Received: 30 May 2023 | Revised: 2 December 2023 | Accepted: 11 January 2024

DOI: 10.1002/jbt.23655

RESEARCH ARTICLE

WILEY

18β-Glycyrrhetinic acid exerts cardioprotective effects against BPA-induced cardiotoxicity through antiapoptotic and antioxidant mechanisms

Ibrahim Bayav[1] | Ekrem Darendelioğlu[2] | Cuneyt Caglayan[3]

[1]Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

[2]Department of Molecular Biology and Genetics, Faculty of Science and Literature, Bingol University, Bingol, Turkey

[3]Department of Medical Biochemistry, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik, Turkey

Correspondence
Cuneyt Caglayan, Department of Medical Biochemistry, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik 11000, Turkey.
Email: cuneyt.caglayan@bilecik.edu.tr

Funding information
Bingöl Üniversitesi

Abstract

Bisphenol A (BPA) is a synthetic environmental pollutant widely used in industry, as well as is an endocrine disrupting chemicals and has a toxic effects on heart tissue. The aim of this study is to reveal the cardioprotective effects of 18β-glycyrretinic acid (GA) against BPA-induced cardiotoxicity in rats. In this study, 40 male rats were used and five different groups (each group includes eight rats) were formed. The rats were applied BPA (250 mg/kg b.w.) alone or with GA (50 and 100 mg/kg b.w.) for 14 days. Rats were killed on Day 15 and heart tissues were taken for analysis. GA treatment decreased serum lactate dehydrogenase and creatine kinase MB levels, reducing BPA-induced heart damage. GA treatment showed ameliorative effects against lipid peroxidation and oxidative stress caused by BPA by increasing the antioxidant enzyme activities (glutathione peroxidase, superoxide dismutase, and catalase) and GSH level of the heart tissue and decreasing the MDA level. In addition, GA showed antiapoptotic effect by increasing Bcl-2, procaspase-3, and -9 protein expression levels and decreasing Bax, cytochrome c, and P53 protein levels in heart tissue. As a result, it was found that GA has cardioprotective effects on heart tissue by exhibiting antioxidant and antiapoptotic effects against heart damage caused by BPA, an environmental pollutant. Thus, it was supported that GA could be a potential cardioprotective agent.

KEYWORDS
18β-glycyrrhetinic acid, Apoptosis, Bisphenol A, Cardiotoxicity, Oxidative stress

1 | INTRODUCTION

Bisphenol A (BPA) is widespread worldwide and is used as an additive in the production of epoxy resins and polycarbonate plastics.[1] BPA, also is used in products that are frequently used in daily life such as phenolic and phenoplast resins, inner linings of food packaging, medical devices, PVC production, carboys, and other water or food containers.[2] People are exposed to BPA when they use these products. BPA is among the most common endocrine disrupting chemicals known, and endocrine disrupting chemicals are associated with leading causes of death such as cardiovascular disease and cancer.[3] BPA has the ability to act like estrogen.[4] With this feature, BPA can trigger various cardiovascular disorders by causing changes in blood pressure and heart rate.[5–7] Several epidemiological studies have found a positive correlation between urinary BPA levels and various diseases, including cardiovascular disease.[8,9] In addition to cardiovascular diseases, BPA also triggers obesity, diabetes, hypertension, brain development anomalies, thyroid dysfunction, breast cancer, and infertility.[10] Although BPA has estrogenic activity, it is known that it generally exhibits its toxic effects by triggering the

formation of oxidative stress in cells or tissues.[11,12] Reactive oxygen species (ROS), which cause oxidative stress, can negatively affect DNA, RNA, and protein synthesis.[13,14] This condition can cause many diseases such as cardiovascular diseases, neurodegenerative diseases, cancer, hypersensitivity, inflammation, and diabetes complications.[15,16] In cells or tissues, there are endogenous enzymatic and nonenzymatic antioxidants that play a role in reducing oxidative stress.[17] These include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH), which efficiently remove excess hydrogen peroxide (H_2O_2) from the cell. Additionally, they neutralize radicals such as superoxide ($O_2\bullet^-$) and hydroxyl (•OH).[18] While these endogenous antioxidants perform this task, they may sometimes be insufficient, and in such cases, they can be supplemented with some exogenously sourced antioxidant compounds.[19] One of the exogenous antioxidants is 18β-Glycyrrhetinic acid (GA).[20]

18β-GA is one of the key bioactive compounds of licorice, one of the most commonly used herbs in traditional Chinese medicine.[21] In the literature data, 18β-GA has been shown a variety of pharmacological actions, including anticancer, antiviral, anti-inflammatory, antioxidant, and hepatoprotective effects.[21–23] In addition, it has been stated that GA has antiapoptotic, neuroprotective, and cardioprotective effects.[20,24] Recently, GA has been reported to enhance the protective effects against doxorubicin-induced cardiotoxicity by activating the Nrf2/HO-1 signaling pathway.[25] However, the underlying cardioprotective mechanism of GA remains unclear.

Therefore, this study was conducted to elucidate the antiapoptotic and antioxidant mechanisms underlying the potentiation effect of GA on BPA-induced cardiotoxicity.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Bisphenol A (CAS-No: 80–05-7), 18β-GA (CAS-No: 471–53-4) (Figure 1A,B), and all other reagents and compounds were of analytical grade and purchased from Sigma-Aldrich Chemicals.

