EFFECT OF TETRAHYDROCURCUMIN AND PTEROSTILBENE ON CHANGES IN GLYCOPROTEIN COMPONENTS IN STREPTOZOTOCIN INDUCED DIABETES

Pidaran Murugan

Assistant professor of Biochemistry,
Centre for Distance Education and Online Education,
Bharathidhasan University, 620024.
Tamil Nadu, India.

Running title: Effect of Tetrahydrocurcumin compare to Pterostilbene in streptozotocin diabetes

***Address Correspondence to: Dr. P.Murugan**
Assistant professor of Biochemistry,
Centre for Distance Education and Online Education,
Bharathidhasan University, 620024.
Tamil Nadu, India.Tel: + 9791620088
E.mail : manomuruganphd@gmail.com
Alternative Email: pmpranithmurugan18@gmail.com

Abstract

Tetrahydrocurcumin (THC) are polyphenolic compounds with para-hydroxyl functional groups and keto functional groups that participate in antioxidant and chemo preventive action (Sugiyama et al. 1996). THC is a hydrogenation product of curcumin produced by reducing curcumin in an organic solvent using a metal catalyst. 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. Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of *Pterocarpus marsupium*. Glucose, plasma insulin and glycoprotein components in plasma and tissues (hexose, hexosamine, fucose and sialic acid) were determined. Oral administration of THC to diabetic rats showed a decrease in the level of blood glucose and plasma glycoproteins. The levels of plasma

insulin and tissue sialic acid were increased where as the levels of tissue hexose, hexosamine and fucose were near normal in diabetic rats treated with THC and Pterostilbene. The present study indicates that the THC and Pterostilbene possesses a significant beneficial effect on glycoprotein moiety in addition to its antidiabetic effect. These biochemical observations were supplemented by histopathological examination of pancreas section.

Keywords: blood glucose, Pterostilbene, diabetes mellitus, glycoproteins components, tetrahydrocurcumin.

Introduction

Diabetes mellitus is a heterogeneous endocrine metabolic disorder characterized by chronic hyperglycemia caused due to insulin deficiency or defects in insulin action with impaired function of carbohydrate, lipid and protein metabolism [1]. Insulin deficiency or defects in insulin action leads to hyperglycemia which affects the functions of various organs due to uncontrolled glucose regulation ultimately leads to various diabetic complications [2]. It is becoming increasingly accepted that the carbohydrate moieties of glycoproteins such as hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover [3]. Glucose level is tightly controlled by insulin secretion from pancreatic β-cells and insulin action on liver, muscle and other target tissues. Insulin promotes the transport of glucose into skeletal muscle and adipose tissue. In the liver, insulin suppresses glycogenolysis and gluconeogenesis and in adipose tissue and inhibits lipolysis. Insulin mediates this control of carbohydrate metabolism in part by directly or indirectly regulating the expression of several enzymes involved in intermediary metabolism (Torneheim, 2004).

Liver plays a pivotal role in regulating the endogenous glucose production from denovo synthesis (gluconeogenesis) or the catabolism of glycogen (glycogenolysis), increased rate of hepatic production that are largely responsible for the development of overt hyperglycemia, in particular fasting hyperglycemia in patients with diabetes. Glycoproteins are conjugated proteins contain prosthetic group of one or more heterosaccharides. The carbohydrate moieties of glycoprotein include neutral sugars,

amino acid and sialic acid. These group of macromolecules carryout numerous biological functions including lipid and hormone transport, haemoglobin binding and blood coagulation. Impaired metabolism of glycoproteins play a major role in the pathogenesis of diabetes mellitus [4]. Jonsson (1976) reported that alterations occur in the concentrations of various membrane bound carbohydrates (glycoproteins) in human diabetes [4].

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 [5]. 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 [6,7]. The reduction of curcumin to THC seems to occur primarily in a cytosolic compartment [8]. Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) [8]. 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 [9]. In our previous study, we have demonstrated the antidiabetic effect of THC in streptozotocin (STZ) induced diabetic rats [10].

