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Research Article

Effect of Tetrahydrocurcumin and Gallic Acid on Tail Tendon Collagen Contents and its Properties in Rats with Streptozotocin Induced Diabetes

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ABSTRACT

Background: Elevated blood sugar levels in diabetes result in significant glycation of collagen, which forms cross-links that contribute to tissue stiffness, decreased joint flexibility, and vascular issues. Although the buildup of advanced glycation end products harms tissues, research indicates that certain marine collagen peptides or collagen hydrolysates could enhance insulin sensitivity, decrease blood sugar levels, and mitigate oxidative stress. **Materials and Methods:** Alterations in the structural and functional characteristics of collagen due to advanced glycation may hold significance for the etiology of late-stage complications associated with diabetes. One of the main reduction metabolites of curcumin, a well-known yellow food pigment from turmeric with potent antibacterial, anti-inflammatory, and antioxidant qualities, is Tetrahydrocurcumin (THC). Compared to curcumin, THC has comparable pharmacological activities and superior solubility, stability, and bioavailability. **Results:** Due to its potent antioxidant and skin-whitening properties, the market for colorless THC has grown significantly in recent years for use in functional foods and cosmetics. 3,4,5-trihydroxybenzoic acid, another name for Gallic Acid (GA), is a naturally occurring secondary metabolite that is frequently extracted from a variety of fruits, plants, and nuts. GA's potent anti-inflammatory qualities have drawn more attention in recent years. As evidenced by increased glycation, collagen-linked fluorescence, neutral salt collagen, and decreased acid and pepsin solubility, diabetic rats had higher hydroxyproline and collagen content as well as a higher degree of cross-linking. **Conclusion:** Collagen accumulation and cross-linking were considerably decreased in diabetic rats given a combination of THC and GA for 45 days. In conclusion, streptozotocin treated diabetic rats' collagen content and characteristics improved when THC and GA were administered together. The combination effect of THC and GA was better when compared with that of THC or GA alone.

Keywords: Collagen, Collagen, Gallic acid, Streptozotocin, Tetrahydrocurcumin.

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INTRODUCTION

Collagen serves as an essential structural protein within the Extracellular Matrix (ECM) of almost all tissues impacted by diabetes, such as the skin, blood vessels, kidneys, tendons, and nerves. In the context of diabetes, hyperglycemia leads to nonenzymatic glycation, a process in which glucose attaches to collagen, resulting in the formation of Advanced Glycation End Products (AGEs). These alterations lead to significant cross-linking, which increases the stiffness of collagen, reduces its solubility, and enhances its resistance to degradation, thereby playing a direct role in the development of diabetic complications (Pari and Murugan, 2007a). In cases of experimental diabetes, the content of collagen typically rises or at the very least, experiences considerable remodeling accompanied by substantial alterations in its properties, such as heightened glycation, increased cross-linking, and augmented collagen-linked fluorescence. These alterations are frequently linked to a reduction in the solubility of collagen, especially in tissues such as the aorta, tail tendon, and skin, because of the buildup of AGEs (Fujimori, 1989).

Increased glycation and collagen-linked fluorescence in the tail tendons of diabetic rats are driven by long-term exposure to high glucose levels, resulting in excessive AGEs accumulation. This hyperglycemia leads to increased cross-linking, reducing structural flexibility and contributing to collagen degradation (Murugan, 2010). Collagen is the most abundant protein in mammals, constituting approximately 25-30% of total body protein and serving as a fundamental structural component of connective tissue (Murugan and Maneemegalai, 2024).

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The interaction between the aldehyde group found in reducing sugars and the amino group present in proteins, known as nonenzymatic glycosylation, is a significant contributor to the complications associated with diabetes and the aging process (Monnier *et al*., 1986). Collagen serves as a vital element of the ECM, significantly contributing to tissue repair and wound healing by offering structural support, modulating inflammation, and promoting tissue regeneration. In the proliferative and maturation stages of wound healing, fibroblasts synthesize collagen and deposit it at the wound site to close the injury and restore the functionality of the tissue (Reddy and Enwemeka, 1996). The nonenzymatic cross-linking of collagen by AGEs represents a significant mechanism that contributes to arterial stiffness and complications associated with diabetes. Hyperglycemia facilitates the nonenzymatic Maillard reaction, leading to the formation of AGEs (such as pentosidine and glucosepane) on collagen, which in turn heightens tissue stiffness, diminishes enzymatic degradation, and results in vascular and myocardial dysfunction (Bucala and Cerami, 1992).

The management of diabetes without experiencing any side effects remains a significant challenge for the medical system. Patients are increasingly seeking natural products that exhibit antidiabetic properties, as insulin and oral hypoglycemic medications often come with undesirable side effects (Pari and Murugan, 2005). Plants that demonstrate antidiabetic effects serve as valuable resources for developing medications aimed at treating diabetes mellitus. Phytochemicals extracted from plant sources are utilized in the prevention and treatment of various conditions, including cancer, heart disease, diabetes, and hypertension, among others (Murugan and Pari, 2007a).

