Original Article

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Quercetin and Adiponectin as Potential Inhibitors of Bisphenol A-Exposed Muscle Cells Toxicity

Davoud Mohammadi[1], Farideh Jalali-Mashayekhi[2, 3], Keivan Nedaei[4], Mina Hemmati[1*], Darya Ghadimi[1], Zahra Rasooli[1]

1. Dept. of Biochemistry, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

2. Dept. of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran

3. Medical and Molecular Research Center, Arak University of Medical Sciences, Arak, Iran

4. Dept. of Medical Biotechnology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

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Corresponding Information:
Mina Hemmati,
Dept.of Biochemistry,Zanjan University of Medical Sciences, Zanjan, Iran
E-Mail: mina1hemmati@yahoo.com

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ABSTRACT

Background & Objective: Bisphenol A [BPA; 2,2-bis-(4 hydroxyphenyl) propane] is an environmental estrogenic and endocrine-disrupting compound that exerts its destructive effects through increasing oxidative stress, but the mechanisms underlying this effect have not yet been fully explained. This study evaluates BPA toxicity in muscle cells and the therapeutic potential of adiponectin (APN) and quercetin (QUER).

Materials & Methods: Confluent L6 rat muscle cells were exposed to BPA (50 and 100 µM) with APN (10 and 100 ng/ml) and QUER (10 and 25 □M) for 24 and 48 hours. Cell viability, pro-oxidant/antioxidant balance (PAB), catalase (CAT) activity, and KEAP1/Nrf2 gene expression were analyzed using desired methods.

Results: BPA in high doses (100 □M) significantly reduced the viability of the muscle cells, while APN and QUER increased cell survival in a time-dependent manner. The effect of QUER also was dose-dependent, while APN in high doses decreased the viability of the muscle cells. APN and QUER also reduced BPA-associated oxidative stress. These changes were significant in the case of QUER. The CAT activity was reduced in BPA-treated cells, which was notably increased with APN and QUER treatment. A decrease in Nrf2 gene expression of BPA-treated muscle cells improved by treatment with QUER and APN in a dose-dependent manner.

Conclusion: Our results hinted that APN and QUER could modulate BPA-induced oxidative stress in muscle cells through KEAP1/Nrf2 pathways. Accordingly, it can also be concluded that APN in low doses and QUER may significantly reduce muscle toxicity caused by BPA.

Keywords: Adiponectin, Flavonoid, Oxidative stress, Muscle cell toxicity, Bisphenol A

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1. Introduction

In the last decade, environmental estrogenic compounds such as bisphenol A (BPA) or [2,2-bis(4-hydroxyphenyl) propane] have raised public health concerns due to their adverse effects in animal models (1).

BPA, a potential endocrine-disrupting chemical, is used in factories that produce polycarbonate plastics, epoxy resins, food packaging and coating, rubber chemicals, and dental sealants (2). Human studies suggest that BPA may be associated with type 2 diabetes and cardiovascular diseases. Recent studies also have shown that oxidative stress induced by BPA can lead to insulin resistance (3). Previous studies have shown that BPA exerts diabetogenic effects by diverting insulin signaling to skeletal muscle and adipose tissues. BPA may induce its toxic effects by increasing oxidative stress and decreasing antioxidants (4).

Evidence shows that cell damage caused by oxidative stress is involved in the pathogenesis of more than a hundred diseases (5, 6). Reactive oxygen species (ROS) are produced in skeletal muscles at rest and during contractile activity. Besides, it has been reported that oxidative stress plays a crucial role in muscle cell pathology through multiple routes, including mitochondrial dysfunction, increased ubiquitin-proteasome system activity, increased myonuclear apoptosis, decreased protein synthesis pathway, and deregulated autophagy. These conditions

finally lead to cachexia-skeletal muscle atrophy (7). Myogenic cells have antioxidant enzymes such as superoxide dismutase, catalase (CAT), glutathione peroxidase, and Heme oxygenase-1. These enzymes affect the viability and proliferation of muscle satellite cells and myoblasts' differentiation (5).

According to the literature, flavonoids such as quercetin (QUER) delay cell death and oxidative damage by scavenging free radicals (8). Previous studies have shown that up to 100 mg of QUER per day is well tolerated in humans and has no side or harmful physiological effects. Moreover, it effectively reduces the risk of cardiovascular disease by reducing oxidative stress (9). Meanwhile, studies have revealed that QUER, as a powerful antioxidant, coordinated its antioxidant effects in the presence of high levels of oxidative stress. QUER can inhibit oxidant biomolecules that can alter antioxidant defense pathways in vivo and in vitro (10, 11). Adiponectin (APN) is an endocrine factor directly released from the adipose tissue and suppresses the harmful effects of oxidative stress (12). APN utilizes protective effects on heart tissue against oxidative stress induced by the remodeling process in cardiomyocytes by activating AMP-activated protein kinase (AMPK) and restraining regulatory kinases with extracellular signals and the NF-κB pathway (13). To cope with stressful conditions, mammalian cells have developed complex defense mechanisms to adapt to these conditions, including the Keap1 / Nrf2 pathway (14). Under stress-free conditions, Nrf2 (nuclear factor erythroid 2–related factor 2) binds to the KEAP1 (Kelch-like ECH-associated protein 1) protein in the cytosol. It is ubiquitinated and degraded after a few hours. Several cysteine amino acids are oxidized during cellular stress to alter KEAP1 conformity. Nrf2 is separated from KEAP1 by the conformation change and transported to the nucleus. It will bind to the ARE promoter region, leading to the specific gene expression that causes increased cellular resistance to various stresses (15, 16). Considering the inevitability of human exposure to BPA in current societies and its related health issues, this study aims to investigate the effect of BPA on muscle cells (Rat skeletal muscle myoblast) and evaluate the therapeutic potential of APN and QUER by studying KEAP1/Nrf2 pathway and downstream enzyme activity.

2. Materials and Methods

2.1 Experimental Design

This study was performed on the L6 (Rat skeletal muscle myoblast) cell line purchased from Pasteur Institute, Iran. The BPA (Merck, Germany), APN (Abnova, Taiwan), and QUER (Sigma, USA) were purchased and prepared in different concentrations. The cells were divided into the following groups: cells with no treatment as the control group, cells treated with BPA (50 and 100 µM) (17), cells treated with BPA (50 and 100 µM) and QUER (10 and 25 □M) (18), cells treated with BPA (50 and 100 µM) and APN (10 and 100 ng/ml) and cells treated with BPA (50 and 100 µM), QUER (10 and 25 µM), and APN (10 and 100 ng/ml)

2.2 MTT Assay

The L6 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin/streptomycin) (Gibco, USA) in a humidified atmosphere containing 5% CO2 at 37 °C to reach 70% confluency. L6 cells were seeded in a 96-well plate at a density of 7×103 cells per well. After 24 hours, culture media was replaced by the new media containing BPA, QUER, and APN according to the mentioned groups, and cells were incubated for 48 hours. After this period, 100 µl of 5 mg/ml concentrated MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and then the plate was incubated in 5% CO2 at 37 °C for 3 h. DMSO was used to dissolve the blue crystals derived from yellow MTT in live mitochondria, leading to a color in each well. The spectrophotometric absorbance of the samples was measured using the microtiter plate (ELISA) reader at the wavelength of 570 nm. Cell viability was calculated as relative absorbance compared with the control and expressed as a percentage of the control.

