Effect of some medicinal plant oils on some physiological parameters in streptozotocin induced diabetic rat

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The present study was aimed to compare the efficacy of neem, sesame, sunflower, evening primrose, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils supplementation in streptozotocin (STZ)-diabetic and control male Wister rats. In comparison with control, highly significant increases in the values of blood glucose (318.7%), triglycerides (62.3%), total cholesterol (99.2%), low density lipoprotein cholesterol (170%), total protein (25.5), creatinine (82.3%), urea (142.8%), uric acid (191.1%), alanine aminotransferase (118.9%) and aspartate aminotransferase (35.3%) were observed in STZ-diabetic rats, while the value of high density lipoprotein cholesterol was markedly decline (66.3%). No significant differences were observed in the above physiological parameters of diabetic rats fed on the examined oils when compared with those rats fed on the control diet after 7 weeks. These data indicate that the diets containing the oils of these medicinal plants improve the examined physiological parameters in STZ-induced diabetic rats. The present data suggest that using the oils of these medicinal plants may improve blood parameters in STZ-induced diabetic rats. The responses in blood parameters in these animals have also demonstrated that oils supplementation may act as antioxidant agents and these oils could be an excellent adjuvant support in the therapy of diabetic mellitus and its complications.

Key words: Medicinal plants, streptozotocin, diabetic rats, blood parameters.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by uncontrolled blood glucose levels. The prevalence of diabetes mellitus in the United States population is increasing in epidemic proportions (Center for Disease Control, 2005). The increasing prevalence merits concern because the disease puts those afflicted at risk for early death and complications resulting from severe damage to many of the body’s systems, in particular the blood vessels and nerves (Gerich, 2001; National Center for Health Statistics, 2005). In 2005, 1.5 million new cases of diabetes were diagnosed in people age 20 or older (Center for Disease Control, 2005). The World Health Organization predicts that by 2030, 366 million people worldwide will have diabetes (World Health Organization, 2005).

Plant foods, the foods derived from plant sources, such as vegetables, fruits, grains, legumes and nuts are generally recommended to be included in the diet mostly to prevent the development of chronic diseases (National Nutrition Council 1999; Franz et al. 2002). These plant foods are rich sources of nutrients that are hypothesized to prevent development of type 2 diabetes such as fiber, antioxidant vitamins, minerals, unsaturated fatty acids, folic acid, phytoestrogens and phenolic compounds (Prior 2003; Slavin 2004). Another important factor thought to mediate the effect of plant foods is reduction of the index in mixed meals (Jenkins 1988).

Besides the nutrients, studies have shown that postprandial glucose responses can be affected by the structure of food (Juntunen et al., 2003). After all, plant foods are usually rather satiating foods and the preventative effect can be mediated by lower energy intake. Other large American prospective studies have demonstrated an inverse association between dietary
magnesium and the risk of type 2 diabetes (Lopez-Ridaura et al., 2004). However, two other studies failed to demonstrate this inverse association (Meyer et al., 2000). In human experimental studies, some (Paolisso et al., 1994), but not all (De Valk et al., 1998) have shown the beneficial result of magnesium supplementation on glucose metabolism or insulin sensitivity. Decreased erythrocyte and plasma potassium concentrations have been associated with glucose intolerance (Modan et al., 1987). Intervention studies of chromium on glucose and insulin responses have shown no effect among persons free of diabetes (Althuis et al., 2002). Vegetable fat has been associated with a reduced diabetes risk in several previous follow-up studies (Meyer et al., 2001; Laaksonen et al., 2002).

However, contradictory findings exist (Van Dam et al., 2002). Available data on the specific type of fatty acids consumed and development of type 2 diabetes suggest a potential beneficial effect of polyunsaturated fatty acids (Salmeron et al., 2001) and a potential adverse effect of saturated fatty acids (Wang et al., 2003). With the exception of one study on patients with impaired glucose tolerance (Marshall et al., 1994), epidemiological studies have failed to find an association between monounsaturated fatty acids and risk of type 2 diabetes (Salmeron et al., 1997). Monounsaturated fatty acids have also been positively associated with insulin concentrations (Mayer et al., 1993), but the association could be due to correlation with saturated fatty acids (Hu et al., 2001).

