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Research Article

Bisphenol A Induces Hepatotoxicity through Oxidative Stress in Rat Model

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Reactive oxygen species (ROS) are cytotoxic agents that lead to significant oxidative damage. Bisphenol A (BPA) is a contaminant with increasing exposure to it and exerts both toxic and estrogenic effects on mammalian cells. Due to limited information concerning the effect of BPA on liver, this study investigates whether BPA causes hepatotoxicity by induction of oxidative stress in liver. Rats were divided into five groups: The first four groups, BPA (0.1, 1, 10, 50 mg/kg/day) were administrated orally to rats for four weeks. The fifth group was taken water with vehicle. The final body weights in the 0.1 mg group showed a significant decrease compared to control group. Significant decreased levels of reduced glutathione, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and catalase activity were found in the 50 mg BPA group compared to control groups. High dose of BPA (50 mg/kg) significantly increased the biochemical levels of ALT, ALP and total bilirubin. BPA effect on the activity of antioxidant genes was confirmed by real time PCR in which the expression levels of these genes in liver tissue were significantly decrease compared to control. Data from this study demonstrate that BPA generate ROS and reduce the antioxidant gene expression that causes hepatotoxicity.

1. Introduction

Exposure to low levels of endocrine disrupting chemicals (EDCs) may be of concern. They interfere with many metabolic processes and cause widespread damage to body tissues and cardiovascular disease [1]. This interference may result in changes in appetite, in food efficiency, and in fat, carbohydrate, and protein metabolism. This is seemingly ubiquitous in today's environment, and consequently the effects of EDCs may manifest primarily in populations (i.e., changes over time) and less with respect to interindividual variation within populations. Recent evidence suggests that endocrine-disrupting chemicals, for example, halogenated aromatic hydrocarbons, may cause perturbations in endogenous hormonal regulation and alter other body mechanisms [2].

Bisphenol A (BPA) is used in plastic and food can liners' manufacture [3]. Some studies reveal the toxic effect of BPA [4] and indicate its possibility in inducing endocrine disorder in organs. It acts as an estrogenic compound in both *in vivo* and *in vitro* studies [5]. *In vitro* studies showed that BPA triggers mouse fibroblasts cells to differentiate into adipocytes [6]. Accelerated maturation of fat pads and a significant increase in the number of adipocytes in mammary glands were observed after exposure of female mice fetuses to BPA [7].

BPA shows potential acute, short-term, and subchronic toxicity [8, 9]. Some studies established BPA effect on the liver, kidney, and body weight at doses of 50 mg/kg bw and higher [9]. A report [10] used different BPA doses and confirmed similar pervious findings [9], with a lowest no observed adverse effect level (NOAEL) of 5 mg/kg bw. There are no chronic organ toxicity studies on BPA. Several studies alert its effect on the reproductive system with inadequate reports on other tissues.

Reactive oxygen species (ROS) are cytotoxic agents causing oxidative damage by attacking cell membrane and DNA [11]. Antioxidants are scavengers by preventing cell and tissue damage that could lead to cellular damage and disease [12]. BPA can cause liver, kidneys, brain, and other organs injury by forming ROS [13, 14]. The liver has a range of antioxidant defense system. ROS are scavenged by the endogenous antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in cells [13, 14]. The liver is a target organ with a NOAEL BPA concentration of 74 mg/kg bw following oral exposure. BPA could induces liver damage, affecting oxidant/antioxidant balance in rat liver [5]. This study aimed to evaluate whether exposure to BPA induce oxidative stress in the liver of male rats.

2. Material and Methods

2.1. Animals. Male Wistar albino rats were purchased from the Experimental Animals, King Saud University, Saudi Arabia. Handling of animals was in compliance with the Guidelines for the Care and Use of Animals for Scientific Purposes. The animals were caged in a well-ventilated animal room with a 12 h dark/light cycle and controlled temperature and all had free access to standard diet and drinking water *ad libitum*.

