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RESEARCH ARTICLE

Effects of ellagic acid in the testes of streptozotocin induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) is a serious and common in the world health problem that leads to different complications. Changes in oxidative stress and antioxidant capacity play an important role in the pathogenesis of DM. The purpose of this study was to investigate ellagic acid (EA) treatment in diabetes induced testicular damage. In our study, 24 male Sprague Dawley rats were divided into four groups. Group 1: Control (n = 6), Group 2: EA (n = 6), Group 3: Diabet (n = 6), Group 4: Diabet + EA (n = 6). Diabetes was induced by intraperitoneal injection of streptozocin (STZ) (55 mg/kg) to group 3 and 4. EA was given 100 mg/kg/day group 2 and 4 for 35 days by oral gavage. We used that Hematoxylen-Eosin (H&E) and Johnsen's scoring to determine histological change. The terminal-deoxynucleoitidyl-transferase mediated nick end-labeling assay (TUNEL) was used for apoptosis. Oxidative stress markers were determined by qRT-PCR and immunexpression of Nrf2 was evaluated in testicular tissue. In conclusion, EA administration on the diabetes model has changed the histopathological features, apoptosis and oxidative stress marker genes in the testis and may have an effect on the reduction of diabetes induced testicular damage.

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KEYWORDS
Testes; ellagic acid; diabetes mellitus; antioxidant system; Nrf2

1. Introduction

DM is a metabolic disorder associated with functional and structural complications (da Rocha Fernandes *et al.* 2016) and has detrimental effects on male and female reproductive system (Sainio-Pöllänen *et al.* 1997, Sexton and Jarow 1997, Baker 1998, Meyer *et al.* 2000). Studies have shown that DM is related to subfertility and infertility, especially in diabetic men (Bener *et al.* 2009, Abdel-Aziz *et al.* 2020, Hasan *et al.* 2020). Diabetes also has detrimental effects on testosterone levels and sperm parameters (Amiri *et al.* 2011, La Vignera *et al.* 2012, du Plessis Stefan 2018).

Oxidative stress plays a critical role in pathogenesis of diabetes (Ding *et al.* 2015) and the count of sperm with nuclear and mitochondrial DNA fragmentation increases dramatically in patients with diabetes due to oxidative damage. Reactive oxygen species (ROS) that accumulate in the cell accelerate the mechanism of apoptosis in the cell and have a negative effect on sperm quality concentration and function (Giugliano *et al.* 1995, West 2000). It is generally agreed today that, ROS may be reduced antioxidants in diabetic complications (Gomez *et al.* 2006, Bucci *et al.* 2011, Bahadoran *et al.* 2013).

Ellagic acid (2,3,7,8-tetrahydroxy [1]-benzopyranol [5,4,3-cde] benzopyran-5,10-dione), EA) is a natural phenol antioxidant found in nuts and many fruits such as strawberries, walnuts, raspberries, grapes and black currants. EA has anti-apoptotic, anti-mutagenic, anti-carcinogenic, anti-genotoxic, anti-viral, anti-bacterial, anti-allergic, anti-inflammatory, cardioprotective, anti-epileptic and anti-diabetic activities (García-Niño and Zazueta 2015). Anti-diabetic feature of EA stimulates insulin secretion on pancreatic cells and reduces glucose intolerance (Malini *et al.* 2011, Fatima *et al.* 2017). EA is like an aromatase inhibitor and improved the serum testosterone level (Mehrzadi *et al.* 2018, Hamza and Al-Baqami 2019). In another study, plasma testosterone concentration increased on adriamycin (ADR)-induced testicular injury after EA administration (Çeribaşı *et al.* 2012).

Nuclear factor erythroid 2-related factor (Nrf2) activates the transcription of antioxidant genes that act as diabetic free radical scavengers such as heme oxygenase-1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). Low Nrf2 mRNA was found to be associated with sperm concentration, motility, viability and morphology (Chen *et al.* 2012, Ruiz *et al.* 2013). We aimed in the present study to evaluate the role of EA in testicular tissue damage in STZ-induced in rats. The novelty of this study is to address efficacy of EA on testis via apoptosis and antioxidant markers in DM.

2. Materials and methods

2.1. Animals and experimental groups

In this study 24 adult 150–200 g male Sprague Dawley rats were used and obtained from the Gazi University. This study was approved by the Ethical Committee of Gazi University

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(Ethical approval number: G.Ü.ET-18.044). The animals were housed at 22 °C ± 2 °C, 30% humidity with a 12 h light:12 h dark cycle and had *ad libitum* access to water and standard chow.

