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Investigation of BPA Toxicity in Male New Zealand White Rabbits

Hayrullah Karabulut, Mehmet Sukru Gulay

ABSTRACT

The current study investigated bisphenol A (BPA) toxicity in rabbits. After the adaptation period of 2 weeks, 24 male New Zealand rabbits were equally assigned to 4 treatment groups. Corn oil was given to the rabbits in the control group, whereas 10, 20, or 100 mg/kg daily BPA in corn oil was administered to the rabbits in the remaining groups orally for 9 weeks. Throughout the experiment, BPA did not cause any clinical symptoms, and serum BPA levels of the rabbits treated with BPA were elevated in a dose-dependent manner. Although no effects of BPA were apparent on feed intake, body weights, or organ weights, BPA altered MDA, CAT, SOD, and GPx levels in all organs examined. Furthermore, BPA treatments negatively affected red blood cell counts, hemoglobin, and serum ALP, AST, ALT, CRP, amylase, creatine, and urea concentrations. The present study concluded that the LOAEL dose of BPA for the hematological and biochemical parameters was 20 mg/kg/day.

Keywords: Biochemical parameters, hematological parameters, histopathology, oxidative stress.

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I. Introduction

In recent decades, the use of artificial products from various chemicals has increased in the production of materials we use at every point of our lives. Therefore, our risk of exposure to endocrine-disrupting chemicals (EDC) has also increased [1]. These chemicals are called EDC because they have negative effects on the endocrine system of humans and animals. In general, various diseases such as fertility disorders, genital malformations, cancer, obesity, increased insulin resistance, and diabetes can be observed due to the effects of EDCs on the hormonal system. Due to the intensive use of materials produced from EDCs, people can be exposed to these substances in various ways. These chemicals can be found in drugs, natural products, pesticides, plastics, perfumes, lotions, dyes, and foods, and may act like hormones, or can bind to hormone receptors and inhibit the functioning of natural hormones when they enter the body [1], [2].

Especially in the last hundred years, in parallel with the rapid increase in industrialization, a significant increase in environmental pollution has been observed. Although there is more than one reason for environmental pollution, the uncontrolled release of factory wastes to nature has been one of the most important causes of soil, water, and air pollution. Bisphenol A (BPA) can also be shown among the EDCs that play a role in this contamination. Approximately 100 tons of BPA are released into the atmosphere every year [3], [4]. In addition, significant amounts of BPA are detected in surface waters and soil. This contamination has made our natural environment one of the sources of BPA exposure for humans and animals. BPA can be exposed orally, by contact, or by inhalation [5]. Despite this, the primary source for humans is food [6], [7].

It is inevitable that BPA has entered our food chain, as it is an industrial chemical that is widely used in the production of polycarbonate and various plastic products today. In addition to its use in the industrial field, its intense use as an additive in feeding bottles, inner coatings of cans, beverage cans, dental products, technological products, and materials used in hospitals have made BPA an important environmental pollutant [8], [9]. However, the current literature lacks the possible effects of BPA in rabbits. Therefore, the current study was carried out to determine the effects of BPA on hematologic and biochemical parameters, oxidant-antioxidant enzymes, and LAOEL value in rabbits.

II. Materials and Methods

The experiment was approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (25.11.2015/159). Eight to 10 months old male rabbits (n=24) were housed individually in galvanized cages at Burdur Mehmet Akif Ersoy University Experimental Animal Research Center (at 22 ± 2 ˚C temperature; 14:10 hour light: dark cycle; 50-55% humidity). Tap water and commercial rabbit feed (17.0% crude protein, 3.67% crude oil, 2.68% crude cellulose, 6.93% crude ash, 10.49% calcium, 0.46% phosphorous; Korkuteli Food Company, Antalya, Turkey) were given *ad libitum* during the entire experimental period. Feed intakes and live body weight measures were taken weekly throughout the experiment.