2.2 | Animals selection and experimental design

In this study, 40 male Wistar albino rats obtained from Bingöl University Experimental Research and Application Center (Bingöl, Turkey) weighing 250–300 g (12–13 weeks) were used. During the experiment, the rats were kept in cages in room under specific conditions on a constant 12-h light/dark (06:00–18:00 light; 18:00–06:00 dark) cycle and at a controlled temperature of 25°C. Rats were fed standard chow pellets and had free access to food and water. The experimental protocols were approved by Bingöl University Animal Experiments Local Ethics Committee (Approval No. 2021-1518).

The rats were randomly selected, eight in each group, and five different groups were formed. The dose of BPA in this study was determined from a previous experimental study by Shirani, et al.[26] It is sufficient to elicit mild or moderate oxidative stress in rats. The doses of GA were selected based on previous study Rashid, et al.[27]

I. Control group; 0.2 mL of olive oil was administered orally daily for 14 days.
II. GA group; GA (100 mg/kg b.w.) in olive oil was administered by oral gavage for 14 days.
III. BPA group; BPA (250 mg/kg b.w.) in olive oil was administered by oral gavage for 14 days.
IV. BPA + GA 50 group; BPA (250 mg/kg b.w.) and GA (50 mg/kg b.w.) were administered by oral gavage for 14 days.
V. BPA + GA 100 group; BPA (250 mg/kg b.w.) and GA (100 mg/kg b.w.) were administered by oral gavage for 14 days.

All treatments were applied in a daily regimen. All rats were killed 24 h after the last treatment (Day 15). The hearts of rats were excised, washed with ice-cold physiological saline, and then used for molecular and biochemical analyses.

2.3 | Determination of cardiac function markers

The blood from rats was transferred to serum tubes and centrifuged at 4000 rpm for 10 min at +4°C to separate serum. Serum creatine

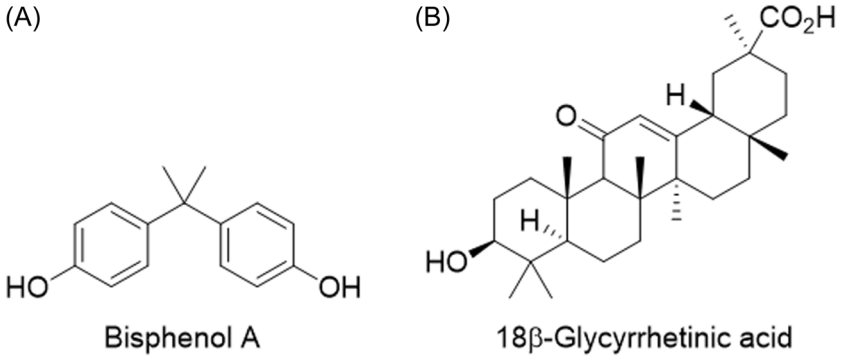

FIGURE 1 (A) Chemical structure of bisphenol A. (B) Chemical structure of 18β-glycyrrhetinic acid.

kinase MB (CK-MB) and lactate dehydrogenase (LDH) levels were measured using the Mindray Perfect Plus 400 device.

2.4 | Oxidative stress and lipid peroxidation markers in the heart tissue

The heart tissues were ground in liquid nitrogen using the TissueLyser II (Qiagen) device. It was then homogenized using suitable buffers for SOD, GPx, CAT, GSH, malondialdehyde (MDA) and protein analysis. For SOD, CAT, and MDA analysis, homogenates were centrifuged at 3500 rpm for 15 min at +4°C. For GSH and GPx analyzes, they were centrifuged at 10,000 rpm for 20 min at 4°C. In heart tissue, CAT enzymatic activity was measured according to Aebi,[28] GPx enzymatic activity according to Lawrence and Burk,[29] SOD enzymatic activity according to the method of Sun et al.,[30] MDA levels according to the methods of Placer et al.,[31] and GSH levels measured to Sedlak and Lindsay.[32] The method of Lowry was used as the method of protein determination Lowry et al.[33]

2.5 | Western blot analysis of heart tissue

The rat heart tissues were diluted at a ratio of 1:5 (w/v) using Mammalian Cell Extraction Kit (ab65399; Abcam) and then homogenized. The homogenates incubated on ice for 1 h were centrifuged at 14.000 rpm for 15 min at +4°C and the supernatant was removed to new tubes. Total protein amounts were calculated using the Bradford.[34] Protein samples were mixed with Laemmli buffer for denaturation at 95°C for 5 min. Equal amounts of protein samples were then subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were subsequently transferred to PVDF membrane. The membranes were washed twice for 5 min with Tris-buffered saline containing 1% Tween-20 (TBS-T) and blocked for 1 h before using primary antibodies in 5% skim milk powder. β-Actin (sc-47778), procaspase-3 (ProCAS-3) (sc-271759), procaspase-9 (ProCAS-9) (sc-70505), Bax (sc-20067), Bcl-2 (sc-7382), cytochrome c (CYT-C) (sc-13156), and p53 (sc-71820) was used as primary antibodies. The PVDF membrane was then left overnight at 4°C in the presence of primary antibody. The membranes were then washed five times for 5 min with TBS-T and incubated for 90 min at 37°C in the presence of anti-mouse secondary antibody. Quantification of the protein band density of ECL (Advansta) X-ray film was incubated to identify specific binding and was analyzed with densitometrically using an optical analysis system (GelDoc, Bio-Rad).

2.6 | Statistical analysis

All results were replicated at least three times. Statistical analysis was performed with GraphPad Prism 5.01 software. Statistical differences and significance levels of comparable data groups in all experiments were evaluated with the "ANOVA (one-way analysis of variance)" Newman–Keuls PostHoc Test. Data at the $p < 0.05$ level were considered significant.

