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Effects of Coenzyme Q10, Fullerene C60, and Alpha-Lipoic Acid on Histomorphometric, Antiapoptotic and Insulin Expression in Pancreatic Tissue of Rats Exposed to Bisphenol A *

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The aim of this study was to determine the morphometric changes in the pancreas, apoptosis related pancreatic damage, and differences in insulin expression in male rats exposed to low doses of BPA from early pubertal development and to determine the possible protective effects of CoQ10, FUL, and ALA against the effects of BPA. Sixty, one-month-old male Sprague-Dawley rats were used and divided into 8 groups. The groups were administered BPA, CoQ10, FUL, ALA in 0.2 mL olive oil for 7 weeks. Morphometric measurements of islets of Langerhans in all groups showed that the mean islet number, islet diameter, and area decreased in the BPA group. Administration of CoQ10 and ALA together with BPA caused an increase in islet numbers, while FUL was not effective. CoQ10 and FUL were ineffective against BPA, but ALA had a positive effect on islet diameter and area. Insulin positivity decreased in all BPA-treated groups compared to the control, and insulin expression in islets was not significantly affected by CoQ10, FUL, and ALA against BPA. CoQ10 and ALA were effective against the apoptotic changes induced by BPA, but FUL had no positive effect. In conclusion, while long-term administration of BPA in rats did not result in histopathological changes in the endocrine pancreas, the study revealed significant morphometric alterations, a reduction in insulin secretion within the islet cells, and an increase in apoptosis. We determined that CoQ10 and ALA had partial protective effects against the impact of BPA on the endocrine pancreas.

Key Words: *Alpha-lipoic acid, bisphenol A, coenzyme Q10, fulleren C60, pancreas*

Bisfenol A'ya Maruz Kalan Sıçanların Pankreas Dokusunda Koenzim Q10, Fulleren C60 ve Alfa-Lipoik Asit'in Histomorfometrik, Antiapoptotik ve İnsülin Ekspresyonu Üzerine Etkileri

Bu çalışmada, erken pubertal gelişim döneminden itibaren düşük dozda BPA'ya maruz kalan erkek sıçanlarda pankreasta meydana gelen morfometrik değişimler, apoptozis temelli pankreatik doku hasarı ile insülin ekspresyonundaki farklılıkların ortaya konulması ve BPA'nın olumsuz etkilerine karşı KoQ10, FUL, ALA'in muhtemel koruyucu özelliklerinin belirlenmesi amaçlandı. Çalışmada; 1 aylık, 60 adet Sprague-Dawley ırkı erkek sıçan kullanıldı. Hayvanlar, canlı ağırlıkları dikkate alınarak rastgele toplam 8 gruba ayrılarak özel kafeslere yerleştirildi. Gruplara, 7 hafta süreyle uygulamalar oral gavaj yöntemiyle BPA, KoQ10, FUL, ALA 0.2 mL zeytinyağına katılarak gerçekleştirildi. Tüm gruplarda Langerhans adacıklarında yapılan morfometrik ölçümler sonucu ortalama adacık sayı, adacık çap ve alanlarının BPA grubunda azaldığı dikkati çekti. BPA ile birlikte KoQ10 ve ALA uygulamasının adacık sayılarında artışa neden olduğu, FUL'un ise etkili olmadığı gözlendi. KoQ10 ve FUL'un adacık çapı ve alanlarında BPA'ya karşı etkisiz olduğu ancak ALA'nın olumlu etkisinin olduğu belirlendi. İnsülin pozitifliğinin BPA uygulanan tüm gruplarda kontrol grubuna göre azaldığı, adacıklarda insülin ekspresyonu KoQ10, FUL ve ALA'nın, BPA'ya karşı anlamlı bir etkisinin olmadığı saptandı. BPA'nın öncülük ettiği apoptotik değişimlere karşı KoQ10 ve ALA'nın etkili olduğu ancak FUL'in olumlu etkisinin olmadığı izlendi. Sonuç olarak sunulan çalışmada sıçanlarda uzun süreli BPA uygulaması ile endokrin pankreasta histopatolojik değişimler gözlenmemekle birlikte morfometrik açıdan önemli değişimler ile adacık hücrelerinde insülin salınımının azaldığı, apoptozisin ise tetiklendiği belirlenmiş; BPA'nın endokrin pankreastaki etkisine karşı ise KoQ10 ve ALA'in kısmen koruyucu etkilerinin olduğu ortaya konmuştur.