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 [11]. 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* [12]. An aqueous extract of heartwood of *P.marsupium* has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients [13].

Streptozotocin-nicotinamide type 2 model shares a number of features with human type 2 diabetes, and is characterized by moderate stable hyperglycaemia, glucose intolerance, 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 [14].

To our knowledge, so far no other biochemical investigation has been carried out on the effect of THC compare to Pterostilbene in plasma and tissue glycoproteins of experimental diabetic rats. In this view the present investigation was carried out to study the effect of THC and Pterostilbene on plasma and tissue glycoproteins in rats with STZ and nicotinamide induced diabetes.

1. 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.

Induction of diabetes

Non-insulin dependent diabetes mellitus was induced [5] in overnight fasted rats by a single intraperitonial injection of 65 mg/kg body weight STZ, 15 min after the intraperitonial administration of 110 mg/kg body weight of nicotinamide. STZ was dissolved in citrate buffer (0.1M, 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 were 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 [14].

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 and glycoproteins. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Analytical methods

Measurement of blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) [15]. Plasma insulin was assayed by ELISA using a Boehringer - Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

Determination of levels of glycoproteins components

For the estimation of glycoprotein components, the tissues were defatted by the method of Folch *et al*. [16] and the defatted tissues were treated with 0.05 M H_2SO_4 and hydrolysed at 80°C and aliquot was used for sialic acid estimation. To the remaining solution, 0.1 M NaOH was added. The aliquots were used for fucose, hexose, and hexosamine estimation. Hexose was estimated by the method of Niebes [17]. The reaction

mixture contained, 0.5 ml of aliquot/ plasma, 0.5 ml of 5% phenol and 2.5 ml of conc. H_2SO_4 and boiled for 20min and absorbance was read at 490 nm.

Hexosamine was estimated by the method of Elson and Morgan (1933) with slight modifications by Niebes [17]. Briefly, the reaction mixture contained, 0.5 ml plasma / 1.0 ml aliquot, 2.5 ml of 3 M HCl and boiled over 6 h and neutralized with 6 M NaOH. To 0.8 ml of neutralized sample added 0.6 ml of acetyl acetone reagent and boiled for 30 min. The mixture was treated with 2.0 ml of Ehrlich's reagent. The absorbance was read at 540 nm.

Sialic acid was determined by the method of Warren [18]. In brief, 0.5 ml of aliquot / plasma was treated with 0.5 ml of water, 0.25 ml of periodic acid and incubated at 37°C for 30 min. To the reaction mixture added 0.2 ml of sodium meta arsenate and 2.0 ml of thiobarbituric acid were added and heated for 6 min and added 5.0 ml of acidified butanol. The absorbance was read at 540 nm.

Fucose was estimated by the method of Winzler [19]. Briefly, 1.0 ml of precipitated glycoprotein from platelet membrane and 1.0 ml of processed serum were dissolved in 1 ml of 0.1 M NaOH and placed in an ice-bath and 4.5 ml of cold H2SO4 was added and mixed well. The tubes were heated in a boiling water bath for 3 min and cooled and then 0.1 ml of 3% cysteine was added and mixed immediately. The tubes were allowed to stand at room temp. for 60–90 min. The absorbance of the solution at 396 and 430 nm was measured in a spectrophotometer and the difference in the absorbances was taken for the calculation.

Analysis of fluorescence of collagen

Fluorescence of collagen in the tail tendon was determined by the method described by Monnier et al. [20]. AGEs exhibit a yellow-brown pigmentation and a characteristic fluorescence pattern, with excitation in the range 350-390 and fluorescence emission at 440-470.

Histopathological study

The 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

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 $p<0.05$ [21].

2. Results

Fig.1 shows the level of blood glucose and plasma insulin of different experimental groups. The diabetic control rats showed a significant increase in the level of blood glucose with significant decrease in the level of plasma insulin. Oral administration of THC to diabetic rats significantly reversed the above biochemical changes. In our previous study [22] we have reported that THC at 80 mg/kg body weight showed better effect than 20 and 40 mg/kg body weight, therefore the 80 mg/kg body weight was used in this study. The administration of THC and Pterostilbene to normal rats showed a significant effect on blood glucose and plasma insulin levels. The THC administration showed more effective than Pterostilbene.