2.3 Prooxidant-Antioxidant Balance (PAB) Assay

The PAB test has been performed in two different reactions: enzymatic and chemical. In the enzymatic reaction, the pro-oxidants oxidized the chromogen TMB to a color cation, while in the chemical reaction, the antioxidants reduced the TMB cation to a colorless compound. First, by dissolving the TMB powder in DMSO, the TMB/DMSO solution was prepared. Next, 1 ml of TMB/DMSO solution was added to the acetate buffer (50 mL, pH 4.5). Chloramine T (175 µL, 100 mM) was then incubated in a dark place for 2 hours at room temperature to prepare the TMB cation solution. 16.5 µL of peroxidase enzyme was added to the solution and placed at -20 °C. The TMB solution was prepared by adding 200 µL of TMB/DMSO to the acetate buffer (10 mL, pH 5.6). As the working solution, the third solution was prepared by adding 1 ml of TMB cation solution to 10 ml of TMB solution and, after 6 minutes of incubation in a dark place at room temperature, was freshly used. Also, the standard solutions are obtained by mixing different ratios of hydrogen peroxide at a concentration of 500 µM (as peroxidants) with a solution of 3 mM Uric acid in 10 mM NaOH (as antioxidants). To perform the PAB test and evaluate the oxidant and antioxidant activity of the cells, 50,000 cells were cultured in 24 well plates according to the mentioned groups for 24 and 48 hours. 200µL of the working solution was added to each well and incubated in a dark place at 37oC for 12 minutes. Then, 5 µL of HCL 2N was added to the wells, and the optical density (OD) was read at 450 nm (the reference wavelength was 620 nm).

2.4 Catalase Activity Assay

For CAT activity evaluation, hydrogen peroxide and ammonium molybdate solutions were prepared. Briefly, 102 µl of 30% hydrogen peroxide solution (Mw: 34.01, 9.79 N) was diluted with PBS to 50 ml, and 10 g of ammonium molybdate was diluted to 250 ml with distilled water to prepare 20 mM of hydrogen peroxide solution and 32.4 mM of ammonium molybdate, respectively. The reagents were gently mixed with the vortex. After 3 minutes of incubation at 37 °C, 4 ml of ammonium molybdate (32.4 mM) was added to the tubes, and absorbance was read at 374 nm using a spectrophotometer. Formula 1 is used to obtain the activity of the CAT enzyme based on (KU / L) KU. CAT activity=2.303/t ×(log (S○)/(S-M))×VT/VS (19) Formula 1. Calculating CAT activity, in which t is time, VT is the total volume of test tube reagents, S○ is standard absorption, S is sample absorption,and VS is the test sample volume

2.5 qReal Time-PCR

L6 cells were cultured and treated with BPA, APN, and QUER for 24 hours. According to the protocol instruction, the total RNA was extracted using the trizol Reagent (Total RNA Isolation Reagent). The purity and amount of RNA was measured by NanoDrop 2000c (Thermo Scientific, Pittsburgh, PA, USA). Complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Yektatajhiz, Co, Iran) for real-time PCR. RealQ Plus 2x Master Mix green was used for real time PCR analysis based on its instruction. RTqPCR was done using Applied Biosystems Real-Time PCR Instrument. The expression levels of Nrf2 and KEAP1 genes were normalized against GAPDH. PCR primer for target cDNAs was: Nrf2: 5'ACAACTGGATGAAGAGACCG3' (forward) and 5'TGTGGGCAACCTGGGAGTAG3' (reverse); KEAP1: 5'CAGCGTGGAGAGATATGAG3' (forward) and 5'AGTAACATTCTGCCGAGTT3' (reverse). GAPDH: 5'TGACTGTGCCGTTGAACTTG3' (forward) and 5' GAGACAGCCGCATCTTCTTG3' (reverse).

Real time-PCR was performed for 10 minutes at 95 °C for enzyme activation, followed by 35 cycles of 15 seconds for denaturation at 95 °C, 60 seconds for annealing at 54.5 °C, and 25 seconds for the extension at 72 °C. The ΔΔCT method was employed to compare the relative gene expression of different groups.

2.6 Data Analysis

PSS version 19 was used to analyze the data reported as mean ± standard deviation. The one-way analysis of variance (ANOVA) and Tukey's test (for Post Hoc analysis) were carried out to compare the means of different groups. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1 Viability of L6 Muscle Cells Under BPA Condition

The viability of L6 cells in the presence of BPA (50 and 100 µM) before and after treatment with APN and QUER was evaluated using the MTT assay, and the results are shown in Figure 1. Based on the results, 50 µM of BPA had no significant effect on the L6 cells' viability after 24 hours. The results also showed that the viability of L6 cells treated with 50 µM of BPA in the presence of APN 10 and 100 ng/ml, QUER 10, and 25 µM after 24 hours was not remarkably different from the control group (Figure 1A).

After 48 hours of L6 cells being treated with the mentioned groups, the results showed that only BPA (50 µM) + APN (100 ng/ml) significantly reduced the cells' survival compared to the control group ($p < 0.05$) (Figure 1 B). In actuality, BPA (50 µM) in the presence of APN 100 ng/ml after 24 and 48 hours, had the lowest cell viability. According to Figure 1 (A, B), APN in low concentration and QUER in high doses, showed the highest viability among experimental groups.

According to the results shown in Figure 1C, it was found that BPA (100 µM) led to no significant effect on cell survival in any APN and QUER treatments after 24 hours, except BPA (100 µM) with APN (100 ng/ml) which decreased cell viability strikingly.

After 48 hours of L6 cells treatment with BPA (100 µM), the cell survival in BPA (100 µM), BPA (100 µM) + APN (10 ng/ml), and BPA (100 µM) + APN (100 ng/ml) had significantly decreased compared to the control group ($p < 0.05$) (Figure 1D). Furthermore, BPA (100 µM) + QUER (10 µM) with APN (100 ng/ml) showed a decrease in cell survival after 48 hours.

According to the results in Figure 1, APN (10 ng/ml) with QUER (25 µM) showed the highest cell viability among experimental groups after 24 and 48 hours.

