BMC Pharmacology and Toxicology

RESEARCH

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Protective effects of Alamandine against doxorubicin-induced liver injury in rats

Ava Soltani Hekmat[1], Maryam Hekmat[2], Sepehr Ramezanipour[3] and Kazem Javanmardi[1*]

Abstract

Background Doxorubicin (DOX), a common chemotherapeutic agent, is often associated with dose-limiting hepatotoxicity. Alamandine, a peptide of the renin-angiotensin system, has shown antioxidant and anti-inflammatory properties that may counteract these adverse effects.

Objective This study investigated the protective effects of alamandine on DOX-induced liver injury in rats.

Methods Male Wistar rats received DOX (3.75 mg/kg intraperitoneally) on days 14, 21, 28, and 35, reaching a cumulative dose of 15 mg/kg. Alamandine (50 µg/kg/day) was administered continuously via mini-osmotic pumps for 42 days. Liver toxicity was assessed through biochemical measurements of oxidative stress markers, inflammatory cytokines, and liver enzymes, as well as histological examination.

Results DOX administration significantly increased serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and malondialdehyde (MDA) levels while reducing superoxide dismutase (SOD) and catalase (CAT) activity. Histological analysis revealed hydropic degeneration and hepatocyte necrosis. Alamandine co-treatment restored SOD and CAT activity, reduced MDA and inflammatory markers, and normalized liver enzyme levels, indicating significant hepatoprotection. Furthermore, treatment with alamandine reduced the expression of pro-inflammatory cytokines IL-6, IL-1, and NF-κB induced by DOX, while p53 expression remained unchanged.

Conclusion Alamandine effectively mitigates DOX-induced hepatotoxicity, demonstrating its therapeutic potential as an adjunctive agent in chemotherapy through its antioxidant and anti-inflammatory mechanisms.

Keywords Alamandine, Doxorubicin, Hepatotoxicity, Liver injury, Chemotherapy, Oxidative stress, Antioxidant, Anti-inflammatory

Introduction

In recent years, chemotherapy has become a cornerstone in cancer treatment, significantly improving patient survival rates and quality of life [1, 2]. Doxorubicin (DOX), an anthracycline chemotherapeutic agent, is highly effective against various malignancies [3]. DOX remains a key cancer treatment, but its use is often limited by dose-related side effects, especially liver toxicity, which has been well-documented in animal studies [4, 5]. DOX-induced liver injury is primarily driven by oxidative stress and inflammation. Through redox cycling, DOX generates reactive oxygen species (ROS), including superoxide

*Correspondence:
Kazem Javanmardi
kjavanmardi@gmail.com
[1]Department of Physiology, Fasa University of Medical Sciences, Fasa, Iran
[2]Department of Dermatology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Student Research Committee, Fasa University of Medical Sciences, Fasa, Iran

anions and hydrogen peroxide, which trigger lipid peroxidation and hepatocyte membrane damage [6, 7]. Concurrently, DOX exposure depletes endogenous antioxidants including superoxide dismutase (SOD), and catalase (CAT), and elevates malondialdehyde (MDA) levels and liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) [8, 9], reflecting extensive hepatic injury. These oxidative insults activate inflammatory pathways, including nuclear factor kappa B (NF-κB), resulting in increased pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and further liver damage [10, 11].

Given this dual oxidative and inflammatory insult, agents with antioxidant and anti-inflammatory properties have been explored to mitigate DOX hepatotoxicity [12]. Alamandine, a novel peptide of the renin-angiotensin system, has gained attention for its protective properties against oxidative stress and inflammation [13, 14]. It is produced through the conversion of Angiotensin A, a variation of Angiotensin II, and is processed via ACE2, or directly from Ang-(1–7). In contrast to Angiotensin II, which induces vasoconstriction and oxidative damage, alamandine acts as a vasodilator with anti-inflammatory properties. The effects are mediated by the Mas-related G protein-coupled receptor D (MrgD), which regulates oxidative and inflammatory responses [15]. While its cardioprotective role is increasingly recognized [16, 17], its potential effects on liver function and DOX-induced hepatotoxicity remain to be investigated.

In light of its pharmacological profile, this study aimed to evaluate the protective effects of alamandine against DOX-induced hepatotoxicity in rats. We investigated whether alamandine's antioxidant and anti-inflammatory mechanisms could attenuate hepatic oxidative stress, inflammatory cytokine expression, and histopathological damage caused by DOX.

Material and method

Chemicals and drugs

Alamandine was sourced from Phoenix Pharmaceuticals Inc. (CA, USA) and prepared as a saline solution for administration. DOX was procured from Tocris Bioscience.

