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Research Article

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Cardiac Histopathological Changes Induced by N-acetyl-para-aminophenol and the Protective Effect of Vitamin C in Albino Rats

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Abstract

N-acetyl-para-aminophenol (NAPA), or paracetamol, is a widely used antipyretic and analgesic; however, high doses can harm the heart, mainly through oxidative stress. This study aimed to examine the cardiac histopathological changes induced by NAPA and the protective effect of vitamin C (VC) in albino rats. Rats were randomly divided into five groups: control, VC (500 mg/kg b.w. for two weeks), NAPA (500 mg/kg b.w. for two weeks), (NAPA + VC) co-treated for two weeks, and a protective group that received VC for one week before NAPA exposure for one week. Histological examination showed that NAPA induced significant myocardial alterations, including disorganized myocardial fibers, cellular deterioration, and vascular congestion. Moreover, the co-treated rats displayed reduced histopathological alterations, while the protective group showed even stronger protection for cardiac tissue. These results suggest that VC exerts a cardioprotective effect against NAPA-induced heart injury, possibly due to its antioxidant properties. Furthermore, the findings highlight the potential of VC as both a preventive and therapeutic strategy against NAPA-related cardiotoxicity.

Keywords: N-Acetyl-Para-Aminophenol, Vitamin C, Cardiac Tissue, Rats, Histopathology.

INTRODUCTION

Cardiovascular diseases remain a major global health concern. Among the contributing factors, drug-induced myocardial injury has become increasingly recognized as an important cause of cardiovascular morbidity and mortality (Mladěnka et al., 2018; Netala et al., 2024; Destere et al., 2024). N-acetyl-para-aminophenol (NAPA), commonly known as paracetamol, is one of the most widely used analgesic and antipyretic agents. Its popularity is due to its effectiveness and its generally safe profile when used at therapeutic doses (Prescott, 2000; Ayoub, 2021). However, excessive or prolonged use of NAPA can result in significant organ toxicity (Kennon-McGill & McGill, 2017). While hepatotoxicity is well-documented (Ramachandran & Jaeschke, 2019), emerging evidence suggests that NAPA can also induce cardiotoxicity, resulting in structural and functional myocardial alterations (Jin & Park, 2012; Elduob et al., 2023A). Moreover, the cardiotoxic effects of NAPA are largely mediated through oxidative stress (OS) (Jin & Park, 2012). Overproduction of reactive oxygen species (ROS) can overwhelm endogenous antioxidant defenses, leading to lipid peroxidation (LPO), protein oxidation, DNA damage, and ultimately, cardiomyocyte death (D'Oria et al., 2020). Additionally, histopathological studies in an-

imal models have demonstrated myocardial deterioration, necrosis, inflammatory penetration, and disruption of normal cardiac architecture following high-dose or chronic NAPA exposure (Ralapanawa et al., 2016; Elduob et al., 2023A). This oxidative injury underscores the need for interventions that can modulate redox balance and protect the myocardium.

Antioxidants have been extensively investigated as protective agents against drug-induced myocardial injury (Han et al., 2024). Vitamin C (VC; ascorbic acid), a water-soluble antioxidant, neutralizes free radicals, regenerates other antioxidants, and stabilizes cellular membranes (Carr & Frei, 1999). Experimental studies have shown that supplementation with VC can reduce the histopathological changes caused by NAPA in both hepatic and cardiac tissues (Adeneye & Olagunju, 2008; Shati et al., 2022). These findings suggest that VC may exert cardioprotective effects (Zheng et al., 2024).

Although previous studies have documented NAPA-induced organ toxicity and the protective effects of antioxidants in hepatic and renal tissues (Modo et al., 2015; Shati et al., 2022; Okiljević et al., 2024), limited research has specifically addressed the histopathological impact of NAPA on cardiac tissue and the potential cardioprotective role of VC. Therefore, this study aims to fill this knowledge gap by systematically evaluating myocardial alterations and the efficacy of vitamin C intervention in albino rats.