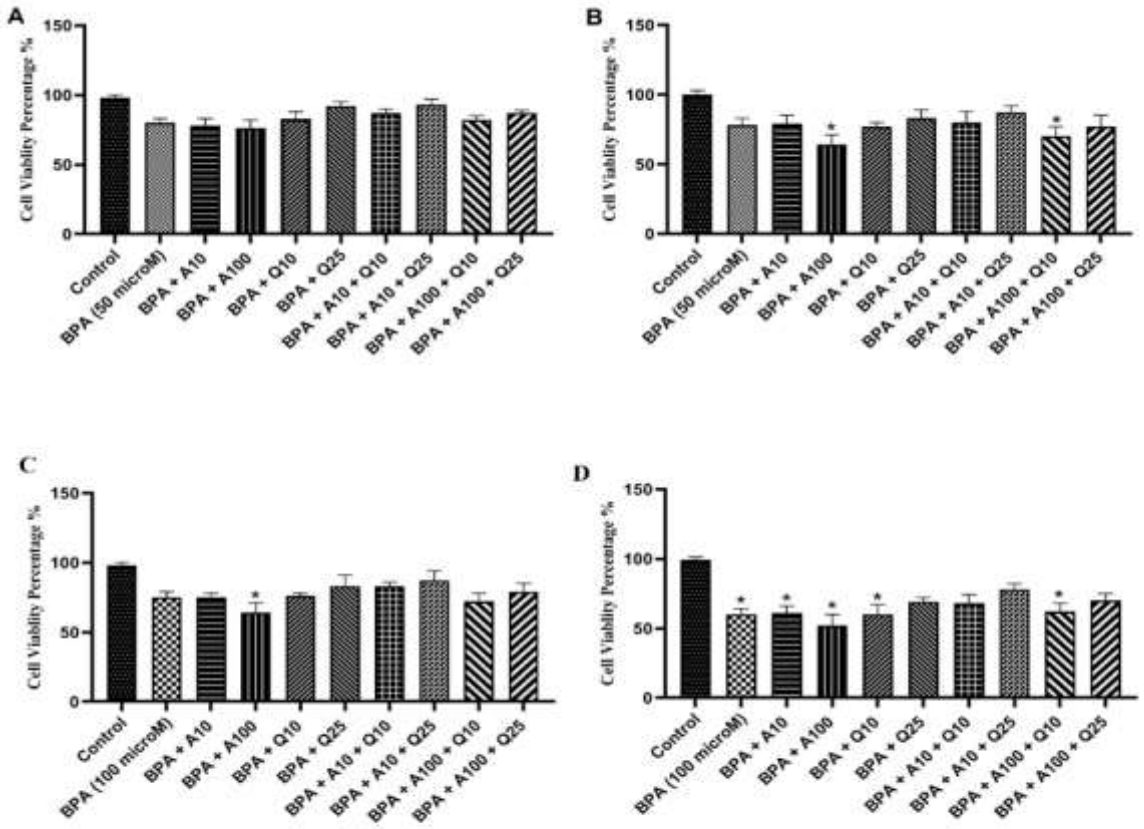

Figure 1. Investigation of cell viability by MTT assay. (A) L6 cells viability treated with 50 µM BPA in the presence of APN (A) and QUER (Q) after 24 hours. (B) L6 cells viability treated with 50 µM BPA in the presence of APN and QUER after 48 hours. (C) L6 cells viability treated with 100 µM BPA in the presence of APN (A) and QUER (Q) after 24 hours. (D) L6 cells viability treated with 100 µM BPA in the presence of APN and QUER after 48 hours. Data expressed as mean ± SD. * Present significant difference at P≤ 0.05 compared to control.

3.2 Effects of APN and QUER on Catalase Activity in L6 Muscle Cells

The CAT activity of L6 cells was examined, and the results are shown in Figure 2. The results have revealed that BPA decreased the CAT activity of L6 cells in all treated groups. However, QUER (25 mM) and APN (10 ng/ml), along with QUER (25 µM), had the opposite effect on BPA impact (Figure 2 A).

The toxic effect of BPA on CAT activity also was seen after 48 hours. In this situation, QUER (25 mM) and APN (10 ng/ml) with QUER (10, 25 µM) diminished BPA effects (Figure 2 B).

CAT activity after 24 hours of treatment with 100 µM BPA in all groups was significantly reduced compared to the control group ($p < 0.05$) (Figure 2 C). Furthermore, 48 hours after treatment, all groups demonstrated significantly less CAT activity than the control group ($p < 0.05$) except BPA (100 µM) + APN (10 ng/ml) + QUER (10, 25 µM) ($p < 0.05$) (Figure 2 D).