3 | RESULTS

3.1 | Serum CK-MB and LDH levels

In this study, CK-MB and LDH levels in serum as markers of heart damage were investigated. When serum LDH levels were examined, it was determined that there was a significant ($p < 0.001$) increase in LDH levels in all BPA-treated groups compared to the control group. However, therapeutically administered GA attenuated this effect in a dose-dependent manner, and a significant reduction in LDH levels was observed in the GA-treated group compared with the BPA group (Figure 2A).

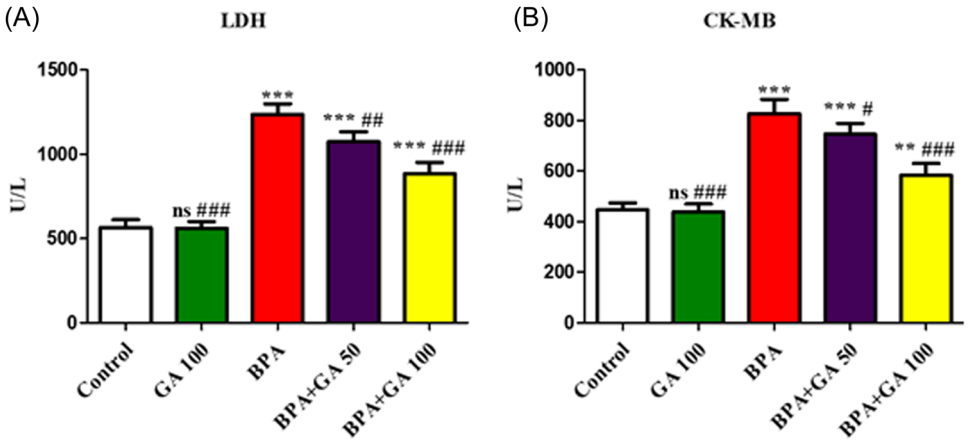

FIGURE 2 Ameliorative effects of glycyrretinic acid (GA) and bisphenol A (BPA) treatments on serum lactate dehydrogenase (LDH) and creatine kinase MB (CK-MB) levels. (A) Ameliorative effect of GA on BPA-induced LDH level. (B) Ameliorative effect of GA on BPA-induced CK-MB level. Values are expressed as mean ± SD. $^{***}p < 0.001$ control versus others, $^{**}p < 0.01$ control versus others, $^{###}p < 0.001$ BPA versus others, $^{##}p < 0.01$ BPA versus others, $^{#}p < 0.05$; BPA versus others; ns: not significant.

CK-MB levels were examined and found that all BPA-treated groups had significantly increased CK-MB levels compared to the control group ($p < 0.01$ and $p < 0.001$). However, therapeutically administered GA attenuated this effect in a dose-dependent manner, with groups receiving GA doses of 50 and 100 mg/kg having lower CK-MB levels compared with the BPA group, significantly improvement was seen. It was observed ($p < 0.05$ and $p < 0.001$) (Figure 2B).

3.2 | Antioxidant and oxidative stress condition in the heart tissue

We examined oxidative stress and several antioxidant biomarkers from cardiac tissue homogenates. Examining the results of MDA obtained as a result of the study, while the MDA level was significantly increased in the BPA-administered group compared to the control group ($^{***}p < 0.001$). Compared to the group receiving BPA alone, a significant decrease in MDA levels was observed in the BPA + GA-50 and BPA + GA-100 groups. It was found that the reduction in MDA levels ($^{###}p < 0.001$) was particularly higher in the BPA + GA 100 group than in the other treatment group, the BPA + GA 50 group ($^{##}p < 0.01$).

When the activity of CAT, SOD, and GPx, which are important antioxidants, and the content of GSH were examined, it was found that only the BPA-induced group significantly decreased compared to the control group ($^{***}p < 0.001$). In the BPA + GA 100 and BPA + GA 50 groups, CAT, SOD, GPx activities and GSH levels increased significantly compared to the BPA group. No significant changes were observed when comparing the GA 100 group with the control group (Figures 3A,B and 4A–C).

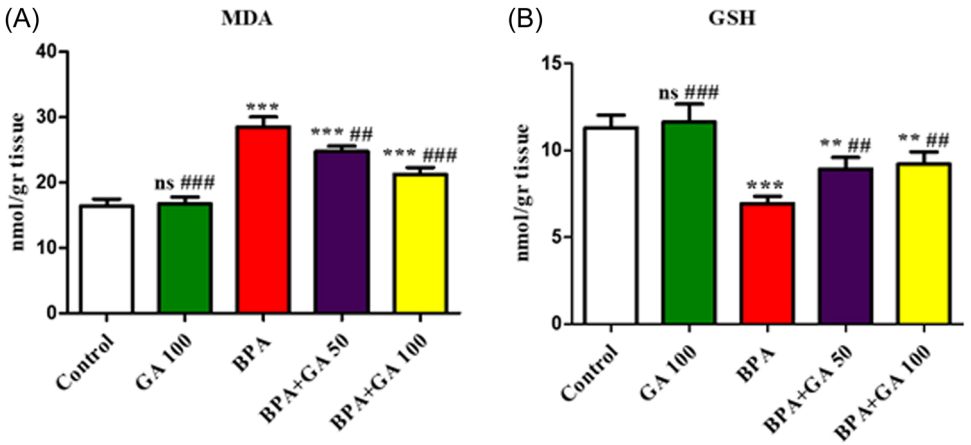

FIGURE 3 Ameliorative effects of glycyrretinic acid (GA) and bisphenol A (BPA) treatments on malondialdehyde (MDA) and glutathione (GSH) levels in heart tissue. (A) Ameliorative effect of GA on BPA-induced MDA level. (B) Ameliorative effect of GA on BPA-induced GSH level. Values are expressed as mean ± SD. $^{***}p < 0.001$ control versus others, $^{**}p < 0.01$ control versus others, $^{###}p < 0.001$ BPA versus others, $^{##}p < 0.01$ BPA versus others, $^{#}p < 0.05$ BPA versus others; ns: not significant.

