Sains Malaysiana 52(2)(2023): 579-588
http://doi.org/10.17576/jsm-2023-5202-19

Hypoglycemic Effect of Flavonoid Glabridin Prevents Homeostatic Disruption of Native Achilles Tendon in Streptozotocin-Induced Type 1 Diabetic Rats

(Kesan Hipoglisemik Flavonoid Glabridin Mencegah Gangguan Homeostatik Tendon Achilles Asal pada Tikus Diabetik Jenis 1 Aruhan Streptozotosin)

JONGDEE NOPPARAT[1,2], WIPAPAN KHIMMAKTONG[1], CHUMPOL PHOLPRAMOOL[3] & CHITTIPONG TIPBUNJONG[1,*]

[1]*Department of Anatomy, Division of Health and Applied Sciences, Faculty of Science, Prince of Songkhla University, Songkhla 90110, Thailand*
[2]*Trace Analysis and Biosensor Research Center, Prince of Songkhla University, Songkhla 90110, Thailand*
[3]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Received: 9 June 2022/Accepted: 16 November 2022

ABSTRACT

Diabetic mellitus is a complex and serious disorder characterized by poor glycemic control leading to tendon architectural alterations and inflammation. This study aimed to investigate the protective effects of glabridin, a polyphenolic flavonoid, on architecture and inflammation of the Achilles tendon in streptozotocin-induced type 1 diabetic rats. Type 1 diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg b.wt.). After confirmation of the diabetic state, the rats were divided into four groups; normal control, diabetic control, diabetic + glabridin (40 mg/kg b.wt.), and diabetic + glyburide (5 mg/kg b.wt.) as a positive control group. After 8 weeks of treatment, the Achilles tendons were collected and subjected to histopathological examinations with hematoxylin and eosin, Masson's trichrome, periodic acid Schiff, and toluidine blue staining. Immunohistochemical staining (IHC) was also performed to study the inflammation of the tendon tissues. Histopathological examinations showed the protective effects of glabridin against hyperglycemia-induced collagen disorganization and deposition of glycoproteins in the extracellular matrix of the tendon. Treatment with glabridin significantly decreased the interfibrillar length, interfibrillar space, and number of infiltrated mast cells in the tendon tissue of diabetic rats. In addition, IHC staining showed that administration of glabridin drastically attenuated advance glycation end products (AGEs) formation and accumulation, and decreased the IL-1β and TNF-α positive stains compared to the non-treated diabetic control group. Taken together, this study showed glabridin prevents architectural alterations and suppresses inflammation in the Achilles tendon of diabetic rats.

Keywords: Achilles tendon; collagen fibre; hyperglycemia; inflammation; phytoestrogen

ABSTRAK

Diabetes mellitus merupakan gangguan yang kompleks dan serius dan dicirikan oleh kawalan glisemik yang lemah yang membawa kepada perubahan dan keradangan struktur tendon. Kajian ini bertujuan untuk mengkaji kesan perlindungan glabridin, flavonoid polifenol pada tendon Achilles pada tikus diabetes jenis 1 aruhan streptozotosin. Diabetes jenis 1 diaruh oleh satu suntikan intraperitoneum streptozotosin (60 mg/kg b.wt.). Selepas pengesahan keadaan diabetes, tikus dibahagikan kepada empat kumpulan; kawalan normal, kawalan diabetes, diabetes + glabridin (40 mg/kg b.wt.) dan diabetes + glyburide (5 mg/kg b.wt.) sebagai kumpulan kawalan positif. Selepas 8 minggu rawatan, tendon Achilles dikumpul dan tertakluk kepada pemeriksaan histopatologi dengan hematoksilin dan eosin, trichrome Masson, asid berkala Schiff dan pewarnaan toluidina biru. Pewarnaan imunohistokimia (IHC) juga dilakukan untuk mengkaji keradangan tisu tendon. Pemeriksaan histopatologi menunjukkan kesan perlindungan glabridin terhadap gangguan kolagen aruhan hiperglisemia dan pemendapan glikoprotein dalam matriks ekstrasel tendon. Rawatan dengan glabridin mengurangkan panjang antarafibril, ruang antarafibril dan bilangan sel mast yang menyusup ke dalam tisu tendon tikus diabetes. Di samping itu, pewarnaan IHC menunjukkan bahawa penyuntikan glabridin secara drastik melemahkan pembentukan dan pengumpulan produk akhir glikasi awal (AGEs) dan mengurangkan kesan positif IL-1β dan TNF-α berbanding kumpulan kawalan diabetes yang tidak dirawat. Secara keseluruhan, kajian ini menunjukkan glabridin menghalang perubahan struktur dan menyekat keradangan pada tendon Achilles tikus diabetes.

