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Research Article

NOVEL PRE-CLINICAL EVIDENCE OF *CENTELLA ASIATICA* AS CARDIOPROTECTIVE AGENT FOR CARDIOMYOCYTES IN AZITHROMYCIN-INDUCED RATS: A HISTOPATHOLOGICAL PERSPECTIVE

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ABSTRACT

Objectives: Azithromycin, a macrolide class of antibiotics, is widely used as a first-line and prophylactic treatment during the COVID-19 pandemic. Despite its therapeutic effects, azithromycin has life-threatening side effects related to the cardiovascular system such as QT-interval prolongation, ventricular arrhythmias, and sudden death. At present, antioxidant agents are needed as cardioprotective agents for cardiac cells to protect against oxidative stress induced by azithromycin. *Centella asiatica* is one of the natural ingredients currently known to have antioxidant effects. This study aims to evaluate the cardioprotective potential of *C. asiatica* against azithromycin-induced cardiotoxicity through histopathological examination using routine H and E staining.

Methods: This *in vivo* experimental study involved four groups of Wistar rats treated orally every day for 14 days: K1 (control group), K2 (azithromycin 15 mg/kg body weight [BW]), K3 (azithromycin 15 mg/kg BW+*C. asiatica* extract 500 mg), and K4 (*C. asiatica* extract 500 mg). Histopathological staining was used to evaluate myocardial interstitial edema, inflammatory cells, and venous dilatation and congestion. Statistical analyses were performed to evaluate significance.

Results: Histopathological results showed significant improvement in myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins in the group treated with *C. asiatica* following azithromycin induction. A statistically significant difference exists among all groups ($p<0.001$; Kruskal-Wallis). Statistically significant differences exist across the groups (K1 vs. K2, K1 vs. K3, K2 vs. K3, K2 vs. K4, K3 vs. K4) ($p<0.001$; Mann–Whitney). No statistically significant difference exists between K3 and K4 for inflammatory cell parameters and venous dilatation and congestion ($p>0.05$), suggesting that the preventive effect of *C. asiatica* is nearly equivalent to normal levels.

Conclusion: These findings demonstrate the efficacy of *C. asiatica* as a cardioprotective agent against azithromycin-induced cardiotoxicity.

Keywords: *Centella asiatica*, Cardioprotective, Cardiomyocytes, Azithromycin, Cardiotoxicity.

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INTRODUCTION

Azithromycin, an antibiotic belonging to the macrolide class, is widely used as a first-line and prophylactic treatment in respiratory tract infections, especially in immunocompromised conditions. The use of azithromycin as an antibiotic increased significantly during the COVID-19 pandemic and is known to be the most frequently prescribed antibiotic in hospitals during that time. Among several macrolides, azithromycin has a superior epithelial barrier effect. Despite its therapeutic effects, azithromycin has life-threatening side effects related to the cardiovascular system. Twenty instances of torsade de pointes (TdP) have been documented after 5 days of azithromycin consumption. Other studies have also proven that azithromycin consumption is associated with sudden death due to ventricular arrhythmia. Electrocardiogram (ECG) abnormalities in the form of QT-interval prolongation have also been observed, so one of the conditions for administering azithromycin is an ECG examination to rule out abnormalities in cardiac electricity. The food and drug administration (FDA) recommends consulting a doctor before using azithromycin, rather than advising discontinuation of this drug. The FDA also issues warnings regarding the use of this drug in combination with other medications, particularly those that also cause cardiotoxicity, such as QT interval prolongation. Due to the high toxicity of azithromycin to the cardiovascular system, further warnings have been written on the product label [1-6].

Numerous investigations on rats have demonstrated the cardiotoxicity of azithromycin. Besides inducing QT interval prolongation, the cardiotoxicity of azithromycin is significantly linked to an elevation in reactive oxygen species (ROS) [7]. Under physiological conditions, ROS serves a vital function in cellular signaling pathways as a secondary messenger that regulates the body's redox reactions, contributing to various cellular activities such as growth and development, adaptation, and cell function. However, in pathological conditions where ROS are excessively produced, an imbalance occurs, rendering the body incapable of neutralizing them, leading to a state of oxidative stress. This condition results in cellular dysfunction and, ultimately apoptosis [8-10]. Azithromycin has been demonstrated to harm cardiac myocytes by augmenting the rigidity of myocardial tissue via the mechanism of myocardial fibrosis. In addition to myocardial fibrosis, azithromycin induces oxidative stress, release of inflammatory mediators, and apoptosis. Necrosis and hypertrophy of heart muscle cells, infiltration of inflammatory cells, and interstitial edema are observed in the histological part of the heart tissue. Until now, no drug has been found to overcome this cardiotoxicity. Consequently, a natural cardioprotective substance is required to mitigate the cardiotoxic effects of azithromycin [1,7,11].

