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Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female rat offspring

Jehane I. Eid [a,b], Shaymaa M. Eissa [a,*], Akmal A. El-Ghor [a]

[a] *Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt*
[b] *Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Makkah Al-Mukarramah, Kingdom of Saudi Arabia*

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Abstract Bisphenol A (BPA) is an endocrine disrupting compound widely spread in our living environment. It is a contaminant with increasing exposure to it and exerts both toxic and estrogenic effects on mammalian cells. Due to the limited information concerning the effect of BPA on the liver, the present study was designed to assess hepatic tissue injury induced by early life exposure to BPA in female rat offspring. Rat dams (n = 9) were gavaged with 0.5 and 50 mg of BPA/kg b.w./day throughout lactation until weaning. The sham group received olive oil for the same duration while the control group did not receive any injection. The liver tissue was collected from female pups at different pubertal periods (PND50, 90 and 110) to evaluate oxidative stress biomarkers, extent of DNA damage and histopathological changes. Our results indicated that early life exposure to BPA significantly increased oxidative/nitrosative stress, decreased antioxidant enzyme activities, induced DNA damage and chronic severe inflammation in the hepatic tissue in a time dependent manner. These data suggested that BPA causes long-term adverse effects on the liver, which leads to deleterious effects in the liver of female rat offspring.

Introduction

Bisphenol A (BPA) is an environmental chemical that has been widely used in the manufacture of polycarbonate plastics and epoxy resins for many years. Due to its major applications in the production of plastic food or beverage containers and the coating of food cans, people of different ages are inevitably exposed to BPA in daily life. BPA has been detected in the human placenta (Schonfelder et al., 2002), cord blood (Wan et al., 2010), amitotic fluid (Ikezuki et al., 2002; Yamada et al., 2002), fetal liver (Cao et al., 2012) and breast milk (Sun et al., 2004), making exposure of human neonates and infants a very real concern. According to studies, BPA is estrogen mimic compound resulted in an array of health impacts including prostate and breast cancer (Prins et al., 2008; Pupo et al., 2012). The adverse effects of BPA are largely related to its estrogenic activity (Hiroi et al., 1999; Kurosawa et al., 2002). However, BPA has other effects such as inflammatory cytokine dysregulation (Wetherill et al., 2007; Ben-Jonathan et al., 2009), and mitochondrial mediated apoptosis in the hepatic tissue (Xia et al., 2014).

It was reported that, BPA is a xenoestrogen compound that has adverse health effects on the developing reproductive

* Corresponding author.
E-mail address: shaymaa.doh2011@gmail.com (S.M. Eissa).

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organs, especially if BPA exposure occurred during critical period of development, fetal BPA exposure has been shown to decrease the efficiency of sperm production in male mice offspring (Takahashi and Oishi,2001; Chitra et al., 2003). Recent data of Park et al. showed that BPA increased human ovarian cancer cell proliferation in a dose-dependent manner (Park et al., 2009).

In addition, BPA is capable of inducing toxic effect on non-reproductive vital organs; *several studies* in the literature have *reported* absorption of large amounts of BPA through skin which has been shown to cause extensive damage to the liver and kidney in humans (Suarez et al., 2000). Moreover, it was evidenced that there was a significant relationship between urine concentration of BPA and cardiovascular disorders, type 2 diabetes and liver enzyme abnormalities in a representative sample of US population (Lang et al., 2008).

In the current study, we decided to evaluate the effect of BPA on the hepatic tissue of female rat offspring, as a recent study revealed that low doses of BPA cause oxidative stress in livers of male rats (Bindhumol et al., 2003), and several BPA researches have focused on female reproductive and estrogen target organs, despite the ability of non-reproductive organs, such as the liver, to express estrogen receptors and respond to steroid hormone signaling (Cui et al., 2013). The liver is the primary organ responsible for BPA metabolism in humans and animals (Knaak and Sullivan, 1966). Therefore, it could be largely exposed to BPA, and could be susceptible to lower doses, than other organs (Moon et al., 2012).

Pharmacokinetic data suggested that an adult human is capable of rapid elimination of estrogenic free BPA into none-strogenic BPA glucuronide and BPA sulfate metabolites by the action of hepatic glucuronyl transferase enzyme, however, in human and rodent fetuses and neonates have reduced or altered capacity for BPA detoxification (Hanioka et al., 2008a,b; Nishiyama et al., 2002; Ikezuki et al., 2002). BPA has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes, DNA adduct and induce the production of free radicals in rat hepatocytes in vitro (Nakagawa and Tayama, 2000; Atkinson and Roy, 1995).

Being the main gate of the body, the liver is affected by hazardous substances, exhibiting different degrees of toxicity (Venukumar and Latha, 2002). In chronic liver injury, the injured cells release a number of cytokines and stimulate the kupffer cells to release more inflammatory mediators and various free radicals (Hori et al., 1997). Massive ROS production in the hepatic tissue induce oxidative stress, moreover, oxidative stress can induce many kinds of negative effects including membrane peroxidation, protein cleavage, and DNA strand breakages, which could lead to cancer (Collins and Harrington, 2002; Mittler, 2002).

