochemical determinations

Serum activities of AST, ALT and ALP were determined by using the reagents of kits supplied by Bayer Health Care (France). Serum fructosamine level was determined by using the reagents of kits supplied by Bayer Health Care (France). Serum concentration of urea and creatinine were determined by using the reagents of kits supplied by Bayer Health Care (France). Serum activities of AST, ALT and ALP were determined by using the reagents of kits supplied by Sera-Pak of Bayer (France). Serum concentration of urea and creatinine were determined by using the reagents of kits supplied by HPLC technique according to method of Little et al. (1983) by using the Kit supplied from Bio-Rad D-10 (France) using the Bio-Rad D-10 hemoglobin testing system.

Serum total cholesterol was determined enzymatically according to the method of Allain et al. (1974) using Biocon kit (Germany), after 15 h fasting of the patients. Serum HDL–cholesterol was determined according to the method of Friedewald et al. (1972) and serum LDL–cholesterol was calculated from the equation developed by Friedewald et al. (1972). Serum triglyceride was determined enzymatically according to the method of Wahlfeld and Bergmeyer (1974) by using the reagents of kit supplied by Biocon (Germany).

The blood reduced glutathione (GSH) and protein thiols (PSH) levels were determined colorimetrically according to the methods of Beutler et al. (1963) and Koster et al. (1986). Malondialdehyde (MDA) as an index of lipid peroxidation was assessed colorimetrically according to the method of Mihara and Uchiyama (1978). A high correlation between endogenous nitric oxide (NO) production and nitrite/nitrate level in plasma and serum has been established. Therefore, measurement of these levels provides a reliable and quantitave estimate of NO output in vivo (Granger et al., 1996). In our study, levels of serum nitrite and nitrate were estimated as an index for NO levels according to Miranda et al. (2001).

C-reactive protein (CRP) was determined by ELISA technique according to the method of Gewurz et al. (1982) using the kit supplied from Diamed Eurogen (Belgium). Statistical analysis of the results are expressed as mean ± SEM. The data were analyzed by ANOVA followed by Tukey test and the significant level was chosen at $P < 0.05$.

3. Results

3.1. Patients' data collection

The patients’ age ranges from 40.00 to 69.00 years (mean of 50.67 ± 3.01), weight ranges from 63.00 to 95.00 kg (mean of 84.60 ± 5.63), all of the patients were house-wives, disease history ranges from 0.20 to 7.00 years (mean of 3.08 ± 0.87).

Concerning the medication history it was found that all of the patients were receiving hypoglycemic drug {second generation of sulfonylurea (Glimiprider tablets 20 mg) + anti-hyperglycemic drug {Bigu-nides (Metformin tablets 500 mg)}. Most of the patients were receiving Vitamin B complex as neuromtoric to alleviate the neuropathy complications of diabetes, and some of the patients were administering aspirin tablets (300 mg). It is worthy to mention that, the majority of the patients, about 78%, were suffering from hypertension and taking antihypertensive drugs.

During the follow-up of the patients and their counseling it was found that some of the complications especially joint and leg pain with neuropathy were highly diminished after DF administration.

3.2. Serum activities of ALT, AST, ALP and serum concentrations of urea and creatinine

It is apparent from the results in Table 1 that the administration of DF for a period of 45 days neither significantly change the activities of ALT, and AST, ALP nor the serum concentrations of urea and creatinine.

3.3. Serum glucose, fructosamine and glycosylated hemoglobin ($HbA_1c$)

Serum glucose level was significantly increased in female diabetic patients group A and B reaching 221.92% and 200.60%, respectively, of the value obtained in the control group. Administration of DF for 45 days in both groups results in a significant decrease of these high levels in such groups of patients.

Concerning the results of serum fructosamine, its value significantly increased in the diabetic patients by 24.34% and 23.15% for groups A and B, respectively, than that of the normal control group and then decreased upon administration of
DF in both groups. Glycosylated hemoglobin was significantly increased the diabetic groups compared to the healthy control one, while, on DF administration, both groups, showed a non-significant decrease in HbA1c level (Table 2).

3.4. Serum lipid profile

The data presented in Table 3 indicates that total cholesterol was significantly higher in the diabetic groups A and B, reaching to 135.07% and 136.52% of the value obtained in normal control, respectively. Administration of DF significantly decreases these values in group A and B by 22.02% and 28.01%, respectively.

