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18β-glycyrrhetinic acid Mitigates bisphenol A-induced liver and renal damage: Inhibition of TNF-α/NF-κB/p38-MAPK, JAK1/STAT1 pathways, oxidative stress and apoptosis

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ABSTRACT

Bisphenol A (BPA) has been commonly used in various consumer products, including water bottles, food containers, and canned food linings. However, there are concerns about its potential toxicity to human health, particularly its impact on the liver and kidneys. The objective of this research was to investigate the potential ameliorative effects of 18β-glycyrrhetinic acid (GA) against BPA-induced hepatotoxicity and nephrotoxicity in rats. The animals were supplemented with BPA (250 mg/kg b.w.) alone or with GA (50 and 100 mg/kg b.w.) for 14 days. GA treatment alleviated the BPA-induced hepato-renal tissue injuries through reducing the serum ALT, AST and ALP levels, and urea and creatinine levels. GA co-treatment also increased activities of SOD, CAT and GPx enzymes and levels of GSH, and suppressed MDA levels in BPA induced tissues. BPA also induced inflammation by increasing the levels of TNF-α, NF-κB, JAK1, STAT1, P38 MAPK and JNK in liver and kidney tissues and GA treatment ameliorated these effects. BPA triggered apoptosis by increasing caspase-3, Bax, and cytochrome *c* at protein levels and also by decreasing the antiapoptotic Bcl-2 level. However, treatment with GA (50 and 100 mg/kg) decreased apoptosis. Overall, our results have revealed the potential ameliorative mechanisms of GA, as a possible agent for BPA-induced hepatotoxicity and nephrotoxicity.

1. Introduction

Bisphenol A (BPA) is a chemical compound that has been used for decades in the production of certain plastics and epoxy resins. It is primarily used in the manufacturing of polycarbonate plastics and epoxy resins, which are commonly found in various consumer products and food and beverage containers (Liu et al., 2021). BPA has been a topic of concern and study due to its potential health effects. Research has indicated that BPA can mimic the hormone estrogen in the body and may have endocrine-disrupting properties (Cimmino et al., 2020). It has been associated with various health issues, including reproductive disorders, developmental problems, and an increased risk of certain cancers (Tarafdar et al., 2022). BPA was demonstrated to induce severe oxidative damage in various tissues, including the kidneys, liver, brain, and other crucial organs (Caglayan et al., 2022; Peerapanyasut et al., 2019b; Zahra et al., 2020). Shielding the tissues from unwanted effects is an important strategy to lessen the unfavorable impacts of BPA in medical practice. Therefore, the therapeutic benefits of natural compounds with anti-oxidant capabilities that may lessen the severity of BPA-induced toxicities might be advantageous.

18β-glycyrrhetinic acid (also known as glycyrrhetinic acid or GA) is a natural substance obtained from licorice root (Glycyrrhiza glabra). It is classified as a triterpenoid and has been used for various medicinal and therapeutic purposes (Kowalska and Kalinowska-Lis, 2019). GA is known for its anti-inflammatory effects. It has been shown to inhibit the activity of certain enzymes, including cyclooxygenase that is

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participated in the generation of inflammatory mediators (Shinu et al., 2023). These properties make it potentially useful in conditions where inflammation plays a role. It also exhibits antioxidant properties, meaning it can help neutralize free radicals. Free radicals are very active substances, which can cause oxidative stress and damage cells, contributing to several diseases. By acting as an antioxidant, GA may help protect against oxidative damage (Qing et al., 2022). GA has been studied for its potential protective effects on the liver. Research suggests that it may help prevent liver injury via lessening severity of inflammation, oxidative injury, apoptosis and lipid accumulation in the liver (Pan et al., 2022; Zhang et al., 2022). Some studies have explored the anti-cancer properties of GA. It has been shown to inhibit the growth of certain cancer cells and induce apoptosis (programmed cell death) in several cancer types, containing breast and liver cancers (Hsu et al., 2023; Liu et al., 2023).

Nonetheless, not much is known related how GA works as a protective mechanism to combat against BPA-induced toxicities. The antioxidant, anti-inflammatory and anti-apoptotic properties of GA were examined in this work in relation to BPA-induced toxicity in the rat liver and kidney tissues.

2. Material and method

2.1. Chemicals

All reagents and chemical compounds including GA (CAS-No: 471-53-4) and BPA (CAS-No: 80-05-7), were acquired from Sigma-Aldrich chemicals (St. Louis, MO, USA) and were of highest purity. Sunred Biological Technology (Shanghai, China) provided complete biochemical rat ELISA tests kits.