Animal studies suggest that BPA exposure, even at low doses, several reports estimated a no-observed-adverse-effect-level (NOAEL) for BPA in rats and mice of 5 mg/kg b.w./day (NTP, 1985). Hence, we aimed to evaluate the effect of early life exposure to BPA low dose of 0.5 mg/kg b.w./day below the NOAEL and a high dose of 50 mg/kg b.w./day through maternal milk from postnatal day (PND 1–21) lactation period in the female offspring during three different pubertal periods (PND 50, 90 and 110) by evaluating the parameters of oxidative/nitrosative status (catalytic activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) and measuring levels of malondialdehyde (MDA), nitric oxide (NO) and total antioxidant capacity (TAC)) and the extent of DNA damage in liver homogenate. Finally histological examination of liver sections from all studied groups was performed.

Materials and methods

Chemical reagents

Bisphenol A, agarose, absolute ethanol, formaldehyde, glacial acetic acid, tris -(hydroxymethyl)-amino methane (Tris-base), ethylenediaminetetracetic acid disodium salt (Na2EDTA), triton X-100, ethidium bromide (EtBr) were purchased from Sigma (St. Louis, MO, USA).

BPA doses

Doses of 0.5 and 50 mg/kg b.w. were selected, based on national toxicological report, 1985. The toxicity study was not performed as the doses were reported to be nontoxic in an animal study (SCF, 2002). BPA, a water insoluble powder was dissolved in olive oil, for daily uptake by gastric gavage to rat mothers.

Ethics statement

Animals were treated humanely and with regard to alleviation of suffering for the care and use of laboratory animals, the experimental protocol was approved by the institutional animal care and use committee (IACUC) at the department of zoology faculty of science, Cairo University (CUFS/F/mol/13/14). Sexually mature female rats (120 days old) were bred at the department of zoology (in the animal house). Animals were maintained in a controlled environment (22 ± 2C; 14 h of light from 06:00 h to 20:00 h) and had free access to pellet laboratory chow rat-mouse diet obtained from Nutrition Animal, NRC, Egypt.

Animals and treatments

Adult female rats in proestrus cycle were caged overnight with males of proven fertility. The day on which the sperm was found in the vagina was designated as day 1 of gestation (GD), each pregnant female was isolated in a cage to stay until the day of birth (PND0), injection was started from (PND 1–21) the lactation period, pups were isolated , female offsprings were sacrificed along three different pubertal periods (PND 50, 90 and 110) by cervical dislocation to isolate the liver. We have three groups: control, BPA0.5 and BPA50, in each group we had 9 pregnant female rat.

Histopathological investigation of the hepatic tissue

Parts of liver tissues were fixed in buffered 10% formalin, dehydrated through graded alcohols series and paraffin embedded sections (4 μm) were deparaffinized, rehydrated through graded alcohols, and stained with hematoxylin and eosin, epithelial structures were histologically examined by a high resolution light microscope.

Tissue homogenation and total protein content measurement in the liver

The liver tissue was homogenized (10% w/v) in a phosphate buffer (0.1 M, pH7.4) and then centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was used for investigating total protein, activities of antioxidant enzymes, and markers of oxidative/nitrosative stress and each sample was tested in triplicate.

Total protein content in homogenate, was determined by the biuret method where in the presence of alkaline cupric sulfate the protein produces a violet color the intensity of which is proportional to their content.

Oxidative/nitrosative stress biomarker measurement

MDA levels were estimated by the thiobarbituric acid reaction as described by Ohkawa et al., 1979 using saturated thiobarbituric acid and trichloroacetic acid (20%) solutions.

NO levels were measured based on (Montgomery and Dymock, 1961) method, in acid medium and in the presence of nitrite the formed nitrous acid diazotize sulfanilamide and the product are coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has bright reddish-purple color which can be measured at 540 nm.

Evaluation of antioxidant enzyme activities

CAT activity was measured based on the spectrophotometric method described by (Aebi, 1984). Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

SOD was measured as described by Nishikimi et al., 1972. SOD enzyme had the ability to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye then absorbance was measured at 560 nm for 5 min at 25 °C.

GPx activity was measured based on (Paglia and Valentine, 1967) method. One unit of GPx is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol NADPH per minute at 25 °C.

Evaluation of total antioxidant capacity

TAC was measured as described by Koracevic et al., 2001 method. The principle was based on the reaction of total antioxidants in the sample with defined amount of exogenously provide hydrogen peroxide (H_2O_2), the antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. Then the residual H_2O_2 was determined calorimetrically.

