

# Cardioprotective Effects of $\alpha$ -Asarone Against Hexavalent Chromium-Induced Oxidative Damage in Mice

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**Introduction:** This comprehensive study investigated the therapeutic potential of  $\alpha$ -asarone in mitigating myocardial oxidative damage, primarily induced by hexavalent chromium (Cr(VI)) exposure in mice.

**Methods:** In this experiment, 24 mice were divided into four groups to assess the cardioprotective role of  $\alpha$ -asarone. The study focused on two treatment groups, receiving 25 mg and 50 mg of  $\alpha$ -asarone, respectively. These groups were compared against a control group subjected to Cr(VI) without  $\alpha$ -asarone treatment, and a normal control negative group. The key biochemical parameters evaluated included serum levels of Creatine Kinase-MB (CK-MB) and Troponin I, markers indicative of myocardial damage. Additionally, the levels of Malondialdehyde (MDA) were measured to assess lipid peroxidation, alongside the evaluation of key inflammatory biomarkers in cardiac tissue homogenates, such as Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-1 $\beta$  (IL-1 $\beta$ ).

**Results** Remarkably,  $\alpha$ -asarone treatment resulted in a significant reduction in these markers compared to the control group. The treatment also elevated the activity of cardinal antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD), and reduced the glutathione (GSH). Furthermore, a notable upregulation of Peroxisome Proliferator-Activated Receptor Gamma (PPAR- $\gamma$ ) in cardiac tissue homogenates was observed, highlighting a potential pathway through which  $\alpha$ -asarone exerts its protective effects. Histopathological analysis of cardiac tissues revealed that  $\alpha$ -asarone ameliorated the structural lesions induced by Cr(VI). The study thus provides substantial evidence that  $\alpha$ -asarone ameliorates Cr(VI)-induced cardiotoxicity through a multifaceted approach. It enhances cardiac enzyme function, modulates free radical generation, improves antioxidant status, and mitigates histopathological damage in cardiac tissues. Given these findings,  $\alpha$ -asarone emerges as a promising agent against Cr(VI)-induced myocardial injury.

**Purpose:** This study paves the way for further research into the cardioprotective properties of  $\alpha$ -asarone and its potential application in clinical settings by specifically exploring the protective efficacy of  $\alpha$ -asarone against Cr(VI)-induced cardiotoxicity and delineating the underlying biochemical and molecular mechanisms involved.

**Keywords:**  $\alpha$ -asarone, chromium, mice, cardiotoxicity, oxidative stress, histopathological studies

## Introduction

Cardiovascular disease (CVD) is a pressing global health concern, with its prevalence continually on the rise. Traditional risk factors like high blood pressure, smoking, diabetes, and high cholesterol are well-established contributors to the development of CVD.<sup>1,2</sup> However, these factors do not account for all instances of the disease. Increasingly, research is uncovering the significant influence of environmental, nutritional, and lifestyle behavioral factors on the development of CVD. These aspects play crucial roles in either mitigating or exacerbating the risk of cardiovascular events, underscoring the need for a broader understanding of CVD etiology that encompasses these elements.<sup>3,4</sup>

Another emerging area of concern is the potential link between chronic exposure to heavy metals and cardiovascular disease. Heavy metals such as lead, mercury, cadmium, and arsenic, ubiquitous in the environment due to industrial and technological activities, pose a significant health risk.<sup>5,6</sup> The exact mechanisms through which heavy metals contribute to CVD are not fully understood, but prevailing theories suggest that impaired antioxidant metabolism and oxidative stress might be key pathways. Oxidative stress, resulting from an imbalance between the production of reactive oxygen species and the body's ability to detoxify these harmful intermediates, is a recognized contributor to the pathogenesis of CVD. The hypothesis that long-term exposure to heavy metals can increase cardiovascular risk thus necessitates further investigation into these potential mechanisms.<sup>7</sup>

The implications of this hypothesis are far-reaching. If proven, it could lead to significant shifts in public health policies, particularly in areas with high exposure to heavy metals.<sup>8–10</sup> It also opens up new avenues for preventive strategies and therapeutic interventions targeting heavy metal detoxification and antioxidant defense systems. Future research should focus on elucidating the precise biological mechanisms by which heavy metals influence cardiovascular health and developing effective strategies to mitigate these risks.<sup>11,12</sup> Such studies will be critical in shaping our approach to reducing the burden of CVD, particularly in populations most vulnerable to heavy metal exposure.<sup>13,14</sup>

Chromium, a naturally occurring element found in rocks, soil, and living organisms, is the 21st most abundant element in the Earth's crust.<sup>15</sup> This metal, known for its silvery-gray hue and lustrous quality, presents itself primarily in two forms: trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)).<sup>16,17</sup> Trivalent chromium is an essential micronutrient required for the proper functioning of insulin in the body, playing a pivotal role in carbohydrate, fat, and protein metabolism. It is abundantly found in diverse environmental sources, including natural deposits, and is also introduced into the environment through various industrial processes. Due to its unique physical and chemical properties,<sup>3</sup> chromium is extensively utilized in several industries, ranging from stainless steel production and leather tanning to wood preservation and pigment production. Additionally, chromium compounds are used in the textile and refractory industry, showcasing the metal's versatility and industrial significance.<sup>18</sup>

However, the widespread use of chromium has led to environmental and health concerns, particularly regarding its more toxic form, hexavalent chromium. Unlike trivalent chromium, hexavalent chromium is a potent human carcinogen and is known to cause severe health issues upon exposure.<sup>19</sup> The primary routes of chromium exposure are inhalation, ingestion, and dermal contact, posing risks to both occupational workers and the general population. Environmental contamination with chromium compounds, particularly in water sources, has become a growing concern globally.<sup>20</sup> Studies have shown that hexavalent chromium can cause various adverse health effects, including skin irritation, respiratory problems, and increased risk of lung cancer. The toxicity of chromium, especially in its hexavalent form, is a significant public health concern, necessitating stringent control measures and effective remediation strategies.<sup>21</sup>

Cardiotoxicity, a lesser-known but equally concerning aspect of chromium toxicity, has gained attention in recent scientific studies. Research indicates that exposure to high levels of chromium, particularly hexavalent chromium, can lead to cardiovascular problems.<sup>22</sup> This form of chromium can induce oxidative stress, inflammation, and cellular damage, leading to various cardiac disorders. The mechanism of chromium-induced cardiotoxicity involves oxidative damage to cardiac cells, disturbance in calcium homeostasis, and disruption of normal heart function.<sup>23</sup> This emerging area of research highlights the need for a deeper understanding of chromium's impact on cardiovascular health and the development of protective measures to mitigate its cardiotoxic effects. The growing body of evidence underscores the importance of addressing chromium exposure's environmental and health implications, particularly in areas with high industrial activity.<sup>19</sup>