The levels of plasma and tissue glycoproteins in control and experimental rats are shown in the Table 4. There was a significant increase in the level of plasma glycoproteins in diabetic rats. In liver and kidney of diabetic rats, the level of hexose, hexosamine and fucose were significantly increased whereas the level of sialic acid was significantly decreased. Oral administration of THC and pterostilbene significantly reversed the changes in plasma, liver and kidney glycoproteins of diabetic rats. The effect of THC was better than Pterostilbene.

Figures 2 and 3 illustrate the SDS-gel pattern of acid soluble and pepsin-soluble collagen in tail tendon of control and experimental rats. The increased bandwidth of β components in diabetic collagen, while THC and Pterostilbene supplemented diabetic group showed lesser bandwidth.

Histopathological observations

Histopathological studies (compared to normal Fig. 4 A) demonstrate fatty infiltration and islet shrinkage in pancreas of STZ diabetic rats (Fig. 4B) and these changes were markedly reduced in diabetic rats treated with THC and Pterostilbene (Fig. 4C and 4D).

3. Discussion

Glycoproteins were chiefly distributed proteins with one or more covalently linked carbohydrate molecules which act as enzymes, hormones, constituents of extracellular membranes and blood group antigens [23]. Glycoproteins involve in recognition, membrane transport, and absorption of macromolecules, cell differentiation, excretion and the adhesion of macromolecules to the cell surface [24]. It is reported that hyperglycemia results in structural and functional changes of both circulating and membrane bound glycoproteins [25]. In hyperglycemic condition utilization of glucose by insulin independent pathways leads to the synthesis of glycoproteins which may be a predictor of angiopathic complications [26]. So, the elevated levels of glycoproteins are the principle cause for the pathogenesis of liver and kidney diseases in diabetic condition.

The function of glycoproteins in stabilizing the tissue may be involved in maintaining the structural stability of collagen fibrils. Glycoproteins are important components of intracellular matrix, cell membrane, and membranes of sub-cellular organelles [27]. During diabetes, synthesis of glycoproteins was decreased because of reduced incorporation of glucose caused by insulin deficiency. Increased glycosylation of various proteins in diabetic patients have also been reported earlier and the elevated of glycoproteins in diabetics may also be a predictor of angiopathic complications [28].

Formation of some AGE combines both the glycation and oxidative steps in a process termed glycoxidation [29]. Glycation occurs inside and outside of cells; Glycation of cellular proteins produces changes in structure and loss of enzymatic activity. These effects are countered by protein degradation and renewal. Glycation of the extracellular matrix produces changes in macromolecular structure affecting cell - cell and cell - matrix interactions

associated with decreased elasticity and increased fluid filtration across arterial wall and endothelial cell adhesion [30]. When the concentration of AGE increased above a critical level, cell surface AGE receptors become activated. This is associated with increased expression of extracellular matrix proteins, vascular adhesion molecules, cytokines and growth factor. Depending on the cell type and concurrent signaling this is associated with chemo taxis, angiogenesis, oxidative stress, and cell proliferation or apoptosis. These processes are thought to contribute to disease mechanisms associated with the development of diabetic complications [31].

Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in plasma [32]. Therefore, vascular complications that involve complex protein-carbohydrate molecules could contribute to increase in plasma and tissue glycoproteins. Coulson and Hernandez [33] have demonstrated that glucosamine in rats increased the glucose level to a height as great as that of found in severe diabetes.

Fucose is present in serum and is a normal constituent of glycoproteins, which may be differentially expressed in pathological condition. Serum fucose levels have also been shown to increase in diabetes, particularly bound to the serum proteins α1-antitrypsin, α1-acid glycoprotein and hepatoglobin [34]. Administration of THC and pterostilbene to diabetic rats significantly reversed all these changes to near normal levels. Our results suggest that the increased in the synthesis and/or decrease in degradation of these proteins.