Comet assay

The alkaline comet assay was performed as described by Singh et al. (2003).The liver tissue was homogenized within minced solution then 5–10μ homogenate was dissolved in 0.75% low melting-point agarose and immediately spread onto a glass microscope slide pre-coated with a layer of 1% normal melting-point agarose. The slides were then incubated in ice-cold lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% Triton X-100, and 10% DMSO, pH 10.0) at 4 °C for at least 1 h in order to remove cellular proteins and membranes. Slides were removed from the lysis solution and placed on a horizontal electrophoresis unit. The unit was filled with fresh buffer (300 mM NaOH, 1 mM EDTA, pH13.0), which covered the slides for 20 min at 4 °C in order to allow unwinding of DNA and expression of alkali-labile sites. Electrophoresis was conducted for 20 min at 25 V (74 V/cm). All the steps listed above were performed in the dark. Slides were then neutralized (0.4 M Tris, pH 7.5), fixed in absolute ethanol for 10 min and then slides were left to dry at room temperature, The neutral comet assay was performed at pH 8.5, essentially according to the same procedure as the alkaline version, except at lower pH. In the neutral version, electrophoresis was carried out in a buffer consisting of 100 mM Tris and 300 mM sodium acetate at pH 8.5.

Slides were stained with ethidium bromide and analyzed under a fluorescent microscope. One hundred cells (50 cells from each of two replicate slides) per concentration of each test substance were selected and analyzed visually with an optical microscope for tail length and amount of DNA present in the tail. When selecting cells, the areas around air bubbles or at the edges were avoided (Asahi et al., 2010). The parameters selected for presentation were: (1) the tail length (TL) - has been defined as the distance between the center of the comet head and the last non-zero pixel of the comet profile, (2) percentage of DNA in tail (% DNA) - defined as the intensity of all tail pixels divided by the total intensity of all pixels in the comet, expressed as percentage, (3) tail moment (TM) - equivalent to the % DNA in the comet tail multiplied by the tail length and (4) olive tail moment (OTM) - computed as the summation of each tail intensity integral value, multiplied by its relative distance from the center of the head, the point at which the head integral was mirrored, and divided by the total comet intensity.

Statistical analysis

All results were expressed as mean ± standard error (SE). The results of oxidative stress and DNA damage parameters were analyzed in BPA treated groups and compared with the control using a two-way ANOVA and Bonferroni post test (at significance level $p < 0.05$) using the GraphPad Prism 5 (GraphPad Software, USA).

Results

Histopathological changes in the hepatic tissue after BPA exposure

Light microscopic study of control rat liver revealed the characteristic hepatic architecture with the central vein and radiating cords of normal hepatocytes possessing central rounded nuclei and showed no histopathological alteration (Plate 1A). However, hepatic histopathological sections of the rat group administrated to BPA0.5 revealed vacuolar

degeneration of most hepatocytes, the congested hepatoportal vein and focal area of necrosis throughout the investigated periods (Plate 1B–D). In BPA50, liver sections showed widening of the blood sinusoids; vacuolized swelled hepatocytes, massive hepatocytic necrosis and pyknotic nuclei all with the congested hepatic portal vein throughout the pubertal period (Plate 1E–G).

Oxidative/nitrosative stress

MDA and NO concentrations representing oxidative/nitrosative stress were significantly ($p < 0.001$) increased compared to the control, even in BPA (0.5 and 50 mg/kg b.w.) treated groups. Moreover, levels of MDA and NO in BPA50 compared to BPA0.5 were significantly ($p < 0.001$) increased with the time of the three different pubertal periods (Fig. 1).

Antioxidant enzymes activities

The activities of antioxidant enzymes (SOD, CAT, and GPX) in BPA (0.5 and 50 mg/kg b.w.) treated groups were significantly ($p < 0.001$) decreased with the time of the three different pubertal periods. Moreover, the activities of CAT and GPX antioxidant enzymes in BPA50 compared to BPA0.5 showed a significant ($p < 0.001$ and $p < 0.05$) decrease respectively at PND 50 while the activity of SOD antioxidant enzyme in BPA50 compared to BPA0.5 showed a significant ($p < 0.001$) decrease with the time of the three different pubertal periods (Fig. 2).