*Acorus calamus* Linn, belonging to the Acoraceae family, is a perennial, aromatic herb recognized for its medicinal properties that have been harnessed in traditional medicine for centuries.<sup>24</sup> This species, a member of the *Acorus* genus, is particularly renowned for its effectiveness in treating respiratory illnesses and a variety of neurological disorders. The therapeutic potency of *Acorus calamus* primarily resides in its rhizomes, which are rich in bioactive phytochemicals, notably alpha ( $\alpha$ )- and beta ( $\beta$ )-asarone. These compounds are the cornerstone of the plant's pharmacological profile, contributing to its widespread use in traditional healing practices. The presence of  $\alpha$ - and  $\beta$ -asarone in the *Acorus* genus highlights the plant's significant role in ethnomedicine, underpinning its historical and contemporary relevance in natural therapeutics.<sup>25</sup>

The pharmacological activities of  $\alpha$ -asarone, one of the key bioactive constituents of *Acorus calamus*, are diverse and potent, making it a molecule of substantial interest in the field of pharmacognosy. Clinical studies, particularly those conducted in China, have validated the efficacy of  $\alpha$ -asarone in managing respiratory diseases and epilepsy, aligning with its traditional use. The broad spectrum of pharmacological activities attributed to  $\alpha$ -asarone encompasses sedative, neuroprotective, antioxidative, and anticonvulsive effects.<sup>26</sup> These multifaceted properties corroborate the historical use of *Acorus calamus* in traditional medicine and open new avenues for modern therapeutic applications. The neuroprotective and anticonvulsive activities, in particular, suggest its potential utility in managing various neurological disorders, while its antioxidative properties point towards its role in combating oxidative stress-related diseases.<sup>27</sup>

The therapeutic potential of  $\alpha$ -asarone extends beyond its traditional scope, suggesting its applicability in a wide range of disorders. Its sedative properties implicate its use in treating anxiety and sleep disorders, while the neuroprotective effects indicate potential benefits in neurodegenerative diseases like Alzheimer's and Parkinson's.<sup>27</sup> The antioxidative action of  $\alpha$ -asarone offers protection against cellular damage caused by free radicals, a common pathway for many chronic diseases. Furthermore, its anticonvulsive impact provides a basis for developing novel antiepileptic drugs. The extensive range of pharmacological impacts of  $\alpha$ -asarone positions *Acorus calamus* as a valuable resource in drug discovery and development.<sup>28</sup> Integrating traditional knowledge with modern scientific research could lead to developing new therapeutic agents, highlighting the importance of ethnomedicine in contemporary pharmacology.

Given the escalating incidence of Cr(VI) contamination and the consequential health hazards it poses, there exists an urgent requirement for efficacious preventive and therapeutic methodologies. Hence, it is crucial to explore the therapeutic capabilities of  $\alpha$ -asarone in reducing the oxidative damage to the myocardium induced by Cr(VI). This research has substantial clinical implications and could potentially lead to the discovery of innovative cardioprotective substances.

## Materials and Methods

### Chemicals

$\alpha$ -asarone (Sigma Aldrich, St. Louis, MO), CAS number: 2883–98-9/5273-86-9), hexavalent chromium Cr (VI), and all other chemicals used in the experiment were purchased from sigma Aldrich (St. Louis, MO).

### Experimental Animals

The male Swiss albino mice were kept in compliance with the rules set by the Faculty of Pharmacy at Suez Canal University and College of Science, Princess Nourah bint Abdulrahman University. All experimental techniques followed the guidelines for the care and handling of laboratory animals. The ethics council of the Faculty of Pharmacy at Suez Canal University has granted authorization for the study, under license number #202110MA1. A total of 24 mice, aged 7–8 weeks and weighing 20–25 g, were placed in polycarbonate cages and maintained at a constant temperature of  $22 \pm 2$  °C and humidity of 55%. The mice were kept in a regulated environment with a 12-hour cycle of light and darkness, and they had unrestricted access to food and water. Following a one-week period of adjustment to their new surroundings, the mice were divided into four groups at random, with each group consisting of six animals ( $n = 6$  per group).

### Sample Size Calculation

The sample size was calculated using resource equation method (REM) based on ANOVA. According to sample size a minimum of two samples per group was calculated, accordingly, the sample size for each cohort was 6 mice and a total of 24 mice for all experiment, an acceptable range of error degree was calculated ( $X=N-T-B+1= 17$ ), where, N = total number of observations, T = number of treatments, B = number of blocks, X = should be between 10 and 20 for ANOVA.<sup>29,30</sup>

The principal outcome was characterized as a reduction in cardiotoxicity. Standard deviation of cardiotoxicity ranged from 3.20 to 5.20 in the majority of studies assessing the effect of  $\alpha$ -asarone on cardiotoxicity decrease.

## Study Design

In order to generate the Cr(VI) solution, the intended concentration of potassium dichromate is dissolved in distilled water. In order to prepare  $\alpha$ -asarone solution for intraperitoneal injection, it is dissolved in dimethyl sulfoxide (DMSO) or sterile saline solution.

The mice were separated randomly into 4 groups (n =6).

Group 1 healthy mice were supplied with normal food and water (negative control).

Group 2 (Positive Cr(VI) group): Mice were given 0.5 mL of the prepared solution of Cr(VI) via oral gavage daily for 45 days.<sup>1</sup>

Group 3 (25 mg  $\alpha$ -asarone): Mice were given 0.5 mL of the prepared solution of Cr(VI) via oral gavage. Then after 30 minute animals administrated 25 mg of  $\alpha$ -asarone via i.p injection daily for 45 days.<sup>31</sup>

Group 4 (50 mg  $\alpha$ -asarone): Mice were given 0.5 mL of the prepared solution of Cr(VI) via oral gavage. Then after 30 minutes animals administrated 50 mg of  $\alpha$ -asarone via i.p injection daily for 45 days.<sup>31</sup>

## Assembling of Specimens and Formation of Tissue Homogenate

At the conclusion of the trial, the mice were rendered unconscious by administering an intraperitoneal (I.P.) dosage of 100mg/kg ketamine and 10 mg/kg xylazine, and subsequently euthanized by cervical displacement. Venous blood samples were collected, allowed to undergo coagulation, and thereafter subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 15 minutes. The serum samples were stored at  $-20^{\circ}\text{C}$  until they were utilized for subsequent analysis. The heart tissues were excised. The cardiac tissues that were separated were divided into two portions. The first portion of cardiac tissue homogenate was utilized to assess inflammatory biomarkers, including TNF- $\alpha$ , IL-1 $\beta$ , and PPAR- $\gamma$ . The second portion was immersed in a 10% formalin solution at room temperature for 24 hours and thereafter subjected to histopathological analysis for cardiac components after being stained with hematoxylin-eosin (H&E).

