

Original Article

QUERCETIN MITIGATES TOXICITY AND OXIDATIVE STRESS MOTIVATED BY BISPHENOL A IN LIVER OF MALE RATS

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ABSTRACT

Objective: Epidemiological reports have indicated a correlation between the increasing of bisphenol A (BPA) levels in the environment and the incidence of hepatotoxicity. The present study aimed to evaluate the protective effect of quercetin on oxidative stress, inflammatory markers, apoptotic and antiapoptotic markers in the liver tissue of the bisphenol A treated rats.

Methods: Forty-eight male Wistar rats were divided into six groups; Group(1): Negative control group (Con), Group(2): Corn oil control group orally administered 1 ml of corn oil/rat daily for two months (Corn), Group(3): Olive oil control group orally administered 1 ml olive oil/rat daily for two months (Olive), Group(4): Quercetin (Qu) control group orally received Qu dissolved in olive oil (50 mg/kg b. wt.) daily for two months (Qu). Group(5): Positive control group orally received Bisphenol A (BPA) dissolved in corn oil in a dose of 50 mg/kg b. wt. daily for two months (BPA), Group(6): Quercetin treated group orally administered 50 mg/kg b. wt. of BPA and treated with Qu (50 mg/kg b. wt. Orally) daily for two months (BPA+Qu).

Results: BPA exposure resulted in significant elevations of oxidative stress, as evidenced by the increased malondialdehyde level and glutathione-S-transferase activity associated with significant decrease in glutathione peroxidase activity in the liver tissue. Moreover, BPA caused an up regulation in the values of liver function enzymes. Also, BPA produced a significant elevation in the hepatic Interleukin-6 (IL-6) and caspase-3 levels with a significant decline in antiapoptotic protein B-cell lymphoma 2 (Bcl2) level in liver tissue. Quercetin significantly attenuated the BPA-evoked liver oxidative stress and modulated the activities of liver function enzymes. In addition, treatment of quercetin with BPA resulted in an improvement of IL-6 and caspase-3 levels associated with a significant increase in hepatic protein Bcl2 expression.

Conclusion: These data suggest that quercetin protects rat liver from BPA-induced oxidative stress, probably *via* its antioxidant activity, anti-inflammatory and antiapoptotic effects. So, Quercetin is a promising pharmacological agent for preventing the potential hepatotoxicity of BPA following occupational or environmental exposures.

Keywords: Bisphenol A, Quercetin, hepatotoxicity, Antioxidant, Anti-inflammatory effect, Antiapoptotic effect

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INTRODUCTION

Bisphenol A (BPA 2, 2-bis (4-hydroxyphenyl) propane), a xeno-estrogen, is an important monomer of polycarbonate plastics and a constituent of epoxy and polystyrene resins [1]. It is commonly used to line metal cans, water pipes, baby bottles, drinking cups [2, 3], dental sealants [4] and many other household appliances [5]. Studies have shown that for incomplete polymerization and for degradation of the polymer, bisphenol A can leach out from food and beverage containers [6]. Bisphenol A has been found not only in environmental samples, including air, water, sewage sludge, soil, and dust but also in specimens of human body fluids, such as plasma, umbilical cord blood, placental tissue, amniotic fluid, follicular fluid, and breast milk [7]. Due to the widespread use and building toxicological database, a need arises to investigate the mechanism of bisphenol A-induced toxicity. Studies showed that Bisphenol A causes adverse effects on the brain, reproductive system, and liver by forming reactive oxygen species (ROS) [8]. Reactive oxygen species (ROS) are cytotoxic agents causing oxidative damage by attacking the cell membrane and DNA [9]. ROS are scavenged by the endogenous antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in cells [10, 11]. BPA could induce liver damage, affecting oxidant/antioxidant balance in rat liver [12].

Antioxidants are scavengers by preventing cell and tissue that could be expected to result in cellular damage and disease [13]. Herbal medicines derived from plants are being increasingly utilized to treat a wide variety of clinical diseases. Quercetin (3, 3',4',5,7-pentahydroxyflavone) is a natural flavonoid, distributed in various fruits, vegetables, tea, red wine, and medicinal herbs [14]. Quercetin has been reported to have antioxidative capacity *in vitro* and *in vivo*

[14]. Meanwhile, such antioxidative capacity of quercetin affords its protection of the brain, heart, liver, and other organs against the oxidative stress injury induced by ischemia-reperfusion, toxic compounds, or other factors [14].

