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Investigation of the Effects of Syringic Acid on Oxidative Stress, Cytokines, Apoptosis and Inflammation Against Cadmium-Induced Hepatotoxicity in Rats

Abstract

Cadmium (Cd) is a toxic heavy metal and a significant environmental contaminant known to cause hepatotoxicity through mechanisms involving oxidative stress, inflammation, and apoptosis. Syringic acid (Sa), a naturally occurring phenolic compound, exhibits potent antioxidant and anti-inflammatory activities. This study aimed to evaluate the protective effects of SA against Cd-induced liver injury in rats. Fifty adult male Sprague-Dawley rats were randomly allocated into five groups (n=10 per group): Control, Sa, Cd, Sa 50 + Cd, and Sa 100 + Cd. Cadmium and/or SA were administered orally for 7 consecutive days. Biochemical markers of oxidative stress (SOD, GSH, CAT, and MDA), pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), and molecular indicators of inflammation (TLR-4, NF-κB) and apoptosis (Bax, Bcl-2, Caspase-3) were measured in liver tissue homogenates. Cadmium exposure significantly decreased the hepatic antioxidant enzyme activities (SOD, GSH, and CAT) and increased MDA and TLR-4 levels, indicating enhanced oxidative damage and inflammatory activation. Additionally, NF-κB, TNF-α, IL-1β, and IL-6 levels were markedly elevated following Cd administration. Apoptotic changes were evident by increased Bax and Caspase-3 expression and decreased Bcl-2 levels. Syringic acid co-administration dose-dependently ameliorated these pathological alterations, restoring oxidative balance, suppressing inflammation, and modulating apoptotic signaling. Syringic acid effectively attenuates cadmium-induced liver toxicity in rats by mitigating oxidative stress, suppressing pro-inflammatory responses, and regulating apoptosis-related pathways. These findings suggest that SA may serve as a promising hepatoprotective agent in conditions of heavy metal-induced hepatic injury.

Keywords: Antioxidant, cadmium, cytokine, inflammation, oxidative stress.

Ratlarda Kadmiyum Kaynaklı Hepatotoksisiteye Karşı Syrinjik Asidin Oksidatif Stres, Sitokinler, Apoptozis ve İnflamasyon Üzerindeki Etkilerinin Araştırılması

Öz

Kadmiyum (Cd), toksik bir ağır metal olup, oksidatif stres, inflamasyon ve apoptoz gibi mekanizmalar yoluyla hepatotoksisiteye neden olan önemli bir çevresel kirleticidir. Doğal olarak bulunan bir fenolik bileşik olan syrinjik asit (Sa), güçlü antioksidan ve anti-inflamatuar etkilere sahiptir. Bu çalışmanın amacı, syrinjik asidin kadmiyum kaynaklı karaciğer hasarına karşı olası koruyucu etkilerini değerlendirmektir. Elli adet erişkin erkek Sprague-Dawley rat rastgele olarak beş gruba ayrıldı (her grup için n=10): Kontrol, Sa, Cd, Sa 50 + Cd ve Sa 100 + Cd. Kadmiyum ve syrinjik asit, ardışık yedi gün boyunca oral

yolla uygulandı. Karaciğer doku homojenatlarında oksidatif stres belirteçleri (SOD, GSH, CAT ve MDA), pro-inflamatuar sitokinler (TNF-α, IL-1β, IL-6) ve inflamasyon (TLR-4, NF-κB) ile apoptoza (Bax, Bcl-2, Caspase-3) ilişkin moleküler göstergeler değerlendirildi. Kadmiyum uygulaması, karaciğerdeki antioksidan enzim aktivitelerinde (SOD, GSH ve CAT) anlamlı bir azalmaya, MDA ve TLR-4 düzeylerinde ise belirgin bir artışa neden olarak oksidatif hasar ve inflamatuar yanıtların arttığını gösterdi. Ayrıca, NF-κB, TNF-α, IL-1β ve IL-6 düzeyleri Cd maruziyeti sonrasında önemli ölçüde yükseldi. Apoptotik değişiklikler ise Bax ve Caspase-3 düzeylerinde artış ve Bcl-2 düzeyinde azalma ile belirlendi. Syrinjik asit uygulaması, bu patolojik değişiklikleri doz-bağımlı olarak iyileştirdi; oksidatif dengeyi yeniden sağladı, inflamasyonu baskıladı ve apoptoz ile ilişkili sinyalleri düzenledi. Syrinjik asit, kadmiyum kaynaklı karaciğer toksisitesini azaltmada etkili olup; bu etkisini oksidatif stresi hafifleterek, pro-inflamatuar yanıtları baskılayarak ve apoptozla ilişkili yolları modüle ederek göstermektedir. Bu bulgular, syrinjik asidin ağır metal kaynaklı hepatik hasarlarda potansiyel bir hepatoprotektif ajan olarak değerlendirilebileceğini göstermektedir.