Animals

The study utilized 35 male Wistar rats weighing between 180 and 220 g, which were obtained from the animal facility at Fasa University of Medical Sciences, Fasa, Iran. The care and handling of the animals, as well as all experimental procedures, adhere to the ARRIVE guidelines (https://arriveguidelines.org) to ensure rigorous and transparent reporting of animal research. The study was approved by the Research Ethics Committee of the Faculty of Physiology, Fasa University of Medical Sciences (Ethics committee number: IR.FUMS.REC.1400.018). Rats were housed in standard conditions under a 12-hour light-dark cycle with unrestricted access to food and water. Animals were monitored daily for signs of distress, including severe weight loss (> 20% body weight reduction), lethargy, respiratory distress, and failure to eat or drink. If these conditions were met, humane euthanasia was performed using sodium thiopental (60 mg/kg, intraperitoneally) to ensure deep anesthesia and minimize distress. Throughout the study, animals remained under close observation to ensure their well-being.

Experimental design

Male Wistar rats were randomly divided into five experimental groups ($n = 7$ per group):

- **Group I (Control)**: Rats remained untreated with no surgical or pharmacological intervention.
- **Group II (Sham)**: Animals were administered normal saline as a vehicle through mini-osmotic pumps (model 2006; ALZET Osmotic Pumps, CA, USA) subcutaneously placed between the scapulae for 42 days and received intraperitoneal (i.p.) normal saline on days 14, 21, 28, and 35.
- **Group III (DOX)**: Animals received DOX (3.75 mg/kg, i.p.) on days 14, 21, 28, and 35, reaching a total cumulative dose of 15 mg/kg.
- **Group IV (Alamandine)**: Animals were treated with alamandine for 42 days via mini-osmotic pumps delivering 50 μg/kg/day at a rate of 0.15 μL/h.
- **Group V (DOX + Alamandine)**: Animals received both treatments: alamandine via mini-osmotic pumps (50 μg/kg/day) for 42 days and DOX (3.75 mg/kg, i.p.) on days 14, 21, 28, and 35, with a cumulative dose of 15 mg/kg.

The DOX dosing regimen (cumulative 15 mg/kg over 4 weeks) was chosen based on established rodent models demonstrating reliable induction of hepatotoxicity at doses in a similar range [18]. Our preliminary pilot experiment further confirmed that this regimen produced consistent hepatic injury, validating its use for the current study. The dosage of alamandine used in this study was determined based on our preliminary findings as well as prior research which indicated that this dose confers significant cardiopulmonary and renal protection without toxicity [13, 19]. At the end of the experimental period (day 42), the rats were weighed and euthanized using sodium thiopental. Blood samples were then collected via cardiac puncture under deep anesthesia, adhering to standard and validated protocols to ensure animal welfare and sample reliability. The collected blood samples were centrifuged at 4000 rpm for 10 min, and the

serum was stored at − 80 °C for subsequent biochemical analysis. Key parameters, including ALT, and AST were measured. The liver was excised, washed with phosphate-buffered saline (PBS) containing 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl (pH 7.4), dried with filter paper, and weighed. A portion of the liver was homogenized in PBS and centrifuged at 10,000×g for 20 min at 4 °C. The supernatant was stored at − 20 °C for subsequent analysis of oxidative stress markers, antioxidants, and cytokines using ELISA kits.

Determination of oxidative stress markers

Markers of oxidative stress, MDA, SOD, and CAT were determined in liver tissue homogenates using available kits according to the manufacturer's protocol (Zellbio GmbH, Ulm, Germany).

MDA measurement

MDA levels were determined using the thiobarbituric acid reaction method, employing the MDA assay kit (Cat No: ZB-MDA) from ZellBio GmbH, Germany. For the procedure, 0.15 ml of the sample was added to a sample tube, 0.15 ml of a standard solution was placed in a standard tube, and 0.15 ml of dehydrated alcohol was used in the blank tube. To each tube, 4 ml of a prepared mixed reagent was added, followed by thorough mixing. The tubes were incubated at 95 °C for 40 min, then cooled under running water and centrifuged at 3500 rpm for 10 min. The supernatant from each tube was subjected to colorimetric analysis at 532 nm, with distilled water used for zero calibration [20].

SOD measurement

The activity of SOD was measured using the SOD assay kit (Cat No: ZB-SOD) from ZellBio GmbH, Germany, utilizing the WST-1 method. Control wells were prepared by combining double-distilled water, enzyme working solution, and substrate application solution. Blank wells contained a mixture of double-distilled water, enzyme dilution solution, and substrate application solution. Measurement wells were set up by combining these solutions as per the kit protocol. After incubation at 37 °C, absorbance was measured at 450 nm using a microplate reader [20].

CAT measurement

CAT activity was assessed using the ELISA kit (Cat NO: ZB-CAT). Assay buffer was first added to the wells, followed by a mixture of methanol and the sample. Diluted hydrogen peroxide was then introduced to initiate the enzymatic reaction. The plate was incubated at room temperature for 20 min. The reaction was stopped using potassium hydroxide and catalase periodate. After further incubation, catalase potassium periodate was added to the wells, and absorbance was measured at 540 nm [20].

