Effects of Chard (Beta vulgaris L. var cicla) on the Liver of the Diabetic Rats: A Morphological and Biochemical Study

Ozlem OZSOY-SACAN,1 Ömür KARABULUT-BULAN,2 Sehnaz BOLKENT,2 Refiye YANARDAG,1,1 and Yasemin OZGEY1

1Department of Chemistry, Faculty of Engineering, Istanbul University, 34850-Avcilar, Istanbul, Turkey
2Department of Biology, Faculty of Science, Istanbul University, 34459-Vezneciler, Istanbul, Turkey

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Chard (Beta vulgaris L. var cicla) is one of the medicinal herbs used by diabetics in Turkey. It has been reported to reduce blood glucose. We have investigated the effect of chard extracts on the liver by biochemical and morphological investigation. The plant extract was administered by the gavage technique to rats at a dose of 2 g/kg every d for 28 d, 14 d after experimental animals were made diabetic. In the diabetic group, some degenerative changes were observed by light and electron microscope examination, but degenerative changes decreased or were not observed in the diabetic group given chard. In the diabetic group, blood glucose levels, serum alanine, aspartate transaminase, alkaline phosphatase activities, total lipids, sialic and uric acid levels, liver lipid peroxidation (LPO), and nonenzymatic glycosylation (NEG) levels increased, while blood glutathione, body weight, and liver glutathione (GSH) levels decreased. The diabetic group given chard, serum alanine, aspartate transaminase, alkaline phosphatase activities, total lipid level, sialic and uric acid levels, blood glucose levels, and liver LPO and NEG levels decreased, but the other values increased. As a result of all the morphological and biochemical findings obtained, it was concluded that the extract of this plant has a protective effect on the liver in diabetes mellitus.

Key words: Beta vulgaris L. var cicla; streptozotocin; liver; chard; hepatic regeneration

Materials and Methods

Plant material. Chard leaves were purchased from open markets. They were carefully washed with tap water, then sliced into small pieces and dried under shade at room temperature. They were stored in well closed cellophane bags.

Preparation of aqueous plant extract. Dried chard leaves (50 g) were extracted by adding 500 ml distilled water and boiling for 30 min. The extract was then filtered and the filtrates were evaporated using a rotary evaporator under reduced pressure until drying. Then the...
extract was dissolved in distilled water before administration to normal and diabetic rats.

Preparation of diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) in a single dose of 65 mg/kg body weight. STZ was dissolved in a freshly prepared 0.01 M citrate buffer (pH = 4.5).

Animals. The experiments were reviewed and approved by the Institute’s Animal Care and Use Committee of the University of Istanbul. Six–6.5 months old female Swiss albino rats weighing 150–200 g were used. The animals were fed laboratory pellet chow and given water ad libitum. All rats were clinically healthy. The animals were randomly divided into four groups. Group I: Intact animals (normal, control). Group II: Animals given chard extract (control). Group III: Diabetic animals. Group IV: Diabetic animals given chard extract. Beginning on d 14 of the study, chard extract was given to the group II (normal + chard) and IV (diabetic + chard) animals at a dose of 2 g/kg daily for 28 days by gavage through an intragastric tube. Twenty-four female rats were used for light and electron microscopic studies and 96 for biochemical analyses.

Histological assays. Small pieces of liver tissue taken from fasted animals for one night under ether anesthesia in the 42nd d, were fixed by Bouin’s fixative. Paraffin sections of 6 μm thickness were taken from liver tissue and stained by Haematoxylin-eosin and Masson’s triple dyes and examined with a light microscope.

Cytologic assays. Small liver tissues at +4 °C were prefixed for two hours in Sörensen’s phosphate buffered (pH = 7.2) 2% glutaraldehyde. Then the tissues were postfixed in phosphate buffered osmium tetroxide for one hour (pH = 7.2), dehydrated in a graded series of ethanol solutions, and embedded in epon. Ultrathin sections were cut using a Reichert Om U3 ultramicrotome with glass knives, stained with uranyl acetate and lead citrate, and examined using a Carl Zeiss EM 9 S-2.

