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MODULATORY EFFECT OF PTEROSTILBENE ON TAIL TENDON COLLAGEN IN TYPE 2 DIABETIC RATS

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Abstract

Introduction: The research was determined to investigate on the effects of pterostilbene and tetrahydrocurcumin (THC) on the collagen content and its physical and chemical characteristics in the tail tendon of streptozotocin (STZ) induced diabetic rats.

Purpose: Ptrerostilbene and THC has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients.

Experimental design: In the experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each, after the induction of STZ diabetes. The experimental period was 45 days. Group 1: Normal rats. Group 2: Diabetic control rats. Group 3: Diabetic rats treated with THC (80 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group 4: Diabetic rats treated with pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days

Results: In the case of diabetic rats treated with pterostilbene and THC, the collagen content was significantly decreased. The extent of glycation, cross-linking and collagen linked fluorescence were significantly reduced. In addition to that, the collagen content in the □ region as well as in the high molecular weight region in tail tendon was near normal when compared to diabetic control groups. The reduction in the advanced glycation and cross-linking of collagen in pterostilbene and THC treated rats to their antiperoxidative activity directly influence the collagen cross-linking and advanced glycation end products formation.

Conclusion: The administration of pterosilbene and THC had a positive influence on the content of collagen and its properties in streptozotocin and nicotinamide diabetic rats. The pterosilbene administration was found to be more effective than THC.

Keywords: tetrahydrocurcumin, pterostilbene, collagen, streptozotocin, nicotinamide, blood glucose, plasma insulin

INTRODUCTION

Collagen is present in all types of multicellular animals and is probably the most abundant animal protein in nature. It is estimated that collagen accounts for about 30% of the total human body protein. Collagen is located in the extracellular matrix of connective tissues [1]. Collagen interacts with cells through the integrin cell receptors and mediates cellular adhesion and migration. Important roles for collagen have been identified in development, wound healing, platelet aggregation and aging. Its commercial importance in leather and the production of gelatin and glue have long been recognized. More recently, it is being used as a basis for biomaterials. Examples of its biomedical applications include injectable collagen to lessen facial wrinkles and defects; surgical collagen sponges to increase blood clotting and artificial skin for the treatment of burns [2].

The classification of an extracellular matrix protein as a collagen is based on the presence of a domain with the distinctive triple-helical conformation. The collagen triple helix consists of three polypeptide chains, each of which adopts an extended polyproline II-like helix. The three chains are supercoiled about a common axis and linked by hydrogen bonds [3]. Recently, the detailed features of this conformation determined by x-ray crystallography, confirming the general structure and showing an extensive network of ordered water hydrogen bonded to the triple helix [4].

The triple-helical conformation requires unique amino acid sequence features. Glycine is the smallest amino acid, must be present as every third residue. A high content of the sterically restricted imino acids, proline and hydroxyproline, is also necessary to stabilize the extended helix. Hydroxyproline provides additional stability through water-mediated hydrogen bonds. Collagen is the only animal protein other than elastin, with significant amounts of hydroxyproline and hydroxylysine residues. It is easy to identify a collagen triple helix from its amino acid sequence pattern of repeating glycine-X-Y sequences and glycine-proline-hydroxyproline is the most frequent tripeptide sequence [5].

Collagen is a protein containing several dibasic amino acids and has a slow turnover rate and is a strong candidate for extensive modification by glycation. Crosslinking is important in stabilizing the collagen fibrils. Crosslinking contributes to the tensile strength of tissues such as tendon by decreasing the permeability and elasticity of extracellular matrix. Extensive crosslinking of collagen however causes changes in its structure and the mechanical properties can be pathological [6]. Such changes have been reported in aging and in various pathological conditions including diabetes.

Glucose and other sugars also act as cross-linking agents of the extracellular matrix (ECM). Collagen has a long biological half-life and the level of non enzymic glycosylation or glycation, increases gradually with aging or in hyperglycemic conditions such as diabetes [7]. Reducing sugars (glucose, fructose, etc.) bound to free protein amino groups and go through a series of reactions to form a class of heterogeneous, non enzymic sugar-amino adducts that are called AGEs [8].

