Songklanakarin J. Sci. Technol.
44 (1), 223-230, Jan. - Feb. 2022

http://www.sjst.psu.ac.th

Original Article

Can polydatin have a beneficial role against bisphenol an exposure in rats?

Recep Aslan[1*], Hasan Hüseyin Demirel[2], Fatih Avdatek[3], Aziz Bülbül[4], Abdullah Eryavuz[1], and Mehmet Şükrü Gulay[5]

[1] *Department of Physiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, 03200 Turkey*

[2] *Bayat Vocational School, Afyon Kocatepe University, Afyonkarahisar, 03200 Turkey*

[3] *Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, 03200 Turkey*

[4] *Department of Physiology, Faculty of Veterinary Medicine, Muğla Sıtkı Kocman University, Muğla, Milas, 48000 Turkey*

[5] *Department of Physiology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, 15030 Turkey*

Received: 15 April 2021; Revised: 28 June 2021; Accepted: 2 July 2021

Abstract

The present study was conducted to evaluate the effects of chronic bisphenol A (BPA) exposure and the possible protective effects of polydatin (PD) on different organs, oxidant-antioxidant status and spermatological parameters in male Wistar rats. Male Wistar rats (2 to 3 months old) were randomly divided into 5 groups (n = 6). Group 1 was the control (daily 0.5ml distilled water). Groups 2, 3, 4 and 5 daily received 0.5 ml olive oil (OO), 25 mg/kg bisphenol A in 0.5 olive oil (BPA), 150 mg/kg polydatin (PD), and a mixture of 25 mg/kg bisphenol A in 0.5 olive oil + 150 mg/kg polydatin (BPA-PD), respectively, daily for 45 days. BPA had negative effects on percent abnormal sperm and Hypo-osmotic swelling-Eosin (HE) test ($P<0.01$). BPA caused negative histopathological alterations to the brain, heart, liver, kidney, and testis tissues. PD administration was able to ameliorate the negative effects of BPA. Moreover, PD had positive effects on percent motility ($P<0.02$), head defects of sperm ($P<0.01$), and HE test ($P<0.01$) when compared to the controls. The results indicate that PD decreases BPA-induced sperm defects and repaired tissue damage in the various organs in rats.

Keywords: BPA toxicity, oxidative stress, polydatin, spermatological parameters

1. Introduction

2,2 - bis (4 - hydroxyphenyl) propane, commonly known as bisphenol A (BPA), is one of the major chemicals used in the plastics industry (Rochester, 2013). About 70 % of BPA is used in the production of polycarbonate plastics such as automotive, electronics, household appliances, medical products, and packaging materials. Additionally, approximately 20 % of the BPA is used in epoxy resins to coat the inner surface of metal beverage cans and food packaging (Zalko *et al*., 2011).

BPA is a very important toxic pollutant that is used worldwide. As a result, BPA has been a major concern over

*Corresponding author
Email address: raslan@aku.edu.tr

the past decades due to the risk of food exposure from BPA-coated containers or plastics (Czub, 2011). Exposure to BPA occurs through environmental (contaminated air and water), local (household products, cosmetics), medical (contaminated equipment and devices), and occupational sources (inhalation, skin contact, industrial use, or swallowing associated with a production process). Consequently, it usually enters the body through contaminated food or water (Choi, Ha, & Kim. 2017; Demierre, Peter, Oberli, & Bourqui-Pittet, 2012). Thus, people and animals can be exposed to a small amount of BPA (Zalko *et al*., 2011). The US Environmental Protection Agency has set the maximum dose for BPA as 50 µg/kg/day (Leranth, Hajszan, Szigeti-Buck, Bober & MacLusky, 2008). However, more recently tolerable daily intake (TDI) for BPA was recommended as 4 µg/kg/day by the European Food Safety Authority (EFSA, 2015).

It has been previously reported that BPA may cause many diseases such as diabetes, obesity, cardiovascular problems, chronic respiratory and kidney disorders, breast cancer, behavioral disorders, and reproductive disorders in both sexes (Rezg, El-Fazaa, Gharbi & Mornagui, 2014; Vandenberg, Chahoud, Heindel, Padmanabhan, & Paumgartten, 2012). BPA is known to cause infertility or spermatogenic defects and metabolic disorders in males by causing oxidative stress, altering enzyme activity or DNA methylation, and mimicking the effects of estrogen hormone. The detrimental effect of BPA on male reproductive function may occur during embryonic, pubertal, or adult life (He *et al*., 2009).

Resveratrol (3, 4, 5 - trihydroxy - trans - stilbene) is a naturally occurring polyphenol found in more than 70 plant species, especially in grapes and polygonum cuspidatum (Salehi *et al*., 2018). Polydatin (PD) is the natural precursor for resveratrol. Because it has an active transmembrane absorption, PD can easily enter the cytosol, organelles, and nucleus. As a result, PD has almost 100 % bioavailability. As a powerful antioxidant, PD has been used to treat different diseases caused by elevated oxidative stress. However, to this date, there is no study on the effects of polydatin in various organs and spermatological parameters of rats exposed to BPA. Therefore, our study aimed at determining the possible positive effects of Polydatin on histopathological alterations, oxidant-antioxidant parameters, and semen quality affected by BPA exposure in male rats.

2. Materials and Methods

2.1 Animals and treatments

The methods used in the current study were approved by Afyon Kocatepe University Animal Experiments Ethics Committee (AKUHAYDEK). Thirty male Wistar rats with ages from 2 to 3 months were used in this study, and they were obtained from the Experimental Animal Research and Application Center of Afyon Kocatepe University. The acclimatization and experimental phases of the study were carried out at this center. Body weights of the rats at the time that trial started were 250 ± 50 g.