Anahtar kelimeler: Antioksidan, inflamasyon, kadmiyum, oksidatif stress, sitokin.

❋ ❋ ❋

Introduction

Hepatotoxicity, the adverse effect on the liver caused by chemicals, drugs, or biological agents, is a significant concern in both clinical and environmental toxicology. Among various hepatotoxic agents, cadmium (Cd) stands out due to its widespread environmental presence and its profound impact on human health. Cadmium is a heavy metal that is primarily released into the environment through industrial activities, mining, and the burning of fossil fuels (Renu et al. 2021; Yang et al. 2021a). Its accumulation in the liver can lead to a range of toxic effects, including oxidative stress, inflammation, and ultimately, liver dysfunction. As a consequence, understanding the mechanisms of cadmium-induced hepatotoxicity and exploring potential protective agents is of paramount importance (Niture et al. 2021).

The liver is a vital organ responsible for numerous physiological functions, including metabolism, detoxification, and the synthesis of essential proteins. Given its central role, liver damage can have far-reaching implications for overall health. Cadmium is known to cause hepatotoxicity through various mechanisms, including the generation of reactive oxygen species (ROS), disruption of cellular homeostasis, and induction of apoptosis. These mechanisms not only compromise the structural integrity of hepatocytes but also impair the liver's ability to perform its critical functions (Shi et al. 2024). Consequently, the search for effective therapeutic agents that can mitigate cadmium-induced liver damage has become a focal point of research.

Although the precise mechanisms underlying cadmium toxicity have not been fully elucidated, accumulating evidence suggests that oxidative stress plays a pivotal role in its pathogenesis (Yesildag et al. 2022). Cd has a strong affinity for binding to glutathione and the sulfhydryl groups of proteins, leading to the generation of ROS and the subsequent inactivation of critical cellular proteins. The elevated levels of ROS are also associated with oxidative DNA damage and the activation of signaling pathways that trigger cellular apoptosis. Given these mechanisms, antioxidant agents are increasingly considered promising candidates for mitigating Cd-induced toxicity (Wang et al. 2013; Yesildag et al. 2022). In this context, there is growing interest in the therapeutic potential of naturally occurring phytochemicals due to their strong antioxidant and cytoprotective properties (Okkay et al. 2022).

Syringic acid (Sa), a phenolic compound found in various plants, has garnered attention for its potential hepatoprotective properties (Akarsu et al. 2023a; Gheena et al. 2022). Sa is a natural antioxidant that exhibits a range of biological activities, including anti-inflammatory, anti-cancer, and hepatoprotective effects. Its ability to scavenge free radicals and modulate oxidative stress pathways positions it as a promising candidate for counteracting the detrimental effects of cadmium on liver health. Recent studies have suggested that Sa may enhance the antioxidant defense system, thereby reducing oxidative damage and improving liver function in the context of hepatotoxicity (Gheena et al. 2022).

The objective of this study is to investigate the effects of syringic acid on cadmium-induced hepatotoxicity in rat models. By examining biochemical markers of liver function, histopathological changes, and the underlying molecular mechanisms, this research aims to provide a comprehensive understanding of the protective role of syringic acid against cadmium toxicity. Additionally, this study will contribute to the growing body of literature on the potential health benefits of natural compounds in mitigating the effects of environmental toxins.

Materials and Methods

Chemicals and Reagents

Syringic acid (≥98% purity) was obtained from BLD Pharm (Shanghai, China), while cadmium chloride ($CdCl_2$; 99.99% purity) was sourced from Sigma-Aldrich (St. Louis, MO, USA).

