Research Article

Effect of Pterostilbene on cardiac oxidative stress on high-fat diet-fed and Streptozotocin-induced type 2 diabetic mice

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Abstract

Objective: Diabetes is a metabolic disorder characterized by enhanced production of free radicals hence oxidative stress. The aim of this study was to evaluate the activity of cardiac and antioxidant enzymes in high-fat diet-fed and streptozotocin-induced type 2 diabetic mice. **Material and methods:** The experimental study period was 16 weeks. C57BL6/J mice were fed a normal diet, normal diet with Pterostilbene (PTS), high-fat diet (HFD) and streptozotocin injection (10^{th} week only), diabetic mice with PTS for last 6 weeks. Diabetic mice had high level of cardiac total cholesterol, triglycerides and free fatty acids level. **Results and conclusion:** Cardiac markers such as AST, CK-MB and LDH levels were significantly increased in diabetic mice. The activity level of enzymic (SOD, CAT and GPx) and non-enzymic antioxidant (Vitamin C, E and GSH) were lower diabetic mice. The levels of lipid peroxidation markers significantly increased in heart tissues. Treatment with PTS at a dose of 40mg/Kg BW significantly improved the parameters. This study suggests that PTS could be effective in improving cardiac dyslipidemia and antioxidant status in type 2 diabetes.

***Keywords*:** Pterostilbene, high-fat diet, streptozotocin, cardiac markers, type 2 diabetes

Introduction

Diabetes is a common disease in the whole world, which has an increasing prevalence (Mousavi et al., 2012). Type 2 diabetes mellitus is a metabolic disorder that reduces the body's ability to glucose uptake. Factors causing diabetes are still unknown, although genetic factors, obesity and the absence of physical activity have an important role in diabetes (Beckert et al., 2006). Diabetes mellitus leads to hyperglycemia, blood lipid disorders, including hypertriglyceridemia and low HDL-cholesterol and increased oxidative stress (Murarka and Movahed, 2010). Uncontrolled diabetes can cause diabetic complications, which increase medical costs and decrease quality of life (Goldberg, 2003).

Cardiovascular diseases (CVDs) are among the most prevalent diabetic complications and leading causes of premature mortality among patients with type 2 diabetes (Garg and Grundy, 1990). Control of hyperglycemia and dyslipidemia is essential in reducing the risk for cardiovascular complications (Giacco and Brownlee, 2010). In addition, evidence has accumulated that oxidative stress in diabetes contributes to the development of complications, including macro and microvascular complications, and improvement of antioxidant status can be beneficial to protection against diabetic complications (Rahimi et al., 2005). Therefore, agents with hypoglycemic, lipid-lowering, and antioxidant activity could be very promising in the management of diabetes and prevention of cardiovascular complications.

Pterostilbene (PTS) is a naturally derived compound found primarily in blueberries and *Pterocarpus marsupium* heartwood (Yue and Ho, 2009). 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 (Chakraborty et al., 2010). PTS is structurally similar to

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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 (Kapetanovic et al., 2011). Hence, our objective of this study was to evaluate cardioprotective activity of PTS on high-fat diet-fed and streptozotocin-induced type 2 diabetic mice.

Materials and Methods

Animals

Male C57BL/6J mice were purchased from BioGen Laboratory Animal Facility, Bangalore, India, at 5 weeks of age. The mice were housed in a room maintained at a controlled temperature (23±1°C) and 12-hour light/12-hour dark cycle at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were given free access to water and food. 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), Annamalai University, Annamalai nagar.

Experimental induction of diabetes

Diabetes was induced in male C57BL/6J mice by feeding a high fat diet containing 35% fat (Beef tallow mixed with the diet) for 60 days, in addition Streptozotocin (STZ) was injected at a low dose in 5th 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 four groups with 6 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 Streptozotocin (STZ) was injected at 10th week.