Prior to the experiment, the rats were divided into 5 groups randomly and kept for 7 days for acclimatization. Group 1 was the control (C; daily 0.5 ml distilled water). Groups 2, 3, 4 and 5 received daily olive oil (OO; daily 0.5ml olive oil), bisphenol A (BPA; daily 25 mg/kg BPA in 0.5 ml olive oil; Sigma-Aldrich Co, St. Louis, MO), polydatin (PD; daily 150 mg/kg; Sigma-Aldrich, Interlab Inc., Istanbul, Turkey), and BPA-PD mixture (BPA + PD; daily 25mg/kg BPA in 0.5 ml olive oil + 150 mg/kg PD), respectively. The experiment lasted for 45 days, and the treatments were given by gavage orally. The rats were accommodated at room temperature (25 ºC) containing 50 - 55 % moisture, fed standard rodent feed, and tap water ad libitum.

2.2 Blood collection

Twenty-four hours after the last treatment, the rats were anesthetized with a combination of xylazine (87mg/kg) and ketamine (13mg/kg) by intramuscular injection (i.m.). After that, 5 cc of blood was collected, by cardiac puncture, into tubes containing EDTA. Following the blood collection, rats were euthanized by cervical dislocation. Blood samples were centrifuged at 3000 rpm for 10 minutes, and plasma samples were stored in 1.5 ml Eppendorf tubes at - 80 ºC until the analyses.

2.3 Determination of the oxidant-antioxidant parameters

Serum malonyl dialdehyde (MDA) was determined by the double boiling method of Draper *et al*. (1993). The method is based on the principle that MDA reacts with TBA to give the maximum absorbance at a wavelength of 532 nm, observed by use of a spectrophotometer.

Serum nitric oxide (NO) concentrations were determined according to the procedure of Miranda, Espey & Wink (2001). Nitrate was reduced to nitrite with vanadium (III) and then nitrite level measured by using Griess reagents. Serial dilutions to 0.5 - 200 µM of Sodium nitrate (Merck, Germany) were used as standards. The results are expressed in µM.

Antioxidant potential (AOP) was measured by the method described by Durak *et al*. (1998). Briefly, in the reaction medium enriched with fish oil, samples were exposed to a superoxide radical (O^{2-}) produced by the xanthine-xanthine oxidase system for 1 h, and then AOP levels were measured as described by Draper *et al*. (1993).

2.4 Spermatological parameters

Immediately after the sacrifice, the left epididymis from each rat was dissected and put into a Petri dish. The progressive motility of the semen samples was evaluated subjectively by using a phase-contrast microscope with a heated stage (37 ºC; Olympus CX 31, Olympus Optical Co., Ltd., Japan). A pre-warmed slide was placed on the microscope. Then, a few drops of 2.9% sodium citrate solution and samples from the left cauda epididymis were placed onto the warm slide. The mixture was covered with a glass coverslip. The progressive motility of the spermatozoa was determined at 3 different microscopic sites under × 400 magnification (Hafez, 1987).

The abnormal spermatozoa ratio was determined by the liquid fixation method in semen samples. Three drops of the semen samples were added into1 ml of Hancock solution for the fixation. Two drops of the mixture were placed on a

slide and a total of 400 spermatozoa were counted under × 1000 magnification to determine abnormal spermatozoa ratios (Schafer & Holzmann, 2000).

The proportion of dead-live spermatozoa and membrane integrity were determined by Hypo-osmotic swelling-Eosin test (HE test) (Gündoğan, Yeni, Avdatek & Fidan 2010). Semen samples were diluted at 1:10 ratio (v / v) with HOST solution (100 mOsm). Then, the eosin dye (1 %, w / v) was added, and the mixture was incubated at 37 °C for 30 minutes. The smears were prepared, and 400 sperm cells were viewed in each smear sample under x 400 magnification. According to the curling of the sperm tails and staining status of the sperm heads, the sperms were classified into four groups: the tail is non-swollen and the head is unstained (HOS- / E-), the tail is swollen and the head is unstained (HOS+ / E-), the tail is swollen and the head is stained (HOS+ / E+), and the tail is non-swollen and the head is stained (HOS- / E+).

2.5. Histopathological examination

At necropsy, the brain, heart, liver, kidney, and testis tissues were fixed in 10% formaldehyde. The tissues were passed through the routine procedures and were blocked in paraffin. The paraffin-embedded blocks were cut by using a microtome to 5µm thickness. The samples were stained with hematoxylin-eosin and examined under a light microscope. Histopathological alterations in different tissues were classified as none= -, mild= +, medium= ++, and severe=+++.

2.6. Statistical evaluation

All values are expressed as mean and standard deviation of the mean (± SD). Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's *post hoc* test using SPSS (11.5) statistical software program (SPSS Inc., Chicago, IL, USA). A difference was considered statistically significant at $P < 0.05$.

3. Results

Serum MDA, NO, and AOP levels are in Table 1. Serum NO levels were not affected by the treatments ($P<0.190$). Serum MDA levels were lower in controls and PD-treated rats. On the other hand, serum MDA levels were similar among the OO, BPA, and BPA + PD groups. The serum AOP levels were declined in BPA-treated rats. However, OO and PD treatments were able to increase the serum AOP levels. PD treatments also had a positive influence on serum AOP levels when coupled with BPA.

