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Syringic acid pretreatment potentiates antioxidant mechanism and downregulates inflammatory signaling towards doxorubicin-related oxidoinflammatory hepatorenal damage in rats

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Abstract:

Although doxorubicin (DOX) is a first-line antineoplastic drug, its clinical use is limited due to its widespread side effects. Hepatic and renal metabolic clearance causes DOX accumulation in these organ systems, ultimately leading to oxidative-inflammatory damage. This study investigated the potential impact of syringic acid (SA), a natural antioxidant phenolic compound, against in vivo DOX-evoked hepatorenal damage. SA was orally administered to rats at 100 mg/kg body weight for 10 consecutive days. On the 8th day of the study, DOX was administered at 20 mg/kg by single-dose intraperitoneal injection to prompt hepatorenal toxicity. Hepatorenal toxicity was assessed by measuring serum alanine transferase (ALT), aspartate transferase (AST), blood urea nitrogen (BUN), and creatinine (Cre). Histopathological evaluation of kidney and liver tissues was also performed to observe DOX-related damage. The concentrations of malondialdehyde (MDA) and glutathione (GSH), along with the activities of catalase (CAT) and superoxide dismutase (SOD), were measured in the hepatorenal tissues, in addition to the concentration of 8-OHdG in serum, to determine the protective effect of SA against oxidative stress. Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) levels were investigated with molecular and immunohistochemical analysis in hepatorenal tissues, evaluating the inflammation. SA treatment prevented DOX-aberrant elevation of hepatorenal biomarkers in serum, besides histopathological alterations in rats' liver and kidney. Additionally, SA markedly counteracted DOX-provoked oxidative damage as evidenced by outstanding reduction in lipid peroxidation and 8-OHdG, conjointly promoted GSH contents, and amelioration of activities of SOD and CAT. Similarly, SA-treated rats exhibited attenuation of hepatorenal inflammation via diminished TNF- α and IL-6 levels. SA exerted a hepatorenal protective effect in rats by exhibiting antioxidant and anti-inflammatory activities.

Keywords: Doxorubicin; antioxidant; rat; hepatorenal toxicity; inflammation; oxidative stress; syringic acid.

Introduction

In contemporary oncotherapy, doxorubicin (DOX) is the first-line chemotherapy agent of choice in the treatment of solid tumors and hematologic malignancies [1, 2]. Despite its therapeutic benefits, side effects such as dose-related organ toxicities may lead to morbidity or a decrease in the patient's quality of life [3]. Although the most well-known side effects of DOX therapy are related to the cardiovascular system [3], its toxic effects on the hepatorenal system have recently attracted attention [4]. Current reports emphasize that the hepatic toxic effects of DOX is manifested by vascular congestion, sinusoidal dilatation, mononuclear cell infiltration, and hepatocyte degeneration, as well as increased activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) [5]. Similarly, it has been reported that elevated serum creatinine (Cre) and urea levels accompany an increase in tubular atrophy and glomerular capillary permeability in the nephrotoxic effect of DOX [6]. The mechanisms underlying DOX-associated hepatorenal toxicity have not been fully elucidated. However, reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals are also considered to be key factors in DOX-induced organ toxicity [5, 6]. Studies have shown that ROS is formed in the liver through the redox cycle of DOX semiquinone or NADPH oxidase [7]. It suggests that oxidative damage to the kidney is probably caused by the formation of a highly reactive iron-DOX complex [7]. As a result, it has been suggested that the imbalance between ROS and antioxidants leads to lipid peroxidation (LPO) and oxidative tissue damage, indicated by protein oxidation in these tissues [3]. In addition, increased oxidative stress in hepatorenal tissues induces hepatorenal inflammation by triggering the release of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6), especially in hepatocyte and renal tubular epithelial cells [8, 9]. Failure to control these side effects of DOX may cause patients to leave treatment, reduce the treatment dose, or terminate the therapy [10]. Recently, further evidence has presented that the side effects of anthracyclines

can be reduced by using novel therapeutic approaches such as controlled drug delivery systems [11] and less toxic analogs [12]. Despite the promising benefits of these applications, they also present several challenges and potential adverse effects in clinical practice [13].

For this reason, the use of plant-derived natural products is supported by various studies, as they may reduce the toxic effects of chemotherapeutic drugs without compromising their antitumor efficacy [14]. Syringic acid (SA), which we will investigate its effect against DOX toxicity in the current report, is a phenolic compound synthesized in plants and found extensively in fruits and vegetables [15-17]. SA has been shown to possess numerous pharmacological properties, including antiproliferative, potent antioxidant, anticancer, hepatoprotective, anti-inflammatory, and anti-apoptotic effects [16]. SA has been reported to enhance cellular regeneration following renal and hepatic injury, as well as other pathological conditions [15-17]. It can also regulate enzyme activity, transcription factors, growth factors, and signaling pathways [18]. A previous study demonstrated that SA alleviated oxidative stress and inflammatory biomarkers while improving liver and kidney function parameters in neonatal diabetic rats [19]. Besides, no adverse effects were observed in toxicological studies conducted with SA [17]. Besides, no adverse effects were observed in toxicological studies conducted with SA. Elucidating the therapeutic targets and related molecular pathways of the antioxidant components of SA in various organ toxicities may contribute to its broader recognition and utilization as a highly valuable pharmacological agent. Although the protective properties of SA have been investigated in different experimental models, its mechanistic role against DOX-induced oxidative-inflammatory hepatorenal injury remains elucidated. Therefore, the present study was designed to investigate the potential protective effects of SA pretreatment against DOX-induced oxidative-inflammatory hepatorenal damage and dysfunction in rats, with special emphasis on antioxidant mechanisms and inflammatory signaling modulation.

Materials and methods

Chemicals

The chemotherapeutic drug doxorubicin hydrochloride employed in the current experiment was acquired from Saba Drug Industries (Adrimisin®, Istanbul, Turkey) and SA ($\geq 95\%$ purity) from Sigma-Aldrich (Darmstadt, Germany). A 0.5 g vial of sodium thiopental was purchased from IE Ulagay (Istanbul, Turkey).

Animals

Adult male Wistar Albino rats (230 ± 20 g; 3-4 months, $n=24$) were provided by the Erzincan Binali Yildirim University Experimental Animals Application and Research Center (EBYU-DEHAM). The animals were housed in a standard laboratory environment (12-h light/dark cycle, $24 \pm 2^\circ\text{C}$). Food and water were provided ad libitum.

Animal groups and experimental procedure

The animals were randomly allocated into four groups of six rats each.

- i) **Control group (n=6):** Rats were administered 0.9% NaCl solution orally for 10 days and intraperitoneally (i.p.) on the 8th day.
- ii) **SA group (n=6):** Rats were administered 100 mg/kg SA orally for 10 days, and 0.9% NaCl solution was given i.p. on the 8th day.
- iii) **DOX group (n=6):** Rats were administered 0.9% NaCl solution orally for 10 days, and 20 mg/kg DOX was given as i.p. on the 8th day,
- iv) **SA+DOX group (n=6):** Rats were administered 100 mg/kg SA orally for 10 days and 20 mg/kg DOX given i.p. on the 8th day.

In the present study, SA pretreatment was employed as a protective approach against DOX-induced hepatorenal toxicity, and the dose of SA was determined according to previously reported therapeutic efficacy [20]. Besides, the DOX dose was chosen based on previously published data [21]. In the present study, a single-dose DOX-induced model was employed. In the literature, it has been reported that a single DOX dose ranging from 5 to 30 mg/kg can

induce oxidative damage in the liver, kidneys, and heart. The 20 mg/kg single dose used in this study corresponds to a relatively high single dose used in clinical settings [22]. DOX and SA were dissolved in 0.9% NaCl solution. The administered volume was adjusted to 1 mL/kg for each drug. To minimize the stress that may develop due to drug administration between the control, DOX, and SA-administered groups, the same volume of 0.9% NaCl drug solvents was applied. All procedures were accomplished between 09:30 and 10:00 in the morning to minimize circadian alterations.

Collection of samples

On the 10th day, 30 minutes after administering the final dose of syringic acid, the animals were anesthetized using a single dose of 30 mg/kg sodium thiopental by i.p. injection [21]. Under deep anesthesia, terminal blood samples were obtained by left ventricular cardiac puncture for biochemical evaluation of hepatic and renal function markers. Complete exsanguination following cardiac puncture was accepted as the method of euthanasia [22]. Then, liver and kidney tissues were expeditiously excised and preserved in 10% buffered formalin for subsequent histopathological and immunohistochemical analysis. Tissue samples obtained for biochemical analysis were meticulously washed with cold phosphate-buffered saline and rapidly frozen in liquid nitrogen. Tissue samples were subjected to tissue homogenisation in phosphate-buffered saline (PBS, pH = 7.4), after which centrifugation at $2,300 \times g$ for 20 min was performed at 4°C [23]. The overlying fluids were utilised to evaluate the content of various biomarkers of oxidative stress, and protein content was determined using the Bradford MM method [24]. Additionally, tissue samples taken for molecular analysis were stored in RNeasy Lysis Buffer (Thermo Fisher Scientific, Carlsbad, CA, USA) stabilization reagent. The collected blood samples from the rats were used in vacutainer tubes specifically intended for biochemical analysis. The samples were left to rest at room temperature for 20 minutes, then

subjected to centrifugation at 21,000 $\times g$ for 15 min [21]. The collected serum samples were then used to measure biomarkers of hepatic and renal injury.