Tetrahydrocurcumin (THC) is a colorless, bioactive metabolite of curcumin that is obtained from turmeric. It provides enhanced stability, solubility in water, and bioavailability. Renowned for its strong antioxidant, anti-inflammatory, and skin-brightening effects, it is widely utilized in skincare products and functional foods (Pari and Murugan, 2007b). THC demonstrates superior effectiveness compared to curcumin in the management of diabetes, the reduction of lipid peroxidation, and the protection against photoaging induced by UVB exposure (Murugan and Pari, 2006). It is commonly utilized in skincare for the purposes of skin lightening and antiaging, attributed to its ability to inhibit tyrosinase. Additionally, it serves as a dietary supplement aimed at managing cardiovascular, metabolic, and neurodegenerative disorders (Murugan and Pari, 2007b).

Gallic Acid (GA) is a naturally occurring phenolic acid present in gallnuts, sumac, witch hazel, tea leaves, and oak bark. It serves as a powerful antioxidant and anti-inflammatory substance, with uses in pharmaceuticals, cosmetics, and ink manufacturing. Recognized for its health advantages, it is frequently located in nuts and berries (Samad, 2018). GA is a naturally occurring phenolic acid known for its strong antioxidant, anti-inflammatory, and antimicrobial characteristics. It finds extensive application in the pharmaceutical industry for the potential treatment of metabolic and cardiovascular disorders, serves as a natural preservative in food products, is utilized in skincare formulations to mitigate aging effects, and is also employed in industrial sectors for the production of inks and dyes (Roberts *et al*., 2007). GA demonstrates considerable promise in the management of diabetes and its associated complications by lowering hyperglycemia, improving insulin sensitivity, and addressing oxidative stress. Research indicates that it enhances glucose metabolism, safeguards pancreatic beta-cells and alleviates diabetic complications such as nephropathy and cardiovascular disease (Gajendhini and Murugan, 2025a). In our previous study, we have demonstrated the antidiabetic combination effect of THC and GA in STZ induced diabetic rats (Gajendhini and Murugan, 2025b; S and P, 2026). To the best of our knowledge, this study represents the initial report regarding the combined effects of THC and GA on the collagen content, as well as its physical and chemical properties, in the tail tendon of rats with Streptozotocin (STZ)-induced diabetes.

MATERIALS AND METHODS

Animals

Adult male albino Wistar rats, aged 8 weeks and weighing between 180 and 220 g, were bred in the Central Animal House of JJ College of Arts and Science, Pudukkottai, India, which is affiliated with Bharathidasan University, Tiruchirappalli, India. All animal experiments received approval from the ethical committee (JJC/BC/AH/002/2025) of JJ College of Arts and Science and adhered to the guidelines set forth by the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The rats were housed in polycarbonate cages within a room that maintained a 12-hr day-night cycle, a temperature of 24±2°C, and humidity levels ranging from 45 to 64%. Throughout the experimental duration, the animals were provided with a balanced commercial diet from Hindustan Lever Ltd., Mumbai, India, along with access to water ad lib.

Drugs and chemicals

THC was given as a present by Sabinsa Corporation, which is situated in the United States. GA was obtained from Sigma Chemical Company, headquartered in St. Louis, United States. All other chemicals and biochemicals were of analytic grade.

Induction of diabetes

Experimental animals received a freshly prepared solution of STZ (55 mg/kg) in 0.1m citrate buffer, pH 4.5, through intraperitoneal injection at a volume of 1 mL/kg. Control rats were administered 1 mL of citrate buffer as a vehicle. Blood glucose levels were assessed 72 hr after the STZ administration. The rats that displayed moderate diabetes, indicated by glycosuria and hyperglycemia

(i.e., blood glucose levels between 200 and 300 mg/100 mL), were chosen for the experiment.

Experimental design

In the experiment, a total of 30 rats were utilized, comprising 24 diabetic surviving rats and 6 normal rats. The body weight of the animals was documented, and they were categorized into 5 groups, each consisting of 6 animals, as detailed below.

Group I. Normal rats. Group II: Diabetic control rats (Diabetes was induced through intraperitoneal STZ injection at a dosage of 55 mg/kg, pH 4.5 in a citrate buffer solution). Group III: Diabetic rats treated with a combination of phytochemicals THC (60 mg/kg body weight) and GA (15 mg/kg body weight) in an aqueous solution, administered daily via an intragastric tube for a duration of 7 weeks (S and P, 2026). Group IV: Diabetic rats receiving THC (80 mg/kg body weight) in an aqueous solution daily through an intragastric tube for 7 weeks (Murugan *et al*., 2008). Group V: Diabetic rats administered GA (20 mg/kg body weight) in an aqueous solution daily via an intragastric tube for 7 weeks (Yu *et al*., 2021).

