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QUERCETIN AMELIORATES BISPHENOL A-INDUCED TOXICITY IN MICE

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The plastic monomer and plasticizer bisphenol A [BPA – 2,2-bis(4-hydroxyphenyl)propane], is one of the highest volume chemicals produced worldwide, with over six billion pounds produced each year (1). BPA is used in the production of polycarbonate plastics, epoxy resins used in lining metal cans, and in many plastic consumer products including toys, water pipes, drinking containers, eyeglass lens, sports safety equipment, dental monomers, medical equipment and tubing as well as consumer electronics (2). BPA mimics estrogen activity and is known as an "endocrine disruptor", a chemical that interferes with the hormonal system in animals and humans and contributes to adverse health effects. The estrogenic properties of BPA were reported as early as 1936 by Dodds and Lawson (3). Since 1996, the European Commission has classified BPA as "external-derivative chemical influencing human health and offspring". Animal experimentation for BPA toxicity revealed some effects like increase in prostate weight and cancer, mammary gland organization and cancer, protein induction in the uterus, organization of sexually dimorphic circuits in the hypothalamus, onset of estrus cyclicity and earlier puberty, genital malformation and others (4). Various studies have proven BPA as mutagenic and carcinogenic component of plastics. Also, it leads to diabetes and many cardiovascular problems. Recent reports also indicate the potential of BPA to disrupt thyroid hormone action (5), to cause proliferation of human prostate cancer cells (6) and to block testosterone synthesis (7). Cytotoxic effect of BPA on human red blood cells was also studied by Verma and Sangai (8). The ubiquity of BPA has been highlighted by measurements in human fluids and tissues (9).

BPA has been shown to reduce mitochondrial function in hepatocytes (10). It has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes (10, 11). The activities of antioxidant enzymes, superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase were decreased in mitochondrial and microsome rich fractions of liver. The levels of hydrogen peroxide and lipid peroxidation increased in the liver of treated rats when compared with the corresponding group of control animals. The results indicated that BPA induces oxidative stress in the liver of rats by decreasing the antioxidant enzymes (12).

Among the tested bioflavonoids, quercetin is shown to have the highest antioxidant activity (13). Quercetin, the most abundant flavonoid in nature, present in large amounts in vegetables, fruits, tea and olive oil, is considered to be potent antioxidant. Quercetin contains a number of phenolic hydroxyl groups in the B ring and conjugation between the A and B rings and has an antioxidant potential four times that of vitamin E (14). It was reported that quercetin has many beneficial effects in human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects (15, 16). These effects of quercetin are due to its antioxidant properties of scavenging free radicals directly (17).

The aim of the present study was to evaluate the toxic effects of BPA in serum parameters and its possible amelioration by quercetin.

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MATERIALS AND METHODS

Chemicals and reagents

Analytical grade of BPA and quercetin were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India. The other chemicals used in the present study were procured from Hi Media Laboratories Pvt. Ltd. and Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Olive oil was obtained from Figaro, Madrid, Spain.

Animals

All experiments were performed on inbred healthy, adult male albino mice of Swiss strain weighing approximately 30–35 g. Animals were obtained from Zydus Research Centre, Ahmedabad, India. They were housed in polypropylene cages in an air-conditioned animal house at 25 ± 2°C maintained at 12 h light/dark cycle in central animal house facility of Zoology Department, Gujarat University, Ahmedabad, India. Animals were fed with certified pelleted rodent feed (supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India) and water *ad libitum* throughout the experiment. Guidelines for Care and Use of Animals in Scientific Research 1991 published by Indian National Science Academy, New Delhi, India were followed. The research protocols were reviewed and approved by the Committee for the Purpose of Control and Supervision of Experiments for Animals (Reg-167/1999/CPCSEA), New Delhi, India.

Experimental design and treatment schedule

Seventy animals were randomized in seven groups as follows: Group I – animals were not exposed to any treatment and were served as untreated control. Group II – animals received olive oil (0.2 mL/animal /day) and served as vehicle control. Group III – animals received quercetin (60 mg/kg b. w./day) and served as antidote control. Groups IV and V – animals were given low dose (LD – 60 mg/kg b. w./day) and high dose (HD – 120 mg/kg b. w./day) of BPA. Groups VI and VII – animals received conjoint treatment of LD and HD of BPA along with quercetin (60 mg/kg/b. w./day).

