

Effect Of Pterostilbene Compared To Tetrahydrocurcumin On Liver And Kidney Functional Markers And Protein Levels In Experimental Diabetes

MURUGAN P^{1*}, SAKTHIVEL V²

¹Assistant professor, Department of Biochemistry, Government arts and science college, Vedharanyam-614810. Tamil Nadu, India.

²Assistant professor, Department of Biotechnology, Government arts and science college, Thiruthuraipoondi-614715. Tamil Nadu, India.

*Corresponding Author: Dr. P.Murugan

*Assistant professor, Department of Biochemistry, Government arts and science college, Vedharanyam-614810. Tamil Nadu, India.
E.mail :manomuruganphd@gmail.com, Alternative Email:pmpranithmurugan18@gmail.com

DOI: 10.47750/pnr.2021.12.01.16

Abstract

Pterostilbene (PTS), a compound most abundantly found in blueberries, is a natural analog of resveratrol. Several plant species, such as peanuts and grapes, produce PTS. It has increased bioavailability in comparison to other stilbene compounds, which may enhance its dietary benefit and possibly contribute to a valuable clinical effect. PTS is a naturally-derived stilbenoid structurally related to resveratrol, with potential antioxidant, anti-inflammatory, pro-apoptotic, antineoplastic and cytoprotective activities. Upon administration, PTS exerts its anti-oxidant activity by scavenging reactive oxygen species (ROS), thereby preventing oxidative stress and ROS-induced cell damage. Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin which exhibits many of the same physiologic and pharmacological activities as curcumin and in some systems may exert greater antioxidant activity than curcumin. Oral administration of PTS (40 mg/kg body weight) THC at 80 mg/kg body weight to diabetic rats for 45 days resulted in a significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, the diabetic rats had decreased the levels of plasma total protein, albumin, globulin and albumin/globulin ratio as compared to control rats. After treatment with PTS and THC total protein, albumin, globulin and albumin/globulin ratio were brought back to near normal levels. The activities of hepatic and renal markers were significantly elevated in diabetic rats as compared to control rats, and treatment with PTS and THC have reversed these parameters to near normal levels. In diabetic rats, the decreased levels of urea, uric acid and creatinine with increased levels of was observed, and treatment with PTS and THC have reversed these parameters to near normal levels. THC had a better protective effect when compared with PTS.

Keywords: pterostilbene, tetrahydrocurcumin, streptozotocin, hepatic and renal marker enzymes

Introduction

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Cardiovascular and renal complications share common risk factors such as blood pressure, blood lipids, and glycemic control. Thus, chronic kidney disease may predict cardiovascular disease in the general population. The impact of diabetes on renal impairment changes with increasing age. Serum markers of glomerular filtration rate and microalbuminuria identify renal impairment in different segments of the diabetic population, indicating that serum markers as well as microalbuminuria tests should be used in screening for nephropathy in diabetic older people. The American Diabetes Association and the National Institutes of Health recommend estimated glomerular filtration rate calculated from serum creatinine at least once a year in all people with diabetes for detection of kidney dysfunction (Dabla, 2010).

The liver and kidney plays a major role in the pathogenesis of type 2 diabetes. It contributes to insulin resistance, along with muscle and adipose tissues, and it has a major impact on the incidence of hyperglycaemia. Hepatic diseases such as cirrhosis, viral hepatitis and nonalcoholic fatty liver disease are associated with altered glucose metabolism and a higher prevalence of diabetes mellitus (DeFronzo et al., 1992).

All of the liver function tests, with the exception of bilirubin, are measurements of enzyme levels. These enzymes are normally present in liver cells, and there is a certain "normal" level of these enzymes circulating in the bloodstream. When the liver becomes inflamed or damaged, these enzymes are released into the bloodstream in abnormal amounts by the damaged cells. This results in elevated levels in the bloodstream. Bilirubin, another substance commonly measured in the blood to detect liver disease, is produced from the breakdown of red blood cells (Pari and Murugan, 2004). Again, a

normal bilirubin level is maintained as the liver continually removes bilirubin from the bloodstream for further processing. If the liver is impaired, however, bilirubin is not removed, and the level in the bloodstream will rise (Murugan and Pari, 2007).

The kidney function tests do not measure enzymes, but measure breakdown products of normal body processes. Blood urea nitrogen (BUN) is produced as a result of protein breakdown. BUN is formed in the liver and carried via the bloodstream to the kidneys to be eliminated (Murugan and Pari, 2005). Creatinine is a breakdown product of a specific type of muscle protein. It is formed in certain muscles and is carried via the bloodstream to be eliminated by the kidneys. If the kidneys become impaired and unable to eliminate the usual amount of these substances, the BUN and creatinine blood levels will rise (Pari and Murugan, 2006).

