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ORIGINAL ARTICLE

Effect of pterostilbene on lipids and lipid profiles in streptozotocin–nicotinamide induced type 2 diabetes mellitus

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Summary
Hyperlipidaemia is one of the major risk factors of cardiovascular complication in diabetes. A study was undertaken to evaluate the antihyperlipidaemic activity of pterostilbene. Oral administration of pterostilbene (40mg/kg bodyweight) to streptozotocin-nicotinamide induced diabetic rats for 6 weeks significantly reduced the elevated serum very low density lipoprotein (VLDL) and low density lipoprotein (LDL)-cholesterol levels and significantly increased the serum high-density lipoprotein (HDL)-cholesterol level. In addition, pterostilbene also significantly lowered the levels of triglycerides, phospholipids, free fatty acids and total cholesterol in the serum, liver and kidney of diabetic rats.

Keywords: pterostilbene – lipoproteins – lipids – diabetes

INTRODUCTION

Dyslipidaemia, plays a significant role in the manifestation and development of premature atherosclerosis leading to cardiovascular (CV) disease, and together, they are the major cause of CV morbidity and mortality in diabetes. Diabetes mellitus is a major risk factor for the development of cardiovascular complications, and cardiovascular disease now accounts for 80% of all diabetic mortality (WHO 2004). Lipid-lowering therapy in diabetes is effective in reducing the risk of vascular complications (Deedwania 2004).

Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. *Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus (Warrier et al. 1995). Pterostilbene (Fig. 1) was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium* (Maurya et al. 2004), and it is suggested that it might be one of the principal anti-diabetic constituents of *Pterocarpus marsupium* (Manickam et al. 1997). An aqueous extract of heartwood of *P. marsupium* has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients (ICMR 1998).

The streptozotocin-nicotinamide type 2 model shares a number of features with human type 2

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diabetes, and is characterized by a moderate stable hyperglycaemia, glucose intolerance, and an altered but significant glucose-stimulated insulin secretion, *in vivo* and *in vitro*. In our previous study we found that pterostilbene (40mg/kg) effectively reduced the blood glucose in diabetic rats (Pari and Amarnath Satheesh, 2006). However, no scientific studies have been done to establish the hypolipidemic effect of pterostilbene in experimental Type 2 diabetes. The present investigation was conducted to evaluate the hypolipidaemic activity of pterostilbene against STZ-nicotinamide induced diabetic rats.

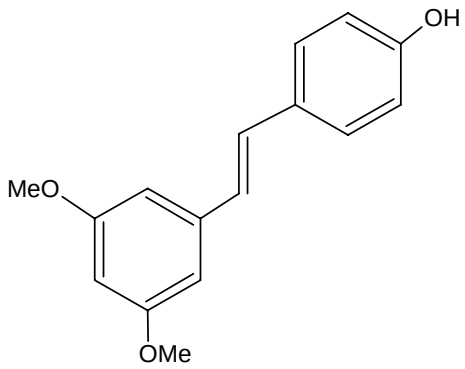

Fig. 1. **Chemical structure of pterostilbene**

MATERIALS AND METHODS

Animals

Male albino Wistar rats obtained from the Central Animal house, Rajah Muthiah Medical College, Annamalai University were used in this study at the age of 8 weeks (200 – 220 g). They were housed in an animal room, temperature 24±2 °C under controlled conditions on a 12 h light/12 h dark cycle. They all received a standard pellet diet (Lipton India Ltd., India) and water *ad libitum*. The animals used in the present study were cared for as per the principles and guidelines of the Institutional Animal Ethical committee of Annamalai University, Annamalainagar (Vide No. 158, 2003).

Chemicals

Streptozotocin (STZ) was purchased from the Sigma Chemical Co., St Louis, MO, USA. Pterostilbene was received as a gift sample from the Sabinsa Corp., USA. Nicotinamide was purchased from Himedia, Mumbai, India. The commercial diagnostic kits from Qualigens Diagnostics (Mumbai, India) were used for the estimation of cholesterol, HDL-Cholesterol and triglycerides. All the other chemicals and reagents used were of analytical grade.

Experimental induction of type 2 diabetes

STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Type 2 diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg STZ, 15 min after the i.p. administration of 110 mg/kg of nicotinamide (Masiello et al. 1998). Hyperglycaemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. The animals with a blood glucose concentration of more than 250 mg/dl were used for the study.