Before the experiment, the rabbits were acclimatized to the laboratory conditions for 2 weeks. After the adaptation

period, the animals were randomly assigned to 4 treatment groups (n=6 per group). Rabbits in different BPA groups received 10 (B10), 20 (B20), and 100 (B100) mg/kg daily oral BPA (Sigma-Aldrich Co., St. Louis, MO; Lot: MKBQ5209V, CAS 80-05-7; 99% purity) in corn oil, whereas the control group (C) was given daily oral corn oil. The detailed experimental protocol was also explained previously [10]. Oral BPA and corn oil doses were adjusted weekly according to the bodyweight changes.

The oral treatments were continued for 9 weeks. At the end of the experiment, the rabbits were not given any food for 12 hours and blood samples were taken from the ear artery with the aid of a 24G IV cannula. Blood samples were directly collected into tubes containing K3-EDTA and gel and clot activator tubes. Whole blood parameters (such as hematocrit, hemoglobin, total erythrocytes, platelets, leukocytes, etc.) were analyzed from freshly collected blood within 30 min with a hematology analyzer (Abacus Junior Vet SN-100702). The remaining blood samples were immediately centrifuged at 1457 × g for 20 min. Serum glucose, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine analyzes were analyzed from fresh serum on the same day (Gesan Chem 200). The remaining serum samples were stored at -80 ˚C. Serum amylase, lipase, c-reactive protein (CRP), and gamma-glutamyl transferase (GGT) levels were measured from the thawed samples by an autoanalyzer (Achitech C8000). Serum BPA levels were determined by the HPLC method at Burdur Mehmet Akif Ersoy University, Scientific and Technology Application and Research Center Laboratory (LC-20AT, Shimadzu, Kyoto, Japan).

Following blood collection, rabbits were euthanized under general anesthesia with isoflurane. After the euthanasia, the organs were washed with chilled PBS, and organ weights were recorded. The left lobe of the liver and left kidney were fixed in 10% formaldehyde for histopathological examination. The caudal lobe of the liver and the right kidney were collected and frozen immediately at -80 ˚C for the determination of oxidant-antioxidant parameters.

Before the analysis of some oxidant and antioxidant parameters, the tissue samples were prepared according to the manufacturer's instructions. Serum and tissue levels of superoxide dismutase (SOD; Cat No: SG-0061Rb), catalase (CAT; Cat No: SG-50185), glutathione peroxidase (GPx; Cat No: SG-0120Rb), and malondialdehyde (MDA; Cat No: SG-50252) were analyzed by ELISA kits (SinoGeneClon Biotech Co., Ltd., China), and the results were read at 450 nm.

For histopathological examination, the tissues were placed into cassettes and fixed in 10% formaldehyde solution for 24 hours. After the fixation, tissues passed through graded alcohols and xylol and were then embedded in paraffin. The paraffin blocks were cut at a thickness of 5 µ and stained with hematoxylin-eosin stain. Tissues were examined under a light microscope in a single-blinded fashion.

The data were evaluated using the SAS statistical program. ANOVA test was carried out to verify the statistical differences among the groups. For the comparisons of the individual treatments, the Tukey test was performed. The difference was considered significant when the differences were $P < 0.05$.

TABLE I: THE EFFECT OF ORAL BPA ON BODY WEIGHT, FEED INTAKE AND ORGAN WEIGHTS

	C			B10			B20			B100			P=
Body Weight (kg)	3.52	±	0.57	3.58	±	0.50	3.40	±	0.46	3.44	±	0.36	0.517
Feed Intake (g)	168.5	±	29.1	164.7	±	40.5	162.6	±	30.7	151.3	±	37.1	0.559
Lung (g)	14.6	±	2.24	17.6	±	3.20	18.64	±	4.44	24.9	±	4.81	0.120
Liver (g)	111.1	±	21.8	124.0	±	18.9	157.2	±	26.1	140.0	±	31.1	0.244
Right Kidney (g)	8.74	±	1.05	10.0	±	1.32	13.6	±	3.59	10.6	±	2.13	0.101
Left Kidney (g)	8.58	±	1.29	11.0	±	1.55	14.0	±	3.92	10.6	±	2.12	0.090
Spleen (g)	0.90	±	0.29	1.41	±	0.53	1.32	±	0.15	1.39	±	0.38	0.488
Brain (g)	5.93	±	0.89	7.05	±	1.22	6.12	±	1.28	6.50	±	0.57	0.534
Heart (g)	9.15	±	0.55	9.93	±	1.53	10.9	±	1.69	10.2	±	2.82	0.617

C=control; B10=10mg/kg/day BPA; B20=20mg/kg/day BPA; B100=100mg/kg/day BPA. Values are given as mean ± standard deviation.