In diabeties, sialic acid level of hepatocytes was found decreased and insulin therapy simultaneously restored the blood glucose and hepatic sialic acid level [35]. Sato et al. [36] observed a decrease in the contents of sialic acid in diabetic glomerualr basement membrane. Alterations in membrane protein glycosylation may also be important in membrane protein turnover. Since carbohydrate groups such as sialic acid render protection against proteolysis of glycoproteins, a decrease in superficial carbohydrate moieties might increase proteolysis and hence membrane protein degradation [37].

The cleavage of sialic acid residue from circulatory and membrane glycoproteins might be the cause of high serum levels. In case of circulatory proteins, loss of sialic acid moiety targets them for degradation by liver cells that have sialoglycoprotein receptors [38]. Desialylation imparts its effect not only by altering the structure and functions of glycoproteins and also increase the free sialic acid level in circulation. The decrease in the content of sialic acid in tissues may also be due to its utilization for the synthesis of fibronectin, which contains sialic acid residues in the core structure. The synthesis of fibronectin was also reported to increase significantly in various tissues of diabetic animals and patients [39,40].

The SDS-gel pattern of collagen confirms the structural alterations in collagen in diabetic rats. The band size of β-component of collagen in the diabetic rat was increased as compared to that of control rat. The relative abundance of high molecular weight collagen chain was demonstrated by the decreased ratio of α to β chains. β-chains are dimers in which the inter chain crosslinks are not disulfide bridges [41]. In the present study, a significant decrease in the ratio of α to β components of tail tendon collagen of diabetic rats was also observed. The increased intensity of β-component observed in diabetic rats in our study suggests that collagen chain are capable of enhanced intramolecular crosslinking since the β-component is a dimer of α-chains.

4. Conclusion

The data in our study suggests that THC and Pterostilbene reversed the abnormalities in the levels of glycoprotein components. THC may have beneficial effects in diabetes mellitus, by the enhancement of insulin action, as evident by the increased level of insulin in diabetic rats treated with THC and Pterostilbene, which may be responsible for the reversal of glycoprotein changes. The THC administration showed more effective than Pterostilbene.

