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ARTICLES

Research Articles

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Whole lung lavage therapy: Treatment for lung injury caused by paraquat poisoning

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Paraquat poisoning is characterized by multi-organ failure and pulmonary fibrosis with respiratory failure, resulting in high mortality and morbidity. To serious paraquat patients, the effectiveness of conventional treatments is unsuccessful. Whole lung lavage is a technique that was developed in the 1960s with the purpose of removing lipoproteinaceous material that accumulates in the bronchi of patients with alveolar proteinosis, leading to clinical and functional improvement. Pneumoconioses are characterized as irreversible, progressive respiratory diseases. No effective therapy exists to prevent progression of these diseases. Whole lung lavage might limit the rate of disease progression through the removal of dust, inflammatory cells, and cytokines. Whole lung lavage is also used successfully to treat other lung diseases such as endogenous lipoid pneumonia and mineral oil lipoid pneumonia. Paraquat poisoning could not be controlled by only one method and combined therapies are needed. So, we hypothesized that whole lung lavage will provide a new therapy of acute lung injury caused by paraquat. On the base of conventional therapy for paraquat poisoning, whole lung lavage could be considered in the early time of poisoning and then followed by glucocorticoid for patients with moderate to severe paraquat poisoning.

Key words: Paraquat, poisoning, acute lung injury, whole lung lavage.

INTRODUCTION

Paraquat is one of the most widely used herbicides in the world, and has been approved for use by authorities in more than 120 countries, and plays an important role in controlling weed in plantation estates. It is very popular in China countryside and widely used by Chinese farmers (Jian et al., 2008). On the other hand, paraquat is also a lethal poison. In China, paraquat is available and inexpensive, making poisoning prevention difficult. However, most of the people who become poisoned from paraquat have taken it as a means of suicide. So, paraquat is also a controversial herbicide, for it is highly toxic for humans (Kan et al., 2012). Intentional self-poisoning is the major reason for paraquat exposure and usually causes serious consequences in China (Shi et al., 2012). Paraquat poisoning is characterized by multi-organ failure and pulmonary fibrosis with respiratory failure,
resulting in high mortality and morbidity (Weng et al., 2012). Conventional therapy for paraquat poisoning both prevents further absorption and reduces the load of paraquat in the blood through haemoperfusion or haemodialysis. To serious paraquat patients, the effectiveness of standard treatments is unsuccessful (Liu et al., 2011). Paraquat mainly accumulates in the lung, and the main molecular mechanism of paraquat toxicity is based on redox cycling and intracellular oxidative stress generation (Huang et al., 2011). Immunosuppressive treatment using glucocorticoid and cyclophosphamide in combination is being developed and studied. But the effects of glucocorticoid with cyclophosphamide for patients with moderate to severe paraquat poisoning is limited (Li et al., 2010). Paraquat is actively taken up against a concentration gradient into lung tissue leading to pneumonitis and lung fibrosis. Paraquat also causes renal and liver injury. Activated charcoal and Fuller's earth are routinely given to minimize further absorption. Antioxidants such as acetylcysteine and salicylate might be beneficial through free radical scavenging, anti-inflammatory and NF-κB inhibitory actions. However, there are no published human trials. The case fatality is still very high in all centres despite large variations in treatment (Gawarammana et al., 2011).