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). PTS 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 PTS 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 (NIDDM) patients. When administered to STZ-induced hyperglycemic rats, PTS and marsupin, two of the major phenolic constituents in aqueous decoction of the heartwood of *P. marsupium*, significantly decreased plasma glucose (Pari and Amarnath Satheesh, 2006).

To our knowledge, so far no other biochemical investigations have been carried out on the effect of PTS compared to THC in hepatic and renal functional markers and protein levels status of experimental diabetic rats. The present investigation was carried out to study the effect of PTS and THC on hepatic and renal functional markers and protein levels 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

Type 2 diabetes mellitus was induced (Masiello et al., 1998) in overnight fasted rats by a single intraperitoneal 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 this experiment, rats were divided into 4 groups of 6 rats each.

Group 1: Normal rats.

Group 2: Diabetic control rats.

Group 3: Diabetic rats given aqueous extract of THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days (Pari and Murugan, 2005).

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).

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. Collection of urine by using metabolic cages animals kept in overnight.

Analytical methods

Total protein and albumin kit, Biuret and BCG Dye Binding method for *in vitro* estimation- Diagnostics. Serum enzymatic activities of aspartate transaminase, alanine transaminase, alkaline phosphatase, and gamma glutamyl transpeptidase were determined on fully automated chemistry analyzer Roche/Hitachi-912 (Roche Diagnostics, Mannheim, Germany) using Roche Diagnostics GmbH kits. The values were expressed as IU/L serum.

The levels of urea, uric acid, creatinine, albumin and total protein were estimated spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India).

Statistical analysis

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

Table 1. Effect of THC and PTS 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 (μ U/ml)	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)
Normal	100.23 \pm 5.32 ^a	12.12 \pm 0.44 ^a	12.35 \pm 0.36 ^a	0.33 \pm 0.03 ^a
Diabetic control	298.32 \pm 7.30 ^b	3.95 \pm 0.30 ^b	8.72 \pm 0.35 ^b	0.80 \pm 0.04 ^b
Diabetic + THC (80 mg/kg)	117.25 \pm 6.15 ^c	9.57 \pm 0.73 ^c	11.36 \pm 0.56 ^c	0.40 \pm 0.03 ^c
Diabetic + Pterostilbene (40 mg/kg)	138.58 \pm 6.25 ^d	8.21 \pm 0.25 ^d	10.87 \pm 0.50 ^d	0.48 \pm 0.03 ^d

Values are given as mean \pm S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Table 2. Effect of THC and PTS total protein, albumin, globulin and A/G ratio in plasma of control and experimental animals.

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control rats	6.85 \pm 0.36 ^a	3.58 \pm 0.20 ^a	3.25 \pm 0.15 ^a	1.15 \pm 0.02 ^a
Diabetic control	4.03 \pm 0.27 ^b	1.78 \pm 0.12 ^b	2.14 \pm 0.14 ^b	0.87 \pm 0.03 ^b
Diabetic + THC (80 mg/kg)	6.48 \pm 0.30 ^c	3.45 \pm 0.13 ^c	3.09 \pm 0.15 ^c	1.15 \pm 0.01 ^c
Diabetic + PTS (40mg/kg)	5.90 \pm 0.35 ^d	3.12 \pm 0.15 ^d	2.85 \pm 0.16 ^d	1.07 \pm 0.01 ^d

Values are given as mean \pm S.D for 6 rats in each group. A/G ratio- albumin/globulin ratio.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Duncan's multiple range test).

Table 3. Effect of THC and PTS on serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) in control and experimental diabetes

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Control rats	75.52 \pm 5.12 ^a	29.53 \pm 1.77 ^a	75.54 \pm 5.12 ^a	11.47 \pm 0.48 ^a
Diabetic control	125.02 \pm 7.25 ^b	65.84 \pm 3.71 ^b	149.32 \pm 8.58 ^b	28.75 \pm 1.55 ^b
Diabetic + THC (80 mg/kg)	85.45 \pm 6.25 ^c	35.22 \pm 2.55 ^c	87.52 \pm 6.35 ^c	14.45 \pm 1.09 ^c
Diabetic + PTS (40mg/kg)	94.19 \pm 5.32 ^d	41.25 \pm 2.31 ^d	95.21 \pm 5.35 ^d	18.58 \pm 1.05 ^d