TABLE II: THE EFFECT OF ORAL BPA ON SOME HEMATOLOGICAL PARMETERS

	C			B10			B20			B100			P=
WBC ($x10^9$/l)	7.61	±	1.15	7.89	±	1.59	8.12	±	2.07	7.30	±	1.45	0.861
Lymphocyte ($x10^9$/l)	3.59	±	1.95	2.91	±	1.87	3.90	±	1.97	3.15	±	1.98	0.780
Monocyte ($x10^9$/l)	0.49	±	0.33	0.32	±	0.15	0.45	±	0.27	0.30	±	0.21	0.576
Granulocyte ($x10^9$/l)	3.17	±	0.97	4.65	±	1.68	3.83	±	2.11	3.84	±	0.85	0.489
Lymphocyte (%)	50.0	±	18.3	36.0	±	19.3	47.4	±	23.4	40.6	±	20.4	0.697
Monocyte (%)	6.5	±	4.3	4.4	±	2.6	6.4	±	4.55	4.2	±	2.46	0.628
Granulocyte (%)	43.5	±	18.2	59.6	±	17.4	46.2	±	29.7	55.2	±	20.1	0.612
RBC ($x10^{12}$/l)	6.93^{a}	±	0.34	6.60^{ab}	±	0.85	5.90^{b}	±	0.40	5.95^{b}	±	0.75	0.049
Hemoglobin (g/dl)	13.64^{a}	±	0.71	12.7^{ab}	±	0.86	11.9^{b}	±	1.38	11.8^{b}	±	0.83	0.021
Hematoctit (%)	44.9	±	1.8	45.5	±	1.6	45.5	±	2.89	47.4	±	3.69	0.484
MCV (fl)	65.0	±	5.0	69.7	±	8.2	75.0	±	5.7	77.9	±	11.5	0.106
MCH (pg)	19.72	±	1.59	19.40	±	1.75	20.05	±	1.58	19.88	±	1.36	0.923
MCHC (g/dl)	30.4^{a}	±	2.37	27.9^{ab}	±	1.92	26.7^{ab}	±	3.40	25.2^{b}	±	2.46	0.035
Platelet ($x10^9$/l)	352	±	121	413	±	144	430	±	133	432	±	109	0.737

C=control; B10=10mg/kg/day BPA; B20=20mg/kg/day BPA; B100=100mg/kg/day BPA. WBC= White blood cells, RBC=Red blood cells, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration. Values have given as mean ± standard deviation .

