RESEARCH ARTICLE
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Prophylactic Effect of Apigenin Against Bisphenol A-Induced Hepatotoxicity

ABSTRACT

Background: Bisphenol A (BPA) is the most widely used endocrine disruptor in the world. As the liver is the main organ involved in the detoxification and metabolism of BPA, it is one of the organs most affected by BPA poisoning. No study has investigated the potential effect of apigenin (APG) on BPA-induced hepatotoxicity. This study was conducted to investigate the possible hepatoprotective effects of APG against subacute BPA intoxication in rats.

Methods: Twenty-eight rats were randomly divided into 4 groups: (i) control, (ii) BPA (130 mg/kg), (iii) BPA (130 mg/kg) + APG100 (100 mg/kg), and (iv) BPA (130 mg/kg) + APG200 (200 mg/kg). Both BPA and APG were administered daily by gavage for a total of 28 days. At the end of the experiment, all animals were sacrificed and serum liver function parameters, markers of oxidative stress and inflammation in liver tissue, and histopathological analysis of the liver were assessed.

Results: Bisphenol A pre-treatment impaired liver function tests, induced oxidative stress and inflammatory response, and disrupted liver microarchitecture. Apigenin pre-treatment improved liver function tests, suppressed oxidative stress and inflammation, and, especially high doses, normalized liver microarchitecture.

Conclusion: It is observed that apigenin pre-treatment has a hepatoprotective effect, both biochemical and histological, in eliminating the hepatotoxicity caused by BPA administration.

Keywords: Apigenin, Bisphenol A, hepatotoxicity, inflammation, oxidative stress

What is already known on this topic?

- Bisphenol A is a well-established endocrine-disrupting chemical.
- Bisphenol A causes hepatotoxicity through oxidative stress and inflammation.
- Phytochemicals such as apigenin are being investigated for their potential to mitigate toxin-induced organ damage.

What this study adds on this topic?

- First in vivo evidence of the protective role of apigenin against bisphenol A-induced hepatotoxicity.
- Dose-dependent hepatoprotective effects of apigenin.
- Demonstration of the anti-inflammatory and antioxidant mechanisms of apigenin.

INTRODUCTION

Bisphenol A (BPA) [2,2-bis (4-hydroxyphenyl) propane] is a substance widely used as an industrial additive, particularly in the manufacture of epoxy resins and other non-polymer plastics, and has adverse effects on the endocrine system due to its pseudo-estrogenic action. It causes unpredictable changes in the development and functioning of the endocrine system. Endocrine disruptors, including BPA, are defined as chemicals that block or mimic the action of hormones at the target receptor or tissue, or directly stimulate or inhibit the production of hormones in the endocrine system.[1] Most studies include BPA in this group because of its estrogenic properties. Bisphenol A enters the body mainly through the ingestion of BPA-contaminated food and drinking water.[2] In addition, BPA-contaminated air and municipal water, personal care products, and some medical devices account for a significant portion of the body's intake of BPA, while inhalation and dermal contact are important routes for workers in the production stages. Oral uptake can result from practices such as heating food in plastic bags (especially in microwaves) or packaging food before cooking. The use of epoxy food packaging, water bottles, or plastic baby bottles can also lead to high oral uptake of BPA. In addition, dental products (toothpastes and composite fillings) can be a major source of oral exposure to BPA.[3,4]

Bisphenol A is known to have toxic effects on the reproductive and endocrine systems as a result of its interaction with estrogen and androgen receptors, mimicking natural estrogens.[5,6] It is also known to have toxic effects on the cardiovascular system, central and peripheral nervous systems, liver, kidney and other organs. Bisphenol A is also known to cause an inflammatory response

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by inducing pro-inflammatory cytokines and oxidative stress by inducing lipid peroxidation and free radical formation. To prevent and/or treat BPA-induced liver damage, oxidative stress, and inflammatory processes should be suppressed. The liver is the main organ involved in the prevention, detoxification, and metabolism of xenobiotics such as BPA. Therefore, the liver is one of the most damaged organs in BPA poisoning.[7] In recent years, plant extracts and phytochemicals derived from these extracts have been widely used to halt or reverse liver damage.[8,9]

