GAVIN PUBLISHERS

OPEN ACCESS

Journal of Urology and Renal Diseases

Research Article

Genç Y, et al. J Urol Ren Dis 06: 1227.

DOI: 10.29011/2575-7903.001227

An Examination of the Effects of Ethyl Pyruvate on the Rat Testes in an Experimental Diabetes Model

Yavuz Genç[1], Başak Baykara[1], Burcu Açıkgöz[2], Selma Aydemir[1*], Filiz Yılmaz[1] and Müge Kiray[2]

[1]Department of Histology and Embryology, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey

[2]Department of Physiology, Dokuz Eylül University, Faculty of Medicine, Division of Behavioral Physiology, Izmir, Turkey

[*]**Corresponding author:** Selma Aydemir, Department of Histology and Embryology, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey

Citation: Genç Y, Baykara B, Açıkgöz B, Aydemir S, Yılmaz F, et al. (2021) An Examination of the Effects of Ethyl Pyruvate on the Rat Testes in an Experimental Diabetes Model. J Urol Ren Dis 06: 1227. DOI: 10.29011/2575-7903.001227

Received Date: 10 June, 2021**; Accepted Date:** 24 June, 2021**; Published Date:** 29 June, 2021

Abstract

Diabetes Mellitus (DM), commonly known as diabetes, is an acute and chronic multisystemic disease causing disorders in male reproductive system. Elevated glucose methabolism is associated with the impairment mechanisms of steriodogenesis, spermatogenesis, sperm maturation and sexual erection. Maintenance of male fertility relies on diminished risk of testicel complication development. Ethyl Pyruvate (EP), a highly permeable ethyl ester, is simple derivative of pyruvic acid. EP has a potential scavenger ability against reactive oxygen species owing to its anti-oxidative, anti-inflammatory, anti-apoptotic and cytoprotective functions. Therefore, we investigated the effects of EP on male rat with diabetes induced testicular damage by histochemical and biochemical analysis. In the DM group, we observed with loss of spermatogenetic cells and spermiums, and rupture between basal membrane and spermatogenetic cells, as well as increased connective tissue and basal membrane. Administration of EP reduced testicular histologocal findings compare to the DM group. We determined that the number of apoptotic cells (TUNEL and A. caspase 3 positive cell) significantly increased in the DM group. We observed a significant decrease in the number of apoptotic cells in the DM+EP group compared to DM group. Elevated Malondialdehyde level was obtained in DM group. Glutathione and glutathione peroxidase decreased significantly compared to the control group. The observations suggested that EP treatment alleviates the increased reactive oxygen species levels and inhibits the apoptotic pathway in the DM+EP group.

Keywords: Diabetes mellitus; Ethyl pyruvate; Rat; Testes

Introduction

Diabetes Mellitus (DM), commonly known as diabetes, is an acute and chronic multisystemic disease. Diabetes is associated with autoimmune destruction of the β-cells of pancreas and/or insulin resistance in tissues. High blood glucose is among the prominent markers of diabetes. Deficient insulin action (inadequate insulin secretion) and reduced tissue response to insulin lead to hyperglycemia [1]. Based on epidemiological studies, high prevalance of diabetes and impaired fasting glucose level result from genetic predisposition, environmental, socioeconomic and cultural factors [2]. Diabetes is associated with acute and chronic complications. Major complications include cardiovascular disease, nephropathy and retinopathy [2,3]. Diabetes can also lead to adverse changes in male reproductive system functions It contributes to disturbance in physiological functions of spermatogenesis, testicular steroidogenesis and male infertility. Therefore, diabetic men have decreased sperm quality including motility, viability, concentration and normal morphology [4].

Diabetes is associated with adverse effects of male reproductive system. Increased glucose metabolism may cause dysfunctions and failures of testes [5]. Therefore, effects of diabetes can be seen in the increased prevalence of infertility. Diabetes-induced male infertility can occur via pre-testicular, testicular, and post-testicular mechanisms. At pre-testicular level, hypogonadal patients with diabetes have low levels of serum gonadotropin and testeron because of the changes of Leydig cell function and GnRH hormone levels. At testicular level, diabetes leads to increased ROS and lipoperoxidation, may cause DNA fragmentation, enzymatic glycation and products. [6] In addition, possible mechanisms at post-testicular level lead to sperm damage and male accessory gland infection/inflammation. These effects cause a variety of complications, including erectile and/or ejaculatory dysfunction, by altering semen deconvential sperm parameters and widening the inflammatory process [7-9].

DM1 triggered damages in sperm nuclei results in a high degree of nuclear DNA fragmentation, mitochondrial DNA proliferation, and changes in the mitochondrial respiration chain, and reduced sperm motility. Altered sperm plasma membrane

and acrosome by serum insulin resistance were shown in diabetic patient biopsies. Therefore, testicular changes may occur during spermatogenesis in response to insulin resistance or insulin deficiency. In diabetic human and experimental animal studies, abnormal spermatogenesis in testes due to degenerative and apoptotic changes, impaired blood-testicular barrier, decreased testosterone synthesis and secretion, erectile and ejaculatory dysfunction were reported [10].

Hyperglycemia and inflammation in diabetes stimulate the formation of reactive oxygen or nitrogen species (ROS or RNS). Increased radicals and inadequate endogenous antioxidant defense cause oxidative stress in many organs. ROS accumulation and oxidative damage lead to stimulation of apoptosis and sperm dysfunction. Diabetes-induced testicular apoptosis may be associated with intrinsic cell death pathways such as mitochondrial and Endoplasmic Reticulum (ER) stress [10]. This stress could be caused by increased oxygen radicals and the damages caused by metabolic disorders. Therefore, preventing increased ROS/RNS formation or increasing endogenous antioxidants can be important to prevent many diabetic complications such as DM-associated male infertility [6]. However, in animal experiments and clinical studies, exogenous antioxidant supplementation has been shown as no significant therapeutic effect on human diabetic complications [1]. Therefore, up-regulation of endogenous antioxidants may be a more effective approach to prevent or treat diabetic complications [6-10].