All treatments were given orally for 30 days using a feeding tube attached to hypodermic syringe. Olive oil was administrated to Group II animals (vehicle control) as it was used to dissolve BPA and quercetin. Low dose (LD – 1/20th of LD_{50} value) and high dose (HD – 1/10th of LD_{50} value) of bisphenol A was based on study of Kimura et al. (18). Dosage of quercetin was based on a previous report (19).

Preparation of serum samples

On completion of treatment, mice were weighed and were sacrificed by cervical dislocation. The blood was collected by cardiac puncture. Blood samples were then centrifuged at 1000 × g for 10 min at 4°C to obtain non-hemolyzed serum which was used for biochemical analysis.

Measurement of serum enzyme activities

Serum samples were used for measuring liver marker enzymes. The activities of serum enzymes such as alanine aminotransferase (ALT; E.C. 2.6.1.2) and aspartate aminotransferase (AST; E.C. 2.6.1.1) activities were assayed according to the method of Reitman and Frankel (20). Alkaline phosphatase (ALP; E.C. 3.1.3.1) and acid phosphatase (ACP; E.C. 3.1.3.2) were analyzed by the method described by Bessey et al. (21). According to the International Union of Biochemistry, the serum ALT, AST were expressed in mU/mL and ALP, AST were expressed in IU/mL. Serum samples were also used as indicators of kidney function tests. The serum creatinine was estimated according to the method of Varley (22). Serum protein was measured by the method of Lowry et al. (23) using bovine serum albumin as a standard.

Statistical analysis

All of the data are expressed as the mean ± standard error of the mean (SEM). The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. The level of significance was accepted with $p < 0.05$.

RESULTS

Effect of bisphenol A on serum enzyme activities and its possible mitigation by quercetin

The results shown in Figures 1–4 revealed no significant changes between untreated and vehicle control groups (Group I and II). Similarly, quercetin treatment (Group III) for 30 days caused no significant alteration as compared to vehicle control. However, BPA treatment in mice (Group IV and V) for 30 days caused significant ($p < 0.05$) elevation in ALT (LD: 29.61%; HD: 65.28%), AST (LD: 42.39%; HD: 62.39%), ALP (LD: 47.48%; HD: 62.32%) and ACP (LD: 19.03%; HD: 40.3%) activities as compared to vehicle control. The effect was dose-dependent in ALT and ACP activities. Co-treatment of quercetin and BPA (Group VI and VII) for 30 days caused significant mitigation, as compared to BPA treated mice. Extent of mitigation in ACP activity in serum of high dose of BPA plus

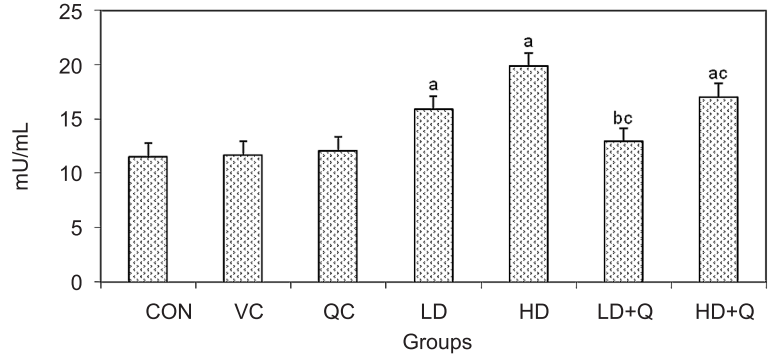

Figure 1..Effect of bisphenol A and its possible mitigation by quercetin in serum ALT activity. The values were expressed as the means ± SEM, n = 10. The ALT activity is expressed as mU/mL at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

