RESTORATION ON TISSUE ANTIOXIDANTS BY FENUGREEK SEEDS (TRIGONELLA FOENUM GRAECUM) IN ALLOXAN-DIABETIC RATS

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Abstract: The influence of fenugreek seed powder supplementation in the diet on lipid peroxidation and antioxidant status was studied in normal and alloxan-diabetic rats. The protective effect of the aqueous extract of the seeds on the activity of calcium-dependent adenosinetriphosphatase (Ca\textsuperscript{2+} ATPase) in liver homogenate in the presence of Fe\textsuperscript{2+}/ascorbate in vitro was also investigated. Normal and diabetic rats were provided with a diet supplemented with fenugreek seed powder for 30 days at a dosage of 2 g/kg body weight. The diabetic rats exhibited enhanced lipid peroxidation and increased susceptibility to oxidative stress associated with depletion of antioxidants in liver, kidney and pancreas. However, treatment with fenugreek seed powder normalised the alterations. In normal rats supplementation resulted in increased antioxidant status with reduction in peroxidation. Ca\textsuperscript{2+} ATPase activity in liver was protected by the aqueous extract to nearly 80% of the initial activity. The findings suggest that the soluble portion of the seeds could be responsible for the antioxidant property.

Key words: experimental diabetes tissue lipid peroxidation fenugreek seeds antioxidants

INTRODUCTION

Increased free radical generation and oxidative stress are hypothesised to play an important role in the pathogenesis of diabetes and its later complications (1). The scavenging ability of the cells decline as free radicals accumulate. Diabetic state is shown to be associated with depletion of antioxidants (2). Dietary supplementation of vitamin E, an antioxidant, can mitigate the peroxidation reactions and oxidative stress in diabetic animals (3).

Fenugreek seeds display hypoglycaemic and hypocholesterolaemic effects and are considered to be potentially useful for glucose control and in the treatment of hyperlipidaemia and atherosclerosis in diabetic subjects (4). The antioxidant

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property exhibited by the seeds has also been reported (5). This prompted us to study whether fenugreek seed supplementation restores the antioxidants and mitigates the oxidative stress in diabetic animals.

In the present study, we determined the effect of supplementation of whole fenugreek seeds in the diet of normal and alloxan-diabetic rats on tissue lipid peroxidation and antioxidant status. The protective effect of aqueous extract of the seeds of Ca$^{2+}$ ATPase activity in liver homogenate during iron-induced peroxidation was also investigated.

**METHODS**

**Plant material**

Fenugreek seeds were purchased from the local market and identified by a botanist from the Department of Botany, Annamalai University. The seeds were cleaned, dried and finely powdered. The powder was used for feeding the animals. The powder was mixed with the diet at a level of 2 g/kg body weight and used for feeding the animals.

For *in vitro* studies, an aqueous extract of the seeds was prepared and used. The seed powder was mixed with distilled water (1% w/v). This was vortexed and then centrifuged. The supernatant represented the aqueous extract and was prepared freshly.

**Chemicals**

Alloxan monohydrate, 1,1',3,3',-tetraethoxy propane, reduced glutathione (GSH) and 5,5' dithio-bis-2-nitro benzoic acid (DTNB) were purchased from the Sigma Chemical Co, St Louis MO, USA. Other chemicals used were of analytical grade and were obtained from Central Drug House, New Delhi, India.

**Animals**

Adult male albino rats of the Wistar strain weighing 150–170 g were obtained from the Department of Experimental Medicine, Raja Muthiah Medical College and Hospital, Annamalai Nagar and were kept in polypropylene cages in a controlled environment (22–24°C) under 12 h light-dark cycle. Commercial rat chow (Hindustan Lever Ltd., India) and water were provided *ad libitum*.

**Induction of diabetes**

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (5% w/v) in physiological saline at a dose of 100 mg/kg body weight in a volume of 0.5 ml. The diabetic state was confirmed 48 h after alloxan injection by weight loss, glucosuria (6) and hyperglycaemia (7). There was 75% mortality in animals treated with alloxan. Surviving rats with a fasting blood glucose level higher than 200 mg/dl were included in the study. Five groups consisting
of six animals each were maintained as follows.

**Experimental groups**

Group 1 - Normal rats injected with 0.5 ml of physiological saline formed the control group. These were fed commercial rat chow.

Group 2 - Diabetic rats maintained on commercial rat chow for 30 days.

Group 3 - Diabetic rats maintained on commercial rat chow mixed with fenugreek seed powder (2 g/kg body weight) for 30 days.

