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QUERCETIN ALLEVIATES BISPHENOL A-INDUCED CHANGES IN NUCLEIC ACID AND PROTEIN CONTENTS IN MICE

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Abstract: The present study was an attempt to examine toxic effects of bisphenol A in liver and kidney of mice and its alleviation by quercetin. Oral administration of bisphenol A (60 and 120 mg/kg b. w./day) for 30 days caused, as compared to vehicle control significant, dose-dependent decrease in DNA, RNA and protein contents in liver and kidney of mice. Supplementation of quercetin (60 mg/kg b. w./day) along with bisphenol A for 30 days caused, as compared to bisphenol A alone treated groups, significant alleviation in DNA, RNA and protein contents. The amelioration was comparatively higher for high dose bisphenol A plus quercetin treated group than that of low dose plus quercetin treated group.

Keywords: bisphenol A, quercetin, liver, kidney, toxicity

Bisphenol A (BPA) is used as a monomer in the manufacture of polycarbonate (infant feeding bottles, tableware, microwave, returnable water and milk bottles etc.) and epoxy resins used in internal protective lining for food and beverage cans, coating on metal lids for glass jars and bottles and surface-coating on drinking water storage tanks and wine vats (1). The extensive use of bisphenol A-based polymers, with ester bonds subjected to hydrolysis and non-polymerized monomer residues, has led to widespread environmental contamination. Bisphenol A concentration ranges from 5–320 mg/L in river water (2), 20–700 ng/L in surface effluents (3), 2–208 ng/m^3 in air, 0.2–199 ng/g in dust (4) and 0.1–384 ng/g in food stuffs (5). Its presence in food is of special concern, since it constitutes the primary source of human exposure.

The ubiquity of bisphenol A has been highlighted by measurements in human fluids and tissues i.e., 10^{-9} to 10^{-12} M (6). Reports also indicate the potential of bisphenol A to disrupt thyroid hormone action (7), to cause proliferation of human prostrate cancer cells (8) and to block testosterone synthesis (9). Cytotoxic effect of bisphenol A on human RBC was also studied (10). Various researches have proved its adverse effect on male and female reproductive organs in experimental animals. However, there are lesser scientific studies on its toxic action on liver and kidney.

Bioflavonoids have attracted attention as a new class of hepatoprotective and renoprotective agents *via* their antioxidative properties. Among the tested bioflavonoids, quercetin is shown to have the highest antioxidant activity (11). Quercetin, the most abundant flavonoid in nature, is present in large amounts in vegetables, fruits, tea and olive oil, and contains a number of phenolic hydroxyl groups. It exhibits its therapeutic potential against many diseases, including ischemic liver fibrosis, renal injury and chronic biliary obstruction (12–15). Antioxidant effects of quercetin are due to its property to scavenge directly free radicals (16), to inhibit xanthine oxidase, lipid peroxidation (17, 18) and to alter the antioxidant defence pathway *in vivo* and *in vitro* (19).

The aim of the present study was to evaluate the toxic effects of bisphenol A in liver and kidney of mice and its possible amelioration by quercetin.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade bisphenol A and quercetin were used in all experiments. All other chemicals and reagents were of analytical grade and purchased

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from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

Animals

All experiments were performed on inbred healthy, adult male mice of Swiss strain weighing approximately 30–35 g. Animals were obtained from Zydus Research Centre, Ahmedabad, India. They were, housed in stainless steel cages in an air-conditioned room at 25 ± 2°C and were maintained in 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 experiment (Reg-167/1999/CPCSEA), New Delhi, India.

Experimental design and treatment schedule

Seventy animals were randomized in seven groups and caged separately. Group I animals were not-exposed to any treatment and were served as untreated control. Animals of Group II and III received olive oil (0.2 mL/animal/day) and quercetin (60 mg/kg b. w./day), respectively, and served as vehicle control as well as antidote control. Animals of Group IV and V were orally administered with low dose (LD – 60 mg/kg b. w./day) and high dose (HD – 120 mg/kg b. w./day) of bisphenol A. Group VI and VII animals received conjoint treatment of LD and HD of bisphenol A as in (group IV and V) along with quercetin (60 mg/kg b. w./day.) as in group III.

All treatments were given orally using a feeding tube attached to hypodermic syringe for 30 days. Olive oil was administered to Group II animals (vehicle control) as it was used to dissolve bisphenol A and quercetin. Low dose (LD – $1/20^{th}$ of LD_{50} value) and high dose (HD – $1/10^{th}$ of LD_{50} value) of bisphenol A was based on study of Kimura et al. (20). Dosage of quercetin was based on the previous report (21).

