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THE SENSETIVITY OF LIVER, KIDNEY ANDTESTIS OF RATS TO OXIDATIVE STRESS INDUCED BY DIFFERENT DOSES OF BISPHENOL A

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ABSTRACT

Bisphenol A (BPA) is a world wide used endocrine disruptor that is incorporated in many plastic industries. The exposure of humans to such substances starts early during the fetal life, postnatal life and extends throughout the life of the individual. Many agencies raised warnings against the excessive use of such substances. The aim of the present study is to evaluate the extent to which BPA can affect the liver, kidney and testis by measuring the oxidative stress induced by two different doses of BPA in these organs. Adult male rats were subjected to different oral doses of BPA (25 mg/kg for 6 weeks and 10mg/kg for 6 and 10 weeks, 5 days a week). The oxidative stress arising from BPA was evaluated in liver, kidney and testis tissues. In addition, serum uric acid level as a marker of kidney function together with the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as markers of liver function were measured. The results of the present study showed significant changes in the antioxidant mechanisms in the liver and testis with the high dose of BPA (25 mg/kg). The low dose showed significant changes after 10 weeks in the case of the liver and 6 weeks in the case of the testis. In addition, significant increases in serum uric acid level were observed after the administration of the two doses of BPA for 6 weeks and in AST and ALT activities after the high dose (25 mg/kg). It could be concluded that BPA-induced toxicity is mediated by oxidative stress which was prominent in liver and testis after the short and long term exposure to both the high and low dose.

Keywords : Bisphenol A – oxidative stress – liver – kidney - testis

INTRODUCTION

Bisphenol A [BPA, 2,2-bis (hydroxyphenyl) propane] is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic (e.g, water bottles, baby bottles), epoxy resins(e.g, inside coating in metallic food cans) and as a non–polymer additive to other plastics (Chitra et al., 2002; Hernandez- Rodriguez et al., 2007). Hence, it becomes an integrated part of the food chain (Vandenberg et al., 2010; Huang et al., 2011). BPA is also found in polymers that are used in dental materials (Chapin et al., 2008).

The ubiquitous and extensive use of BPA-containing products results in a high human exposure worldwide (Vandenberg et al., 2010). It is thought that human exposure mainly occurs through diet as polymers containing BPA can be hydrolyzed under high temperature and acidic or basic conditions leading to leaching into food and drink containers (Welshons et al., 2006). However,

recent evidence also indicates that exposure may occur through dermal contact with thermal papers used widely in cash register receipts (Biedermann et al., 2010).

The majority of studies on BPA have focused on their endocrine disrupting and potential adverse effects on the developing reproductive system.

There are several reports suggesting testicular toxicity of BPA in rats and mice (Tohei et al., 1999; Takahashi and Oishi, 2001). Accumulation of BPA in male reproductive organs have some clinical implications since exposure to low doses of BPA during fetal life has been shown to decrease the efficiency of sperm production in the offspring of male mice (Chitra et al.,2003).

Lang et al. (2008) reported 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. Moreover, two studies on laboratory animals have shown adverse effects of BPA on brain, reproductive system, metabolic processes, including alterations in insulin homeostasis and liver enzymes (Richter et al., 2007; Lang et al., 2008). In addition, absorption of large amounts of BPA through skin has been shown to cause extensive damage to liver, kidney and other vital organs in human (Sax, 1985; Suarez et al., 2000).

BPA has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes. It also causes degeneration of renal tubules in kidney of rat and mice ((National Toxicology Program, 1982; Nakagawa and Tayama, 2000). BPA semiquinone, a radical intermediate, was shown to be involved in DNA adduct formation along with peroxidase and hydrogen peroxide in rat hepatocytes *in vitro* (Atkinson and Roy, 1995).

Several studies reported the occurrence of oxidative toxicity after BPA exposure in rats and mice (Chitra et al., 2003; Gong and Han, 2006). It was suggested that BPA caused tissue injury in the liver, kidney, brain and other organs by the formation of reactive oxygen species (ROS) (Bindhumol et al., 2003, Kabuto et al., 2004). Moreover, the study of Bindhumol et al. (2003) revealed that low doses of BPA generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress in liver of rats.