WHOLE LUNG LAVAGE

Whole lung lavage is a technique that was developed in the 1960s with the purpose of removing lipoproteinaceous material that accumulates in the bronchi of patients with alveolar proteinosis, leading to clinical and functional improvement. There has been an evolution in the technique; initially, it was performed under local anesthesia to each segment of the lung and currently it is performed under general anesthesia sequentially to both lungs (Aguiar et al., 2009). In brief, it involves the induction of general anesthesia followed by isolation of the two lungs with a double-lumen endotracheal tube and performance of single-lung ventilation, while large-volume lavages are performed on the non ventilated lung. Warmed normal saline solution in 1-L aliquots (total volumes up to 20 L) was instilled into the lung, and chest physiotherapy was performed. The proteinaceous effluent is drained with the aid of postural positioning. The sequence of events was repeated until such time as the effluent becomes clear. This procedure results in significant clinical and radiographic improvement secondary to the washing out of the proteinaceous material from the alveoli (Michaud et al., 2009). Whole lung lavage is considered the golden standard of pulmonary alveolar proteinosis treatment (Stoica et al., 2012; Rebelo et al., 2012). However, not all patients respond to this treatment. Based on the current literature, a stepwise treatment plan is suggested starting with WLL, continuing to inhaled GM-CSF, and then to rituximab if the former treatment regimes are unsuccessful (Leth et al., 2013; Yamamoto et al., 2008). Some authors think that the whole-lung lavage is a safe and effective palliative procedure in pulmonary alveolar proteinosis and in the treatment of patients with pulmonary disease, such as cystic fibrosis or asthma, in which filling of the lung acini by liquid or solid material impairs oxygenation of the pulmonary capillary blood (Lippmann et al., 1977). Pneumonitis is another lung disease that used whole-lung lavage as one of the major therapy in China (Zhang et al., 2012). Pneumoconioses are characterized as irreversible, progressive respiratory diseases. No effective therapy exists to prevent progression of these diseases. Whole lung lavage might limit the rate of disease progression through the removal of dust, inflammatory cells, and cytokines. Whole lung lavage is also used successfully to treat other lung diseases such as endogenous lipid pneumonia and mineral oil lipid pneumonia (Nicholson et al., 2002; Ceruti et al., 2007; Chang et al., 1993; Ciravegna et al., 1997).

HYPOTHESIS

Paraquat poisoning is an extremely frustrating clinical condition with a high mortality and with a lack of effective treatments in humans up to now. It is impossible for us stop person making use of paraquat, but some new therapy must be considered to control paraquat poisoning. Paraquat poisoning could not be controlled by only one method and combined therapies are needed (Lin et al., 2011). So, it was hypothesized in this study that whole lung lavage will provide a new therapy of acute lung injury caused by paraquat. Conventional therapy for paraquat poisoning both prevents further absorption and reduces the load of paraquat in the blood by using gastric lavage, catharsis, activated carbon adsorption, Fuller’s earth inactivation, transfusion, dieresis, antioxidant, haemoperfusion or haemodialysis, etc. On the base of the earlier mentioned methods, whole lung lavage could be considered in the early time of poisoning and then followed by glucocorticoid for patients with moderate to severe paraquat poisoning.

DISCUSSION

Deliberate self-harm with pesticides is a significant public health problem in rural China. Even though many paraquat poisoning cases died in the past ten years in China, the pathological mechanisms of paraquat poisoning-induced acute lung injury were not well understood. A lot of clinical and basic research work has been done on paraquat poisoning in the past decade in our department (Zhao et al., 2010; Ning et al., 2010). This study developed and characterized a mouse model of paraquat-induced acute lung injury and studied the role of
cytokines in the pathogenesis of paraquat poisoning (Xiangdong et al., 2011). Acute lung injury is characterized by three consecutive phases: exudative, proliferative, and fibrotic. In the exudative phase alveoli contain proteinaceous fluid, red blood cells, neutrophils, and macrophages. Edema and neutrophils accumulate in the interstitium, and alveolar ducts contain hyaline membranes. Microatelectasis is present, endothelial cells are swollen, and focal destruction of endothelial cells occurs (Meduri et al., 1996). The pathogenesis of acute lung injury involves various cytokines and growth factors (Gauldie et al., 1993). In the exudative phase, a number of presentations addressed the importance of the early response of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and interleukin (IL)-6 for their role in initiation of inflammation. The release of these cytokines into the alveolar space with diffusion to the vascular space in turn triggers diverse effects, including activation of the endothelium and circulating and resident leukocytes (Baughman et al., 1996; Metz et al., 1991). Studies have provided evidence for the importance of these cytokines in the pathogenesis of acute lung injury induced with paraquat (Ishida et al., 2006; Satomi et al., 2004). Abnormal expression of inflammatory cytokines is believed to play an important role in the pathogenesis of pulmonary fibrosis. So, thorough removal of inflammatory cytokines maybe a useful strategy to prevent further injury to the lungs. Whole lung lavage can remove the inflammatory seepage content from the lung tissue of paraquat poisoning at early stage, especially inflammatory factors. Therefore, it is recommend along with conventional therapies in the treatment of acute paraquat poisoning.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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injury induced by paraquat in a rat model. Hum. Exp. Toxicol. 30: 460-469.
Bisphenol A induced reactive oxygen species (ROS) in the liver and affect epididymal semen quality in adults Sprague-Dawley rats

