Post-Weaning Exposure to Bisphenol A Induces Histological Changes in the Liver

Siti Sarah Mohamad Zaid\textsuperscript{1}, Siti Nur Hajar Rohim\textsuperscript{1}, Goh Yong Meng\textsuperscript{2} and Noordin Mohamed Mustapha\textsuperscript{3}

\textsuperscript{1}Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
\textsuperscript{2}Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
\textsuperscript{3}Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT

Bisphenol A (BPA) is an endocrine disrupting chemical (EDC) widely used in industry as a plasticizer for the production of polycarbonate plastics and epoxy resins. The liver is highly sensitive to BPA, even at low doses. The objective of the study is to investigate the effect of BPA on histo-architecture of the liver in post-weaning rats. Post-weaning female rats were exposed to BPA by oral gavage over a six weeks period. The results showed that even at low environmental doses, BPA exposure had adverse effects on the liver histo-architecture, thereby disrupting the functions of cellular. The administration of BPA resulted in severe hepatocytes necrosis, dilated sinusoid, and depicting features of conspicuous Kupffer cells. The results may be due to the generation of reactive oxygen species (ROS) by BPA. In conclusion, post-weaning exposure of BPA resulted in significant histological alterations due to ROS generation.

Keywords: Bisphenol A, histology, liver, oxidative stress, plastic

INTRODUCTION

In the last few decades, exposure to environmental toxicants has become a serious health concern (Monisha et al., 2014). Bisphenol A (BPA) is a type of xenobiotic or endocrine disrupting chemical (EDC) widely used in industry as...
a plasticizer (Lim et al., 2017; Von et al., 2010). EDCs are substances in the environment which may disrupt the normal function and development of body system (Miao et al., 2011).

The liver is one of the major organs for the detoxification of xenobiotics and metabolism, and is thus highly sensitive to effects of BPA even at low levels of exposure (Moon et al., 2012). EDCs bind to a nuclear receptor (NR) and interfere with a hormone action by altering the hormone responsive tissues (Diamanti-Kandarakis et al., 2009). According to the Center for Disease Control and Prevention (CDC), children are highly exposed and more susceptible to BPA than the adults (Diamanti-Kandarakis et al., 2009). In children, BPA exposure may cause liver abnormalities, diabetes, hormone and brain disruption (Brouard et al., 2016; Frederica & Julie, 2011). A study has shown that BPA can induce liver inflammation through the formation of reactive oxygen species (ROS) (Eid et al., 2015). ROS plays a key role in pathological conditions and can cause tissue damage, inactivation of many enzymes, and the alteration of receptor proteins involved with cell signaling (Prahalathan et al., 2004).

In this study, we investigated the effects of BPA on liver histo-architecture in pre-pubertal rats. The rats were exposed to BPA by oral gavage over a six-week period. Using an image analyzer, histological changes in the liver were measured to assess the degree of abnormalities at the cellular level. This study provides scientific information regarding the degree of disruptive effects of BPA on the liver, a major metabolizing and excretory organ for toxicants, particularly at the cellular level. In addition, this study is intended to create awareness among students, scholars, academics, and NGOs working with environmental issues about the effects of BPA on human health.

MATERIALS AND METHODS

Animals

The study was performed with 24 of prepubertal female Sprague Dawley (SD) rats at the age of 28 days (P28) with a range of 100-120 g of body weight, obtained from the Animal Resources Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. SD rats were chosen because they are relatively easy to handle and are widely used in toxicological experiments worldwide. At the Animal House, Comparative Medicine and Technology Unit (COMeT), Institute of Biological Science, the rats were maintained under standard laboratory conditions (temperature 25±2°C, 50±15% relative humidity and normal photoperiod of 12 h dark and 12 h light). They had ad libitum access to rat chow (Gold Coin Feedmills Pte. Ltd, Malaysia) and water. To minimize exposure to EDC, drinking water was provided in glass bottles with rubber stoppers surrounded by a steel ring.
Experimental Design

The experimental design and procedures were conducted under protocols in compliance with EU Directive 2010/63/EU that was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Approval no: UPM/IACUC/AUP-U004/2017). After one week of acclimatization, the rats were randomly divided into three groups (n=8 in each group). Group I (NC) served as control and received a treatment of palm oil alone at 0.2 ml. Group II (BPA LD-low dose) and group III (BPA HD- high dose) were treated with 10 and 100 mg/kg body weight of BPA, respectively. The dose selection of BPA at 10 mg/kg body weight was based on a previous study in which BPA was shown to induce histological changes on the liver at this dose (Richter et al., 2007). Additionally, the dose selection of 100 mg/kg body weight was intended to investigate the disruptive changes of BPA at a 10× higher dose. Administration was performed every day (between 09:00 am to 10:00 AM) by oral gavage (to mimic the most likely route of human exposure) for six consecutive weeks. After the last dose of treatment, all rats were sacrificed at the onset of the diestrous phase.