Kata kunci: Fitoestrogen; hiperglisemia; keradangan; serat kolagen; tendon Achilles

Introduction

Diabetic mellitus (DM), a major public health problem worldwide, is a complex and serious disorder characterized by poor glycemic control leading to high blood sugar levels termed hyperglycemia. The persistently elevated plasma glucose level in DM leads to oxidative stress and is associated with numerous secondary diseases including, but not limited to, osteoporosis (Wongdee & Charoenphandhu 2011), nephropathy (Gross et al. 2005), neurodegeneration (Madhusudhanan, Suresh & Devanathan 2020), retinopathy (Fong et al. 2004), and foot ulcers (Kruse & Edelman 2006). Recently, several publications reported people with DM are more susceptible to developing problems with the musculoskeletal system especially in tendon tissue (Leung et al. 1986; Spanheimer 1992; Rao et al. 2006).

Tendon is a highly organized connective tissue joining muscle to bone and has a primary function of transmitting forces to produce joint movement or fixation. Epidemiological studies show that sustained hyperglycemia in DM is associated with a marked disruption of tendon homeostasis by impaired collagen production (Leung et al. 1986; Spanheimer 1992), altered extracellular matrix composition, infiltration of new cells, and increased stiffness leading to decreased joint mobility (Rao et al. 2006). Hyperglycemia causes the downregulation of the AMP-activated protein kinase (AMPK)/Egr1 pathway, which, in turn, leads to the alteration of tendon homeostasis (Wu, Jin & Jin 2017). The abnormality and/or loss of tendon integrity can lead to major disability that would affect the quality of life. Therefore, effective prevention or treatment of these DM-associated tendon complications would have a strong impact on improving quality of life in diabetic patients.

Glabridin is a polyphenolic flavonoid, a naturally occurring antioxidant, originally isolated from the root of licorice (*Glycyrrhiza glabra* L.) (Chin et al. 2007; Vaya, Belinky & Aviram 1997), which has been widely used in traditional Thai medicine for a long time as an expectorant, amelioration of coughing and fever. It has been reported to exhibit multiple pharmacological activities such as regulation of energy expenditure and metabolism, anti-inflammation, antibacterial, anti-osteoporotic, antinephritic, anti-cancer and chemo-preventive properties (Simmler, Pauli & Chen 2013). In 2013, Wu, Jin and Jin found that glabridin can exert a hypoglycemic effect in animal models of DM which, in turn, restores the body weight and decreases relative internal organ weights. It also affects the immune system of the animal model by decreasing pro-inflammatory cytokines and increasing antioxidant activity in serum (El-Ghffar 2016; Wu, Jin & Jin 2013), suggesting the anti-inflammation property of glabridin. In addition, glabridin has been reported to upregulate antioxidant enzymatic gene expression thereby attenuating hyperglycemia-induced osteoblastic cell damages, which is useful for the treatment of diabetes-related bone disease (Kim et al. 2013). Furthermore, with AMPK activation property, glabridin possesses the abilities to alleviate adiposity and hyperlipidemia (Lee et al. 2012), and induce glucose uptake in skeletal muscle cells (Sawada et al. 2014).

Although there are several lines of evidence for the beneficial effects of glabridin on adipose tissue, bone, skeletal muscle, liver, and pancreas in animal models of DM, its effect on tendon tissue has not yet been explored. In view of the previous report that glabridin is a potent antioxidant among naturally occurring flavonoids, we hypothesized that this compound would protect tendon tissue against hyperglycemia-induced oxidative stress and inflammation. The present study, therefore, was carried out to elucidate the long-term modulatory effects of glabridin administration on tendon tissue in streptozotocin-induced type 1 diabetic rats.

Materials and Methods

Experimental animals

Adult male Wistar rats weighing 200-250 g body weight, eight weeks old, were obtained from the Southern Laboratory Animal Facility, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. They were maintained at a constant temperature (23 ± 2 ºC) and humidity (50 ± 10%) on a 12 h light/dark cycle. The experimental protocols described in this study were approved and guided by the Animal Ethics Committee of Prince of Songkla University. All animals were acclimatized under laboratory conditions for one week prior to experiments.

Experimental induction of type 1 diabetes and design

Type 1 diabetes mellitus (T1DM) was induced by a single intraperitoneal injection of freshly prepared streptozotocin, STZ (Sigma-Aldrich, St. Louis, MO, USA) dissolved in citrate buffer (0.1 M, pH 4.5), at a dose of 60 mg/kg b.wt. to overnight-fasted animals, while the control animals were injected with the same volumes of isotonic saline. After 72 h, blood glucose levels were

measured from the tail vein using a blood glucose meter (Accu-Check Active ® and test strips, Roche diagnostic, Mannheim, Germany) and those rats with fasting blood glucose above 250 mg/dL were considered to be diabetic and were used in the present study. This day was assigned as the first day of the treatment.

After the confirmation of T1DM status, rats were randomly assigned into four groups, comprising 7 animals in each group (7 normal control rats, 21 diabetic surviving rats). Group 1: normal control; Group 2: untreated diabetic control (STZ); Group 3: glabridin-treated diabetic rats (STZ+glabridin) receiving glabridin (purity > 98% by HPLC analysis, Shaanxi Green Bio-Engineering Co., Ltd, China) at a dose of 40 mg/kg b.wt. dissolved in 0.5 mL of 0.5% Tween-80 solution for 8 weeks; Group 4: glyburide-treated diabetic rats (STZ+glyburide) receiving glyburide (Sigma) at a dose of 5 mg/kg b.wt. dissolved in 0.5 mL of 0.5% Tween-80 solution) as a positive control.