Values are given as mean \pm 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 4. Effect of THC and PTS on serum Urea, Uric acid and Creatinine in control and experimental diabetes

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control rats	24.19 \pm 1.25 ^a	1.17 \pm 0.06 ^a	0.93 \pm 0.05 ^a
Diabetic control	38.50 \pm 2.69 ^d	2.30 \pm 0.15 ^b	2.30 \pm 0.15 ^b
Diabetic + THC (80 mg/kg)	26.56 \pm 1.13 ^c	1.27 \pm 0.05 ^c	1.07 \pm 0.05 ^c
Diabetic + PTS (40mg/kg)	30.26 \pm 1.65 ^d	1.39 \pm 0.05 ^d	1.23 \pm 0.04 ^d

Values are mean \pm SD 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 5. Effect of THC and PTS on Urea in urine, Ureic acid, Creatinine and Albumin, Urine volume in control and experimental diabetes

Groups	Urea in urine (mg/dl)	Ureic acid (mg/dl)	Creatinine (mg/dl)	Albumin (μ g/dl)	Urine volume (ml/day)
Control rats	144.52 \pm 8.02 ^a	7.57 \pm 4.62 ^a	2.79 \pm 0.15 ^a	145.21 \pm 7.12 ^a	9.21 \pm 0.36 ^a
Diabetic control	105.54 \pm 7.21 ^b	5.54 \pm 0.26 ^b	1.70 \pm 0.11 ^b	310.77 \pm 13.02 ^b	20.02 \pm 1.21 ^b
Diabetic + THC (80 mg/kg)	130.21 \pm 8.12 ^c	7.21 \pm 0.54 ^c	2.54 \pm 0.15 ^c	160.21 \pm 9.21 ^{ac}	11.05 \pm 0.55 ^c
Diabetic + PTS (40mg/kg)	115.40 \pm 7.15 ^d	7.15 \pm 0.11 ^d	2.35 \pm 0.15 ^d	185.14 \pm 8.78 ^c	12.24 \pm 0.54 ^d

Values are mean \pm SD for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Duncan's multiple range test).

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 diabetic rats, compared with normal rats. Administration of PTS and THC tended to bring blood glucose and plasma insulin towards normal. 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 PTS and THC to diabetic rats significantly restored total haemoglobin and glycosylated haemoglobin levels. The effect of THC was more prominent when compared with PTS.

The levels of total protein, albumin, globulin and albumin/globulin ratio in the plasma of normal and diabetic rats are presented in the table 2. The diabetic rats had decreased levels of plasma total protein, albumin, globulin and albumin/globulin ratio when compared with control rats. After treatment with PTS and THC total protein, albumin, globulin and albumin/globulin ratio were brought back to near normal levels. The protective effect of THC was more prominent compared with PTS.

Table 3 represents the effect of PTS and THC on changes in the activities of serum aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma glutamyl transpeptidase of normal and experimental rats. The activities of hepatic markers were significantly elevated in diabetic rats when compared to control rats. PTS and THC treatment to diabetic rats reversed the above changes in a significant manner when compared to diabetic control rats. The effect of THC was more prominent compared with PTS.

The level of urea, uric acid and creatinine in the serum of control and diabetic rats are represented in the table 4. In our study, the levels of urea, uric acid and creatinine are elevated remarkably in the serum of diabetic rats as compared with control rats. Diabetic rats treated with PTS and THC showed the reversed of these parameters near normal levels. The effect of THC was more potent than PTS.

Table 5 shows urine output and the levels of urea, uric acid, creatinine and albumin in the urine of diabetic rats. Diabetic rats have decreased levels of urea, uric acid, creatinine and increased levels of albumin was observed, and treatment with PTS and THC has reversed these parameters to near normal levels.