Ethical Approval

Fifty Sprague Dawley rats (10–12 weeks old, 250–280 g) were obtained from the Experimental Animal Center of XXX University (Turkey). Following one week of acclimatization, rats were housed under standard conditions (24 ± 1°C, 45 ± 5% humidity, 12-hour light/dark cycle) and given *ad libitum* access to food and water. Animals were randomly divided into five groups. All procedures were approved by the Animal Ethics Committee of XXX University (Approval No: 14.03.2025/55) and complied with international laboratory animal care guidelines.

Experimental Groups

The study included five groups, each composed of ten male rats. Dosage selections for Syringic acid and cadmium were based on previous studies (Akarsu et al., 2023; Sengül et al., 2024). The experimental protocol was as follows:

Control Group: Received intraperitoneal (i.p.) physiological saline for 7 consecutive days, followed 30 minutes later by oral administration of normal saline.

Sa Group: Treated with 100 mg/kg syringic acid via oral gavage daily for seven days.

Cd Group: Administered cadmium chloride (6.5 mg/kg, i.p.) once daily for seven days.

Sa 50 + Cd Group: Received cadmium chloride (6.5 mg/kg, i.p.) followed 30 minutes later by 50 mg/kg syringic acid orally, once daily for seven days.

Sa 100 + Cd Group: Same treatment as above, but with 100 mg/kg syringic acid.

On the eighth day, 24 hours after the final treatment, animals were anesthetized with sevoflurane and sacrificed by decapitation. Liver tissues were promptly harvested and processed for biochemical and Western blot analyses. Liver samples were snap-frozen in liquid nitrogen and subsequently ground into fine powder using a mechanical tissue disruptor.

Analysis of Oxidant and Antioxidant Markers in Liver Tissue

Due to the high sensitivity of the commercial assay kits employed, the analysis of oxidative and antioxidative parameters in liver tissue was carried out using the ELISA technique. For this purpose, powdered liver samples were diluted in phosphate-buffered saline at a 1:20 (w/v) ratio and homogenized using a tissue homogenize (Qiagen, TissueLyser II). The resulting homogenates were centrifuged at 3500 rpm for 15 minutes at 4 °C. The supernatants were collected and analyzed for malondialdehyde (MDA; Cat No: 201-11-0157), reduced glutathione (GSH; Cat No: 201-11-5134), superoxide dismutase (SOD; Cat No: 201-11-0169), and catalase (CAT; Cat No: 201-11-5106) levels, following the protocols provided by the respective ELISA kit manufacturers (Sunred Biological Technology, Shanghai, China).

Analysis of Cytokine Markers in Liver Tissue by ELISA Method

Levels of tumor necrosis factor α (TNF-α) (Cat No: 201-11-0765), Interleukin 1 beta (IL-1β) (Cat No: 201-11-0120) and Interleukin 10 (IL-10) (Cat No: 201-11-0109) in liver tissue were determined using commercial ELISA kits from Sunred Biological Technology (Shanghai, China). The analyses were carried out in strict accordance with the manufacturer's instructions.

Analysis of Inflammatory Markers in Liver Tissue by ELISA Method

Levels of nuclear factor kappa B (NF-κB) (Cat No: 201-11-0288) and Toll Like Receptor 4 (TLR-4) (Cat No: 201-11-0081) in liver tissue were determined using commercial ELISA kits from Sunred Biological Technology (Shanghai, China). The analyses were carried out in strict accordance with the manufacturer's instructions.

Western Blot Analysis in Liver Tissue

Liver tissues were homogenized in RIPA buffer (Santa cruz- sc-24948) containing PMSF and a protease inhibitor cocktail. After centrifugation, the supernatants were collected, and protein concentrations were determined using the BCA assay (Rockford, IL, USA), with BSA as the standard. Equal amounts of protein (40 µg) were mixed with Laemmli buffer, separated on 10% SDS-PAGE gels, and transferred onto PVDF membranes. Membranes were blocked with 5% BSA for 90 min at room temperature and incubated overnight at 4 °C with primary antibodies against caspase-3, Bax, Bcl-2, and β-tubulin. After washing with PBST, membranes were incubated with HRP-conjugated secondary antibody (1:2000, sc-2005) for 90 min. Bands were detected using ECL substrate (Bio-Rad, USA) and visualized via the GelDoc XR imaging system. Protein band intensities were quantified using Image Lab v6.1 and normalized to β-tubulin. Results were expressed as fold changes relative to the control group (Bass et al. 2017).

