Evaluation of Antihyperglycemic Effect of Sinapic Acid in Normal and Streptozotocin-Induced Diabetes in Albino Rats

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Abstract: Diabetes is a major public health problem. The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great study. The present study was designed to evaluate the effect of sinapic acid on physiological and biochemical parameters in streptozotocin-induced diabetic rats. Streptozotocin treatment (45 mg/kg, ip) caused hyperglycemia, that led to various physiological and biochemical alterations. The findings were compared among normal, diabetic and sinapic acid treated diabetic rats. Plasma glucose, insulin and C-peptide and the levels of blood hemoglobin and glycosylated hemoglobin and the activities of carbohydrate metabolizing enzymes hexokinase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were markedly altered in STZ-induced diabetic animals. Oral administration of sinapic acid for a period of 35 days restored all these biochemical parameters to near normal. The results of the present study revealed that sinapic acid has a potential antihyperglycemic effect in streptozotocin -induced diabetic rats.

Key words: Streptozotocin • Diabetes • Sinapic acid and insulin

INTRODUCTION

Diabetes mellitus (DM) is a group of heterogenous, hormonal and metabolic disorders characterized by hyperglycemia and glucosuria, with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1].

Diabetes mellitus, a chronic disorder is associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy. DM is considered to be a major risk factor for cardiovascular disorders namely ischemic heart disease, cerebral stroke and peripheral artery disease leading to increased mortality of diabetics [2]. Globally, the estimated incidence of DM and projection for year 2010, as given by International Diabetes Federation is 239 million [3].

Insulin is a major anabolic hormone in the body. Pancreatic insulin reserve is an important parameter of islet function, with tight coupling between insulin secretion and production being necessary for adequate functioning of pancreatic β-cells [4]. Streptozotocin (STZ) (2-deoxy-2-([methyl (nitroso) amino] carbonyl] amino)-β-D-glucopyranose) is a naturally occurring compound [5]. The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin [6].

The chronic hyperglycaemia in diabetes enhances the production of reactive oxygen species (ROS) from glucose oxidation, protein glycation and glycoxidation [7].

In diabetes, protein glycation and glucose oxidation may generate free radicals, which in turn, cause lipid peroxidation [8]. Moreover, ROS have also been implicated in the mechanism of damage to the red blood cells [9]. The concentration of ROS is modulated by antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase and by non-enzymatic antioxidant such as glutathione [10]. In diabetes, oxidative stress seems to be caused by increased production of ROS, a sharp reduction in antioxidant defenses and altered cellular redox status [11].

Antioxidants provide protection to living organism from damage caused by uncontrolled production of ROS concomitant lipid peroxidation, protein damage and DNA strand breaking [12, 13]. The management of diabetes without any side effects is still a challenge to the medical
system. There is an increasing demand from patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects [14]. In the indigenous Indian system of medicine (Ayurveda), many herbal medicines have been recommended for the treatment of diabetes or ‘madhumeha’ and some of them have been experimentally evaluated.

In recent years, flavonoids have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals and against oxidative stress [15]. Flavonoids cannot be produced by the human body and have taken in through the daily diet. The evidence reported that flavonoids play a vital biological role, including the function of scavenging reactive oxygen species [16].

Sinapinic acid or sinapic acid is a small naturally occurring carboxylic acid. It is a member of the phenylpropanoid family. Sinapic acid is a cinnamic acid derivative which possesses 4-hydroxy-3, 5-dimethoxy cinnamic acid is one of the phenolic acids widely distributed in edible plants such as cereals, nuts, oil seeds and berries [17]. Sinapic acid is a major free phenolic acid in rapseed meal, with the majority found in the esterified form of sinapine [18]. Sinapic acid has demonstrated potent antioxidant capacity and its efficiency is always higher than ferulic acid and sometimes comparable to that of caffeic acid [19]. Sinapic acid has also presented strong inhibition of tyrosine nitration by per nitrate. It is well known that phenolic acids exist in the bound form by an ester linkage to other molecule in a plant body [20].

To the best of our knowledge, no research has been conducted on antihyperglycemic effect of sinapic acid in STZ-induced diabetes in rats. Hence, the present study was aimed to evaluate the antihyperglycemic effect of sinapic acid in STZ-induced diabetes in albino wistar rats.

**MATERIALS AND METHODS**

**Experimental Animals:** Female albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in the present investigation. The animals were housed in polypropylene cages (47 x 34 x 20cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22°C and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

**Drug and Chemicals:** Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai, India. Sinapic acid was purchased from Sigma-Aldrich, St. Louis, USA. Glucose, hemoglobin and glycosylated hemoglobin kits were purchased from Agappe diagnostics, Kerala, India. Insulin and C-peptide kits were obtained from Monobind Inc, Lake Forest, CA 92630, USA. All other chemicals used in the study were of analytical grade.

