

# Effects of Epigallocatechin Gallate Administration on Diabetic Rat Myocardial Tissue

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## ABSTRACT

This study aimed to investigate the actions of Epigallocatechin Gallate (EGCG) on heart tissue in diabetic rats. In the study, twenty-eight Wistar albino male rats were taken and divided into 4 groups as control group, EGCG, Diabetes, and Diabetes + EGCG. A one dose of 45 mg/kg streptozotocin (STZ) was administered intraperitoneally to the groups to be described with diabetes. EGCG was given to the animals at 20 mg/kg/day for 14 days using an intragastric tube. The heart tissue was stained with Masson trichrome and Hematoxylin-Eosin (H&E). Heart tissue was evaluated by histopathological and immunohistochemical (Collagen type III) methods. In the histological structure of the diabetic group, irregular heart muscle fibers, inflammatory cell infiltration, and necrotic myocytes in the myocardium and increased collagen type III expression were observed, and these findings were found to decrease in the Diabetes + EGCG group. It was found that the density of collagen content increased in the Diabetes group. This finding decreased in the Diabetes + EGCG group. The findings of our study suggest that EGCG may have ameliorative effects on induced cardiotoxicity in diabetic rats.

**Keywords:** Diabetes, Epigallocatechin Gallate, Heart, Immunohistochemistry, Rat

## Introduction

Diabetes mellitus (DM), a common global illness, is a heterogeneous metabolic disease featured by chronic hyperglycemia and causes long-term harm and dysfunction of many organs (1, 2). Diabetic cardiomyopathy (DCM) is one of the most grave and prevalent reasons of morbidity and mortality in patients with diabetes owing to heart failure (2). The molecular mechanism of diabetic cardiac harm involves increased oxidative stress, apoptosis and inflammation, finally leading to heart failure and cardiac dysfunction (2). The pathophysiology underlying diabetes-induced cardiac harm is multifactorial and complex, with oxidative stress being a major contributor (1). The formation of free radicals in diabetes speed the development of heart damage (2). Moreover, cardiac fibrosis is associated with the pathogenesis of DCM (3). Cardiac fibrosis is characterized by collagen deposition in the interstitial space of cardiac tissue and contributes to cardiac failure (4).

Plant-based medicines, including flavonoids, have been used in the management of various diseases. Flavonoids, an essential part of the human diet, have also been found in plant extracts used in

oriental medicine for centuries (5). Epigallocatechin-3-gallate (EGCG), one of the well-known polyphenols, is the main component of green tea. It is known to show a wide range of biological activities such as hypoglycemic, hypocholesterolemic, antioxidative, antibacterial, antiviral, anticancer and chemopreventive (6). The antioxidant properties of flavonoids, ROS scavenging and cell function modulation could explain for the major section of their pharmacological activity (5). EGCG could protect against oxidative damage by reacting with free radicals, increasing the endogenous antioxidant enzymes and inhibiting pro-oxidant enzymes (2). Antioxidant activity of EGCG is considerably stronger than vitamins E and C (7). EGCG also acutely activates special intracellular signaling pathways organizing metabolic activity of insulin. EGCG inhibits gluconeogenesis via activation of PI3-kinase addicted pathways in hepatocytes (5).

As understood from the literature review, cardiac fibrosis plays an important role in understanding the pathogenesis of DM-induced cardiomyopathy. Therefore, our study aimed to evaluate cardiac fibrosis and cardiac morphological changes in DM-induced cardiomyopathy and investigate the protective effects of EGCG.

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## Material and Methods

**Experimental animals:** In this work, twenty-eight Wistar albino male rats weighing 200-250 g were used, provided from Van Yüzüncü Yıl University. This study was approved by the Ethical Committee of Van Yuzuncu Yil University (Ethical confirmation number: 2025/06-09). The animals were sheltered at 21-24 °C with a 12h light:12h dark period and had ad libitum reach to standard rat food and water. The experimental protocol was performed in conformity with the National Institutes of Health guide for the maintenance and use of laboratory animals (NIH Publication No. 8523, revised 1996) and was adapted to the guidelines of the local experimental animal ethics committee.