The diabetes associated changes in collagen function in the basement membranes are documented to be the biochemical link between persistent hyperglycemia and diabetic microvascular disease. These modifications are associated with decreased solubility, increases in fluorescence, thermal stability and mechanical strength. An increase in AGE-modified collagen has been detected in diabetic and ageing rats [9]. The alterations caused by AGE- modifications of collagen may play a role in the pathogenesis of various complications in poorly controlled diabetic patients [10].

During diabetes, collagen undergoes an extensive modification and finally leads to the development of diabetic complications. Two main factors play an important role in the post translational modifications of collagen. Firstly, the accumulation of browning product or AGE's by the long half-life period or slow turnover of collagen, rendering it highly susceptible to advanced glycation [11]. Secondly, the structural and functional alterations of collagen that occur in tissues like joints, arteries, retina and renal glomerular system that are severely affected during diabetes [12]. Atherosclerosis, thickening of basement membranes, increased arterial wall stiffness, decreased lung elasticity, sclerosis of renal glomeruli, stiffening of heart, periarticular rigidity and osteoarthritis [13] are the pathological changes which involve collagen in diabetes.

Accelerated aging occurs in diabetes when collagen becomes increasingly cross-linked, less soluble, thermally stable and resistant to enzyme digestion with advancing age. It has been reported that collagen in various tissues from diabetic individuals is less soluble in acid and pepsin [14] and less digested by collagenase and CNBr [15]. Several experimental diabetic studies revealed the abnormalities in mechanical properties of tail tendons such as tensile behaviour, stress-strain behaviour, crimp length and tendency towards a larger fibril diameter [16]. The extensive crosslinking of collagen play a main role in functional and structural alterations of renal, vascular and cardiac tissue [17].

Several medicinal phytochemicals such as THC [18], Curuma longa [19], Cassia auriculata [20], etc. were reported to possess antihyperglycemic effects [21]. The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes [22]. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels [23]. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects [24].

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 [25]. Pterostilbene was found to be one of the active constituents in the extracts of the heartwood of Pterocarpus marsupium. it is suggested that pterostilbene might be one of the principal anti-diabetic constituents of Pterocarpus marsupium [26]. An aqueous extract of heartwood of P.marsupium has been tested clinically and found to be effective in non-insulin dependent diabetes mellitus patients [27].

THC was one of the major colourless metabolite of curcumin. THC has been reported to exhibit the same physiological and pharmacological properties of curcumin [28]. Curcumin was rapidly metabolized during absorption from the intestine, yielding THC [29], which had shown the strongest antioxidant activity among all curcuminoids [30]. THC thought to play a pivotal role in protecting the cell membrane against lipid peroxidation, which exhibits its protective effect by means of □-diketone moieties and phenolic hydroxyl groups [31]. Several studies in experimental animals indicated that THC also preventes cancer, protect the inflammation, atherosclerotic lesions and hepatotoxicity [32].

Recently it was found that THC improves plasma insulin, decrease glucose levels, scavenging free radical and also antioxidant activity in type 2 diabetic rats [33]. THC reverses the changes in the levels of the carbohydrate moieties of glycoprotein [34] and also antihyperlipidemic effect [35]. The diabetic rats have reduced capacity answer to oxidative status and that this reduction is associated with hyperglycemia driven non-enzymatic glycation and oxidation of lipids, which enhances the formation of erythrocyte membrane enzymes in STZ -nicotinamide, induced diabetic rats.

EXPERIMENTAL DESIGN

In the experiment, rats were divided into 4 groups of 6 rats each. The effect of THC was compared with pterostilbene drug.

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.

Group 4: Diabetic rats given Pterostilbene (40 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days.