Apigenin is a flavone of the flavonoid subclass and its chemical structure is 4′, 5, 7-trihydroxyflavone. Apigenin is a compound with a wide range of biological activities, including antioxidant, anti-inflammatory, antitumor, antigenotoxic, antiallergic, hepatoprotective, nephroprotective, neuroprotective, cardioprotective and antimycytobial, and is widely distributed in many fruits and vegetables such as parsley, chamomile, celery, Indian spinach, artichoke, orange, onion, wheat sprouts. In recent years, the hepatoprotective effects of apigenin have been extensively studied.[10-12] Apigenin is a promising agent in the treatment of liver diseases due to its ability to regulate lipid metabolism, reduce insulin resistance, inhibit reactive oxygen species (ROS) generated in hepatocytes, suppress the inflammatory process and inhibit cell proliferation, differentiation, migration, invasion, angiogenesis, and metastasis in liver cancer and induce apoptosis of cancer cells.[13,14]

It is known that phytochemicals with antioxidant activity can help reduce the potential toxic effects of environmental pollutants.[15] Although the effects of various antioxidant compounds have been investigated against liver damage caused by BPA intoxication,[9,8] there is no *in vivo* study investigating the potential effect of apigenin on BPA-induced hepatotoxicity. Therefore, this study was conducted to investigate the possible hepatoprotective effects of apigenin pre-treatment against subacute BPA intoxication in rats.

MATERIAL AND METHODS

Chemicals

Bisphenol A (Cat. No: 239658) and apigenin (Cat. No: AKI-06) were supplied by Sigma-Aldrich (St. Louis, MO, USA) and Aktin Chemicals, Inc. (Chengdu, P.R. China), respectively. Other chemicals used in this work were purchased from usual commercial sources.

Animals

This study was carried out in February 2021 at Van Yüzüncü Yıl University Experimental Application and Research Centre Directorate after the approval of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee dated June 25, 2020 and numbered 2020/06-03 decision. In this study, 28 male Wistar Albino rats (200-250 g), 6-8 weeks old, obtained from Van Yüzüncü Yıl University Experimental Application and Research Centre Directorate were used. The rats were housed in special cages with each group in separate cages and fed ad libitum with standard rat chow. The rats were treated at 20 ± 2°C in a 12 h light/darkness period. Rats were quarantined for adaptation 10 days before the experiment. During the experiment, drinking water was changed every other day and the cage was cleaned.

Bisphenol A used in the study was prepared by dissolving in corn oil. Apigenin (APG) was prepared by dissolving in 1% carboxymethylcellulose (CMC) solution. Bisphenol A and all phytochemicals were administered orally for 28 days and at least 2 hours were allowed between administrations. Rats were randomly selected and divided into 4 groups. The groups were formed by making sure that the groups were homogeneous within themselves and that the total weight of the rats in the groups was approximately the same. A total of 4 experimental groups were formed as follows;

Control Group (n = 7): The animals in this group were administered only 1 mL corn oil and 1 mL 1% CMC solution by gavage for 28 days without any medication.

BPA Group (n = 7): Rats in this group were administered 1 mL BPA (130 mg/kg/day)[9] and 1 mL 1% CMC solution by gavage for 28 days.

BPA + APG100 Group (n = 7): Rats in this group were administered 1 mL BPA (130 mg/kg/day)[9] and 1 mL APG (100 mg/kg)[16] by gavage for 28 days.

BPA + APG200 Group (n = 7): Rats in this group were administered 1 mL BPA (130 mg/kg/day)[9] and 1 mL APG (200 mg/kg)[8] by gavage for 28 days.