2.2. Animals and experimental design

For this investigation, 40 male Wistar albino rats weighing 250–300 g and 12–13 weeks old were employed. Animals were purchased from Bingol University's Experimental Research Center in Bingol, Turkey. The rats were housed in cages in a climate-controlled space that was kept at a constant 24–25 °C and had a 12-h cycle of light and darkness (07:00–19:00 light; 19:00–07:00 dark). Standard food and unlimited amounts of water were offered. The Bingol University's Animal Experimentation Ethics Committee reviewed all procedures involving animals (Protocol No. 2020-01/02).

The rats were separated into 5 groups at random with 8 rats in each group.

Group I (Control): Olive oil at dosage of 0.2 ml was administered orally daily for 14 days.
Group II (GA); Oral gavage administration of GA (100 mg/kg b.w.) in olive oil was given to the group for 14 days (Rashid et al., 2017).
Group III (BPA): BPA (250 mg/kg b.w.) in olive oil was given orally by gavage for 14 days (Shirani et al., 2019).
Group IV (BPA + GA 50): BPA (250 mg/kg b.w.) and GA (50 mg/kg b.w.) were administered orally for 14 days.
Group V (BPA + GA 100): BPA (250 mg/kg b.w.) and GA (100 mg/kg b.w.) were administered orally for 14 days.

24 h after the last dose (day 15), the animals were anesthetized with sevoflurane and killed. Blood samples from neck veins were collected by decapitation and centrifuged at 3500 rpm for 10 min to collect serum. The Serum were kept at −80 °C until evaluation of liver and kidney function tests. Liver and kidney tissues were rapidly collected and stored at −80 °C for biochemical and molecular analyses.

2.3. Measurement of liver and kidney function markers

In the Mindray Perfect Plus 400 autoanalyzer, the levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate (ALP) were examined. The results for these enzymes were displayed as U/L. The manufacturer's recommendations were followed while measuring renal function parameters (urea and creatinine) using colorimetric kits from Diasis Diagnostic Systems in Istanbul, Turkey.

2.4. Oxidative stress indicators

The liver and kidney tissues were homogenized in a homogenizer (Tissue Lyser II, Qiagen, Netherlands) in a solution of 1.15% KCl to produce the homogenate. Glutathione peroxidase (GPx) activity was assessed using the Lawrence and Burk (1976) method. The technique described by Sun et al. (1988) was used to measure the activity of superoxide dismutase (SOD). Both enzymes' activity has been expressed as U/g protein. The Aebi (1984) procedure was used to test the catalase (CAT) activity, which has been reported as katal/g protein. The Sedlak and Lindsay (1968) method has been used to analyze the glutathione (GSH) content. The MDA level was tested in accordance with Placer et al. (1966). The amounts of GSH and MDA were calculated in nmol/g of tissue. The liver and kidney tissue's protein content were determined by the method developed by Lowry et al. (1951).

2.5. Analysis of inflammation markers with ELISA kits

Pro-inflammatory cytokine levels were examined with commercial rat ELISA kits. All measurements were made in liver and kidney tissues according to the manufacturer's instructions. In the study, signaling pathway molecules and inflammation markers such as p38 mitogen-activated protein kinase (p38 MAPK), signal transducer and activator of transcription-1 (STAT1), janus kinase-1 (JAK1), c-Jun NH2-terminal kinase (JNK), tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB) were analyzed.

2.6. Western blot analysis

Tissues from rats treated with BPA and GA were homogenized and protein levels analyzed via Western blot. The same amounts of protein were run on 12–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following this, the samples were transferred to nitrocellulose membranes. The membrane was then washed for 5 min in TBS-0.05% Tween-20 (TBS-T) and blocked for 1 h before using the primary antibody in 5% bovine serum albumin. Bax, Bcl-2, cytochrome *c*, procaspase-3 and β-Actin were used as primary antibodies. The membranes were then incubated with primary antibodies at 4 °C overnight. Afterwards, the membranes were washed 5 × 5 minutes in TBS-T. After washing, the membranes were conjugated with horse radish peroxidase.

2.7. Statistical analysis

Data on biochemical reactions were statistically analyzed using the package program SPSS 20.0. The "One-way Analysis of Variance (ANOVA)" test was used to establish statistical differences and significance levels, and the Tukey test was utilized to determine group differences. Results were judged significant at the $p < 0.05$ level, and all data were presented as mean ± standard error (SEM).

3. RESULTS

3.1. Liver and kidney function markers

Table 1 displays the serum AST, ALT, and ALP enzyme activity. It was observed that BPA application augmented levels of serum AST, ALP and ALT in comparison to the control ($p < 0.05$). It was determined that GA (50 and 100 mg/kg) given for treatment with BPA significantly

Table 1
Effect of GA on BPA-induced serum liver and kidney function tests. Different letters (a-d) on the columns show a statistical difference ($p < 0.05$).