The present study was undertaken to elucidate the antioxidant property of quercetin against Bisphenol A-induced hepatotoxicity in male Wistar rats along with the expression of lipid peroxidation, antioxidant enzymes, inflammatory, apoptotic and antiapoptotic markers in male rats.

MATERIALS AND METHODS

Chemicals and drugs

Bisphenol A and quercetin were purchased from Sigma Chemical Co., USA. Corn oil and olive oil were purchased from a local market, Egypt. All other reagents and chemicals used for analysis met the quality criteria in accordance with international standards.

Experimental design

Animals and treatment

All experiments involving animals and tissue samples were conducted in accordance with the principles and guidelines for the care and use of laboratory animals in the National Institute of Health (NIH) (USA). This study was approved by the Ethical Committee for animal experimentation, National Research Centre, Egypt.

Forty-eight adult male albino rats of Wistar strain weighing 130±10g at 90 d of age were enrolled in the present study. Animals were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. Animals were housed throughout the

experiment (8 rats/cage) in polypropylene cages under specific pathogen-free (SPF) conditions with controlled illumination (12 h light/12 h dark cycle), relative humidity (30-50%) and temperature (18-22 °C). Animals were fed with standard laboratory rat diet and water provided *ad libitum*. Animals were allowed to adapt to their environment for two weeks before the commencement of the experiment.

After the acclimatization period, the animals were randomly divided into six groups (n=8) and orally administered daily for two months as: Group (1) (Con): rats served as normal control group. Group (2) (Corn): rats were inoculated orally with 1 ml corn oil. Group (3) (Olive): rats were administered orally with 1 ml olive oil. Group (4) (Qu): rats were administered orally with quercetin (50 mg/kg b. wt.) dissolved in olive oil [15]. Group (5) (BPA): rats were inoculated orally with Bisphenol A (50 mg/kg b. wt.) dissolved in corn oil [16]. Group (6) (BPA+Qu): rats were inoculated orally with BPA (50 mg/kg b. wt.) dissolved in corn oil and treated with QU (50 mg/kg b. wt.) dissolved in olive oil.

Sample collection

At the end of experimental period, orbital blood samples were obtained from the retro-orbital venous plexus using micro capillaries. The blood samples were collected in clean, dry centrifuge tubes and allowed to clot to obtain sera. Serum samples were separated by centrifugation at 1800 xg for 10 min at 4 °C. Aliquots of serum samples were frozen and stored at -20 °C pending further analysis. Following blood collections, animals were sacrificed by cervical dislocation, and a midline abdominal incision was performed, and whole liver of each animal was rapidly dissected out, thoroughly washed with ice-cold isotonic saline, blotted dry and then weighed. After that, the liver was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH 7.4). The homogenate was centrifuged at 1800 xg for 10 min at 4 °C. The supernatant (10%) was separated and stored at -20 °C for determination of different biochemical determinations.

Biochemical determinations

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity was determined by a colorimetric method

using Salucea kit (Netherlands) according to the method described by Young [17]. Hepatic malondialdehyde (MDA), glutathione-S-Transferase (GST) and glutathione peroxidase (GPx) contents were determined by colorimetric methods using Bio diagnostic kit (Egypt) following the methods of Satoh [18], Habig and Jakoby [19] and Peglia and Valentine [20], respectively. Interleukin-6 (IL-6) and Caspase-3 were estimated using ELISA technique using a kit purchased from Uscn life science Inc., USA. B-cell lymphoma 2 (Bcl2) determined by using ELISA technique using a kit purchased from Glory Science Co., Ltd, USA, according to manufacturer's instruction.