No detectable irritation or restlessness was observed after each drug or vehicle administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animals after the drug administration. At the end of 45 days, all the rats were killed by decapitation after inducing anaesthesia (Pentobarbitone sodium, (60 mg/kg)). Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and plasma was separated for assay of insulin.

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.

Chemicals

THC and Pterostilbene was a gift provided by Sabinsa Corporation, USA. STZ, insulin powder and pepstatin A were purchased from Sigma Chemical Co., (St Louis, MO), USA.

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. 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.

Preparation and purification of type I collagen

Collagen was purified by the method described by Chandrakasan et al. [36]. Briefly, tail tendons were dissected free, washed extensively in phosphate-buffered saline (PBS) and extracted. The entire procedure was carried out at 0-5ºC. Tail tendons obtained from rats were washed with neutral 1% NaCl and extracted by stirring overnight in eight volumes (v/v) of 0.5 M acetic acid. The suspension was centrifuged at 28,330 g for 30 min and the pellet was re-extracted with acetic acid. The supernatant solutions were combined and solid NaCl was added slowly with stirring to reach 20% concentration (w/v). The precipitate obtained was collected by centrifugation at 17,000 g for 30 min and the pellet was washed three times by resuspending in 20% NaCl and recentrifuged. The washed pellet was then suspended in three volumes of 0.5 M acetic acid, stirred for a few hours and then dialyzed overnight against several volumes of 0.5 M acetic acid with at least one change. The solution was centrifuged at 28,330 g for 3 min. Collagen was precipitated from the supernatant by slow addition with stirring of 0.2 volumes 30% NaCl in 0.5 M acetic acid. The washed pellet was suspended in two volumes of 0.5 M acetic acid and dialyzed overnight against several changes of 20 mM Na2HPO4. The precipitate was collected by centrifugation at 17,000 g for 30 min, washed once with 20 mM Na2HPO4 and redissolved in two volumes of 0.5 M acetic acid. The solution was centrifuged at 28,330 g for 60 min and dialyzed for 2 days against 0.1 M acetic acid with three changes. The purified collagen was lyophilized and stored in a freezer in containers sealed under vacuum.

Estimation of collagen content

Weighed tail tendon tissue was hydrolyzed in 6.0 N HCl for 18 h at 110º C. The collagen content was determined by measuring hydroxyproline, as described by Woessner [37].

Extent of glycation

The extent of glycation was determined by the method described by Rao and Pattabiraman [38] in which 1.0 ml of purified collagen (containing 1.0 mg collagen) was mixed with 3.0 ml of

concentrated H_2SO_4, vortexed, cooled on ice, mixed with 0.5 ml of 80% phenol, and left to stand at room temperature for 30 min. Absorbance was measured at 485 nm using glucose as standard.

Collagen-linked fluorescence

Collagen-linked fluorescence was measured by the method of Monnier et al. [7]. Approximately 3.0 mg tissue was finely minced in PBS and centrifuged at 3,330 g for 10 min. The pellet was washed with distilled water and the lipids were extracted with 5.0 ml of chloroform: methanol (2:1, v/v) overnight. The samples were rehydrated by the addition of 2.0 ml methanol and 0.5 ml distilled water and centrifuged at 3,330 g for 10 min and the pellet was washed twice with methanol, three times with distilled water, twice with 20 mM N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), pH 7.5, containing 0.12 M $CaCl_2$ (buffer H) and stored overnight at 4ºC in buffer H. The buffer was then removed by centrifugation at 3,330 g for 10 min and the pellet resuspended in 3.5 ml of buffer H containing 120 units of type VII collagenase. Four drops of toluene were added to prevent bacterial growth and the material was digested for 48 h at 37ºC. A blank containing collagenase in buffer H was included. The digest was centrifuged at 3,330 g for 30 min and the clear supernatant containing digested collagen was used for the fluorescence assay. Fluorescence was measured with a Hitachi spectrofluorometer (Hitachi, Tokyo, Japan) against distilled water at 440 nm after excitation at 370 nm and was corrected for the collagenase blank.