Statistical Analysis

Statistical analyses were performed using the SPSS version 20.0 software package. Data for all groups were presented as mean ± standard error. Normality of the data distribution was assessed with the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. Following confirmation of these assumptions, one-way ANOVA was employed to determine intergroup differences, and Tukey's post hoc test was applied for multiple comparisons.

Results

Effects of Cadmium and Syringic Acid on Oxidant and Antioxidant Markers in Rat Liver Tissues

Lipid peroxidation and MDA content increased following Cd administration (Figure 1). Malondialdehyde levels in the Sa 50+Cd ($P=0.007$) and Sa 100+Cd ($P<0.001$) groups decreased significantly compared to the Cd group. The difference between Sa 50+ Cd and Sa 100+ Cd groups was found to be significant ($P=0.0162$).

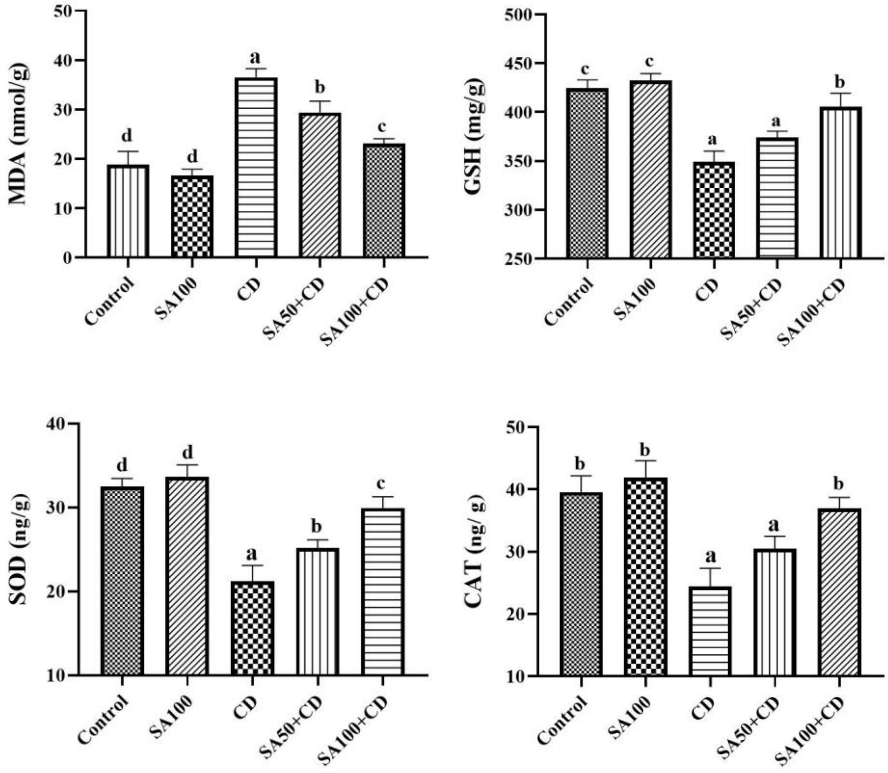

Figure 1: The effects of SA and CD on the MDA and GSH levels, SOD and CAT activitiesin the experimental groups (significant differences between the control group and the other groups are shown with letters). [a,b,c,d] Means superscripted with different row are significantly different

*** P<0.001, **P<0.01, *P<0.05, n=10.

As shown in Figure 1, the GSH levels of the Cd group were significantly decreased compared to the C (P<0.001) and SA 100 (P<0.001) groups. While no significant difference was found between the Cd group and the Sa 50+Cd group (P=0.065), a significant difference was found between the Sa 100+Cd group (P=0.003). When the Sa 50+Cd group was compared with the Sa 100+Cd group, the difference was found to be significant (P=0.019).

CAT and SOD activities decreased following Cd administration. A significant difference was found in CAT activity between the control and SA 100 groups compared to Cd (P<0.001). While no significant difference was found between Sa 50 treatment and Cd (P=0.075), a significant difference was found between Sa 100 treatment and Cd (P=0.001). The difference between Sa 50+Cd and Sa 100+Cd groups was found to be significant (P=0.049). A significant difference was found in SOD activity between the control and Sa 100 groups compared to Cd (P<0.001). It was observed that SOD activity increased with Sa 50 treatment compared to the Cd group (P=0.030), but this difference was greater with Sa 100 treatment (P<0.001). The difference between Sa 50+Cd and Sa 100+Cd groups was found to be significant (P=0.015). Figure 1 summarizes the ELISA results.