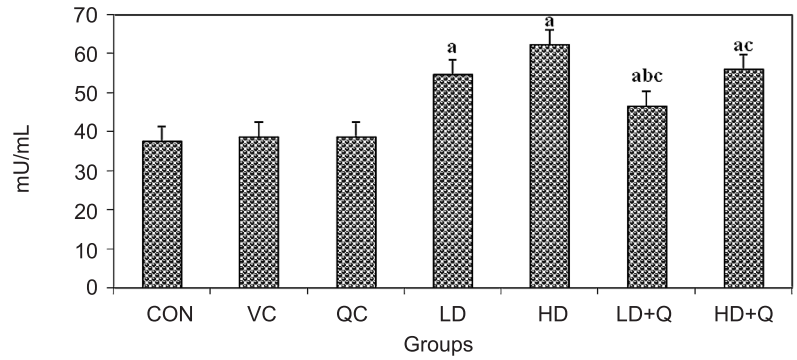

Figure 2. Effect of bisphenol A and its possible mitigation by quercetin in serum AST activity. The values were expressed as the means ± SEM, n = 10. The AST activity is expressed as mU/ mL at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

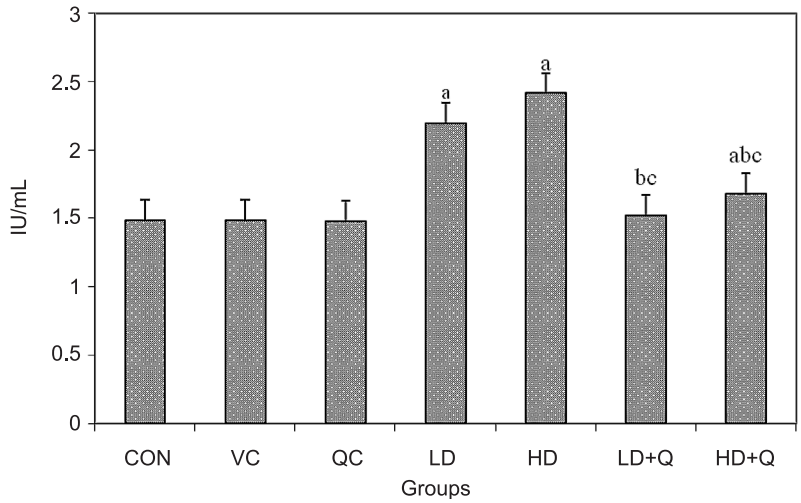

Figure 3. Effect of bisphenol A and its possible mitigation by quercetin in serum ALP activity. The values were expressed as the means ± SEM, n = 10. The enzyme activity is expressed as IU/mL min at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

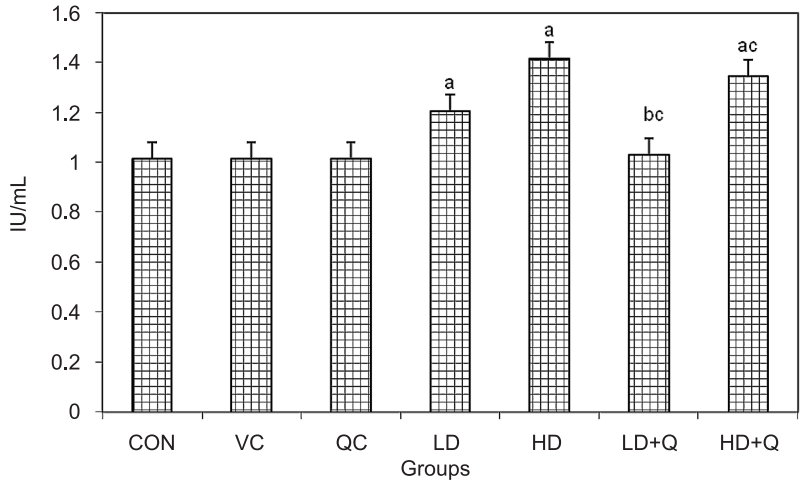

Figure 4. Effect of bisphenol A and its possible mitigation by quercetin in serum ACP activity. The values were expressed as the means ± SEM, n = 10. The enzyme activity is expressed as IU/mL at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

