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HEPATOPROTECTIVE EFFECT OF QUERCETIN ON BISPHENOL A-INDUCED TOXICITY

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Abstract: One of the natural antioxidant flavonols -Quercetin is found in various food products and plants. Its anticancer properties have been proved by *in vivo* and *in vitro* experiments; it shows an attempt to examine toxic effects of Bisphenol A in the liver of mice and its alleviation by quercetin. For this inbred Swiss strain male albino mice were orally administered with quercetin (30, 60 and 90 mg/kg body weight/day) along with BPA (240 mg/kg body weight/day) for 45 days. On the completion of the treatment period, animals were sacrificed; organs were isolated and used for biochemical analysis. All these effects were dose-dependent. Co-treatment with quercetin (30, 60 and 90 mg/kg body weight) and BPA (240 mg/kg body weight) alleviates the changes in body weight, absolute and relative organ weights of mice. Biochemical analysis revealed significant ($p < 0.05$) and dose-dependent reduction in enzymatic antioxidants such as superoxide dismutase, Catalase and glutathione peroxidase and non-enzymatic antioxidants such as Glutathione and Total ascorbic acid content were also observed in Bisphenol A - treated groups as compared to control. The present results revealed that graded doses of BPA caused oxidative damage in the liver of mice, which is mitigated by quercetin.

***Keywords**:* Quercetin, Bisphenol A (BPA), liver, toxicity

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Introduction

Quercetin belongs to a widespread class of polyphenolic flavonoid compounds almost everywhere in plants and food sources, including fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. Its anticancer properties have been proved by *in vivo* and *in vitro* experiments. It is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation.[1] It has a beneficial effect on human health like cardiovascular protection, anti-ulcer effects, cataract prevention and antiviral activity; moreover it has anticancer, anti-allergic and anti-inflammatory properties due to its potent antioxidant property.[2,3]

Bisphenol A (BPA) is one of the most widely used synthetic chemical in the world and the major components of plastic products. It is also known as an endocrine disruptor and its estrogenic properties are reported since 1936.[4] It is used in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastic is widely used to make housings for electronic products such as cell phones, CDs, DVDs, food containers like milk, water and baby bottles and epoxy resins are used as the interior protective lining for food and beverage cans and other metal containers.[5,6] The most important human exposure route to BPA is diet, including ingestion of contaminated food and water.[7] The toxic effect of Bisphenol A on vital organs is due to the production of ROS[8,9] and decreasing the activity of antioxidant enzymes.[10,11] Bisphenol A may interfere with endocrine transduction mechanisms at very low doses[12] and the exposure to this contaminant has been correlated with a wide variety of adverse health effects in both male and female including birth defects, reproductive, developmental, metabolic, immune, and neuro-behavioral disorders.[13,14]

The aim of the present study was to estimate the defending effect of quercetin on BPA induced subchronic toxicity in the liver of adult male mice of Swiss strain.

Materials and methods

Chemicals

Bisphenol A and quercetin was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Olive oil was obtained from Figaro, Madrid, Spain. All the other chemicals used in the study were of AR grade.

Animals

All experiments were performed on inbred healthy, adult male mice of Swiss strain from Cadila Research Center, Ahmedabad, India, weighing approximately 30-35 g. They were housed in an air-conditioned room at a temperature 25±2 °C, relative humidity 50-55% with 12 h light/dark cycle throughout the experiment. Animals were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and potable water ad libitum. Animals were handled according to the guidelines published by the Indian National Science Academy, New Delhi, India (1991). The experimental protocols of animals were sanctioned by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad and approved by the committee for the purpose of control and supervision of experiment on Animals (Reg – 167/1999/CPCSEA), New Delhi, India.

Experimental Design

Mice were randomly divided into seven groups, 10 animals each. Treatment schedule of the animals was as follows.

Animals from Group I (untreated control) were kept without any treatment and given free access to feed and water. Group II (vehicle control) animals were treated with olive oil (0.2 ml/animal/day), as olive oil was

used to dissolve Bisphenol A. Group III (Antidote control) animals were treated with a maximum amount of quercetin (90 mg quercetin/kg body weight/day). Animals of Group IV (BPA dose) were treated with the dose of BPA. Animals of group V, VI and VII received three different doses of quercetin (30, 60 and 90 mg/kg body weight/day) along with BPA dose respectively for 45 days. All treatments were given orally using a feeding tube attached to a hypodermic syringe.