Tissue preparation

On completion of treatment, animals were sacrificed by cervical dislocation. The liver and kidney were quickly isolated, blotted free of blood and used for biochemical analysis. The estimation of DNA and RNA in the liver and kidney was carried out by the method of Giles and Meyer (22) and Mejboum (23), respectively. The protein content was measured by the method of Lowry et al. (24) using bovine serum albumin as a standard.

Statistical analysis

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

RESULTS

Effect of bisphenol A on DNA, RNA and protein contents in liver of mice and its possible mitigation by quercetin

No significant alterations in DNA, RNA and protein contents were observed between different control groups (untreated and vehicle control, Groups I and II). Oral administration of bisphenol A to group IV (LD) and V (HD) for 30 days caused, as compared to vehicle control (Group II), a significant ($p < 0.05$) decrease in DNA (LD: 29.03%; HD: 61.49%), RNA (LD: 30.32%; HD: 72.04%) and protein (LD: 30.42%; HD: 48.93%) contents in the liver of mice. The effect was dose-dependent in DNA and RNA contents. Oral administration of quercetin alone (Group III) for 30 days did not cause significant change in all parameters as compared to vehicle control. However, conjoint treatment of quercetin and bisphenol A (Group VI and VII) caused considerable mitigation in the liver, as compared to bisphenol A treated mice. Mitigation was more in all parameters in high dose bisphenol A treated group with quercetin as compared to low dose bisphenol A treated group with quercetin [DNA (HD *vs*. HD + Q: 25.79%: LD *vs*. LD + Q: 5.79%), RNA (HD *vs*. HD + Q: 49.15%: LD *vs*. LD + Q: 15.47%) and protein (HD *vs*. HD + Q: 37.97%: LD *vs*. LD + Q: 28.36%)] (Figs. 1–3).

Effect of bisphenol A on DNA, RNA and total protein contents in kidney of mice and its possible mitigation by quercetin

No significant alterations in DNA, RNA and protein contents were observed between Group I (untreated control), Group II (vehicle control) and Group III (quercetin treated animals) (Figs. 4–6). Oral administration of bisphenol A for 30 days in animals of Group IV and V caused, significant ($p < 0.05$) reduction in DNA (LD: 30.54%; HD: 59.88%), RNA (LD: 34.49%; HD: 72.42%) and pro-

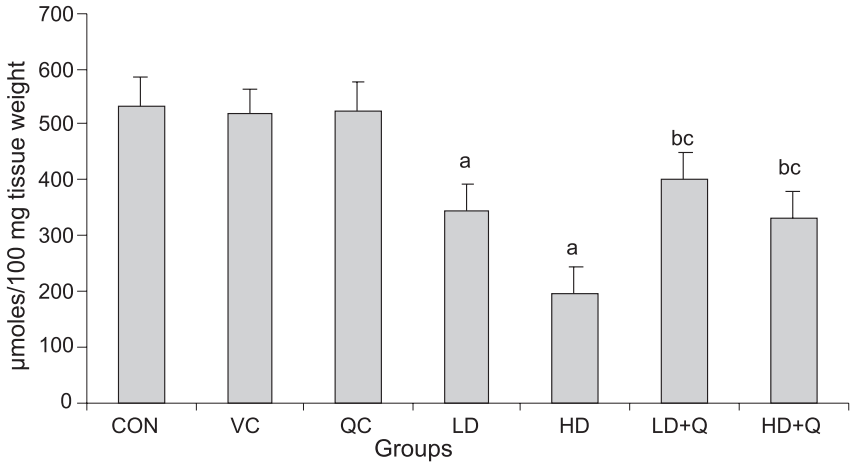

Figure 1. Effect of bisphenol A on DNA content in mice liver and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The DNA content was expressed as µmoles/100 mg tissue weight. [a]p < 0.05 *vs*. vehicle control group, [b]p < 0.05 *vs*. low dose treated group and [c]p < 0.05 *vs*. high dose treated group