## Biochemical Analysis

Based on manufacturing instructions, the serum CK-MB was assessed using Mouse Creatine Kinase MB isoenzyme, CK-MB ELISA Kit (Cat.No: MBS705293, MyBioSource, USA). Serum troponin I was assessed using Mouse CTNI (Cardiac Troponin-I) ELISA Kit (Cat.No: MBS766175, MyBioSource, USA). Serum ATP was assessed using Mouse Adenosine Triphosphate ELISA Kit (Cat.No: MBS724442, MyBioSource, USA). Serum MDA was assessed using Mouse Malondialdehyde (MDA) ELISA Kit (Cat.No: MBS269473, MyBioSource, USA). Serum SOD was assessed using Mouse Superoxide Dismutase ELISA Kit (Cat.No: MBS034842, MyBioSource, USA). Serum GSH was assessed using Mouse reduced glutathione, GSH ELISA Kit (Cat.No: MBS267424, MyBioSource, USA). TNF- $\alpha$  in cardiac tissue homogenate was assessed using Mouse TNF alpha ELISA Kit (Cat.No: MBS825075, MyBioSource, USA). The IL-1 $\beta$  concentration in cardiac tissue homogenate was assessed using Mouse IL-1 $\beta$  ELISA Kit (Cat.No: ab197742, abcam, USA). PPAR- $\gamma$  in cardiac tissue homogenate was assessed using Mouse PPAR- $\gamma$  ELISA Kit (Cat.No: MBS2501353, MyBioSource, USA).

## Histopathology Examination

Cardiac tissue samples were fixed in 10% buffered formalin solution, paraffin-embedded, and sectioned at 5 $\mu\text{m}$  for histological analysis. Light microscopy (Olympus Optical Corp., Tokyo, Japan) was used to inspect the sections for histopathological alterations after being deparaffinized and stained with hematoxylin and eosin (H&E).

## Statistical Analysis

Data were collected and organized in tables and Figures using Microsoft Excel. Data normality, to determine whether the data were parametric or nonparametric, was checked using the Shapiro–Wilk test, Q-Q plot, and boxplot, all at the 0.05 significance level. Accordingly, the data were found to be normally distributed (ie, parametric data) and were presented as mean values and standard deviations. The effects of the four treatment groups on the various biochemical markers were compared using Welch one-way analysis of variance (ANOVA) with Games-Howell multiple comparisons at 0.05

level. The statistical analysis and chart generation were performed using SPSS 24.0 (IBM Corp., Armonk, NY) and GraphPad Prism 8.0.2 (San Diego, CA) software, (GraphPad Software, Inc).

## Results

### Evaluation of Cardiac Enzyme Activity in Serum

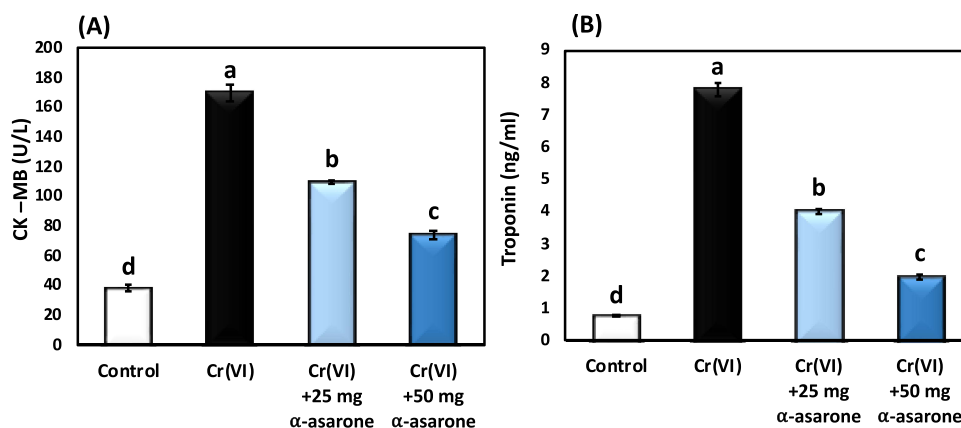
Our data revealed that serum CK-MB, mean (CI95%) (169.39; 155.2–183.6) and Troponin-I (7.78; 7.2–8.4) activities were the highest significant ( $p < 0.001$ ) in the positive control group (Cr (IV)). On the other hand, CK-MB and Troponin I levels were the lowest in the control negative group, and the levels of CK-MB and Troponin I were significantly ( $p < 0.001$ ) decreased in the group administered 25 mg  $\alpha$ -asarone and the group treated with 50 mg  $\alpha$ -asarone in comparison with the control positive group (Cr(IV)). Our data revealed that the group treated with 50 mg  $\alpha$ -asarone showed the most improvement in CK-MB (74.30; 66.5–82.1) and Troponin I (1.98; 1.8– 2.2) levels compared to groups treated with Cr(IV) and 25 mg  $\alpha$ -asarone (Figure 1A and B; Table 1).

### Evaluation of Adenosine Triphosphate (ATP) in Serum

Data showed that the ATP level was the highest significant ( $p < 0.001$ ) in the negative control group. Conversely, the ATP level (Mean; CI) was the lowest in the control positive group (Cr(IV)) (19.20; 18.3–20.1). The level of ATP was increased in the group administered 25 mg  $\alpha$ -asarone (38.19; 18.3–20.1) and the group treated with 50 mg  $\alpha$ -asarone (51.04; 48.6–53.5) in comparison with the positive control group (Cr(IV)). The group treated with 50 mg  $\alpha$ -asarone showed more improvement than the group treated with 25 mg  $\alpha$ -asarone (Figure 2).