Epididymal sperm motility, percent sperm defects, and plasma membrane integrity rates are in Table 2. The results of the current study indicate that BPA had negative effects on the spermatological parameters studied in rats. Although there was no significant decrease in percent motility of the epididymal sperm due to BPA, BPA increased the abnormal sperm number by more than 21 %. Parallel to these findings, a significant decrease in plasma membrane integrity (HE test) was observed in the BPA group. On the other hand, PD administration had positive effects on percent motility, head defects, and HE test when compared to the controls. Moreover, daily oral PD was able to ameliorate the negative effects of BPA on abnormal sperm percent and HE test, when these two treatments were combined.

The detailed histopathological alterations in the different organs studied are in Table 3. BPA caused focal gliosis and neuronophagia around neurons in brain tissues (Figure 1). Hyaline degeneration and necrobiotic changes were apparent in cardiac muscle cells (Figure 2). There was expansion, hyperemia, and degenerative changes in hepatocytes with activated Kupfer cells around the sinusoids (Figure 3). The kidney tissues showed necrotic areas around the tubules and degeneration in the glomerulus (Figure 4.). In the testis, vacuolar degeneration and a decrease in sperm concentration were observed due to BPA treatment (Figure 5). While no considerable histopathological changes were detected in control, OO, and PD groups, PD treatment tended to have positive effects on the histopathological alterations caused by BPA when coupled with BPA (Table 3, Figures 1, 2, 3, 4, and 5).

4. Discussion

In the past century, the industrial progress has increased the risks for all living beings in nature to face endocrine disruptors (Sharpe, 2010). Although endocrine disruptors are external chemicals, they can disrupt normal hormonal activity (Zoeller *et al*., 2012). BPA is one of the most widely used endocrine disruptors in commercial products and is one of the most produced chemicals in the world (Rochester, 2013; Vandenberg, Hauser, Marcus, Olea & Welshons, 2007). Along with the production of polycarbonate and various plastic products, BPA is also widely used in medical products for dentistry, in coating the inner surfaces of canisters, bottles, beverage cans, and in additives (Dekant & Völkel, 2008; Vom Saal *et al*., 2005). BPA is very common in our environment, so BPA exposure can stem both from food and non-food sources (Geens *et al*., 2012). Detection of BPA in human serum, urine, placental tissue, umbilical cord blood, and breast milk is evidence of how widespread this chemical is (Geens *et al*., 2012; Rochester, 2013).

Table 1. Serum malonyldialdehyde (MDA), nitric oxide (NO) and antioxidant potential (AOP) levels of rats treated with distilled water (control), olive oil (OO), polydatin (PD), bisphenol A (BPA), or bisphenolA and polydatin (BPA +PD).

	Control	OO	PD	BPA	BPA + PD	P<
MDA (nmol/L)	1.49± 0.18^{b}	1.92± 0.01^{a}	1.46± 0.19^{b}	1.98± 0.02^{a}	2.04± 0.03^{a}	0.002
NO (µmol/g)	12.3± 0.24	12.7± 0.21	12.8± 0.36	13.1± 0.21	13.5± 0.58	0.190
AOP (nmol/L)	3.27± 0.10^{c}	5.45± 0.27^{a}	4.39± 0.24^{b}	2.78± 0.12^{d}	4.35± 0.29^{b}	0.0001

a-c: Different superscripts in the same row indicate statistically significant differences ($P < 0.05$).

Table 2. Spermatological parameters of rats treated with distilled water (control), olive oil (OO), polydatin (PD), bisphenol A (BPA), or bisphenolA and polydatin (BPA +PD).

	Control	OO	PD	BPA	BP + PD	P<
Motility (%)	64.3 ± 4.3^{b}	80.0 ± 2.2^{a}	81.4 ± 3.4^{a}	58.6 ± 3.4^{b}	65.7 ± 6.9^{b}	0.002
Sperm defects (%)						
Head	3.14 ± 0.37^{b}	2.85 ± 0.23^{b}	1.28 ± 0.18^{c}	3.50 ± 0.66^{ab}	4.35 ± 0.28^{a}	0.001
Mead Piece	1.14 ± 0.09^{c}	2.00 ± 0.30^{b}	1.00 ± 0.15^{c}	2.42 ± 0.20^{b}	3.07 ± 0.13^{a}	0.001
Tail	7.64 ± 0.62^{b}	8.00 ± 0.26^{b}	7.21 ± 0.28^{b}	15.85 ± 0.67^{a}	8.64 ± 0.40^{b}	0.001
Total	11.9 ± 0.61^{c}	12.9 ± 0.44^{c}	9.50 ± 0.44^{d}	21.6 ± 1.07^{a}	16.1 ± 0.58^{b}	0.001
Hypo-osmotic swelling-Eosin test (%)						
H+/E-	59.6 ±1.93^{b}	66.0 ± 1.19^{a}	68.1 ± 2.02^{a}	19.4 ± 1.41^{c}	64.3 ± 1.14^{a}	0.001
H-/ E-	33.4 ± 2.39^{a}	18.3 ± 1.78^{bc}	18.1 ± 0.55^{bc}	15.9 ± 1.48^{c}	22.3 ± 0.89^{b}	0.001
H+/ E+	3.6 ± 1.06^{b}	7.00 ± 1.32^{b}	7.1 ± 2.08^{b}	36.1 ± 2.28^{a}	6.8 ± 0.88^{b}	0.001
H- / E+	3.4 ± 0.52^{c}	8.7 ± 1.59^{b}	7.4 ± 1.30^{b}	28.6 ± 1.64^{a}	6.6 ± 0.84^{bc}	0.001

a-d: Different superscripts in the same row indicate statistically significant differences (P < 0.05). HOS+ / E− = tail is swollen and head is unstained, HOS− / E−=tail is non-swollen and head is unstained, HOS+ / E+= tail is swollen and head is stained, and HOS− / E+ = tail is non-swollen and head is stained.