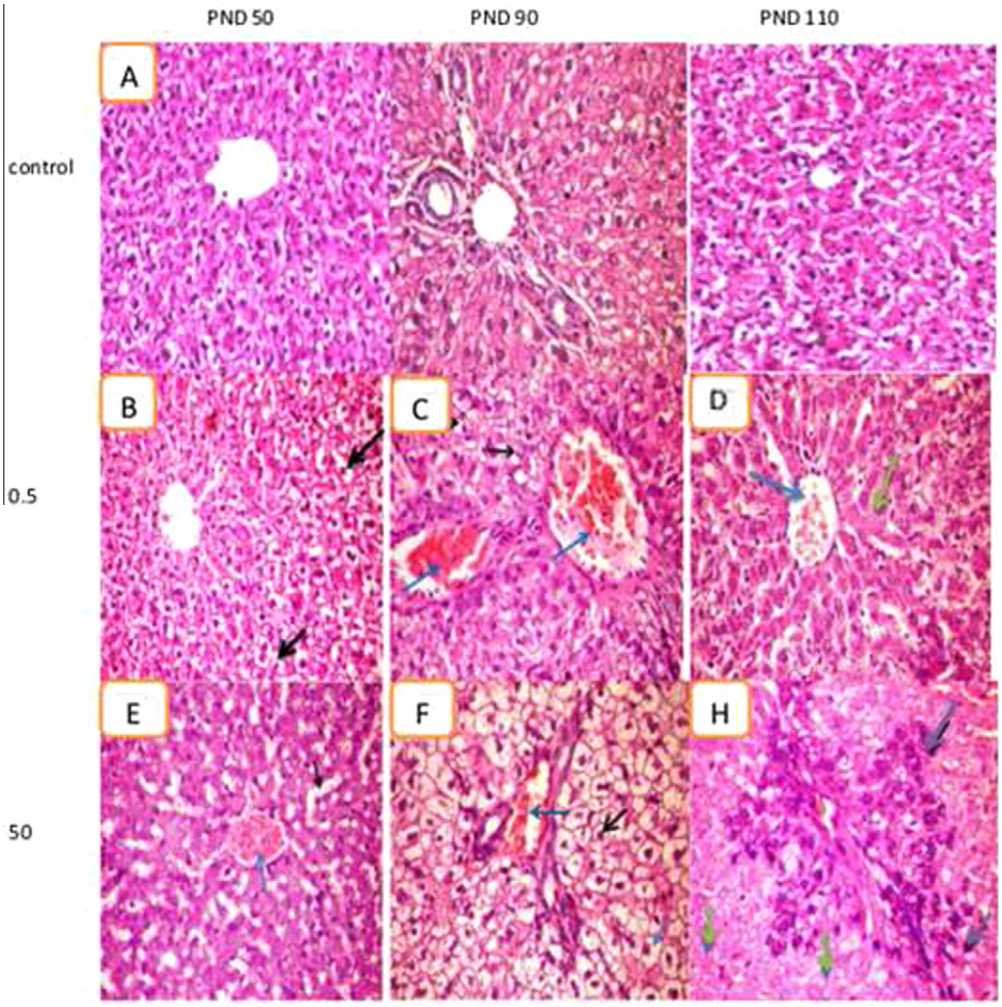

Plate 1 Light photomicrograph of liver sections obtained from female rat offspring showing BPA (0.5 and 50 mg/kg b.w.) treated groups compared to the negative control throughout three different pubertal periods (PND 50, 90 and 110) stained with (H&E) (×200). Liver sections (A) control showing apparently healthy parenchyma, (B) BPA0.5 at PND 50 showing vacuolar degeneration of most hepatocytes (black arrow), (C) BPA0.5 at PND 90 showing congestion of hepatoportal blood vessels (blue arrows) with hepatocyte vacuolation (black arrows), (D) BPA0.5 at PND 110 showing focal areas of necrosis (green arrows), (E) BPA50 at PND 50 showing the congested central vein (blue arrow) and widening of the blood sinusoids (black arrow), (F) BPA50 at PND 90 showing swelled hepatocytes vacuolized (black arrow) together with the congested hepatoportal blood vessel (blue arrow), (H) BPA50 at PND 110 showing massive hepatocytic necrosis (green arrows) with pyknotic nuclei (violet arrows).

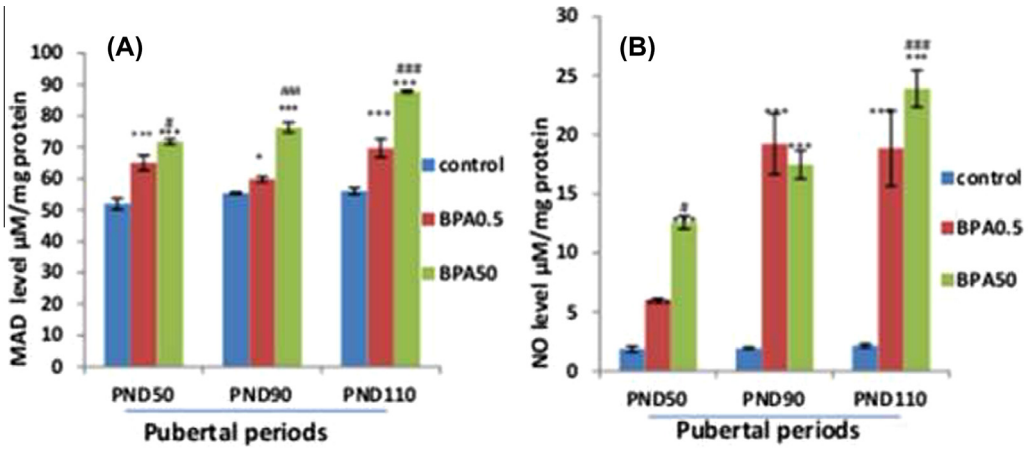

Figure 1 Bar diagram showing the levels of (A) NO and (B) MDA in the liver of female rat offspring treated with BPA (0.5 and 50 mg/kg b.w.) during the lactational period and evaluated at PND 50, 90 and 110. Data represented as mean ± SEM for three animals from each group are shown. *Significant difference between BPA treated groups compared to control at $^{***}P < 0.001$, $^{\#}$significant difference in BPA50 compared to BPA0.5 at $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$.