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Reactive oxygen species (ROS) generation, induced by Bisphenol A (BPA) may cause mammalian sperm damage according to research findings. BPA is a known contaminant that with increased exposure in the body can exert both toxic and estrogenic effects in mammalians cells. The aim of this study was to evaluate the effect of BPA-induced oxidative stress in the liver on epididymal semen quality in adult rat. BPA was mixed in corn oil and intra-peritoneally administered for 20 days in dose dependent manner. After 24 h of the last treatment, rats were weighed, sacrificed and organs harvested for analysis. BPA caused a reduction in the epididymal semen quality and sperm count in a dose dependent manner. Sperm analyses results showed that there was oligozoospermia (<20 × 10⁶ spermatozooids/ml) and asthenozoospermia (motility <50%) in the treatment group compared to the control groups. The levels malondialdehyde (MDA) and superoxide dismutase (SOD) increased significantly in the treatment group compared to the control group (P < 0.05; P < 0.01, respectively). While, the levels of glutathione peroxidase (GSH-Px) decreased in the treatment group compared to the control group (P < 0.01). These results indicate that exposure of graded doses of BPA may elicit depletion of antioxidant system and induce oxidative stress in epididymal sperm of rat thereby decreasing sperm count and quality. These findings provide a possible toxicological evidence of an adverse effect of BPA on semen quality.

Key words: Bisphenol A (BPA, 2, 2-bis (4-hidroxyphenyl) propane), semen quality, oxidative stress, sperm count, rat, reactive oxygen species (ROS).

INTRODUCTION

During the last two decades, it has become evident that environmental contaminants disrupt male reproduction in wildlife and humans and play an important role in the decline of quality and quantity of human semen. Bisphenol A [BPA: 2, 2-bis (4-hydroxyphenyl) propane] is a well known estrogenic endocrine disruptor used as a
epoxy resin lining of canned foods; it is also released from epoxy resin lining of canned foods, beverages, dental sealants and a multitude of consumer products (Vandenberg et al., 2009). The detection of BPA in biological fluids like maternal plasma, fetal plasma, placental tissue, amniotic fluid and umbilical cord blood have indicated that it can easily transverse the placental barrier (Tsutsumi, 2005; Vandenberg et al., 2007). Numerous toxicological studies have shown that rodents exposed to BPA during the prenatal and perinatal period show a marked negative change in the reproductive system, including decreased epididymal weight and daily sperm production (vom Saal et al., 1998; Salian et al., 2009a,b), and an increase in prostate weight (Nagel et al., 1997). Similarly, BPA was also reported to significantly increase anogenital distance (AGD) and prostate weight, and decrease epididymal weight in postnatal offspring among CD-1 mice fed BPA at 50 μg/kg on day 16 to 18 of pregnancy (Gupta, 2000). Moreover, it interferes with the function of androgen receptors and the production of male sex hormones (Richter et al., 2007; vom Saal et al., 2007). Recently, high risk of male sexual dysfunction associated with exposure to BPA has been reported (Li et al., 2010a, b). Concurrently, other studies have reported that exposure to low doses of BPA causes reproductive toxic effects (Nagel et al., 1997; vom Saal et al., 1998). A significant decrease in the efficiency of sperm production and a constant increase in weight were also observed when male rats were fed BPA at 20 μg/kg, and after feeding pregnant CF-1 mice with BPA at 2 and 20 μg/kg on days 11 to 17 of pregnancy, a decrease in epididymal weight was observed among their offspring up to six months after birth (Cagen et al., 1999). In addition, vom Cooke et al. (1998) demonstrated toxic effects and reduced daily sperm production per gram of testes in male offspring of mice fed with BPA and octylphenol during pregnancy. One cannot rule out the possibility that BPA can affect spermatogenesis in rodents; problems with spermatogenesis are associated with varying degrees of oxidative stress (Menezo et al., 2007) and environmental factors in general have doubtlessly been identified as likely causes of these disorders.