**Experimental Induction of Diabetes:** Rats were made diabetic by a single intraperitoneal injection of freshly prepared STZ (45 mg/ kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5) in a volume of 1 ml/ kg [21]. After 48 hours, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Albino rats with a blood glucose level above 240 mg/dl were considered diabetic and selected for the study.

**Experimental Design:** In the experiment, a total of 36 rats (18 diabetic surviving rats, 18 control rats) were used. The rats were divided into 6 groups of 6 rats in each group.

- **Group 1:** Normal control rats (animals that received only normal diet and water).
- **Group 2:** Normal rats given Sinapic acid (15 mg/kg)
- **Group 3:** Normal rats given Sinapic acid (30 mg/kg)
- **Group 4:** Diabetic control rats
- **Group 5:** Diabetic rats treated with Sinapic acid (15 mg/kg)
- **Group 6:** Diabetic rats treated with Sinapic acid (30 mg/kg)

Sinapic acid was dissolved in 0.2% dimethyl sulfoxide (DMSO) and administrated to rats orally using an intragastric tube daily for a period of 35 days.
Sample Collection: At the end of the treatment period, all rats were fasted for 12 hours and sacrificed by cervical decapitation. The blood was collected into heparinized tubes and plasma was separated by centrifugation. Liver and kidney were dissected out, washed in ice-cold physiological saline, patted dry and weighed. The tissues were then homogenized in 0.1M Tris-Hcl buffer, pH 7.4. The homogenate was used for the estimation of carbohydrate metabolic enzymes.

Biochemical Measurements: Plasma glucose was estimated by the method of Trinder [22]. Glycosylated hemoglobin was estimated by the method of Tietz [23] and hemoglobin in blood was estimated by the method of Samuel [24] using kit from Agappe diagnostics. Plasma insulin and C-peptide were determined by the method of Kann and Rosenthal [25] using an immunoenzymatic assay kit. Hexokinase was assayed by the method of Brandstrup [26]. Glucose-6phosphatase was assayed by the method of Koide and Oda [27]. Fructose-1, 6-bisphosphatase was assayed by the method of Gancedo and Gancedo [28].

Statistical Analysis: Results were expressed as mean±SD for six rats in each experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one-way analysis of variance (ANOVA) and group means were compared with Duncan’s Multiple Range Test (DMRT). P-values < 0.05 were considered as significant.

RESULTS

Effect of Sinapic Acid on Body Weight, Food Intake, Water Intake and Organ Weight: The effect of sinapic acid on body weight, food intake, water intake and organ weight in normal and STZ-induced diabetic rats are presented in table 1. Induction of STZ to rats caused significant reduction (p<0.05) in body weight as compared to normal rats. Oral administration of sinapic acid to STZ-induced diabetic rats significantly increased the body weight to near normal. Food and water intake was significantly (p<0.05) increased in STZ-induced diabetic rats when compared with normal rats. Oral administration of sinapic acid exerted a significant effect on food and water intake compared to diabetic control group.

Table 1: Effect of Sinapic acid on body weight, food intake, water intake and organ weight in normal and STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Organ weight (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Food intake (g)</td>
<td>Water intake (ml)</td>
</tr>
<tr>
<td>Normal control</td>
<td>162.46±2.7</td>
<td>195.24±4.5a</td>
<td>48.16±2.3a</td>
<td>92.71±3.6a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (15mg/kg)</td>
<td>163.30±2.9</td>
<td>192.08±4.3a</td>
<td>48.69±2.8a</td>
<td>93.03±4.9a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (30mg/kg)</td>
<td>164.00±3.4</td>
<td>193.96±4.1a</td>
<td>48.46±2.6a</td>
<td>94.33±3.8a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>156.41±6.4</td>
<td>170.30±2.0b</td>
<td>110.89±3.4b</td>
<td>222.61±7.4b</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (15mg/kg)</td>
<td>167.75±6.7</td>
<td>193.21±3.4c</td>
<td>83.59±3.08c</td>
<td>137.59±3.5c</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (30mg/kg)</td>
<td>168.72±6.8</td>
<td>205.98±4.4d</td>
<td>65.14±1.5d</td>
<td>129.40±2.7d</td>
</tr>
</tbody>
</table>