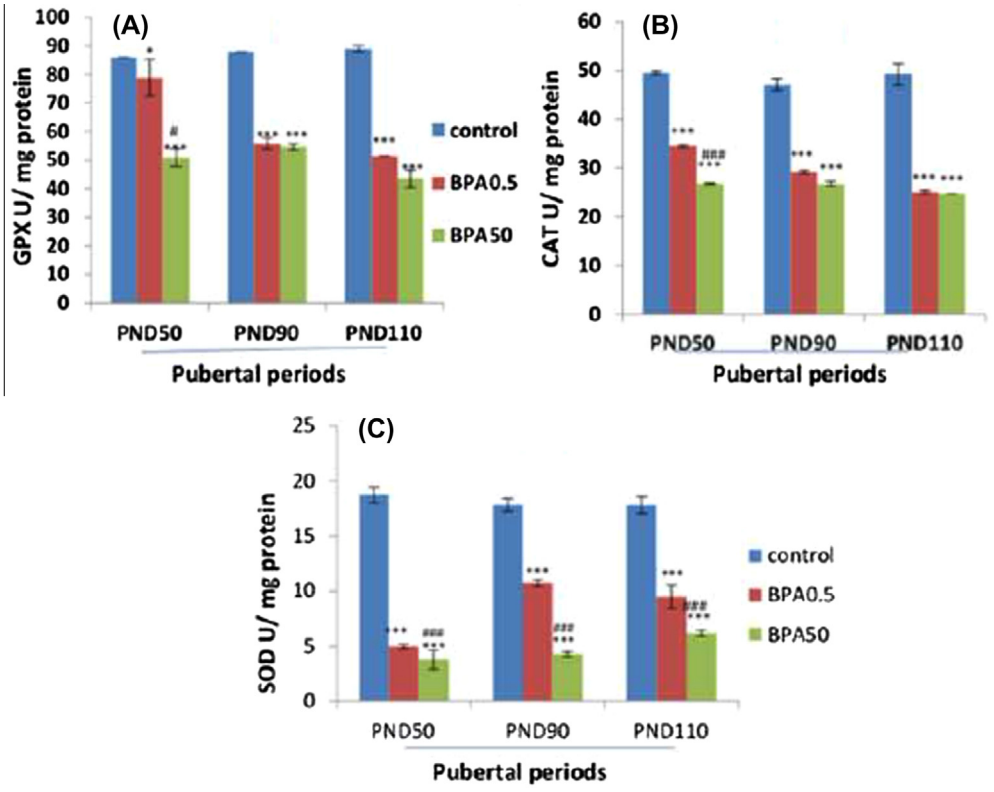

Figure 2 Bar diagram showing the levels of antioxidant enzyme activities (A) GPX, (B) CAT and (C) SOD in the liver of female offspring rats treated with BPA (0.5 and 50 mg/kg b.w.) during the lactation period and evaluated at PND 50, 90 and 110. Data represented as the mean ± SEM for three animals from each group are shown. *Significant difference between BPA treated groups compared to control at $^{***}P < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. $^{\#}$Significant difference in BPA50 compared to BPA0.5 at $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$ (using ANOVA).

Total antioxidant capacity

Levels of total antioxidant capacity in BPA (0.5 and 50 mg/kg b.w.) were significantly ($p < 0.001$) decreased compared to the control in the three different pubertal periods. Moreover, BPA50 compared to BPA0.5 showed a significant decrease in levels of antioxidant capacity in all estimated periods (Fig. 3).

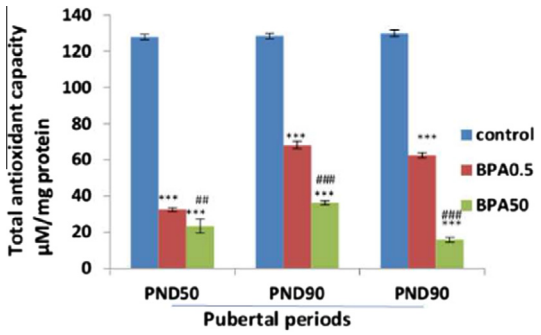

Figure 3 Levels of TAC in the liver of female rat offspring treated with BPA (0.5 and 50 mg/kg b.w.) during the lactation period and evaluated at PND 50, 90 and 110. Data represented as the mean ± SEM for three animals from each group are shown. *Significant difference between BPA treated groups compared to control at $^{***}P < 0.001$, #significant difference in BPA50 compared to BPA0.5 at $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$.

Comet assay

Alkaline comet assay

The exposure to BPA caused an increase in DNA strand breaks leading to greater DNA migration out of the nucleus into the tail of the comet in the hepatic tissue (Plate 2). A quantitative assessment of DNA damage in an individual cell was performed using the comet score.exe (Tri Tek Comet Score). (Fig. 4) represents the results of TL, TM, OTM and % DNA parameters used as a measure of primary DNA damage and compared with the control. A significant increase in DNA damage parameters was observed in the BPA (0.5 and 50 mg/kg b.w.) treated group with the time of the three different pubertal periods while DNA damage parameters in BPA50 compared to BPA0.5 showed a significant increase with the time of the three different pubertal periods.