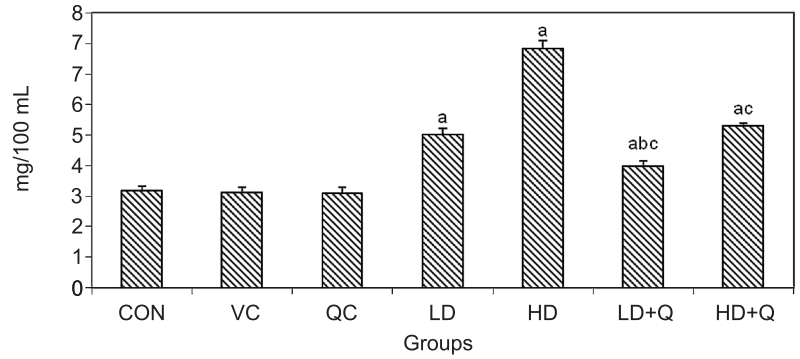

Figure 5. Effect of bisphenol A and its possible mitigation by quercetin in serum creatinine content. The values were expressed as the mean ± SEM, n = 10. The creatinine content is expressed as mg/100 mL at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

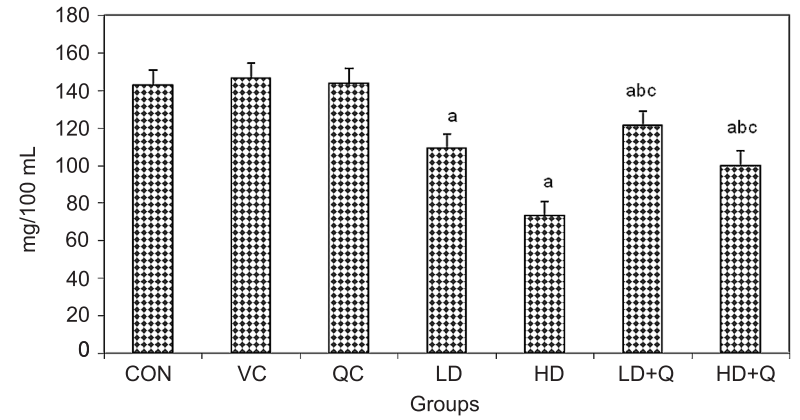

Figure 6. Effect of bisphenol A and its possible mitigation by quercetin in serum protein content. The values were expressed as the means ± SEM, n = 10. The protein content is expressed as mg/100 mL/30 min at 37°C. [a]p < 0.05 *versus* vehicle control group, [b]p < 0.05 *versus* low dose treated group and [c]p < 0.05 *versus* high dose treated group

quercetin treated mice was comparatively higher than that of low dose of BPA and quercetin treated group [ACP (HD *vs*. HD + A: 17.25%: LD *vs*. LD + A: 7.1%)]. However, extent of mitigation in ALT, AST and ALP activities in serum of low dose BPA plus quercetin treated mice was comparatively higher than that of high dose BPA plus quercetin treated group [ALT (LD *vs*. LD + A: 20.85%; HD *vs*. HD + A: 16.4%:), AST (LD *vs*. LD + A: 16.65%; HD *vs*. HD + A: 15.61%)] and ALP (HD *vs*. HD + A: 45.20%: LD *vs*. LD+A:48.95 %).

Effect of BPA on serum creatinine and protein contents and its possible mitigation by quercetin

Oral administration of BPA for 30 days caused non-significant changes between untreated and vehicle control groups (Group I and II). Similarly, quercetin treatment (Group III) show no significant alterations as compared to vehicle control. As compared to vehicle control, BPA treatment in mice (Groups IV and V) for 30 days caused significant ($p < 0.05$) rise in creatinine content (LD: 50.75%; HD: 135.44%), whereas protein content reduced significantly (LD: 25.61%; HD: 50.14%) in serum of mice (Figs. 5, 6). The effect was dose-dependent. However, in Groups VI and VII oral administration of BPA along with quercetin, caused significant mitigation, as compared to BPA alone treated mice (Groups IV and V). Extent of mitigation in creatinine and protein contents in serum, of high dose BPA plus quercetin treated mice was comparatively more than that of low dose of BPA and quercetin treated group [creatinine content (HD *vs*. HD + A: 76.59%: LD *vs*. LD + A: 10.51 %) and protein content (HD *vs*. HD + A: 18.24 %: LD *vs*. LD + A: 5.52 %)].