MATERIALS AND METHODS

Experimental Animals and Design

Adult albino rats of comparable body weights were obtained from the Central Animal House, College of Veterinary Medicine, University of Omar Al-Mukhtar, Al-Beyda, Libya. Animals were housed under standard laboratory conditions (22–25 °C, 12 h light/12 h dark cycle) with free access to food and water. All experimental procedures were conducted in accordance with institutional and international ethical guidelines for animal experimentation. The experimental protocol was performed in accordance with our previous studies on hepatic and testicular tissues (Alshailabi et al., 2021; Alshailabi et al., 2024), using the same procedures to evaluate cardiac tissue. After a one-week acclimatization, rats were randomly assigned to five groups (n = 7 each):

- Control group: received distilled water orally throughout the experimental period.
- VC group: received vitamin C (500 mg/kg/day, orally) for two weeks (Adeneye and Olagunju, 2008).
- NAPA group: received NAPA (500 mg/kg/day, orally) for two weeks (Modo et al., 2015).
- VC + NAPA group: received both VC and NAPA simultaneously at the above doses for two weeks.
- Protective group: received VC (500 mg/kg/day, orally) for one week, then NAPA was administered (500 mg/kg/day) for one week. At the end, animals were humanely euthanized, and heart samples were collected for histopathological investigation.

Histopathological Examination of Cardiac Tissue

Heart tissue samples were collected from all experimental groups and immediately fixed in 10% formalin. The tissues were then processed using routine methods and embedded in paraffin. Sections of 5 µm thickness were observed under a light microscope (Lillie, 1954). Myocardial lesions were also assessed using a semiquantitative scoring system: absent (–), mild (+), moderate (++), or severe (+++) according to Moshaie-Nezhad et al. (2021).

RESULTS

Histopathological Findings

Control and VC Groups: Under light microscopy, cardiac sections from control rats displayed the typical histological structure of cardiomyocytes, which included limited interstitial connective tissue (CT) and a sparse distribution of interstitial fibroblasts between well-organized and branched cardiomyofibers with centrally located oval nuclei (Figure 1). Similarly, rats treated with VC alone for two weeks presented myocardial histology comparable to that of control rats (Figure 2).

NAPA Group: Rats treated with N-acetyl-p-aminophenol (NAPA) alone demonstrated pronounced histopathological alterations. Observed changes included necrotic or damaged tissue, cellular oedema, irregular arrangement of cardiac fibers, perivascular mononuclear cell infiltration, sarcoplasm fragmentation, and blood vessel congestion. Additionally, hyaline deterioration of myocardial fibers and focal infiltration of inflammatory cells were also noted in Figures 3-5.

VC + NAPA Group: Co-administration of VC with NAPA moderately ameliorated these histopathological changes. Cardiac sections revealed fewer deteriorating fibers, mild interstitial hyperemia, limited necrosis, and minor inflammatory cell infiltration (Figures 6–8). Some sections still showed fragmented or necrotic fibers and hyaline deterioration, but overall tissue integrity was improved compared with the NAPA group.

Protective Group: Rats pretreated with VC before NAPA administration revealed the most preserved myocardial architecture. Myofibers displayed improved organization with minimal degenerative changes, accompanied by mild hyperemia of interstitial vessels. Only limited focal necrosis was detected (Figures 9–10), indicating substantial defense against NAPA-induced cardiotoxicity.

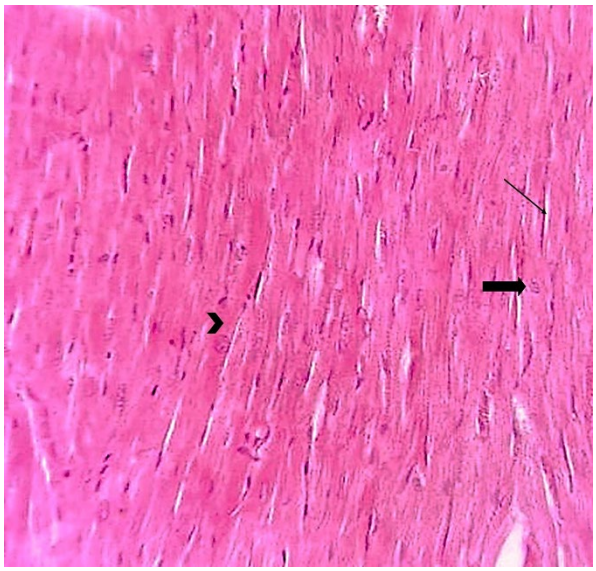

Figure (1). The heart of control rats displays the typical histological structure of cardiomyocytes, which included limited interstitial CT and a sparse distribution of interstitial fibroblasts (thin arrow) between well-organized and branched cardiac myofibers (head arrow) with centrally located oval nuclei (thick arrow). (H&E-stained section, ×400).