Anahtar Kelimeler: *Alfa-lipoik asit, bisfenol A, koenzim Q10, fulleren C60, pankreas*

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Introduction

Endocrine-disrupting chemicals (EDCs) are natural or synthetic compounds that bind to specific receptors and mimicking biological activities (1,2). Bisphenol A (BPA) is classified into the xenoestrogen group due to its action on the estrogen receptor (ER) and is classified into the endocrine-disrupting chemical category because of its effects on hormones and receptors (3). Humans are primarily exposed to BPA through food and food-contact materials, and to a lesser extent through epoxy coatings of metal cans, kitchen utensils, medical devices, and dental composites and adhesives (4). BPA has been found to play a role in the development of obesity, diabetes, endocrine and immune system disorders, polycystic ovary syndrome, hypertension, atherosclerosis,

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liver damage, irregular thyroid hormone secretion, and breast and prostate cancers in both humans and experimental animals (5-7). Furthermore, BPA has been reported to increase insulin secretion from the pancreas (8), disrupt blood glucose homeostasis (9), and induce insulin resistance (10).

While it has long been recognized that BPA exhibits estrogen-like effects, it has been stated that this effect occurs not only through classical ERs and mechanisms but also through different pathways (11). In experimental studies, BPA has been shown to mimic the effects of 17β-estradiol, leading to excessive insulin stimulation and insulin resistance (12). In mice, both BPA and estrogen have been found to increase insulin secretion at low doses and decrease plasma glucose concentration. Prolonged exposure to BPA has been observed to cause chronic hyperinsulinemia and subsequent insulin resistance (10). Furthermore, BPA has been found to reduce the number of macrophages in the pancreas of mice and increase suppressive T lymphocytes and apoptotic cell counts (13,14). Additionally, BPA has been shown to decrease the number of β cells and their proliferative capacity in the pancreas (15), as well as reduce the viability of insulin-secreting cells (16). Moreover, BPA has been reported to slowdown the body's energy metabolism and disrupt insulin signaling in skeletal muscle. Therefore, BPA is considered a risk factor for the development of diabetes (17). On the other hand, it has been determined that BPA weakens Ca^{2+} signals in α cells of the pancreas (10) and increases glucagon expression (18).

Coenzyme Q10 (CoQ10) is an important coenzyme with strong antioxidant effects. It is required for the activities of enzyme systems involved in redox reactions in the electron transfer chain and is located in the hydrophobic parts of all membranes and the inner mitochondrial membrane (19-21). It is found at the highest concentrations in the heart, liver, kidney, and pancreas. CoQ10 is a potent antioxidant in the mitochondrial respiratory chain, serving as a membrane stabilizer and an essential co-factor, facilitating the production of adenosine triphosphate (ATP), the main cellular energy source. CoQ10 acts directly by protecting cellular components from free radicals and indirectly by regenerating other antioxidants such as α-tocopherol and ascorbate (22). Besides maintaining membrane stability, CoQ10 is also involved in cellular signaling, gene expression, cell growth, and apoptosis control (23). Fullerene C60 (FUL) has been recognized as a biocompatible agent that does not exhibit toxic effects on normal tissues at low concentrations and possesses strong free radical scavenging and antioxidant properties (24, 25). Studies have revealed its anticancer, neuroprotective, anti-inflammatory, antiatherogenic, and radioprotective properties (26, 27). Alpha-lipoic acid (ALA) is an antioxidant molecule with both hydrophilic and lipophilic properties, synthesized in various tissues of living organisms and found in certain vegetables, mainly broccoli and spinach (28, 29). Due to its antioxidant properties, ALA has been reported to be used as a supportive therapy in conditions such as diabetes, ischemia, heavy metal poisoning, radiation exposure, neurodegeneration, and HIV infection (30).