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 2: Effect of Sinapic acid on the levels of plasma glucose, insulin and C-Peptide in normal and STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (µU/ml)</th>
<th>C-Peptide (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>85.61±0.24a</td>
<td>14.18±0.80a</td>
<td>8.68±0.014a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (15mg/kg)</td>
<td>86.62±0.20a</td>
<td>14.44±0.05a</td>
<td>6.57±0.13a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (30mg/kg)</td>
<td>85.29±0.21a</td>
<td>14.57±0.05a</td>
<td>6.61±0.17a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>263.63±5.29b</td>
<td>8.31±0.14b</td>
<td>2.14±0.21b</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (15mg/kg)</td>
<td>135.00±1.35c</td>
<td>10.95±0.16c</td>
<td>4.34±0.22c</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (30mg/kg)</td>
<td>114.50±1.20d</td>
<td>12.45±0.12d</td>
<td>4.72±0.19d</td>
</tr>
</tbody>
</table>

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 3: Effect of Sinapic acid on the levels of blood hemoglobin and glycosylated hemoglobin in normal and STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemoglobin (g/dl)</th>
<th>Glycosylatedhemoglobin (mg/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>14.60±2.16a</td>
<td>6.58±0.50a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (15mg/kg)</td>
<td>14.69±2.19a</td>
<td>6.33±0.15a</td>
</tr>
<tr>
<td>Normal+Sinapic acid (30mg/kg)</td>
<td>15.97±2.15a</td>
<td>6.55±0.28a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>8.71±1.06b</td>
<td>14.33±0.36b</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (15mg/kg)</td>
<td>13.41±1.12c</td>
<td>11.62±0.56c</td>
</tr>
<tr>
<td>Diabetic+Sinapic acid (30mg/kg)</td>
<td>17.34±1.08d</td>
<td>11.22±0.59d</td>
</tr>
</tbody>
</table>

Each value is mean±S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).
Table 4: Effect of Sinapic acid on the activities of carbohydrate metabolic enzymes in liver and kidney of normal and STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase (UnitA/h/mg protein)</th>
<th>Glucose-6-phosphatase (UnitB/min/mg protein)</th>
<th>Fructose-1,6-bisphosphatase (UnitC/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.74±0.09a</td>
<td>0.24±0.05a</td>
<td>0.32±0.02a</td>
</tr>
<tr>
<td>Normal + Sinapic acid (15mg/kg)</td>
<td>0.74±0.08a</td>
<td>0.23±0.03a</td>
<td>0.31±0.02a</td>
</tr>
<tr>
<td>Normal + Sinapic acid (30mg/kg)</td>
<td>0.72±0.09a</td>
<td>0.22±0.01a</td>
<td>0.31±0.01a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.33±0.04b</td>
<td>0.54±0.07b</td>
<td>0.54±0.06b</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (15mg/kg)</td>
<td>0.44±0.05c</td>
<td>0.44±0.03c</td>
<td>0.47±0.08c</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (30mg/kg)</td>
<td>0.62±0.06d</td>
<td>0.35±0.06d</td>
<td>0.42±0.09d</td>
</tr>
</tbody>
</table>

A - mmoles of glucose phosphorylated, B - mmoles of inorganic phosphorous liberated, C - mmoles of inorganic phosphorous liberated. Each value is mean±S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

on water and food intake in diabetic rats. Diabetic rats showed significant (p<0.05) decrease in liver weight and increase in kidney weight when compared to normal rats. Sinapic acid restored the liver weight and kidney weight to near normal.

**Effect of Sinapic Acid on Plasma Glucose, Insulin and C-peptide:** The effect of sinapic acid on plasma glucose, insulin and C-peptide levels of normal and STZ-induced diabetic rats are depicted in table 2. The diabetic rats showed a significant (p<0.05) increase in plasma glucose and significant (p<0.05) decrease in plasma insulin and C-peptide. Oral administration of sinapic acid significantly (p<0.05) reduced the level of plasma glucose and increased the levels of plasma insulin and C-peptide in STZ-induced diabetic rats.

**Effect of Sinapic Acid on Blood Total Hemoglobin and Glycosylated Hemoglobin:** Table 3 shows the levels of total hemoglobin and glycosylated hemoglobin of normal and STZ-induced diabetic rats. Rats induced with STZ, showed a significant (p<0.05) decrease in the level of total hemoglobin and a significant (p<0.05) increase in the level of glycosylated hemoglobin. Oral administration of sinapic acid reversed the changes in hemoglobin and glycosylated hemoglobin levels to near normalcy.

**Effect of Sinapic Acid on Carbohydrate Metabolic Enzymes in Tissues:** Table 4 illustrates the effect of sinapic acid on carbohydrate metabolic enzymes in liver and kidney of normal and STZ-induced diabetic rats. The activity of hexokinase in liver was significantly (p<0.05) decreased, while the activities of glucose-6-phosphatase and fructose-1, 6-phosphatase was significantly (p<0.05) increased in liver and kidney of STZ-induced diabetic rats when compared to control group of rats. Oral administration of sinapic acid significantly minimized the alterations in the activities of these carbohydrate metabolic enzymes in STZ-induced diabetic rats.

