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EFFECT TETRAHYDROCURCUMIN AND PTEROSTILBENE ON LIPID PEROXIDATION AND LIPIDS IN STREPTOZOTOCIN - NICOTINAMIDE INDUCED DIABETIC RATS

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Abstract
Pterocarpus marsupium has been used in the treatment of toothache, diarrhoea, heartburn, urinary tract infections, boils, sores and skin diseases. *P. 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, a phenolic compound derived from resveratrol, possesses greater bioavailability than its parent compound due to the presence of two methoxyl groups. In this review, the beneficial effects of pterostilbene on diabetes, liver steatosis and dyslipidemia are summarized. Pterostilbene is a useful bioactive compound in preventing type 1 diabetes, insulin resistance and type 2 diabetes in animal models. Hyperlipidemia is an associated complication of diabetes mellitus. We recently reported that Tetrahydrocurcumin (THC) and pterostilbene lowered the blood glucose in diabetic rats. THC 80mg/kg body weight and Pterostilbene 40 mg/kg body weight was orally administered to diabetic rats for 45 days, resulted a significant reduction in blood glucose and significant increase in plasma insulin in diabetic rats, which proved its antidiabetic effect. THC and pterostilbene also caused a significant reduction in lipid peroxidation (thiobarbituric acid reactive substances and hydroperoxides) and lipids (cholesterol, triglycerides, free fatty acids and phospholipids) in serum and tissues, suggesting its role in protection against lipid peroxidation and its antihyperlipidemic effect. THC showed a better effect when compared with pterostilbene.

Key words: lipids, lipid peroxidation, tetrahydrocurcumin, curcumin

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 (WHO, 2003). Hyperlipidemia in diabetes mellitus is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL) and changes in lipoprotein composition (Haffner, 1991). 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 (Eder and Bergman, 1990). 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 (UKPDS, 1998). Lipid-lowering therapy in diabetes was effective in reducing the risk of vascular complications (Deedwania et al., 2000). In this chapter, the effects of pterostilbene on lipids and fatty acid composition in normal and experimental rats are described.

THC, produced from curcumin by hydrogenation, are colorless which render these products useful in non-colored food and cosmetic applications that currently employ synthetic antioxidants (Murugan and Pari, 2006). THC is one of the major metabolites of curcumin, with potential bioactivity. This metabolite was identified in intestinal and hepatic cytosol from humans and rats (Murugan and Pari, 2007; Murugan and Pari, 2008). The reduction of curcumin to THC seems to occur primarily in a cytosolic compartment (Ireson, 2002). Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) (Ireson, 2002). Recently, attention has focused on THC, as one of the major metabolites of curcumin, because this compound appears to exert greater antioxidant activity in both in vitro and in vivo systems (Pari and Murugan, 2004). In our previous study, we have demonstrated the antidiabetic effect of THC in streptozotocin (STZ) induced diabetic rats ((Pari and Murugan, 2005).

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, 1995). Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of Pterocarpus marsupium (Maurya et al. 2004). It is suggested that pterostilbene might be one of the principal anti-diabetic constituents of Pterocarpus marsupium (Maurya et al. 2004). 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).

To our knowledge, so far no other biochemical investigations has been carried out on the effect of THC compared pterostilbene in lipid peroxidation and lipids of experimental diabetic rats. The present investigation was carried out to study the effect of THC compared pterostilbene on tissue lipid peroxides and lipids in rats with STZ and nicotinamide induced diabetes.

Materials and methods

Drugs and chemicals

THC and Pterostilbene was a gift provided by Sabinsa Corporation, USA. All other chemicals and biochemicals were of analytical grade.

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced (Masiello *et al*., 1998) in overnight fasted rats by a single intraperitonial injection (i.p) of 65 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design

In the experiment, 24 rats were divided into 4 groups of 6 each, after the induction of STZ diabetes. The experimental period was 45 days.

Group 1: Normal untreated rats. Group 2: Diabetic control rats. Group 3: Diabetic rats given THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Muugan and Pari, 2006). Group 4: Diabetic rats given Pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Pari and Amarnath Satheesh, 2006). Group 5: Diabetic rats given glibenclamide (600 μg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the

estimation of blood glucose. Plasma was separated for the estimation of insulin. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Analytical procedure

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) (Lott and Turner 1975). 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 (1932). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982).

Thiobarbituric acid reactive substances were measured by the method of Fraga et al. (1988). Hydroperoxides were determined by the method of Jiang et al. (1992).

Extraction of lipids from serum and tissues was carried out according to the procedure of Folch *et al*. (1957) by using chloroform - methanol (2:1 v/v) mixture. From this, the total cholesterol, triglycerides, free fatty acids and phospholipids were estimated by the method of Zlatkis *et al*. (1953), Foster and Dunn (1973) Falholt *et al*. (1973) and Zilversmit and Davis (1950), respectively.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan 1957).

