

Effect of an aqueous extract of *Phaseolus vulgaris* on the properties of tail tendon collagen of rats with streptozotocin-induced diabetes

L. Pari and
S. Venkateswaran

Department of Biochemistry, Faculty of Science,
Annamalai University, Annamalainagar, Tamil Nadu, India

Abstract

Changes in the structural and functional properties of collagen caused by advanced glycation might be of importance for the etiology of late complications in diabetes. The present study was undertaken to investigate the influence of oral administration of aqueous pod extract (200 mg/kg body weight) of *Phaseolus vulgaris*, an indigenous plant used in Ayurvedic Medicine in India, on collagen content and characteristics in the tail tendon of streptozotocin-diabetic rats. In diabetic rats, collagen content (117.01 ± 6.84 mg/100 mg tissue) as well as its degree of cross-linking was increased, as shown by increased extent of glycation (21.70 ± 0.90 μ g glucose/mg collagen), collagen-linked fluorescence (52.8 ± 3.0 AU/ μ mol hydroxyproline), shrinkage temperature ($71.50 \pm 2.50^\circ\text{C}$) and decreased acid (1.878 ± 0.062 mg hydroxyproline/100 mg tissue) and pepsin solubility (1.77 ± 0.080 mg hydroxyproline/100 mg tissue). The α/β ratio of acid- (1.69) and pepsin-soluble (2.00) collagen was significantly decreased in streptozotocin-diabetic rats. Administration of *P. vulgaris* for 45 days to streptozotocin-diabetic rats significantly reduced the accumulation and cross-linking of collagen. The effect of *P. vulgaris* was compared with that of glibenclamide, a reference drug administered to streptozotocin-diabetic rats at the dose of 600 μ g/kg body weight for 45 days by gavage. The effects of *P. vulgaris* (collagen content, 64.18 ± 1.97 ; extent of glycation, 12.00 ± 0.53 ; collagen-linked fluorescence, 33.6 ± 1.9 ; shrinkage temperature, 57.0 ± 1.0 ; extent of cross-linking - acid-soluble collagen, 2.572 ± 0.080 , and pepsin-soluble collagen, 2.28 ± 0.112) were comparable with those of glibenclamide (collagen content, 71.5 ± 2.04 ; extent of glycation, 13.00 ± 0.60 ; collagen-linked fluorescence, 38.9 ± 2.0 ; shrinkage temperature, 59.0 ± 1.5 ; extent of cross-linking - acid-soluble collagen, 2.463 ± 0.078 , and pepsin-soluble collagen, 2.17 ± 0.104). In conclusion, administration of *P. vulgaris* pods had a positive influence on the content of collagen and its properties in streptozotocin-diabetic rats.

Key words

- Collagen
- Diabetes
- *Phaseolus vulgaris*
- Cross-linking
- Glibenclamide
- Collagen glycation

Correspondence

L. Pari
Department of Biochemistry
Faculty of Science
Annamalai University
Annamalainagar-608 002
Tamil Nadu
India
Fax: +91-4144-23-8145
E-mail: paribala@sanchamnet.in

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Introduction

Collagen is a major component of mammalian connective tissue and is present in all major tissues that require strength and flexibility such as skin, bone and tendon (1). Type I collagen is the principal extracellular matrix protein (2). Collagens are characterized by the presence of one or more triple helix domains. The helical structure consists of polypeptide chains containing Gly-x-y repeats, where x and y typically represent proline or hydroxyproline residues. Collagen molecules, after being secreted by the cells, assemble into characteristic fibers responsible for the functional integrity of tissues such as bone, cartilage, skin and tendon. They provide a structural framework for other tissues, such as blood vessels, aorta and most organs. Cross-links between adjacent molecules are a prerequisite for the collagen fibers to withstand the physical stresses to which they are exposed (3,4).