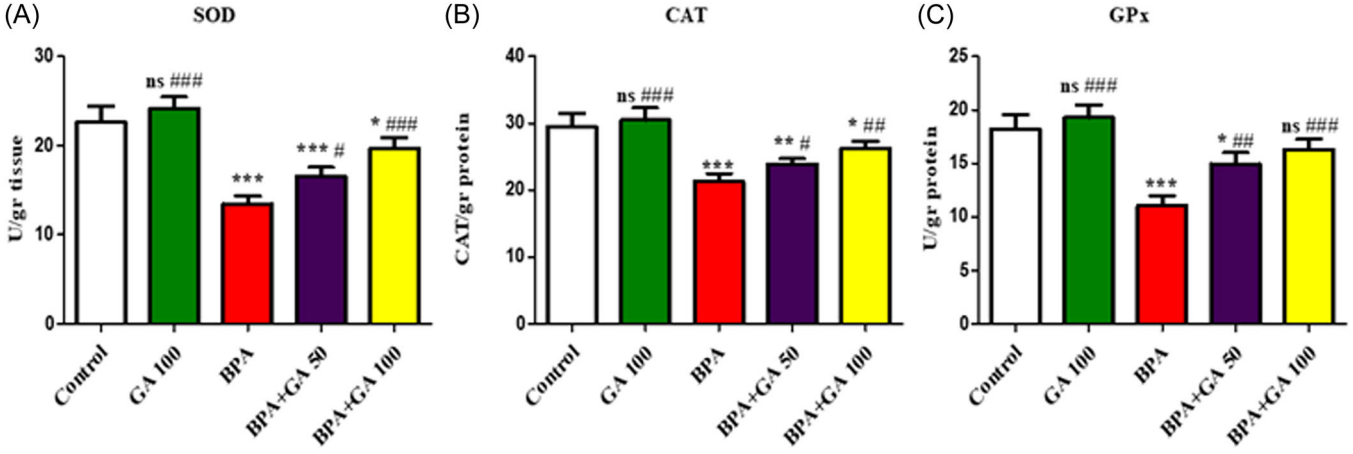

FIGURE 4 Ameliorative effects of glycyrretinic acid (GA) and bisphenol A (BPA) treatments on antioxidant enzyme activities in heart tissue. (A) Ameliorative effect of GA on BPA-induced superoxide dismutase (SOD) activity. (B) Ameliorative effect of GA on BPA-induced catalase (CAT) activity. (C) Ameliorative effect of GA on BPA-induced glutathione peroxidase (GPx) activity. Values are expressed as mean ± SD. $^{***}p < 0.001$ control versus others, $^{**}p < 0.01$ control versus others, $^{*}p < 0.05$ control versus others; $^{###}p < 0.001$ BPA versus others, $^{##}p < 0.01$ BPA vs others, $^{#}p < 0.05$ BPA vs others; ns: not significant.