In this study, we aimed to identify the morphometric changes in the pancreas, apoptosis-based pancreatic tissue damage, and differences in insulin expression in male rats exposed to low doses of BPA during the early pubertal period. Additionally, the potential protective properties of CoQ10, FUL, and ALA against the adverse effects of BPA were investigated.

Materials and Methods

Research and Publication Ethics: The study was conducted with the approval of the Fırat University Local Ethics Committee for Animal Experiments (dated 12.10.2022, approval number 2022/17-10).

Laboratory Animals: Sixty male Sprague-Dawley rats with an average weight of approximately 100 grams and aged 1 month were used. The animals were placed in special cages and the light schedule was adjusted to 12 hours of light and 12 hours of darkness. The rats were also provided with ad libitum access to food and water.

Experimental Procedure: The animals were randomly divided into eight groups based on their live weights and placed in cages. The administrations to the groups were carried out for seven weeks, every day, using the oral gavage method. BPA, CoQ10, FUL, and ALA were administered by mixing them with 0.2 ml of olive oil. Accordingly, the groups received the following treatments: **Control group:** 0.2 mL of olive oil; **BPA group:** 25 mg/kg of BPA; **CoQ10 group:** 40 mg/kg; **FUL group:** 8 µg/kg of FUL in aqueous form; **ALA group:** 70 mg/kg of ALA; **BPA + CoQ10 group:** 25 mg/kg of BPA with 40 mg/kg of CoQ10; **BPA + FUL group:** 25 mg/kg of BPA with 8 µg/kg of FUL in aqueous form; **BPA + ALA group:** 25 mg/kg of BPA with 70 mg/kg of ALA.

Collection of Tissue Samples: Pancreas samples were taken from the decapitated rats and fixed in a formaldehyde solution for 48 hours. Following fixation, the samples underwent routine processing to prepare paraffin blocks. Sections with a thickness of 5 microns (µm) were obtained from the paraffin blocks and stained using the Hematoxylin and Eosin (H&E) method. Subsequently, the stained sections were examined under a light microscope (31).

Histomorphometric Analysis: For histological examinations, Langerhans islets, which are the endocrine components of the pancreas, were examined in H&E-stained pancreas sections. The number of islets was determined by counting them in ten different randomly selected microscopic fields at 10x magnification. Additionally, in H&E-stained sections, the number of islets in the pancreas and the diameter and area of 20 different randomly selected islets at 40x magnification were calculated using the Image J (Wayne Rasband and contributors, National Institutes of Health, USA, Version 1.53t) analysis program.

Immunohistochemical Analysis: The Avidin-Biotin-Peroxidase method was used for immunohistochemical analysis (32). For this purpose, the universal immunoperoxidase kit (Ultravision Detection System, Antipolyvalent, HRP/DAB, Thermo Scientific, Cat No: TP-015-HD) was employed. Sections with a thickness of 4 microns obtained from paraffin blocks were deparaffinized with xylene and dehydrated through a graded series of alcohols to remove water. To expose the antigenic receptors, the sections were incubated in 0.01 M sodium citrate for 20 minutes, then washed with PBS, and incubated in 3% H_2O_2 prepared with methanol for 10 minutes to block endogenous peroxidase activity. To prevent nonspecific binding, the sections were incubated in 1% normal goat serum for 1 hour. Subsequently, the sections were incubated overnight at 4°C with primary antibodies for insulin (diluted at 1/100, 1/200; Abcam, Kimera Medical Laboratory Supplies, Cat No: ab-63820) and caspase 3 (Bioss Inc Cat No: bs-0081R). After washing with PBS, the sections were incubated with biotinylated secondary antibodies (Thermo Scientific, Cat No: TP-015-BNS) for 10 minutes. Then, the sections were incubated with streptavidin peroxidase (Thermo Scientific, Cat No: TS-015-HRS) for 10 minutes. The color-developing substrate used was 3-amino-9-ethylcarbazole (AEC) (Thermo Scientific, Cat No:001122). After applying AEC to the sections, the reaction was stopped as soon as the color change began. In the final step, the sections were counterstained with Mayer's Hematoxylin (MH) for 2 minutes, washed in tap water, covered with immu-mount, and examined under a light microscope at appropriate magnifications.