Evaluation of oxidative stress parameters

MDA level, which is a measure of lipid peroxidation, was determined by spectrophotometric measurement at 532 nm with a pink complex formed in the method described by Ohkawa et al. [25]. SOD activity was determined by measuring it at 560 nm, by the technique of Sun et al., which is based on the principle that superoxide anion reduces nitro blue tetrazolium (NBT) to form a blue formazan compound [26]. The GSH level was determined by spectrophotometric measurement of the yellowish-green complex formed according to the method described by Ellman et al. 412 nm [27]. CAT activity was measured spectrophotometrically at 240 nm using H_2O_2 as the substrate, according to Aebi's method [26]. 8-OHdG levels were determined using a commercial kit, and the absorbance was analyzed at 450 nm according to the manufacturer's instructions (Sunred Biological Technology, Shanghai, China).

Measurement of liver function parameters

ALT and AST activities were determined spectrophotometrically using a Cobas 8000 autoanalyzer (Roche Diagnostics GmbH, Germany) with kits (Roche Diagnostics).

ALT determination: ALT in serum samples catalyzes the reaction between L-alanine and 2-oxoglutarate, producing pyruvate and L-glutamate. NADH subsequently reduces the generated pyruvate in a reaction catalyzed by lactate dehydrogenase. Pyridoxal phosphate serves as a coenzyme in the aminotransfer reaction. The catalytic activity of ALT is directly proportional to the rate of NADH oxidation. The decrease in absorbance was measured spectrophotometrically at 340 nm, and ALT activity was determined accordingly.

AST determination: AST in serum samples catalyzes the reaction between L-aspartate and 2-oxoglutarate, leading to the formation of oxaloacetate and L-glutamate. The produced oxaloacetate subsequently reacts with NADH in a reaction catalyzed by malate dehydrogenase,

forming L-malate and NAD^+ . Pyridoxal phosphate serves as a coenzyme in the aminotransfer reaction. The catalytic activity of AST is directly proportional to the rate of NADH oxidation. The decrease in absorbance was measured spectrophotometrically at 340 nm, and AST activity was determined accordingly.

Measurement of kidney function parameters

The Roche Cobas® 8000 autoanalyzer (Mannheim, Germany) was used to spectrophotometrically measure the levels of serum Cre and BUN.

Cre determination: Creatinine levels were measured using a colorimetric assay based on the Jaffe method [28]. In an alkaline solution, Cre reacts with picrate to form a yellow–orange complex. The absorbance of this complex was measured at 505 nm, and the rate of color formation is directly proportional to the Cre concentration in the sample. To minimize bilirubin interference, a rate-blanking method was applied. To correct for non-specific reactions caused by pseudo-Cre chromogens in serum or plasma, a correction factor of $-26 \mu\text{mol/L}$ (-0.3 mg/dL) was applied to the measured values.

BUN determination: Urea was hydrolyzed by urease to produce ammonium and carbonate. Subsequently, ammonium reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase and the coenzyme NADH to form L-glutamate. In this reaction, two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed. The rate of decrease in NADH concentration is directly proportional to the urea concentration in the sample. Absorbance was measured at 340 nm. The formula $\text{BUN} = \text{URE} \times 0.48$ was used to calculate BUN.

Histopathological evaluation

Liver and kidney tissues taken from rats were fixed in a 10% neutral formalin solution and then subjected to routine alcohol-xylol follow-up procedures. Afterward, the samples were placed in paraffin blocks and stained with hematoxylin-eosin in 4μ sections. For histopathological evaluation, all slides were randomly coded before examination to ensure blinded assessment.

Histopathological findings, including necrotic and degenerative alterations, were semiquantitatively scored as absent (0), mild (1), moderate (2), or severe (3), based on a modification of previously defined criteria [29]. All evaluations were performed by an experienced pathologist who was blinded to the experimental groups. To enhance scoring reliability, each section was independently examined twice by the same observer at different time points, and the final score was determined based on intra-observer consistency.

Molecular analysis

The relative mRNA transcript levels of the genes TNF- α and IL-6, whose sequences are given in Table 1 were analyzed by RT-PCR. For this, the quantitative detection of homogenization of the liver and kidney tissues, RNA isolation, cDNA synthesis, and mRNA expression measurements were done. Total RNA was isolated using an RNeasy kit (Thermo Fisher, Waltham, MA, USA) relative to the manufacturer's directions in the first RT-PCR analysis stage. Total mRNA concentration was evaluated by spectrophotometry (EPOCH Take 3 Plate, Biotek) at 260 nm. In the second stage, cDNA synthesis was performed with a high-capacity cDNA Reverse Transcription Kit (Thermo Fisher, Waltham, MA, USA) from total RNA. The amount of cDNA was measured with nanodrop spectrophotometry (EPOCH Take 3 Plate, Biotek), and the cDNA was stored at -20°C . As an internal control, β -actin transcript levels were measured, and relative fold changes were calculated using cycle threshold (Ct) values obtained from the instrument, according to the $2^{-\Delta\Delta\text{Ct}}$ method of Livak and Schmittgen [30].

Immunohistochemical evaluation

Sections obtained from liver and kidney tissues (4 μm) were exposed to primary antibodies against TNF- α (Santa Cruz, Catalog no. sc-133192) and IL-6 (Affbiotech, Catalog no. DF6087), which were diluted 1:200. To observe the immune reaction, diaminobenzidine tetrachloride and 3-Amino-9-Ethylcarbazole (AEC) were employed. After staining, the sections were contrasted with Mayer's Hematoxylin, sealed with a water-based adhesive, and examined under

a light microscope. During the examination, immunoreactivity was evaluated as none (0), mild (1), moderate (2), severe (3), and very severe (4) [31]. For the negative control, sections were processed using the same protocol; however, the primary antibody was omitted and replaced with PBS. No specific immunostaining was observed in the negative control sections.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistical for Windows (IBM Corp., v.27.0, Armonk, NY, USA). Data presented as mean $\pm$ standard deviation (SD). For analysis of ELISA and RT-PCR numerical data, one-way ANOVA was used, followed by a post hoc Tukey test. Results were reported using F statistics (F), p-values, effect size expressed as eta-squared (η^2), and corresponding 95% confidence intervals. On the other hand, histopathological and immuno-histochemical findings were performed with the Kruskal-Wallis test, afterward Man-Whitney U test. P less than 0.05 was accepted as statistically significant.

Results

Effect of SA on oxidative stress induced by DOX in liver and kidney tissue

Fig. 1 represents the consequence of DOX intoxication and oral pretreatment with SA on hepatic LPO and antioxidant activities. According to the statistical analysis, significant differences were detected among the groups in terms of liver MDA and tGSH levels, SOD, and CAT activities (MDA: $F(3, 20) = 41.191$, $p < 0.001$; $\eta^2 = 0.861$, 95% CI: 0.672–0.903, tGSH: $F(3, 20) = 33.965$, $p < 0.001$; $\eta^2 = 0.836$, 95% CI: 0.619–0.886, SOD: $F(3, 20) = 19.742$, $p < 0.001$; $\eta^2 = 0.748$, 95% CI: 0.445–0.825, CAT: $F(3, 20) = 15.257$, $p < 0.001$; $\eta^2 = 0.696$, 95% CI: 0.355–0.789). MDA levels were determined to be 1.84 ± 0.10 in the control group and 4.05 ± 0.39 in the DOX group. DOX intoxication was associated with a remarkable ($p < 0.001$) rise in the hepatic MDA levels (Fig. 1A). Hepatic tGSH, SOD, and CAT levels were determined to be 4.93 ± 0.52 , 3.89 ± 0.66 , and 30.54 ± 3.78 , respectively, in the control group, whereas they were found to be 2.58 ± 0.55 , 2.08 ± 0.27 , and 17.80 ± 2.77 , respectively, in the DOX group. As

shown in Fig. 1B, a single dose of 20 mg of DOX treatment with i.p injection led to a marked depletion in the hepatic GSH concentration ($p<0.001$) and a noteworthy reduction in the hepatic SOD (Fig. 1C) and CAT (Fig. 1D) activities ($p<0.001$). In the SA+DOX group, MDA and tGSH levels, as well as SOD and CAT activities, were 3.20 ± 0.75 , 4.16 ± 0.46 , 3.52 ± 0.35 , and 25.17 ± 2.62 , respectively. Pretreatment with SA at a dose of 100 mg/kg significantly reduced hepatic MDA levels in DOX-treated tissues ($p=0.015$). In addition, SA pretreatment prompted serious increases in the hepatic tissue GSH ($p<0.001$) level and SOD ($p<0.001$) and CAT ($p=0.016$) activities, restoring their concentrations to normal ($p>0.05$). In rats receiving SA alone, MDA, tGSH, SOD, and CAT levels were 1.65 ± 0.18 , 5.18 ± 0.43 , 3.98 ± 0.56 , and 17.80 ± 2.77 , respectively, and were comparable to those of the control group, with no statistically significant differences observed between the groups ($p > 0.05$).