Effects of Cadmium and Syringic Acid on Cytokine Markers in Rat Liver Tissue

Cytokine levels in rat liver tissue, specifically TNF-α and IL-1β, were quantified using ELISA. The data indicated that cadmium exposure led to a marked elevation in both TNF-α and IL-1β levels compared to the control group (P = 0.002 for both markers), as shown in Figure 2. Co-treatment with 50 mg/kg syringic acid (Sa 50+Cd) did not significantly alter these cytokine levels relative to the cadmium-only group. However, the group receiving 100 mg/kg syringic acid (Sa 100+Cd) exhibited a statistically significant reduction in both TNF-α and IL-1β concentrations when compared to the Cd group, suggesting a dose-dependent anti-inflammatory effect of syringic acid. Furthermore, a notable

decrease in IL-10 levels was observed in the cadmium group versus both the control and Sa 100+Cd groups ($P < 0.001$). Treatment with syringic acid appeared to partially restore IL-10 levels in a dose-responsive manner.

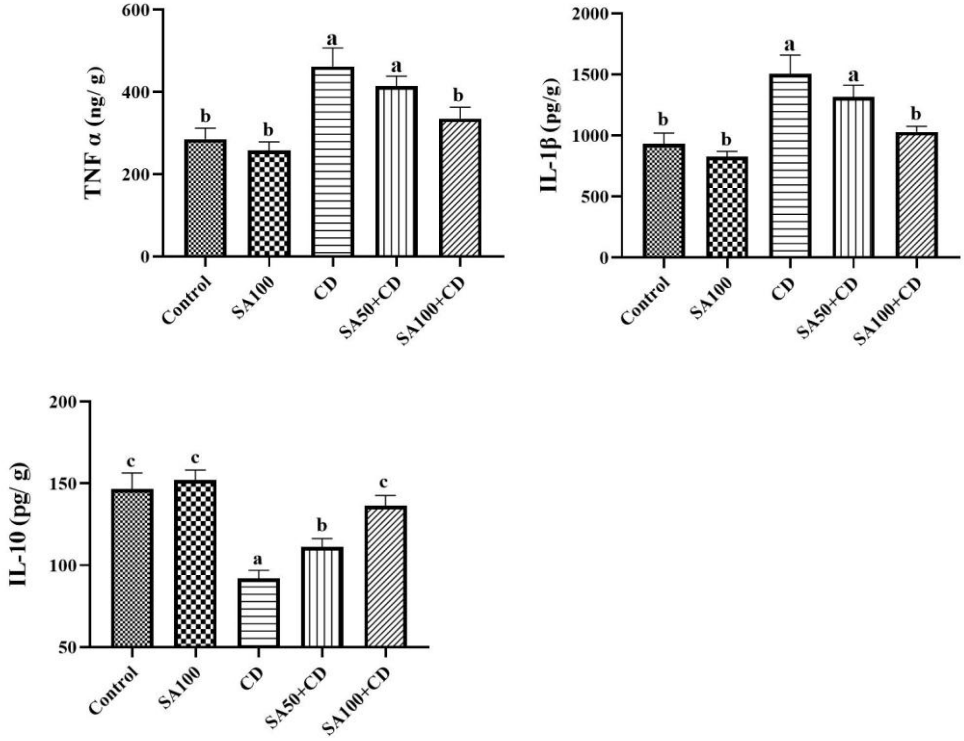

Figure 2: The effects of SA and CD on the TNF-α, IL-1β and IL_10 levels the experimental groups (significant differences between the control group and the other groups are shown with letters). [a,b,c] Means superscripted with different row are significantly different *** P<0.001, **P<0.01, *P<0.05, n=10.