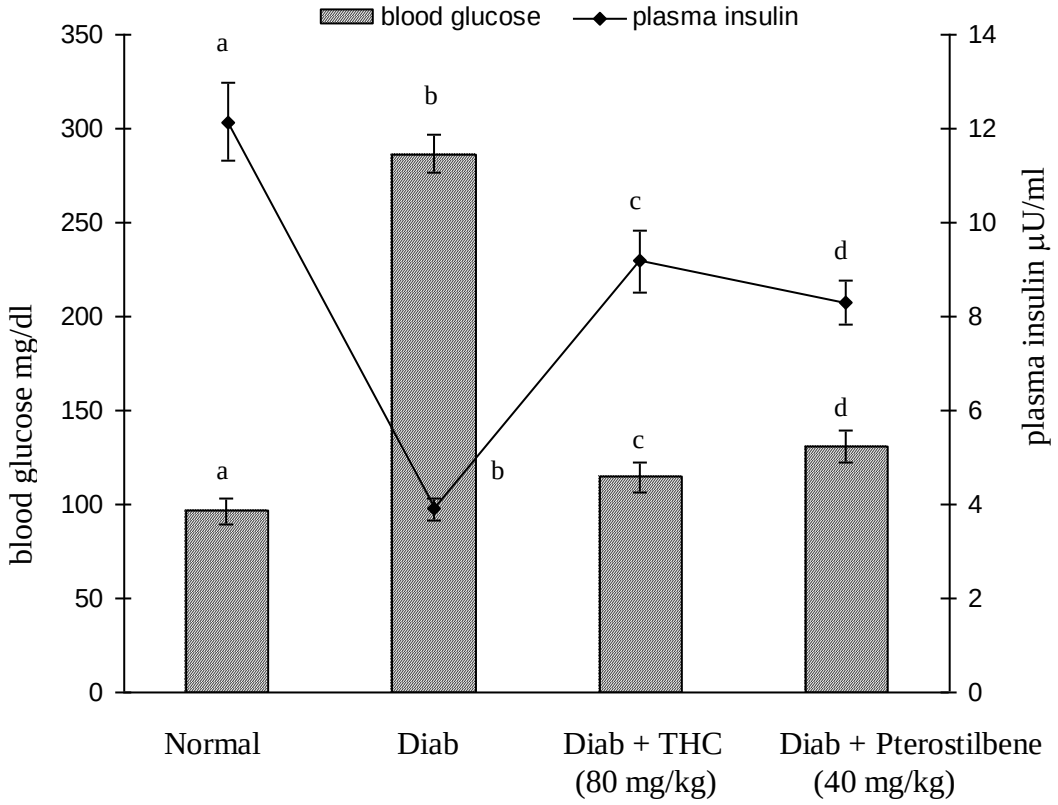


Figure 1. Effect of tetrahydrocurcumin (THC) on the levels of blood glucose and plasma insulin in normal and experimental rats.

Diab - Diabetic control.

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).

Figure 2. SDS gel pattern of acid soluble collagen from tail tendon in control and experimental rats

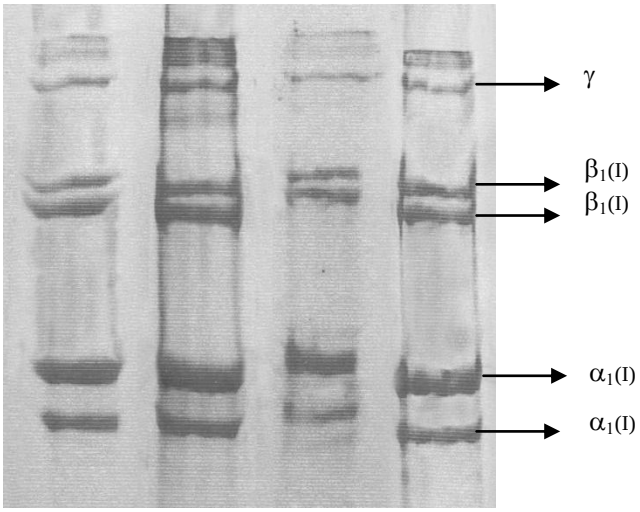
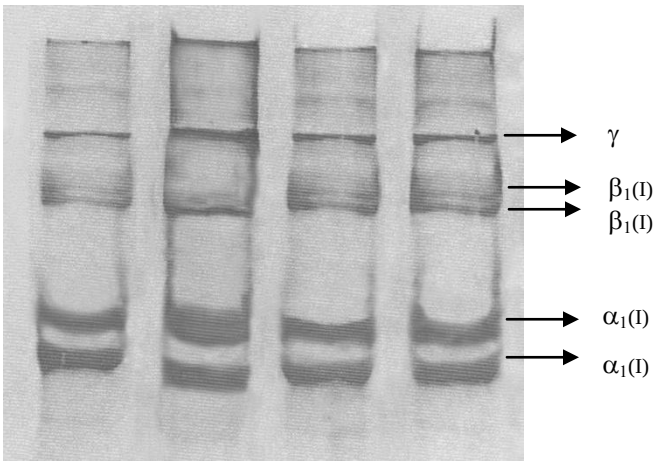


Figure 3. SDS gel pattern of pepsin soluble collagen from tail tendon in control and experimental rats



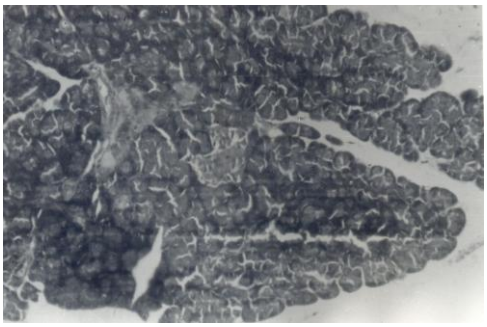


Figure 4 A. Normal rats pancreas H&E x 20
Pancreas showing β-islets (→)