Twenty-four rats were divided into four experimental groups: Group 1: Control ($n = 6$), Group 2: EA ($n = 6$), Group 3: Diabet ($n = 6$), Group 4: Diabet + EA ($n = 6$). After the experiments, all the rats were euthanized by intraperitoneal injection of xylazine (Bayer, Turkey) and ketamine (Eczacibasi, Turkey). Testes were rapidly removed. The right testes were used for histological and immunohistochemical procedures and the other testis was used for quantitative real-time polymerase chain reaction (qRT-PCR).

2.2. Diabetes induction and EA treatment

Diabetes was induced by a single injection by intraperitoneal (0.1 mol/L; 55 mg/kg) streptozotocin (Santa Cruz, CA) dissolved in 0.9% sodium citrate buffer (pH 4.5). A 5% dextrose solution then was given for the first 24 h. After 48 h STZ administration, DM was detected by measuring the blood glucose level (Srinivasan and Ramarao 2007, Akarca *et al.* 2012, AktugA *et al.* 2013, Seedevi *et al.* 2020). Blood glucose levels for DM groups were $\geq$ 250 mg/dl and these animals were considered diabetic.

The diabetic state was confirmed throughout the 8 weeks. Glucose levels and body weights were measured weekly in all experimental groups. EA (Santa Cruz, CA) (100 mg/kg/day) dissolved in 0.2% dimethyl sulfoxide (DMSO) was administered orally to group EA and DM + EA for 35 days using an intragastric tube (Tasaki *et al.* 2008, Malini *et al.* 2011).

2.3. Histomorphologic analyses

Right testis of experimental groups removed and fixed in formalin solution for histopathologic assay. The testis were embedded in paraffin and cut 4 μm thickness by microtome (Leica SM 2000, Germany). The slides were stained with H&E for Johnsen's scoring and measurement of the diameters of seminiferous tubules (STs).

Johnsen scoring was used to evaluate spermatogenesis and testicular damage in experimental groups. At least 70 STs were examined and scored per section (Johnsen 1970). STs were scored from 1 to 10 according to Johnsen criteria. According to these criteria:

1. No cells observed;
2. Sertoli cells without germ cells were observed;
3. Only spermatogonia were present;
4. Only a few spermatocytes were present;
5. Spermatozoa or spermatid were not present, but many spermatocytes were present;
6. Only a few spermatids were present;
7. Spermatozoa were not present, but there are many spermatids;
8. Only a few spermatozoa were present;
9. Many spermatozoa were present, but the organization of spermatogenesis was disturbed;
10. Complete spermatogenesis and perfect tubules were observed.

In addition, the diameters of 50 STs from each section were randomly evaluated by using an ocular micrometer with a light microscope (Fazelipour *et al.* 2014).

2.4. Immunohistochemical analyses

For immunohistochemical analyses, the sections were deparaffinized, rehydration and incubated with pH 6.0 citrate buffer and treated with 3% H_2O_2 followed by an Ultra V block (Thermo Scientific). Then, the sections were incubated with primary antibody Nrf2 (bs-1074R; Bioss, China) overnight. The antibody was diluted 1: 100 to 24 hours incubation at 4 °C. Subsequently, secondary antibody, anti-mouse IgG anti-rabbit IgG (TP-125-BN, Thermo Scientific) was applied. Sections were incubated with 3,3′-diaminobenzidine tetrahydrochloride (DAB) chromogen (90824B; Spring Bioscience, CA) to make immunpositivity visible. It was counterstained with Mayer's hematoxylin (Zymed Laboratories, CA) and coverslipped using Entellan. Negative control samples were processed identically, but the primary antibody was replaced by PBS. Nrf2 staining was analyzed using a computer imaging system (Leica DM 4000B, LAS V4.9, Wetzlar, Germany).

Percentage of Nrf2 immunopositivity from 10 non-overlapping of each rat randomly determined and analyzed by using Image J (1.51n; National Institute of Health; NIH, Bethesda, MD, USA) program. Briefly, first, the total tissue area was calculated by ignoring the empty areas of the tissue with the Image J program. A threshold value was determined in the control tissue by selecting the DAB chromogen from the program. This threshold value determined was kept the same for all sections. The percentage of immunopositivity was determined by proportioning the uptake intensity in each section with the whole area.

2.5. Tunel assay

The apoptosis detection kit (ApopTag Peroxidase in Situ Apoptosis Detection kit, Merck Millipore) was used to identify the cells of DNA fragmentation. Hematoxylen was used for background. The nuclei of TUNEL-positive apoptotic germ cells were stained and counted examined in Leica DM 4000B (Germany) light microscope with Leica Las V 9 soft ware.