Pyruvic acid is an organic acid, conjugate of acid of pyruvate in glycosis, which has many physiological properties, including potent anti-inflammatory and antioxidative effects. Ethyl Pyruvate (EP) is a derivative ethyl ester of pyruvic acid, which was firstly described as a potentially good compound by Yang, et al. (2016) [11]. In addition, EP were approved as a safe compound from the Food and Drug Administration (FDA). EP has been known as a strong anti-inflammatory, anti-bacterial, anti-viral, anti-allergic and vasodilator agent. In addition, several experimental studies have confirmed its potent antioxidant and antiapoptotic effects. EP is a scavenger of ROS such as hydrogen peroxide, and has been shown as an anti-apoptotic, a necroptotic and a cytoprotective compound against cellular damage [12]. Based on the many recent studies suggest that EP may be a pluripotent pharmacological agent due to its anti-inflammatory, anti-coagulation and anti-oxidative properties. In this study, we aimed to investigate whether EP has potential benefical effect on testes with histochemical (Hematoxylin-Eosin, Masson Trichrome, Periodic Acid Schiff (PAS), immunohistochemical activate Caspase-3, and TUNEL, and biochemical analyses [Malondialdehyde (MDA), Glutathione (GSH) and Glutathione Peroxidase (GPx)].

Methods

Ethics Statement

This study was approved by the Dokuz Eylul University Animal Experiments Local Ethics Committee (Protocol no. 19/2016). All experiments were performed in March 2017 in Dokuz Eylül University Experimental Animal Laboratory.

Animals

The experiment was performed using 28 healthy 10–12-week-old Wistar Albino male rats (weight:200-250 g). The rats were obtained from the Medicine Experimental Animal Laboratory of Dokuz Eylül University. All animals were housed in the standard animal room (20–22°C). The animals were kept under 12/12 hours of dark/light periods and a standard rat diet ad libitum.

Experimental design

Male Wistar Albino rats (n=28) were randomly divided into four groups. Control group (C, n=7) was not received any treatment; Ethyl Pyruvate Group (EP, n=7) was received Ethyl pyruvate 50mg/kg i.p enjection twice a day for 2 weeks; Diabetes Mellitus Group (DM, n=7); and Ethyl Pyruvate-treated Diabetes Mellitus Group (DM+EP, n=7) induced by a single dose injection streptozotocin (45 mg/kg, i.p.) (STZ, Sigma, USA). Ethyl pyruvate 50 mg/kg i.p injection twice a day for 2 weeks. All animals were (anesthetized with an i.p injection of ketamine (45 mg/kg) and xylazine (10 mg/kg) [13-18].

Histological methods

Testes tissues were fixated in 10% formaldehyde for 48-hours. Then by applying routine tissue processing procedure, the tissues were embedded in paraffin blocks. 5 μm sections were stained Hematoxylin-Eosin (H&E); testicular connective tissue, capsule (tunica albigunea), seminiferous tubule diameter, seminiferous tubule epithelium, leydig cells and general testicular morphology were examined [18]. Sections were stained with PAS in order to examine basement membrane thickness of seminiferous tubules. Then, Masson's-Trichrome staining was performed to examine the capsule thickness. The activities of Active caspase-3 and germ cell apoptosis were detected by TUNEL assay. Johnsen's score used to categorize the spermatogenesis, followed by light microscopy analysis (Olympus BX51, Japan) and seminiferous tubules were evaluated using Image J program. The number of TUNEL positive nuclei were examined in 10 random fields at 20x magnification [16].

Immunohistochemical methods

Immunohistochemical staining was performed on 5 μm sections. Slides were dehydrated in graded alcohol series. All slides were incubated with proteinase K for 15 min at 37 °C and applied to heat antigen retriveal with H_2O_2 for 30 min at room temperature. All slides were incubated with Active caspase-3 (1:100, cat.; AB3623, Merck Millipore, Italy) and biontinylated secondary antibody (cat.;856743, ThermoFisher, Germany) for overnight period. And then, slides were incubated with avidin-biotin prediluted complex for 20 min at room temperature. Antigenic sites were revealed by Diaminobenzidine solution (DAB) and counterstained with hematoxylin. The slides were

cover-slipped with entellan mounting medium, followed by light microscopy analysis (Olympus BX51, Japan) [17].

Apoptotic cell detection

Terminal deoxynucleotidyl Transferase (TdT) dUTP nick end-labeling (TUNEL) (Roche, Manheim, Germany) assay was used to detect apoptotic cells. The assay kit was applied to 5 µm paraffin sections. After dehydration, slides were treated with Proteinase K for 15 min at 37°C incubator. All slides were washed with Phosphate-Buffered Saline (PBS) and incubated with fluorescent labelled deoxy-UTP at 18-24°C for 5 min. And then, the slides were incubated for 1h at 37°C with TdTdNTP. After PBS washing, the slides were incubated with POD solution for 30 min. The number of apoptotic TUNEL- positive cells per seminiferous tubules was randomly selected in 10 different fields of each testes [18].

Biochemical analysis

GSH levels and GPx enzyme activity were measured in the supernatant. MDA level was measured spectrophotometrically in homogenates of the testes tissues. The tissues were homogenized using a homogenizer (Bandelin Sonoplus, Germany) containing 0.15 M KCl solution at 600 rpm for 10 min. Homogenization was performed in an ice container. The supernatant was obtained by centrifugation of the homogenate at 10000 g for 20 min. (+4°C), and was kept at – 80°C until the time of analysis (1 week). All analysis were measured spectrophotometrically (T80, PG instruments, UK). MDA levels were measured using Bioxytech MDA-586 (Oxis International, USA) assay kit. The method of the kit is based on the reaction of MDA with a chromogenic reagent at 45ºC. MDA levels were determined from the standard curve by measuring the absorbance at 586 nm. The results were expressed as µM. GSH levels were determined with Bioxytech GSH-420 (Oxis International, USA) assay kit. The method of the kit is based on the formation of chromophoric thione. The oxidized glutathione is converted into the reduced form by adding the reducing agent to the supernatant mixed with the buffer. After adding the chromogen, pH is increased to form chromophoric thione. GSH levels were determined by measuring the absorbance at 420 nm. The results were expressed as µM/mg protein. The activity of GPx was determined using Bioxytech GPx-340 (Oxis International, USA) assay kit. GPx catalyzes the oxidation of GSH with tert-butyl hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, oxidized glutathione (GSSG) is converted to reduced form GSH, while NADPH is oxidized to $NADP^+$. GSH-Px activities were determined by measuring the decrease in absorbance at 340 nm monitored by a spectrophotometer. The results were expressed as mU/mg protein. In addition, blood was taken from the tail vein at the beginning of the experiment, on the 3rd day of experiment and on the 18th day. Blood glucose levels of the blood samples were measured with a glucometer (Optima).