BPA has been shown to cause injury in the liver, kidney, brain, epididymal sperm in rodents and other organs by forming reactive oxygen species (ROS) (Bindhumol et al., 2003; Chitra et al., 2003; Kabuto, 2003; Kabuto et al., 2004). The liver has a range of antioxidant defense systems. ROS are scavenged by the endogenous antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in cells. ROS have been shown to play an important role in the defense mechanisms against pathological conditions, but excessive generation of free oxygen radicals may damage tissues (Kitas et al., 1991). The fundamental notion that spermatozoa could generate ROS, specifically hydrogen peroxide, was confirmed by Tosic and Walton (1946). However, Aitken and Clarkson (1987) demonstrated that the notion that oxidative stress might also be factor in the etiology of defective sperm function in our species (Alvarez et al., 1987).

BPA is currently a very controversial subject; any factor that exacerbates the production of oxygen free radicals in the mitochondria is a major source of oxidative stress and apoptosis among sperm cells. Several studies have shown BPA effect on the ARs, on male sex hormone levels, on male reproductive organs including testes, epididymal sperm and seminal vesicles and prostate gland, and on sperm production (Richter et al., 2007; National Toxicology, 2008). However, the mechanisms of the adverse effect on semen quality are not yet completely understood. The liver has a range of antioxidant defense systems. The purpose of this study was to evaluate the relationship between the presence of oxidative stress indicators in the liver during exposure to BPA and its effects on sperm quality.

MATERIALS AND METHODS

Animals

Twenty four healthy male Sprague-Dawley rats (50-days olds, weighing 170 to 185 g) were purchased from the Tongji Medical College Animal Laboratory (Wuhan, China) and kept in accordance with the Guide for the Care and Use of Laboratory Animals published by Ministry of Health of People’s Republic of China (Permit Number: 2011-s2456).

Treatments

The animals were housed in plastic cages under a well-regulated light and dark schedule (12 h light:12 h dark) at 24±3°C, humidity (50 ± 5%) environment, and free access to chow and tap water ad libitum. The rats were randomly divided into four groups, each group containing six rats. Each group (e.g. control group, low dose group, middle dose group and high dose group) was fed different doses of BPA: 0, 2, 10, and 50 mg/kg body weight, respectively in corn oil every other day by intra-peritoneal injection for 20 days. After 20-days of treatment, the rats were sacrificed; the testes, epididymis, seminal vesicles and ventral prostates were removed, freed of the adhering tissues and weighed. Ethical clearance for the use of animals in the study was obtained from the Institutional Animal Ethics Committee prior to the initiation of the study, and the experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals published by Ministry of Health of People’s Republic of China.

Dose selection and preparation

The doses and time used for the present study were derived from published data (You et al., 1998; Yamasaki et al., 2009) and the results of our preliminary experiment. BPA was dissolved in a in corn oil to obtain the desired concentration of BPA dose range, that is, 0, 2, 10 and 5 mg/kg. An additional control group that had received only corn oil. Dose formulations were well mixing and stored in crystal bottles at 37°C overnight and were subsequently kept at room temperature throughout the study. Solutions were mixed thoroughly before use.
Chemicals and reagents

BPA (2,2-Di (4-hydroxyphenyl) propane) was purchased from DR Co., Augsburg, Germany, purity: 98.5%. Corn oil was obtained from Sigma-Aldrich, St. Louis, MO, USA. Sigma Chemical Co. (St. Louis, MO) USA. Collagenase, Trypsin-EDTA were obtained from GIBCO (Grand Island, NY, USA), sodium lauryl sulphate from SRL. Eosin stain, Hematoxylin stain, Orange G stain from HiMedia (Mumbai). GSH-Px, MDA and SOD assay kit (Jiancheng Bioengineering Ltd., Nanjing, China).

Body weight and organ collection

The weight of each animal was recorded every two days and any gross abnormality was noted. The animals were fasted overnight, weighed and killed by cervical dislocation. Testes, epididymis, liver and other organs, were isolated from adhering tissues and weighed independently. The liver and testes were quickly frozen at -70°C for later use for biochemical assays, while epididymal sperm was used immediately for sperm analysis (CASA).