Nonenzymatic glycation of collagen leading to the formation of advanced glycation end products is increased in patients with diabetes mellitus and in animals with experimental diabetes (5). Changes in the structural and functional properties of proteins caused by advanced glycation might be of importance for the etiology of late complications in diabetes. The process of nonenzymatic glycation is initiated by covalent binding of the reactive aldehyde moiety of glucose to free amino groups of protein (6). Collagenous proteins are especially exposed to glycation because they contain several lysine, hydroxyl lysine and arginine residues with free amino groups, have a very slow turnover rate and are exposed to ambient levels of glucose (7,8). The content of advanced glycation end products in collagen increases with age and diabetes (8).

In recent years the popularity of alternative medicine has increased for various reasons. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and

other indigenous medicine systems are commonly used in India (9). The beneficial actions of these diets on the amelioration of diabetic symptoms are well documented (10,11).

Phaseolus vulgaris L. (Leguminosae), commonly known as kidney bean, is a food item of mass consumption in Asia and Eastern countries. The various parts of the plant have been extensively used in Ayurvedic and Unani practice on the Indian subcontinent for the treatment of diabetes mellitus (12). In 1995, Roman-Ramos et al. (13) showed that the aqueous extract of *P. vulgaris* pods possessed antihyperglycemic activity. *P. vulgaris* has also been reported to contain nearly 50 mg of flavonoids per 100 g (14). Recently we have demonstrated the antioxidant (15) and hypolipidemic (16) effects of *P. vulgaris* pods in diabetic rats.

To our knowledge, the present investigation is the first report on the effects of *P. vulgaris* extracts on the collagen content and its physical and chemical characteristics in the tail tendon of streptozotocin-diabetic rats.

Material and Methods

Experimental animals

Male albino Wistar rats weighing 170-200 g bred in the Central Animal House, Rajah Muthiah Medical College, were used. The animals received a normal laboratory pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The study was approved by the Ethics Committee of Annamalai University.

Drugs and chemicals

All drugs and biochemicals used in this study were purchased from Sigma (St. Louis,

MO, USA). The chemicals were of analytical grade or equivalent.

Plant material

P. vulgaris was purchased on the local market in Chidambaram, Cuddalore District, Tamil Nadu, India. The plant was identified in the herbarium of the Botany Directorate of Annamalai University. A voucher specimen (No. 2387) was deposited in the Botany Department of Annamalai University.

Preparation of plant extract

One hundred and thirty-two grams of dried *P. vulgaris* pods was extracted with 1.0 liter of water for 2 h at 60-70°C by continuous hot extraction and evaporated to dryness in a rotary evaporator at 40-50°C under reduced pressure. A semisolid material was obtained (20 g) and stored at 0-4°C until the time for use. When needed, the residual extract was suspended in distilled water and used in the study (13).

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M citrate buffer, pH 4.5, was injected intraperitoneally in a volume of 1 ml/kg (17). Forty-eight hours after streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose of 200-300 mg/dl) were used for the experiment.

Experimental procedure

A total of 40 rats (30 surviving diabetic rats and 10 normal rats) were used. The rats were divided into 4 groups of 10 rats each. Group 1: normal untreated rats; group 2: diabetic control rats; group 3: diabetic rats receiving the *P. vulgaris* pod extract (200 mg/kg body weight) in 1 ml aqueous solution daily by gavage for 45 days (13); group

4: diabetic rats receiving glibenclamide (600 µg/kg body weight) in aqueous solution daily by gavage for 45 days (18).

After 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected with potassium oxalate and sodium fluoride as anticoagulants for the determination of blood glucose. Plasma was separated by centrifugation for insulin assay. The tail was removed and stored frozen at -80°C and tail tendon tissue was prepared as described below.

Blood glucose and plasma insulin

Fasting blood glucose was measured by the O-toluidine method (19) and plasma insulin was assayed with an ELISA kit (Boehringer-Mannheim, Mannheim, Germany).

Preparation and purification of type I collagen

Collagen was purified by the method described by Chandrakasan et al. (20). Briefly, tail tendons were dissected free, washed extensively in phosphate-buffered saline (PBS) and extracted. The entire procedure was carried out at 0-5°C. 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 110°C. The collagen content was determined by measuring hydroxyproline, as described by Woessner (21).