Biochemical assays. Blood samples were collected through the tail vein of the experimental animals at 0, 14 and 42 d. In all samples, the 18 h fasting blood glucose and glutathione levels were determined by o-toluidine and the Beutler, Duron, Kelly method respectively. Serum AST and ALT activities were determined by Reitman and the Beutler, Duron, Kelly method respectively. Serum ALP activity and serum total lipid levels were determined by two point and sulfophosphovanilin respectively. At 42d, serum sialic acid and uric acid levels were determined by the Lorentz and Caraway method respectively.

At the end of the experimental period, liver tissues were taken from animals sacrificed under ether anesthesia after an overnight fast. They were homogenized in cold 0.9% serum physiologic by means of a glass homogenizer to make up 10% (w/v) homogenize. The homogenates were centrifuged and the clear supernatants were used for glutathione (GSH), lipid peroxidation (LPO), nonenzymatic glycosylation (NEG), and protein analysis. Liver glutathione (GSH) levels were determined according to the method of Beutler using Ellman’s reagent. LPO levels were determined with malondialdehyde (MDA) by the method of Ledwozyw et al. NEG levels were determined by thiobarbituric acid methods. Protein content in the supernatants was determined by the Lowry method.

Statistical analysis. The results were evaluated using an unpaired t-test and ANOVA variance analysis using the NCSS statistical computer package.

Results

Light microscope results

In the hepatocytes of the diabetic group in comparison to the control group (Fig. 1), picnotic nuclei, vacuolization, many big cytoplasmic granules, rupturing in the central vein, moderate hyperemia and dilation in some sinusoids were detected (Figs. 2A, B, C), while in the hepatocytes of diabetic group given the plant extract, it was observed that vacuolization, picnotic nuclei, and hyperemia decreased and that little sinusoidal dilation was present in some places (Figs. 3A, B).

Electron microscope results

In the diabetic group in comparison to the control group, an expansion of the granular endoplasmic reticulum (GER) cisterns, picnotic nuclei, an increase in smooth endoplasmic reticulum, lipid accumulations, a decrease in glycogen amount in some cells, mitochondria surrounded by dilated granular endoplasmic reticulum, dark cells including picnotic nuclei, many

Fig. 1. Liver of the Control Rat. Hematoxylin-eosin. × 400.
Fig. 2. Liver of the Diabetic Rat.

Vacuolization (▲), picnotic nuclei (→), many large cytoplasmic granules (▲), rupturing in the central vein (CV), moderate hyperemia (♦), dilations in sinusoids (+). A, B: Haematoxylin-eosin. C: Masson’s tri-dye. ×400.

Fig. 3. Liver of the Diabetic Rat Given Chard Extract.

mitochondria, and expanded intercellular spaces were detected (Figs. 4A, B), and in diabetic group given the plant extract, an increase in the glycogen amount peroxisomes, a decrease in the lipid amount, an increase in granular endoplasmic reticulum containing many ribosomes, dark cells including dense cytoplasm, picnotic nuclei, and many mitochondria (Figs. 5A, B).

Biochemical results

The mean body weights of the four groups are given in Table 1. Body weight was significantly lower in rats with STZ diabetes than in the control group during the experiment (Table 1). The body weight was significantly higher in the diabetic + chard group than in the diabetic group at 42 d (Table 1).

The mean blood glucose levels of the groups are given in Table 2. Before induction of diabetes, the blood glucose levels of all groups were similar (pANOVA = 0.441). Fourteen days after administration of STZ, the diabetic and diabetic + chard groups had significantly higher blood glucose levels than at 0 d. At 42 d blood glucose levels were still high in the diabetic animals, but in the diabetic + chard group it decreased significantly (p = 0.0001). The maximum reduction in blood glucose was noted 42 d after the administration of chard extract. At the end of the study, blood glucose levels were similar in all groups except for the diabetic group (Table 2). The control rats did not show any significant variation in the blood glucose throughout the experimental period (p = 0.163).

Before diabetes was induced there were no significant differences in blood glutathione levels among the groups (Table 3). After administration of chard to the diabetic + chard animals, blood glutathione levels were found to increase significantly compared with the diabetic animals (Table 3). At the end of the study, blood glutathione levels were similar in all groups except for the diabetic group (Table 3).

