PKP
PUBLIC KNOWLEDGE PROJECT

Journal of Biological Studies
ISSN 2209-2560

JBS
An Open Access Peer Reviewed Journal

The Comparative Effects of Aqueous Extracts of Green Tea and Catechin on Inflammation, Apoptosis and Oxidative Stress in the Testicular Tissue of Diabetic Rats

Fatemeh Noori Roshnavand[1], Vida Hojati[1*], Gholamhassan Vaezi[1],Raheleh Rahbarian[2]

[1]*Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran*
[2]*Department of Biology, Faculty of Science, Payame Noor University, Tehran, Iran*

Received: December 30, 2021; **Accepted:** February 10, 2022; **Published online:**March 30, 2022.

Abstract: Diabetes mellitus causes damage to testicular tissue through inflammation and oxidative stress. Catechin has been reported to have anti-oxidant and anti-inflammatory properties. This study was performed to compare the effects of aqueous extracts of green tea and catechin on inflammation, apoptosis and oxidative stress in the testicular tissue of type-1 diabetic rats. In this experimental study, 48 male Wistar rats were divided into control group, and diabetic rats treated with aqueous extract of green tea (100 and 200 mg/kg) and diabetic rats treated with catechin (100 and 200 mg/kg) (n = 6/each group) for 4 weeks. Diabetes was induced by an intraperitoneal injection of 240 mg/kg Aloxan. Levels of glucose, CRP, TNF-, IL-1 and IL-6, SOD, CAT, GPX, MDA, Bax and Bcl-2 were measured by Elisa in testicular tissue. Histomorphometric changes in testicular tissue were evaluated using EH staining method. Treatment with aqueous extract of green tea (200 mg/kg) and catechin (100 and 200 mg/kg) resulted in a significant increase in Bcl-2, SOD, CAT, GPX levels and a significant decrease in serum glucose, Hs-CRP, TNF-, IL-1, IL-6, Bax and MDA levels, and significantly increased seminiferous tubules diameter and germinal cells count in testicular tissue compared to the diabetic control group. Treatment of diabetic rats with catechin had more improving effects on blood glucose, apoptosis, inflammation, lipid peroxidation, antioxidant enzymes, and histological structure of testis than green tea aqueous extract.

Keywords: Diabetes; Green tea; Catechin; Inflammation; Oxidative Stress; Apoptosis; Testicular tissue; Rat.

*e-mail: V.Hojati@damghaniau.ac.ir

1 Introduction

Diabetes is a heterogeneous set of metabolic diseases characterized by hyperglycemia due to defective insulin secretion or insulin resistance. Due to the chronic increase in blood glucose concentration, normal metabolic functions are disturbed (Cryer et al., 2003) and oxidative stress increases in tissues including testicular tissue. Although a small quantity of active oxygen species is needed for cellular processes, its accumulation leads to oxidative stress condition, which negatively influence metabolic pathways (Asmat et al., 2016). Oxidative stress increases in diabetic animals due to decreased serum levels of SOD (Superoxide dismutase), GPX (Glutathione peroxidase), CAT (Catalase), protein glycation, and increased production of active oxygen species (Ighodaro and Akinloye, 2018) and MAD (Malondialdehyde), and increased lipid peroxidation and protein oxidation (Cohen et al., 2013; Ruiz et al., 1999).

It has been shown that chronic systemic inflammation is a significant sign of type-1 and type-2 diabetes (Xiao et al., 2014). Increased levels of cytokines such as TNF- (Tumor necrosis factor-), IL-1 (Interleukin-1) and IL-6 (Interleukin-6) indicate systemic inflammation of the body (King G.L., 2008). CRP (C-reactive protein) levels, which reflect the presence and severity of inflammation, also increase in diabetic patients (Doi et al., 2005).

Diabetes may also induce apoptosis in several tissues, leading to impaired tissue structure. Apoptosis is characterized by morphological changes such as cell wrinkling, chromatin condensation, and DNA degradation (Laulier and Lopez, 2012). The process of apoptosis is regulated by anti-apoptotic Bcl-2 and pro-apoptotic Bax genes. The Bcl-2 family can play both an inductive and an inhibitory role in the apoptosis process. Some members of this family are involved in inhibition (Bcl-2 and Bcl-xl) and others have a role in inducing apoptosis (Bad, Bax, Bax, Bcl-xs) (Laulier and Lopez, 2012, Elmore, 2007). Bax protein acts as an inducer of apoptosis (Renault and Manon, 2011; Ohtsuka et al., 2004).

Medicinal herbs have been reported to treat diabetes as complementary supplements or an appropriate alternative to reduce the side effects of chemical drugs (Giovannini et al., 2016). Green tea (*Camellia sinensis*) has been shown to accelerate the healing process of wounds in rats, attributing these effects to the antioxidant properties of green tea compositions (Asadi et al., 2011; Choi et al., 2016). Catechins, which are polyphenol compounds are an important component of tea leaves, are strong anti-oxidants (Bae et al., 2020). Catechin is an efficient scavenger of reactive oxygen species (ROS) in higher concentrations. (Chobot et al., 2009).

The decrease in weight due to the consumption of catechins in green tea has been previously confirmed. In addition, catechins can reduce the risk of cardiovascular diseases and diabetes. Moreover, it plays an important role in reducing complications in patients with metabolic syndrome by reducing serum blood glucose and lipid levels (Thieleckea and Boschmann, 2009). It has also been reported that consuming green tea can decrease the serum levels of inflammatory factors such as CRP, IL-6, and TNF- in women with type II diabetes (Banitalebi et al., 2016).