After a period of seventh weeks, the animals were denied food overnight and subsequently sacrificed through decapitation. Blood was gathered in tubes that contained a mixture of potassium oxalate and sodium fluoride for the purpose of estimating blood glucose levels. Plasma was then separated for the assessment of insulin. The tails of both normal and experimental rats were excised and preserved in a frozen state at -80°C until required.

Analytic procedure

Estimation of blood glucose and plasma insulin

Blood glucose levels were measured colorimetrically utilizing commercial diagnostic kits from Sigma Diagnostics (St. Louis, United States) (I) Pvt. Ltd., Baroda, India (Lott and Turner, 1975). Plasma insulin was evaluated through an enzyme-linked immunosorbent assay employing a Boehringer Mannheim kit in conjunction with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of collagen content

The total collagen content in the tail tendon was assessed by hydrolyzing the weighed tissues with 5 mL of 6 N HCl at 110°C for 20 hr in sealed tubes. The hydrolyzed samples were then evaporated to dryness using a boiling water bath. The resulting residue was dissolved in water and adjusted to a known volume. Subsequently, decolorization was performed using activated charcoal, followed by filtration through Whatmann No.1 filter paper. The filtrate obtained was analyzed for hydroxyproline. The hydroxyproline content in the collagen samples was determined using the method established by Woessner (1961).

Extent of glycation

The extent of glycation was determined by the method described by Rao and Pattabiraman (1989). In this method, 1.0 mL of purified collagen (which contains 1.0 mg of collagen) was combined with 3.0 mL of concentrated H_2SO_4, vortexed, cooled on ice, then mixed with 0.5 mL of 80% phenol, and allowed to stand at room temperature for 30 min. The absorbance was recorded at 485 nm, using glucose as the standard.

Collagen-linked fluorescence

Collagen-linked fluorescence was measured by the method of (Murugan and Pari, 2007c). Approximately 3.0 mg of tissue was finely chopped in phosphate-buffered saline and subjected to centrifugation at 3,330 g for 10 min. The resulting pellet was rinsed with distilled water, and the lipids were extracted using 5.0 mL of chloroform: methanol (2:1, vol/vol) overnight. The samples were rehydrated by adding 2.0 mL of methanol and 0.5 mL of distilled water, followed by centrifugation at 3,330 g for 10 min. The pellet was then washed twice with methanol, 3 times with distilled water, and twice with 20 mm N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), pH 7.5, containing 0.12m $CaCl_2$ (referred to as Buffer H), and stored overnight at 4°C in Buffer H. Subsequently, the buffer was removed through centrifugation at 3,330 g for 10 min, and the pellet was resuspended in 3.5 mL of Buffer H containing 120 units of Type VII collagenase. To inhibit bacterial growth, 4 drops of toluene were added, and the material was digested for 48 hr at 37°C. A blank sample containing collagenase in Buffer H was also included. The digest underwent centrifugation at 3,330 g for 30 min, and the clear supernatant, which contained the digested collagen, was utilized for the fluorescence assay. Fluorescence measurements were conducted using a Hitachi (Tokyo, Japan) spectrofluorimeter (Hitachi) against distilled water at 440 nm after excitation at 370 nm, with corrections made 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 (1982).

Neutral salt-soluble collagen

The tail tendon tissue was finely minced and then homogenized in a neutral salt solvent consisting of 10 volumes (1.0m NaCl, 50 mm Tris, pH 7.5) that included 20 mm EDTA and 2.0 mm N-ethyl maleimide, followed by stirring for 24 hr. Subsequently, the suspension underwent centrifugation at 35,000 g for 1 hr at a temperature of 4°C, and the extraction process was repeated with the resulting pellet. The supernatants were combined, and an aliquot was taken for the hydroxyproline assay (Woessner, 1961).

Acid-soluble collagen

The residue that was obtained was resuspended in 10 volumes of 0.5m acetic acid and extracted for a duration of 24 hr with continuous stirring. Following this, the mixture was subjected to centrifugation. The pellet was then re-extracted using acetic acid, the supernatants were combined, and a portion was utilized for the measurement of hydroxyproline.

Pepsin-soluble collagen

The residue acquired following acid extraction was resuspended in 0.5m acetic acid with 100 mg of pepsin per gram of wet tissue. Digestion was performed for 24 hr, after which centrifugation and re-extraction were conducted. Aliquots of the combined supernatant were utilized for the measurement of hydroxyproline.

Statistical analysis

The analysis of variance was employed to evaluate the data for different biochemical parameters, and Duncan multiple-range test was utilized to compare the group means. A *p*-value of less than 0.05 was deemed statistically significant (Duncan, 1957).

