

Testicular toxicity of orally administrated bisphenol A in rats and protective role of taurine and curcumin

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Abstract: Bisphenol A (BPA) is an endocrine disrupting chemical widely used in the world. Curcumin, the yellow bioactive compound of turmeric has demonstrated its antioxidant activities. Taurine is a low-molecular weight organic compound in living organisms. The present study was aimed to investigate the adverse effects of BPA and its protection by taurine and curcumin. Oral BPA, curcumin and taurine administration in adult male rats at 130mg/kg bw, 100mg/kg bw and 100mg/kg bw, respectively for four weeks. Pathology and oxidative damages were investigated. The results show that BPA increased malondialdehyde (MDA) levels and decreased antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)] in testes of rats compared to the control group. Co-treatment with curcumin or taurine with BPA led to reduce in MDA and increased GPx, GST, CAT, SOD activities compared to BPA group. Furthermore, while some pathological findings were observed in testis tissues in BPA treated group, less histopathological findings were shown in BPA plus curcumin and/or taurine treated groups. Consequently, curcumin and taurine significantly protect BPA induced testicular damage in rats.

Keywords: Bisphenol A, taurine, curcumin, oxidative stress, histopathology.

INTRODUCTION

Bisphenol A (BPA) [2,2-(4,4-dihydroxydiphenol) propane] is a chemical that is generally used in many product of the environment (Takahashi and Oishi, 2003; Chitra *et al.*, 2003). BPA epoxy resins are used as coating for food and beverages. However, because food and beverages account generally for human oral exposure, the most important source of BPA is human exposure (Santamaria *et al.*, 2015). Moreover, BPA exposure induces alterations in the brain cells (Kubo *et al.* 2003), behavioral effect (Farabollini *et al.*, 2002), hepatotoxicity (Eid *et al.*, 2015), and thyroid disorders. It was reported that BPA induced such as estrogen by inducing prolactin increase and also it acts like a hormone, changing cellular functions. There is evidence that, similar to other estrogens, BPA can bind to androgen receptors and causes inhibition of androgen action (Richter *et al.*, 2007; Gonzales-Parra *et al.*, 2013).

Oxidative stress consists of with the increasing of reactive oxygen species (ROS) can cause to the induced of a lot of histopathological damages (Apaydin *et al.*, 2016). Antioxidants have also been performed for their ability to overcome oxidative stress in the testes created by several mechanisms (Apaydin *et al.*, 2015). Nowadays, the use of herbal antioxidants is very important in the treatment of various diseases. It is reported that has been related to negative effects of oxidative damages are related to use dietary antioxidants like vitamins, flavonoids and polyphenols (Durak *et al.*, 2010; Kalender *et al.*, 2015).

Supplementation of antioxidant compounds could be a promising therapeutic effect in protecting the BPA induced testicular pathophysiology. For this reason, taurine (2-aminoethanesulfonic acid) might be useful. Curcumin extracted from the *Curcuma longa* rhizome is the active component of dietary spice turmeric. Curcumin is scavenger of free radicals and also shows on the antioxidant effects (Chandra *et al.*, 2007; Ghosh *et al.*, 2015). Previous studies showed that curcumin has been inhibitor role to the proliferation of several tumor cell types, anti-fungal and anti-viral effects (Joe *et al.*, 2004). Taurine (2-aminoethane sulphonic acid) is the cell free beta-amino acid in humans and animals (Das *et al.*, 2009). In addition, it may act as an antioxidant via prevents lipid per oxidation in many tissues (Aly and Khafagy, 2014). It is reported that taurine has various physiological functions including bile acid conjugation, pharmacological actions, and pathological states (Lallemand and De Witte, 2004). Some studies demonstrate beneficial effects of curcumin and taurine on testicular damage but not BPA-induced (Khorsandi *et al.*, 2013; Wei *et al.*, 2007). The present objective was to investigate the possible adverse effects of BPA-induced testicular damage of rats in terms of oxidative stress and histopathological changes and to investigate the therapeutic effects of curcumin and taurine.