Histopathological Analysis of Liver

The formalin-fixed tissue (liver) was processed by dehydration in a graded series of ethanol, cleared by xylene, and infiltrated in paraffin using an automatic tissue processor (Leica 1020, Germany). Subsequently, these processed tissues were embedded in paraffin blocks at the embedding centre (Leica EG1150H & Leica EG1159C, Germany). Sections of about 5 μm thickness using microtome (Jung Multicut 2045, Germany) were deparaffinized in xylene, dehydrated in a graded series of ethanol, cleared in xylene and stained with Harris hematoxylin and eosin (H&E) for histological study. Histological analysis was conducted under a light microscope attached to an image analyser.

Statistical Analysis

All values have been expressed as median (IR) for eight animals (n = 8) in each group. Significant differences between the groups were determined using Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, USA, version 22.0) by performing Kruskal-Wallis test for multiple comparisons, followed by Mann-Whitney U-test to compare differences between two groups. A difference was considered significant at p < 0.05.

RESULTS AND DISCUSSION

Liver Weight

To determine whether BPA exposure induces toxicity effects, the liver weights of all rats were taken at the end of experiment (Table 1). Figure 1 shows the relative weight of the liver in all experimental groups. The significant decline in relative weight of liver (1.03%)
was observed in the HD group compared to the NC group after six weeks of BPA exposure. This is because some studies have shown that high doses of BPA exposure may alter liver weight in rats (Moon et al., 2012).

Table 1

<table>
<thead>
<tr>
<th>Weight of liver in all experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Liver wet weight (g)</td>
</tr>
<tr>
<td>Liver relative weight (wet weight (mg)/body weight (kg))</td>
</tr>
</tbody>
</table>

All values are expressed as the median (IR). There is no significant difference between all the groups. NC, control group; LD, low dose group (10 mg/kg); HD, high dose group (100 mg/kg).

Figure 1. Liver relative weight in all experimental groups NC, control group; LD, low dose group (10 mg/kg); HD, high dose group (100 mg/kg)

**Histopathological Findings of Liver**

Histopathological analysis of the representative sections of liver from all experimental groups are shown in Figures 2, 3 and 4. In Figure 2, a photomicrograph of a rat liver in control group shows normal limits feature where there is an absence of massive necrosis, congestion or widened sinusoids. The liver in the low dose of BPA 10 mg/kg (LD BPA group) shows an abnormally extended sinusoidal cavity, a feature of conspicuous Kupffer cell (KC), in addition to necrotic hepatocytes, granularly degenerated hepatocytes and dilated sinusoid (Figure 3). In the high dose of BPA 100 mg/kg (Figure 4), a photomicrograph of the liver shows massive loose spaces due to cytoplasmic vacuolation arising from hepatocytic degeneration, massive hepatocytes with either cytoplasmic vacuolation or granulation, and conspicuous Kupffer cells.
Bisphenol A Induces Histological Changes in the Liver

Figure 2. (a) Normal limit features were observed in control group (H&E, ×10). (b) Normal limit features except for the slightly more congested central vein (H&E, ×40) (NC group)

Figure 3. (a) Abnormally extended sinusoidal cavity (H&E, ×10). (b) Features of conspicuous Kupffer cell in addition to necrotic hepatocytes (arrow), granularly degenerated hepatocytes and dilated sinusoid (H&E, ×40) (LD group)

Figure 4. (a) Massive loose spaces due cytoplasmic vacuolation arising from hepatocytic degeneration (H&E, ×10). (b) Massive hepatocytes with either cytoplasmic vacuolation (blue arrow) or granulation (green arrow) and conspicuous Kupffer cells (arrow) (H&E, ×40) (HD group)
Bisphenol A (BPA) is used in the production of polycarbonate plastics and epoxy resins. Thus, BPA is present all around us in the environment. BPA is an endocrine disruptor that can imitate the body’s hormones, and it may disrupt the secretion, transport, action, production, function, and elimination of hormones (Von et al., 2010). Young children are highly sensitive to the BPA (Diamanti-Kandarakis et al., 2009). The liver is one of the major organs for the detoxification of xenobiotics and metabolism (Knaak & Sullivan, 1996). The liver is highly sensitive to BPA, even at low doses. For that reason, the present study has investigated the disruptive effects of BPA on the post-weaning of female rat (as an animal model) that occur in the liver.

