Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i4.2398

MODULATORY EFFECT OF PTEROSTILBENE ON CHANGES IN THE FATTY ACID COMPOSITION IN STREPTOZOTOCIN - NICOTINAMIDE INDUCED DIABETES.

Pidaran Murugan[1]*

[1]*Assistant professor of Biochemistry, Centre for Distance and Online Education, Bharathidhasan University, 620024. Tamil Nadu, India. India. Tel: + 9791620088
Email: manomuruganphd@gmail.com, Email: pmpranithmurugan18@gmail.com

***Corresponding Author:** Dr. P. Murugan
Email: manomuruganphd@gmail.com, Email: pmpranithmurugan18@gmail.com

Abstract
P.marsupium has been used in the treatment of toothache, diarrhoea, heartburn, urinary tract infections, boils, sores and skin diseases. *Pterocarpus marsupium* has been used for many years in the treatment of diabetes mellitus. Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium*. Pterostilbene is a useful bioactive compound in preventing type 1 diabetes, insulin resistance and type 2 diabetes in animal models. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances. The aim of this study was to evaluate the effect of Pterostilbene on blood glucose, plasma insulin and fatty acid composition of total lipids in liver, kidney and brain of control and streptozotocin (STZ)-nicotinamide diabetic rats. The analysis of fatty acids showed that there was a significant increase in the concentrations of palmitic acid (16:1), stearic acid (18:0) and oleic acid (18:1) acid in liver, kidney and brain, whereas the concentrations of linolenic acid (18:3) and arachidonic acid (20:4) were significantly decreased. Oral administration of the Pterostilbene (40 mg/kg body weight) for 45 days to diabetic rats decreased the concentrations of fatty acids, viz., palmitic, stearic, and oleic acid whereas linolenic and arachidonic acid were elevated.

Keywords: Pterostilbene, Tetrahydrocurcumin, palmitic, stearic, oleic acid linolenic acid, arachidonic acid, Diabetes

Introduction
Diabetes mellitus is a major risk factor for the development of cardiovascular complications and cardiovascular disease now accounts for 80% of all diabetic mortality (1). Hyperlipidemia in diabetes mellitus is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL) and changes in lipoprotein composition (2). Dyslipidemia plays a significant role in the manifestation and development of premature atherosclerosis leading to cardiovascular disease (CVD), and together, they are the major cause of CVD morbidity and mortality in diabetes. Elevation of lipids and lipoproteins are the characteristic of uncontrolled diabetes mellitus (3). High glucose is associated with increased oxidative stress and glycosylation of virtually every protein in the body, including lipoproteins, apolipoproteins and clotting factors (Laakso, 1999). Improved blood glucose control delays the onset and slows the progression of microvascular complications in

diabetic patients (4). Lipid-lowering therapy in diabetes was effective in reducing the risk of vascular complications (5). In this chapter, the effects of pterostilbene on lipids and fatty acid composition in normal and experimental rats are described.

During the process of injury, repair and cell growth, the fatty acids in phospholipids undergo severe modification (6). Earlier report showed that there is an alteration in the fatty acid composition in plasma and erythrocyte membrane of diabetic patients (6). Seigneur et al. (8) reported that there is a significant alteration in the fatty acid composition of serum and variety of tissues in experimental diabetes.

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 (9). Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium* (10). it is suggested that pterostilbene might be one of the principal anti-diabetic constituents of *Pterocarpus marsupium* (11). An aqueous extract of heartwood of *P.marsupium* has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients (12).

Tetrahydrocurcumin (THC) was one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin (13). Curcumin was rapidly metabolized during absorption from the intestine, yielding THC (14), which had shown the strongest antioxidant activity among all curcuminoids (15). THC thought to play a pivotal role in protecting the cell membrane against lipid peroxidation, which exhibits its protective effect by means of β-diketone moieties and phenolic hydroxyl groups (16). Several studies in experimental animals indicated that THC also preventes cancer, protect the inflammation, atherosclerotic lesions and hepatotoxicity (17).

Recently in our lab, we found that THC improves plasma insulin, decrease glucose levels, scavenging free radical and also antioxidant activity in type 2 diabetic rats (18,19). THC reverses the changes in the levels of the carbohydrate moieties of glycoprotein (20,21) and also antihyperlipidemic effect (22). The diabetic rats have reduced capacity answer to oxidative status and that this reduction is associated with hyperglycemia drives non-enzymatic glycation and oxidation of lipids, which enhances the formation of erythrocyte membrane enzymes in STZ - nicotinamide, induced diabetic rats.

To our knowledge, no other biochemical investigations had been carried out on the effect of THC in control and STZ - nicotinamide induced diabetic rats. So, the present investigation was carried out to study the effect of THC on fatty acid composition and lipids in control and diabetic rats.

Materials and methods

Animals

Studies were performed on adult male albino rats of Wistar strain weighing 180-220g. According to the experimental protocol approved by the Committee for Research and Animal Ethics of Annamalai University, animals were housed in cages and maintained in 24 ± 2; ^{o} C normal temperature and a 12 hour light/dark cycle. The animals were fed on pellet diet (Lipton India Ltd., Mumbai) and water *ad libitum*.

Drugs and chemicals

THC and Pterostilbene was a gift provided by Sabinsa Corporation, USA. All other chemicals and biochemicals 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 intraperitoneal administration of 110 mg/kg nicotinamide (23). Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h. Animals with a 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 (24) 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 (25). Group V: Diabetic rats received THC (80 mg/kg body weight) in1 ml of saline (26) for six weeks.

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose and the plasma was separated for the estimation of insulin. Liver, kidney and brain were dissected out, patted dry and weighed.

