

PROTECTIVE EFFECT OF QUERCETIN ON BISPHENOL A-CAUSED ALTERATIONS IN SUCCINATE DEHYDROGENASE AND ADENOSINE TRIPHOSPHATASE ACTIVITIES IN LIVER AND KIDNEY OF MICE

NEHA P. SANGAI and RAMTEJ J. VERMA*

Department of Zoology, University School of Sciences, Gujarat University,
Ahmedabad-380 009, Gujarat, India.

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Bisphenol A, a monomer of polycarbonate plastics and epoxy resins, is found in food packaging, can-coating chemicals and in dental sealants. Bisphenol A is released from polycarbonate flasks during autoclaving or from lacquer coatings in food cans, showing estrogenicity for human breast cancer MCF-7 cells (1, 2). This compound may be released into food, reach circulating blood and can cause genotoxic and cytotoxic effects (3). Bisphenol A with a weak estrogen-like feature has been implicated to cause a variety of effects on reproduction and development in animals, including effects at doses well below those showing adverse effects in routine toxicity studies (4). Early exposure of rodents to BPA caused increased susceptibility to both mammary and prostate tumorigenesis (5, 6). Because of its widespread use and ubiquitous presence in the environment, the potential for human exposure to BPA is high. Indeed, BPA has been detected in multiple human tissue compartments including serum, follicular fluid, and amniotic fluid (7, 8), urine (9), breast milk (10), saliva (11) and adipose tissue (12). Quercetin belongs to an extensive class of polyphenolic flavonoid compounds almost ubiquitous in plants and plant food sources. Quercetin is the major bioflavonoid in the human diet. Its reputation as an antioxidant stems from the reactivity of phenolic compounds with free radical species to form phe-

noxy radicals which are considerably less reactive. Quercetin is helpful in the recovery of N-diethylnitrosamine induced carcinogenesis (13), human leukemia cell (14), streptozotocin induced diabetes (15), chronic renal failure and reactive oxygen species (ROS) induced DNA damage (16).

Since the flavonoids are well-tolerated, widely studied and least toxic, we had chosen quercetin to ameliorate the changes caused by bisphenol A in energy metabolism in liver and kidney of mice.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade of bisphenol A [BPA – 2,2-bis(4-hydroxyphenyl)propane] and quercetin (3,3',4',5,7-pentahydroxyflavone dehydrate) were used in all experiments. All other chemicals and reagents were of analytical grade and purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

Animals

Inbred Swiss strain male albino mice (*Mus musculus*) weighing 30–35 g (6–8 weeks old) used in the present study were obtained from Zydus Research Centre (Ahmedabad, India). They were kept under controlled condition (temperature 25 ±

* Corresponding author: e-mail: ramtejverma2000@yahoo.com; phone: +91 9825323077; +91 9924375363

2°C, relative humidity 50–55%; 12 h light/dark cycle) in the animal house of Zoology Department, Gujarat University (Ahmedabad, India). Animals were maintained on certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd. (Pune, India) and water *ad libitum*. The experimental procedures were assessed and approved by “Committee for the Purpose of Control and Supervision of Experiment on Animals” (Reg-167/1999/ CPCSCA), New Delhi, India. All animal care guidelines imposed by Prevention of Cruelty to Animals Act, 1960 (59 of 1960; Govt. of India, New Delhi) were strictly followed during the entire course of study.

Experimental design and treatment schedule

Seventy animals were divided into seven groups (n = 10) and caged separately. All treatments were given orally for 30 days using a feeding tube attached to hypodermic syringe. The experimental protocol is shown in Table 1. Group I animals (untreated control) were given free access to food and drinking water. Group II received olive oil (0.2 mL/animal/day). Olive oil was used as a vehicle to dissolve bisphenol A and quercetin. Animals of group III were given quercetin (60 mg/kg b. w./day) in 0.2 mL of olive oil, which served as antidote control group. Dosage of quercetin was based on a previous report by Mishra and Flora (17). Group IV and V received bisphenol A (LD –120 and HD – 240 mg/kg b. w./day), respectively. The dosage of bisphenol A administered (corresponding to 1/10th and 1/20th of LD₅₀ value of bisphenol A) was based

on our earlier study, Tyl et al. (18). Groups VI and VII animals were orally treated with LD and HD of bisphenol A along with quercetin (60 mg/kg b.w./day).

Tissue homogenate 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. A hundred milligrams of the tissue was homogenized in 5 mL of distilled water. The estimation of SDH and ATPase activities in the liver and kidney was done by the standard method as described below.

Succinic dehydrogenase (SDH) (E.C.1.3.99.1) activity

The SDH activity in the liver and kidney was assayed by the method of Beatty et al. (19) using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) as an electron acceptor. The electrons released by the enzyme SDH from the substrate are taken up by INT, which was reduced to a red colored formazan. This was extracted in ethyl acetate, and the absorbance was read at 420 nm. The enzyme activity was expressed as µg formazan formed/mg protein/min.

