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Modulatory effect of dry orange (*citrus sinensis*) peel powder on bisphenol A-induced hepatic and splenic toxicity in rats

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Abstract

Background: Bisphenol A (BPA) is used as monomer in polycarbonate synthesis, and it acts as plasticizer in baby and water bottles and the production of epoxy resins which are used as inner coatings of many food and beverage cans. This study was carried out to evaluate the possible modulatory effect of dry orange peels powder (OPP) to attenuate the toxic effects of BPA on liver and spleen in rats.

Method: Sixty male Spargue–Dawley rats weighing 130 ± 10 g were randomly divided into six groups ($n = 10$ for each group). Group 1: negative control, fed on balanced diet and received corn oil. Group 2: positive control, fed on balanced diet, received BPA (350 mg/kg b.w. per orally; *p.o* twice weekly) suspended in corn oil. Groups from 3 to 6 fed on balanced diet supplemented with OPP in the tested doses of 12.5, 25, 50, and 100 g/Kg diet respectively, and received BPA (350 mg/kg b.w. twice weekly).

Results: There was a significant increase in liver sterol regulatory element-binding transcription factor 1 gene expression (SREBF1), serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, nitric oxide (NO), total cholesterol (TC), triacylglycerols (TAGs), low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c), interluken-4 (IL-4), immunoglobulin-E (IgE)levels, and total leukocytes count (TLC) in BPA group as compared to negative control group ($P < 0.05$).On the other hand, BPA caused a significant decrease in liver catalase activity, serum high-density lipoprotein cholesterol (HDL-c), serum immunoglobulin-M (IgM) levels, blood hemoglobin% (Hb), and red blood cell (RBCs) counts compared to the negative control group ($P < 0.05$). Also, the histopathological examination of liver and spleen sections supported biochemical parameters showed a significant destruction in the BPA group as compared to the negative control group.

Conclusion: It is observed that OPP dietary supplementation in the tested doses ameliorates deleterious effects induced by BPA. The improvement in these altered parameters in OPP supplemented groups was in a dose-dependent manner.

Keywords: Bisphenol A (BPA), Orange peel powder (OPP), Hepatic, Splenic, Toxicity, Rats

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Background

Bisphenol A (BPA) (2,2-bis (4-hydroxyphenyl) propane or 4,4′-isopropylidenediphenol) is an organic synthetic compound. It is endocrine disrupting chemical that exhibits estrogen hormone-like properties (FDA, 2014 Gabr et al., 2017). It is used as plasticizer in a wide range of polycarbonate plastic industries like baby and water bottles, sport equipment, medical and dental devices, dental filling sealants, household electronics, and eyeglass lenses (Erickson, 2008Inadera, 2015). Also, the lining of water pipes and epoxy resins which is used as coatings on the inside of many food and beverage cans (Bae & Hong, 2015).

Contact with heat and acidic or basic conditions accelerates the hydrolysis of the ester bonds between BPA molecules, which results in human and domesticated animal exposure from heating of cans to sterilized food. Also, the presence of acidic or basic food or beverages in cans and polycarbonate plastic and repeated heating of these products lead to leaching of BPA from the container into the media (Von Goetz, Wormuth, Scheringer, & Hungerbu¨hler, K., 2010).

Bisphenol A affects estrogenic receptors, which may be responsible for risk of some types of cancers, and it was proven that this substance was able to bind to androgen receptors (AR), at which excessive stimulation may cause prostate cancer development (Michalowicz, 2014).

Citrus fruit peels could be interesting not only for its important fiber content but also because of its high antioxidant capacity including bioactive compounds such as flavonoids (naringin, hesperidin, and neohesperidin) which are a group of naturally occurring polyphenols (Aschoff et al., 2015Boeing et al., 2012).Also, orange peels rich in methylated derivatives such as polymethoxyflavones (PMFs) which exhibit a strong anti-inflammatory effect both at the level of gene expression and enzyme activity (Pan, Li, & Lai, 2012).

The purpose of this study was to investigate the ameliorative effect of orange peels dry powder dietary supplementation against genetic, biochemical, and histological alterations induced by oral administration of BPA in adult male albino rats.

Materials and methods

Chemicals

Bisphenol A (BPA) was purchased from the Sigma-Aldrich Company for Chemicals (St. Louis, MO, USA).

Plant material

Navel orange fruits (*Citrus sinensis*) were purchased from local market, Cairo, Egypt, considering the quality attributes like uniformity in color, shape, and size.

