Antinutritional effects of soya bean lectin derived from glycine Max (L.) Merrill on diabetes induced albino rats

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ABSTRACT

Soya bean lectin (SBL), a major antinutrient factor isolated from yellow coat soya seeds showed higher haemagglutination activity. The maximum activity was observed with blood type AB. Diabetes was induced by a single injection of streptozotocin (STZ) (50 mg/kg) into the intra peritoneum. The experiment was performed on male albino wistar rats fed ad libitum and control diet or the same diet supplemented with SBL at a rate of 15 mg/day for 14 days. Short term (14 days) dietary exposure to purified SBL affects feed intake, body weight and enzyme activity in pancreas and pancreatic juice of both non-diabetic (ND-SBL) and diabetic (D-SBL) group to a greater extent. Fasting blood glucose and total cholesterol were decreased in both diabetic (D-SBL) and non-diabetic rats (NDSBL) when compared with their control. There was 5-7 fold increase in serum cholecystokinin (CCK) levels in both groups ((D-SBL and NDSBL). Increased plasma insulin levels and serum protein levels were observed in both groups. Pancreatic weight was greatly increased and increased amylase, trypsin activity, pancreatic protein, DNA and RNA content of pancreas (D-SBL: ND-SBL). SBL in diet resulted in decreased trypsin activity while protein levels and amylase activity were increased in pancreatic juice of both diabetic and non diabetic group.

Key words: Soya bean lectin, streptozotocin-induced diabetic rats, non-diabetic rats, agglutination, amylase activity, trypsin activity, toxicity.

Introduction

Soya bean is a stable food of good nutritional value, and can be consumed in the form of whole soya beans, processed soya products (Enwere, 1998; Batal et al., 2002), high quality vegetable protein and oil used in animal foodstuffs and for human consumption (Hancock et al., 2000) and also important diet in diabetic cookery to reduce complications associated with diabetes (Adams et al., 2002; Clarkson, 2002; Nestel, 2002). Raw soya beans contain a number of bioactive or antinutritional factors that can possibly alter the body metabolism of consumers (Liener, 1989; Grant et al., 1990). Of these, the protease inhibitors and the lectins are generally present in seeds at very high levels which show diverse biological activities. Soya-bean agglutinin is one of the major anti-nutritional components (Maenz et al., 1999) and it accounts for approximately half of the inhibition of growth observed in rats given unheated soya bean (Liener, 1996). It has been shown that feeding raw soya-bean flour to rats can stimulate pancreatic growth by hypertrophy and hyperplasia (Crass and Morgan, 1982).

Diabetes is prevalent worldwide and is stated to be one of the important causes of death worldwide. Diabetes mellitus (DM) is a chronic; widely spread human disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both (Amos et al., 1997). Soya products are used as an important protein rich source for normal as well as diabetics and have been investigated. It remains a possibility that soya has a positive and direct effect on the management of diabetes by some yet unrecognized mechanism. However, the interaction between soya protein and its components on diabetic complications is little known. In the present study, the possible effects of soya bean lectin on the basis of performance, pancreatic juice parameters, pancreatic trypsin activity and plasma amylase activity of streptozotocin-induced adult diabetic rats have been evaluated.

Materials and methods

Lectin extraction and agglutination test

Lectins were extracted from raw soya bean seeds (Glycine max.) according to Rudiger protocol (1993). Lectin activity was estimated by the agglutination test (Liener, 1989) with human red blood cells of groups A, B, AB and O and rabbit blood cells.

Animals and diets

Healthy male Wistar strain albino rats (6-8 weeks old) were used throughout the study. The animals were
purchased from King Institute of Preventive Medicine, Chennai, Tamil Nadu, India, and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 hr light/dark cycles. All animals were fed standard rat pellet diet (Lipton India Ltd., Bangalore, India) and water ad libitum. This work was approved by the Institutional animal ethical committee (IAEC 03/P.NO 8/2007).

Induction of diabetes in rats
Diabetes was induced by a single injection of streptozotocin (STZ) (50 mg/kg BW; Sigma, USA) freshly dissolved in a 0.1 mol/L saline buffer (pH 4.5) into the intra peritoneum. The control rats were only injected with the citrate buffer. Diabetes was confirmed in the STZ-treated rats by measuring the fasting blood glucose concentration 48 hr post-injection. The rats with blood glucose level above 350 mg/dl were considered to be diabetic and were used in the experiment. The diabetic rats were randomly divided into two subgroups, diabetic controls (D), diabetic rats given soybean lectin (DSBL: 15 mg/day).

Sample collection
Blood samples were taken from the heart approximately 2 hr after feeding and collected with ethylene diamine tetra acetic acid (EDTA) and without anticoagulant for the separation of plasma and serum respectively. The pancreas was excised and freed from blood, fat, and other tissues, washed with ice-cold saline, blotted on filter paper and organ weights were measured and then freeze-dried followed by homogenization in ice-cold tris-HCL (pH 8.0) buffer. The homogenate was centrifuged at 3000 rpm for 30 min at 4°C and the supernatant was stored at -70°C until needed for protein, nucleic acid and digestive enzyme assay. The gut was dissected and a catheter introduced into the common bile-pancreatic duct. Pancreatic juice was collected for 1 hr. Pancreatic juice, blood plasma, and pancreatic supernatant were frozen at -20°C until analysis.