Animals were sacrificed on 46th day by using anesthetic ether then the liver was dissected out, blotted free from blood and used for biochemical analysis.

Biochemical analysis

Enzymatic antioxidants

Catalase (E.C.1.11.1.6) activity:

The Catalase (CAT) activity was assayed in the liver by the method of Luck (1963)[15]. The assay mixture consisted of 50 mM phosphate buffer (pH 7.0); aliquot and 10 mM H_2O_2 which was added to aliquots for initiate the reaction. A decrease in absorbance was noted every 5 seconds at 240 nm. The enzyme activity was expressed as μmoles H_2O_2 consumed mg/min protein.

Superoxide dismutase (E.C.1.15.1.1) activity:

The superoxide dismutase (SOD) activity in the liver was assayed by the method of Kakkar*et al*. (1984)[16] with slight modification. The method is based on the NADH- phenazine methosulfate nitroblue tetrazolium formazon inhibition. The formazon formed at the end of the reaction was extracted into butanol layer, upon inactivation of the reaction with acetic acid. The enzyme activity was expressed as units/mg protein. One unit of

enzyme activity is defined as the enzyme concentration required inhibiting chromogen production (at 560 nm) by 50% in 1 min under the assay condition. The enzyme activity was expressed as units/mg protein.

Glutathione peroxidase (E.C.1.11.1.9) activity:

The glutathione peroxidase (GSH-Px) activity in the liver was assayed by the modified method of Pagila and Valentine (1967).[17] The decrease in absorbance was recorded every 10 s for 3 min at 340 nm. The enzyme activity was expressed as units/mg protein/min, where 1 unit of GSH-Px equals to nmoles of NADPH consumed/mg protein/min.

Non-enzymatic antioxidants

Glutathione content:

The glutathione (GSH) content in the liver was measured by the method of the Grunert and Philips (1951).[18] In saturated alkaline medium, the GSH present in the tissues react with sodium nitroprusside to give a red colored complex which was measured at 520 nm. The glutathione content was expressed as μg/100 mg tissue weight.

Total ascorbic acid content

Total ascorbic acid (TAA) content was estimated in the liver by the method of Roe and Kuether (1943).[19] Total ascorbic acid is oxidized to dehydroascorbic acid (DHA) by Norit reagent in the presence of TCA. This couples with 2, 4-dinitrophenyl hydrazine in the presence of thiourea and sulphuric acid to yield a red coloured complex which was read at 540 nm. The TAA content was expressed as mg/gm tissue weight.

Statistical analysis

The Data is statistically analysed using graph pad prism software, version 5.03 (trial version). The results are expressed as the mean ± SEM.

Hypothesis testing method included one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The level of significance was accepted with $p<0.05$.

Results

Body weight and liver weight

Table 1. Effect of Quercetin treatment on BPA induced toxicity in body weight (g) and liver weight of mice.

	Experimental groups	Body weight (g)		Liver Weight	
		0 day	45th day	Absolute	Relative
I	Untreated control	40.00 ± 0.70	40.20 ± 0.44	2.248±0.43	5.62±0.16
II	Vehicle control (0.2 ml olive oil/animal/day)	39.60± 0.89	40.00± 1.58	2.242±0.26	4.60±0.14
III	Antidote control (90mg quercetin/kg body weight/day)	40.00±0.63	39.83± 0.75	2.234±0.27	5.59±0.69
IV	BPA dose (240 mg/kg body weight/day)	39.87 ± 0.64	30.13± 1.13	3.115±0.37	7.79±0.92
V	BPA + quercetin (30 mg/kg body weight/day)	40.17 ± 0.98	32.83± 1.83	2.704±0.39 (47.50)	6.76±0.98* (47.11)
VI	BPA + quercetin (60 mg/kg body weight/day)	40.14 ± 0.37	36.14± 1.95	2.526±0.29 (64.77)	6.32±0.73* (67.39)
VII	BPA + quercetin (90 mg/kg body weight/day)	40.13 ± 0.83	39.37± 1.06	2.391±0.24 (83.07)	5.98±0.60* (82.8)

Results are expressed as mean ± S.E.M; n=10.