The TUNEL-positive germ cells in 10 seminiferous tubules randomly selected in each section were counted by two researchers. The apoptosis index was calculated by dividing the numbers of positive staining nuclei of the total number of nuclei in the cells and presented as a percentage (Kong *et al.* 2016).

2.6. RNA isolation and qRT-PCR

Total RNA was extracted from rat testicular tissue using WizPrep Total RNA Mini Kit (WizBio, Korea). RNA concentrations were measured by Genova Nano Micro-volume Spectrophotometer (Jenway, UK). Also, the total RNA

structure was verified by gel electrophoresis in terms of fragmentation. The mRNA was converted into cDNA using HyperScript™ First strand Synthesis cDNA Synthesis Kit for real-time PCR (GeneAll, Korea). Nrf2, CAT, Mn-SOD, HO-1, NQO1 and GAPDH mRNA levels in rat testicular tissues were determined using SensiFAST™ SYBR® No-ROX Kit (Bioline, Germany). Rat GAPDH was used as a housekeeping gene and internal reference control. The PCR condition was as follows: polymerase activation at 95 °C for 2 minutes; 35 cycle, denaturation at 95 °C for 5 seconds, annealing at 55–60 °C for 10 seconds as touchdown, extension at 72 °C for 20 s. All procedures were performed according to the manufacturer's protocol. Expressions of mRNA were evaluated by real-time PCR (Roche light cycler 480). In Table 1, the primers specific to the genes used in the study are summarized. Fold changes in the mRNA levels of the target genes determined were calculated according to the endogenous GAPDH control. The cycle threshold (Ct) values of each target gene were subtracted from the Ct values of the housekeeping gene GAPDH (ΔCt). Target gene ΔΔCt was calculated as ΔCt of target gene minus ΔCt of control. The fold change in mRNA expression was calculated as $2^{-\Delta\Delta Ct}$.

2.7. Statistical analysis

Data distribution was evaluated by Shapiro–Wilk test. Paired Samples *T*-Test and post-hoc paired ANOVA tests were used while evaluating dependent and independent data with normal distribution. Related-Samples Wilcoxon Signed Rank Test and post-hoc paired Kruskal–Wallis test were used to evaluate dependent and independent data that do not normally distribute. Statistical analyzes were made using IBM SPSS Statistics 21. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Blood glucose levels

At the end of the treatment, as shown in Table 2, higher blood glucose levels in the DM and DM + EA groups compared to the control and EA groups.

Table 1. RT-PCR Primer Sequences.

	Primer sequences	
Gene	Forward	Reverse
GAPDH	5′-GGCACAGTCAAGGCTGAGAATG-3′	5′-ATGGTGGTGAAGACGCCAGTA-3′
NRF-2	5′-GAGACGGCCATGACTGAT-3′	5′-GTGAGGGGATCGATGAGTAA-3′
CAT	5′-GGAGGCAGAAACTTTCCCATT-3	5′-GGCCAAACCTTGGTCAGATC-3′
Mn-SOD	5′-CACGACCCACTGCAAGGAA-3′	5′-GCGTGCTCCCACACATCA-3′
HO-1	5′-CAGAAGGGTCAGGTGTCCAG-3′	5′-GAAGGCCATGTCCTGCTCTA-3′
NQO1	5′-GTGAGAAGAGCCCTGATTGT-3′	5′-CCTGTGATGTCGTTTCTGGA-3′

3.2. The body and testis weights

Although an increase in body weight of the rats in the DM and DM + EA groups was observed, this increase was not statistically significant ($p = 0.092$; 0.059, respectively). However, this increase in control and EA groups was statistically significant ($p = 0.001$; 0.001, respectively) (Table 3).

When the testicular weights were compared at the end of the experiment, although the testicular weight averages of the rats in the DM group decreased compared to other groups, no statistical difference was found in any group (Table 3).

3.3. Johnsen testicular scoring results

Johnsen scoring in Table 4 was used to evaluate spermatogenesis in the experimental groups. In the testicle tissue belonging to each group, 50 seminiferous tubule profiles were scored between 1–10. When Johnsen testicular scoring is evaluated between groups; DM and DM + EA scores were statistically significantly lower than the control group ($p < 0.001$, $p < 0.001$, respectively). Also, no statistically significant difference was found between the control and EA groups ($p = 1.000$). When DM group and EA group were compared, statistically significant increase was observed in the score values of EA group ($p < 0.001$). When the DM group and the DM + EA group were compared, there was an increase in the score values of the DM + EA group, but this increase was not statistically significant. ($p = 1.000$).