Discussion

Diabetes mellitus (DM) is the most common disorder of endocrine system in humans, characterized by hyperglycemia over a prolonged period of time. Hyperglycemia usually occurs due to insulin secretion impairment, defects in the metabolic action of insulin, or both. The long term hyperglycemia in diabetes is related to both micro and macrovascular disorders. Various organs, e.g., the liver, eyes, kidneys and nerves, are negatively affected by chronic hyperglycemia, which also causes an increased risk of cardiovascular disease (CVD) (Punthakee et al., 2018). The liver has an essential role in glucose homeostasis through its ability for storing glycogen in the fed state and producing glucose by both processes of glycogenolysis and gluconeogenesis in the fasting state. An abnormal liver function test in itself may be a biochemical indicator for the diagnosis of metabolic disorders. For instance, lipid and glucose abnormalities, fatty liver disease, and increased activities of liver enzymes are markers of liver tissue damage. Increased levels of these markers was linked with type 2 diabetes, metabolic syndrome, and insulin resistance (Elkrief et al., 2016). The scope of liver disease in type 2 diabetes includes abnormal liver enzymes and non-alcoholic fatty liver disease (NAFLD), liver cirrhosis, hepatocellular carcinoma (HCC), and acute liver failure (Mahran et al., 2017). Most patients with type 2 DM have some form of nonalcoholic fatty liver disease. However, it is becoming clear that the link between non-alcoholic fatty liver disease and type 2 DM is more complex than previously believed. Moreover, numerous pathophysiological and cardiometabolic risk factors (proinflammatory and profibrotic pathways) are shared between these two diseases. Additionally, strong

epidemiological evidence suggests a bidirectional relationship between these two disease conditions and that non-alcoholic fatty liver disease may precedes and/or promotes the development of type 2 DM (Mantovani et al., 2018).

New drugs are investigated in animals both for desired for desired effects and for the undesired (toxic) effects. This is important because any hepatic and renal damage will alter structure and function of these vital organs and have serious effects on over all metabolisms. Liver is the most important organ in the metabolism of drugs and other substances. Liver cell destruction shows its effects mostly as important in the liver cell membrane permeability, which results in the leaking out of tissue content into the blood stream (Ozsoy-Sacan *et al.* 2006). In several organs, cell membrane damage is followed by release of a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basis for clinical diagnosis. Abnormal levels in serum of aspartate transaminase, alanine transaminase and alkaline phosphatase are of clinical and toxicological importance, being indicative of tissue damage by toxicants or disease condition (Singh *et al.* 2001).

Moreover, the appearance of abnormally low levels of microalbumin in the urine is the first clinical renal complication in diabetes mellitus. Diabetic nephropathy is one of the main causes of morbidity and mortality in diabetes mellitus. Actually, the increase of death rate of diabetes mellitus occurs mainly in people with DM and proteinuria, not only from the end-stage renal disease but also from CVD, with the latter being particularly common in type 2 diabetic patients. Clinically, diabetic nephropathy is characterized by the development of kidney damage, which is reflected by increased albuminuria, renal function impairment (reduction in glomerular filtration rate GFR), high blood pressure, and increased morbidity and mortality rates related to complications of the cardiovascular system (Persson and Rossing, 2018). The results of the present study reported that all serum levels of renal function markers were significantly elevated in DM patients in comparison to healthy controls. These results are in accordance with those of other investigations (Chutani and Pande, 2017) which found increases in the levels of renal function markers in DM patients in comparison to non diabetic subjects.

Patients with Nonalcoholic fatty liver disease (NAFLD) have been characterized by elevated ALT, AST and GGT. Therefore, these hepatic enzymes were used as markers for liver fat accumulation to predict incident diabetes before direct measurements of liver fat became easily available (Ohlson et al., 1998; Sattar et al., 2004; Vozarova et al., 2004). However, prediction powers of ALT, AST and GGT for incident diabetes have been inconclusive. In addition, hepatocellular damage or cholestasis may also contribute to the elevation in the activity. Increased activity of gamma glutamyl transpeptidase in streptozotocin -induced diabetic rats was lowered to near normal by PTS and THC treatment that indicates the possible prevention of necrosis by PTS and THC treatment.

ALP elevation has been commonly seen in patients with bones diseases and/or renal hyper filtration, and bone diseases and renal hyperfiltration have been associated with diabetic patients. In particular, renal hyperfiltration has been observed in patients with newly diagnosed type 2 diabetes. However, many studies reported no association between ALP level and diabetes risk (Pari and Murugan, 2004; Vozarova et al., 2002). In our study, the activities of serum transaminases were found to be elevated in diabetic rats. In this context, several investigators reported increases in aspartate transaminase and alanine transaminase in the liver and serum of streptozotocin diabetic rats (Singh et al. 2001; Murugan and Pari, 2005; Murugan and Pari, 2007). The changes in levels of serum enzymes are directly related to changes in the metabolism in which the enzymes are involved. The increased protein catabolism accompanying gluconeogenesis in diabetic state might be the reason for the elevated activities of these enzymes, which were brought back to near normal by PTS and THC treatment. This result shows that the normalizing effects of PTS and THC on hepatocellular damage and suppression of gluconeogenesis.