No significant difference was noted between untreated and vehicle control groups.

Level of significant *$p < 0.05$, as compared to vehicle control.

Values shown in parenthesis indicate organo protective index.

Unit Body weight - g, Unit; Absolute weight - g; Relative weight - g/100 mg body weight.

Table 1 shows the effect of Quercetin treatment on BPA induced toxicity in body weight of mice. No significant change in the body weight of control animals (group I, II, III) was observed. As compared to vehicle control, oral administration of Bisphenol A caused reduction in body weight (groups IV) after 45 days of treatment. Quercetin along with the dose of BPA increased the body weight of mice.

Absolute and relative weight

Oral administration of a dose of BPA (group IV) for 45 days increased the absolute and relative weight of liver (138.938%) and (138.963%) respectively. Co-treatment of quercetin along with the dose of BPA resulted in significant ($p < 0.05$) and dose-dependent ($r = 0.94$ and 0.90), decreased in absolute and relative weights of the liver as compared to BPA treated animals. The Hepato-protective index calculated for quercetin for absolute weight was Q30:47.50%, Q60:64.77% and Q90:83.07% in case of relative weight. Highest protection was achieved at 90 mg/kg bw/day dose treated groups of mice (group VII). No significant difference was observed in control groups I- III.

Table 2. Effect of Quercetin treatment on BPA induced alterations in enzymatic and non enzymatic antioxidants in theliver of mice.

Sr.	Experimental groups	CAT	SOD	GSH-Px	GSH	TAA
I	Untreated control	15.07±0.85	4.09±0.16	58.62±1.40	4.95±0.05	4.01±0.13
II	Vehicle control (0.2 ml olive oil/animal/day)	14.85±0.78	4.08±0.10	58.70±1.45	4.91±0.06	4.05±0.03
III	Antidote control (90 mg quercetin/kg body weight/day)	15.00±0.34	4.11±0.12	57.88±0.92	4.95±1.91	4.11±0.07
IV	BPA- dose (240 mg/kg body weight/day)	07.68±0.34*	1.35±0.07*	32.67±1.04	1.91±0.13	1.73±0.04*

Table 2. Continued

V	BPA + quercetin (30 mg/kg body weight/day)	10.49±0.34* (39.16)	2.20±0.07* (31.10)	38.87±1.42* (23.82)	2.69±0.10* (26.17)	2.35±0.20* (26.63)
VI	BPA + quercetin (60 mg/kg body weight/day)	12.90±0.19* (72.71)	3.39±0.09* (74.98)	45.97±0.74* (51.09)	3.54±0.07* (54.37)	3.01±0.05* (55.04)
VII	BPA + quercetin (90 mg/kg body weight/day)	13.93±0.17* (87.11)	3.71±0.09* (86.43)	53.07±1.80* (78.37)	4.72±0.15* (93.51)	3.86±0.08* (91.78)

Results are expressed as mean ± S.E.M; n=10.

No significant difference was noted between untreated, vehicle control and antidote control groups.

Level of significant *$p < 0.05$, as compared to untreated control

Values shown in parenthesis indicate organo protective index.

Unit: CAT: μmoles H_2O_2 consumed/mg protein/min; SOD: U/mg protein; GR: nmoles NADPH consumed/mg protein/min; GPx: nmoles NADPH consumed/mg protein/min, GSH: μg/100 mg tissue weight; TAA: mg/g tissue weight.

Enzymatic antioxidants

Activities of enzymatic antioxidants were severely affected by BPA treatment, which was brought back to normal by co – treatment of quercetin as shown in Table 2 Activities of hepatic CAT and SOD were reduced by BPA treatment and found to elicited significantly ($p<0.05$) by various doses of quercetin in a dose – dependent manner (CAT: $r = 0.89$, SOD: $r = 0.95$). Hepatic protection by quercetin for CAT activity was Q30:39.16 %, Q60:72.71%, Q90:87.11 % and for SOD activity was Q30:31.10 %, Q60:74.98% Q90:86.43 % respectively.