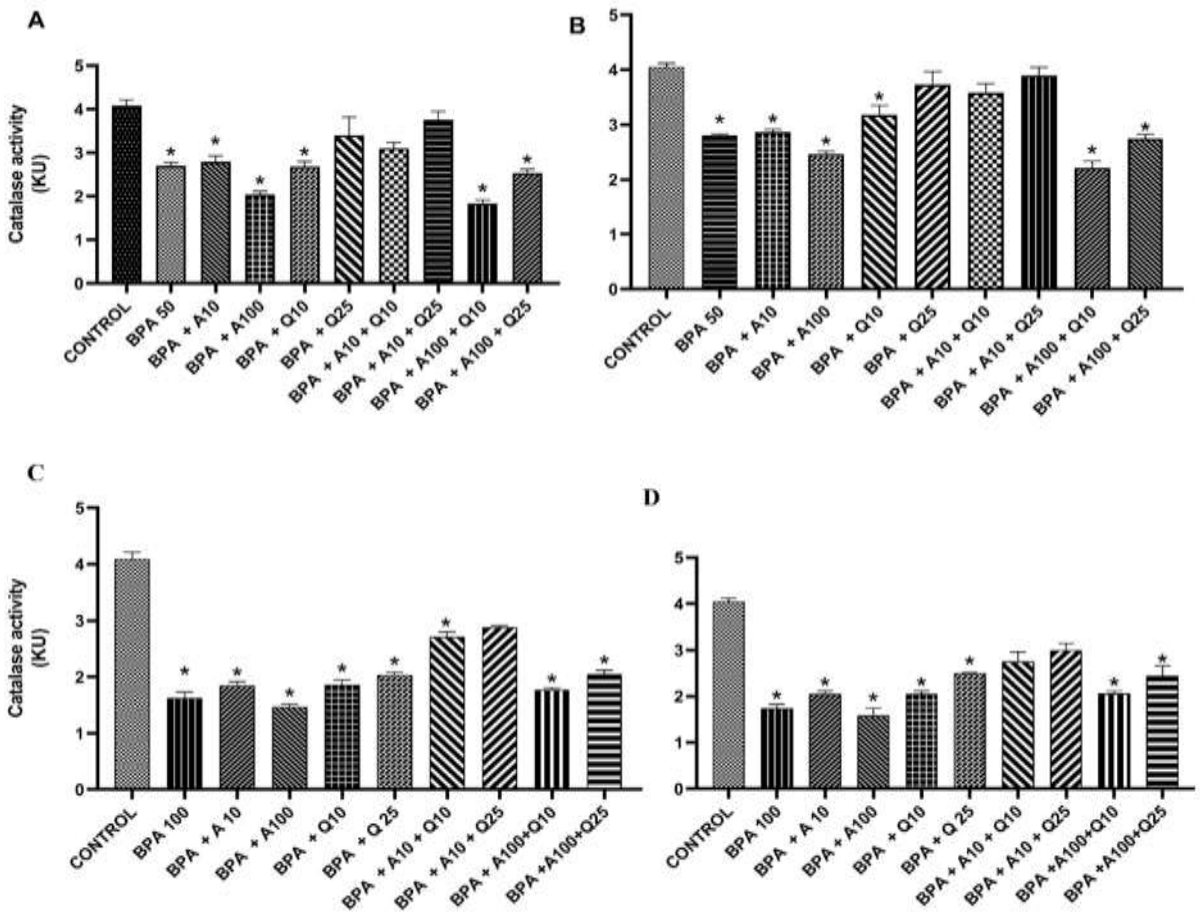

Figure 2. CAT activity in experimental groups. (A) L6 cells CAT activity with 50 µM BPA in the presence of APN (A) and QUER (Q) after 24 hours. (B) L6 CAT activity treated with 50 µM BPA in the presence of APN and Q after 48 hours. (C) L6 cells CAT activity treated with 100 µM BPA in the presence of APN and QUER (Q) after 24 hours. (D) L6 cells CAT activity treated with 100 µM BPA in the presence of APN and QUER after 48 hours. Data expressed as mean ± SD. * Present significant difference at P≤ 0.05 compared to control.

3.3 Effects of APN and QUER on Pro-oxidant/Antioxidant Balance in L6 Muscle Cells

The results of the PAB analysis of BPA-treated L6 cells (50 and 100 µM) in the presence of APN and QUER are shown in Figure 3. The results showed that 24 hours after cell treatment with BPA (50 µM), PAB had a significant increase compared to the control group ($p < 0.05$). According to the results, the presence of APN in high concentration showed a significant increase in PAB compared to the control group ($p < 0.05$) (Figure 3 A). The minimum amount of PAB was seen in APN (10 ng/ml) with QUER (25 µM).

BPA (50 µM) leads to a significant increase in PAB compared to the control group after 48 hours ($p < 0.05$). A combination of BPA (50 µM) + APN (10 ng/ml), BPA (50 µM) + APN (100 ng/ml), BPA (50 µM) + APN (100 ng/ml) + QUER (10 µM) and BPA (50 µM) + APN (100 ng/ml) + QUER (25 µM) also showed a significant increase in PAB compared to the control group ($p < 0.05$) (Figure 3 B). The maximum amount of PAB was seen in APN (100 ng/ml), and the minimum prooxidant status was seen in QUER (25 µM) + APN (10 ng/ml).

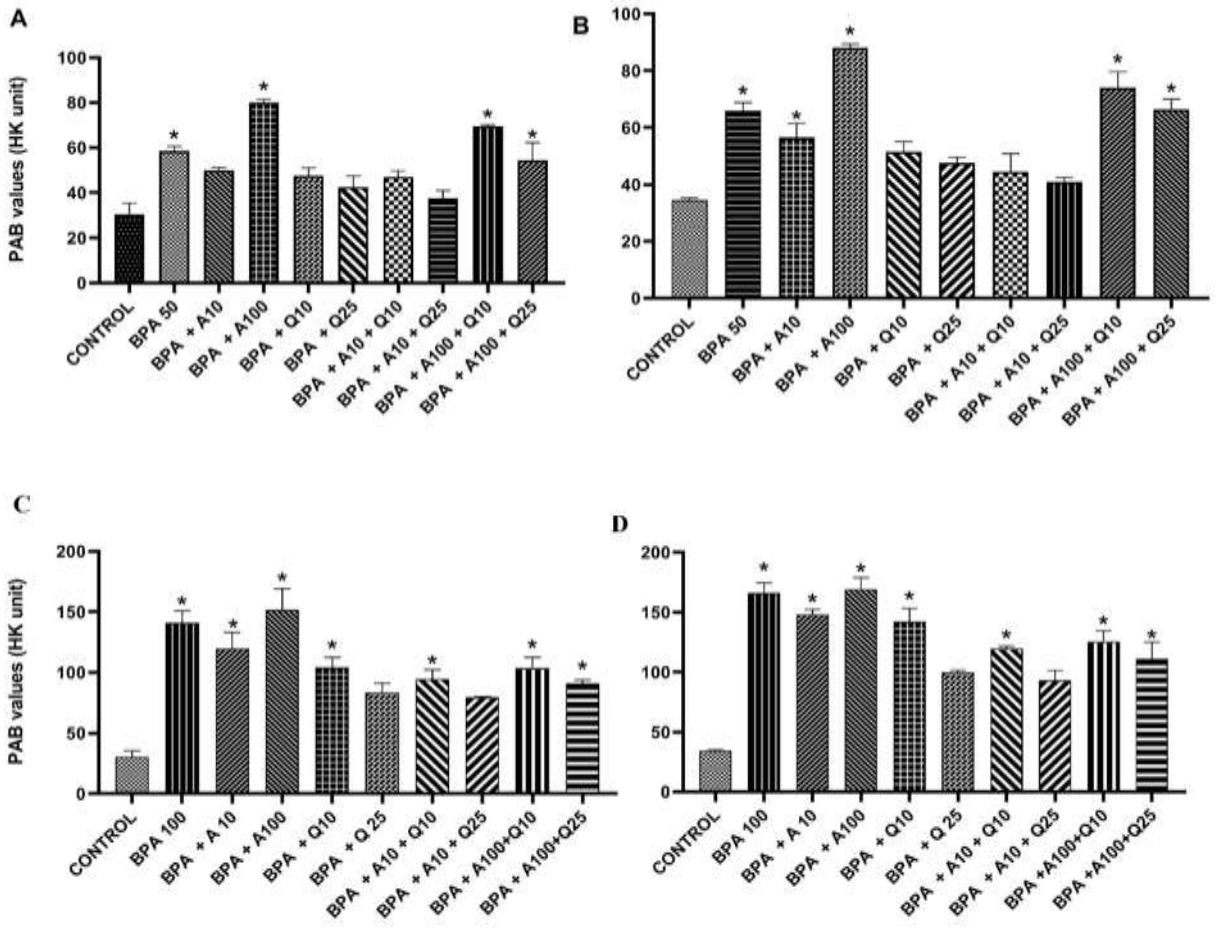

Figure 3. Pro-oxidant/antioxidant balance in experimental groups. (A) L6 PAB treated with 50 µM BPA in the presence of APN (A) and QUER (Q) after 24 hours. (B) L6 PAB treated with 50 µM BPA in the presence of APN and QUER after 48 hours. (C)) L6 PAB treated with 100 µM BPA in the presence of APN (A) and QUER (Q) after 24 hours. (D) L6 PAB treated with BPA (100 µM) in the presence of APN and QUER after 48 hours. Data expressed as mean ± SD. * Present significant difference at P≤ 0.05 compared to control.