Group 4: Diabetic mice received PTS (40 mg/kg BW) for last 6 weeks

At the end of the experimental period, mice were sacrificed by cervical dislocation. Blood was collected by cutting the jugular vein into heparinised glass tubes. Plasma was obtained from blood samples after centrifugation (1500×g for 10 min) and stored at 4 °C until analysis.

Tissue homogenate preparation

Heart tissues was sliced into pieces and homogenized in phosphate buffer (pH 7.0) in cold condition to give 20% homogenate (w/v). The homogenates were centrifuged at 1000 rpm for 10 min at 0 °C in a cold centrifuge. The supernatant were separated and used to measure the lipid peroxidative markers, enzymic and nonenzymic antioxidants system.

Analysis of cardiac markers

Levels of various cardiac enzymes including Aspartate aminotransferase (AST), creatine kinase-isoenzyme (CK-MB) and lactate dehydrogenase (LDH) were assessed using commercial available kits (Agappe Pharmaceutical, Kerala, India), using autoanlyzer.

Heart lipid profile

The level of total cholesterol (TC) was estimated by the method of Allain et al. (1974). The level of triglycerides (TG) was estimated by the method of McGowan et al. (1983). Free fatty acids (FFA) levels in the liver were estimated by the method of Falholt et al. (1973).

Estimation of lipid peroxidation products

Thiobarbituric acid reactive substances (TBARS) were measured by method of Niehaus and Samuelson (1968) by their reactivity with thiobarbituric acid in an acidic condition to generate pink colored chromophore which was read at 535 nm. Lipid hydroperoxides (LOOH) was estimated by the method of Jiang et al. (1992) based on the oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange that led to the formation of a chromophore with an absorbance maximum at 560 nm.

Determination of enzymic antioxidants

The activity of superoxide dismutase (SOD) was estimated by the method of Kakkar et al. (1984) based on 50% inhibition of the formation of NADH-phenazine methosulphate nitroblue tetrazolium (NBT) formazan at 520 nm. The activity of catalase (CAT) was assayed by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The reaction was stopped by the addition of dichromate - acetic acid mixture and the chromic acetate formed was measured colorimetrically at 620 nm. Glutathione peroxidase (GPx) activity was assayed by the method of Rotruck et al. (1973). A known amount of enzyme preparation was allowed to react with H_2O_2 in the presence of GSH for a specified time period. GPx utilize GSH for the decomposition of H_2O_2. After a specific time, the remaining GSH content was measured by Ellman's method (1959).

Determination of non-enzymic antioxidants

The level of vitamin C was assessed by the method of Roe

and Kuether (1980). The ascorbic acid was converted to dehydroascorbic acid by mixing with norit and then coupled with 2, 4-dinitrophenylydrazine (DNPH) in the presence of thiourea, a mild reducing agent. The coupled dinitrophenylhydrazine was converted into a red colored compound when treated with sulfuric acid and read at 520 nm. Vitamin E was estimated by the method of Baker and Frank (1980). The method involves the reduction of ferric ions to ferrous ions by α-tocopherol and the formation of a red colored complex with 2, 2-dipyridyl. Absorbance of the chromophore was measured at 520 nm. Reduced glutathione (GSH) was estimated by the method of Ellman (1959). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow color compound) when dithionitrobenzoic acid was added to compounds containing sulfhydryl groups. The yellow colour developed was read at 412 nm.

Statistical analysis

Values are given as means ± S.D. for six readings from ten mice in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 11.5 (SPSS, Chicago, IL). The limit of statistical significance was set at $p \leq 0.05$.