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TABLE III: THE EFFECT OF ORAL BPA ON SOME BIOCHEMICAL PARMETERS

	C			B10			B20			B100			P=
Amylase(IU/l)	143.3^{a}	±	41.6	203.7^{ab}	±	42.1	215.4^{b}	±	38.7	220.6^{b}	±	27.3	0.049
Lipase (IU/l)	102.8^{a}	±	38.7	141.4^{ab}	±	24.3	162.6^{b}	±	22.9	169.1^{b}	±	25.2	0.043
CRP (mg/dl)	1.09^{a}	±	0.57	3.65^{ab}	±	1.27	4.43^{b}	±	2.11	4.85^{b}	±	1.98	0.022
GGT (IU/l)	4.84^{a}	±	2.96	13.01^{b}	±	2.11	15.17^{b}	±	2.74	16.40^{b}	±	3.10	0.021
Ürea (mmol/l)	27.0^{a}	±	9.30	32.8^{ab}	±	5.89	41.0^{b}	±	4.47	40.5^{b}	±	9.22	0.025
Creatinine (mg/dl)	0.73^{a}	±	0.09	0.81^{ab}	±	0.10	0.96^{b}	±	0.06	0.98^{b}	±	0.12	0.034
AST (IU/l)	23.7^{a}	±	6.63	31.8^{ab}	±	10.9	37.3^{b}	±	8.26	40.5^{b}	±	8.98	0.017
ALT (IU/l)	67.4^{a}	±	14.9	73.6^{a}	±	7.09	99.4^{b}	±	14.2	99.3^{b}	±	20.3	0.004
ALP (IU/l)	81.6^{a}	±	16.2	102.2^{ab}	±	15.7	117.4^{b}	±	16.2	113.2^{b}	±	16.8	0.012
Glucose (mg/del)	92.2	±	11.0	94.4	±	8.84	93.6	±	7.76	95.8	±	9.47	0.932
Cholesterol (mg/dl)	30.0	±	5.38	41.8	±	12.8	35.2	±	4.14	33.0	±	8.53	0.188
Triglyceride (mg/del)	127.2	±	25.5	129.8	±	22.7	125.8	±	24.3	127.0	±	37.6	0.997

C=control; B20=20 mg/kg/day BPA; B20=20 mg/kg/day BPA; B100=100 mg/kg/day BPA. CRP= C reactive protein, GGT=Gamma glutamyl transferase,AST= Aspartate aminotransferase , ALT= Alanine aminotransferase, ALP= Alkaline phosphatise. Values have given as mean ± standard deviation.

TABLE IV: THE EFFECT OF ORAL BPA ON SOME OXIDANT-ANTIOXIDANT PARMETERS

	C			B10			B20			B100			P=
Serum													
MDA (ng/ml)	30.3^{a}	±	3.61	46.4^{b}	±	2.94	49.2^{b}	±	13.8	49.3^{b}	±	10.7	0.016
SOD (pg/ml)	9.86^{a}	±	3.28	4.05^{b}	±	2.05	3.73^{b}	±	1.51	2.98^{b}	±	1.73	0.004
CAT (pg/ml)	28.7^{a}	±	8.82	20.2^{b}	±	2.28	14.3^{bc}	±	5.38	11.7^{c}	±	3.05	0.002
GPx (pg/ml)	383.9^{a}	±	23.4	338.2^{ab}	±	23.6	318.4^{b}	±	53.4	306.8^{b}	±	61.2	0.071
Liver													
MDA (ng/ml)	28.8^{a}	±	3.25	38.3^{b}	±	5.44	42.7^{b}	±	5.23	47.2^{b}	±	5.81	0.012
SOD (pg/ml)	7.14^{a}	±	1.45	4.43^{b}	±	0.96	3.93^{b}	±	1.69	2.30^{b}	±	1.23	0.066
CAT (pg/ml)	14.1^{a}	±	2.46	9.99^{b}	±	1.94	7.95^{bc}	±	1.56	5.43^{c}	±	2.14	0.005
GPx (pg/ml)	286.0^{a}	±	39.6	242.7^{ab}	±	43.2	217.0^{b}	±	15.9	209.7^{b}	±	20.6	0.070
Kidney													
MDA (ng/ml)	32.5^{a}	±	2.33	45.5^{b}	±	1.58	51.6^{b}	±	8.93	54.6^{b}	±	10.2	0.018
SOD (pg/ml)	9.07^{a}	±	3.85	5.83^{ab}	±	1.66	3.33^{b}	±	1.14	2.51^{b}	±	0.51	0.025
CAT (pg/ml)	20.7^{a}	±	3.77	13.9^{b}	±	3.33	12.4^{b}	±	5.19	4.83^{c}	±	1.73	0.005
GPx (pg/ml)	296.0^{a}	±	9.16	219.3^{b}	±	22.7	210.3^{b}	±	41.4	236.4^{b}	±	26.7	0.024

C=control; B20=20 mg/kg/day BPA; B20=20 mg/kg/day BPA; B100=100 mg/kg/day BPA. MDA=malondialdehyde; SOD=superoxide dismutase; CAT= catalase; GPx= glutathione peroxidase.Values have given as mean ± standard deviation.