Immunohistochemical Evaluation: The images of 20 randomly selected islets from the pancreatic sections of each animal in the microscope field at 40x magnification were captured with a camera. Subsequently, using the Image J program, the positive cells for insulin and caspase 3 were counted, and the obtained group mean values were used as the β-cell index and apoptotic cell index.

Statistical Analyses: The data were statistically evaluated using IBM SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). Shapiro–Wilk and Levene's tests were performed to analyze the normality of the data and the homogeneity of the variances, respectively. The data were tested using one-way analysis of variance (ANOVA). Post hoc comparisons between the groups were conducted using Tukey's test. The data were presented as mean ± standard error, and a P-value of less than 0.05 was considered significant.

Results

Histomorphometric Analysis: The endocrine pancreas regions in all groups maintained normal histological appearance. The average values of morphometric measurements (count, diameter, and area of islets) in Langerhans islets are summarized in Table 1 for all groups. When comparing the groups based on the mean islet count, the BPA group had the lowest number of islets. There was a statistically significant difference in islet count between the control, CoQ10, FUL, and ALA groups compared to the BPA group ($P \leq 0.001$). A similar difference was also observed between the BPA group and the BPA+CoQ10 and BPA+ALA groups. Although there was no statistical difference between the BPA and BPA+FUL groups, there was a noticeable numerical difference (Figure 1a). Except for in the BPA and BPA+FUL groups, the mean islet counts in the other experimental groups were found to be similar to the control group ($P \geq 0.05$).

The average islet diameters were found to be similar in the control, CoQ10, FUL, and ALA groups. Additionally, the diameter values in the BPA+ALA group were statistically similar to those in the control, CoQ10, FUL, and ALA groups. The smallest diameters were found in the BPA, BPA+CoQ10, and BPA+FUL groups, respectively. While there was no difference between the BPA and BPA+CoQ10 groups in terms of diameter ($p>0.05$), a significant difference was detected between the BPA and BPA+FUL groups (Figure 1b). A similar difference was also observed between the BPA+ALA and BPA+CoQ10 groups ($P \leq 0.001$).

The islet areas were similar in the control, CoQ10, FUL, ALA, and BPA+ALA groups. However, the islet areas were significantly decreased in the BPA, BPA+CoQ10, and BPA+FUL groups compared to the aforementioned groups ($P \leq 0.001$). There was no statistical difference in islet areas between the BPA, BPA+CoQ10, and BPA+FUL groups (Figure 1c).

β cell index and apoptotic index values in islets of Langerhans in control and experimental groups are presented in Table 1. It was observed in immunostaining that insulin positivity was homogeneously distributed in the islets except for the peripheral parts of the islets in the BPA group, and it was cytoplasmic and granular in the islet cells. In the BPA group, areas devoid of insulin expression were observed near the islet center (Figure 2). According to the groups, the β cell index was found to be lowest in BPA, BPA+FUL, BPA+ALA, BPA+CoQ10 groups and highest in control, FUL, ALA, CoQ10 groups. There was no statistical difference between BPA and BPA treatment groups. β cell index values were similar in control and CoQ10, FUL, and ALA groups (Figure 1d).

Caspase 3 positivity was generally cytoplasmic and granular in the cells located at the periphery of the islets except for the BPA and BPA+FUL groups. In BPA and BPA+FUL groups, positivity was also observed towards the center of the islets (Figure 3). Apoptotic index was highest in BPA+FUL, BPA, BPA+ALA, and BPA+CoQ10 groups. It was noteworthy that there was a statistically significant difference between the aforementioned groups. The groups with the lowest apoptotic index were determined as CoQ10, control, ALA, and FUL. Apoptosis was significantly increased in the FUL group compared to the CoQ10 and control groups. A similar difference was observed between the CoQ10 and ALA groups. It was noteworthy that both BPA and FUL treatment alone increased apoptosis (Figure 1e).