Parameters of oxidative stress

Glutathione peroxidase (GSH-Px) activity, SOD activity and malondialdehyde (MDA) level were measured.

The liver was homogenized using lysis buffer (containing 1 mM Na2EDTA, 150 mM NaCl, 10 mM PMSF, 10 mM Tris, 1 mM aprotin). The homogenates was centrifuged at 10,000 rpm at 10 min at 4°C and the supernatant was recovered for use to evaluate oxidative stress following the protocol of GSH-Px, SOD and MDA assay kit (Jiancheng Bioengineering Ltd., Nanjing, China).

GSH-Px

GSH-Px activities were assayed by quantifying the rate of oxidation of the reduced glutathione to the oxidized glutathione by H2O2. One unit of GSH-Px was defined as the amount that reduced the level of GSH by 1 µM in 1 min/mg protein at 412 nm absorbance.

MDA

MDA level were assessed to determine the concentration of MDA, measuring thiobarbituric acid (TBA) reacting substances at 532 nm. The level of MDA was expressed as nmol MDA per milligram protein. Protein content was measured according to Bradford method.

SOD

SOD activity in supernatant was determined by determining the reduction of nitro blue tetrazolium (NBT) by O2⁻ produced from the xanthine-xanthine oxidase system. One unit of SOD was defined as the amount of protein inhibited in the rate of NBT reduction by 50%. Results were defined as U/mg protein.

Analysis of semen quality

Semen quality analysis was performed simultaneously using the CASA system (CFT-9200 computer-aided sperm and microorganism test and analysis system). After the animals were sacrificed, the epididymis was immediately removed and the tissues were minced with surgical scissors to extract the sperm cells into 2 ml of 0.9% NaCl solution at 37°C and kept for 15 min to allow the sperm to disperse. The sperms were counted with CASA to evaluate the specific parameters of sperm quality, sperm motility, density and motion including beat cross frequency VCL, straight line velocity (VSL), average path velocity (VAP), linearity (LIN=VSL/VCL), and straightness (STR=VSL/VAP). The CASA settings were followed according to the manufacturer’s instructions.

Morphology and sperm normality criterion

A small amount of sperm suspension was smeared on to a slide using a pipette and fixed with methanol; after drying for 10 min, it was stained with 2% Eosin for 1 h. Each of the stained slides was analyzed. The images were captured by a color by light microscopy (Olympus IX-71, Tokyo, Japan) for high quality image production. Morphological evaluation was accomplished on a monitor screen and the total calculated magnification was (×400). For a spermatozoon to be considered normal, the sperm head, neck, mid piece and tail must be considered normal. The head should be oval in shape. The percentage of normal sperm cells was calculated. It showed normal looking hook-shaped heads and the shape and thickness of the tail was thin uniform. Abnormal sperm cells included headless and hookless cells; amorphous shapes and forms; folded, short and double Y tail and other aberrations.

Statistical analysis

Data were presented as the mean ± standard error of mean (SEM) and were analyzed using the GraphPad PrismTM software version 5.0 (San Diego, USA) and SPSS statistical package 17.0 (SPSS Inc, Chicago, IL, USA). Comparison of means for treatment and control groups were done by independent-sample T-test. semen quality analysis was performed simultaneously using the CASA system (CFT-9200 computer-aided sperm and microorganism test and analysis system). Levels of significance were set at P ≤ 0.05.

RESULTS

The results are illustrated as shown in Figure 1. The body weights of BPA-treated rats did not show significant changes as compared to the corresponding control groups except for a slight difference with the low dose group. The same behavior was observed in testicular weight; in this case, a significant difference was observed (P < 0.05) (Figure 2). However, the weights of the liver, decreased significantly when the concentration of BPA was gradually increased to 50 mg/kg, P < 0.05 (Figure 3). Among the BPA treated rats, the activities of superoxide dismutase (SOD), malondialdehyde (MDA) increased significantly (**P < 0.01, *P < 0.05), respectively (Table 1). A dose dependent decrease in the levels of glutathione peroxidase (GSH-Px) was observed in response to BPA treatment when compared with the control group (**P < 0.01) (Table 1).

