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RESEARCH ARTICLE

Potential effects of punicalagin on New Zealand White rabbits exposed to bisphenol A

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ABSTRACT

The possible effects of punicalagin on some oxidant-antioxidant enzymes and biochemical parameters in bisphenol A (BPA)-treated rabbits were investigated. Animals were randomly divided into 4 groups, each containing 6 rabbits: Control (C; corn oil and distilled water), BPA (BPA; 20 mg/kg BPA in corn oil and distilled water), the punicalagin (PUN; corn oil and 2 mg/kg punicalagin in distilled water), and BPA-punicalagin (B+P; 20 mg/kg BPA in corn oil and 2 mg/kg PUN in distilled water) groups. Daily treatments continued for 9 weeks and doses were adjusted according to body weights measured for each week. At the end of the study, hematological, biochemical, and oxidant-antioxidant parameters were measured from blood and tissue samples. The difference in the levels of plasma bilirubin, albumin, total plasma protein, Mg, P, Ca, Na, K, and levels of glutathione peroxidase in plasma, liver, and kidney were non-significant ($p>0.1$). However, oral BPA administration adversely affected serum cholesterol, LDL, HDL, amylase, lipase, CRP, and GGT concentrations. Likewise, malondialdehyde, catalase, and superoxide dismutase levels in the kidney and liver were also negatively altered by BPA ($p<0.05$). Significant improvements in these parameters were apparent in the B+P group. The data generated here showed that punicalagin possessed a beneficial impact on potentially reducing the possible toxic effects of BPA in rabbits.

Keywords: Antioxidants, Cholesterol, Oxidant-Antioxidant enzymes, Polyphenols, Toxication

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Bisfenol A'ya maruz kalan erkek Yeni Zelanda tavşanlarında punikalajinin potansiyel etkileri

ÖZ

Çalışmada, BPA verilen Yeni Zelanda tavşanlarında punikalajininbazı oksidan-antioksidan enzimler ile bazı biyokimyasal parametreler üzerine olası etkilerin incelendi. Bu amaçla 2 hafta boyunca laboratuvar koşullarına alıştırılan tavşanlar, her grupta 6 tavşan olacak şekilde rastgele 4 gruba ayrıldı: Kontrol (C; mısır yağı ve distile su), BPA (BPA; mısır yağı içerisinde 20 mg/kg BPA ve distile su), punikalajin (PUN; mısır yağı ve distile su içerisinde 2 mg/kg punikalajin), ve BPA-punikalajin grubu (B+P; mısır yağı içerisinde 20 mg/kg BPA ve distile su içerisinde 2 mg/kg PUN). Uygulamalar 9 hafta boyunca günlük olarak yapıldı ve haftada bir kez yapılan tartımlara göre dozlar ayarlandı. Çalışma sonunda alınan kan ve doku örneklerinden hematolojik, biyokimyasal ve oksidan-antioksidan parametrelerin ölçümleriyapıldı. Analizler neticesinde plazma bilirubin, albümin ve toplam plazma protein düzeyleri ile Mg, P, Ca, Na, K, seviyelerinde herhangi bir istatistiki farka rastlanmazken, farklı gruplardaki plazma, karaciğer ve böbrek glutatyon peroksidaz değerleri de önemsiz bulundu ($p>0,1$). Oral BPA uygulamaları serum kolesterol, LDL, HDL, amilaz, lipaz, CRP, GGT seviyeleri ile karaciğer ve böbrek dokusundaki malonildialdehit, katalaz ve süperoksit dismütaz seviyelerini olumsuz etkiledi ($p<0,05$). B+P grubunda ise bu parametrelerde önemli ölçüde iyileşme gözlendi. Elde edilen sonuçlar, BPA'nın erkek tavşanlarda yol açtığı olası toksik etkilerin punikalajin tedavisi ile önemli ölçüde düzeltilebileceğini gösterdi.

Anahtar kelimeler: Antioksidanlar, Kolesterol, Oksidan-Antioksidan enzimler, Polifenoller, Toksikasyon

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INTRODUCTION

Antioxidants can be helpful in preventing the formation of reactive oxygen species (ROS) and reducing the potential damage caused by ROS. Thus, it is a major defense mechanism that regulates the detoxification process (Şener and Yeğen 2009). Antioxidants are either produced by the organism itself (endogenous) or taken from the diet or other outer sources (exogenous). When exposed to high levels of oxidants, the body's natural antioxidant mechanism may be insufficient. This phenomenon is called oxidative stress and occurs due to the imbalance between pro-oxidants and antioxidants reserves (Karabulut and Gulay 2016a). Substances such as superoxide, hydroperoxyl, and hydroxyl radical are some of the sources of oxidative stressors for the body. Such substances can cause both lipid peroxidation in cell membranes and DNA damage (Aydemir et al. 2009). Therefore, live organisms require certain levels of antioxidants to prevent the detrimental effects of free radicals on tissues (Karabulut and Gulay 2016b).