2.2. Methods. To investigate the toxicity of BPA, a total of 25 rats were randomly divided into five groups consisting of five rats. Different groups of rats were administered freshly prepared BPA (Sigma-Aldrich, USA) orally via gavage at specific concentrations between 0.1 and 50 mg/L with 0.02% ethanol (vehicle) in water [16]. The animals were treated via oral gavage once daily for four weeks as follows. Group one was administred orally daily 0.1 mg/Kg/day of BPA. Group two was administred daily 1 mg/Kg/day of BPA. Group three was administred daily 10 mg/Kg/day of BPA. Group four was administred daily 50 mg/Kg/day of BPA. In the fifth group, the control group, all rats were taken water with 0.02% ethanol (vehicle) in water. Body weights and feed consumption were recorded, and clinical observations were made daily. At 24 hours after receiving the last dose, animals were anesthetized with ether and blood samples were obtained from the retroorbital sinus of the eye. Sera were separated for measurement of alanine transaminase (ALT), alkaline phosphatase (ALP). Immediately after blood samples were collected, animals were then sacrificed by decapitation after exposure to ether in desiccators and their livers were rapidly excised and part of it was immediately frozen at −80°C for gene expression studies.

2.3. Processing of Tissues and Assays. The liver was homogenated (10% w/v) in Tris-HCl (0.1 M, pH 7.4). Homogenates were centrifuged at 1000 ×g for 10 minutes at 4°C. The supernatant was used for investigating total protein, activities of antioxidant enzymes, and markers of oxidative stress. Each sample was tested in triplicate.

Total glutathione and GSSG were estimated using GSH : GSSG ratio assay kit according to the manufacturer's instructions. GSH was then calculated. Glutathione Peroxidase (GPx) activity was measured using Cayman (USA) assay kit according to manufacturers' instructions. One unit of GPx is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25°C. Glutathione Reductase (GR) activity was assayed by ELISA kit (Life Science) based on sandwich enzyme immunoassay. Glutathione-S-Transferase (GST) activity was assayed according to Habig et al. [17].

Catalase (CAT) activity was measured using Cayman (USA) assay kit according to the manufacturer's instructions. Total nitrate/nitrite (NOx), an index of nitric oxide (NO) production, was measured basing on the reduction of nitrate by vanadium trichloride combined with detection by the acidic Griess reaction according to the method of Miranda et al. [18]. Total Antioxidant Status (TAS) was measured according to Koracevic et al. [19]. TAS levels in the liver homogenates were calculated using uric acid as standard. TAS was expressed as nmol uric acid equivalent per mg protein. Superoxide Dismutase (SOD) activity was measured using Cayman (USA) assay kit according to the manufacturer's instructions. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of $O_2^{\bullet -}$. Protein Assay: protein concentration was estimated using Bio-Rad protein assay kit based on the method of Bradford [20]. A standard curve was generated using bovine serum albumin as standard.

2.4. Gene Expression Profile by Real-Time PCR in Liver Tissues. Total RNAs were extracted from liver tissue by Trizol method according to the manufacturer's protocol as previously explained [15]. The quantity and integrity were characterized using a UV spectrophotometer. The isolated RNA has an A 260/280 ratio of 1.9–2.1. cDNA synthesis and real-time PCR methods. First-strand cDNA was synthesized from 1 μg of total RNA by reverse transcription with a SuperScriptTM first-strand synthesis system kit (Invitrogen, CA USA), according to the manufacturer's instructions. Real-time PCR was done according to our previous study [15]. We used GAPDH gene as endogenous control. All primers were listed in Table 1. Following amplification, melting curve analysis was performed to verify the correct product according to its specific melting temperature (T_m) [15].

2.5. Statistical Analysis. Differences between obtained values (mean ± SEM, $n = 5$) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. A P value of 0.05 or less was taken as a criterion for a statistically significant difference.

3. Results

The absolute organ weights, relative organ weights and final body weights of male rats in control and treatment groups, are presented in Table 2. There were no differences in

Table 1: The primers sequences for GSHPx, CAT, GR, and GST genes.

Gene name	Forward primer	Reverse primer
GSHPx	5′-GGG CAA AGA AGA TTC CAG GTT-3′	5′-AGA GCG GGT GAG CCT TCT-3′
Catalase	5′-AGG TGA CAC TAT AGA ATA GTG GTT TTC ACC GAC GAG AT-3′	5′-GTA CGA CTC ACT ATA GGG ACA CGA GGT CCC AGT TAC CAT-3′
GR	5′-TTC TGG AAC TCG TCC ACT AGG-3′	5′-CCA TGT GGT TAC TGC ACT ACT TCC-3′
GST	5′-GCC TTC TAC CCG AAG ACA CCT T-3′	5′-GTC AGC CTG TTC CCT ACA-3′

Primers sequence for glutathione peroxidase (GSHPx), catalase (CAT), glutathione reductase (GR), and glutathione-s-transferase (GST) genes used in real-time PCR [15].