Immunohistochemistry (IHC)

Immunohistochemistry was employed to assess the local expression and distribution of cytokines and NF-κB in hepatic tissue, providing insight into tissue-specific responses that complement the quantitative data obtained from serum and liver homogenates. Interleukin 6 (IL-6), interleukin 1 (IL-1), P53, and NF-κB expressions were assessed in paraffin-embedded tissues using a standard immunostaining protocol. Briefly, deparaffinization was achieved using xylene followed by rehydration through a graded alcohol series. Subsequently, the slides underwent a 30-minute incubation in a blocking reagent containing 1.5% hydrogen peroxide in methanol. Antigen retrieval was performed using a microwave-based method, followed by a 30-minute incubation in serum. The tissue sections were then immunostained with primary antibodies against IL-6 (catalog no. sc-28343; Santa Cruz Biotechnology, Inc.), IL-1 (catalog no. sc-32294; Santa Cruz Biotechnology, Inc.), P53 (catalog no. sc-81168; Santa Cruz Biotechnology, Inc.), and NFκB (catalog no. sc-48366; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Subsequently, the slides were subjected to three PBS washes and then incubated with secondary antibodies for 30 min. Staining was visualized using 3, 30-diaminobenzidine (Dako liquid DAB color solution), and the slides were counterstained with hematoxylin. An Olympus BX51 microscope (Olympus, Tokyo, Japan) was employed to observe the results. Five random microscopic fields were selected from each slide, and positive staining within each slide was quantified using Image-Pro plus 6.1. Finally, quantitative analysis was conducted in a blinded fashion.

Histopathological analysis

Liver samples were collected from a specific lobe, fixed in 10% formaldehyde, and embedded in paraffin. Section (5 µm thick) were prepared from paraffin blocks and stained with hematoxylin and eosin (H&E). A blinded pathologist examined the histological slides under light microscopy (Olympus BX51; Olympus, Tokyo, Japan). Observations included acute and chronic inflammatory responses, fatty changes, coagulative necrosis, hemorrhage, and hyperemia [21].

Data and statistical analysis

Statistical analyses were conducted using GraphPad Prism software (GraphPad Prism software v6 Inc., La Jolla, CA, USA). All data are presented as mean ± standard deviation (SD). To compare all three groups, a one-way Analysis of Variance (ANOVA) was employed, followed by a Tukey post-hoc test. For the comparison of

Table 1 Effects of DOX, Almandine, or both on liver enzyme levels in male rats

Groups	Control (Group I)	Sham (Group II)	DOX (Group III)	Alamandine (Group IV)	Alamandine + DOX (Group V)
ALT (SGPT), U/L	79.87 ± 7.73	85.70 ± 8.10	286 ± 68.79*	84.27 ± 35.40	109.14 ± 12.17**
AST (SGOT), U/L	110.14 ± 5.40	113.11 ± 5.32	235.12 ± 21.32*	108.28 ± 4.60	137.36 ± 6.72**
ALP, U/L	203 ± 2.78	183 ± 5.85	290 ± 5.61*	189 ± 5.32	198.6 ± 8.25**

Values are expressed as mean ± SD ($n = 7$ per group). *$p < 0.05$ vs. Control group (Group I); **$p < 0.05$ vs. DOX group (Group III)

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase

Table 2 Effects of DOX, Alamandine, or their combination on liver oxidative stress markers in rats

Groups	Control (Group I)	Sham (Group II)	DOX (Group III)	Alamandine (Group IV)	Alamandine + DOX (Group V)
SOD (U/mg protein)	65.7 ± 3.44	63.9 ± 3.97	32.2 ± 6.22*	68.27 ± 3.65	52.04 ± 9.52**
MDA (nmol/mg protein)	6.20 ± 1.85	6.4 ± 2.67	11.06 ± 0.03*	6.7 ± 2.1	8.2 ± 0.012**
CAT (U/mg protein)	62 ± 5.82	65 ± 4.50	93.32 ± 34.61*	59 ± 6.61	72.34 ± 8.31**

Data are expressed as mean ± SD ($n = 7$ per group). *$p < 0.05$ vs. Control group (Group I); **$p < 0.05$ vs. DOX group (Group III)

Abbreviations: SOD: superoxide dismutase; MDA: malondialdehyde; CAT: catalase

Table 3 Body weight, and liver weight in different groups

Groups	Control (Group I)	Sham (Group II)	DOX (Group III)	Alamandine (Group IV)	Alamandine + DOX (Group V)
Final body weight (g)	280 ± 16.14	281 ± 15.08	228 ± 53.17*	284 ± 25.41	260 ± 34.91**
Liver weight (g)	10.32 ± 1.85	10.63 ± 0.40	17.13 ± 2.67*	11.17 ± 0.53	11.2 ± 0.0.0317**

Values are expressed as mean ± SD ($n = 7$ per group). *$p < 0.05$ vs. Control group (Group I); **$p < 0.05$ vs. DOX group (Group III)

baseline and terminal body weights within and between groups, a two-way ANOVA was utilized, followed by a Tukey post-hoc test. Statistical significance was determined at $p < 0.05$.