Over the last two decades, there has been a considerable scientific effort to evaluate the toxic effect of bisphenol A (BPA). People are exposed to man-made industrial chemical products, particularly plastics in daily life. It is thought that the intensive use of BPA resulted in environmental pollution that could adversely affect human and animal health, and lead to an increase in oxidative stress in the body (Hamed and Abdel-Tawwab 2017). BPA is closely associated with oxidative stress in different tissues and organs, and further cause a number of metabolic problems (Ogo et al. 2017, Karabulut 2019, Karabulut and Gulay 2020). Today, reducing the use of BPA and other external oxidative stress sources is extensively discussed elsewhere (ECHA, 2021). Moreover, it is evaluated that endogenous antioxidants alone may not be sufficient against increased oxidative stress (Suhendi et al. 2018).

Pomegranate (Punica granatum L.) is a fruit that is frequently consumed in our country and the Mediterranean region. This fruit has a very high antioxidant capacity and punicalagin is thought to be responsible for the most important part of the antioxidant properties in pomegranate juice (Gil et al. 2000). Due to its high antioxidant capacity, it is possible to see studies suggesting anti-inflammatory (Jean Gilles et al. 2013, Lin et al. 1999), antimicrobial (Machado et al. 2002, Silva et al. 2015), and tissue-protective effects (Lin et al. 2001, Yildiz-Gulay and Gulay 2019) of punicalagin. Moreover, it is possible that punicalagin can be used to reduce or prevent the possible toxic effects of BPA (Yildiz-Gulay et al. 2020). Therefore, our study aimed to investigate the potential protective effect of this natural antioxidant in male New Zealand rabbits given oral BPA.

MATERIALS and METHODS

The experiment was supported partly by TUBITAK (116O027) and Mehmet Akif Ersoy University Scientific Research Projects Unit (BAP-0474YL-17) and approved by the Ethics Committee of the Mehmet Akif Ersoy University (25.11.2015/159). Individually housed male New Zealand White rabbits (8 to 10 months old, n=24) were kept at 22 ± 2 ºC, 50-55% humidity, and 10 hours of dark - 14 hours of light cycle in the Experimental Animals Unit of Mehmet Akif Ersoy University, Faculty of Veterinary Medicine. Water was freely available at all times. The rabbits were fed ad libitum with commercial rabbit feed (0,49% calcium, 0.46% phosphorous, 3,67% crude oil, 6,93% crude ash, 12.68% crude cellulose, 17,0% crude protein; Korkuteli Food Company, Antalya, Turkey). The initial weights of the rabbits prior to the experiment were 2,8-3,7 kg. Bodyweight and feed intake measurements were made weekly. BPA and punicalagin amounts were adjusted weekly according to the weekly body weight changes of each individual rabbit. After the adjustment period of 2 weeks, the rabbits were randomly divided into 4 groups (n=6 per treatment group). Rabbits in the control group (C) received daily corn oil (1 mL corn oil for 1 kg live weight) + daily distilled water (1 mL distilled water for 1 kg live weight). Rabbits in the bisphenol A group (BPA) were treated with daily BPA (20 mg/kg live weight) in corn oil (1 mL corn oil contained 20 mg BPA) and daily distilled water (1 mL distilled water for 1 kg live weight), whereas Punicalagin group (PUN) received daily punicalagin (2 mg/kg live weight) in distilled water (1 mL distilled water contained 2 mg punicalagin) and daily corn oil (1 mL corn oil for 1 kg live weight). The last group (Bisphenol A+Punicalagin group: B+P) was given the same amounts of daily BPA (20 mg/kg live weight) in corn oil and daily punicalagin (2 mg/kg live weight) in distilled water. The current oral doses for BPA (Karabulut and Gulay 2020) and punicalagin (Yildiz-Gulay and Gulay 2019) were selected according to the previous studies. Daily BPA and punicalagin doses were administered orally before the morning feedings between 08:00 and 09:30 hours.

