EFFECT OF SINAPIC ACID ON BIOCHEMICAL MARKERS AND HISTOPATHOLOGICAL STUDIES IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETES IN WISTAR RATS

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ABSTRACT
The objective of this study is to investigate the effect of sinapic acid on certain biochemical markers and histology of liver and kidney in normal and streptozotocin (STZ)-induced diabetes in wistar rats. STZ treatment (45 mg/kg/ip) caused a hyperglycemic state that led to various physiological and biochemical alterations. Blood urea, serum creatinine, uric acid, total protein, albumin and A/G ratio were markedly altered in STZ induced diabetic rats. Oral administration of sinapic acid for a period of 35 days restored all these biochemical parameters and histopathological changes that occurred in liver and kidney to near normalcy. Thus, our results revealed that the administration of sinapic acid may have protective effects on the liver and kidney of STZ-induced diabetic rats.

Keywords: Diabetes mellitus, Protein metabolism, Sinapic acid, Streptozotocin

INTRODUCTION
Oxidative stress is currently suggested as one of the mechanism underlying diabetes mellitus, which affects carbohydrate, lipid and protein metabolism. Diabetes mellitus (DM) is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs. Increased urea nitrogen production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins. As per WHO report, approximately 150 million people have diabetes mellitus worldwide and this number may be double by 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in 2025, making India apart the country with the highest number of diabetics in the world.

Decomposition of pancreatic β cells by streptozotocin produces diabetes in experimental animals. Glucose homeostasis involves the co-ordinate regulation of several metabolic pathways, including gluconeogenesis and glycolysis, which are due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response. The effect of diabetes is not limited to carbohydrate metabolism. Lipid and protein metabolism play an important role in the progression of the diseases. Impairment of kidney function is a prominent feature of diabetes.

Over time diabetic nephropathy will develop, characterized by proteinuria, loss of renal function and a rapid progression to end-stage renal failure. Formation of reactive oxygen species (ROS) is thought to be a mediator of the cytotoxic actions of streptozotocin. Organisms have developed several defense mechanisms to protect their cells against ROS. Such mechanisms include the use of antioxidant enzymes and small antioxidant molecules such as vitamins C, E and flavonoids. Antioxidant enzymes metabolize ROS into non-toxic products as the first line of defense against toxic free radicals. Many plants reported useful for the treatment of diabetes mellitus in the Ayurvedic system of medicine have been tested for their hypoglycemic activity in experimental animals.

Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables. The flavonoids are frequently components of the human diet and intake may reach 800 mg/day. Flavonoids are able to indirectly participate in the reduction of oxidative stress in diabetic patients by improving glycemic control and/or are able to exert antioxidant activity. Sinapic 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. Sinapic acid is a major free phenolic acid in rapseseed meal, with the majority found in the esterified form of sinapine. Sinapic acid has demonstrated potent antioxidant capacity and its efficiency is always higher than ferulic acid and sometimes comparable to that of caffeic acid.

In view of the above facts, the present study was designed to investigate the efficiency of sinapic acid on biochemical markers and histopathological disturbances that occur in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS
Experimental Animals
Female albino wistar rats (150-200 g) obtained from Venkataseswara Enterprises, Bangalore were used in this study. They 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, 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. Total protein, albumin, uric acid and creatinine kits were purchased from Agappe diagnostics, Kerala, India. All other chemicals used in the study were of analytical grade.

Experimental Induction of Diabetes
Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body weight) in citrate buffer (pH 4.5) in a volume of 1 ml/kg. Streptozotocin injected animals were given 10% glucose solution for 5 days to prevent initial drug induced hyperglycemic mortality. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, after 48 hours of injection with STZ. Albino rats with a blood glucose level above 240 mg/dl were considered to be diabetic and used in the experiment.