Table 1. Mean Langerhans islet number, diameter, area values, and β cell and apoptotic cell indexes in the control and experimental groups

	Control	BPA	BPA+CoQ10	BPA+FUL	BPA+ALA	CoQ10	FUL	ALA	SE	P
Number of islets	1.73 ± 0.11 [a]	0.86 ± 0.12 [c]	1.43 ± 0.15 [ab]	1.13 ± 0.12 [bc]	1.37 ± 0.13 [ab]	1.53 ± 0.14 [ab]	1.71 ± 0.16 [a]	1.61 ± 0.14 [a]	0.05	0.001
Islet diameter (μ)	119.82 ± 7.42 [a]	85.77 ± 3.61 [d]	95.76 ± 4.25 [cd]	102.43 ± 3.21 [bc]	112.52 ± 4.28 [ab]	119.87 ± 3.80 [a]	124.89 ± 4.44 [a]	117.18 ± 4.49 [a]	1.67	0.001
Islet area (μ^2)	12607.49 ± 1128.91 [a]	7464.84 ± 626.70 [b]	9000.03 ± 769.97 [b]	9403.70 ± 575.71 [b]	11942.49 ± 847.39 [a]	12996.53 ± 832.86 [a]	14357.02 ± 991.90 [a]	12922.08 ± 1037.76 [a]	314.15	0.001
β Cell Index	86.86 ± 0.66 [a]	78.42 ± 1.67 [b]	81.24 ± 1.43 [b]	79.24 ± 1.14 [b]	81.20 ± 0.96 [b]	85.48 ± 1.09 [a]	86.27 ± 0.76 [a]	85.84 ± 1.04 [a]	0.43	0.001
Apoptotic Cell Index	13.34 ± 1.52 [ef]	35.40 ± 2.16 [b]	18.85 ± 1.60 [d]	42.94 ± 2.15 [a]	29.77 ± 1.82 [c]	10.55 ± 1.37 [f]	21.46 ± 1.82 [d]	16.89 ± 1.80 [de]	0.80	0.001

[a,b,c,d,e,f]: Different letters on the same line are statistically significant (P<0.001). **SE:** Standard error

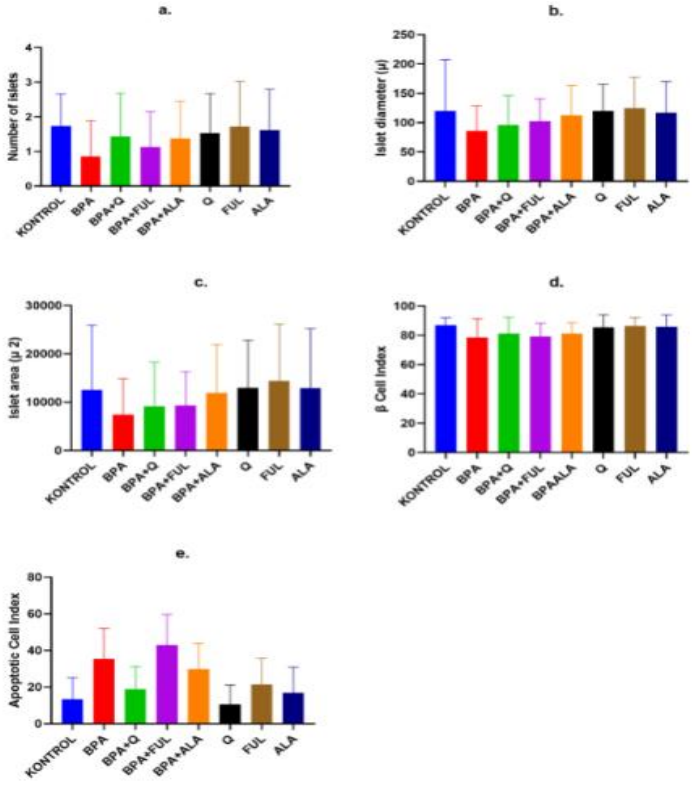

Figure 1. a. Number of islets, **b.** Islet diameter, **c.** Islet area, **d.** β Cell Index, **e.** Apoptotic Cell Index