Analytical methods
Feed intake and body weight were measured for 14 days. Blood plasma was analysed for protein and glucose content. Serum cholesterol and triglyceride were also determined. Plasma insulin and serum cholecystokinin were assayed by using an RIA kit according to the manufacturer's instructions. The sensitivity of the assay was 35 pmol/L and intra and inter assay coefficients of variation were 0\,\cdot\,030 and 0\,\cdot\,048, respectively. Pancreas supernatant was analysed for protein content by the method of dye-concentrate solution (Bio-Rad, Hertfordshire, UK), nucleic acids by Schneider (1957), amylase activity by hydrolysis of starch as previously described by Hue\’re\’ou et al. (1990), trypsin activity (Erlanger et al., 1961). The pancreatic juice was analysed for protein content by the method of Lowry et al. (1951), trypsin activity (Erlanger et al., 1961), α-amylase activity (Hue\’re\’ou et al., 1990).

Statistical Analysis
Results are expressed in mean ± S.E.M. Data were analyzed using one-way analysis of variance. Statistical analyses were performed using Graph pad Prism for windows (Graph pad vision 4, San Diego, CA). P-value ≤ 0.05 was considered significant.

Results
Soybean lectin showed higher haemagglutination activity. In the agglutination test with various types of human erythrocytes, the maximum activity was observed with blood type AB, 50% moderate activity with types A and 0, and the minimum activity, with type B. Soybean lectin in diets (15mg/day) decreased the food intake and promoted weight gain in diabetic rats (D-SBL) as compared to their controls, whereas non diabetic rats (ND-SBL) showed increased food intake and loss of weight. There was no significant change in feed efficiency ratio and protein efficiency ratio.

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Discussion
Agglutination activity depends on the blood group and type of lectin. The SBL exhibited greater agglutination activity with various types of human erythrocytes. The highest agglutination activity was found with blood type AB. This is in agreement with the earlier reports by Czerwiński et al. (2005). The results of the current experiment indicate that adding soya bean lectin to the diet at a concentration of 15mg/day, decreased the food...
intake and promoted weight gain in diabetic rats as compared to their controls, whereas non diabetic rats showed increased food intake and loss of weight (Grant et al., 1986, 1993; Douglas et al., 1999 and Li et al., 2003a). However, the dosage of lectin to the experimental rats in this study is higher than that used by others (Leontowicz et al., 2000; Czerwiński et al., 2005). It is interesting to note that feeding the STZ induced diabetic rats with soya bean lectin for 14 days resulted in gaining weight by these rats without increasing the food intake. Hence, there was no significant change in feed efficiency ratio and protein efficiency ratio. This observation is not in agreement with the previous findings data or the (Grant, et al 1986; Grant, 1989). Nevertheless, the present study on feed efficiency ratio and influence on rat performance are in agreement with that reported by Li et al. (2003a, b), Leontowicz et al. (2004) and Czerwiński et al. (2005).

The levels of glucose in blood decreased marginally in both diabetic induced and non-diabetic rats when fed with soya bean lectin (Table.1). The exact mechanism by which soya bean lectin is acting upon the blood glucose and plasma insulin levels are not clear. Increase in the plasma insulin levels due to soya bean lectin could be explained in terms of utilization of glucose by liver and extra hepatic tissues.

In the present study, the supplementation of the diet with soya bean lectin in both STZ-induced diabetic rats and non-diabetic rats showed no significant changes in total cholesterol levels (Ali et al., 2000). On the other hand, the triglyceride levels increased in non-diabetic rats when fed with SBL (Anwar et al., 2003).

Lectin can modulate hormone secretion like insulin, CCK, gastrin (Liener, 1994; Herzig et al., 1997; Grant et al., 2000; Radberg et al., 2001; Kelsall et al., 2002). Supplementation of soya bean lectin increased the plasma insulin level of the STZ-induced diabetic rats by about 43%. Similarly, serum cholecystokinin levels in both STZ-induced diabetic rats and non-diabetic rats was also increased, which is nearly 5–7 folds greater than the control rats. Cholecystokinin in the blood stream is mainly derived from endocrine cells in the intestinal mucosa (Miyasaka et al.,1997) and it is synthesized and processed by endocrine cells in the upper small intestine, as well as in the nervous system (Liddle, 1997). Jordison et al. (1997) also reported an increase in the cholecystokinin levels in rats fed with lectins and attributed the phenomenon to the binding of lectins to complex motifs in intestine.

In the present study, rats given soya-bean agglutinin also had a marked increase in pancreatic weight (Grant et al., 1986; Grant, 1989; Li et al., 2003a, 2003b; Czerwiński et al., 2005), and pancreatic activity of amylase and trypsin (Grant et al., 1997). One potential explanation for these changes is the fact that the enzymes trypsin and chymotrypsin contain high concentrations of the sulphur containing amino acids methionine and cystine (Lienen, 1995). Lienen (1995) suggested that an increase in pancreatic growth and enzyme secretion may divert these amino acids from the synthesis of body tissue protein towards enzyme synthesis, resulting in a depression in growth. Excess secretion of these enzymes may result in poor performance through an increase in endogenous nitrogen loss, which was previously noted, may be one of the reasons for the anti-nutritional effects of soya-bean agglutinin.