Fig. 2 demonstrates the effect of DOX intoxication and oral SA pretreatments on the renal tissue LPO and antioxidant profile. Statistical analysis revealed significant differences among the groups in kidney MDA, tGSH levels, and SOD, and CAT activities (MDA: $F(3, 20) = 90.011$, $p<0.001$; $\eta^2=0.931$, 95% CI: 0.832–0.952, tGSH: $F(3, 20) = 49.334$, $p<0.001$; $\eta^2=0.881$, 95% CI: 0.716–0.917, SOD: $F(3, 20) = 38.716$, $p<0.001$; $\eta^2=0.853$, 95% CI: 0.655–0.898, CAT: $F(3, 20) = 99.811$, $p<0.001$; $\eta^2=0.937$, 95% CI: 0.847–0.956). Kidney MDA levels were determined to be 0.98 ± 0.21 in the control group and 2.72 ± 0.32 in the DOX group. As demonstrated in Fig. 2A, taking a single dose of 20 mg of DOX with i.p injection resulted in an elevation of MDA levels in the renal tissue compared to control ($p<0.001$). Kidney tGSH level, SOD, and CAT activities were 6.05 ± 0.28 , 2.98 ± 0.10 , and 28.05 ± 1.31 in the control group, respectively, whereas the corresponding values in the DOX group were 3.66 ± 0.34 , 1.65 ± 0.45 , and 15.35 ± 1.96 , respectively. DOX injection related to depletion in the renal GSH (Fig. 2B), and a decrease in the renal SOD (Fig. 2C) and CAT (Fig. 2D) activities ($p<0.001$). In the SA+DOX group, MDA and tGSH levels, as well as SOD and CAT activities, were 1.51 ± 0.21 , $5.26 \pm$

0.47, 2.38 ± 0.18 , and 20.90 ± 1.65 , respectively. 100 mg/kg SA pretreatment resulted in a significant reduction in renal tissue MDA levels ($p < 0.001$). Similarly, oral pretreatment with 100mg/kg SA also significantly elevated the renal tissue GSH levels and marked increased renal tissue SOD and CAT activities when contrasted to the untreated DOX-intoxicated values ($p < 0.001$). In rats treated with SA alone, MDA, tGSH levels, and SOD and CAT activities were 0.81 ± 0.12 , 6.15 ± 0.48 , 3.01 ± 0.11 , and 28.88 ± 1.24 , respectively, and were comparable to those of the control group, with no statistically significant differences between the groups ($p > 0.05$).

Effect of SA on oxidative DNA damage caused by DOX in serum

Fig. 3 represented the effect of oral SA pretreatment on 8-OHdG in serum of DOX-treated rats. Significant differences were observed among the groups in serum 8-OHdG levels $F(3, 20) = 35.529$, $p < 0.001$; $\eta^2 = 0.842$, 95% CI: 0.631–0.890). 8-OHdG levels in the control, SA, DOX, and SA + DOX groups were 51.32 ± 9.65 , 49.09 ± 7.62 , 88.25 ± 5.60 , and 63.04 ± 5.95 , respectively. In the DOX group, elevations in the levels of measured 8-OHdG were noted compared to control rats ($p < 0.001$). Oral SA pretreatment resulted in a reduction in serum 8-OHdG concentration ($p < 0.001$). and reverting the value to the control group ($p = 0.055$).

Effect of SA on DOX-induced hepatic and renal dysfunctions

In this experiment, we evaluated serum levels of ALT (Fig. 4A) and AST (Fig. 4B) as indicators of liver function, and BUN (Fig. 4C) and Cre (Fig. 4D) levels as indicators of renal function. The statistical analysis revealed significant differences in serum ALT, AST, BUN, and creatinine levels (ALT: $F(3, 20) = 78.668$, $p < 0.001$; $\eta^2 = 0.922$, 95% CI: 0.810–0.945, AST: $F(3, 20) = 41.606$, $p < 0.001$; $\eta^2 = 0.862$, 95% CI: 0.674–0.904, BUN: $F(3, 20) = 10.445$, $p < 0.001$; $\eta^2 = 0.610$, 95% CI: 0.277–0.729, Cre: $F(3, 20) = 97.470$, $p < 0.001$; $\eta^2 = 0.936$, 95% CI: 0.844–0.955). ALT activities in the control, SA, DOX, and SA + DOX groups were 41.48 ± 4.15 , 40.92 ± 4.12 , 85.33 ± 7.50 , and 63.47 ± 6.78 , respectively, whereas AST activities were 65.42 ± 5.02 ,

60.27 $\pm$ 6.12, 114.53 $\pm$ 13.98, and 84.12 $\pm$ 9.45, respectively. A single intraperitoneal dose of 20 mg/kg DOX resulted in marked increases in ALT and AST levels compared to the control group ($p < 0.001$). However, oral 100 mg/kg SA pre-treatment resulted in a noteworthy reduction in the serum ALT and AST when compared with the values for DOX-intoxicated rats ($p < 0.001$). BUN levels in the control, SA, DOX, and SA + DOX groups were 19.91 $\pm$ 5.82, 17.71 $\pm$ 4.71, 32.82 $\pm$ 3.61, and 24.19 $\pm$ 5.76, respectively, whereas Cre levels were 0.58 $\pm$ 0.12, 0.50 $\pm$ 0.11, 3.04 $\pm$ 0.53, and 2.44 $\pm$ 0.33, respectively. Similar to hepatic function test results, a single dose of 20 mg of DOX administration with intraperitoneal injection resulted in significant increases in the serum BUN and Cre when compared to the control values ($p < 0.001$). With oral SA 100 mg/kg SA pre-treatment, there was a significant reduction in BUN and Cre when compared to the only DOX-injected group values ($p < 0.05$).

SA pre-treatment ameliorated histopathological lesions in rats' liver and kidney against administration with DOX

Control rats (Fig. 5A) and rats treated with SA alone (Fig. 5B) had typical liver morphology and histological structure. However, DOX application to the rats was associated with severe mononuclear cell infiltration in the periportal areas of the liver, severe hepatocyte necrosis, and moderate hydropic degeneration, compared with the livers from control rats, which showed normal hepatic histoarchitecture (Fig. 5C). Oral SA pre-treatment in the DOX-intoxicated rats mitigated hepatic mononuclear cell infiltration, hydropic degeneration, and necrosis (Fig. 5D). The statistical analysis results of the histopathological scoring data in liver tissues are presented in Figure 5E-G and Table 2.

Control rats (Fig. 6A) and SA-only-treated rats (Fig. 6B) exhibited typical kidney morphology with no marked lesions. DOX injection resulted in interstitial nephritis consisting of severe mononuclear cell infiltrations and impaired renal histology with tubular degeneration in the rat kidney (Fig. 6C) compared with the control rat kidneys that have normal renal histoarchitecture.

In contrast, with oral pretreatment with SA to DOX-intoxicated rats, there were significant improvements in the DOX-related deteriorations in histopathological structures in the renal tissues of DOX-only treated rats (Fig. 6D). The statistical analysis results of the histopathological scoring data in kidney tissues are presented in Figure 6E-F and Table 2.

Effect of SA on DOX-induced inflammatory response in liver and kidney tissues.

The mRNA expression levels of TNF- α and IL-6 genes in liver tissues differed significantly among the groups (TNF- α : $F(3, 20) = 137.264$, $p < 0.001$; $\eta^2 = 0.954$, 95% CI: 0.886–0.968, IL-6: $F(3, 20) = 10.411$, $p < 0.001$; $\eta^2 = 0.610$, 95% CI: 0.226–0.728). Hepatic TNF- α and IL-6 relative mRNA expression levels in the control group were 1.68 ± 0.12 and 0.94 ± 0.26 , respectively, whereas in the DOX group these values were 3.36 ± 0.27 and 1.45 ± 0.15 , respectively. In the DOX-treated group, there was a marked rise in the hepatic levels of the measured pro-inflammatory cytokines, including TNF- α (Fig. 7A) and IL-6 (Fig. 7B) mRNA expressions, which were recorded compared to the control ($p < 0.001$). In the SA+DOX group, TNF- α and IL-6 relative mRNA expression levels were 2.61 ± 0.16 and 1.11 ± 0.10 , respectively. Oral SA pre-treatment caused a significant decrease in hepatic expressions of TNF- α and IL-6 ($p < 0.05$). In the SA group, TNF- α and IL-6 were 1.60 ± 0.08 and 0.89 ± 0.22 , respectively, and were found to be comparable to those of the control group ($p > 0.05$).

The mRNA expression levels of TNF- α and IL-6 genes in kidney tissues differed significantly among the groups (TNF- α : $F(3, 20) = 22.968$, $p < 0.001$; $\eta^2 = 0.775$, 95% CI: 0.496–0.844, IL-6: $F(3, 20) = 58.416$, $p < 0.001$; $\eta^2 = 0.898$, 95% CI: 0.754–0.929). Renal TNF- α and IL-6 relative mRNA expression levels were 1.93 ± 0.46 and 1.26 ± 0.23 in the control group, respectively, whereas in the DOX group these values were 4.90 ± 1.05 and 2.81 ± 0.28 , respectively. DOX intoxication was associated with a marked increase in renal tissue TNF- α (Fig. 7C) and IL-6 (Fig. 7D) mRNA expressions ($p < 0.001$). In the SA+DOX group, TNF- α and IL-6 relative mRNA expression levels were determined to be 3.32 ± 0.80 and 1.86 ± 0.25 , respectively. Oral

SA pre-treatment significantly reduced TNF- α and IL-6 mRNA expression levels in renal tissues ($p < 0.05$). In the SA group, TNF- α and IL-6 levels (1.85 ± 0.46 and 1.17 ± 0.21 , respectively) were consistent with those observed in the control group ($p > 0.05$).

Immunohistochemical findings

Figure 8 represents the effect of DOX intoxication and oral pre-treatment with SA on the hepatic and renal TNF- α and IL-6 levels. Control (Fig. 8A, B; respectively) and SA-only treated rats (Fig. 8C, D; respectively) consistently displayed no significant immunopositivity with TNF- α and IL-6 levels in liver tissues. DOX poisoning led to severe TNF- α and moderate IL-6 immunopositivity in hepatic tissue (Fig. 8E, F; respectively). However, oral SA pretreatment mildly reduced liver tissue TNF- α and IL-6 immunopositivity (Fig. 8G, H; respectively). The results of the statistical analyses of immunohistochemical scoring in liver tissues are shown in Figure 8I, 8J, and Table 3.

Control (Fig. 9A, B; respectively) and SA-only treated rats (Fig. 9C, D; respectively) demonstrated no marked immunopositivity with TNF- α and IL-6 levels in renal tissues. Intraperitoneal injection of DOX induced severe TNF- α and IL-6 immunopositivity in kidney tissues (Fig. 9E, F). In contrast, oral SA pretreatment reduced immunopositivity to mild for TNF- α and moderate for IL-6 (Fig. 9G, H; respectively). Statistical evaluation of the immunohistochemical scoring data obtained from kidney tissues is presented in Figure 9I, 9J, and Table 3.