Table 3. Histopathological changes in various organs of rats treated with distilled water (control), olive oil (OO), polydatin (PD), bisphenol A (BPA), or bisphenolA and polydatin (BPA +PD).

Organ	Histopatological changes	Control	OO	PD	BPA	BPA+PD
Brain	Focal gliosis and neuronophagia around neurons	- (6/6)	- (6/6)	- (6/6)	+ (3/6) ++ (2/6) +++ (1/6)	+ (4/6) - (2/6)
Heart	Hyaline degeneration and necrobiotic changes in cardiac muscle cells	- (6/6)	- (6/6)	- (6/6)	+ (5/6) ++ (1/6)	- (3/6) + (3/6)
Liver	Expansion, hyperemia and degenerative changes in hepatocytes	- (6/6)	- (6/6)	- (6/6)	+ (5/6) ++ (1/6)	- (3/6) + (3/6)
	Kupfer cell activation.	- (6/6)	- (6/6)	- (6/6)	+ (4/6) ++ (2/6)	- (2/6) +(4/6)
Kidney	Necrobiotic changes in tubulus epithelial cells	- (6/6)	- (6/6)	- (6/6)	+ (1/6) ++ (5/6)	- (3/6) + (3/6)
	Expansion in bowman capsule cavities and vacuolar degeneration areas in glomerulus	- (6/6)	- (6/6)	- (6/6)	- (1/6) + (4/6) ++ (1/6)	- (5/6) + (1/6)
Testis	Reduced spermatozoa density in tubulus seminiferus countortus lumen	- (6/6)	- (6/6)	- (6/6)	+ (6/6)	- (4/6) + (2/6)
	Vacuoler degeneration areas in the lumens of tubulus seminiferus countortus	- (6/6)	- (6/6)	- (6/6)	+ (4/6) ++ (2/6)	- (4/6) + (2/6)

Histopathological alterations = - : none, + : mild, ++ : medium, +++ : severe

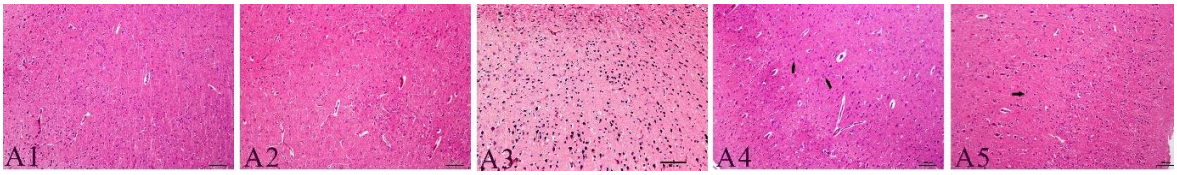

Figure 1. Brain tissues. A1: Control, A2: Olive Oil, A3: Polydatin, A4: Bisphenol A-Focal gliosis and neuronophagia around neurons in brain tissues (arrows), A5: Bisphenol A+ Polydatin-Low degree of focal gliosis in brain tissues (arrows). The tissue samples were stained with hematoxylin-eosin (10X; scale bar=100 µm).

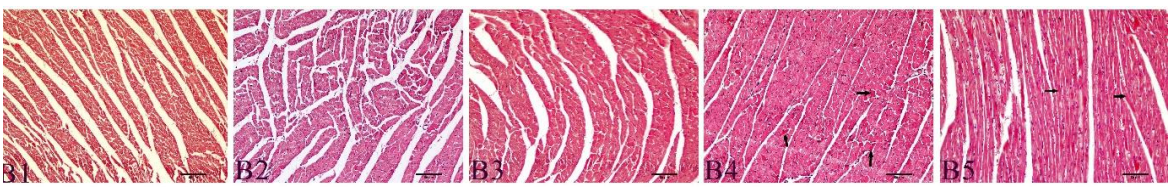

Figure 2. Cardiac muscle. B1: Control, B2: Olive Oil, B3: Polydatin, B4: Bisphenol A Hyaline degeneration and necrobiotic changes in cardiac muscle (arrows), B5: Bisphenol A+ Polydatin-Low degree of hyaline degeneration in cardiac muscle (arrows). The tissue samples were stained with hematoxylin-eosin (10X; scale bar=100 µm).

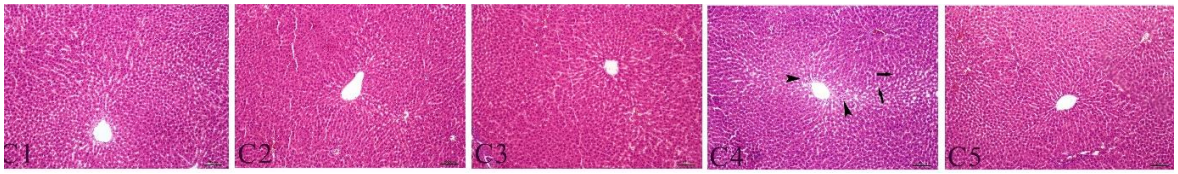

Figure 3. Liver tissues. C1: Control, C2: Olive Oil, C3: Polydatin, C4: Bisphenol A- Expansion, hyperemia and degenerative changes in hepatocytes (arrows) and activated Kupfer cells around the sinusoids (arrow heads), C5: Bisphenol A+ Polydatin. The tissue samples were stained with hematoxylin-eosin (10X; scale bar=100 µm).