MATERIALS AND METHODS

Animals and test chemicals

Male Wistar rats (sexually mature and weighing approximately 250-300g) were used after ten days of quarantine in the animal house conditions (12h lighting

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cycle and $20\pm 2^{\circ}\text{C}$ temperature, 40% humidity) and fed standard rodent cow and water *ad libitum*. Experimental studies were confirmed by University of Gazi Animal Ethics Committee (G.U.ET-14.075). Bisphenol A, curcumin and taurine were used with 99% purity.

Experimental design

The experimental animals were divided into two groups; the control (n=12) and experimental groups (n=30). The rats in the control were divided into two groups, namely, the distilled water group (treated with 1mg/kg bw distilled water per day) (n=6), the olive oil group (treated with 1 mg/kg bw olive oil per day) (n=6). The rats at the experimental groups were separate into 5 groups, namely, the taurine-treated group (100mg/kg bw taurine dissolved in distilled water) (n=6), the curcumin-treated group (100mg/kg bw curcumin dissolved in olive oil) (n=6), the bisphenol A-treated group (130mg/kg bw 1/25 LD₅₀ BPA dissolved in olive oil) (n=6), bisphenol A plus taurine-treated group (130mg/kg bw BPA and 100 mg/kg bw taurine, respectively) (n=6) and bisphenol A plus curcumin (130mg/kg bw BPA and 100mg/kg b.w curcumin, respectively) (n=6) treated group. All chemicals were exposed to rats orally via gavage in the morning (between 09:00-10:00 hrs.). Finally of subacute exposure, the rats were dissected using xylazin and ketamin the testes were removed for investigations for analysis of enzymatic activities, lipid peroxidation levels and microscopic histopathological examinations.

Assessment of testes oxidative stress parameters and statistical analysis

The testes were removed and storage -80 deep freezer, then tissues were homogenized (Heidolph Silent Crusher M). MDA levels and SOD, CAT, GPx, GST activities were investigated by detecting the levels of the homogenates with spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). Tissue protein level was analyzed (Lowry *et al.*, 1951).

MDA levels were analyzed at 532 nm to detect the MDA content (Ohkawa *et al.*, 1979). MDA was shown as nmol/mg protein of protein at 440nm (Marklund and Marklund, 1974) for 3min. The activity of SOD was determined as U/mg protein at 240nm (Aebi, 1984). Activity of GPx was measured using H₂O₂ and it was shown as nmol/mg protein (Paglia and Valentine, 1967). The activity of GST was shown as $\mu\text{mol/mg}$ protein (Habig *et al.*, 1974). The data was analyzed by using SPSS 13.0 for Windows. Evaluation calculated by ANOVA test based on Tukey. $P<0.05$ was accepted as statistically significant.

Histopathological examination

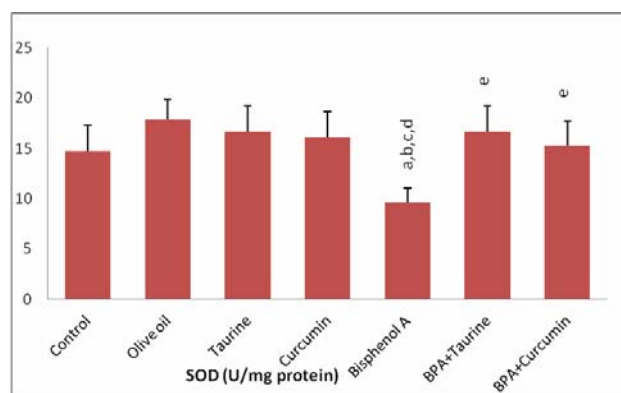
For light microscopy, testicular specimens were fixed in 10% neutral formalin and processed for paraffin sections

of 6-7 μ thickness. Sections were stained with hematoxylin and eosin for routine histological investigations.