Analytical procedure

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (27). Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). Haemoglobin was estimated using the cyanmethaemoglobin method described by Drabkin and Austin (28). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman (29) with modifications according to Bannon (30).

Fatty acid composition was performed according to the method of Morrison and Smith (31). Fatty acid analysis was performed using a Tracer 540 gas chromatograph equipped with a column 2 cm long × 2 mm internal diameter, packed with 10% Cilar on chromosorb W, 80/100 mesh. Fatty acids separated were identified by the comparison of retention times with those obtained by the separation of a mixture of standard fatty acids. Measurements of peak areas and data processing were carried out by electronic integrator. Individual fatty acids were expressed as percentage of total fatty acids of 100mg tissue.

Histopathological study

The liver, kidney and pancreas samples fixed for 48h in 10% formal-saline were dehydrated by passing successfully in different mixture of ethyl alcohol – water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (4-5 μm thick) were prepared and then stained with hematoxylin and eosin dye, which mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

Statistical analysis

All data were expressed as mean ± SD of number of experiments. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (SPSS, Cary, NC, USA) and the individual comparison were obtained by Duncan's Multiple Range Test (DMRT) (32). A value of $p<0.05$ was considered to indicate a significant difference between groups. Values sharing a common superscript do not differ significantly with each other at $p<0.05$. The data on insulin

binding studies were analyzed by competition curve, Scatchard plot and average affinity profiles. All values are expressed as mean ± SD.

Results

Plasma glucose

Table 1 shows the effect of pterostilbene and THC on glucose levels for 0, 2, 4 and 6 weeks. In the pterostilbene-treated groups (all doses), although a significant antihyperglycemic effect was evident from the second week onwards, the decrease in glucose was maximum (56.54%) by the end of sixth week in the group receiving 40 mg/kg body weight of pterostilbene. Based on these data, the higher dose, 40 mg/kg body weight of pterostilbene was selected for further evaluation.

Table 1. Changes in the levels of glucose in normal and experimental rats for 2, 4 and 6 weeks

Groups	Glucose (mg/dl)			
	O day	2nd week	4th week	6th week
Normal control	73.31 ± 5.48	80.46 ± 5.38^{a}	77.6 ± 7.58^{a}	74.61 ± 5.41^{a}
Normal + Pterostilbene (40 mg/kg)	81.61 ± 6.56	77.41 ± 5.61^{a}	72.65 ± 6.58^{a}	69.51 ± 4.57^{a}
Diabetic control	315.35 ± 24.21	325.45 ± 27.31^{b}	362.75 ± 29.55^{b}	397.31 ± 33.56^{b}
Diabetic + Pterostilbene (10 mg/kg)	291.54 ± 25.85	265.35 ± 24.58^{c}	234.20 ± 20.55^{c}	185.75 ± 14.58^{c}
Diabetic + Pterostilbene (20 mg/kg)	285.58 ± 22.32	245.58 ± 22.31^{cd}	206.31 ± 15.62^{c}	167.21 ± 13.41^{d}
Diabetic + Pterostilbene (40 mg/kg)	278.47 ± 24.55	225.25 ± 18.33^{d}	177.41 ± 13.77^{d}	124.41 ± 11.02^{e}
Diabetic + THC (80 mg/kg)	284.41 ± 23.41	251.21 ± 21.01^{c}	196.41 ± 17.35^{c}	141.75 ± 11.21^{f}

Values are mean ± SD for 6 rats in each group. $^{a-f}$ In each column, means with different superscript letter differ significantly at $p<0.05$ (DMRT).

Plasma insulin, hemoglobin, glycated hemoglobin and urine sugar

Table 2 presents the levels of plasma insulin, total hemoglobin, glycated hemoglobin and urine sugar of normal control and experimental rats. There was a significant elevation in glycated Hb levels, whereas plasma insulin and total hemoglobin levels were decreased significantly in diabetic rats when compared with normal rats. In diabetic control rats, urine sugar was more than 2%, but in the case of rats treated with pterostilbene and THC, there was no urine sugar. The administration of pterostilbene to normal control rats showed that the levels of hemoglobin, glycated Hb and increase in plasma insulin.

Table 2. Changes in the levels of insulin, haemoglobin, glycosylated hemoglobin and urine sugar in normal and experimental rats.

Groups	Insulin (μ U/ml)	Haemoglobin (g/dl)	Glycosylated Hb (mg/gHb)	Urine sugar
Normal control	16.41 ± 1.32^{a}	11.55 ± 0.92^{a}	0.25 ± 0.02^{a}	Nil
Normal + Pterostilbene (40 mg/kg)	17.31 ± 1.41^{a}	12.54 ± 0.71^{a}	0.24 ± 0.02^{a}	Nil
Diabetic control	6.35 ± 0.48^{b}	9.05 ± 0.60^{b}	0.50 ± 0.04^{b}	+++
Diabetic + Pterostilbene (40 mg/kg)	14.31 ± 1.49^{c}	10.58 ± 0.68^{c}	0.34 ± 0.02^{c}	Nil
Diabetic + THC (80 mg/kg)	11.39 ± 0.48^{c}	10.54 ± 0.78^{c}	0.37 ± 0.03^{c}	Trace

Values are mean ± SD for 6 rats in each group. $^{a-c}$ In each column, means with different superscript letter differ significantly at $p<0.05$ (DMRT).

Table 3,4 and 5 depicts the effect of pterostilbene and THC on changes in the fatty acid composition in liver, kidney and brain of control and experimental rats. There was a significant increase in the palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1) in liver and kidney of diabetic rats. In contrast, there was a significant decrease in the concentration of linolenic acid (18:3) and arachidonic acid (20:4) in tissues of diabetic rats. Treatment with pterostilbene and THC significantly decreased the concentration of palmitic acid, stearic acid and oleic acid whereas linolenic and arachidonic acids were significantly increased in diabetic rats.