Results and discussion

Results showed in table 1 and 2 describe the activities of marker enzyme such as AST, CK-MB and LDH in serum samples of control and diabetic mice. The activities of AST, CK-MB and LDH showed significant increase in diabetic animals (Group III) when compared to that of Group I animals. These marker activities are significantly decreased in PTS treated (Group IV) animals as compared to diabetic mice (Group II). Diagnosis of cardiac enzymes is prerequisite in case of diabetic cardiomyopathy. AST, CK-MB and LDH are commonly used as biomarkers for myocardial infarction (Maghamiour and Safaie, 2014). Serum creatine phosphokinase activity is a more sensitive indicator in early stage of myocardial ischemia, while peak rise in LDH is roughly proportional to the extent of injury to the myocardial tissue. Diabetic animals show increased levels of AST, CK-MB and LDH in serum (Fatima Ali et al., 2016). In this study, PTS (40 mg/kg) significantly reduced levels of cardiac enzymes such as CK-MB, LDH, and AST compared with diabetic control indicating prevention of cardiac damage and offering cardioprotection.

The levels of TC, TG and FFA in the heart of HFD and STZ induced diabetic mice shown in table 2. The concentrations of hepatic lipids (TC, TG and FFA) were significantly increased in HFD and STZ induced mice as compared to the normal mice. Treatment with PTS significantly reduced the concentrations of cardiac lipids (TC, TG and FFA), as compared to diabetic mice. Coronary heart disease (CHD) is a life threatening diabetes complication and its risk increases two or more folds in diabetes. Dyslipidemia in diabetes has been reported to be strongly associated with CHD (Haffner, 2000). Increased triglycerides, total cholesterol and free fatty acid levels in heart represent atherogenic lipid profile, which leads to the development of

Table 1. Effect of Pterostillbene (PTS) on the AST, CK-MB and LDH level in HFD + STZ induced diabetic mice

Groups	AST (IU/L)	CK-MB (IU/L)	LDH (IU/L)
Control	73.16 ± 0.49	84.92 ± 7.14	465.47 ± 22.53
Control + PTS (40 mg/kg BW)	69.52 ± 0.51	80.27 ± 7.18	461.66 ± 21.74
HFD + STZ	162.59 ± 13.62	152.75 ± 11.72	683.36 ± 25.04
HFD + STZ + PTS (40 mg/kg BW)	93.62 ± 7.53	118.47 ± 8.50	512.62 ± 11.83

Each value is mean ± SD for mice in each group. In each group, means with different superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

Table 2. Effect of Pterostillbene (PTS) on the cardiac lipids level in HFD + STZ induced diabetic mice

Groups	TC (mg/g wet tissue)	TG (mg/g wet tissue)	FFA (mg/g wet tissue)
Control	3.48 ± 0.26^{a}	5.48 ± 0.36^{a}	6.17 ± 0.44^{a}
Control + PTS (40 mg/kg BW)	3.16 ± 0.25^{a}	5.18 ± 0.30^{a}	6.05 ± 0.45^{a}
HFD + STZ	9.64 ± 0.66^{b}	12.42 ± 0.82^{b}	10.26 ± 0.84^{b}
HFD + STZ + PTS (40 mg/kg BW)	5.24 ± 0.41^{c}	7.39 ± 0.94^{c}	8.14 ± 0.68^{c}

Each value is mean ± SD for mice in each group. In each group, means with different superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

CHD (Garber, 2002.). As a favorable effecton lipid profile was observed following treatment with PTS, this indicated that it might help prevent the progression of CHD.

Table 3 shows the effect of PTS on the levels of TBARS and LOOH in the cardiac tissue of HFD and STZ induced C57BL/6J mice. The HFD and STZ mice exhibited a significant increase in TBARS and LOOH (Group III). PTS treatment showed significant reduction in the levels of TBARS and LOOH in cardiac tissue (Group IV). Lipid peroxidation is an important pathogenic event in diabetes mellitus. TBARS and Lipid hydroperoxides are the most commonly used markers of lipid peroxidation in plasma and tissues (de Souza Bastos et al., 2016). The data presented in this study showed that HFD and STZ caused a marked increase in TBARS and lipid hydroperoxides level in cardiac tissues. The marked increase in the production of TBARS could be due to the superoxide radical overload, indicating the presence of oxidative stress and a subsequent increase in the production of hydrogen peroxide (Bartels, Davidson and Gong, 2007). This is consistent with previous reports that HFD and STZ induced critical oxidative damage in plasma and liver, kidney and heart tissues (Kaviarasan and Pugalendi, 2011.). Though treatment with PTS reduced the lipid peroxidation level significantly, this may be due to PTS scavenging excessive free radicals produced in diabetic condition.