Solubility pattern of tail tendon collagen

The solubility pattern of tail tendon collagen was determined as described by Miller and Rhodes [39].

Neutral salt-soluble collagen

Tail tendon tissue was thoroughly minced, homogenized in 10 volumes of neutral salt solvent (1.0 M NaCl, 50 mM Tris, pH 7.5) containing 20 mM EDTA and 2.0 mM N-ethyl maleimide and stirred for 24 h. The suspension was then centrifuged at 35,000 g for 1 h at 4ºC and the extraction was repeated with the pellet. The supernatants were pooled and an aliquot was used for the assay of hydroxyproline [37].

Acid-soluble collagen

The residue obtained was resuspended in 10 volumes of 0.5 M acetic acid and extracted for 24 h with constant stirring, after which the contents were centrifuged. The pellet was re-extracted with acetic acid, the supernatants were pooled and an aliquot was used for the determination of hydroxyproline.

Pepsin-soluble collagen

The residue obtained after acid extraction was resuspended in 0.5 M acetic acid containing 100 mg pepsin per g wet tissue. Digestion was carried out for 24 h, followed by centrifugation and re-extraction. Aliquots of pooled supernatant were used for hydroxyproline measurement.

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). 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

Figure 1 shows the level of plasma glucose and plasma insulin in normal and experimental groups. The level of plasma glucose was significantly increased whereas the level of plasma insulin was significantly decreased in diabetic rats. Oral administration of THC and pterostilbene to diabetic animals significantly reversed all these changes significantly.

The levels of hydroxylproline and total collagen content in the tail tendon of control and diabetic rats are given in table 1. The levels of hydroxylproline and total collagen content are elevated in the tail tendon of diabetic control as compared with normal control rats. Treatment with THC and pterostilbene has reversed the levels of hydroxylproline and total collagen content to near normal. Table 1 represents the levels of extent of glycation and fluorescence of collagen in the tail tendon of control and diabetic rats. Increased levels of extent of glycation and fluorescence of collagen are observed in the tail tendon of diabetic rats when compared with normal control rats. Treatment with THC and pterostilbene has brought back the extent of glycation and fluorescence of collagen.

The levels of acid, pepsin and neutral soluble collagens of tail tendon of control and diabetic rats are represented in table 2. Diabetic rats have decreased levels of acid, pepsin and neutral soluble collagen in the tail tendon as compared with normal control rats, and treatment with THC and pterostilbene has reversed the levels of acid, pepsin and neutral soluble collagens.

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 □ components in diabetic collagen, while THC and pterostilbene supplemented diabetic group showed lesser bandwidth.

The □□□ ratio of both acid and pepsin soluble collagen in tail tendon (Table 2) of control and experimental animals are presented. The □□□ratio is significantly decreased in diabetic rats as compared to control rats. THC and Pterostilbene administration to diabetic rats significantly increased the □□□ ratio.

DISCUSSION

Non enzymic glycation and subsequent AGE formation inevitably occur during chronic diabetes and have been suggested as a primary cause of diabetic late complications. In the present investigation, treatment with THC and pterostilbene showed significant antihyperglycaemic activity [40]. The antihyperglycaemic activity of pterostilbene is due to release of insulin from the

existing □-cells of pancreas. In our study, the levels of hydroxylproline and total collagen are elevated in the tail tendons of diabetic rats, which could be due to increased glucose and non enzymic glycation. In addition, prolyl hydroxylase, an ascorbic acid dependent enzyme, is required to maintain the normal properties of collagen. The activity of prolyl hydroxylase has been reported to alter in diabetic rats. This alteration is mainly due to the reduction in the concentration of plasma and tissue ascorbic acid in diabetes [41]. In our study, we have also observed a significant reduction in the concentration of ascorbic acid in plasma and tissue of diabetic rats. The decrease in ascorbic acid concentration and thereby altered prolyl hydroxylase could be responsible for the alterations of collagen observed in diabetic rats. Significant increase in the concentration of ascorbic acid in THC and pterostilbene treated diabetic rats may be responsible for the activation of prolyl hydroxylase, which inturn maintain the collagen content.