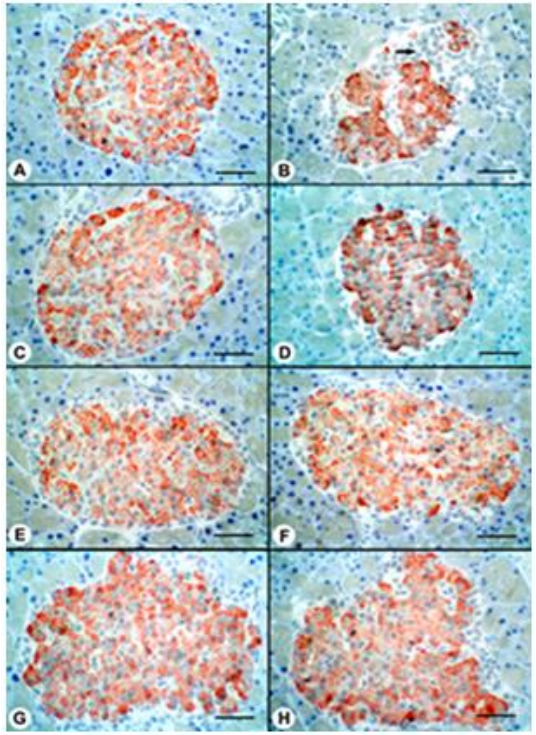

Figure 2. Insulin positivity in β cells of Langerhans islets **A.** Control group, **B.** The appearance of insulin positive cells (arrow) in islets in BPA group, **C.** BPA+CoQ10 group, **D.** BPA+FUL group, **E.**BPA+ALA group, **F.** CoQ10 group, **G.** FUL group, **H.** ALA group; MH × 100, Bar= 50 μ.