Centella asiatica is a natural substance recognized for its cardioprotective properties. *C. asiatica* (L.) Urban, often known as Gotu kola, Indian

Pennywort, or Tiger Grass, is recognized in traditional Chinese medicine (TCM) as "ji xue cao" or "luei gong gen." *C. asiatica* is a botanical component from the *Apiaceae* family that has demonstrated efficacy as a therapeutic herb for over three millennia [12]. This plant is prevalent in tropical nations, including Southeast Asia (Indonesia and Malaysia) and Asia (India, China, Nepal). This plant contains active chemicals such as Asiatic acid, madecassic acid, asiaticoside, and madecassoside. This plant also includes flavonoids, brahmoside, brahminoside, glycosides, isothankuniside, and thankuniside. *C. asiatica* exhibits significant antioxidant effects due to its rich presence of triterpenoids and flavonoids. Asiatic acid has demonstrated the ability to safeguard myocardial and cardiomyocytes against ischemia/reperfusion damage by inhibiting glycogen phosphorylase. The presence of additional active chemicals is associated with oxidative stress in cardiovascular disorders, including hypertension and heart failure. Moreover, its cardioprotective impact is associated with its anti-atherosclerotic, antihyperlipidemic, antioxidant, antiapoptotic, and anti-inflammatory properties in both *in vitro* and *in vivo* settings [13-17].

We propose that the toxicity of azithromycin, which induces QT interval prolongation and elevated ROS generation, can be alleviated by the antioxidative and anti-inflammatory characteristics of the active chemicals in *C. asiatica*. Consequently, the risk of death resulting from azithromycin-induced cardiotoxicity can be mitigated or averted. At now, experimental investigations on the cardiotoxic effects of azithromycin remain significantly constrained. This study especially demonstrates the cardioprotective benefits of *C. asiatica* through the administration of azithromycin. This study employed Wistar rats categorized into four groups: a control group (untreated), a group administered azithromycin only, a group receiving an amalgamation of azithromycin and *C. asiatica* extract, and a group exposed exclusively to *C. asiatica*. The intervention was conducted every day for a duration of 14 days. The research utilized cardiac muscle tissue slices from rats to assess shape and structure by standard H&E staining during histopathological analysis. This study sought to evaluate the existence of cardiac interstitial edema, inflammatory cells, venous dilation, and congestion.

METHODS

Animal test

This research included 30 male Wistar rats, with weights ranging from 151 to 270 g, and aged 10–12 weeks. The test participants were obtained and examined in the Laboratory of Pharmacology at the Faculty of Medicine, University of North Sumatra, Medan, Indonesia. The test subjects were situated in a specified standard location, namely, within a stainless steel enclosure separated from the cage (three rats per cage) under regulated conditions of temperature (20±2°C), humidity (50±10%), and lighting (12 h of light and 12 h of darkness). All mice were housed in pathogen-free environments with unlimited access to food and water. All study was conducted in compliance with the procedures set out by the Animal Study Ethics Committee at the Faculty of Mathematics and Natural Sciences, under trial number 0855/KEPH-FMIPA/2024.

Chemicals

1. The extract of *C. asiatica* is acquired online from the Herbana brand, manufactured by PT Deltomed Laboratories in Indonesia. This extract is derived from a singular plant extract processed using the Quadra Extraction System. Each capsule has 500 mg of *C. asiatica* extract. Production code: DL002C24.
2. Azithromycin is sourced from local pharmacies manufactured by PT. Kimia Farma, Indonesia. Each film-coated tablet contains 500 mg of azithromycin. Azithromycin is solubilized in distilled water for oral administration in experimental animals. Production code: I32179J.

All doses used are taken based on previous research [7,15].

Experimental protocol

The test subjects were haphazardly assigned to four experimental categories:

1. K1 (8 male Wistar rats)=This group is the control group. The mice received an equivalent amount of distilled water and food orally.
2. K2 (8 male Wistar rats)=Group given azithromycin treatment. Rats were given azithromycin as much as 15 mg/kg body weight (BW) orally daily for 14 days.
3. K3 (8 male Wistar rats)=Group treated with azithromycin and *C. asiatica* extract. Rats were given azithromycin as much as 15 mg/kg BW and *C. asiatica* extract as much as 500 mg orally every day for 14 days.
4. K4 (8 male Wistar rats)=Group that was given only *C. asiatica* extract. Rats were given 500 mg of *C. asiatica* extract orally every day for 14 days.