III. RESULTS

During the experiment, no significant health problem was observed due to BPA treatment. Serum BPA levels of the groups were statistically significant depending on the oral dose given (P<0.0001). While no measurable level of BPA was found for the rabbits in the C group (0.00±0.00 µg/ml), the serum BPA levels increased paralleled with the increase in the daily oral BPA dose (B10=0.24±0.02; B20 =0.58±0.09; B100=0.89±0.12 µg/ml). However, the increase in serum BPA levels had no significant effects on the body weights, feed intakes, and organ weights of the rabbits in different treatment groups (Table I).

The hematological parameters are in Table II. Overall, the oral administration of BPA did not cause a significant change in hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). In addition, the oral BPA administration did not alter total WBC, lymphocyte, monocyte, granulocyte, and platelet levels. However, a significant difference was observed in RBC, hemoglobin, and mean corpuscular hemoglobin concentrations (MCHC) among the groups. Especially, oral administration of 20 and 100 mg/kg/day BPA resulted in a significant decrease in red blood cell counts and hemoglobin levels in male rabbits. However, MCHC decreased significantly in only the highest dose BPA treatment (B100) compared to C (Table II).

The serum biochemical parameters are in Table III. There were no significant changes in serum glucose concentrations at the end of the study. Similarly, no significant difference was found for serum cholesterol and triglyceride levels due to BPA treatments. On the other hand, oral BPA administration increased serum urea, creatinine, amylase, lipase CRP, and GGT levels, especially in B20 and B100 groups compared to the controls. Similarly, although the serum AST, ALT, and ALP levels of C and B10 groups were not significant, the serum levels of these enzymes were increased significantly in B20 and B100 groups compared to the rabbit in the C group.

MDA, SOD, CAT, and GPx levels from serum, liver, and kidney samples of the treatment groups are in Table 4. In general, BPA treatments significantly increased serum, liver, and kidney MDA levels. The different BPA doses were also caused a decrease in SOD and CAT levels compared to controls. The serum and liver GPx levels decreased in the B20 and B100 groups, whereas there is no statistical difference in the kidney GPx levels between the rabbits in C and B10 groups.

For histopathological examination of the liver and kidney tissues, the study by [11] was taken into consideration. In the liver tissues of the BPA group, it was observed that the vena centralis, vena porta, and sinusoids were dilated and filled with erythrocytes. Severe degenerative changes ranging from cloudy swelling to hydropic were observed in liver epithelial cells. Hepatocytes with pycnotic hyperchromatic nuclei were noticed in some areas. Mild focal mononuclear cell infiltrations, mostly mid-zonal and occasionally centriacinar localized, were observed in the parenchyma. Kuppfer in stellate cells were prominent. There were edema and hyalinizations around the portal veinin some areas (Fig 1 and 2). In BPA-treated rabbits, the nuclei of the tubular epithelial cells were pycnotic or completely obliterated. In some areas, proteinous masses were found in the lumens of the proximal convoluted and distal tubules

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(Fig.3). In some areas of the interstitium, edema, bleeding and focal mononuclear cell infiltrations were apparent. In addition, fibrotic changes and atrophic glomeruli were found in the interstitium (Fig 4).

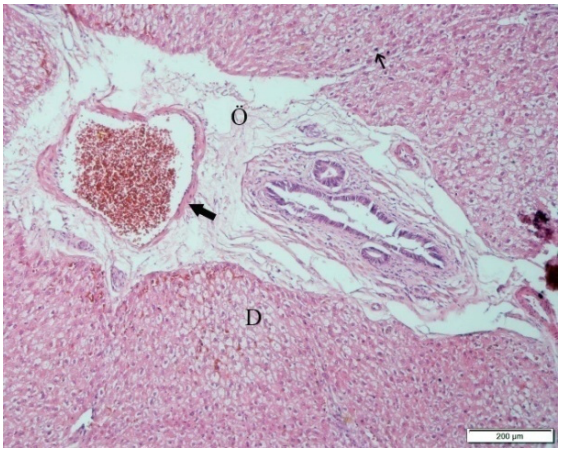

Fig.1. Liver-BPA: Severe degeneration of the liver (D), Hepatocytes with pycnotic nuclei (Thin arrow), Perivascular edema in the portal region (O), Dilated portal vein (Bold arrow), HE, X100.