A review of the literature revealed that prescribing the extract of green tea can significantly improve male reproductive system function, sperm motility, the diameter of seminiferous tubules and reproductive epithelium thickness in rats. It was found that as a strong antioxidant, green tea extract reduces lipid peroxidation in sperm by inhibiting oxidative stress caused by sodium

arsenite (Shariatzadeh and Mohammadi, 2015; Sadat et al., 2010). The treatment of aqueous extract of green tea and catechin, have been shown to have a significant dose-dependent increase in the serum level of LH, FSH, estrogen, testosterone, and dihydrotestosterone in diabetic rats (Noori-Roshnavand et al., 2019) .

Studies have shown that catechins contribute to the improvement of diabetes type II, and can be a good supplement for metformin due to its ability to reduce blood glucose before and after diabetes (Park et al., 2019). It has been reported that the administration of green tea to rat models of type 1 diabetes significantly reduced serum glucose levels (Takechi et al., 2016). Consumption of green tea increases catalase and glutathione peroxidase enzymes in patients with diabetes type II, indicating the antioxidant effect of green tea, which is mainly attributed to one of its components called catechin (Spadiene et al., 2014).

Although large body of clinical and experimental studies have reported the antioxidant and improving effects of green tea and its effective compound, catechin, on diabetes, few studies have focused on comparing the whole leaf extract of green tea and catechin effects on subjects with diabetes. This study was performed to compare the effects of aqueous leaf extracts of green tea and catechin on inflammation, apoptosis and oxidative stress in the testicular tissue of diabetic rats.

2 Materials and Methods

In this laboratory-experimental research, 48 male Wistar rats weighting 160 ± 5 g were maintained in standard transparent polycarbonate cages with a temperature of 24 ± 3 °C, relative humidity of 35 ± 4 % and a 12/12 light-darkness cycle. In addition, the animals had free access to water and food. All animal experiment protocols were performed in accordance with the Ethical Committee Acts of Damghan Branch, Islamic Azad University, Damghan, Iran (IR.IAU.DAMGHAN.REC.1398.001).

2.1. Protocol of study:

Rats were divided into 6 groups (8 rats/each group): control group, diabetic group, diabetic groups treated with 100 mg/kg and 200 mg/kg aqueous extract of green tea, diabetic groups treated with 100 mg/kg and 200 mg/kg of catechin (Sigma-Aldrich, USA) for 4 weeks. Green tea extract and catechin were injected intraperitoneally. Control and diabetic groups received 0.5 ml of saline solution intraperitoneally.

2.2. Preparation of green tea extract:

Green tea leaves were confirmed by a botanist. The leaves were ground by a grinder after drying in the shade at 36 ± 3 °C. To prepare the extract, 100 g of dried green tea powder was poured into the percolator and ethyl alcohol 80% was added. It was placed in a laboratory environment for 72 hours and then filtered by using a filter paper. The extract was placed at 45 °C for 48 hours to dry (Sadoughi et al., 2017). After removal of the solvent, aqueous extracts were prepared at concentrations of 100 and 200 mg/kg.

2.3. Induction of diabetes in rats:

The empirical model of diabetes was induced in rats by one intraperitoneal injection of alloxan monohydrate (240 mg/kg) (Sigma-Aldrich, Germany). In addition, citrate buffer (pH = 5.4) was applied as an alloxan solvent. 30 days after injection, plasma glucose was measured using the IGM-0002A glucometer (EasyGluco, Korea). Rats with blood glucose levels higher than 300 mg/dl were considered as diabetic animals (Sadoughi, 2016).

2.4. Blood collecting:

At the end of the treatment, rats were anesthetized with diethyl ether and blood samples were taken from the left ventricle of the heart. The blood was placed at 37 °C for 12 minutes in an incubator (Memmert INB400, Germany). After coagulation, they were placed in a centrifuge (Hettich EBA280, Germany) (5,000 rpm) for 12 minutes. Then, blood serum was isolated.

2.5. Measurement of serum glucose, hs-CRP, TNF-, IL-1 and IL-6:

Glucose, hs-CRP, TNF-, IL-1 and IL-6 levels measured using by ELISA kits (Finetest, China).

2.6. Testicular tissue analysis:

The testes were removed from the body for tissue analysis. After washing with saline solution, they were homogenized with Tris buffer at 5,000 rpm for 2 minutes by Homogenizer (IKA Ultra turrax T25, Germany). The cellular cytoplasm then was the isolated from the homogenized tissue by centrifuge (Hermle Z366, Germany) and used for evaluation. To prevent the degradation of enzymes and proteins, all steps were carried out at 4 °C (refrigerated centrifuge) and a solution of 0.5 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich, Germany) was used as the inhibitor of cell proteases. The levels of SOD, CAT, GPX, MDA, Bax, and Bcl-2 in testicular tissue were measured by ELISA kits (Finetest, China). The testis tissue was then isolated and fixed with 10% formalin for histomorphological evaluations. After the passage of the tissue, 10 tissue sections were prepared randomly at 5µ in thickness and stained with hematoxylin-eosin (Rashid and Sil, 2015). Subsequently, images with 100x magnification were prepared with an optical microscope (Olympus CX21FS1, Japan) equipped with a camera (XC30, Olympus) and a photographing software (CellSens Dimension v1.6, Olympus). From each section, 10 round seminiferous tubules were randomly selected and the average thickness of the germinal epithelium was calculated (4 sites per each seminiferous tubules randomly). The mean of two diameters which were perpendicular to each other was measured in each of 10 seminiferous tubules and the average diameter of 10 tubules was determined. These steps were repeated for each of the tissue sections prepared from the testes of each rat, and the mean of the results were calculated.

