Mitigatory Effects of Quercetin on Bisphenol A-Induced Oxidative Stress in Testis of Swiss Albino Mice

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Abstract

**Background:** Bisphenol A is a well-known xenoestrogen, mainly used in plastics and food can liners manufacture. Hence, it becomes an integral part of the food chain. Experimental studies shows negative health effects. Quercetin is one of the most effective antioxidants of the flavonoids, generally present in food stuff. The present study was an attempt to assess the mitigatory effects of quer on BPA–induced reduction in fertility of mice.

**Materials and methods:** Inbred male Swiss strain albino mice were orally administered with various doses of BPA for 45 days. In another experiment quer along with high dose of BPA was administered to study the mitigatory consequence.

**Result:** Administration of BPA caused significant (P<0.05) and dose-dependent increase in oxidative stress compared to control groups. Administration of quer along with high dose of BPA caused amelioration in enzymatic and non-enzymatic antioxidant. Histopathological alteration was also seen in BPA treated groups, which were ameliorated on administration of quer.

**Conclusion:** Quer is potent enough to mitigate the toxic effect of BPA. Biochemical alteration was mitigated by quer and dose dependent increase in fertility was observed.

**Keywords:** Quercetin (Quer); Bisphenol A (BPA); Sub chronic toxicity; Oxidative stress; Amelioration

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**Competing Interests:** The authors have declared that no competing interests exist.

**Consent:** We confirm that the patients have given the informed consents for the case reports to be published.

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1. Introduction

Flavonoids belong to a group of polyphenolic compounds that are produced exclusively in plants [1, 2]. Quercetin (Quer) (3,3’, 4’, 5, 7-pentahydroxyflavone) is naturally-occurring dietary flavones compounds, existing in large amounts in vegetables, fruits and tea [3-5]. Which acts as an antioxidant, due to definite chemical assembly, counteracts oxidative stress generated as a result of reactive oxygen species, which contributes to the lipid peroxidation [6-8]. Various studies have shown the potential effect of quer as antioxidant, anti-inflammatory, anti-carcinogenic and cardioprotective [7-10].

Bisphenol A (BPA) (4-4’-dihydroxy-2, 2-diphenylpropane) is a well-known xenoestrogen which is used in manufacture of a various consumer products like epoxy resins and polycarbonate plastics [10, 11]. BPA is used in coating of food and beverage containers, So food is said to be the main source of BPA exposure as it leaches out from it [12, 13]. Effects of BPA are largely related to its estrogenic activity and oxidative stress [14]. Experimental studies revealed that, very low doses of BPA mimics estrogen, resulting in an array of health maladies including prostate [15], breast cancer [16], genotoxic effect [17] and other health related problems [18-20]. However, there is no data available on the mitigatory effect of quer on BPA induced testicular toxicity.

The present investigation was an attempt to evaluate mitigatory effects of quer on BPA–induced reduction in fertility by estimation of oxidative stress in testis of mice.

2. Materials and methods

Chemicals

Quer and BPA and was acquired from Hi Media Laboratories Pvt. Ltd., Mumbai, India. Olive oil was acquired from Figaro, Madrid, Spain. All the other chemicals used in the experiments were of AR grade.

Experimental animals

In present study, inbred healthy adult Swiss strain male albino mice weighing 30-35 gm were acquired from Cadila pharmaceutical research Center, Ahmedabad, India. Animals were preserved in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India. They were kept in an air conditioned room at a temperature of 25±2 °C and 50 - 55% relative humidity with a 12 h light and 12 h dark cycle during the experiment. Animals were fed with pelleted rodent feed and drinkable water ad libitum. All the experimental procedures were sanctioned by the Committee for the Purpose of Control and Supervision of Experiment on Animals (Reg. – 167/1999/CPCSEA), New Delhi, India. Animals were handled According to the guidelines published by Indian National Science Academy, New Delhi, India (1991).

Dose selection

Different doses of BPA was selected on the bases of LD$_{50}$ value [21], which is 1/10$^{th}$ (HD), 1/20$^{th}$ (MD) and 1/30th (LD). (240,120 and 80 mg/kg bw/day respectively) of LD$_{50}$ for 45 days. For the mitigation of toxicity, different doses of quer (30, 60 and 90 mg/ kg bw/ day) was selected on the basis of the study of Sangai and Verma (2014) [22].
Experimental Plan

Mice were haphazardly divided into nine groups, each containing 10 animals. Treatment schedule of the animals was as follows. Animals from group I (untreated control) were kept untreated and given free access to feed and water. Vehicle control mice (Group II) were administered with olive oil (0.2 ml olive oil /animal/day), which has been used as vehicle to dissolve BPA. Mice of antidote control group (Group III) were treated with quer (90 mg/kg bodyweight/day), which has been used for amelioration of BPA-induced toxicity.