The observation of an increased serum activity of alkaline phosphatase in diabetes has been interpreted as a manifestation in serum of the increased phosphatase activity that may occur in tissues in the diabetic state (Belfiore *et al.* 1972). In this condition, increased activity has been reported for glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver. These phosphatases are enzymes distinct from alkaline phosphatase. Belfiore *et al.* (1973) also reported that owing to some overlapping substrate specificity shows by the phosphatases, and the possibility that an enhanced alkaline phosphatase might be present in tissues of diabetics, it can also be ruled out that phosphatases released from tissues, mainly liver, might contribute to the elevated serum alkaline phosphatase activity (Geidam *et al.* 2004).

The activity of serum alkaline phosphatase was observed to increase in streptozotocin diabetic rats. Supporting our finding, it has been found that the liver was necrotized in streptozotocin diabetic rats (Mansour *et al.* 2002). Therefore, the increase of the activity of alkaline phosphatase in serum is mainly due to the leakage of the enzyme from the liver into circulation (Mansour *et al.* 2002). On the other hand, the administration of PTS and THC to diabetic rats reduced alkaline phosphatase activity towards its normal.

In accordance with above findings, streptozotocin - induced diabetes has a significant role in the alteration of liver functions since the activities of spartate transaminase, alanine transaminase, alkaline phosphatase and gamma glutamyl transpeptidase were significantly increased. However, the PTS and THC treatment to diabetic rats significantly reduced the activities of serum enzymes. In our previous reported that THC treatment, as there was no significant change in the

activities of serum enzymes in normal rats (Pari & Murugan, 2004; Pari and Murugan, 2006), it can be stated that the drug is non-toxic to the mammalian system.

Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine nucleotides, adenosine and guanosine; creatinine is endogenously produced and released into body fluids and its clearance rate. The diabetic hyperglycemia and creatinine which are considered as significant markers of renal function (Perone et al. 1992) and decreased urea, uric acid and creatinine and increased urine output which resemble the present result. Treatment with PTS and THC resembles the present result. Treatment with PTS and THC reversed these parameters to near normal level which could be due to decreased metabolic disturbances of other pathway such as protein and nucleic acid metabolism as evidenced by improved glycemic control. The clinical manifestation of diabetic nephropathy is the development of microalbuminuria. Gomes *et al.* (1997) observed animals; developed albuminuria may be due to impaired tubular reabsorption or leakage of albumin due to damaged glomerular membrane ascribed to alterations in size or changes in selective barriers of the glomerular or both.

In conclusion, our study suggests that the liver and kidney functions are highly altered in diabetic state. Treatment with PTS and THC reversed these changes in diabetic rats, which indicates that PTS and THC protect the hepatic and renal function in diabetic condition.