3.4. Histomorphological findings

In testicular tissue of control and EA groups, integrity of seminiferous tubules was maintained and spermatogenic cell line supported by Sertoli cells was found to be normal in structure and morphology. It was noted that interstitial connective tissue containing Leydig cells and vascularized structures were of normal density (Figure 1(A,B)). In the DM group, atrophy and degeneration of seminiferous tubules, vacuolization, irregularity in spermatogenic cell line, germ cell degeneration and interstitial edema were observed in testicular tissue. The decrease in sperm content in the seminiferous tubule lumens was remarkable (Figure 1(C)). In the testis of the DM + EA group, germinal epithelial irregularity was decreased in

Table 2. Blood glucose levels (mg/dl) of experimental groups.

Parameter	Control	EA	DM	DM + EA
Blood glucose level	87.83 ± 6.11*	94.33 ± 10.29*	541.50 ± 47.41	386.17 ± 106.57

Values are presented Mean (SD, *$p < 0.05$ vs. DM group; post-hoc paired comparisons after Kruskal–Wallis test (The number of subjects (n) for each group: $n = 6$).

Table 3. The body and testis weights.

Parameters	Control	EA	DM	DM + EA
First body weight (g)	151 ± 33.30	162.5 ± 33.28	181.5 ± 22.73	176.83 ± 27.76
Final body weight (g)	286.5 ± 29.70*	291.17 ± 21.07*	232.83 ± 41.22	221.33 ± 52.29
Testicular weights (g)	1.64 ± 0.19	1.55 ± 0.13	1.46 ± 0.16	1.56 ± 0.15

Values are presented Mean (SD., *$p < 0.05$ vs. The rat weights at the beginning of the experiment; paired samples T test (The number of subjects (n) for each group: $n = 6$).

Table 4. The percentage of Johnsen's score in each group.

Johnsen's score (%)						
Group	Score 6	Score 7	Score 8	Score 9	Score 10	Sig. relation*
Control	–	–	2	20	78	$p < 0.001$, vs. DM group
DM	6	38	40	12	4	$p < 0.001$, vs. Control and EA group
EA	–	–	6	26	68	$p < 0.001$, vs. DM group
DM + EA	2	18	62	14	4	$p < 0.001$, vs. Control and EA group

*Post-hoc paired comparisons after Kruskal–Wallis test (The number of subjects (n) for each group: $n = 6$).