2.7. Data analysis:

Data analysis was performed in SPSS version 20 using the Kolmogorov-Smirnov test (to determine the normal frequency distribution of the data) and one-way analysis of variance (ANOVA) and Tukey's post hoc test. Data was presented as mean ± standard deviation, and a P-value of 0.05 was considered statistically significant.

3 Results and Discussions

Serum glucose and CRP levels significantly increased in the diabetic group compared to the control group ($p < 0.05$), however, serum glucose and CRP levels significantly decreased in diabetic groups treated with 100 mg/kg and 200 mg/kg of green tea and catechin compared to diabetic group ($p < 0.05$). Serum glucose and CRP levels decreased significantly in diabetic rats treated with 200 mg/kg of green tea and 100 or 200 mg/kg of catechin compared with the diabetic group treated with 100 mg/kg of green tea ($p < 0.05$) (Fig. 1).

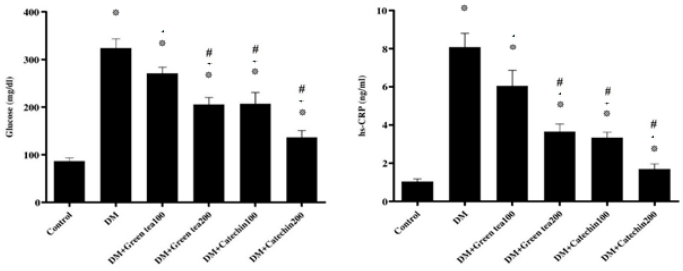

Figure 1: Mean serum glucose and CRP levels in control and experimental groups ($n = 8$). $p < 0.05$ compared to control group, $p < 0.05$ compared to the DM (Diabetes Mellitus) group, $p < 0.05$ compared to the DM + Green tea100 (diabetic rats treated with 100 mg/kg of green tea extract) group.

Serum levels of IL-1, IL-6, and TNF- significantly increased in diabetic group compared to control group ($p < 0.05$) and decreased in diabetic rats treated with 100 and 200 mg/kg of green tea and catechin compared with diabetic group ($p < 0.05$). Serum levels of IL-1, IL-6, and TNF- significantly decreased in diabetic rats treated with 200 mg/kg green tea and rats receving100 and 200 mg/kg of catechin compared to diabetic group treated with 100 mg/kg of green tea ($p < 0.05$) (Fig. 2).

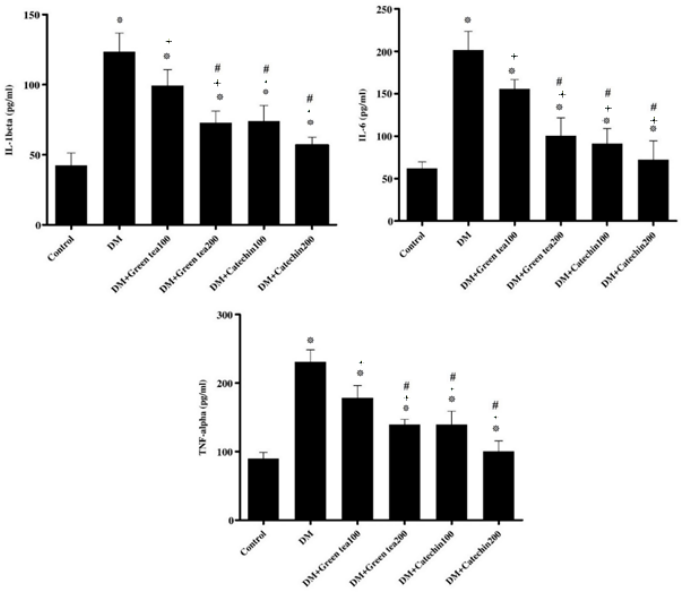

Figure 2: Mean serum IL-1, IL-6, and TNF- levels in control and experimental groups ($n = 8$). *$p < 0.05$ compared to control group, + $p < 0.05$ compared to the DM (Diabetes Mellitus) group, $p < 0.05$ compared to the DM + Green tea100 (diabetic rats treated with 100 mg/kg of green tea extract) group.

Testicular tissue SOD, CAT, and GPX levels significantly decreased and MDA level significantly increased in the diabetic group compared to the control group ($p < 0.05$). Treatment of diabetic rats with 100 and 200 mg/kg green tea and catechin led to significantly increased SOD, CAT, and GPX and decreased MDA level in testicular tissue compared with the diabetic group ($p < 0.05$). There were also higher SOD, CAT, and GPX levels and lower MDA in the testicular tissue of diabetic rats treated with 200 mg/kg of green tea and 100 and 200 mg/kg of catechin than in the diabetic group treated with 100 mg/kg of green tea ($p < 0.05$) (Figure 3).