At the end of the experiment (day 63), no food was given to the rabbits for 12 hours. K3 EDTA and gel-activated blood collection tubes were used for a blood sample collection from the ear arteries. Hematologic parameters (such as red blood cells-RBC, white blood cells-WBC, hematocrit, hemoglobin, etc.,) were measured by using an autoanalyzer (Abacus Junior Vet SN-100702) right after blood collection. The remaining blood samples were centrifuged (20 min, at 1457 x g). Following centrifugation, serum and plasma samples were stored at -80 °C. Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine values were analyzed on the same day from fresh serum by an

autoanalyzer (Gesan Chem 200). Other biochemical parameters (Mg, P, Ca, Na, K, bilirubin, albumin, total plasma protein levels, serum cholesterol, LDL, HDL, Amylase, Lipase, gamma-glutamyl transferase-GGT, and C-reactive protein-CRP) were analyzed from the thawed serum samples (Achitech C8000).

The lungs, liver, kidney, brain, and spleen of each rabbit were collected immediately after sacrifice and washed with PBS chilled to 5ºC, and weights of the organs were recorded. The right kidney and the caudal lobe of the liver were stored at -80 ºC until used for oxidant and antioxidant parameters. Before the analysis for oxidant-antioxidant parameters with ELISA kits (SinoGeneClon Biotech Co., Ltd., China), the tissue samples were prepared according to the manufacturer's instructions. Superoxide dismutase (SOD; Cat No: SG-0061Rb; detection range of 30-1000 pg/mL; intra- and inter-assay precision of <10%), glutathione peroxidase (GPx; Cat No: SG-0120Rb; detection range of 33-3000 pg/mL; intra- and inter-assay precision of <10%), catalase (CAT; Cat No: SG-50185; detection range of 1-36 pg/mL; intra- and inter-assay precision of <10%), and malondialdehyde (MDA; Cat No: SG-50252; detection range of 0.3-7 mmol/L; intra- and inter-assay precision of <10%), concentrations of the tissue and serum samples were read at 450 nm.

Statistical analysis of the data was evaluated using the SAS statistical program. ANOVA test was used to determine the statistical differences among the groups. The Tukey test was used for the comparisons of the individual treatment groups.

RESULTS

During the experiment, no apparent health problems were encountered in any of the study groups. Data on feed consumption, body weights, and wet organ weights of rabbits are in Table 1. There was no statistical difference in feed consumption, body weights, and the weights of various organs among the groups.

Considering the hematological parameters, no significant changes were observed in the total WBC and platelet counts, WBC percentages, hematocrit, and MCH values. On the other hand, RBC, hemoglobin, MCV, and MCHC were affected significantly (Table 2). While RBC count did not differ in the C, PUN, and B+P groups, there was a significant decrease in the BPA group compared to the C and PUN groups. (Table 2). The MCV was also affected by BPA treatment. Moreover, MCHC was the highest in the PUN group. Although MCHC decreased significantly in the BPA group, the decrease in MCHC was corrected by punicalagin in the B+P group (Table 2).

The serum biochemical parameters from the treatment groups were in Table 3. Results indicated that orally administered punicalagin and BPA applications did not have any effect on serum minerals, glucose, and triglyceride levels. Similarly, punicalagin and BPA applications did not have any effect on plasma bilirubin, total bilirubin, albumin, and total protein levels. However, serum lipase, CRP, and GGT levels were significantly affected by the treatments. BPA treatment caused a significant increase in serum amylase, lipase, CRP, and GGT levels while punicalagin treatments were able to lower these parameters up to control levels when given with BPA in the B+P group. In addition, the administration of BPA alone caused a significant increase in serum urea, creatinine, AST, and ALT levels when compared with the C and PUN groups, while this increase was inhibited in the B+P group. A similar trend was apparent in serum cholesterol levels. Serum total cholesterol and LDL levels were higher in the BPA treated group than in the C and PUN groups. Conversely, HDL levels were not affected by the BPA and punicalagin treatments. On the other hand, BPA alone caused a decrease in the serum HDL/LDL percentages (Table 3).

The effects of BPA and punicalagin treatments on some antioxidant parameters are in table 4. In general, GPx levels in plasma, kidney, and liver tissues were not statistically affected by the treatments studied. However, the SOD enzyme was decreased due to BPA treatments at tissue and plasma levels, and punicalagin administration reduced this decrease to control levels in the B+P group (Table 4). A similar trend was evident for the CAT enzyme. CAT levels were decreased significantly in the BPA group, but the decrease in CAT levels was statistically inhibited in the B+PUN group when punicalagin was administered with BPA. In addition, the use of BPA increased MDA levels in all tissues and blood compared to the C group. However, punicalagin applications in the B+P group were effective in reducing the MDA levels to the levels of the C group and reduced the negative effect of BPA.