Statistical analysis

All results of the present study were expressed as mean±S. E. of the mean. The statistical Package for the Social Sciences (SPSS) program, version 14.0 was used to compare the significance between each two groups. The difference was considered significant when $P < 0.05$. Percentage difference representing the percent of variation with respect to the corresponding control group was calculated according to the following formula:

$$\% \text{ Difference} = \frac{\text{Treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS

The data illustrated in table 1 represent the effect of BPA and its treatment with quercetin on MDA level and the activities of antioxidant enzymes in liver tissue of rats. The results revealed that hepatic malondialdehyde (MDA) content and GST activity displayed significant increase ($P < 0.05$) in BPA group (257.57% and 27.61%, respectively) versus the normal control group. In the contrary, treatment of BPA group with Qu reverted this increase as indicated by the significant drop ($P < 0.05$) in hepatic MDA and GST (-37.51% and -16.06%) contents as compared with BPA group. Meanwhile, hepatic GPx activity in BPA group showed significantly ($P < 0.05$) decline (-22.39%) as compared to the control group. On the other hand, the treatment of BPA group of Qu recorded significant ($P < 0.05$) elevation (26.05%) in the activity of GPx as compared to the BPA group.

Table 1: Effect of quercetin on hepatic MDA level and antioxidant enzymes activities of rats treated with BPA

Groups parameters	Con group	Corn group	Olive group	Qu group	BPA group	BPA+QU group
MDA (nmol/mg protein)	32.88±0.66	38.73±0.84	41.95±0.55	32.38±0.23	117.57±3.15 ^a 257.57%	73.46±0.30 ^b -37.51%
GST (U/g tissue)	6.88±0.71	6.91±0.22	6.90±0.30	6.50±0.87	8.78±0.24 ^a 27.61%	7.37±0.12 ^b -16.06%
GPx (U/g tissue)	9803.56±106.86	9506.46±181.10	9024.67±79.59	9136.83±128.20	7600.08±161.23 ^a -22.39%	9590.50±311.70 ^b 26.05%

Data were represented as mean±S.E. of 8 rats/group, a: Significant change at $P > 0.05$ in comparison with normal control group, b: Significant change at $P > 0.05$ in comparison with BPA group.

Table 2: Effect of quercetin on liver functions of rats treated with BPA

Groups Parameters	Con group	Corn group	Olive group	Qu group	BPA group	BPA+QU group
ALT (U/l)	12.50±0.26	14.12±0.39	13.62±0.37	14.00±1.10	26.12±0.74 ^a 108.96%	16.87±0.51 ^b -35.41%
AST (U/l)	97.57±1.29	107.76±2.14	106.25±1.76	109.11±2.79	144.37±2.51 ^a 47.96%	123.51±4.10 ^b -14.44%

Data were represented as mean±S. E. of 8 rats/group, a: Significant change at $P > 0.05$ in comparison with normal control group, b: Significant change at $P > 0.05$ in comparison with BPA group.

The results in table 2 showed the effect of BPA and treatment with quercetin on liver functions of Wistar rats. In comparison with a normal control group, there was a significant increase ($P < 0.05$) in the activity of serum ALT (108.96%) and AST (47.96%) in group

induced with BPA. On the other hand, the treatment of BPA group with Qu produced a significant reduction ($P < 0.05$) in the activities of serum ALT and AST (-35.41% and -14.44% respectively) as compared to BPA group.

The data illustrated in table 3 revealed that hepatic IL-6 and caspase 3 levels showed a significant increase ($P < 0.05$) in BPA group (100.67% and 164.51%, respectively) compared with normal control group.

However, treatment of BPA group with Qu exerted a significant decrease ($P < 0.05$) in hepatic IL-6 and caspase 3 levels (-38.30%

and -32.85%, respectively) versus BPA group (table 3). In contrast, the hepatic Bcl2 recorded significant ($P < 0.05$) decrease (-79.3%) with BPA group as respect to control group. On the other side, the treatment of BPA group with Qu led to significant increment ($P < 0.05$) in Bcl2 (119.71%) as compared to BPA group.

