Antihyperglycaemic Effect of ‘Ilogen-Excel’, an Ayurvedic Herbal Formulation in Streptozotocin-Induced Diabetes Mellitus

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Abstract: ‘Ilogen-Excel’, an Ayurvedic herbal formulation is composed of eight medicinal plants (Curcuma longa, Strychnos potatorum, Salacia oblonga, Tinospora cordifolia, Vetiveria zizanioides, Coscinium fenestratum, Andrographis paniculata and Mimosa pudica). The present study evaluates the antihyperglycemic effect of ‘Ilogen-Excel’ in streptozotocin induced diabetic rats. Rats were rendered diabetic by streptozotocin (STZ) (45 mg/kg body weight). Oral administration of ‘Ilogen-Excel’ (50 mg/kg and 100 mg/kg) for 60 days resulted in significantly lowered levels of blood glucose and significantly increased levels of plasma insulin, hepatic glycogen and total hemoglobin. ‘Ilogen-Excel’ administration also decreased the levels of glycosylated hemoglobin, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and vitamin E in diabetic rats. Plasma reduced glutathione and vitamin C were significantly elevated by oral administration of ‘Ilogen-Excel’. Administration of insulin normalized all the biochemical parameters studied in diabetic rats. The effect at a dose of 100 mg/kg was more pronounced than 50 mg/kg and brought back all the parameters to near normal levels. Thus, our study shows the antihyperglycemic effects of ‘Ilogen-Excel’ in STZ-induced diabetic rats. Our study also shows that combined therapy is better than individual therapy.

Keywords: ‘Ilogen-Excel’, streptozotocin diabetes, plasma lipid peroxides.

In traditional practice, medicinal plants are widely used in many countries for the treatment of diabetes mellitus. The antihyperglycemic effect of several plant extracts and herbal formulations has been confirmed (1). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (2). Combined extracts of herbs are used as the drug of choice rather than individual plant extracts. Studies have shown that herbal formulations such as D-400 (3) and Hyponidd (4) have antidiabetic and antioxidant effects. ‘Ilogen-Excel’, an Ayurvedic herbal formulation is widely used in Indian medicine for the treatment of diabetes mellitus. It contains eight plant constituents such as Curcuma longa L., Strychnos potatorum L., Salacia oblonga Wall, Tinospora cordifolia Wild., Vetiveria zizanioides L., Coscinium fenestratum Colebr., Andrographis paniculata Nees and Mimosa pudica L. There is no available reports pertaining to the antihyperglycemic activity of ‘Ilogen-Excel’, in streptozotocin-induced diabetic rats. This study evaluates the antihyperglycemic activity of ‘Ilogen-Excel’ in streptozotocin-induced diabetic rats.

Experimental

‘Ilogen-Excel’ was purchased from local pharmacy, Cuddalore District, Tamil Nadu, India. (Manufactured by PANKAJA KASTHURI HERBALS INDIA (P) LTD. Poovachal, Thiruvananthapuram-75, Kerala, India).

Male albino Wistar rats, body weight of 180-200 g bred in the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University were used in the present study. The animals were housed in polypropylene cages (47×34×18 cm) lined with husk. It was renewed every 24 h. They were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra) and water was freely available. The animals were maintained in a controlled environment. This study was approved by the Animal Ethical Committee of Annamalai University (Approval number: 252, 16.12.2004).

Chemicals

Streptozotocin and thiobarbituric acid were...
purchased from Sigma Chemical Company Inc., St. Louis, USA. 2,2'-Dipyridyl, D-fructose 1,6-diphosphate trisodium salt, D-glucose-6-phosphate and 2,4-dinitrophenylhydrazine were purchased from S.D. Fine Chemicals, Mumbai. Butanol and petroleum ether were purchased from Ranbaxy, New Delhi. All other chemicals used were of analytical grade.

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M citrate buffer, (pH 4.5) was injected intraperitoneally in a volume of 1 mL/kg (5). After 72 h of streptozotocin administration, the rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with a blood glucose range of 230-280 mg/dL) were taken for the experiment.

In our study, a total of 42 rats were used. The rats were divided into 7 groups and each group consisted of 6 rats. Group I: normal untreated rats; Group II and III: normal rats treated with ‘Iogen-Excel’ (50 mg and 100 mg/kg body weight) orally in physiological saline, using an intragastric tube, daily for 60 days; Group IV: STZ (45 mg/kg body weight) induced diabetic rats. Group V and VI: STZ treated rats orally given ‘Iogen-Excel’ (50 mg and 100 mg/kg body weight) in physiological saline, using an intragastric tube, daily for 60 days; Group VII: STZ treated rats administered insulin (6 units/kg) intraperitoneally, daily for a period of 60 days.