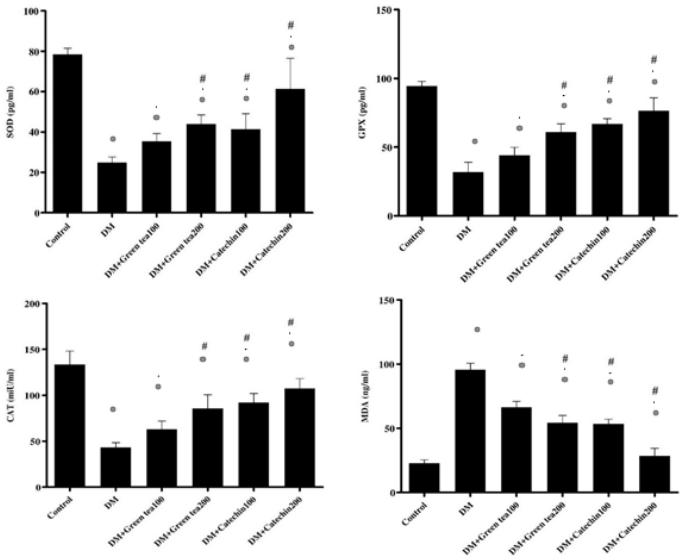

Figure 3: SOD, CAT, GPX, and MDA levels in testicular tissue of control and experimental groups (n = 8). *p $<$ 0.05 compared to the control group, + p $<$ 0.05 compared to the DM (Diabetes Mellitus) group, p $<$ 0.05 compared to the DM + Green tea100 (diabetic rats treated with 100 mg/kg of green tea extract) group.

Testicular tissue Bcl-2 and Bax levels significantly decreased and increased compared to the control group, respectively (p $<$ 0.05). Bcl-2 levels significantly increased in the testicular tissue of diabetic rats treated with 100 and 200 mg/kg of green tea and catechin, however, Bax levels significantly decreased compared with diabetic group (p $<$ 0.05). There were higher testicular tissue Bcl-2 levels in diabetic rats treated with 200 mg/kg of green tea and 100 or 200 mg/kg catechin and lower Bax levels than in the diabetic group treated with100 mg/kg of green tea (p $<$ 0.05) (Fig. 4).

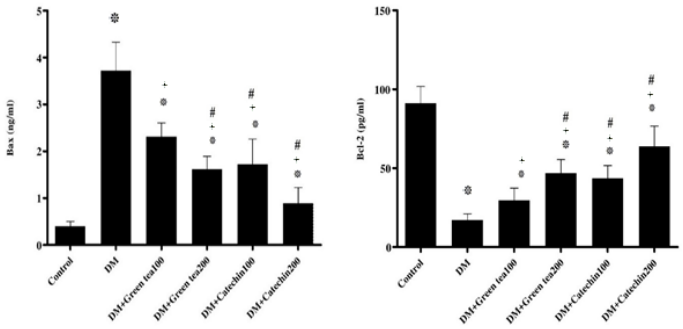

Figure 4: Bax and Bcl-2 levels in testicular tissue of control and experimental groups (n = 8). *p $<$ 0.05 compared to control group, + p $<$ 0.05 compared to the DM (Diabetes Mellitus) group, p $<$ 0.05 compared to the DM + Green tea100 (diabetic rats treated with 100 mg/kg of green tea extract) group.

Seminiferous tubules diameter and testicular germinal epithelium thickness of the diabetic group significantly decreased compared to control group (p $<$ 0.05). Significantly increased seminiferous tubules diameter and germinal epithelium thickness were observed in diabetic rats treated with 100 and 200 mg/kg of green tea extract and in diabetic groups treated with 100 and 200 mg/kg of catechin compared with the diabetic group (p $<$ 0.05). There was also higher seminiferous tubules diameter and germinal epithelium thickness in diabetic rats treated with 200 mg/kg of green tea and 100 and 200 mg/kg of catechin than in the diabetic group treated with 100 mg/kg of green tea (p $<$ 0.05) (Fig. 5).

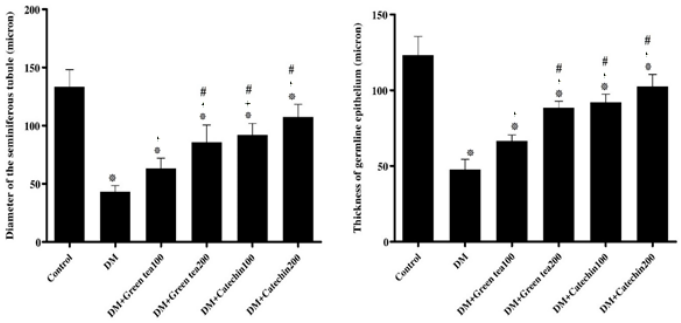

Figure 5: Seminiferous tubules diameter and germinal epithelium thickness in testicular tissue of control and experimental groups (n = 8). *p $<$ 0.05 compared to the control group, + p $<$ 0.05 compared to the DM (Diabetes Mellitus) group, p $<$ 0.05 compared to the DM + Green tea100 (diabetic rats treated with 100 mg/kg of green tea extract) group.

From testicular tissue histopathology results, the highest germinal epithelium thickness was observed in control group. The thickness of the germinal epithelium was considerably reduced in the diabetic group, however, treatment with 200 mg/kg of green tea and 100 and 200 mg/kg of catechin led to improved germinal epithelium thickness in diabetic rats (Figures 5 and 6).