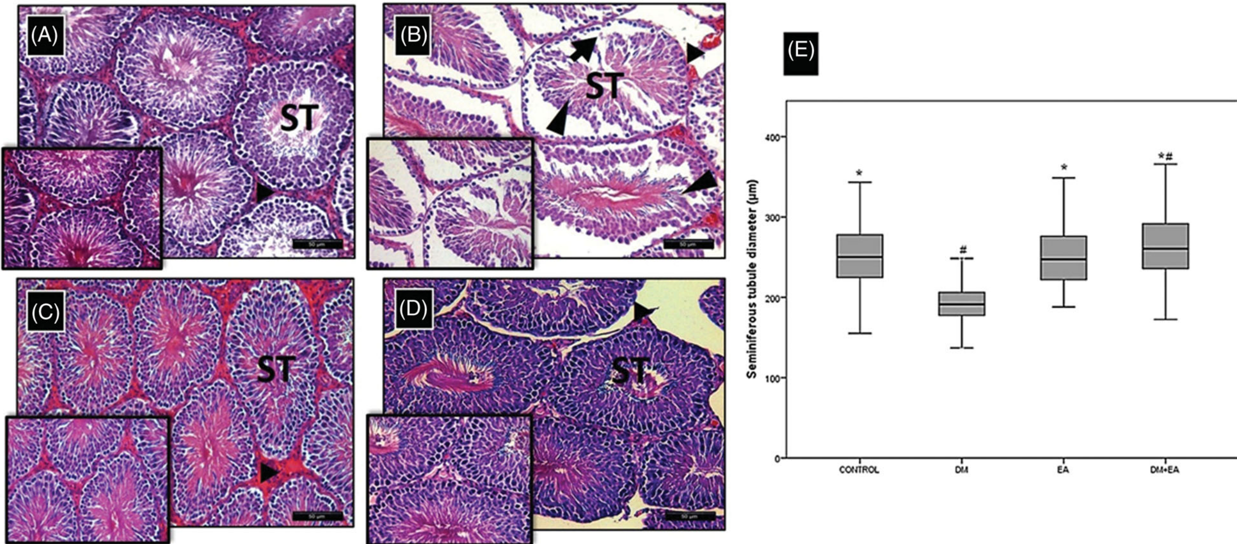

Figure 1. Histological structure of rat testis. (A) Control; (B) DM; (C) EA and (D) DM + EA groups. Seminiferous tubules (ST), interstitial connective tissue (Δ), germ cell degeneration (➔), irregularity in spermatogenic cell line (Δ).(HE, Magnifications: A–D × 200; inset × 400) (E) The seminiferous tubule diameter in each group. *$p < 0.05$ vs. DM group; # $p < 0.05$ vs. control group, for 6 animals in each group. Post-hoc paired comparisons after Kruskal–Wallis test.

seminiferous tubules compared to DM group, whereas spermatogenic series cells were in normal arrangement in some areas. Interstitial connective tissue integrity was observed in some places (Figure 1(D)).

3.5. Seminiferous tubule diameter measurement results

The seminiferous tubule diameters were compared between the groups, no statistically significant difference was observed between the control group and ellagic acid ($p = 1.000$). There was statistically significant difference between the control group and all other groups. When the DM group was compared with DM + EA group, a statistically significant difference was observed ($p < 0.001$) (Figure 1(E)).

3.6. Apoptotic results

When TUNEL positive cell ratios were evaluated statistically, DM group increased significantly when compared with the control group, EA group and DM + EA group ($p < 0.001$; $p < 0.001$ and $p = 0.044$, respectively). Also, control group decreased significantly when compared with DM group and DM + EA group ($p < 0.001$ and $p = 0.047$, respectively). The difference between the control and EA groups was not statistically significant ($p = 1.000$) (Figure 2).

3.7. Immunohistochemical results

When the Nrf2 findings were evaluated, the immunopositivity of Nrf2 appeared in spermatocytes and spermatids. In the DM group, the immunopositivity rate was lower than the control group, but the difference between them was statistically insignificant ($p > 0.05$). Same way, when the DM + EA group, the immunopositivity rate was lower than the control group, but the difference between them was statistically insignificant ($p > 0.05$). However, the closest value to the control group was in the DM + EA group (Figure 3). Nrf2 findings presented immunohistochemically showed compatibility with gene expression findings.

3.8. mRNA transcript levels of antioxidant-related genes

The mRNA levels of target genes (CAT, HO1, Mn-SOD, NQO1 and Nrf2) were evaluated by qRT-PCR (Figure 4). CAT, HO1, MnSOD and NQO1 mRNA levels of the DM group showed a statistically significant decrease compared to the control group ($p < 0.001$). There was no difference in expression of mRNA of these genes between control and other groups ($p > 0.05$). A statistically significant decrease was observed in the expression levels of genes belonging to the DM group compared to other groups. CAT, HO1, MnSOD and NQO1 mRNA levels of DM group showed a statistically significant decrease compared to EA group ($p = 0.012$; <0.001; 0.032; 0.044, respectively). Likewise, the CAT, HO1, MnSOD and