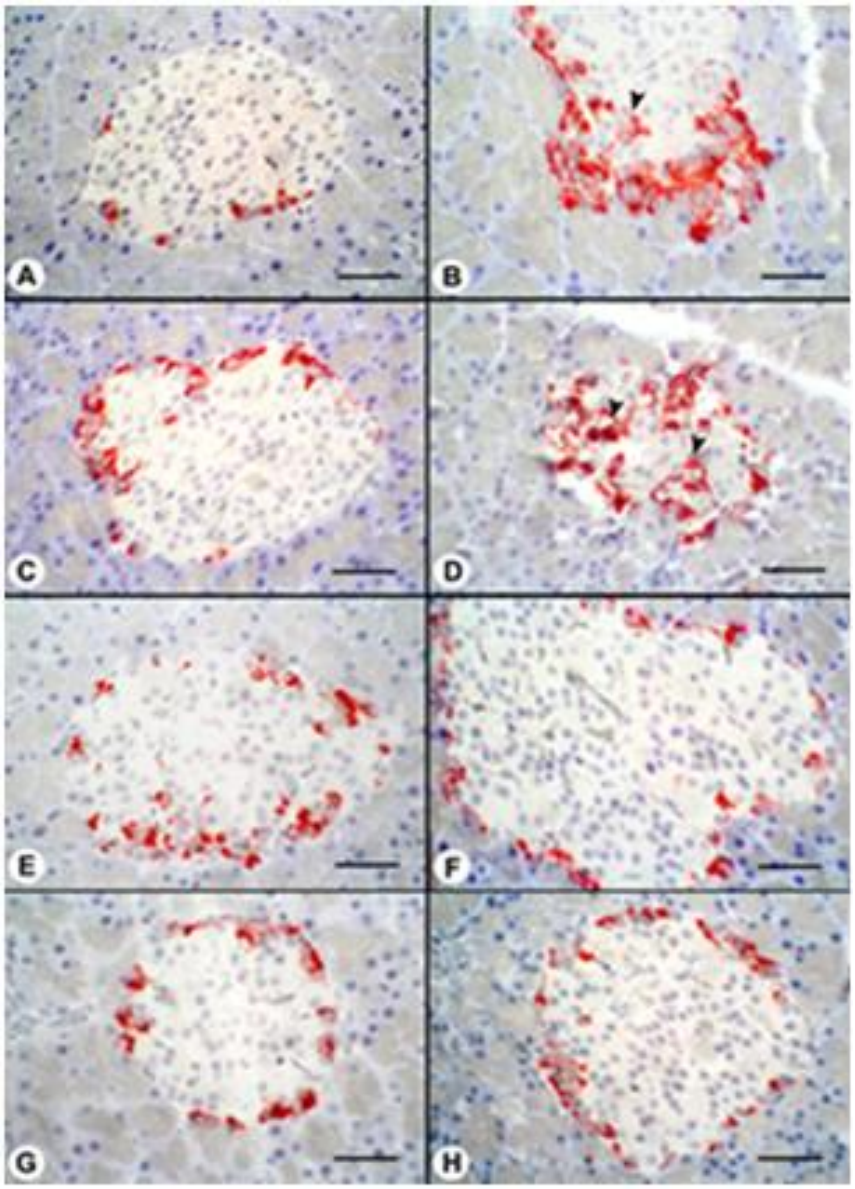

Figure 3. Caspase 3 positivity in islets of Langerhans **A.** Control, **B.** BPA group, increase in Caspase 3 positive cell density in islets (arrowhead), **C.** BPA+CoQ10 group, **D.** BPA+FUL group pronounced Caspase 3 positivity in the islet center (arrowheads), **E.**BPA+ALA group, **F.** CoQ10 group, **G.** FUL group, **H.** ALA group; MH × 100, Bar= 50 µ.

Discussion

In recent years, it was shown that BPA can adversely affect the endocrine system, even at low doses (11). Previous studies have shown that BPA can induce excessive insulin stimulation in the endocrine pancreas and contributing to insulin resistance (12, 33). It has been reported that BPA may increase the risk of developing type II diabetes by directly affecting pancreatic cells, impairing insulin and glucagon secretion, inhibiting cell growth, triggering cell apoptosis, affecting muscle, liver, and fat cell function, and activating insulin resistance (14, 33, 34). Similarly, it was reported that 100 mg/kg intraperitoneal injection of BPA into female mice decreased the size and cell number of islets of Langerhans (35). In the present study, statistical differences were determined between the groups in terms of islet number, diameter, and area. Accordingly, we determined that the number of islets decreased by 50.29%, 17.34%, 37.68% and 20.81% in BPA, BPA+CoQ10, BPA+FUL, and BPA+ALA groups, respectively, compared to the control group. In the BPA, BPA+CoQ10, BPA+FUL, and BPA+ALA groups, islet diameters decreased by 28.42%, 20.08%, 14.51%, and 6.09% and islet areas decreased by 40.79%, 28.61%, 25.41% and 5.27%, respectively. These observed changes in islets of Langerhans were evaluated as an indication that BPA can directly affect pancreatic cells and lead to the development of type I diabetes. It was

reported that insulitis developed at the end of 11 weeks and type 1 diabetes developed at the end of 28 weeks in offspring born from pregnant mice administered BPA at a dose of 10 mg/l in daily drinking water (14).

A previous study determined that no histopathological changes occurred in the islets of Langerhans of male rats exposed to 5, 50, or 500 µg/kg/day oral doses of BPA for 8 weeks. However, in the same study, the percentage of insulin-positive β cells in the islets of Langerhans increased significantly in the group administered BPA at a dose of 500 µg/kg/day compared to the other groups (2). Another study in which rats were administered Bisphenol F (BPF) at 20, 100, or 500 mg/kg/day for 28 days, insulin positive β cell index values decreased in BPF groups compared to the control group (33). In the present study, no histologic changes were observed in the pancreas of male rats after oral administration of 25 mg/kg/day BPA for 7 weeks, and insulin positive β cell index was significantly decreased in BPA-treated groups compared to control and antioxidant-only groups. It was determined that β cell index decreased by 9.72%, 6.47%, 8.77%, and 6.52% in BPA, BPA+CoQ10, BPA+FUL, and BPA+ALA groups, respectively, compared to the control group. BPA dose, animal species, and exposure time seem to be the main factors determining whether histopathologic changes will occur in the pancreas.