Group 4 - Normal rats maintained on commercial rat chow mixed with fenugreek seed powder (2 g/kg body weight) for 7 days, made diabetic and continued on the same diet (pretreated diabetic animals) for 30 days.

Group 5 - Normal rats injected with 0.5 ml of physiological saline maintained on the commercial rat chow mixed with fenugreek seed powder (2 g/kg body weight) for 30 days.

**General procedure**

Blood sugar levels were determined periodically by sino ocular puncture. The body weights of animals were recorded every third day and food intake was measured daily.

At the end of 30 days, the rats were fasted overnight and killed by cervical dislocation. Tissues liver, kidney and pancreas were excised immediately and processed for analysis.

**Biochemical measurements**

**Thiobarbituric acid reactive substances (TBARS)**

Lipid peroxidation in tissue homogenates was determined by quantitating the thiobarbituric acid reactive-substances (TBARS) after incubation in the presence and absence of inducers (8). The incubation mixtures contained requisite amount of tissue homogenate and either ascorbate-iron or hydrogen peroxide as inducers (9). The release of TBARS was determined after incubation at 37°C for 20 minutes with vigorous shaking. 1,1′3′3′-Tetra ethoxy propane was used as the standard.

**Diene conjugates**

Diene conjugates were determined by the method of Klein 1970 (10). Aliquots of lipid extract obtained from tissues were evaporated to dryness and suspended in the methanol. The absorbance at 215 nm and 233 nm were measured against a solvent blank. "Oxidation index", the ratio of the absorbance at 233 nm to absorbance at
233 nm (A233/215), was computed which reflected the extent of peroxidation in lipid samples.

Superoxide dismutase, catalase and glutathione peroxidase

Superoxide dismutase (SOD) (EC 1.15.1.1) was assayed by the inhibition of the formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan (11). The method of Rotruck et al. (12) was used for the assay of glutathione peroxidase (EC 1.11.1.9) by the disappearance of reduced glutathione and catalase (EC 1.11.1.6) was assayed by the method of Sinha, (13) using hydrogen peroxide as substrate.

Thiols, ascorbic acid and vitamin E

Non-protein and protein-bound thiols were estimated in the tissue homogenate by the method of Sedlack and Lindsay (14). Tissue contents of ascorbic acid (15) and α-tocopherol (16) were also measured.

Ca²⁺ ATPase

Ca²⁺ ATPase (E.C.3.6.1.3) in the tissue homogenates was determined according to the method of Hjertan and Pan (17). Protein content of the homogenates was assayed by the method of Lowry et al. (18).

In vitro studies

The influence of oxidants on the activity of Ca²⁺ ATPase and protective effect of the seed extract in vitro was studied as follows.

Liver homogenates from normal animals were preincubated with 1 ml of the aqueous extract for 10 minutes after which Fe²⁺/ascorbate (50 µmol/L and 0.2 µmol/L) were added. The samples were incubated with vigorous shaking for 30 and 60 minutes. Control samples were incubated in the absence of seed extract. After the requisite time the enzyme activity was determined and expressed as µmoles of phosphorous liberated/min/mg protein at 37°C.

Statistical analysis

The quantitative measurements were made on six animals in each group and the values were expressed as means ± SD. The statistical significance of differences between two independent groups was performed by the Student's t-test. A value of P<0.05 was considered significant. The data was subjected to the analysis of variance (ANOVA) to determine the significance of changes between and within the experimental groups. The protective effect for Ca²⁺ ATPase activity was calculated as
\[
1 - \left( \frac{T_c - T_f}{T_c - T_a} \right) \times 100
\]

Where \( T \) is the parameter level, \( a, f \) and \( c \) are alloxan-treated, fenugreek-treated and control rats respectively.

**RESULTS**

**Body weight changes and food intake**

The mean initial body weight of each group was 150–170 g. The control and animals treated with fenugreek seeds (group 1, 3, 4 and 5) registered a weight gain, while the untreated diabetic animals (group 2) showed a progressive reduction in body weight. The final body weights of untreated diabetic animals (137 ± 3.4 g) were significantly lower \((P<0.001)\) than that of control animals (211 ± 4.36 g). Fenugreek-treated diabetic animals (groups 3 and 4) showed a significant gain in weight \((P<0.001)\) during the experimental period compared with diabetic animals. Group 5 animals gained weight similar to that of control animals. Food intake was similar in all groups (Table I).