Discussion

It is already known that treatment with DOX usually causes hepatorenal damage by disrupting physiological homeostasis [8, 9]. Due to its importance in cancer treatment, studies are being carried out to prevent or reduce its side effects [4, 1]. One of the recently developed treatment protocols is the co-administration of anticancer drugs with antioxidants to reduce oxidative damage [4-6]. This investigation focused on the protective effect and potential molecular

mechanisms of SA, a powerful antioxidant, in the treatment of DOX-induced hepatorenal injury. The findings demonstrated that SA suppressed adverse biochemical and morphological alterations in DOX-related hepatorenal tissues.

Although the molecular mechanisms underlying the hepatorenal toxicity of DOX have been attempted to be elucidated, the factors responsible for this toxicity are not fully understood. Nevertheless, the formation of toxic DOX semiquinone metabolites may play a role in the development of hepatorenal toxicity. Even though the half-life of the formed semiquinone radical is short, when it interacts with molecular oxygen, it initiates a series of reactions that generate ROS [32]. It has been documented that DOX leads to an increased production of ROS, such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which react rapidly with membrane lipids to cause LPO [32]. MDA, the end product of LPO, is an endogenous genotoxic product [33]. Elevated LPO and MDA levels disrupt membrane functions by decreasing membrane fluidity and membrane-bound enzyme and receptor activities [34]. In earlier studies, an increment in MDA concentrations was observed in the liver and kidney tissues of rats as a result of DOX administration [35]. Our results agree with reports in the existing literature that DOX is a trigger of elevation of MDA concentrations in off-target healthy organs, including the liver and kidney. Interestingly, DOX-evoked LPO was attenuated due to the modulatory effects of SA. Indeed, previous data have delineated SA's ability to scavenge free radicals by dint of the existence of two methoxy groups attached to the aromatic ring at positions 3 and 5 [36].

Organ toxicities caused by DOX are known to be associated with depression of oxidant/antioxidant balance and consequent oxidative stress damage [8, 32]. Therefore, the current report considered enzymatic and non-enzymatic antioxidant defense systems in the hepatic and renal tissues of rats. It is a known fact that cellular antioxidant homeostasis and the deactivation and removal of ROS are mediated by GSH, SOD, and CAT. GSH plays a role in the detoxification of hydroperoxyl radicals and suppresses LPO by protecting lipids and

proteins from being targets of ROS through its sulfhydryl group. Meanwhile, this molecule also acts as a part in the conjugation and excretion of toxic compounds [37]. SOD, which takes a role in the first-line antioxidant defense system against ROS, provides catalytic conversion of superoxide radical or singlet oxygen radical into H_2O_2 and molecular oxygen (O_2). This highly toxic H_2O_2 is catabolized into water and O_2 by the CAT enzyme to reduce the damage caused by ROS [38]. Therefore, one of the most impressive defense mechanisms as provision for diverse diseases may be to scavenge superoxide ions and hydroxyl radicals [39]. It is thus biochemically evident that DOX-evoked depression of GSH, SOD, and CAT would promote LPO as manifested by outstanding elevation of MDA in the current experiment as well as in previous research [4, 35]. However, oral pretreatment with SA reversed the hepatic damage by suppressing the elevation of MDA and reducing the levels of GSH, SOD, and CAT in hepatorenal tissues. Our findings emphasize the preventive feature of SA administration against DOX damage by strengthening the endogenous antioxidant defense system in the hepatorenal system. A similar conclusion was reached by Zhang et al., who reported that the increase in MDA and the decrease in GSH, SOD, and CAT in experimentally induced non-alcoholic liver disease in rats were suppressed by SA and had a hepatoprotective effect [40]. A similar pattern of results was obtained in another study, which announced that elevated MDA formation and diminished antioxidant levels were markedly reversed in oxidative renal damage in rats with diabetic nephropathy as a finding of SA treatment [41]. The other marker of oxidative damage that we scrutinized at this firm is 8-OHdG, which is a useful marker of oxidative DNA damage [42]. The toxicity induced by DOX is associated with oxidative DNA damage, as demonstrated by increased 8-OHdG levels [43]. It is emphasized that there is a correlation between 8-OHdG and ROS production, and that ROS can lead to 8-OHdG formation [42]. These results parallel previous reports [44], showing that 8-OHdG levels increased significantly in the serum samples of rats after DOX application. Pretreatment of SA seriously prevented the rise of 8-OHdG

levels. These findings signified a possible mechanism by which SA may prevent oxidative DNA damage caused by DOX administration and may also protect against diseases related to DNA oxidation. Many *in vivo* studies mentioned that treatment of SA alleviates oxidative DNA damage [20].

Determination of alterations in biomarkers indicating hepatorenal functions are excellent indicator for investigating hepatorenal toxicity [21]. The increase in hepatospecific enzymes such as ALT and AST in serum has been attributed to hepatic cell damage. It has been predicted that enzymes enter the circulation with increased membrane permeability in the damaged cell [45]. Additionally, a rise in serum Cre level demonstrated impaired kidney function. Further, an elevation in serum urea level is a diagnostic marker of kidney dysfunction caused by rapid protein degradation or disruption of nitrogen homeostasis. Together, these markers are diagnostic indicators of glomerular filtration rate reduction and nephrotic damage [46, 47]. In this report, the acute injection of DOX evoked hepatorenal injury exhibited by pronounced elevation in serum markers of AST, ALT, BUN, and Cre, as earlier reported by systematic experimental studies [20, 35, 47]. The histopathology findings depicted pathological changes coinciding with hepatorenal necrosis, detection of inflammatory cells, and degeneration that might have destroyed the cell membrane, causing extracellular efflux of the enzymes and a noticeable depletion of hepatic and renal functions. Noticeably, it was observed that SA pretreatment ameliorated the altered serum enzyme activities and biomarker levels. Contributing to the overall protective effects observed in our study, it highlights the potential benefits of SA with potent antioxidant effects in the management of DOX-induced liver and kidney damage.

In addition to all this, DOX-evoked aggravating oxidative stress also contributes to hepatorenal damage by triggering proinflammatory cytokine release [8, 9]. It is also worth noting that oxidative stress and inflammation exert synergistic effects, aggravating DOX-induced

hepatorenal damage through a vicious cycle [7, 35]. TNF- α , a key proinflammatory cytokine in this process, initiates the cascade that stimulates cytokines such as IL-1 β and IL-6, which are essential for inflammation and ultimately promote extrinsic and intrinsic cell death [32]. In addition, these pro-inflammatory cytokines are well-recognized downstream targets of the NF- κ B signaling pathway and are commonly used as indirect markers of its activation [17]. In the current study, a significant increase in hepatic and renal TNF- α and IL-6 mRNA levels was demonstrated following injection of DOX in consonance with previous studies [48, 49]. Our immunohistochemistry findings, which demonstrated elevated immunopositivity in TNF- α and IL-6 in response to DOX in the liver and kidney tissues, further confirm molecular results. Therefore, the observed elevation in TNF- α and IL-6 levels suggests the involvement of NF- κ B-mediated inflammatory signaling in DOX-induced tissue injury. In our study, IL-6 levels were found to be higher in renal tissue than in the liver. The more pronounced IL-6 expression in the kidney may be associated with the ability of renal tubular epithelial cells to actively produce IL-6 during injury, thereby contributing to local cytokine amplification [50]. This may explain the sustained elevation in oxidative damage and inflammatory response characterised by a sustained hepatic/cellular increase in TNF- α and IL-6, a concomitant decrease in antioxidant levels such as GSH, SOD, and CAT, and an increase in MDA. In addition, growing evidence highlights a functional interplay between the Nuclear Factor kappa-B (NF- κ B) signaling pathway and the Nuclear factor erythroid 2-related factor 2/Heme oxygenase-1 (Nrf2/HO-1) pathway, which plays a critical role in maintaining redox homeostasis [51]. Activation of the Nrf2/HO-1 pathway enhances antioxidant defenses and suppresses inflammation. This response is partly mediated by suppression of the NF- κ B signaling pathway, resulting in reduced expression of pro-inflammatory cytokines, including TNF- α and IL-6 [51, 52]. Although Nrf2/HO-1 signaling was not directly evaluated, previous studies have shown that syringic acid can exert protective effects via upregulation of Nrf2/HO-1 and concomitant

inhibition of NF- κ B-mediated inflammation [53]. In line with these reported mechanisms, SA administration significantly curbed the activation of hepatic and renal TNF- α and IL-6 expression and immunopositivity, protecting kidney and liver tissues from inflammatory damage. Based on these data, we assumed that the anti-inflammatory properties of SA can be attributed to its antioxidant properties. Although this is the first evidence that demonstrates these outcomes in DOX-treated liver and kidney, many other similar preventive features of SA were previously declared in the liver and other tissues of rats. Indeed, SA administration in rats treated with methyl cellosolve attenuated the elevation of TNF- α and IL-6 levels in hepatotesticular tissue [15]. Likewise, SA protected against experimental diabetic nephropathy and cisplatin-induced renal damage, respectively, by suppressing the elevation of IL-6, TNF- α , and IL-1 β concentrations [54, 55].