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 22.0 for Windows software. All the data were presented as the mean ± standard deviation and median value. The difference between the groups were analyzed with Kruskal Wallis and Mann Whitney-U test. Analysis of variance was analyzed by one-way analysis of variance (ANOVA) with the Least Significant Difference (LSD) post-hoc test. The statistically significance level was considered as $p<0.05$.

Results

Final body weight increased compare to the initial body weight in all group ($p<0.05$) (Figure 1A). Ethyl piruvat treated diabetes group showed significant difference compared to the control group ($p=0.021$). There are no significant differences on rest of the groups ($p>0.05$) (Figure 1A).

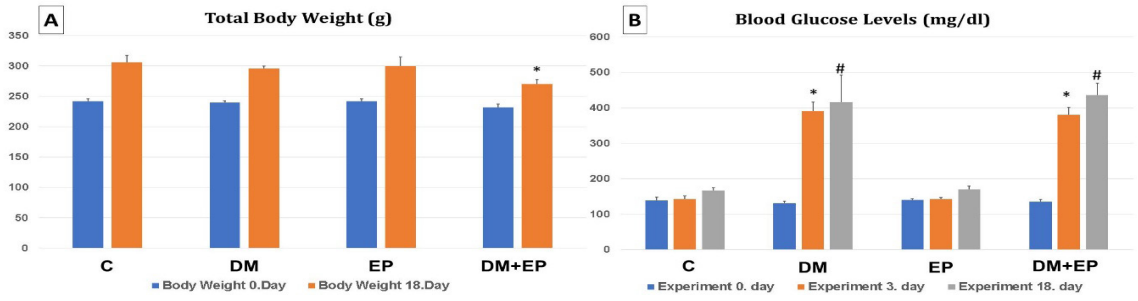

Figure 1: Comparison of the body weights and blood glucose levels in all groups (**A, B**). **(A)** Evaluation of total body weight. Asterisk (*) symbol; significant differences with comparison to to the control group. **(B)** Evaluation of the blood glucose levels. Asterisk (*) symbol; significant differences compared to the control group about the 3rd day, square (#) symbol; significant differences compared to the control group about the 18th day. (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabeties Mellitus; **EP**, Ethyl Pyruvate and **DM+EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

Biochemical Findings

Between the C group and the EP group, no significant difference was observed according to the blood glucose values on the third day and at the end of the experiment. DM and DM+EP were found to be a significantly different compared to the C group ($p<0.05$) (Figure 1B). In addition, DM and DM+EP groups showed a significant increase in blood glucose levels at the end of the experiment compared to the C group ($p= 0.02$). And then, it was shown that there was a significant difference between the DM group and the DM+EP group ($p=0.004$) (Figure 1B). MDA, GSH and GPx values of all the groups are shown in (Figure 3). MDA values of the DM group were significantly higher than those obtained in the C group ($p=0.025$) while it was dimished in the EP group compared to the DM group. There was no significant difference in the MDA values of the EP and DM+EP groups compared to the C group (Figure 2A).

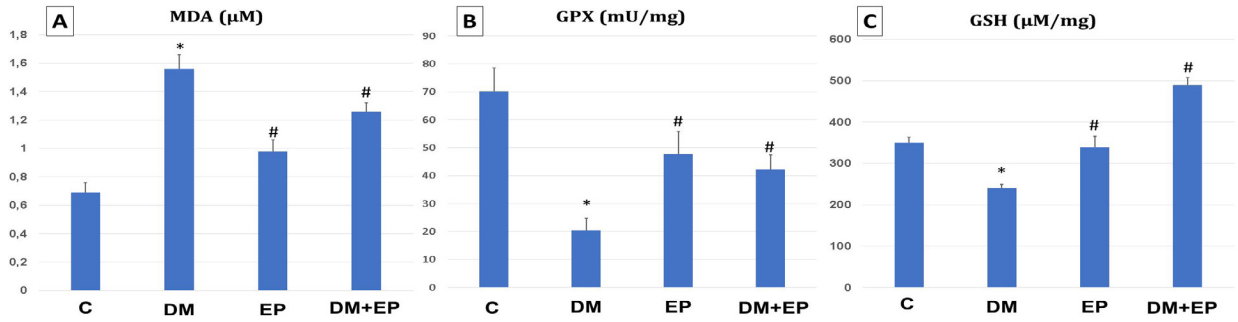

Figure 2: Biochemical analysis results of **(A)** MDA, **(B)** GPX and **(C)** GSH values. Asterisk (*) symbol; significant differences compared to the control group, square (#) symbol; significant differences compared to the DM group (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabetes Mellitus; **EP**, Ethyl Pyruvate and **DM+EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

GSH values in the DM group were significantly lower than the C group ($p<0.001$). In addition, GSH values in the DM+EP group were significantly higher than tose obtained in the DM group ($p<0.001$), and GSH values of EP group were significantly higher than DM group ($p= 0.001$) (Figure 2C). There was no significant difference in GSH values of the EP and the DM+EP groups compared to the C group (Figure 2C). Additionaly, in the DM group, the GPx values were significantly lower than the C group ($p<0.001$). Nevertheless, in the DM+EP group, the GPx values were significantly higher than the DM group ($p<0.05$), and in the EP group GPx values were significantly higher than the DM group ($p<0.05$) (Figure 2B). There was no significant difference in the GPx values of EP and DM+EP groups compared to the C group ($p>0.05$) (Figure 2B).