Epidemiological evidence on phytoestrogens and glucose metabolism is almost absent. In one cross-sectional study, dietary isoflavone consumption has been inversely associated with fasting and 2-h postload insulin levels among postmenopausal women (Goodman-Gruen & Kritz-Silverstein, 2001). A human intervention study demonstrated a beneficial effect of a soy phytoestrogen supplementation on glucose metabolism among persons with diabetes (Jayagopal et al., 2002), although no change in glycedated hemoglobin level was observed in another study (Hermansen et al., 2001). Elevated plasma homocystein level has been related to diabetes complications (Colwell et al., 1997) and prospective studies have suggested a stronger relationship between homocystein and mortality in persons with diabetes than without diabetes (Audelin and Genest 2001). Higher plasma concentrations of homocystein have been found in persons with diabetes in some studies (Chico et al., 1998; Cronin et al. 1998; Hofmann et al., 1998).

However, several studies have reported normal homocystein concentrations in persons with diabetes (Audelin and Genest 2001). To date, there are no prospective studies that have examined the relationship between elevated levels of homocystein and the incidence of type 2 diabetes. Epidemiologic evidence suggests that replacing high glycemic index forms of carbohydrate with low ones will reduce the risk of chronic diseases (Jenkins et al., 2002). Among persons with diabetes, the consumption of foods with a low glycemic index has been shown to improve glycemic control (Opperman et al., 2004). Large-scale cohort studies have shown direct association between the glycemic index and glycemic load with diabetes incidence (Schulze et al., 2004).

However, no association was found either for the glycemic index or glycemic load in the Iowa Women Study (Meyer et al., 2001). This research aimed to evaluate the effects of administration of some plant oils (neem oil, sesame oil, evening primrose oil and sunflower oil) on some physiological responses in streptozotocin-induced diabetic rat.

MATERIALS AND METHODS

Materials

Streptozotocin (STZ) was purchased from Sigma Chemical Company, St. Louis, MO, USA. The oils of neem (Azadirachta indica), sesame (Sesamum indicum) and sunflower (Helianthus annuus) were obtained from Dreams Essential Oils Est., Alexandria, Egypt. Evening primrose (Oenothera biennis) oil was obtained from Martinez Nieto, S.A., Cartagena, Spain. Nature neem oil was obtained by cold pressing the seed kernel of good quality seeds and sesame oil was obtained by cold pressing the seeds (Biswa, 2002).

Animals

Healthy young adult male Wister rats weighing (225–252 g) were obtained from The Animal Experimental Unit of Faculty of Agriculture, Alexandria University. The rats were housed in well-aerated individual cages and maintained in a temperature-controlled room (24 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. They were fed with normal commercial chow and water ad libitum. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals.

Methods

Induction of diabetes

The experimental animals were fasted for 12 hours and then diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma Chemical Company, St. Louis, MO, USA), dissolved in a freshly prepared physiological saline solution (0.9% NaCl) at a dose of 65
mg/kg body. Normal rats received only the saline solution (0.9% NaCl) in the same volume and through the same route. After injection, all animals were returned to their cages and given free access to food and water.

After 3 days, the fasting blood glucose levels were measured from tail blood samples by using a One Touch Ultra® glucometer (Lifescan; Johnson & Johnson Company, Milpitas, CA, USA). Animals with blood glucose levels more than 277 mg/dl were considered diabetic and used for the experiment.

**Experimental design**

A total of 160 rats were used in the experiment. The rats were divided into 16 groups of 10 animals each as follows: Group 1: Normal control (Non-diabetic normal rats) received normal commercial chow and water ad libitum. Group 2: STZ-Control (diabetic control rats) received the same diet given in group 1. Group 3: STZ + neem oil received diet containing 5% neem oil. Group 4: STZ + sesame oil received diet containing 5% sesame oil. Group 5: STZ + evening primrose oil received diet containing 5% evening primrose oil (EPO). Group 6: STZ + sunflower oil received diet containing 5% Sunflower oil. Group 7: STZ + neem oil plus sesame oil received diet containing 2.5% neem oil plus 2.5% sesame oil. Group 8: STZ + neem oil plus EPO oil received diet containing 2.5% neem oil plus 2.5% primrose oil (EPO).