RESULTS

Blood glucose and plasma insulin

Table 1 presents the blood glucose levels and plasma insulin concentrations across different experimental groups. The diabetic control rats showed a significant increase in blood glucose and leptin, along with a marked decrease in plasma insulin and QUICKI levels. The oral administration of a combination of THC and GA to diabetic rats effectively reversed the aforementioned biochemical changes. In normal rats, the administration of THC and GA had a considerable effect on blood glucose, leptin, HOMA, QUICKI, and plasma insulin levels. The combination therapy of THC and GA proved to be more effective than either THC or GA administered alone.

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

Table 2 illustrates the concentrations of hydroxyproline, total collagen, the degree of glycation, and the intensity of collagen-associated fluorescence in the tail tendons of both normal and experimental rats. During the course of diabetes, there was a notable increase in the levels of hydroxyproline and total collagen. The administration of a combination of THC and GA to diabetic rats resulted in a significant reduction in the levels of hydroxyproline and collagen when compared to the diabetic control group. Furthermore, elevated levels of glycation and collagen fluorescence were noted in the tail tendons of diabetic rats in contrast to the normal control rats. The combination treatment of THC and GA also led to a significant decrease in fluorescence intensity among diabetic rats. This combination of THC and GA demonstrates greater efficacy than treatments involving THC or GA alone.

Pattern of collagen solubility

The levels of acid, pepsin and neutral soluble collagens of tail tendon of control and diabetic rats are represented in Table 3. Compared to normal control rats, diabetic rats exhibit reduced levels of acid, pepsin, and neutral soluble collagen in their tail tendons. Furthermore, the combined treatment of THC and GA has restored the levels of these collagens. The administration of THC in conjunction with GA proved to be more effective than either THC or GA alone.

DISCUSSION

Collagen is the predominant structural protein found in the body, constituting approximately 30% of the total protein content, mainly located within the ECM. It plays a crucial role in providing tensile strength, maintaining structural integrity, and ensuring rigidity in connective tissues, including skin, tendons, ligaments, and bone (Brown and Timpl, 1995). Oxidation reactions act as a critical mechanism in the nonenzymatic glycation of collagen, leading to the formation of cross-links and AGEs (Fu *et al*., 1992).

The cross-linking of collagen in the presence of air is influenced by the concentration of glucose; however, it is suppressed under antioxidative conditions, such as a nitrogen atmosphere combined with transition metal chelators. Glycation could be a necessary condition for glucose-induced cross-linking, although it may not dictate the degree of cross-linking (Sajithlal *et al*., 1999). The oxidation of glycated collagen, or glyoxidation, might be the key element that leads to collagen cross-linking and the associated complications observed in diabetes mellitus (Turk, 1997). In the present study, diabetes mellitus induced by STZ, which is marked by hyperglycemia, resulted in a notable increase in hydroxyproline levels and collagen content within the tail tendon. The relationship between collagen concentration and intracellular degradation is noteworthy, as it may play a role in the regulation of collagen levels.

Golub, Greenwald, Zebrowski, and Ramamurthy (1978) have noted that experimental diabetes results in heightened degradation of newly formed collagen in tissues such as gingiva, skin, and tendons. This phenomenon is driven by increased activity of collagenolytic enzymes, which can result in swift degradation of collagen, thereby contributing to a remodeling process and, paradoxically, a dysfunctional buildup of cross-linked collagen during the initial phases of diabetic connective-tissue complications. The combination therapy involving THC and GA in diabetic rats led to a notable reduction in total collagen levels compared to untreated diabetic rats. This reduction can be linked to the significant decline in blood glucose levels, which in turn reduced nonenzymatic glycation and collagen deposition in diabetic rats receiving THC and GA. The noted rise in the level of

glycation within the tail tendon of diabetic rats can be primarily attributed to the extended, elevated exposure of tissues to glucose (hyperglycemia) characteristic of the diabetic condition (Meng *et al*., 1996). They have additionally indicated that glucose plays a direct role in the expedited cross-linking of collagen in diabetes.

In diabetic rats, tail tendon collagen shows decreased solubility in neutral salt, acid, and pepsin due to increased cross-linking, likely caused by AGEs. Elevated collagen-linked fluorescence and increased shrinkage temperature indicate enhanced collagen maturity and reduced enzymatic digestibility. Highly cross-linked collagen exhibits reduced solubility in the aforementioned solution, which can be indicated by a notable rise in glucose-mediated cross-linking of collagen in the tail tendons of diabetic rats. It is possible that the covalent interaction between glucose and collagen is not the sole contributor to the cross-linking observed in diabetes.