Parameters	Control	GA	BPA	BPA + GA-50	BPA + GA-100
ALP (U/L)	93.70 ± 6.09^{a}	93.09 ± 6.76^{a}	182.53 ± 7.88^{d}	161.55 ± 7.36^{c}	139.63 ± 8.46^{b}
ALT (U/L)	37.19 ± 3.12^{a}	35.58 ± 2.71^{a}	65.79 ± 4.85^{d}	57.24 ± 3.56^{c}	46.33 ± 4.15^{b}
AST (U/L)	72.04 ± 4.89^{a}	71.62 ± 4.47^{a}	135.51 ± 6.17^{d}	119.75 ± 5.47^{c}	96.22 ± 5.08^{b}
Urea (mg/dL)	21.13 ± 1.94^{a}	20.86 ± 1.64^{a}	44.32 ± 2.28^{d}	38.41 ± 2.56^{c}	31.19 ± 2.07^{b}
Creatinine (mg/dL)	0.49 ± 0.02^{a}	0.51 ± 0.01^{a}	1.07 ± 0.04^{d}	0.85 ± 0.03^{c}	0.67 ± 0.03^{b}

reduced serum AST, ALT and ALP enzyme activities compared to the toxic group caused by BPA ($p < 0.05$). When serum urea and creatinine levels, which are indicators of kidney damage, were examined, it was found that these levels increased in the BPA supplemented group in comparison to the untreated group, and GA administration together with BPA caused a decrease in these levels ($p < 0.05$).

3.2. Effects of GA on BPA-induced liver and kidney oxidative damage and antioxidant status

It was determined that BPA application in liver and kidney tissues considerably lessened activities of antioxidant enzymes containing SOD, CAT and GPx and GSH level compared to the untreated group ($p < 0.05$). Tables 2 and 3 show that GA (50 and 100 mg/kg) doses used in combination with BPA increase antioxidant enzyme activities such as SOD, CAT and GPx, and GSH level ($p < 0.05$). The results of the current investigation showed that BPA caused oxidative stress to develop in the liver and kidney, which was shown by a significant ($p < 0.05$) rise in MDA levels while GA co-administration at 50 and 100 mg/kg doses considerably alleviated this effect.

3.3. Inflammation parameters in liver and kidney tissues

Liver and kidney tissues NF-κB, TNF-α, JAK1, STAT1, P38 MAPK and JNK levels increased in the BPA group compared to the control group ($p < 0.05$). GA (50 and 100 mg/kg) given in two different doses (50 and 100 mg/kg) for therapeutic purposes decreased the parameter levels in these tissues compared to the BPA group ($p < 0.05$) (Figs. 1 and 2).

3.4. Western blot analysis

To better understand the molecular mechanisms of GA's anti-apoptotic effects on BPA-induced apoptosis in liver and kidney, the protein expression levels of Bax, Bcl-2, cytochrome *c*, and procaspase-3 were examined. It was determined that the Bax/Bcl-2 ratio, which is an important parameter in defining apoptosis, increased significantly with BPA application, and decreased in both liver and kidney tissues with GA treatment (Figs. 3 and 4). Also, a significant increase in cytochrome *c* level was observed after BPA administration, which was significantly reduced in the BPA + GA 50 and 100 groups. In addition, protein levels of procaspase-3 were significantly decreased in the BPA-treated group and increased in the BPA + GA 50 and 100 groups in both tissues.

4. Discussion

Bisphenol A has been the subject of a myriad of studies and research investigating its potential toxicities. BPA is known to have endocrine-disrupting properties. It can mimic the hormone estrogen in the body and interfere with the normal hormonal signaling. This disruption can have various adverse effects on the endocrine system, including reproductive disorders, altered development of the reproductive organs, and hormonal imbalances (Xing et al., 2022). BPA has been linked to reproductive toxicity in both males and females. In animal studies, it has been associated with reduced fertility, impaired sperm quality, and altered reproductive organ development. In females, BPA exposure has been linked to menstrual cycle irregularities, reduced fertility, and potential effects on fetal development (Liu et al., 2022). There is evidence suggesting that BPA exposure, particularly during critical periods of development (such as prenatal and early childhood), may have detrimental effects on neurodevelopment. Animal studies have indicated that BPA exposure can lead to behavioral changes, impaired learning and memory, and altered brain development. Human studies have reported associations between prenatal BPA exposure and neurobehavioral issues, such as hyperactivity and attention problems in children (Yoo et al., 2020). BPA has been linked with increased risk of metabolic disorders, containing insulin resistance and diabetes. Animal studies have reported that BPA exposure can disrupt metabolic processes, alter fat metabolism, and contribute to weight gain (Amjad et al., 2020). Some studies have suggested a probable link between BPA administration and increased risk of certain cancers (Salamanca-Fernández et al., 2021). Despite presence of several articles related BPA toxicity, the ameliorative effects of GA on BPA-induced liver and kidney toxicities are yet to be studied. Therefore, the objective of the current work was to examine and identify the molecular and biochemical mechanisms by which GA protects against BPA-induced liver and kidney damages.