The mean serum AST and ALT levels of the fasting animals at 0, 14, and 42 d are shown in Tables 4 and 5 respectively. When AST values were determined, there was no significant difference between the 4 groups on d 0 (pANOVA = 0.268). Fourteen days after administration
of STZ, the diabetic and diabetic + chard groups had significantly higher AST activities than at d 0. But at 42 d AST activities were still high in the diabetic rats, but in the diabetic + chard group it decreased. Treatment with chard extract for 28 d decreased serum AST activities in the diabetic rats.

* Mean ± SD.
** n = Number of animals.

Table 1. Mean Levels of Body Weights for All Groups (g)*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 42</th>
<th>PValues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>179.8 ± 13.1</td>
<td>184.3 ± 25.1</td>
<td>179.4 ± 22.2</td>
<td>0.783</td>
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<tr>
<td>Control + Chard</td>
<td>23</td>
<td>182.9 ± 30.5</td>
<td>179.0 ± 22.6</td>
<td>175.6 ± 20.9</td>
<td>0.627</td>
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<tr>
<td>Diabetic</td>
<td>25</td>
<td>164.8 ± 14.9</td>
<td>144.9 ± 18.7</td>
<td>139.5 ± 20.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic + Chard</td>
<td>25</td>
<td>175.0 ± 14.4</td>
<td>156.1 ± 20.0</td>
<td>155.1 ± 23.9</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

PANOVA: 0.058 0.0001 0.0001

Table 2. Mean Levels of Blood Glucose for All Groups (mg/dl)*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 42</th>
<th>PValues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>66.7 ± 12.8</td>
<td>59.7 ± 15.4</td>
<td>59.1 ± 15.9</td>
<td>0.163</td>
</tr>
<tr>
<td>Control + Chard</td>
<td>23</td>
<td>66.6 ± 17.2</td>
<td>59.5 ± 18.7</td>
<td>61.4 ± 13.6</td>
<td>0.353</td>
</tr>
<tr>
<td>Diabetic</td>
<td>25</td>
<td>62.3 ± 11.8</td>
<td>206.1 ± 63.0</td>
<td>141.3 ± 54.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic + Chard</td>
<td>25</td>
<td>69.2 ± 16.6</td>
<td>180.4 ± 59.5</td>
<td>79.7 ± 22.9</td>
<td>0.0001</td>
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</tbody>
</table>

PANOVA: 0.441 0.0001 0.0001

Table 3. Mean Levels of Blood Glutathione for All Groups (mg/dl)*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 42</th>
<th>PValues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>36.2 ± 10.6</td>
<td>38.9 ± 16.3</td>
<td>38.5 ± 20.2</td>
<td>0.839</td>
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<tr>
<td>Control + Chard</td>
<td>23</td>
<td>38.9 ± 13.8</td>
<td>33.4 ± 14.2</td>
<td>47.1 ± 23.3</td>
<td>0.050</td>
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<tr>
<td>Diabetic</td>
<td>25</td>
<td>42.5 ± 9.3</td>
<td>36.5 ± 20.0</td>
<td>36.5 ± 21.6</td>
<td>0.529</td>
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<tr>
<td>Diabetic + Chard</td>
<td>25</td>
<td>45.1 ± 13.1</td>
<td>31.0 ± 17.1</td>
<td>50.7 ± 17.4</td>
<td>0.001</td>
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PANOVA: 0.118 0.5 0.075

Table 4. Mean Levels of Serum AST for All Groups (U/l)*

<table>
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<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 42</th>
<th>PValues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>63.7 ± 8.1</td>
<td>60.7 ± 26.7</td>
<td>70.3 ± 31.7</td>
<td>0.516</td>
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<tr>
<td>Control + Chard</td>
<td>23</td>
<td>60.4 ± 7.5</td>
<td>69.6 ± 28.5</td>
<td>105.4 ± 15.5</td>
<td>0.0001</td>
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<tr>
<td>Diabetic</td>
<td>25</td>
<td>60.8 ± 9.0</td>
<td>91.3 ± 36.5</td>
<td>113.3 ± 17.5</td>
<td>0.0001</td>
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<tr>
<td>Diabetic + Chard</td>
<td>25</td>
<td>66.7 ± 14.3</td>
<td>88.3 ± 6.7</td>
<td>72.0 ± 37.6</td>
<td>0.229</td>
</tr>
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</table>