Lysyl oxidase is an amine oxidase that depends on copper and plays a vital role in the stabilization of collagen and elastin by facilitating the oxidative deamination of lysine residues into reactive aldehydes, specifically allysine. This enzyme, which operates outside of cells, begins the process of covalent cross-linking, a critical step for the formation, maturation, and tensile strength of collagen fibrils (Smith-Mungo and Kagan, 1998). The heightened cross-linking of collagen observed in diabetic rats may result from the elevated activity of the enzyme lysyl oxidase. It has been suggested that both free glucose and protein-glucose adduct (Ahmed *et al*., 1986) undergo oxidation when trace amounts of ions are present, leading to the generation of free radicals and reactive carbonyls. These reactive oxygen species and carbonyls could play a significant role in the increased cross-linking of collagen. Elgawish, Glomb, Friedlander, and Monnier (1996) have shown that hydrogen peroxide is directly implicated in the glucose-induced cross-linking of collagen. Additionally, the metal oxidation of protein-glucose adducts results in the formation of fragmentation products such as 3-deoxyglucosone, which further enhances the cross-linking process.

The suppression of collagen cross-linking in antioxidative environments (such as a nitrogen atmosphere and the presence of metal chelators) strongly suggests that oxidative processes are crucial for cross-linking induced by glucose. Although glycation (the initial Maillard reaction) takes place without the need for oxygen, the later formation of fluorescent, irreversible collagen cross-links depends on oxidative mechanisms (Aoki *et al*., 1992). Malondialdehyde is a final product of lipid peroxidation that has the ability to interact with the free amino groups of collagens, thereby promoting cross-linking. Fu *et al*. (1996) noted that the *in vitro* peroxidation of lipids, when proteins are present, leads to the creation of glycoxidation products, specifically carboxymethyl lysine.

Table 1: Effect of Tetrahydrocurcumin (THC) and Gallic Acid (GA) on hormone and glucose index parameters in normal and experimental animals.

Parameters	Normal	Diabetic control	Diabetic + THC and GA	Diabetic + THC	Diabetic + GA
Glucose (mg/100 mL)	91.15±5.62^{a}	289.72±5.75^{b}	95.35±4.65^{a}	118.87±4.35^{c}	122.55±4.65^{c}
Leptin (ng/mL)	0.62±0.03^{a}	1.99±0.03^{b}	0.67±0.04^{a}	0.85±0.03^{c}	0.89±0.04^{c}
Insulin (µU/mL)	12.45±0.70^{a}	3.99±0.27^{b}	11.78±0.50^{a}	9.57±0.49^{c}	9.69±0.44^{c}
HOMA	11.36±1.25^{a}	33.56±2.34^{b}	12.45±1.47^{a}	17.73±1.52^{c}	17.81±1.40^{c}
QUICKI	1.63±0.15^{a}	0.98±0.03^{b}	1.59±0.12^{a}	1.25±0.05^{c}	1.20±0.05^{c}

Values are expressed as Mean±SD for 6 rats. Statistical analysis of one-way ANOVA, *post hoc* followed by DMRT test using SPSS ver. 24. Mean values are within the row followed by different letters, superscript (homogeneous subsets) is mentioned as statistically significant (p<0.05), while same letter was statistically nonsignificant (p>0.05) from each other groups. Abbreviations: GA: Gallic Acid; THC: Tetrahydrocurcumin.

Table 2: Effect of Tetrahydrocurcumin (THC) and Gallic Acid (GA) on hydroxyproline, total collagen, extent of glycation and levels of fluorescence measured in the tail tendon in normal and experimental rats.

Groups	Hydroxyproline (mg/100 mg tissue)	Total collagen (mg/100 mg tissue)	Extent of glycation (µg of glucose/mg collagen)	Fluorescence (AU/µmol hydroxyproline)
Normal	9.15±0.55^{a}	68.76±4.45^{a}	11.68±0.65^{a}	30.21±1.47^{a}
Diabetic control	16.87±0.84^{b}	127.31±6.25^{b}	25.47±1.25^{b}	59.45±3.47^{b}
Diabetic + THC and GA	8.95±0.53^{a}	76.25±4.58^{a}	12.55±0.55^{a}	32.24±1.90^{a}
Diabetic + THC	10.55±0.52^{c}	89.45±5.21^{c}	14.45±0.77^{c}	40.22±2.41^{c}
Diabetic + GA	10.89±0.55^{c}	95.15±4.45^{c}	14.78±1.14^{c}	41.14±2.24^{c}

Values are expressed as Mean±SD for 6 rats. AU- arbitrary units. Statistical analysis of one-way ANOVA, *post hoc* followed by DMRT test using SPSS ver. 24. Mean values are within the row followed by different letters, superscript (homogeneous subsets) are mentioned as statistically significant (p<0.05) while same letter was statistically nonsignificant (p>0.05) from each other groups. Abbreviations: GA: Gallic Acid; THC: Tetrahydrocurcumin.