Group 9: STZ + neem oil plus sunflower oil received diet containing 2.5% neem oil plus 2.5% sunflower oil. Group 10: STZ + sesame oil plus EPO oil received diet containing 2.5% sesame oil plus 2.5% primrose oil (EPO). Group 11: STZ + sesame oil plus sunflower oil received diet containing 2.5% sesame oil plus 2.5% sunflower oil. Group 12: STZ + primrose oil (EPO) plus sunflower oil received diet containing 5% primrose oil (EPO) plus 2.5% sunflower oil. Group 13: Normal group (Non-diabetic normal rats) received diet containing 5% neem oil. Group 14: Normal group (Non-diabetic normal rats) received diet containing 5% sesame oil. Group 15: Normal group (Non-diabetic normal rats) received diet containing 5% primrose oil (EPO). Group 16: Normal group (Non-diabetic normal rats) received diet containing 5% sunflower oil. All of the experimental groups received the treatments for a period of 7 weeks.

**Blood collection and determination of physiological parameters**

After 7 weeks, the rats were fasted for 8 h before blood sampling, water was not restricted. Blood samples were collected from the orbital venous plexus of the rat under mild ether anaesthesia by heparinized capillary tube and into non-heparinized tubes, indicated that brief exposure and little amount of anesthetic used do not influence the activity of hepatic cytochrome P450 2E1 and P450 reeducates in the rat. Clear serum samples were separated by centrifugation at 3000 rpm for 20 min and then collected and stored at -20 °C for different biochemical analyses, prior immediate determination of glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol (HDL-C), low density lipoprotein LDL-cholesterol (LDL-C), total protein, creatinine, urea, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST). All of these parameters were measured using an automatic analyzer (Architect c8000 Clinical Chemistry System, USA).

**Statistical analysis**

Statistical analyses were performed using SPSS package for Windows version 13.0. Data are expressed as mean ± SE. One-way ANOVA and two-way ANOVA were used to analyze differences among groups. Post-hoc analyses of significance were made using least-significant difference (LSD) test. Differences between groups were considered statistically significant at p<0.05.

**RESULTS**

**Blood glucose**

The mean values of blood glucose of both control and experimental groups are presented in Table 1. STZ-induced diabetic rats showed a highly significant (p<0.001) increase in the levels of blood glucose, registering increases of 318.7% after 7 weeks compared to the controls. Administration of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils to diabetic rats resulted in a significant (p<0.001) decrease in blood glucose levels after 7 weeks compared to untreated diabetic rats. On the other hand, the administration of sesame oil produced the most significant reduction (p<0.001) among the test oils in the blood glucose levels of 73.1% after 7 weeks of treatment. No significant differences were observed in blood glucose level of normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood glucose levels than those of the control group.
Blood triglyceride, cholesterol, LDL-C and HDL-C

The changes in the levels of serum lipids in control and experimental groups are illustrated in Table 1. There was a significant (p<0.001) decrease in the level of HDL-cholesterol (66.3%) and significant (p<0.001) increases in the levels of cholesterol, LDL-cholesterol and triglycerides in STZ-induced diabetic rats, with percentages of 170%, 99.2% and 62.3% respectively, compared to the controls. However, treatment of STZ-induced diabetic rats with neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils resulted in a significant (p<0.01) decrease in the levels of triglycerides, cholesterol and LDL-cholesterol compared to untreated diabetic rats. While HDL-cholesterol level was significantly (p<0.01) increased.

No significant differences were observed in the levels of triglycerides, cholesterol and LDL-cholesterol of healthy rats fed on diets containing the oil of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet. Moreover, no significant differences were observed in HDL-cholesterol level of healthy rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood cholesterol, LDL-cholesterol and triglycerides levels than those exposed to the same diets for 3 weeks (p<0.05). STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had higher blood HDL-cholesterol than those of the control group (p<0.05). (Figure 2), (figure 3), (figure 4) and (figure 5).