Liver function enzymes, also known as liver enzymes, are proteins produced by liver cells (hepatocytes) that play a crucial role in various metabolic processes within the liver. These enzymes are involved in the synthesis, breakdown, and transformation of substances in the body. They are also released into the bloodstream, and their levels can be measured through blood tests to assess liver function and detect liver diseases or abnormalities. ALT levels are specifically used to evaluate liver health and are considered one of the most reliable markers of liver function. Elevated levels of AST and ALP indicate liver damage (Holt and Ju, 2006). The findings of this study demonstrated that BPA intoxication causes liver and kidney damage, which is evident by an increase in blood levels of urea and creatinine for the kidneys and an increase in ALP, ALT, and AST, which indicates hepatocellular damage. The increase in serum ALT, AST, and ALP activity may be caused by hepatocyte damage and enzyme leaking into the blood. The results of our study showed that GA improved blood levels of serum and creatinine in the kidney, as well as serum activities of liver enzymes. In several earlier studies, GA showed protective effects for the hepatic and renal systems (Alekhya Sita et al., 2019; Vahdati Hassani et al., 2017). In a study, the researchers have

Table 2
The oxidative stress markers of GA protection against BPA-induced hepatotoxicity. Different letters (a-d) on the columns show a statistical difference ($p < 0.05$).

Parameters	Control	GA	BPA	BPA + GA-50	BPA + GA-100
MDA (nmol/g tissue)	25.82 ± 1.76^{a}	25.96 ± 1.90^{a}	40.94 ± 1.77^{d}	36.34 ± 1.22^{c}	32.45 ± 2.04^{b}
GSH (nmol/g tissue)	7.82 ± 0.37^{d}	7.88 ± 0.27^{d}	3.85 ± 0.20^{a}	4.82 ± 0.29^{b}	6.04 ± 0.33^{c}
CAT(Catal/g protein)	64.37 ± 3.76^{c}	67.99 ± 2.66^{c}	39.40 ± 2.20^{a}	48.80 ± 1.42^{b}	52.53 ± 2.80^{b}
SOD (U/g tissue)	37.50 ± 2.10^{d}	36.93 ± 1.73^{d}	23.95 ± 1.81^{a}	28.26 ± 1.42^{b}	31.82 ± 1.58^{c}
GPx (U/g tissue)	45.01 ± 2.70^{d}	47.88 ± 2.28^{d}	27.16 ± 1.57^{a}	31.83 ± 2.18^{b}	38.10 ± 2.20^{c}

Table 3
The oxidative stress markers of GA protection against BPA-induced nephrotoxicity. Different letters (a-d) on the columns show a statistical difference ($p < 0.05$).

Parameters	Control	GA	BPA	BPA + GA-50	BPA + GA-100
MDA (nmol/g tissue)	44.76 ± 2.33^{a}	44.22 ± 1.62^{a}	75.66 ± 3.37^{d}	64.65 ± 2.29^{c}	56.48 ± 3.91^{b}
GSH (nmol/g tissue)	5.74 ± 0.31^{d}	5.82 ± 0.26^{d}	2.43 ± 0.17^{a}	2.92 ± 0.14^{b}	3.72 ± 0.31^{c}
CAT (Catal/g protein)	38.37 ± 1.90^{c}	39.13 ± 2.22^{c}	23.26 ± 2.12^{a}	30.14 ± 1.35^{b}	32.08 ± 2.30^{b}
SOD (U/g tissue)	17.80 ± 1.64^{c}	18.71 ± 1.96^{c}	9.13 ± 1.49^{a}	12.00 ± 0.86^{b}	14.03 ± 1.63^{b}
GPx (U/g tissue)	37.33 ± 2.15^{d}	37.20 ± 2.55^{d}	22.47 ± 1.80^{a}	26.77 ± 1.15^{b}	31.13 ± 1.82^{c}