Experimental evidence indicates that collagen in diabetes undergoes extensive chemical modifications that results in decreased solubility, decreased susceptibility to enzymes, increased stability and accelerated cross-linking [42]. These modifications of collagen have received considerable attention, since collagen is an important constituent of most of the tissues that are damaged in diabetes. Collagens are especially exposed to glycation because they contain several lysine, hydroxylysine and arginine residues with free amino groups. Further they have a slow turnover rate and are exposed to ambient level of glucose [43]. AGE crosslinking causes proteins that are normally flexible to become rigid. The cells, tissues and blood vessels become stiff and increasingly dysfunctional. In healthy individuals, this process occurs slowly as the body ages. In diabetic patients, the rate of AGE accumulation and the extent of protein cross-linking are accelerated due to exposure to highly elevated concentrations of glucose [44].

ROS acts as a fixative that couples both glycation and cross-linking of collagen. Oxygen radicals play an important role in the formation and accumulation of AGE [45]. Thus it is well documented that free radical scavengers and metal chelators can inhibit the formation of AGE and cross-linking of proteins both in vitro and in vivo.

Glycation may be the prerequisite for glucose induced cross-linking and oxidation of glycated collagen was proposed to be a critical factor responsible for collagen cross-linking and diabetic complications. THC and Pterostilbene treatment reduced the extent of glycation and the effect of THC was comparable to Pterostilbene, a known antiglycating agent. Oxidation reactions play a critical role in the glycation of proteins. One of the steps in the glycation of protein is tautomerisation of sugar from enediol form of glucose to dicarbonyl intermediate and oxidants facilitate this conversion. It was proposed that increased oxygen radicals can stimulate glycation of protein associated with hyperglycemia and antioxidants that prevent lipid peroxidation reactions may be able to modulate glycation of proteins in diabetes [46]. Thus it is well documented that antioxidants reduce in vitro and in vivo protein glycation [47]. The decrease in the extent of glycation in pterostilbene treated tail tendon collagen may be ascribed to the antioxidant effect of pterostilbene.

Several reports indicate the increase in collagen-linked fluorescence during exposure to high glucose levels in vitro and in vivo [44]. In the present study, the increase in fluorescence in

collagen incubated with glucose is an indication of increased advanced glycation and it is also quantified as a measure of increased AGE. AGE modifies and damage tissues in various ways in addition to forming cross-links. These modification and cross-linking actions of AGE, contribute to numerous complications associated with diabetes. ROS formed during glucose oxidation and glycated protein oxidation involved directly in the formation of AGE [48]. Many antioxidants have some AGE-inhibitory activity primarily by preventing the autoxidative pathways of AGE formation. It is well documented that the chelating activity of AGE inhibitors and AGE breakers contribute to the inhibition of AGE formation and protection against diabetic complication [46]. Pterostilbene reduced the collagen-linked fluorescence indicating its role in reducing AGE. Pterostilbene was shown to antioxidant activity and provide protection from free radical damage. In the present study, THC compare to pterostilbene ameliorate the AGE linked fluorescence, which may be due to its antioxidant and free radical scavenging effect.

The collagen cross-linking in aging and hyperglycemia results in dysfunction of collagenous tissues those are responsible for the morbidity and mortality in age and diabetes, primarily renal, cardiovascular and retinal tissues [17]. Studies indicate that collagen cross-links significantly contribute to cardiovascular stiffening in human and experimental animals [49]. Free radicals generated by the oxidation of free glucose and protein–glucose adducts in the presence of trace amounts of metal ions may contribute significantly to increase in the cross linking of collagen [50]. H2O2 [51] and lipid peroxidation [52] may also play an important role in the cross-linking of collagen. THC and Pterostilbene increase the solubility of tail tendon, which could be due to the decrease in the cross-linking of collagen.