Adenosine triphosphatase (ATPase) (E.C.3.6.1.3) activity

The ATPase activity in the liver and kidney was assayed by the method of Quinn and White (20). ATPase causes hydrolysis of adenosine

Table 1. Treatment plan.

Experimental groups	Treatments	No. of animals used	Duration (days)	Day of autopsy
1.	Control (untreated)	10	30	31 st
2.	Vehicle control (olive oil) (0.2 mL/animal/day)	10	30	31 st
3.	Quercetin (antidote control) (60 mg/kg b.w./day)	10	30	31 st
4.	Low dose bisphenol A (120 mg/kg b.w./day) treated	10	30	31 st
5.	High dose bisphenol A (240 mg/kg b.w./day) treated	10	30	31 st
6.	Low dose bisphenol A (120 mg/kg b. w./day) + quercetin (60 mg/kg b.w./day) treated	10	30	31 st
7.	High dose bisphenol A (240 mg/kg b. w./day) + quercetin (60 mg/kg b.w./day) treated	10	30	31 st

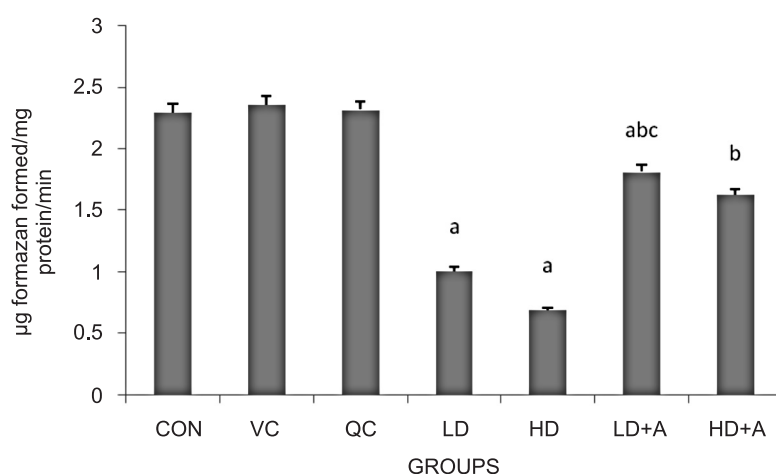


Figure 1. Effect of quercetin on bisphenol A-caused alterations in SDH activity in liver of mice. The values are expressed as the means \pm SEM, n = 10; *p < 0.05 vs. vehicle control group, ^bp < 0.05 vs. low dose treated group and ^cp < 0.05 vs. high dose treated group

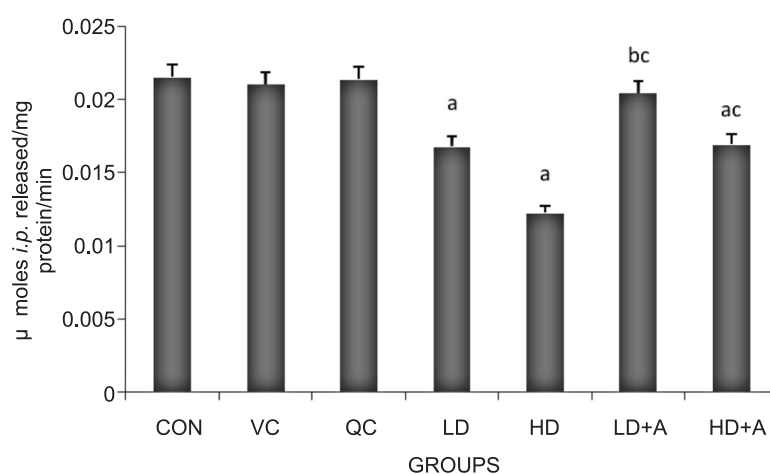


Figure 2. Effect of quercetin on bisphenol A-caused alterations in ATPase activity in liver of mice. The values are expressed as the means \pm SEM, n = 10; *p < 0.05 vs. vehicle control group, ^bp < 0.05 vs. low dose treated group and ^cp < 0.05 vs. high dose treated group

triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (*i.p.*). The liberated inorganic phosphate was estimated by the method of Fiske and Subbarow (21). The optical density was read at 660 nm. The enzyme activity was expressed as micromoles of inorganic phosphate released/mg protein/min.

Statistical analysis

All the data are expressed as the means \pm standard error of the mean (SEM). The data were statistically analyzed using one-way analysis of variance (ANOVA) by SPSS 17th version followed by Tukey

test. The level of significance was accepted with p < 0.05.