RESULTS

Effects on oxidative stress parameters

There were no important changes between the control, olive oil, curcumin and taurine treated groups. When the BPA treated group was compared with the control group at the end of the study, there was an important increase in the MDA levels in the testis tissues. Treatment with Curcumin or taurine plus BPA caused a significant decrease in the MDA levels.



^aComparison of control and other groups.

^bComparison of olive oil-treated group and other groups.

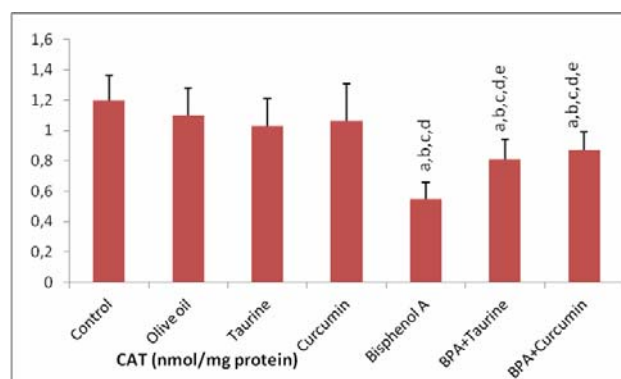
^cComparison of taurine-treated group and other groups.

^dComparison of curcumin-treated group and other groups.

^eComparison of BPA-treated group and other groups.

Values are mean $\pm$ SD of six rats in each group. Significance at $P<0.05$.

Fig. 1: Effects of treatment of BPA on SOD content in the testicular tissues of rats.



^aComparison of control and other groups.

^bComparison of olive oil-treated group and other groups.

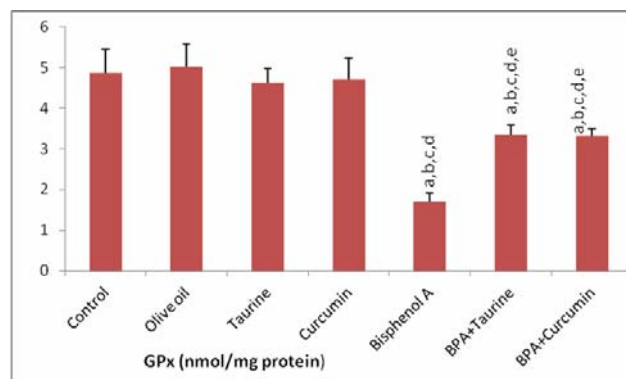
^cComparison of taurine-treated group and other groups.

^dComparison of curcumin-treated group and other groups.

^eComparison of BPA-treated group and other groups.

Values are mean $\pm$ SD of six rats in each group. Significance at $P<0.05$.

Fig. 2: Effects of treatment of BPA on CAT content in the testicular tissues of rats.



^aComparison of control and other groups.

^bComparison of olive oil-treated group and other groups.

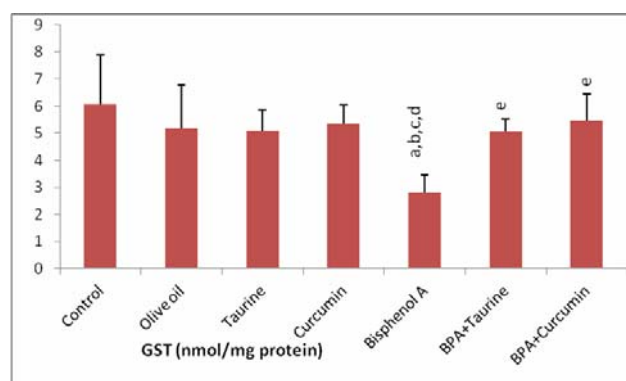
^cComparison of taurine-treated group and other groups.

^dComparison of curcumin-treated group and other groups.

^eComparison of BPA-treated group and other groups.

Values are mean $\pm$ SD of six rats in each group. Significance at $P < 0.05$.

Fig. 3: Effects of treatment of BPA on GPx content in the testicular tissues of rats.



^aComparison of control and other groups.

^bComparison of olive oil-treated group and other groups.

^cComparison of taurine-treated group and other groups.