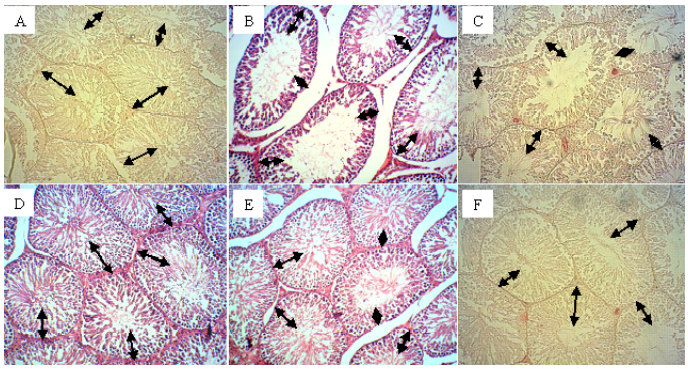

Figure 6: Photomicrograph of the transverse section of testicular tissue with 100x magnification in control (A), diabetic (B), diabetic treated with 100 mg/kg aqueous extract of green tea (C), diabetic treated with 200 mg/kg aqueous extract of green tea (D), diabetic treated with 100 mg/kg of catechin (E), and diabetic treated with 200 mg/kg of catechin (F) groups.

The main aim of this study was to evaluate and compare the effects of aqueous leaf extracts of green tea and catechin on inflammation, apoptosis and oxidative stress in the testicular tissue of diabetic rats. The results of this study showed that anti-apoptotic Bcl-2 levels decreased and pro-apoptotic Bax levels increased in testicular tissue of diabetic rats leading to cell death (apoptosis) and impaired testicular structure. Previous studies have shown that high concentrations of glucose can cause apoptosis. This has been demonstrated by the fragmentation of DNA and an increase in the ratio of Bax to Bcl-2 (Sharifi et al., 2007). Also, it has been shown that the exposure of myocardial cells with high concentrations of glucose causes an increase in the Bax protein (Su et al., 2017). It has been reported that the chronic hyperglycemia in diabetic patients causes glutathione levels to reduce in mitochondria, and increases the production of free oxygen radicals, and eventually induces apoptosis (Tuorkey et al., 2015). Indeed, increased Bax protein levels causes the mitochondrial membrane permeability to increase, by which cytochrome C is released from the mitochondria and makes the apoptosome complex leading to apoptosis (Scorrano and Korsmeyer, 2003).

Our findings revealed that there was increased TNF-, IL-1, IL-6, and CRP levels in testicular tissue of diabetic rats. In consistent with our finding, researchers have shown that serum levels of TNF- are significantly higher in patient with type-1 diabetes than healthy subjects. TNF- has also been reported to destroy pancreatic cells by inducing apoptosis and eventually leads to the development of type-1 diabetes (Tavakkoli Bazzaz et al., 2004). Type-1 diabetes causes elevated levels of IL-1 and TNF- in skeletal muscle (Molanouri Shamsi et al., 2014). Previous

studies have shown that inflammatory mediators, such as inflammatory cytokines, increase in diabetic patients. Further, high concentrations of glucose stimulate phosphorylation of the insulin receptors by increasing inflammatory cytokines such as IL-1 and IL-6 and exposing cells to high concentrations of TNF- (Tilg and Moschen, 2008). CRP levels also increase in type-1 diabetic patients, implying systemic and interstitial inflammation (Heier et al., 2015).

We have shown that levels of SOD, CAT, and GPX decreased and MDA level increased in the testicular tissue of rats in diabetic group. It has been reported that the activity of antioxidant enzymes in testicular tissue of diabetic rats has been reduced (Tafakkor et al., 2017). A significant decrease in the activity of SOD, CAT and GPX enzymes has been observed in the RBCs of diabetic rats (Rahbarian et al., 2016). CAT, which is one of the important antioxidants in the detoxification of free radicals, has been reported to be reduced in diabetic cases (Salimnejad et al., 2017). It has been proven that lipid peroxidation increases in heart tissue of streptozotocin-induced diabetic rats, and high levels of MDA, which is a lipid peroxidation product, causes damage to cell membranes and other lipid structures (38). A study also revealed that MDA levels increase in the testicular tissue of type-1 diabetic rats (Tafakkor et al., 2017).

Our findings revealed that treatment of diabetic rats with aqueous extract of green tea and catechin reduced apoptosis in testicular tissue. It has been shown that polyphenols in green tea, by regulating Bcl-2 expression, reduce apoptosis and improve hepatotoxicity due to acetaminophen (Oz and Chen, 2008). A study has shown that the combination of 12 weeks of aerobic exercise with the supplementation of green tea extract could reduce apoptosis in rats (Norouzi Kamareh et al., 2018). Researchers have reported that catechin has a protective and anti-apoptotic effect on hepatotoxicity due to D-galactosamine, by reducing the expression of Bax and increasing Bcl-2 levels (Vasanth et al., 2010). It has also been found that after induction of cataract by N-methyl-N-nitrosourea, catechin inhibited ocular lens epithelial cells apoptosis in rats by reducing the Bax/Bcl-2 ratio (Lee et al., 2010).

Our findings revealed that treatment of diabetic rats with aqueous extract of green tea and catechin reduced apoptosis in testicular tissue. It has been shown that polyphenols in green tea, by regulating Bcl-2 expression, reduce apoptosis and improve hepatotoxicity due to acetaminophen (Oz and Chen, 2008). A study has shown that the combination of 12 weeks of aerobic exercise with the supplementation of green tea extract could reduce apoptosis in rats (Norouzi Kamareh et al., 2018). Researchers have reported that catechin has a protective and anti-apoptotic effect on hepatotoxicity due to D-galactosamine, by reducing the expression of Bax and increasing Bcl-2 levels (Vasanth et al., 2010). It has also been found that after induction of cataract by N-methyl-N-nitrosourea, catechin inhibited ocular lens epithelial cells apoptosis in rats by reducing the Bax/Bcl-2 ratio (Lee et al., 2010).