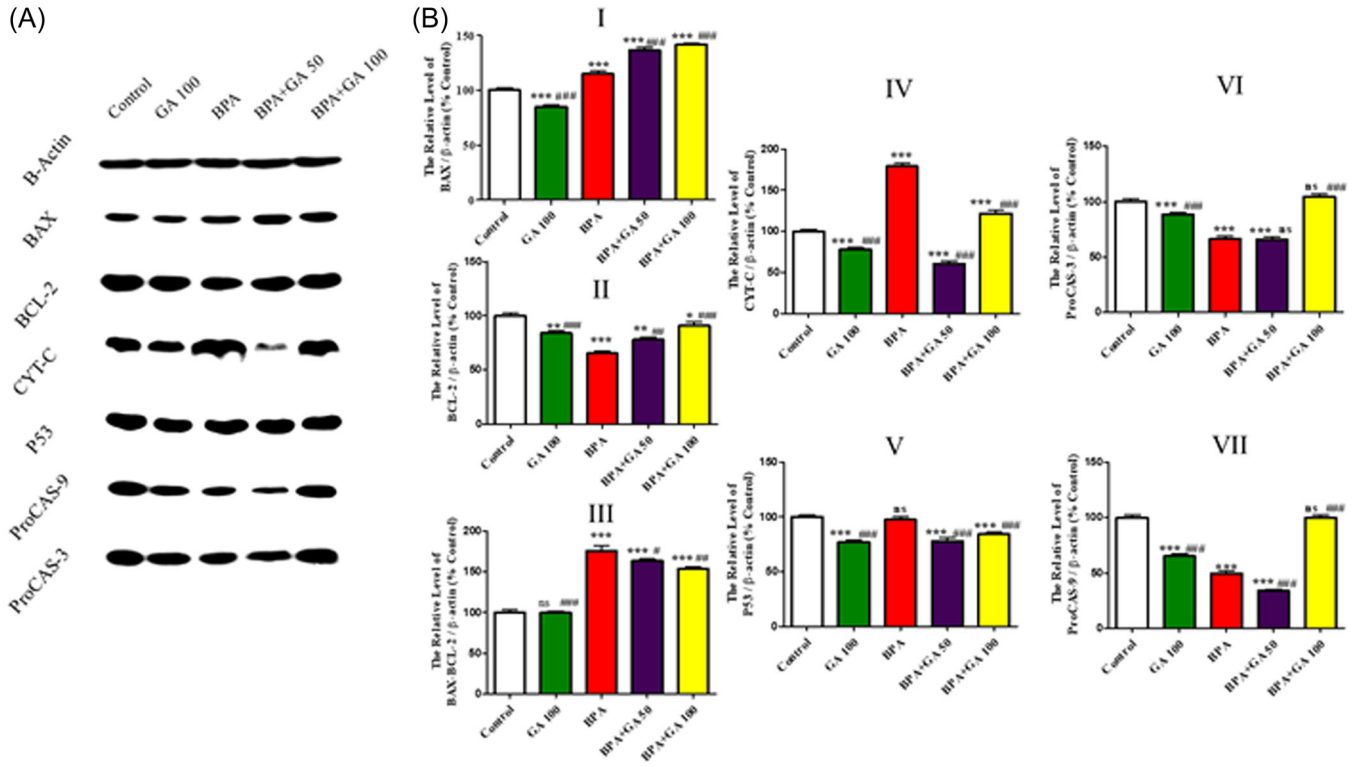

FIGURE 5 Ameliorative effects of glycyrretinic acid (GA) and bisphenol A (BPA) treatments on apoptotic protein levels in heart tissue. (A) β-Actin (43 kDa), Bax (23 kDa), Bcl-2 (26 kDa), cytochrome C (15 kDa), P53 (53 kDa), procaspase-3 (32 kDa), and procaspase-9 (46 kDa) protein levels were measured by Western blot analysis analysis. β-Actin was used as housekeeping protein. (B-I) Bax protein expression level. (B-II) Bcl-2 protein expression level. (B-III) The ratio of the Bax-Bcl-2 protein expression levels. (B-IV) cytochrome C protein expression level. B-V) P53 protein expression level. (B-VI) procaspase-3 protein expression level. (B-VII) procaspase-9 protein expression level. Values are expressed as mean ± SD. $^{***}p < 0.001$ control versus others, $^{**}p < 0.01$ control versus others, $^{*}p < 0.05$ control versus others; $^{\#\#\#}p < 0.001$ BPA versus others, $^{\#\#}p < 0.01$ BPA versus others, $^{\#}p < 0.05$ BPA versus others; ns: not significant.

3.3 | The effect of GA on mechanism of programmed cell death (apoptosis)

To study the detailed biomolecular mechanism of the antiapoptotic effect of the GA on BPA-induced in heart tissue, the protein expressions levels of Bcl-2, Bax, CYT-C, ProCAS-3, ProCAS-9 and p53 were investigated. In the findings obtained, it was found that GA significantly suppressed the Bax/Bcl-2 ratio and CYT-C expression level, while it significantly protected the levels of ProCAS-3 and ProCAS-9, and was also effective in reducing the level of p53 expression. These results idea that GA may play an anti-apoptotic role in BPA-applied rats (Figure 5A,B).

4 | DISCUSSION

BPA is an environmental pollutant and a synthetic compound used to make epoxy resins and polycarbonate plastics.[35] BPA causes multiorgan toxicity in humans by entering the body mostly through gastrointestinal tract, skin absorption, and respiratory tract.[36] BPA also causes nephrotoxicity, hepatotoxicity, neurotoxicity, and cardiotoxicity by triggering apoptosis and ROS formation in various tissues.[20,37–39] Studies are increasing day by day to reduce these negative effects of BPA.[26,40] GA has important antioxidant, anticancer, anti-inflammatory, and antiapoptotic properties.[41] As the ameliorative effects of GA on BPA-caused cardiotoxicity had not been previously studied, this study demonstrated cardioprotective effects of GA on several antioxidant and antiapoptotic signaling pathways.

In cases where various tissue damages occur in biological organisms, changes are observed in some enzyme and protein levels in the serum. These enzymes may increase or decrease specific to tissue damage. CK-MB and LDH, which are important biomarkers for cardiac tissue, are located in the cytoplasm of cells. These two important enzymes are secreted into the blood when contractile elements are broken down or during myocardial damage due to oxidative stress. Measurement of high levels of both enzymes in the blood is considered a sign of cardiac dysfunction.[42–44] Looking at similar studies in the literature, it has been reported that BPA causes a significant rise in serum CK-MB and LDH levels in various rat studies.[38,45] In this study, CK-MB and LDH levels in serum as markers of cardiac damage were investigated. When serum CK-MB

and LDH levels were analyzed, it was determined that there was a significant rise in CK-MB and LDH levels in all BPA-applied groups compared to the control group. However, important reductions in CK-MB and LDH levels were observed in the GA-treated groups compared to the BPA group. A previous study reported that GA improved CK-MB and LDH levels in mice with isoproterenol-induced myocardial infarction.[46]

BPA mediates the increase of various oxidant molecules (H_2O_2, $O_2\bullet^-$, •OH etc.) in cells or tissues, causing an increase in oxidative stress.[47,48] Biological organisms have antioxidant defense mechanisms that balance these oxidant molecules, including enzymes such as GPx, SOD, and CAT, as well as non-enzyme molecules such as GSH.[49] In a recent study, Apaydın et al. indicated that BPA exposure decreased GPx, SOD, CAT enzymes activities, and GSH levels in rat hearts, while significantly increasing MDA levels.[50] In our study, it was stated that BPA significantly decreased GPx, SOD, CAT enzyme activities, and GSH in heart tissue, while it caused a important increase in MDA levels. It was determined that these parameters were preserved at a significant level in the GA groups applied together with BPA. It is thought that GA shows these effects by supporting the antioxidant defense in the cardiac tissue and reducing the oxidative stress caused by BPA. Supporting these results, a similar study stated that GA has a preventive effect to global cerebral ischemia/reperfusion in mice.[24]