HISTOLOGICAL AND IMMUNOHISTOCHEMISTRY STUDIES

After 8 weeks of DM treatment, rats were sacrificed by an overdose of thiopental (150 mg/kg), and the Achilles tendons were carefully excised and collected. Tissue samples were then fixed in 10% formalin, dehydrated in a graded ethanol series and finally paraffin-embedded. Tissue sections (5 μm) for histological and immunohistological stainings were deparaffinized in xylene and rehydrated in a graded ethanol series. For tissue morphological examinations, sections were stained with hematoxylin-eosin (H&E), and 6 separate fields from 4 specimens were analysed by ImageJ software. For collagen and glycoprotein content observations, sections were stained with Masson's trichrome (MT) and periodic acid-Schiff (PAS) reagent, respectively.

For immunohistochemistry, prior to the detection of IL-1β, TNF-α, and advance glycation end products (AGEs), tissue sections were incubated in tri-sodium citrate-buffer (pH 6.0) for 3 h at 60 °C, cooled to room temperature (RT) and equilibrated in phosphate-buffered saline (PBS) and processed for immunodetection. Subsequently, tissue sections were washed in PBS with 0.5% Tween-20 and endogenous peroxidase was quenched with 3% H_2O_2 in methanol for 30 min at RT followed by incubation with a primary antibody. After 3 washes, incubation of secondary peroxidase-conjugated antibodies was performed for 30 min at RT. Finally, slides were washed in PBS with 0.5% Tween-20 and peroxidase activity was detected with 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, Vienna, Austria) and stopped in deionized water. Tissue sections were counterstained with hematoxylin (Leica, Vienna, Austria) for 2 min and mounted for microscopic examinations under an Olympus DP73 microscope equipped with Cellsens software.

Positive staining was represented by a brown signal and photographed at 200X magnification. A total of 36 fields per experimental group (3 fields per section, 3 sections per rat, 4 rats per group) were randomly selected. The mean intensity in the captured representative fields (a standard area, 460,096 μm^2) was recorded using ImageJ software. The mean optical density of positive staining was calculated with the following formula: optical density = log (max intensity/mean intensity), where max intensity = 255 for 8-bit images.

QUANTIFICATION OF MAST CELL (MC) NUMBERS

To assess the numbers of infiltrated MCs, the tissue samples were stained with toluidine blue. Briefly, tissue samples were deparaffinized in xylene and rehydrated in a graded ethanol series. The tissues were then incubated with toluidine blue solution, 0.1% toluidine blue in 0.17 mM NaCl at pH 2.3, for 3 min. After several washed with distilled water, quickly dehydrated in graded ethanol series and clearing in xylene, the tissues were mounted for microscopic examinations under an Olympus DP73 microscope. Quantitative analysis of MC number was performed by counting the number of mast cells per field at 100X magnification.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard error of mean (SEM). One-way ANOVA was performed to evaluate the statistical differences, followed by Tukey's multiple comparisons or an independent t-test with the GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). Differences with a P-value less than 0.05 were considered to be significant.

RESULTS

GLABRIDIN PREVENTS THE ALTERATION OF TENDON ARCHITECTURE IN TYPE 1 DM RATS

On H&E staining, we observed that the Achilles tendons in the STZ group exhibited poorly organized architecture. The collagen fibres were not tightly arranged causing spaces between collagen fibres (Figure 1(B)). In contrast, the collagen fibres in the normal control group were tightly arranged in parallel with slight waves (Figure

the other hand, those in the glabridin (STZ+glabridin) and glyburide (STZ+glyburide) treatment groups showed tightly and parallelly arranged patterns similar to normal controls (Figure 1(C) and 1(D)). In addition, there are an evidently higher number of nuclei present in the STZ group. The nuclear shape became rounded compared to the oval or long shape in the normal control and glabridin (STZ+glabridin) and glyburide (STZ+glyburide) treated groups. Quantitative analysis of the nuclear numbers (Figure 1(G)) and interfibrillar spaces (Figure 1(E) & 1(F)) showed significant increases in the STZ group compared to normal controls. Treatments with glabridin (STZ+glabridin) or glyburide (STZ+glyburide) diminished the changes and brought these parameters close to normal control. Taken together, treatment with glabridin exhibited a preventive effect on the architecture of diabetic tendon by reducing the alteration of fiber organization.