**Lipid peroxidation products – TBARS**

The release of TBARS in the liver, kidney and pancreas homogenates in the presence and absence of stimulants is presented in Figures 1, 2 and 3. The level of TBARS is significantly higher in the diabetic animals than in the control animals basally and in the presence of stimulants. Fenugreek treatment restored the peroxidation reaction promoted by ascorbate and hydrogen peroxide systems in all the three tissues studied (groups 3 and 4). The amount of TBARS released was significantly lower in group 5 animals than in group 1 under both unstimulated and stimulated conditions.

**TABLE I:** Body weight and food intake of control and experimental animals. Values are given as means ± SD for six animals in each group.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
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<tr>
<td>Body weight (g)</td>
<td></td>
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</tr>
<tr>
<td>Initial</td>
<td>158.5±7.2</td>
<td>162.2±6.3</td>
<td>167.4±2.1</td>
<td>162.0±4.2</td>
</tr>
<tr>
<td>Final</td>
<td>211.2±4.4</td>
<td>137.1±3.4*</td>
<td>195.3±3.1b</td>
<td>190.5±5.1b</td>
</tr>
<tr>
<td>Food intake (g/kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>83.5±3.2</td>
<td>84.1±2.3</td>
<td>83.8±6.1</td>
<td>84.2±2.5</td>
</tr>
<tr>
<td>Final</td>
<td>98.8±4.6</td>
<td>98.2±5.2</td>
<td>99.7±3.8</td>
<td>97.5±3.6</td>
</tr>
</tbody>
</table>

Treatment and comparisons: Group 1-Control; Group 2-Diabetic; Group 3-Diabetic + Fenugreek; Group 4-Diabetic pretreated with Fenugreek; Group 5-Control + Fenugreek.

* a-significantly different compared to control \((P<0.05)\);

* b-significantly different compared to control \((P<0.05)\).
**FIGURE 1**

Fig. 1: The levels of TBARS in liver of control and experimental rats in the presence of promoters of peroxidation. Values are means ± SD for 6 animals in each group. *-Significantly different (P<0.05) from control; #-significantly different (P<0.05) from diabetic Group 1-control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-Diabetic pretreated with fenugreek; Group 5-Control + fenugreek.

**FIGURE 2**

Fig. 2: The levels of TBARS in kidney of control and experimental rats in the presence of promoters of peroxidation. Values are means ± SD for 6 animals in each group. *-Significantly different (P<0.05) from control; #-significantly different (P<0.05) from diabetic Group 1-control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-Diabetic pretreated with fenugreek; Group 5-Control + fenugreek.
The levels of TBARS in pancreas of control and experimental rats in the presence of promoters of peroxidation values are means ± SD for 6 animals in each group. *-Significantly different (P<0.05) from control; #-Significantly different (P<0.05) from diabetic. Group 1-control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-Diabetic pretreated with fenugreek; Group 5-Control + fenugreek.

**Diene conjugates**

Table II presents the concentration of diene conjugates in liver and kidney of control and experimental animals. Increased level of diene conjugates was observed in the diabetic rat liver and kidney as compared to control animals (P<0.01). The levels were near normal in group 3, 4 and 5 animals, which were fed fenugreek seed powder diet.

**TABLE II:** Contents of diene conjugates in liver and kidney of control and experimental animals. Values are ratio between A_{233} and A_{19} and are given as means ± S.D for six animals in each group.

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.67±0.08</td>
<td>0.9±0.12</td>
<td>0.61±0.15</td>
<td>0.63±0.09</td>
<td>0.51±0.11</td>
<td>13.30</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.60±0.09</td>
<td>0.87±0.02</td>
<td>0.65±0.11</td>
<td>0.72±0.08</td>
<td>0.58±0.09</td>
<td>35.49</td>
</tr>
</tbody>
</table>

Treatment and comparisons: Group 1-Control; Group 2-Diabetic; Group 3-Diabetic + Fenugreek; Group 4-Diabetic pretreated with Fenugreek; Group 5-Control + Fenugreek.

a-significantly different compared to control (P<0.05);

b-significantly different compared to diabetic (P<0.05); x - significant at 5% level.
Antioxidant enzymes

Table III presents the activities of the antioxidant enzymes in the tissues in the control and experimental animals. Significant reduction in the activity of SOD in liver and kidney of diabetic animals was observed. Diabetic rats treated with fenugreek seed powder showed normal enzyme activity. Increased activity was observed in tissues of normal animals treated with fenugreek seeds (group 5).