Regarding BPA (100 µM), the results after 24 and 48 hours showed that the PAB value of all treatments increased compared to the control group ($p < 0.05$) except BPA (100 µM) + APN (10 ng/ml) with QUER (25 µM) (Figure 3 C and D).

3.4 KEAP1 and Nrf2 Gene Expression in Response to APN and QUER Under BPA Condition

The expression of KEAP1 and Nrf2 genes in L6 cells was examined after the treatment with BPA (50 and 100 µM) and in the presence of different concentrations of APN and QUER, and the results are shown in Figures 4 and 5. The results showed that the expression of the KEAP1 gene treated with BPA (50 µM) had a significant increase only in the BPA (50 µM) + APN (100 ng/ml) group compared to the control group ($p < 0.05$) (Figure 4 A).

Nrf2 gene expression in BPA (50 µM) + QUER (25 µM) and BPA (50 µM) + APN (10 ng/ml) with QUER (25 µM) groups was significantly higher than the control group ($p < 0.05$) (Figure 5 A).

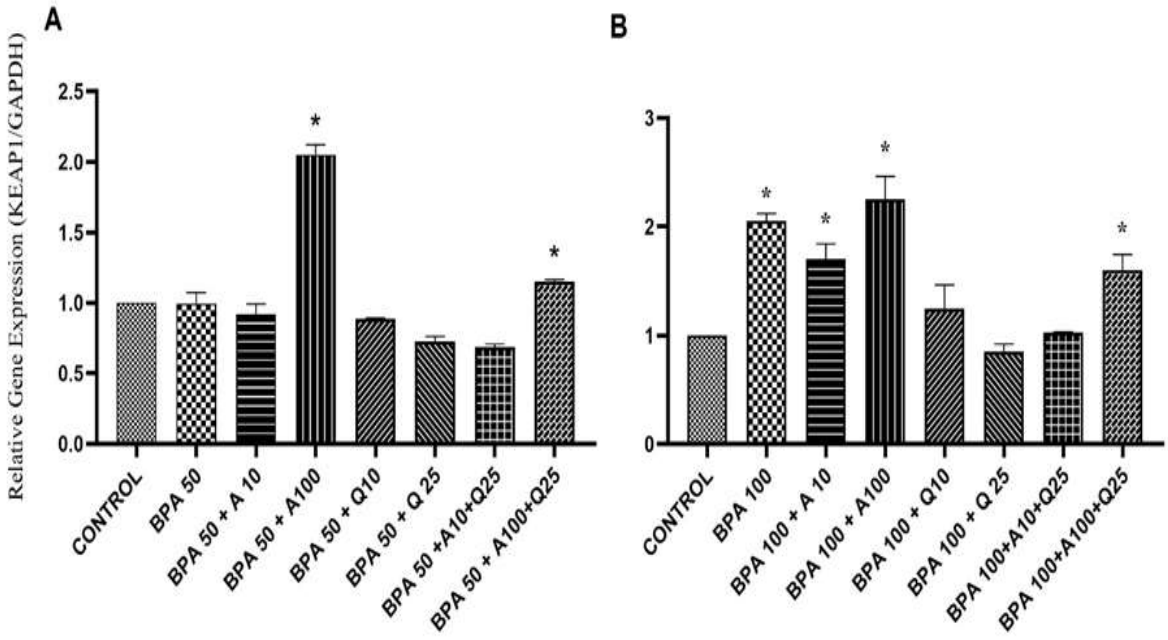

Figure 4. (A) Expression of the KEAP1 gene in L6 treated with 50 µM of BPA in the presence of APN (A) and QUER (Q). (B) expression of KEAP1 genes in L6 cells treated with BPA (100 µM) in the presence of APN and QUER. Data expressed as mean ± SD. * Present significant difference at P≤ 0.05 compared to control.

However, cells with BPA (100 µM), BPA (100 µM) + APN (10 ng/ml), BPA (100 µM) + APN (100 ng/ml), and BPA (100 µM) + APN (100 ng/ml) with QUER (25 µM) showed a higher expression of KEAP1 genes than the control group (p <0.05) (Figure 4 B). Nevertheless, obvious differences were observed in the expression of KEAP1 genes in cells treated with 100 µM of BPA. The results showed that in BPA (100 µM), BPA (100 µM) + APN (100 ng/ml), and BPA (100 µM) + QUER (10 µM) Nrf2 expression was exceptionally reduced compared to the control group (p <0.05) (Figure 5 B).

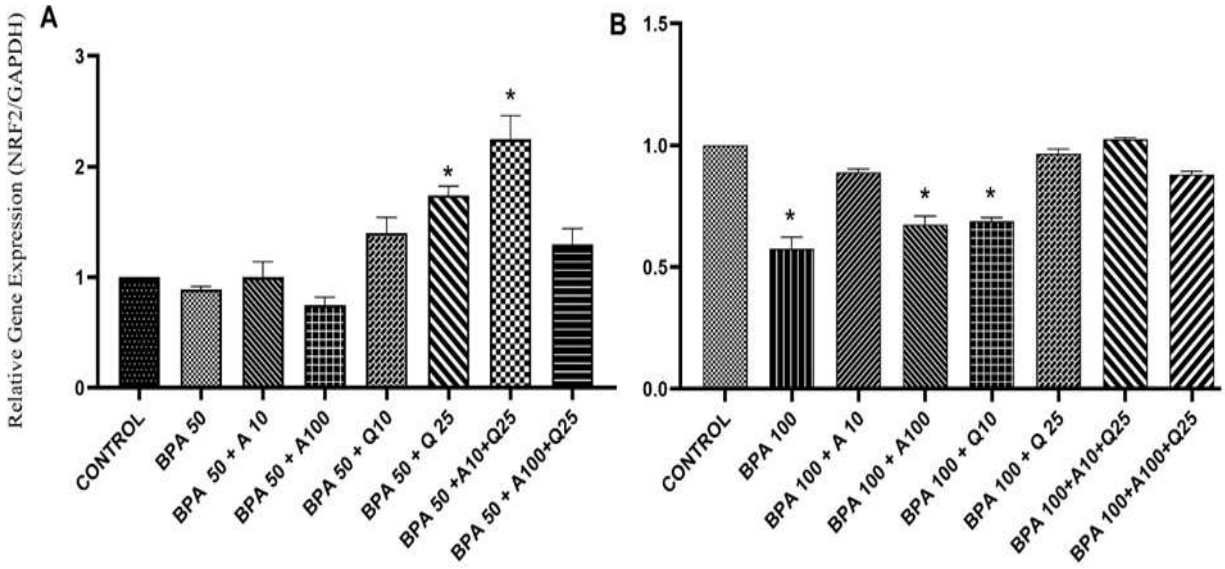

Figure 5. (A) Expression of the Nrf2 gene in L6 treated with 50 µM of BPA in the presence of APN (A) and QUER (Q). (B) Expression of the Nrf2 gene in L6 cells treated with BPA (100 µM) in the presence of APN and QUER. Data expressed as mean ± SD. * Present significant difference at P≤ 0.05 compared to control.