Serum HDL–C did not significantly differ or changed among the three studied groups, moreover, LDL–C values were significantly higher in both diabetic groups A and B from those of control group, besides, this parameter was significantly lower than the respective values after administration of DF in both groups.

Serum triglycerides were significantly higher in female diabetic patients group A and B reaching 264.57% and 206.74%, respectively, of the value obtained in the control group. Although these values significantly decreased in group A only (P-value <0.05), after DF administration compared to those before treatment, they were significantly higher than the control ones, in both groups A and B, even after DF treatment (Table 3).

3.5. Blood glutathionne (GSH) and serum protein thiol

The results in Table 4 Blood GSH content was significantly decreased by 35.59% and 28.86% in groups A and B, respectively, compared to the normal healthy group. Administration of DF resulted in a significant increase in

---

Table 1  Clinical characteristics of type 2 diabetic female patients before and after administration of Daflon®.

<table>
<thead>
<tr>
<th>Group</th>
<th>Alkaline phosphatase (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy</td>
<td>123.12 ± 10.47</td>
<td>22.5 ± 2.18</td>
<td>19.85 ± 2.45</td>
<td>38.56 ± 2.31</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Diabetic group (A) before treatment</td>
<td>117.75 ± 10.35</td>
<td>29.91 ± 3.02</td>
<td>23.16 ± 1.32</td>
<td>46.51 ± 5.07</td>
<td>1.1 ± 0.04</td>
</tr>
<tr>
<td>Diabetic group (A) after treatment</td>
<td>129.70 ± 11.92</td>
<td>26.56 ± 3.56</td>
<td>25.75 ± 3.06</td>
<td>43.74 ± 3.75</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>Diabetic group (B) before treatment</td>
<td>134.51 ± 12.05</td>
<td>19.76 ± 2.21</td>
<td>26.72 ± 4.32</td>
<td>43.59 ± 2.14</td>
<td>1.19 ± 0.06</td>
</tr>
<tr>
<td>Diabetic group (B) after treatment</td>
<td>126.86 ± 9.65</td>
<td>23.49 ± 2.05</td>
<td>23.04 ± 2.23</td>
<td>43.32 ± 3.56</td>
<td>1.23 ± 0.07</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE.
Number of patients in group A = 12, and number of patients in group B = 12.

Table 2  Serum glucose, fructosamine and percentage of HbA1c in female diabetic patients before and after administration of Daflon®.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dl)</th>
<th>Fructosamine (µmol/L)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy</td>
<td>87.97 ± 5.79</td>
<td>235.30 ± 7.13</td>
<td>5.78 ± 0.14</td>
</tr>
<tr>
<td>Diabetic group (A) before treatment</td>
<td>195.22 ± 7.31a</td>
<td>292.58 ± 8.18a</td>
<td>8.15 ± 0.53a</td>
</tr>
<tr>
<td>Diabetic group (A) after treatment</td>
<td>145.25 ± 7.72b</td>
<td>259.12 ± 2.54b</td>
<td>6.44 ± 0.57a</td>
</tr>
<tr>
<td>Diabetic group (B) before treatment</td>
<td>176.47 ± 6.36a</td>
<td>289.78 ± 8.53a</td>
<td>8.16 ± 0.43a</td>
</tr>
<tr>
<td>Diabetic group (B) after treatment</td>
<td>143.39 ± 7.06ab</td>
<td>271.79 ± 6.07a</td>
<td>7.57 ± 0.52a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
Number of patients in group A = 12, number of patients in group B = 12, and number of normal healthy = 12.

a There is a significant difference between the mean values of the different variables before and/or after Daflon Administration Compared to the Normal Healthy Group by using ANOVA at p < 0.05.

b There is a significant difference between the mean values of the different variables before and after Daflon Administration Compared to the diabetic female patients by using ANOVA at p < 0.05.