Results

Effect of DOX and Alamandine on liver enzymes

DOX administration caused significant hepatotoxicity, evidenced by elevated serum liver enzymes and oxidative stress indices. DOX-treated rats (Group III) showed a marked increase in serum ALT, AST, and ALP levels compared to the control group ($p < 0.05$ vs. Group I), whereas the DOX + alamandine group (Group V) had significantly lower enzyme levels than DOX group ($p < 0.05$ vs. Group III). Alamandine treatment alone (Group IV) showed no significant differences in enzyme levels in comparison to the control (Group I), indicating its safety profile. These results are summarized in Table 1.

Effects of different treatments on liver oxidative stress markers

DOX treatment (Group III) resulted in a significant increase in liver MDA levels ($p < 0.05$ vs. Group I), indicating enhanced lipid peroxidation. This was accompanied by significant reductions in SOD, and CAT activities compared to the control group ($p < 0.05$ vs. Group I). Co-administration of alamandine (Group V) significantly lowered MDA levels and improved SOD and CAT activities compared to the DOX group ($p < 0.05$ vs. Group III), suggesting a protective antioxidant effect. Alamandine alone (Group IV) did not significantly affect oxidative stress markers relative to the Control. These findings are summarized in Table 2.

Effect of DOX and Alamandine co-administration on body weight and liver weight

Table 3 summarizes the effects of different treatments on final body weight and liver weight after 42 days. No significant differences in baseline body weight were observed among the groups. However, by the end of the study, rats in the DOX group (Group III) showed a significant reduction in final body weight compared to the control group ($p < 0.05$ vs. Group I). Co-treatment with alamandine (Group V) significantly attenuated this weight loss compared to DOX alone ($p < 0.05$ vs. Group III), indicating a protective effect. Additionally, liver weight was markedly increased in the DOX group ($p < 0.05$ vs. Group I), along with visible signs of hepatomegaly such as puffiness and rounded edges (Fig. 1). Alamandine co-administration significantly reduced liver weight compared to the DOX group ($p < 0.05$ vs. Group III). No significant changes were observed in the Alamandine-only group (Group IV).

Immunohistochemical analysis of cytokines

Representative immunohistochemical staining of liver sections is shown in Fig. 2. The Control group (Group I) exhibited minimal positive staining for IL-1, IL-6, NF-κB, and P53. In contrast, the DOX group (Group III) demonstrated a significant increase in IL-1, IL-6, and NF-κB

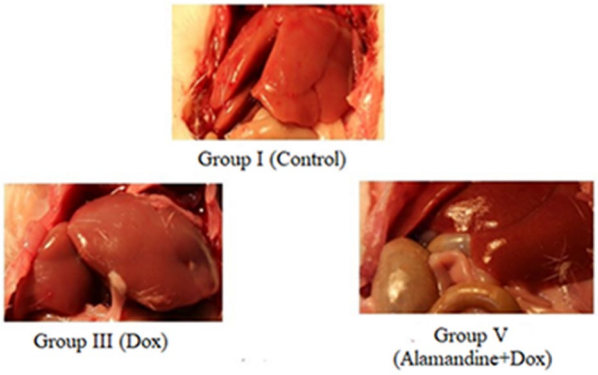

Fig. 1 Gross liver images showing size and weight differences among experimental groups

immunoreactivity ($p < 0.001$ for all), indicating a robust pro-inflammatory response. The DOX + Alamandine group (Group V) also showed elevated expression of these markers compared to the control (Group I), but with significantly lower levels than the DOX group (IL-6: $p < 0.05$; NF-κB: $p < 0.01$; IL-1: $p < 0.001$), suggesting that alamandine mitigates DOX-induced hepatic inflammation. The Alamandine-only group (Group IV) showed staining patterns comparable to the control, with negligible expression of inflammatory markers. P53 expression remained low and statistically non-significant across all groups, indicating it may not play a major role in either DOX-induced liver injury or the protective effect of alamandine. Figure 3 provides a detailed analysis of the immunohistochemical analysis of IL-1, IL-6, P53, and NF-κB in liver tissue.

Histopathological study

All liver sections stained with H&E from different experimental groups were histologically assessed (Fig. 4). The Control group (Group I) showed normal hepatic architecture with well-arranged hepatocyte cords and intact cellular morphology. Similarly, liver sections from the Alamandine group (Group IV) displayed no significant histopathological alterations and closely resembled the control group, indicating no adverse structural effects from alamandine alone. In contrast, the DOX group (Group III) exhibited substantial liver injury, characterized by ballooning degeneration of hepatocytes and evident areas of cell necrosis, as marked by thin arrows in Fig. 4. Notably, the DOX + Alamandine group (Group V) demonstrated only mild hydropic degeneration (thick arrows), with largely preserved tissue structure compared to the DOX group. These findings suggest that alamandine confers a degree of hepatoprotection against DOX-induced toxicity in liver tissue.