Limitations

Despite the promising data obtained in our study, it must be acknowledged that there are some limitations. Although the current report demonstrates the potential protective properties of SA, it needs to be investigated in more detail to provide an empirical basis for its consumption by DOX-treated individuals. Therefore, the oxido-inflammatory signaling of SA and its ability to improve the hepatorenal system should be evaluated with detailed mechanisms. Meanwhile, another limitation of this research is that we did not use inhibitors or knock-out models to suppress the molecular mechanisms involved in DOX-evoked hepatorenal damage to confirm the liver and kidney protective effect of SA. Therefore, further research will focus on investigating the detailed protection mechanism of SA in healthy cells and how it preserves the anticancer potential of DOX in tumor models. In addition, only male rats were used in this study, which may limit the generalizability of the findings due to potential sex-related biological differences. Future studies should include both sexes.

Conclusion

The obtained data confirms the hypothesis that the hepatorenal toxicity mechanism of DOX is closely related to ROS production and proinflammatory cytokine release, leading to antioxidant depletion. Our results suggest, for the first time, that SA pretreatment exerts a protective effect against DOX-induced hepatotoxicity by modulating oxidative DNA damage and inflammatory responses.

Ethics approval and consent to participate:

All experiments were carried out in accordance with European Parliament Directive 2010/63/EU (Approval Number 2016-24-199) and the ARRIVE guidelines. The use of animals and all experimental protocols were approved by the Erzincan Binali Yıldırım University Animal Experiments Local Ethics Committee (Meeting date/number: December 26, 2024 / 53).

Data availability

The data in this report is available from the corresponding author upon request.

Competing Interests

No conflicts of interest are disclosed by the authors

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None

Authors' contributions:

MTK: Conceptualization, writing-original draft, writing-review and editing. **YY:** Investigation, project administration, data curation, resources, writing-review, and editing. **BY:** Data curation, formal analysis, resources, and writing-review and editing. **AG:** Investigation, data curation, formal analysis, and writing-review and editing. **DA:** Validation, writing-review, and editing. **FT:** Writing-review and editing. **HS:** Conceptualization, methodology, project administration, investigation, writing-original draft, and writing-review and editing.

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Table 1. Primer sequences

Genes	Forward sequence (5'-3')	Reverse sequence (3'-5')
TNF- α	CACACGAGACGCTGAAGTAG	AACAGTCTGGGAAGCTCTGA
IL-6	AGCGATGATGCACTGTCAGA	GGAAGTCCAGAAGACCAGAGC
β -actin	CAGCCTTCCTTCTTGGGTATG	AGCTCAGTAACAGTCCGCCT

Table 2. Results of histopathological evaluation of liver and kidney tissues

		Histopathological analyses			
		Mean $\pm$ Standard deviation			
		Control	SA	DOX	SA+DOX
Liver Tissues	Mononuclear cell infiltration	0	0	2,66 $\pm$ 0,66 ^a	1,33 $\pm$ 0,51 ^b
	Necrosis	0	0	2,83 $\pm$ 0,40 ^a	2,00 $\pm$ 0,00 ^b
	Hydropic degeneration	0	0	1,83 $\pm$ 0,40 ^a	1,16 $\pm$ 0,40 ^b
Kidney Tissues	Interstitial nephritis	0	0	2,83 $\pm$ 0,40 ^a	1,33 $\pm$ 0,51 ^b
	Tubular degeneration	0	0	2,66 $\pm$ 0,51 ^a	1,00 $\pm$ 0,00 ^b

Histopathological damage scoring: 0; absent, 1; mild, 2; moderate, 3; severe. a,b; Shows the difference between groups ($p < 0.05$). SA, syringic acid, DOX, doxorubicin. Kruskal-Wallis test was used for statistical analysis, followed by post hoc Mann-Whitney U test.

Table 3. Results of immunohistochemical evaluation of liver and kidney tissues.

Immunohistochemical analyses					
Mean $\pm$ Standard deviation					
		Control	SA	DOX	SA+DOX
Liver Tissues	TNF- α	0	0	2,83 $\pm$ 0,40 ^a	1,16 $\pm$ 0,40 ^b
	IL-6	0	0	1,66 $\pm$ 0,51 ^a	0,83 $\pm$ 0,40 ^b
Kidney Tissues	TNF- α	0	0	3,66 $\pm$ 0,51 ^a	1,16 $\pm$ 0,40 ^b
	IL-6	0	0	2,83 $\pm$ 0,40 ^a	2,33 $\pm$ 0,51 ^b

Immunohistochemical scoring: 0; absent, 1; mild, 2; moderate, 3; severe, 4; very severe. a,b; Shows the difference between groups ($p < 0.05$). NS: Not significant; SA, syringic acid, DOX, doxorubicin. The Kruskal-Wallis test was used for statistical analysis, followed by the Mann-Whitney U test.

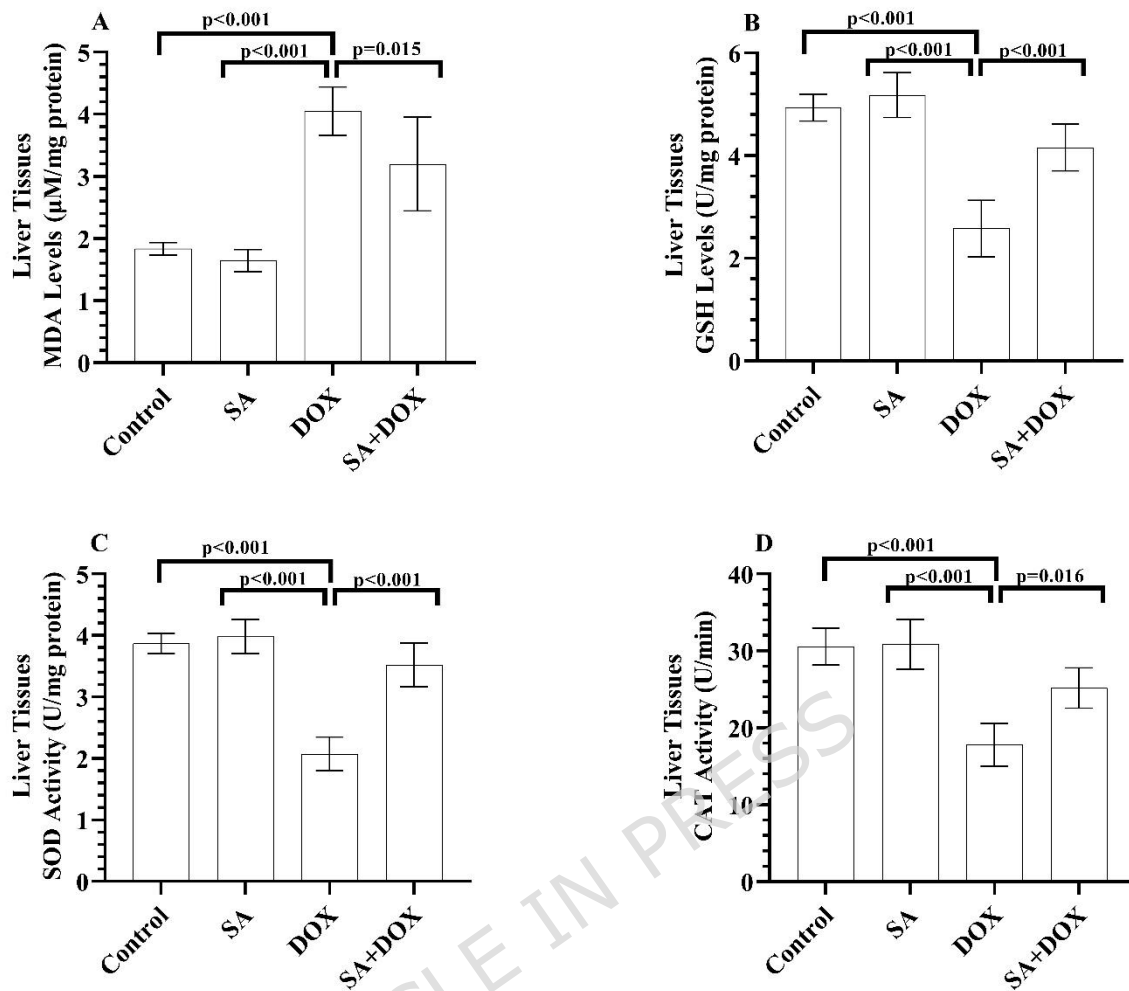


Fig. 1 (A-D). Effect of oral SA pretreatment on LPO and antioxidant profile in the liver DOX-treated rats. Each bar represents the mean $\pm$ SD of 6 rats. The significance level was set at $p < 0.05$. DOX: doxorubicin; SA: syringic acid; MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; CAT: catalase.