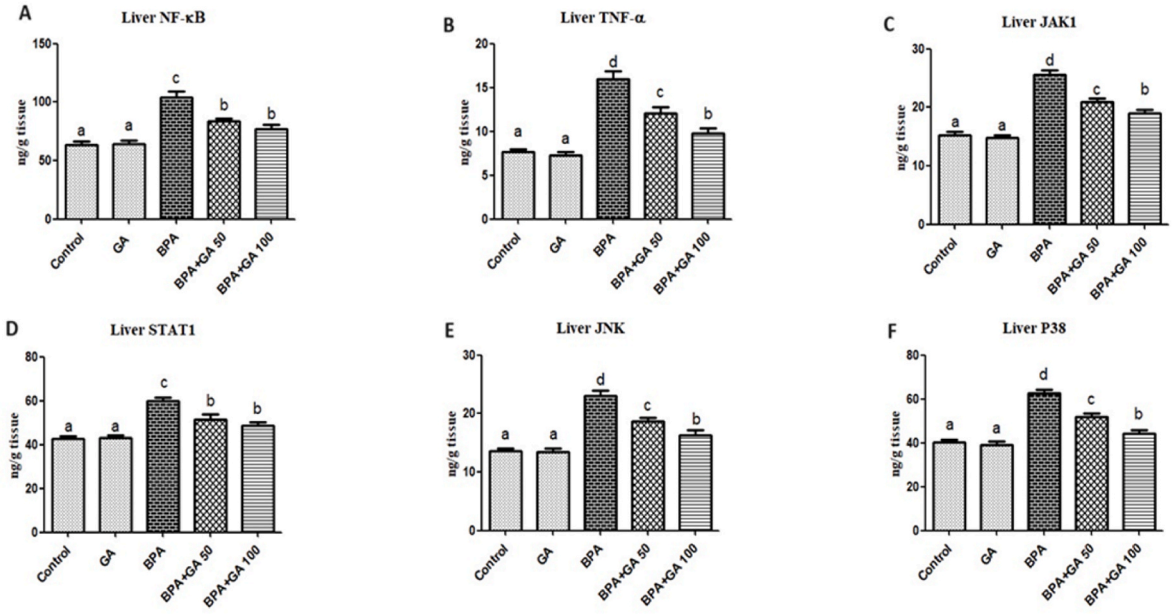

Fig. 1. (A) Effect of GA on BPA-induced liver NF-κB levels. (B) Effect of GA on BPA-induced liver TNF-α levels. (C) Effect of GA on BPA-induced liver JAK1 levels. (D) Effect of GA on BPA-induced liver STAT1 levels. (E) Effect of GA on BPA-induced liver JNK levels. (F) Effect of GA on BPA-induced liver p38 levels. Values are expressed as mean ± SEM. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

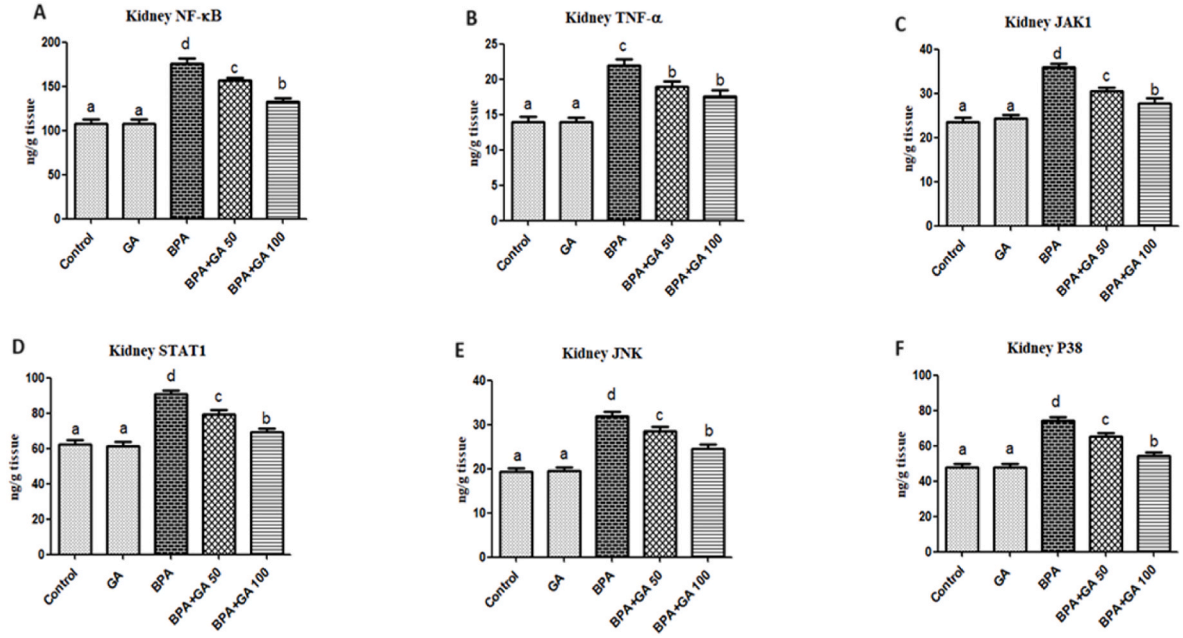

Fig. 2. (A) Effect of GA on BPA-induced kidney NF-κB levels. (B) Effect of GA on BPA-induced kidney TNF-α levels. (C) Effect of GA on BPA-induced kidney JAK1 levels. (D) Effect of GA on BPA-induced kidney STAT1 levels. (E) Effect of GA on BPA-induced kidney JNK levels. (F) Effect of GA on BPA-induced kidney p38 levels. Values are expressed as mean ± SEM. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