Catalase activity was significantly (P<0.01) lower in liver and kidney while in pancreas the activity was significantly (P<0.01) higher in diabetic animals as compared to control animals. The activity was near normal in fenugreek treated and pre-treated diabetic rats.

Glutathione peroxidase activity was reduced by 33% in the liver and elevated by 62% and 64% in kidney and pancreas respectively in the diabetic animals as compared to control animals. Activity was near-normal in treated and pre-treated animals.

Thiols, ascorbic acid and α-tocopherol

The levels of the non-enzymic antioxidants in tissues are presented in Figures 4, 5 and 6. Diabetic animals showed

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>SOD</td>
<td>4.24±0.09</td>
<td>2.32±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.64±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.40±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.11±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>71.40±3.08</td>
<td>41.9±2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.6±3.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.5±3.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.7±3.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSHPx</td>
<td>3.82±0.47</td>
<td>2.53±0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.47±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
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<tr>
<td>SOD</td>
<td>2.39±0.34</td>
<td>1.62±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.53±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.48±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.48±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>53.30±3.40</td>
<td>36.2±2.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.5±3.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.8±2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.6±8.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSHPx</td>
<td>2.71±0.28</td>
<td>4.40±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.87±0.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.62±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.73±0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>1.42±0.46</td>
<td>0.59±0.36</td>
<td>1.29±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.37±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.46±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>8.90±1.10</td>
<td>19.03±2.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.23±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.78±1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2±2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSHPx</td>
<td>2.10±0.37</td>
<td>3.45±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.99±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.98±0.49</td>
<td>2.45±0.25</td>
<td>17.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Treatment and comparisons: Group 1-Control; Group 2-Diabetic; Group 3-Diabetic + Fenugreek; Group 4-Diabetic pretreated with Fenugreek; Group 5-Control + Fenugreek.

<sup>a</sup>-significantly different compared to control (P<0.05);
<sup>b</sup>-significantly different compared to diabetic (P<0.05); 
<sup>x</sup>-significant at 5% level.
Fig. 4: Levels of total, non-protein and protein bound thiols, ascorbic acid and vitamin E in liver of control and experimental animals. The values are means ± SD for 6 animals in each group. Group 1-Control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-Diabetic pretreated with fenugreek; Group 5-control + fenugreek *-significantly different (P<0.05) from control; #-significantly different (P<0.05) from diabetic.

Fig. 5: Levels of total, non-protein and protein bound thiols, ascorbic acid and vitamin E in kidney of control and experimental animals. The values are means ± SD for 6 animals in each group. Group 1-Control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-Diabetic pretreated with fenugreek; Group 5-control + fenugreek *-significantly different (P<0.05) from control; #-significantly different (P<0.05) from diabetic.
Fig. 6: Levels of total, non-protein and protein bound thiols and ascorbic acid in pancreas of control and experimental animals. The values are means ± SD for 6 animals in each group. Group I-Control; Group 2-diabetic, Group 3-Diabetic + fenugreek; Group 4-diabetic pretreated with fenugreek; Group 5-control + fenugreek. *-significantly different (P<0.05) from control; #-significantly different (P<0.05) from diabetic.

a significant reduction in total, protein bound and non protein thiols, ascorbic acid and α-tocopherol levels. The levels were normal in animals treated with fenugreek seeds. Further it is noted that the antioxidant status in tissue of normal animals which were fed with fenugreek supplemented diet was improved as compared to control animals.

### Ca\(^{2+}\) ATPase

Table IV shows the activities of Ca\(^{2+}\) ATPase in the tissues of animals. The

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.83±0.21</td>
<td>0.60±0.24*</td>
<td>1.80±0.33* (97%)</td>
<td>1.77±0.30* (95%)</td>
<td>1.83±0.32</td>
<td>22.02*</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.70±0.45</td>
<td>0.66±0.023*</td>
<td>1.63±0.34* (97%)</td>
<td>1.65±0.28* (97%)</td>
<td>1.61±0.30</td>
<td>13.06*</td>
</tr>
</tbody>
</table>

Treatment and comparisons: Group 1-Control; Group 2-Diabetic; Group 3-Diabetic + Fenugreek; Group 4-Diabetic pretreated with Fenugreek; Group 5-Control + Fenugreek. Values given in parenthesis indicate percentage protection.

* - significantly different compared to control P<0.05;
* - significantly different compared to diabetic P<0.05; x - significant at 5% level.
activity was significantly declined in diabetic animals. Fenugreek seed administration for 30 days exerted a protection of 97% in liver and kidney in group 3 and 95% and 97% in liver and kidney respectively in group 4 animals.