Discussion

DOX, a widely used chemotherapeutic agent, is known for its potent anticancer effects. However, its therapeutic benefits are often overshadowed by severe side effects, especially hepatotoxicity, which poses a significant challenge in clinical settings [22]. In our rat model, a cumulative dose of 15 mg/kg DOX caused significant liver injury, aligning with clinical observations that a substantial proportion of patients receiving DOX experience some degree of hepatotoxicity [23]. Consistent with previous studies, we found that doxorubicin DOX-induced oxidative stress and inflammation in the liver, Notably, various antioxidant agents have been shown to mitigate such effects by modulating inflammatory, oxidative, and apoptotic pathways [9, 12, 24]. For instance, Rašković et al. demonstrated that silymarin, a flavonolignan complex extracted from Silybum marianum (milk thistle) and widely used for its hepatoprotective properties, reduced DOX-induced hepatotoxicity by decreasing lipid peroxidation and modulating oxidative stress-related enzymes, including CAT, xanthine oxidase (XO), and glutathione peroxidase (GPX) [25]. Likewise, Nagai et al. reported that taurine, a sulfur-containing amino acid with antioxidant and cytoprotective properties, effectively normalized serum liver enzymes such as AST, and ALT, restored antioxidant defenses including SOD and glutathione (GSH), and downregulated pro-apoptotic markers such as Bcl-2-associated X protein (Bax), Fas cell surface death receptor (Fas), and cleaved caspase-3 in DOX-treated models [24]. Additionally, Motamedi et al. reported that Zingerone, a phenolic compound from ginger, significantly improved liver function in DOX-treated rats by lowering serum AST and ALT levels, reducing MDA content, and restoring the activity of key antioxidant enzymes such as SOD and GPX, alongside improvements in hepatic histology [26]. Furthermore, crocin, a carotenoid derived from saffron, demonstrated pronounced antioxidant and anti-inflammatory effects in a comparable DOX-induced hepatotoxicity model [12]. These findings support the idea that agents like alamandine, with antioxidant and anti-inflammatory properties, can offer meaningful protection against DOX-related liver injury.

Building on this rationale, our objective in this analysis was to investigate the protective effects of alamandine against DOX-induced hepatotoxicity. In this study, we successfully established a rat model through DOX administration. DOX injection resulted in a significant elevation of serum marker enzymes AST, ALT, and ALP compared to the control group, as indicated in Table 1. Notably, ALP, a membrane-associated enzyme, serves as an indicator of liver damage [27]. Serum levels of AST, ALT, and ALP were mitigated with the administration of 50 µg/kg/day of alamandine. These changes in serum enzyme levels were consistent with the observed extent