Table 2: The body weight (gm), and selected absolute (gm), and relative organ weights (mg/g) of male rats in control and BPA groups.

	Control	BPA (0.1 mg)	BPA (1 mg)	BPA (10 mg)	BPA (50 mg)
Initial body weight	285 ± 10.3	234 ± 21.4	230 ± 15.9	235 ± 11.4	256 ± 13.6
Liver					
Absolute	9.23 ± 0.2	8.50 ± 0.4	8.08 ± 0.36	8.79 ± 0.1	6.62 ± 0.2
Relative	36.4 ± 0.2	32.5 ± 2.25	34.0 ± 0.65	32.8 ± 1	32.3 ± 0.7
Final body weight	330 ± 12.3	168 ± 21.0*	237 ± 5.8	357 ± 9.4*	316 ± 5.6

*$P < 0.05$ significantly different from control group.

absolute organ weights of BPA groups when compared to the control group. There was a significant decrease in the body weights of BPA 0.1 mg groups when compared to control group. During treatment, a reddish secretion was observed around the nose of BPA 0.1 mg group but not in all of the other groups.

Serum ALT and AST levels were significantly higher in BPA groups when compared to control group ($P < 0.05$). Significantly higher serum ALT levels where observed in the BPA-50 mg group when compared to other BPA groups ($P < 0.05$). A high significant difference in bilirubin levels was observed in the 50 mg group when compared to all other groups and the control group. The cumulative hepatotoxicity of BPA was clearly featured by dose-dependent increase in serum biochemical markers, ALT, ALP, and bilirubin (Table 3).

The effect of BPA on the oxidative and the nitrosative stress biomarkers is presented. In the BPA-50 mg group, significant decreases in GSH and SOD in liver tissues are shown in Table 4. In the BPA-50 mg group, liver TBARS and NO were significantly higher compared to control group ($P < 0.05$).

Table 5 showed that the BPA-50 mg group resulted in significant decrease of antioxidant enzymes activity of GSHPx, CAT, GR, and GST compared to normal group ($P < 0.05$).

The effect of BPA on the antioxidant genes expression level of GR, GSHPx, Catalase, and GST in liver was confirmed by real-time PCR. Table 6 showed the effect of BPA on mRNA expression of antioxidant genes GR, GSHPx, catalase, and GST in liver tissue by quantitative detection of the gene expression. The highest concentration of BPA 50 mg/kg showed a significant downregulation in all of the studied genes. On the other hand, all doses of ≤10 mg/kg showed nearly the same gene expression level of the control group.

Table 3: Effect of BPA on liver enzymes indices, ALT, ALP, and total bilirubin.

Treatment groups	Serum ALT (U/dL)	Serum ALP (U/dL)	Serum bilirubin (mg/dL)
Control	30 ± 1.5	304 ± 5.6	0.59 ± 0.01
BPA 0.1 mg/kg	32 ± 0.81	312 ± 5.8	0.62 ± 0.04
BPA 1 mg/kg	42 ± 1.7	321.5 ± 3.1	0.84 ± 0.06
BPA 10 mg/kg	47 ± 7.9*	366 ± 1.7*	1.17 ± 0.02*
BPA 50 mg/kg	340 ± 1.5*	626.6 ± 9.6*	1.75 ± 0.1*

Data are presented as the mean ± SEM.
*Indicates significant change from control at $P < 0.05$ using ANOVA test.

4. Discussion

BPA is an endocrine disorderly chemical released in environment, so most studies are focused on its effect on reproduction [3, 21]. Due to limited information considering the toxic effect of different concentrations on liver, our study used four different concentrations for evaluating the toxic effect of BPA on male Wister Albino rat. The Center for the Evaluation of Risks to Human Reproduction group on BPA stated the lowest oral LD50s for rat is 3.25 g/kg bw for the acute toxicity [22]. Owing to the debates around the low-dose and high-dose effects of BPA, this study included low and high doses according to several reports and there was no record for any animal death.

Liver function tests, including ALT, AST, and bilirubin, evaluated the presence of liver damage or disease. Data presented in our study demonstrate that high dose of BPA 50 mg/kg significantly increased the serum indices of liver function and near normal in the lower concentration groups. The high levels of ALT and ALP are attributed to damage in liver. Similar elevated levels of serum indices for liver

Table 4: The effect of BPA on oxidative and nitrosative stress biomarkers.