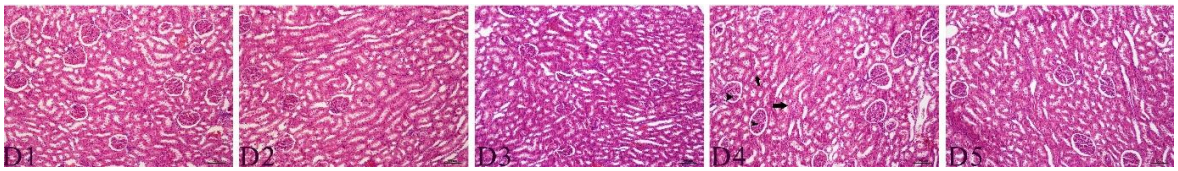

Figure 4. Kidney tissues. D1: Control, D2: Olive Oil, D3: Polydatin, D4: Bisphenol A- necrotic areas around the tubules (arrows) and vacuolar degeneration in the glomerulus (arrow heads), D5: Bisphenol A+ Polydatin. The tissue samples were stained with hematoxylin-eosin (10X; scale bar=100 µm).

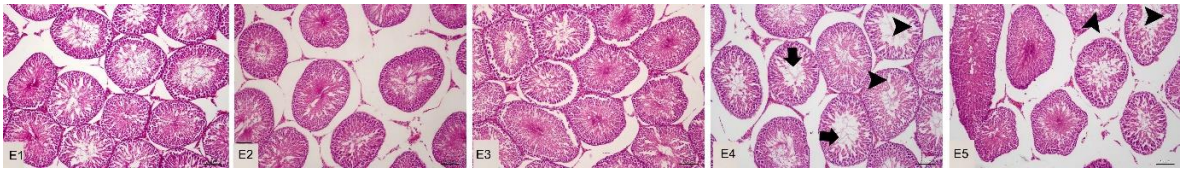

Figure 5. Testis tissues. A: Control, B: Olive Oil, C: Polydatin, D: Bisphenol A- decrease in sperm concentration (arrows) and vacuolar degenerations in the tubulus seminiferus contortus (arrow heads), E: Bisphenol A+ Polydatin- vacuolar degenerations in the tubulus seminiferus contortus (arrow heads). The tissue samples were stained with hematoxylin-eosin (10X; scale bar=100 µm).

It was previously reported that BPA could accumulate in several organs, such as liver, kidneys, gonads, and brain (Rezg *et al*., 2014; Vandenberg *et al*., 2012). The results of the present study agree with the previous findings. The current study suggests that daily intake of 25 mg/kg BPA resulted in cell damage in the various tissues studied in rats. Focal gliosis was apparent in brain tissues of the BPA-treated group. Hyaline degeneration and necrobiotic changes were observed in the myocardial cells. Degenerative changes were seen in the liver tissues. Necrotic areas around the tubules and degeneration in the glomerulus were observed in the kidney tissues. In the testis, BPA treatment caused a decline in the sperm concentration and vacuolar degeneration. Acaroz *et al*. (2019) also reported similar alterations in liver, kidney, brain, and gonads due to 25 mg/kg oral BPA treatments in rats. Moreover, liver tissues of the BPA-treated rats (10 mg/kg) showed serious hepatocellular damage (Amraoui *et al*., 2018). Comparably, oral BPA treatments from 10 up to 100 mg/kg induced degenerative changes such as swelling of proximal tubules in kidneys, a proliferation of Kupfer cells and necrosis in hepatocytes, inflammatory cell infiltration and extravasated erythrocytes around the alveolar lumen in lungs, as well as epithelial cell degeneration, low sperm density and cytoplasmic vacuolization in testis tissues (Kamel, Foaud & Moussa, 2018; Kanwal, Amina, Iqbal & Munir, 2018; Korkmaz, Ahbab, Kolankaya & Barlas, 2010; Morgan, El-Ballal, El-Bialy & ElBorai, 2014,).

Morphological evaluation of semen is an important marker for determining male fertility. Ejaculate quality can be affected by different factors such as progressive motility, abnormal sperm ratio, and the number of dead spermatozoa. Moreover, head and tail defects are the typical indicators of testicular injuries. In the present study, BPA exposure had negative effects on sperm quality in rats. The number of sperm defects and dead/live sperm ratio were significantly altered due to BPA exposure. BPA is well known as a male reproductive toxicant, and negative alterations of the male reproductive system can be seen due to both experimental and environmental exposures to BPA. BPA exposure often results in decreased semen quality. BPA could cause an increase in abnormal spermatozoa, dead sperm count, decline in sperm number, and infertility in man and laboratory animals (Chitra, 2003). Several experimental studies on rats concluded BPA exposure could negatively alter the semen quality in rats (Liu *et al*., 2013; Richter *et al*., 2007; Saian *et al*., 2011; Wisniewski *et al*., 2015). Moreover, environmental contact with BPA resulted in an abnormal testicular function (Tinwell, Haseman, Lefevre, Wallis & Ashby, 2002), reduced testicular steroidogenesis (Akingbemi, Sottas, Koulova, Klinefelte &, Hardy, 2004), spermatogenesis (Salian, Doshi & Vanage, 2011), daily sperm production (Rochester, 2013), and the sperm and embryo quality (Bloom *et al*., 2011) in adult men.