According to previous studies, in vivo and in vitro animal data revealed that BPA is able to increase the ROS generation and disrupt the enzyme activity in rat livers, as well as induce cytotoxicity effect at high doses on rat hepatocytes (Diel et al., 2000; Hanioka et al., 1998). In toxicological studies, analysis of weight of selected organs is a very sensitive indicator for adverse effects of toxicants, as reflected in histological findings. The present study shows that the organ weight in the BPA-exposed rats was higher compared to the normal group. The result is in agreement with a previous study that found that fat starts to accumulate in the liver of BPA-exposed rats by disrupting the metabolic functions (Adams et al., 2009). In addition, another study has shown that high dose of BPA exposure can alter liver weight and decrease the viability of hepatocytes (Moon et al., 2012).

Some studies have revealed that BPA exposure in perinatally or juvenile-exposed animals can affect liver homeostasis, causing altered gene expression and accumulation of fat (Marmugi et al., 2012; Ronn et al., 2013). In this study, the administration of BPA resulted in severe hepatocytes necrosis, dilated sinusoid, and depicting features of conspicuous Kupffer cells. These results were similar to the acute and chronic effects of BPA as documented in previous studies (Marmugi et al., 2012; Ronn et al., 2013). Since the liver is the primary site for getting rid of xenobiotics, the observed increase in its weight in the present study may represent a homeostatic mechanism to deliver more BPA into the liver for detoxification. Liver enlargement may be due to a combination of hepatocyte hypertrophy and smooth endoplasmic reticulum proliferation, which is presumed to stimulate a hepatic physiological adaptation to an increased workload demand.

From the histological findings, morphological changes in the liver of BPA-exposed rats are associated with oxidative stress. BPA is a phenolic compound which may cause abnormalities, DNA damage, and genotoxicity in the liver of rats and mice (Iso et al., 2006). It has been evidently reported by previous studies (Marmugi et al., 2012; Ronn et al., 2013). A previous study stated that abnormal liver function and altered insulin homeostasis was found to associate with BPA (Abdel-Wahab, 2014). The hepatocytes are approximately 80% of the total liver (Kmiec, 2001). One of the functions of hepatocytes is the production of...
proteins, including albumin (25% of hepatic protein production), lipoproteins, globulins, clotting factors, and certain hormones (Talwar & Srivastava, 2002). When DNA is broken at late stages of necrosis, it is easier to distinguish from apoptotic cells on morphological grounds when morphological alterations are unambiguous. These may be explained by BPA-induced apoptosis in the liver cell. The greater extent of hepatocytes apoptosis seen in the liver sections of BPA-exposed rats is in agreement with previous studies.

In this study, histological findings revealed that even at low dose, the liver cells were affected. It has been observed that BPA can cause degenerative changes in the hepatic cells as observed by vacuolated hepatocytes, congested blood vessels, dilated sinusoids, Kupffer cell, and necrosis. The findings are in agreement with a previous study which found that BPA exposure led to membrane damage and cell rupture, necrosis, cell infiltration of erythrocytes, vacuolated hepatocytes (Hanioka et al., 1998).

CONCLUSION
Liver weight in the group that had been exposed to BPA was higher compared to the normal group because high doses of BPA exposure could alter liver weight in rats. Histological analysis revealed vacuolated hepatocytes, congested blood vessels, dilated sinusoids, Kupffer cell, and necrosis. It can be concluded that even at low dose exposure of environmentally relevant concentrations, BPA can cause significant histological alterations in the liver that consequently may cause deleterious hazardous effects on human health. Moreover, exposure to high dose of BPA could have severe effects on the liver. Awareness should be built among stakeholders including students, scholars, academics, and NGOs working with environmental issues about the effects of BPA on human health. Furthermore, the results of the present study suggest that in a population of high use of plastics where there is a chance of exposure to BPA, the population may suffer adverse health effects. Thus, the use of BPA in other industries and plasticizers should be limited, and incorrect handling of plastic containers must be avoided, to reduce the health risks resulting from exposure to endocrine disruptors such as BPA.

ACKNOWLEDGEMENTS
The first author would like to express special thanks to Assoc. Prof. Dr. Goh Yong Meng from the Faculty of Veterinary for financial support, and Prof. Dr. Noordin for his guidance in histopathology examination. A special appreciation should be given to Dr. Hafidz bin Mohd Izhar and En. Mohamad Ismail bin Baharom from the Comparative Medicine and Technology Unit (COMeT) who provided animal room facility, extensive training and consistent guidance for this study.
REFERENCES