### Evaluation of Oxidant/Antioxidant Markers in Serum

Our data revealed that the MDA level (Mean; CI 95%) (Figure 3A) in serum was the highest significant level ( $p < 0.001$ ) in the control positive groups (Cr(IV)) (29.85; 28.1–31.6). Conversely, the MDA level was the lowest in the control negative group. The MDA level was significantly decreased in the group administered 25 mg  $\alpha$ -asarone and the group treated with 50 mg  $\alpha$ -asarone (11.38; 11.0–11.7) in comparison with the positive control group (Cr(IV)). Our data revealed that the group treated with 50 mg  $\alpha$ -asarone showed the most significant ( $P < 0.001$ ) decrease in the MDA level compared to groups treated with Cr(IV) and 25 mg  $\alpha$ -asarone. On the other hand, CAT (12.20; 11.3–13.1), SOD (16.34; 14.5–18.2), and GSH levels (14.29; 12.6–15.9) (Figure 3B and D) in serum were the lowest significant level ( $p < 0.001$ ) in the positive control groups (Cr(IV)). The CAT, SOD, and GSH levels were the highest in the negative control group, while the CAT, SOD, and GSH levels were significantly increased in the group administered 25 mg  $\alpha$ -asarone and the group treated with 50 mg  $\alpha$ -asarone in comparison with the positive control group (Cr(IV)). Our data revealed that the



**Figure 1** Impact of administration of  $\alpha$ -asarone on the activities of (A) creatine kinase-MB (CK-MB) (U/L) and (B) troponin I (ng/mL) in Cr(VI)-induced cardiotoxicity in mice. Results are presented as Mean  $\pm$  SEM (n=6) and tested by Welch-one-way ANOVA followed by Games-Howell multiple comparisons at 0.05 level. a,b bars followed by different letter are significantly different according to DMRTs at 0.05 level.

**Table I** Description of Study Variables in Terms of Mean, Standard Deviation (SD), Confidence Interval for Mean 95%

Variable		Mean	SD	95% Confidence Interval for Mean		ANOVA P-value
				Lower	Upper	
MDA ng/g tissue	C	7.3	1.0	6.2	8.3	<0.001***
	M	29.9	1.7	28.1	31.6	
	T25 mg	17.8	0.7	17.0	18.6	
	T50 mg	11.4	0.3	11.0	11.7	
GSH Pg/tissue	C	51.2	2.8	48.2	54.1	<0.001***
	M	14.3	1.6	12.6	15.9	
	T25 mg	25.1	1.4	23.6	26.5	
	T50 mg	40.2	1.3	38.8	41.6	
SOD U/tissue	C	68.5	4.4	63.9	73.0	<0.001***
	M	16.3	1.8	14.5	18.2	
	T25 mg	28.1	1.6	26.4	29.7	
	T50 mg	47.2	1.9	45.2	49.2	
Catalase U/g tissue	C	53.8	3.9	49.6	57.9	<0.001***
	M	12.2	0.9	11.3	13.1	
	T25 mg	24.7	1.2	23.4	25.9	
	T50 mg	36.1	2.1	33.9	38.3	
TNF - $\alpha$ Pg / g tissue	C	13.8	1.0	12.8	14.8	<0.001***
	M	56.0	2.3	53.6	58.4	
	T25 mg	30.2	1.4	28.8	31.7	
	T50 mg	20.6	1.1	19.4	21.7	
IL - I $\beta$ Pg / g tissue	C	17.7	1.3	16.3	19.1	<0.001***
	M	74.6	3.6	70.8	78.4	
	T25 mg	42.0	3.2	38.6	45.3	
	T50 mg	27.6	1.6	25.9	29.3	
ATP ng / g tissue	C	80.4	3.1	77.2	83.6	<0.001***
	M	19.2	0.9	18.3	20.1	
	T25 mg	38.2	1.3	36.9	39.5	
	T50 mg	51.0	2.3	48.6	53.5	

(Continued)



Table I (Continued).

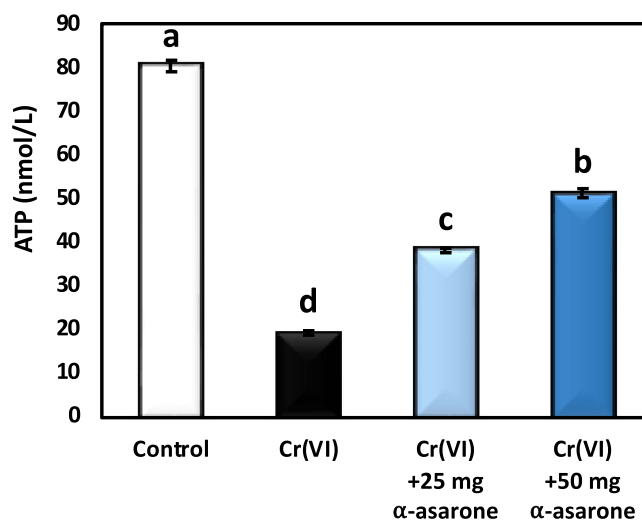
Variable		Mean	SD	95% Confidence Interval for Mean		ANOVA P-value
				Lower	Upper	
PPAR- $\gamma$ ng / g tissue	C	8.5	0.8	7.6	9.3	<0.001***
	M	2.1	0.2	1.9	2.2	
	T25 mg	4.2	0.3	3.9	4.4	
	T50 mg	6.1	0.2	5.9	6.3	
Troponin ng/mL	C	0.8	0.1	0.7	0.8	<0.001***
	M	7.8	0.6	7.2	8.4	
	T25 mg	4.0	0.2	3.8	4.2	
	T50 mg	2.0	0.2	1.8	2.2	
CK -MB U/L	C	38.0	5.3	32.4	43.6	<0.001***
	M	169.4	13.5	155.2	183.6	
	T25 mg	109.6	2.9	106.6	112.7	
	T50 mg	74.3	7.4	66.5	82.1	

Note: \*\*\*mean significant difference at  $P < 0.05$ .