Table 4 shows the effect of PTS on the activities of SOD, CAT and GPx in the heart tissue of HFD and STZ induced C57BL/6J mice. The activities of SOD, CAT and GPx decreased in heart tissues of diabetic mice. PTS treatment significantly increased the activities of these enzymic antioxidants towards normality. In diabetics, hyperlipidemia and hyperglycemia-induced oxidative stress has been regarded as contributors to progression of MI (Ansley and Wang, 2013). The oxidative stress results in disturbance between free radicals and antioxidant defense mechanism. SOD, one of the important defense enzymes catalyzes the dismutation of superoxide radicals into either oxygen (O^2) or hydrogen peroxide. GPx or CAT catalyzes the reduction of hydrogen peroxide (H_2O_2) into H_2O, CAT catalyzes this reduction independently without any cofactor, whereas GP_X relies on GSH, GSH also inhibits lipid peroxidation (Vessal et al., 2003). Depletion in the activities of theses antioxidant enzymes can be owed to an enhanced radical production. Our data showed that diabetes decreased the activities of SOD, CAT and GPx in heart tissues. Suggesting that HFD and STZ induced an imbalance in the antioxidant defense systems and eventually leading to enhanced lipid peroxidation. Treatment with PTS increased the SOD, CAT and GPx activities brought to normality. The additive effect of the PTS on increasing SOD, CAT and GPx activities could be partially explained by their combined action on scavenging free radicals.

Table 5 shows the levels of non-enzymic antioxidants (vitamin C, vitamin E and GSH) in control and

Table 3. Effect of Pterostillbene (PTS) on the heart lipid peroxidation level in HFD + STZ induced diabetic mice

Groups	TBARS (mmol/100 g wet tissue)	Lipid hydroperoxides (mmol/100 g wet tissue)
Control	3.62 ± 0.31^a	5.72 ± 0.42^a
Control + PTS (40 mg/kg BW)	3.48 ± 0.28^a	5.28 ± 0.36^a
HFD + STZ	10.44 ± 0.74^b	10.75 ± 0.83^b
HFD + STZ + PTS (40 mg/kg BW)	5.61 ± 0.37^c	7.62 ± 0.53^c

Each value is mean ± SD for mice in each group. In each group, means with different superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

Table 4. Effect of Pterostillbene (PTS) on the heart antioxidant level in HFD + STZ induced diabetic mice

Groups	SOD (U*/mg protein)	CAT (U**/mg protein)	GPx (U***/mg protein)
Control	5.74 ± 0.32^a	68.25 ± 5.31^a	12.94 ± 0.45^a
Control + PTS (40 mg/kg BW)	5.96 ± 0.38^a	69.46 ± 4.27^a	13.58 ± 0.74^a
HFD + STZ	2.14 ± 0.16^b	28.63 ± 2.94^b	4.52 ± 0.35^b
HFD + STZ + PTS (40 mg/kg BW)	3.98 ± 0.27^c	47.51 ± 3.62^c	10.49 ± 0.72^c

U* = Enzyme concentration required for 50% inhibition of NBT reduction/minute. U**= µmole of hydrogen peroxide consumed/minute. U***=µmol of GSH utilized/minute. Values are means ± S.D. for six assays from ten mice. Values not sharing a common superscript differ significantly at $p \leq 0.05$. Duncan's multiple range test (DMRT).