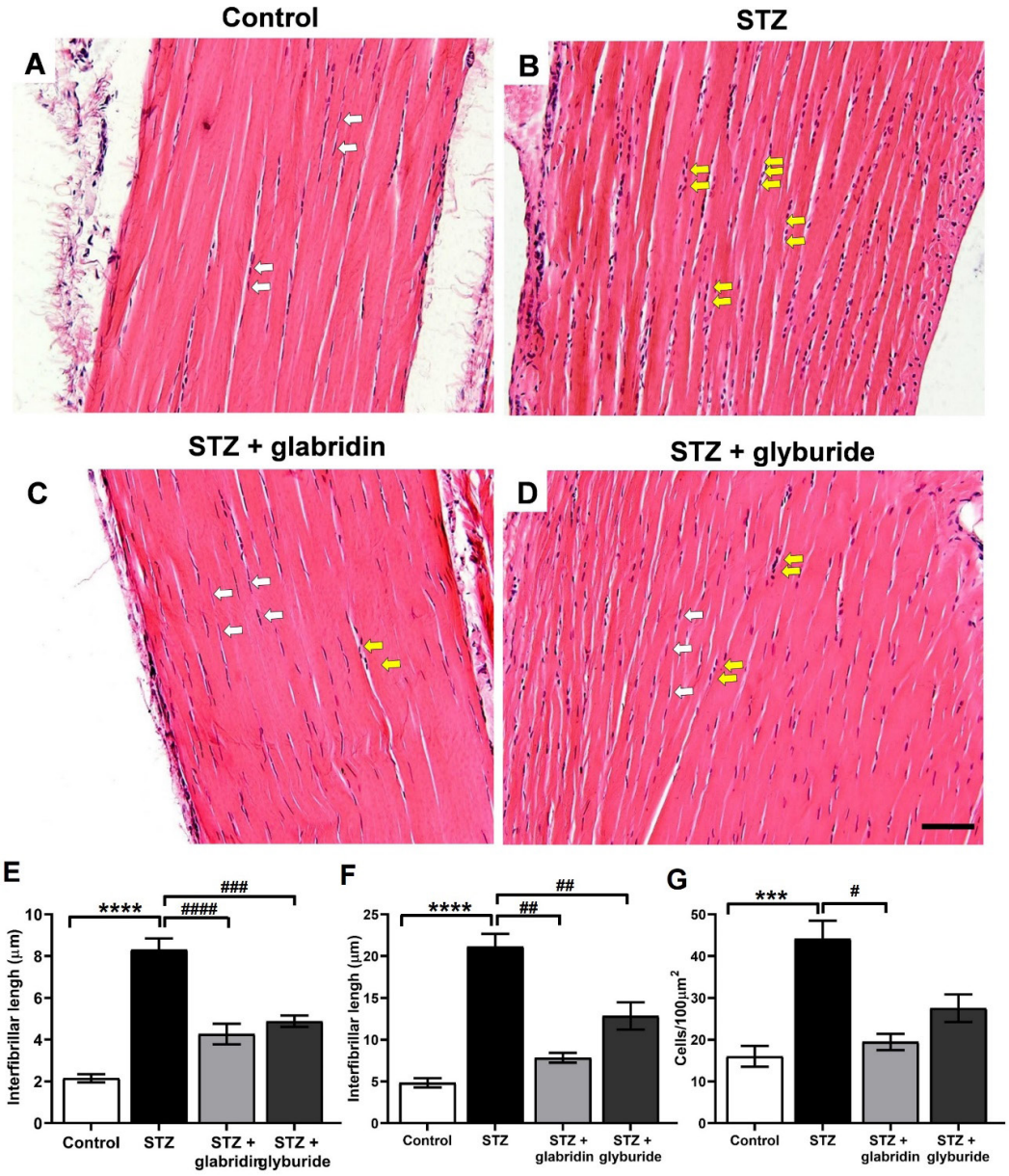

FIGURE 1. The longitudinal section of Achilles tendon from normal control (A), STZ (B), STZ+glabridin (C), and STZ+glyburide (D) stained with haematoxylin and eosin (H&E). Quantitative analyses of interfibrillar length (E), interfibrillar areas (F), and cell density (G) were calculated from H&E-stained micrographics. White arrows denote spindle shaped tenocyte nuclei, and yellow arrows identify abnormal plentiful chondrocyte like-cells. Magnification, 200x; scale bar, 100 µm. $^{****}P<0.0001$, $^{***}P<0.001$ vs. the corresponding control group, $^{\#\#\#\#}P<0.0001$, $^{\#\#\#}P<0.001$, $^{\#\#}P<0.01$, $^{\#}P<0.05$ vs. the corresponding STZ group

GLABRIDIN PREVENTS COLLAGEN DISORGANIZATION IN TENDON OF TYPE 1 DM RATS

The arrangement and deposition of collagen fibres in experimental rat tendon were further determined by MT staining. The tendons of the STZ group (Figure 2(B)) showed disorganization of collagen fibres (stained blue) and increases in the cellular area (stained red) compared to parallel organization of fibres and only spot of cells in the tendon of the normal control group (Figure 2(A)). Treatment with glabridin (Figure 2(C)) and glyburide (Figure 2(D)) dramatically improved the disorganization of collagen fibres and reduced the cellular area in the tendon.