Table 3. Changes in the fatty acid composition of total liver lipids in normal and experimental rats.

Group	Percentage of fatty acid / 100 mg tissue				
	16:0 Palmitic acid	18:0 Stearic acid	18:1 Oleic acid	18:3 Linolenic acid	20:4 Arachidonic acid
Normal	19.51 ± 1.11[a]	10.75 ± 0.75[a]	8.87 ± 0.41[a]	6.87 ± 0.40[a]	19.25 ± 1.25[a]
Diabetic Control	26.65 ± 1.85[b]	18.41 ± 1.25[b]	13.71 ± 0.85[b]	2.75 ± 0.15[b]	12.54 ± 0.78[b]
Diabetic + Pterostilbene (40 mg/kg)	23.47 ± 1.54[ac]	14.31 ± 0.69[c]	10.75 ± 0.59[c]	5.75 ± 0.31[c]	18.41 ± 1.31[c]
Diabetic + THC (80 mg/kg)	25.32 ± 1.29[c]	14.54 ± 0.65[d]	11.41 ± 0.59[d]	3.48 ± 0.15[d]	16.71 ± 0.75[d]

Values are given as mean ± S.D from ten rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 4. Changes in the fatty acid composition of total kidney lipids in normal and experimental rats.

Group	Percentage of fatty acid / 100 mg tissue				
	16:0 Palmitic acid	18:0 Stearic acid	18:1 Oleic acid	18:3 Linolenic acid	20:4 Arachidonic acid
Normal	23.41 ± 1.35[a]	13.41 ± 0.65[a]	5.65 ± 0.31[a]	6.65 ± 0.35[a]	12.54 ± 0.65[a]
Diabetic Control	32.75 ± 2.25[b]	24.12 ± 1.75[b]	12.57± 0.54[b]	2.58 ± 0.15[b]	5.65 ± 0.35[b]
Diabetic + Pterostilbene (40 mg/kg)	25.39 ± 1.51[c]	16.41 ± 1.01[c]	7.78 ± 0.41[c]	5.12 ± 0.35[c]	10.54 ± 0.65[c]
Diabetic + THC (80 mg/kg)	28.74 ± 1.45[d]	17.36 ± 0.84[d]	9.11 ± 0.40[d]	3.48 ± 0.17[d]	9.21 ± 0.41[d]

Values are given as mean ± S.D from ten rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Table 5. Changes in the fatty acid composition of total brain lipids in normal and experimental rats.

Group	Percentage of fatty acid / 100 mg tissue				
	16:0 Palmitic acid	18:0 Stearic acid	18:1 Oelic acid	18:3 Linolenic acid	20:4 Arachidonic acid
Normal	23.54 ± 1.01[a]	13.45 ± 1.07[a]	9.41 ± 0.51[a]	8.21 ± 0.41[a]	16.05 ± 0.84[a]
Diabetic Control	37.314 ± 2.51[b]	22.45 ± 1.55[b]	16.47 ± 1.12[b]	3.75 ± 0.23[b]	7.47 ± 0.44[b]
Diabetic + Pterostilbene (40 mg/kg)	26.31 ± 1.49[c]	14.55 ± 0.85[c]	11.44 ± 0.66[c]	6.12 ± 0.33[c]	14.21 ± 0.88[c]
Diabetic + THC (80 mg/kg)	30.32 ± 1.55[d]	16.12 ± 0.65[d]	13.22 ± 0.63[d]	5.22 ± 0.13[d]	12.52 ± 0.56[d]

Values are given as mean ± S.D from ten rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

Histopathological observations in liver, kidney and pancreas of normal and experimental rats

Liver

Histopathological section of diabetic control rats liver showed portal triad with mild inflammation and cell infiltration, sinusoidal congestion and fatty degeneration in the form of fat lake and fatty change predominantly microvesicular type (Fig. 1B-D). Diabetic rats treated with 10 and 20 mg/kg body weight of pterostilbene revealed focal granuloma with macrovesicular fatty generation and mild sinusoidal dilatation and congestion (Fig. 1E and F). Administration of 40 mg/kg body weight of pterostilbene to diabetic rats showed sinusoidal dilatation and focal kupffer cell hyperplasia (Fig. 1 H). Treatment of diabetic rats with 40 mg/kg body weight of pterostilbene documented mild portal inflammation (Fig. 1 G).

Doses of 10 and 20 mg/kg of pterostilbene caused a decrease in the glycemia however, they showed liver damage at lower doses, which may be due to decreased effect of pterostilbene to protect the tissue against STZ-induced toxicity. Higher dose of pterostilbene 40 mg/kg, although caused a significant decrease in blood glucose showed liver damage that might be due to some toxic effect, by the presence of some other substances, which hide the hypoglycemic effects with damage to liver cells. 40 mg/kg of pterostilbene showed reduced liver damage.