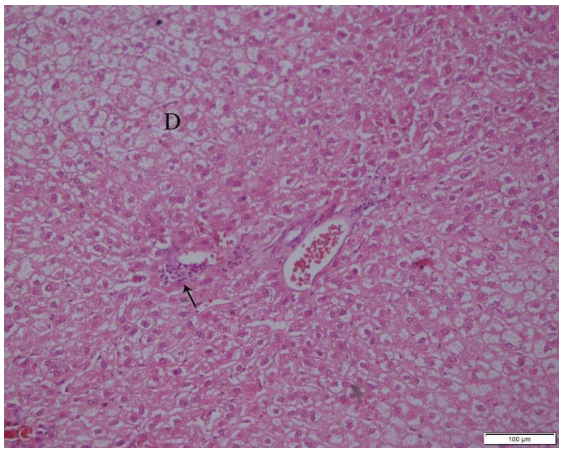

Fig.2. Liver-BPA: Severe degenerative changes (D) and focal mononuclear cell infiltration (Thin arrow) in hepatocytes, HE, X200.

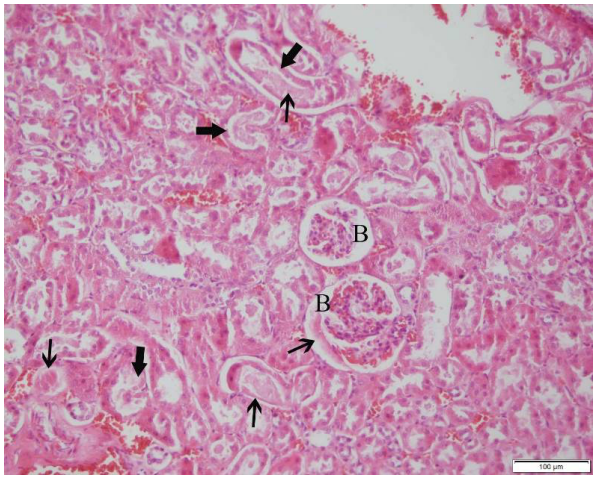

Fig.3. Kidney-BPA: Dilatation in proximal and distal tubules, severe degenerative and necrotic changes (Thick arrows), Enlargement of Bowman's space (B), Proteinous masses in Bowman's space and tubular lumens (Hyaline caste), (Thin arrows). HE, X200.

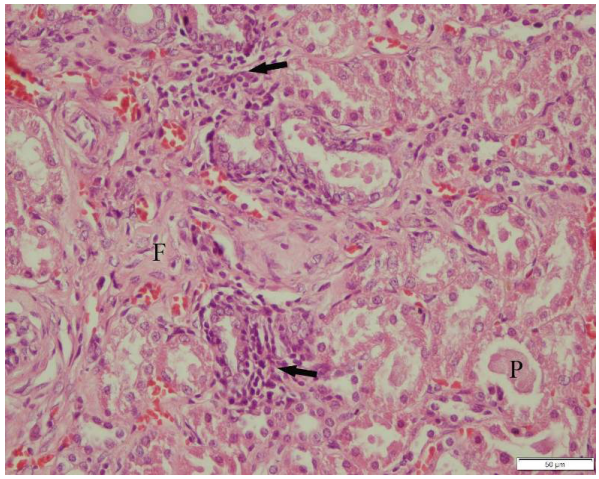

Fig.4. Kidney-BPA: Proteinous masses (P) in degenerated tubular lumens, focal mononuclear cell infiltrates (Arrows) and fibrosis (F) in interstitium. HE, X400.