Experimental Design
In the experiment, a total of 36 rats (18 diabetic surviving rats and 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: Control rats administrated orally with Sinapic acid (1.5mg/kg)
Group 3: Control rats administrated orally with Sinapic acid (30mg/kg)
Group 4: Diabetic control rats
Group 5: Diabetic rats treated orally with Sinapic acid (15mg/kg)
Group 6: Diabetic rats treated orally with Sinapic acid (30mg/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
After 35 days of treatment, the animals were fasted for 12 hours, and then sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation and utilized for biochemical parameters.

Biological Studies
Blood urea was estimated by the method of Chaney and Marbach27 and Searcy et al.28. Uric acid in serum was estimated by the method of Fossati et al.29. Serum creatinine was estimated by the method of Henry et al.30. Total protein, albumin and globulin in serum were estimated by the method of Doumas et al.31 and Webster32.

Effect of sinapic acid on serum total protein, albumin and A/G ratio

The effect of sinapic acid on serum total protein, albumin and A/G ratio in normal and diabetic rats are shown in Table 2. STZ-induced diabetic rats showed a significant (P<0.05) decrease in the levels of serum total protein, albumin and A/G ratio when compared to normal control rats. The lowered levels of total protein, albumin and A/G ratio in STZ-induced diabetic rats were increased to near normal levels due to sinapic acid treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.40 ± 0.40</td>
<td>4.68 ± 0.38</td>
<td>1.21 ± 1.28</td>
</tr>
<tr>
<td>Normal + Sinapic acid (15mg/kg)</td>
<td>8.75 ± 0.58</td>
<td>4.65 ± 0.31</td>
<td>1.13 ± 1.31</td>
</tr>
<tr>
<td>Normal + Sinapic acid (30mg/kg)</td>
<td>8.63 ± 0.54</td>
<td>4.50 ± 0.25</td>
<td>1.19 ± 1.32</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>4.40 ± 0.43</td>
<td>1.44 ± 0.35</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (15mg/kg)</td>
<td>6.25 ± 0.21</td>
<td>3.20 ± 0.39</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (30mg/kg)</td>
<td>7.60 ± 0.38</td>
<td>3.81 ± 0.35</td>
<td>1.12 ± 0.15</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).

Histopathological study of liver and kidney

The liver and kidney were quickly dissected out and washed in ice-cold saline to remove the blood. After dissection, the tissues were preserved in 10% formalin and stained with haematoxylin and eosin and examined under high power microscope (400x) and photomicrographs were taken.

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 blood urea, serum creatinine and uric acid

The levels of blood urea, serum creatinine and uric acid in normal and STZ-induced diabetic rats are shown in Table 1. Rats induced with STZ, showed a significant (P<0.05) increase in the levels of blood urea, serum creatinine and uric acid when compared to normal rats. Diabetic rats treated with sinapic acid showed significant (P<0.05) reduction in the levels of blood urea, serum creatinine and uric acid when compared with diabetic control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>25.34 ± 2.90</td>
<td>3.61 ± 0.89</td>
<td>1.35 ± 0.10</td>
</tr>
<tr>
<td>Normal + Sinapic acid (15mg/kg)</td>
<td>25.23 ± 2.85</td>
<td>3.51 ± 0.76</td>
<td>1.38 ± 0.14</td>
</tr>
<tr>
<td>Normal + Sinapic acid (30mg/kg)</td>
<td>25.19 ± 2.10</td>
<td>3.50 ± 0.71</td>
<td>1.32 ± 0.15</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>37.58 ± 2.67</td>
<td>5.73 ± 1.96</td>
<td>2.59 ± 0.52</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (15mg/kg)</td>
<td>26.61 ± 1.23</td>
<td>4.72 ± 1.36</td>
<td>2.13 ± 0.21</td>
</tr>
<tr>
<td>Diabetic + Sinapic acid (30mg/kg)</td>
<td>25.05 ± 1.12</td>
<td>4.28 ± 1.24</td>
<td>1.95 ± 0.14</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).
Histopathology of Liver

Fig. 1: Normal control rat liver shows normal architecture

Fig. 2: Normal with sinapic acid (15 mg/kg) treated rat liver shows hepatocytes within normal limits