Light Microscope Findings

The control group's sections were examined as a normal morphology and structure. In the diabetic group, testicular tissues were wrapped with capsule on the outermost side. But large thicked wall vessels were observed around the capsule. The diameter and shape of the seminiferous tubules were found to be impaired. In addition, we were observed with loss of spermatogenetic cells and spermiums, and rupture between basal membrane and spermatogenetic cells, presence of multinucleated cells between spermatogenetic serial cells and vacuole in seminiferous tubules. Spilled cell remains were detected in many seminiferous tubule lumens. When the sections of ethyl pyruvate group were examined, testicular tissues were encapsulated from outside and seminiferous tubule diameters were similar to the control group. Normal spermatogenetic cell lines were observed in seminiferous tubules, and interstitial space was similar to the control group. The DM+EP group's sections were observed as surrounded by testicular tissue capsules like other groups (Figure 3A). Johnsen score, there was a significant diminished in the DM, EP and DM+EP groups compared to the C group (Figure 3B). Diameters and shapes of seminiferous tubules were observed similar to normal appearance (Figure 3C). In the DM group, spermatogenetic serial cells were found to be fully present and increasing number of seminiferous tubules (Figure 3). Also, leydig cells, connective tissue cells, blood and lymph vessels were found in the interstitial space (Figure 3).

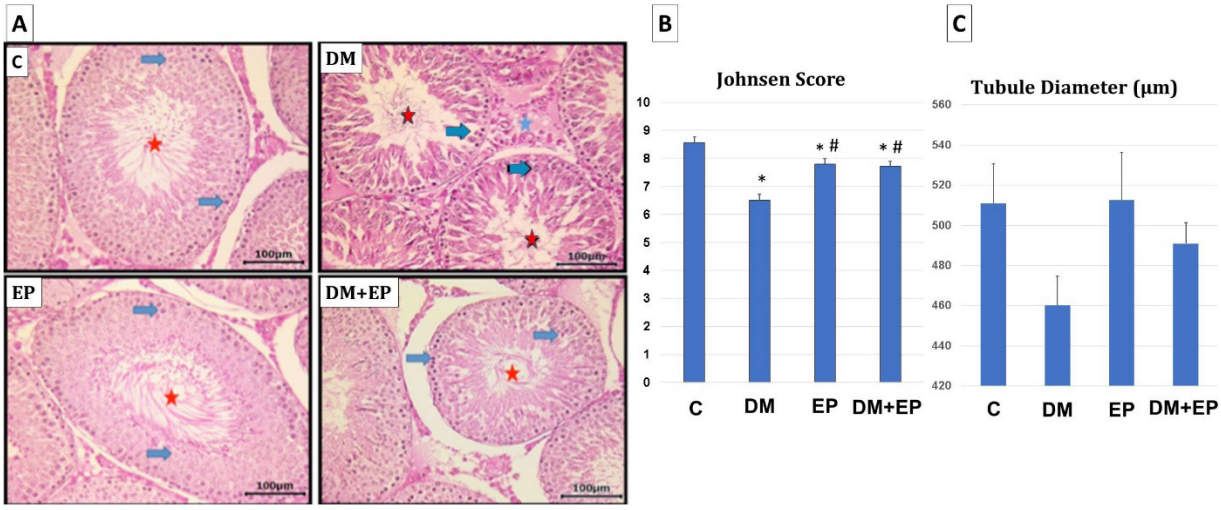

Figure 3: (A) Testes histological images show normal seminiferous tubulles, reguler spermatogenic serial cells and mature spermiums. There are vacuolations between spermatogenic series cell- spermatogenic series cell, spermatogenic series cell loss and intertisial edema (H&E). Red stars show seminiferous tubul lumen, blue arrow show intertisial area, where arrows show spermatogenic serial cells. **(B)** Johnsen score, (**C**) tubule diameter. Asterisk (*) symbol; significant differences compared to the control group, square (#) symbol; significant differences compared to the DM group. (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabetes Mellitus; **EP**, Ethyl Pyruvate and **DM+EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

When basal membrane thicknesses were evaluated; there was a significant increase in the DM group compared to the C group (p<0.001) (Figure 4A-B). No statistically significant difference was observed in the DM+EP and EP groups compared to the C group. Furthermore, DM+EP and EP groups basal membrane decreased significantly compared to the DM group (p<0.001) (Figure 4A-B). When the data obtained from all groups were evaluated, it was determined that tunica albuginea thickness increased significantly in DM group compared to the C group (p=0.018).

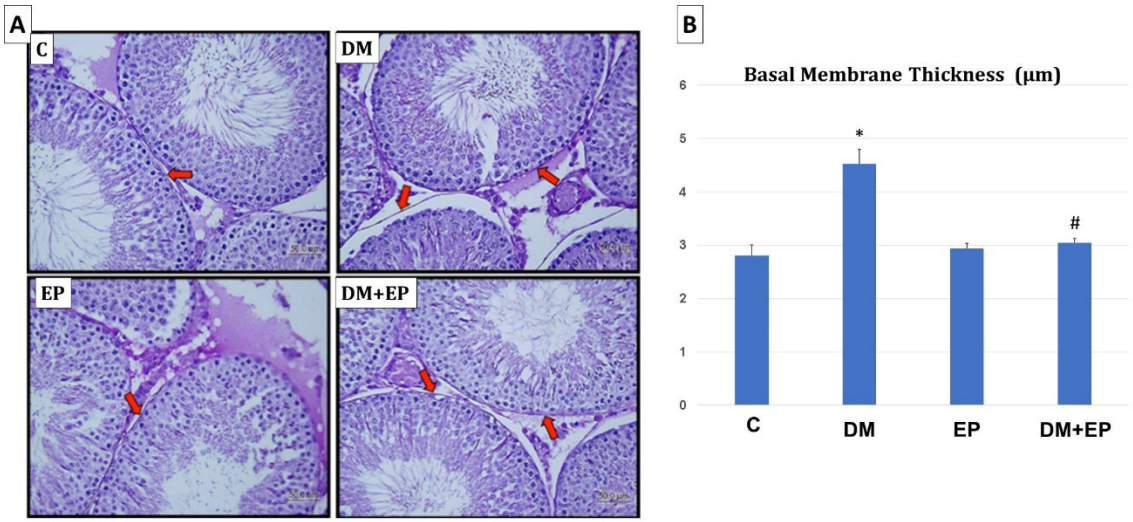

Figure 4: (A) Basement membrane structure is shown on histological images of testes. There is normal thickness membrane structure in the control and EP group sections, but basal membrane thickness increased in the DM group. The DM+EP group is similar structure to the Control group, (PAS). Red arrows show basement membrane. **(B)** Basal membrane thickness, Asterisk (*) symbol; significant differences compared to the control group, square (#) symbol; significant differences compared to the DM group. (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabetes Mellitus; **EP**, Ethyl Pyruvate and **DM+EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