Extent of glycation

The extent of glycation was determined by the method described by Rao and Pattabiraman (22) in which 1.0 ml of purified collagen (containing 1.0 mg collagen) was mixed with 3.0 ml of concentrated H₂SO₄, 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. (23). 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 CaCl₂ (buffer H) and stored overnight at 4°C 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 37°C. 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 (24).

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 4°C and the extraction was repeated with the pellet. The supernatants were pooled and an aliquot was used for the assay of hydroxyproline (21).

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.

Shrinkage temperature

The shrinkage temperature of tail tendon collagen was determined as described by Nutting and Borasky (25). Small strips of collagen fibers were cut from the tail tendon and placed on microscope slides mounted on a holder for viewing under the microscope. An electric bulb with heat-producing capacity was placed under the holder. A thermometer was inserted into the hole available in the holder to monitor the temperature. The electric bulb was then switched on and the heating rate was set at 3°C/min. The shrinkage temperature of collagen fibers was monitored through the microscope and the exact shrinkage temperature was recorded during the shrinkage process (26).

SDS-PAGE

Acid- and pepsin-soluble collagen was prepared from tail tendon as described by Miller and Rhodes (24). Collagen samples were investigated by SDS-PAGE using a 3% stacking gel with a 5% running gel and Coomassie brilliant blue staining. The gels were scanned with a densitometer and the α/β ratio of acid- and pepsin-soluble collagen was calculated.

Statistical analysis

The data for the various biochemical parameters were analyzed by analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (27). Values were considered statistically significant when $P < 0.05$.

Results

Blood glucose and plasma insulin

Blood glucose and plasma insulin levels of normal and experimental rats are given in Table 1. The diabetic rats showed a significant increase in blood glucose and a significant decrease in plasma insulin levels. The administration of *Phaseolus* pod extract and glibenclamide to diabetic rats caused a significant decrease in blood glucose levels and a significant increase in plasma insulin. However, neither the plant extract nor glibenclamide normalized glucose or insulin levels completely.

The dose of *P. vulgaris* (200 mg/kg body weight) was selected based on our previous studies in which a dose-dependent effect of *P. vulgaris* on blood glucose was obtained. *P. vulgaris* was studied with three different doses - 50, 100 and 200 mg/kg body weight. Among the three different doses, *P. vulgaris* showed a highly significant effect at 200 mg/kg body weight (15,16). So we have chosen

Table 1. Effect of *Phaseolus vulgaris* pod extract (PPEt) on blood glucose and plasma insulin levels in normal and diabetic rats.

Group	Blood glucose (mg/dl)	Plasma insulin (μ U/ml)
Normal	79.12 \pm 5.42 ^a	14.41 \pm 0.70 ^a
Diabetic control	278.40 \pm 22.00 ^b	4.13 \pm 0.26 ^b
Diabetic + PPEt	91.20 \pm 4.65 ^c	7.78 \pm 0.40 ^c
Diabetic + glibenclamide	98.20 \pm 6.50 ^d	7.50 \pm 0.34 ^c

PPEt (200 mg/kg) and glibenclamide (600 μ g/kg) were administered by gavage daily for 45 days. Data are reported as means \pm SD for 6 rats in each group. Values not sharing a common superscript letter differed significantly at $P < 0.05$ (Duncan multiple range test).

the dose for our present study. The effect of the dose was compared with glibenclamide at a dose of 600 µg/kg body weight, which is a well-accepted standardized dose in experimental animals. Glibenclamide is also known to cause severe side effects at doses higher than 600 µg/kg (18).

Hydroxyproline, total collagen, extent of glycation, collagen-linked fluorescence and shrinkage temperature

The data in Table 2 show that the levels of hydroxyproline and total collagen and the extent of glycation and collagen-linked fluorescence were significantly increased in diabetic animals. The shrinkage temperature was also increased in the diabetic group

compared to the normal group. Administration of both *Phaseolus* pod extract and glibenclamide significantly reduced collagen levels, extent of glycation and collagen-linked fluorescence in diabetic rats. The increased shrinkage temperature was also significantly prevented in diabetic rats treated with *Phaseolus* pod extract and glibenclamide. The effect of the extract was almost similar to that of glibenclamide.