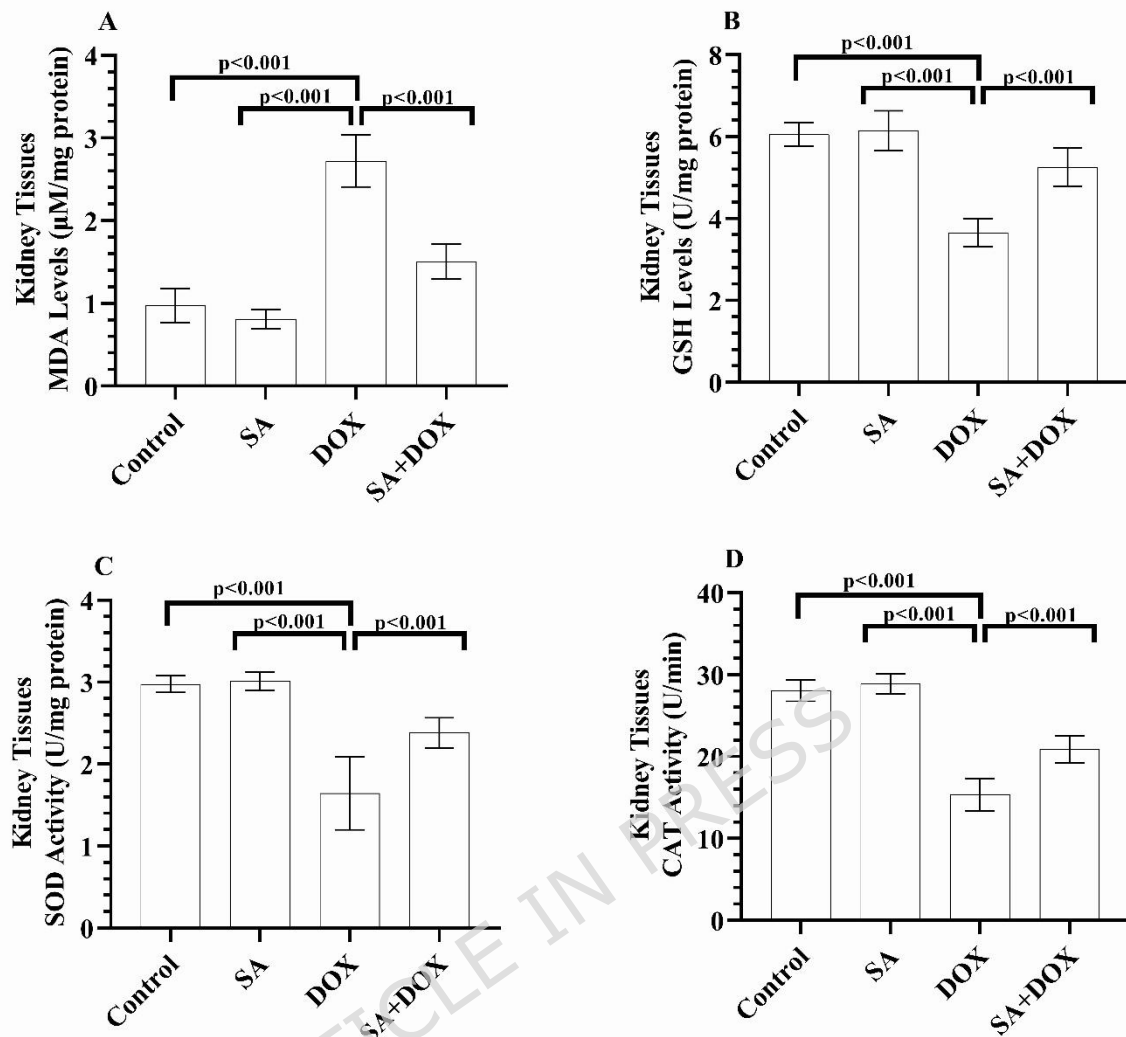


Fig. 2 (A-D). Effect of oral SA pretreatment on LPO and antioxidant profile in the kidney DOX-treated rats. Each bar represents the mean $\pm$ SD of 6 rats. The significance level was set at $p < 0.05$. DOX: doxorubicin; SA: syringic acid; MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; CAT: catalase.

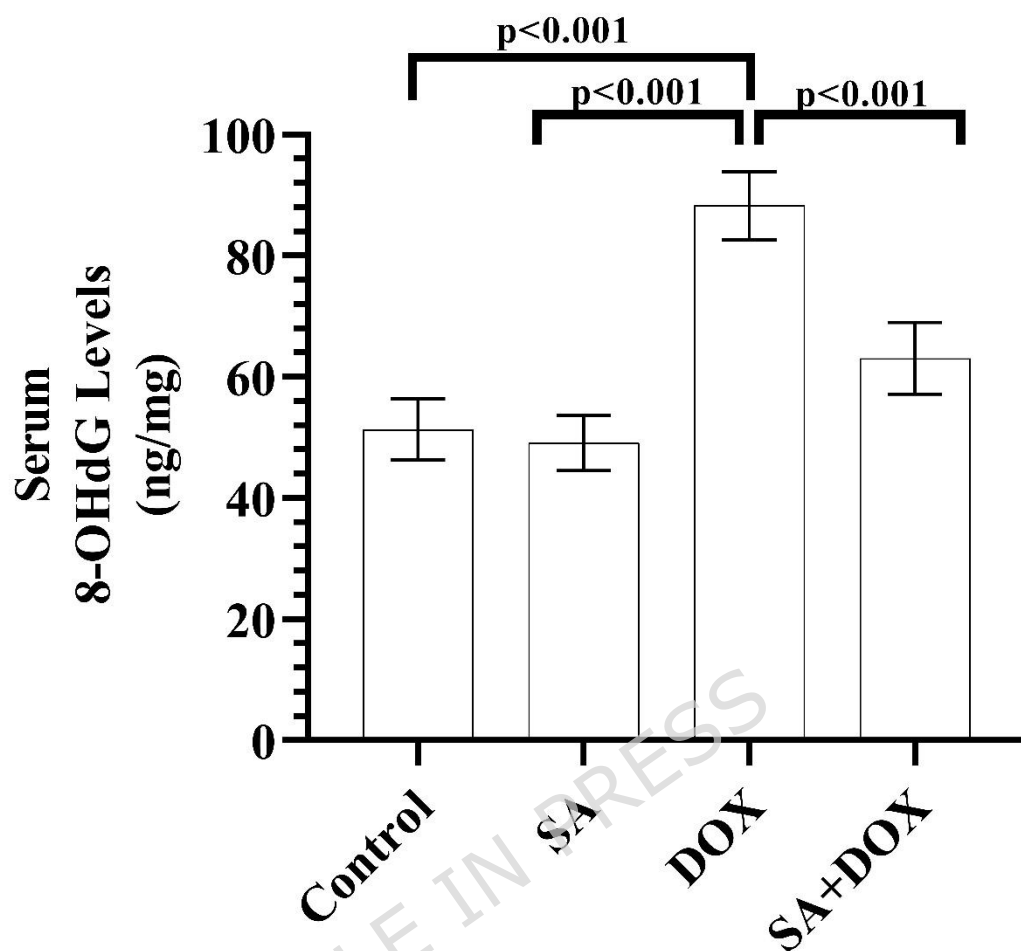


Fig. 3. Effect of oral SA pretreatment on serum 8-OHdG levels in DOX-treated rats. Each bar represents the mean $\pm$ SD of 6 rats. The significance level was set at $p < 0.05$. DOX: doxorubicin; SA: syringic acid; 8-OHdG: 8-hydroxy-2'-deoxyguanosine

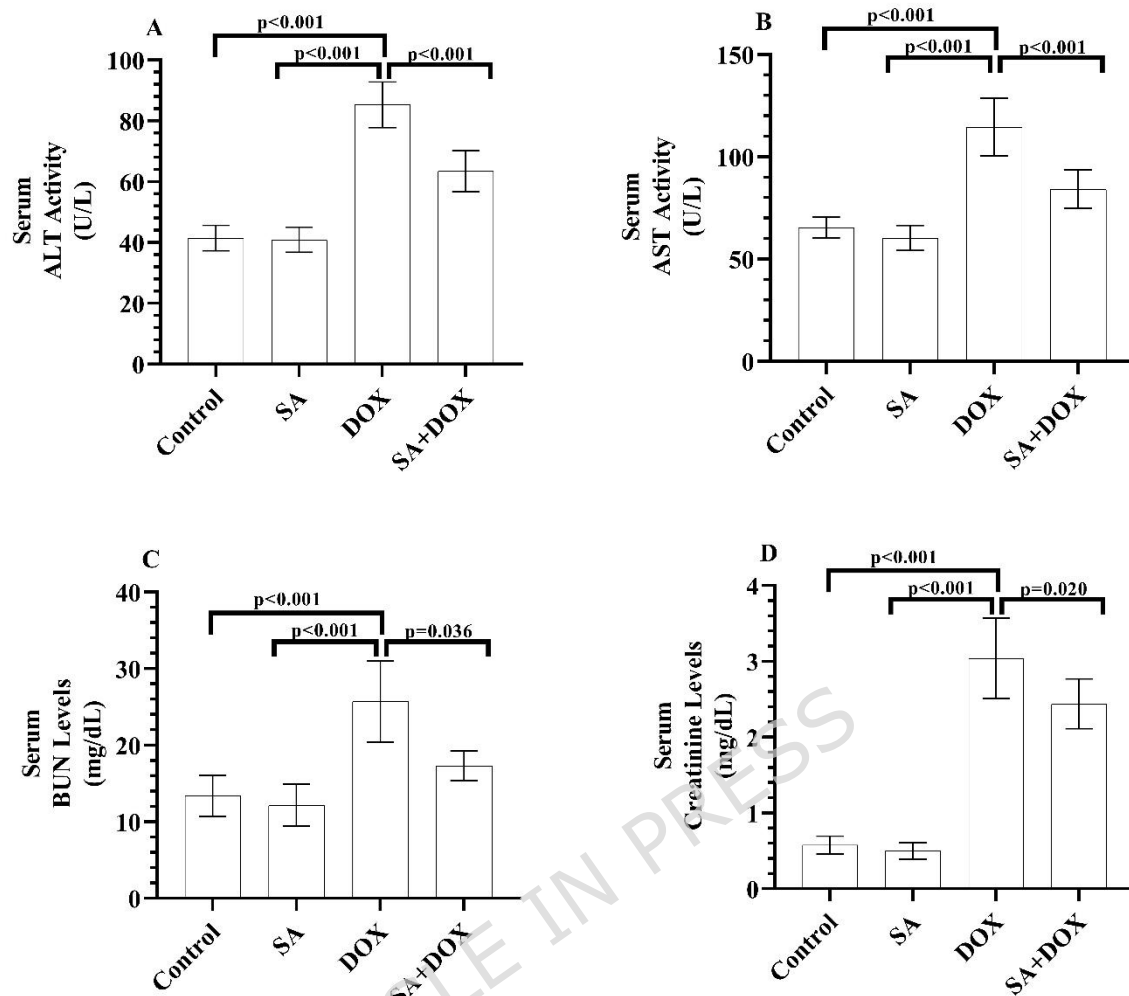


Fig. 4 (A-D). Effect of oral SA pretreatment on hepatic and renal function parameters in the serum DOX-treated rats. Each bar represents the mean $\pm$ SD of 6 rats. The significance level was set at $p < 0.05$. DOX: doxorubicin; SA: syringic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; Cre: creatinine