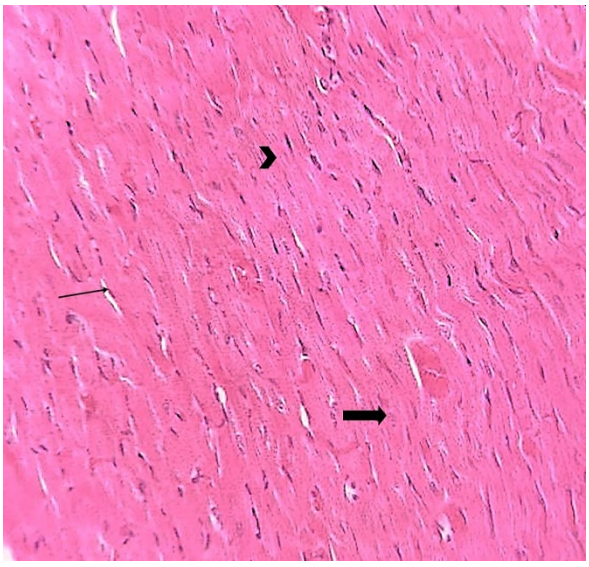

Figure (2). The heart of VC rats displays the typical histological structure of cardiomyocytes, which included limited interstitial CT and a sparse distribution of interstitial fibroblasts (thin arrow) between well-organized and branched cardiac myofibers (head arrow) with centrally located oval nuclei (thick arrow). (H&E-stained section, ×400).

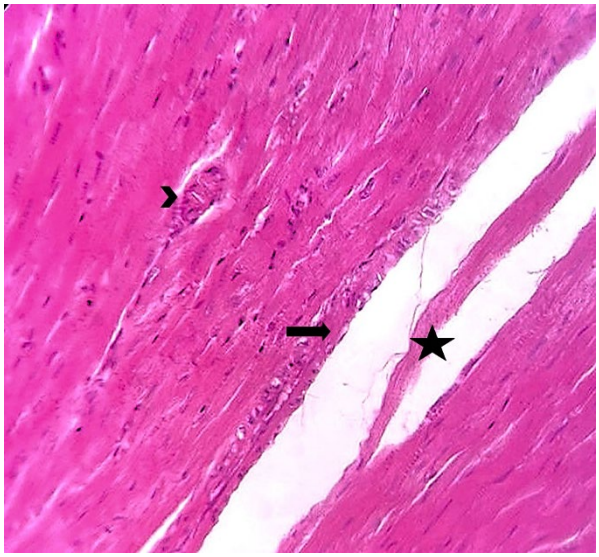

Figure (3). The heart of NAPA rats displays broken or necrotic tissue, and there were cellular oedema and an irregular arrangement of cardiac fibers (star). Moreover, fragmentation of sarcoplasm and degenerative changes with perivascular mononuclear cell infiltration (thick arrows), and blood vessel congestion (head arrows). (H&E-stained section, ×400).

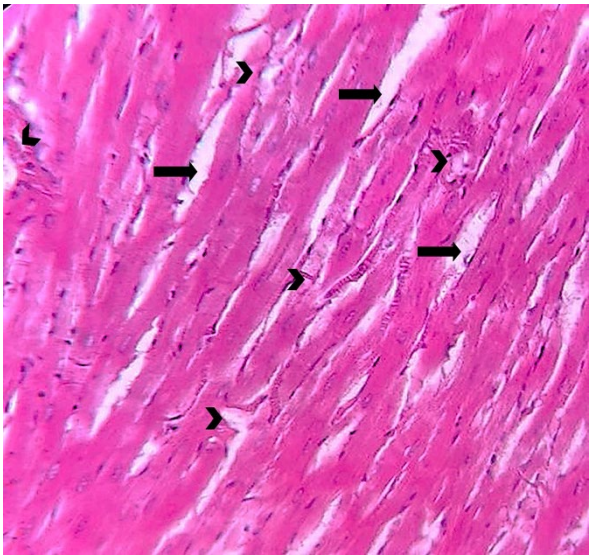

Figure (4). The heart of NAPA rats displays hyaline deterioration of cardiac fibers (head arrows), oedema with sarcoplasm fragmentation and disintegration alterations (thick arrow). (H&E-stained section, ×400).