At the end of the experimental period, all rats were fasted for 12 hours before being sacrificed by cardiac puncture under anesthesia of ketamine hydrochloride (15 mg/kg/i.p.) and xylazine (10 mg/kg/i.p.) and blood samples and liver tissues were isolated for biochemical and histological analysis.

Collection of Blood Sample

Blood samples were collected from jugular vein of each animal of all the groups separately in a centrifuge tubes and left to clot at room temperature for 45 minutes. Serum was separated by centrifugation at 3000 rpm for 15 minutes at 30°C and kept frozen at −40°C for analysing the liver function tests (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin and albumin levels) using standard diagnostics kits in Cobas Integra 800 autoanalyzer (Roche Diagnostics GmbH, Manheim, Germany).

Liver Tissue Homogenate Sampling and Preparation

After blood sampling, animals were sacrificed and liver tissues were isolated. Liver tissues were homogenized in ice-cold 0.01 mol/L sodium-potassium phosphate with 1.15% KCl buffer (pH 7.4). The homogenate was centrifuged at 10 000 x *g* (4°C) for 20 minutes then the supernatants

were collected and used for the determination of oxidative stress parameters and cytokine levels.[17]

Oxidative Stress Parameters

Malondialdehyde (MDA) Levels: Since MDA is a degradation product of peroxidized lipids, the development of pink color after reaction with thiobarbituric acid is measured at 532 nm.[18]

Superoxide Dismutase Activities: The assay is based on the principle of inhibitory effect of superoxide dismutase (SOD) on the reduction of nitro blue tetrazolium (NBT) by superoxide radical, which is generated by auto oxidation of hydroxylamine hydrochloride. The reduction of NBT to blue formazone in the reaction mixture induces an increase in optical density at 560 nm.[19]

Catalase Activities: Hydrogen peroxide decomposition by catalase is monitored spectrophotometrically by following the decrease in O.D at 240 nm.[20]

Reduced Glutathione Levels: In this method 5,5′-dithiobis(2-nitrobenzoic acid) is reduced by sulfhydryl group of glutathione to form 1 mole of 2-nitro-5-thiobenzoic acid per mole of –SH which produces an intense yellow color and is used to measure Sulfhydryl group groups at 412 nm.[21]

Cytokine Levels

Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) levels of liver tissue were measured using ELISA kits in accordance with the manufacturer's instructions. Absorbance was measured at 450 nm with a VersaMax microplate reader. The concentrations were calculated from a standard curve.[22]

Total Tissue Protein Levels

Total protein contents in liver tissue supernatants were measured according to the method of Lowry et al.[23]

Histopathological Evaluation

For histopathological analyses, rat liver tissues were divided into 30-35 mm small pieces. The pieces were placed in plastic tissue tracking cassettes and fixed in 10% formaldehyde for 24 hours. After fixation, the tissues were washed in running tap water for 24 hours. They were then dehydrated in graded alcohols, made transparent in xylene and embedded in paraffin. Paraffin blocks were divided into 5 micron sections by microtome. Hematoxylin and eosin (H&E) staining was utilised to examine the general histological structure. Liver damage was determined according to the number of affected inflammation areas with necrotic, apoptotic, and inflammatory cells in the parenchyma. For the evaluation of inflammation areas, 10 areas were examined at ×20 magnification from each section.[24] According to the degree of damage; 0 = no affected area, 1 = 1 affected area, 2 = 2 affected areas, 3 = 3 affected areas were scored.[25] The preparations were examined and photographed with Olympus BX43 light microscope (Tokyo, Japan).

Statistical Analysis

Statistical analysis of the experimental results was performed in IBM SPSS Statistics v22.0 programme (IBM SPSS Corp.; Armonk, NY, USA). For histopathological analyses, one-way analysis of variance and significance test were used to determine the differences between the groups. The results were given as arithmetic mean ± standard error. For the intergroup comparisons of the variables analysed by one-way analysis of variance, conformity to normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene's test) were checked. The difference between group means for the related variables was analysed by one-way ANOVA and multiple comparisons were performed by Tukey honestly significant difference test. $P < .05$ was considered statistically significant.