Pattern of collagen solubility

Data concerning the solubility pattern of tail tendon collagen of normal rats and of the experimental groups after extraction with neutral salt and acid solution and by pepsin digestion are presented in Table 3. The per-

Table 2. Effect of *Phaseolus vulgaris* pod extract (PPEt) on hydroxyproline, total collagen, extent of glycation, fluorescence and shrinkage temperature of rat tail collagen from normal and diabetic rats.

Group	Hydroxyproline (mg/100 mg tissue)	Total collagen (mg/100 mg tissue)	Extent of glycation (µg glucose/mg collagen)	Fluorescence (AU/µmol hydroxyproline)	Shrinkage temperature (°C)
Normal	8.87 ± 0.233 ^a	66.13 ± 1.14 ^a	10.30 ± 0.42 ^a	27.7 ± 1.3 ^a	60.5 ± 1.0 ^a
Diabetic control	15.69 ± 0.917 ^b	117.01 ± 6.84 ^b	21.70 ± 0.90 ^b	52.8 ± 3.0 ^b	71.5 ± 2.5 ^b
Diabetic + PPEt	9.68 ± 0.265 ^c	64.18 ± 1.97 ^c	12.00 ± 0.53 ^c	33.6 ± 1.9 ^c	57.0 ± 1.0 ^c
Diabetic + glibenclamide	9.59 ± 0.254 ^d	71.5 ± 2.04 ^d	13.00 ± 0.60 ^d	38.9 ± 2.0 ^d	59.0 ± 1.5 ^d

For drug administration see Table 1 legend. AU = arbitrary units. Data are reported as means ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at P < 0.05 (Duncan multiple range test).

Table 3. Effect of *Phaseolus vulgaris* pod extract (PPEt) on neutral salt-, acid- and pepsin-soluble collagen content (hydroxyproline/100 mg tissue) of tail tendon in normal and diabetic rats.

Group	Neutral salt-soluble collagen (µg/100 mg)	Acid-soluble collagen (mg/100 mg)	Pepsin-soluble collagen (mg/100 mg)
Normal	137.56 ± 9.2 ^a	2.895 ± 0.091 ^a	2.96 ± 0.100 ^a
Diabetic control	71.99 ± 4.2 ^b	1.878 ± 0.062 ^b	1.77 ± 0.080 ^b
Diabetic + PPEt	113.60 ± 7.8 ^c	2.572 ± 0.080 ^c	2.28 ± 0.112 ^c
Diabetic + glibenclamide	101.40 ± 7.4 ^d	2.463 ± 0.078 ^d	2.17 ± 0.104 ^d

For drug administration see Table 1 legend. Data are reported as means ± SD for 6 rats in each group. Values not sharing a common superscript letter differed significantly at P < 0.05 (Duncan multiple range test).

centage of neutral salt-, acid- and pepsin-soluble collagen was significantly decreased in diabetic animals. Administration of the *Phaseolus* pod extract and glibenclamide significantly increased collagen solubility in diabetic rats.

SDS-PAGE

The gel patterns obtained by SDS-PAGE of acid- and pepsin-soluble collagen from the tail tendon of normal and experimental rats are shown in Figure 1. The α/β ratio of both acid- and pepsin-soluble collagen was decreased significantly in diabetic rats (Table 4). Administration of *Phaseolus* pod extract and glibenclamide significantly increased the α/β ratio to near normal values.

Discussion

We determined the influence of an ex-

tract of *P. vulgaris* on collagen content and characteristics in diabetic rats.

The capacity of the *Phaseolus* pod extract to decrease the elevated blood sugar to normal levels is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which the plant extract exerts its hypoglycemic action in diabetic rats may be by potentiating the plasma insulin effect by increasing either the pancreatic secretion of insulin from the existing β -cells or its release from the bound form, as demonstrated by the significant increase in insulin levels induced by the plant extract in diabetic rats (Table 1).