Body weight was measured once a week. Water intake and food intake were monitored daily during the experimental period (60 days). After 60 days of treatment, all the rats were decapitated after an overnight fast. Blood was collected in potassium oxalate and sodium fluoride containing tubes for the estimation of fasting blood glucose. Serum and plasma were also separated from blood after centrifugation. Tissues such as liver and kidney were collected in ice-cold containers for various biochemical estimations. Tissue samples from rats were weighed and homogenized using appropriate buffer and teflon pestle.

Blood glucose was estimated using a reagent kit (Product No. 72081). Plasma insulin was performed by the ELISA method using a Boehringer Mannheim kit (Boehringer analyser ES 300). Glycosylated hemoglobin was estimated using a reagent kit (Product No. 11017001). Total hemoglobin was estimated using a reagent kit (Product No. 72091).

Liver glycogen content was estimated by the method of Morales et al. (7). A weighed amount of the tissue was subjected to alkali digestion in a boiling water bath for 20 min after the addition of 5 mL of 30% potassium hydroxide. The tubes were cooled and 3 mL of absolute ethanol and a drop of ammonium acetate were added and then placed in a freezer overnight to precipitate the glycogen. The precipitated glycogen was collected after centrifugation at 3000 rpm for 10 min. The precipitate was washed thrice with alcohol and dissolved in 3 mL of water. Aliquots were taken and made up to 1 mL with water. Four mL of anthrone reagent was added to the tubes kept in ice bath, mixed and heated in a boiling water bath for 20 min. The green color developed was read at 640 nm. Working standard glucose and a blank were treated similarly. The values are expressed as mg/g tissue.

Hexokinase activity was assayed by the method of Brandstrup et al. (8). The reaction mixture in a total volume of 5.2 mL contained the following: 1 mL of glucose solution, 0.5 mL of ATP solution, 0.1 mL of magnesium chloride solution, 0.4 mL of potassium dihydrogen phosphate, 0.4 mL of potassium chloride, 0.4 mL of sodium fluoride and 2.5 mL of Tris- HCl buffer (pH 8.0). The mixture was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of 0.1 mL serum/2 mL of tissue homogenate. One mL of the reaction mixture was immediately removed to the tubes containing 1 mL of 10% TCA which was considered as zero time. A second aliquot was removed after 30 minutes incubation at 37°C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated by the method of o-toluidine (9). The enzyme activity is expressed as mmoles of glucose phosphorylated/h/mL serum and mmoles of glucose phosphorylated/h/mg protein.

Glucose-6-phosphatase activity was assayed by the method of Koide and Oda (10). Incubation mixture contained 0.7 mL of citrate buffer, 0.3 mL of substrate and 0.1 mL serum/0.3 mL of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. Addition of 1 mL of 10% TCA to the reaction tubes terminated the enzymatic reaction. The suspension was centrifuged and the phosphorus (P) content of the supernatant was estimated by the method of Fiske and Subbarow (11). The supernatant was made up to a known volume. To this, 1 mL of ammonium molybdate was added followed by 0.4 mL of ammonium sulfonic acid (ANS). The blue color developed after 20 min was read at 640 nm. The enzyme activity is expressed as mmoles of P liberated/min/mL serum and mmoles of P liberated/min/mg protein.

Fructose-1,6 bisphosphatase was assayed by the method of Gancedo and Gancedo (12). The assay mixture in a total volume of 2 mL contained
1.2 mL of buffer, 0.1 mL of substrate, 0.25 mL of magnesium chloride, 0.1 mL of KCl solution, 0.25 mL of EDTA solution and 0.1 mL of serum/0.1 mL of tissue homogenate. The incubation was carried out at 37°C for 15 min. The reaction was terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus estimation by the method of Fiske and Subbarow (11). The supernatant was made up to a known volume. To this, 1 mL of ammonium molybdate was added followed by 0.4 mL of ANSA. The blue color developed after 20 min was read at 660 nm. Enzyme activity is expressed as µmoles of P liberated/min/mL serum and µmoles of P liberated/min/mg protein.