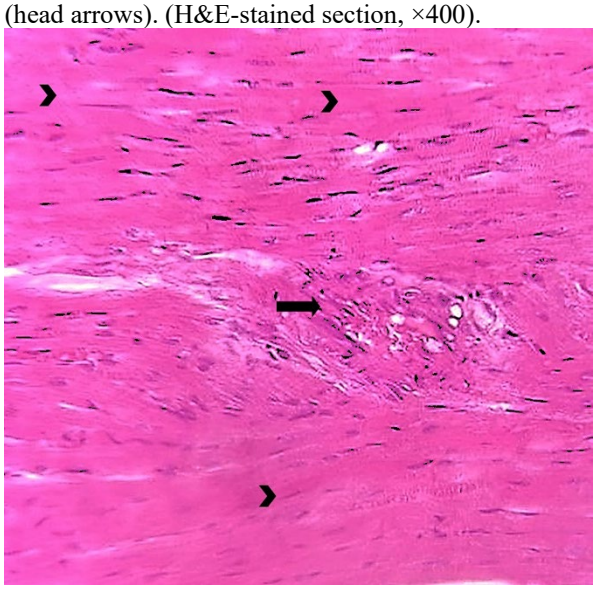

Figure (5). The heart of NAPA rats displays degenerative changes with focal inflammatory cell infiltration (thick arrow) and hyalinization of myocardium (head arrows). (H&E-stained section, ×400).

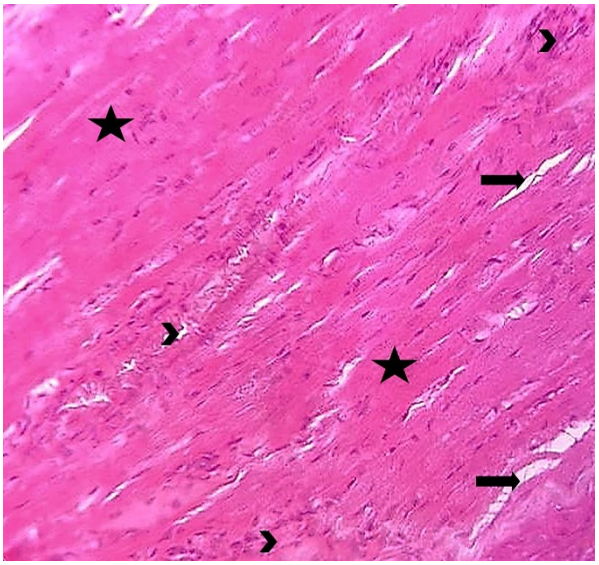

Figure (6). The heart of VC + NAPA rats displays a few degenerating fibers, hyperemic interstitial blood vessels with some inflammatory cell infiltration (head arrows), mild necrosis (thick arrows), and hyalinization of myocardium (stars). (H&E-stained section, ×400).

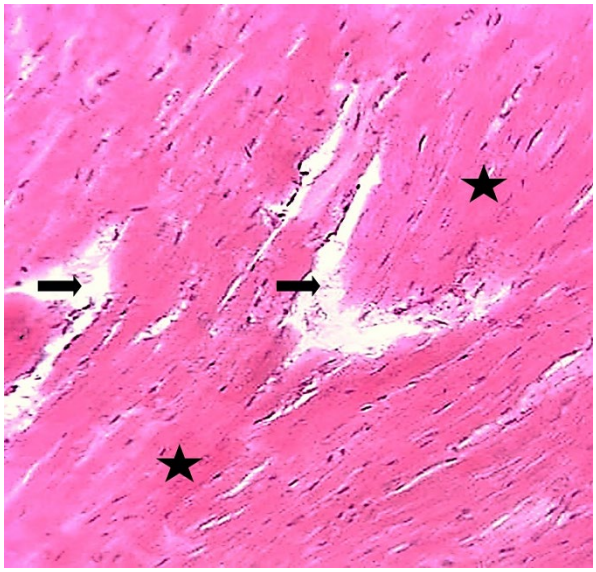

Figure (7). The heart of VC + NAPA rats displays broken or necrotic tissue, and there was cellular oedema and an irregular arrangement of cardiac fibers (thick arrows), and hyaline deterioration of myocardial fibers (stars). (H&E-stained section, ×400).