Sacrifice of rats and specimens collection

All mice from each group were anesthetized with ketamine at a dosage of 60 mg/kg BW by intraperitoneal injection. The cardiac tissue was subsequently preserved in a 10% formalin buffer solution for 24 h.

Histopathological study

Following a 1-day fixation in 10% formal salts, the cardiac tissue is then preserved in formalin and embedded in paraffin. The 5 µm thick sections were stained with hematoxylin-eosin (H and E) and examined under a light microscope, followed by the acquisition of photomicrographs to evaluate their histological alterations. Histopathological examination is conducted using the Olympus BH-2 microscope (Olympus Corp., Tokyo, Japan). The degree of myocardial interstitial edema, inflammatory cell presence, and venous dilatation and congestion detected in the staining findings is classified semi-quantitatively on an ordinal scale (Table 1).

Statistical analysis

Statistical analysis evaluates the cardioprotective properties of *C. asiatica* against azithromycin induction by measuring myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins, with data processed using IBM Statistical Packages for the Social Sciences version 25. The Kruskal–Wallis statistical correlation test was employed to assess fibrosis levels across four groups. If the results were significant ($p<0.05$), the Mann–Whitney test was subsequently utilized to evaluate differences in fibrosis levels between each group, applying Bonferroni correction. The Bonferroni adjustment adjusts the significance threshold for multiple comparisons, establishing a significant p-value at $p<0.0083$ (0.05 divided by 6 comparisons). Nevertheless, if the bulk of the acquired p-values are much below the threshold for significance, the Bonferroni correction is not used, since the likelihood of a Type I mistake is deemed negligible.

RESULTS

This study is an *in vivo* experimental investigation aimed at examining the cardioprotective effects of *C. asiatica* on cardiomyocytes generated by azithromycin, through the evaluation of myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins histopathological findings.

Histopathological examination

Examination outcomes in K1 (control group)

This group served as the control group. The mice were administered a comparable amount of distilled liquid and nourishment orally. Fig. 1

Table 1: Myocardial interstitial edema, inflammatory cell, and dilatation and congestion of vein scale

Score	Interpretation
0	No changes (none)
1	Mild myocardial interstitial edema/inflammatory cell/ dilatation and congestion of vein
2	Moderate myocardial interstitial edema/inflammatory cell/ dilatation and congestion of vein
3	Severe myocardial interstitial edema/inflammatory cell/ dilatation and congestion of vein

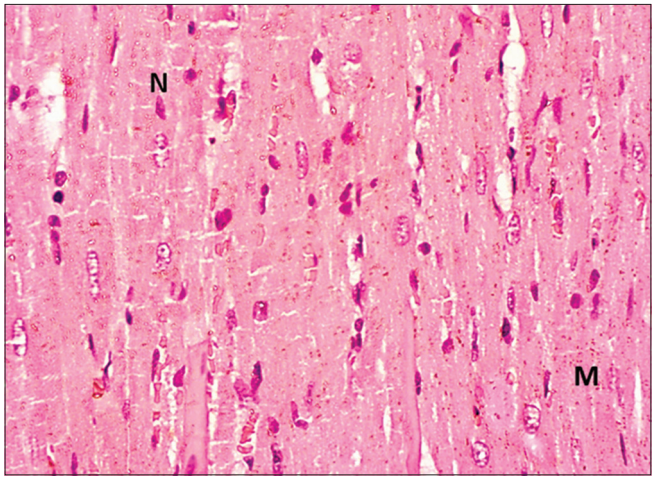

Fig. 1: Histopathological results of K1 (Control Group)

shows histopathological examination of cardiac tissue stained with H&E at ×400 magnification in the control group revealed normal myofibrillar (M) architecture and nuclei (N).

Examination outcomes in K2 (azithromycin treatment)

This cohort underwent azithromycin therapy. Rats received azithromycin at a dosage of 15 mg/kg BW orally every single day for 14 days. Fig. 2 shows histopathological examination of cardiac tissue stained with H and E at ×400 magnification in the azithromycin-induced group revealed extensive infiltration of inflammatory cells (black arrows) inside the myofibrils (M), interstitial edema (blue arrows), and venous dilatation and congestion (green arrows).