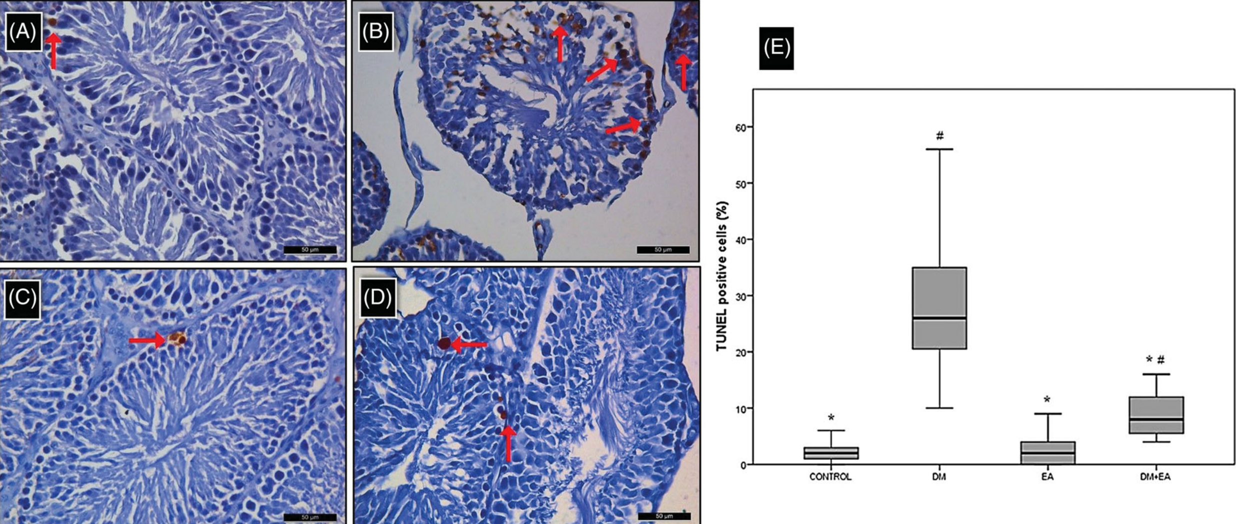

Figure 2. Representative photomicrographs of apoptotic cells by TUNEL method in the rat testis of experimental groups. (A)Control; (B) DM; (C) EA; (D) DM + EA groups. The number of TUNEL positive cells (arrow) was reduced in the DM + EA group compared with the DM group. (Magnifications: A–F × 400) (E) The percentage of TUNEL positive cells in each group, for 6 animals in each group. $*p < 0.05$ vs. DM group; # $p < 0.05$ vs. control group; post-hoc paired comparisons after Kruskal–Wallis test.

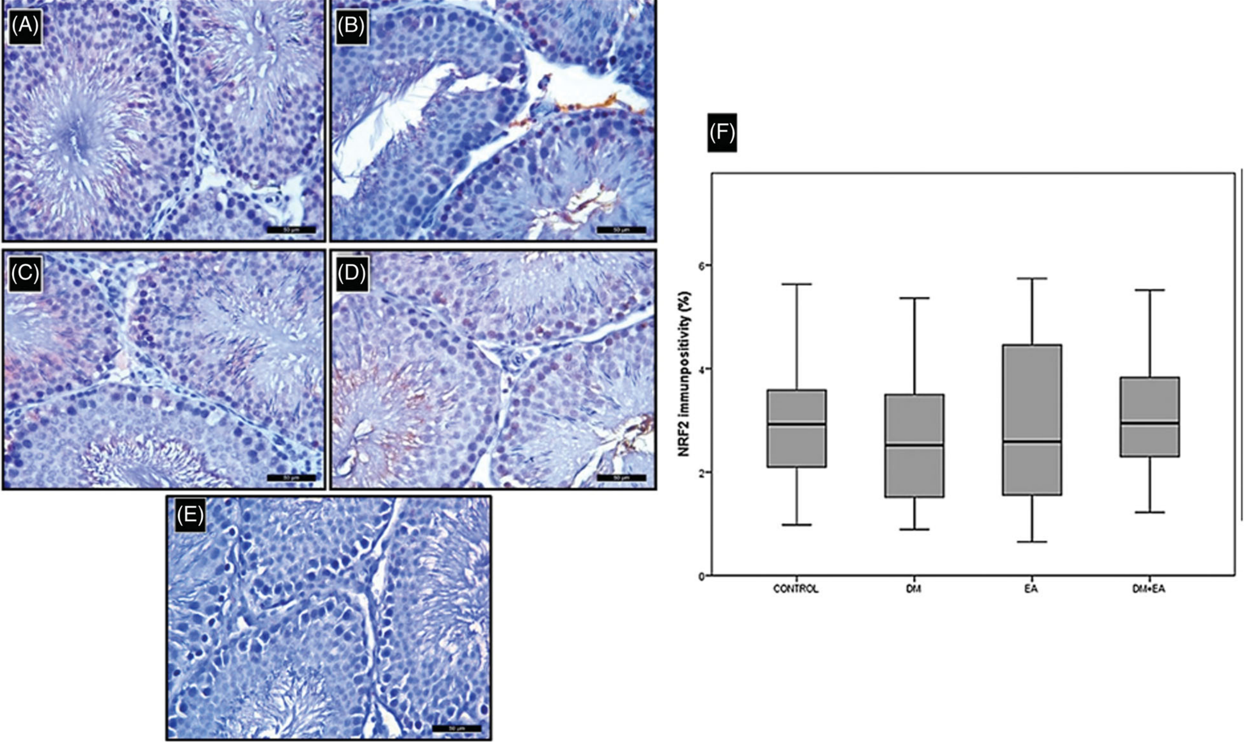

Figure 3. Testicular sections of immunohistochemical staining of Nrf2. (A)Control; (B) DM; (C) EA; (D) DM + EA groups. (E) Negative control (no primary antibody). (F) The percentage of Nrf2 immunpositivity in 6 animals each group. The difference between groups was not statistically significant (Kruskal–Wallis Test).