To date, there is a controversy about the toxicity of BPA. Although FDA has labeled BPA as a safe agent (FDA, 2008), Newly emerging data has stirred discussion on urgency of more studies to make human health risk assessment of BPA exposure (Hugo et al., 2008) especially in developing countries where plastic usage has increased exponentially and certain population groups such as those suffering from malnutrition may be at higher risk than other populations (Aslan et al., 2006; Lahera et al., 2006).

Accordingly, the aim of the present study was to investigate the effect of the administration of two doses of BPA (10 mg/kg for 6 and 10 weeks and 25 mg/kg for 6 weeks, 5 days a week) on the oxidant/antioxidant status of the liver, kidney and testis of adult male rats.

MATERIALS AND METHODS

Animals:

Adult male wistar albino rats weighing 120-180 g were used as experimental animals. The animals were obtained from the animal house of the National Research Center (Cairo, Egypt). They were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center (Cairo, Egypt), which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Chemicals:

Pure bisphenol A (BPA) powder was purchased from Sigma (USA). Phosphate buffer and reagent kits were purchased from Bio-diagnostic Company, Giza, Egypt

Experimental design:

The animals were divided into 4 groups. Animals of group (1) served as control and received a daily oral administration of distilled water throughout

the experimental protocol. Animals of group (2) were administered orally 25 mg/kg of BPA for six weeks. Animals of groups (3) and (4) were administered orally 10mg/kg of BPA for 6 and 10 weeks, respectively. The doses of BPA were administered five days a week. The higher dose of BPA (25mg/kg) in this study was chosen on the base of previous studies (Bian et al., 2006; Richter et al., 2007).

Preparation of serum

At the end of the experiment the animals were sacrificed after being fasted overnight. Blood samples were drawn from the retro-orbital venous plexus according to the method of Sorg and Buckner (1964). Blood samples were left to coagulate at room temperature, then centrifuged at 986 g for 15 minutes, the clear non-hemolyzed supernatant serum was quickly removed and used for the estimation of serum enzymes.

Handling of tissue samples:

The liver, kidney and testes of each animal was quickly removed and rapidly weighed and frozen until analyzed. The liver and testes are homogenized in 10 ml and kidney in 6ml of ice cold phosphate buffer (50 mM pH 7.4, 0.1% triton X and 0.5 mM EDTA). The homogenates were centrifuged at 1753 g for 15 min at 4°C using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany). The clear supernatants were separated and used for analysis.

Determination of lipid peroxidation and reduced glutathione levels:

Lipid peroxidation was assayed by determining the level of malondialdehyde (MDA) by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al. (1994). The thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex that has peak absorbance at 532nm using a helios alpha thermospectronic (UVA111615, Cambridge, UK).

Reduced glutathione (GSH) was assayed by Ellman's method (1959). The procedure is based on the reduction of Ellman's reagent (5, 5-dithio-bis-2-nitrobenzoic acid) by –SH groups of GSH to form 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color. The absorbance was measured at 412 nm and the GSH concentration was calculated by comparison with a standard curve.

Determination of enzyme activities

Glutathione-S-transferase (GST) activity was determined according to the method of Habig et al. (1974). 0.4 ml potassium phosphate buffer (50 mmol/l; pH 6.5), 0.1 ml of supernatant, 1.2 ml water and 0.1 ml CDNB (1-chloro-2, 4 di-nitrobenzene, 30 mmol/l) were added and incubated in a water bath at 37°C for 10 min. After incubation, 0.1 ml of reduced glutathione (30 mmol/l) was added. The change in absorbance was measured at 340 nm at 1 min interval.

Catalase activity was measured using Biodiagnostic Kit No. CA 25 17 (Giza, Egypt) which is 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.

Serum enzymes assay

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by reagent kits No. CA10 34(45) based on the method of Reitman and Frankel (1957) and purchased from Bio-diagnostic Company (Giza, Egypt). The concentration of uric acid was measured by Barham and Trinder (1972) method using reagent kits No CA21 20 purchased from Bio-diagnostic Company (Giza, Egypt).

Statistical analysis

The data were expressed as means ± standard error of mean (S.E.M.) All variables were tested for normal distribution and compared using the independent t-test. All analyses were performed

using the Statistical Package for Social Sciences (SPSS) software in a PC-compatible computer and the significance was set at $p < 0.05$.