In addition, there was an increase in connective tissue in the interstitial area in the DM group compared to the C group. (Figure 5A-B). No significant difference was observed in the EP and DM+EP groups compared to the C group ($p>0.05$) (Figure 5A-B). Additionally, no significant difference was observed between the groups when the DM group compared to the EP and DM+EP groups ($p>0.05$) (Figure 5A-B).

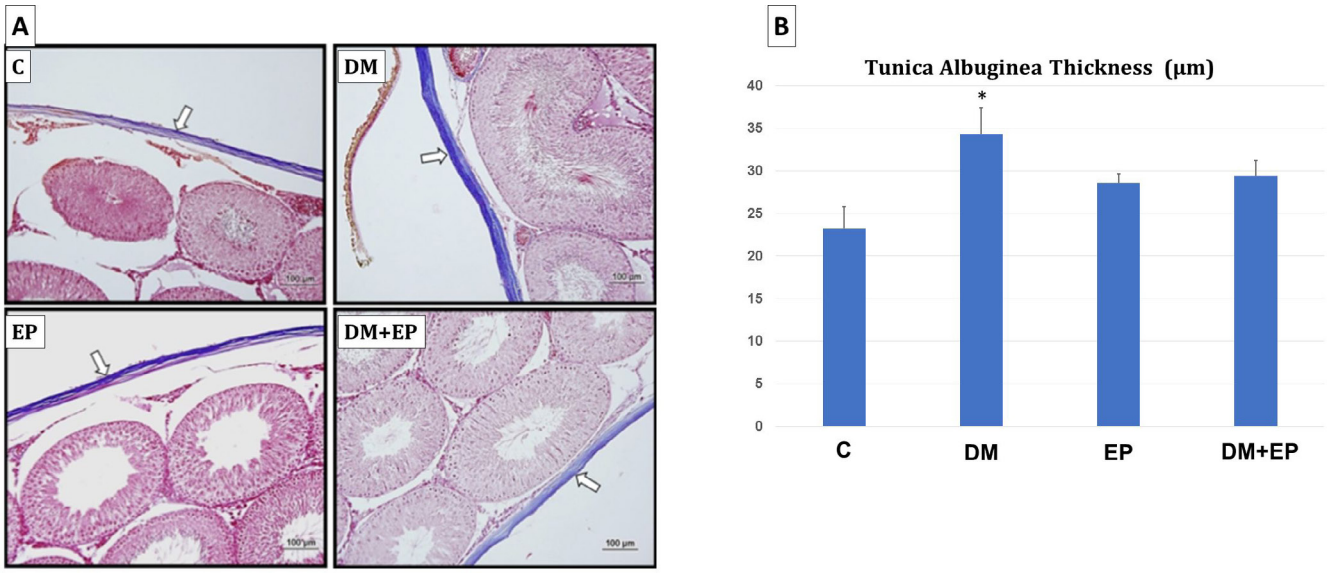

Figure 5: (A) Connective tissue structure (testicular capsule) is shown by histological images of testes. Testicular capsule is normal thickness in the control and EP groups, but testicular capsule thickness increased in the DM group. In the DM+EP group is similar thickness to the control group. (Masson's Trichrome). White arrows show testicular capsule. **(B)** Tunica albuginea thickness**,** Asterisk (*) symbol; significant differences compared to the control group. (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabetes Mellitus; **EP**, Ethyl Pyruvate and **DM**+**EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

Immunohistochemical Findings

When all the groups were compared statistically; the number of TUNEL positive cells increased significantly in the DM group compared to the C group, and TUNEL (+) cell number decreased significantly in the DM+EP group compared to the DM group ($p<0.001$) (Figure 6). When the groups were evaluated statistically; Active caspase-3 (+) cells were significantly increased in the DM, EP and EP and DM+EP groups compared to the C group ($p <0.001$) (Figure 6). We determined that Active caspase-3 (+) cell number of DM+EP and EP groups decreased significantly compared to DM group ($p<0.001$). And then, in the EP group that is the number of Active caspase-3 (+) cells significant difference was observed compared to DM+EP group ($p <0.001$) (Figure 6).