Neutral comet assay

In neutral comet assay the hepatic tissue showed no significant variation in BPA treated cells than in the control (Plate 3) Moreover, the measured parameters of TL, TM, % DNA and OTM showed no significant variations from the control (data not shown).

Discussion

The current study demonstrated that BPA caused hepatoxicity by decreasing the activities of antioxidant enzymes (SOD, CAT, GPX), elevating levels of NO and MDA and a significant decrease in total antioxidant capacity. Moreover, incidence of severe DNA damage in BPA (0.5 and 50 mg/kg b.w.) treated groups was investigated using alkaline comet assay in the three different pubertal periods. However, in neutral comet assay the hepatic tissue showed no significant variation in BPA treated groups compared to the control. In addition, early life exposure to BPA induces leucocytic cell infiltration, appearance of necrotic areas and other histopathological changes. All these findings suggest that a dose dependent hepatotoxic effect of BPA exposure on female rat offspring.

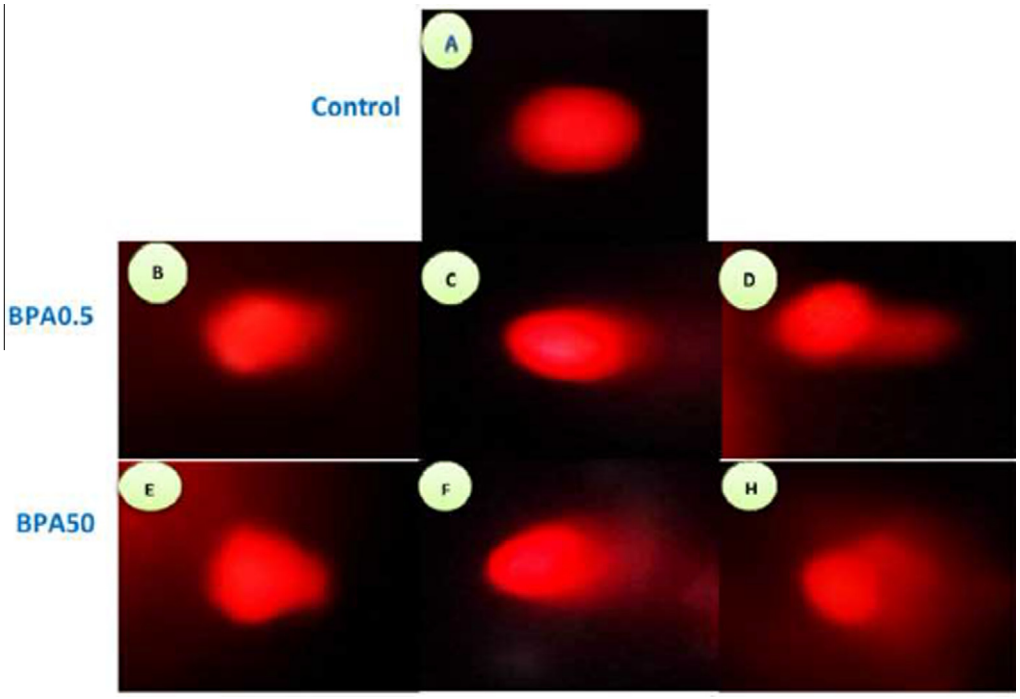

Plate 2 Fluorescent microscope photomicrographs of hepatic cells obtained from female rat offspring showing the toxic effect of BPA (0.5 and 50 mg/kg b.w.) treated groups compared to the negative control throughout the three different pubertal periods (PND50, 90 and 110) demonstrating the extent of DNA damage assessed by alkaline comet assay (A) negative control; (B–D) BPA0.5 treated throughout the different pubertal periods at (B) PND50; (C) PND90; (D) PND110. (E–F) BPA50 treated throughout different pubertal periods at (E) PND50; (F) PND90 and (H) PND110.