Preparation of orange peels powder

Navel orange fruits were washed, then peeled, and peels were cut into small pieces. Then, they were distributed in trays and dried by the hybrid solar convective drying system, belonging to the Solar Energy Department, National Research Center, Dokki, Egypt, at 40–50 °C for three consecutive days. After that, the dried peels were ground into powder using an electric dry mill and added to the balanced diet as supplement.

Chemical composition of orange peel powder (Citrus sinensis)

Protein, fat, carbohydrate, and fiber contents as well as ash and moisture were measured according to AOAC (2000).

Phytochemical and antioxidant analysis of orange peel powder (OPP)

Dried powdered samples were extracted at room temperature by percolation with methanol. All extracts were concentrated over a rotary vacuum evaporator until a solid extract sample was obtained. The resulting crude extract was freeze-dried. Total phenolic content was determined by using Folin-Ciocalteau method according to (Singleton & Rossi, 1995), while aluminium chloride colorimetric method was used for determination of total flavonoids according to Arvouet-Grand, Vennat, Pourrat, and Legret (1994).

Diet

The experimental diet used in the present study was standard diet which was prepared according to the American Institute of Nutrition (AIN-93) as adjusted by Reeves, Nielsen, and Fahey (1993).

Experimental animals

Healthy adult male albino rats (Sprague–Dawely strain) weighing 130 ± 10 g were used for the experimental study, obtained from the National Research Center, Cairo, Egypt. The animals were acclimated for 1 week prior to the experiment. During the adaptation period and the biological experiment, standard diet and water were available ad libitum, and they were maintained under 12-h light/dark cycles at 25 ± 2 °C. An animal experiment was conducted according to the guidelines of the institutional animal ethics committee in accordance with the Faculty of Science protocol, Ain Shams University.

Experimental design

In the present study, sixty male albino rats were divided into 6 groups each of 10. All international and local rules and regulation for handling animals throughout

the experimental period were followed. The experimental groups illustrated as follows:

- Group 1 (negative control): Rats were fed on balanced diet, received corn oil.
- Group 2 (positive control or BPA group): Rats were fed on balanced diet + BPA (350 mg/Kg b.w. per orally; *p.o.* twice weekly) suspended in corn oil (Sauerwein, Wittig, Moss, & Nakagawa, 2012).
- Group 3: Rats were fed on balanced diet supplemented with OPP (12.5 g/Kg diet) + BPA (Ebrahimi et al., 2015).
- Group 4: Rats were fed on balanced diets supplemented with OPP (25 g/Kg diet) + BPA.
- Group 5: Rats were fed on balanced diet supplemented with OPP (50 g/Kg diet) + BPA.
- Group 6: Rats were fed on balanced diets supplemented with OPP (100 g/Kg diet) + BPA.

The abovementioned treatments were continued for 4 weeks, after which the animals were sacrificed after 12 h fasting under sodium barbital anesthesia. Blood samples were taken from the hepatic portal vein divided into two tubes. The first tube contained EDTA for determination of Hb%, RBCs, and TLC count. Blood sample in the second tube was left for 15 min at 37 °C for serum separation, then centrifuged at 3000 rpm for20 min, then sera were separated, and kept in plastic vials at − 20 °C until analyses. Liver and spleen were removed and washed twice with ice cold saline solution. Then parts of the liver and spleen were taken and immediately fixed in 10% formalin for microscopic examination. The remaining sections of liver were stored frozen at − 80 °C until used for the genetic and biochemical analyses.

Preparation of liver homogenate

Liver was perfused with phosphate buffered saline, at pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Liver was homogenized in 5 ml cold buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA and 1 ml/L Triton X-100) per gram tissue, then centrifuged at 4000 rpm for 15 min at 4 °C. Finally, the supernatant was removed for assay and stored in ice.

Detection of Sterol regulatory element-binding transcription factor 1 gene expression (SREBF1) by real-time quantitative polymerase chain reaction (RT-qPCR)

Sterol regulatory element-binding transcription factor 1 gene expression (SREBF1) was analyzed in liver using Qiagen tissue extraction kit (Qiagen, USA) and high capacity cDNA reverse transcription kit (Fermentas, USA). RT-qPCR includes reaction fluorescent reporter molecules that increase proportionally with the increase of DNA amplification in thermo-cycler. The fluorescent chemistries for this purpose are double strand DNA binding dyes as SYBR Green. The key equipment for qPCR is a specialized thermo-cycler with fluorescence detection modules which is used to monitor and record the fluorescence in real time as amplification occurs. A typical workflow of qPCR for gene expression measurement involves RNA isolation, reverse transcription, qPCR assay development, qPCR experiment, and data analysis.