Table 3  Lipid profile in female diabetic patients before and after administration of Daflon®.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total-cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>LDL-cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy</td>
<td>176.52 ± 12.62</td>
<td>47.39 ± 4.77</td>
<td>108.90 ± 9.08</td>
<td>101.13 ± 24.36</td>
</tr>
<tr>
<td>Diabetic group (A) before treatment</td>
<td>238.43 ± 12.49a</td>
<td>35.12 ± 6.29</td>
<td>150.3 ± 7.26a</td>
<td>267.56 ± 33.07a</td>
</tr>
<tr>
<td>Diabetic group (A) after treatment</td>
<td>185.93 ± 11.69b</td>
<td>44.48 ± 4.88</td>
<td>107.45 ± 4.15b</td>
<td>169.28 ± 18.54b</td>
</tr>
<tr>
<td>Diabetic group (B) before treatment</td>
<td>240.99 ± 26.90a</td>
<td>35.98 ± 4.83</td>
<td>163.22 ± 7.32a</td>
<td>209.08 ± 15.08a</td>
</tr>
<tr>
<td>Diabetic group (B) after treatment</td>
<td>173.49 ± 13.99b</td>
<td>48.82 ± 4.98</td>
<td>89.67 ± 7.98a</td>
<td>175.68 ± 7.49a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.
Number of patients in group A = 12, number of patients in group B = 12, and number of normal healthy = 12.

a There is a significant difference between the mean values of the different variables before and/or after Daflon Administration Compared to the Normal Healthy group by using ANOVA at p < 0.05.

b There is a significant difference between the mean values of the different variables before and after Daflon Administration Compared to the diabetic female patients by using ANOVA at p < 0.05.

3.5. Blood glutathionne (GSH) and serum protein thiol

The results in Table 4 Blood GSH content was significantly decreased by 35.59% and 28.86% in groups A and B, respectively, compared to the normal healthy group. Administration of DF resulted in a significant increase in
blood GSH in both groups ($p > 0.05$). On the other hand, serum protein thiol concentration was not significantly changed among the studied groups.

### 3.6. Effect on serum nitric oxide (nitrite/nitrate) and malondialdehyde

The data reported in Table 4 shows that serum nitric oxide values were significantly lower by 39.20% and 49.59% in the diabetic groups A and B respectively compared to the normal healthy group. On DF administration, nitric oxide (nitrite/nitrate) values significantly increased in both groups, however, nitric oxide values still significantly lower than those of the control group in patients administered DF only.

Serum MDA levels were significantly higher in groups A and B compared to the control group ($0.43 \pm 0.04$, and $0.42 \pm 0.02$ versus $0.22 \pm 0.02$, respectively, $P > 0.05$), however these values significantly decreased after DF treatment compared to values obtained before treatment, but still significantly higher than that of healthy group in patients administered DF only.

### 3.7. Serum C-reactive protein

The results in Table 5 shows that serum C-reactive protein levels in the diabetic groups A and B were significantly higher than that of the normal healthy group. Although these values significantly decreased after DF administration, they remained significantly higher than the normal group values.

### 4. Discussion

Diabetic patients showed an increased mortality and morbidity compared with non-diabetics and are more likely to develop cardiovascular diseases (Boemi et al., 1993). This high risk cannot be completely predicted by known risk factors such as hypertension and plasma lipids. Serum C-reactive protein (CRP) is a biomarker for chronic inflammation and is a sensitive risk factor for cardiovascular diseases. It is released in response to inflammatory markers present within atherosclerotic plaques and may be a stronger predictor of cardiovascular disease than LDL cholesterol (Ridker et al., 2004; Prasad, 2006).

The present study revealed a highly significant increase in serum CRP in diabetic female patients compared to non-diabetic control, suggesting increase in cardiovascular disease risk. Administration of Daflon® either alone or with oral hypoglycemic drug produced significant decrease in its level, this decreasing effect of Daflon® may be referred to its anti-inflammatory effect as proved in a model of inflammatory granuloma in rat where Daflon® 500 mg reduced edema formation and inhibited the synthesis for prostaglandin E2 (PGE2) and thromboxane B2 (TXB2) (Jean and Bodinier, 1994). Moreover, diosmin was reported as a potent inhibitor of PGE2 and thromboxane A2 (TXA2) (Labrid, 1994).