PANOVA: 0.268 0.011 0.0001

Table 5. Mean Levels of Serum ALT for All Groups (U/l)*

<table>
<thead>
<tr>
<th>Group</th>
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<th>Day 14</th>
<th>Day 42</th>
<th>PValues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>20.3 ± 8.2</td>
<td>22.1 ± 9.8</td>
<td>28.6 ± 7.3</td>
<td>0.202</td>
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<tr>
<td>Control + Chard</td>
<td>23</td>
<td>20.6 ± 4.3</td>
<td>17.0 ± 8.1</td>
<td>27.2 ± 7.2</td>
<td>0.001</td>
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<tr>
<td>Diabetic</td>
<td>25</td>
<td>27.4 ± 5.7</td>
<td>50.5 ± 14.8</td>
<td>89.4 ± 15.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic + Chard</td>
<td>25</td>
<td>25.3 ± 7.9</td>
<td>59.2 ± 14.8</td>
<td>27.9 ± 5.8</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

PANOVA: 0.130 0.0001 0.0001

* Mean ± SD.
** n = Number of animals.
Serum ALT activities and total lipids levels were high in the diabetic rats compared with the controls. These changes were reversed in diabetic animals that were given the chard extract (Tables 5 and 7).

The changes in serum ALP values at 42 d are given in Table 6. There was a significantly difference in the serum ALP values between groups (Table 6). A significant increase was observed in the values for the diabetic groups ($P_{\text{ANOVA}} = 0.001$). The ALP value in the diabetic + chard group exhibited a significant decrease compared to the diabetic group ($p = 0.011$) (Table 6).

The serum sialic acid and uric acid levels in the STZ diabetic rats significantly, whereas no significant changes in normal rats were detected (Table 8).

Table 6 shows the content of GSH, NEG, and LPO in the livers of the normal and experimental groups. In diabetic rats a significant decrease in liver GSH levels was observed ($p = 0.0001$). Administration of chard extract caused elevation in liver GSH levels in the diabetic and control groups ($p = 0.0001$). The liver LPO content in the diabetic group was significantly increased as compared to the control group ($p = 0.0001$). In the diabetic rats a significant increase in liver NEG levels was observed. Administration of chard extract was found to reduce liver NEG levels in them (Table 9).

**Discussion**

In many countries, medicinal plants are used in traditional practice to control diabetes.32) Plant drugs are frequently considered to be less toxic and free of side effects than synthetic ones.32) In our previous study, chard showed a hypoglycemic effect on diabetic rats.7–10) The functions of the liver and the kidney may also be affected by changes in the level of insulin, which provides rapid uptake, storage as glycogen, and usage of glucose, especially in the liver. In diabetes, as the activities of glycogen synthase and hexokinase are diminished as a result of insulin deficiency, glucose cannot be transformed into glycogen and glycogenesis is reduced and thus the amount of glucose increases.33) In another study done by us in which the pancreases of diabetic rats were examined under light and electron microscopes, it was found that chard extract increased insulin synthesis and release and that blood glucose...
levels were reduced. In our present study, with administration of chard extract, it was observed that blood glucose levels decreased in diabetic rats. The increase in the amount of liver glycogen can be explained by an increase of glucose intake which resulted in storage as glycogen.

Insulin is associated with protein as well as carbohydrate metabolism. In the diabetic group given chard extract, an increase in regular parallel GER cisterns containing many ribosomes showed an increase in protein synthesis. In diabetes, protein synthesis ceases and protein catabolism increases. Liver cell destruction shows itself as an impairment in the permeability of liver cell membranes. As a result, cytoplasmic enzymes such as AST and ALT pass into blood plasma and their activities in serum increase. The increase in serum activities of secretion enzymes (AST, ALT, ALP) which were formed in the liver and released into the bile is a sign of a loss of the secretion function of the liver. In our study, a decrease in the serum AST, ALT, and ALP activities of secretion enzymes (AST, ALT, ALP) which were formed in the liver and released into the bile is a sign of a loss of the secretion function of the liver.

In diabetes mellitus, the serum concentration of sialic acid was found to increase significantly, especially in poorly controlled and long term cases. In our diabetic rats, a significant increase in serum total sialic acid levels were observed when compared with the control group. Various factors might cause an elevation in the concentration of serum sialic acid. Among these factors are an increase in the synthesis of sialic acid in insulin-independent tissues such as the liver and the brain and an increase in the activity of sialytransferase, which transfers the sialic acid residues to the glycolipids and glycoproteins. In our study, administration of chard extract decreased the content of sialic acid in serum of diabetic rats.