Biochemical assays

Serum enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to Burtis and Ashwood (1999) using Diamond Diagnostics kit. Serum nitric oxide (NO) level was determined according to Grisham, Johnson, and Lancaster Jr. (1996) using Biodiagnostic kits. Total cholesterol (TC) and triacylglycerols (TAGs) were determined in serum according to Young and Friedman (2001) using Spin react kits. HDL-c was determined in serum according to Lopes-Virella, Stone, Ellis, and Colwell (1977) using Spectrum kit, while very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were calculated according to Friedewald, Levy, and Fredrickson (1972).

Interleuken-4 (IL-4) was determined in serum by ELISA according to Toi, Harris, and Bicknell (1991) using IB49709 ELISA kits. Serum IgE was determined according to Young (1995), and catalase (CAT) enzyme activity was determined in liver homogenate according to Aebi (1984) using Biodiagnostic kits.

Hematological analysis

Hb%, RBCs, and TLCs were analyzed with an automatic VetabcTM Animal Blood Counter (Horiba ABX, Montpellier, France) using the hematology kits specified in that instrument (HoribaABX, France) according to the manufacturer's instructions.

Histopathological examination

Liver and spleen were dissected and fixed at 10% neutral formalin. Fixative tissues were processed and stained with hematoxylin and eosin (H&E) and examined under alight microscope.

Statistical analysis

The data were presented as means ± SE. One-way analysis of variance (ANOVA) was performed using the statistical package for social science (SPSS) version 16 to compare all treated groups (Levesque, 2007). Differences were considered to be significant when $p < 0.05$.

Results

Results of chemical composition analyses showed that each 100 g of dry orange peels contain 5.1 g protein, 5.61 g fat, 65.67 g carbohydrate, 2.9 g ash, 8.5 g moisture, and 12.22 g fiber. The values of total polyphenols were measured in m as gas gallic acid equivalent (GAE %) and total flavonoids in mg as quercetin (QE %) equivalent. Data showed that 100 g of dry orange peel extract contains 567.93 mg GAE and 162.12 mg QE.

Results reported in Table 1 showed that BPA induced significantly overexpression of SREBF1 gene with a significant increase in TC, TAGs, LDL-c, and VLDL-c with concomitant reduction in HDL-c as compared to the normal control group ($p < 0.05$). Orange peel powder dietary supplementation significantly reversed all these altered parameters.

From the results presented in Table 2, it is clear that BPA induced oxidative stress in the liver and significantly altered liver enzyme markers levels in serum ($P < 0.05$).The evidence for oxidative stress was the significant increase in serum nitric oxide concomitant with a significant reduction in hepatic catalase activity. On the other hand, OPP dietary supplementation attenuated the oxidative stress and liver enzyme markers induced by BPA ($P < 0.05$).

Data in Table 3 suggested that BPA administration induced a significant increase inserum IL-4 and IgElevels as compared to the normal control group ($p < 0.05$). Considering serum IgM levels, there was a significant decrease in the BPA group when compared to the negative control group. On the other hand, there was a significant decrease in serum IL-4 and IgE levels in a dose-dependent manner in OPP supplemented groups as compared with the BPA group. Results also demonstrated that OPP supplemented groups showed a significant increase in IgM levels when compared with the BPA group ($p < 0.05$).

Results in Table 4 showed that there was a significant decrease in blood Hb % and RBC count, while there was a significant increase in total leucocyte count in the BPA group as compared to negative control ($p < 0.05$). Dietary supplementation with OPP restored blood Hb%, RBC count, and total leucocyte count near to the normal values.

Microscopically, the hepatic tissue of rats kept as –ve control represented by Fig. 1 a1 showed normal configuration, while liver sections of BPA-treated group revealed severe hepatic alterations, as massively hyperplasia bile duct, portal artery hypertrophy, dilatation of portal vein, vacuolar degeneration, and fatty changes all over the portal zone as noticed in Fig. 1 a2.

On the other hand, rats administered with BPA and treated with OPP (12.5 g/Kg diet) illustrated slightly pathological lesions as blood sinusoid hemorrhage, fibroid portal artery with lymphocytes infiltration, and with remarkable improvement in some parenchymal area (Fig. 1 a3), whereas Fig. 1 a4, a5, and a6 represented tissue sections from rats administered with BPA and OPP (25, 50&100 g/Kg diet) respectively clarified slightly improvement in the hepatic tissue with little apoptotic body, in spite of the peri-portal and centriolobular area significant regeneration, beside a little activated Kupffer cell and rupture of endothelial lining of central vein.