RESULTS

The data revealed that lipid peroxidation level was significantly increased in the liver tissue after the daily oral administration of 25mg/kg of BPA for six weeks, recording +47.81 % above the control level (Table 1). However, a significant decrease in the lipid peroxide level occurred after 6 weeks of the oral administration of BPA (10mg/kg) and returned to nearly control level after 10 weeks. On the other hand, the only significant decrease in liver catalase activity was obtained after 10 weeks of daily BPA administration at the dose level of 10 mg/kg. A significant decrease in the level of liver GSH was also evident after the administration of both the high (25 mg/kg) and low (10 mg/kg) dose of BPA for 6 weeks and for 6 and 10 weeks, respectively. BPA also resulted in an increase in the activity of GST after 6 weeks of administration of the high dose (+31.74 %) and after 10 weeks of treatment with the low dose level (+15.45 %).

In the kidney (Table 2), non significant changes in lipid peroxidation and GSH levels and in the activity of catalase occurred after the daily oral administration of BPA at the two dose levels. The only significant change obtained was an increase in GST activity after 6 weeks of oral administration of 25 mg/kg of BPA.

Table (3) shows that the daily oral administration of BPA at both the high (25 mg/kg) and low (10 mg/kg) dose resulted in a significant increase in lipid peroxidation level in the testis after 6 weeks, recording +32.07 % and +26.41 %, respectively. This was accompanied by a significant decrease in catalase activity and GSH levels at the same time interval. The decrease in GSH levels continued significantly (-44.88 %) after 10 weeks of BPA administration at the low dose level. However, no significant changes were recorded in GST activity in the testis after BPA administration.

It is clear from table (4) that the oral administration of BPA at the dose of 25mg/kg for 6 weeks caused a significant increase in ALT and AST activities as compared to the control. However, the dose level of 10mg/kg had no significant effect on the activities of both enzymes after 6 and 10 weeks compared to the control values. On the other hand, a significant increase in uric acid concentration occurred after 6 weeks of the daily oral administration of BPA at both the high (25 mg/kg) and low (10 mg/kg) dose levels.

Table(1): ***Effect of oral administration of bisphenol A on some oxidative stress parameters in the liver of male albino rats***

	Control	BPA 25 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (10 weeks)	%d
MDA (nmol/gtissue)	57.90±2.15 (6)	85.58±9.01*(6)	47.81	36.09±.3.08*(6)	**--37.67**	59.75±3.55 (6)	-3.20
Catalase (U/g tissue)	8.01±0.17 (6)	8.41±014 (6)	-4.99	7.46±0.17 (6)	-6.87	6.86±0.47*(6)	-14.36
GSH (mmol/g tissue)	1.50 ±0.19 (6)	0.87 ±0.12*(6)	**-42.00**	0.70 ±0.11*(6)	-53.33	0.77±0.08*(6)	-48.67
GST (U/g tissue)	19.03±0.66 (6)	25**.07** ±0.69*(6)	31.74	18.11 ±0.89 (6)	-4.83	21.97±1.05*(6)	15.45

Data are expressed as Mean ± S.E.M., wih the number of animals between parantheses.
**** : Significant at p < 0.05***
%d : percentage difference

Table(2): ***Effect of oral administration of bisphenol A on some oxidative stress parameters in the kidney of male albino rats***

	Control	BPA 25 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (10 weeks)	%d
MDA (nmol/gtissue)	47.65 ± 4.95(6)	49.89 ±2.92(6)	-4.70	46.89 ±2.58(6)	-1.59	47.14±3.10(6)	-1.07
Catalase (U/g tissue)	5.87 ± 0.14(6)	5.68 ± 0.19(6)	-3.24	5.32 ± 0.30(6)	-9.37	5..80 ±0.30(6)	-1.19
GSH (mmol/g tissue)	0.72 ±0.11(6)	0.61 ±0.08(6)	-15.28	0.60 ± 0.06(6)	-16.67	0.52 ±0.03(6)	-27.78
GST (U/g tissue)	18.15 ± 0.14(6)	21.80±0.13*(6)	20.11	16.69±0.09(6)	**-8.04**	17.39 ±0.09(6)	- 4.19

Data are expressed as Mean ± S.E.M., wih the number of animals between parantheses.
**** : Significant at P < 0.05***
%d : percentage difference

Table(3): ***Effect of oral administration of bisphenol A on some oxidative stress parameters in the testis of male albino rats***