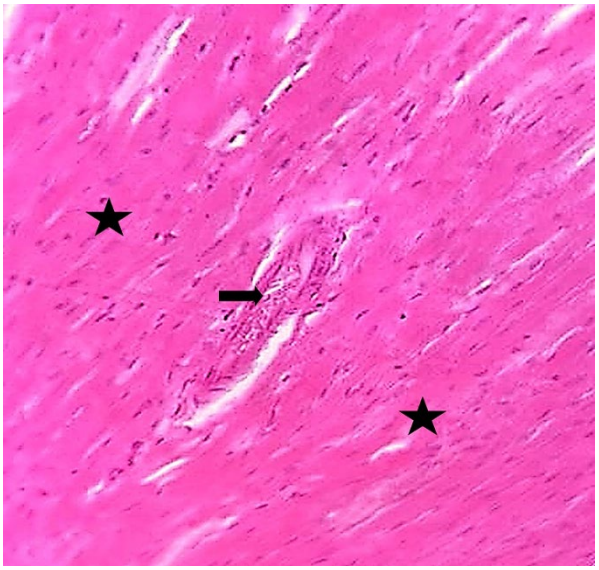

Figure (8). The heart of VC + NAPA rats displays hyaline deterioration of myocardial fibers (stars), and blood vessel congestion with significantly thickened walls (thick arrow). (H&E-stained section, ×400).

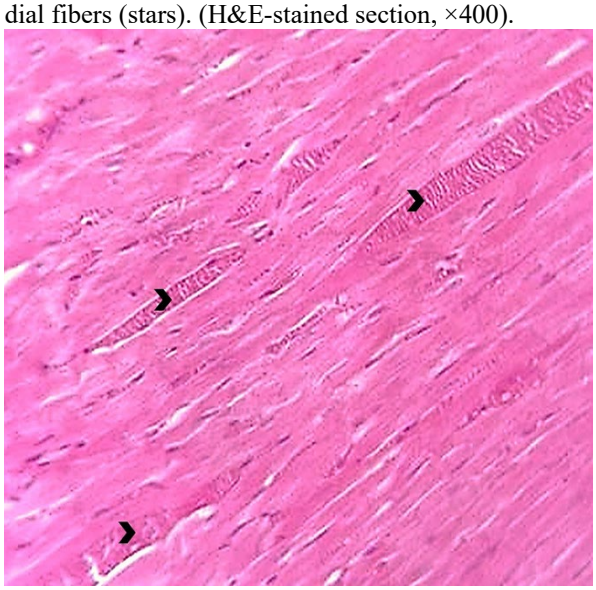

Figure (9). The heart of protective rats displays a significant improvement in the cardiac fibers, with few fiber deterioration, hyperemic interstitial blood vessels (head arrows). (H&E-stained section, ×400).

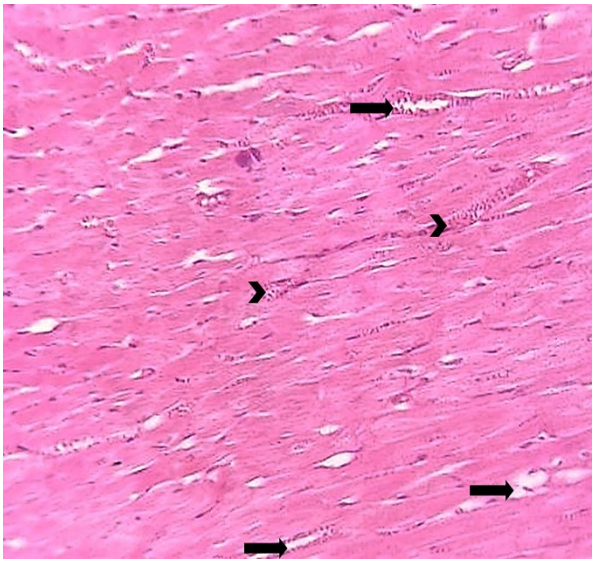

Figure (10). The heart of protective rats displays a significant improvement in the cardiac fibers, with few fiber deterioration, hyperemic interstitial blood vessels (head arrows), and mild necrosis (thick arrows). (H&E-stained section, ×400).