Treatment groups	TBARS	NO (x)	GSH	SOD
Control	300 ± 9	40.3 ± 0.95	4.8 ± 0.2	3.8 ± 0.07
BPA 0.1 mg/kg	329.1 ± 6.2	44 ± 0.5	4.5 ± 0.2	3.52 ± 0.01
BPA 1 mg/kg	340.8 ± 8.7	54.5 ± 1.2	3.81 ± 0.12	3.17 ± 0.02
BPA 10 mg/kg	352.5 ± 3.5*	56.2 ± 1	3.65 ± 0.02	2.93 ± 0.02*
BPA 50 mg/kg	540 ± 15.49*	83.2 ± 1.9*	0.97 ± 0.04*	0.56 ± 0.01*

Data are presented as the mean ± SEM.
*Indicates significant change from control, at $P < 0.05$ using ANOVA.

Table 5: The effect of BPA on antioxidant enzymes, GSHPx, CAT, GR, and GST activity on rat liver tissues.

Treatment groups	GSHPx (μmol/g wet tissue)	CAT (μmol/min/g tissue)	GR (ng/mL)	GST (μmol/min/g tissue)
Control	204.2 ± 11.1	86.1 ± 0.34	1.09 ± 0.02	37.2 ± 0.5
BPA 0.1 mg/kg	200 ± 0.8	84.4 ± 0.22	0.97 ± 0.01	36.8 ± 0.7
BPA 1 mg/kg	197.5 ± 1.5	77.4 ± 0.40	0.91 ± 0.01	35.9 ± 0.9
BPA 10 mg/kg	174.6 ± 0.29	72.2 ± 0.32	0.88 ± 0.06	30.7 ± 1.08
BPA 50 mg/kg	50.6 ± 1.4*	29.5 ± 0.32*	0.3 ± 0.09*	16.2 ± 0.8*

Effect of BPA on the activity of the antioxidant enzymes, glutathione peroxidase (GSHPx), catalase (CAT), glutathione transferase (GST), and glutathione reductase (GR) in liver tissues. Data are presented as the mean ± SEM.
*Indicate significant change from control, at $P < 0.05$ using ANOVA followed by Tukey-Kramer as a post-ANOVA test.

damage have been previously reported considering the BPA toxicity that resulted from 5 mg BPA/kg/day in rats with no effects at ≤5 mg. Comparable study reported increase in the serum ALT and AST levels in BPA groups compared to control group [5]. Yamasaki et al. reported an increase in AST activity in males treated with BPA ≥ 200 mg/kg/day and increased ALP, γ-glutamyl transpeptidase activity in males treated with 600 mg/kg/day [23, 24].

In the current study, a significant decrease in the body weight was observed in the least dose used (0.1 mg/kg BPA) with no differences in absolute organ weights with a remarkable bloody nasal secretion when compared to the control group. Similar reddish secretion around the nose was observed in a study for chronic exposure to low doses bisphenol A [25]. Another study showed a statistically significant decrease in body weight in the group treated with ≥466 mg/kg/day BPA. Statistically significant and dose-related decreases in absolute (>22%) and relative liver weights (>10%) were observed to be ≥466 mg/kg/day, compared with controls [23, 24].

Antioxidants reduce the cellular damage resulting from interaction between lipid, protein and DNA molecules and ROS. Regardless of the presence of this antioxidant system, an over or unbalanced production of ROS due to contact with chemicals may resulted in a number of clinical disorders. BPA can cause oxidative stress by disturbing the redox status in cells [26]. Due to limited information concerning the effects of BPA on liver, the present study investigates whether BPA causes hepatotoxicity by induction of oxidative stress in liver.

The levels of nonenzymatic antioxidants GSH and enzymatic antioxidant (SOD) activity were measured to evaluate the stability of ROS production in liver. SOD protects tissues from oxidative stress and damage by catalyzing the conversion of $O_2^{\bullet -}$ to H_2O_2, a more stable ROS [27]. Therefore, the damage at the cellular level by oxidants is attenuated by antioxidant enzyme such as SOD, GSHPx, GSP, CAT and GR [28]. The current studyshowed that 50 mg/kg BPA caused significant decrease in the levels of GSH, along with decrease in the activity of SOD. In concordance with our results, Wu et al. showed significant decrease in the levels of GSH and SOD in BPA group; this decrease indicated liver tissues damage [29]. Similarly, in other organs ROS were induced when BPA was administered during the embryonic and infancy [11, 14, 30]. Similarly, others demonstrated that BPA generates ROS that causes oxidative damage in the brain, reproductive tract, and kidney of rats [5, 31, 32].