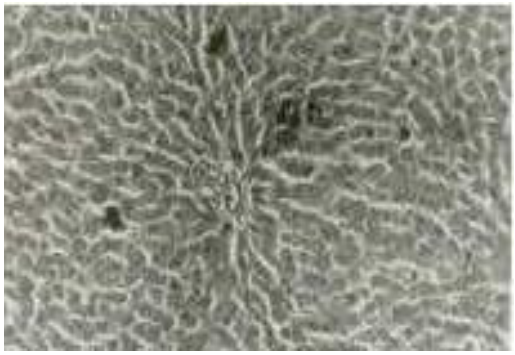

Figure 1 A. Normal rats liver H&E x 20

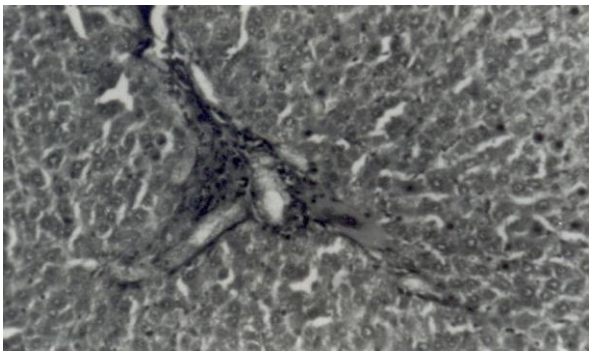

Figure 1 B. Diabetic control rats liver H&E x 20. Portal triad with mild inflammation and cell infiltration

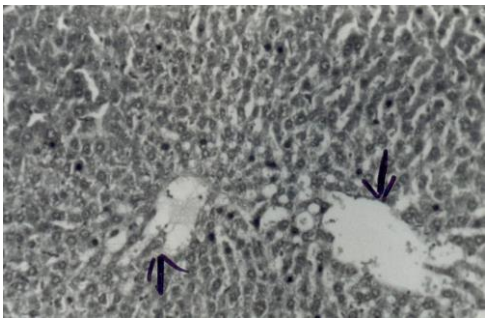

Figure 1 C. Diabetic control rats liver H&E x 20. Sinusoidal congestion and fatty degeneration in the form of fat lake (→)

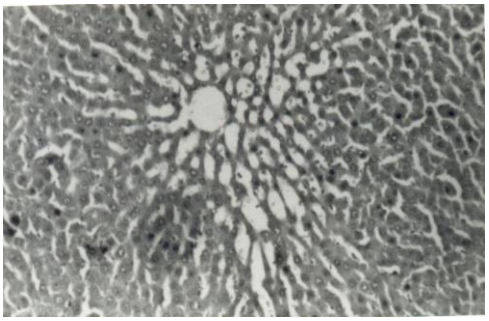

Figure 1 D. Diabetic control rats liver H&E x 20 Another area of fatty change predominantly microvesicular

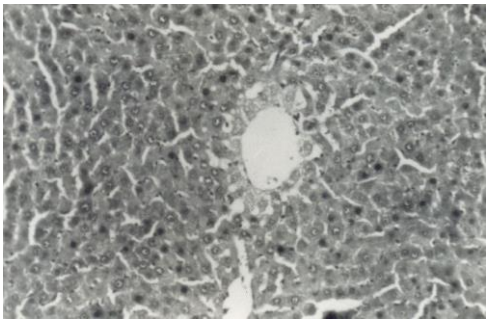

Figure 1 E. Diabetic + Pterostilbene (10 mg) treated rats liver H&E x 20 Focal granuloma and macrovesicular fatty degeneration

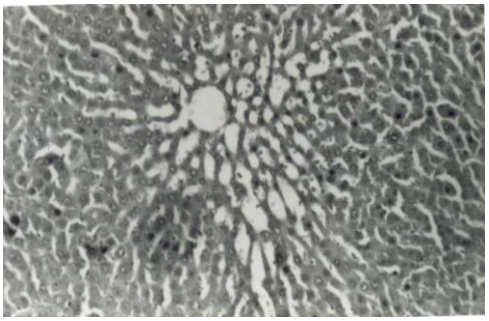

Figure 1 F. Diabetic + Pterostilbene (20 mg) treated rats liver H&E x 20 Mild sinusoidal dilatation and congestion

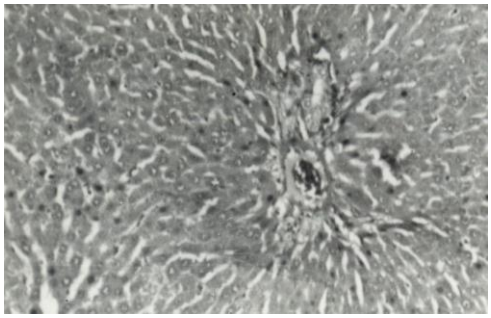

Figure 1 G. Diabetic + Pterostilbene (40 mg) treated rats liver H&E x 20 Mild portal inflammation with near normal appearance

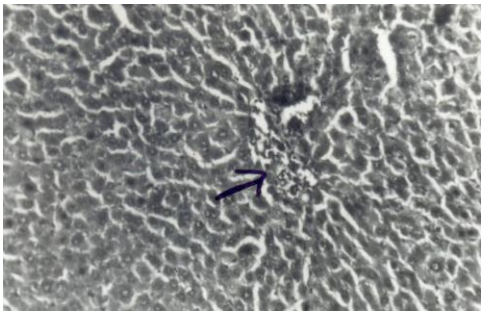

Figure 1 H. Diabetic + THC (80 mg) treated rats liver H&E x 20 Mild sinusoidal dilatation and focal kupffer cell hyperplasia

Kidney

Kidney of diabetic control rats (Fig. 2 B-D) showed fatty infiltration, parenchymal inflammation and haemorrhages. Diabetic rats treated with10, 20 and 40 mg/kg of pterostilbene (Fig. 2 E, F, H and I) revealed parenchymal inflammation, fatty infiltration, necrotic areas and cloudy swelling of tubules, whereas treatment with 40 mg/kg pterostilbene (Fig. 2 G) showed mild parenchymal inflammation.

The histological evidence of diabetic control rats suggest that structural alterations at the end of 3 weeks are due to STZ-induced free radical generation quite early in diabetes. Damage to the kidney was significantly reduced in diabetic rats treated with 200 mg/kg of pterostilbene. Pterostilbene at 10, 20 and 40 mg/kg caused damage to kidney of diabetic rats whereas 40 mg/kg of pterostilbene reduced the toxic effects of STZ.