Histopathological Scoring:

At least ten microscopic fields per section were examined using a semiquantitative grading system for myocardial lesions: absent (–), mild (+), moderate (++), and severe (+++), as summarized in Table 1. No lesions were observed in the control and VC-treated groups, whereas the NAPA-treated group exhibited moderate to severe cardiac damage, including myocardial fiber disorganization, cellular oedema, inflammatory cell infiltration, and vascular congestion. In contrast, the protective group showed a marked attenuation of NAPA-induced cardiac injury, with most alterations graded as mild and only occasional focal necrotic changes.

Table (1). Semi-quantitative histopathological scoring of myocardial lesions in experimental rats

Histopathological lesion	Control	VC	NAPA	VC + NAPA	Protective
Myocardial fiber disorganization	–	–	++ / +++	+	+
Cellular oedema	–	–	++	+	–
Myocardial necrosis	–	–	++ / +++	+	– / +
Inflammatory cell penetration	–	–	++	+	–
Vascular congestion/hyperemia	–	–	++	+ (hyperemia)	+ (mild hyperemia)
Hyaline deterioration	–	–	++ / +++		+

Scoring system: Absent (–), Mild (+), Moderate (++), Severe (+++)

DISCUSSION

The present study demonstrates that NAPA induces pronounced histopathological alterations in cardiac tissue, including myocardial fiber disorganization, cellular oedema, fiber fragmentation, necrosis, vascular congestion, and inflammatory cell infiltration. These findings are consistent with Jin & Park (2012), Elduob et al. (2023A), and Okiljević et al. (2024), who reported the known cardiotoxic effects of NAPA mediated by OS. Moreover, co-administration of VC significantly attenuated these alterations, improving fiber organization, reducing necrosis, and limiting inflammatory penetration. On the other hand, pretreatment with VC provided the greatest defense, with myocardial architecture nearly normal and minimal degenerative changes. These protective effects are probably attributable to the antioxidant properties of VC, including scavenging of ROS, stabilization of cell membranes, and inhibition of LPO, which collectively reduce oxidative damage (Elduob et al., 2023B; Zheng et al., 2024; Xu et al., 2025). Additionally, the semiquantitative scoring indicated that myocardial lesions were most pronounced in the NAPA group, while VC administration, particularly as pretreatment, markedly reduced these lesions. This aligns with reports of antioxidants modifying NAPA-induced tissue injury in animal models (Rahaman et al., 2021; Moshaie-Nezhad et al., 2021; Indumathi et al., 2024), who suggest that antioxidant therapy can serve as a general protective strategy against drug-induced toxicity. These explanations also support our previous studies indicating OS-mediated organ damage in hepatic and testicular tissues under similar NAPA exposure conditions (Alshailabi et al., 2022; Alshailabi et al., 2024), highlighting a consistent mechanism of toxicity affecting different organs.

Overall, vitamin C appears to offer significant protection against NAPA-induced heart injury, and these results highlight its potential as an antioxidant supplementation for preventive and therapeutic strategies for drug-related cardiotoxicity. Moreover, future studies should focus on evaluating cardiac biomarkers, heart function, and OS indicators over longer periods. Additionally, investigating dose-response relationships in controlled clinical studies would help translate these findings to human applications.

CONCLUSION

N-acetyl-para-aminophenol induces marked histopathological alterations in the myocardium of albino rats, including fiber disorganization, cellular oedema, necrosis, inflammatory cell infiltration, vascular congestion, and hyaline deterioration. Moreover, co-administration of VC significantly attenuated these alterations, improving fiber organization, reducing necrosis, and limiting inflammatory penetration, while pretreatment with VC provided the greatest protection, with myocardial architecture that was relatively normal and minimal degenerative changes. These results suggest that VC exerts a cardioprotective effect against NAPA-induced heart injury, possibly due to its antioxi-

dant properties. Further experimental and clinical studies are necessary to clarify the long-term efficacy and clinical relevance of VC in preventing drug-induced tissue toxicity.

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Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Eda Alshailabi developed theoretical formalism, prepared the histological sections and performed the analysis. Ahlaam Khalid and Ola Abdalally contributed to the final version of the manuscript. Eda Alshailabi supervised the project.

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