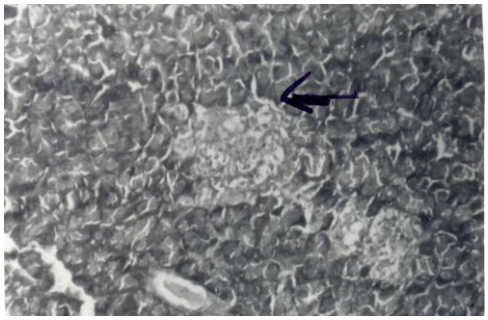


Figure 4 B. Diabetic control rats pancreas H&E x 20
Fatty infiltration of islet cells and shrinkage (→)

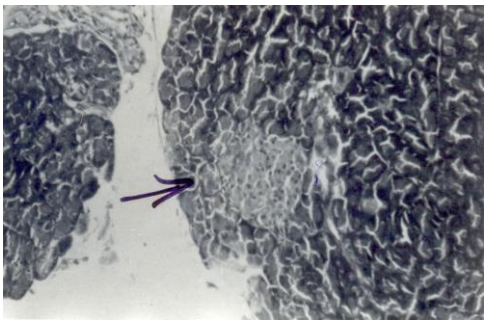


Figure 4C. Diabetic + THC (80 mg) treated rats pancreas H&E x 20
Normal appearance of islets (→)

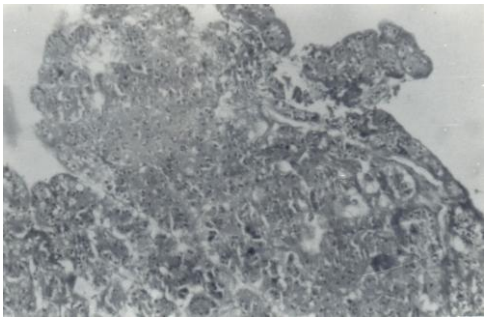


Figure 4 D. Diabetic + Pterostilbene (40 mg) treated rats pancreas H&E x 20
Parenchymal inflammation and necrosis

5. References

1. Kar M, "Chakraborti AS, Release of iron from haemoglobin-a possible source of free radicals in diabetes mellitus" Indian Journal of Experimental Biology. 37, Page 190–192, 1999.
2. Zimmet P, "Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic" Nature. 414, Page 6865, 2001.
3. Wiese TJ, Dunlap JA, Yorek MA, "Effect of L-fucose and L-glucose concentration on L-fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and□□□□□□L fucosidase activity in liver of diabetic rats" Biochim Biophys Acta. Page 61 – 72, 1997.
4. Jonsson A, Wales JK, "Blood glycoprotein levels in diabetes mellitus" Diabetologia. 12(3), Page 245–250, 1976.
5. Murugan P, Pari L, "Antioxidant effect of tetrahydrocurcumin in streptozotocin - nicotinamide induced diabetic rats" Life sciences. 79, Page 1720-1728, 2006.
6. Murugan P, Pari L, "Protective role of tetrahydrocurcumin on changes in the fatty acid composition in streptozotocin - nicotinamide induced type 2 diabetic rats" Journal of Applied Biomedicine. 5, Page 31-38, 2007.
7. 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.
8. Ireson CR, "Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine" Cancer Epidemiology, Biomarkers and Prevention. 11, Page 105–111, 2002.
9. Pari L, Murugan P, "Protective role of tetrahydrocurcumin against erythromycin estolate induced hepatotoxicity" Pharmacological Research. 49 (5), Page 481-486, 2004.
10. Murugan P, Pari L, "Effect of tetrahydrocurcumin on erythromycin estolate-induced lipid peroxidation in rats" Journal of Basic and Clinical Physiology and Pharmacology. 16, Page 1-15, 2005.
11. Warrier PK, Nambiar VPK, Ramankutty C, 'Indian Medicinal Plants" Orient Longman Limited, Madras, Page. 381–383, 1995.