	Control	BPA 25 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (10 weeks)	%d
MDA (nmol/gtissue)	26.47±1.32(6)	34.96±**1.58*(**6)	32.07	33.46±2.42*(6)	26.41	30.12±1.39(6)	-13.79
Catalase (U/g tissue)	6.56 ± 0.23(6)	5.75 ±0.11*(6)	-12.35	5.73 ±0.14*(6)	-12.65	6.19 ± 0.21(6)	-5.64
GSH (mmol/g tissue)	1.27± 0.08(6)	1.03±0.06* (6)	-18.90	0.78 ±0.04* (6)	-38.58	0.70±0.08* (6)	-44.88
GST (U/g tissue)	16.92±0.93(6)	17.09 ±0.75(6)	-1.00	18.09 ± 0.51(6)	-6.91	19.50±1.30(6)	-15.25

Data are expressed as Mean ± S.E.M., wih the number of animals between parantheses.
**** : Significant at P < 0.05***
%d : percentage difference

Table(4): ***Effect of oral administration of bisphenol A on serum uric acid level, and the activities of ALT and AST***

	Control	BPA 25 mg/kg/d (6 weeks)	%d	BPA 10 mg/kg/d (6 weeks)	%d	BPA10 mg/kg/d (10 weeks)	%d
ALT (U/ml)	13.80 ± 0.97(5)	34.50±2.62* (5)	150	14.20 ±1.24(5)	-2.90	14.00 ± 0.32(5)	-1.45
AST (U/ml)	26.00 ± 2.66 (5)	34.36±2.98*(5)	32.15	25.92 ± 1.32(5)	-0.31	23.75 ± 2.20(5)	-8.65
Uric acid (mg/dl)	1.53 ± 0.12 (5)	2.22 ± 0.56* (5)	45.10	2.14 ± 0.08* (5)	39.87	1.95 ± 0.63 (5)	27.45

Data are expressed as Mean ± S.E.M., wih the number of animals between parantheses.
**** : Significant at P < 0.05***
%d : percentage difference

DISCUSSION

The widespread consumption of BPA-containing products has raised concerns among scientists and regulatory agencies that human exposure to BPA may have adverse effects on different vital organs. In the present study, the recorded significant increase in lipid peroxidation and GST activity which was accompanied by a significant decrease in GSH in the liver of rats treated with BPA (25 mg/kg) for 6 weeks reflects a state of oxidative stress in liver cells.

GSH is the most important freely available antioxidant, which acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as glutathione peroxidase, glutathione reductase and glutahthione-S-transferase (Circu and Aw, 2008; Biswas and Rahman, 2009). In addition, GST catalyzes the conjugation of reduced glutathione – via a sulfhydryl group – to electrophilic centers on a wide variety of substrates (Douglas, 1987). This activity detoxifies endogenous compounds such as peroxidized lipids (Leaver and George, 1998) as well as breakdown of xenobiotics.

Atkinson and Roy (1995) reported that BPA is oxidized to a reactive metabolite 4, 5-bisphenol-O-quinone and major DNA adduct increased in rat liver DNA at the presence of peroxidase activation. BPA has been shown to decompose to many kinds of metabolites probably including BPA radical by a reaction of radical oxygen (Sajiki, 2001). Previous studies showed that macrophages generate ROS such as superoxide anion and hydrogen peroxide in liver after exposure to hepato-toxicants (Pilaro and Laskin, 1986; McCloskey et al., 1992).

In rat liver, BPA decreases the activity of the male–specific cytochrome P450 isoforms, testosterone 2α and 6β hydroxylase in liver of rats (Hanioka et al., 1998). The reduced cytochrome P450 has been shown to provoke ROS that impair sperm function (Griveau et al., 1995).

Accordingly, it could be concluded that the oxidative stress induced by BPA in liver of rats treated with 25 mg/kg of BPA may be due to the formation of ROS arising from reduced mitochondrial fractions and the formation of quinine radical, one of the BPA metabolites. The raised activity of GST may be at the expense of the content of reduced GSH that acts as a catalyst for GST. At the same time, GST catalyzes the conjugation of reduced glutathione to electrophilic centers of endogenous compounds to detoxify peroxidized lipids that recorded a significant increase in the present study. This could explain the decreased content of GSH and the increased activity of GST that were recorded in the liver after the high dose.

In the liver of rats treated with BPA at a dose level of 10 mg/kg for 10 weeks, a significant decrease in GSH content and catalase activity has been observed. However, GST activity showed a significant increase.