Mice of group IV, V and VI given three different doses of BPA (80, 120 and 240 mg/kg body weight /day). Animals of group VII, VIII and IX were administered with three different doses of quer (30, 60 and 90 mg/kg bw/day) along with high dose of BPA. All treatments were given orally via a feeding tube attached to hypodermic syringe for 45 days. Animals were sacrificed on 46th day by using anesthetic ether and the testis was dissected out, blotted free from blood and used for biochemical analysis.

Biochemical analysis

Lipid peroxidation

Lipid peroxidation was assayed by determining the level of malondialdehyde (MDA) by measuring thiobarbituric acid reactive species using the method of Ohkawa et al. (1979) [23].

Enzymatic antioxidants and Non-enzymatic antioxidants

Catalase (E.C.1.11.1.6) activity was analyzed using the method of Luck (1963) [24], utilizing hydrogen peroxide as a substrate. Decrease in absorption was noted at 240 nm. The enzyme action was articulated as μ moles H₂O₂ consumed/ mg protein/min. Superoxide dismutase (SOD) activity was measured by the method of Kakkar et al. (1984) method [25]. The enzyme activity was articulated as units/mg protein. The activity of glutathione peroxidase (GSH-Px) in the testis of mice was analyzed by the reformed method of Pagila and Valentine (1967) [26]. 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. The method defined by Grunert and Philips (1951) was used to estimate of glutathione content [27]. Total ascorbic acid content was quantified according to the method as described by Roe and Kuether (1943) [28].

Statistical analysis

The results were articulated as the mean ± SEM. The data were statistically evaluated via one way analysis of variance (ANOVA) followed by Turkey’s post hoc test in Graph pad prism 6 (graph pad, software, USA). Statistical significance was accepted with p<0.05. Correlation coefficient was measured to estimate the strength of linear association among two variables. Pearson’s correlation analysis was used to find the correlation between lipid peroxidation and other biochemical parameters.

Histopathological analysis

Histopathological studies were carried out by the standard technique of hematoxylin and eosin staining. The testis of all control and treated groups of animals were dissected out, blotted free of blood and fixed in 10% neutral buffered formalin immediately afterwards the autopsy. The preserved tissues were dehydrated by passing over ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (58 to 60°C mp). 5 μm thick sized sections were cut on a rotary microtome and stained with H & E,
dehydrated in alcohol, cleared in xylene, mounted in DPX and examined in a light microscope (Labomed vision 2000).

**Fertility index**

The fertility index of vehicle control and treated groups of animals were calculated by formula [29] as mentioned below:

\[
\text{Male fertility index} = \frac{\text{Number of males impregnating female}}{\text{Number of males cohabitated}} \times 100
\]

### 3. Results

**Biochemical changes**

The consequence of BPA treatment on lipid peroxidation as well as enzymatic antioxidants in testis and their possible amelioration by co-treatment with quer is shown in table 1. No significant variance was noted in LPO and enzymatic antioxidants among different control groups of animals (Groups I-III). BPA administration (Groups IV-VI) for 45 days caused significant (p<0.05), dose-dependent (r=0.867) increase in LPO (LD: 42.33 %, MD: 68.42 % and HD: 102.14 %) as compared to vehicle control (Group II). Enzymatic antioxidants activities were significantly lowered in BPA-treated mice as compared to vehicle control. These effects were in dose-dependent manner (r= 0.876, 0.809, 0.932 respectively). The maximum reduction was observed with BPA-HD.

As compared to BPA-HD (Group VI), quer co-treatment along with BPA-HD (Groups VII-IX) caused significant (p<0.05) dose-dependent reduction (r=0.789) in LPO (LD: 41.86, MD: 63.37 and HD: 86.29) as calculated by organoprotective index. Similarly, co-treatment of quer along with BPA-HD caused significant (p<0.05), dose-dependent increase in activities of CAT (r=0.830), SOD (r=0.849) and GSH-Px (r=0.923) as compared to BPA-HD alone treated group (Group VI). Organoprotective index was highest in high dose quer along with BPA-HD-treated animals (Group-XI).