Blood total protein

The mean values of blood total protein concentrations in normal and STZ-induced diabetic rats are presented in Table 2. STZ-induced diabetic rats showed significant (p<0.01) increases in blood total protein concentrations with percentage of 25.5% compared to control after 7 weeks. On the other hand, the treatment with sesame, neem plus primrose and sesame plus sunflower oils lead to significant (p<0.05) decreases in the levels of blood total protein in STZ-induced diabetic rats by 18.4%, 18.6% and 18.6% respectively, compared with untreated STZ-induced diabetic rats after 7 weeks. Moreover, the administration of sesame oil produced the most significant reduction (p<0.001) among the test oils in the blood total protein levels of 18.9% after 7 weeks of treatment. No significant differences were observed in blood level total protein of normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood total protein levels than those of the control group (p<0.05). (Figure 6)

Blood creatinine

The mean values of blood creatinine of both control and experimental groups are presented in Table 2. STZ-induced diabetic rats showed a highly significant (p<0.001) increase in the levels of blood creatinine, registering increases of 82.3% after 7 weeks compared to the controls. Administration of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils to diabetic rats resulted in a significant (p<0.001) decrease in blood creatinine levels after 7 weeks compared to untreated diabetic rats. On the other hand, the administration of sesame plus evening primrose oils produced the most significant reduction (p<0.001) among the test oils in the blood creatinine levels of 45.2% after 7 weeks of treatment. No significant differences were observed in blood level of creatinine normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood creatinine levels than those of the control group (p<0.05). (Figure 7)

Blood uric acid

The mean values of blood uric acid concentrations in
Table 1. Effects of neem, sesame, sunflower, evening primrose, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils supplementation on blood glucose, triglyceride, cholesterol, LDL-C and HDL-C in normal and STZ-induced diabetic rats after 7 weeks of treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>97.19±2.3</td>
<td>62.12±2.5</td>
<td>64.38±3.3</td>
<td>42.55±0.2</td>
<td>33.01±1.3</td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td>406.88±5.2</td>
<td>100.79±5.4</td>
<td>128.23±5.4</td>
<td>14.34±0.4</td>
<td>89.26±6.6</td>
</tr>
<tr>
<td>STZ + neem oil</td>
<td></td>
<td>127.53±4.4</td>
<td>70.38±5.0</td>
<td>79.11±3.2</td>
<td>38.18±0.2</td>
<td>45.31±2.2</td>
</tr>
<tr>
<td>STZ + sesame oil</td>
<td></td>
<td>108.51±5.2</td>
<td>63.02±4.1</td>
<td>67.18±2.1</td>
<td>39.31±0.4</td>
<td>35.32±2.4</td>
</tr>
<tr>
<td>STZ + evening primrose oil</td>
<td></td>
<td>118.38±5.1</td>
<td>75.17±3.8</td>
<td>70.31±2.4</td>
<td>38.23±0.5</td>
<td>38.54±3.2</td>
</tr>
<tr>
<td>STZ + sunflower oil</td>
<td></td>
<td>135.16±4.2</td>
<td>72.51±3.1</td>
<td>78.19±3.2</td>
<td>37.32±0.1</td>
<td>45.22±2.1</td>
</tr>
<tr>
<td>STZ + neem plus sesame oils</td>
<td></td>
<td>100.26±2.2</td>
<td>65.28±3.2</td>
<td>66.21±1.3</td>
<td>38.00±0.5</td>
<td>35.00±1.2</td>
</tr>
<tr>
<td>STZ + neem plus primrose oils</td>
<td></td>
<td>130.11±4.8</td>
<td>72.74±3.6</td>
<td>72.66±3.2</td>
<td>40.61±0.1</td>
<td>38.35±3.2</td>
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<tr>
<td>STZ + neem plus sunflower oils</td>
<td></td>
<td>138.57±2.1</td>
<td>74.16±2.2</td>
<td>83.62±3.1</td>
<td>38.36±0.2</td>
<td>32.11±3.1</td>
</tr>
<tr>
<td>STZ + sesame plus evening primrose oils</td>
<td></td>
<td>102.86±4.2</td>
<td>65.24±3.5</td>
<td>72.51±3.3</td>
<td>41.00±0.2</td>
<td>31.12±2.1</td>
</tr>
<tr>
<td>STZ + sesame plus sunflower oils</td>
<td></td>
<td>121.33±5.9</td>
<td>64.46±1.8</td>
<td>73.15±2.5</td>
<td>38.21±0.2</td>
<td>35.25±2.6</td>
</tr>
<tr>
<td>STZ + evening primrose plus sunflower oils</td>
<td></td>
<td>145.72±6.3</td>
<td>74.88±5.6</td>
<td>78.23±3.6</td>
<td>36.62±0.1</td>
<td>40.98±3.1</td>
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<td>Neem oil</td>
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<td>93.13±4.1</td>
<td>64.90±2.1</td>
<td>61.21±1.5</td>
<td>41.62±0.2</td>
<td>33.51±2.9</td>
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<td>sesame oil</td>
<td></td>
<td>97.63±2.3</td>
<td>61.62±2.8</td>
<td>62.62±2.3</td>
<td>40.22±0.2</td>
<td>34.12±2.2</td>
</tr>
<tr>
<td>evening primrose oil</td>
<td></td>
<td>93.21±2.0</td>
<td>62.53±1.9</td>
<td>63.20±1.3</td>
<td>42.15±0.1</td>
<td>31.56±2.4</td>
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<tr>
<td>sunflower oil</td>
<td></td>
<td>94.88±2.5</td>
<td>61.42±2.6</td>
<td>61.92±2.7</td>
<td>40.26±0.1</td>
<td>30.21±1.8</td>
</tr>
</tbody>
</table>

