ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 5, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

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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Received: 06 March 2019, Revised and Accepted: 04 April 2019

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 (10^{th} 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.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i5.32940

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 develop 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 numerous 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/6J mice 3 weeks of age were obtained from BioGen Laboratory Animal Facility, Bengaluru, India, and housed in polypropylene cages. The animals were housed in well-ventilated polypropylene 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), Annamalainagar.

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 10th 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 at 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 recentrifuged, 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 homogenized with 14.0 ml of chloroform. This was filtered and the residue was successively homogenized in chloroform-methanol (2:1v/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, hexosamine, and SA.

Determination of glycoproteins levels

Hexose was estimated by the method of Niebes [13]. The reaction mixture contained 0.5 ml of tissue homogenate/plasma, 0.5 ml of 5% phenol, and 2.5 ml of concentrated H_2SO_4 and 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 3N HCl. It was boiled for 6 h and neutralized with 6N 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-arsenate and 2.0 ml of thiobarbituric 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 H_2SO_4 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% formalin and embedded in paraffin. The prepared paraffin blocks were later sectioned (5 μm) using a microtome, deparaffinated 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 the 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,

Table 1: Effect of PTS on plasma glycoproteins in normal and experimental mice

Groups	Hexose (mg/dl)	Hexosamine (mg/dl)	Fucose (mg/dl)	Sialic acid (mg/dl)
Normal control	90.23±6.87^a	69.72±5.30^a	29.33±2.23^a	56.36±4.29^a
Normal+PTS (40 mg/kg b.w)	89.47±6.84^a	66.32±5.07^a	29.16±2.23^a	54.45±4.16^a
HFD+STZ	141.35±10.76^b	92.42±7.03^b	48.43±3.68^b	75.43±5.74^b
HFD+HFD+PTS (40 mg/kg b.w)	103.22±7.90^c	73.56±5.63^c	33.85±2.59^c	62.39±4.77^c

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 2: Changes in the levels of liver glycoproteins in normal and experimental mice

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Fucose (mg/g)	Sialic acid (mg/g)
Normal control	41.64±3.17^a	17.37±1.32^a	15.82±1.20^a	10.42±0.79^a
Normal+PTS (40 mg/kg b.w)	38.60±2.95^a	18.83±1.43^a	16.94±1.29^a	10.02±0.76^a
HFD+STZ	58.13±4.42^b	43.42±3.30^b	34.87±2.65^b	04,27±0.32^b
HFD+HFD+PTS (40 mg/kg b.w)	45.80±3.50^c	22.34±1.71^c	19.44±1.48^c	07,33±4.77^c

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

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Fucose (mg/g)	Sialic acid (mg/g)
Normal control	27.81±2.11[a]	14.81±1.12[a]	11.88±0.90[a]	7.82±0.59[a]
Normal+PTS (40 mg/kg b.w)	24.19±1.85[a]	12.61±0.96[a]	11.06±0.84[a]	7.23±0.55[a]
HFD+STZ	52.30±3.98[b]	27.08±2.06[b]	28.60±2.17[b]	4.78±0.36[b]
HFD+HFD+PTS (40 mg/kg b.w)	32.65±2.50[c]	17.13±1.30[c]	15.82±1.21[c]	6.12±0.46[c]