GLABRIDIN AMELIORATE UNEVEN DEPOSITION OF GLYCOPROTEINS AND PROTEOGLYCANS IN TENDON OF TYPE 1 DM RATS

The depositions of glycoproteins and proteoglycans in the experimental rat tendons were examined by PAS staining. Achilles tendons from normal control rats (Figure 2(E)) showed a normal pattern for PAS staining, smoothly stained glycoproteins and proteoglycans in the extracellular matrix with pale pink color. In contrast, the tendon from STZ group (Figure 2(F)) showed an increased PAS-positive stain and showed uneven glycoproteins and proteoglycans depositions around the nuclei. Interestingly, glabridin- and glyburide-treatment groups (Figure 2(G) and 2(H), respectively) exerted a beneficial effect on diabetic rat tendons by preventing the accumulation of glycoproteins and proteoglycans.

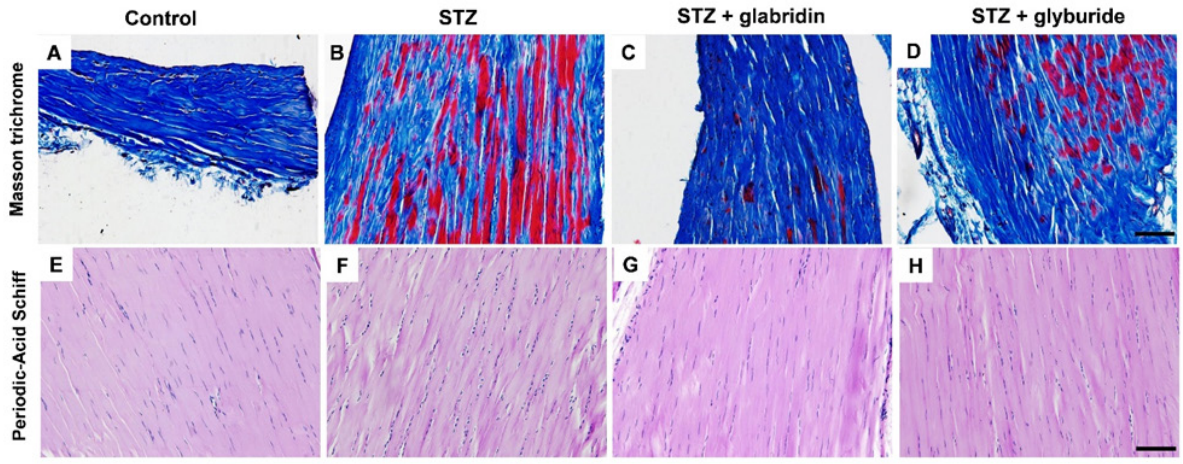

FIGURE 2. The longitudinal section of Achilles tendon from normal control (A, E), STZ (B, F), STZ+glabridin (C, G), and STZ+glyburide (D, H) stained with Masson trichrome (MT, upper row) and periodic-acid Schiff (PAS, lower row). Magnification, 200×; scale bar, 100 μm

GLABRIDIN SUPPRESSES THE INFLAMMATORY MARKERS IN TENDON OF TYPE 1 DM RATS

Inflammation has been proposed to involve with collagen degradation and disorganization. To examine whether hyperglycemia results in tendon inflammation and finally leads to tendon architecture damaging, the expression of inflammatory markers including IL-1β and TNF-α, were observed by IHC staining. Tendon tissue of normal control group (Figure 3(A) and 3(E)) showed only pale staining for IL-1β and TNF-α and only several positive staining cells while intense staining and abundant distribution of IL-1β and TNF-α positive cells were observed in the STZ group (Figure 3(B) & 3(F), red arrow). Interestingly, the numbers and distribution of IL-1β and TNF-α positive cells were dramatically decreased in glabridin (Figure 3(C) and 3(G)) and glyburide (Figure 3(D) and 3(H)) treatment groups. Optical density measurement showed significant increases in IL-1β and TNF-α positive cells in the STZ group compared to normal control. Treatment with glabridin further decreased the IL-1β and TNF-α positive cells (Figure 3(I) and 3(J)). Altogether, glabridin showed a suppressive effect on the expression of inflammatory cytokines in diabetic conditions.