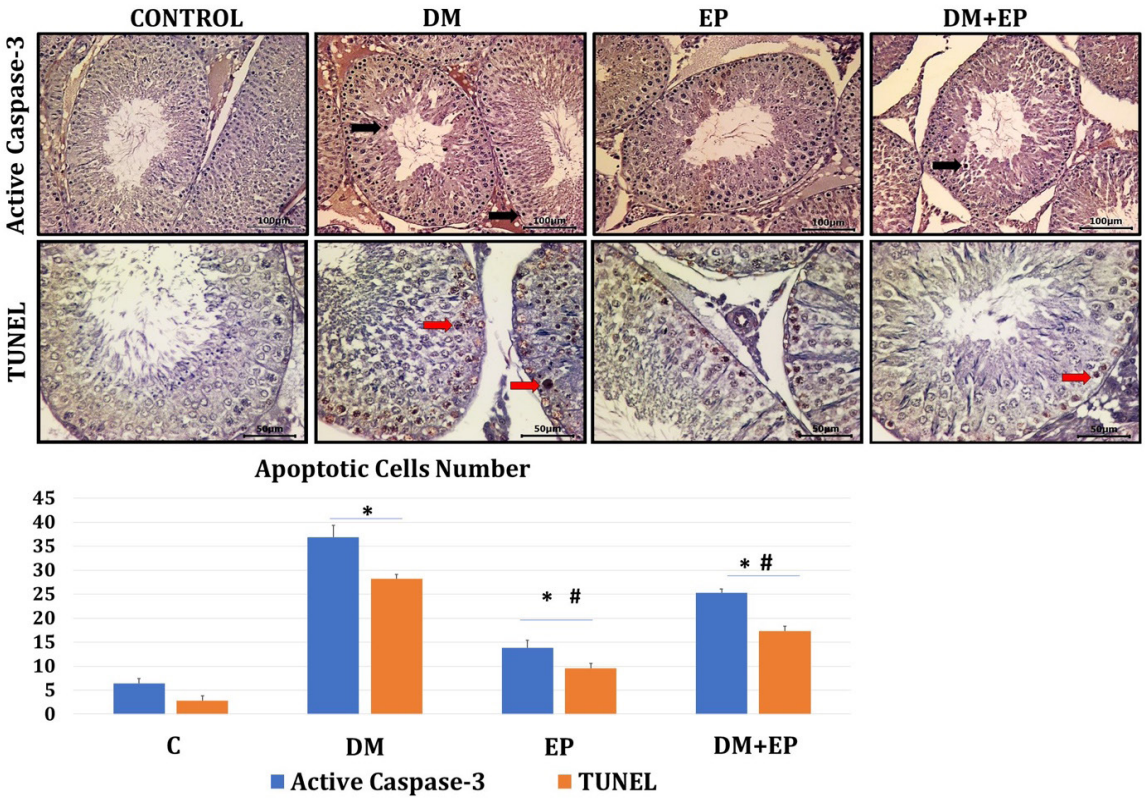

Figure 6: Active caspase-3 positive cells were shown on the histological images of testes. Apoptotic cells increased in the DM group. In the DM+EP group, apoptotic cells numbers were reduced. (Active caspase-3). Black arrows show Active caspase-3 positive cells. TUNEL positive cells were shown on histological images of testes. Positive cells increased in DM group. In the DM+EP group, TUNEL positive cells numbers were reduced (TUNEL). Red arrows show TUNEL positive cells. Apoptotic cells number, Asterisk (*) symbol; significant differences with compare to control group, square (#) symbol; significant differences with compare to the DM group. (The values are expressed as mean ± S.E.M, p <0.05). **C**, Control; **DM**, Diabetes Mellitus; **EP**, Ethyl Pyruvate and **DM**+**EP**, Ethyl Pyruvate-treated Diabetes Mellitus group.

Discussion

EP plays an important role in both hemostasis of blood glucose levels and male reproductive health. The underlying of EP mechanism is not fully known. Researchers found anti-apoptotic effects of EP *in vitro* and *in vivo* models [19]. All of this study, EP treatment is a proctective effects against the increased blood glucose level in diabetic rats. It has been revealed that it inhibits hepatic apoptosis and autophagy via the anti-inflammatory and anti-apoptotic mechanisms [20,21]. Furthermore, in the hypoxic brain injury, neuroprotective properties of ethyl pyruvate are related to the anti-apoptotic and anti-necrotic mechanisms [22]. In the cyclophosphamide study, EP was shown a protective effect on testicular diorders and improve of increased germ cell apoptotic activity [23]. These results suggest that EP may regulate the production of free radicals in inflammatory/inflamation of testicular injury caused by diabetes. Therefore, we can control oxidative stress due to homeostasis of balance between antioxidants and oxidants [24-26].

In the previous studies, the ratio of testes weight to body weight and body weight were found decreased in DM group. When we compare to 0-day body weight to 18-day body weight, there was a significant difference all the groups. Over-production of ROS plays a crucial role on damaging of male reproductive system [27]. Especially, these effects cause damage to the spermatogenetic cells, sertoli cell and capillary endothelium. The most common histological changes in male infertility, including degradation of seminiferous tubules, increased polynucleated cells and degenerated cells, vacuole formation, desquamation, interstitial edema, loss of leydig cells, decrease in spermatogenetic serial cells and number of spermatids in the lumen of seminiferous tubules [28-30]. We observed similar finding in DM group, increased basal membrane and connective tissue of the tunica albuginea. These observations indicate that testicular damage might cause by production of mainly proinflammatory cytokines and chemokines. High lipid peroxidation and excessive production of ROS may play a role in the mechanism of testicular degeneration of male infertility.

ROS plays an important role in many physiological processes such as sperm capacitation and hyperactivation, and sperm-oocyte fusion. Increased ROS production causes extensive sperm DNA damage and sperm dysfunction [31]. Numerous previous studies have demonstrated significantly increased apoptotic cell in the diabetes group compared to low levels of testicular apoptotic cell death during normal spermatogenesis. The negative effects of diabetes, we found a significant increase in the number of apoptotic cells in the diabetic group compared to the control group [32-34]. In diabetic group treated with strong antioxidant ethyl pyruvate, apoptotic index was significantly decreased compared to diabetic group. Previous studies have demonstrated, ethyl pyruvate may inhibit systemic inflammation by reducing TNF alpha and IL-8 production due to blocked p65NF- κB pathway in macrophages cells. These results demonstrated that EP inhibits NF- κB/STAT3 pathway [35]. For this reason, we used EP antioxidant that can inhibit DNA-binding due to actived intrinsic apoptotic pathway in the diabetes group. In additon to these, the risk of diabetes could be reduced by EP providing anti-inflammatory effects as an antioxidant agent. Diabetes lead to high glucose due to impaired pancreatic beta cell, which results in dysfuntion of many organs. STZ-induced diabetes model studies consistenly exhibited a high glucose levels in diabetic group compared to the control group. Similarly, we found significantly high blood glucose levels in the DM group [32].