RESULTS

The serum ALT, AST, ALP and total bilirubin levels of animals in the BPA group were found to be higher than in the control group ($P < .05$). On the other hand, pre-treatment of high-dose apigenin (200 mg/kg) to BPA-induced rats caused a decrease in serum total bilirubin levels, while both doses of apigenin (100 mg/kg and 200 mg/kg) pre-treatment decreased serum ALT and AST levels ($P < .05$). But, pre-treatment of apigenin at both doses to BPA-treated animals did not cause a significant decrease in serum ALP levels ($P > .05$). There was no statistically significant change in serum albumin levels in BPA-treated rats ($P > .05$), also pre-treatment of BPA-induced rats with

Table 1. Effect of Bisphenol A and Apigenin on Liver Function Tests in Rats

Liver Function Tests	Control	BPA	BPA + APG100	BPA + APG200
ALT (IU/L)	52.63 ± 2.8	67.21 ± 4.02[a]	60.17 ± 2.24[a,b]	58.83 ± 3.49[a,b]
AST (IU/L)	73.46 ± 3.91	88.03 ± 4.64[a]	79.53 ± 3.92[a,b]	76.64 ± 3.57[b]
ALP (IU/L)	317.55 ± 17.09	386.25 ± 16.3[a]	381.56 ± 17.84[a]	378.19 ± 20.13[a]
Total bilirubin (mg/dL)	0.26 ± 0.01	0.38 ± 0.02[a]	0.31 ± 0.02[a,b]	0.30 ± 0.02[a,b]
Albumin (g/dL)	3.11 ± 0.59	3.06 ± 0.46	3.09 ± 0.43	3.08 ± 0.5

Data represent the means ± SD of 7 independent experiments.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; APG, Apigenin; AST, aspartate aminotransferase; BPA, Bisphenol A.
[a]$P < .05$ Significantly different in comparison with the control group.
[b]$P < .05$ Significantly different in comparison with the BPA group.

apigenin at both concentrations did not cause any change in serum albumin levels ($P > .05$) (Table 1).

Although MDA levels in the liver tissue of rats in the BPA group were found to be higher than in the control group, apigenin pre-treatment at both doses caused a decrease in liver MDA levels in BPA-treated rats ($P < .05$). Catalase and SOD activities and reduced glutathione (GSH) levels were also found to be lower in BPA-treated rat liver tissue compared to the control group ($P < .05$). On the other hand, apigenin pre-treatment at both doses caused an increase in liver GSH levels and SOD activity in BPA-treated rats ($P < .05$). However, high-dose apigenin pre-treatment increased catalase activity in BPA-treated rats ($P < .05$), whereas low-dose apigenin pre-treatment had no statistically significant effect on catalase activity ($P > .05$) (Figure 1).

TNF-α and IL-6 levels in the liver tissue of rats in the BPA group were higher than in the control group ($P < .05$), but apigenin pre-treatment at both doses caused a decrease in the levels of TNF-α and IL-6 in the liver of BPA-treated rats ($P < .05$). IL-10 levels were found to be lower in the liver tissue of BPA-treated rats compared to the control group ($P < .05$). However, high-dose apigenin pre-treatment caused an increase in IL-10 levels in BPA-treated rats ($P < .05$), whereas low-dose apigenin pre-treatment did not cause a significant increase in IL-10 levels ($P > .05$) (Figure 2).