Results

Table 1 shows the level of blood glucose, total haemoglobin, glycosylated haemoglobin and plasma insulin of different experimental groups. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in streptozotocin diabetic rats, compared with normal rats. The effect of THC was more prominent when compared with pterostilbene. The diabetic control rats showed a significant decrease in the level of total haemoglobin and significant increase in the level of glycosylated haemoglobin. Oral administration of THC and pterostilbene to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. In the case of normal rats, the level of haemoglobin and glycosylated haemoglobin remained unaltered.

Table 2 represents the concentration of TBARS and hydroperoxides in tissues of normal and experimental rats. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes, when compared to the corresponding normal group. Administration of THC and pterostilbene significantly decreased the lipid peroxidation in diabetic rats. The THC was more potent than pterostilbene and glibenclamide.

The levels of serum and tissues cholesterol, free fatty acids, triglycerides and phospholipids of normal and experimental rats are given in table 3. Cholesterol, free fatty acids, triglycerides and phospholipids were significantly decreased in THC treated rats as compared to diabetic rats. Oral administration of THC and pterostilbene significantly decreased the levels of serum and tissue lipids as compared to untreated diabetic rats. The THC administration showed more effective than pterostilbene and glibenclamide.

Discussion

Hypercholesterolemia and hypertriglyceridemia are independent major risk factors that alone or together, can accelerate the development of CAD (McKenney, 2001). The cause of hyperlipidemia has been related to increased lipid synthesis, decreased lipid clearance from the blood or a combination of these two processes. Generally, hepatic and muscle tissues lose their sensitivity to the action of insulin (Porte and Kahn, 2001). In the early stages of the disease, the □-cells of the pancreatic islets compensate for decreased insulin sensitivity by increasing insulin secretion. As the disease progresses, diabetes ensues when β-cell is no longer able to compensate for insulin resistance. It has been suggested that raised lipid levels play a key role in the development of type 2 diabetes (Boden and Shulman, 2002).

Serum lipids, lipoproteins and tissue lipids

The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of FFA from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamines and other hormones enhance the lipolysis. The marked hyperlipemia 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 et al., 1994).

Studies on STZ-induced diabetes in experimental animals have suggested that an increase in circulatory VLDL and their associated TG are largely due to defective clearance of these particles from circulation (Babu and Srinivasan 1997). As there is a close relationship between elevated serum TC level and the occurrence of atherosclerosis, the ability of the pterostilbene in selective reduction of TC through the reduction of VLDL and LDL components could be beneficial in preventing the atherosclerotic conditions and thereby reduce the possibility of CHD in general. As regards to 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). Hypertriglyceridemia in diabetes can result from an increased hepatic VLDL over production and impaired catabolism of TG-rich particles. Dysfunction of LPL also contributes to hypertriglyceridemia in the fasting and postprandial state (Kanters et al., 2001). The increased level of cholesterol observed in diabetic liver and kidney might also be due to the decreased levels of HDL-cholesterol.

During diabetes, kidney exhibits a characteristics pattern of changes in the glomerulus producing initial hyper filtration with a marked thickness of glomeruli basement membrane which eventually leads to renal insufficiency or complex kidney failure. Changes in the fatty acids during diabetes are closely associated with the activity of Na+/K+-ATPase in the kidney. Accumulation of fatty acids results in higher levels of their metabolities such as acyl-carnitine and long chain acyl-CoA. This interferes with Na+/K+-ATPase action leading to impairment in the action of Na+/K+ ions, which may finally results in diabetic nephropathy (Bergman and Ader, 2000). Thus, the diabetic complications associated with renal tissue may be partly due to abnormalities in lipid metabolism.

The PPARα isoform is predominantly involved in fatty acid and lipid catabolism. It also involved in the import and activation of genes involved in fatty acid oxidation in the liver, heart, kidney and skeletal muscles (Gilde and Van Bilsen, 2003). In the liver, activation of PPARα leads to increased β-oxidation of fatty acids and decreased TG and VLDL synthesis (Fruchart and Duriez, 2004). Activation of PPARα also leads to reduction of TG because of repression of hepatic apolipoprotein C-III and an increase in lipoprotein lipase gene expression (Gervois et al. 2000). Furthermore, PPARα activation causes 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 an activity of hypolipidemic drug, there by provides a possible alternative for the treatment of dyslipidemia (Rimando et al., 2005). In this context, the decreased levels of cholesterol, TG, FFA and PL were found in plasma and tissues of diabetic rats treated with pterostilbene could be due to an activation of PPARα by the administration of pterostilbene.

Conclusion

It can be concluded from the data that THC and pterostilbene significantly reduces the level of serum and tissue lipids and lipid peroxidation marker, which are actively raised in streptozotocin diabetic rats. THC and pterostilbene has beneficial effect on plasma insulin and blood glucose level. Moreover it was a prevention of lipid metabolism defects could represent a protective mechanism against the development of atherosclerosis. The THC administration showed more effective than pterostilbene and glibenclamide.