Plasma thiobarbituric acid reactive substances were estimated by the method of Yagi (13). To 0.5 mL of plasma, 4 mL of 0.415 M H₂SO₄ was added. To this mixture, 0.5 mL of 10% phosphotungstic acid was added and mixed. The mixture was allowed to stand for 5 min and then was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 2 mL of H₂SO₄ and 0.3 mL of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3000 rpm for 10 minutes. The sediment was suspended in 4 mL of distilled water and 1 mL of thiobarbituric acid (TBA) reagent was added. The reaction mixture was heated at 95°C for 60 min. After cooling, 5 mL of n-butanol was added and the mixture was shaken vigorously and centrifuged at 3000×g for 15 minutes. The color extracted in butanol layer was read at 530 nm. Standard solution (1-5 nmol) in 4 mL volumes and blank containing 4 mL of distilled water were processed along with the test samples. The values are expressed as nM/mL plasma.

Lipid hydroperoxides were estimated by the method of Jiang et al. (14). 1.8 mL of the Fox reagent was mixed with 0.2 mL of plasma and incubated for 30 min at room temperature. The color developed was read at 560 nm. Lipid hydroperoxides are expressed as 10ⁿM/dL plasma.

Reduced glutathione (GSH) was estimated by the method of Ellman (15). 0.5 mL of plasma was precipitated with 2.0 mL of 5% TCA. 0.1 mL of supernatant was taken and to this added 0.5 mL of Ellman’s reagent and 3 mL of phosphate buffer. The yellow color developed was read at 412 nm. A series of standards were also treated in a similar manner along with a blank containing 3.5 mL of buffer. The concentration of GSH is expressed as mg/dL plasma.

Vitamin C was estimated by the method of Omaye et al. (16). 0.5 mL of plasma was mixed thoroughly with 1.5 mL of 6% TCA and centrifuged for 20 min. To 0.5 mL of the supernatant, 0.5 mL of 2,4-dinitrophenylhydrazine (DNPH) reagent was added and mixed well. The tubes were allowed to stand at room temperature for 3 h, removed and then placed in ice-cold water. To this, 2.5 mL of 85% H₂SO₄ was added and allowed to stand for 30 minutes. A set of standards containing 10-50 µg of ascorbic acid were taken and processed similarly along with a blank containing 0.5 mL of 4% TCA. The color developed was read at 530 nm. Ascorbic acid values are expressed as mg/dL plasma.

Vitamin E was estimated by the method of Baker and Frank (17). To 0.1 mL of plasma, 1.5 mL of ethanol and 2 mL of petroleum ether were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80°C. To this, 0.2 mL of 2,2'-dipyridyl solution and 0.2 mL of ferric chloride were added. They were mixed well and kept in darkness for 5 min, and then 2 mL of butanol was added. The intense red color developed was read at 520 nm. Standards of tocopherol in the range of 10 to 100 µg were taken and treated similarly along with a blank containing only the reagent. The values are expressed as mg/dL plasma.

Ceruloplasmin (CP) was estimated by the method of Ravin (18). To 8 mL of buffer in two tubes marked control and test, 0.05 mL of plasma was added. One mL of sodium azide was added to control tubes and mixed. To both the tubes, 1 mL of p-phenylenediamine was added, mixed and kept at 37°C for 1 hr. One mL of sodium azide was then added to the test. All the tubes were kept at 4-10°C for 30 min. The color developed was read at 540 nm with control as blank. The values are expressed as mg/dL plasma.

The protein content of tissue homogenate was estimated by the method of Lowry et al. (19). 0.5 mL of tissue homogenate was precipitated with 0.5 mL of 10% TCA, centrifuged for 10 min and the precipitate was dissolved in 1.0 mL of 0.1 M sodium hydroxide. 0.1 mL of aliquot was taken and made up to 1.0 mL with distilled water. Then added, 4.5 mL of alkaline copper reagent and allowed to stand at room temperature for 10 min. After incubation, 0.5 mL of Folin-Ciocalteau reagent was added and the blue color developed after 20 min was read at 620 nm. A standard curve was obtained using BSA. The protein levels are expressed as mg/g tissue.

Statistical analysis was done by using SPSS package version 6.0 one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). All the results were expressed as mean ± SD for six rats in each group; p values < 0.05 were considered as significant.
RESULTS

Table 1 shows the changes in the levels of blood glucose and plasma insulin of normal and STZ-induced diabetic rats. The levels of blood glucose were significantly elevated, while the levels of plasma insulin were significantly decreased in diabetic rats. Oral administration of ‘Ilogen-Excel’ at doses of 50 mg and 100 mg/kg for a period of 60 days showed a significant decrease in blood glucose and a significant increase in plasma insulin in diabetic rats.