Microscopically, the spleen of the –ve control group shown in Fig. 1 b1 was illustrating normal architecture, while rats administered with BPA, manifested a dysfunction of splenic tissues, and included the disfabric trabecular tissues that contain both arteries and veins, whereas those of intermediate size contain only veins. Scattered smooth muscle fibers were seen within the trabecular and surrounding connective tissues. Also, hemorrhaged bulge and lymphocyte infiltrations in the peri-arteriolar were showed in Fig. 1 b2.

Table 1 The effect of orange peel powder on liver SREBF1 expression and serum lipid profile in BPA-treated rats

	Liver SREBF1	Serum TC (mg/dl)	Serum TAGs (mg/dl)	Serum HDL-C (mg/dl)	Serum VLDL (mg/dl)	Serum LDL-C (mg/dl)
Group 1: -ve control	1.00 ± 0.002^{a}	77.65±0.73 ^{a}	118.15± 2.32 ^{a}	43.85 ± 0.30 ^{a}	23.63 ± 0.46 ^{a}	10.17±0.23 ^{a}
Group 2:+ve control (BPA group)	4.74 ± 0.11^{b}	118.01±0.71^{b}	224.85± 6.78 ^{b}	29.20 ± 0.53^{b}	44.97 ± 1.36^{b}	43.84± 1.87^{b}
Group3: (BPA + 12.5g/kg diet OPP)	3.66 ± 0.12^{c}	90.60 ± 0.41^{c}	172.95± 2.62 ^{c}	32.85 ± 0.57 ^{c}	34.59 ± 0.52 ^{c}	23.16±0.72^{c}
Group4: (BPA + 25g /kg diet OPP)	3.35 ± 0.06^{d}	85.90 ± 0.55^{d}	164.25± 2.17 ^{c}	37.45 ± 0.81 ^{d}	32.94± 0.41^{c}	15.51± 0.96^{d}
Group5: (BPA + 50g/kg diet OPP)	2.68 ± 0.15^{e}	81.5 ± 0.56^{e}	135.80± 1.91^{d}	41.90 ± 0.50 ^{e}	27.16 ± 0.38^{d}	12.44± 0.82 ^{a}
Group6: (BPA + 100g/kg diet OPP)	2.53 ± 0.07^{e}	79.44 ± 0.70^{f}	127.80± 1.20^{d}	41.85 ± 1.00 ^{e}	25.56 ± 0.24^{d}	12.93± 0.24 ^{ad}

Values are represented as mean ±SE ($n = 10$)
There was a significance difference between means have different letters in the same column ($P < 0.05$)

Table 2 The effect of orange peel powder on serum nitric oxide level, ALT, AST, and liver catalase activities in BPA-treated rats

	Nitric oxide (μ mol/L)	ALT (U/L)	AST (U/L)	Liver Catalase (U/g tissue)
Group 1: -ve control	4.34±0.06 [a]	10.71±0.13 [a]	15.83± 0.38 [a]	1.18 ± 0.03 [a]
Group 2:+ve control (BPA group)	27.70 ± 0.24[b]	18.06±0.34[b]	41.78± 1.38 [b]	0.86 ± 0.02[b]
Group3: (BPA + 12.5g/kg diet OPP)	12.93 ± 0.2[c]	11.77 ± 0.34[c]	19.75± 0.59 [c]	1.07 ± 0.02 [c]
Group4: (BPA + 25g/kg diet OPP)	7.45 ± 0.06[d]	11.55 ± 0.18[c]	18.63± 0.22 [cd]	1.09± 0.02 [c]
Group5: (BPA + 50g/kg diet OPP)	7.30 ± 0.10[de]	11.49 ± 0.12[c]	18.38± 0.25[cd]	1.09 ± 0.01 [c]
Group6: (BPA + 100g/kg diet OPP)	6.95 ± 0.1 [e]	11.39 ± 0.08[c]	17.76± 0.15[d]	1.12 ± 0.01 [c]

As legend in Table 1

The splenic tissues from rats administered with BPA and treated with low OPP doses (12.5 and 25 g/Kg diet) revealed slightly regeneration accompanied by atrophied peri-arteriolar lymphatic sheath (PALS) and fibroid bands invasion with inflammation. Malfunction and inconsistency both of splenic cords and sinuses in red pulp region and dissociation in white pulp, but still atrophied follicular arteriole, were evident in Fig. 1 b3 and b4. Noteworthy, the supplementation of OPP at high doses (50 and 100 g/Kg diet) revealed markedly improvement of splenic sinuses manifested by significant red and white pulp recovery with rearranged splenic medullary tissues, and a little irregularity pattern in white core was showed in Fig. 1 b5 and b6 respectively with rarely necrotic lesions.