IV. Discussion

During the current study, no detectable BPA was apparent in the blood samples of the rabbits in the C group as expected. On the other hand, the blood BPA concentrations statistically increased paralleling with the BPA dose applied. The LOAEL dose of BPA was 20 mg/kg/day in most of the parameters measured. This oral dose of BPA corresponds to the serum BPA concentrations of 0.58 µg/ml. The previous animal studies are usually concerned with the dose-related effects of BPA administered by different routes and have not dealt with what serum levels of BPA would cause toxic effects. The current study is unique in this sense, as it shows after which serum levels the negative effects of BPA could occur.

The BPA doses that were used in the current study did not affect the body weight and daily feed intake. Unlike our findings, there are different results regarding the effects of BPA on body weights [12]. Reference [13] reported that male adult mice exposed to BPA at doses of 0.5 and 2 mg/kg/day for 4 weeks increased body weights. As a synthetic estrogen, BPA could increase body weight during the perinatal and prepubertal periods [14]-[18]. Although there are numbers of studies that show an increase in body weight due to BPA exposure, there are also studies that report the opposite. BPA could cause a reduction in body weight in mice [19] and rats [20]. However, there are other studies implying that BPA had no effect on body weight in adults. BPA administered at a dose of 25 [21], and 150mg/kg/day [11] had no effect on body weight in rats. The difference in the literature can be explained by the age of the animals, the dose, and the duration of BPA, the sex of the animals, and the species that were used.

Our data failed to reveal an overall negative association between BPA and cholesterol. Studies examining the relationship between BPA and cholesterol are limited. In one study, a short-term (4 days) oral BPA administrations at doses of 0.1, 1, and 10 mg/kg/day did not affect total cholesterol levels [22]. Similarly, BPA given at low doses (0.033 and 0.333 mg/kg) for three months did not affect cholesterol levels statistically [23]. However, different studies are showing the negative effects of BPA on fat

metabolism. Reference [24] reported that BPA applications increased cholesterol and triglyceride levels in rats. In another study conducted with elderly people, there was a positive correlation between high serum BPA and cholesterol levels, but no effect on triglycerides[25]. Metabolic problems such as glucose intolerance, dyslipidemia, increased cholesterol, and triglyceride accumulation in the liver was observed in mice given BPA for a total of 10 months. In addition, exposure to BPA increased the expression of key enzymes that stimulate fatty acid and cholesterol synthesis in the liver [26].

Erythrocytes are the most abundant cell type in the blood, and they have very important physiological functions such as oxygen transport. Many xenobiotics are carried in the blood and therefore RBC can be affected by these xenobiotics [27]. In the current study, BPA had a negative effect on RBC, hemoglobin, and MCHC. Similarly, BPA triggered the formation of free radicals in the RBC membrane [28]. BPA could be cytotoxic to RBC due to its lipophilic property. BPA might bind to the iron in hemoglobin and cause the iron to dissociate from hemoglobin, and free iron could stimulate lipid peroxidation. Moreover, free radical formation and peroxidation could cause damage to RBC, shortening their lifespan and causing early hemolysis [29]. Phenolic compounds such as BPA cause the formation of superoxide radicals and thus the oxidation of iron in the heme molecule, leading to methemoglobin conversion [30]. Erythrocytes containing methemoglobin are rapidly removed from the circulation [31]. It has been shown that the decrease in erythrocyte count may be due to disruption of erythropoiesis and/or increased destruction of red blood cells [32]. In an in vitro study, a dose of 150 µg/ml BPA caused 96.20%hemolysis on human erythrocytes [33]. BPA also causes oxidative damage in the bone marrow [34]. In our study, disruption of erythropoiesis and/or destruction of RBC as a result of hemolysis could explain the negative effects of BPA on hematologic parameters.