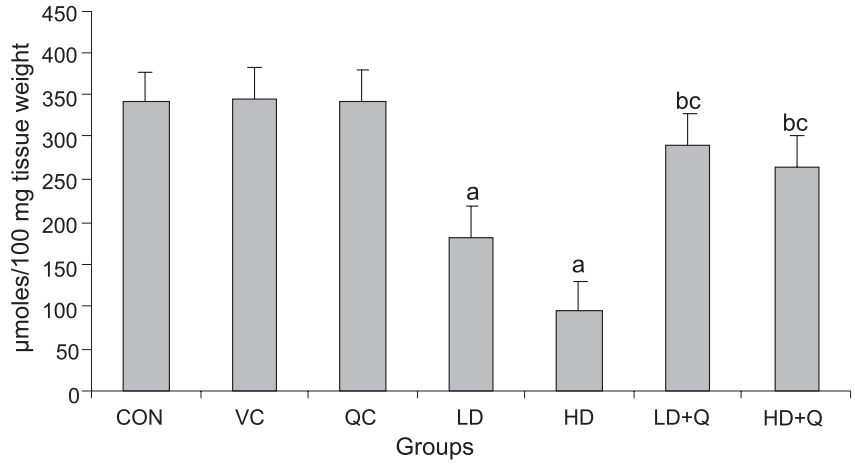

Figure 2. Effect of bisphenol A on RNA content in mice liver and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The DNA content was expressed as µmoles/100 mg tissue weight. [a]p < 0.05 *vs*. vehicle control group, [b]p < 0.05 *vs*. low dose treated group and [c]p < 0.05 *vs*. high dose treated group

tein (LD: 30.12%; HD: 46.10%) contents in the kidney of mice. The effect was found to be dose-dependent in DNA and RNA contents. In Groups VI and VII, combined treatment of bisphenol A and quercetin lead to considerable alleviation in the kidney as compared to bisphenol A alone treated group. In high dose bisphenol A plus quercetin group (Group VII) improvement was higher in all parameters as compared to low dose bisphenol A treated group with quercetin [DNA (HD *vs*. HD + Q: 40.14%: LD *vs*. LD + Q: 23.27%), RNA (HD *vs*. HD + Q: 59.40%: LD *vs*. LD + Q: 26.12%) and protein (HD *vs*. HD + Q: 40.91%: LD *vs*. LD + Q: 28.90%)].

DISCUSSION

The present study revealed significant reduction in DNA, RNA and protein contents in bisphenol A treated animals. This might be due to BPA-DNA adduct formation (Fig. 1 and 4). De Flora et

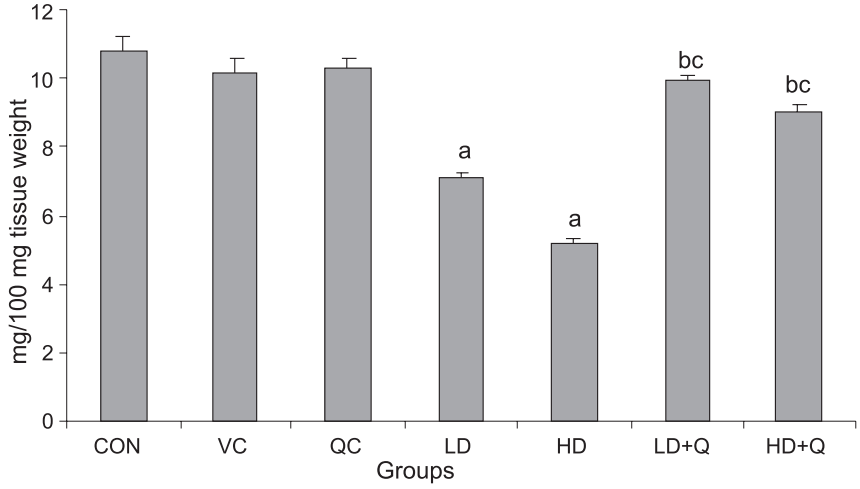

Figure 3. Effect of bisphenol A on protein content in mice liver and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The protein content was expressed as mg/100 mg tissue weight. [a]$p < 0.05$ *vs*. vehicle control group, [b]$p < 0.05$ *vs*. low dose treated group and [c]$p < 0.05$ *vs*. high dose treated group

