PROTECTIVE ROLE OF PTEROSTILBENE ON PLASMA AND TISSUE GLYCOPROTEIN COMPONENTS IN HIGH-FAT DIET-FED AND STREPTOZOTOCIN-INDUCED TYPE 2 DIABETIC MICE

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ABSTRACT

Objective: The aim of the current study is to evaluate the effect of pterostilbene (PTS), on deranged plasma and tissue glycoprotein components in high-fat diet (HFD) and streptozotocin (STZ)-induced type 2 diabetic mice.

Methods: The experimental duration was 16 weeks. The C57BL6/J mice were fed a normal diet, normal diet with PTS, HFD, and STZ injection (10th week only), and diabetic mice with PTS for the past 6 weeks.

Results: A significant increase in glycoprotein components such as hexose, hexosamine, fucose, and sialic acid (SA) in plasma was observed in diabetic mice. In hepatic and renal tissues, a significant decrease in SA with an increase in other glycoprotein components was detected in diabetic mice when compared with control mice. Oral administration of PTS significantly improved the glycoprotein levels in plasma and tissues of diabetic mice to near normal level.

Conclusion: In this study, we resolved that PTS improves disturbed glycoprotein metabolism in HFD and STZ-induced type 2 diabetic mice.

Keywords: Pterostilbene, High-fat diet, Streptozotocin, Hexosamine, Fucose, Sialic acid.

INTRODUCTION

Diabetes mellitus is a multifaceted disease characterized by unusual metabolism of carbohydrates, lipids, and proteins, and type 2 diabetes mellitus (T2DM) is the most dominant form of diabetes, which presents in more than 90% of diabetic cases [1]. Diabetes has developed one of the most challenging health problems on a global scale. The International Diabetes Federation (IDF) estimated that, in 2017, there were 451 million (age 18–9 years) people with diabetes worldwide and this number was predicted to rise to 693 million by 2045 [2]. Diabetes is now showing an alarming increase in prevalence in developing countries. India alone, the prevalence of diabetes is expected to 79.4 million in 2030, i.e. one-sixth of the world total [3].

Hyperglycemia and insulin deficiency, the hallmarks of diabetes mellitus, alter glycoprotein components in various tissues. Several studies reported that impaired metabolism of glycoproteins plays a major role in the pathogenesis of diabetic complications [4]. Under diabetic conditions, reactive oxygen species is produced mainly through glycation reaction, which occurs in various tissues and may play an important role in the development of diabetic complications [5]. Advanced glycation end-products modify galactose, fucose, and sialic acid (SA) contents of specific cellular glycoproteins [6]. This process leads to long-term damage, dysfunction, and failure of various organs, especially the eyes, kidney, nerves, heart, and blood vessels, and creates a huge economic burden related to the management of diabetic complications [7].

Glycoproteins are conjugated proteins that have one or more covalently connected carbohydrate chains which donate to the structure of extracellular matrix in animal cells. The principle forms of sugar present are hexose, hexosamine, fucose, and SA, which encrypt the diverse biological roles of glycoproteins [8]. Glycoproteins are main components of intracellular matrix, cell membrane, and membranes of the subcellular organelles. They play a vital role in the maintenance of structural integrity of the membrane bilayer. Cell surface glycoproteins have important roles in the transport of vitamins and lipids, in signal transduction as hormone receptors and in immunological specificity [9].

Pterostilbene (PTS) is a naturally derived compound found primarily in blueberries and Pterocarpus marsupium heartwood [10]. Substantial evidence suggests that PTS may have various preventive and therapeutic properties in a vast range of human diseases that include neurological, cardiovascular, metabolic, and hematologic disorders. Further benefits of PTS have been reported in preclinical trials, in which PTS was shown to be a potent anticancer agent in several malignancies [11]. PTS is structurally similar to resveratrol, a compound found in red wine that has comparable antioxidant, anti-inflammatory, and anticarcinogenic properties; however, PTS exhibits increased bioavailability due to the presence of two methoxy groups which cause it to exhibit increased lipophilic and oral absorption. In animal studies, PTS was shown to have 80% bioavailability compared to 20% for resveratrol making it potentially advantageous as a therapeutic agent [12].

The present study is aimed to investigate the ameliorative potential of PTS on glucose, insulin, and glycoprotein components (hexose, hexosamine, fucose, and SA) in plasma and tissues (the liver and kidney) of high-fat diet (HFD)/STZ-induced diabetic mice.