The results of the present study clearly show that treatment of diabetic rats with aqueous extract of green tea and catechin decreases inflammation by reducing serum levels of CRP, TNF-, IL-1, and IL-6 in a dose-dependent manner. Compatible with the results of the present study, green tea reduced the serum levels of CRP, TNF- and IL-6 and in inflammatory conditions. It exerted its anti-inflammatory effects by reducing the release of inflammatory cytokines (Singh et al., 210). It has also been revealed that oral administration of epigallocatechin-3-gallate in aged mice with a high-fat diet significantly reduced CRP and TNF-. It can also exert its anti-inflammatory effects by reducing serum IL-6 levels (Senthil Kumaran et al., 2009). Studies have shown that the use of

green tea improves inflammatory factors in women with type-2 diabetes and has a beneficial role in preventing systemic inflammation of diabetic patients by preventing the increase of inflammatory cytokines (Banitalebi et al., 2016).

This study showed that treatment of diabetic rats with aqueous extract of green tea and catechin was associated with increased levels of antioxidant enzymes SOD, CAT, and GPX, and also reduced MDA levels and decreased lipid peroxidation in testicular tissue. Previous tudies have shown that catechin prevents tissue degeneration as a vigorous antioxidant by inhibiting oxidative stress and lipid peroxidation. It can also increase the quantities of endogenous antioxidants and the activity of antioxidant enzymes (Senanayake, 2013; Crespy and Williamson, 2004). The antioxidant properties of green tea compounds in inhibiting free radicals were attributed to the structure of the catechol and the hydroxyl groups in their structure. By linking with free radicals, these compounds cause them to be inactive. It has also been shown that catechin deactivates superoxide, hydroxyl, nitric oxide, and hydrogen peroxide radicals by increasing the activity of antioxidant enzymes (Chobo et al., 2009). Green tea consumption has been reported to increase the activity of CAT and GPX anti-oxidant enzymes in male mice with liver damage induced by tioacetamide (Sharifi et al., 2014). Catechin prevents the expanse of lipids peroxidation by inhibiting the accumulation of free radicals (Azam et al., 2004).

The results obtained from our study revealed an improvement in seminiferous tubules diameter and germinal epithelium thickness in diabetic rats treated with aqueous extract of green tea and catechin. Previous studies showed that green tea hydroalcoholic extract could significantly increase the mean diameter of seminiferous tubules, germinal epithelium thickness, basal membrane thickness, as well as increasing the number of spermatocyte cells, round spermatids, long spermatids, and Sertoli cells in the testis of rats poisoned with sodium arsenite (Soleimani Mehrnjani and Shokuhande, 2015). Indeed, green tea polyphenols reduce oxidative stress and testicular tissue and germinal epithelium damage by activating antioxidant enzymes (Soleimani Mehrnjani and Shokuhande, 2015). Catechin, because of its antioxidant properties, can also significantly increase the average number of seminiferous tubules and their diameter, the thickness of the base membrane of seminiferous tubules, the germinal epithelium height, and the average number of spermatocytes, spermatids, and Sertoli cells in the paranonylphenol-contaminated group (Shariatzadeh et al., 2015), which is consistent with the results of our study.

4 Conclusions

In conclusion, the results of this study suggest that catechin is more effective in reducing blood glucose, apoptosis, inflammation, oxidative stress and lipid peroxidation in testicular tissue of diabetic rats than aqueous extract of green tea. Catechin has more advantageous effects on the healing of diabetes-induced testicular tissue damage compared with aqueous extract of green tea. Further research is required to evaluate the exact mechanism behind catechin's improving action on apoptosis and inflammation caused by diabetes.

5 Acknowledgements

All authors of this article would like to thank the laboratory personnel of Islamic Azad University, Damghan Branch and Payame Noor University of Mashhad. In.

Conflict of interests

The authors declare that there are no competing interests.

References

Asadi, S., Zamiri, A., Ezzati, S., Parsaei, P., Rafieian, M., & Shirzad, H. (2011). Effect of alcoholic extract of green tea (Camellia sinensis) on the healing process in surgical and burn wounds in rats. Journal of Birjand University of Medical Sciences, 18(1): 1–9.

Asmat, U., Abad, K., & Ismail, K. (2016). Diabetes mellitus and oxidative stress-A concise review. Saudi Pharmaceutical Journal, 24(5): 547–553.

Azam, S., Hadi, N., Khan, N.U., & Hadi, S.M. (2004). Prooxidantproperty of green tea polyphenols, epicatechin and epicatechin-3-gallate: implications of anticancer properties. Toxicology in Vitro, 18(5): 555–561.

Bae, J., Kim, N., Shin, Y., Kim S.Y., & Kim Y.J. (2020). Activity of catechins and their applications. Biomedical Dermatology, 4: 8. https://doi.org/10.1186/s41702-020-0057-8.

Banitalebi, E., Razavi, T., Norian, M., & Bagheri, L. (2016). The effect of combined aerobic exercise training and green tea extract on serum TNF- and IL-6 levels in obese women with type 2 diabetes. Daneshvar Medicine, 23(123): 11–20. (In Farsi).