MDA has been protective function to cell survival and signaling mechanism of mitochondrion. High level MDA plays essential cytotoxic role in inhibition of gene expression that leads to cell death. Indeed, elevated level of free radicals or ROS may inflict damage to lipids. Several studies have shown that MDA levels significantly increased in STZ-induced diabetic rats compared to the control group [32,33]. Researchers evaluating the efficacy of EP as a protective agent against the side effects of chemotherapeutic agents. MDA could prevent the damage by inhibiting lipid peroxidation and increasing the activity of antioxidant enzymes [36]. EP as a non-enzymatic antioxidant can protect the reproductive organs against the negative effects of DNA damage, reduced sperm count and decreased sperm viability and reduced testosterone levels by removing free radicals [37]. In our study, the MDA values of the diabetes group increased significantly compared to the other groups. Furthermore, there is no significant difference between the EP groups (EP, DM+EP) and control group. High levels of MDA in the diabetes group showed that diabetes caused oxidative damage to testicular tissue. EP treatment reduced oxidative damage to EP treated DM group.

Some studies revealed that GPx is one of the antioxidant defense system enzymes. GPx activity decreased due to increased oxidative stress with diabetes [38]. In addition, the administration of ethyl pyruvate in diabetic rats has been shown to increase the regulation of glutathione peroxidase mRNA activation in antioxidant mechanisms, activation of different anti-apoptotic pathways, suppression of mitochondrial DNA damage and protein degradation [38,39]. The GPx analyze values of our study were examined, we demonstrated that the diabetes group was significantly lower than the control group. We observed an increase in enzyme levels in rats treated with ethyl pyruvate. In particular, GSH is the most important antioxidant of the cell in defence against ROS. Studies have shown that increased ROS and nitrogen species are effective in rapidly decreasing cellular GSH levels with diabetes [38-40]. So, reduced GSH that caused impairment balance of antioxidant and oxidant, and increased hydrogen peroxide and wide range of many reactive radicals. In our study, we found a significant decrease in GSH level in the diabetes mellitus group compared to the control group. Unlike, the EP treated diabetes group gave high level of GSH. More integrative approaches were shown that EP is pro- and antioxidant reactions inside the cell.

Conclusion

In our study, EP treatment ameliorate diminish increased ROS, and inhibit apoptotic pathway in the diabetes groups. In addition, EP function may able to potentional prevent the lipid peroxidation and defense in against oxidant system due to indicated by enzymatic and nonenzymatic antioxidant system. Also, EP improves the oxidative/antioxidant imbalance caused by diabetes in testicular damage.

Acknowledgements

This research was a part of Master thesis, which was granted by the Dokuz Eylül University of Medicine Faculty. The authors especially appreciate TUBITAK to financial support this study and who helped that providing useful information and the laboratory facilities.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, autorship, and/or publication of this article.

Funding

The author(s) discolsed receipts of the following financial support for the research, authorship and/or publication of this article; this work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (Funding number: 216S259)

References

1. Jiang X, Bai Y, Zhang Z, Xin Y, Cai L (2014) Protection by sulforaphane from type 1 diabetes-induced testicular apoptosis is associated with the up-regulation of Nrf2 expression and function. Toxicol Appl Pharmacol 279: 198-210.
2. International Diabetes Federation (2017) IDF Diabetes Atlas 8th edition.
3. Ersoy O, Kizilay G (2018) Effects of fucoidan on diabetic rat testicular tissue. Biotech Histochem 93: 277-285.

4. Sampannang A, Arun S, Burawat J, Sukhorum W, Lamsaard S (2018) Testicular histopathology and phosphorylated protein changes in mice with diabetes induced by multiple-low doses of streptozotocin: An experimental study. Int J Reprod Biomed 16: 235-246.

5. Ghanbari E, Nejati V, Najafi G, Khazaei M, Babaei M (2015) Study on the effect of royal jelly on reproductive parameters in streptozotocin-induced diabetic rats. Int J Fertil and Steril 9: 113-120.

6. Liu J, Litt L, Segal MR, Peltın JG, Kim M (2011) Metabolomics of oxidative stress in recent studies of endogenous and exogenously administered intermediate metabolites. Int J Mol Sci 12: 6469-6501.

7. Condorelli RA, La Vignera S, Mongioì LM, Alamo A, Calogero AE (2018) Diabetes Mellitus and Infertility: Different Pathophysiological Effects in Type 1 and Type 2 on Sperm Function. Front Endocrinol (Lausanne) 9: 268.

8. Pomjunya A, Ratthanophart J, Fungfuang W (2017) Effects of Vernonia cinerea on reproductive performance in streptozotocin-induced diabetic rats. J Vet Med Sci 79: 572-578.

9. Sefidgar SM, Ahmadi-Hamedani M, Jebelli Javan A, Narenji Sani R, Javaheri Vayghan A (2019) Effect of crocin on biochemical parameters, oxidative/antioxidative profiles, sperm characteristics and testicular histopathology in streptozotocin-induced diabetic rats. Avicenna J Phytomed 9: 347-361.

10. El-Behery EI, El-Naseery NI, El-Ghazali HM, Elewa YHA, Mahdy EAA, et al. (2019) The efficacy of chronic zinc oxide nanoparticles using on testicular damage in the streptozotocin-induced diabetic rat model. Acta Histochem 121: 84-93.

11. Yang R, Zhu S, Tonnessen TI (2016) Ethyl pyruvate is a novel anti-inflammatory agent to treat multiple inflammatory organ injuries. J Inflamm (Lond) 13: 37.

12. Bahadir MV, Yildirim Y, Baran OP, Polat S, Akkoc H, et al. (2016) The potential beneficial effects of ethyl pyruvate on diabetic nephropathy: an experimental and ultrastructural study. Pol J Pathol 67: 250-257.

13. Navarro-Casado L, Juncos-Tobarra MA, Cháfer-Rudilla M, de Onzoño LÍ, Blázquez-Cabrera JA, et al. (2010) Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. Journal of Andrology 31: 584–592.

14. Büyükleblebici O, Karagül H (2012) The biochemical effects of dietary chromium in experimental diabetic rats with streptozotocin. Kafkas Univ Vet Fac Journal 18: 21-26.

15. Ramzy MM, El-Sheikh AA, Kamel MY, Abdelwahab SA, Morsy MA (2014) Mechanism of testicular protection of carvedilol in streptozotocin-induced diabetic rats. Indian J Pharmacol 46: 161-165.