Table 1. The effects of oral administration of BPA and Punikalagin on body weight, feed consumption and organ weights in male New Zealand rabbits.

	C	BPA	PUN	B+P	P=
Body Weight (kg)	3.65 ± 0.49	3,61 ± 0.36	3.45 ± 0.42	3.39 ± 0.45	0.625
Feed Intake (g)	196.3 ± 39.3	168.5 ± 27.0	180.5 ± 38.0	173.9 ± 28.5	0.129
Lung (g)	14.9 ± 2.09	15.0 ± 1.62	13.9 ± 1.64	15.8 ± 2.06	0.395
Liver (g)	105.5 ± 15.5	98.4 ± 17.5	106.2 ± 18.1	99.9 ± 17.2	0.675
Right Kidney (g)	10.5 ± 1.20	9.59 ± 1.19	9.22 ± 1.53	9.98 ± 1.14	0.392
Left Kidney (g)	10.3 ± 1.21	9.58 ± 1.34	9.07 ± 1.60	10.0 ± 1.00	0.429
Spleen(g)	1.02 ± 0.32	1.12 ± 0.39	1.18 ± 1.41	1.13 ± 0.39	0.859
Brain (g)	7.18 ± 0.80	6.61 ± 0.35	6.75 ± 0.35	6.78 ± 0.67	0.398
Heart (g)	9.62 ± 1.07	10.6 ± 1.20	9.81 ± 1.39	9.65 ± 0.98	0.435

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin.Values have given as mean ± standard deviation.

Table 2. The effect of oral administration of BPA and Punikalagin on some blood parameters in male New Zealand rabbits.

	C	BPA	PUN	B+P	P=
WBC ($x10^9$/l)	7.16 ± 1.42	6.41 ± 1.80	7.69 ± 2.17	7.81 ± 1.92	0.470
Lymphocyte($x10^9$/l)	1.97 ± 1.20	2.34 ± 1.14	2.36 ± 1.91	2.74 ± 2.01	0.875
Monocyte ($x10^9$/l)	0.24 ± 0.18	0.42 ± 0.30	0.46 ± 0.36	0.50 ± 0.28	0.456
Granulocyte($x10^9$/l)	4.95 ± 1.09	3.64 ± 1.20	4.88 ± 2.07	4.55 ± 2.28	0.554
Lymphocyte (%)	26.6 ± 16.1	36.1 ± 16.6	30.0 ± 17.0	34.1 ± 20.4	0.789
Monocyte (%)	3.2 ± 2.56	6.8 ± 4.98	5.9 ± 4.25	7.1 ± 4.11	0.350
Granulocyte (%)	70.2 ± 0.49	57.1 ± 18.2	64.1 ± 17.8	58.8 ± 33.6	0.623
RBC ($x10^{12}$/l)	6.88^a ± 0.91	6.17^b ± 0.16	7.00^a ± 0.90	6.64^{ab} ± 0.21	0.035
Hemoglobin (g/dl)	13.5^a ± 0.45	11.9^b ± 0.34	13.4^a ± 1.34	12.9^{ab} ± 1.28	0.042
Hematocrit (%)	46.1 ± 3.68	47.2 ± 1.98	43.6 ± 3.87	44.7 ± 4.57	0.246
MCV (fl)	67.1^a ± 2.16	76.5^b ± 4.39	62.6^a ± 5.10	67.3^a ± 5.79	0.001
MCH (pg)	19.6 ± 0.39	19.3 ± 0.74	19.2 ± 1.00	19.3 ± 1.66	0.892
MCHC (g/dl)	29.3^{ab} ± 1.09	25.3^c ± 1.20	30.7^a ± 1.06	28.7^b ± 0.56	0.001
Platelet ($x10^9$/l)	418 ± 60	430 ± 99	351 ± 80	395 ± 109	0.457

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin.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.

Table 3. The effect of oral administration of BPA and Punikalagin on some biochemical parameters in male New Zealand rabbits.