Chobot, V., Huber, C., Trettenhahn, G., & Hadacek, F. (2009). Catechin: Chemical weapon, antioxidant, or stress regulator? Journal of Chemical Ecology, 35(8): 980–996.

Choi, S.J., Park, S.Y., Park, J.S., Park, S.K., & Jung, M.Y. (2016). Contents and compositions of policosanols in green tea (Camellia sinensis) leaves. Food Chemistry, 204: 94–101.

Cohen, G., Riahi, Y., Sunda, V., Deplano, S., Chatgilialoglu, C., & Ferreri, C. (2013). Signaling properties of 4-hydroxyalkenals formed by lipid peroxidation in diabetes. Free Radical Biology and Medicine, 65: 978–987.

Crespy, V., & Williamson, G. (2004). A review of the health effects of green tea catechins in in vivo animal models. Journal of Nutrition, 134(12): 3431–3440.

Cryer, P.E., Davis, S.N., & Shamoon, H. (2003). Hypoglycemia in diabetes. Diabetes Care, 26(6): 1902–1912.

Doi, Y., Kiyohara, Y., Kubo, M., Ninomiya, T., Wakugawa, Y., & Yonemoto, K. (2005). Elevated C-reactive protein is a predictor of the development of diabetes in a general Japanese population: the Hisayama Study. Diabetes Care, 28(10): 2497–2500.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. Toxicology and Pathology, 35(4): 495-516.

Giovannini P., Howes M.R., & Edwards S.E. (2016). Medicinal plants used in the traditional management of diabetes and its sequelae in Central America: A review. Journal of Ethnopharmacology, 184: 58–71.

Heier, M., Margeirsdottir, H.D., Brunborg, C., Hanssen, K.F., Dahl-Jørgensen, K., & Seljeflot, I. (2015). Inflammation in childhood type 1 diabetes; influence of glycemic control. Atherosclerosis, 238(1): 33–37.

Ighodaro, O.M., & Akinloye, O.A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54(4): 287–293.

King, G.L. (2008). The role of inflammatory cytokines in diabetes and its complications. Journal of Periodontology, 79(8): 1527–1534.

Laulier, C., & Lopez, B.S. (2012). The secret life of Bcl-2: apoptosis-independent inhibition of DNA repair by Bcl-2 family members. Mutation Research, 751(2): 247–257.

Lee, S.C., & Pervaiz, S. (2007). Apoptosis in the pathophysiology of diabetes mellitus. International Journal of Biochemistry and Cell Biology, 39(3): 497–504.

Lee, S.M., Ko, I.G., Kim, S.E., Kim, D.H., & Kang, B.N. (2010). Protective effect of catechin on apoptosis of the lens epithelium in rats with N-methyl-N-nitrosourea-induced cataracts. Korean Journal of Ophthalmology, 24(2): 101–107.

Molanouri Shamsi, M., Mahdavi M., Gharakhanlou, R., & Hassan, M.Z. (2014). Effect of intensive resistance exercise training on protein expression of IL-6, IL-1 and TNF- myokines in fast twitch skeletal muscle of diabetic rats. Journal of Sport and Biomotor Sciences, 6(11): 69–77.

Noori Roshnavand, F., Hojati, V., Vaezi, G., & Rahbarian, R. (2019). The Comparison of Green Tea Aqueous Extract and Catechin Effect on Pituitary-Gonadal Axis in Rat Models of Type 1 Diabetes. Journal of Advances in Medical and Biomedical Research, 27(125): 9–15.

Norouzi Kamareh, H., Zolfaghari, M.R., Ghaderi Pakdel, F., & Tolouei Azar, J. (2018). Effect of 12 weeks aerobic training and oral green tea extract on cardiac caspase-3 expression in aged male rats. Sport Biosciences, 10(2): 221–235.

Ohtsuka, T., Ryu H., Minamishima, Y.A., Macip, S., Sagara, J., & Nakayama, K.I. (2004). ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. Nature Cell Biology, 6: 121–128.

Oz, H.S., & Chen, T.S. (2008). Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity. Digestive Diseases and Sciences, 53(11): 2980–2988.

Park, J.H., Bae, J.H., Im, S.S., & Song, D.K. (2014). Green tea and type 2 diabetes. International Medical Research, 3(1): 4–10.

Rahbarian, R., SepehriMoghadam, H., & Sadooghi, S.D. (2015). Effect of aqueous extract of Launaea acanthodes on testicular tissue and sperm parameters in alloxan-induced diabetic rats. Horizon Medical Sciences, 21(1): 21–29.

Rahbarian, R., Sepehri-Moghadam, H., & Sadoughi, S.D. (2016). The effects of aqueous extract of Launaea acanthodes on oxidative stress parameters of red blood cells in diabetic rats. Journal of Rafsanjan University of Medical Sciences, 14(10): 865–878.

Rashid, K., & Sil, P.C. (2015). Curcumin ameliorates testicular damage in diabetic rats by suppressing cellular stress-mediated mitochondria and endoplasmic reticulum-dependent apoptotic death. Biochimica et Biophysica Acta, 1852(1): 70–82.

Renault, T.T., & Manon, S. (2011). Bax: Addressed to kill. Biochimie, 93(9): 1379–1391.

Ruiz, C., Alegría, A., Barberá, R., Farré, R., & Lagarda, M.J. (1999). Lipid peroxidation and antioxidant enzyme activities in patients with type 1 diabetes mellitus. Scandinavian Journal of Clinical and Laboratory Investigation, 59(2): 99–105.