Oxidative stress could be an important factor for poor semen and embryo quality due to BPA contact. BPA is known to stimulate oxidative stress and deplete antioxidant enzymes, which in turn could decrease the number of sperm and percent motility (Chitra *et al*., 2003). In the current study, BPA significantly altered serum MDA levels when compared to controls. BPA could stimulate MDA production in different tissues (Karabulut & Gulay, 2020). Testicular and serum MDA concentrations were elevated due to BPA treatment (Karabulut & Gulay, 2020). Oral BPA exposure could stimulate the production of more reactive oxygen species (ROS) in the testis tissues, increase oxidative stress and

elevate total MDA (Yonar *et al*., 2014). Therefore, BPA can promote ROS production by increasing free radical formation in mammals and exposure may lead to oxidative stress (Chitra *et al*., 2003). The oxidative stress induced by exposure to BPA could adversely affect the enzymatic antioxidant defense system, leading the oxidative damage to testicular tissue and sperm cells, and causing problems in both sperm development and survival.

The current study investigated the possible positive effects of PD on the changes to the different organs and spermatological parameters when exposed to BPA. Antioxidants protect our body against the harmful effects of free radicals (Fridovich, 1976) and PD is known to have a powerful antioxidant capacity. It has many activities described in previous studies: it reduces the lipid profile in hyperlipidemic rabbits and causes weight loss (Xing *et al*., 2009), possesses a neuroprotective effect in ischemia/ reperfusion-induced cerebral injury (Cheng *et al*., 2006), attenuates spinal cord injury (Lv *et al*., 2019), provides an anti-osteoporotic activity (Zhou, Qin, Yang, Huang & Yang, 2016), has protective roles in primary hepatocyte cultures of CCl4 - induced rats (Huang *et al*., 1999), and decreases lipid oxidation (Pn *et al*., 2007). Moreover, it has a strong growth inhibitory effect on different cancer types (Wu, Li, Feng & Ji, 2018).

In the current study, PD treatment significantly improved BPA-induced tissue damage and augmented semen quality. As a very powerful antioxidant, PD supplementation can revise the results of oxidative stress. It can increase cellular antioxidant properties and/or reduce the reactive oxygen species in the cells and act as a free radical scavenging agent. PD also has an important role in diminishing caspase-3 and 9 activities showing its anti-apoptotic effects (Gao *et al*., 2016). In addition, there are reports that PD can effectively improve the testes injury after irradiation through reducing oxidative stress (Ma & Jia, 2018).

Protective effects of antioxidants on ejaculate quality, motility, and vitality had been verified previously in different animal models (Akarca *et al*., 2020; Ata, Hatipoglu, Yildiz-Gulay & Gulay, 2007; Evcimen, Aslan & Gulay 2020; Motamedi, Asghari, Jahandideh, Abedi & Mortazavi, 2018). *In vitro* studies have also confirmed that antioxidants such as Vit E, Vit C, and glutathione induced positive outcomes on sperm functions (Shah *et al*., 2017; Verma & Kanwar, 1998; Zlata *et al*., 2014). In accordance with earlier reports, the results of the current study show that the negative effects of BPA on spermatologic parameters were mitigated by an antioxidant. Decreased AOP due to BPA could lead to permanent injuries to sperm, increase the number of morphological problems and alter the fertilizing ability (Agarwal *et al*., 2014). In the present study, PD was able to increase total antioxidant status when coupled with BPA. Thus, it is possible that PD could scavenge the excessive ROS due to BPA, lowering the oxidative stress, and maintaining the activity of endogenous antioxidant enzymes (Ince *et al*., 2014).

5. Conclusions

Daily oral PD administration was able to ameliorate the negative effects of BPA on semen quality. Positive ameliorating effects of PD on tissue levels were also remarkable. Thus, the results of the current study indicate a beneficial role of PD in neutralizing the harmful effects of BPA in rats.

References

Acaroz, U., Ince, S., Arslan-Acaroz, D., Gurler, Z., Demirel, H. H., Kucukkurt, I., . . . Zhu, K. (2019). Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. *Toxicological Research*, *8*(2), 262-269.

Agarwal, A., Mulgund, A., Alshahrani, S., Assidi, M., Abuzenadah, A. M., Sharma, R., & Sabanegh, E. (2014). Reactive oxygen species and sperm DNA damage in infertile men presenting with low level leukocytospermia. *Reproductive Biology and Endocrinology*, *12*, 1-8.

Akarca-Dizakar, S. O., Erdogan, D., Peker, T., Akcay, N. C., Turkoglu, I., Esmekaya, M. A., & Omeroglu, S. (2020). Effects of co-administered melatonin, fructose and bisphenol A (BPA) on rat epididymis and sperm characteristics. *Biotechnic and Histochemistry*, *95*(1), 18-26. doi:10.1080/10520295.2019.1627418

Akingbemi, B. T., Sottas, C. M., Koulova, A. I., Klinefelte, G. R., & Hardy, M. P. (2004). Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat leydig cells. *Endocrinology*, *145*(2), 592-603.

Amraoui, W., Adjabi, N., Bououza, F., Boumendjel, M., Taibi, F., Boumendjel, A., . . . Messarah, M. (2018). Modulatory role of selenium and vitamin e, natural antioxidants, against bisphenol a-induced oxidative stress in wistar albinos rats. *Toxicological Research*, *34*(3), 231-239.

Ata, A., Hatipoglu, F. S., Yildiz-Gulay, O., & Gulay, M. S. (2007). Protective role of ascorbic acid on subacute sperm toxicity in male New Zealand white rabbits treated with endosulfan. *Drug and Chemical Toxicology*, *3*(3), 181-195.