**Induction of Diabetes and EGCG Application:** Diabetes was induced by a one injection of intraperitoneal (0.1 mol/L; 45mg/kg) streptozotocin dissolved in 0.9% sodium citrate tampon (pH 4.5). After 72h of STZ application, DM was identified by measuring the tail vein blood glucose level (8). Blood glucose grades for DM groups were 250 mg/dl and these animals were accepted diabetic (9). EGCG was administered using an intragastric tube at 20 mg/kg/day for 14 days (10).

**Experimental design:** Twenty-eight rats were splitted into four groups of seven rats each. Experimental groups:

**Group 1:** Control

**Group 2:** EGCG

**Group 3:** Diabetes

**Group 4:** Diabetes + EGCG

At the end of the experiment, all the rats were euthanized by intraperitoneal injection of ketamine (50 mg/kg, Eczacibasi, Turkey) and xylazine (10 mg/kg, Bayer, Turkey). The heart tissue was fixed in 10% formalin for histological and immunohistochemical studies.

**Histologic Analysis:** The heart of experimental groups was excised and fixed in 10% formalin solution for histopathologic examination. The tissue was embedded in paraffin and cut 5 µm thick by a microtome (Leica SM 2000, Germany). The samples were stained with Hematoxylin-Eosin (H&E), explored under a light microscope (Olympus BX53 Digital Microscope, Japan), and photographed.

**Masson Trichrome Staining Analysis:** The samples were stained with Masson Trichrome (MT) with aniline blue (Bio-Optica, lot/cat# 04-010802) with a ready-to-use kit. The staining steps were performed according to the manufacturer's instructions.

Histological images obtained after MT staining, performed to evaluate collagen content density in the heart tissue sections, were analysed using FIJI (ImageJ, NIH, USA) software. Five fields were randomly selected for each sample and evaluated under a light microscope (Olympus BX53 Digital Microscope, Japan) at 40x magnification. The percentage of positive staining (% Area) in the selected area was recorded in the images. The average area density was calculated for each animal (11).

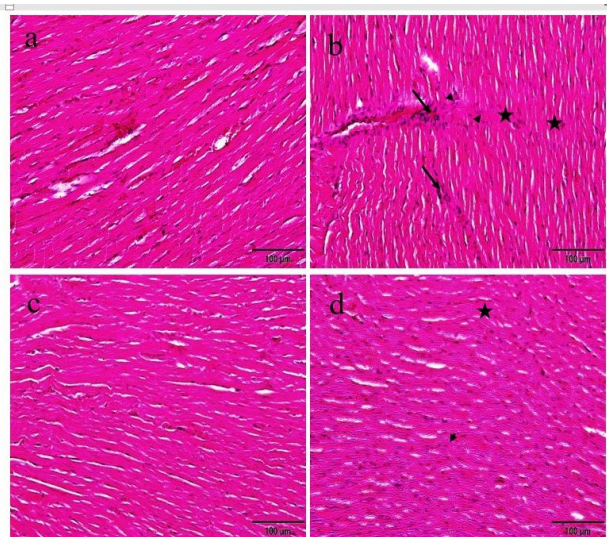
**Immunohistochemical Analysis:** 5 µm-thick sections from the tissues embedded in paraffin were taken onto slides coated with poly-L-Lysin. After the slides were deparaffinized, they were crossed through xylene and alcohol series. Then, the immunohistochemical method was applied (12). Collagen III primary antibody (bs-0948R, 1:200, Bioss) was used for immunohistochemical evaluation. For immunohistochemical evaluation, different areas randomly selected from the heart were examined at 40x magnification, and the staining intensity was evaluated.

**Statistical Analysis:** Statistical evaluations and graphical plots of histological data were accomplished with GraphPad Prism 8.01 (GraphPad, USA). Normality distributions of the data were examined with the Shapiro-Wilks test. A one-way ANOVA was conducted to compare the group means. Following the ANOVA test, the Duncan Test was used to determine the groups that made a difference. Descriptive statistics for the continuous variables were offered as standard error of the mean (SEM) and the mean. The significance levels between all groups were reported as \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , and \*\*\*\* $p \leq 0.0001$ , indicating the degree of confidence in the observed differences.