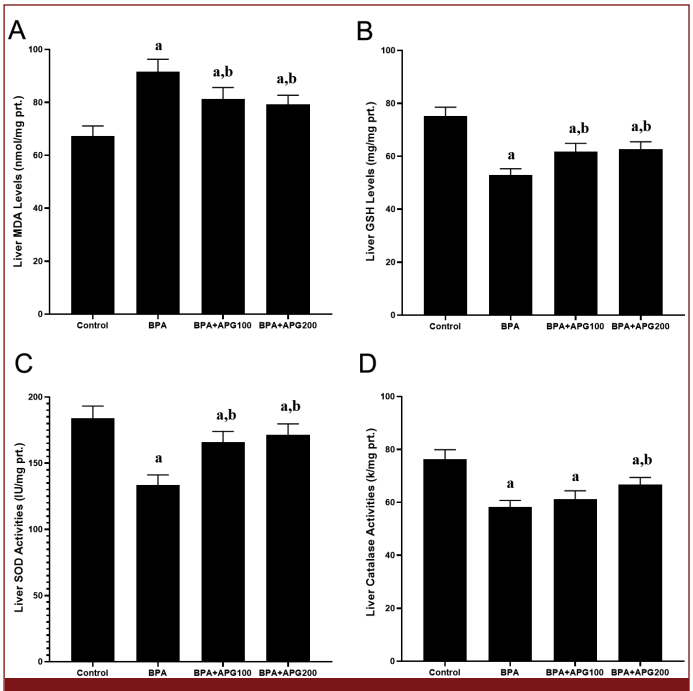

Figure 1. Effect of apigenin and BPA on (A) MDA level, (B) GSH level, (C) SOD activity, and (D) catalase activity in liver tissue. Data represent the means ± SD of 7 independent experiments. (APG, Apigenin; BPA, Bisphenol A; GSH, reduced glutathione; MDA, malondialdehyde; SOD, superoxide dismutase). [a]$P < .05$ Significantly different in comparison with the control group. [b]$P < .05$ Significantly different in comparison with the BPA group.

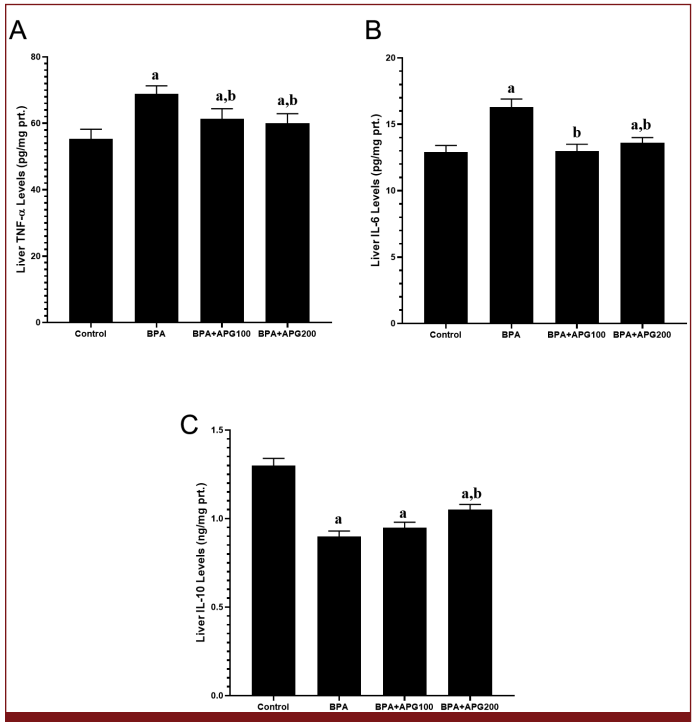

Figure 2. Effect of apigenin and BPA on (A) TNF-α level, (B) IL-6 level, and (C) IL-10 level in liver tissue. Data represent the means ± SD of 7 independent experiments. (APG, Apigenin; BPA, Bisphenol A; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor-alpha). [a]$P < .05$ Significantly different in comparison with the control group. [b]$P < .05$ Significantly different in comparison with the BPA group.