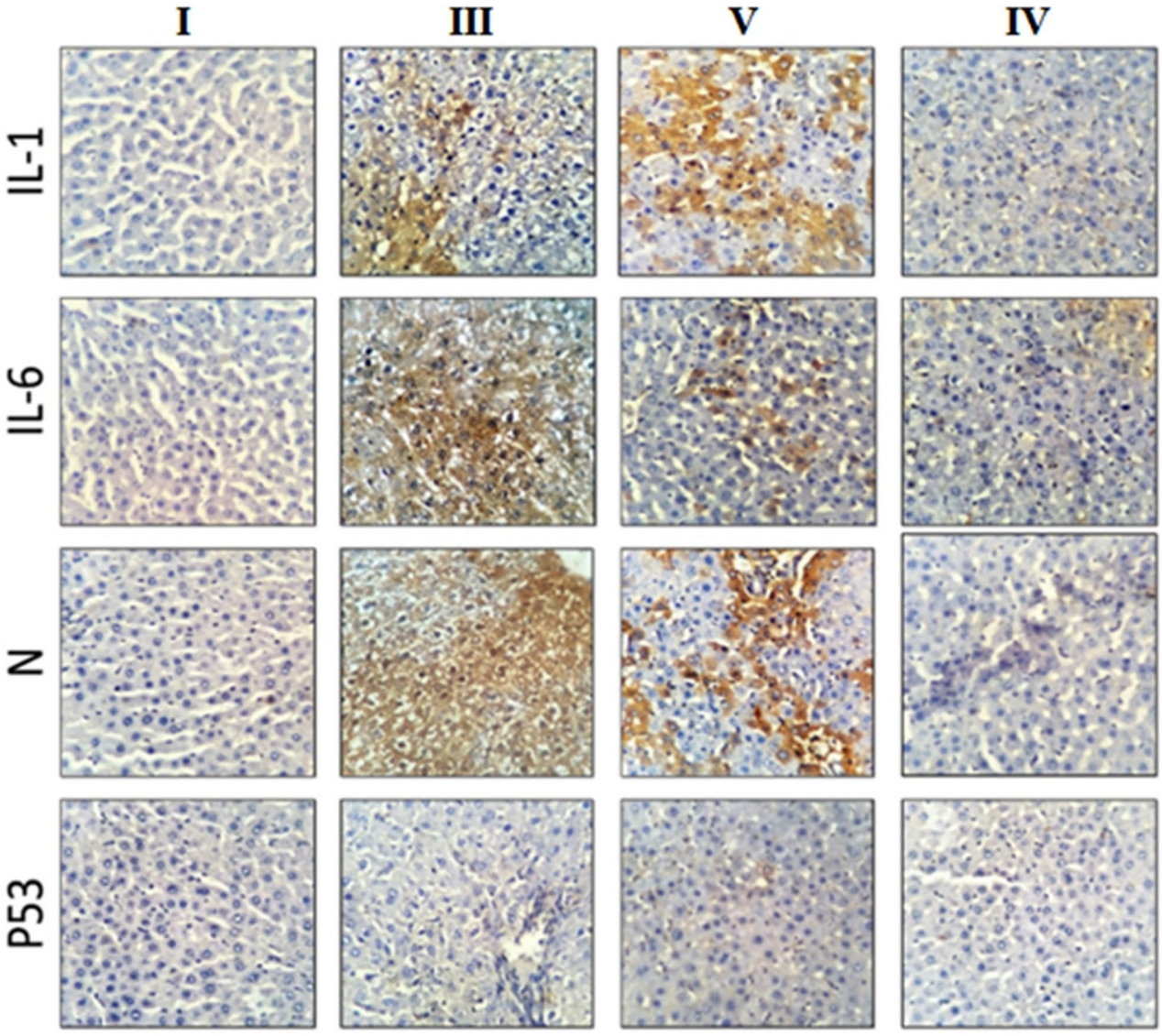

Fig. 2 Immunohistochemical staining of IL-1, IL-6, NF-κB (N), and P53 in liver tissue sections from different experimental groups (magnification ×400). Positive immunoreactivity is indicated by brown staining. Group I (Control) exhibits minimal expression of inflammatory markers. Group III (DOX) demonstrates strong IL-1, IL-6, and NF-κB expression. Group V (DOX + Alamandine) shows reduced staining intensity, indicating the anti-inflammatory effects of alamandine. Group IV (Alamandine) displays staining patterns comparable to the control group

of histological alterations in liver tissue. These elevated levels suggested DOX-mediated hepatic damage, which was further confirmed by the microscopic examination of histological specimens from liver tissue, these findings align with previous reports of histopathological alterations in rat livers caused by DOX [26]. Alamandine demonstrated a significant capacity to ameliorate histological damage, including hepatocyte ballooning degeneration and cell necrosis induced by DOX. These findings collectively suggest that alamandine possesses the capability to protect against DOX-induced hepatotoxicity. Additionally, we observed a significant decrease in the final body weights of DOX-treated rats, despite evident thoracic and abdominal ascites, which are consistent with the findings of a previous study indicating DOX-induced inhibition of protein synthesis, loss of appetite, skeletal muscle wasting, and adipose tissue lipolysis [28–31]. In contrast, a marked improvement in body weight was recorded in animals administered with alamandine. Recent studies have highlighted alamandine's significant role in protecting against hepatic fibrosis [32]. Consequently, alamandine's antioxidant activity could represent one of the potential mechanisms involved in hepatic damage protection. MDA is considered a major oxidative agent of peroxidized polyunsaturated fatty acids, and increased

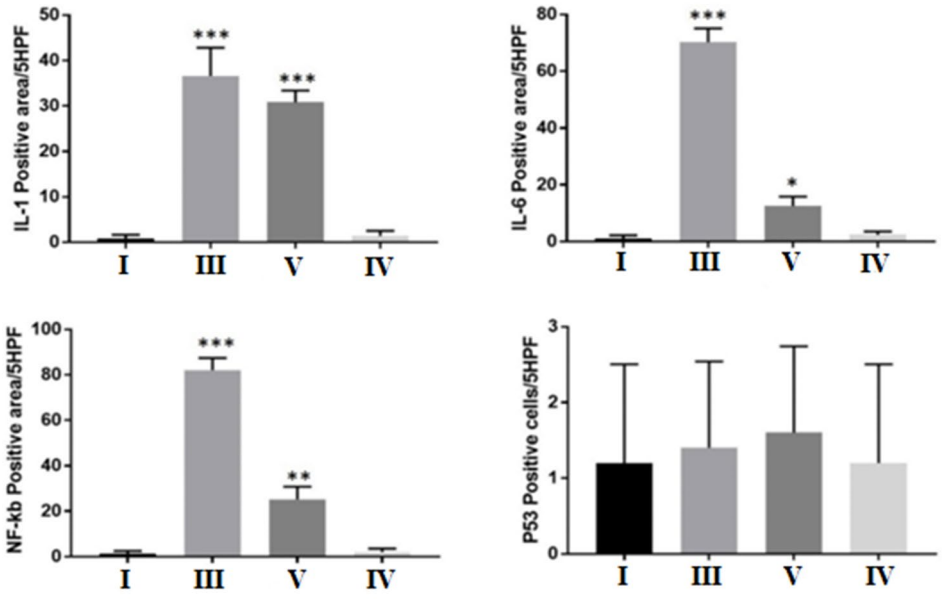

Fig. 3 Immunohistochemical analysis of the IL1, IL-6, P53, and NF-κB in liver. DOX treatment (Group III) significantly increased IL-1, IL-6, and NF-κB immunoreactivity. Co-treatment with alamandine (Group V) markedly reduced IL-6 and NF-κB expression. No significant differences in P53 levels were observed.*Statistical significance: ***$p < 0.001$, **$p < 0.01$, $p < 0.05$ vs. control group