Examination outcomes in K3 (treatment with azithromycin+C. asiatica)

The group received treatment with azithromycin and *C. asiatica* extract. Rats received azithromycin at a dosage of 15 mg/kg BW and *C. asiatica* extract at a dosage of 500 mg orally every single day for 14 days. Fig. 3 shows histopathological examination of heart tissue stained with H&E at ×400 magnification in the group subjected to *C. asiatica* following azithromycin induction revealed enhancements in the infiltration of inflammatory cells (black arrows) within the myofibrils (M), interstitial edema (blue arrows), and venous dilation and congestion (green arrows).

Examination outcomes in K4 (treatment with C. asiatica)

This group received just *C. asiatica* extract. Rats received *C. asiatica* extract at a dosage of 500 mg orally every single day for 14 days. Fig. 4 shows histopathological examination of cardiac tissue stained with H&E at ×400 magnification in the group alone exposed to *C. asiatica* yielded results identical to those of the control group. This result indicates the absence of inflammatory cell infiltration in the myofibrils (M), as well as interstitial edema and venous dilation and congestion.

Statistical analysis

Statistical analysis is employed to assess variations in histopathological outcomes across all treatment groups concerning myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins. The study employed the Kruskal–Wallis test to assess differences among all treatment groups and the Mann–Whitney test to evaluate differences between individual treatment groups.

Table 2 presents the outcomes of the Kruskal–Wallis test, revealing a statistically significant difference in the histopathological results across the four groups across all evaluated parameters, with a $p<0.05$ ($p<0.001$).

Subsequently, the Mann–Whitney test was performed following the Kruskal–Wallis test due to the significant findings obtained. Table 3

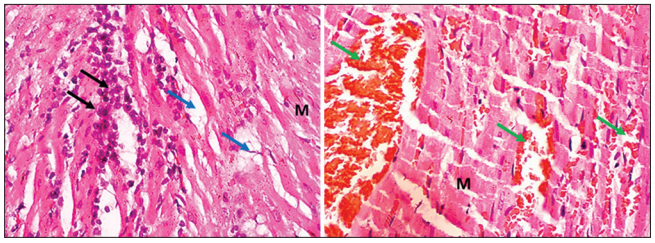

Fig. 2: Histopathological results of K2 (azithromycin)

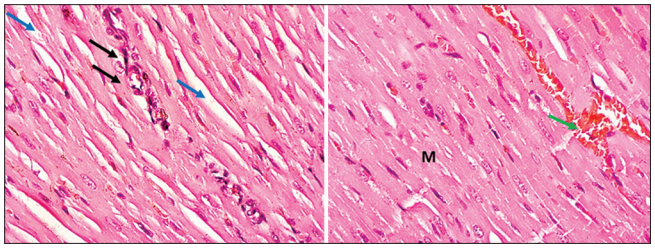

Fig. 3: Histopathological results of K3 (azithromycin+*Centella asiatica*)

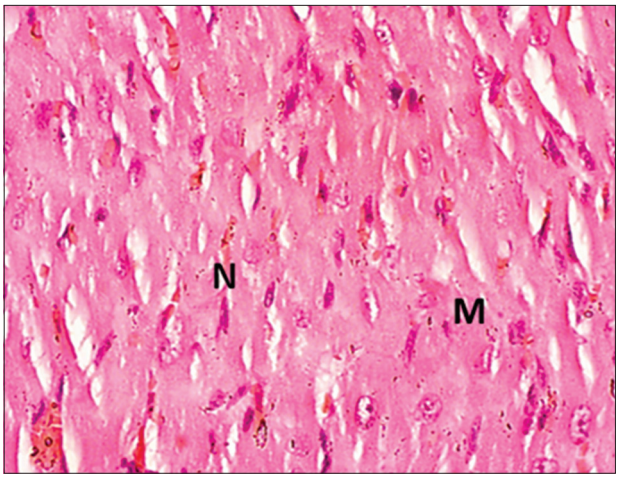

Fig. 4: Histopathological results of K4 (*Centella asiatica*)

Table 2: The outcomes of the statistical analyses across all treatment groups concerning three parameters

Parameter	Kruskal-Wallis H	df	p-value
Myocardial interstitial edema	24.634	3	<0.001*
Inflammatory cells	29.254	3	<0.001*
Dilatation and congestion of vein	25.461	3	<0.001*

Data shown from *Kruskal-Wallis* analysis, *p<0.05 was statistically significant, K1: Control group, K2: Azithromycin 15 mg/kg BW, K3: Azithromycin 15 mg/kg BW+*Centella asiatica* extract 500 mg, K4: *Centella asiatica* extract 500 mg

demonstrates statistically significant changes in the histopathological results for all group comparisons, with the exception of the K3 versus K4 group. The disparity in inflammatory cells and dilatation and congestion of vein between the K3 (p=0.535) and K4 (p=0.084) groups is not statistically significant.