4. Discussion

It is well known that environmentally toxic compounds such as BPA have a role in the pathogenesis of many metabolic disorders. This suggests that antioxidants may be an optimal option for encountering them. The ROS produced in response to toxic compounds can disrupt metabolic regulation and enhance metabolic diseases such as insulin resistance locally in adipose tissues and insulin-target tissues such as skeletal muscle and the liver (20). Flavonoids like QUER can postpone cell death and oxidative damage through free radical scavenging (21-24). According to previous studies, APN plays a protective role in metabolizing many tissues, including heart tissue, skeletal muscle, the liver, and adipose tissue, by reducing ROS levels (25, 26).

Based on the results of our study, the toxicity effect of BPA on muscle cells was dependent on concentration and time. 50 µM of BPA did not significantly impact cell viability for up to 48 hours. On the other hand, while 100 µM did not show any toxicity on the cells for 24 hours, it significantly reduced cell survival after 48 hours. Increased BPA concentration is associated with an increased risk of cardiovascular, hepatic, and renal diseases such as coronary heart disease, fatty liver disease, and chronic kidney disease. In this regard, BPA has been shown to cause various cytotoxic, genotoxic, and carcinogenic effects in vitro and in vivo. In line with our findings, Podlich et al. studied the cytotoxic effects of BPA on HEK293 cells. They showed that the incubation of HEK293 with 100 µM or 1000 µM of BPA resulted in a significant dose-dependent reduction in renal cell viability (27).

Moreover, BPA-treated cells incubated with APN and QUER showed that treatments with QUER (at both concentrations) could overcome BPA-induced toxicity after 48 hours. Sangai NP et al. showed that QUER decreased ROS production and increased total antioxidant capacity in rat kidneys (28). Using QUER can reduce the production of free radicals and play an essential role in improving the antioxidant/oxidant balance (29). However, QUER has another therapeutic property that inhibits lipid peroxidation and has been shown to inhibit H2O2-induced lipid peroxidation even at a low concentration (2 µM) (30).

Our study also evaluated CAT activity, and the results showed that cell treatment with BPA (50 and 100 µM) momentously reduced the cells' CAT activity at 24 and 48 hours. Shirani et al. also showed that BPA reduced catalytic activity in rats (31). CAT is an enzyme found in almost all living organisms exposed to oxygen that catalyzes the breakdown of hydrogen peroxide into water and oxygen. This enzyme is crucial in protecting cells against oxidative damage by ROS. Our study showed that QUER is almost more compelling than APN in increasing cell CAT. Shirani et al. reported similar results on the effect of QUER on CAT activity (31). Demkovych et al. also reported increased catalytic activity due to QUER treatment in rats (32).

PAB, as an index of oxidative stress, was evaluated in BPA-treated cells. The results showed that BPA at both concentrations significantly increased PAB. BPA disrupts cellular oxidative homeostasis by altering the dynamic balance between oxidative mediators and the activity of antioxidant enzymes, thereby predisposing the cell to apoptosis (33). Moghaddam et al. reported that exposure to BPA (0.5 and 2 mg/kg /day) for four weeks in male mice significantly disrupts the dynamic balance of enzymatic antioxidants (34). Regardless of the presence of the antioxidant system, excessive or unbalanced production of ROS due to exposure to chemicals may lead to several clinical disorders. For example, BPA can cause liver damage due to disrupting the oxidation state of cells (35). Our study showed that BPA exposure induces oxidative stress by upsetting the balance between ROS and the antioxidant defense system in muscle cells, which aligns with other studies. Our results also showed that QUER reduced the effects of BPA on PAB. It seems that using compounds with potent antioxidant properties, such as QUER, can reduce BPA-induced production of free radicals and toxicity in the body and play an essential role in improving the antioxidant/oxidant balance (36). Mahdavinia et al. examined the effect of QUER on the oxidative toxicity of BPA in the liver. They found that QUER could prevent BPA-related hepatotoxicity and mitochondrial stress in rats (36).

In the present study, we also investigated the expression of KEAP1 and Nrf2 genes and found that BPA at 100 µM concentration increased KEAP1 expression and decreased Nrf2 expression. Nrf2 is a transcription factor that mediates an essential signaling pathway that prevents injury and disease. Under normal circumstances, Nrf2 is found and degraded in the cytoplasm attached to KEAP1. Following oxidative stress, Nrf2 is cleaved from KEAP1 and transported to the nucleus, activating the transcription of protective genes to defend against organ damage (37). Our results found that BPA in L6 cells causes oxidative stress by activating KEAP1 and suppressing Nrf2. Similar results were reported by Zhangshan Gao et al., regarding the effect of BPA on laying hens and acknowledged that BPA disrupts ovarian redox homeostasis by activating Keap1 and suppressing the Nrf2 signaling pathway (38). Our study showed that combining QUER (25 µM) and APN (10 ng/ml) could increase Nrf2 expression to a normal level. In a parallel study, Li-LiJi et al. investigated the effect of QUER on the Nrf2 pathway expression in hepatotoxicity toxicity. They showed that QUER increased the expression of Nrf2-dependent antioxidant genes by inhibiting Keap1 binding to Nrf2 and inducing p62 expression (39). In another study, Ghazizadeh Darband et al. showed that QUER reduced oxidative DNA damage through the Nrf2 signaling pathway in rats with colorectal cancer (40). According to the results, APNs in low concentration played an effective protective role in muscle cells.

On the contrary, a high concentration of APN (100 ng/ml) imposed a toxic effect on muscle cells (decline in cell viability, CAT activity, and Nrf2 gene expression), which can be ascribed to the pro-

inflammatory feature of APN at high concentration. According to the literature, inflammatory diseases, such as chronic kidney disease (CKD), type 1 diabetes, cystic fibrosis, and systemic lupus erythematosus can be associated with high doses of APN (41). Choi et al. showed that the CKD patients with high concentrations of APN also had high levels of Toll-like receptor 4 (TLR-4), a C-reactive protein, and IL-6 compared to the healthy subjects. Since TLR-4 is a critical mediator of innate immunity, activation of TLR-4, which could be associated with high concentrations of APN, enhanced inflammatory responses in CKD patients (42). According to a recent study and considering the results of other studies mentioned above, APNs at high concentrations can have proinflammatory potential. This issue should be considered in studying various metabolic diseases in human populations.