Catalase and GSHPx catalyze dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) which then convert hydrogen peroxide to water, in this manner, providing protection against reactive oxygen species [33]. In our study, the 50 mg/kg BPA group significantly increased the NOx and TBARS and decreased the GSHPx, GR, and GST activities which were confirmed by the gene expression levels of antioxidant enzymes in liver tissues. Our results showed a decrease in CAT activity in the liver of 50 mg/kg BPA group. Hence, this enzyme protects tissues from highly reactive hydroxyl radical ($^{\bullet}OH$), derived from H_2O_2 [34]. Thus, the decrease in CAT activity in the liver increased the toxic effect of the free radicals formed from the BPA effect.

GSH acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as glutathione peroxidase and glutathione reductase. The reduction of GSH shows the failure of primary antioxidant system to act against free radicals [35]. The increased TBARS level and decreased GSH concentration indicate an increased generation of ROS, which cause lipid peroxidation in the liver [36]. In our study, the BPA-50 mg group significantly

Table 6: The effect of BPA on the gene expression level of antioxidant enzymes, GSHPx, CAT, GR, and GST, in rat liver tissues.

Treatment groups	GSHPx	CAT	GR	GST
Control	9.3 ± 1.43	8.34 ± 1.5	10.3 ± 1.2	12.3 ± 1.4
BPA 0.1 mg/kg	8.7 ± 0.9	7.82 ± 0.91	9.26 ± 1.2	11.97 ± 0.6
BPA 1 mg/kg	8.45 ± 0.3	7.25 ± 0.3	8.65 ± 0.6	11.4 ± 0.4
BPA 10 mg/kg	6.63 ± 0.02	6.5 ± 0.2*	6.54 ± 0.3*	8.8 ± 0.5
BPA 50 mg/kg	0.52 ± 0.01*	0.54 ± 0.01*	0.57 ± 0.01*	0.7 ± 0.05*

The effect of BPA on the gene expression of antioxidant enzymes, glutathione peroxidase (GSHPx), catalase (CAT), glutathione transferase (GST), and glutathione reductase (GR) in liver tissues. Data were presented as the mean of fold expression ± SEM.
*indicates significant change from control $P < 0.05$ using ANOVA followed by Tukey-Kramer as a post-ANOVA test.

decreases in GSH and SOD in liver tissues. Korkmaz et al. [5] showed an increase in TBARS level in liver of rats exposed to BPA. Similarly, previous studies show increased in the TBARS levels in the brain, testes, and kidneys of male rats exposed to BPA dose [5, 31, 32]. The increase in GSH level is important for GSHPx, which requires GSH as a cofactor, and the elevation in GSH level increases activity of GSHPx. The current study showed reduction in both GSH and GSHPx levels. Our results are consistent with previous study, reportes the decrease in GSH concentration in the liver in BPA administrated rats [5]. Therefore, our study confirmed that the exposure to high concentrations of BPA causes oxidative stress by disturbing the balance between ROS and antioxidant defenses system in liver.

GST protects cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes [37]. The current study showed a significant downregulation in the GST gene expression levels by 11 folds and also its protein level in the tissue in the 50 mg/kg BPA group compared to the control group. The current data showed a decrease activity of GST in the liver in all groups but not significant compared to control. A number of compounds lead to induce activity and expressions of GST isoenzymes [38, 39]. In contrast to our data, it has been reported that increased GST activity and upregulated GST-Π expression correlate with increased oxidative stress and apoptosis in breast cancer [35]. Our results showed that high dose of BPA not only increases the free radical formation but also decreases its ability to detoxify reactive oxygen species. The formation of superoxide radicals together with NO might form peroxynitrite induced by high doses of BPA causes tissue damage leading to an increase in the levels of TBARs and NOx.

5. Conclusion

In conclusion, our results indicate that high dose of BPA 50 mg/kg induces liver damage, affecting oxidant/antioxidant balance, as a result of reactive oxygen species in rat liver and the dose of 10 mg/kg could cause damage and must be taken in consideration.

Conflict of Interests

All authors declare that there is no conflict of interests.

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