Bloom, M. S., Vom Saal, F. S., Kim, D., Taylor, J. A., Lamb, J. D., & Fujimato, V. Y. (2011). Serum un conjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. Environ. *Toxicological Pharmacology*, *32*(2), 319-323.

Cheng, Y., Zhang, H. T., Sun, L., Guo, S., Ouyang, S., Zhang, Y., & Xu, J. (2006). Involvement of cell adhesion molecules in polydatin protection of brain tissues from ischemia-reperfusion injury. *Brain Research*, *1110*(1), 193-200.

Chitra, K. C., Latchoumycandane, C., & Mathur, P. P. (2003). Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. *Toxicology*, *185*(1-2), 119-127.

Choi, Y. J., Ha, K. H., & Kim, D. J. (2017). Exposure to bisphenol A is directly associated with inflammation in healthy Korean adults. *Environmental Science Pollution Research*, *24*(1), 284-290.

Czub, P. (2011). Bisphenol-A, In S. Thomas, & P. M. Visakh, (Eds.), *Handbook of engineering and speciality thermoplastics: Polyethers and polyesters*, *Volume 3* (pp.221-269). Beverly, MA: Scrivener Publishing.

Dekant, W., & Völkel W. (2008). Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures. *Toxicology and Applied Pharmacology*, *228*(1), 114-134.

Demierre, A. L., Peter, R., Oberli, A., & Bourqui-Pittet, M. (2012). Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. *Toxicology Letters*, *213*(3), 305-308.

Draper, H. H., Squires, E. J., Mahmoodi, H., Wu, J., Agarwal, S., & Hadley, M. (1993). A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radical Biology and Medicine*, *15*(4), 353-363.

Durak, I., Karabacak, H. I., Buyukkocak, S., Cimen, M. Y., Kacmaz, M., Omeroglu, E., & Ozturk, H. S. (1998). Impaired antioxidant defence system in the kidney tissues from rabbits treated with cyclosporine. Protective effects of vitamins E and C. *Nephron*, *78*, 207-211.

EFSA-European Food Safety Authority. (2015). Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *European Food Safety Authority Journal, 13*, 3978.

Evcimen, M., Aslan, R., & Gulay, M.S. (2020). Protective effects of polydatin and grape seed extract in rats exposed to cadmium. *Drug and Chemical Toxicology*, *43*(3), 225-233.

Fridovich, I. (1976). Free radical in biology. In W. A. Pryor (Ed.) Academic: New York. 1, 239-271.

Gao, Y. G., Chen, T., Lei, X. H., Li, Y. F., Dai, X. G., Cao, Y. Y., . . . Lin, X. Z. (2016). Neuroprotective effects of polydatin against mitochondrial-dependent apoptosis in the rat cerebral cortex following ischemia/reperfusion injury. *Molecular Medicine Reports, Part: B, 14*(6), 5481-5488.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J. P., Goeyens, L., Lecomte, P., & Covaci, A. (2012). A review of dietary and non-dietary exposure to bisphenol-A. *Food and Chemical Toxicology*, *50* (10), 3725-3740.

Gündoğan, M., Yeni, D., Avdatek, F., & Fidan, A.F. (2010). Influence of sperm concentration on the motility, morphology, membrane and DNA integrity along with oxidative stress parameters of ram sperm during liquid storage. *Animal Reproduction Science*, *122*, 200-207.

Hafez, E. S. E. (1987). Semen evaluation (5th ed.). In Hafez, E. S. E. (Ed.), *Reproduction in farm animals*. Lea and Fabiger, Philadelphia.

He, Y., Miao, M. H., Herrinton, L. J., Wu, C. H., Yuan, W., Zhou, Z. J., & Li, D. K. (2009). Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. *Environmental Research*, *109*(5), 629-633.

Huang, Z. S., Wang, Z. W., Liu, M. P., Zhong, S. Q., Li, Q. M., & Rong, X. L. (1999). Protective effects of polydatin against CCl4- induced injury to primarily cultured rat hepatocytes. *World Journal of Gastroenterology*, *5* (1), 41-44.

Ince, S., Acaroz, D. A., Neuwirth, O., Demirel, H. H., Denk, B., Kucukkurt, I., & Turkmen, R. (2014). Protective effect of polydatin, a natural precursor of resveratrol, against cisplatin-induced toxicity in rats. *Food and Chemical Toxicology*, *72*, 147-153.

Kamel, A. H., Foaud, M. A., & Moussa, H. M. (2018). The adverse effects of bisphenol A on male albino rats. *Journal of Basic and Applied Zoology*, *79*, 6-15.

Kanwal, Q., Amina, A., Iqbal, H. H., & Munir, B. (2018). Healing potential of Adiantum capillus-veneris L. plant extract on bisphenol A-induced hepatic toxicity in male albino rats. *Environmental Science and Pollution Research*, *25*(12), 11884-11892.

Korkmaz, A., Ahbab, M. A., Kolankaya, D., & Barlas, N. (2010). Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Food and Chemical Toxicology*, *48*(10), 2865-2871.

Leranth, C., Hajszan, T., Szigeti-Buck, K., Bober, J., & MacLusky, N. J. (2008). Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(37), 14187-14191.

Liu, C., Duan, W., Li, R., Xu, S., Zhang, L., Chen, C., . . . Zhou, Z. (2013). Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity. *Cell Death Disorders*, *4*(6), e676.

Ma, Y., & Jia, X. J. (2018). Polydatin Alleviates Radiation-Induced Testes Injury by Scavenging ROS and Inhibiting Apoptosis Pathways. *Medicinal Sciences*, Mon. *24*, 8993-9000.