Effect of BPA on sperm counts

Figure 4 demonstrates the results obtained after exposure to BPA on epididymal sperm counts of adult male rats. Outcomes according to the percentage were strictly
strictly normal morphology. Total sperm counts were reduced at all doses, but whilst a significant decrease was observed at a dose of 50 mg/kg. The semen characteristics from a total of twenty four fresh semen samples were examined by CFT-9200 computer-aided sperm analyzer (Table 2 and Figure 5). The mean ± standard deviation (SD) of total sperm concentration, density, motility, and sperm motion variables (LIN=VSL/VCL and STR=VSL/VAP; P > 0.05) were analyzed by SPSS Student’s t-test.

Sperm morphology

After observation under the microscope, a significant reduction in the number of normal sperm was observed compared to the control group (Table 2). Sperm analyses showed oligozoospermia (<20 x 10^6 spermatozoids/ml) and asthenozoospermia (progressive motility <50%) in all groups treated by BPA including control groups. Meanwhile, in the 2, 10, and 50 mg/kg dose groups, percentage of sperm normality decreased gradually to 15.00, 6.50 and 2.33%, respectively; compared with the control group, the differences were statistically significant (P < 0.05 and P < 0.01). Finding on sperm abnormalities showed that, headless sperm cells were the most common abnormality followed by amorphous cells, bent tail, coiled tail, pyriform head abnormal midpiece detached head and highly unusual double tail (Figures 4 and 5). Sperm with deformed heads were observed in all four groups.

Figure 1. Effect of BPA on body weight of adult SD rats at 20 days. Data represent as means ± standard error of mean (SEM) (n= 6 rats per group). *P < 0.05 denotes significant difference when compared with controls.

Table 1. The effect of BPA on antioxidant enzymes, GSH-Px, MDA and SOD on rat SD liver tissues.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>n</th>
<th>GSH-Px (U/mg prot)</th>
<th>MDA (nmol/mg prot)</th>
<th>SOD (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>47.83 ± 4.08</td>
<td>2.47 ± 0.39</td>
<td>49.94 ± 2.82</td>
</tr>
<tr>
<td>Low 2 mg/kg</td>
<td>6</td>
<td>42.93 ± 2.83*</td>
<td>2.85 ± 0.18</td>
<td>54.81 ± 6.61*</td>
</tr>
<tr>
<td>Middle 10 mg/kg</td>
<td>6</td>
<td>37.13 ± 2.17*</td>
<td>3.30 ± 0.82</td>
<td>65.71 ± 8.74**</td>
</tr>
<tr>
<td>High 50 mg/kg</td>
<td>6</td>
<td>29.16 ± 2.35**</td>
<td>3.32 ± 0.72*</td>
<td>73.38 ± 6.97**</td>
</tr>
</tbody>
</table>

Effect of BPA on the activity of the antioxidant enzymes, glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and superoxide dismutase (SOD) in liver tissues. Data are presented as the mean ± standard deviation (SD). *Indicate significant change compared with control group, by means of Independent-Samples T test. *P < 0.05 versus control; **P < 0.01 versus control.
Table 2. This table shows caudal epididymal semen characteristics in the experimental adult male rats SD using CASA CFT-9200.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=6)</th>
<th>Low 2 mg/kg (n=6)</th>
<th>Middle 10 mg/kg (n=6)</th>
<th>High 50 mg/kg (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (M/ml)</td>
<td>21.23 ± 2.44</td>
<td>20.02 ± 1.51</td>
<td>12.35 ± 2.62*</td>
<td>9.33 ± 2.77**</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>55.21 ± 6.57</td>
<td>44.82 ± 9.86</td>
<td>34.72 ± 3.04**</td>
<td>32.51 ± 5.88**</td>
</tr>
<tr>
<td>VSL (µm/s)</td>
<td>40.30 ± 6.07</td>
<td>30.53 ± 7.67**</td>
<td>34.42 ± 3.66</td>
<td>34.89 ± 2.04</td>
</tr>
<tr>
<td>VCL (µm/s)</td>
<td>19.82 ± 6.15</td>
<td>15.65 ± 4.02</td>
<td>19.09 ± 6.17</td>
<td>18.41 ± 4.50</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>20.85 ± 6.08</td>
<td>17.96 ± 5.18</td>
<td>22.93 ± 6.35</td>
<td>21.56 ± 3.03</td>
</tr>
<tr>
<td>LIN</td>
<td>2.18 ± 0.75</td>
<td>2.05 ± 0.66</td>
<td>1.93 ± 0.48</td>
<td>1.98 ± 0.41</td>
</tr>
<tr>
<td>STR</td>
<td>2.05 ± 0.53</td>
<td>1.72 ± 0.17</td>
<td>1.57 ± 0.31</td>
<td>1.64 ± 0.17</td>
</tr>
</tbody>
</table>