5. Conclusion

Due to the destructive effect of BPA in metabolism, and because BPA plays as an endocrine-disrupting agent, understanding the precise mechanism of action will be beneficial for applying useful preventive or treatment strategies. According to our results, BPA targets antioxidant system in muscle cells and uses QUER as an antioxidant, suppressing BPA-associated oxidative stress in low and high doses. APN also acts as a suppressor of BPA-induced oxidative stress. However, considering that APN had harmful effects in high concentrations (100 ng/ml), using different doses of APNs in invivo and invitro experiments should be done with caution. In conclusion, QUER and APN are increasingly looked upon as support against toxic compounds. However, additional clinical investigations are needed to be accomplished to prove the effectiveness and safety of APN and QUER.

6. Declaration

6.1 Acknowledgements

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6.2 Authors' Contribution

DM and MH have made substantial contributions to the conception and design. FJM, KN and ZR have also participated in drafting the article or revising it critically for important intellectual content. All authors have given the final approval for the version which will be submitted and any revised versions following after it.

6.3 Conflict of Interest

The authors declare that they have no conflict of interest.

6.4 Funding

No Funding.

6.5 Ethics Approval

This study has been approved by vice president of research and technology and ethics committee of Zanjan University of Medical Sciences [ethical code: IR.ZUMS.RES.1399.021]. Ethical considerations were observed in all steps of the research.

References

1. Carwile JL, Michels KBJEr. Urinary bisphenol A and obesity: NHANES 2003-2006. Environ Res. 2011;111(6):825-30.

https://doi.org/10.1016/j.envres.2011.05.014

PMid:21676388 PMCid:PMC3747781

2. Groff TJCoip. Bisphenol A: invisible pollution. Curr Opin Pediatr. 2010;22(4):524-9.

https://doi.org/10.1097/MOP.0b013e32833b03f8

PMid:20489636

3. Song S, Zhang L, Zhang H, Wei W, Jia L. Perinatal BPA exposure induces hyperglycemia, oxidative stress and decreased adiponectin production in later life of male rat offspring. Int J Environ Res Pub Health. 2014;11(4):3728-42.

https://doi.org/10.3390/ijerph110403728

PMid:24705360 PMCid:PMC4025022

4. Alonso-Magdalena P, Ropero AB, Soriano S, Quesada I, Nadal A. Bisphenol-A: a new diabetogenic factor? Hormones. 2010;9(2):118-26.

https://doi.org/10.1007/BF03401277

PMid:20687395

5. Kozakowska M, Pietraszek-Gremplewicz K, Jozkowicz A, Dulak J. The role of oxidative stress in skeletal muscle injury and regeneration: focus on antioxidant enzymes. J Musc Res Cell Motil. 2015;36(6):377-93.

https://doi.org/10.1007/s10974-015-9438-9

PMid:26728750 PMCid:PMC4762917

6. Maritim A, Sanders a, Watkins Iii J. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Molec Toxicol. 2003;17(1):24-38.

https://doi.org/10.1002/jbt.10058

PMid:12616644

7. Ábrigo J, Elorza AA, Riedel CA, et al. Role of oxidative stress as key regulator of muscle wasting during cachexia. Oxid Med Cell Long. 2018;2018:2063179.

https://doi.org/10.1155/2018/2063179

PMid:29785242 PMCid:PMC5896211

8. Xu D, Hu MJ, Wang YQ, Cui Y. Antioxidant activities of quercetin and its complexes for medicinal application.Molecules. 2019;24(6):1123.

https://doi.org/10.3390/molecules24061123

PMid:30901869 PMCid:PMC6470739

9. Morand C, Crespy V, Manach C, Besson C, Demigne C, Remesy . Plasma metabolites of quercetin and their antioxidant properties. Am J Physiol. 1998;275(1):R212-R9.

https://doi.org/10.1152/ajpregu.1998.275.1.R212

PMid:9688981

10. Song X, Wang Y, Gao L. Mechanism of antioxidant properties of quercetin and quercetin-DNA complex. J Mol Model. 2020;26:1-8.

https://doi.org/10.1007/s00894-020-04356-x

PMid:32399900

11. Lesjak M, Beara I, Simin N, et al. Antioxidant and anti-inflammatory activities of quercetin and its derivatives.J Func Food. 2018;40:68-75.

https://doi.org/10.1016/j.jff.2017.10.047

12. Straub LG, Scherer P. Metabolic Messengers: adiponectin. Nat Metab. 2019;1(3):334.

https://doi.org/10.1038/s42255-019-0041-z

PMid:32661510 PMCid:PMC7357716

13. Essick EE, Ouchi N, Wilson RM, et al. Adiponectin mediates cardioprotection in oxidative stress-induced cardiac myocyte remodeling. Am J Physiol Heart Circul Physiol.2011;301(3):H984-H93.

https://doi.org/10.1152/ajpheart.00428.2011

PMid:21666115 PMCid:PMC3191107

14. Matzinger M, Fischhuber K, Heiss EH. Activation of Nrf2 signaling by natural products-can it alleviate diabetes? Biotechnol Adv. 2018;36(6):1738-67.

https://doi.org/10.1016/j.biotechadv.2017.12.015

PMid:29289692 PMCid:PMC5967606

15. Suzuki T, Motohashi H, Yamamoto MJ. Toward clinical application of the Keap1-Nrf2 pathway.Trends Pharmacol Sci. 2013;34(6):340-6.

https://doi.org/10.1016/j.tips.2013.04.005
PMid:23664668

16. Baird L, Yamamoto M. The molecular mechanisms regulating the KEAP1-NRF2 pathway. 2020;40(13):e00099-20.

https://doi.org/10.1128/MCB.00099-20

PMid:32284348 PMCid:PMC7296212

17. Xin F, Jiang L, Liu X, et al. Bisphenol A induces oxidative stress-associated DNA damage in INS-1 cells. Mutat Res Genet Toxicol Environ Mutagen. 2014; 769: 29-33.

https://doi.org/10.1016/j.mrgentox.2014.04.019

PMid:25344109

18. Ben Salem I, Boussabbeh M, Graiet I, Rhouma A, Bacha H, Abid Essefi S. Quercetin protects HCT116 cells from Dichlorvos-induced oxidative stress and apoptosis. Cell Stress Chaperones, 2016. 21(1): 179-86.

https://doi.org/10.1007/s12192-015-0651-7

PMid:26476661 PMCid:PMC4679746

19. Hadwan MH, Najm Abed H. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief. 2016; 6:194-199.

https://doi.org/10.1016/j.dib.2015.12.012

PMid:26862558 PMCid:PMC4707181

20. Li Z, Chen B, Dong W, et al. Epigenetic activation of PERP transcription by MKL1 contributes to ROS-induced apoptosis in skeletal muscle cells. Biochim