Table 5. Effect of Pterostillbene (PTS) on the heart antioxidant level in HFD + STZ induced diabetic mice

Groups	Vitamin C (µg/mg protein)	Vitamin E (µg/mg protein)	GSH (µg/mg protein)
Control	0.93 ± 0.05^{a}	5.37 ± 0.31^{a}	12.73 ± 0.86^{a}
Control + PTS (40 mg/kg BW)	1.25 ± 0.06^{a}	5.84 ± 0.42^{a}	13.62 ± 0.97^{a}
HFD + STZ	0.31 ± 0.02^{b}	2.04 ± 0.19^{b}	5.34 ± 0.59^{b}
HFD + STZ + PTS (40 mg/kg BW)	0.81 ± 0.03^{c}	4.21 ± 0.38^{c}	9.74 ± 0.83^{c}

Values are means ± S.D. for six assays from ten mice. Values not sharing a common superscript differ significantly at $p \leq 0.05$. Duncan's Multiple Range Test (DMRT)

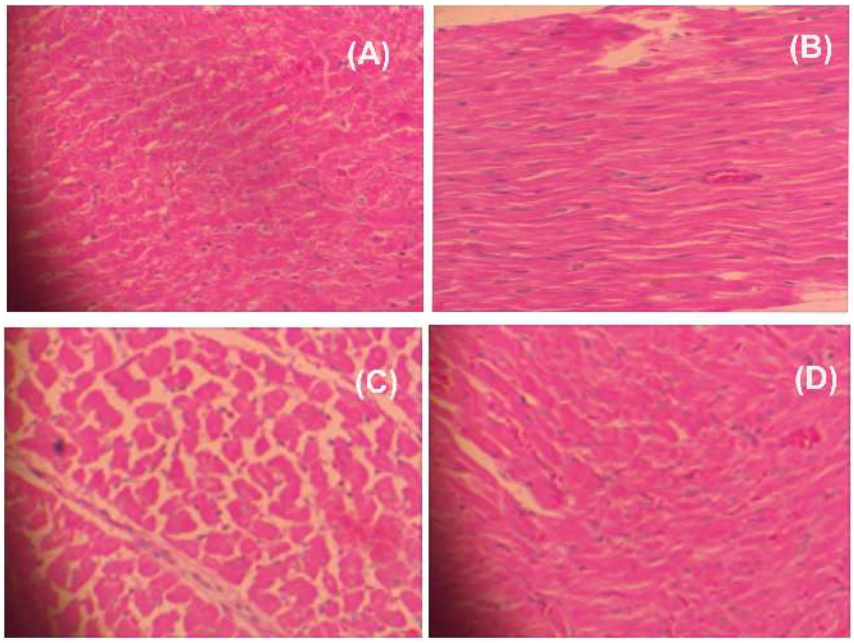

Figure 1. Histology of heart on treatment with PTS. (A) Normal, (B) Normal + PTS, (C) HFD + STZ, (D) HFD + STZ +PTS. Figure (A) and (B) showing normal cardiac fibers, figure (C) mice showing cardiac damage and fatty infiltration in the myocardium, figure (D) showing reduced damage of cardiac muscle fibres. Stained by Haematoxylin and eosin.

experimental animals. The levels of vitamin C, vitamin E and GSH reduced in the heart of HFD and STZ induced C57BL/6J mice. Supplementation of PTS significantly increased these levels, when compared to diabetic group. Apart from the enzymatic antioxidants, nonenzymatic antioxidants such as vitamin C, vitamin E and GSH play an excellent role in preventing the cells from oxidative threats. Vitamin E is the most ancient antioxidant in the lipid phase (Sundaresan and Pugalendi, 2012). In the present study, we have observed a significant decrease in the levels of nonenzymatic antioxidants in the heart tissues of diabetic mice. Administration of PTS to diabetic mice significantly increased the levels of these nonenzymatic antioxidants.

Conclusion

In conclusion, our current study demonstrated that PTS improved alleviated lipid abnormalities in cardiac tissues and reduces the risk of myocardial infraction, as well as reduced cardiac oxidative stress in type 2 diabetic mice. Thus, may be useful in the management of diabetes mellitus and prevention of diabetic complications.

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Conflict of Interest

All the authors stated that there is no conflict of interest.

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