NQO1 mRNA levels of the DM group showed a statistically significant decrease compared to the DM + EA group ($p < 0.001$; 0.004; <0.001; 0.004, respectively).

The mRNA levels of Nrf2 decreased in the DM group compared to the control, EA and DM + EA groups, but this decrease was not statistically significant ($p > 0.05$). Although the mRNA levels of Nrf2 increased in DM + EA group, when the control group was compared to DM + EA group, the difference between them was not statistically significant ($p > 0.05$) (Figure 4).

4. Discussion

Diabetes has several detrimental effects on male reproductive system associated with maturation of sperm, spermatogenesis and fertilization (Ding *et al.* 2015, Shi *et al.* 2017). Previous studies have shown that the increase in oxidative stress has an important factor in diabetes (Evans *et al.* 2005). Although the antioxidant properties of EA had been shown in some previous studies (Barch *et al.* 1994, Han *et al.* 2006, Aslan *et al.* 2020), we did not find a study examining the

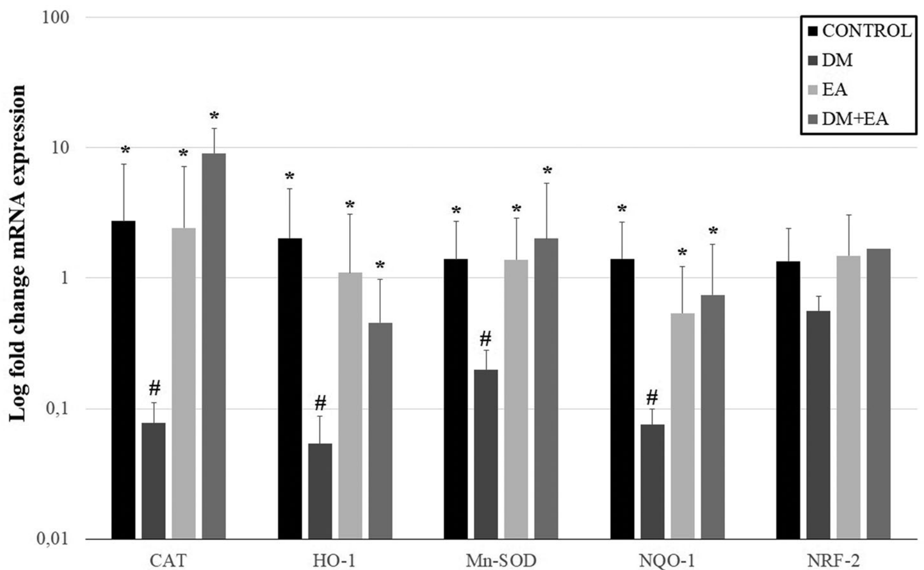

Figure 4. The transcription of CAT, HO1, MnSOD, NQO1 and Nrf2 were measured by real-time PCR. $*p < 0.05$ vs. DM group; # $p < 0.05$ vs. control group, for 6 animals in each group, post-hoc paired comparisons after Kruskal–Wallis test.

therapeutic effect of EA on testicular damage caused by diabetes in our literature review. Thus, it was thought that EA could be used as a potential new antioxidant agent to protect against testicular damage caused by diabetes. The present study was designed to investigate the effects of EA in the testes.

The effect of EA in on glucose tolerance STZ-induced model of DM has been reported before. (Chao *et al.* 2010, da Silva Pinto *et al.* 2010, Malini *et al.* 2011, Panchal *et al.* 2013, Polce *et al.* 2018). In our study, when blood glucose levels were evaluated before STZ injection and 3 months after STZ injection no statistically significant was found in the control and EA groups. However, after STZ administration, a statistically significant increase was observed in the DM group compared to the initial values. Although this increase was statistically significant in the DM + EA group, this value was lower than in the DM group. The results of our study showed that the with administration of EA decreases blood-glucose levels in the diabetic rats.

Histopathological examination showed that the diameter of the seminiferous tubule decreased, disordered arrangement, degeneration in the seminiferous tubules and germ cells. The Johnsen score in the diabetic rat testicles was noted to be significantly low. Previous studies on STZ-induced diabetes have shown similar histomorphological and Johnsen score results in testicular tissue (Guneli *et al.* 2008, Karaca *et al.* 2015, Rashid and Sil 2015, Roshankhah *et al.* 2019, Tian *et al.* 2021). In our study, in the testicular tissues of the diabetic group treated with ellagic acid, germinal epithelial irregularity was reduced in the seminiferous tubules compared to the DM group, while in some areas spermatogenic serial cells were in normal order. It was found that tubular diameter values and the Johnsen score in diabetic testicular tissues increased with EA treatment.