RESULTS

Effect of bisphenol A on succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase) activities and its amelioration by quercetin in mice liver

No significant changes were observed in the activities of SDH and ATPase in liver of vehicle (oil) and quercetin control groups (Groups II–III) in comparison with untreated control group (Group

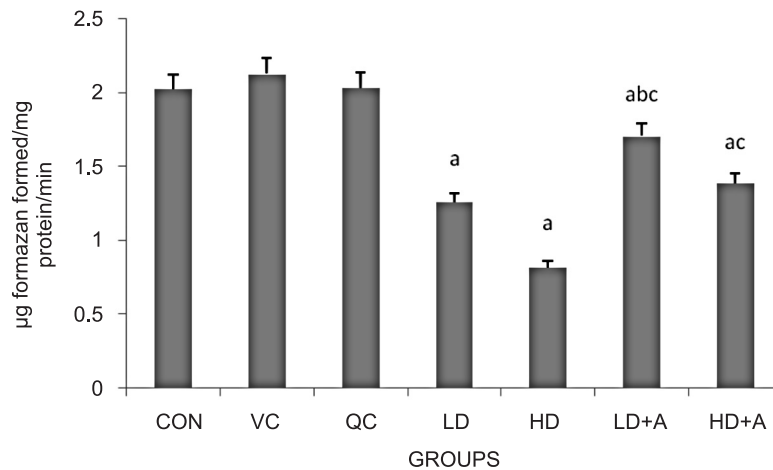


Figure 3. Effect of quercetin on bisphenol A-caused alterations in SDH activity in kidney of mice. The values are expressed as the means \pm SEM, n = 10; *p < 0.05 vs. vehicle control group, ^bp < 0.05 vs. low dose treated group and ^cp < 0.05 vs. high dose treated group

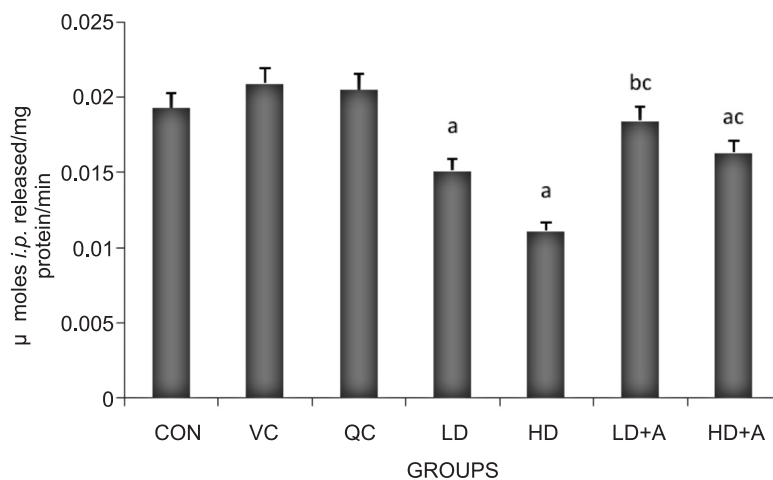


Figure 4. Effect of quercetin on bisphenol A-caused alterations in ATPase activity in kidney of mice. The values are expressed as the means \pm SEM, n = 10; *p < 0.05 vs. vehicle control group, ^bp < 0.05 vs. low dose treated group and ^cp < 0.05 vs. high dose treated group

I). However, in bisphenol A treated groups (Groups IV and V) significant ($p < 0.05$) reduction was found in the activities of SDH (LD: -57.14%; HD: -70.60%, Fig. 1) and ATPase (LD: 20.25%; HD: 41.72%, Fig. 2) as compared to Group II animals. Results revealed a significant amelioration in the activities of SDH and ATPase activity when quercetin and bisphenol A were concurrently administered. Alleviation found was higher in Group VII as compared to Group VI (SDH: HD vs. HD + Q: 39.48%; LD vs. LD + Q: 34.37% and ATPase: HD vs. HD + Q: 22.15%; LD vs LD+Q: 17.405%) (Figs. 1, 2)

Effect of bisphenol A on succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase) activities and its amelioration by quercetin in mice kidney

No significant changes were observed in the activities of SDH and ATPase in liver of vehicle (oil) and quercetin treated control group animals (Groups II-III) in comparison with untreated control group (Group I). As compared to animals of Group II, in bisphenol A-treated animals (Groups IV and V) significant ($p < 0.05$) reduction was found in the activities of SDH (LD: -40.77%; HD: -61.39%; Fig. 3) and ATPase (LD: -27.24%; HD: -46.47%;

Fig. 4). Addition of quercetin and bisphenol A conjointly revealed a significant ($p < 0.05$) amelioration in the activities of SDH and ATPase (Groups VI and VII) (Figs. 3, 4). When quercetin and bisphenol A was concurrently administered, alleviation found was more in Group VII as compared to Group VI SDH (HD vs. HD + Q: 26.04%: LD vs. LD + Q: 21.04%) and ATPase (HD vs. HD+Q: 35.25%: LD vs. LD+Q: 16.02%) (Figs. 3, 4).