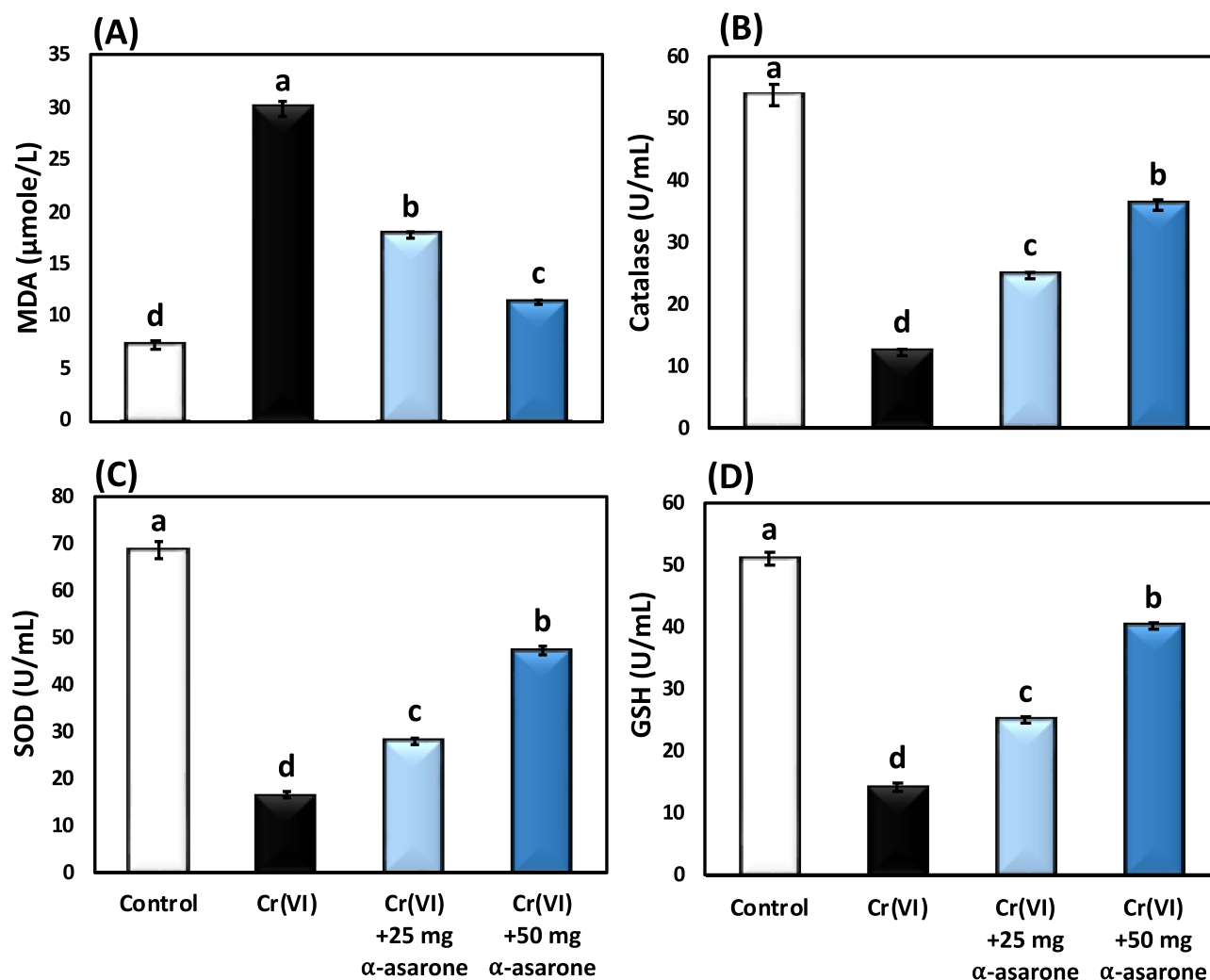
group treated with 50 mg  $\alpha$ -asarone showed a more significant increase in CAT (36.08; 33.9–38.3), SOD (47.23; 45.2–49.2) and GSH (40.20, 38.8–41.6) levels in comparison with groups treated with Cr(IV) and 25 mg  $\alpha$ -asarone.

## Evaluation of Inflammatory Biomarkers in Cardiac Tissue Homogenate

Data revealed that TNF- $\alpha$  (55.98; 53.6–58.4) and IL-1 $\beta$  levels (74.63; 70.8–78.4) (Figure 4A and B) in cardiac tissue homogenate were the highest significant levels ( $p < 0.001$ ) in the control positive groups (Cr(IV)). Conversely, TNF- $\alpha$  and IL-1 $\beta$  levels were the lowest in the control negative group. TNF- $\alpha$  and IL-1 $\beta$  levels were significantly decreased in the group administered 25 mg  $\alpha$ -asarone and the group treated with 50 mg  $\alpha$ -asarone in comparison with the positive control



**Figure 2** Impact of administration of  $\alpha$ -asarone on the level of adenosine triphosphate (ATP) (nmol/L) in Cr (VI)-induced cardiotoxicity in mice. Results are presented as Mean  $\pm$  SEM (n=6) and tested by Welch-one-way ANOVA followed by Games-Howell multiple comparisons at 0.05 level. a,b bars followed by different letter are significantly different according to DMRTs at 0.05 level.



**Figure 3** Impact of administration of  $\alpha$ -asarone on the levels of oxidant/antioxidant markers (A) Malondialdehyde (MDA) ( $\mu$ mol/L); (B) Catalase (CAT) (U/mL); (C) Superoxide dismutase (SOD) (U/mL); (D) Reduced glutathione (GSH) (U/mL) in Cr(VI)-induced cardiotoxicity in mice. Results are presented as Mean  $\pm$  SEM (n=6); and tested by Welch-one-way ANOVA followed by Games-Howell multiple comparisons at 0.05 level. a,b bars followed by different letter are significantly different according to DMRTs at 0.05 level.

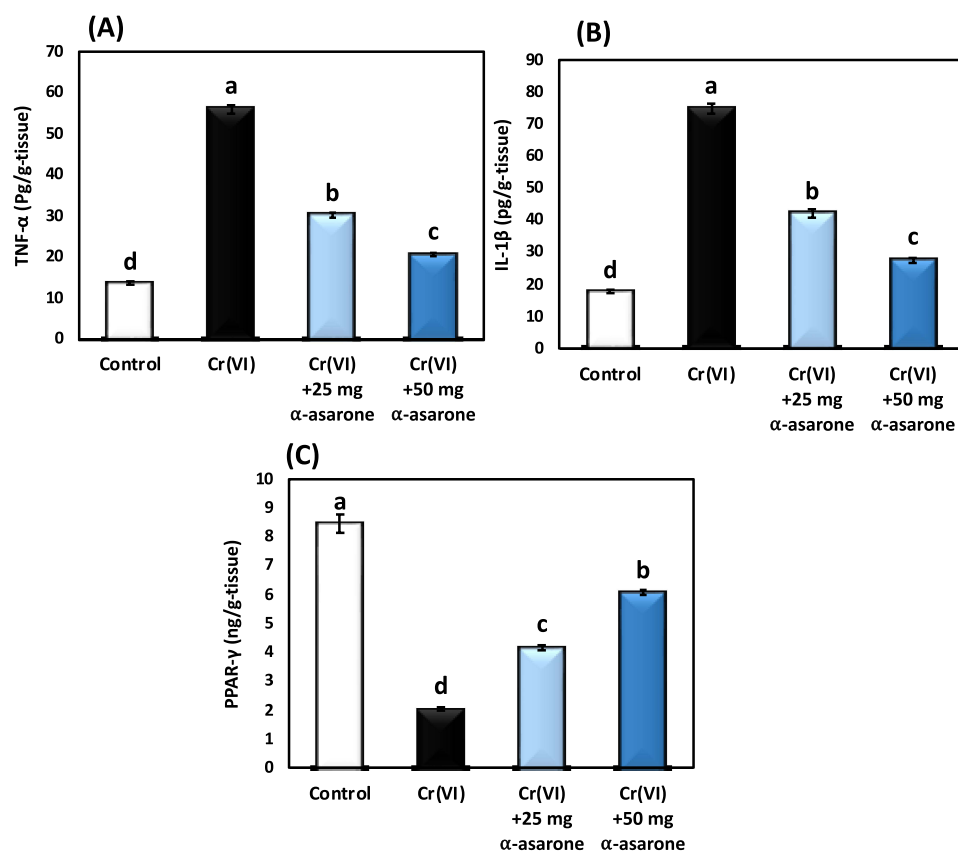
group (Cr(IV)). Our data revealed that the group treated with 50 mg  $\alpha$ -asarone showed more significant ( $p < 0.001$ ) improvement in TNF- $\alpha$  (20.55; 19.4–21.7) and IL-1 $\beta$  (27.59, 25.9–29.3) levels in comparison with groups treated with Cr(IV) and 25 mg  $\alpha$ -asarone. On the other hand, the PPAR- $\gamma$  level (Figure 4C) in cardiac tissue homogenate was the lowest significant level in the control positive groups (Cr(IV)) (2.06; 1.9–2.2).