The cross linking of tail tendon collagen was assessed by the solubility of collagen. The percentage of neutral salt, acid and pepsin soluble collagen was decreased in the tail tendons of diabetic rats. As cross-linking proceeds, the solubility of collagen in neutral buffer and acid solution also changes. Highly cross-linked collagen becomes less soluble in the above solutions and can be released only by limited pepsin digestion [53]. Treatment with THC and pterostilbene had increased the solubility of collagen in neutral, acid and pepsin digestion, which could be associated with decreased cross-linking of collagen. This is evidenced by improved glycemic control and decreased extent of glycation.

The SDS-gel pattern of collagen confirms the structural alterations in collagen in diabetic rats. The band size of □-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 □ to □ chains.

□-chains are dimers in which the inter chain crosslinks are not disulfide bridges (6). In the present study, a significant decrease in the ratio of □ to □ components of tail tendon collagen of diabetic rats was also observed [20]. The increased intensity of □-component observed in diabetic rats in our study suggests that collagen chain are capable of enhanced intramolecular crosslinking since the □-component is a dimer of □-chains.

CONCLUSION

Pterostilbene and THC significantly reduced the glycation, AGE and cross-linking of tail tendon. On the basis of these observations, it is clear that the THC and pterostilbene had a positive influence on the content of collagen and its characteristics in STZ and nicotinamide diabetic rats. The THC administration was observed to be more effective than pterostilbene in treating diabetic mellitus.

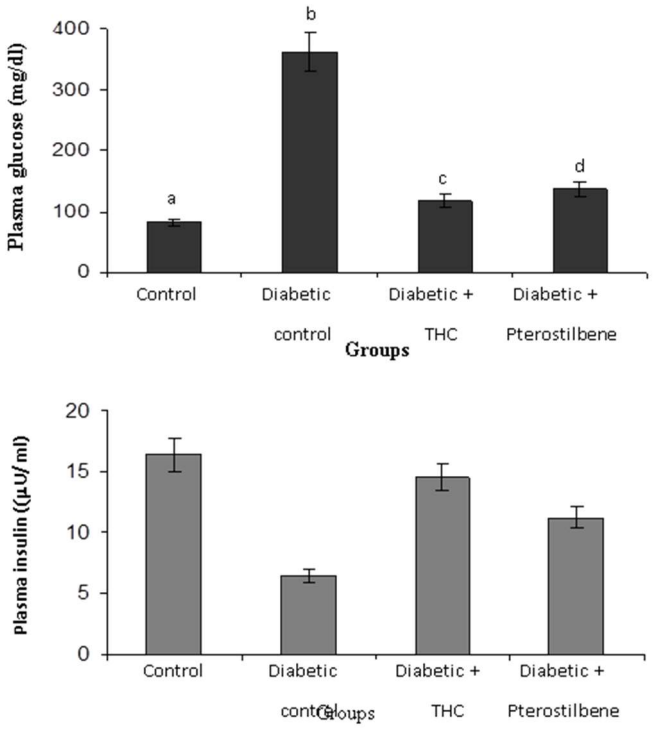

Figure 1. Changes in the levels of glucose and insulin in normal and experimental rats

Values are mean □ SD from 6 rats in each group. Values not sharing a common letter (a-d) differ significantly at $p < 0.05$ (DMRT).