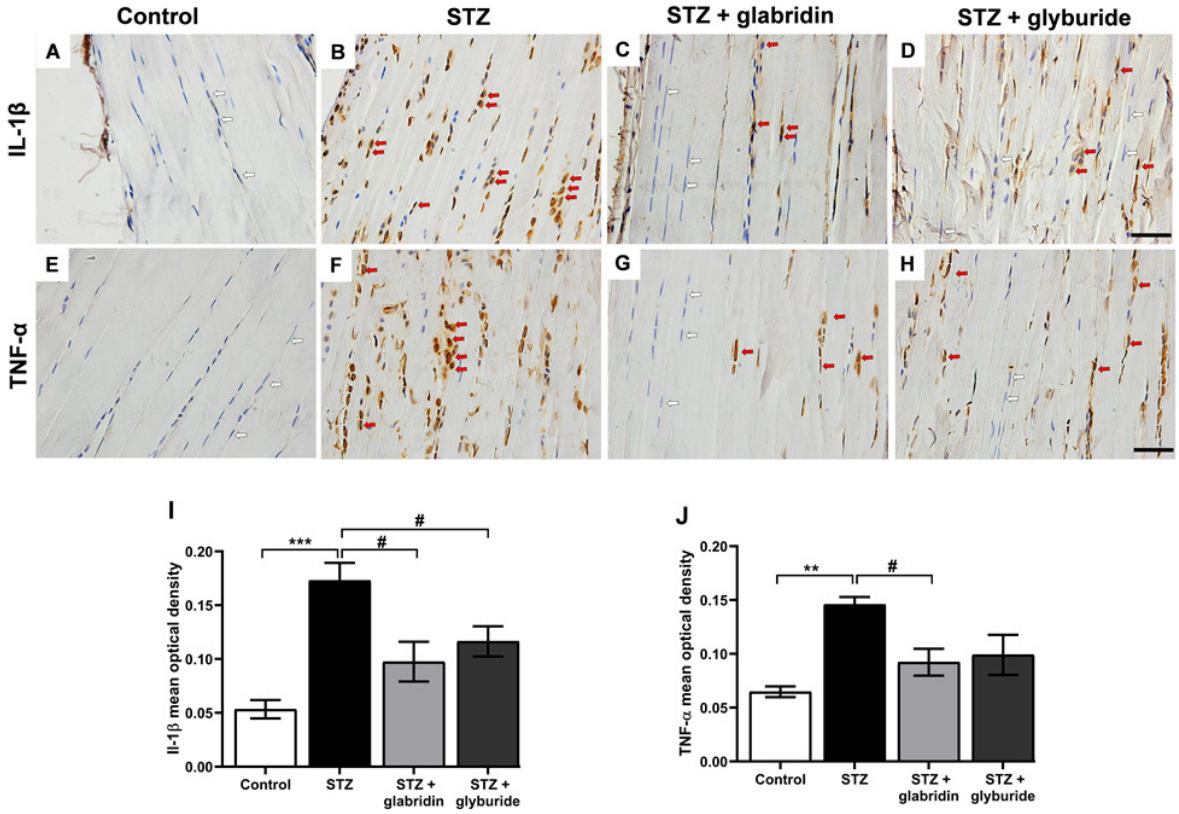

FIGURE 3. The longitudinal section of Achilles tendon from normal control (A, E), STZ (B, F), STZ+glabridin (C, G), and STZ+glyburide (D, H) immunostainned with anti-IL-1β (upper row) and anti-TNF-α (lower row) antibodies. Optical density of the IL-1β (I) and TNF-α (J) positive cells were measured. White arrows denote spindle shaped tenocyte nuclei, red arrows indicated IL-1β and TNF-α positive cells. Magnification, 200×; scale bar, 100 μm. $^{***}P<0.001$, $^{**}P<0.01$ vs. the corresponding control group, $^{\#}P<0.05$ vs. the corresponding STZ group

EFFECT OF GLABRIDIN ON AGES FORMATION IN TENDON OF TYPE 1 DM RATS

Advance glycation end products (AGEs) are proteins or lipids that become glycated after prolonged exposure to glucose. These AGEs play an important role in the pathogenesis of tendon. The accumulation of AGEs in Achilles tendons of experimental rats was observed by IHC staining. The Achilles tendons in the STZ group (Figure 4(B)) clearly showed positive staining with AGEs around the nuclei while only a small amount of AGEs positive area was seen in the normal control group (Figure 4(A)). Administration of glabridin (STZ+glabridin, Figure 4(C)) or glyburide (STZ+glyburide, Figure 4(D)) showed a slight decrease in AGEs positive area. Optical density measurement confirmed a significant increase in AGE positive staining in the STZ group compared to the normal control group. Treatment with either glabridin or glyburide showed only a trend to decrease in the expression of AGE compared to the STZ group (Figure 4(E)).

EFFECT OF GLABRIDIN ON MAST CELLS INFILTRATION IN TENDON OF TYPE 1 DM RATS

Mast cells play a critical role in the inflammatory responses of tissues in diabetic condition. In the STZ group (Figure 5(B)), MCs infiltrated into and accumulated in the surrounding connective tissue of Achilles tendon. The number of MCs in the STZ group was about 3-folds higher than the normal control group (Figure 5(A)). Administration of glabridin (STZ+glabridin, Figure 5(C)) or glyburide (STZ+glyburide, Figure 5(D)) restored the number of MCs closed to normal. These results suggest that administration of glabridin can