Liver tissue sections were taken from each group and examined with H&E staining to evaluate histological changes (Figure 3 and Table 2). Microscopic examination of the sections in the control group showed that the parenchymal hepatocytes forming the liver lobules were healthy. These cell cords (remark cords), which were located in a radial arrangement towards the periphery of the lobulus through the vena centralis, were observed to have a normal structure. Close examination of the sinusoids (intra-lobular blood capillaries) between the remark cords did not reveal any structural abnormality. Observations of the portal areas revealed no abnormalities in their vascular structures (Figure 3A and Table 2). In the BPA-treated group, marked necrotic foci were observed in the examination of liver tissue. In other words, an intense eosinophil increase in the cytoplasm of many hepatocytes in the parenchyma, marked hyperchromasia in the nuclei, and irregularities in the cell membranes were detected. Disorganization of remark cords, dilatation of sinusoids, and intense erythrocyte aggregation were among the symptoms. In addition, congestion of the vascular structures in both the central vein and portal area were among the findings observed (Figure 3B and Table 2). In the BPA + APG100 group, a very limited improvement was observed, especially a partial reduction in necrotic cell foci and a slight improvement in dilatation of sinusoids. However, the improvement was not significant in this group (Figure 3C and Table 2). In

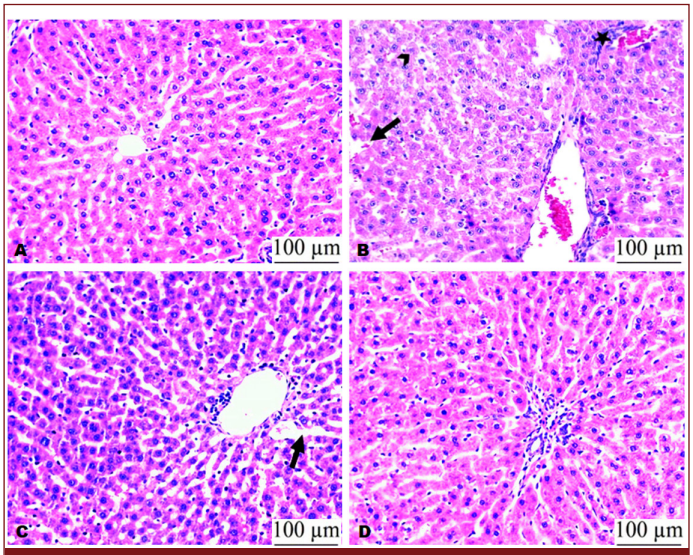

Figure 3. Histological alterations in liver tissue (A: Control group, B: BPA group, C: BPA + APG100 group, D: BPA + APG200 group). Liver sections were stained with hematoxylin and eosin and imaged at a magnification of ×20. Sinusoidal dilatation and congestion (black arrow), degenerative changes in hepatocytes (arrowhead) and inflammatory cell infiltration (asterisk). (APG, Apigenin; BPA, Bisphenol A)

the BPA + APG200 group, a more pronounced reduction in necrotic cell foci, a visible improvement in dilatations in the sinusoids, and a significant improvement in tissue architecture were observed (Figure 3D and Table 2).

DISCUSSION

In order to reduce or eliminate the effects of BPA and similar environmental pollutants, many studies have focused on the use of many substances with antioxidant activity. Apigenin has been widely used in alternative medicine in recent years due to its broad therapeutic activities and its antioxidant and anti-inflammatory activity against environmental toxins. Although apigenin is a lipophilic molecule, it has a low solubility in water, which limits its absorption. Apigenin is usually present in glycoside form and must therefore be hydrolyzed in the intestine before absorption. As apigenin can be cleared from the cell by transporters such as P-glycoprotein, its absorption may also be limited by these mechanisms. After absorption, apigenin undergoes phase II reactions such as glucuronidation and sulphation in the liver. Apigenin and its phase II metabolites are excreted in urine and bile. The terminal half-life of apigenin after oral administration has been reported to be approximately 2-5 hours. For these reasons, the low oral bioavailability and rapid elimination of apigenin limit its clinical use, and repeated dosing and/or the development of new delivery systems are required to achieve therapeutic/prophylactic effects. Apigenin is a natural compound with a wide range of therapeutic potential and there is strong preclinical evidence that it may be particularly effective in processes such as diabetes, cancer, atherosclerosis, inflammation, and neurodegeneration, but clinical trials with apigenin are very limited. Pharmaceutical research is underway to develop several oral formulations of apigenin. The long-term health effects of dietary apigenin are supported by some epidemiological studies. There are many studies showing that apigenin, which is known to have highly effective antioxidant and anti-inflammatory properties, reduces MDA levels by suppressing oxidative stress, increases the antioxidant activities of enzymes such as GSH and SOD and prevents inflammation.[11,12]