It has been reported that BPA causes oxidative stress and has detrimental effects on organs such as the pancreas, liver, brain, kidney, and testis (35). In addition, it was reported that BPA injection at a dose of 2mg/kg/day in male rats caused a decrease in antioxidative effective enzymes in the pancreas at the end of four weeks and an increase malondialdehyde levels, a product indicative of oxidative stress (36). In the present study, caspase 3 positivity increased by 265.37%, 141.30%, 321.89%, and 223.16% in the BPA, BPA+CoQ10, BPA+FUL, and BPA+ALA groups, respectively, compared to the control group. In the islets of Langerhans in rats, β-cells make up 60-80% of the total number of cells, α-cells 15-20%, δ-cells less than 10%, and PP-cells approximately 1% (37-39). It was reported that a significant increase in caspase 3 positivity was observed in the islets of Langerhans in 11-week-old offspring of female mice exposed to BPA (3000 µg/kg/day) during pregnancy (14). In the present study, we interpreted that the decreases in the number, diameter, and area of islets detected in the BPA-exposed groups compared to the control group may be due to oxidative stress-based apoptotic cell deaths in islets, especially β cells.

CoQ10 is a fat-soluble compound that acts as a coenzyme in important enzymatic reactions during energy production in cells and can be found in every cell (40, 41). Although CoQ10 is at a lower concentration than other antioxidants in plasma, it is the first antioxidant to react with plasma oxidants. It is also known to play a role in the regeneration of other antioxidants (40, 42). When CoQ10 was administered with metformin in diabetic rats, it was found to reduce the number of apoptotic cells in the pancreas and provided a significant improvement in insulin staining intensity in islets (43). In addition, it was reported that CoQ10 reduced changes such as interstitial edema, inflammatory cell infiltration, and acinar necrosis in an experimental pancreatitis model in rats (44). In rats with diabetes induced by sirolimus, CoQ10 administration at a dose of 20 mg/kg/day for two weeks caused a decrease in the number of TUNEL positive cells in islets of Langerhans (43). In another study, there was no significant difference observed in terms of β cell area determined by histopathological and immunohistochemical insulin positivity between the group administered 100 mg/kg/day dose of BPA alone and the group administered 10 mg/kg/day dose of CoQ10 with the same dose of BPA for 14 days (45). In the present study, CoQ10 administration at a dose of 40 mg/kg/day significantly ameliorated the apoptotic effects of BPA on the endocrine pancreas.

FUL treatment was found to be effective for the prevention of oxidative stress against increased lipid peroxidation and changes in antioxidant enzyme activities in doxorubicin-treated male rats (46). In male rats with acute cholangitis, development of acute pancreatitis characterized by necrosis, degeneration, edema, and thrombosis in the acinus was observed, and degenerative changes in the pancreas were suppressed by oral or intraperitoneal 0.5 mg/kg FUL administration for 3 days (47). In the present study, it was surprisingly noted that FUL administration at a dose of 8 µg/kg/day together with 20 mg/kg/day BPA exacerbated the apoptotic effect of BPA in islets. In addition, it was found to have no effect on morphometric changes. Consistent with the present study, the cell number and volume in the islets decreased by approximately 50% at the end of 5 days in the endocrine pancreas of rats with streptozotocin (STZ)-induced diabetes; 25.00 mg/kg FUL treatment daily with STZ injection did not improve the condition in the pancreas and caused further deterioration of periductal inflammatory changes (48).

It has been emphasized that ALA has suitable properties to treat tissue damage caused by free oxygen radicals due to its strong antioxidative effects (49). It was reported that administration of 100 mg/kg/day ALA for 8 weeks to STZ-induced diabetic rats prevented necrotic and degenerative destruction in endocrine pancreatic cells (50). Consistent with previous studies, we found in the present study that administration of 70 mg/kg/day ALA together with 25 mg/kg/day BPA for 7 weeks significantly improved BPA-induced morphometric changes in the pancreas and decreased the number of apoptotic cells in islets. In previous studies, ALA was shown to prevent oxidative damage and cell death by scavenging oxygen radicals, metal ion chelates, and various mechanisms protective against lipid peroxidation (50-52).

In conclusion, while long-term BPA administration in rats did not result in observable histopathological changes in the endocrine pancreas, significant morphometric alterations, decreased insulin secretion, and apoptosis in islet cells were identified in the present

study. Notably, CoQ10 and ALA exhibited partial protective effects against the impact of BPA on the endocrine pancreas. Given the absence of studies exploring the effects of ALA and FUL on BPA-induced pancreatic tissue damage, it is suggested that future research should focus on unraveling the detailed interaction of these antioxidant substances with BPA.

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