Discussion

Orange peels have been found to contain high amounts of antioxidants and many phytochemicals with strong potential; therefore, it can be used in drug production or as food supplements (Benamrouchea & Madani, 2013). Results of Uraku (2015) are in harmony with the results of the present study, as they reported that OPP contains protein (3.73 g%), fat (10.34 g%), fiber (13.51 g%), moisture (9.78 g%), ash (1.57 g%), and carbohydrate (61.07 g%) in levels slightly different from those reported in the current study. Also, Ghasemia, Ghasemia, and Ebrahimzadeh (2009) illustrated that total phenols and flavonoids contents were usually high in *citrus sinensis* peels.

Serum total cholesterol, triacylglycerols, LDL-c, and VLDL-c levels were significantly increased in BPA-treated animals while HDL-c was ignificantly reduced as compared to the normal control group ($P < 0.05$). However, in the OPP-treated group, these altered levels were recovered. These changes in lipid profile are explained by the overexpression of SREBF1 gene which is responsible for fat de novo lipogenesis in liver. The obtained results in the present study confirmed that orange peel powder dietary supplementation significantly suppressed the induction of SREBF1 gene expression, resulting in a reduction of both TC and TAG synthesis. Also, pectin content of OPP augments the reduction of TC and TAGs that led to an improvement in the lipid profile of OPP supplemented groups in a dose-dependent manner.

In parallel with the present results, Marmugi et al. (2012) and Lin et al. (2017) showed that oral exposure to BPA affects hepatic mRNA in mice and increases lipogenic gene expression related to lipid biosynthesis. The same trend results of Abdel-Wahab (2014) reported that oral administration of 10 mg/kg/day BPA for 4 weeks caused a significant increase in TC, TAGs, and LDL levels and a decrease in the HDL-c level. An explanation suggested by Ahmed, Hassan, Abdel-Twab, and Abdel Azeem (2017) illustrated that navel orange peel contents (naringin and naringenin) potentially ameliorated the elevation of serum TC, TAGs, LDL-c, and VLDL-c, while increased HDL-c. Assini et al. (2013) illustrated that naringenin markedly attenuates dyslipidemia and hepatic lipid accumulation. Furthermore, naringenin alleviates the cholesterol-induced inflammatory response in liver, adipose tissue, and aorta, collectively resulting in an attenuation of atherogenesis. Naringenin reduced hepatic Srebf1c expression and increased Fgf21, Pgc1a, and Cpt1a mRNA in concert with

Table 3 The effect of orange peel powder on serum IL-4, IgM, and IgE levels in BPA-treated rats

	IL-4 (Pg/ml)	IgM (mg/dl)	IgE (mg/dl)
Group 1: -ve control	31.04±0.74 [a]	141.52±1.02 [a]	100.75± 0.99 [a]
Group 2:+ve control (BPA group)	119.62 ± 1.73[b]	129.62±1.84[b]	146.35± 2.67 [b]
Group3: (BPA + 12.5g/kg diet OPP)	65.29 ± 1.94[c]	133.95 ± 0.95[c]	121.90± 1.18 [c]
Group4: (BPA + 25g/kg diet OPP)	60.18 ±1.50[d]	134.15 ± 0.96[c]	109.52± 1.80 [d]
Group5: (BPA + 50g/kg diet OPP)	45.40 ± 2.31[e]	135.20 ± 1.47[c]	106.23± 1.13[d]
Group6: (BPA + 100g/kg diet OPP)	39.09 ± 0.84 [f]	136.15 ± 0.81[c]	105.45± 1.38[d]

As legend in Table 1

Table 4 The effect of orange peel powder against BPA action on blood Hb, RBCs, and TLCs in BPA-treated rats