Effects of Cadmium and Syringic Acid on Inflammatory Markers in Rat Liver Tissue

The impact of Cd and Sa on inflammatory mediators, specifically NF-κB and TLR-4, in rat liver tissue was assessed via ELISA. As presented in Figure 3, exposure to Cd resulted in a notable increase in both NF-κB and TLR-4 protein levels compared to controls. However, administration of 100 mg/kg Sa in combination with Cd significantly attenuated these elevations. A marked reduction in NF-κB concentration was observed in the Sa 100+Cd group relative to the Cd group alone ($P = 0.002$). Similarly, TLR-4 levels were significantly lower in the Sa 100+Cd group compared to the Cd group ($P = 0.001$). These findings highlight a dose-dependent anti-inflammatory effect of syringic acid on Cd-induced hepatic inflammation.

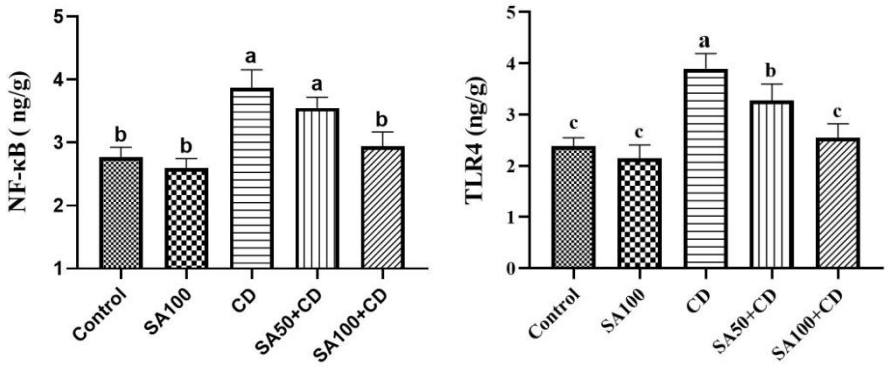

Figure 3: The effects of SA and CD on the NF-KB and TLR-4 levels the experimental groups (significant differences between the control group and the other groups are shown with letters). [a,b,c] Means superscripted with different row are significantly different *** P<0.001, **P<0.01, *P<0.05, n=10.

Effects of Cadmium and Syringic Acid on Apoptosis Markers in Rat Liver Tissue

Western Blot analysis results of Bax, Bcl-2 and Caspase-3 levels, which are apoptosis biomarkers belonging to the experimental groups, were presented in Figure 4. Study findings showed that Cd significantly increases Bax and Caspase-3 levels proteins levels (P=0.001). It was observed that Bax and Caspase-3 levels proteins levels decreased in a dose-dependent manner in Cd+Sa groups (P<0.001). Bcl-2 levels decreased significantly after Cd application. In the Sa 100 treatment group, the increase in Bcl-2 level was remarkable (P<0.001).