Streptozotocin has been widely used to induce diabetes in experimental animals. It causes selective destruction of pancreatic B cells, probably by a free radical-mediated mechanism. Free radicals formed during the reduction of O₂, such as peroxy-, hydroxy-, and superoxide radicals, are known to cause cell damage directly or indirectly. Previous animal studies show that persistent hyperglycemia causes increased production of reactive oxygen species through glucose autoxidation and NEG, which in turn causes increased oxidative stress.

GSH, the most important biomolecule against chemically induced toxicity, can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GP. GSH also functions as a free radical scavenger and in the repair of radical-caused biological damage. We observed a decrease in the level of GSH in the liver and blood in diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress. Administration of chard extract increased the content of GSH in the liver and blood of the diabetic rats.

Nonenzymatic glycosylation (NEG) of liver proteins causes alteration in their structure and function. Levels of NEG were found to increase in the STZ-diabetic groups in comparison with the untreated controls. Various means of preventing this increase have been investigated both in vitro and in vivo. It has been reported that GSH, various antioxidant vitamins and compounds, trace elements such as vitamins C and E, flavanoids, saponins, vanadium, and selenium prevent

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (nmol GSH/ mg protein)*</th>
<th>P_{Values}</th>
<th>NEG (nmol Fructose/ mg protein)*</th>
<th>P_{Values}</th>
<th>LPO (nmol MDA/ mg protein)*</th>
<th>P_{Values}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.4 ± 0.3</td>
<td>0.0001</td>
<td>18.2 ± 3.0</td>
<td>0.546</td>
<td>0.7 ± 0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Control + Chard</td>
<td>5.0 ± 0.1</td>
<td>0.0001</td>
<td>16.9 ± 5.6</td>
<td>0.548</td>
<td>1.3 ± 0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2.9 ± 0.6</td>
<td>0.0001</td>
<td>32.2 ± 6.6</td>
<td>0.548</td>
<td>1.94 ± 0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetic + Chard</td>
<td>5.1 ± 0.0</td>
<td>0.0001</td>
<td>30.4 ± 8.5</td>
<td>0.548</td>
<td>1.79 ± 0.1</td>
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<tr>
<td><strong>PANOVA</strong></td>
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<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
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</tr>
</tbody>
</table>

*Mean ± SD
n = Number of animals
* p = 0.001 versus control groups.
**p = 0.0001 versus control groups.
the increase of tissue NEG levels. We found a reduction of the NEG increase in the tissues of the animals to which chard extract was administered, producing a significant decrease in the diabetic group.

Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders, including chemically induced diabetes. In diabetes, increases in lipid peroxidation can cause tissue damage. Lipid peroxide levels increased in STZ diabetic rat tissue. In our study, we have observed an increase in the levels of LPO in the liver in diabetes. Administration of chard extract significantly decreased the level of LPO in the diabetic rats. This indicates that the extract may be helpful in the prevention of damage caused by oxygen free radicals.

Hypoglycemic effects have been reported for some plants that contain saponins, alkaloids, tannins, and quinovic acid. Flavonoids have also been found to possess various other biological properties, for example, hepatoprotective, antithrombotic, and antiviral activity. Many actions have been correlated with their ability to scavenge oxygen-generated free radicals and to inhibit lipid peroxidation in vitro.

Phytochemical results showed that the chard extracts were rich in flavonoids and saponins, which are known to have strong antioxidant and free radical scavenging properties. Our results indicate that chard extract is effective in controlling hyperglycemia in STZ diabetic rats. In our other study, we observed the hypoglycemic and antidiabetic effects and the regenerating effect on B cells of the endocrine pancreas, both morphologically and biochemically. Increased oxidative stress in diabetic rats was successfully reduced by the extract. It is possible that the antihyperglycemic effects and the prominent improvement in the tissues may be related to the flavonoid and saponin content of this plant. In conclusion, due to the hypoglycemic, antioxidant, and improving effect of chard on liver, we can say that chard extract shows an ameliorating effect on liver injury caused by diabetes.