12. 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.
13. Indian Council of Medical Research (ICMR). "Collaborating centers, New Delhi, Flexible open trial of vijayasar in case of newly diagnosed non-insulin dependent diabetes mellitus" Indian Journal of Medical Research. 108, Page 24-29, 1998.
14. 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.
15. Lott JA, Turner K, "Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine" Clinical Chemisty. 21/12, Page 1754-1760, 1975.
16. Folch J, Lees M, Solane SGH, "A simple method for isolation and purification of total lipids from animal tissues" Journal of Biological Chemistry. 26, Page 497-509, 1957.
17. Niebes P, "Determination of enzymes and degradation products of glycosaminoglycans metabolism in the serum of healthy and varicose subjects" Clin Chim Acta. 42, Page 399 – 408, 1972.
18. Warren L, "The thiobarbituric acid assay of sialic acids" Journal of Biological Chemistry. 234, Page 1971-1975, 1959.
19. Winzler RJ. "Determination of serum glycoproteins" Methods of Biochemical Analysis. 2, Page 279-311, 1955
20. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR, "Relation between complications of type I diabetes mellitus and collagen-linked fluorescence" The New England Journal of Medicine. 314, Page 403-408, 1986.
21. Duncan BD, "Multiple range tests for correlated and heteroscedastic means" Biometrics. 13, Page 359 - 364. 1957.
22. Pari L, Murugan P, "Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats" Journal of Basic and Clinical Physiology and Pharmacology. 16, 257-274, 2005.

23. Kumar GS, Shetty AK, Salimath PV, "Modulatory effect of bitter gourd (Momordica charantia LINN.) on alterations in kidney heparan sulfate in streptozotocin-induced diabetic rats" Jornal of Ethnopharmacology. 115(2), Page 276–283, 2008.
24. Parillo F, Arias MP, Supplizi AV, "Glycoprofile of the different cell types present in the mucosa of the horse guttural pouches" Tissue Cell. 41(4):257–265. 2009.
25. Ciftci G, Yarim GF, "Evaluation of IGF-I levels and serum protein profiles of diabetic cats and dogs" Journal of Veterinary Science. 12(4), Page 325–331, 2011.
26. Neerati P, Barla R, Bedada S, "Influence of diabetes mellitus on P-glycoprotein function in rat intestine" Pharmacologia. 2(10), Page 293–298, 2011.
27. Zachariah, B, Basu D, "Surface carbohydrates in cell-biology" Indian Journal of Biochemistry and Biophysics, 30, Page 422–425, 1993.
28. Konukoglu D, Serin, O, Akcay T, "Hatemi H, Relationship between diabetic angiopathic complications and serum total and lipid associated sialic acid concentrations" Medical Science Research. 27, Page 53–55, 1999.
29. Vlassara H, Palace MR, "Diabetes and advanced glycation endproducts" Journal of Internal Medicine. 251, Page 87-101, 2002.
30. Thornalley PJ, "Glycation diabetic neurpathy; characteristics, consensus causes, and therapeutic options" International Review of Neurobiology. 50, Page 37-57, 2002.
31. Guillot R, Liassab JP, Ogneva U, Andre J, Durussel SS, "Relation between pancreatic islet cellular infiltration and plasma fibrinogen or alpha-1 acid glycoprotein levels in spontaneously and streptozotocin diabetic rats. An increase in these protein levels is not necessary for inducing microcirculatory and erythrocyte alteration" Pancreas. 9, Page 336-343, 1994.
32. Berenson GS, Radhakrishnamurthy, Dalferes ER, Jr., Ruiz H, Srinivasan SR, Plavidal F, Brickman F "Connective tissue macromolecular changes in rats with experimentally induced diabetes and hyperinsulinism" Diabetes. 21:733-743, 1972.