	C			BPA			PUN			B+P			P=
Chol (mg/dl)	41.2^{ab}	±	1.30	46.8^{c}	±	1.92	39.8^{a}	±	3.11	43.6^{bc}	±	2.70	0.002
LDL (mg/dl)	16.22^{a}	±	1.57	20.44^{b}	±	2.42	14.36^{a}	±	1.90	16.94^{a}	±	2.02	0.002
HDL (mg/dl)	10.56	±	1.59	9.64	±	1.62	10.46	±	0.77	10.54	±	0.89	0.627
H/LDL (%)	65.10^{a}	±	10.89	47.16^{b}	±	8.77	72.84^{a}	±	9.04	62.21^{ab}	±	5.99	0.036
Trig (mg/dl)	95.8	±	18.5	97.2	±	14.4	100.1	±	12.8	99.8	±	15.6	0.953
Glucose(mg/dl)	119.3	±	13.1	113.2	±	8.81	120.6	±	12.4	118.7	±	6.80	0.634
Mg (mmol/l)	1.72	±	0.33	1.35	±	0.17	1.46	±	0.21	1.52	±	0.40	0.284
P (mmol/l)	5.48	±	1.20	5.16	±	1.56	4.65	±	0.65	5.0	±	0.91	0.770
Ca (mg/dl)	13.22	±	0.68	12.06	±	2.31	12.96	±	1.69	12.92	±	0.41	0.639
Na (mmol/l)	139.2	±	4.20	138.6	±	2.50	140.4	±	4.77	139	±	2.23	0.873
K (mmol/l)	4.24	±	0.26	4.15	±	0.13	3.97	±	0.28	4.13	±	0.24	0.379
AST (IU/l)	19.8^{a}	±	4.94	34.6^{b}	±	12.6	17.3^{a}	±	4.20	21.9^{a}	±	9.93	0.012
ALT (IU/l)	58.3^{a}	±	12.9	82.5^{b}	±	16.9	51.5^{a}	±	5.54	60.5^{a}	±	19.9	0.009
ALP (IU/l)	70.2^{a}	±	26.9	123.3^{b}	±	33.7	78.3^{a}	±	21.6	93.8^{ab}	±	17.4	0.019
Urea (mmol/l)	29.3^{a}	±	3.82	40.1^{b}	±	7.96	31.7^{a}	±	7.44	31.8^{a}	±	3.76	0.029
Creat (mg/dl)	0.67^{a}	±	0.15	0.82^{b}	±	0.10	0.62^{a}	±	0.07	0.69^{a}	±	0.08	0.024
Amylase (IU/l)	155.6^{a}	±	50.9	228.8^{b}	±	52.4	148.0^{a}	±	41.1	169.6^{ab}	±	29.1	0.043
Lipase (IU/l)	119.6^{a}	±	47.5	173.2^{b}	±	22.9	112.8^{a}	±	21.3	136.8^{ab}	±	26.3	0.004
CRP (md/dl)	0.98^{a}	±	0.59	4.75^{b}	±	2.38	0.73^{a}	±	0.46	2.74^{a}	±	1.58	0.002
GGT (IU/l)	6.40^{a}	±	2.96	15.40^{b}	±	2.30	6.40^{a}	±	1.14	9.80^{a}	±	3.49	0.001
Bil (µg/dl)	0.12	±	0.04	0.18	±	0.13	0.12	±	0.04	0.14	±	0.09	0.654
TotBil (µg/dl)	0.51	±	0.38	1.04	±	0.64	0.42	±	0.24	0.69	±	0.26	0.167
Albümin (g/dl)	3.16	±	0.45	2.84	±	0.70	3.10	±	0.40	3.16	±	0.26	0.608
TotP (g/dl)	6.06	±	0.32	5.38	±	1.22	6.16	±	0.43	5.76	±	0.42	0.323

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin. Chol=Cholesterol, LDL=Low density lipoprotein, HDL=High density liploprotein, H/LDL=HDL/LDL ratio (HDL/LDLx100), Trig=Triglyceride, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, Creat=Creatinine, CRP= C reactive protein, GGT=Gamma glutamyl transferase, Bil=Billirubin, TotBil=Total billirubin, TotP=Total plasma protein. Values have given as mean ± standard deviation.

Table 4. The effect of oral administration of BPA and Punicalagin on some oxidant-antioxidant enzymes in blood and various tissues in male New Zealand rabbits.