BPA exposure caused changes in non-enzymatic antioxidants and its mitigation by quer is shown in Table 1 No significant alterations were observed in level of testicular GSH and TAA contents between different control groups of animals (Groups I-III). Administration of BPA (Groups IV-VI) caused significant (p<0.05), dose-dependent (r=0.950) decrease in GSH (LD: 21.05%, MD: 33.56% and HD: 48.77%) as compared to vehicle control group (Group II). Similarly, as compared to untreated control, BPA caused significant, dose-dependent (r=0.876) decrease in TAA content (LD: 14.49%, MD: 33.756% and HD: 55.641%).

Outcomes revealed that co-treatment with different doses of quer along with BPA (Group VII-IX) caused significant (p<0.05), dose-dependent increase in GSH (r=0.948) and TAA (r=0.850) contents, as compared to BPA-HD alone treated group (Group VI). Organoprotective index was highest for (GSH=91.88, TAA= 89.14) in 90 mg/kg bw/day dose of quer along with BPA HD- treated animals (Group IX).
Table 1 Effect of BPA on lipid peroxidation, enzymatic and non-enzymatic antioxidants in testis of mice and amelioration by quercetin

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>LPO</th>
<th>Enzymatic antioxidants</th>
<th>Non-enzymatic antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Catalase</td>
<td>SOD</td>
</tr>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>2.04±0.14</td>
<td>19.98±3.55</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>2.02±0.08</td>
<td>20.07±3.13</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>1.99±0.07</td>
<td>20.14±0.65</td>
</tr>
<tr>
<td>BPA-treated groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>2.36±0.09</td>
<td>17.89±3.32</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>3.13±0.09</td>
<td>14.04±0.45</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>3.69±0.13</td>
<td>10.67±0.38</td>
</tr>
<tr>
<td>HD BPA(240 mg/kg body weight) + quercetin treated groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td>2.93±0.13</td>
<td>13.53±0.52</td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td>2.59±0.07</td>
<td>15.84±0.61</td>
</tr>
<tr>
<td>IX</td>
<td></td>
<td>2.23±0.11</td>
<td>18.54±0.69</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., n=10

Values shown in parenthesis indicate:

Italicics- Percent change in BPA-treated from vehicle control

Bold- Organoprotective index from BPA-HD

Level of significance; \( \text{ap}<0.05 \) as compared to vehicle control

bp<0.05 as compared to BPA-HD -treated

No significant difference was noted between different control groups (Groups I-III).

Units: LPO- nmoles MDA formed/mg protein/60 min; Catalase- μmoles H2O2 consumed/mg protein/min; SOD-units/mg protein; GSH-Px- nmoles of NADPH consumed/mg protein/min; GSH- μg/100 mg tissue weight; TAA- mg/gm tissue weight

Histopathological analysis

The transverse section of testis of control groups (Groups I-III) of animals showed normal histological features; seminiferous tubules were intact, exhibiting normal spermatogenesis, lumen with sperm bundles and normal Leydig cells (Figure-1). BPA administration (Group VI) caused cellular pyknosis, degeneration of seminiferous tubules, depletion of spermatogenic and lower number of sperms in seminiferous tubules (Figure-2). Degeneration of Leydig cells were also evident. The effect was more pronounced in BPA-HD-treated group (Group VI). However, co-treatment with quer along with BPA (Group IX) lowers histopathological abnormalities (Figure3). The recovery was almost complete at the dose of 90 mg/kg bw/day of quer along with BPA-HD (Group IX).
Figure 1 T.S. of testis of untreated control mice showing typical seminiferous tubules with various stages of spermatogenic cells and sperm bundles in the lumen. The Leydig cells are observed normal in the intertubularinterstitium with distinct nucleus (H & E staining, X225)

Figure 2 T.S. of testis of BPA-HD-treated mice showing pyknosis and highly degenerative seminiferous tubules, depletion of spermatogenic cells, lumen with devoid of sperms and degeneration of Leydig cells (H & E staining, X225)

Figure 3 T.S. of testis of HD BPA+ HD Quercetin mice showing normal histoarchitecture as mentioned above (H & E staining, X225)
Fertility Index

Oral administration of BPA shows dose dependent reduction in fertility (Graph 1). Fertility rate of BPA exposed groups (IV-VI) had reduced to 80%, 50% and 20% respectively as compared to vehicle control groups. The supplementation of quer along with BPA for 45 days restored fertility almost to control levels (LD-30%; MD-60; HD-90). Reduction in fertility could be related to reduction in sperm function parameters.