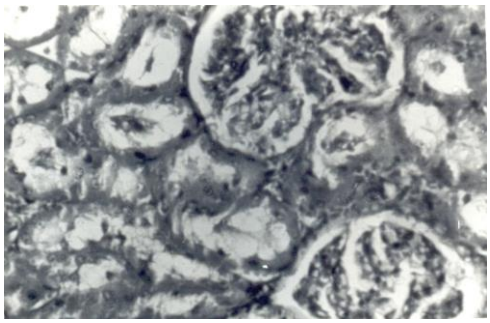

Figure 2 A. Normal rats kidney H&E x 20

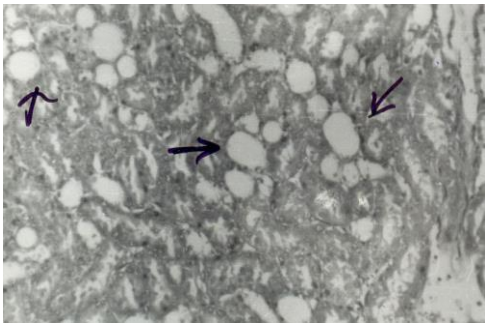

Figure 2 B. Diabetic control rats kidney H&E x 20. Fatty infiltration (→)

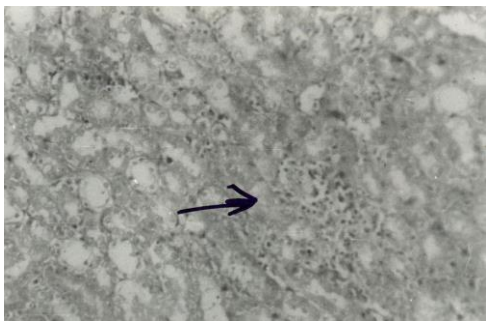

Figure 2 C. Diabetic control rats kidney H&E x 20. Parenchymal inflammation →)

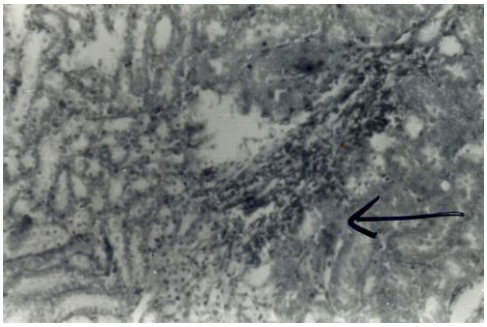

Figure 2 D. Diabetic control rats kidney H&E x 20. Haemorrhages (→)

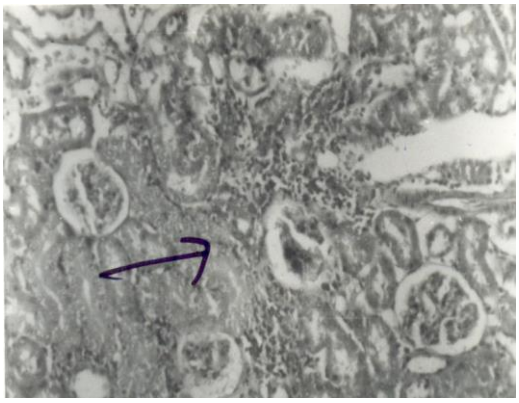

Figure 2 E. Diabetic + Pterostilbene (10 mg) treated rats kidney H&E x 20 Parenchymal inflammation (→)

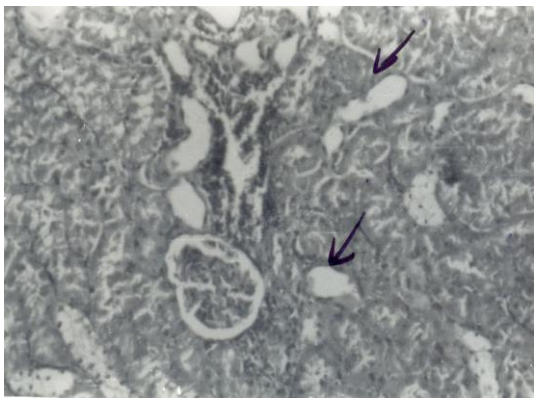

Figure 2 F. Diabetic + Pterostilbene (20 mg) treated rats kidney H&E x 20 Fatty infiltration (→)

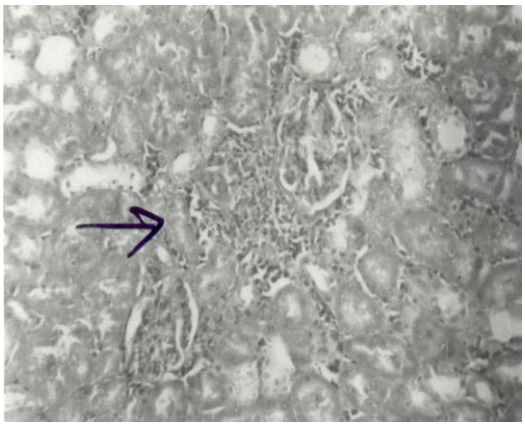

Figure 2 G. Diabetic + Pterostilbene (40 mg) treated rats kidney H&E x 20. Mild parenchymal inflammation (→)

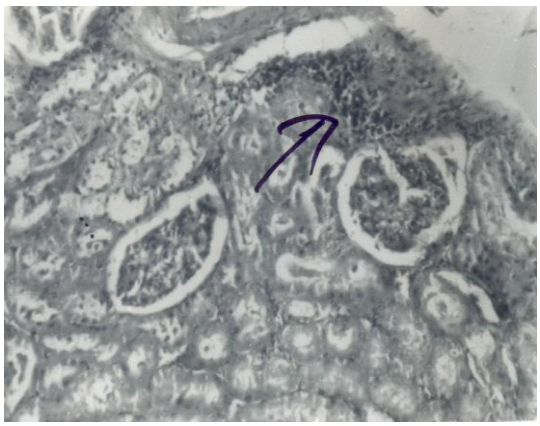

Figure 2 H. Diabetic + THC (80mg) treated rats kidney H&E x 20 Parenchymal inflammation and necrotic areas (→)