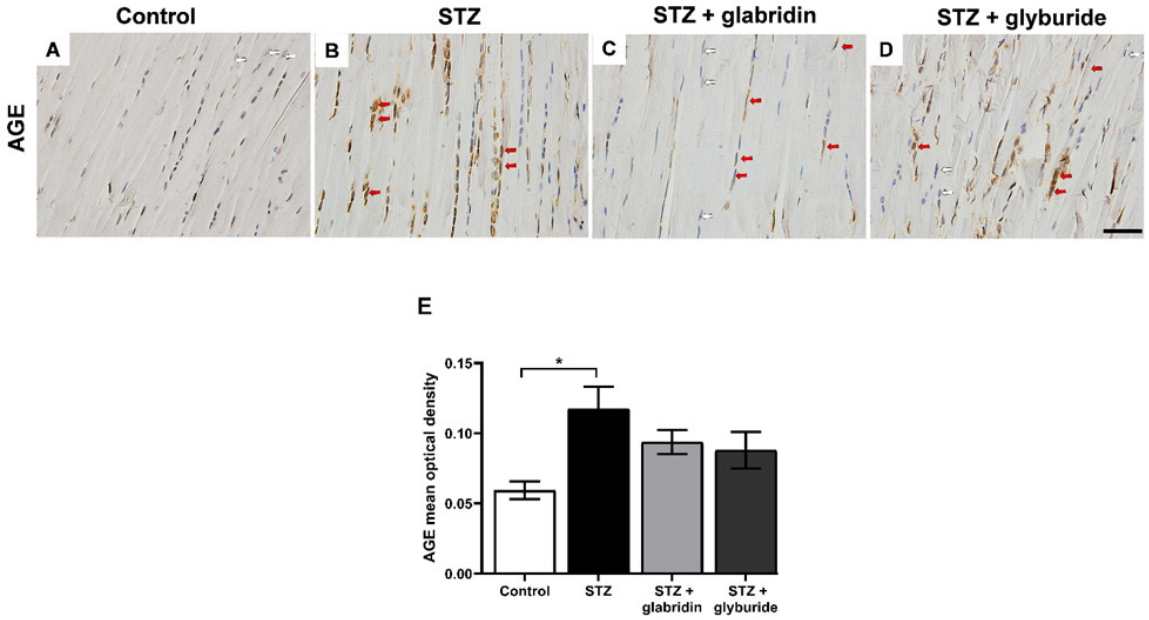

FIGURE 4. The longitudinal section of Achilles tendon from normal control (A), STZ (B), STZ+glabridin (C), and STZ+glyburide (D) immunostainned with anti-AGE antibody. Optical density of AGEs positive cells was measured (E). White arrows denote spindle shaped tenocyte nuclei, red arrows identify AGEs positive cells. Magnification, 200×; scale bar, 100 μm. *P<0.05 vs. the corresponding control group

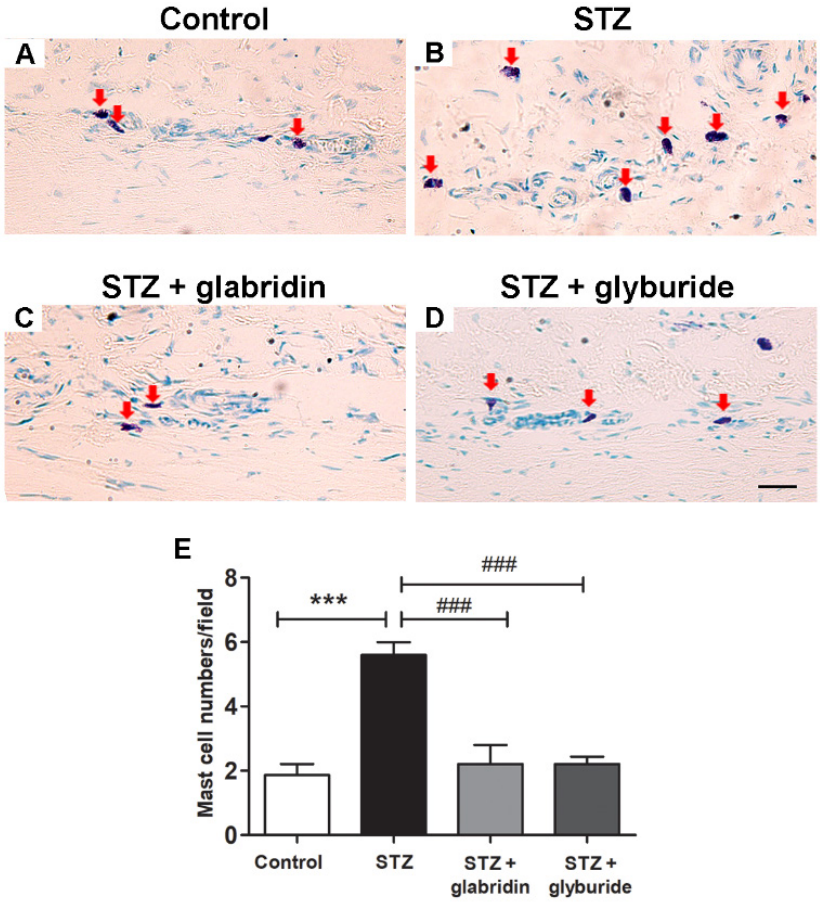

FIGURE 5. The longitudinal section of Achilles tendon from normal control (A), STZ (B), STZ+glabridin (C), and STZ+glyburide (D) stained with toluidine blue. The number of mast cells were counted and expressed as cells per field (E). Red arrows indicate mast cells. Magnification, 100×; scale bar, 100 μm; ***P<0.001 vs. the corresponding control group, ###P<0.001 vs. the corresponding STZ group

prevent the infiltration and accumulation of MCs in the Achilles tendon of diabetic rats.