Table 3: Effect of Tetrahydrocurcumin (THC) and Gallic Acid (GA) 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/100 mg)	Acid soluble collagen (mg/100 mg)	Pepsin soluble collagen (mg/100 mg)
Normal	155.35±8.52a	3.15±0.25a	3.25±0.20a
Diabetic control	75.65±4.47b	1.85±0.14b	1.65±0.08b
Diabetic + THC and GA	162.21±9.42a	2.97±0.21a	3.07±0.19a
Diabetic + THC	135.75±9.31c	2.22±0.13c	2.64±0.14c
Diabetic + GA	125.20±6.55c	2.12±0.12c	2.55±0.13c

Values are expressed as mean±SD for 6 rats. Statistical analysis of one-way ANOVA, *post hoc* followed by DMRT test using SPSS ver. 24. Mean values are within the row followed by different letters, superscript (homogeneous subsets) are mentioned as statistically significant ($p<0.05$) while same letter was statistically nonsignificant ($p>0.05$) from each other groups. Abbreviations: GA: Gallic Acid; THC: Tetrahydrocurcumin.

The solubility patterns observed for collagen from the tail tendons of diabetic rats' combination treated with THC and GA indicate that this treatment led to an increase in solubility in neutral, acidic, and pepsin environments. This suggests a reduction in cross-linking levels within the collagen of the treated groups. The decrease in advanced glycation and collagen cross-linking in diabetic rats treated with the combination of THC and GA may be attributed to their antiperoxidative properties, as lipid peroxidation products have been demonstrated to directly affect collagen cross-linking and the formation of AGEs (Odetti *et al*., 1994).

Diabetic rats demonstrate elevated collagen-linked fluorescence in their tail tendons and various other tissues, indicating a buildup of AGEs. This increased fluorescence, typically observed with 365-370-nm excitation and 416-440-nm emission, correlates with enhanced cross-linking, reduced solubility, and greater resistance to collagenase digestion, which signifies an accelerated process of nonenzymatic browning (Monnier *et al*., 1986). The fluorescence at 370/440 nm (with excitation at 370 nm and emission at 440 nm) is widely acknowledged as a significant marker for advanced Maillard reaction products and Advanced Glycation End Products (AGEs). This specific wavelength pair is employed to observe the later phases of the Maillard reaction-a nonenzymatic browning process involving amino groups and reducing sugars-before or during the development of brown-hued melanoidins (Njoroge and Monnier, 1989).

In addition to cross-linking, the fluorescence associated with collagen is modified by glycation. While the underlying mechanisms remain unclear, a relationship has been documented between the severity of diabetic complications and collagen-linked fluorescence (Sajithlal *et al*., 1999). Likewise, pentosidine demonstrates a strong correlation with the severity of complications in patients with prolonged insulin-dependent diabetes. Nevertheless, the role of fluorescent products in the cross-linking of collagen remains ambiguous, as collagen-linked fluorescence does not reliably correspond to the degree of cross-linking (Brennan, 1989).

Fujimori (1989) illustrated that when collagen is incubated with glucose, particularly in the presence of chemically produced singlet oxygen, the formation of cross-linked products is significantly expedited, leading to a greater accumulation of blue-fluorescent materials. The research indicates that singlet oxygen functions as a catalyst, linking the glycation process (nonenzymatic glucosylation) with enhanced oxidation (glycoxidation) to facilitate collagen cross-linking.

Odetti *et al*. (1994) conducted separate studies on glycation-induced fluorescence and malondialdehyde-induced fluorescence, discovering that the accumulation of malondialdehyde-induced fluorescence occurs at a slower pace compared to glycation-induced fluorescence. In our research, we observed that collagen extracted from the tail tendons of diabetic rats exhibited heightened fluorescence, likely attributable to an increased formation of AGEs. This finding aligns with the earlier report by Odetti *et al*. (1994). Furthermore, combination treatment with THC and GA resulted in a reversal of fluorescence, which may be linked to a reduction in AGEs formation, as indicated by enhanced glycemic control.

CONCLUSION

Based on findings from research investigating the combined effects of THC and GA in STZ diabetic rats, it is concluded that these substances exert a beneficial therapeutic impact on collagen metabolism.

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None.

ABBREVIATIONS

AGEs: Advanced glycation end products; **THC:** Tetrahydrocurcumin; **GA:** Gallic acid; **ECM:** Extracellular matrix; **STZ:** Streptozotocin; **ELISA:** Enzyme-linked immunosorbent assay; **HOMA:** Homeostatic Model Assessment; **QUICKI:** Quantitative Insulin Sensitivity Check Index; **DMRT:** Duncan's multiple range test; **MRPs:** Advanced Maillard reaction products;

AU: Arbitrary Units; **EDTA:** Ethylenediaminetetraacetic acid; **NaCl:** Sodium chloride; **HCl:** Hydrochloric acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Ahmed, M. U., Thorpe, S. R., and Baynes, J. W. (1986). Identification of N epsilon-carboxymethyllysine as a degradation product of fructose lysine in glycated protein. *Journal of Biological Chemistry*, *261*(11), 4889-4894. doi: 10.1016/S0021-9258(19)89188-3.