The female patients conducted in this study showed highly levels in serum glucose, fructosamine and HbA1c, indicating sustained hyperglycemia in these patients. In patients with type 2 diabetes, previous studies have shown an association between the degree of hyperglycemia and increased risk of microvascular complications, myocardial infarction (Klein, 1995; Lehto et al., 1996) and macrovascular mortality (Uusitupa et al., 1993). The relative risk for myocardial infarction seems to increase with any increase in glycaemia above the normal range (Fuller et al., 1983), whereas the risk for microvascular disease is thought to occur only with more extreme concentrations of glyceria (Jarrett and Keen, 1976).

### Table 4: Blood glutathione (GSH), serum protienthiol (PSH), malondialdehyde (MDA) and nitric oxide (NO) levels in female diabetic patients before and after Daflon® administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal healthy group</th>
<th>Diabetic group A</th>
<th>Diabetic group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>22.14 ± 2.36</td>
<td>14.26 ± 0.73a</td>
<td>25.07 ± 1.61b</td>
</tr>
<tr>
<td>PSH (g/mol/ml)</td>
<td>504.08 ± 32.01</td>
<td>486.31 ± 46.48</td>
<td>509.74 ± 30.49</td>
</tr>
<tr>
<td>MDA (g/mol/ml)</td>
<td>0.22 ± 0.02</td>
<td>0.43 ± 0.04a</td>
<td>0.27 ± 0.04b</td>
</tr>
<tr>
<td>NO (nitrite/nitrate) (g/mol/ml)</td>
<td>48.98 ± 4.20</td>
<td>29.78 ± 1.47a</td>
<td>41.74 ± 2.14a,b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

Number of patients in group A = 12, number of patients in group B = 12, and number of normal healthy = 12.

a There is a significant difference between the mean values of the different variables before and/or after Daflon Administration Compared to the Normal Healthy group by using ANOVA at $p < 0.05$.

b There is a significant difference between the mean values of the different variables before and after Daflon Administration Compared to the diabetic female patients by using ANOVA at $p < 0.05$.

### Table 5: Mean ± serum level of C-reactive protein in diabetic female patients before and after Daflon® administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>C-reactive protein (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal healthy</td>
<td>2.97 ± 0.75</td>
</tr>
<tr>
<td>Diabetic group A</td>
<td>9.01 ± 1.47$^a$</td>
</tr>
<tr>
<td>Diabetic group A</td>
<td>5.09 ± 0.51$^{a,b}$</td>
</tr>
<tr>
<td>Diabetic group B</td>
<td>10.12 ± 2.24$^a$</td>
</tr>
<tr>
<td>Diabetic group B</td>
<td>6.26 ± 0.58$^{a,b}$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

Number of patients in group A = 12, number of patients in group B = 12, and number of normal healthy = 12.

a There is a significant difference between the mean values of the different variables before and/or after Daflon Administration Compared to the Normal Healthy group by using ANOVA at $p < 0.05$.

b There is a significant difference between the mean values of the different variables before and after Daflon Administration Compared to the diabetic female patients by using ANOVA at $p < 0.05$. 

Evaluation of clinical activity and safety of Daflon 500 mg in type 2 diabetic female patients 203
In the present study, administration of Daflon® to female diabetic patients resulted in significant decrease in serum glucose level which could be attributed to hesperidin content in Daflon® tablets. Jung et al. (2004) found that the improvement in hyperglycemia associated with hesperidin is attributed to increase in glucose utilization via elevation in glycolysis and hepatic glycogen concentration and this was mediated through activation of glucokinase enzyme. Jung et al. (2006) found that hesperidin significantly increased the glucokinase mRNA level. Although the decrease in HbA1c in the current study after Daflon® administration is not significant, but it could be promising in decreasing cardiovascular disease risk. Stratton et al. (2000) stated that each 1% reduction in haemoglobin HbA1c was associated with a 37% decrease in risk for microvascular complications and a 21% decrease in the risk of any end point or death related to diabetes.