Streptozotocin-induced diabetes mellitus characterized by hyperglycemia caused a significant increase in hydroxyproline levels and collagen content. The correlation between collagen and intracellular degradation is of interest and may have a role in the

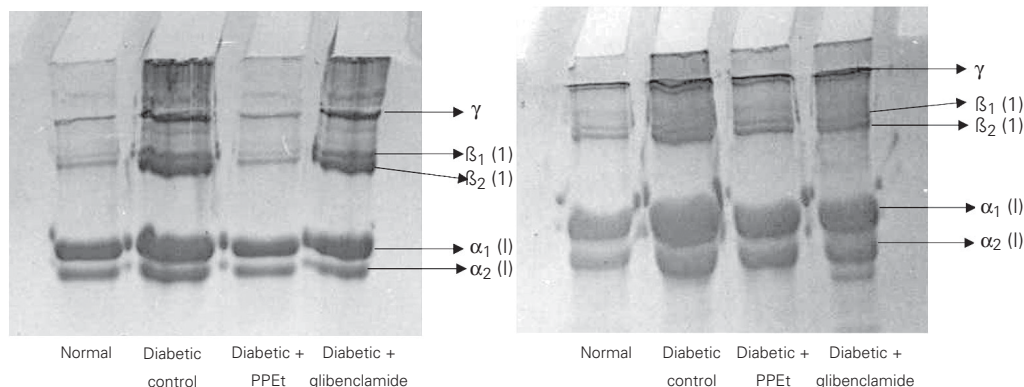


Figure 1. Effect of *Phaseolus vulgaris* pod extract (PPEt) on SDS-PAGE pattern of acid-soluble (left) and pepsin-soluble (right) collagen of tail tendon in normal and diabetic rats.

Table 4. Effect of *Phaseolus vulgaris* pod extract (PPEt) on the α/β ratio of acid- and pepsin-soluble collagen in tail tendon of normal and diabetic rats.

Group	Acid-soluble collagen component			Pepsin-soluble collagen component		
	α	β	α/β ratio	α	β	α/β ratio
Normal	69.5	30.3	2.27 ^a	73.1	26.9	2.71 ^a
Diabetic control	62.9	37.1	1.69 ^b	66.7	33.3	2.00 ^b
Diabetic + PPEt	66.8	33.2	2.00 ^c	69.4	30.6	2.26 ^c
Diabetic + glibenclamide	65.5	34.5	1.89 ^d	66.5	33.5	1.98 ^d

For drug administration see Table 1 legend. Data are reported as means \pm SD for 6 rats in each group. Values not sharing a common superscript letter differed significantly at $P < 0.05$ (Duncan multiple range test).

regulation of collagen content. Golub et al. (28) have suggested that increased degradation of newly synthesized collagen during streptozotocin-induced diabetes might contribute to collagen deposition in the early stages. Diabetic rats treated with the plant extract and glibenclamide showed a significant decrease in total collagen content when compared to untreated diabetic rats. This decrease may be attributed to the significant decrease in blood glucose and consequent decrease in nonenzymatic glycation and deposition of collagen in diabetic rats treated with the *Phaseolus* pod extract and glibenclamide. The extract exhibited effects similar to those of glibenclamide.

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 change in diabetic rats (29). This alteration is mainly due to the reduction in the concentration of ascorbic acid in diabetes (29). In a previous study, we also observed a significant reduction in the concentration of ascorbic acid in streptozotocin-diabetic rats (16). We also observed a significant increase in the concentration of ascorbic acid in diabetic rats treated with the plant extract and glibenclamide (16). The decrease in the ascorbic acid concentration and consequently altered prolyl hydroxylase could also be responsible for the alteration of collagen observed in streptozotocin-diabetic rats. A significant increase in the concentration of ascorbic acid in diabetic rats treated with the plant extract and glibenclamide (16) may also be responsible for the significant reduction in collagen content.