Due to PPAR- $\gamma$  function in modulating inflammatory pathways and regulating the expression of pro-inflammatory genes, it is regarded as an inflammatory marker. It has been demonstrated that PPAR- activation inhibits the synthesis of inflammatory cytokines and reduces inflammatory responses in a variety of tissues. Due to its participation in the regulation of immune responses and inflammation, it represents a pertinent target for therapeutic interventions that seek to alleviate inflammatory disorders. The PPAR- $\gamma$  level was the highest in the negative control group, and the PPAR- $\gamma$  level was significantly increased in the group administered 25 mg  $\alpha$ -asarone and the group treated with 50 mg  $\alpha$ -asarone in comparison with the positive control group (Cr(IV)). Our data revealed that the group treated with 50 mg  $\alpha$ -asarone (6.07; 5.9–6.3) showed more significant improvement ( $p < 0.001$ ) in the PPAR- $\gamma$  level compared to groups treated with Cr(IV) and 25 mg  $\alpha$ -asarone.

## Histopathological results

Our results in the negative control group revealed uniform cardiac tissue with no evidence of injury (Figure 5A and B). In the control positive (Cr(VI)) group, about 75% of cardiac tissue showed evidence of injury: edema and splitting of





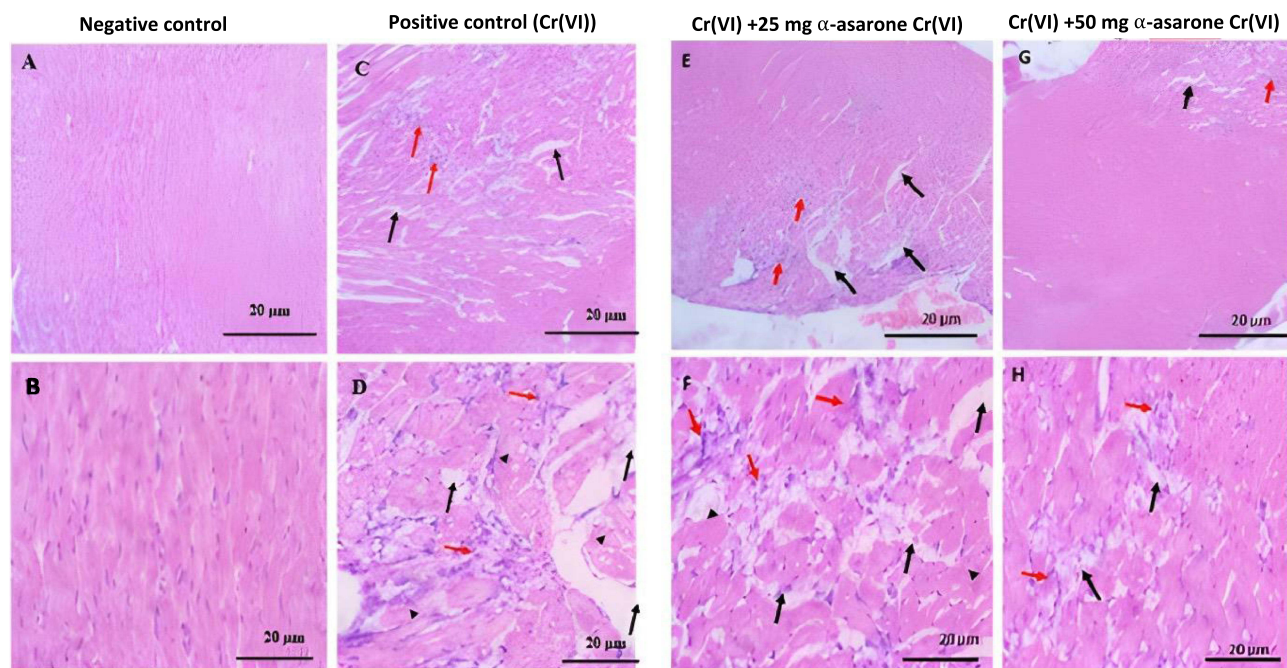
**Figure 4** Impact of administration of  $\alpha$ -asarone on the levels of (A) Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (pg/g tissue), (B) Interleukin-1 $\beta$  (IL-1 $\beta$ ) (pg/g tissue), and (C) Peroxisome proliferator activated receptor gamma (PPAR- $\gamma$ ) (ng/g tissue) in Cr(VI)-induced cardiotoxicity in mice. Results are presented as Mean  $\pm$  SEM (n=6); and tested by Welch-one-way ANOVA followed by Games-Howell multiple comparisons at 0.05 level. a,b bars followed by different letter are significantly different according to DMRTs at 0.05 level.

myofibers, and mononuclear cells infiltrated (Figure 5C and D). In the 25 mg  $\alpha$ -asarone-treated group, there was some reduction in the extent of injury in cardiac tissue, with a slight reduction in myocyte edema and mononuclear cells infiltrate (Figure 5E and F). In the 50 mg  $\alpha$ -asarone-treated group, there was a significant reduction in the extent of injury in cardiac tissue, with a marked reduction in myocyte edema and mononuclear cells infiltrate (Figure 5G and H).