Serum amylase, lipase, CRP, GGT, urea, and creatinine levels were elevated in the two highest BPA doses in the current study. An increase in serum levels of these tissue markers is generally perceived as tissue and organ damage. Thus, changes in these tissue markers suggest liver, kidney, and pancreas damage due to BPA exposure. It has been suggested that there was a positive correlation between BPA and inflammation indicators such as serum CRP [35]. Thus, elevated serum CRP concentrations imply that there was a general inflammation related to BPA exposure. Furthermore, BPA adversely affected urea and creatinine levels in lab animals [11], [36]-[38]. Generally, BPA is excreted by the kidneys after conjugation by the liver. Oxidative stress during the excretion of conjugated BPA can cause a problem in the kidneys. Mesangial cell proliferation in kidney tissues, presence of pink homogeneous proteinous masses in Bowman spaces, edema and hemorrhage observed in some areas, and pycnotic tubular epithelial cell nuclei suggested kidney damage for the rabbits in the BPA groups. The possible damage to the kidney tissues due to BPA exposure might be the main cause of increased serum urea-creatinine levels. In addition, the increase in serum creatinine levels could imply that the kidneys were not sufficient to effectively remove toxic metabolites from the body due to BPA.

Serum ALT, AST, and ALP levels are important enzymes that provide information about liver functions. When there is a degeneration in the liver tissue, an increase in serum levels of these enzymes is observed [39]. It has been reported that high urinary BPA levels may cause adverse changes in liver functions [40], [41]. Based on our findings, the two highest doses of BPA increased serum levels of liver enzymes in male rabbits. Damage seen in the liver tissues also supports the elevated levels of these liver enzymes. Histopathological findings of liver tissue show severe degenerative changes due to BPA. Hepatocytes with pycnotic hyperchromaticnuclei, focal mononuclear cell infiltrations, edema, and hyalinization around the portal vein were observed in some areas of the liver tissues examined in the current study. Epidemiological studies in humans also suggested a positive relationship between urinary BPA and liver enzymes such as ALT and AST [42]. BPA was also reported to cause liver damage and alter the liver enzymes in rats [43]. In addition, the increase in liver enzymes can be perceived as a marker of a general inflammation phenomenon [44]. The degree of elevation of these enzymes increases in direct proportion to the loss of hepatocellular function. BPA can increase oxidative [45]. Thus, elevated BPA levels can stimulate the tissue levels of reactive oxygen species (ROS) and free radical formation, disrupt the prooxidant/antioxidant balance, and increase the risk of causing damage to the liver tissue [46].

In the current study, BPA exposure caused an elevation in MDA levels, while SOD, CAT, and GPx levels declined. It is generally accepted that oxidative stress occurs as a result of the imbalance between the production of free radicals and/or ROS, and their elimination by antioxidants, and this is thought to be a pathological mechanism that contributes to the initiation and progression of tissue damage. MDA, a widely used indicator of lipid peroxidation, is considered a potential biomarker for oxidative stress. SOD catalyzes the breakdown of superoxide radicals into hydrogen peroxide, while CAT and GPx are enzymes that convert hydrogen peroxide into hydrogen oxide. BPA-induced increase in MDA in the liver and kidney tissues is a sign of increased lipid peroxidation in the liver and kidneys. In addition, the decrease in SOD, CAT, and GPx levels in the liver and kidney tissues suggested increased oxidative stress. Similar conclusions were reported stating that oxidative stress increased in BPA-related tissues; [3], [4], [48]. BPA stimulated oxidative stress in the liver and is responsible for the release of marker enzymes related to hepatotoxicity and mitochondrial dysfunction by causing cell membrane damage [49]. Thus, BPA causes depletion of the antioxidant defense system and oxidative stress in tissues by decreasing SOD, CAT, and GPx activities.

V. Conclusion

The present BPA study concluded that the LOAEL value of oral BPA for hematologic and biochemical parameters was 20 mg/kg/day (0.58 µg/ml plasma). Even though BPA, up to 100mg/kg/day dose level, did not cause any clinical problems and did not cause any negative effects on body

weight gain, oral BPA exposure for 9 weeks could alter the oxidative status, cause oxidative stress, and develop tissue degeneration in New Zealand White rabbits.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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