Several experimental, epidemiologic and clinical studies support the notion that oxidative stress plays significant role in type 2 diabetes and in the development of cardiovascular diseases (Jacob and Burri, 1996). In the present study, the increased levels of MDA clearly show that the diabetic patients were exposed to an increased oxidative stress via lipid peroxidation (Mahboob et al., 2005), while decreased level of glutathione indicates decreased scavenging capacity against elevated lipid peroxidation process in these patients. This finding could be referred to the fact that hyperglycemia triggers the generation of free radicals and oxidant stress (Hunt et al., 1990). Hyperglycemia also leads to activation of sorbitol pathway and contributes to the formation of triose phosphate which generates oxaloaldehydes with high capacity to produce protein glycation and oxidative stress (Diaz-Flores et al., 2004). Furthermore, hyperglycemia has been shown not only to generate ROS, but also inactivate some of the scavenging enzymes through non-enzymatic glycation (Blaktny and Harding, 1992). Administration of Daflon® either alone or with oral hypoglycemic drugs to female patients with type 2 diabetes showed decrease in serum MDA with subsequent increase in GSH levels, this ameliorating effect of Daflon® could attribute to decrease in the cardiovascular diseases risk. Hesperidin in combination with diosmin has been shown to inhibit the reactive oxygen radicals production in Zymosan-stimulated human polymorphnuclear neutrophils (Jean and Bodnirer, 1994), it also decreased the level of TBARS in kidney homogenate of CCl4 treated rats (Tirkey et al., 2005).

Abnormalities in lipid metabolism often present in patients with type 2 diabetes (Rosenson, 2005). The current study revealed significant elevation in total-cholesterol, LDL-cholesterol and triglycerides levels in type 2 diabetes female patients compared to non-diabetic control group (Garvey et al., 2003). Type 2 diabetes is characterized by increased secretion of apolipoprotein (apo) B which is a key component of VLDL assembly process leading to increased serum levels of triglycerides (Krauss and Siri, 2004; Mayerson et al., 2002).

The increased levels of LDL-cholesterol with concomitant increase in free radicals increase the probability for oxidation of LDL forming oxidized-LDL which was proposed to stimulate the expression of adhesion molecules of endothelial cells and might cause adhesion of blood monocytes to vascular endothelium, the early stage of atherosclerosis (Steinberg, 1995; Steinberg et al., 1989).

Administration of Daflon® to type 2 diabetic female patients resulted in significant decrease in total- and LDL-cholesterol when given either alone or with oral hypoglycemic drugs; while significant decreased in triglyceride level was observed only in patients administered Daflon® with oral hypoglycemic drugs. In support of this results, serum cholesterol decreased and in vitro activities of hydroxymethylglutaryl-CoA reductase and sterol O-acyltransferase were inhibited in cholesterol-fed rats supplemented with mixtures of principal citrus flavonoids (Bok et al., 1999). Additionally, treatment of HepG2 cells with hesperetin, a metabolite of hesperidin, reduced the net secretion of apolipoprotein B (apo B), the LDL protein (Borradaile et al., 1999). This response could provide an additional cardio-protective effect.

Diosmin could also help in reducing the risk for cardiovascular diseases as it is previously reported to causes a significant decrease in plasma levels of endothelial adhesion molecules, which increased by ox-LDL, thus providing protection against microcirculatory damage (Ramelet, 2000).

A decline in NO bioactivity and the resultant endothelial dysfunction occurs in many disease settings including; hyperlipidemia, hypertension, metabolic syndrome, and diabetes (Mueller et al., 2005). The present study showed a significant decrease in serum NO (nitrite/nitrate) level in the diabetic group compared to the non-diabetic one. Impairment in the bioavailability of nitric oxide (NO) results in compromised vasodilatation with subsequent increase in the cardiovascular risk (Endemann and Schiffrin, 2004). Both endothelium-derived and platelet-derived NO contribute to preventing platelet adhesion to the vascular wall and platelet aggregation, therefore playing a key role in the prevention of thrombus formation, which is a major trigger of coronary accidents (Le Mellèdo et al., 2004). The decrease in NO (nitrite/nitrate) level is observed in the current study may be correlated to the hyperglycemia recorded in such patients (Williams et al., 1998). High glucose-induced NO deficiency is partially attributable to the uncoupling of the endothelial NO synthase (eNOS) reaction resulting in the net synthesis of superoxide and to the oxidative inactivation of NO (Hayden and Tyagi, 2003; Wadham et al., 2007). Considerable evidence indicates that atherogenic oxidized low-density lipoprotein (ox-LDL) may impair signal transduction activation of nitric oxide synthase enzyme (NOs), thus diminishing the synthesis of NO (Liao et al., 1995). In addition, CRP at concentrations known to predict CVD has been found to down regulate eNOS and destabilize eNOS mRNA, with resultant decreases in both basal and stimulated NO release (Järvisalo et al., 2002). Daflon® administration in the current study revealed a significant increase in serum NO (nitrite/nitrate) level attributed to its hypoglycemic and hypolipidemic effect. Increase in NO (nitrite/nitrate) level by Daflon® treatment could decrease the risk for cardiovascular disease.