Similarly the protective effect of quercetin on the activities of GPx was also significant ($p<0.05$) and dose – dependent (GPx: $r = 0.96$) as compared to the dose of BPA treated group (Group IV). Treatment of BPA alone reduced activities of GPx 42.86%.

Non-enzymatic antioxidants

Quercetin is known to possess strong anti-oxidative potency and was used to combat BPA – induced oxidative stress in this study. Table 2 shows the protective effects of quercetin on BPA – induced changes in non-enzymatic antioxidants levels in the liver of mice. No significant differences were noted in GSH and TAA contents between control groups (Group I – III). Oral administration of the dose of BPA for 45 days caused significant ($p < 0.05$) reduction in GSH (44.34%) and TAA (38.34%) contents as compared to vehicle control.

Recovery in the content of non-enzymatic antioxidants (GSH and TAA) was also achieved by concurrent administration of quercetin along with the dose of BPA for 45 days. Hepatoprotective index calculated for GSH content for quercetin was Q30 : 23.82 %, Q60 : 51.09 %, and Q90 : 78.37 % and for TAA content was Q30 : 26.17 %, Q60 : 54.37 % and Q90 : 93.51% respectively. The effect was significant ($p < 0.05$) and dose – dependent (GSH: $r = 0.93$, TAA: $r = 0.97$) as compared to various control groups (Group I-III).

Discussion

The present study aimed to estimate whether exposure to BPA induces oxidative stress in the liver of male rats. Health promoting effects of plants are primarily denoted by the presence of bioactive phytochemicals acting as potent antioxidants having nutritional and pharmacological properties.[20,21]

The liver plays an important role in metabolism and removal of a toxic substance from the portal circulation, placing it in the first line and prone to be attacked by foreign materials.[22,23] Results of in vivo evaluation of BPA for 45 days caused significant changes in body and organ weight. The present study indicated reduction food intake, dullness and lethargy in mice treated with BPA for 45 days. Also, frequent body hair fall was observed after 10-15 days of dosing period. Treatment related mortality was absent in all the treated groups. No treatment related clinical signs were observed in untreated, vehicle and antidote control group animals. No other abnormal clinical symptoms were observed.

There is significant, dose-dependent reduction in enzymatic antioxidants such as SOD, Catalase and GSH-Px, which constitutes the first line defense against ROS, induced damage. An increase of quercetin concentration in rats treated with Bisphenol A (240 mg/kg bodyweight/day), caused significant reduction in oxidative stress as evidenced by a significant increase in enzymatic antioxidants SOD, CAT and GPx enzymatic activities and restored these levels close to corresponding control values. Quercetin increases the body's endogenous antioxidants to reduce oxidative damage. SOD protects tissues from oxidative stress and damage by catalyzing the conversion of peroxide to H_2O_2, a more stable ROS. Catalase catalyzes the decay of hydrogen peroxide to water and oxygen. It is an extremely important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). The damage at the cellular level by oxidants is attenuated by an antioxidant enzyme such as SOD. Our results are consistent with the previous study showing the decrease in enzymatic antioxidants concentrations in the liver of BPA administered mice.[24]

Content of non-enzymatic antioxidants of the hepatocytes were found to increase with co-treatment of quercetin. Glutathione is an important non-enzymatic antioxidant defense required to maintain the normal redox state of cells and to counteract deleterious effects of oxidative stress. Our result showed significant ($p < 0.05$) decrease in GSH contents in animals exposed to BPA was restored back by free radical scavenging and sulfhydryl (thiol) group protecting effects of quercetin, which indicated the potential of quercetin to counteract the oxidative damage induced by BPA and to reinforce the antioxidant defense in normal condition. Ascorbic acid content also found increase due to proton donating effect of quercetin sparing body's natural antioxidants from getting oxidized. Increased level of glutathione could be the reason for the reduction in lipid peroxidation as in the presence of GSH, lipid peroxidation are converted to less toxic alcohol derivatives rather than MDA.[25]

Conclusions

These data suggest that quercetin protects mice liver from BPA induced oxidative stress, presumably via its antioxidant activity. So quercetin is a promising pharmacological agent for proscriptive the potential hepatotoxicity of BPA following occupational or environmental exposures.

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