	Blood Hb (g/dl)	Blood RBCs ($x10^6$/μg)	Blood TLCs ($x10^3$/μg)
Group 1: -ve control	14.07±0.07 [a]	8.07±0.12 [a]	7.70± 0.20 [a]
Group 2:+ve control (BPA group)	12.4 ± 0.10 [b]	6.41±0.03[b]	10.16± 0.20 [b]
Group3: (BPA + 12.5g/kg diet OPP)	13.32 ± 0.04 [c]	6.84 ± 0.01[c]	9.42± 0.41 [c]
Group4: (BPA + 25g/kg diet OPP)	13.56 ±0.05[d]	7.05 ± 0.01[d]	8.92± 0.34 [cd]
Group5: (BPA + 50g/kg diet OPP)	13.64 ± 0.04 [de]	7.39 ± 0.03[e]	8.73± 0.09 [cd]
Group6: (BPA + 100g/kg diet OPP)	13.76 ± 0.04 [e]	7.66 ± 0.08 [f]	8.47± 0.10 [d]

As legend in Table 1

significantly decreased hepatic FA synthesis and enhanced FA oxidation. Also, naringenin maintains its ability to shift hepatic gene expression to prevent hepatic TG accumulation. Previous study of Yen et al. (2011) suggested that polymethoxyflavones (PMFs) also may lower TC and TAGs, also modifying LDL-c uptake by cells and increased HDL-c.

Bisphenol A can cause liver, spleen, and other organs injury by forming reactive oxygen species (ROS). The significant increase in serum nitric oxide level with the significant decrease in hepatic catalase activity in BPA-treated group indicates the increase in oxidative stress in comparison with the control group ($p < 0.05$). Oxidative stress causes lipids and protein peroxidation, DNA modification, and liver cell injury. The damage of hepatic cells with destruction of their membranes resulted in releasing of the liver enzymes ALT and AST into the blood. However, dietary supplementation with OPP restored these altered parameters near to their normal levels. This may be due to the anti-oxidative activity of OPP which is reflected in a significant decrease in serum nitric oxide and restoration of hepatic catalase activity. Suppression of oxidative stress resulted also in an improvement in liver function markers (ALT and AST) activities in serum.

The previous results obtained by Hassan et al. (2012) and Mahajan et al. (2018) supported the results of the current study which confirmed that BPA administration induced oxidative stress and significantly altered liver enzyme markers levels in serum ($P < 0.05$). In the same line, the results of La Pantsulaia, Iobadze, Pantsulaia, and Chikovani (2014), and Ahmed et al. (2019) showed that *C. sinensis* peel extract (CPE) significantly suppresses oxidative stress, which in turn resulted in evident reduction of the liver damage.

Oral administration of BPA induced allergic immune response which is manifested by a significant increase in serum IL-4 and IgE levels concomitant with a significant reduction in IgM levels ($p < 0.05$). This allergic immune response may be due to oxidative stress induced by BPA. On the other hand, there was a significant decrease in serum IL-4 and IgE values with a significant increase in IgM in OPP supplemented groups as compared with the BPA group. The improvement in immunological parameters was in a dose-dependent manner. This may be attributed to the antioxidant activity of OPP.

In parallel with the results of this study, Lee et al. (2003) and Ellis, Hong, Zaghouani, and &Braley-Mullen, H. (2013) reported that BPA administration resulted in an increase in serum IL-4 production and antigen-specific IgElevels. Palacios-Arreola, Nava-Castro, Río-Araiza, Pérez-Sánchez, and Morales-Montor, 2017) explained the decrease in circulating IgM may be due to the decrease in IFN-gamma concentration which subsequently inhibits B cell proliferation and directing the differentiation of B terminal cells towards the IgM. Also, results of Palacios-Arreola et al. (2017a) similar to the results of the current study demonstrating that BPA administration resulted in a significant reduction in IgMlevel. In the same trend, Pourhossein et al. (2015) reported that flavonoids especially PMFs contained in *C. sinensis* peels are stimulating agents to the immune system through the increase of IgM antibody production that reinforce the humoral immune system.

In the present study, it was observed that BPA induced hematological changes which were ameliorated in the OPP-treated groups. The decrease in RBC count and subsequent decrease in Hb% may be attributed to hemolysis induced by ROS generated from BPA, while the increase in TLCs may be attributed to a stimulation of the immune system response caused by BPA. The improvement in these altered parameters levels in OPP supplemented groups is explained by the antioxidant activity of OPP.