	C			BPA			PUN			B+P			P=
SOD (nmol/L)													
Plasma	501.2^{ab}	±	99.2	410.4^{c}	±	76.2	567.8^{a}	±	44.5	458.4^{bc}	±	45.1	0.012
Kidney	386.2^{a}	±	98.4	272.6^{b}	±	78.1	420.6^{a}	±	64.7	347.4^{ab}	±	63.8	0.045
Liver	400.1^{ab}	±	52.4	280.8^{c}	±	64.8	437.2^{a}	±	66.0	335.2^{bc}	±	69.8	0.011
MDA (nmol/L)													
Plasma	3.37^{ab}	±	0.65	4.88^{b}	±	1.76	2.51^{a}	±	0.77	3.68^{ab}	±	0.87	0.028
Kidney	2.10^{ab}	±	0.80	3.24^{b}	±	1.37	1.16^{a}	±	0.44	2.36^{ab}	±	1.11	0.033
Liver	2.72^{ab}	±	0.96	4.84^{c}	±	1.91	1.69^{a}	±	0.54	3.78^{bc}	±	0.82	0.004
CAT (pg/ml)													
Plasma	4.81^{ab}	±	1.43	2.59^{c}	±	0.48	5.81^{a}	±	2.04	3.32^{bc}	±	1.45	0.013
Kidney	17.32^{a}	±	4.82	11.97^{b}	±	1.73	19.21^{a}	±	1.81	16.16^{a}	±	2.81	0.012
Liver	16.80^{a}	±	4.74	11.71^{b}	±	2.05	19.1^{a}	±	2.30	14.98^{ab}	±	2.86	0.014
GPx(pg/ml)													
Plasma	2742	±	171	2784	±	236	2764	±	262	2879	±	437	0.887
Kidney	2347	±	687	1880	±	618	2133	±	268	2183	±	404	0.573
Liver	2144	±	633	1779	±	316	2176	±	299	1988	±	394	0.470

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin. SOD=superoxide dismutase; MDA=malondialdehyde; CAT= catalase; GPx= glutathione peroxidase.Values have given as mean ± standard deviation.

DISCUSSION

In the present study, BPA applications had no effect on body weight and feed consumption in male rabbits. There are different results regarding the effects of BPA on body weight in laboratory animals (Liu et al. 2013). Unlike our findings, Moghaddam et al. (2015) reported that BPA administered intraperitoneally up to 2mg/kg/day for 4 weeks caused an increase in body weight in adult mice. However, there are also findings that BPA given at a dose of 5000µg/kg/day in adult mice had no effect on body weight (Marmugi et al. 2012). Similarly, Avcı et al. (2016) stated that BPA administered at a dose of 25 mg/kg/day had no effect on body weight in rats. It has been reported that there may be a relationship between the increase in blood BPA levels and obesity in humans and rodents and that high BPA may cause an increase in body weight by accelerating fat storage due to oxidative stress (Moghaddam et al. 2015). However, Marmugi et al. (2014) reported that there was no change in body weight in 6-week-old mice exposed to BPA for 31 weeks. This difference in the literature can be explained by the duration of application of BPA, the age of the animals, and the different species.

Red blood cells are the most abundant cell type in the blood and have very important physiological functions. Many xenobiotics are carried by our blood and therefore it is possible for RBC to be affected by these xenobiotics (Stasiuk et al. 2009). In our study, oral BPA had a negative effect on the RBC, hemoglobin amount, and MCHC. Similarly, Macczak et al. (2016) reported that BPA could affect RBC levels and cause hemolysis. In a different study, BPA triggered the formation of free radicals in the RBC membrane (Suthar et al. 2014). It has been reported that BPA was cytotoxic to RBC due to its lipophilic property. It is suggested that BPA might bind to the iron in hemoglobin and cause the iron to dissociate from hemoglobin. Then, the free iron could pass into the RBC cytoplasm and cause lipid peroxidation. Free radical formation and peroxidation cause damage to RBCs, shortening their lifespan and causing early hemolysis (Macczak et al. 2016). The literature reveals that 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 (Bukowska and Kowalska 2004). Consequently, RBCs that contain methemoglobin will rapidly be removed from the circulation.

Punicalagin applied at different doses in our study did not have a positive or negative effect on the blood parameters measured in this study. In a study in rats, punicalagin administered orally at a dose of 4800 mg/kg/day for 37 days had no effect on hematological parameters (Cerda et al. 2003). Moreover, pomegranate extract at doses of 60, 200 and 600 mg/kg/day given orally in rats did not have a negative effect on the number of RBCs and the amount of hemoglobin (Patel et al. 2008). Although oral administration of punicalagin to rabbits alone did not affect blood parameters in our study, when applied together with BPA, it improved the negative effect of BPA on RBC. The favorable effects of punicalagin, when administered with BPA, may be due to the superb antioxidant properties of this phytochemical. As previously shown, punicalagin was responsible for more than 50% of the high antioxidant properties found in pomegranate juice (Gil et al. 2000). Exogenous antioxidants, together with endogenous antioxidants, are a vital system that works both outside and inside the cell to prevent the deterioration of the oxidant-antioxidant balance. Thus, the number of free radicals was reduced and oxidative stress was suppressed, thereby increasing resistance to diseases (Berger 2005).