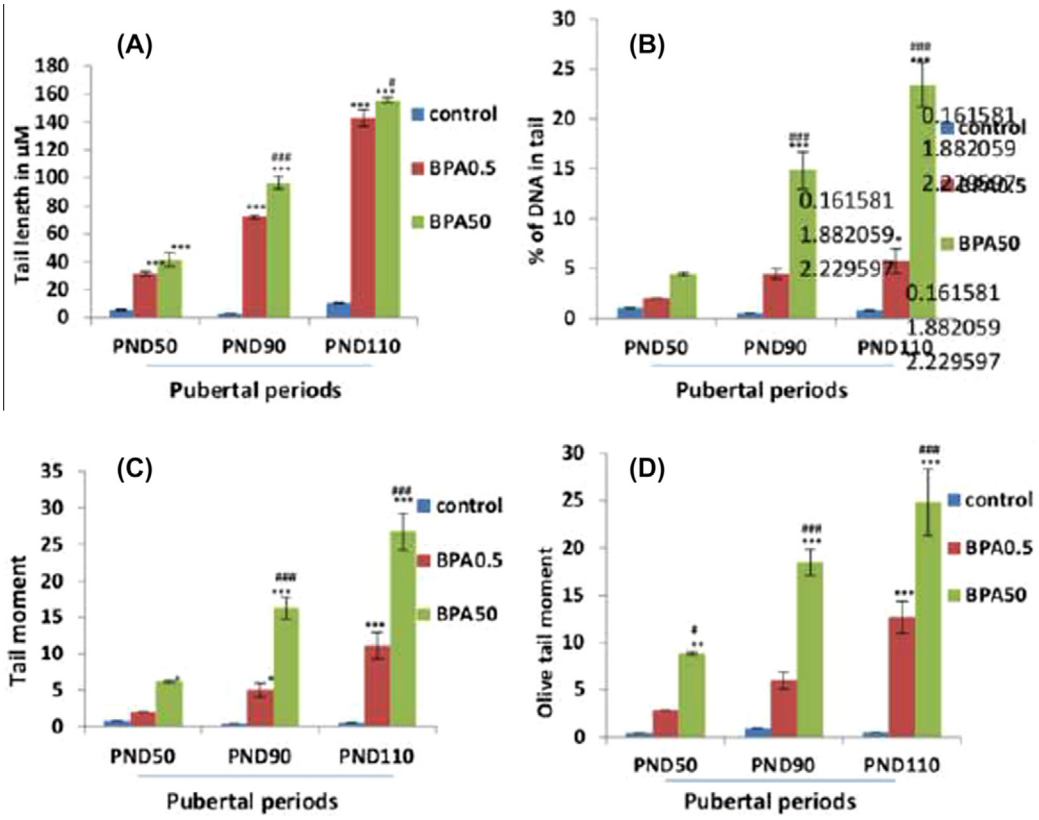

Figure 4 Bar diagrams showing the levels of (A) TL, (B) % DNA, (C) TM and (D) OTM parameters demonstrating the extent of DNA damage in the liver of female rat offspring treated with BPA (0.5 and 50 mg/kg b.w.) during the lactation period and evaluated at PND 50, 90 and 110. Data represented as the mean ± SEM for three animals from each group are shown. *Significant difference between BPA treated groups compared to control at $^{***}P < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. #Significant difference in BPA50 compared to BPA0.5 at $^{\#}P < 0.05$; $^{\#\#\#}p < 0.001$ (using ANOVA).

It was reported that BPA increases the generation of reactive oxygen species (ROS) and induced hepatic damage and mitochondrial dysfunction (Moon et al., 2012; Asahi et al., 2010). In the current study, BPA increased ROS production as assessed by the measurement of the end product of lipid peroxidation, MDA concentration which increased with the time of the three different pubertal periods. These results are in agreement with the previous studies which demonstrated that BPA administration increases MDA levels in the tissue (Kabuto et al., 2003). Moreover, it was evidenced that, MDA could induce hepatotoxicity, inhibit protective enzymes and act as a co-carcinogenic agent (Bauer, 2000).

Nitric oxide (NO) is a highly diffusible free radical. In the present study levels of NO production increased after BPA exposure in the three different pubertal periods. It was demonstrated that, NO reacts with superoxide ($O2^{\cdot-}$) to form peroxynitrite ($ONOO^{-}$) a highly reactive free radical, therefore, NO causes increased nitrosative stress (Grattagliano et al., 1999). Moreover, NO is a potent oxidant and nitrating agent is capable of attacking and modifying proteins, lipids, and DNA as well as depleting antioxidant defenses (Sayed-Ahmed et al., 2010).

BPA induced a significant increase in oxidative/nitrusative stress, which is accompanied by marked alterations in TAC (fridovich, 1997). TAC in the present study decreased after BPA administration. Because BPA caused induction of free radicals in the hepatic tissue, in consequence, it leads to disruption in the antioxidant defense system.

The cells have various defense mechanisms against oxidative stress, including enzymatic scavengers (such as SOD, CAT and GPX) that protect the system from deleterious effects of ROS. Our data revealed that BPA caused marked oxidative impact by decreasing the activities of antioxidant enzyme compared to their activities in the control group in the three different pubertal periods. These data are in agreement with the previous results of Chitra et al. (2003) who illustrated that treatment of rats with BPA increases levels of ROS production. Also, other results of Karafakioglu et al. (2010) evidenced that concentrations and activities of antioxidant enzymes significantly decreased in rats after nonylphenol administration.

In addition, severe oxidative stress resulted from early life exposure to BPA could lead to DNA damage and mutation of tumor suppressor genes. The alkaline comet assay was used as a quantitative and visual method to measure DNA damage in the form of single strand breaks, double strand breaks, alkali labile sites (primarily a purinic and a pyrimidinic sites), incomplete excision repair sites and DNA cross links (Collins and Harrington, 2002).