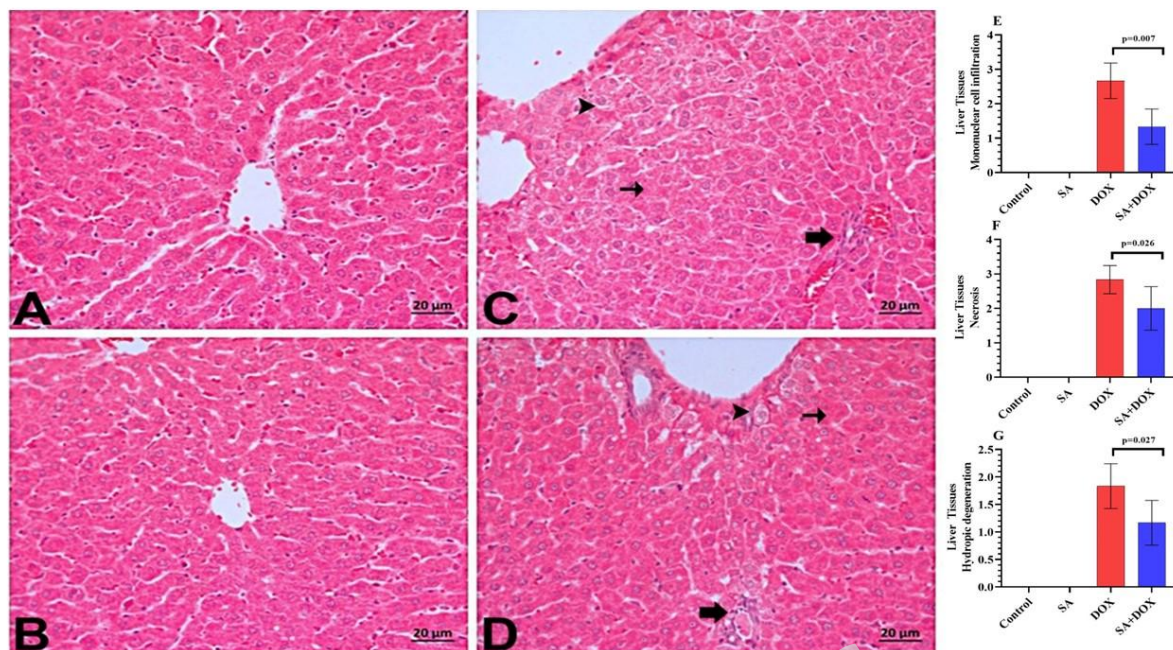


Fig. 5 (A-G). The liver's representative photographic section from control, SA alone, DOX alone, and DOX+SA experimental rats. Control (A) and SA (B) alone indicate no important lesion, characterized by structurally and functionally normal hepatocytes. The hepatic structure of rats administered with DOX alone demonstrated severe mononuclear cell infiltration in the periportal areas (bold arrow), severe necrosis (arrow), and moderate hydropic degeneration (arrowhead) (C). The morphological attributes of the liver treated with SA +DOX appeared to be improving with mild mononuclear cell infiltration in the periportal areas (bold arrow), moderate necrosis (arrow), and mild hydropic degeneration (arrowhead) (D). Histopathological scores (E-G). Each bar represents the mean $\pm$ SD. Hematoxylin and Eosin stained, DOX: doxorubicin; SA: syringic acid.

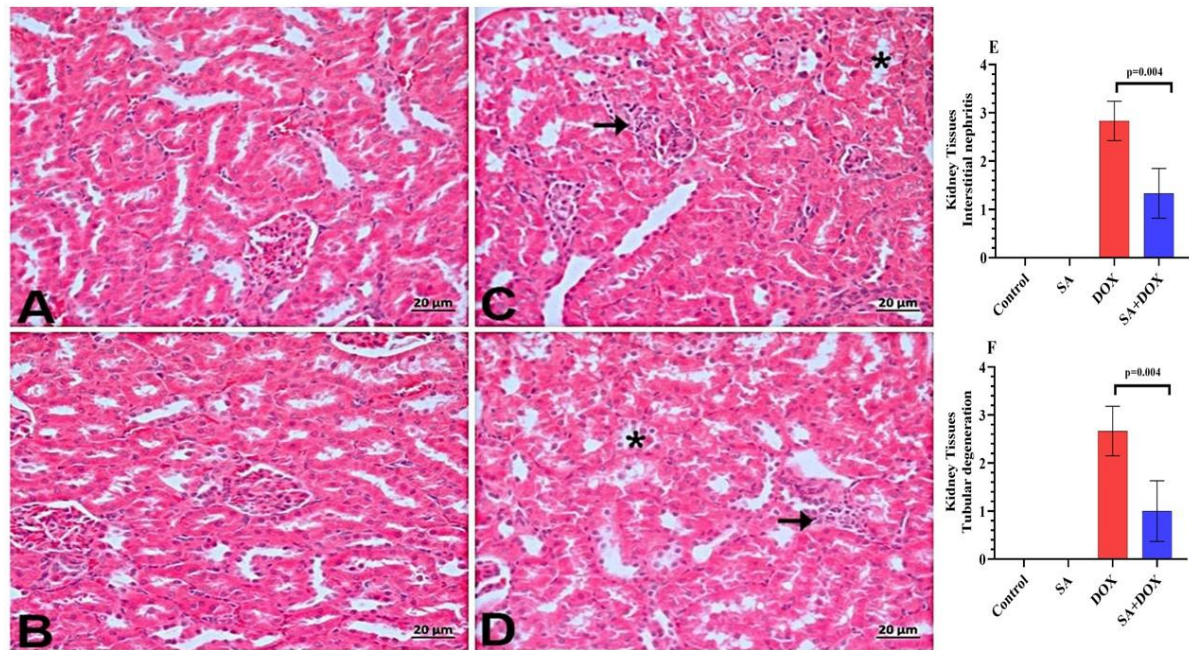


Fig. 6 (A-F). The kidney's representative photographic section from control, SA alone, DOX alone, and DOX+SA experimental rats. Control (A) and SA (B) alone indicate no important lesion. The renal structure of rats administered with DOX alone demonstrated severe mononuclear cell infiltrations in the interstitial areas (arrow) and severe tubular degeneration (asterisk) (C). The morphological attributes of the kidney treated with SA + DOX appeared to improve with mild mononuclear cell infiltration in the interstitial areas (arrow), and mild tubular degeneration (asterisk) (D). Histopathological scores (E-F). Each bar represents the mean $\pm$ SD. Hematoxylin and Eosin stained, DOX: doxorubicin; SA: syringic acid

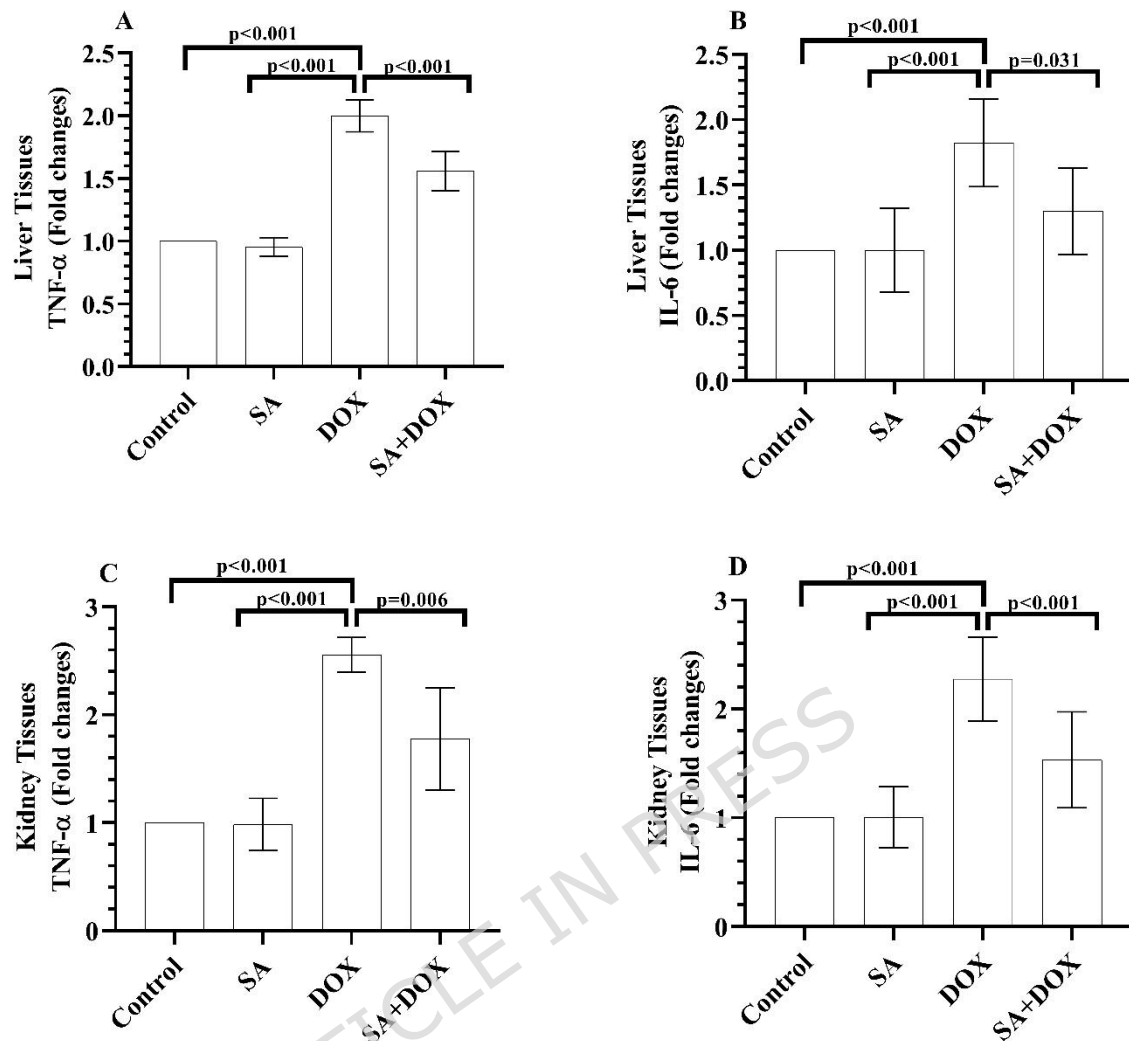


Fig. 7 (A-D). Effect of SA on TNF- α and IL-6 mRNA expression levels in the liver and kidney of DOX-treated rats. Each bar represents the mean $\pm$ SD of 6 rats. The significance level was set at $p < 0.05$. DOX: doxorubicin; SA: syringic acid; TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6.