METHODS

Experimental animals and animal ethical

Healthy adult male C57BL/6 mice 3 weeks of age were obtained from BioGen Laboratory Animal Facility, Bengaluru, India, and housed in polypolyene cages. The animals were housed in well-ventilated polypolyene cages and controlled environment (temperature 23±2°C, humidity 65–70%, and 12 h light/dark cycle) in the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University. Animals were maintained under standard conditions with a 12 h light/dark cycle and were provided with
standard pellet diet and water ad libitum. All studies were conducted in accordance with the National Institute of Health, "guide for the care and use of laboratory animals" and CPCSEA guidelines. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg. No. 160/1999/CPCSEA, Proposal number: 972, Anamalainagar).

**Experimental induction of diabetes**

Diabetes was induced in male C57BL/6J mice by feeding a HFD containing 35% fat (beef tallow mixed with the diet) for 60 days; in addition, streptozotocin (STZ) was injected at a low dose in the 16th week.

**Experimental design**

Test animals were fed initially, before the study, with standard diets for 2 weeks. Then, these mice were assigned to one of the four groups with six mice in each group:

- **Group 1**: Mice received standard pellet diet for 16 weeks
- **Group 2**: Mice received PTS (40 mg/kg BW) for 16 weeks
- **Group 3**: Mice received HFD for 16 weeks and STZ was injected in the 10th week
- **Group 4**: Diabetic mice received PTS (40 mg/kg BW) for the last 6 weeks

**PTS**: Pterostilbene, **HFD**: High-fat diet, **STZ**: Streptozotocin

**Extraction of glycoproteins**

To 0.1 ml of plasma, 5.0 ml of methanol was added, mixed well, and centrifuged for 10 min at 3000× g. The supernatant was decanted and the precipitate was again washed with 5.0 ml of 95% ethanol and the supernatant was decanted to obtain the precipitate of glycoproteins. This was used for the estimation of hexose and hexosamine.

For extraction of glycoproteins from the tissues, a known weight of the tissue was homogenized in 7.0 ml of methanol. The contents were filtered and then acidified with 14.0 ml of chloroform. This was filtered and the residue was then successively homogenized in chloroform-methanol (2:1 v/v), and each time, the extract was filtered. The residue (defatted tissues) was obtained and the filtrate was decanted. A weighed amount of defatted tissue was suspended in 3.0 ml of 2 N HCl and heated at 90°C for 4 h. The sample was cooled and neutralized with 3.0 ml of 2 N NaOH. Aliquots from this were used for the estimation of hexose and hexosamine.

**Determination of glycoproteins levels**

Hexose was estimated by the method of Niebes [13]. The reaction mixture contained 0.5 ml of tissue homogenous/plasma, 0.5 ml of 5% phenol, and 2.5 ml of concentrated H₂SO₄, and was boiled for 20 min, and absorbance was read at 490 nm. Hexosamine was estimated by the method of Elson and Morgan [14], with slight modifications by Niebes. Briefly, the reaction mixture contained 0.5 ml plasma/1.0 ml tissue homogenate and 2.5 ml of 3 N HCl. It was boiled for 6 h and neutralized with 6 N NaOH. To 0.8 ml of the neutralized sample, add 0.6 ml of acetylacetone reagent and boiled for 30 min. The mixture was treated with 2.0 ml of Ehrlich’s reagent. The color developed was read at 540 nm colorimetrically.

SA was determined by the method of Warren [15]. In brief, 0.5 ml of tissue homogenate/plasma was treated with 0.5 ml of deionized water and 0.25 ml of periodic acid, and incubated at 37°C for 30 min. 0.2 ml of sodium meta-arsenite and 2.0 ml of thioarbituric acid were added to the reaction mixture which was heated for 6 min. 5.0 ml of acidified butanol was then added and the absorbance was read at 540 nm.

Fucose was estimated by the method of Dische and Shettles [16]. Briefly, 0.5 ml of tissue homogenate/plasma was treated with 4.5 ml of H₂SO₄ and boiled for 3 min. 0.1 ml of cysteine hydrochloride reagent was then added. After 75 min in the dark, the absorbance was read at 393 and 430 nm. The glycoprotein levels were expressed as mg/100 g for defatted tissue and mg/dl for plasma.

**Histopathological examination of the kidney**

For histopathological studies, the kidney was excised from each group. Each tissue was fixed in 10% formaldehyde and embedded in paraffin. The prepared paraffin blocks were later sectioned (5 μm) using a microtome, deparaffinized in xylene, passed through alcohol, stained with hematoxylin-eosin, and examined under a light microscope (Nikon, Tokyo, Japan).