DISCUSSION

Previously, we reported the hypoglycemic effect of glabridin in STZ-induced T1DM in adult male Wistar rats by decreasing blood glucose levels up to fifty percent, leading to the prevention of hepatocyte destruction and collagen deposition in the liver (Komolkriengkrai et al. 2019). In the present study, STZ-induced T1DM rats presented a marked alteration of Achilles tendon architecture and upregulation of the expression of inflammatory marker proteins. Collagen separation and disorganization and the presence of the inflammatory response are the markers for the disrepair stage of tendinopathy (McCreesh & Lewis 2013). Administrations of glabridin as well as glyburide, which is an antidiabetic drug used as a positive control, significantly prevented these deleterious effects.

These results are in accordance with the previous report that long-sustained high blood glucose state induced alterations of the architecture and disorganization of collagen in the tendon of diabetic rat model without affecting growth and apoptosis (Wu et al. 2017). These changes in tendon structure occurred through the disruption of tendon homeostasis via the down-regulation of the AMPK/Egr1 pathway (Wu et al. 2017). Early growth response factor 1 (Egr1) has been shown to regulate the expression of matrix molecule genes including type 1 collagen, to promote tendon differentiation and repair (Guerquin et al. 2013). Apart from the collagen organization examination, the quantitative analysis of total collagen should be investigated to explain the effect of glabridin in collagen synthesis or degradation inhibition in diabetic conditions.

Since glucose is the reducing sugar, prolonged exposure to hyperglycemic conditions would provoke cellular oxidative stress leading to oxidative damage to many tissues (Mohamed et al. 2016; Obafemi et al. 2017) including tendon (Wu et al. 2017). The major mechanism of hyperglycemia-induced tissue damage involves generating a high level of free radicals by glucose autoxidation, followed by oxidative degeneration (Hunt, Dean & Wolff 1988). As previously reported, the beneficial effects of glabridin and other flavonoids in preventing tissue damage in the animal model of DM are due to their hypoglycemic effect (Komolkriengkrai et al. 2019; Obafemi et al. 2017). This effect has been proposed to be due to the potentiation of insulin secretion from the remaining pancreatic β-cells and/or an increase in the utilization of glucose by tissues (Nopparat, Nuallaong & Phongdara 2019; Obafemi et al. 2017). Moreover, the administration of flavonoids protects the body against free radicals (Rice-Evans, Miller & Paganga 1996) and increases the antioxidant enzyme activity which, in turn, protects tissue against oxidative stress-induced cell death (Maher & Hanneken 2005).

The deposition of AGEs, glycoproteins and proteoglycans in the extracellular matrix altered protein physical properties and affected tendon function (Li et al. 2013). Formation and accumulation rates of AGEs have been reported to be increased in the DM condition (Singh et al. 2014). Accumulation of AGEs would inhibit biomechanical plasticity (Lee & Veres 2019) and diminish tendon collagen fibre sliding by a loss of tissue viscoelasticity (Li et al. 2013), which, in turn, adversely affected tendon function. However, our results show only a trend in decreasing the deposition of AGEs after treatment with glabridin. The biomechanical responses of the Achilles tendon after treatment of diabetic rats with glabridin should be investigated to confirm the beneficial effect on tendon function improvement.

In addition, our data demonstrated the inhibitory effect of glabridin on inflammation of the tendon of T1DM rats by attenuating mast cell infiltration and suppressing the expression of inflammatory markers. These results are in accordance with the previous reports that glabridin has potent anti-inflammation activity by inhibiting the expression of inflammatory markers in macrophages (Liu et al. 2017), colon (Kwon, Oh & Kim 2008), and liver (Ma et al. 2021; Maksoud et al. 2019) via the PI3K/Akt/Nrf2 pathway. Moreover, it also has the potential to decrease the proinflammatory cytokines and increase antioxidant activity in serum (El-Ghffar 2016; Wu et al. 2013). Indeed, our results show significant reductions in the expressions of IL-1β and TNF-α. The potent anti-inflammation may exert through their estrogenic activity, as the glabridin has been reported to possess the estrogenic property (Simmler, Pauli & Chen 2013; Somjen et al. 2004) which exerts anti-inflammatory effects through the activation of Nrf2 protein (Song et al. 2019). Moreover, the flavonoids have been reported to have an interrelationship with the mast cells (Shaik et al. 2018) by being a potent anti-cytokine and chemokine which in turn inhibit immunological and non-immunological conditions mediated by mast cells (Peluso et al. 2015).

CONCLUSIONS

In conclusion, this study demonstrates for the first time

the preventive effect of glabridin on the derangement of structure and inflammation of the Achilles tendon due to hyperglycemia in STZ-induced T1DM rats suggesting the potential application for therapeutic uses in DM patients.

ACKNOWLEDGEMENTS

This research was supported by the Faculty of Science Research Fund (2020), Prince of Songkla University.

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*Corresponding author; email: chittipong.t@psu.ac.th