Aoki, Y., Yanagisawa, Y., Yazaki, K., Oguchi, H., Kiyosawa, K., and Furuta, S. (1992). Protective effect of vitamin E supplementation on increased stability of collagen in diabetic rats. *Diabetologia*, *35*(10), 913-916. doi: 10.1007/BF00401418.

Brennan, M. (1989). Changes in solubility, non enzymatic glycation and fluorescence of collagen in tail tendons from diabetic rats. *Journal of Biological Chemistry*, *264*(35), 20947-20952. doi: 10.1016/S0021-9258(19)30028-6.

Brown, J. C., and Timpl, R. (1995). The collagen superfamily. *International Archives of Allergy and Immunology*, *107*(4), 484-490. doi: 10.1159/000237090.

Bucala, R., and Cerami, A. (1992). Advanced glycosylation: Chemistry, biology, and implications for diabetes and aging. *Advnced Pharmacology*, *23*, 1-34.

Duncan, D. B. (1957). Multiple range tests for correlated and heteroscedastic means. *Biometrics*, *13*(2), 164. doi: 10.2307/2527799.

Elgawish, A., Glomb, M., Friedlander, M., and Monnier, V. M. (1996). Involvement of hydrogen peroxide in collagen cross-linking by high glucose *in vitro* and *in vivo*. *Journal of Biological Chemistry*, *271*(22), 12964-12971. doi: 10.1074/jbc.271.22.12964.

Fu, M. X., Knecht, K. J. W., Thorpe, S. R., and Baynes, J. W. (1992). Role of oxygen in crosslinking and chemical modification of collagen by glucose. *Diabetes*, *41*(Suppl. 2), 42-48. doi: 10.2337/diab.41.2.S42.

Fu, M. X., Requena, J. R., Jenkins, A. J., Lyons, T. J., Baynes, J. W., and Thorpe, S. R. (1996). The advanced glycation end product, N epsilon-(carboxymethyl) lysine, is a product of both lipid peroxidation and glycoxidation reactions. *Journal of Biological Chemistry*, *271*(17), 9982-9986. doi: 10.1074/jbc.271.17.9982.

Fujimori, E. (1989). Cross-linking and fluorescence changes of collagen by glycation and oxidation. *Biochimica et Biophysica Acta*, *998*(2), 105-110. doi: 10.1016/0167-4838(89)90260-4.

Gajendhini, S., and Murugan, P. (2025a). Combined and comparative antioxidant effect of tetrahydrocurcumin and gallic acid in streptozotocin induced diabetic rats. *Current Topics in Peptide and Protein Research*, *26*, 47-59. doi: 10.31300/CTPPR.26.2025.47-59.

Gajendhini, S., and Murugan, P. (2025b). Comparative and combined effect of tetrahydrocurcumin and gallic acid on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin-induced diabetic rats. *Current Topics in Peptide and Protein Research*, *26*, 21-32. doi: 10.31300/CTPPR.26.2025.21-32.

Golub, I. M., Greenwald, R. A., Zebrowski, E. J., and Ramamurthy, K. (1978). The effect of experimental diabetes on the molecular characteristics of soluble rat collagen. *Biochimica Biophysics Acta*, *534*, 73-81.

Kesava Reddy, G., and Enwemeka, C. S. (1996). A simplified method for the analysis of hydroxyproline in the biological tissues. *Clinical Biochemistry*, *29*(3), 225-229. doi: 10.1016/0009-9120(96)00003-6.

Lott, J. A., and Turner, K. (1975). Evaluation of trinder's glucose oxidase method for measuring glucose in serum and urine. *Clinical Chemistry*, *21*(12), 1754-1760. doi: 10.1093/clinchem/21.12.1754.

Meng, J., Sakata, N., Takebayashi, S., Asano, T., Futata, T., Araki, N., and Horiuchi, S. (1996). Advanced glycation end products of the Maillard reaction in aortic pepsin-insoluble and pepsin-soluble collagen from diabetic rats. *Diabetes*, *45*, 1037-1043.

Miller, E. J., and Rhodes, R. K. (1982). Preparation and characterization of different types of collagen. *Methods in Enzymology*, *82*, 33-64.

Monnier, V. M., Vishwanath, V., Frank, K. E., Elmets, C. A., Dauchot, P., and Kohn, R. R. (1986). Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *New England Journal of Medicine*, *314*(7), 403-408. doi: 10.1056/NEJM198602133140702.

Murugan, P. (2010). Taner's Cassia (*Cassia auriculata* L.) extract prevents hemoglobin glycation tail tendon collagen properties in experimental diabetic rats. *Cell and Tissue Research*, *10*(1), 2109-2114.

Murugan, P., and Maneemegalai, S. (2024). Modulatory effect of pterostilbene on tail tendon collagen in type 2 diabetic rats. *Chelonian Conservation and Biology*, *19*: 01(1).111-125.