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Table 1. Effect of THC and Pterostilbene on the levels of blood glucose, plasma insulin, haemoglobin and glycosylated haemoglobin in normal and experimental rats

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	99.53 ± 5.12^{a}	12.65 ± 0.53^{a}	12.21 ± 0.54^{a}	0.31 ± 0.03^{a}
Diabetic control	293.41 ± 7.52^{b}	3.92 ± 0.34^{b}	8.54 ± 0.35^{b}	0.79 ± 0.04^{b}
Diabetic+ THC (80 mg/kg)	114.21 ± 6.25^{c}	9.92 ± 0.75^{c}	11.65 ± 0.66^{c}	0.42 ± 0.03^{c}
Diabetic + Pterostilbene (40 mg/kg)	135.54 ± 6.63^{d}	8.52 ± 0.33^{d}	10.98 ± 0.58^{d}	0.47 ± 0.03^{d}
Diabetic+Glibencalamide (600 μg/ mg)	142.87 ± 7.51^{d}	8.87 ± 0.35^{d}	10.87 ± 0.45^{d}	0.52 ± 0.04^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Table 2. Influence of THC and Pterostilbene on the content of TBARS and hydroperoxides in rats liver and kidney.

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
TBARS					
Liver (mM/100g tissue)	0.79 ± 0.05^{a}	1.88 ± 0.11^{b}	1.42 ± 0.04^{c}	1.17 ± 0.03^{d}	1.23 ± 0.03^{d}
Kidney (mM/100g tissue)	1.82 ± 0.13^{a}	3.94 ± 0.23^{b}	2.31 ± 0.12^{c}	1.99 ± 0.15^{d}	2.18 ± 0.03^{e}
Hydroperoxides					
Liver (mM/100g tissue)	82.15 ± 5.25^{a}	105.43 ± 5.73^{b}	94.84 ± 4.53^{c}	91.35 ± 4.54^{ac}	87.54 ± 0.05^{d}
Kidney (mM/100g tissue)	59.12 ± 3.15^{a}	81.53 ± 4.54^{b}	71.58 ± 4.47^{c}	63.45 ± 4.24^{c}	72.23 ± 4.25^{d}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (Duncan's Multiple Range Test).

Table 3. Effect of THC and Pterostilbene on the levels of cholesterol, free fatty acids, triglycerides and phospholipids in serum, liver and kidney of normal and experimental rats.

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
Cholesterol					
Serum (mg/100ml)	87.51 ± 5.32^{a}	170.65 ± 12.02^{b}	109.54 ± 7.25^{c}	129.25 ± 6.25^{d}	139.54 ± 6.35^{d}
Liver(mg/100g wet tissue)	325.77 ± 14.25^{a}	515.35 ± 32.52^{b}	419.54 ± 23.25^{c}	449.25 ± 27.58^{c}	449.45 ± 27.55^{c}
Kidney(mg/100g wet tissue)	375.32 ± 22.67^{a}	535.12 ± 30.15^{b}	415.85 ± 20.34^{c}	445.24 ± 25.15^{c}	455.62 ± 26.13^{c}
Free fatty acids					
Serum (mg/100ml)	79.32 ± 4.45^{a}	142.77 ± 10.52^{b}	88.54 ± 5.15^{c}	106.21 ± 5.66^{d}	119.54 ± 6.47^{d}
Liver (mg/100g wet tissue)	575.35 ± 30.74^{a}	825.45 ± 45.15^{b}	710.65 ± 40.32^{c}	778.16 ± 35.26^{c}	775.11 ± 35.21^{c}
Kidney(mg/100gwet tissue)	410.52 ± 19.54^{a}	600.31 ± 39.65^{b}	535.61 ± 34.62^{c}	565.21 ± 25.25^{d}	515.13 ± 20.15^{d}
Triglycerides					
Serum (mg/100ml)	57.21 ± 3.35^{a}	92.32 ± 7.21^{b}	63.51 ± 4.21^{c}	77.25 ± 4.47^{d}	81.32 ± 4.25^{d}
Liver (mg/100g wet tissue)	335.58 ± 18.35^{a}	620.32 ± 35.45^{b}	418.32 ± 18.89^{c}	510.32 ± 20.77^{d}	500.11 ± 25.77^{d}
Kidney(mg/100gwet tissue)	265.54 ± 17.12^{a}	410.25 ± 21.12^{b}	365.32 ± 25.88^{c}	430.42 ± 17.17^{d}	435.42 ± 17.11^{d}
Phospholipids					
Serum (mg/100ml)	115.67 ± 6.56^{a}	175.12 ± 13.12^{b}	130.31 ± 8.34^{c}	108.32 ± 8.65^{d}	157.650 ± 8.54^{d}
Liver (g/100g wet tissue)	1.57 ± 0.10^{a}	3.12 ± 0.16^{b}	1.91 ± 0.15^{c}	2.19 ± 0.13^{d}	2.25 ± 0.12^{d}
Kidney (g/100g wet tissue)	1.59 ± 0.13^{a}	2.30 ± 0.13^{b}	1.75 ± 0.13^{c}	1.96 ± 0.12^{d}	1.99 ± 0.13^{d}

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).