American Journal of Toxicology

http://www.ivyunion.org/index.php/ajt/

Research Article

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

Sanman Samova, Hetal Doctor and R.J. Verma*

Department of Zoology, BMTC and HG, School of Sciences,

Gujarat University, Ahmedabad-380009, India

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

**Received**: May 13, 2018; **Accepted**: June 28, 2018; **Published:** August 20, 2018

**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.



***Correspondence to:** R.J. Verma, Department of Zoology, BMTC and Human Genetics, School of Sciences, Gujarat University, Ahmedabad-380009, India

**Email**: ramtejverma2000@yahoo.com

# 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 ℃ 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 containing10 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 45days. Animals were sacrificed on $46^{th}$ 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 $H_2O_2$ 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:

$$Male\ fertility\ index = \frac{Number\ of\ males\ impregnanting\ female}{Number\ of\ males\ cohabitated} \times 100$$

# 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**

| Experimental group | | LPO | Enzymatic antioxidants | | | Non- enzymatic antioxidants | |
|---|---|---|---|---|---|---|---|
| | | | Catalase | SOD | GSH-Px | GSH | TAA |
| **Control groups** | | | | | | | |
| **I** | **Untreated control** | 2.04±0.14 | 19.98±3.55 | 2.09±0.16 | 1.96±0.08 | 49.74±1.64 | 7.12±0.20 |
| **II** | **Vehicle control** (0.2 ml olive oil /animal/day) | 2.02±0.08 | 20.07±3.13 | 2.02±0.09 | 1.93±0.07 | 49.85±0.79 | 7.09±0.03 |
| **III** | **Antidote control** (90 mg quer/kg body weight/day) | 1.99±0.07 | 20.14±0.65 | 2.07±0.09 | 1.95±0.03 | 49.56±0.52 | 7.12±0.13 |
| **BPA-treated groups** | | | | | | | |
| **IV** | **BPA-Low dose** (80mg/kg bodyweight/day) | 2.36±0.09[a] *(17.24)* | 17.89±0.32[a] *(13.65)* | 1.52±0.12[a] *(24.87)* | 1.48±0.04[a] *(23.64)* | 39.36±0.63[a] *(21.05)* | 6.07±0.17[a] *(14.49)* |
| **V** | **BPA-Medium dose** (120 mg/kg bodyweight/day) | 3.13±0.09[a] *(55.44)* | 14.04±0.45[a] *(32.26)* | 1.02±0.05[a] *(49.45)* | 1.10±0.06[a] *(42.85)* | 33.12±0.50[a] *(33.56)* | 4.70±0.27[a] *(33.76)* |
| **VI** | **BPA-High dose** (240 mg/kg bodyweight/day) | 3.69±0.13[a] *(83.13)* | 10.67±0.38[a] *(48.51)* | 0.60±0.02[a] *(70.31)* | 0.70±0.05[a] *(63.63)* | 25.54±0.84[a] *(48.77)* | 3.15±0.22[a] *(55.64)* |
| **HD BPA(240 mg/kg body weight) + quercetin treated groups** | | | | | | | |
| **VII** | **BPA-HD + quercetin** (30 mg/kg body weight/day) | 2.93±0.13[b] **(41.86)** | 13.53±0.52[b] **(30.45)** | 1.07±0.03[b] **(32.79)** | 1.01±0.05[b] **(24.92)** | 35.84±0.93[b] **(42.37)** | 4.11±0.18[b] **(24.35)** |
| **VIII** | **BPA-HD + quercetin** (60 mg/kg body weight/day) | 2.59±0.07[b] **(63.37)** | 15.84±0.61[b] **(55.03)** | 1.44±0.09[b] **(59.23)** | 1.43±0.04[b] **(59.38)** | 40.06±0.49[b] **(59.73)** | 5.44±0.23[b] **(58.17)** |
| **IX** | **BPA-HD + quercetin** (90 mg/kg body weight/day) | 2.23±0.11[b] **(86.29)** | 18.54±0.69[b] **(88.74)** | 1.85±0.16[b] **(87.79)** | 1.81±0.04[b] **(89.86)** | 47.88±0.76[b] **(91.88)** | 6.67±0.16[b] **(89.14)** |

Values are mean ± S.E.M., n=10

Values shown in parenthesis indicate:

Italics- Percent change in BPA-treated from vehicle control

Bold- Organoprotective index from BPA-HD

Level of significance; *a*p<0.05 as compared to vehicle control

*b*p<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 cells 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).