Experimental design

In the experiment, a total of 40 rats (24 diabetic surviving rats, 16 normal rats) were used. The rats were divided into five groups of 8 each, after the induction of type 2 diabetes. Two rats from each group were randomly selected and used for histopathological studies. Group I: Normal control (vehicle treated). Group II: Normal rats received pterostilbene (40 mg/kg body weight) in 1 ml of 0.5% methylcellulose suspension (Klimes et al. 1998) for six weeks. Group III: Diabetic control. Group IV: Diabetic rats received pterostilbene (40 mg/kg body weight) in 1 ml of 0.5% methylcellulose suspension for six weeks. Group V: Diabetic rats received metformin (500 mg/kg body weight) in 1 ml of saline (Soon and Tan 2000) for six weeks.

At the end 6 weeks, plasma was separated for the estimation of glucose. Tissues (liver and kidney) were dissected out, washed in ice-cold saline and stored at – 20 °C until used. The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer, pH 7.5. After centrifugation at 2000 rpm for 10 min, the clear supernatant was used for biochemical assays.

Analytical Methods

The level of plasma glucose was estimated by using a reagent kit from Qualigens Diagnostics kit (Mumbai, India) according to the method of Trinder (1969). Lipids were extracted from plasma and tissues by the method of Folch et al. (1957) using chloroform:methanol mixture (2:1 v/v). For the lipid extraction, the liver was rinsed in cold physiological saline thoroughly and dried by pressing between the folds of filter paper. From the lipid extract, the levels of total cholesterol and triglycerides (TG) were estimated by using reagent kits from Qualigens Diagnostics kit (Mumbai, India) according to the method of Zlatkis et al. (1953) and Fossati and Lorenzo (1982), respectively. The levels of free fatty acids (FFA) and phospholipids (PL) were estimated by the method of Falholt et al. (1973) and Zilversmit and Davis (1950), respectively. The phosphorus content in the extract was determined by the method of Fiske and Subbarow (1925).

The high density lipoprotein cholesterol (HDL-C) content in plasma was estimated by using a reagent kit (Qualigens Diagnostics, Mumbai, India). Very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) fractions were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol – (HDL-C + VLDL-C), respectively. The activity of hydroxy 3-methylglutaryl-coenzyme A (HMG CoA) reductase in the liver and kidney was assayed by the method of Philipp and Shapiro (1970). The ratio between HMG CoA and mevalonate in the liver was taken as an index of the activity of HMG CoA reductase. The decrease in HMG CoA/mevalonate ratio indicates the increased activity of the enzyme.

Statistical analysis

The data for various biochemical parameters were analyzed using one way analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (Duncan 1957) at the significance level $2\alpha=0.05$.

Table 1. **Changes in the levels of plasma glucose in normal and experimental rats**

Groups	Glucose (mg/dl)	
	Intial	Final
Normal control	78.35 ± 5.48	82.50 ± 6.33[a]
Normal + Pterostilbene (40 mg/kg)	80.48 ± 6.26	75.48 ± 6.08[a]
Diabetic control	293.49 ± 28.12	396.61 ± 33.82[b]
Diabetic + Pterostilbene (40 mg/kg)	284.16 ± 23.52	123.65 ± 10.63[c]
Diabetic + Metformin (500 mg/kg)	285.98 ± 25.41	142.27 ± 12.89[c]

Values are given as mean ± SD from 6 rats in each group. Values not sharing a common superscript letter differ significantly at (DMRT).

RESULTS

The levels of plasma glucose are shown in normal and experimental rats (Table 1). There was a significant elevation in the level of glucose observed in diabetic rats compared to normal rats. The administration of pterostilbene and metformin significantly decreased the levels of glucose in diabetic rats when compared to non-diabetic rats.

Table 2 demonstrates the level of total cholesterol and lipoproteins in serum and tissue, and the activity of HMG-CoA reductase in normal and experimental rats. The levels of total cholesterol, LDL-C and VLDL-C and hepatic HMG-CoA reductase activity were significantly increased whereas the level of HDL-C was significantly decreased in diabetic control rats. The administration of pterostilbene to diabetic rats significantly decreased the levels of total cholesterol, LDL-C, VLDL-C and the activity of HMG-CoA reductase alone significantly increased in the levels of HDL-C.