Apoptosis is one of the well-known cellular death pathways and is controlled by many metabolic pathways.[51] Many studies indicate that ROS triggers apoptosis by inducing oxidative stress and causing changes in mitochondrial membrane potential.[52,53] The internal pathway of apoptosis, also known as the mitochondrial pathway, is regulated by the proapoptotic Bax and antiapoptotic Bcl-2 proteins. The imbalance between these two proteins causes disruption of the pores in the outer mitochondrial membrane and the release of cytochrome c into the cytoplasm.[54] Cytochrome c provides activation of procaspase-9 in the presence of Apaf-1. Thus, it converts procaspase-9s into active caspase-9. Caspase-9 activates procaspase-3s and initiates the apoptosis process.[55] In addition, p53 protein is a potent inducer of apoptosis and is present at low levels in healthy cells.[56] In addition, the p53 protein can affect the apoptotic pathway by changing the levels of the Bcl-2 family of proteins.[57] Some studies suggest that BPA has significant effects on caspase-3 and -9 activation.[58,59] It has also been stated that BPA is more effective on the internal pathway of apoptosis by increasing caspase-3, -8, -9, and -10 gene expression levels.[60] In our study, BPA caused a important increase in Bax/Bcl-2 ratio and cytochrome c expression levels, while it caused a significant decrease in procaspase-9 and procaspase-3 levels. The expression of those proteins have been considerably reversed through our GA treatment. Many researchers have shown that different doses of GA on various cell groups and various tissues can suppress some proapoptotic proteins, while some antiapoptotic proteins can be supported.[61-63] While GA increase Bcl-2 activity, it has also been reported to suppress the activities of Bax and caspase-3.[63,64] It has also been reported that GA can inhibit apoptosis by reducing caspase-9 and cytochrome c expression levels.[65,66]

5 | CONCLUSION

In summary, this research conducted in rats aimed to evaluate the effects of GA application against BPA-induced cardiotoxicity. The obtained results demonstrate that GA effectively mitigates apoptotic and oxidative stress mechanisms induced by exposure to BPA, which adversely affects the cardiovascular system. These findings underscore the potential of GA as a protective agent for maintaining cardiac health. The revealed protective effects in our study provide significant insight into reducing BPA-induced cardiotoxicity and preserving cardiac tissue from oxidative stress. In this context, the cardioprotective properties of GA may serve as a crucial step in future research, offering valuable implications for developing potential therapeutic strategies in situations involving exposure to similar environmental toxins. However, further research is needed to elucidate the mechanism at the molecular level and propose clinical applications.

AUTHOR CONTRIBUTIONS

Ekrem Darendelioğlu and Cuneyt Caglayan designed the research. Ibrahim Bayav and Cuneyt Caglayan conducted experiments. Ekrem Darendelioğlu, Ibrahim Bayav, and Cuneyt Caglayan analyzed data. Ibrahim Bayav and Cuneyt Caglayan wrote the manuscript. All authors have read the final version and approved it for publication.

ACKNOWLEDGMENTS

We thank Prof. Dr. Fatih Mehmet Kandemir and Assoc. Prof Sefa Küçükler for their contributions to this study. We are also grateful to Bingöl University Scientific Research Projects Coordination Unit for their financial support. This study was supported by Scientific Research Projects Coordination Unit of Bingol University.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data are available with the corresponding author. The data will be provided on request.

ORCID

Cuneyt Caglayan http://orcid.org/0000-0001-5608-554X

REFERENCES

[1] J. Gu, H. Wang, L. Zhou, D. Fan, L. Shi, G. Ji, A. Gu, *Sci. Total Environ.* **2020**, *731*, 139190.
[2] Y. Q. Huang, C. K. C. Wong, J. S. Zheng, H. Bouwman, R. Barra, B. Wahlström, L. Neretin, M. H. Wong, *Environ. Int.* **2012**, *42*, 91.
[3] V. Quagliariello, C. Coppola, D. G. Mita, G. Piscopo, R. V. Iaffaioli, G. Botti, N. Maurea, *Environ. Toxicol. Pharmacol.* **2019**, *69*, 1.
[4] B. L. Cooper, N. G. Posnack, *Cardiovasc. Toxicol.* **2022**, *22*(3), 273.