Data represent as means ± SEM (n= 6 rats per group). *P ˂ 0.05 and **P ˂ 0.01 denotes significant difference compared with controls.

Figure 2. Effect of BPA on weight of the testis of adult SD rats. Data represent as means ± standard error of mean (SEM) (n= 6 rats per group). *P < 0.05 denotes significant difference when compared with controls.

The groups, but most notable in the groups treated with BPA (10 and 50 mg/kg). As related to tail abnormalities, some had no flagella, and others had proximal and distal cytoplasmic droplets.

DISCUSSION

It has been shown that BPA may have effect on liver enzymes and also affect sperm quality. Less is known about effects of BPA on the liver, and there are only a few animal studies done to show for instance formation of DNA adducts and impaired mitochondrial functioning (Ronn et al., 2013) exposure route. These studies are not compared with our study. Our study suggests that ROS may be associated with biochemical markers of liver damage. In this study, a weight gain was observed during the administration of BPA as well as testicular volume of rats which was statistically significant between low dose and control groups (P ˂ 0.05). BPA has been reported to interfere with the function of Leydig cells resulting in a reduction of testosterone biosynthesis (Akingbemi et al., 2004).

In this study, it was observed that BPA treatment did not affect the body weight of rats except the low dose, and testis. In the liver, a decrease in weight of rats treated with BPA compared with those of control group was observed (*P < 0.05) (Figure 3). Thus, the tissues (liver)
antioxidant evaluation seems to have important role in the etiology of semen quality. The levels of enzymatic antioxidants GSH-Px, SOD and MDA activity were determined to evaluate the stability of ROS production in liver. In this study, the BPA (10 to 50 mg/kg) groups significantly increased in MDA and SOD in liver tissues. Whilst GSH-p decreased GSH-Px levels in liver tissues. This reduction in activities of antioxidant enzymes shows the failure of primary antioxidant system to act against free radicals. Decrease in the activity of GSH-Px indicates either reduced synthesis, may be elevated degradation or inactivation of the enzyme and excessive ROS production. So, the GSH-Px deficiency can result in the emergence of morphologic abnormalities in sperm cell mitochondria (Imai et al., 2001; StradaioI et al., 2009). The increase in the activity of SOD, may be due to higher enzyme activity, but do not mean better antioxidative protection of spermatozoa. The over expression of SOD may reflect a defect in the development or maturation of spermatozoa, as well as sperm cellular damage, resulting in decreased sperm fertilization potential (Sinha et al., 1991; Gavella et al., 1996). The beneficial effect of SOD activity may concern only sperm movement, but has no influence on sperm count. It has been reported that there is relationship between the rate of lipid peroxidation and some morphological characteristics of spermatozoa, such as motility loss (Alvarez et al., 1987) or occurrence of midpiece defects (Rao et al., 1989; Aitken et al., 1993), which could explain the distortion level of sperm flagella in our study (Figure 5).