^dComparison of curcumin-treated group and other groups.

^eComparison of BPA-treated group and other groups.

Values are mean $\pm$ SD of six rats in each group. Significance at $P < 0.05$.

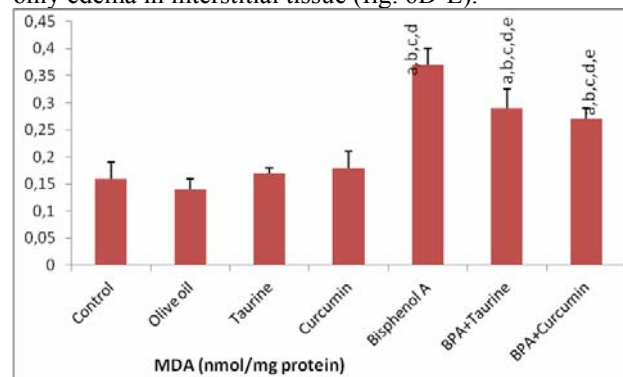
Fig. 4: Effects of treatment of BPA on GST content in the testicular tissues of rats.

Treatment with BPA statistically decreased the enzyme (GPx, GST, SOD and CAT) levels compared with the control groups in testes. However, BPA plus curcumin and BPA plus taurine treated groups showed that a significant increase shown in antioxidant enzyme levels than the BPA treated group.

Effects on histopathological changes of testicular tissues

The histopathological findings observed in testicular sections from the control and other groups are presented in fig. 6. Both distilled water control and olive oil control groups showed normal histological appearance. Furthermore, no pathological changes were seen in

taurine and curcumin treated rat testis tissues similar as control rats (fig. 6A). Rats treated with BPA alone exhibited necrosis, edema in interstitial tissues and undulation in seminiferous tubules (Fig 6B-C). BPA plus curcumin and BPA plus taurine treated groups shown that only edema in interstitial tissue (fig. 6D-E).



^aComparison of control and other groups.

^bComparison of olive oil-treated group and other groups.

^cComparison of taurine-treated group and other groups.

^dComparison of curcumin-treated group and other groups.

^eComparison of BPA-treated group and other groups.

Values are mean $\pm$ SD of six rats in each group. Significance at $P < 0.05$.

Fig. 5: Effects of treatment of BPA on MDA content in the testicular tissues of rats.

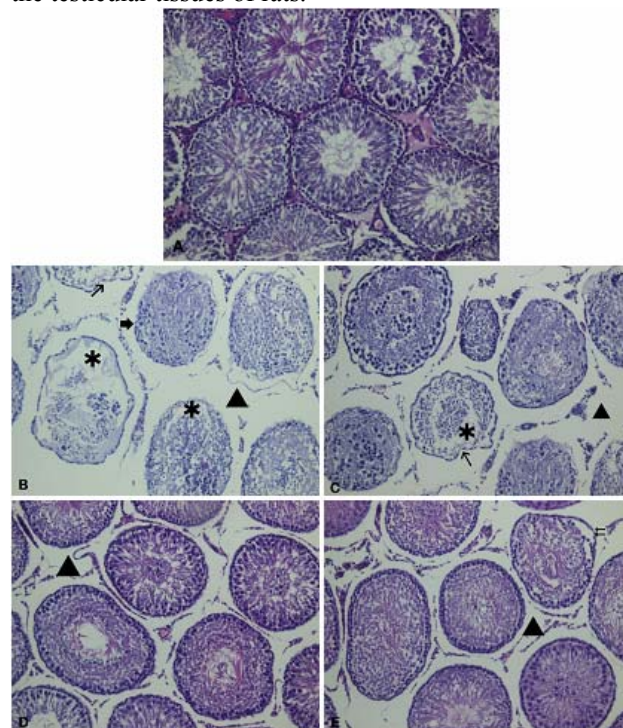


Fig. 6: (A) Testicular section of control rats showing seminiferous tubules and interstitial tissue. (B-C) Testicular sections of BPA-treated rats showing necrosis (*) in seminiferous tubules, edema in interstitial tissues (▲) and undulation in seminiferous tubules (→) x200. Testicular sections of BPA plus taurine treated rats and BPA plus curcumin treated rats showing (D-E) edema (▲) in interstitial tissues x200.