Figure 1 shows the change of body weight in normal and STZ-induced diabetic rats. Diabetic rats showed a significant decrease in body weight as compared to normal rats. Oral administration of ‘Ilogen-Excel’ at doses of 50 mg and 100 mg/kg for a period of 60 days showed a significant increase in body weight in diabetic rats.

Table 2 shows the changes in liver glycogen, total hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats. A significant decrease in liver glycogen and total hemoglobin, and a significant increase in glycosylated hemoglobin was observed in diabetic rats. Oral administration of ‘Ilogen-Excel’ at doses of 50 mg and 100 mg/kg for a period of 60 days showed a significant decrease in the water intake and food intake in diabetic rats.

Table 3 shows the effect of ‘Ilogen-Excel’ on the activities of hexokinase, glucose-6-phosphatase and fructose 1,6-bisphosphatase in serum, liver and kidney in normal and STZ-induced diabetic rats. The activity of hexokinase in serum, liver and kidney were significantly decreased, while the activities of glucose-6-phosphatase and fructose 1,6-bisphosphatase were significantly increased in serum, liver and kidney in diabetic rats as compared to normal rats. Oral administration of ‘Ilogen-Excel’ at doses of 50 mg and 100 mg/kg for a period of 60 days showed a significant increase in the activity of hexokinase and a significant decrease in the activities of glucose-6-phosphatase and fructose 1,6-bisphosphatase in serum, liver and kidney in diabetic rats.

Table 4 shows the levels of TBARS, HP, GSH, ceruloplasmin vitamin C and vitamin E in plasma in normal and STZ-induced diabetic rats. The concentration of TBARS, HP, ceruloplasmin and vitamin E were significantly increased in diabetic rats, when compared to normal rats. The levels of GSH and vitamin C were significantly decreased in plasma in normal and STZ-induced diabetic rats, when compared to normal rats. Oral administration of ‘Ilogen-Excel’ at doses of 50 mg and 100 mg/kg for a period of 60 days showed a significant increase in the levels of GSH and vitamin C and a significant decrease in the concentration of TBARS, HP, ceruloplasmin and vitamin E in diabetic rats.

DISCUSSION

Streptozotocin-induced diabetes mellitus is a valuable model for induction of diabetes mellitus (20). It causes disturbance in the uptake of glucose as well as glucose metabolism. The use of a lower dose of STZ (45 mg/kg) produced an incomplete destruction of pancreatic β-cells even though the rats became permanently diabetic (21). After treatment with a low dose of STZ, there should be many surviving β cells, and regeneration is also possible (22). The increased levels of blood glucose in STZ-induced diabetic rats was lowered by ‘Ilogen-Excel’ administration. The antihyperglycemic action of ‘Ilogen-Excel’ results from the potentiation of insulin from existing β-cells of the islets of Langerhans. This is evident from the significant increase in plasma insulin concentration in STZ-diabetic rats. The levels of blood glucose levels was increased and the levels of plasma insulin was decreased in STZ-induced diabetic rats. An increase in insulin secretion may lead to the inhibition of lipid peroxidation due to a decrease in blood glucose levels. As ‘Ilogen-Excel’ comprises many antioxidant phytochemicals, these are also responsible for scavenging reactive oxygen species and inhibiting lipid peroxidation. In this context, oral administration of ‘Hyponidd’, an Ayurvedic herbomineral formulation lowered blood glucose in STZ-induced diabetic rats (4). Oral administration of ‘Ilogen-Excel’ significantly decreased the blood glucose and significantly increased plasma insulin levels in diabetic rats.

A decrease in the body weight of diabetic rats was observed in diabetes mellitus (23). Rajkumar et al. (1991) have reported that increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by the diabetic rats (24). Moreover, the increased secretion of
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insulin, due to its catabolic effect may also result in increased synthesis of proteins. Decreased levels of blood glucose could improve the body weight in STZ-diabetic rats (4). Oral administration of ‘Ilogen-Excel’ significantly increased the body weight in diabetic rats.