At doses 10 and 50 mg/kg of BPA, there was decreased epididymal sperm count which may have been due to increased MDA (Thiele et al., 1995; Bindhumol et al., 2003). It is well known that sperm cell membranes are rich in polyunsaturated fatty acids and are very susceptible to free radical attack. Lipid peroxidation affects the sperm concentration, motility, morphology and related with poor sperm quality. Increased MDA level might represent the pathologic lipid peroxidation of spermatozoa membrane and inhibition of sperm motility (Hsieh et al., 2006) which may corroborate our findings of the low percent active sperm motility in rats. MDA is one of the major end products of lipid peroxidation, especially the poly-unsaturated fatty acid peroxidation. Lipid peroxidation is used to monitor the oxidative stress in cells and tissues and it is a well developed way of describing cellular injury which causes endothelial damage, vascular inflammation and cell membrane injury (Subermaniam et al., 2014). Other studies have shown that BPA in combination with carbohydrates can affect fat mass or liver fat content during prenatal and perinatal periods (Marmugi et al., 2012; Ronn et al., 2013). In rats, the main route of elimination of conjugated BPA is by biliary and fecal elimination which enables enterohepatic recirculation.
Figure 4. Effect of BPA on the epididymal sperm count of adult rats SD. (A) Outcomes according to the percentage strictly normal morphology. (B) Mean and standard error of normal sperm cells (%) of the semen of adult rats SD after 20 days treatment with BPA.

Data represent as means ± SEM (n= 6 rats per group). *P < 0.05 and **P < 0.01 denotes significant difference compared with controls.

(Volkel et al., 2002). Atkinson and Roy (1995) have reported that BPA accumulates in fatty tissues and is metabolized to 5-hydroxybisphenol by Cytochrome P-450 dependent enzymes and further converted to 4,5-bisphenol-O-quinone. Cytochrome P-450 has been shown to induce ROS that permanently impairs sperm function thereby resulting in decline of sperm counts in men and laboratory animals. Cytochrome P-450 once activated, inactivates, and facilitates the excretion of most xenobiotics, thus modulating the intensity and duration of their toxicity (Aitken et al., 1989) such as drugs and environmental chemicals as well as endogenous compounds such as steroids and fatty acids (Hanioka et al., 2000).

It has been shown that CASA is likely to be of greater value in predicting male fertility than the routine semen examination (Suzuki et al., 2002). The common sperm parameters of CASA have shown significant correlation of sperm concentration in all groups treated with BPA against control group (Table 2). Observed values are below the values of references of semen analysis (Cooper et al., 2010). Sperm density <20 × 10^6/ml, sperm motility <50%, VCL <70 µm/s in most cases except VSL >25 µm/s; this result is similar to the findings of a previous in vivo study on murine, but they are different with respect to the dose and time of exposure to BPA (Ashby et al., 2003; Bindhumol et al., 2003) and in humans (Meeker et al., 2010; Li et al., 2011). Majority of the epididymal sperm from adult rat had normal morphology (77.44%). This study strictly considered only the percentage normal morphology to be the outcomes. However, there was a decrease in the epididymal sperm count among the animals treated with BPA dose dependent manner (Figure 4). The morphological study showed abnormalities related to spermatozoon. The most abundant abnormalities were bent tail, coiled tail, detached head and a highly unusual double tail (Figure 5; M). The coiled tail was seen in control group and low dose group (Figure 5; C, L). An increasing number of kinked sperm were seen in these rats treated with BPA when compared with the control group (Figure 5, C). Additionally, an increase in detached head sperm was seen in rats treated with BPA of 50 mg/kg (Figure 5, H). These abnormalities may be attributed to damage of DNA by BPA during the process of spermatogenesis. This study provides toxicological evidence that exposure to BPA has an adverse effect on semen male rat. Also, the interesting remark in our study was the observation of significant difference in the sperm morphology between the groups treated with BPA against the control groups. The high prevalence of
oxidative stress in the spermatozoa may have effect on male infertility and implications in reproductive health. High ROS in the liver due to high dose of BPA could cause damage to sperm production and fertility and need to be taken into consideration when handling the interpretation of such results.

Conclusion

Conclusively, this study provides evidence that exposure of adult male rats to low dose of BPA induces oxidative stress in the liver, and impairs spermatogenesis through decreasing epididymal sperm count. However, the differences between humans and animals in terms of kinetics may make it difficult to transpose the effects observed in animals to humans directly. The analyzed semen parameters using CASA might be useful in planning the strategy of screening for semen quality. A clear understanding of the potential mechanisms of observed adverse effects of BPA exposure in the liver and on male reproductive organs including semen quality may help to explain the observed abnormalities and exploration of future treatments.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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