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

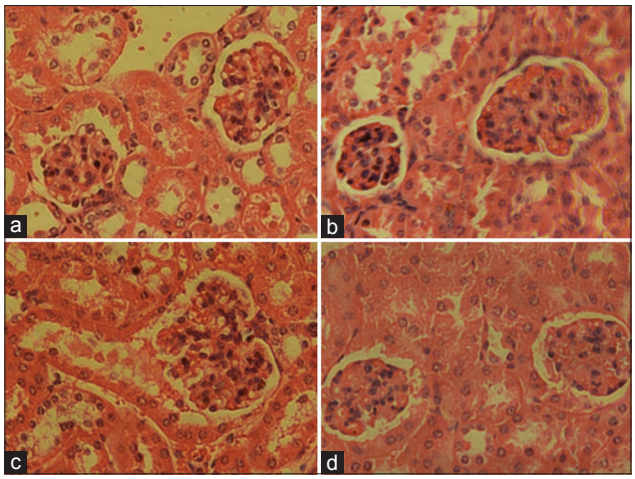

Fig. 1: Photomicrographs of hematoxylin and eosin stained section of mice kidney (100× standard light magnification). (a) Normal renal tubular architecture and glomeruli, (b) normal + pterostilbene (PTS) showing normal architecture and glomeruli, (c) high-fat diet (HFD) +streptozotocin (STZ)-induced kidney parenchyma showing significant vacuolar degeneration of renal tubular (→) shows severe degeneration along with necrosis of tubular vacuolization and dilation of cells, (d) HFD+STZ+ PTS showed normal glomeruli maintaining structure similar to observed near normal and mild fatty infiltration

whereas an increase in protein-bound hexose, hexosamine, and fucose was observed. Oral administration of PTS to HFD-induced diabetic mice significantly reversed these changes in tissues to near normal.

Fig. 1 shows the histopathological changes in the kidney of different groups of mice. In HFD/STZ-fed diabetic mice, segmented glomerulonephritis and fat accumulation in the tubules were observed (1c). Administration of PTS showed cloudy swelling and mild fat accumulation in tubules.

DICUSSION

Diabetes is a progressive disease whose biochemical basis for progression and complications is poorly understood [17]. In the diabetic state, glucose is redirected through insulin-independent pathways resulting in enhanced production of carbohydrate moieties of glycoproteins. Therefore, there is an elevation in the levels of protein-bound hexose, hexosamine, fucose, and SA in plasma of diabetic animals. Sustained hyperglycemia increases the expression of glutamine: Fructose-6-phosphate aminotransferase, the rate-limiting enzyme of the hexosamine pathway leading to an increase in the levels of hexose and hexosamine in plasma and tissues [18].

Protein-bound hexose contributes hydrophilic nature to the cell membrane and hexosamine through its cationic charges makes cell membrane polarized [19]. In our study, diabetic mice showed increased levels of hexose and hexosamine in plasma, hepatic, and renal tissues. Administration of PTS to diabetic mice significantly reduced their levels to near normal. Our results are in concordance with Saravanan and Pari, who reported that administration of thymol improved hexose and hexosamine levels in diabetic mice [20].

Fucose is a member of a group of eight essential sugars that the body requires for the optimal functioning of cell-to-cell communication and its metabolism appears to be altered in various diseases such as diabetes mellitus [21]. Fucose and sialic acid form specific structures called glycanic chains covalently linked to lipids or proteins which are present on the cell surface. The fucose level could be increased due to increased glycosylation in diabetic condition [22]. In the present study, a rise in fucose levels could be due to increased glycosylation in diabetic condition. Oral administration of PTS has restored the level of fucose to near normal which could be due to improved glycemic control.

SA is widely distributed in tissues and circulation. It is an acetylated derivative of neuraminic acid and is an essential component of glycoproteins and glycolipids. It is covalently bound to glycoproteins as the terminal sugar of oligosaccharide chains. Elevated levels of serum SA are considered to be a good predictor of cardiovascular disease [23]. Diabetic mice had increased levels of SA in the plasma and a decrease in tissues [24]. The increased levels of SA reported in diabetic mice might be due to either enhanced SA synthesis or decreased sialidase activity. However, decreased level of SA is observed in the tissues of diabetic mice which may be related to increased synthesis of fibronectin, which contains SA in its core structure. Further, the decreased tissue SA levels are associated with oxidative stress-induced desialylation of glycoproteins [25]. Treatment with PTS has normalized SA levels in the plasma and tissues of diabetic mice, which could be due to the regulation of sialidase activity by insulin, since insulin is a more likely mediator of SA changes.

Histopathological examination of the kidney of diabetic mice revealed morphological changes. The kidney of HFD/STZ-induced mice showed segmented glomerulonephritis and tubule shows fat accumulation. Treatment with PTS improved the above histopathological changes toward normality. This histological observation showed the protective role of PTS in HFD/STZ-induced oxidative stress in insulin resistance mice.

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.

ACKNOWLEDGMENT

We gratefully acknowledge the Indian Council of Medical Research (ICMR), New Delhi, for providing financial assistance to this project.

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.

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