33. Coulson RA, Hernandez T "Hyperglycemic effect of glucosamine" American Physiological Society Journal. 203:243-247, 1962.
34. McMillan DE "Elevation of glycoprotein fucose in diabetes mellitus" Diabetes. 21:863-871, 1972.
35. Belce A, Uslu E, Kucur M, Umut M, Ipbuker A, Seymen HO "Evaluation of salivary sialic acid level and Cu-Zn superoxide dismutase activity in type 1 diabetes mellitus" The Tohoku Journal of Experimental Medicine. 219-225, 2000.
36. Sato T, Munakata H, Yoshinaga K, Yosizawa Z "Comparison of the chemical composition of glomerular and tubular basement membranes obtained from human kidneys of diabetics and non-diabetics" Clin Chim Acta. 61:145-150, 1975.
37. Jacobs LR "Alterations in labeling of cell-surface glycoproteins from normal and diabetic rat intestinal microvillous membranes" Biochim Biophys Acta. 649:155-161, 1981.
38. Mazzanti L, Rabini RA, Salvolini E, Tesei M, Martarelli D, Venerando B, Curatola G "Sialic acid, diabetes, and aging: a study on the erythrocyte membrane" Metabolism. 46:59-61, 1997.
39. Noh H, Ha H, Yu MR, Kang SW, Choi KH, Han DS, Lee HY "High glucose increases inducible NO production in cultured rat mesangial cells" Possible role in fibronectin production. Nephron. 90:78-85, 2002.
40. Isono M, Chen S, Hong SW, Iglesias-de la Cruz MC, Ziyadeh FN "Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF-beta-induced fibronectin in mesangial cells" Biochemical and Biophysical Research Communications. 296:1356-1365, 2002.
41. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR, "Relation between complications of type I diabetes mellitus and collagen-linked fluorescence" The New England Journal of Medicine: Research & Review.314, Page 403-408, 1986.

Table 1. Effect of tetrahydrocurcumin (THC) and Pterostilbene on the levels of plasma and tissue hexose, hexosamine, sialic acid and fucose in normal and experimental rats

Groups	Normal	Diabetic control	Diabetic + THC (80 mg/kg)	Diabetic + Pterostilbene (40 mg/kg)
Hexose				
Plasma (mg/dl)	96.83 ± 5.51^{a}	138.71 ± 6.41^{b}	104.59 ± 5.15^{ac}	112.21 ± 6.83^{c}
Liver (mg/g defatted tissue)	29.15 ± 1.41^{a}	48.76 ± 2.48^{b}	33.51 ± 2.31^{c}	38.21 ± 2.01^{d}
Kidney (mg/g defatted tissue)	23.42 ± 1.51^{a}	43.13 ± 2.32^{b}	28.43 ± 3.12^{c}	33.16 ± 1.68^{d}
Hexosamine				
Plasma (mg/dl)	78.12 ± 6.32^{a}	99.35 ± 5.77^{b}	82.71 ± 3.78^{ac}	88.51 ± 5.59^{c}
Liver (mg/g defatted tissue)	11.21 ± 0.76^{a}	20.37 ± 1.51^{b}	12.41 ± 0.97^{c}	14.52 ± 1.14^{d}
Kidney (mg/g defatted tissue)	17.15 ± 1.32^{a}	32.15 ± 2.12^{b}	18.52 ± 1.25^{ac}	19.75 ± 1.32^{c}
Sialic acid				
Plasma (mg/dl)	56.47 ± 4.58^{a}	71.39 ± 4.47^{b}	58.25 ± 2.28^{ac}	64.31 ± 3.59^{c}
Liver (mg/g defatted tissue)	9.68 ± 0.49^{a}	4.59 ± 0.21^{b}	7.55 ± 0.35^{c}	6.80 ± 0.48^{d}
Kidney (mg/g defatted tissue)	8.59 ± 0.41^{a}	4.09 ± 0.21^{b}	6.99 ± 0.61^{c}	5.82 ± 0.44^{d}
Fucose				
Plasma (mg/dl)	29.51 ± 1.71^{a}	42.41 ± 2.03^{b}	33.56 ± 1.49^{c}	36.74 ± 2.78^{d}
Liver (mg/g defatted tissue)	18.05 ± 1.67^{a}	28.12 ± 1.48^{b}	20.75 ± 1.16^{c}	22.12 ± 1.12^{d}
Kidney (mg/g defatted tissue)	13.54 ± 0.68^{a}	28.51 ± 1.71^{b}	16.22 ± 0.99^{c}	19.94 ± 1.05^{d}

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