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

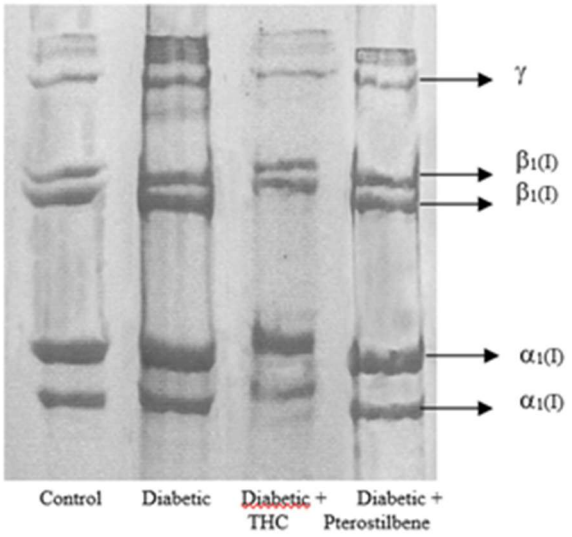

Figure 3. SDS gel pattern of pepsin soluble collagen from tail tendon in control and experimental rats

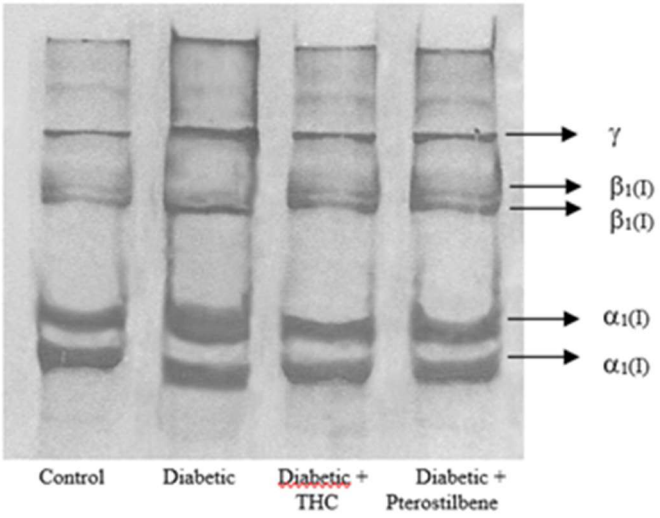

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Table 1. Effect of pterostilbene and THC on hydroxyproline, total collagen, extent of glycation and levels of fluorescence measured in the in tail tendon in normal and experimental rats

Groups	Hydroxyproline (mg/100mg tissue)	Total collgen (mg/100 mg tissue)	Extent of glycation (µg of glucose/ mg collagen)	Fluorescence (AU/µmol hydroxyproline)
Normal	8.84 ± 0.57^{a}	66.42 ± 4.35^{a}	11.69 ± 0.59^{a}	29.19 ± 1.78^{a}
Diabetic control	16.32 ± 0.78^{b}	123.21 ± 6.37^{b}	24.28 ± 1.48^{b}	57.58 ± 3.21^{b}
Diabetic + THC (80 mg/kg)	9.35 ± 0.62^{c}	74.59 ± 5.25^{c}	13.01 ± 0.85^{c}	33.31 ± 2.41^{c}
Diabetic+ Pterostilbene (40 mg/kg)	10.32 ± 0.43^{d}	82.35 ± 4.54^{d}	14.89 ± 1.21^{d}	39.31 ± 2.39^{d}

Values are given as mean ± S.D from six rats in each group. AU- Arbitrary Units, THC- tetrahydrocurcumin.
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

Table 2. Effect of pterostilbene and THC on neutral salt, acid and pepsin soluble collagen content (hydroxyprolone/100 mg tissue) of tail tendon in normal and experimental rats

Groups	Neutral salt soluble collagen (µg/100mg)	Acid soluble collagen (mg/100mg)	Pepsin soluble collagen (mg/100mg)
Normal	144.54 ± 7.58^{a}	3.05 ± 0.19^{a}	2.92 ± 0.19^{a}
Diabetic control	79.54 ± 5.64^{b}	1.81 ± 0.09^{b}	1.44 ± 0.07^{b}
Diabetic + THC (80 mg/kg)	130.71 ± 8.74^{c}	2.44 ± 0.13^{c}	2.63 ± 0.17^{c}
Diabetic + Pterostilbene (40 mg/kg)	115.34 ± 5.62^{d}	2.03 ± 0.13^{d}	2.39 ± 0.13^{d}

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