Fig. 3: Normal with sinapic acid (30 mg/kg) treated rat liver shows hepatocytes within normal limits

Fig. 4: Diabetic control rat liver shows congestion in sinusoidal space and hepatic necrosis

Fig. 5: Sinapic acid (15 mg/kg) treated diabetic rat liver shows moderate congestion in sinusoidal space

Fig. 6: Sinapic acid (30 mg/kg) treated diabetic rat liver shows the normal radiating pattern of hepatocytes

Histopathology of Kidney

Fig. 7: Normal control rat kidney shows intact tubules and glomeruli

Fig. 8: Normal with sinapic acid (15 mg/kg) treated rat kidney shows mild dilation of tubules
Histopathology of kidney

Figure 7-12 shows the histopathology of kidney. The kidney of normal rats showed the intact tubules and glomeruli (Fig 7). The kidney of normal rats treated with sinapic acid 15 mg/kg and 30mg/kg did not show any alterations in the normal architecture of the kidney (Fig 8 & 9). The kidney of streptozotocin diabetic rats showed degenerating tubules with desquamated epithelial cells in the lumen (Fig 10). Streptozotocin induced diabetic rats treated with sinapic acid with 15 mg/kg showed intertubular as well as periglomerular congestion (Fig 11). Streptozotocin induced diabetic rats treated with sinapic acid with 30 mg/kg showed the intact tubules and glomeruli (Fig 12).

DISCUSSION

Insulin dependent diabetes is usually accompanied by high urinary glucose concentration, which produces an osmotic diuresis and therefore polyuria. Abnormalities in lipid and protein metabolism including enhanced lipolysis and increased breakdown of protein may be secondary to insulin deficiency.

A significant increase in the levels of blood urea and serum creatinine was observed in diabetic rats when compared with respective normal control rats. The diabetic hyperglycemia induces elevation of the serum levels of urea, creatinine and uric acid which are considered as significant markers of renal dysfunction. Degradation of protein and nucleic acid results in the formation of non-protein nitrogenous compound such as urea and creatinine. The elevated levels of serum urea and creatinine in diabetic rats are due to catabolism of the protein and nucleic acids. Similar result was also reported by Yasin et al. Sinapic acid treatment significantly decreased the levels of blood urea and serum creatinine in diabetic rats, which could be due to the prevention of protein and nucleic acid degradation by sinapic acid.

Serum uric acid level was found to be significantly increased in STZ-induced diabetic animals. Uric acid clearance has been associated with insulin resistance. Changes in the levels of serum urea, creatinine and uric acid concentrations strongly suggested impairment of kidney function in diabetes. Administration of sinapic acid decreased the levels of serum uric acid in STZ-induced diabetic rats. The present work also showed a significant reduction in the serum total protein, albumin and A/G ratio in diabetic rats. The decreased level of total protein observed in diabetic rats coincides with the findings of Peavy et al. and Wanke and Wong. This decline may be due to the inhibited oxidative phosphorylation processes which lead to decrease in protein synthesis, increase in the catabolic process and reduction of protein absorption. Rasch and Mogenson reported that the plasma A/G ratio was lower in diabetic animals. Increased protein catabolism in diabetes might have induced a direct adverse effect on the synthesis and secretion of albumin. Diabetic rats treated with sinapic acid brought back total protein, albumin and A/G ratio to near normal levels. This could be due to the effect of sinapic acid on the improvement of oxidative phosphorylation process in cells.

The presence of degenerated hepatocytes and necrotic cells are possibly associated with the generation of free radicals in the liver of diabetic rats. There is increasing evidence that free radicals play an important role in the initiation and progression of liver injury and causes apoptosis, necrosis, and regeneration in hepatocyte and endothelial cells. Pyachaturavat et al. reported that STZ exhibits nephrotoxic and hepatotoxic activity. Histology of liver in diabetes condition showed that there was structural alternation in the liver as a result of absence of insulin. The diabetic liver showed degeneration and congestion. In diabetes, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose-6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzymes