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10.
All values are expressed as means ± SE.
Significantly different from untreated STZ-induced diabetic rats (* p < 0.05, ** p < 0.01 and *** p < 0.001).
Significantly different from normal control (# p < 0.05, ## p < 0.01 and ### p < 0.001).

Figure 1. Blood glucose levels (means ± SE) of different treatments in normal and STZ-induced diabetic rats after 7 weeks.
normal and STZ-induced diabetic rats are presented in Table 2. STZ-induced diabetic rats showed significant (p<0.01) increased in blood uric acid concentrations with percentage of 191.1% compared to control after 7 weeks. On the other hand, the treatment with sesame, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils lead
Figure 4. Blood LDL-cholesterol levels (means ± SE) of different treatments in normal and STZ-induced diabetic rats after 7 weeks.

Figure 5. Blood HDL-cholesterol levels (means ± SE) of different treatments in normal and STZ-induced diabetic rats after 7 weeks.

to significant (p<0.01) decreases in the levels of blood uric acid in STZ-induced diabetic rats compared with untreated STZ-induced diabetic rats after 7 weeks. Moreover, the treatment with neem, evening primrose and sunflower oils lead to significant (p<0.05) decreases in the levels of blood uric acid in STZ-induced diabetic rats compared with untreated STZ-induced diabetic rats after 7 weeks. STZ-induced diabetic rats exposed to the
Table 2. Effects of neem, sesame, sunflower, evening primrose, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils supplementation on blood total protein, urea, uric acid, creatinine, AST and ALT in normal and STZ-induced diabetic rats after 7 weeks of treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>Total protein (g/L)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
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<td>Normal control</td>
<td></td>
<td>5.11± 0.1</td>
<td>1.13± 0.0</td>
<td>3.04± 0.1</td>
<td>19.45± 1.5</td>
<td>37.54± 2.8</td>
<td>80.21± 1.1</td>
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<td>STZ</td>
<td></td>
<td>6.36± 0.1**</td>
<td>2.06± 0.1***</td>
<td>8.85± 0.1**</td>
<td>47.23± 1.8***</td>
<td>82.21± 4.43##</td>
<td>108.54± 3.2##</td>
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<tr>
<td>STZ + neem oil</td>
<td></td>
<td>5.36± 0.1***</td>
<td>1.30± 0.0***</td>
<td>7.11± 0.1*</td>
<td>31.91± 1.2***</td>
<td>48.54± 3.4**</td>
<td>93.72± 4.1**</td>
</tr>
<tr>
<td>STZ + sesame oil</td>
<td></td>
<td>5.19± 0.1*</td>
<td>1.18± 0.1***</td>
<td>6.32± 0.1**</td>
<td>29.12± 1.1***</td>
<td>42.21± 3.5**</td>
<td>84.29± 3.2**</td>
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<td>STZ + evening primrose oil</td>
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<td>1.24± 0.1***</td>
<td>7.03± 0.1*</td>
<td>30.84± 1.6***</td>
<td>44.21± 4.6**</td>
<td>93.60± 4.8**</td>
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<td>35.08± 1.5***</td>
<td>49.65± 3.8*</td>
<td>91.41± 3.7**</td>
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<td>5.98± 0.1**</td>
<td>26.64± 1.9***</td>
<td>39.23± 3.9***</td>
<td>87.94± 4.8**</td>
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<tr>
<td>STZ + neem plus evening primrose</td>
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<td>5.18± 0.1*</td>
<td>1.31± 0.1***</td>
<td>5.70± 0.1**</td>
<td>30.02± 1.7***</td>
<td>38.28± 4.1***</td>
<td>90.08± 3.1**</td>
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<tr>
<td>oil</td>
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<td>5.20± 0.1</td>
<td>1.35± 0.1***</td>
<td>5.42± 0.1**</td>
<td>32.66± 1.1***</td>
<td>50.12± 5.5*</td>
<td>89.20± 1.9**</td>
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<td>STZ + sesame plus evening primrose oils</td>
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<td>5.24± 0.1</td>
<td>1.13± 0.0***</td>
<td>5.19± 0.1*</td>
<td>23.19± 1.4***</td>
<td>40.75± 4.6***</td>
<td>85.19± 3.9***</td>
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<td>Neem oil</td>
<td></td>
<td>5.18± 0.1***</td>
<td>1.16± 0.1***</td>
<td>5.34± 0.1**</td>
<td>30.34± 1.0***</td>
<td>47.21± 4.9**</td>
<td>89.85± 1.2**</td>
</tr>
<tr>
<td>sesame oil</td>
<td></td>
<td>5.20± 0.1</td>
<td>1.34± 0.1***</td>
<td>6.11± 0.1**</td>
<td>33.63± 1.4***</td>
<td>42.12± 3.0**</td>
<td>92.72± 4.2**</td>
</tr>
<tr>
<td>evening primrose oil</td>
<td></td>
<td>5.10± 0.1***</td>
<td>1.11± 0.0***</td>
<td>2.99± 0.1***</td>
<td>22.05± 1.6***</td>
<td>39.98± 2.1***</td>
<td>79.52± 1.1***</td>
</tr>
<tr>
<td>sunflower oil</td>
<td></td>
<td>5.14± 0.1***</td>
<td>1.13± 0.0***</td>
<td>3.04± 0.1***</td>
<td>20.64± 1.0***</td>
<td>36.54± 1.1***</td>
<td>80.28± 1.5***</td>
</tr>
<tr>
<td>Neem oil + evening primrose oil</td>
<td></td>
<td>5.11± 0.1***</td>
<td>1.11± 0.1***</td>
<td>2.98± 0.1***</td>
<td>18.11± 1.1***</td>
<td>39.75± 2.1***</td>
<td>83.52± 1.1***</td>
</tr>
<tr>
<td>sesame oil + evening primrose oil</td>
<td></td>
<td>5.14± 0.1***</td>
<td>1.09± 0.0***</td>
<td>3.00± 0.1***</td>
<td>21.58± 1.6***</td>
<td>38.23± 1.1***</td>
<td>81.44± 2.3***</td>
</tr>
</tbody>
</table>

The number of animals was 5 for each treatment except for the control and STZ, in which it was 10.
All values are expressed as means ± SE.
Significantly different from untreated STZ-induced diabetic rats (* p < 0.05, ** p < 0.01 and *** p < 0.001).
Significantly different from normal control (# p < 0.05, ## p < 0.01 and ### p < 0.001).

Figure 6. Blood total protein levels (means ± SE) of different treatments in normal and STZ-induced diabetic rats after 7 weeks.
diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose and evening primrose plus sunflower for 7 weeks had lower blood uric acid levels (p<0.05) than control (Figure 8).

**Blood urea**

The mean values of blood urea of both control and experimental groups are presented in Table 2. STZ-induced diabetic rats showed a highly significant (p<0.001) increase in the levels of blood urea, registering increases of 142.8% after 7 weeks compared to the controls. Administration of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower oils to diabetic rats resulted in a significant (p<0.001) decrease in blood urea levels after 7 weeks compared to untreated diabetic rats.

On the other hand, the administration of sesame plus evening primrose oil produced the most significant reduction (p<0.001) among the test oils in the blood urea levels of 50.9% after 7 weeks of treatment. No significant differences were observed in blood urea level of normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood urea levels than those of control group (p<0.05) (Figure 9).

**Blood AST**

The mean values of blood ALT of both control and experimental groups are presented in Table 2. STZ-induced diabetic rats showed a significant (p<0.001) increase in the levels of blood ALT, registering increases of 118.9% after 7 weeks compared to the controls. Administration of sesame, neem plus sesame and sesame plus evening primrose oils to diabetic rats resulted in a significant (p<0.001) decrease in blood ALT levels after three weeks compared to untreated diabetic rats.