References

1. Belfiore, F., E. Napoli & L. Lo Vecchio: Increased activity of some enzymes in serum in cases of severely decompensated diabetes with and without ketoacidosis. *Clin. Chem.* 1972, **18**, 1403-1406.
2. Belfiore, F., L.L. Vecchio & E. Napoli. Serum enzymes in diabetes mellitus. *Clin Chem.* 1973, **19**, 447-452.
3. Chutani, A. and Pande, S. 2017. 'Correlation of serum creatinine and urea with glycemic index and duration of diabetes in type 1 and type 2 diabetes mellitus: A comparative study', *National Journal of Physiology, Pharmacy and Pharmacology*, 7(9): 914-9.
4. Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes.* 2010 May 15; **1**(2): 48-56.
5. DeFronzo, R.A, Bonadonna, R.C, Ferrannini, E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care.*1992, **15**, 318-368.
6. Duncan, BD (1957). Multiple range test for correlated and heteroscedastic means. *Biometrics.* **13**, 359-364.
7. Elkrief, L., Rautou, P.E., Sarin, S., Valla, D., Paradis, V. and Moreau, R. 2016. 'Diabetes mellitus in patients with cirrhosis: clinical implications and management', *Liver International*, 36(7): 936- 48. 3.
8. Geidam, M.A., G.I. Adoga & F.A. Sanda: Effects of aqueous stem bark extract of *Cissus populnea* on some serum enzymes in normal and alloxan induced diabetic rats. *Pakistan J. Biol. Sci.* 2004, **7**, 1427-1429.
9. Gomes, M.B., M.R. Lucchetti & M.F.R. Goncalvez: Influence of first morning urine volume, fasting blood and glycosylated Hb on first morning urine albumin excretion. *Braz. J. Med. Biol. Res.* 1997, **30**,191-196.
10. Ireson CR, "Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine" *Cancer Epidemiology, Biomarkers and Prevention.* **11**, Page 105-111, 2002.
11. Mahran, H.N., Saber, L.M., Alghaithy, A.A. and Elareefy, A.A. 2017. 'The role of elevated alanine aminotransferase (ALT), FasL and atherogenic dyslipidemia in type II diabetes mellitus', *Journal of Taibah University Medical Sciences*, 12(1): 8-13. 4.
12. Mansour, H.A., A. Newairy, S.A. Yousef & M.I. Sheweita. Biochemical study on the effects of some Egyptian herbs in alloxan - induced diabetic rats: *Toxicology* 2002, **170**, 221-228.
13. Mantovani, A., Byrne, C.D., Bonora, E. and Targher, G. 2018. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. *Diabetes care*, 41(2): 372-382.
14. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* 1998;47:224-229.
15. Maurya R, Singh R, Deepak M, Handa SS, Yadav PP, Mishra PK, "Constituents of *Pterocarpus marsupium*: an ayurvedic crude drug" *Phytochemistry.* **65**, Page 915-920, 2004.
16. Murugan P, Pari L, "Antioxidant effect of tetrahydrocurcumin in streptozotocin - nicotinamide induced diabetic rats" *Life sciences.* **79**, Page 1720-1728, 2006.
17. Murugan P, Pari L, "Effect of tetrahydrocurcumin on insulin receptors status in type 2 diabetic rats: Studies on insulin binding to erythrocytes" *Journal of Biosciences.* **33**(1), Page 63-72, 2008.
18. Murugan P, Pari L. Effect of tetrahydrocurcumin on erythromycin estolate- induced lipid peroxidation in rats. *Journal of Basic & Clinical Physiology & Pharmacology.* 2005; **16**:1-15.
19. Murugan P, Pari L. Influence of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in experimental type 2 diabetic rats. *Basic & Clinical Pharmacology & Toxicology.* 2007; **101**: 241-245.
20. Ohlson, L. O. et al. Risk factors for type 2 (non-insulin-dependent) diabetes mellitus. Thirteen and one-half years of follow-up of the participants in a study of Swedish men born in 1913. *Diabetologia* **31**, 798-805 (1988).
21. Ozsoy-Sacan, O., R. Yanardag, H. Orak, Y. Ozgey, A. Yarat & T. Tunali: Effects of parsley (*Patroselinum crispum*) extract versus glibornuride on the liver of streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 2006, **104**, 175-181.
22. Pari L, Amarnath Satheesh M, "Effect of pterostilbene on hepatic key enzymes of glucose metabolism in streptozotocin- and nicotinamide-induced diabetic rats" *Life Sciences.* **79**, Page 641-645, 2006.
23. Pari L, Murugan P. Protective role of tetrahydrocurcumin against erythromycin estolate induced hepatotoxicity. *Pharmacological Research.* 2004; **49** (5): 481-486.
24. Pari L, Murugan P. Tetrahydrocurcumin: Effect on chloroquine-mediated oxidative damage in rat kidney. *Basic & Clinical Pharmacology & Toxicology.* 2006; **99**: 329-334.
25. Perone, R.D., N.E. Madias & A.S. Levey: Serum creatinine as an index of renal function. New insights into old concepts. *Clin. Chem.* 1992, **38**, 1933-1953.
26. Persson, F. and Rossing, P. 2018. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney international supplements*, **8**(1): 2-7. 26. Ufuoma, C., Ngozi, Jc., Kester, Ad. and Godwin, Y. 2016.
27. Prevalence and risk factors of microalbuminuria among type 2 diabetes mellitus: A hospital-based study from, Warri, Nigeria. *Sahel Medical Journal*, **19**(1): 16. 27.
28. Punthakee, Z., Goldenberg, R. and Katz, P. 2018. 'Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome', *Canadian Journal of Diabetes*, **42**: S10-5.
29. Sattar, N. et al. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes* **53**, 2855-2860 (2004).
30. Singh, S.N., P. Vats, S. Suri, R. Shyam, M.M.L. Kumria, S. Ranganathan & K. Sridharan: Effects of an antidibetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. *J. Ethnopharmacol.* 2001, **76**, 269-277.

31. Vozarova, B. et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51, 1889–1895 (2002).
32. Warriar PK, Nambiar VPK, Ramankutty C, 'Indian Medicinal Plants' Orient Longman Limited, Madras, Page. 381–383, 1995.