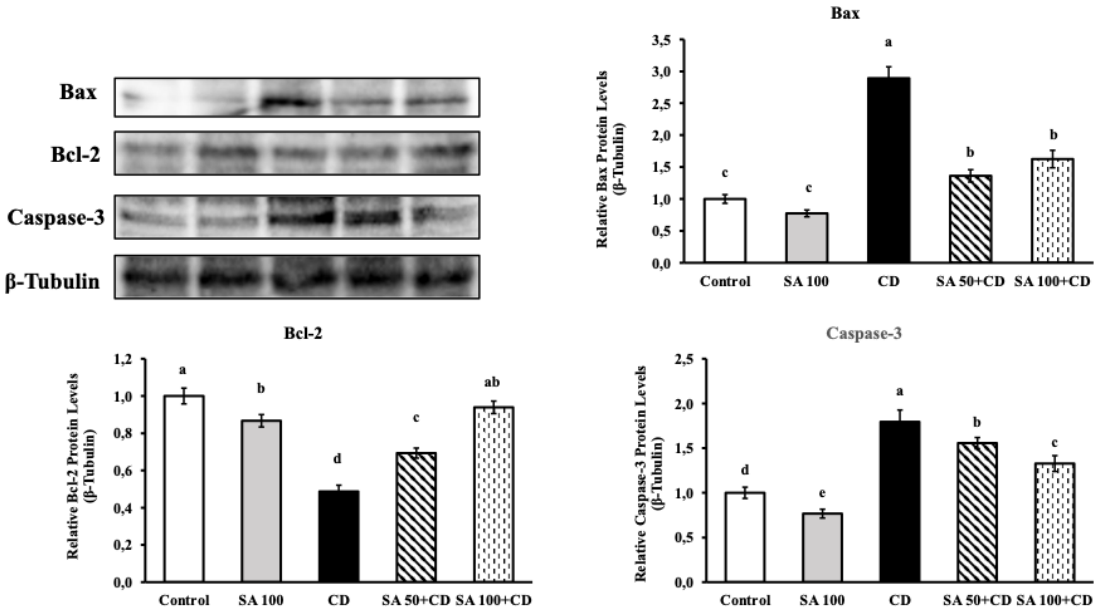

Figure 4: Representative western blot bands in different groups. [a,b,c,d,e] Means superscripted with different row are significantly different (***P<0.001; **P<0.01; *P<0.05 NS: Non-significant). Statistical analysis of Western blotting revealed the amount of expression of Bax, Bcl-2 and Caspase-3 proteins in all groups. The data are expressed as the ratio to β-actin. Data are mean ± S.E. n=10 for each group. P<0.001 compared with Intact group.

Discussion

The present study aimed to investigate the protective effects of syringic acid against cadmium-induced hepatotoxicity in rats. Cadmium is a well-known environmental pollutant that poses significant health risks, particularly affecting the liver, an organ crucial for detoxification and metabolic processes (Niture et al., 2021). The findings of this research contribute to the growing body of evidence regarding the hepatotoxic effects of cadmium and the potential therapeutic role of natural compounds like Sa.

Multiple factors contribute to the toxic effects of cadmium in biological tissues (Vijiyakumar and Prince, 2024). Although Cd itself is not a redox-active metal, oxidative stress is widely regarded as a fundamental mechanism underlying its toxicity. Cd disrupts redox balance primarily by displacing essential metal cations from the active sites of antioxidant enzymes. Furthermore, it interacts with protein sulfhydryl groups including those of antioxidant enzyme leading to their functional inhibition and further compromising the cellular antioxidant defense system (Bhattacharyya et al., 2023; Niture et al., 2021).

SOD and CAT are among the key enzymes involved in the antioxidant defense system (Jomova et al., 2024). Cadmium exposure has been shown to deplete intracellular GSH, a critical non-enzymatic antioxidant, thereby exacerbating oxidative stress. These alterations collectively result in the excessive accumulation of ROS and disrupt the delicate balance between pro-oxidants and antioxidants in cells (Qu and Zheng, 2024; Vijiyakumar and Prince, 2024). In the current study, treatment with Sa appeared to restore antioxidant capacity, as evidenced by enhanced activities of SOD, and CAT potentially through its ability to neutralize Cd-induced ROS. Furthermore, it was discovered that GSH, which has a sulfhydryl group, raises its levels by shielding it from the effects of Cd. According to earlier research, Cd reduces the amounts of enzymatic and non-enzymatic antioxidants, which results in oxidative stress and organ damage (Qu and Zheng, 2024; Xu et al., 2021; Yang et al., 2021b). However, it has been found that many antioxidant agents used to combat Cd protect against toxicity by lowering oxidative stress. One of the most important markers of oxidative stress is lipid peroxidation, and as MDA is a byproduct of lipid peroxidation, it is a crucial signal for determining the oxidative status (Cordiano et al., 2023; Sun et al., 2021). Antioxidant defense mechanisms lower lipid peroxidation by shielding mammalian cells from reactive oxygen species, whereas a number of variables, including obesity, alcohol, smoking, stress, and air pollution, increase free oxygen radicals and promote lipid peroxidation (Cordiano et al., 2023; Sun et al., 2021). In line with other research, we found that Cd increased MDA levels and caused lipid peroxidation in our study (Xu et al., 2021; Yang et al., 2021b). However, via decreasing MDA levels and lipid peroxidation, Sa was found to shield cell membranes from the impacts of Cd.