A significant increase in the parameters of TL, TM, OTM and % DNA damage in tail was observed in the rat hepatic tissue exposed to BPA. These results are in agreement with the

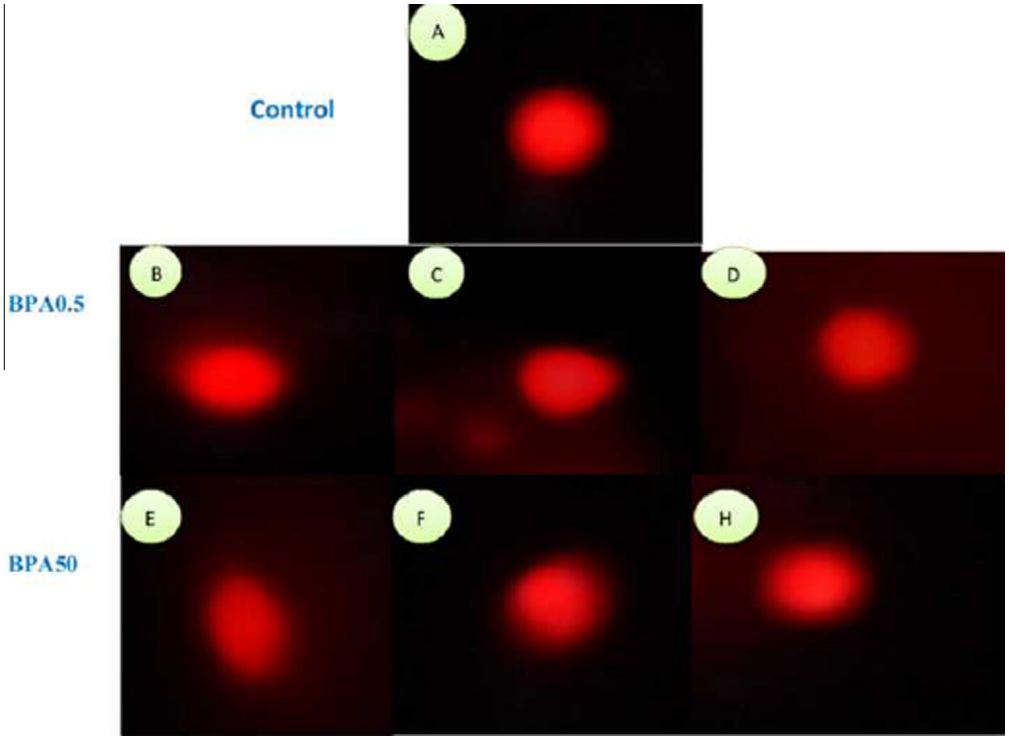

Plate 3 Fluorescent microscope photomicrographs of hepatic cells obtained from female rat offspring showing the toxic effect of BPA (0.5 and 50 mg/kg b.w.) treated groups compared to the negative control in three different pubertal periods (PND 50, 90 and 110) demonstrating no variation between the BPA treated group and control assessed by neutral comet assay (A) negative control; (B–D) BPA0.5 treated throughout different pubertal periods at (B) PND50; (C) PND90; (D) PND110. (E–H) BPA50 treated throughout different pubertal periods at (E) PND50; (F) PND90 and (H) PND110.

previous studies demonstrating DNA damage in human breast adenocarcinoma cell line (MCF) after E2 and BPA exposure (Parry et al., 2002) and also consistent with the findings in cell line from hamster ovary (CHO K1) (Mourad and Khadrawy, 2012). Hence, DNA damage detected by comet assay in the present study revealed that BPA is potent enough to cause DNA damage in the hepatic tissue. However, neutral comet assay showed no significant variation from the control, which may indicate that DNA damage induced by several factors such as single strand break, double strand breaks, free radical attack and necrotic factors and not through the apoptotic process. Therefore, all these consequences could be a step in the incidence of liver cancer later in life.

In consistent with the mentioned data, we observed histopathological changes in the liver indicating variable damage due to early life exposure to BPA. Our microscopic examination revealed that the liver could be susceptible to low doses, this result was reported by several authors (Boshra and Moustafa, 2011). In present study; it has been observed that BPA showed degenerative changes in hepatic cells and this also was reported by recent studies of Roy et al., 2011; Verma and Sangai, 2009. Previous studies showed that treatment with bisphenol A leads to cell rupture and membrane damage of human erythrocytes which may be due to the oxidative stress (Hassan et al., 2012). In the current study, light microscopic examination revealed inflammatory cellular infiltration, vacuolated hepatocytes, dilated sinusoids, congested blood vessels, pyknotic nuclei and necrosis. Previous findings reported that BPA causes cell infiltration and necrosis (Boshra and Moustafa, 2011; Verma and Sangai, 2009), vacuolated hepatocytes and liver damage (Hanioka et al., 2008a,b).

Conclusion

Therefore from the obtained results, we concluded that neonatal exposure to environmental relevant concentrations of BPA resulted in a significant DNA damage due to high free radical production and the disruption in ROS elimination mechanism in the hepatic tissue. Moreover, the genetic DNA damage may be an initiation to multistep carcinogenesis later in life. Although, the selected doses of BPA were relevant to their concentration in environment, we revealed that their levels, even at very low concentrations, may cause deleterious hazardous effects on human health.

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