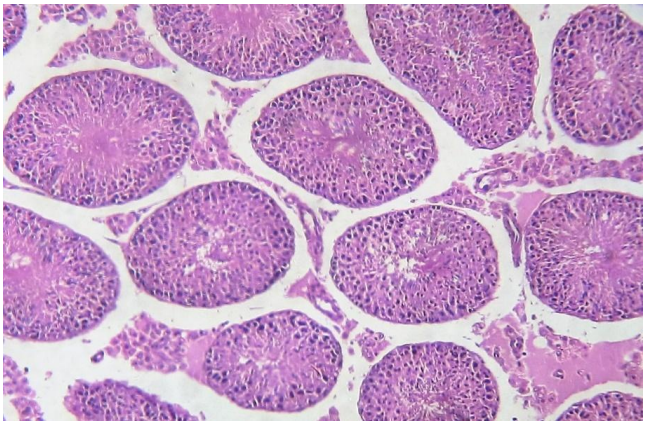

**Figure 1** T.S. of testis of untreated control mice showing typical seminiferous tubules with various stages of spermatogenic cells and sperm bundles in the lumen. The Leydig cells are observed normal in the inter tubularinterstitium with distinct nucleus (H & E staining, X225)

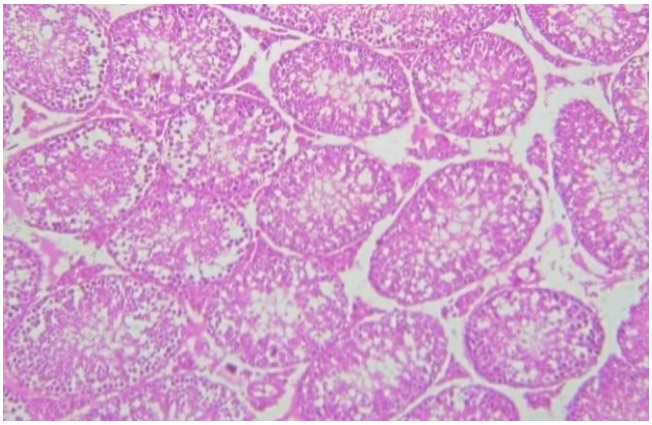

**Figure 2** T.S. of testis of BPA-HD-treated mice showing pyknosis and highly degenerative seminiferous tubules, depletion of spermatogenic cells, lumen with devoid of sperms and degeneration of Leydig cells (H & E staining, X225)

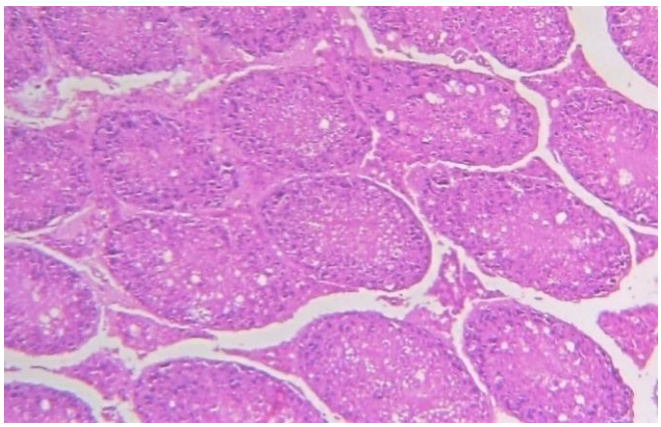

**Figure 3** T.S. of testis of HD BPA+ HD Quercetin mice showing normal histoarchitecture as mentioned above (H & E staining, X225)

## 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 Chiou [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 •− 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.

# 6. Acknowledgement

The authors are grateful to the University Grants Commission (UGC), (RGNF) New Delhi for Financial assistance. We are also indebted to the Department of zoology, school of Sciences, Gujarat University, India, for providing the space and facilities to complete the above Research work. The authors are grateful in this regard.

# 7. Conflict of interest

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

# Reference

1. Brunetti C, Di Ferdinando M, Fini A, Pollastri S, Tattini M. Flavonoids as antioxidants and developmental regulators: relative significance in plants and humans. ***International journal of molecular sciences***. 2013, 14(2):3540-55
2. Wanyo P, Meeso N, Siriamornpun S. Effects of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk. ***Food chemistry***. 2014, 157:457-63

3. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ***The Scientific World Journal***. 2013, 162750, doi:10.1155/2013/162750
4. Manach C, Mazur A, &Scalbert A. Polyphenols and prevention of cardiovascular diseases. ***Current Opinion in Lipidology***.2005, 16:77-84
5. Wang W, Sun C, Mao L, Ma P, Liu F, Yang J, Gao Y. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. ***Trends in Food Science & Technology***. 2016, 56:21-38
6. Mariee AD, Abd-Allah GM, El-Beshbishy HA. Protective effect of dietary flavonoid quercetin againstlipemic-oxidative hepatic injury in hypercholesterolemic rats. ***Pharmaceutical Biology***. 2012, 50 (8):1019-1025
7. Harwood M, Danielewska-Nikiel B, Borzelleca J, Flamm G, Williams G, Lines T. A critical review ofthe data related to the safety of quercetin and lack of evidence of in vivo toxicity, includinglack of genotoxic/carcinogenic properties. ***Food and Chemical Toxicology***. 2007, 45(11):2179-2205
8. Yetuk G, Pandir D, Bas H. Protective role of catechin and quercetin in sodium benzoate-induced lipid peroxidation and the antioxidant system in human erythrocytes in vitro. ***The Scientific World Journal***. 2014, 874824. doi: 10.1155/2014/874824
9. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. ***Pharmacological reviews***. 2000, 52(4):673-751
10. Erlund I. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. ***Nutrition research***. 2004, 24(10):851-74
11. D'Cruz SC, Jubendradass R, Jayakanthan M, Rani SJ. andMathur PP. Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: an *in vivo* and in silico study. ***Food Chemical Toxicol***. 2012, 50:1124-33
12. Zota AR, Phillips C, Mitro SD. Recent fast food consumption and Bisphenol A and phthalates exposures among the US population in NHANES, 2003-2010. ***Environmental health perspectives***. 2016, 124(10):1521-1528
13. Manoli E, Voutsa D. Food Containers and Packaging Materials as Possible Source of Hazardous Chemicals to Food. ***Springer***, Berlin, Heidelberg, 2016
14. Chen D, Kannan K, Tan H, Zheng Z, Feng YL, Wu Y, Widelka M. Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity A Review. ***Environmental science & technology***. 2016, 50(11):5438-53
15. Di Donato M, Cernera G, Giovannelli P, Galasso G, Bilancio A, Migliaccio A, Castoria G. Recent advances on bisphenol-A and endocrine disruptor effects on human prostate cancer. ***Molecular and Cellular Endocrinology***. 2017, 457:35-42
16. Giulivo M, de Alda ML, Capri E, Barcel ó D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. ***Environmental Research***. 2016, 151:251-64
17. Seachrist DD, Bonk KW, Ho SM, Prins GS, Soto AM, Keri RA. A review of the carcinogenic potential of bisphenol A. ***Reproductive Toxicology***. 2016, 59:167-82
18. M ńguez-Alarc ón L, Hauser R, Gaskins AJ. Effects of bisphenol A on male and couple reproductive health: a review. ***Fertility and Sterility***. 2016, 106(4):864-70
19. Karim Z, and Husain Q. Application of fly ash adsorbed peroxidase for the removal of bisphenol A in batch process and continuous reactor: Assessment of genotoxicity of its product. ***Food and Chemical Toxicology***. 2010, 48(12):3385-3390

20. Ejaredar M, Lee Y, Roberts DJ, Sauve R, Dewey D. Bisphenol A exposure and children's behavior: A systematic review. ***Journal of exposure science and environmental epidemiology***. 2017, 27(2):175-83
21. Hussein RM, Eid JI. Pathological mechanisms of liver injury caused by oral administration of bisphenol A. ***Life Science Journal***. 2013, 10(1):663
22. Sangai NP, Verma RJ, Trivedi MH. Testing the efficacy of quercetin in mitigating bisphenol A toxicity in liver and kidney of mice. ***Toxicol. Ind. Health***. 2014, 30:581-597
23. Ohkawa H, Ohishi N. and yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. ***Anal. Biochem***.1979, 95: 351-58
24. Luck H. Catalase. In Methods of Enzymatic Analysis. Edited by H. U. Bergmeyer. New York: ***Academic Press.*** 1963, 1:885
25. Kakkar P, Das B. and Vishwanathan PN. A modified spectrometric assay of superoxide dismutase. ***Indian J. Biochem. Biophys***.1984, 21:130-132
26. Pagila DE. and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte peroxidase. ***J. Lab. Cli. Med***. 1976, 70:158-169
27. Grunert RR and Philips PH. A modification of the nitroprusside method of analysis for glutathione. Arch. ***Biochem***.1951, 30: 247-225
28. Roe JH. and Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem.1943, 147:399-407
29. Parker RM.Testing for reproductive toxicity. In: Developmental and Reproductive Toxicology. Hood, R. D. (ed.) ***Taylor & Francis***, Boca Raton, 2006, 425-488
30. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, AlOlayan EM. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. ***Oxidative medicine and cellular longevity***. 2012, 194829. doi: 10.1155/2012/194829
31. Kovacic P. How safe is bisphenol A? Fundamentals of toxicity: metabolism, electron transfer and oxidative stress. ***Med Hypotheses***. 2010, 75(1):1-4
32. Suthar H. and Verma RJ. Bisphenol A induces hepatotoxicity through oxidative stress in mice. ***International journal of advanced life sciences***. 2014, 194829. doi: 10.1155/2012/194829
33. Ullah H, Ambreen A, Ahsan N, Jahan S. Bisphenol S induces oxidative stress and DNA damage in rat spermatozoa in vitro and disrupts daily sperm production in vivo. ***Toxicological & Environmental Chemistry***. 2017, 99:1-3
34. Tiwari D, Vanage G. Bisphenol A Induces Oxidative Stress in Bone Marrow Cells, Lymphocytes, and Reproductive Organs of Holtzman Rats. ***International Journal of Toxicology***. 2017, 36(2):142-52
35. Wahbby MM, Abdallah ZM, Abdou HM, Yousef MI, Newairy AS. Mitigating potential of Ginkgo biloba extract and melatonin against hepatic and nephrotoxicity induced by Bisphenol A in male rats. ***Egyptian Journal of Basic and Applied Sciences***. 2017, 4(4): 350-357
36. Korkmaz A, Ahbab MA, Kolankaya D. and Barlas N. Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. ***Food and Chemical Toxicology***. 2010, 48(10): 2865-2871
37. Manfo FP, Jubendradass R, Nantia EA, Moundipa PF, MathurPP.Adverse effects of bisphenol A on male reproductive function.***Rev Environ Contam Toxicol***. 2014, 228:57-82
38. Samova S, Doctor H. and Verma RJ. Spermatotoxic effect of bisphenol A and its amelioration using quercetin. ***World Journal of Pharmacy and Pharmaceutical Sciences***. 2016, 5(5):1161-1175