Apoptotic cell death in the seminiferous tubules is an important cellular event on testicular dysfunction in diabetic rat (Cai *et al.* 2000). Based on previous studies, TUNEL-positive cells were shown to be significantly higher in diabetic rats than in the control rats (Guneli *et al.* 2008, Zhao *et al.* 2011, Roshankhah *et al.* 2019). In our study, apoptosis was demonstrated by TUNEL method. With these findings, apoptosis showed a statistically significant increase in the DM group compared to other groups; it was also noted that with the treatment of ellagic acid, apoptosis are reduced.

There are many studies evaluating the activity and expression of antioxidant enzymes in STZ-induced diabetes. In these studies, the activity of antioxidants in the DM group was found to be low (Horie *et al.* 1997, Matés *et al.* 1999, Sindhu *et al.* 2004). There are some members of the antioxidant response element group such as HO1, MnSOD, NQO1 and Nrf2 to investigate the effect of antioxidants on the diabetic condition in testis (Jiang *et al.* 2014, Artimani *et al.* 2018, Nna *et al.* 2019). Some researchers have shown that Nrf2 signaling pathways are a key signal pathway in the process of oxidative stress injuries (Bae *et al.* 2016). Nrf2 signaling pathways are inhibited in diabetic conditions and this condition exacerbates oxidative stress damages in diabetic patients (Wang *et al.* 2014). Nrf2 activates the transcription of antioxidant genes that work as a free radical scavenger from diabetes, such as heme oxygenase-1 (HO-1) and NAD (P) H dehydrogenase quinone 1 (NQO1). Nrf2 interacts with antioxidant sensitive elements (ARE), regulating the transcription of antioxidant enzymes. ARE domains (quinone oxidoreductase, NQO1) contain hemeoxygenase-1 (HO-1) and glutamylcysteine ligase (GCL) (Ha *et al.* 2006). HO-1 is one of the most important endogenous antioxidant protective factors in the body and plays a regulatory role in protection and reduction of oxidative stress injuries in many pathophysiological processes (Zhao *et al.* 2018a). In one study, testicular oxidative stress was evaluated in diabetes. When they evaluated Nrf2 protein expression in testicular tissue, it was stated that the

Nrf2 expression in the DM group decreased compared to the control group (Zhao *et al.* 2011).

In a study examining the antioxidative effect of Resveratrol on the diabetic testicular tissue, the expression of markers such as SOD, CAT, NQO1 and HO1 was analyzed. In this study, researchers showed that mRNA expressions of these genes were significantly reduced in diabetic testicular tissue. This change was the same for Nrf2 expression. They found that antioxidant markers and Nrf2 expression increased with Resveratrol application and apoptotic index decreased compared to diabetes group (Zhao *et al.* 2018b).

In our experiment we used CAT, HO1, MnSOD, NQO1 and Nrf2 markers as antioxidants. When the mRNA levels of the markers in the antioxidant pathway in our study were evaluated, it was found that CAT, HO-1, Mn-SOD, NQO1 and Nrf2 mRNA expressions in the DM group decreased compared to the control group. This decrease in the DM group was statistically significant compared to all other groups in the expression of CAT, HO-1, Mn-SOD and NQO1 genes. In addition, it was observed that all these markers increased statistically significantly in DM + EA group compared to the DM group with EA treatment. It was noteworthy that the levels of antioxidant markers evaluated in the study approached normal levels as a result of ellagic acid administration. Nrf2 mRNA expression decreased in the DM group; It was found that expression increased with EA treatment. However, this decrease and increase was not statistically significant.

Following treatment of EA in STZ-induced diabetic rats, increased the testicular levels of antioxidants markers suggesting EA's ability to neutralize or minimalize STZ induced oxidative stress. EA may be helpful in the protection against diabetes associated testis injury via the the antioxidant capacity and reduced of apoptosis.

5. Conclusion

In conclusion, it has been shown that antioxidant activities increase both in gene expression and protein expression in testicular tissue with EA treatment. The results of this study support the possibility that EA can be used as a potential agent for the treatment of diabetic testicular dysfunction. It was observed that the expression of HO1 and NQO1 decreased with the decrease in Nrf2 in the DM group, and the increase in the expression of these genes compared to the DM group with the increase in the Nrf2 in the treatment group. Therefore, it can be said that Nrf2 plays a role in the increase of HO1 and NQO1 with EA treatment. It is thought that the applied dose and duration of ellagic acid may be effective on this finding or it would be more informative to investigate different signal pathway mechanisms in the relationship between diabetes and ellagic acid.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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