DISCUSSION

Oral administration of bisphenol A for 30 days caused significant reduction in activities of SDH and ATPase in liver and kidney of mice. The effect was comparatively more pronounced in high-dose bisphenol A-treated group than that of low dose. SDH is a key enzyme in mitochondrial Krebs cycle which is mainly concerned with the aerobic oxidation of acetyl-CoA and the generation of ATP. Putiliana and Eschanko (22) explained that among the Krebs cycle dehydrogenases, SDH is the most active enzyme; therefore, reduction in SDH activity clearly indicates reduction in aerobic metabolism, which might be a result of reduced oxygen transport to tissues. A detailed study by Nakagawa and Tayama (23) explained the relation between the metabolism and the cytotoxic effects of bisphenol A has been studied in freshly isolated rat hepatocytes and isolated hepatic mitochondria. The incubation of hepatocytes with BPA (0.25–1.0 mM) elicited a concentration- and time-dependent cell death, accompanied by losses of intracellular ATP and total adenine nucleotide pools. BPA at a low-toxic level (0.25 mM) in the hepatocyte suspensions was rapidly converted to its major conjugate, BPA-glucuronide, and other minor products without marked loss of cell viability, although at a toxic level (0.5 mM), more than 65% of the compound was present in an unaltered form 2 h after the incubation. The addition of BPA to isolated hepatic mitochondria caused a concentration (0–0.5 mM)-dependent increase in the rate of state 4 oxygen consumption in the presence of an FAD-linked substrate (succinate), indicating an uncoupling effect, whereas the rate of state 3 oxygen consumption was inhibited by BPA. Further, the addition of BPA (0.25 mM) reduced state 3 respiration with NAD⁺-linked substrates (pyruvate plus malate) and/or with the FAD-linked substrate, whereas state 3 respiration with ascorbate plus tetramethyl-*p*-phenylenediamine (cytochrome oxidase-linked respiration) was not significantly affected by BPA. These results indicate that the onset of cytotoxicity caused by BPA may depend on

the intracellular energy status and that mitochondria are important targets of the compound. The toxicity caused by the inhibition of ATP synthesis may be related to the concentration of unmetabolized free BPA remaining in the cell suspensions. In addition, the toxic potency of bisphenols to hepatocytes and mitochondria depends on the relative elongation and/or molecular size of the hydrocarbon bridge between the phenolic groups. Thus, bisphenol A causes cytotoxicity by impairing mitochondrial function and a consequent decrease in the cellular levels of ATP. Reduction in ATPase activity in liver and kidney suggests reduced utilization of ATP produced in the cell. Thus, reduced aerobic oxidation and ATP generation could be responsible for the reduction in ATPase activity. Bisphenol A glycerolate (1 glycerol/phenol) dimethacrylate (Bis-GMA) induced both cellular differentiation and decrease in oxygen consumption. Cells treated with Bis-GMA showed a significant enhancement of glucose consumption and lactate production. Thus, Bis-GMA affects the metabolism of HL-60 cells and shows differentiating activity. The changes in energy metabolism and glutathione redox balance could be considered as potential mechanisms for inducing clinical and sub-clinical adverse effects by bisphenol A (24).

Treatment with quercetin along with bisphenol A significantly ameliorates bisphenol A-caused changes in the activities of SDH and ATPase in liver and kidney of mice (Figs. 1–4). Quercetin co-treatment ameliorated the biomarkers of kidney function. Renal cortical phospholipid and lipid peroxidation (LPO) contents were reduced while Na⁺, K⁺-ATPase activity in comparison with gentamycin-diclofenac (GM-DC) treated group. Thus, quercetin acts as renoprotective against nephrotoxicity caused by GM-DC therapy (25). Shah et al. (26) showed that Na⁺/K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase are the membrane bound enzymes and the level of Na⁺/K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase were reduced in heart of doxorubicin-treated rats. Since these membrane bound enzymes are 'SH' group containing enzymes, so are lipid dependent. Quercetin significantly improved the level of Na⁺/K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase. The restoration of ATPase activity suggests the ability of quercetin to protect the sulfhydryl group from oxidative damage through inhibition of lipid peroxidation. Quercetin treatment prevented the daunorubicin-induced decrease in GPx activity, furthermore, quercetin treatment has also been reported to significantly improve the decrease of GPx activity induced by oxidative stress (27, 28).

CONCLUSIONS

Oral administration of bisphenol A caused dose-dependent, significant reductions in the activities of succinic dehydrogenase and adenosine triphosphatase, as compared to the controls. Treatment with quercetin along with bisphenol A caused significant amelioration in all parameters as compared to bisphenol A alone treated groups in liver and kidney of mice.

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