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycation is proportional to the concentration of blood glucose (25). The lowered levels of total hemoglobin observed in diabetic rats might be due to the increased formation of glycosylated hemoglobin (26). In diabetes, protein synthesis is decreased in all tissues due to a relative insulin deficiency and thus the synthesis of hemoglobin is also suppressed (27). Increased glycation of proteins has been found to be a consequence of diabetic complications. A number of proteins including hemoglobin, are glycated to a greater degree in diabetes mellitus (28). Oral administration of ‘Ilogen-Excel’ significantly decreased the levels of glycosylated hemoglobin and significantly increased the levels of total hemoglobin in diabetic rats.

Glycogen content in liver is reduced in STZ-induced diabetic rats (29). The lack of insulin causes decrease in hepatic glycogen content in diabetic state, which results in inactivation of the glycogen synthase system (30). Oral administration of ‘Ilogen-Excel’ significantly improved hepatic...
glycogen levels in STZ diabetic rats, possibly because of the reactivation of glycogen synthase system as a result of increased insulin secretion.

In our study, we have noticed increased water and food consumption in STZ-induced diabetic rats. This indicates polyphagic condition and loss of weight due to excessive breakdown of tissue proteins (31). Oral administration of ‘Ilogen-Excel’ significantly decreased the water and food consumption in diabetic rats.

In diabetic state, decreased activity of hexokinase was seen in serum, liver and kidney. A relative deficiency of insulin caused a decrease in the activity of this enzyme in STZ-induced diabetic rats (32). The activity of glucose 6-phosphatase significantly increased in serum, liver and kidney of diabetic rats. Streptozotocin has been shown to increase the expression of glucose 6-phosphatase mRNA, which contributes to the increased activity of this enzyme in diabetes mellitus (33). A state of hyperglycemia increased the activity of fructose 1,6-bisphosphatase in serum, liver and kidney of STZ-diabetic rats. Similar results have been reported by other researchers in STZ-induced diabetic rats (34). Oral administration of ‘Ilogen-Excel’ significantly increased the activity of hexokinase in serum, liver and kidney and significantly decreased the activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase in serum, liver and kidney of diabetic rats.

We have also observed elevated levels of plasma TBARS and HP in STZ-induced diabetes rats. Elevated levels of lipid peroxides observed in plasma are thought to be a consequence of lipid peroxides into the circulation due to pathological changes (35). Similar findings were reported in STZ-induced diabetic rats (36). Oral administration of ‘Ilogen-Excel’ significantly increased the levels of plasma TBARS and HP in diabetic rats.

Table 2. Effect of ‘Ilogen-Excel’ on liver glycogen, total hemoglobin and glycated hemoglobin in normal and STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver glycogen (mg/g)</th>
<th>Total hemoglobin (mg/dL)</th>
<th>Glycated hemoglobin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.09 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>4.08 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.8 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>4.09 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>2.20 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.02 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>2.73 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.70 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.76 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>3.67 ± 0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.22 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.59 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VII</td>
<td>3.91 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for 6 rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05 (DMRT)

We have observed a decrease in the concentration of GSH in plasma in diabetic rats. It appears that generation of oxygen radicals by increased levels of glucose causes utilization of GSH and thus diminishes GSH levels in the plasma in diabetes mellitus (36). In this context, Sajithal et al. (1998) also reported diminished levels of serum GSH in STZ-induced diabetic rats (37). Oral administration of ‘Ilogen-Excel’ significantly increased the concentration of GSH in diabetic rats.

Vitamin E is a non-enzymic antioxidant that resides in the lipid bilayer of the cell membrane. It reduces lipid hydroperoxides generated during the process of peroxidation and protects the cell structures against damage (38). The level of vitamin E was elevated in diabetic rats. Similar reports have been observed by other workers in STZ-induced diabetic rats (39). The observed elevated levels of vitamin E in diabetic rats might be due to the increased production of free radicals. Oral administration of ‘Ilogen-Excel’ significantly decreased the levels of vitamin E in diabetic rats.

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species (ROS) (40). Further, vitamin C plays an important role in detoxification of reactive intermediates produced by cytochrome P<sub>450</sub> that detoxifies xenobiotics (41). A decrease in the levels of vitamin C have been observed in diabetic rats. This decrease could have been due to increased utilization of vitamin C as an antioxidant against increased ROS. Similar observations were reported in STZ-induced diabetes (37). Oral administration of ‘Ilogen-Excel’ significantly increased the levels of vitamin C in diabetic rats.

Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than
Table 3. Effect of 'Ilogen-Excel' on the activities of hexokinase, glucose-6 phosphatase and fructose-1,6 bisphosphatase in serum, liver and kidney in normal and STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexokinase*</td>
<td>Glucose-6 phosphatase**</td>
<td>Fructose-1,6 bisphosphatase**</td>
</tr>
<tr>
<td>Group I</td>
<td>0.18 ± 0.06a</td>
<td>0.28 ± 0.02a</td>
<td>0.34 ± 0.01a</td>
</tr>
<tr>
<td>Group II</td>
<td>0.18 ± 0.06a</td>
<td>0.29 ± 0.02a</td>
<td>0.35 ± 0.01a</td>
</tr>
<tr>
<td>Group III</td>
<td>0.19 ± 0.07a</td>
<td>0.29 ± 0.03a</td>
<td>0.36 ± 0.02a</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.10 ± 0.04a</td>
<td>0.60 ± 0.05a</td>
<td>0.69 ± 0.05a</td>
</tr>
<tr>
<td>Group V</td>
<td>0.12 ± 0.05a</td>
<td>0.50 ± 0.06a</td>
<td>0.54 ± 0.04a</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.15 ± 0.09a</td>
<td>0.42 ± 0.02a</td>
<td>0.42 ± 0.02a</td>
</tr>
<tr>
<td>Group VII</td>
<td>0.17 ± 0.90a</td>
<td>0.34 ± 0.03a</td>
<td>0.37 ± 0.03a</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for 6 rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05 (DMRT).

* µmoles of glucose phosphorylated/h/ml serum and µmoles of glucose phosphorylated/h/mg protein
** µmoles of P liberated/min/ml serum and µmoles of P liberated/min/mg protein.

Table 4. Effect of 'Ilogen-Excel' on the concentration of TBARS, HP, GSH, ceruloplasmin, vitamin C and vitamin E in plasma in normal and STZ-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS (nm/mL)</td>
</tr>
<tr>
<td>Group I</td>
<td>2.26 ± 0.11a</td>
</tr>
<tr>
<td>Group II</td>
<td>2.25 ± 0.12a</td>
</tr>
<tr>
<td>Group III</td>
<td>2.23 ± 0.12a</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.30 ± 0.26a</td>
</tr>
<tr>
<td>Group V</td>
<td>4.48 ± 0.22a</td>
</tr>
<tr>
<td>Group VI</td>
<td>3.06 ± 0.19a</td>
</tr>
<tr>
<td>Group VII</td>
<td>2.53 ± 0.16a</td>
</tr>
</tbody>
</table>

Each value is mean ± SD for 6 rats in each group.
Values not sharing a common superscript differ significantly at p < 0.05 (DMRT).
other antioxidants when plasma is exposed to reactive oxygen species (ROS) (40). Further, vitamin C plays an important role in detoxification of reactive intermediates produced by cytochrome P450 that detoxifies xenobiotics (41). In this context, we have observed a decrease in the levels of vitamin C in diabetic rats. This decrease could have been due to increased utilization of vitamin C as an antioxidant against increased ROS. Similar observations were reported in STZ-induced diabetes (37). Oral administration of 'Ilogen-Excel' significantly increased the levels of vitamin C in diabetic rats.

Ceruloplasmin forms a major part of the extracellular antioxidant defense. It also inhibits iron and copper dependent lipid peroxidation and also has a superoxide radical scavenging activity (42). The observed increase in plasma ceruloplasmin in diabetic animals might be due to elevated lipid peroxides. Dorrman has shown that ceruloplasmin levels increase under conditions leading to the generation of superoxide radicals and hydrogen peroxide (43). Analogous findings were also observed in STZ-induced diabetes mellitus (36). Oral administration of 'Ilogen-Excel' significantly decreased the levels of ceruloplasmin in diabetic rats.

Phytochemical studies of the constituent plants of Ilogen Excel revealed the presence of a number of alkaloids, sterols and polyphenolic compounds. The following plants of 'Ilogen-Excel' exhibit antihyperglycemic effects. Curcuma longa (44), Strychnos potatorum (45), Salacia oblonga (46), Coscinium fenestratum (47), Tinospora cordifolia (48), Andrographis paniculata (49) and Mimosa pudica (50). The antioxidant effect of 'Ilogen-Excel' is also due to the presence of Curcuma longa (51), Strychnos potatorum (45), Salacia oblonga (46), Coscinium fenestratum (52) Tinospora cordifolia (53), Vetiveria zizanioides (54), and Andrographis paniculata (49).

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