Bindhumol et al. (2003) suggested that the reduction in the activity of catalase may reflect the inability of liver mitochondria and microsomes to eliminate hydrogen peroxide produced after exposure to BPA. This may be due to the enzyme inactivation caused by excess ROS production in mitochondria (Pigeolet et al., 1990).

On comparing the effect of the two doses of BPA on liver lipid peroxidation, we observed that there was no significant change after the low dose of BPA, while the high dose of BPA induced a significant increase in liver lipid peroxidation.

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen.

Thus the reduced activity of catalase after 10 weeks in the liver of rats treated with 10 mg/kg of BPA which was accompanied by reduced GSH content and increased GST activity could explain the ability of these antioxidant mechanisms to prevent the increase in lipid peroxidation and maintain its normal level at this time interval.

The results of the present study are in agreement with the study of Bindhumol et al. (2003) who found that BPA significantly decreased the activities of antioxidant enzymes and increased lipid peroxidation in the liver thereby increasing oxidative stress.

In the present study, both AST and ALT showed a significant increase over control values in rats treated daily with 25 mg/kg for 6 weeks. However, the lower dose (10 mg/kg) did not affect

the activities of both enzymes that reflect liver function after the two studied time intervals.

The present results are in agreement with those of Korkmaz et al. (2010) who reported a significant increase in ALT and AST activities in rats treated with 25 mg/kg BPA for 50 days.

AST and ALT are two enzymes of the most reliable markers of hepatocellular injury or necrosis. Their levels are elevated in a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in liver cytosole and in low concentration elsewhere (Giboney, 2005). When the liver hepatocytes are damaged, these enzymes are released into the blood where the significant increase in AST and ALT activities indicates the damage to the cytosole and also to mitochondria (Mathuria and Verma, 2008). Therefore, it could be suggested that the oxidative stress induced by the high dose of BPA (25 mg/kg/day for 6 weeks) may mediate the disturbance in hepatic function which is reflected by the present increase in ALT and AST. The absence of any effect on hepatic function after the lower dose (10 mg/kg for 6 and 10 weeks) which was accompanied by normal values of lipid peroxidation may support this explanation.

In the testis, the present data revealed a significant increase in lipid peroxidation that was accompanied by a significant decrease in GSH content and catalase activity due to BPA treatment. This effect was observed after 6 weeks of daily administration of both the high (25 mg/kg) and low dose (10 mg/kg) of BPA.

These effects reflect a state of oxidative stress in testicular tissue. This effect of BPA may be the mechanism by which BPA induced testicular toxicity in rat and mice (Tohei et al., 1999; Takahashi and Oishi, 2001). In addition, the effect of BPA on the epididymas and epididymal sperms could be due to the oxidative stress caused by BPA in the testis.

It is clear from the present data that both the high and low doses of BPA could induce oxidative stress in the testis. This may be due to the fact that the testicular membranes are rich in polyenoic fatty acids that are prone to undergo peroxidative decomposition (Rosenblum et al., 1989).

The present data showed non significant changes in oxidative stress parameters in the kidney due to BPA treatment. However, serum uric acid increased significantly after 6 weeks of the daily oral administration of BPA at both the high (25 mg/kg) and low (10 mg/kg) dose levels. In the light of the present results, this increase in uric acid could not be attributed to impairment in kidney function.

It has been demonstrated that serum uric acid levels are significantly elevated in non alcoholic fatty liver patients (Li et al., 2009).

Marmugi et al. (2011) suggested that low doses of BPA may influence de novo fatty acid synthesis thereby contributing to hepatic steatosis. Furthermore, uric acid increase may be due to the effect of BPA on the heart as several studies showed an association between elevated uric acid levels and cardiovascular diseases (Culleton et al., 1999; Fang and Alderman, 2000).

In the light of the present results, it could be concluded that high doses of BPA have serious effects on the liver and testis. These effects are mediated by the oxidative stress induced by BPA. Although low doses of BPA induced minor changes in oxidative stress parameters after 6 weeks in liver tissue, significant changes were obtained after 10 weeks suggesting that low doses may produce drastic effects after long term exposure. Furthermore, the results of the present study suggest that in a population of high use of plastics where there is a great chance of exposure to BPA, the males may suffer from sexual disturbances due to the oxidative stress induced in the testis. Thus, the use of BPA in different plasticizers and other industries should be limited and the erroneous handling of plastic containers should be avoided to reduce the health risks resulting from exposure to these endocrine disruptors including BPA.

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