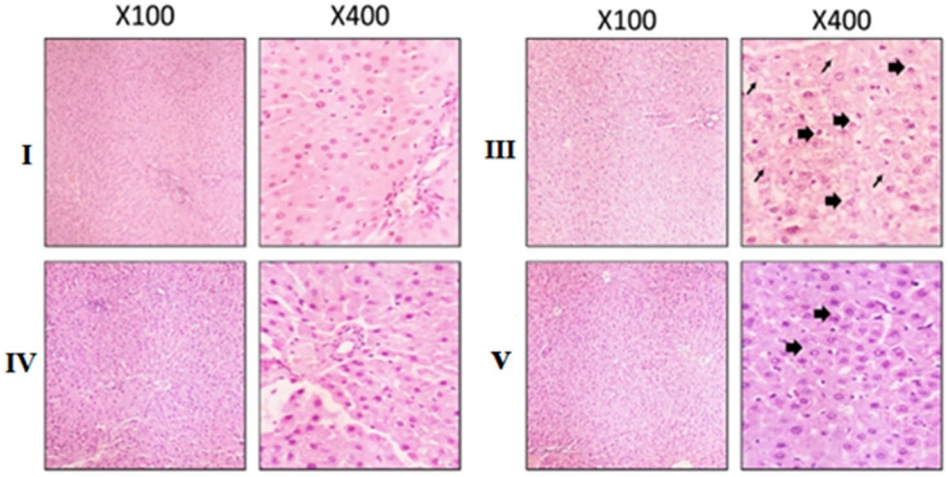

Fig. 4 Histopathologic sections of the liver in different experimental groups (H&E stain, ×100 and ×400). Control (I) and Alamandine (IV) groups show normal hepatic structure. DOX-treated group (III) displays hepatocyte ballooning and necrosis (thin arrows). Co-treatment with alamandine (V) shows mild hydropic degeneration (thick arrows), indicating hepatoprotection. Thick black arrows: hydropic degeneration of hepatocytes; Thin arrows: cell necrosis

MDA levels serve as a crucial predictor of oxidative stress and lipid peroxidation [33, 34].

In our study, MDA levels significantly increased in DOX-treated rats, whereas co-administration of alamandine with DOX led to significantly lower MDA levels compared to DOX-treated rats. Furthermore, DOX not only promotes the production of free radicals within tissues but also diminishes the tissue's ability to detoxify reactive oxygen species (ROS) [35, 36]. Liver tissue is particularly susceptible to free radical damage due to reduced levels of free radical detoxifying enzymes, such as SOD and CAT, following DOX administration [37]. As a result, an imbalance between oxidant and antioxidant agents can contribute to liver damage [38]. In the current study, we observed a significant decrease in SOD and CAT levels following DOX treatment, consistent with previous findings [9]. Consequently, alamandine may act as an effective antioxidant in protecting the liver against damage induced by DOX administration. In this study, we also explored the impact of alamandine on inflammatory

factors released following DOX administration in hepatic tissue. It's important to note that inflammation and oxidative stress are closely intertwined, with oxidative stress triggering the expression of various transcription factors, including NF-κB, as well as inflammatory cytokines [39]. Previous research has indicated that DOX induces an inflammatory reaction by promoting the infiltration of mononuclear cells into liver tissue [6].

Our study demonstrated a consistent inflammatory response in the DOX-treated groups, characterized by significantly elevated levels of IL-6, IL-1, and NF-κB compared to groups treated with alamandine. Notably, alamandine significantly reduced these inflammatory markers, while no significant changes were observed in P53 expression levels between the treated and untreated groups. These findings suggest that alamandine's anti-inflammatory effects may operate through mechanisms independent of P53, possibly involving other pathways of inflammation and oxidative stress. The observed variations in protein levels and gene expression further emphasize the complexity of the inflammatory response, warranting additional investigations to fully elucidate the molecular interactions influenced by alamandine.

One plausible mechanism for the observed hepatoprotective effects of alamandine involves its activation of the Mas-related G protein-coupled receptor D (MrgD). This signaling pathway has been shown to mediate alamandine's antioxidant, anti-inflammatory, and anti-apoptotic actions in various tissues [16, 40, 41]. For instance, Santuchi et al. demonstrated that alamandine, via MrgD signaling, promoted an anti-inflammatory phenotype in macrophages by significantly reducing lipopolysaccharide (LPS)-induced expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1β, and C-C motif chemokine ligand 2 (CCL2) [42]. Additionally, activation of the MrgD receptor by alamandine has been shown to stimulate nitric oxide release and counteract angiotensin II-induced oxidative stress in cardiovascular tissues, highlighting a key mechanism underlying its protective effects [43, 44]. These mechanisms may contribute to alamandine's ability to attenuate NF-κB activation and reduce pro-inflammatory cytokine expression in hepatic tissue, consistent with our observed reductions in IL-6, IL-1, and NF-κB levels in alamandine-treated rats. Moreover, alamandine has demonstrated anti-apoptotic effects in doxorubicin-induced cardiotoxicity models, as evidenced by its ability to reduce caspase-3 expression and preserve myocardial tissue integrity [40]. This anti-apoptotic influence may also play a role in hepatic protection, although direct evidence in liver tissue remains limited. Collectively, alamandine appears to exert its hepatoprotective effects through a combination of antioxidant and anti-inflammatory actions, with a possible contribution from anti-apoptotic mechanisms, primarily mediated via the MrgD receptor pathway.