With regard to novelty, although prior research has examined the antioxidant and anti-inflammatory characteristics of  $\alpha$ -asarone, this study adds to the body of knowledge by focusing on its effectiveness in ameliorating myocardial injury induced by Cr(VI). A comprehensive analysis of numerous biochemical, inflammatory, oxidative stress, and histopathological markers enables a profound comprehension of the mechanisms by which  $\alpha$ -asarone exerts its cardioprotective effects. Furthermore, the research emphasizes the potential therapeutic utility of  $\alpha$ -asarone in mitigating cardiovascular damage caused by environmental pollutants. This is an area of natural product-based therapeutics that has received relatively little attention thus far. In general, the results of this research provide significant knowledge regarding the potential therapeutic application of  $\alpha$ -asarone in the treatment of cardiovascular disorders that are linked to exposure to environmental toxins.

## Discussion

In this study, we demonstrated for the first time that  $\alpha$ -asarone protected against Cr(VI)-induced cardiac oxidative damage in mice. The Cr(VI) ion is extremely hazardous and is present in many environments. Recently, this metal's presence in polluted environments has become a growing ecological and public health concern. It has been documented that Cr(VI) has cardiotoxic impacts and may be responsible for other clinical problems. The cardiotoxicity of this metal has been linked to oxidative stress and other variables.<sup>5,21</sup> Our study revealed that Cr(VI) increased CK-MB, Troponin,



**Figure 5** Photomicrograph of cardiac sections showing normal histological pictures in the control negative group (**A** and **B**) (H&E, 10x, 40x), and while in control positive (Cr(VI)) (**C**) about 75% of cardiac tissue showed evidence of injury: edema (Black arrows) and splitting of myofibers, and mononuclear cells infiltrate (Red arrows) (H&E, 10x, 40x). Higher magnification of the control positive (Cr (VI)) (**D**) showed myocyte edema (Black arrows), mononuclear cells infiltrate (Red arrows) and necrotic myocytes (Black arrowheads) (H&E, 40x). (**E** and **F**) showing some reduction in the extent of injury in cardiac tissue, with slight reduction in myocyte edema (black arrows) and mononuclear cells infiltrate (Red arrows) (H&E, 10x, 40x) in the 25 mg  $\alpha$ -asarone treated group. (**G** and **H**) and necrotic myocytes (Black arrowheads). In 50 mg  $\alpha$ -asarone treated group there was significant reduction in the extent of injury in cardiac tissue, with marked reduction in myocyte edema (black arrows) and mononuclear cells infiltrate (Red arrows) (H&E, 10x, 40x). Scale bar in 10x=20  $\mu$ m, in 40x=20  $\mu$ m.

MDA, IL-1 $\beta$ , and TNF- $\alpha$  levels, and decreased GSH, CAT, SOD, ATP, and PPAR- $\gamma$  levels in mice; the following reports<sup>18–36</sup> explain the protective impacts of  $\alpha$ -asarone on the different diseases, and most of these reports are in agreement with our results. Mitochondrial homeostasis ensures steady ATP generation and cellular energy homeostasis. Mitochondrial fusion and fission maintain normal mitochondrial architecture. When mitochondria die, reactive oxygen species (ROS) generation goes up.<sup>32</sup> Many of the most important steps in the development of atherosclerosis and its clinical symptoms are triggered by oxidative stress caused by ROS. The primary enzymes responsible for mopping up oxygen free radicals are CAT and SOD. GSH can prevent peroxides from disrupting and damaging the structure of cell membranes.<sup>33,34</sup>

The research offers valuable understanding regarding the cardioprotective properties of  $\alpha$ -asarone in the context of myocardial infarction induced by Cr(VI). Through the assessment of various biochemical indicators (eg, PPAR- $\gamma$  expression), cardiac enzymes (CK-MB, Troponin I), ATP levels, oxidative stress indicators (MDA, CAT, SOD, GSH), and inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ ), the research investigated the diverse mechanisms by which  $\alpha$ -asarone imparts its protective properties.<sup>35</sup>

The study emphasizes the dose-response relationship and the detrimental impact of  $\alpha$ -asarone on myocardial injury. Greater improvements in biochemical and histological markers were observed in response to higher dosages of  $\alpha$ -asarone (50 mg) administered in comparison to lower doses (25 mg), indicating the presence of a dose-response relationship.

Cr(VI)-induced oxidative damage in the heart is characterized by several distinct features, one of which is lipid peroxidation.<sup>36</sup> This reaction is set in motion by the highly reactive hydroxyl radical, which removes allylic hydrogen from the polyunsaturated fatty acids found in cell membranes.<sup>37</sup> This work confirms the prior findings of Soudani et al,<sup>22</sup> which found that K<sub>2</sub>Cr<sub>2</sub>O treatment raised MDA levels in the hearts of rats. This finding represented oxidative degradation of cardiac membrane lipids caused by elevated hydroxyl radical production due to chromium reduction. Non-specific anion transporters allow Cr(VI) to cross cell membranes easily. Cr(VI) is metabolically converted to Cr(V), Cr(IV), and Cr(III) within the cell. Molecular oxygen is broken down into superoxide anion, then dismuted into hydrogen

peroxide ( $\text{H}_2\text{O}_2$ ).<sup>38</sup> The resulting intermediates react with  $\text{H}_2\text{O}_2$  to produce hydroxyl radicals, which have various harmful impacts, via a Haber-Weiss- or Fenton-like process.<sup>39</sup> Zheng et al<sup>40</sup> found that MDA content in the kidneys of the Cr(VI) groups increased dose-dependency, whereas GSH content and SOD activity decreased. The Cr(VI) treatment groups showed dose-dependent decreases in kidney ATP levels compared to the controls.

Another study aimed to test the theory that chromium exposure causes cardiac oxyradical alterations in rats. Changes in ECG patterns and higher blood levels of CK-MB, lactate dehydrogenase, and cardiac Troponin I were observed in female Wistar rats after chronic Cr(VI) oxide administration for 15 days.<sup>41</sup> Increased lipid peroxidation was revealed to be the mediator of chromium-induced cardiotoxicity, which also resulted in a decrease in GSH levels and the activity of SOD, CAT, and GPx.<sup>42</sup> Elevated levels of enzyme markers of cardiotoxicity (CK, LDH, CK-MB, and Troponin I) in the blood provide biochemical evidence supporting the aforementioned ECG abnormalities.<sup>43,44</sup> Although the lack of Troponin I, a structural protein of foetal and adult skeletal muscle, verifies its cardiac specificity, making it a highly sensitive marker of cardiac damage, all of these enzymes are exceedingly selective for myocardial injury.<sup>45,46</sup> In this approach, an increase in the CK and LDH levels in the blood indicates that these enzymes are being secreted by cardiac myocytes and entering the bloodstream.<sup>35,47</sup> These alterations in enzymatic indicators of damage have been connected to heavy metal-induced cardiotoxicity and other experimental models of heart injury.<sup>45</sup>

Myocardial disease was found to have strong associations with TNF- $\alpha$  and IL-1 $\beta$ , a major proinflammatory cytokine.<sup>48</sup> The study conducted by Mitrov et al<sup>49</sup> assessed the effects of Cr(VI) on platelet activation, inflammation, and lipid peroxidation in rats. In line with our results, the levels of IL-1 $\beta$ , TNF- $\alpha$ , and creatinine in the plasma were considerably higher in the group treated with Cr(VI) compared to the control group.