5. Conclusion

Administration of Daflon® as a co-therapy with oral hypoglycemic drugs to patients with type 2 diabetes might be decrease the incidence for cardiovascular diseases, which evidenced by decrease in CRP level. Such decrease in the cardiovascular disease risk could be attributed to its hypoglycemic, anti-oxidative stress, hypolipidemic effects with concomitant increase in
Acknowledgements

We would like to thank The Review Board and/or the Ethical Committee of the National Institute of Diabetes in Egypt for their precious and great advice for the acceptance of our study protocol to be performed in the Institute. We would like also to express our deepest gratitude and profound thanks to Prof. Dr. Sameh Abdel Shokour, Head Manager of the National Institute of Diabetes in Egypt and to Dr. Ashraf Ismail, Assistant Professor of Clinical Pathology and Head of Clinical Pathology Department in The National Institute of Diabetes in Egypt for their sincere help, continuous support and valuable cooperation to achieve this work.

References


Rosenson, R.S., 2005. Assessing risk across the spectrum of patients with the metabolic syndrome. Am. J. Cardiol. 96 (4A), 8E–10E.


تقييم الفاعلية الإكلينيكية والمأمونية للدافلون 500 مغ في مرضى السكري النوع الثاني

شيرين نجوى علي صبري

ملخص البحث

الخلفية: يبلغ معدل الإصابة بالأمراض القلبية الوعائية في مرضى السكري-النوع الثاني ضعف غير المصابين بالسكري.

المهدف: دراسة التأثيرات الجانبية للاستعمال المستمر للفايبرول في الدم لدى مرضى السكري، 500 مغ، ودراسة احتماليات وفاعليته في التقليل من عوامل الخطورة القلبية الوعائية المتعددة في مرضى السكري، النوع الثاني في الإناث.

الطريقة: في دراسة مضمودية وأحادية التسمية، 300 مغ، ذات تصميم متوازي تحت دراسة احتمالية وفاعلية دواء دافلون (500 مغ) منفردا أو مع الادواء الخاضع لسكر الدم مرتين يوميا لمدة 45 يوما، في 36 مريضة بالسكري-النوع الثاني.

النتائج: لم يتم الإبلاغ عن أي مريضة في مجموعة الدراسة بالإصابة بأي عوارض ضارة للدواء طوال فترة العلاج (45 يوما) وكانت اختبارات الوظيفة الكلوية والكبدية ضمن الحدود الطبيعية ولم يكن هناك أي فرق معنوي بين قيم ما قبل العلاج وما بعد. وأظهرت المريضات اللواتي كن يتلقين داء دافلون إما منفردا أو مع الادواء الخاضع لسكر الدم إخفاضا في مستويات الجلوكوز المصل، وفي مادة فرمونزامين، والكوليسترول الكلي، والكوليسترول المنخفض الكثافة، والجلوكوز ومستويات الكلي، والبروتينات (كديل على فوق أكسة الشحم) والبروتينات المتفاعلة مع زيادة في مستويات أكسيد البيكريبول والبروتينات.

الاستنتاج: أظهرت هذه الدراسة أن داء دافلون (500 مغ مرتين يوميا لمدة 45 يوما) يساعد على خفض مستوى الجلوكوز ومخاطر المرض القلبية الوعائية في مرضى السكري.

التوصيات: من الضروري إجراء المزيد من الدراسات الإكلينيكية للحصول على براهين أقوى عن دور الادواء في المخاطر القلبية الوعائية في مرضى السكري.

المؤلف المراسل.

1. قسم الكيمياء الحيوية ، كلية الصيدلة ، جامعة القاهرة ، القاهرة ، مصر.
2. قسم الصيدلة الإكلينيكية ، كلية الصيدلة ، جامعة عين شمس، القاهرة ، مصر.

*