In this study, apigenin was evaluated in rats exposed to BPA serum liver function tests, liver tissue cytokines, and oxidative stress parameters.[12] Histopathological examination of liver tissue was also performed. In this study, the dose, duration, and route of administration were determined for the concentrations of BPA and apigenin administered to the volunteers, taking into account previous studies on this topic in laboratory animals.[9,16] AST and ALT are cytosolic enzymes of the hepatocyte and their increased activity in the circulation, i.e., due to disruption of membrane structure, reflects the presence of cell damage by entering the circulation. ALP is found in the bile duct of the liver.[26] There are many studies in the literature showing that AST, ALT, and ALP enzyme levels increase in rats with xenobiotic-induced liver damage.[27] Most serum proteins are produced in the liver. The most important of these proteins is albumin, which is an indicator of the synthesis capacity of the liver. Therefore, albumin is low in liver deficiency. Bilirubin is produced by the breakdown of haem formed as a result of the destruction of erythrocytes in the reticuloendothelial system. Bilirubin binds to plasma albumin and is transported to the liver, where it is conjugated and converted to conjugated bilirubin and excreted in the bile and intestine. Therefore, unconjugated bilirubin and total bilirubin levels increase in liver failure. Bilirubin is formed by the breakdown of the haem molecule and

Table 2. Effect of Bisphenol A and Apigenin on the Score of Histopathological Changes in Liver Tissue

Histopathological Parameters	Control	BPA	BPA + APG100	BPA + APG200
Dispersed remark cords	–	+++	++	+
Sinusoidal dilation	–	+++	+	–
Inflammatory cell infiltration	–	+++	++	+
Cell membrane disruption	+	+++	+++	–
Hemorrhage	–	+++	++	+

–, normal; +, mild; ++, moderate; +++, severe.
APG, Apigenin; BPA, Bisphenol A.

is conjugated by the liver and excreted in the bile. As this process is impaired in liver failure, bilirubin levels increase.[28] Studies have shown that the administration of BPA at various doses increases AST, ALT, and ALP activities, and total bilirubin levels and decreases albumin levels.[7] In this study, although serum AST, ALT, and ALP activities, and total bilirubin levels were increased, no change was observed in serum albumin levels. It was believed that serum albumin levels may change at a later time. Apigenin pre-treatment decreased serum AST, ALT, and ALP activities, and total bilirubin levels in BPA-treated rats but did not change serum albumin levels. Consistent with these findings, apigenin pre-treatment decreased serum AST, ALT, and ALP activities in experimental animals in which liver damage was induced by methotrexate, cyclophosphamide, and N-nitrosodiethylamine.[29-31]

Oxidative stress is caused by an increase in the production of free radicals and/or ROS in the body and an imbalance in their neutralization or elimination by antioxidants. The result is a pathological condition that stimulates the onset and progression of tissue damage.[32] In this study, when the data obtained at the end of the experiment were examined to determine the level of oxidative stress that could be caused by BPA, it was observed that the level of MDA, the end product of lipid peroxidation, increased in the liver tissue of rats in the BPA group. The increase in MDA levels in the BPA group can be considered as an indicator of increased lipid peroxidation in the organism due to BPA. The results obtained in the study support other studies reporting that BPA toxicity causes oxidative stress in various tissues.[7] In these studies, the investigators reported that oxidative stress is caused not only by the increase in lipid peroxidation products, but also by the depletion or suppression of the antioxidant defence system by decreasing the levels of enzymatic and non-enzymatic antioxidants.[7,33] In this study, it was observed that BPA intoxication caused a decrease in catalase and SOD activities and GSH levels in liver tissue. It was believed that this is due to oxidant damage at the cellular level, which weakens cellular antioxidants. Apigenin pre-treatment caused a decrease in liver MDA levels and an increase in catalase and SOD activities and GSH levels in BPA-treated rats. Studies have shown that apigenin pre-treatment decreased lipid peroxidation in liver tissue and increased levels of enzymatic and non-enzymatic antioxidants in experimental animals in which liver damage was induced by methotrexate, a chemotherapeutic agent, and environmental pollutants such as furan and carbon tetrachloride (CCl4).[31,34,35] Literature data were consistent with the results that were obtained.