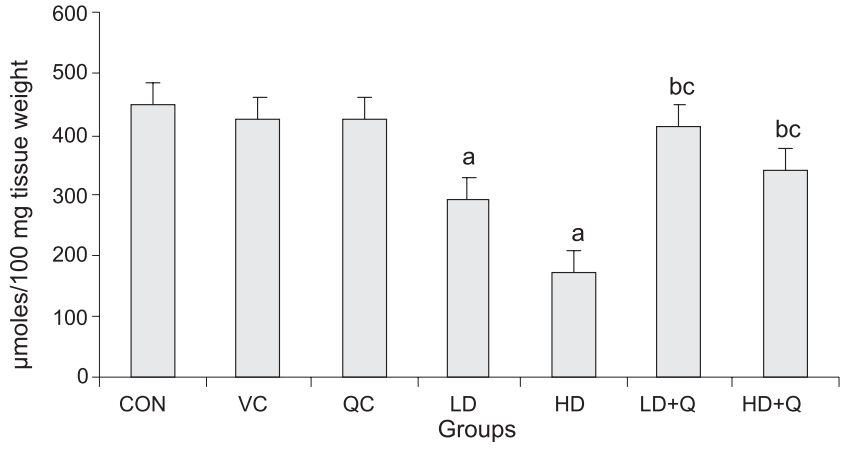

Figure 4. Effect of bisphenol A on DNA content in mice kidney and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The DNA content was expressed as µmoles/100 mg tissue weight. [a]$p < 0.05$ *vs*. vehicle control group, [b]$p < 0.05$ *vs*. low dose treated group and [c]$p < 0.05$ *vs*. high dose treated group

al. (25) studied and confirmed the ability of bisphenol A to form DNA adducts both *in vitro*, in an acellular system and *in vivo* in the rodent liver. Topical treatment of skin in C3H mice with diglycidyl ether, a bisphenol A derivative used as a component of epoxy resins, resulted in the formation of radioactive (26) and fluorescent (27) DNA adducts. The compound also formed DNA adducts to calf thymus DNA detectable by nanoflow electrospray mass spectrometry (28). Further, Aktinson and Roy (29) found that bisphenol A is converted to bisphenol O-quinone. The semiquinone and/or quinone intermediates of bisphenol A may be the ultimate DNA binding metabolites. 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 (30). Significant and dose-dependent reduction in mRNA expression and protein content was observed by Ramos et al. (31) in AvPv (anteroventral periventricular nucleus) region of hypothalamus of female pups treated with BPA. Thus, alterations in DNA, RNA and protein contents affect the overall process of protein synthesis.

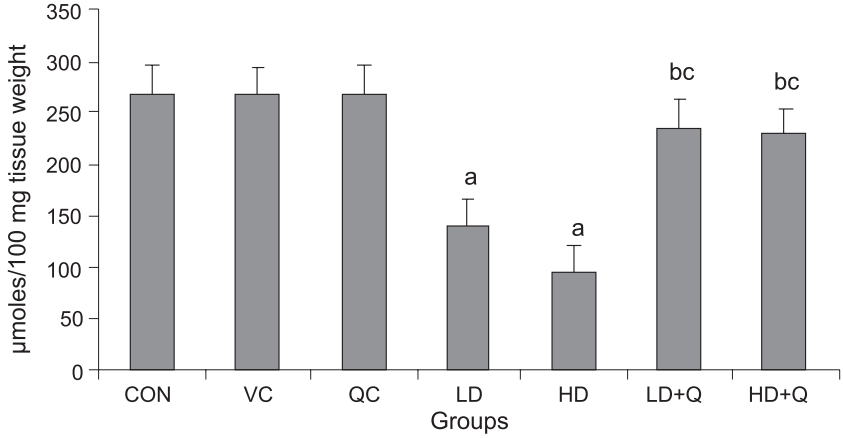

Figure 5. Effect of bisphenol A on RNA content in mice kidney and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The DNA content was expressed as µmoles/100 mg tissue weight. [a]p < 0.05 *vs*. vehicle control group, [b]p < 0.05 *vs*. low dose treated group and [c]p < 0.05 *vs*. high dose treated group

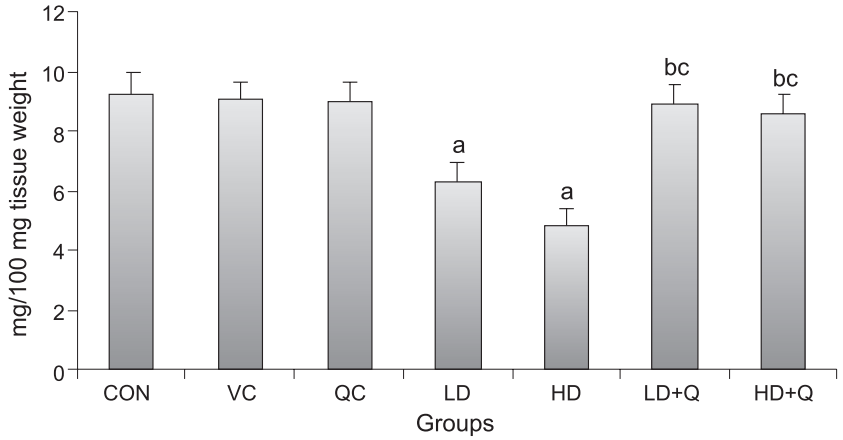

Figure 6. Effect of bisphenol A on protein content in mice kidney and its possible alleviation by quercetin. The values were expressed as the mean ± SE, n = 10. The protein content was expressed as mg/100 mg tissue weight. [a]p < 0.05 *vs*. vehicle control group, [b]p < 0.05 *vs*. low dose treated group and [c]p < 0.05 *vs*. high dose treated group