Table 3: Effect of quercetin on hepatic IL-6, caspase 3 and Bcl2 levels of rats treated with BPA

Groups Parameters	Con group	Corn group	Olive group	Qu group	BPA group	BPA+QU group
IL-6 (Pg/mg protein)	62.64±4.07	66.22±2.63	67.30±6.97	60.14±0.99	125.70±11.13 ^a 100.67%	77.55±3.35 ^b 38.30%
Caspase 3 (ng/mg protein)	4.96±0.27	6.02±0.35	4.98±0.59	3.96±0.17	13.12±1.7 ^a 164.51%	8.81±0.45 ^b -32.85%
Bcl2 (ng/mg protein)	5.91±0.13	4.51±0.21	6.58±0.83	4.51±0.1	1.42±0.21 ^a -79.3%	3.12±0.23 ^b 119.71%

Data were represented as mean±S. E of 8 rats/group., a: Significant change at $P > 0.05$ in comparison with normal control group, b: Significant change at $P > 0.05$ in comparison with BPA group.

DISCUSSION

Liver toxicity is still a major problem for clinicians, pharmaceutical companies, and regulators [21, 22]. Hence, the goal of this work is to evaluate the potential effect of Qu against BPA-induced hepatotoxicity. The present study recorded that treatment with BPA significantly increased liver MDA value and GST activity whereas showed a significant decrease in GPx activity as compared to the normal control group. The increased MDA level and GST activity, besides, the decreased GPx activity indicated an increasing in the generation of ROS, which causes lipid peroxidation in the liver [23]. Korkmaz *et al.* [12] showed an increase in MDA level in the liver of rats exposed to BPA. Similarly, previous studies show increased in the MDA levels in the brain, testes, and kidneys of male rats exposed to BPA dose [12, 24]. GST protects cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes [25]. A number of compounds lead to induce activity and expressions of GST isoenzymes [26]. It has been reported that increased GST activity and upregulated GST- Π expression correlate with increased oxidative stress and apoptosis in breast cancer [27]. Hassan *et al.* [28] suggested that high dose of BPA not only increases the free radical formation but also decreases its ability to detoxify reactive oxygen species. The formation of superoxide radicals together with nitric oxide (NO) might form peroxynitrite that induced by high doses of BPA and causes tissue damage leading to an increase in the levels of MDA and NO. Moreover, the present results are consistent with the previous study of Hassan *et al.* [28] who reported that GSH level is important for GPx activity, which requires GSH as a cofactor, and the elevation of GSH level increases the activity of GPx. So, it has been suggested that BPA exposure produces ROS by inhibiting antioxidant enzymes [29].

The treatment of BPA group with Qu showed a significant reduction in the hepatic level of MDA and GST activity associated with significant improvement in the values of the antioxidant enzyme (GPx) versus to the normal control group. Quercetin, which behaves as a powerful antioxidant and free radical scavenger, can decrease MDA level induced by many hepatotoxins in rat liver [30]. This may be explained by its higher diffusion into the membranes allowing it to scavenge free radicals at several sites throughout the lipid bilayer [31]. The present findings suggest that quercetin could be at least partly attenuate oxidative stress by decreasing the lipid peroxide level in BPA-treated rat liver. Moreover, this result is in agreement with several studies on hepatocytes exposure to oxidants [32, 33]. It was pointed out that quercetin could improve the antioxidant potentials in a cell by enhancing hepatic Mn-superoxide dismutase, Cu/Zn-superoxide dismutase, catalase and GPx mRNA expression [34, 35]. These findings indicated that quercetin could enhance the expression of antioxidant enzymes in the liver.

The present study revealed that both AST and ALT recorded significant increase over control values in rats treated daily with BPA. These results are consistent with those of Korkmaz *et al.* [12]

who reported a significant increase in ALT and AST activities in rats treated with 25 mg/kg BPA for 50 d. When the liver hepatocytes are damaged, these enzymes are released into the blood where the significant increase in AST and ALT activities indicates the damage to the cytosol and also to mitochondria [36]. Therefore, it could be suggested that the oxidative stress induced by BPA in the present study may mediate the disturbance in hepatic function which is reflected by the increase in ALT and AST. On the other hand, the treatment of BPA group with Qu caused significant depletion in the activity of liver enzymes ALT and AST. The ability of the hepatoprotective agent to reduce the injurious effect or to preserve the physiological normal hepatic function which has been disturbed by hepatotoxin is an index of its protective effect. The lowering of enzymes levels is a definite indication of hepatoprotective action of quercetin. It is also known to reduce toxicant-induced liver damage [37].