Biophysica Acta (BBA)-Gene Regul Mech. 2018;1861(9):905-15.

https://doi.org/10.1016/j.bbagrm.2018.07.011

PMid:30056131

21. Rezaei-Sadabady R, Eidi A, Zarghami N, Barzegar A. Intracellular ROS protection efficiency and free radical-scavenging activity of quercetin and quercetin-encapsulated liposomes. Artif Cells Nanomed Biotechnol. 2016;44(1):128-34.

https://doi.org/10.3109/21691401.2014.926456

PMid:24959911

22. Chen TJ, Jeng JY, Lin CW, Wu CY, Chen YC. Quercetin inhibition of ROS-dependent and-independent apoptosis in rat glioma C6 cells. Toxicol. 2006;223(1-2):113-26.

https://doi.org/10.1016/j.tox.2006.03.007

PMid:16647178

23. Abharzanjani F, Hemmati M.Protective effects of quercetin and resveratrol on aging markers in kidney under high glucose condition: in vivo and in vitro analysis.Molec Biol Rep. 2021; 48(7): 5435-5442.

https://doi.org/10.1007/s11033-021-06550-3

PMid:34273031

24. Aramjoo H, Ebrahimzadeh H, Hemmati M. Investigation of protective effects of quercetin on Oxidative stress induced by Vinblastine in bone marrow of Rats.Pharmaceut Sci. 2021; 27(2): 194-200.

https://doi.org/10.34172/PS.2020.77

25. Karamian M, Moossavi M, Hemmati M. From diabetes to renal aging: the therapeutic potential of adiponectin. J Physiol Biochem. 2021; 77(2): 205-214.

https://doi.org/10.1007/s13105-021-00790-4

PMid:33555532

26. Esfahani M, Movahedian A, Baranchi M, Goodarzi MT. Adiponectin: an adipokine with protective features against metabolic syndrome. Iran J Basic Med Sci. 2015;18(5):430-42.

27. Podlich S PC, Bohme K, Kreutz R, et al. Comparative analysis of cytotoxic effects of bisphenol A and bisphenol S on human renal HEK293 cells. Arch Sci . 2018;2(1):114

https://doi.org/10.36876/smjnkd.1012

28. Sangai NP, Verma RJ. Protective effect of quercetin on bisphenol A-caused alterations in succinate dehydrogenase and adenosine triphosphatase activities in liver and kidney of mice. Acta poloniae pharmaceutica. 2012;69:1189-94.

29. Fartkhooni FM, Noori A, Mohammadi A. Effects of titanium dioxide nanoparticles toxicity on the kidney of male rats. Int J Life Sci. 2016;10(1):65-9.

https://doi.org/10.3126/ijls.v10i1.14513

30. Abarikwu SO, Simple G, Onuoha SC, Mokwenye I, Ayogu JF. Evaluation of the protective effects of quercetin and gallic acid against oxidative toxicity in rat's kidney and HEK-293 cells. Toxicol Rep. 2020;7:955-62.

https://doi.org/10.1016/j.toxrep.2020.07.015

PMid:32874919 PMCid:PMC7451806

31. Shirani M, Alizadeh S, Mahdavinia M, Dehghani MA. The ameliorative effect of quercetin on bisphenol A-induced toxicity in mitochondria isolated from rats. Environ Sci Pollut Res. 2019;26(8):7688-96.

https://doi.org/10.1007/s11356-018-04119-5

PMid:30666577

32. Demkovych A, Bondarenko Y, Hasiuk P. Effects of quercetin on antioxidant potential in the experimental periodontitis development. Int Med Apply Sci Int Med. 2019;11(1):60-4.

https://doi.org/10.1556/1646.11.2019.06

PMid:32148904 PMCid:PMC7044571

33. Amjad S, Rahman MS, Pang MG. Role of antioxidants in alleviating bisphenol A toxicity. Biomolec. 2020;10(8):1105.

https://doi.org/10.3390/biom10081105

PMid:32722388 PMCid:PMC7465987

34. Moghaddam HS, Samarghandian S, Farkhondeh T. Effect of bisphenol A on blood glucose, lipid profile and oxidative stress indices in adult male mice. Toxicol Mech Methods. 2015;25(7):507-13.

https://doi.org/10.3109/15376516.2015.1056395

PMid:26376105

35. Hassan ZK, Elobeid MA, Virk P, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxid Med Cell Longev. 2012;2012:194829-.

https://doi.org/10.1155/2012/194829

PMid:22888396 PMCid:PMC3409570

36. Mahdavinia M, Alizadeh S, Vanani AR, et al. Effects of quercetin on bisphenol A-induced mitochondrial toxicity in rat liver. Iran J Basic Med Sci. 2019;22(5):499.

37. Wen X, Thorne G, Hu L, Joy MS, Aleksunes LM. Activation of NRF2 Signaling in HEK293 Cells by a First-in-Class Direct KEAP1-NRF2 Inhibitor. J Biochem Molec Toxicol. 2015;29(6):261-6.

https://doi.org/10.1002/jbt.21693

PMid:25683455 PMCid:PMC4713195

38. Gao Z, Gao X, Fan W, et al. Bisphenol A and genistein have opposite effects on adult chicken ovary by acting on ERα/Nrf2-Keap1-signaling pathway. Chem Biol Interact. 2021;347:109616.

https://doi.org/10.1016/j.cbi.2021.109616

PMid:34363818

39. Ji LL, Sheng YC, Zheng ZY, Shi L, Wang ZT. The involvement of p62-Keap1-Nrf2 antioxidative signaling pathway and JNK in the protection of natural flavonoid quercetin against hepatotoxicity. Free Radic Biol Med. 2015;85:12-23.

https://doi.org/10.1016/j.freeradbiomed.2015.03.035

PMid:25881548

40. Darband SG, Sadighparvar S, Yousefi B, et al. Quercetin attenuated oxidative DNA damage through NRF2 signaling pathway in rats with DMH induced colon carcinogenesis. Life Sci. 2020;253:117584.

https://doi.org/10.1016/j.lfs.2020.117584

PMid:32220623

41. Chou IP , Lin YY , Ding ShT, Chen ChY. Adiponectin receptor 1 enhances fatty acid metabolism and cell survival in palmitate-treated HepG2 cells through the PI3 K/AKT pathway. Eur J Nutr. 2014; 53 (3): 907-917.

https://doi.org/10.1007/s00394-013-0594-7

PMid:24129500

42. Choi HM, Doss HM, Kim KS. Multifaceted physiological roles of adiponectin in inflammation and diseases. Int J Mol Sci. 2020; 21: 1219.

https://doi.org/10.3390/ijms21041219

PMid:32059381 PMCid:PMC7072842