Pancreas

Diabetic control rats pancreas exhibited fatty infiltration of islets and shrinkage (Fig. 3 B). Administration of pterostilbene at a doses of 10, 20 and 40 mg/kg (Fig. 3 C, D and F) showed marked reduction in fatty changes of the islets with islet shrinkage, parenchymal inflammation and necrosis, whereas diabetic rats treated with 40 mg/kg pterostilbene (Fig. 3 E) revealed near normal appearance of islets. The shrinkage of islets in diabetic control rats and diabetic rats treated with 10, 20 and 40 mg/kg pterostilbene may be due to excessive destruction of islets by STZ that specifically damages islets. In addition, the above doses of pterostilbene were not able to counteract the effect of STZ. Pterostilbene at 0.40 mg/kg exhibited normal islets, which is probably due to the ability of it to withstand the detrimental effect of STZ at that concentration and protection offered by it to β-cells leading to increased insulin secretion. Thus in addition to blood glucose lowering effect, histopathological observations also supports the notion that pterostilbene at 0.45 mg/kg produced significant antihyperglycemic activity by protecting the tissues against STZ action.

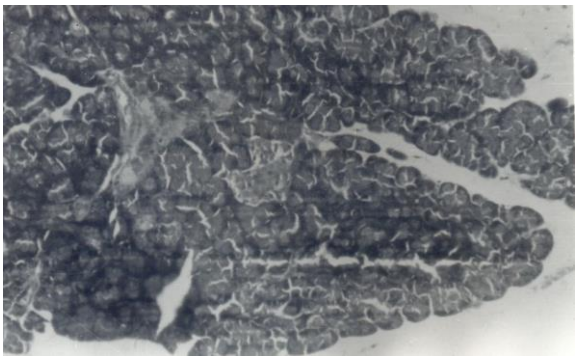

Figure 3 A. Normal rats pancreas H&E x 20. Pancreas showing β-islets (→)

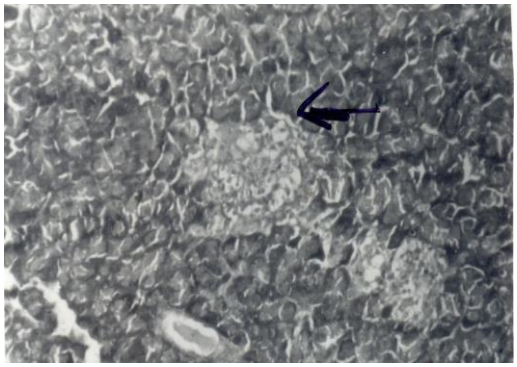

Figure 3 B. Diabetic control rats pancreas H&E x 20.Fatty infiltration of islet cells and shrinkage (→)

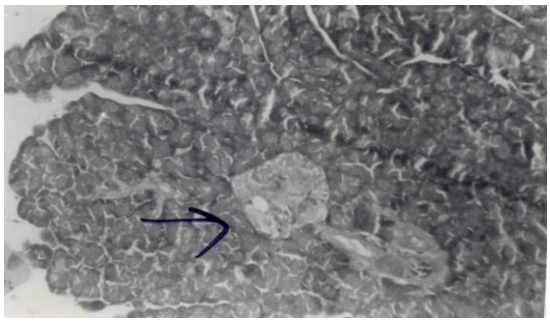

Figure 3 C. Diabetic + Pterostilbene (10 mg) treated rats pancreas H&E x 20. Marked reduction in fatty infiltration of islets (→)

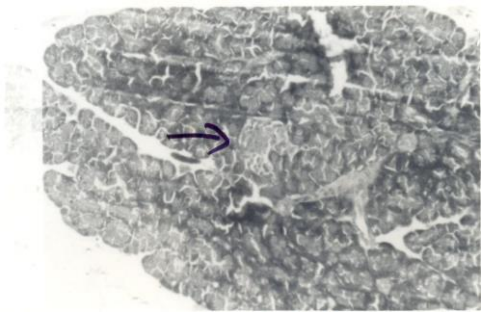

Figure 3 D. Diabetic + Pterostilbene (20 mg) treated rats pancreas H&E x 20 Islet shrinkage (→)

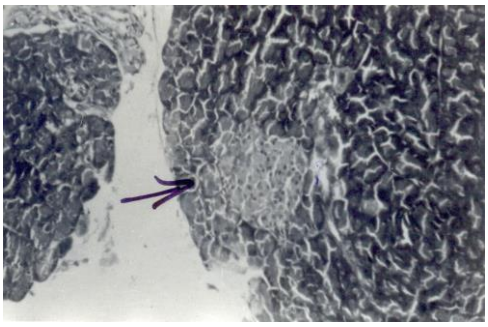

Figure 3 E. Diabetic + Pterostilbene (40mg) treated rats pancreas H&E x 20. Normal appearance of islets (→)

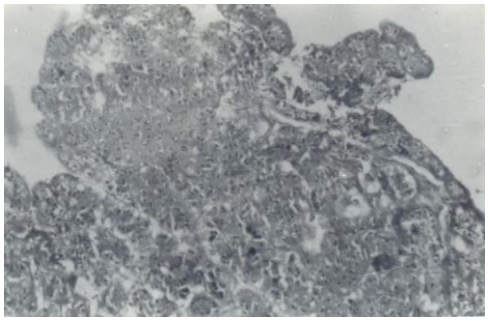

Figure 3 F. Diabetic +THC (80 mg) treated rats pancreas H&E x 20 Parenchymal inflammation and necrosis

Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate and lipid metabolism (33), which leads to hyperglycemia and hyperlipidemia (34). The relationship between diabetes and hyperlipidemia is a well-recognized phenomenon. Insulin deficiency/insulin resistance is considered to be a significant pathogenic factor in diabetes mellitus (35) and an obvious target for antidiabetic medications. Hyperlipidemia in diabetes mellitus is characterized by elevated levels of cholesterol (TC), triglycerides (TG), phospholipids (PL) and changes in lipoprotein composition (36).