In the present study, an increase in the extent of glycation was observed in the tail tendon of diabetic rats, probably due to exposure of the tissues to glucose in the diabetic state. Earlier studies have also reported that glucose is directly involved in the accelerated cross-linking of collagen in the diabetic state. Several studies have also estab-

lished that collagen glycation is increased during exposure to high glucose levels *in vitro* and *in vivo* (30). Flavonoids were reported to have antiglycating activity (31). The decrease in the extent of glycation in diabetic rats treated with the plant extract could be due to the antiglycating property of the flavonoids present in the extract.

The cross-linking of tail tendon collagen was assessed by measuring the shrinkage temperature. The shrinkage temperature of collagen fibers is related to the number of covalent cross-links present in collagen and to the content of imino acids such as proline and hydroxyproline (32). The shrinkage temperature of collagen was reported to increase with age and in diabetes and was explained in terms of intermolecular cross-links (32). Therefore, the measurement of collagen shrinkage temperature may be used to determine the gross tissue changes at the molecular level.

We observed a significant increase in the collagen shrinkage temperature in diabetic rats, clearly indicating the increase in the cross-links of tail tendon collagen in these animals. Administration of the plant extract and glibenclamide induced a significant reduction in the cross-linking of tail tendon collagen.

The percentage of neutral salt-, acid- and pepsin-soluble collagen was decreased in the tail tendon 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 (33). It has been proposed that free radicals and reactive carbonyls generated during diabetes may contribute significantly to the increased cross-linking of collagen (34,35). From the solubility patterns obtained for collagen of tail tendon tissues of *Phaseolus* pod extract-treated and -untreated diabetic rats, it can be seen that the plant extract treatment resulted in increased solubility in

neutral, acid and pepsin digestion. This is an indication of decreased levels of cross-linking in the collagen of the treated groups. The reduction in the advanced glycation and cross-linking of collagen in diabetic rats treated with the plant extract may be due to its antiperoxidative activity (15), since lipid peroxidation products have been shown to directly influence collagen cross-linking and advanced glycation end product formation (36,37). In addition, advanced glycation end products were also reported to induce the upregulation of the expression of type I collagen genes that could result in excess deposition of collagen in diabetes (38). The increase in advanced glycation end product levels in diabetic rats observed in the present study could be responsible for the upregulation of collagen gene expression which results in the increased deposition of collagen and consequent increased cross-linking in streptozotocin-diabetic rats. The *Phaseolus* pod extract is reported to be rich in flavonoids. These flavonoids may contribute by their protective action to the reduction of collagen cross-linking in treated diabetic rats.

Collagen obtained from the tail tendon of diabetic animals showed increased fluorescence, which is a strong indication of increased advanced glycation. Previous studies have also documented an overall increase in the fluorescence of diabetic tissue collagen (39,40). The 370/440 nm fluorescence is usually due to Maillard reaction-related fluorescence (6).

It has been shown that, in addition to glucose, free radicals and lipid peroxides also play an important role in the development of collagen-linked fluorescence (41). It

appears that the reactive radicals formed during glycation and oxidation reactions can also have an influence on the development of fluorescence.

Administration of the *Phaseolus* pod extract and glibenclamide significantly reduced the intensity of fluorescence in diabetic rats. This may be due to the significant reduction in blood glucose and consequent decreased glycation, and to the significant scavenging of free radicals generated during diabetes by flavonoids present in *Phaseolus* pod extract.

The increased band size of β -components in diabetic collagen clearly indicates the increased cross-linking. Golub et al. (28) have also reported that the acid-soluble collagen from streptozotocin-diabetic rats contains higher than normal amounts of β -component and hence exhibits increased cross-linking. The increased intensity of the β -component observed here in diabetic rats suggests that collagen chains are capable of enhanced intramolecular cross-linking since the β -component is a dimer of α -chains. In diabetic rats treated with the *Phaseolus* pod extract, collagen content in the β -region as well as in the high molecular weight region was near normal when compared to diabetic control groups.

On the basis of these observations, it is clear that the *P. vulgaris* pod extract had a positive influence on the content of collagen and its characteristics in streptozotocin-diabetic rats. Further work is currently underway to analyze the components of the aqueous extract of *P. vulgaris* for their beneficial effects on collagen content in streptozotocin-diabetic rats.

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