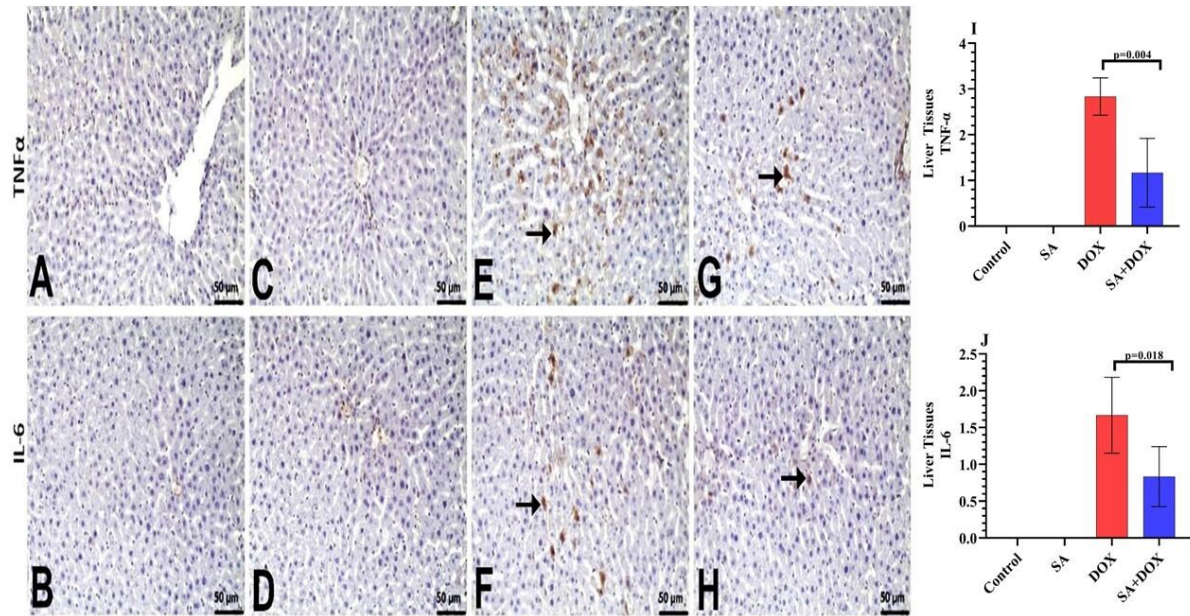


Fig. 8 (A-J). Immunohistochemical analysis of TNF- α and IL-6 in the liver tissues of experimental groups. Scale bar = 50 μ m. (A-D): Immunonegativity in control (A, B) and SA-only treated (C, D) groups. (E-F): DOX group. Severe TNF- α and moderate IL-6 immunopositivity (arrows). (G-H): SA+DOX group. Mild TNF- α and mild IL-6 immunopositivity (arrows). (I, J): Immunohistochemical score. Each bar represents the mean $\pm$ SD. DOX: doxorubicin; SA: syringic acid; TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6.

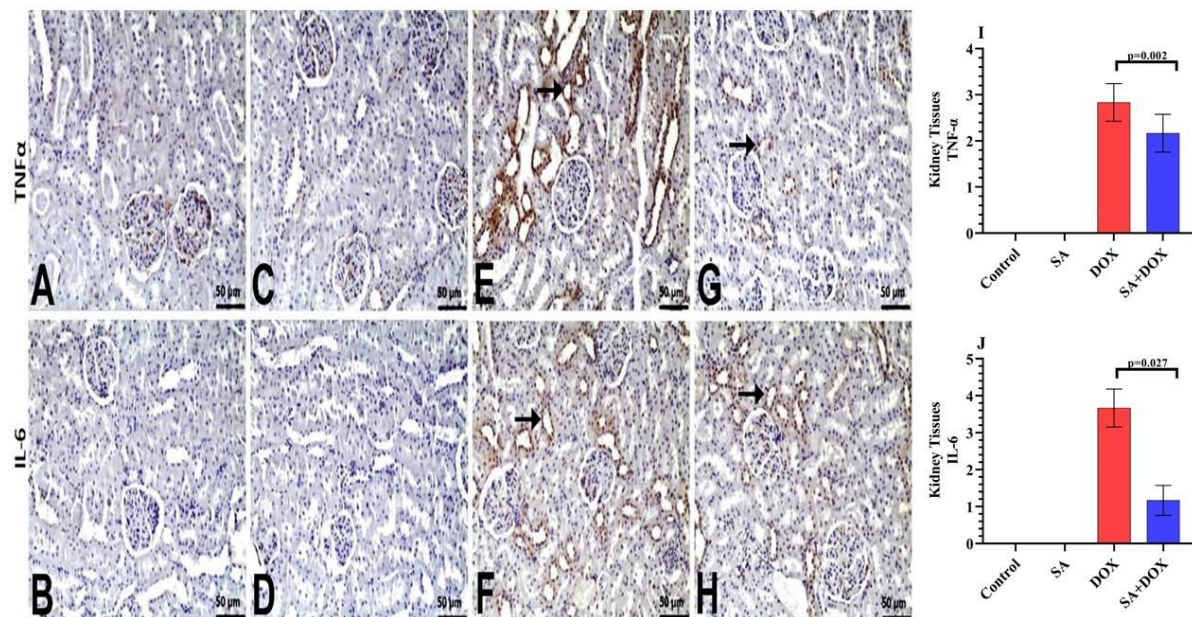


Fig. 9 (A-J). Immunohistochemical analysis of TNF- α and IL-6 in the kidney tissues of experimental groups. Scale bar = 50 μ m. **(A-D):** Immunonegativity in control (**A, B**) and SA-only treated (**C, D**) groups. **(E-F):** DOX group. Severe TNF- α and moderate IL-6 immunopositivity (arrows). **(G-H):** SA+DOX group. Mild TNF- α and moderate IL-6 immunopositivity (arrows). **(I, J):** Immunohistochemical score. Each bar represents the mean $\pm$ SD. DOX: doxorubicin; SA: syringic acid; TNF- α : tumour necrosis factor-alpha; IL-6: interleukin-6.

Table 2. Results of histopathological evaluation of liver and kidney tissues

Histopathological analyses	
Mean $\pm$ Standard deviation	

		Control	SA	DOX	SA+DOX
Liver Tissues	Mononuclear cell infiltration	0	0	2,66±0,66 ^a	1,33±0,51 ^b
	Necrosis	0	0	2,83±0,40 ^a	2,00±0,00 ^b
	Hydropic degeneration	0	0	1,83±0,40 ^a	1,16±0,40 ^b
Kidney Tissues	Interstitial nephritis	0	0	2,83±0,40 ^a	1,33±0,51 ^b
	Tubular degeneration	0	0	2,66±0,51 ^a	1,00±0,00 ^b

Histopathological damage scoring: 0; absent, 1; mild, 2; moderate, 3; severe. a,b; Shows the difference between groups ($p<0.05$). SA, syringic acid, DOX, doxorubicin. Kruskal-Wallis test was used for statistical analysis, followed by post hoc Mann-Whitney U test.

Table 3. Results of immunohistochemical evaluation of liver and kidney tissues.

Immunohistochemical analyses					
Mean ± Standard deviation					
		Control	SA	DOX	SA+DOX
Liver Tissues	TNF- α	0	0	2,83±0,40 ^a	1,16±0,40 ^b
	IL-6	0	0	1,66±0,51 ^a	0,83±0,40 ^b
Kidney Tissues	TNF- α	0	0	3,66±0,51 ^a	1,16±0,40 ^b
	IL-6	0	0	2,83±0,40 ^a	2,33±0,51 ^b

Immunohistochemical scoring: 0; absent, 1; mild, 2; moderate, 3; severe, 4; very severe. a,b; Shows the difference between groups ($p<0.05$). NS: Not significant; SA, syringic acid, DOX, doxorubicin. The Kruskal-Wallis test was used for statistical analysis, followed by the Mann-Whitney U test.

Table 1. Primer sequences

Genes	Forward sequence (5'-3')	Reverse sequence (3'-5')
TNF- α	CACACGAGACGCTGAAGTAG	AACAGTCTGGAAGCTCTGA
IL-6	AGCGATGATGCACTGTCAGA	GGAAGTCCAGAAGACCAGAGC
β -actin	CAGCCTTCCTTCTTGGGTATG	AGCTCAGTAACAGTCCGCCT