DISCUSSION

The significant dose-dependent change in the activities of ALT, AST (Figs. 1, 2) shows the toxic effect of BPA. Elevated levels of serum enzymes (alanine aminotransaminase, aspartate transaminase, alkaline phosphatase, acid phosphatase) are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver (24). These are of major importance in assessing and monitoring functional status of liver. Thus, their increased presence in serum may give information on organ dysfunction (25). It has been reported by Gao et al. (26) that ALT activity is an important index to measure the degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme.

Alkaline phosphatase, a 'marker' enzyme for plasma and endoplasmic reticulum (27, 28), is often employed to assess the integrity of plasma membrane (29). The increase in ALP activities in serum (Fig. 3) may be attributed to either *de novo* synthesis of the enzyme molecules or loss of other proteins from tissues (27, 30). The increase in enzyme activity may have resulted from excess leakage of enzymes from hepatocytes due to BPA treatment in serum. Such increase in ALP activities can constitute a threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate esters of the tissues. This suggests that BPA may act as a plasma membrane labilizer. Acid phosphatase in its own case is a 'marker' enzyme for the lysosomal integrity (31) and increase in ACP is the consequence of damage to lysosomal integrity in the liver and serum. The significant rise seen in the ACP activity after toxin administration may be attributed to an increase in cellular degeneration and other pathological liver injury (32). This also suggested high tissue catabolism and cellular autophagy, which are possible sequences leading to tissue damage (33). This is in accordance with our results shown in Figure 4.

The ability of hepatoprotective agent to reduce the injurious effect or to preserve the normal hepatic physiological function which has been disturbed by hepatotoxin is an index of its protective effect (34). Oral administration of BPA along with quercetin caused significant amelioration in BPA-induced toxicity in mice. The lowering of enzymes levels is a definite indication of hepatoprotective action of quercetin (35). It is also known to reduce toxicant-induced liver damage (36). The protective role of quercetin had been explained by Mandal and Das (37) as galactosylated liposomes against CCl_4-induced hepatocellular damage.

The serum concentration of creatinine is relatively constant under normal circumstances, unless glomerular filteration rate (GFR) changes, as a result of defective renal function. Serum creatinine was examined as indicator for kidney function. Oral administration of BPA for 30 days caused elevation in serum creatinine content (Fig. 5). The increased level of serum creatinine after BPA intoxication might be due to reduced ability of the kidney to eliminate the toxic metabolic substances (38). Supplementation of quercetin brings back the normal level of serum creatinine concentration. Quercetin has for long been used to enhance renal filtration and increase the excretion of xenobiotics. This may be due to antioxidative action of quercetin (39).

A significant decrease was observed in the serum protein in BPA-treated mice (Fig. 6). All the serum proteins are invariably secreted by liver. Decreased biosynthesis and secretion of protein might be due to formation of BPA adducts with DNA, RNA and protein. De Flora et al. (40) studied and confirmed the ability of BPA to form DNA adducts both *in vitro*, in an acellular system and *in vivo* in rodent liver. Aktinson and Roy (41) found that BPA is converted to bisphenol O-quinone, which might be the ultimate DNA binding metabolite. This interaction might prevent RNA polymerase transcribing the DNA and can inhibit the formation of mRNA. A failure in mRNA formation can result in an inhibition of protein synthesis, which may be considered to be the cause of the liver cell necrosis (42). Oral administration of quercetin (60 mg/kg b. w./day) along with BPA for 30 days significantly ($p < 0.05$) alleviates serum protein content. A study by Raju et al. (43) reveals that inclusion of quercetin in the diet improved total protein level. Quantitative measurement of protein oxidation in ethanol treated rats along with quercetin, revealed that supplementation of quercetin decreased protein oxidation or an increase in the protein synthesis and thus maintaining the normal endogenous protein content (44).

CONCLUSION

In conclusion, the present investigation has shown that BPA is capable of producing alterations in serum parameters investigated which were significantly ameliorated by quercetin. This may be due to antioxidative potential of quercetin.

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