[5] E. G. Krause, K. S. Curtis, J. P. Markle, R. J. Contreras, *J. Physiol.* **2007**, *582*(1), 435.
[6] B. B. Patel, M. Raad, I. A. Sebag, L. E. Chalifour, *Toxicol. Sci* **2013**, *133*(1), 174.
[7] M. G. Valokola, G. Karimi, B. M. Razavi, M. Kianfar, A. H. Jafarian, M. R. Jaafari, M. Imenshahidi, *Environ. Toxicol.* **2019**, *34*(3), 319.
[8] I. A. Lang, *JAMA* **2008**, *300*(11), 1303.
[9] D. Melzer, N. E. Rice, C. Lewis, W. E. Henley, T. S. Galloway, *PLoS One* **2010**, *5*(1), e8673.
[10] J. R. Rochester, *Reprod. Toxicol.* **2013**, *42*, 132.
[11] S. Rahman Md, W.-S. Kwon, C. Karmakar Polash, S.-J. Yoon, B.-Y. Ryu, M.-G. Pang, *Environ. Health Perspect.* **2017**, *125*(2), 238.
[12] S. Amjad, M. S. Rahman, M.-G. Pang, *Biomolecules* **2020**, *10*, 1105.
[13] M. S. Rahman, W.-K. Pang, D.-Y. Ryu, Y.-J. Park, M.-G. Pang, *Hum. Reprod.* **2020**, *35*(8), 1740.
[14] M. O. Yıldız, H. Çelik, C. Caglayan, F. M. Kandemir, C. Gür, İ. Bayav, A. Genç, Ö. Kandemir, *Neurotoxicology* **2022**, *90*, 197.
[15] A. Ghosh, N. Shcherbik, *Int. J. Mol. Sci.* **2020**, *21*, 2661.
[16] C. Türkeş, Y. Demir, Ş. Beydemir, *J. Biomol. Struct. Dyn.* **2022**, *40*(1), 77.
[17] İ. Gulcin, *Arch. Toxicol.* **2020**, *94*(3), 651.
[18] F. J. Martínez-Noguera, P. E. Alcaraz, R. Ortolano-Ríos, S. P. Dufour, C. Marín-Pagán, *Antioxidants* **2021**, *10*, 282.
[19] F. Benzer, F. M. Kandemir, M. Ozkaraca, S. Kucukler, C. Caglayan, *J. Biochem. Mol. Toxicol.* **2018**, *32*(2), e22030.
[20] C. Caglayan, F. M. Kandemir, A. Ayna, C. Gür, S. Küçükler, E. Darendelioğlu, *Metab. Brain Dis.* **2022**, *37*(6), 1931.
[21] B. Tu, J. Liang, Y. Ou, X. Zhang, W. Zheng, R. Wu, L. Gan, D. Li, Y. Lu, J. Wu, W. David Hong, K. Zhang, P. Wu, J. Jin, W. L. Wong, *Bioorg. Chem.* **2022**, *122*, 105714.
[22] Y. Xiao, J. Xu, C. Mao, M. Jin, Q. Wu, J. Zou, Q. Gu, Y. Zhang, Y. Zhang, *J. Biol. Chem.* **2010**, *285*(2), 1128.
[23] G. Sharma, S. Kar, S. Palit, P. K. Das, *J. Cell. Physiol.* **2012**, *227*(5), 1923.
[24] N. B. Türkmen, H. Yüce, A. Taşlıdere, Y. Şahin, İ. Ayhan, S. UnÜVar, O. Çiftçi, *J. Pharm. Sci.* **2022**, *58*, e21219.
[25] Y. Cheng, X. Wu, X. Nie, Y. Wu, C. Zhang, S. M.-Y. Lee, K. Lv, G. P.-H. Leung, C. Fu, J. Zhang, J. Li, *Phytomedicine* **2022**, *106*, 154407.
[26] M. Shirani, S. Alizadeh, M. Mahdavinia, M. A. Dehghani, *Environ. Sci. Pollut. Res.* **2019**, *26*(8), 7688.
[27] S. Rashid, S. Nafees, A. Siddiqi, A. Vafa, S. M. Afzal, R. Parveen, N. Ali, S. K. Hasan, P. Barnwal, A. Shahid, S. Sultana, *Pharmacol. Rep.* **2017**, *69*(5), 1007.
[28] H. Aebi, *Methods Enzymol.* **1984**, *105*, 121.
[29] R. A. Lawrence, R. F. Burk, *Biochem. Biophys. Res. Commun.* **1976**, *71*(4), 952.
[30] Y. Sun, L. W. Oberley, Y. Li, *Clin. Chem.* **1988**, *34*(3), 497.
[31] Z. A. Placer, L. L. Cushman, B. C. Johnson, *Anal. Biochem.* **1966**, *16*(2), 359.
[32] J. Sedlak, R. H. Lindsay, *Anal. Biochem.* **1968**, *25*(1), 192.
[33] O. Lowry, N. Rosebrough, A. L. Farr, R. Randall, *J. Biol. Chem.* **1951**, *193*(1), 265.
[34] M. M. Bradford, *Anal. Biochem.* **1976**, *72*(1), 248.
[35] A. Tarafdar, R. Sirohi, P. A. Balakumaran, R. Reshmy, A. Madhavan, R. Sindhu, P. Binod, Y. Kumar, D. Kumar, S. J. Sim, *J. Hazard. Mater.* **2022**, *423*, 127097.
[36] Y. Ma, H. Liu, J. Wu, L. Yuan, Y. Wang, X. Du, R. Wang, P. W. Marwa, P. Petlulu, X. Chen, H. Zhang, *Environ. Res.* **2019**, *176*, 108575.
[37] M. A. Al-Griw, Z. O. Alshibani, R. Alghazeer, M. Elhensheri, R. M. Tabagh, A. A. Eskandrani, W. S. Alansari, M. M. Habibulla, G. Shamlan, *Sci. Rep.* **2022**, *12*(1), 10258.