The results of the current study suggested that oral BPA negatively affected cholesterol levels in male rabbits. Studies examining the effects of BPA on cholesterol levels are limited. Parallel to our study, oral BPA at a dose of 2.72 mg/kg body weight in female mice increased the cholesterol levels (Miyawaki et al. 2007). On the other hand, Dodge et al. (1996) reported that BPA given orally for 4 days (0.1, 1, and 10 mg/kg body weight) did not affect total cholesterol levels in rats. Similarly, Seidlova-Wuttke et al. (2005) did not find any effect on total cholesterol levels of BPA given with feed at doses of 0.033 and 0.333 mg/kg body weight for 3 months. However, in these last two studies, either BPA duration (4 days) or doses (0.033 and 0.333 mg/kg body weight) were kept at limited levels, so BPA might not have had any effect on serum cholesterol.

In different studies examining the effects of punicalagin on cholesterol levels, punicalagin protected macrophage cells from lipid deposition and foam cell formation (Aviram et al. 2002, Kaplan et al. 2001, Rosenblat and Aviram 2011). Similarly, when given along with a cholesterol-lowering drug (statins), punicalagin reduced the required statin dose, strengthened the effect of statin, and inhibited cholesterol biosynthesis (Reiner, 2014; Rosenblat et al., 2013). In atherosclerotic mice supplemented with 6.25 mL/L pomegranate juice in drinking water, macrophage lipid peroxidation, cellular cholesterol accumulation, and the development of atherosclerosis was reduced (Kaplan et al. 2001). It is assumed that punicalagin binds to ApoB100 in close proximity to the LDL receptor binding site. Upon binding, punicalagin could change the conformation of the protein and increase the affinity of LDL for the LDL receptor. Thus, punicalagin might lower serum LDL levels, possibly by interacting with the lipid portion of LDL and protein and by accelerating LDL transport

to macrophages (Atrahimovich et al. 2016). Although the punicalagin dose we applied in our study did not have a statistical effect on cholesterol or LDL levels, it contributed positively by reducing the negative effects of BPA, in line with the information in the literature.

Serum Mg, P, Ca, Na, and K levels were within the normal physiological limits suggested for rabbits (Jones 1975). Furthermore, the oral BPA administration did not significantly alter the levels of these minerals in serum. Although there is no study on the Mg, P, Na, and K levels of BPA in serum, it is thought that it may affect the plasma Ca level due to its potential estrogenic nature (Suzuki et al. 2003). In an 8-day experiment on goldfish, BPA increased the serum Ca level for the first 4 days and decreased it for the next four days. In addition, the serum calcitonin level was also affected in the last 4-day period when the Ca level decreased in these fish (Suzuki et al. 2003). Studies have reported that this was due to the effect of BPA not indirectly on estrogen, but directly by changing osteoblastic and osteoclastic activity (Suzuki and Hattori 2003).

Serum ALT, AST, and ALP are important enzymes that provide information about liver functions. An increase in serum levels of these enzymes is observed when there is a degeneration of liver tissue. (Henderson and Moss 2005). In our study, the daily 20 mg/kg BPA increased the serum levels of these liver enzymes in male rabbits. In similar studies in rats, it was stated that BPA increased liver enzymes (Avcı et al. 2016, Korkmaz et al. 2010). The degree of elevation of these enzymes increases in direct proportion to the loss of hepatocellular function. There are studies that BPA increases oxidative stress and may cause toxicity in organs (Korkmaz et al. 2010, Daidoji et al. 2003). It is thought that this situation occurs because it causes an increase in reactive oxygen products and free radical levels, disrupts the balance of prooxidants and antioxidants, and increases the risk of causing damage to the liver tissue (Videla 2009).