Table 3 shows the level of serum and tissue (liver and kidney) triglycerides, free fatty acid and phospholipids in normal and experimental rats. The levels of all these lipids were significantly increased in diabetic control rats whereas the administration of pterostilbene and metformin to diabetic rats significantly decreased the levels of triglycerides, free fatty acid and phospholipids.

DISCUSSION

In the present investigation, treatment with pterostilbene showed significant antihyperglycaemic activity. The antihyperglycaemic activity of pterostilbene is due to the release of insulin from the existing β-cells of the pancreas (Pari and Amarnath Satheesh 2006).

Table 2. **Effect of pterostilbene on changes in the levels of lipoproteins and cholesterol in normal and experimental rats**

Groups	Normal	Normal + Pterostilbene	Diabetic control	Diabetic + Pterostilbene	Diabetic + Metformin
Serum					
Total cholesterol (mg/dl)	95.61 ± 8.49^{a}	93.24 ± 7.96$^{\alpha}$	173.56 ± 15.34^{b}	114.82 ± 9.91^{c}	123.49 ± 10.74^{c}
HDL-C (mg/dl)	54.86 ± 4.40^{a}	56.12 ± 27.54^{a}	27.54 ± 2.11^{b}	47.69 ± 3.93^{c}	41.86 ± 3.70^{d}
LDL-C (mg/dl)	30.05 ± 2.65^{a}	26.79 ± 2.16^{a}	126.88 ± 10.67^{b}	53.71 ± 4.70^{c}	66.36 ± 6.18^{d}
VLDL-C (mg/dl)	10.69 ± 1.04^{a}	10.32 ± 1.10^{a}	19.13 ± 1.91^{b}	13.41 ± 1.19^{c}	15.27 ± 1.30^{d}
Liver (mg/100 g tissue)	328.46 ± 16.80^{a}	319.67 ± 14.37^{a}	525.84 ± 27.85^{b}	410.35 ± 20.29^{c}	433.56 ± 22.66^{c}
Kidney (mg/100 g tissue)	385.65 ± 17.71^{a}	378.65 ± 16.44^{a}	534.22 ± 28.20^{b}	428.39 ± 21.62^{c}	450.96 ± 23.59^{c}
Hepatic HMG-CoA Reductase^{A}	1.74 ± 0.1^{a}	1.81 ± 0.1^{a}	1.06 ± 0.1^{b}	1.58 ± 0.1^{c}	1.49 ± 0.1^{c}

^{A} – HMG-CoA/mevalonate ratio

Values are given as mean±SD from 6 rats in each group. Values not sharing a common superscript letter differ significantly at (DMRT).

Table 3. **Effect of pterostilbene on changes in the levels of serum and tissue free fatty acids, triglycerides and phospholipids in normal and experimental rats**

Groups	Normal	Normal + Pterostilbene	Diabetic control	Diabetic + Pterostilbene	Diabetic + Metformin
Triglycerides					
Serum (mg/dl)	53.48 ± 4.67^{a}	51.62 ± 4.38^{a}	95.67 ± 8.53^{b}	67.08 ± 5.32^{c}	76.35 ± 6.57^{d}
Liver (mg/100 g tissue)	340.91 ± 18.74^{a}	335.37 ± 17.77^{a}	620.49 ± 23.27^{b}	430.59 ± 19.48^{c}	479.98 ± 20.13^{d}
Kidney (mg/100 g tissue)	275.12 ± 16.54^{a}	270.35 ± 16.36^{a}	462.84 ± 27.78^{b}	371.52 ± 21.65^{c}	420.10 ± 25.23^{d}
Free fatty acids					
Serum (mg/dl)	75.64 ± 4.65^{a}	71.25 ± 4.36^{a}	146.84 ± 8.49^{b}	93.58 ± 5.30^{c}	99.11 ± 6.54^{c}
Liver (mg/100 g tissue)	586.11 ± 25.34^{a}	571.73 ± 23.72^{a}	864.08 ± 37.85^{b}	750.31 ± 31.45^{c}	796.19 ± 33.56^{d}
Kidney (mg/100 g tissue)	430.81 ± 24.58^{a}	424.08 ± 21.58^{a}	712.64 ± 36.84^{b}	540.10 ± 23.27^{c}	605.76 ± 27.32^{d}
Phospholipids					
Serum (mg/dl)	104.86 ± 8.26^{a}	93.95 ± 7.36^{a}	172.33 ± 15.82^{b}	125.42 ± 10.27^{c}	137.60 ± 11.89^{c}
Liver (g/100 g tissue)	1.63 ± 0.12^{a}	1.58 ± 0.11^{a}	2.81 ± 0.13^{b}	1.24 ± 0.12^{c}	1.15 ± 0.12^{c}
Kidney (g/100 g tissue)	1.44 ± 0.10^{a}	1.38 ± 0.11^{a}	2.12 ± 0.15^{b}	1.68 ± 0.11^{c}	1.82 ± 0.12^{c}