Sadat Khorsandi, L., Javadnia, F., Orazizadeh, M., & Abdolahi, M. (2010). Effect of green tea (Camellia sinensis L.) extract on acetaminophen induced acute hepatotoxicity in mice. Iranian Journal of Medicine and Aromatic Plants, 26(1): 22–29. (In Farsi).

Sadoughi, D., Edalatmanesh, M.A., & Rahbarian, R. (2017). Investigating the effect of Avicennia marina aqueous extract on neuronal density of hippocampal CA1, CA2, CA3 and dentate gyrus in diabetic rats. Shafaye Khatam Journal, 5(2): 28–35. (In Farsi).

Sadoughi, S.D. (2016). Investigation the effect of curcumin on the hormones of pituitary-ovarian axis in alloxan-induced diabetic rats. Journal of Ardabil University of Medical Sciences, 16(4): 441–451.

Salimnejad, R., Jalali, M., Nikravesh, M.R., & Fazel, A.R. (2014). Effect of garlic aqueous extract on markers of oxidative stress in diabetic rats testes. Journal of Rafsanjan University of Medical Sciences, 13(4): 371–382. (In Farsi).

Scorrano, L., & Korsmeyer, S.J. (2003). Mechanisms of cytochrome c release by proapoptotic BCL-2 family members. Biochemical and Biophysical Research Communications, 304(3): 437–44.

Senanayake, S.P.J.N. (2013). Green tea extract: chemistry, antioxidant properties and food applications - A review. Journal of Functional Foods, 5(4): 1529–1541.

Senthil Kumaran, V., Arulmathi, K., Sundarapandiyan, R., & Kalaiselvi, P. (2009). Attenuation of the inflammatory changes and lipid anomalies by epigallocatechin-3-gallate in hypercholesterolemic diet fed aged rats. Experimental Gerontology, 44(12): 745–751.

Shariatzadeh, M.A., & Mohammadi, M. (2015). Protective role of green tea (Camellia sinensis) hydroalcholic extract on sperm parameters and testicular tissue in NMRI mice exposed to sodium arsenite. Journal of Birjand University of Medical Sciences, 21(4): 432–443. (In Farsi).

Shariatzadeh, M.A., Soleimani-Mehranjani, M., & Naderi-Noreini, S. (2015). The effect of green tea (Camellia sinensis) on rat testicular tissue following exposure to paranonylphenol. Journal of Kerman University of Medical Sciences, 22(5): 510–523.

Sharifi, A., Razmi, N., & Naghsh, N. (2014). The antioxidant effect of Camellia Sinesison on the liver damage induced by tioacetamide in male mice. Medical Laboratory Journal, 7(4):13–18.

Sharifi, AM, Mousavi, SH, Farhadi, M, & Larijani, B. (2007). Study of high glucose-induced apoptosis in PC12 cells: role of bax protein. Journal of Pharmacological Sciences, 104(3): 258–262.

Singh, R., Akhtar, N., & Haqqi, T.M. (2010). Green tea polyphenol epigallocatechin 3- gallate: Inflammation and arthritis. Life Sciences, 86(25-26): 907–918.

Soleimani Mehrnjani, M., & Shokuhande, M.H. (2015). Stereological study of the protective effect of green tea extract on the testicular tissue of mice treated with sodium arsenite. Journal of Chemical Thermodynamics, 5(4): 429–437.

Spadiene, A., Savickiene, N., Ivanauskas, L., Jakstas, V., Skesters, A., & Silova, A. (2014). Antioxidant effects of Camellia sinensis L. extract in patients with type 2 diabetes. Journal of Food Drug Analusis, 22(4): 505–511.

Tafakkor, S., Sadoughi, S.D., & Rahbarian, R. (2017). Effect of equeous extract of Launaea acanthodes on DNA oxidative damage and antioxidant enzymes activities in diabetic rats testes tissue. Horizon of Medical Sciences, 23(2): 149–155.

Takechi, R., Alfonso, H., Hiramatsu, N., Ishisaka, A., Tanaka, A., & Tan, L. (2016). Elevated plasma and urinary concentrations of green tea catechins associated with improved plasma lipid profile in healthy Japanese women. Nutrition Reseaerch, 36(3): 220–226.

Tavakkoli Bazzaz, J., Pravica, V., Boulton, A., & Hutchinson, I. (2004). Genetics of type 1 diabetes: study of TNF-alpha gene. Iranian Journal of Diabetes and Lipid Disorders, 4(1): 9–18.

Thieleckea, F., & Boschmann, M. (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome - A review. Phytochemistry, 70(1): 11–24.

Tilg, H., & Moschen, A.R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. Molecular Medicine, 14(3-4): 222–231.

Tuorkey, M.J., El-Desouki, N.I., & Kamel, R.A. (2015). Cytoprotective effect of silymarin against diabetes-induced cardiomyocyte apoptosis in diabetic rats. Biomedical and Environmental Sciences, 28(1): 36–43.

Vasanth, Raj P., Nitesh, K., Sagar, Gang S., Hitesh Jagani, V., Raghu Chandrashekhar, H., & Venkata Rao, J. (2010). Protective role of catechin on d-galactosamine induced hepatotoxicity through a p53 dependent pathway. Indian Journal of Clinical Biochemistry, 25(4): 349–356.

Xiao, J., Li, J, Cai, L., Chakrabarti, S., & Li, X. (2014). Cytokines and diabetes research. Journal of Diabetes Research, 2014: 920613. doi:10.1155/2014/920613.