Oxidative stress is one of the factors that starts the inflammatory process, which is recognized to be a significant mechanism for the development of liver damage. Liver cells under oxidative stress emit chemokines and inflammatory cytokines. In the cytoplasm, NF-κB coexists with IκB and is one of the primary regulatory transcription factors controlling inflammatory responses. TNF-α and IL-1β are proinflammatory cytokines that are regulated by NF-kB, which is produced and enters the nucleus when protein kinases phosphorylate IκB. Hepatocellular necrosis brought on by Cd-induced oxidative stress triggers immune cell infiltration and a number of inflammatory reactions (Liu et al., 2024). Consequently, pattern recognition receptors including TLR4 are activated (Cao et al., 2022). Through TLR family members and associated pathways including the NF-κB pathway, chemokines and other cytokines like IL-1β and TNF-α are released when TLR signaling is activated (Zou et al., 2022). These cytokines draw inflammatory cells. In this study, Cd elevated NF-κB p65 downstream genes, such as TNF-α and IL-1β, and markedly increased the phosphorylation of TLR4 and NF-κB p65 proteins. In line with the Gong et al., (2024) study, these findings imply that Cd causes hepatotoxicity by triggering the TLR4-NF-κB inflammatory pathway (Gong et al., 2024).

By inhibiting the expression of pro-inflammatory cytokines, the anti-inflammatory cytokine IL-10 plays a vital role in controlling the immunological response (Zhang and Wang, 2006). The balance between pro-inflammatory and anti-inflammatory systems is upset when IL-10 levels are low, leaving

the liver susceptible to inflammation (Tylutka et al., 2024). Consequently, raising IL-10 expression can offset rising IL-6 and TNF-α expression, lowering Cd-induced toxicity levels. Prior studies have shown that in liver tissue, Cd raises pro-inflammatory cytokines while lowering anti-inflammatory cytokines (Kayama et al., 1995; Yang et al. 2021)). However, it was observed that Sa treatment significantly increased the IL-10 level.

Additionally, there is growing evidence that oxidative stress, inflammation, and apoptosis are closely related (Kannan and Jain, 2000). By upsetting calcium homeostasis, Cd lowers mitochondrial membrane potential in the mechanism linked to oxidative stress. Consequently, it results in apoptosis that is dependent on caspase (Wang et al., 2022). Bcl-2 is an anti-apoptotic protein, while Caspase-3 and Bax are pro-apoptotic. By downregulating Bcl-2 and activating caspase-3, NF-kB also increases apoptosis, according to a prior study (Hassan et al., 2025). It has been demonstrated that pro-inflammatory cytokines activate the caspase family of proteases, which aids in the apoptotic process (Grunnet et al., 2009). According to a prior study, administering $CdCl_2$ raised the levels of Bax and caspase-3 in cortical tisues, while downregulating Cd raised cytochrome c levels and activated caspase-3 in the study that was presented (Hassan et al., 2024; Yuan et al., 2018). Furthermore, it was shown that Cd caused a rise in Bax levels and a fall in Bcl-2 levels, which in turn caused apoptosis in liver tissue. However, it was discovered that Bcl-2 levels rose, shielding the liver against apoptosis, while caspase-3 and Bax levels dropped in the liver tissues of rats given Sa. The apoptotic process was initiated with the help of Bcl-2 levels.

One limitation of this study is the lack of an independent SA 50 group, despite the inclusion of the Silymarin 50 + CD group. This imbalance, inherent to the approved ethical design, should be considered when interpreting the comparative effects of the treatments.

Conclusion

Taken together, it was found that acute Cd exposure mainly caused oxidative stress in liver tissue and accordingly induced oxidative inflammation and apoptosis. However, it was concluded that Sa could protect the liver by suppressing Cd-induced oxidative stress, inflammation, and apoptosis through its antioxidant properties. Future studies involving longer exposure periods, different dosages, and evaluations in other organ systems are warranted to further elucidate the protective mechanisms of Sa. Moreover, these findings may have important implications for veterinary medicine and toxicology, highlighting the potential of Sa as a candidate hepatoprotective agent against heavy metal–induced toxicity.

Peer-review: External, Independent.

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Declarations:

1. Statement of Originality:

This work is original.

2. Author Contributions:

Concept: TK,EAS,OA,MBH; ***Conceptualization:*** TK,EAS,OA,MBH; ***Literature Search:*** TK,EAS,OA,MBH; ***Data Collection:*** TK,EAS,OA,MBH; ***Data Processing:*** TK,EAS,OA,MBH; ***Analysis:*** TK,EAS,OA,MBH; ***Writing – original draft:*** TK,EAS,OA,MBH; ***Writing – review & editing:*** TK,EAS,OA,MBH.

3. Ethics approval:

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5. Competing Interests:

The authors declare no competing interests.

6. GenAI Usage Statement:

No GenAI tools were used at any stage of the study.

7. Sustainable Development Goals:

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