These alterations may be relevant in explaining atleast inpart the increased predisposition of diabetes to atherosclerosis (2).

Hyperlipidemia in diabetes certainly contributes to the high prevalence of accelerated atherosclerosis and coronary artery disease (37). Coronary artery disease, as a result of premature atherosclerosis is a major cause of death both in type 1 and type 2 diabetes (38). It has been suggested that individuals possessing abnormalities in circulating lipids and glucose have strong tendency to develop diabetes (39).

Fatty acids are required for both the structure and function of every cell and they form an important component of cell membranes. Various tissue fatty acid compositions are altered in both experimental and human diabetes (6,40).

In the present study, we have observed a marked alteration in the fatty acid composition of total lipids in the liver and kidney. There was an increase in palmitic acid (16:0) and stearic acid (18:0) in the tissues of diabetic rats. Administration of pterostilbene to diabetic rats significantly decreased the concentration of stearic acid and palmitic acid in liver and kidney. This may represent an attempt by pterostilbene to minimize the toxicity of fatty acid ethyl esters formed from saturated fatty and ethyl ester species.

In our study, we have also observed a significant decrease in linolenic acid and arachidonic acid in diabetic rat tissues. Since these are rich in PUFAs, they are the major targets for ROS damage. The common dietary sources are linoleic acid (n-6) and/or □-linolenic acid (n-3), which are further metabolised by a series of desaturation and elongation steps to produce several PUFAs, including arachidonic acid (n-6) and eicosapentaenoic acid (n-3) that are major precursors of prostanoids, leukotrienes and other mediators. Diabetes reduces the rate limiting desaturation steps, particularly Δ-6-desaturation that converts linoleic acid to γ-linolenic acid and □-linolenic acid to stearidonic acid. Thus, the reduced availability of essential fatty acid intermediates in diabetes is further exacerbated by increased destruction due to elevated ROS (6).

Administration of pterostilbene afforded a significant protection against the changes in the fatty acid composition in diabetic rats. n-6 and n-3 PUFA are known to decrease thrombosis and atherosclerosis, which lower the incidence of CVD (41). This effect may also be due to improved glycemic control and increased plasma insulin that allows the diabetic rats treated with pterostilbene to maintain the tissue fatty acid composition in normal level. Δ-6-desaturase in the liver is a key enzyme of fatty acid desaturation (42), which has been shown to be suppressed in diabetes mellitus and rapidly restored with insulin treatment. The suppression of this enzyme is responsible for the altered fatty acid composition in diabetes mellitus (7,43), which corroborate with the present study. Treatment with pterostilbene brought back linolenic and arachidonic acids to near normalcy, which could be due to elevated level of insulin, which consequently restores Δ-6-desaturase activity in the liver of diabetic rats.

Conclusion

The present investigation shows that the administration of pterostilbene and THC to diabetic rats decreases serum and tissue lipids and maintains the fatty acid composition to near normal level. Moreover, the antihyperlipidemic effect could represent a protective mechanism against the development of atherosclerosis. The effect of tetrahydrocurcumin was more prominent compared with pterostilbene.

References

1. World Health Organization (WHO). (2003). The expert committee on the diagnosis and classification of diabetes mellitus. Diab Care, 26:5-20.
2. Haffner, S.M., Lehto, S., Rönnemaa, T., Pyörälä, K., Laakso, M. (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med, 339: 229-234.