BPA has been suggested to cause immune system disorders by interfering with various cytokine pathways. It has been reported that BPA plays a role in the aetiology of inflammation, allergic reactions, some cancers and autoimmune diseases, particularly as a result of its negative effects on cytokines. In fact, in line with the results of many studies on the subject, it is also reported that oxidative damage occurs as a result of the stimulation of inflammation by BPA.[36] In the present study, it was observed that the levels of TNF-α and IL-6, which play an active role in shaping inflammation and are among the pro-inflammatory cytokines, increased with BPA administration, whereas the levels of IL-10, an anti-inflammatory cytokine, decreased. This is because BPA causes an inflammatory response in liver tissue. Pre-treatment with apigenin at both doses decreased liver TNF-α and IL-6 levels in BPA-treated rats, while only pre-treatment with apigenin at the high-dose increased liver IL-10 levels. In this study, the positive effect of apigenin on the mentioned parameters was attributed to the anti-inflammatory activity of apigenin. Similar to these results, it was observed that apigenin pre-treatment decreased pro-inflammatory cytokine levels and increased anti-inflammatory cytokine levels in liver tissue of experimental animals with liver damage induced by environmental pollutants such as furan and CCl_4.[34,35]

Regional hepatic necrosis is the typical histological feature of BPA-induced liver injury. Studies have reported that BPA induction leads to centrilobular hepatic necrosis, mild to moderate sinusoidal dilatation and congestion, massive neutrophil and lymphocyte infiltration, and hepatocyte degeneration.[37] In this study, BPA poisoning caused marked necrotic foci characterised by intense eosinophilic increase in the cytoplasm of many hepatocytes in the parenchyma, marked hyperchromasia in the nuclei, and cell membrane irregularities. Dilatation of sinusoids, intense erythrocyte aggregation, and congestion of vascular structures around the central and portal veins were also observed. Low-dose apigenin pre-treatment caused a partial reduction in necrotic cell foci and a slight improvement in sinusoidal dilatation in BPA-treated rats, whereas high-dose apigenin pre-treatment caused a more pronounced reduction in necrotic cell foci, a visible improvement in sinusoidal dilatation and a significant improvement in tissue architecture. In the literature review, apigenin pre-treatment was found to improve liver histology in methotrexate-induced liver injury.[38] These results highlight that apigenin pre-treatment has hepatoprotective properties at the cellular and tissue level.

It was observed that BPA toxicity impaired liver function tests in rats, induced oxidative stress and inflammation in the liver and damaged the microarchitecture of the liver tissue, and this damage could be reversed by the high concentration of apigenin. In this study, pre-treatment with apigenin against BPA-induced oxidative stress and inflammation alleviated the negative changes observed in the study, and it is believed that this is due to the antioxidant and anti-inflammatory properties of apigenin. Based on the results obtained, it is concluded that apigenin can be used as a preventive and mitigating agent against the negative effects of BPA and similar toxic substances and the damage that may occur.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received for this study from Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Approval no: 2020/06-03, Date: June 25, 2020).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.B.; Design – M.B.; Super-vision – M.B.; Data Collection – M.B.; Analysis and/or Interpretation – M.B.; Literature Search – M.B.

Declaration of Interests: The authors have no conflicts of interest to declare.

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