**Statistical analysis**

The results were expressed as a mean standard deviation for six mice (n=6) in each group. Data were analyzed by one-way analysis of variance followed by Duncan’s multiple range test using SPSS version 17 (SPSS, Chicago, IL). Post hoc testing was performed for intergroup comparisons using the least significant difference test. p<0.05 was considered as statistically significant.

**RESULTS**

Table 1 shows the changes in the levels of hexose, hexosamine, fucose, and SA in plasma of control and experimental mice. There was a significant increase in plasma glycoproteins in HFD/STZ-induced diabetic mice when compared to normal control mice. Administration of PTS to diabetic mice resulted in a significant reduction of glycoproteins in plasma when compared to diabetic control mice.

The levels of glycoproteins in liver and kidney tissues of control and experimental mice are shown in Tables 2 and 3. The levels of SA were significantly decreased in the tissues of HFD-induced diabetic mice, but the levels of hexose and hexosamine were increased in these tissues when compared to normal control tissues.

**Table 1: Changes in the levels of liver glycoproteins in normal and experimental mice**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexose (mg/g)</th>
<th>Hexosamine (mg/g)</th>
<th>Fucose (mg/g)</th>
<th>Sialic acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>41.64±1.17⁹</td>
<td>17.32±1.32⁹</td>
<td>15.82±1.20⁹</td>
<td>10.42±0.79⁹</td>
</tr>
<tr>
<td>Normal+PTS (40 mg/kg b.w.)</td>
<td>38.60±2.95⁹</td>
<td>19.64±1.20⁹</td>
<td>16.02±0.79⁹</td>
<td>10.02±0.79⁹</td>
</tr>
<tr>
<td>HFD+STZ</td>
<td>58.13±3.42⁹</td>
<td>18.81±3.13⁹</td>
<td>14.07±2.53⁹</td>
<td>7.53±5.74⁹</td>
</tr>
<tr>
<td>HFD+HFD+PTS (40 mg/kg b.w)</td>
<td>45.80±3.50⁹</td>
<td>22.34±1.71⁹</td>
<td>19.44±1.81⁹</td>
<td>6.33±4.47⁹</td>
</tr>
</tbody>
</table>

Values are means±SD for six mice. Values not sharing a common superscript (a, b, and c) differ significantly at p<0.05 (DMRT). **PTS**: Pterostilbene, **HFD**: High-fat diet, **STZ**: Streptozotocin, **SD**: Standard deviation, DMRT: Duncan’s multiple range test.
Table 3: Changes in the levels of renal glycoproteins in normal and experimental mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexose (mg/g)</th>
<th>Hexosamine (mg/g)</th>
<th>Fucose (mg/g)</th>
<th>Sialic acid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>27.8±2.11</td>
<td>14.8±1.12</td>
<td>11.88±0.90</td>
<td>7.82±0.59</td>
</tr>
<tr>
<td>Normal+PTS (40 mg/kg b.w.)</td>
<td>24.1±1.85</td>
<td>12.61±0.96</td>
<td>11.06±0.84</td>
<td>7.23±0.55</td>
</tr>
<tr>
<td>HFD+STZ</td>
<td>5.23±3.96</td>
<td>27.08±2.60</td>
<td>26.60±2.17</td>
<td>4.78±0.36</td>
</tr>
<tr>
<td>HFD+HFD+PTS (40 mg/kg b.w.)</td>
<td>3.65±2.50</td>
<td>17.13±1.30</td>
<td>15.82±1.21</td>
<td>6.12±0.46</td>
</tr>
</tbody>
</table>

Values are means±SD for six mice. Values not sharing a common superscript (a, b, c) differ significantly at p<0.05 (DMRT). PTS: Pterostilbene, HFD: High-fat diet, STZ: Streptozotocin, SD: Standard deviation, DMRT: Duncan’s multiple range test.

CONCLUSION

From the above observations, we concluded that the oral administration of PTS to diabetic mice reversed the changes in glycoprotein components in plasma, liver, and kidney. PTS may have a beneficial effect by the enhancement of insulin action in diabetic mice as evident by the decreased level of serum glucose in PTS-treated diabetic mice. Hence, the PTS may be useful in the preparation of drug ingredient for the prevention and control of diabetes mellitus.

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AUTHORS’ CONTRIBUTIONS

First author (Gunaseelan) conducted animal care and prepared the manuscript. The second author (Dr. L. Pari) initiated and supervises the research.

CONFLICTS OF INTEREST

All the authors stated that there are no conflicts of interest.
REFERENCES