Moreover, the treatment with the oils of administration of neem, evening primrose, sunflower, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose plus sunflower and evening primrose plus sunflower lead to significant (p<0.01) decrease in blood ALT levels after 7 weeks compared to untreated diabetic rats. Moreover, the administration of sesame oil produced the most significant reduction (p<0.001) among the test oils in the blood ALT levels of 22.3% after 7 weeks of treatment. No significant differences were observed in blood ALT level of normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus evening...
primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood ALT levels than those of the control group (p<0.05) (Figure 10).

**Blood ALT**

The mean values of blood ALT of both control and experimental groups are presented in Table 2. STZ-induced diabetic rats showed a significant (p<0.001) increase in the levels of blood ALT, registering increases of 35.3% after 7 weeks compared to the controls. Administration of neem plus sesame, neem plus evening primrose, sesame plus evening primrose oils to diabetic rats resulted in a significant (p<0.001) decrease in blood ALT levels after three weeks compared to...
untreated diabetic rats. Moreover, the treatment with the oils of Administration of neem, sesame, evening primrose, sesame plus sunflower and evening primrose plus sunflower lead to significant (p<0.01) decrease in blood ALT levels after 7 weeks compared to untreated diabetic rats. On the other hand, the treatment with the oils of Administration sunflower and neem plus sunflower, lead to significant (p<0.05) decrease in blood ALT levels after three weeks compared to untreated diabetic rats. Moreover, the administration of neem plus primrose oils produced the most significant reduction (p<0.001) among the test oils in the blood ALT levels of 53.4% after 7 weeks of treatment.

No significant differences were observed in blood AST level of normal rats fed on diets containing the oils of neem, sesame, evening primrose, sunflower when compared with those rats fed on the control diet after 7 weeks of treatment. STZ-induced diabetic rats exposed to the diets containing the oils of neem, sesame, evening primrose, sunflower, neem plus sesame, neem plus evening primrose, neem plus sunflower, sesame plus evening primrose, sesame plus sunflower and evening primrose plus sunflower for 7 weeks had lower blood ALT levels than those of the control group (p<0.05) (Figure 11).

**DISCUSSION**

Several studies demonstrated that a variety of herbal extracts effectively lowered the glucose level in STZ-induced diabetes mellitus rats (Ravi et al., 2004; Rajasekaran et al., 2005; Sekar et al., 2005; Hussein and Abu-Zinadah, 2010).

In the present study, all tested oils significantly reduce blood glucose levels in STZ-induced diabetic rats after 7 weeks of treatment. The mechanism of reducing blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor in case of neem oil. In accordance to the present findings few researchers also reported reduction of blood glucose following administration of insulin and neem extract (Srivastava et al., 1993) On the other hand (Chattopadhyay et al., 1993) reported that *Azadirachta indica* alcoholic Leaf Extract significantly lowered the blood sugar level in glucose-fed and adrenaline induced hyperglycemic rats.

Generally, it is obviously from the present data that the STZ-induced several disturbances on carbohydrate, lipid and protein metabolism in experimental rats. Bolkent et al. (2004), Prakasham et al. (2004), Ravi et al. (2005) and Rajasekaran et al. (2006) reported that in the STZ-diabetic rats, the levels of blood glucose, total lipid, cholesterol, triglycerides, LDL-cholesterol, creatinine, urea, uric acid, ALT and AST activities were significantly increased, while the levels of HDL-cholesterol were markedly decreased. In the study of (Shinde and Goyal, 2003), histopathological investigations of kidney and liver showed several changes included the increases in the intensity and incidence of vacuolations, cellular infiltration and hypertrophy in STZ-diabetic rats. Additionally, they showed that STZ-induced an elevation of serum creatinine and urea levels as well as an elevation of serum level of hepatic enzymes in diabetic rats.

Moreover, (Yoshida et al., 2005) reported that kidney damage in STZ-induced diabetic rats includes glomerular expansion, renal hypertrophy, glycogen degeneration of distal tubules, and fatty degeneration of glomerular endothelium. It is worth to mention that the above previous studies demonstrated that the administrations of several herbal extracts could restore the alterations in the levels of blood and tissue parameters, morphological and histological structure.

In the present investigation, it cannot excluded that the possibility that diabetes-induced liver and kidney damage. However, the increases of serum ALT, AST, creatinine, urea and uric acid levels are considered as obvious indicators for liver and kidney damage and dysfunctions. ALT and AST are directly associated with the conversion of amino acids to keto acids and the increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in diabetic state might be responsible for the elevation of these aminotransferases.

The diabetic hyperglycaemia induces elevations of the blood levels of creatinine, urea, uric acid which are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). It has been documented that several medicinal plants show their hypoglycaemic effects associated with a significant alteration in the activity of liver hexokinase (Santhakumari et al., 2006), glucokinase (Lee et al., 1997). It has been reported that treatment with the herbs caused an improvement in the activities of liver glucose-6-phosphatase, glycogen synthetase, glycogen phosphorylase, glucose-6-phosphate dehydrogenase and phospho-fructokinase. Diabetes mellitus is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs (Almdal and Vilstrup, 1987). Increased urea nitrogen production in diabetes may be accounted by enhanced catabolism of both liver and blood proteins (Jorda et al., 1982). The effect of diabetes mellitus on lipid metabolism is well established.

The association of hyperglycaemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in diabetes. Many secondary plant metabolites have been reported to possess lipid-lowering properties (Rajasekaran et al., 2006). The serum cholesterol and triglycerides were significantly decreased in diabetic rats supplemented with sesame, evening primrose, neem plus sesame, sesame plus evening primrose and sesame plus sunflower oils. These oils
supplementation also result the significant attenuation in the levels of HDL-cholesterol and LDL-cholesterol in serum toward the control level which again strengthen the hypolipidaemic influence of these oils. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, is responsible for the observed accumulation of lipids (Sharma et al., 2003). The impairment of insulin secretion results in enhanced...
metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic rats treated with insulin show normalized lipid levels (Pathak et al., 1981). We suggest that the present effects of these oils-treated diabetic rats may be due to its role in normalization of insulin secretion, lowering activity of lipid biosynthesis enzymes, especially cholesterol and or lowering level of lipolysis.

In the present study revealed that the treatment with the oils of neem (Azadirachta indica), neem plus sesame, neem plus evening primrose, neem plus sunflower returned blood parameters levels to near normal levels. Moreover, many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. Azadirachta indica popularly known as neem is an indigenous plant widely available in India and Burma. Different parts of this plant have been reported to have antiseptic, wound healing and skin disease curing activity (Biswas et al., 2002).

Several studies demonstrated that water soluble portion of alcoholic extract of leaves of Azadirachta indica possesses significant antiinflammatory, antiserotonin, antifertility and hepatoprotective activity (Chattopadhyay et al., 1993). Significant hypolipidemic activity in rats fed on atherogenic diet and antihyperglycemic as well as hypotensive activity have also been reported by us (Chattopadhyay, 1997). Significant blood sugar lowering effect of A. indica in alloxan and streptozotocin induced diabetic rats have also been reported by several workers (Sukla et al., 1973). It is well documented that cardiovascular disease induced by hyperglycemia is associated with alterations in serum lipid profiles (Massing et al., 2001).

The present study revealed that the treatment with sesame returned blood parameters levels to near normal levels. Moreover sesame oil is rich in linoleic acid and oleic acid, which constitutes about 80-85% of total fats in it. Further it is a rich source of vitamin E and the lignan-sesamin, both being good antioxidants. Oleic acid and linoleic acid have proven its role in control of blood glucose and in inducing beneficial changes in lipid profile (Cuninane and Thompson, 1995). (Ramesh et al., 2005) found that diabetic rats which were fed with sesame oil, when compared with controls (diabetic rats not receiving sesame oil), showed a significant reduction in the levels of blood Glucose. Effect of sesame oil on blood pressure may be due to polyunsaturated fatty acids (PUFA) and the compound sesamin— a lignan present in sesame oil. Both compounds have been shown to reduce blood pressure in hypertensive rats. Sesame lignans also inhibit the synthesis and absorption of cholesterol (Ramesh et al., 2005).

In conclusion, the present data suggest that using the oils of sesame, evening primrose, neem plus sesame, sesame plus evening primrose and sesame plus sunflower may improve blood parameters in STZ-induced diabetic rats. The responses in blood parameters in these animals are also demonstrated that oils supplementation may act as antioxidant agents and these oils could be an excellent adjuvant support in the therapy of diabetic mellitus and its complications.

REFERENCES