Our results extend the findings of our previous work on alamandine in DOX-induced nephrotoxicity [19], current study focuses on its role in mitigating hepatotoxicity caused by DOX. While both studies highlight the antioxidant and anti-inflammatory properties of alamandine, this research additionally demonstrates its ability to modulate key hepatic enzymes (AST, ALT, ALP) and reduce oxidative stress markers such as MDA in liver tissue and the role of alamandine in regulating hepatic inflammatory markers such as IL-6, IL-1, and NF-κB, further elucidating its anti-inflammatory mechanisms in liver-specific contexts.

Future directions and study limitations

Although our study provides evidence of the protective effects of alamandine against doxorubicin-induced hepatotoxicity, the potential influence of alamandine on phase I or II enzymes involved in doxorubicin metabolism was not assessed. Future studies are needed to investigate whether alamandine modulates the activity or expression of these enzymes, which could affect doxorubicin's pharmacokinetics and efficacy. Such investigations would provide a deeper understanding of the mechanistic interactions between alamandine and doxorubicin. Additionally, while this study demonstrated the protective effects of alamandine against doxorubicin-induced hepatotoxicity, it is important to address the potential impact of alamandine on the anticancer efficacy of doxorubicin. Although existing evidence suggests that alamandine's antioxidant and anti-inflammatory properties are selective for healthy tissues [14], future studies should directly evaluate its influence on the therapeutic effects of doxorubicin. We recommend conducting investigations in tumor-bearing animal models to assess whether alamandine alters doxorubicin's anticancer activity. These studies could include measuring tumor growth inhibition, apoptosis induction in cancer cells, and long-term survival outcomes. Such experiments would provide definitive insights into the compatibility of alamandine as an adjunctive agent in chemotherapy regimens. This addition would strengthen our understanding of alamandine's therapeutic potential and ensure its broader applicability in clinical settings. Moreover, studies exploring alamandine's impact on the PI3K/Akt pathway, NF-κB signaling dynamics, apoptotic pathways, and fibrogenic pathways in the liver would further clarify how it confers protection.

Conclusion

In conclusion, the present study demonstrates that alamandine can significantly protect the liver from DOX-induced toxicity. By restoring SOD and CAT levels and

reducing MDA, alamandine demonstrated strong antioxidant activity, complemented by its anti-inflammatory effects, including reduced pro-inflammatory cytokines and transcription factors. Its ability to protect liver tissue without altering P53 expression underscores its targeted and potentially safe profile. Alamandine's dual role in enhancing hepatic antioxidant defenses and suppressing inflammation positions it as a promising adjunct to improve the safety of DOX chemotherapy. Future research should explore its broader applications in mitigating chemotherapy-induced organ toxicity and its effects on DOX's anticancer efficacy.

Abbreviations

DOX	Doxorubicin
SOD	Superoxide dismutase
CAT	Catalase
MDA	Malondialdehyde
ALT	Alanine transaminase
ALP	Alkaline phosphatase
AST	Aspartate transaminase
NF-κB	Nuclear factor kappa B
MrgD	Mas-related G protein-coupled receptor D
Ang A	Angiotensin A
PBS	Phosphate-Buffered Saline
H&E	Hematoxylin and eosin
IL-6	Interleukin 6
IL-1	Interleukin 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40360-025-00932-0.

Supplementary Material 1

Acknowledgements

The authors would like to express their sincere gratitude the Fasa University of Medical Sciences, Fasa, Iran for their invaluable support and cooperation throughout the study.

Author contributions

Ava Soltani Hekmat (First Author): Conceptualization, Visualization, Writing- Original draft preparation, Methodology, Project administration. Maryam Hekmat: Data curation, Investigation, Resources, Writing- Original draft preparation. Sepehr Ramezani Pour: Writing- Reviewing and Editing, Visualization, Investigation. Kazem Javanmardi (Corresponding author): Conceptualization, Supervision, Validation, Formal analysis, Project administration.

Funding

The authors declare that no financial support was received for the research, authorship, or publication of this article.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

This study was conducted in full compliance with ethical standards and approved by the Ethics Committee of Fasa University of Medical Sciences (Ethical code: IR.FUMS.REC.1400.018). All animal procedures were performed in accordance with the ARRIVE guidelines (https://arriveguidelines.org/) to ensure rigor, reproducibility, and transparency in animal research.

Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors only used artificially intelligent proofing tools to enhance the lingual experience for the readers. No other generative AI and AI-assisted technology was used in the writing process. After using the tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Competing interests

The authors declare no competing interests.

Received: 16 January 2025 / Accepted: 23 April 2025
Published online: 03 May 2025

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