Values are given as mean±SD from 6 rats in each group.
Values not sharing a common superscript letter differ significantly at (DMRT).

Hyperlipidaemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition. The results of our present study clearly show that pterostilbene has a lowering action on triglyceride, total cholesterol, FFA, PL, VLDL and LDL. There is a substantial evidence that lowering the total cholesterollevel, particularly the LDL level will lead to a reduction in the incidence of coronary heart disease (CHD). Lowering of lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Bishayee 1993).

The abnormally high concentration of serum lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since the insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamines and other hormones enhance lipolysis. The marked hyperlipaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depot (Al-Shamaony 1994). Studies on STZ-induced diabetes in experimental animals have suggested that an increase in circulatory VLDL and their associated triglycerides are largely due to defective clearance of these particles from the circulation (Suresh Babu 1997). As there is a close relationship between the total cholesterol level of elevated serum and the occurrence of atherosclerosis, the ability of pterostilbene in the selective reduction of total cholesterol through the reduction of VLDL and LDL components could be beneficial in preventing atherosclerotic conditions and thereby reducing the possibility of CHD in general. As regards the effect of the pterostilbene on serum HDL, our results clearly show that the level of this lipoprotein fraction increased with pterostilbene treatment.

An increase in cholesterol levels in the hepatic tissue might be due to an increase in the transport of chylomicron cholesterol to the liver (Chauhan et al. 1987). Hypertriglyceridaemia in diabetes can result from an increased hepatic VLDL overproduction and impaired catabolism of TG-rich particles. Dysfunction of LPL also contributes to hypertriglyceridaemia in the fasting and postprandial state (Kanters et al. 2001). The increased level of cholesterol observed in diabetic kidney might be due to the decreased levels of HDL-cholesterol.

The Peroxisome Proliferator-Activated Receptor α-isoform (PPARα) is predominantly involved in fatty acid and lipid catabolism. It is also involved in the import and activation of genes involved in fatty acid oxidation in the liver, heart, kidney, and skeletal muscles (Fruchart et al. 2003, Gilde 2003). In the liver, activation of PPARα leads to increased β-oxidation of fatty acids and decreased triglyceride and VLDL synthesis (Fruchart and Duriez 2004). Activation of PPARα also leads to a reduction in triglyceride because of the repression of hepatic apolipoprotein C-III and an increase in lipoprotein lipase gene expression (Gervois et al. 2000). Furthermore, PPARα activation causes the induction of hepatic apoliporotein A-I and A-II expression in humans, leading to increased plasma HDL cholesterol. PPARα agonists are also known to slow the progression of premature coronary atherosclerosis. Pterostilbene is an agonist for PPARα, which possesses the activity of a hypolipidaemic drug, and thereby provides a possible alternative for the treatment of dyslipidaemia (Rimando et al. 2005). The decreased levels of cholesterol, TG, FFA and PL were found in the plasma and tissues of diabetic rats treated with pterostilbene. This could be due to an activation of PPARα by the administration of pterostilbene.

Since both diabetes and hyperlipidaemia are considered to be major risk factors for premature atherosclerosis and essentially all the cholesterol in atherosclerotic plaques is derived from that of circulatory cholesterol the hypolipidaemic and hypocholesterolaemic effect of pterostilbene in particular could be considered for its possible therapeutic value in diabetic hyperlipidaemia.

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