39. Chiou TJ, Chu ST, Tzeng WF, Huang YC and Liao CJ. Arsenic trioxide impairs spermatogenesis via reducing gene expression levels in testosterone synthesis pathway. ***Chem. Res. Toxicol***. 2008, 8: 1562-1569
40. Williams C, Bondesson M, Krementsov DN, Teuscher C. Gestational bisphenol A exposure and testis development. ***Endocrine disruptors***. 2014, 2(1):e29088.
41. Jakhar R, Paul S, Park YR, Han J, Kang SC. 3, 5, 7, 3′, 4′-Pentamethoxyflavone, a quercetin derivative protects DNA from oxidative challenges: Potential mechanism of action. ***Journal of Photochemistry and Photobiology B: Biology***. 2014, 131:96-103
42. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. ***Food and Chemical Toxicology***. 2013, 51:15-25
43. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. ***Fitoterapia***. 2011, 82(4):513-23
44. Çelik N, Vurmaz A, Kahraman A. Protective effect of quercetin on homocysteine-induced oxidative stress. ***Nutrition***. 2017, 33:291-6
45. Jahadi M, Khosravi-Darani K. Liposomal Encapsulation Enzymes: From Medical Applications to Kinetic Characteristics. ***Mini reviews in medicinal chemistry***. 2017, 17(4):366-70
46. Ali AH. The effect of brucellosis on lipid Profile and oxidant-antioxidants status. ***Iraqi Journal of Pharmaceutical Sciences***. 2017, 18(Suppl.):26-31
47. Mekki BE, Hussien HA, Salem H. Role of glutathione, ascorbic acid and α-tocopherol in alleviation of drought stress in cotton plants. ***International Journal of ChemTech Research***. 2016, 8(4):1573-81
48. Forman HJ. Glutathione–From antioxidant to post-translational modifier. ***Archives of biochemistry and biophysics***. 2016, 595:64-7
49. Masisi K, Beta T, Moghadasian MH. Antioxidant properties of diverse cereal grains: A review on in vitro and in vivo studies. ***Food chemistry***. 2016, 196:90-7
50. Boatright WL. Oxygen dependency of one-electron reactions generating ascorbate radicals and hydrogen peroxide from ascorbic acid. ***Food chemistry***. 2016, 196:1361-7
51. Vandamme EJ, Revuelta JL. Industrial Biotechnology of Vitamins, Biopigments, and Antioxidants. ***John Wiley & Sons***, 2016
52. Foyer CH, Trebst A, Noctor G. Protective and signalling functions of ascorbate, glutathione and tocopherol in chloroplasts. ***Advances in photosynthesis and respiration: photoprotection, photoinhibition, gene regulation, and environment***. 2005, 19:241-68
53. Munne-Bosch S, Pinto-Marijuan M. Free Radicals, Oxidative Stress and Antioxidants. ***Encyclopedia of Applied Plant Sciences***. 2016, 2(H2O2):16
54. Lee ES, Lee HE, Shin JY, Yoon S, Moon JO. The flavonoid quercetin inhibits dimethylnitrosamine-induced liver damage in rats. ***J. Pharm. Pharmacol***. 2003, 55:1169-1174