16. Johsen SG (1970) Testicular biopsy score count-a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1: 2-25.

17. Elgawish RAR, Abdelrazek HMA (2014) Effects of lead acetate on testicular function and Caspase-3 expression with respect to the protective effect of cinnamon in albino rats. Toxilogy Report 2014: 795-801.

18. Cheraghi E, Golkar A, Roshanaei K, Alani B (2017) Aluminium-Induced Oxidative Stress, Apoptosis and Alterations in Testicular Tissue and Sperm Quality in Wistar Rats: Ameliorative Effects of Curcumin. Int J Fertil Steril 11: 166-175.

19. Nagatome M, Kondo Y, Kadowaki D, Saishyo Y, Irikura M, et al. (2018) Ethyl pyruvate attenuates acetaminophen-induced liver injury and prevents cellular injury induced by N-acetyl-p-benzoquinone imine. Heliyon J 4: e00521.

20. Jovanović Stojanov S, Martinović V, Bogojević D, Poznanović G, Petrović A, et al. (2018) Modulation of diabetes-related liver injury by the HMGB1/TLR4 inflammatory pathway. J Physiol Biochem 74: 345-358.

21. Akkoc H, Kelle I, Tunik S, Bahceci S, Sencar L, et al. (2012) M. Protective effect of ethyl pyruvate on liver injury in streptozotocin-induced diabetic rats. Acta Gastroenterol Belg 75: 336-341.

22. Rong Z, Pan R, Chang L, Lee W (2015) Combination treatment with ethyl pyruvate and IGF-I exerts neuroprotective effects against brain injury in a rat model of neonatal hypoxic-ischemic encephalopathy. Inter J Mol Med 36: 195-203.

23. Bakhtiary Z, Shahrooz R, Ahmadi A, Soltanalinejad F (2020) Protective effect of ethyl pyruvate on testicular histology and fertilization potential in cyclophosphamide treated mice. Vet Res Forum 11: 7-13.

24. Rossmann C, Nusshold C, Paar M, Ledinski G, Tafeit E, et al. (2013) Ethyl pyruvate inhibits oxidation of LDL in vitro and attenuates oxLDL toxicity in EA.hy926 cells. PLoS One 13: e0191477.

25. Atashfaraz E, Farokhi F, Najafi G (2013) Protective effect of ethyl pyruvate on epididymal sperm characteristics, oxidative stress and testosterone level in methotrexate treated mice. J Reprodand Infertil 14: 190-196.

26. Turkmen S, Mentese A, Karaguzel E, Karaca Y, Kucuk A, et al. (2012) A comparison of the effects of N-acetylcysteine and ethyl pyruvate on experimental testicular ischemia-reperfusion injury. Int J Fertil Steril 98: 626-631.

27. Kayiran O, Cuzdan SS, Uysal A, Kocer U (2017) Ethyl pyruvate improves skin flap survival after ischaemia reperfusion injury. Indian J Med Res 146: 369-374.

28. Roy S, Rahaman N, Ahmed F, Metya S, Sannigrahi S (2013) Naringenin attenuates testicular damage, germ cell death and oxidative stress in streptozotocin induced diabetic rats: Naringenin prevents diabetic rat testicular damage. J Appl Biomed 11: 195-208.

29. Jiao N, Chen Y, Zhu Y, Wang W, Liu M, et al. (2019) Protective effects of catalpol on diabetes mellitus-induced male reproductive damage via suppression of the AGEs/RAGE/Nox4 signaling pathway. Life Sci 6: 116736.

30. Omolaoye TS, Skosana BT, du Plessis SS (2018) Diabetes mellitus-induction: Effect of different streptozotocin doses on male reproductive parameters. Acta Histochem 120: 103-109.

31. Kong WY, Tong LQ, Zhang HJ, Cao YG, Wang GC, et al. (2016) The calcium-sensing receptor participates in testicular damage in streptozotocin-induced diabetic rats. Asian J Androl 18: 803-808.

32. Koroglu P, Senturk GE, Yucel D, Ozakpinar OB, Uras F, et al. (2017) The effect of exogenous oxytocin on streptozotocin (STZ)-induced diabetic adult rat testes. Peptides 63: 47-54.

33. Zhao L, Gu Q, Xiang L, Dong X, Li H, et al. (2017) Curcumin inhibits apoptosis by modulating Bax/Bcl-2 expression and alleviates oxidative stress in testes of streptozotocin-induced diabetic rats. Ther Clin Risk Manag J 13: 1099-1105.

34. Kanter M, Aktas C, Erboga M (2012) Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testes. Food and Chem Toxicol 50: 719-725.

35. Zhao Y, Song W, Wang Z, Wang Z, Jin X, et al. (2018) Resveratrol attenuates testicular apoptosis in type 1 diabetic mice: Role of Akt-mediated Nrf2 activation and p62-dependent Keap1, degradation. Redox Biol 14: 609-617.

36. Pulathan Z, Altun G, Hemşinli D, Menteşe A, Yuluğ E, et al. (2014) Role of ethyl pyruvate in systemic inflammatory response and lung injury in an experimental model of ruptured abdominal aortic aneurysm. Biomed Res Int 2014: 857109.

37. Demir S, Kazaz IO, Aliyazicioglu Y, Kerimoglu G, Teoman AS, et al. (2020) Effect of ethyl pyruvate on oxidative state and endoplasmic reticulum stress in a rat model of testicular torsion. Biotech Histochem 95: 317-322.

38. Dong W, Cai B, Peña G, Pisarenko V, Vida G, et al. (2010) Ethyl pyruvate prevents inflammatory responses and organ damage during resuscitation in porcine hemorrhage. Shock 34: 205-213.

39. Ding C, Wang Q, Hao Y (2016) Vitamin D supplement improved testicular function in diabetic rats. Biochem Biophys Res Commun 473: 161-167.

40. Bal R, Türk G, Tuzcu M, Yilmaz O, Ozercan I, et al. (2011) Protective effects of nanostructures of hydrated C60 fullerene on reproductive function in streptozotocin-diabetic male rats. Toxicology 282: 69-81.