DISCUSSION

It is well known that BPA is an endocrine disrupter chemical which is commonly found in various products including plastics, water-pipes, thermal paper, electronic equipment, toys and the coating of food and drinks such as bottles (Michalowicz, 2014). Although, many authors have shown several defective effects of BPA, the current study is the first comprehensive study revealing the ameliorative effects of curcumin and taurine against toxicity by BPA.

Takahashi and Oishi (2003), reported that dietary administrated BPA is less toxic than subcutaneous or intraperitoneal injection in male reproductive and genital accessory organs. The oral LD₅₀ dose of BPA for rats is 3250 mg/kg body weight (Michalowicz, 2014). In current study, CPF was given at 1/25 of oral LD₅₀.

BPA is known as a xenoestrogen compound which has negative effect on the reproductive organs (Chitra *et al.*, 2003; Santamaria *et al.*, 2015). Similarly, we have found that treatment with BPA causes various histopathological alterations in testis tissues such as necrosis, edema, and undulation. It is reported that exposure of BPA causes cell damage to human erythrocytes which may be due to the oxidative stress (Hassan *et al.*, 2012). These pathological alterations observed in this study in BPA treated groups are probably due to increased ROS production leading to oxidative stress. Various xenobiotics have also been cause to cellular damage in the testis tissues related to inhibition of antioxidant mechanism (Dirican and Kalender, 2012, Apaydin *et al.*, 2015). Oxidative stress is an important disorder caused by oxygen radicals and cell antioxidant enzymes (Halliwell, 2011). The cells have various defense mechanisms against oxidative stress, including enzymatic antioxidants (SOD, CAT, GPx, and GST) that protect the cell and organs from negative effects of ROS (Kalender *et al.*, 2013; Uzun *et al.*, 2013). Antioxidant enzymes such as SOD, CAT, GPx and GST are the important major line of defense against cellular injury. SOD is an antioxidant enzyme in the cell defense system while CAT and GPx catalyze the toxic H₂O₂ to water and oxygen (Boujbiha *et al.*, 2011; Demir *et al.*, 2011). ROS are supposed to play one of the key roles in the development of testis toxicity. Aly and Khafagy, (2014) reported that production of ROS may cause impaired spermatogenesis and steroidogenesis, and also it associated with male infertility. MDA is the end product of per oxidized polyunsaturated fatty acids. So, if MDA level is increased, lipid peroxidation will be enhanced in cell (Kalender *et al.*, 2015). According to previous investigators, BPA increased MDA levels and also alterations in antioxidant enzyme activities in several rat tissues (Eid *et al.*, 2015; Mahmoudi *et al.*, 2015). According to this study the activities of CAT, SOD, GPx, and GST were significantly decreased in testis in BPA

treated rats compared to the control rats. These adverse effects are probably due to its estrogenic properties. The results of biochemical parameters support the results of histopathological findings in this study.

Curcumin not only shows anti-oxidative and free radical scavenging properties, but also induces the activities of other antioxidants, such as superoxide dismutase, catalase and glutathione per oxidase (Joe *et al.*, 2004). Curcumin inhibited lipid per oxidation of cell membrane (Ghosh *et al.*, 2015). Yang *et al.*, (2010) demonstrated that taurine elevates testicular anti-oxidation and improve sperm quality in their study. They also mentioned about taurine can be biosynthesized by male reproductive organs.

CONCLUSIONS

In conclusion, our results demonstrated that the adverse effects of BPA on rat testes may be due to increase of oxidative stress in cells. In this study, we found that the taurine and curcumin treatment with BPA-treated groups, shown that protective effects. These preventive effects possibly are due to their antioxidant properties on testes.

Besides, during our experimental period supplementation with taurine and curcumin partially protected the adverse effects of BPA on testes.

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