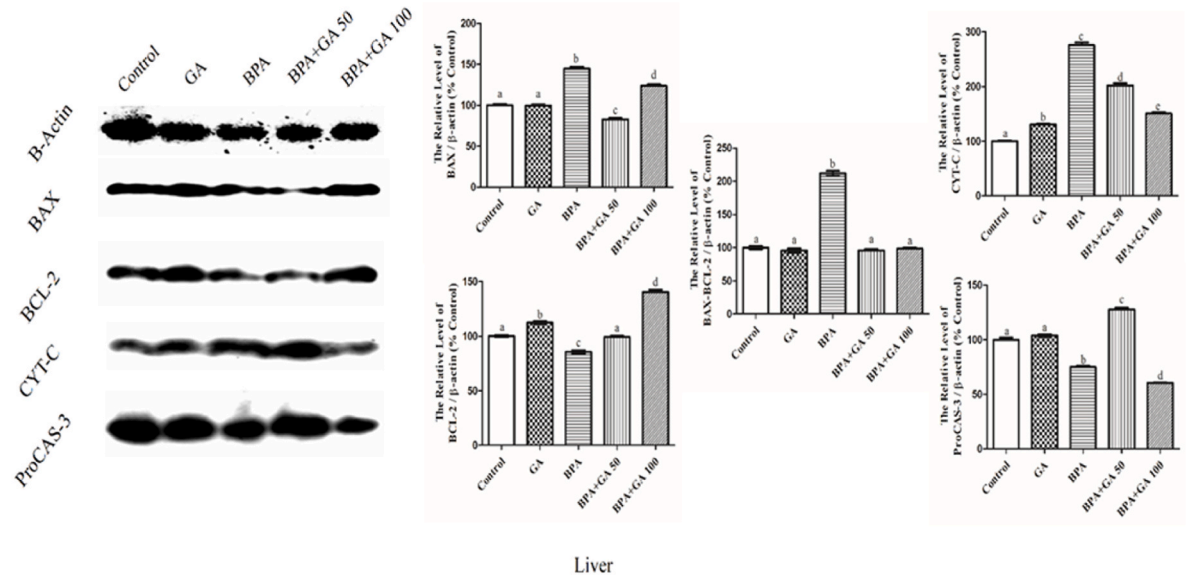

Fig. 3. Anti-apoptotic effects of GA on BPA-induced apoptosis in liver tissue. Protein levels (A) Bax, Bcl-2, Cytchrome c and procaspase-3 were measured by Western blotting analysis. β-Actin was used as reference. (B) Data were presented as mean ± SEM. Different letters (a-d) on the columns show a statistical difference ($p < 0.05$).