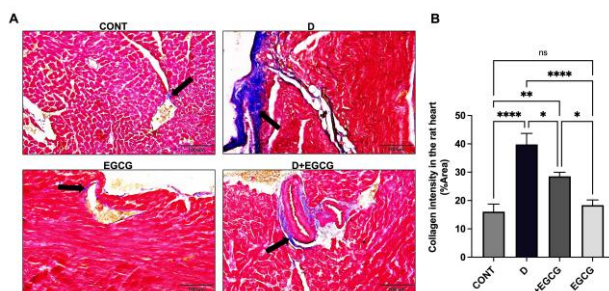
## Results

**Histopathological Findings:** The histological examinations of the heart tissue of the control and EGCG groups revealed normal heart structure with centrally located nuclei and striated muscle fibers. Inflammatory cell infiltration, disorganized cardiac muscle fibers in the myocardium, and necrotic cardiac myocyte damage were observed in heart sections of diabetic animals. However, EGCG treatment significantly reduced these pathological changes (Figure 1).

**Masson Trichrome Staining Finding:** The density of collagen content in heart tissue sections was evaluated as normal in the control and EGCG groups. The collagen content density was observed to be increased in the diabetic group compared to the control group (\*\*\*\* $p \leq 0.0001$ ).



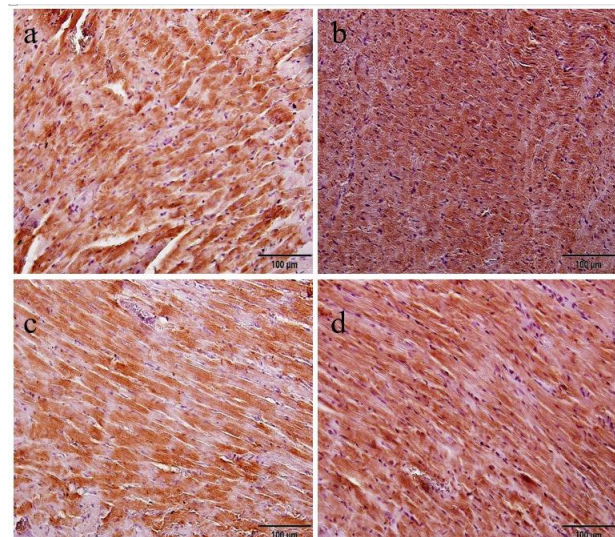
**Fig. 1.** Histological Evaluation of heart tissue a: Cont (Control) b: D (Diabetes) c: EGCG d: D + EGCG, head arrows: change in the core asterisk: increased connective tissue (fibrosis) black arrows: Inflammatory cell infiltration (Hematoxylin-Eosin, Scale bars: 100 µm)



**Fig. 2.** Representative images of all groups in the heart tissue with Masson Trichrome staining. **(A)** Microphotographs of all groups (Cont: Control, D: Diabetes, EGCG, D + EGCG). (Scale bar: 100 µm). **(B)** A graph of collagen intensity (%Area) for all groups. ns: non-significant, \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , and \*\*\*\* $p \leq 0.0001$

The results suggested that prolonged hyperglycemia causes excessive accumulation of collagen fibers in heart tissue. It was observed that collagen fiber accumulation in myocardial tissue was significantly reduced in the EGCG+ Diabetes group compared to the Diabetes group (\* $p \leq 0.05$ ) (Figure 2).

**Immunohistochemical Findings:** Collagen type III expression was evaluated as normal in the control group and EGCG group. Collagen type III expression was observed to be increased in the diabetic group compared to the control group. It was observed that Collagen type III expression in myocardial tissue was significantly reduced in the



**Fig. 3.** Representative photomicrographs of Collagen type III a: Cont (Control) b: D (Diabetes) c: EGCG d: D + EGCG (IHC staining, Scale bars: 100 µm)

Diabetes + EGCG group compared to the Diabetes group (Figure 3).