Similar to the present study, Yamasaki and Okudab (2012) reported that there was a significant decrease in Hb% and RBC counts, while there was a significant increase in TLCs in group administrated BPA compared with the –ve control group ($P < 0.05$). In the same line of the present study, Uribe, Folch, Enriquez, and Moran (2011) suggested that toxicity with BPA caused shorter half-life for RBCs and their degradation as a result of changing in cell membrane permeability that makes RBCs more fragile and prone to hemolysis. Oppositely,

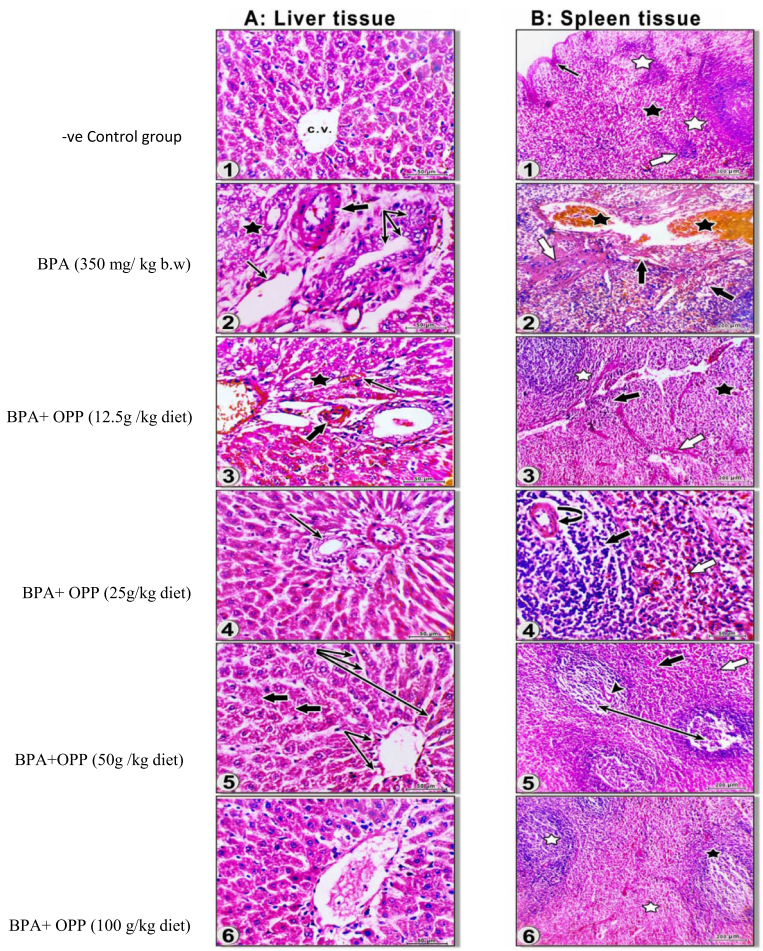

Fig. 1 a Photomicrographs of hepatic micro-anatomical structures. (1) Control group illustrating normal hepatocytes and central vein (cv), (H&E × 400). (2) Rats administered BPA (350 mg/kg b.w/twice weekly) showing necrotic area (star), bile duct hyperplasia (3 arrows), hypertrophy portal artery (thick arrow) dilated vein (thin arrow), and (H&E × 400). (3) Rats administered BPA and orange peel powder (12.5 g/kg diet), illustrating the hemorrhage (thin arrow), fibroid with lymphocytes infiltration (thick arrow), and areas markedly improved (star), (H&E × 400). (4) Rats administered BPA and OPP (25 g/kg diet) showing slightly improvement in periportal area, well-developed duct (arrow), and the little apoptotic body (H&E × 400) (5). Rats administered BPA and OPP (50 g /kg diet) illustrating partially improvement, Kupffer cell (3 arrows), restoring hepatocytes with necrotic cells (thick arrows), and central vein wall rupture (2 arrows) (H&E × 400). (6). Rats administered BPA and OPP (100 g/kg diet) pointed out to clarity of hepatocytes regeneration (H&E × 400). **b** Photomicrographs of splenic micro anatomical structures. (1) Control group capsule of cortex (black arrow) and the white pulp (white stars), red pulp (black star) trabecular, and central artery (white arrow) (H&E × 100). (2) Rats administered BPA (350 mg/kg b.w/twice weekly) showing marginal zones disfaberic (black arrows) fibrotic tissue (white arrow) and dilated vein with hemorrhaged bulge (stars) (H&E × 100) (3). Rats administered BPA and OPP (12.5/kg diet) illustrating PALS atrophy (white star), fibroid bands (white arrow), inflammatory lymphocytes (black arrow), and red pulp reduction (Black star) (H&E × 100) (4). Rats administered BPA and OPP (25 g/kg diet) showing both splenic cords and sinuses malfunction (white arrow), dissociation white pulp (black arrow), and atrophied artery (curved arrow) (H&E × 400) (5). Rats administered BPA and OPP (50 g kg diet) revealing necrotic white pulp (long arrow), atrophied artery (arrow head), and markedly improvement splenic tissue (white + black arrows) (H&E × 100). (6) Rats administered BPA and OPP (100 g/kg diet) pointing out to improvement in pulps, splenic medullary tissue (white stars), and white core rearranged (black star) (H&E × 100)

the increase in TLCs after the exposure of BPA may be explained on the basis of the role of BPA in stress production that stimulate immune system.