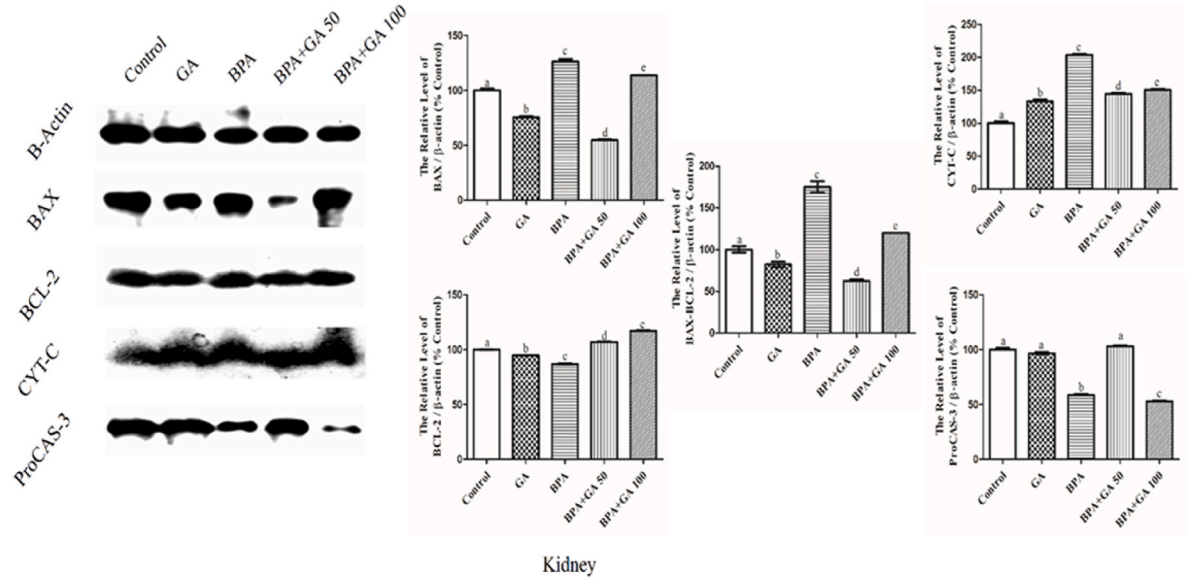

Fig. 4. Anti-apoptotic effects of GA on BPA-induced apoptosis in kidney tissue. Protein levels (A) Bax, Bcl-2, Cytchrome c and procaspase-3 were measured by Western blotting analysis. β-Actin was used as reference. (B) Data were presented as mean ± SEM. Different letters (a-d) on the columns show a statistical difference ($p < 0.05$).

shown that GA shields towards CP-induced liver toxicity in rats, as shown by a decline in the levels of the enzymes ALT, AST, ALP, LDH, GT, and total bilirubin (Mahmoud and Al Dera, 2015). Similar data were obtained in Hasan et al.'s study, which showed that GA reduced serum transaminases in rat liver injury brought on by 2-acetylaminofluorene (Hasan et al., 2014). In addition, Chen et al. found that supplementation with GA reduced serum transaminases in mice with CCl_4-induced chronic liver fibrosis (Chen et al., 2013). In a study, administration of GA orally significantly decreased blood urea nitrogen, creatinine in cyclophosphamide induced kidney toxicities (Wu et al., 2015).

Bisphenol A has been reported to increase the levels of ROS through the enzymatic (H_2O_2/peroxidase and NADPH/CYP450) and non-enzymatic (peroxynitrite/CO_2 and OCl-/HOCl) production of phenoxyl radicals, which react with GSH or NADPH to generate a variety of radical species, containing O_2^-, peroxides (ie. H_20_2), and hydroxyl (OH^-) radicals (Gassman, 2017). Antioxidants lessen the cellular damage brought on by the interaction of DNA, protein, and lipid molecules with ROS. Regardless of the existence of this antioxidant mechanism, exposure to chemicals can lead to an excessive or unbalanced generation of ROS, which can cause a variety of clinical diseases (Jakubczyk et al., 2020). BPA has been shown to lead to oxidative stress through alteration of cells redox status (Kobayashi et al., 2020). The current study examined whether BPA causes hepatorenal toxicity through inducing oxidative stress in the liver and kidney and GA alleviated these effects. Antioxidant enzymes (SOD, CAT, GPx) and nonenzymatic endojen antioxidant (GSH) have all been stated as antioxidant indicators devoted to the control of the redox balance (Caglayan et al., 2022). In this research, BPA administration substantially diminished GSH levels of and

the activities of the endogen antioxidant enzymes (SOD, CAT and GPx), and gave rise to the remarkable increase in MDA levels in liver and kidney tissues whereas GA co-treatment alleviated these effects signifying that GA demonstrates its protective effects against BPA liver and kidney toxicities through enhancing the antioxidant defence mechanisms of these organs. Linillos-Pradillo et al. (2023) findings were in agreement with ours, showing a significant decrease in GSH and activities of SOD, CAT and GPx in the BPA group, which indicated liver tissue injury. It has been reported that the negative effect of BPA on the kidney causes degeneration of renal tubules by changing kidney biochemical profiles as a result of oxidative DNA and ROS production. Under normal conditions, the kidneys have estrogen receptors, which can lead to epithelial cell proliferation by stimulating BPA receptors, and may also cause hydronephrosis by increasing the volume of proximal and distal tubules. It has been reported that BPA directly affects the kidney mitochondria by causing mitochondrial oxidative stress and dysfunction of the kidney in the kidney parenchyma (Sangai et al., 2012). In accordance with our study, the researchers discovered that exposure to BPA decreased the activities of CAT, GPx, and SOD while increasing levels of MDA in kidney (Chen et al., 2022). In a different study carried out by our group, BPA toxicity resulted similar effects in brain tissue while GA ameliorated these effects (Caglayan et al., 2022).

According to Volpe et al. (2018), the formation of ROS has been linked to the activation of pro-inflammatory pathways and stress signaling. NF-κB signaling has been identified as one of these as the primary signal transduction pathway involved in the control of the genes and activation of several pro-inflammatory cytokines including TNF-α. Our research showed that NF-κB and TNF-α gene expression levels were elevated in the liver and kidney after prolonged exposure to BPA. These findings are in line with earlier research showing that BPA exposure dramatically raised the gene expression of the TNF-α (Hong et al., 2022) and NF-κB (Elbakry et al., 2022) in liver and kidney (Peerapanyasut et al., 2019a). In a study, the ameliorative properties of GA against acrylamide-induced cellular damage were investigated in diabetic rats. The results demonstrated that GA treatment alleviated levels inflammatory cytokines in liver and kidney tissues of rats demonstrating the capability of GA to restore the inflammatory defence (Alanazi et al., 2021).