Free radicals can oxidize macromolecules such as DNA, proteins, carbohydrates and lipids (32). Free radical damage can cause unsaturated bonds in membrane lipids to lose fluidity when peroxidized and proteins to denature (33). Thus, in addition to DNA adduct formation, oxidative stress could be another reason for the alterations produced by bisphenol A in DNA, RNA and protein contents. A study by Verma and Sangai (10) showed that treatment with bisphenol A leads to cell rupture and membrane damage of human erythrocytes which may be due to the oxidative stress. In another experiment, Sangai and Verma (34) reported significant and concentration dependent rise in lipid peroxidation in liver and kidney homogenates treated with bisphenol A in *in vitro* experiments. Mathur et al. (35) explained that bisphenol A elicit depletion of antioxidant defense system and induces oxidative stress in epididymal sperm of rats. DNA lesions are constantly being produced in living cells by the deleterious action of both endogenous and environmental DNA damaging agents (36). DNA damage is caused by the oxidation of DNA by reactive oxygen species (ROS) which are generated during normal cell metabolism and in response to exogenous factors (37, 38). Oxidative DNA lesions include the

oxidation of nucleotidic bases, modifications to the sugar moiety of DNA, which may result in base-loss abasic (apurinic/apyrimidinic) sites and/or strand breakage (single and double strand breaks), DNA-DNA intra-strand adducts and DNA-protein cross-links, all of which are cytotoxic and some can be mutagenic (39–41).

The effect of an antioxidant on recovery from oxidative DNA damage can be explained in two ways: it can stimulate the act of repair enzymes or it can directly protect against oxidation (42). Quercetin being a flavonoid might have effectively quenched free radicals and consequently decreases lipid peroxidation. The antioxidant efficacy of quercetin may be due to (i) its higher diffusion into membranes (43) allowing it to scavenge oxyradicals at several sites throughout the lipid bilayer; (ii) its pentahydroxyflavone structure allowing it to chelate metal ions *via ortho*-dihydroxy phenolic sturucture, thereby scavenging lipid alkoxyl and peroxyl radicals (44–46). Antioxidant effects of quercetin *in vitro* were shown by Alia et al. (47) in a human hepatoma cell line. Wilms et al. (48) found protective effects of quercetin against induction of oxidative damage in human lymphocytes caused by ROS. In spite of the free radical scavenging activities, quercetin is also involved in the indirect induction of detoxifying agent (49) which might be involved in detoxification of bisphenol A and its toxicity. A study done by Rimbach et al. (50) shows that quercetin supplementation regulates PON2 mRNA and protein levels in RAW264.7 murine macrophages in culture. It has been reported that flavonoids from pomegranate affect the DNA binding activity of the transcription factor AP-1 (51), which is present in the promoter region of the PON2 gene (52), thereby possibly driving PON1 gene expression. AP-1 DNA binding is partly regulated by NADPH oxidase, which produces superoxide anion free radicals, which has been previously shown to be the molecular target of quercetin (53).

Oral administration of quercetin (60 mg/kg b. w./day) along with bisphenol A for 30 days significantly ($p < 0.05$) alleviates DNA, RNA and total protein content in the liver probably by enhancing the synthesizing function of the liver. A study by Raju et al. (54) 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 total protein content (55).

In conclusion, the present investigation has shown that bisphenol A is capable of producing alterations in biochemical parameters investigated in vital organs i.e., liver and kidney which are the organs responsible of detoxification of xenobiotics and foreign compounds. The alterations in biochemical parameters appeared to be more pronounced with the liver as compared to kidney. The 30 days treatment with bisphenol A revealed that bisphenol A might lead to DNA adduct formation with defect in RNA metabolism, which leads to impaired protein synthesis as DNA, RNA and protein contents decrease in case of bisphenol A treated animals Also, this study clarifies that the damage caused by plasticizer in mice can be recovered by combined treatment of quercetin and bisphenol A for 30 days. This may be attributed to the hepatoprotective, renoprotective and antioxidative properties of quercetin.

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