Murugan, P., and Pari, L. (2006). Antioxidant effect of tetrahydrocurcumin in streptozotocin-Nicotinamide induced diabetic rats. *Life Sciences*, *79*(18), 1720-1728. doi: 10.1016/j.lfs.2006.06.001.

Murugan, P., and Pari, L. (2007a). Influence of tetrahydrocurcumin on erythrocyte membrane bound enzymes and antioxidant status in experimental type 2 diabetic rats. *Journal of Ethnopharmacology*, *113*(3), 479-486. doi: 10.1016/j.jep.2007.07.004.

Murugan, P., and Pari, L. (2007b). Protective role of tetrahydrocurcumin on changes in the fatty acid composition in streptozotocin-Nicotinamide induced type 2 diabetic rats. *Journal of Applied Biomedicine*, *5*(1), 31-38. doi: 10.32725/jab.2007.005.

Murugan, P., and Pari, L. (2007c). Influence of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in experimental type 2 diabetic rats. *Basic and Clinical Pharmacology and Toxicology*, *101*(4), 241-245. doi: 10.1111/j.1742-7843.2007.00109.x.

Murugan, P., Pari, L., and Rao, C.A. (2008). Effect of tetrahydrocurcumin on insulin receptors status in type 2 diabetic rats: Studies on insulin binding to erythrocytes. *Journal of Biosciences*, *33*(1), 63-72. doi: 10.1007/s12038-008-0022-y.

Njoroge, F. G., and Monnier, V. M. (1989). The chemistry of the Maillard reaction under physiological conditions: A review. In J. W. Baynes and V. M. Monnier (Eds.),*The Maillard reaction and aging, diabetes and nutrition*, Ar. Liss. New York.

Odetti, P., Pronzato, M. A., Noberasco, G., Cosso, L., Traverso, N., Cottalasso, D., and Marinari, U. M. (1994). Relationships between glycation and oxidation related flurorescences in rat collagen during ageing. An *in vivo* and *in vitro* study. *Laboratory Investigation*, *70*, 61-67.

Pari, L., and Murugan, P. (2005). 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*(4), 257-274. doi: 10.1515/JBCPP.2005.16.4.257.

Pari, L., and Murugan, P. (2007a). Influence of tetrahydrocurcumin on tail tendon collagen contents and its properties in rats with streptozotocin-Nicotinamide induced type 2 diabetes. *Fundamental and Clinical Pharmacology*, *21*(6), 665-671. doi: 10.1111/j.1472-8206.2007.00542.x.

Pari, L., and Murugan, P. (2007b). Changes in glycoprotein components in streptozotocin-Nicotinamide induced type 2 diabetes: Influence of tetrahydrocurcumin from *Curcuma longa*. *Plant Foods for Human Nutrition*, *62*(1), 25-29. doi: 10.1007/s11130-006-0037-1.

Rao, P., and Pattabiraman, T. N. (1989). Reevaluation of the phenol sulphuric acid reaction for the estimation of hexoses and pentoses. *Analytical Biochemistry*, *181*(1), 18-22. doi: 10.1016/0003-2697(89)90387-4.

Roberts, A. T., Martin, C. K., Liu, Z., Amen, R. J., Woltering, E. A., Rood, J. C., . (2007). The safety and efficacy of a dietary herbal supplement and gallic acid for weight loss. *Journal of Medicinal Food*, *10*(1), 184-188. doi: 10.1089/jmf.2006.272

S, G., and P, M. (2026). Combined and comparative effect of tetrahydrocurcumin and gallic acid on streptozotocin induced diabetic rats. *International Journal of Zoological Investigations*, *12*(1), 400-410. doi: 10.33745/ijzi.2026.v12i01.041.

Sajithlal, G. B., Chithra, P., and Chandrakasan, G. (1999). An *in vitro* study on the role of metal catalysed oxidation in glycation and crosslinking of collagen. *Molecular and Cellular Biochemistry*, *194*(1-2), 257-263. doi: 10.1023/A:1006988719374.

Samad, N. (2018). amd Javed A., Therapeutic Effects of gallic acid: Current Scenario. *Journal of Phytochemistry Biochemistry*, *2*(113), 1000113.

Smith-Mungo, L. I., and Kagan, H. M. (1998). Lysyl oxidase: Properties, regulation and multible function in biology. *Matrix Biology*, *16*(7), 387-398. doi: 10.1016/S0945-053X(98)90012-9.

Turk, Z. (1997). Advanced glycation toxicity and diabetic complications. *Diabetologia Croatica*, *26*, 11-26.

Woessner, J. F. (1961). The determination of hydroxyproline in tissue and protein samples containing small portions of this imino acid. *Archives of Biochemistry and Biophysics*, *93*(2), 440-447. doi: 10.1016/0003-9861(61)90291-0.

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