The punicalagin treatment during our study had no effect on the levels of ALT, AST, and ALP compared to control rabbits. In a similar study conducted on humans, it was reported that ellagitannin-enriched polyphenols were given 1420 mg/day orally for 28 days caused no negative effect on serum ALT, AST, or ALP levels (Haber et al. 2007). Supplementing rats at a dose of 600 mg/kg/day for 90 days did not cause an increase in these enzymes (Patel et al. 2008). In our study, punicalagin reduced the ALT, AST, and ALP levels to normal levels in rabbits treated with BPA. Similar effects of punicalagin were observed in rats given carbon tetrachloride (Vora et al. 2015). There are indications that pomegranate juice protects liver tissue in rats with hepatitis induced by D-galactosamine (Amal et al. 2012). Punicalagin may have shown this effect as an antioxidant indirectly by protecting the antioxidant defense system, removing ROS from the system, or suppressing lipid peroxidation.

In the current study, the levels of different tissue markers such as amylase, lipase, CRP, and GGT increased by BPA applications. An increase in blood levels of these markers suggests the possible negative effects of BPA in organs such as the liver, kidney, and pancreas. In addition, in previous studies in humans, there was a link between the increase in BPA levels and inflammation indicators, and the increase in BPA levels caused an elevation in serum CRP levels (Tarantino et al. 2013). Thus, the increase in serum levels of CRP seen in the current study due to BPA treatments suggests that there was a general inflammation related to BPA. On the other hand, it has appeared that punicalagin applications may be beneficial in alleviating the negative effects of BPA in these organs. The fact that BPA administration caused a raise in all these parameters suggests that this chemical alters the oxidative stress in the main organs and causes tissue damage related to it, and the positive effect of punicalagin on the parameters examined implies that the antioxidant capacity of punicalagin can be effective in overcoming increased oxidative stress.

The intracellular enzymatic antioxidants such as SOD, CAT, and GPx are vital because of their role in scavenging hydrogen peroxide and superoxide radicals. Furthermore, SOD forms the first line of defense against oxygen species (Sen et al. 2010). In the current study, the concentrations of SOD and CAT in the tissues and plasma of the BPA-treated rabbits were lower than for the rabbits in the C group. This may typically be the result of increased lipid peroxidation as a result of oxidative stress.

In different studies, BPA caused oxidative stress in fish and rodents (Andersen 2004, Geetharathan and Peera 2018, Kabuto et al. 2004, Qiu et al. 2016). In carp fish, BPA augmented the lipid peroxidation and suppressed SOD, CAT, and GPx activities when added to the water tanks at the doses of 10 - 1000 μg/L (Qiu et al. 2016). The increase in oxidative stress in rat heart tissues treated with BPA was accompanied by a decrease in the activity of SOD and CAT (Geetharathan and Peera 2018). Furthermore, BPA exposure increased lipid peroxidative damage in neurons by suppressing the enzymatic antioxidant defense, thus, negatively affecting the functional and structural development of the central nervous system (Andersen 2004). The increase in tissue MDA levels due to BPA can also be observed due to the production of ROS more than normal levels (Yonar et al. 2014). Therefore, BPA could boost the formation of additional ROS by inducing free radical formation in rabbits and causing oxidative stress (Chitra et al. 2003).

The data from the current study shows that the increase in MDA activity and the decrease in SOD and CAT concentrations due to BPA approached the levels of the C group with the addition of punicalagin.

Our results with punicalagin on antioxidant parameters are similar to the results obtained with different flavinoids and pomegranate juice in previous studies. In an in vitro study on goat liver tissues, punicalagin showed significant antioxidant activity exposed to oxidative stress (Yaidikar 2001). Similarly, Sun et al. (2017) showed that the application of punicalagin increased the effect of SOD. It shows that these antioxidants obtained from various plants and fruits are powerful superoxide molecule scavengers (Coballase-Urrutia et al. 2011). Various studies have shown that the pharmacological effects of tannins and flavonoids have major antioxidant activities, which may result from their ability to scavenge superoxide, chelate metal ions, and exert synergistic effects with other antioxidant metabolites (Manna et al. 2006, Niki et al. 2005, Raja et al. 2007). Our results suggest that punicalagin can directly or indirectly reduce oxidative damage by preventing the increased formation of free radicals that may occur due to BPA.

CONCLUSIONS

The current study suggests that punicalagin applications have potential ameliorating effects in the blood and various tissues against the oxidative stress caused by BPA in male New Zealand White rabbits. The protective effects of punicalagin may be due to both an increase in the activity of the antioxidant defense system and the inhibition of lipid peroxidation. These findings imply that punicalagin may be effective in preventing the possible negative effects of BPA on various tissues.

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