The results of Khan, Mallick, and Feroz (2016) are in harmony with the result of the current study. Researchers suggested that the antioxidant and anti-inflammatory properties of *C. sinensis* were so important in lowering the level of TLCs, in colitis induced by trinitrobenzenesulphonic acid (TNBS) in rats. *C. sinensis* action leads to decrease the oxidative stress of TNBS, and this aims to reduce the free radical generation, which may lower the level of TLCs (Cestari, Bastos, & Di Stasi, 2011).

In harmony with the present study, Shirisha, Mandava, Batchu, Thammana, and Turpu (2019) reported that *C. sinensis* peel extract leads to increase in Hb concentration and RBC count. The action of *C. sinensis* peel extract may be due to the improvement in iron absorption

from the rat's gut because of its high content of ascorbic acid (Shah, Griffin, Lifschitz, & Abrams, 2003).

The results of current study are agreeing with Huc, Lemarie, Gueraud, and Helies-Toussaint (2012) who attributed the hepatic tissue alterations to caspase-3 significant activity which increases in BPA-treated rats. Investigators suggesting that apoptosis is caspase-3 dependent which is apoptotic key molecular marker, which activated by either mitochondrial cytochrome-c dependent or death receptor of apoptotic pathways (Nakagawa & Tayama, 2000).Orange peel powder and their active components have various biological activities, including anti-inflammatory and antioxidant properties through suppression of inflammatory mediators. The present study demonstrates that OPP has tissue enhanced effects and hepatoprotective and immunosuppressive effect, which is supported with the previous results of Gosslaua, Chenb, Hoc, and Li (2014).

The current results agreed with the previous observations of Kendziorski and Belcher (2015) who found that the action of BPA could induce pathologic fibrosis and inflammatory responses of the splenic tissues. Another study suggested the links between BPA exposures and varied impacts on immune-responsiveness, autoimmunity, and allergic diseases (Zhou et al., 2017).After orange peel powder supplementation at different doses in this study, improvement was observed in some tissue areas, regards to the effect of OPP free radical scavenging's action. It also contains considerable amounts of calcium, copper, magnesium, vitamin A, vitamin C, foliate and other B vitamins, and dietary fiber, and all of these are important for immune system health which went in parallel with (Pourhossein et al., 2015).

Conclusion

From the results of the present study, it can be concluded that dietary supplementation of orange peel powder attenuated the toxic effect of bisphenol A-induced hepatic and splenic dysfunction. This ameliorative effect of orange peel powder may be attributed to its phenol and flavonoid content.

Abbreviations

AIN-93: American Institute of Nutrition; ALT: Alanine aminotransferase; AR: Androgen receptors; AST: Aspartate aminotransferase; BPA: Bisphenol A; Cpt1a: Carnitine palmitoyltransferase I; CSPE: *C. sinensis* peel aqueous extract; DNL: De novo lipogenesis; FA: Fatty acid; Fgf21: Fibroblast growth factor 21; GAEs: Gallic acid equivalents; Hb: Hemoglobin; HDL-c: High-density lipoprotein cholesterol; IFN-γ: Interferon gamma; IgE and IgM: Immunoglobulin E and M; IL-4: Interleukin-4; LDL-cL: Low-density lipoprotein cholesterol; miRNA: MicroRNA; NO: Nitric oxide; OPP: Orange peels powder; PALS: Semi-arterial lymphoid sheath; Pgc1a: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PMFs: Polymethoxyflavones; QE: Quercetin equivalent; RBCs: Red blood cells; ROS: Reactive oxygen species; RT-qPCR: Real-time quantitative polymerase chain reaction; SREBF1: Sterol regulatory element-binding transcription factor 1; TAGs: Triacylglycerol; TC: Total cholesterol; TIBC: Total iron binding capacity; TLCs: Total leucocyte counts; TNBS: Trinitrobenzenesulphonic acid; VLDL-c: Very low-density lipoproteincholesterol; WBCs: White blood cells

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Ethics approval and consent to participate

This study follows guidelines for the care and use of experimental animals established by the Committee for the purpose of control and supervision of experiments on animals. Animal procedures were also made in accordance with the Faculty of Science protocol, Ain Shams University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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