Miranda, K. M., Espey, M. G., & Wink, D. A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, *5*, 62-71.

Morgan, A. M., El-Ballal, S. S., El-Bialy, B. E., & ElBorai, N. B. (2014). Studies on the potential protective effect of cinnamon against bisphenol A- and octylphenol-induced oxidative stress in male albino rats. *Toxicological Reproduction*, *1*, 92-101.

Motamedi, S., Asghari, A., Jahandideh, A., Abedi, G., & Mortazavi, P. (2018). Effects of Echinacea purpurea Extract on Sperm Characteristics and Hematology Following Testicular Ischemia-Reperfusion Injury in Rat. *Crescent Journal of Medicine and Biological Sciences*, *5*(2), 119-122.

Pan, Y. M., Zhang, X. P., Wang, H. S., Liang, Y., Zhu, J. C., Li, H. Y., . . . Wu, Q. (2007). Antioxidant potential of ethanolic extract of Polygonum cuspidatum and application in peanut oil. *Food Chemistry*, *105*, 1518-1524.

Rezg, R., El-Fazaa, S., Gharbi, N., & Mornagui, B. (2014). Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. *Environmental International*, *64*, 83-90.

Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S., Talsness, C. E., . . . Vom Saal, F. S. (2007). In vivo effects of bisphenol A in laboratory rodent studies. *Reproductive Toxicology*, *24*(2), 199-224.

Rochester, J. R. (2013). Bisphenol A and human health: a review of the literature. *Reproductive Toxicology*, 42, 132-155.

Salehi, B., Mishra, A. P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M. . . . Sharifi-Rad, J. (2018). Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicine*, *6*(3), 91. doi:10.3390/bio medicines6030091

Salian, S., Doshi, T., & Vanage, G. (2011). Perinatal exposure of rats to bisphenol A affects fertility of male offspring-an overview. *Reproductive Toxicology*, *31*(3), 359-362.

Schafer, S., & Holzmann, A. (2000). The use of transmigration and Spermac TM stain to evaluate epididymal cat spermatozoa. *Animal Reproduction Science*, *59*, 201-211.

Shah, N., Singh, V., Yadav, H. P., Verma, M., Chauhan, D. S., Saxena, A., . . . Swain, D. K. (2017). Effect of reduced glutathione supplementation in semen extender on tyrosine phosphorylation and apoptosis like changes in frozen thawed Hariana bull spermatozoa. *Animal Reproduction Science*, *182*, 111-122.

Sharpe, R. M. (2010). Environmental/lifestyle effects on spermatogenesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1546), 1697-1712.

Tinwell, H., Haseman, J., Lefevre, P. A., Wallis, N., & Ashby, J. (2002). Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. *Toxicological Science*, *68*(2), 339-348.

Vandenberg, L. N., Chahoud, I., Heindel, J. J., Padmanabhan, V., & Paumgartten, F. J. R. (2012). Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Cien Saude Colet*, *17*(2), 407-434.

Vandenberg, L. N., Hauser, R, Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology*, *24*(2), 139-177.

Verma, A., & Kanwar, K. C. (1998). Human sperm motility and lipid peroxidation in different ascorbic acid concentrations: An in vitro analysis. *Andrologia*, *30*(6), 325-329.

Vom Saal, F. S., & Hughes, C. (2005). An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environmental Health Perspectives*, *113*(8), 926-933.

Wisniewski, P., Romano, R. M., Kizys, M. M. L., Oliveira, K. C., Kasamatsu, T., Giannocco, G., . . . Romano, M. A. (2015). Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary-testicular axis. *Toxico lology*, *329*, 1-9.

Wu, X. N., Li, Q., Feng, Y., & Ji, Q. (2018). Antitumor Research of the Active Ingredients from Traditional Chinese Medical Plant Polygonum. *Cuspidatum. Evidence Based Complement Alternative Medicine*, 2313021. doi:10. 1155/2018/2313021.

Xing, W. W., Wu, J. Z., Jia, M., Du, J., Zhang, H., & Qin, L. P. (2009). Effects of polydatin from Polygonum cuspidatum on lipid profile in hyperlipidemic rabbits. *Biomedicine and Pharmacotherapy*, *63*(7), 457-462.

Yonar, M. E., Yonar, S. M., Çoban, M. Z., & Eroğlu, M. (2014). Antioxidant effect of propolis against exposure to chromium in Cyprinus carpio. *Environmental Toxicology*, *29*(2), 155-164.

Zalata, A., Elhanbly, S., & Abdalla, H. (2014). In vitro study of cypermethrin on human spermatozoa and the possible protective role of vitamins C and E. *Andrologia*, *46*(10), 1141-1147.

Zalko, D., Jacques, C., Duplan, H., Bruel, S., & Perdu, E. (2011). Viable skin efficiently absorbs and metabolizes bisphenol A. *Chemosphere*, *82*(3), 424-430.

Zhou, Q. L., Qin, R. Z., Yang, Y. X., Huang, K. B., & Yang, X. W. (2016). Polydatin possesses notable anti-osteoporotic activity via regulation of OPG, RANKL and beta-catenin. *Molecular Medicine Reports*, *14*(2), 1865-1869.

Zoeller, R. T., Brown, T. R., Doan, L. L., Gore, A. C., Skakkebaek, N. E., Soto, A. M., . . . Vom Saal, F.S. (2012). Endocrine-disrupting chemicals and public health protection: a statement of principles from the Endocrine Society. *Endocrinology*, *153*(9), 4097-4110.