Consistent with our findings, Yang et al<sup>50</sup> proposed that extended exposure to  $\text{K}_2\text{Cr}_2\text{O}_7$  leads to various adverse effects, such as changes in blood cell counts and oxidative stress, cardiac dysfunction and structural abnormalities, and apoptosis of cardiomyocytes. These effects are triggered by activating the inflammatory response through the nuclear factor kappa B (NF- $\kappa$ B) upstream gene IL-1 $\beta$ . These findings led to the hypothesis that Cr(VI) worsened ventricular dysfunction caused by IL-1 $\beta$ /NF- $\kappa$ B-mediated inflammation.<sup>51</sup>

The histological findings of heart tissue are concordant with our biochemical data. In line with our study and Chaâbane et al,<sup>52</sup> there was a hemorrhage and cytoplasmic vacuolization of cardiac muscle cells in cardiac tissue treated with Cr(VI). Elevated production of ROS after Cr(VI) exposure may contribute to certain morphological abnormalities. Soudani et al<sup>22</sup> also had similar findings on the histopathology.

Our study revealed that  $\alpha$ -asarone ameliorated the level of CK-MB, Troponin, MDA, IL-1 $\beta$ , and TNF- $\alpha$  levels, and increased GSH, CAT, SOD, ATP, and PPAR- $\gamma$  levels in mice, and the following studies are in agreement with our results. Antioxidants can reduce the damage caused by free radicals by neutralizing them. This allows damaged cells to repair and renew.<sup>53,54</sup> Multiple in vitro and animal investigations demonstrated the antioxidant properties of *A. calamus* or  $\alpha$ -asarone. It has been established that the plant's bioactive compounds can scavenge free radicals by modulating the activity of particular endogenous enzymes and non-enzymatic components. Asarone's antioxidant properties have been demonstrated against oxidative stress brought on by various factors, such as ischemia-induced brain infarction,  $\beta$ -radiation, and noise stress.<sup>55</sup>

The neurobehavioral impacts of asarone were significantly improved in rats that had undergone brain ischemia and reperfusion. Asarone injection significantly boosted antioxidant activity (GSH, GPx, and CAT), which bolstered the defensive mechanism against cerebral ischemia. Brain ischemia injury recovery may be aided by reperfusion, which restores the brain's antioxidant balance.<sup>56</sup>  $\alpha$ -asarone inhibited pentylene-tetrazol-induced seizures in zebrafish by activating PPAR- $\gamma$ .<sup>57</sup> Furthermore, another study found that  $\alpha$ -asarone induced upregulation of GLT-1 and PPAR- $\gamma$  expression in PWMI rats.<sup>58</sup>

Our results in the negative control group revealed uniform cardiac tissue with no evidence of injury, while in the control positive (Cr(VI)) group, about 75% of cardiac tissue showed evidence of injury. In the  $\alpha$ -asarone-treated group, there was some reduction in the extent of injury in cardiac tissue, with a marked reduction in myocyte edema and mononuclear cell infiltrate. Myocardial tissue slices from Sham group mice showed normal histological structure under light microscopy, supporting our findings. Tissues from the I/R group rats showed myocardial hypertrophy and

mitochondrial loss. Heart muscle structure was well-conserved, and inflammatory cell infiltration was modest in rats administered  $\beta$ -asarone, according to the results of an interesting tissue analysis.<sup>59</sup>

According to another experiment, therapy with  $\alpha$ -asarone dramatically decreased the size of the central lesion area in rats. Patients administered  $\alpha$ -asarone showed less tissue loss and smaller lesions one week after injury compared to those without treatment. The results demonstrated that  $\alpha$ -asarone can prevent tissue damage while also minimizing the size of a lesion cavity.<sup>60</sup>

The primary objective of another investigation was to assess the protective effect of asarone against experimental rat hepatotoxicity induced by CCL4. Serum concentrations of AST, ALT, total bilirubin, and albumin were measured, along with hepatic hydroxyproline, GSH, and MDA. Treatment of rodents with CCL4 led to a reduction in body weight and an increase in liver weight, whereas treatment with asarone maintained the weights of the organs and liver, respectively. Rats that were administered asarone demonstrated a decrease in oxidative stress, a dose-dependent inhibition of cytokine release, and protection against hepatotoxicity. It can be concluded from the study that asarone exhibits a protective effect against the hepatotoxicity induced by CCL4.<sup>61</sup>

When supplied concomitant with cardiotoxicity, intensive studies on the impact of  $\alpha$ -asarone on cardiotoxicity drugs are needed. More efforts need to be done to translate the findings into clinical trials on antioxidant combinations like  $\alpha$ -asarone for the prevention/improvement of oxyradical overload and cardiac diseases caused by metals and other oxidants.

## Conclusions

In summary, our research illuminates the potential protective effects of  $\alpha$ -asarone against heart injury induced by Cr(VI).  $\alpha$ -asarone has been demonstrated to effectively mitigate oxidative stress in the heart, a critical factor in preventing cardiac toxicity associated with Cr(VI). The results of our study illustrate the cardiac protection capacity of  $\alpha$ -asarone in rodents and reveal the underlying mechanisms that drive its effects. This preventive effect is due to the potent antioxidants in  $\alpha$ -asarone. Based on our research, it appears that  $\alpha$ -asarone may have potential as a component in the development of novel supplements aimed at mitigating the adverse cardiovascular effects of Cr(VI) exposure.

The clarification of the underlying mechanisms enhances the therapeutic potential of the subject matter. To facilitate the clinical application of our in vivo findings, it is suggested that additional clinical research be conducted. Further research may be conducted to investigate the effectiveness and safety of supplementing human subjects with  $\alpha$ -asarone when they are exposed to environmental pollutants. This would facilitate the creation of innovative interventions aimed at protecting cardiovascular health. In essence, our research makes a valuable contribution towards the progression of efficacious plant-based remedies that target cardiovascular hazards induced by environmental factors.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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