**DISCUSSION**

STZ selectively destroys the insulin secreting pancreatic β-cells, leaving the less active β-cells and thus, resulting in diabetic state [29]. STZ-induced diabetes is characterized by severe weight loss which was observed in the present study. The reduction in body weight may be attributed to insulin depletion provoking a loss of adipose tissues. The loss in weight in diabetic rats might also be the result of degradation of structural proteins due to unavailability of carbohydrate as energy source [30]. For this reason, weight reduction is being used as a marker of diabetes mellitus induced by STZ [31]. In the present study, oral administration of sinapic acid to STZ-induced diabetic rats caused significant improvement in the body weight. This may be due to the antihyperglycemic effect of sinapic acid thus enhancing glucose metabolism.

STZ-induced diabetes showed a significant increase in the food and water intake. The administration of sinapic acid (15 & 30 mg/kg) to STZ-induced diabetic rats caused statistically significant reduction in food and water intakes. This could be the result of improved glycemic control produced by sinapic acid.

A decrease in the liver weight observed in diabetic animals might be due to an increased breakdown of glycogen / or pronounced gluconeogenesis. After 35 days of treatment with sinapic acid in diabetic animals, a significant increase in the liver weight was observed. The result of the present study coincides with Jefferson et al. [32] who have reported that insulin therapy could increase the accumulation of glycogen in liver. A significant increase in kidney weight was observed in diabetic animals when compared with normal control animals. The kidney enlargement is an early feature in both experimental and human diabetes due to an increase in the capillary length and diameter and as correlated with the degree of glycemic control [33]. This might be due to the glomerular cell proliferation and glomerular enlargement in
diabetic rats. In the present study, oral administration of sinapic acid significantly decreased the kidney weight to near normal value which might be due to the effect of sinapic acid on glomerular cells in STZ-induced diabetic rats.

In the present study, an increase in the plasma glucose level in diabetic rats confirmed the induction of diabetes by STZ. The fundamental mechanism underlying hyperglycemia in diabetes involves the overproduction of glucose by excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization by the tissues [34]. We have observed a significant decrease in plasma glucose in sinapic acid treated diabetic rats, when compared with diabetic control rats. This could be due to the regeneration of existing pancreatic beta cells and enhanced transport of glucose to the peripheral tissues by sinapic acid.

C-peptide and insulin are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone [35]. STZ-induced diabetic rats showed significant reduction in the levels of insulin and C-peptide. This might be due to the destruction of the pancreatic β-cells and thereby induces hyperglycemia [36]. A significant increase in the levels of plasma insulin and C-peptide observed in sinapic acid administered diabetic rats in the present study might be due to the increased pancreatic secretion of insulin from the existing remnant β-cells.

A significant decrease in the level of total hemoglobin and significant increase in the level of glycosylated hemoglobin observed in STZ-induced diabetic rats might be due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting blood glucose level for about 3 months [37]. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin [38]. Therefore, the total hemoglobin level is decreased in diabetic rats. The rate of glycation is directly proportional to the concentration of blood glucose [39]. The level of glycosylated hemoglobin has been shown to provide an index of blood glucose concentration [40]. The decreased level of glycosylated hemoglobin and increased level of total hemoglobin observed in sinapic acid administered diabetic rats may be due to the reduction of blood glucose level.

Hexokinase catalyses the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis [41]. In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose [42]. Streptozotocin-induced diabetic rats showed significant decrease in the activity of hexokinase in liver with subsequent increase in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney. According to the recent report, insulin stimulates and activates hexokinase activity [43]. Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin [44]. Oral administration of sinapic acid to STZ-induced diabetic rats resulted in a significant increase in the activity of hexokinase, which may be associated with reduced blood glucose level. The increased activity of hexokinase suggests that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilization of glucose at peripheral sites.

Glucose-6-phosphatase, a crucial gluconeogenic enzyme, is mainly found as an integral protein in the lumen of the endoplasmic reticulum of hepatocytes that catalyzes the dephosphorylation of glucose-6-phosphate to glucose in the liver [45]. Fructose-1, 6-bisphosphatase is another gluconeogenic enzyme that catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of gluconeogenesis [46]. In our study, the increased activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in liver and kidney of diabetic rats may be due to insulin deficiency. Oral administration of sinapic acid reversed the glucose-6-phosphatase and fructose-1, 6-bisphosphatase activities in STZ-induced diabetic rats which are responsible for the improved glycemic control.

In conclusion, the present study revealed that sinapic acid possesses a potential antihyperglycemic effect, through an increase in insulin production associated with subsequent increase in the activity of glycolytic enzyme, hexokinase and decrease in the activity of gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase.

REFERENCES