3. Eder, H.A., Bergman, M. (1990). Metabolism of plasma lipids and lipoproteins. Diabetes Mellitus: Theory and Practice, 3rd ed. Medical Examination Publishing, New York. 61-72.
4. UKPDS. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet, 352: 837-853.
5. Deedwania, P.C. (2000) Hypertension and diabetes: new therapeutic options. Arch Intern Med, 160:1585-1594.
6. Cameron, N.E., Cotter, M.A. (1999). Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. Diabetes Res Clin Pract, 45:137-146.
7. Faas, F.H., Dang, A.Q., Kemp, K., Norman, J., Carter, W.J. (1988). Red blood cell and plasma fatty acid composition in diabetes mellitus. Metabolism 37:711-713.
8. Seigneur, M., Freyburger, G., Gin, H., Claverie, M., Lardeau, D., Lacape, G. (1994). Serum fatty acid profiles in type I and ii diabetes;metablic alteration of fatty acids of the main serumlipids. Diab Res Clin Pract, 23:169-177.
9. Warrier, P.K., Nambiar, V.P.K., Ramankutty, C. (1995). Indian Medicinal Plants. Orient Longman Limited, Madras, pp. 381–383.
10. Maurya, R., Ray, A.B., Duah, F.K., Slatkin, D.J., Schiff, P.L. (1984). Constituents of Pterocarpus marsupium. J Nat Prod,47:179-181.
11. Manickam, M., Ramanathan, M., Jahromi, M.A., Chansouria, J.P., Ray, A.B. (1997). Antihyperglycemic activity of phenolics from Pterocarpus marsupium. J Nat Prod, 60: 609-610.
12. Indian Council of Medical Research (ICMR). (1998). Flexible open trial of vijayasar in case of newly diagnosed non-insulin dependent diabetes mellitus. Ind J Med Res, 108:24-29.
13. Murugan, P., Pari, L., Chippada Appa Rao. (2008). Effect of tetrahydrocurcumin on insulin receptors status in type 2 diabetic rats: Studies on insulin binding to erythrocytes. J Biosci, 33(1): 63-72.
14. Murugan, P., Pari L. (2006). Effect of tetrahydrocurcumin on lipid peroxidation and lipids in streptozotocin - nicotinamide induced diabetic rats. Basic Clin Pharmacol Toxicol, 99:122-127.
15. Murugan, P., Pari L. (2007). Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type 2 diabetic rats. J Ethanopharmacol, 113: 479–486.
16. Murugan, P., Pari L. (2006). Antioxidant effect of tetrahydrocurcumin in streptozotocin - nicotinamide induced diabetic rats. Life sci, 79: 1720-1728.
17. Pari, L., Murugan P. (2004). Protective role of tetrahydrocurcumin against erythromycin estolate induced hepatotoxicity. Pharmacol Res, 49 (5): 481-486.
18. Murugan, P., Pari L. (2007). Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type 2 diabetic rats. J Ethanopharmacol, 113: 479–486.
19. Murugan, P., Pari L, Chippada Appa Rao. (2008). Effect of tetrahydrocurcumin on insulin receptors status in type 2 diabetic rats: Studies on insulin binding to erythrocytes. J Biosc, 33(1): 63-72.
20. Pari, L., Murugan P. (2005). Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. J Basic Clin Physiol Pharmacol, 16: 257-274.
21. Pari, L., Murugan P. (2007). Changes in glycoprotein components in streptozotocin - nicotinamide induced type 2 diabetes: Influence of tetrahydrocurcumin from Curcuma longa. Plant Foods Hum Nutr, 62(1): 25-29.
22. Pari, L., Murugan P. (2007). Antihyperlipidaemic effect of curcumin and tetrahydrocurcumin in experimental type 2 diabetic rats. Renal Failure, 29: 881-889.

23. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G. (1998). Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes, 47: 224-229.
24. Klimes, I., Sebokova, E., Gasperikova, D., Mitkova, A., Kuklova, S., Bohov, P. (1998). Search for extra pancreatic effects of new oral hypoglycemic agent A-1466. 1: Oral glucose tolerance tests in normal and hereditary insulin resistant rats. Endocrinol Reg, 32: 115-123.
25. Pari L, Amarnath Satheesh M. (2006). Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin- and nicotinamide-induced diabetic rats. Life Sci, 79, 641–645.
26. Pari, L., Murugan, P. (2007). Tetrahydrocurcumin prevents brain lipid peroxidation in streptozotocin-induced diabetic rats. J Med Food, 10 (2): 323-329.
27. Lott, J.A., Turner, K. (1932). Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine. Clin Chem, 21/12: 1754-1760.
28. Drabkin, D.L, Austin, J.M. (1932). Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. J Biol Chem, 98: 719-733.
29. Sudhakar Nayak, S., Pattabiraman, T. (1982). A new colorimetric method for the estimation of glycosylated haemoglobin. Clin Chim Acta 1981; 109: 267-274.
30. Bannon, P. (1982). Effect of pH on the elimination of the labile fraction of glycosylated haemoglobin. Clin Chem, 28: 2183.
31. Morrison, W.R., Smith, L.M. (1964). Preparations of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride methanol. J Lipid Res, 5: 600-607.
32. Duncan, B.D. (1957). Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 359-364.
33. Cowie, C.C., Eberhardt, M.S. (1996). Diabetes: Vital statistics. American Diabetes Association, Alexandria, VA: 1-4.
34. DeFronzo, R.A., Bonadonna, R.C., Ferrannini, E. (1992). Pathogenesis of NIDDM: a balanced overview. Diabetes Care, 15: 318-368.
35. Olefsky, J.M., Nolan, J. (1995). Insulin resistance and NIDDM: Cellular and molecular mechanisms. Am J Clin Nutr, 61: 980S-986S.
36. Haffner, S.M. (1991). Compositional changes in lipoproteins of subjects with non-insulin-dependent diabetes mellitus. J Lab Clin Med, 118: 109-110.
37. Gandhi, H.R. (2001). Diabetes and coronary artery disease: Importance of risk factors. Cardiol Today, 1: 31-34.
38. American Diabetes Association. (1989). Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. Consensus statement. Diabetes Care, 12: 573-579.
39. Zavaroni, I., Bonora, E., Pagliara, M., Dall'Aglio, E., Luchetti, L., Buonanno, G., Bonati, P.A., Bergonzani, M., Gnudi, L., Passeri, M. (1989). Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. New Engl J Med, 320: 702-706.
40. Tilvis, R.S., Miettinen, T.A. (1985). Fatty acid compositions of serum lipids, erythrocytes, and platelets in insulin-dependent diabetic women. J Clin Endocrinol Metab, 61:741-745.
41. Demaison, L., Sergiel, J.P., Moreau, D., Grynberg, A. (1994). Influence of the phospholipid n-6/n-3 polyunsaturated fatty acid ratio on the mitochondrial oxidative metabolism before and after myocardial ischemia. Biochim Biophys Acta, 1227:53-59.
42. Brenner, R.R. (1977). Regulatory function of delta6 desaturate key enzyme of polyunsaturated fatty acid synthesis. Adv Exp Med Biol, 83:85-101.
43. Eck, M.G., Wynn, J.O., Carter, W.J., Faas, F.H. (1979). Fatty acid desaturation in experimental diabetes mellitus. Diabetes, 28:479-485.