**In vitro studies - Protective effect of the seed extract on Ca\(^{2+}\) ATPase activity**

The results of *in vitro* studies on Ca\(^{2+}\) ATPase activity in liver homogenates are depicted in Fig. 7. Ca\(^{2+}\) ATPase activity was observed to decrease by 60% when the homogenate was subjected to lipid peroxidation induced by Fe\(^{2+}/\)ascorbate system. Further the reduction in activity was increased with the time of incubation. The addition of the aqueous extract from the seeds protected the enzyme from inactivation even in the presence of promoters of lipid peroxidation. About 80% of the enzyme activity could be restored by incubating the homogenate with seed extract.

![Fig. 7: The protective effect of aqueous extract (1 mg/ml) of fenugreek seeds on Ca\(^{2+}\) ATPase activity *in vitro* in the presence and absence of Fe\(^{2+}/\)ascorbate system. *-Values are significantly different (P<0.05) from those obtained in the absence of extract. **-Values are significantly different (P<0.05) from those obtained in the absence of stimulants.](image)

**DISCUSSION**

A marked increase in the concentration of TBARS and diene conjugates is observed in tissues of diabetic rats. The tissues also showed an increased susceptibility to peroxidation in the presence of stimulants suggesting that the diabetic tissue are less resistant towards oxidants owing to the depletion of antioxidants.

Among the enzymic antioxidants, SOD activity declined in all three tissues and that of catalase in liver and kidney. Glutathione peroxidase activity declined only in liver. Reduction in antioxidant enzyme activities in diabetic tissues have been reported (19, 20). The reduction in the activities can be attributed to inactivation of the enzymes by glycation. For example Cu, Zn-SOD is inactivated by the glycation of specific lysine residues Lys 122 and Lys (21). Reactive oxygen radicals can themselves reduce the activity of the antioxidant enzymes such as catalase and glutathione peroxidase (22). The impairment of antioxidant system can be correlated to the increased lipid peroxidation products found in tissues.

The levels of total and non-protein thiols are also lower in diabetic animals representing increased utilisation due to oxidative stress. The thiols (predominantly GSH) are important compounds which are required to keep up the cellular levels of the active forms of other antioxidants such as vitamins C and E in their reduced forms. These vitamins exist in interconvertible (reduction and oxidised) forms. Thus reduction in the levels of antioxidant vitamins (C and E) can be attributed to the reduced regeneration from their oxidised forms.
Decreased Ca\(^{2+}\) ATPase can be attributed to increased peroxidation. Inactivation of Ca\(^{2+}\) ATPase activity in sarcoplasmic reticulum by oxygen-mediated lipid peroxidation in cardiac tissue has been reported (23). Alterations in membrane lipid composition (24) and increased sarbitol content (25) are also implicated in the derangement of ATPase activity in diabetes.

Whole fenugreek seeds contain 48% total fibre, which includes 20% gum and 28% neutral detergent fibre (NDF) and about 4% of saponins (26). Further, fenugreek seeds are reported to contain high levels of flavonoids (>100 mg/100 g) (27). It could be possible that saponins, NDF and flavonoids present in high quantities in fenugreek seeds augment the antioxidant potential and thus are responsible for the reduction in lipid peroxidation in tissues. Further supplementation of fenugreek seeds in the diet was found to restore the antioxidants to normal levels in diabetic animals and to enhance the levels in pretreated and normal animals.

Aqueous extract of the seeds prevented Ca\(^{2+}\) ATPase from inactivation in the presence of Fe\(^{2+}\)/ascorbate system, a promoter of lipid peroxidation. We have earlier reported that the aqueous extract showed strong antioxidant activity \textit{in vitro} (5).

Oxidation of sulphhydryl groups of Ca\(^{2+}\) ATPase in the sarcoplasmic reticulum by oxidative stress is partially restored in the presence of reduced thiol reagents (28). Thus the protection of Ca\(^{2+}\) ATPase by seeds extract may be mediated through the protection of –SH groups of the enzyme.

The findings of the present study show that increased tissue lipid peroxidation associated with reduction in antioxidant status in diabetic rats are mitigated by fenugreek seed administration. Supplementation also increased the antioxidant potential in normal rats. Long-term feeding of fenugreek seeds in diabetic patients has been shown to be non-toxic (29). Thus the results of our study are highly significant which demonstrate the potential efficacy on fenugreek seeds in protecting the tissues from peroxidative damage.

REFERENCES

420 Anuradha and Ravikumar