## Discussion

This study aimed to research the influence of EGCG administration on the heart in a rat model of DCM induced by STZ. The data showed that STZ disrupted the histological structure of heart tissue, and EGCG could ameliorate these pathologies.

STZ is one of the widely used compounds for experimental diabetes induction (8). STZ is a glucosamine-nitrosourea reproduced from the microbe *Streptomyces chromogenes* (13). In our study, we also used STZ to induce diabetes.

DCM is one of the grave complications of diabetes. DCM is characterized by the development of fibrosis, cardiomyocyte hypertrophy and apoptosis. Myocardial fibrosis is an essential feature of most cardiac pathologies. It is a dynamic process that is significantly related to the progression of DCM. Cardiac fibrosis occurs as a result of increased fibroblasts and extreme accumulation of extracellular matrix (ECM) constituents such as fibronectin and collagen types I-III (14). In our study, we evaluated collagen type III using immunohistochemical methods. We also performed Masson Trichrome staining to evaluate heart tissue fibrosis. We found that it increased in the diabetic group compared to the control group and decreased in the Diabetes + EGCG group. EGCG has been reported to have various health benefits, including anti-inflammatory, anticancer,

antioxidant, and antifibrotic features (14). Additionally, many animal studies have shown that EGCG has cardioprotective effects (15). Muhammed et al. showed that EGCG can be useful to fight against aging-induced cardiac hypertrophy, apoptosis, and fibrosis, thereby healing cardiac function in rats (16). EGCG has antioxidant activity by directly chelating metal ions and/or directly scavenging ROS and indirectly inducing antioxidant enzymes and indirectly inhibiting pro-oxidant enzymes (7). Many studies suggest that the antidiabetic effects of EGCG are likely due to its ability to suppress appetite, alter the emulsification of dietary fat in the gastrointestinal tract, inhibit gastrointestinal lipolysis, and reduce nutrient absorption (17).

There are many molecular signaling pathway studies in the literature to reveal the antidiabetic effects of EGCG. Jia et al. showed that EGCG could reduce cardiac fibrosis in type 2 diabetic rats and the underlying mechanisms connected with autophagy activation via modulation of the AMPK/mTOR pathway and subsequent suppression of the TGF- $\beta$ /MMP pathway (14). Othman et al. investigated the potential preservative effect of EGCG on type 2 diabetes-induced cardiac damage. They showed that EGCG protected against cardiac damage by healing the increase in metabolic risk factors, apoptosis, inflammation and oxidative stress in male rats with streptozotocin nicotinamide-induced type 2 diabetes (2). In the study of Zhang et al. EGCG experimental heart failure rat focusing on the desensitization of  $\beta$ 1-ARs by GRK2. Their results showed that EGCG improved cardiac function and alleviated myocardial fibrosis. The desensitization of  $\beta$ 1-AR directly affects the cardiac function, which encourages the secretion of myocardial fibrosis markers MMP9, collagen type I-III, PAR-1 and SMA, ultimately resulting in myocardial fibrosis (15). Gui et al. studied the TGF- $\beta$ /JNK signaling pathway in rats with complicated cardiomyopathy induced by STZ. As a result, they showed that EGCG improves myocardial fibrosis from diabetic complications induced by STZ and protects the heart through the TGF- $\beta$ /JNK signaling pathway (18). The results of our study are consistent with all these literature results, supporting that increased collagen type III and fibrosis occur in diabetic cardiomyopathy and that EGCG improves heart function by reducing myocardial fibrosis.

When all the data obtained in our study are evaluated, we think that EGCG can be suggested as a complementary and supplementary option for

conservation against cardiac harm in diabetes mellitus following sufficient clinical studies.

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**Data Availability Statement:** The data that support the findings of current study are available from the corresponding author on request.

**Conflict of Interest:** The authors in this work declare that they have no financial or non-financial interests that could compete with the described work.

**Ethical Approval:** The Van Yuzuncu Yil University Experimental Animal Ethics Committee evaluated the study's compliance with ethical principles and granted ethical approval (Ethical confirmation number: 2025/06-09).

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