[38] M. J. Khodayar, H. Kalantari, M. Mahdavinia, L. Khorsandi, S. Alboghobeish, A. Samimi, S. Alizadeh, L. Zeidooni, *Drug Chem. Toxicol.* **2020**, *43*(1), 85.
[39] A. Aslanturk, M. Uzunhisarcikli, *Environ. Sci. Pollut. Res.* **2020**, *27*(19), 23994.
[40] G. Thayumanavan, S. Jeyabalan, S. Fuloria, M. Sekar, M. Ravi, L. K. Selvaraj, L. Bala, K. Chidambaram, S. H. Gan, N. N. Rani, *Molecules* **2022**, *27*(8), 2572.
[41] A. Kowalska, U. Kalinowska-Lis, *Int. J. Cosmet. Sci.* **2019**, *41*(4), 325.
[42] B. Varışlı, E. Darendelioğlu, C. Caglayan, F. M. Kandemir, A. Ayna, A. Genç, Ö. Kandemir, *Cardiovasc. Toxicol.* **2022**, *22*(8), 727.
[43] S Kasap, A. Gönenç, D.E. Şener, İ. Hisar, *J. Clin. Biochem. Nutr.* **2007**, *41*(1), 50.
[44] S. M. Hazzaa, E. S. El-Roghy, M. A. Abd Eldaim, G. E. Elgarawany, *Environ. Sci. Pollut. Res.* **2020**, *27*(16), 20014.
[45] A. R. Vanani, M. Mahdavinia, M. Shirani, S. Alizadeh, M. A. Dehghani, *Environ. Sci. Pollut. Res.* **2020**, *27*(13), 15093.
[46] S. Chu, W. Wang, N. Zhang, T. Liu, J. Li, X. Chu, S. Zuo, Z. Ma, D. Ma, L. Chu, *Food Sci. Nutr.* **2021**, *9*(12), 6831.
[47] A. Anet, S. Olakkaran, A. Kizhakke Purayil, G. Hunasanahally Puttaswamygowda, *J. Hazard. Mater.* **2019**, *370*, 42.
[48] W. Amraoui, N. Adjabi, F. Bououza, M. Boumendjel, F. Taibi, A. Boumendjel, C. Abdennour, M. Messarah, *Toxicol. Res.* **2018**, *34*(3), 231.
[49] F. M. Kandemir, C. Caglayan, E. Darendelioğlu, S. Küçükler, E. İzol, Ö. Kandemir, *Life Sci.* **2021**, *277*, 119610.
[50] F. G. Apaydin, A. Aslanturk, M. Uzunhisarcikli, H. Bas, S. Kalender, Y. Kalender, *Environ. Sci. Pollut. Res.* **2019**, *26*(12), 12302.
[51] M. A. Savitskaya, G. E. Onishchenko, *Biochemistry (Moscow)* **2015**, *80*(11), 1393.
[52] Q. Zhang, W. Chen, X. Lv, Q. Weng, M. Chen, R. Cui, G. Liang, *Front. Pharmacol.* **2019**, *10*, 1180.
[53] H. U. Simon, A. Haj-Yehia, F. Levi-Schaffer, *Apoptosis* **2000**, *5*(5), 415.
[54] E. Darendelioglu, *Neurochem. Res.* **2020**, *45*(5), 1064.
[55] M. S. D'Arcy, *Cell Biol. Int.* **2019**, *43*(6), 582.
[56] B. J. Aubrey, G. L. Kelly, A. Janic, M. J. Herold, A. Strasser, *Cell Death Differ.* **2018**, *25*(1), 104.
[57] D. Yang, X. Tan, Z. Lv, B. Liu, R. Baiyun, J. Lu, Z. Zhang, *Sci. Rep.* **2016**, *6*(1), 37157.
[58] Q. Liu, W. Wang, Y. Zhang, Y. Cui, S. Xu, S. Li, *Fish. Shellfish. Immunol.* **2020**, *102*, 489.
[59] Y.-W. Chiang, C.-H. Su, H.-Y. Sun, S.-P. Chen, C.-J. Chen, W.-Y. Chen, C.-C. Chang, C.-M. Chen, Y.-H. Kuan, *Environ. Toxicol.* **2022**, *37*(1), 131.
[60] K. Wang, Z. Zhao, W. Ji, *Biomed. Pharmacother.* **2019**, *117*, 109182.
[61] G. Yang, L. Wang, X. Yu, Y. Huang, C. Qu, Z. Zhang, D. Luo, J. Lin, L. Zhou, Z. Su, X. Zhang, H. Chen, *Evid. Based Complement. Alter. Med.* **2017**, *2017*, 1.
[62] L. Yu, Y. He, *Chin. Tradit. Pat. Med.* **2017**, *12*, 2018–2023.
[63] D. Zhang, J. Sun, S. Chang, X. Li, H. Shi, B. Jing, Y. Zheng, Y. Lin, G. Qian, Y. Pan, G. Zhao, *Exp. Ther. Med.* **2021**, *22*(5), 1241.
[64] M. Zhang, Z. Chang, F. Zhao, P. Zhang, Y.-J. Hao, L. Yan, N. Liu, J.-L. Wang, L. Bo, P. Ma, W. Zhou, X. Ma, Q. B. Xu, R. Zhou, *Front. Pharmacol.* **2019**, *10*, 10.
[65] J. C. Yang, S. C. Myung, W. Kim, C. S. Lee, *Mol. Cell. Biochem.* **2012**, *370*(1), 209.
[66] M. Yan, L. Guo, Y. Yang, B. Zhang, Z. Hou, Y. Gao, H. Gu, H. Gong, *Front. Pharmacol.* **2021**, *12*, 12.

How to cite this article: I. Bayav, E. Darendelioğlu, C. Caglayan, *J. Biochem. Mol. Toxicol.* **2024**, *38*, e23655. https://doi.org/10.1002/jbt.23655