Moreover, an increase in the level of IL-6 was noted following BPA treatment. Although ROS can increase proinflammatory cytokines [38], proinflammatory cytokines themselves can induce oxidative stress [39]. The expression of increase IL-6 and MDA contents after BPA injection suggested that IL-6 might have a pathogenic role in BPA-induced ROS generation. On the other hand, present data recorded significant depletion in the hepatic level of IL-6 in BPA group treated with Qu. This could be contributed to the anti-inflammatory effect of quercetin structurally similar flavonoids on the liver, *via* mechanisms likely to involve blockade of NF- κ B activation.

Increasing attention is concentrated on alternative signaling pathways leading to cell death including necrosis, autophagy, and mitotic catastrophe [40]. The accomplishment of apoptosis is mediated by caspases, which constitute a family of aspartate-specific cysteine proteases that cleave their substrate and then activate caspases cascade [41]. The current data revealed that exposure of rats to BPA was found to upregulate caspase 3 level in liver tissue of rats as compared to the normal control group. This effect may be because caspases are part of a highly conserved protein family that is central to the apoptotic pathway. Caspases are proteases activated after a cell has received a signal instructing it to undergo apoptosis. The key components that the caspases break down include DNA repair enzymes and structural proteins of the cytoskeleton. Caspases can also activate other enzymes that degrade other parts of the cellular machinery by cleaving an inhibitory sequence on these enzymes. There is a loss of nuclear membrane integrity after disruption [42].

The Bcl-2 family of proteins, containing both pro-apoptotic and anti-apoptotic members, is known to regulate mitochondrial-mediated apoptosis [43]. Bcl-2, prevent the release of apoptogenic molecules from the intermembrane space of mitochondria [44]. Mitochondria may be one of the most important locations for apoptosis [45], which has a close relationship with the levels of Bcl-2 [46]. The

current data has shown that the expression of Bcl-2 was dropped in BPA group in comparison to the normal control group. It is thought that BPA may cause a reduction in the Bcl-2 level and activates caspase-3. An earlier study showed that BPA-induced germ and Sertoli cell apoptosis through the mitochondrial apoptotic pathway and through the Fas/Fas L signaling pathway [47-49]. Moreover, BPA could foster increased DNA damage, and genotoxicity is by the inhibition of cellular antioxidant activity and increase the oxidative stress [50, 51]. It suggested that BPA can diminish the expression of Bcl-2. Also, cells are susceptible to oxidative stress-induced apoptosis when levels of intracellular antioxidants are down-regulated. GSH-Px is important antioxidant defenses. In the present study, the activity of GSH-Px decreased significantly when exposed to BPA, therefore, this may be the underlying another mechanism by which BPA can cause apoptosis.

On the other hand, the present data recorded a significant decrease in the hepatic level of caspase 3 and a significant increase in hepatic Bcl2 level in the group of BPA treated with Qu as compared to the BPA group. Induction of direct liver cellular damage, ROS and oxidative stress are known as apoptosis triggers and modulators [52]. ROS-induced apoptosis requires the participation of other cell death signaling pathways, including JNK which regulates the expression of various apoptosis proteins implicated in hepatotoxicity [52, 53]. The mechanism of JNK-dependent apoptosis has been suggested to involve activation of caspase 3 via phosphorylation of Bcl-2 family proteins [54]. Previous studies indicated that quercetin was able to attenuate the toxicant-induced apoptosis by the inhibition of JNK activation [55, 56]. Also, same studies indicated that quercetin can prevent apoptosis by altering the expression of Bax, Bcl-2 and caspase 3 [55, 57]. So, the present study suggested that quercetin significantly attenuated the upregulation of Bax and cleaved caspase 3 expression in BPA treated rats. Furthermore, quercetin markedly prevented the downregulation of hepatic Bcl-2 expression in BPA treated rat.

CONCLUSION

In conclusion, the present study indicate that quercetin has a protective effect against BPA-induced hepatotoxicity in rats through attenuating lipid peroxidation, renewing the activities of antioxidant enzymes, ameliorating the activity of liver function enzymes, improving the IL-6 level and alleviating apoptosis by modulating caspase-3 and Bcl-2 expression.

CONFLICT OF INTERESTS

Declare none

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