Pancreatic protein, pancreatic DNA and RNA contents were increased by soya bean lectin in both diabetic and non-diabetic rats as observed by others (Grant, 1989; Pusztai, 1991; Pusztai et al., 1992a; Jordinson et al., 1997). Supplementation soya bean lectin (SBL) decreased trypsin amylase activity in pancreatic juice, while protein levels and amylase activity increased in the pancreatic juice (Table.3). Leontowicz et al. (2004) also found decreased amylase activity in pancreatic juice in rats fed diets supplemented with evening primrose agglutinin.

**Conclusion**

Soya bean lectin, one of the major anti-nutritional factors found in raw soya bean, has been shown to depress growth and cause pancreatic enlargement in rats. Hence it is a relevant issue applicable to animal as well as human nutrition.
Table 1. Effect of soya bean lectin on blood parameters of adult diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ND-SBL</th>
<th>Diabetic</th>
<th>D-SBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic weight (mg)</td>
<td>1385^c</td>
<td>2411^b</td>
<td>1275</td>
<td>2743^a</td>
</tr>
<tr>
<td>Pancreatic protein (mg/pancreas)</td>
<td>280.1</td>
<td>484.9^b</td>
<td>277.4^c</td>
<td>558.7^a</td>
</tr>
<tr>
<td>DNA (mg/g of pancreas)</td>
<td>3.25^c</td>
<td>3.51^b</td>
<td>2.57</td>
<td>3.72^a</td>
</tr>
<tr>
<td>RNA (mg/g of pancreas)</td>
<td>13.34^c</td>
<td>19.67^b</td>
<td>12.36^c</td>
<td>26.41^a</td>
</tr>
<tr>
<td>Pancreatic amylase activity (U/L)</td>
<td>83.13^b</td>
<td>94.97^a</td>
<td>7.89</td>
<td>15.03^c</td>
</tr>
<tr>
<td>Pancreatic trypsin activity (U/L)</td>
<td>14.08^b</td>
<td>17.65^a</td>
<td>2.69</td>
<td>5.59^c</td>
</tr>
</tbody>
</table>

ND- Non diabetic; D- Diabetic. Values are expressed as mean ± SEM for six rats in each group. Values in the same row with no common following superscript are not significantly different (p<0.05).

Table 2. Effect of soya bean lectin on pancreatic parameters of adult diabetic rats. ND- Non diabetic; D- Diabetic. Values are expressed as mean ± SEM for six rats in each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ND-SBL</th>
<th>Diabetic</th>
<th>D-SBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/L)</td>
<td>63.67^c</td>
<td>68.97^b</td>
<td>75.03^a</td>
<td>78.43^a</td>
</tr>
<tr>
<td>Amylase activity (U/L)</td>
<td>78.67</td>
<td>115.8^a</td>
<td>81.50^c</td>
<td>89.77^b</td>
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<tr>
<td>Trypsin activity (U/L)</td>
<td>294.3^a</td>
<td>268.0</td>
<td>283.02^b</td>
<td>276.3^c</td>
</tr>
</tbody>
</table>

Values in the same row with no common following superscript are not significantly different (p<0.05).

Table 3. Effect of soya bean lectin on pancreatic juice parameters of adult diabetic rats. ND- Non diabetic; D- Diabetic. Values are expressed as mean ± SEM for six rats in each group.

<table>
<thead>
<tr>
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<th>ND-SBL</th>
<th>Diabetic</th>
<th>D-SBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>64.68±5.71</td>
<td>73.85±4.3^c</td>
<td>219.5±11.56^a</td>
<td>181.5±8.7^b</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>49.97±8.14^c</td>
<td>63.03±7.35^a</td>
<td>59.32±5.3^b</td>
<td>46.35±6.5^c</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>72.85±4.9^b</td>
<td>68.23±7.9^a</td>
<td>77.30±7.9^a</td>
<td>68.40±3.5^c</td>
</tr>
<tr>
<td>Serum protein (g/L)</td>
<td>69.33±2.2^c</td>
<td>73.10±2.2^b</td>
<td>73.77±2.6^b</td>
<td>78.3±2.2^a</td>
</tr>
<tr>
<td>Plasma insulin (nmol/mL)</td>
<td>10.64±0.26^a</td>
<td>7.13±0.02^b*</td>
<td>4.60±0.07</td>
<td>6.59±0.15^c*</td>
</tr>
<tr>
<td>Serum cholecystokinin (pmol/L)</td>
<td>2.4±0.3</td>
<td>12.5±0.1^b</td>
<td>3.3±0.5^c</td>
<td>16.67±0.2^a</td>
</tr>
</tbody>
</table>

Values in the same row with no common following superscript are not significantly different (p<0.05).
REFERENCES