Acknowledgments

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References

32) Pari, L., and Umamaheswari, J., Antihyperglycaemic
31) Hintze, J. L., Copyright C, 865, East 400, North
28) Ledwozwy, A., Michalak, J., Stepien, A., and Kadziolka,
27) Beutler, E., ''Glutathione in Red Cell Metabolism: A
26) Caraway, W. T., Determination of uric acid in serum by
22) Reitman, S., and Frankel, S. A., Colorometric method for
23) Walter, K., and Schu ¨tt, C., Acid and alkaline phospha-
25) Lorentz, K., Weiss, T., and Kraas, E., Sialic acid in
20) Relander, A., and Raiha, C. E., Differences between the
19) Junod, A., Lambert, A. E., Stafffacher, W., and Renold,
30) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and
29) Parker, K. M., England, J. D., Casto, J. D., Hessel, K.,
21) Beutler, E., Duron, O., and Kelly, B. M., Improved
determination of total serum lipids by the
24) Machlin, L. J., and Bendich, A., Free radical tissue
damage: protective role of antioxidant nutrients. 
36) Baraona, E., Pikkarainen, P., Salaspuro, M., Finkelman,
F., and Lieber, C. S., Acute effects of ethanol on hepatic
protein synthesis and secretion in the rat. Gastroenterol.,
79, 104 (1980).
37) Saladini, M., Menabue, L., and Ferrari, E., Binding
ability of sialic acid towards biological and toxic metal
ions. NMR, potentiometric and spectroscopic study. 
38) Sönmez, H., Sürer, S., Güngör, Z., Kokoğlu, E., and Isbir,
T., Sialidase activities and sialic acid levels of the liver
and serum from streptozotocin induced diabetic rats. 
39) Vannucci, H., Araujo, W. F., Bernardes, M. M., and
Jordao-JR, A. A., Effect of different vitamin E levels on 
lipid peroxidation in streptozotocin-diabetic rats. Int. J. 
40) Machlin, L. J., and Bendich, A., Free radical tissue
damage: protective role of antioxidant nutrients. 
41) Sano, T., Umeda, F., Nawata, H., and Utsumi, H., 
Oxidative stress measurement by in vivo electron spin 
resonance spectroscopy in rats with streptozotocin-
42) Venkatesswaran, S., and Pari, L., Effect of Coccinia 
indica leaves on antioxidant status in streptozotocin-
induced diabetic rats. J. Ethnopharmacol., 84, 163–168 
(2003).
43) Yue, D. K., Mc Lennan, S., and Turtle, J. R., Non-
enzymatic glycosylation of tissue protein in diabetes in the 
44) Anderson, R. A., Roussel, A. M., Zouari, N., Mahjoub,
S., Matheau, J. M., and Kerkeni, A., Potential anti-
oxidant effects of zinc and chromium supplementation in 
people with type 2 diabetes mellitus. J. Am. Coll. Nutr.,
45) Kinalski, M., Sledziewski, A., Telejko, B., Zarzycki, W., 
and Kinalska, I., Lipid peroxidation and scavenging 
46) Prakasam, A., Sethupaty, S., and Pugalendi, K. V., Effect of 
Casearia esculenta root extraction on blood glucose and 
plasma antioxidant status in streptozotocin diabetic 
47) Ravikumar, P., and Anuradha, C. V., Effect of fenugreek 
seeds on blood lipid peroxidation and antioxidants in 
48) Wang, H. X., and Ng, T. B., Natural products with 
hypoglycemic, hypotensive, hypocholesterolemic, anti-
atherosclerotic and antithrombin activities. Life Sci., 6,
49) Sheweteia, S. A., Newairy, A. A., Mansour, H. A., and 
Yousef, M. I., Effect of some hypoglycemic herbs on the 
activity of phase I and II drug-metabolizing enzymes in 
alloxan-induced diabetic rats. Toxicology, 174, 131–139 
(2002).
50) Prashanth Kumar, V., Shashidhara, S., Kumar, M. M., and 
Sridhara, B. Y., Effect of Luffa echinata on lipid 
peroxidation and free radical scavenging activity. J. 
51) Kameswara Rao, B., Renuka Sudarshan, P., Rajasekhar,
M. D., Nagaraju, N., and Appa Rao, C., Antidiabetic 
activity of Terminalia pallida fruit in alloxan induced 