Cytokines control several aspects of hematopoiesis and immunological response. They activate the JAK/STAT signaling pathway to mediate their responses. STATs are key cytoplasmic transcription factors that are suppressed in the cytosol until activated by external cytokines. They are involved in both immune and pro-inflammatory pathways. When a cytokine binds to its appropriate receptor, the JAK/STAT pathway is activated. This causes conformational changes in the receptor's cytoplasm, resulting in the activation of receptor-associated members of the JAK family of kinases (Kisseleva et al., 2002). One of the most exciting discoveries of our investigation was that BPA greatly increased the levels of STAT1 and JAK1. Therefore, we hypothesize that BPA promoted STAT1 activation via potential JAK1 phosphorylation and GA co-treatment alleviated these effects. BPA treatment was also shown to activate the JAK1/STAT1 signaling while co-administration with GA considerably reduced this effect in brain tissue (Caglayan et al., 2022). One of the previous study reported that JAK-STAT pathway was rigorously disrupted after BPA treatment (Liu et al., 2020).

One of the main groups of kinases involved in cellular processes like differentiation, stress reactions, apoptosis, and immunological defense is the MAPK family. Extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases (JNKs), and p38 are the three main MAPK signaling pathways (Pua et al., 2022). It has been observed that natural antioxidant products may have anti-inflammatory effects via blocking NF-κB and MAPK signaling pathways, which are important in the regulation of the inflammatory process (Lee et al., 2022). It has also been reported that ROS stimulate redox-sensitive transcription factors by MAPK signaling pathways (Riemann et al., 2011). In a different study, curcumin was shown to attenuate BPA-triggered insulin resistance in HepG2 cells via suppression of JNK/p38 pathways (Geng et al., 2017). In another study, BPA was reported to disturb MAPK signalling pathway leading to impairments in glucose and lipid metabolism in liver (Vahdati Hassani et al., 2017). Our findings showed that BPA exposure increased p38 MAPK and JNK levels, but that treatment with GA reversed this effect.

In intrinsic apoptosis, the cell initiates the process from within due to various internal signals, often in response to cellular stress, DNA damage, or other detrimental conditions. This pathway is primarily regulated by members of the Bcl-2 family of proteins, which can either promote or inhibit apoptosis (Singh and Lim, 2022). The process begins with the activation of pro-apoptotic Bcl-2 family proteins, such as Bax and Bak. These proteins permeabilize the outer membrane of the mitochondria, leading to the release of several pro-apoptotic factors including cytochrome-c into the cytoplasm. Once in the cytoplasm, cytochrome *c* interacts with other proteins to initiate the formation of the apoptosome that consists of cytochrome *c*, Apaf-1 and procaspase-9. This complex activates procaspase-9, resulting to the synthesis of active caspase-9, an enzyme critical for the execution of apoptosis. Active caspase-9 then starts a caspase cascade by leading the activation of the downstream effector caspases (caspase-3 and caspase-7) which cleave and activate various cellular proteins, leading to cell dismantling and apoptosis (Lossi, 2022). In our study, the anti-apoptotic effects of different doses of GA on BPA-induced liver and kidney apoptosis were investigated by analyzing protein expression levels. While active caspase-3 increases, procaspase-3 decreases, thus inducing apoptosis from the intrinsic (internal) pathway. In our study, we found that Bax/Bcl-2 expression rate and cytochrome *c* protein levels increased significantly with BPA.

5. Conclusion

In conclusion, this investigation shed fresh light on the potential pathways behind GA's hepato and renal ameliorative effects in BPA-induced toxicity. Because of the excessive production of ROS and its connections to inflammation, oxidative damage and apoptosis, BPA consumption is linked to hepato-renal toxicities. According to the findings of this study, co-supplementing GA can reduce these toxicities by reducing oxidative damage, inflammation and apoptosis in hepato-renal tissues of rats.

CRediT authorship contribution statement

Ekrem Darendelioglu: Validation, Methodology, Investigation, Data curation. **Cuneyt Caglayan:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Sefa Küçükler:** Methodology, Formal analysis, Data curation. **İbrahim Bayav:** Writing – review & editing, Investigation, Formal analysis. **Fatih Mehmet Kandemir:** Methodology, Investigation, Formal analysis. **Adnan Ayna:** Writing – review & editing, Supervision. **Sevda Sağ:** Methodology, Investigation.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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