4. Discussion

BPA caused significant increase in the formation of MDA [30]. MDA levels measurement in the tissue is a indicator of lipid peroxidation which is among the chief mechanism of cell damage. Significant raise in MDA level could be due to over production of reactive oxygen species and suppression of antioxidant enzymes (Table-1). BPA is lipophilic in nature due to which it can easily interact with the lipid membrane of the cells. Moreover it causes oxidative stress by disquieting the redox status in cells [31]. Suthar and Verma have also revealed that BPA generate oxidative stress by decreasing the actions of antioxidant enzymes [32]. Similarly, various studies have demonstrated that BPA generates ROS that causes oxidative stress in the vital organs of rats [33-37]. According to Samova et al., BPA treatment caused dose-dependent reduction in testis weight [38]. The testis weight is mainly dependent on the mass of the differentiated spermatogenic cells. The reduction in the testes weight is because of the decreased tubule size, spermatogenic detention and inhibition of steroid biosynthesis of Leydig cells has been reported by Chiu [39]. Histopathological alterations shows pyknosis and highly degenerative seminiferous tubules, depletion of spermatogenic cells, lumen with devoid of sperms and leydig cell degeneration. This may due to the overproduction of ROS. Testicular degenerative changes have also been reported by Williams et al.,2014 [40].

The predominance of quer in preventing both metal and non-metal-induced oxidative damage is partly attributed to its free 3-OH substituent [36,37] which is believed to surge the stability of the flavonoid radical. The catechol group is directly coupled to the chelating action of quer, as has been proven by various studies in which quer suppresses lipid peroxidation by the scavenging free radical [41]. There is significant, dose-dependent reduction in enzymatic antioxidants such as SOD, catalase and GPx, which constitutes the first line defense against ROS induced damage [42]. This may be due to the interaction of LPO products with enzyme molecules causing modification of histidine residues and generation of protein-protein cross-linked derivatives causing reduction in enzyme activity [43]. SOD protects tissues from oxidative stress and damage by catalyzing the conversion of O2 \(-\cdot\) to H2O2, a more stable ROS [28]. The damage at cellular level by oxidants is decreased by antioxidant enzyme such as SOD [44-46]. Our results are consistent with previous study showing the decrease in enzymatic antioxidants concentrations in the liver of BPA administered mice [30, 31].

Glutathione (GSH) and ascorbic acid are important endogenous free radical scavenger and non-enzymatic oxidants. Glutathione metabolism is one of the most essential antioxidative defense mechanisms [47]. It is an intracellular reductant and plays major role in catalysis, metabolism and transport. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. Ascorbic acid is a most powerful antioxidant under physiological conditions [48]. Hydrogen peroxide was converted into water by ascorbic acid via ascorbate peroxidase reaction [49, 50]. The levels glutathione and TAA also significantly reduced after the oral administration of BPA; the
effect was dose dependent. The increased TBARS level and decreased GSH concentration shows an increased generation of ROS, which cause lipid peroxidation in the tissue [51].

The cellular damage resulting from interaction between macromolecules and ROS, can be reduced by antioxidants, such as quer. Different concentrations (30, 60, 90 mg/kg bodyweight/day) of quer when treated with high concentration (240 mg/kg bodyweight/day) of BPA, caused significant reduction in oxidative stress as evidenced by a significant upsurge in enzymatic antioxidants SOD and CAT enzymatic activities and restored these levels close to corresponding control values. Quer increases the body’s endogenous antioxidants to reduce oxidative damage. Similar investigations has reported antioxidant properties of quer [52, 53].

In present study dose dependent reduction in fertility of BPA treated mice as compared to vehicle control groups. Moreover, the supplementation of quer restored fertility almost to control levels. Histopathological and biochemical analysis shows alterations. This could be the possible reason for the decrease in fertility [14, 16, 18]. Reduction in sperm functional parameters are also observed in various study [38].

5. Conclusion

Present study revealed that treatment of BPA for 45 days causes oxidative stress in experimental animals by disturbing the balance between ROS and antioxidant defenses system in testis which leads to reduction in fertility [54], However co-administration of quer for 45 days caused significant amelioration in enzymatic and non-enzymatic antioxidants. The effect was dose dependent, in addition lipid peroxidation was significantly decreased. This study concludes that BPA causes oxidative stress by reducing antioxidative capacity and it can be mitigated by the quer.

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7. Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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