DISCUSSION

Azithromycin is a commonly employed broad-spectrum macrolide antibiotic, especially for the treatment of pneumonia. The cardiotoxicity of macrolides is linked to the inhibition of ether-à-go-go potassium channels in cardiomyocytes [18]. During the COVID-19 pandemic, azithromycin was included in the treatment protocol for COVID-19

Table 3: The outcomes of the statistical analysis comparing two treatment groups across three parameters

Intergroup comparison	Myocardial interstitial space edema	Inflammatory cells	Dilatation and congestion of vein
K1 versus K2	<0.001*	<0.001*	<0.001*
K1 versus K3	<0.001*	<0.001*	<0.001*
K2 versus K3	<0.001*	<0.001*	<0.001*
K2 versus K4	<0.001*	<0.001*	<0.001*
K3 versus K4	0.001*	0.535	0.084

Data shown from Mann–Whitney analysis, *p<0.05 was statistically significant, K1: Control group, K2: Azithromycin 15 mg/kg BW, K3: Azithromycin 15 mg/kg BW+*Centella asiatica* extract 500 mg, K4: *Centella asiatica* extract 500 mg

acute respiratory distress syndrome, either as monotherapy or, more commonly, in combination with hydroxychloroquine [5,19-21]. This is due to azithromycin's immunomodulatory role and its *in vitro* antiviral activity. Azithromycin diminishes viral replication and inhibits its entry into host cells, significantly contributing to the mitigation of SARS-CoV-2 activity [17]. In addition to being used for respiratory tract infections, azithromycin is also effective for treating skin and soft-tissue infections [22].

Azithromycin is known to have toxic effects on the heart, with proarrhythmic effects having been reported repeatedly [23]. This drug can significantly prolong the QT interval and a longer duration of action potential. In addition, azithromycin also induces severe repolarization dispersion, thereby significantly increasing susceptibility to arrhythmias. Azithromycin can cause TdP with a mechanism involving increased spatial dispersion of repolarization, that is, an imbalance in the repolarization time between myocardium cells. This occurs due to a combination of IKr inhibition (which prolongs the QT interval by slowing down repolarization) and INaL induction (This extends the duration of the action potential). This condition creates the coexistence of excited and excitable cardiomyocytes, thus allowing the appearance of abnormal electrical activity that triggers ventricular arrhythmias such as TdP [24,25]. Ventricular arrhythmia leads to sudden deaths were also reported [26,27].

Azithromycin induces oxidative DNA damage at the cellular level by triggering the release of ROSs through the suppression of mitochondrial respiration [27,28]. ROS predominantly affects cardiomyocytes, particularly in scenarios involving pre-existing cardiac damage. Mitochondria function as the primary generator of ROS in cells, making them vulnerable to ROS-induced damage [29]. Damaged mitochondria can disrupt cellular metabolism, decreasing mitochondrial enzyme activity, particularly in cardiac cells exposed to toxins such as azithromycin. This decline in function underlies the occurrence of intrinsic cellular dysfunction [30]. Mitochondrial fluid retention and the emission of cytochrome c in cardiomyocyte mitochondria, and the induction of apoptosis are also associated with increased mitochondrial membrane permeability [27]. These mechanisms induce oxidative stress in cardiac tissue, resulting in modifications to heart performance, ion channel functionality, ionic pumps, ion exchange, and inflammatory responses [31]. ROS unleashes further harm to cell membranes and cytoplasmic organelles by reacting with the phospholipid groups of polyunsaturated fatty acids in the cell plasmalemma [32].

As a result of oxidative stress activity at the tissue level, there is evidence of cell membrane loss in injured cardiomyocytes, cardiac muscle fiber atrophy, and interstitial space dilation [33]. Under healthy conditions, cardiac fibroblasts, comprising over 90% of non-myocytes, support cardioprotective factors and are essential for the establishment of proteins that compose the extracellular matrix and angiogenic substances [34]. In adverse conditions, myocardial fibrosis occurs as fibroblasts are transformed into myofibroblasts, producing excessive fibronectin and collagen [35]. Myocardial remodeling occurs following cardiomyocyte death, resulting in fibrosis and subsequent cardiac dysfunction. This process is propelled by unregulated stimulation of fibroblasts in cardiac tissue and a significant accumulation of the extracellular matrix amino acids [36,37].

Histopathological examination with routine H&E staining at ×400 magnification in K1 (control group) and K4 (*C. asiatica* exposure) showed no changes at all. No damage to myofibrils or nuclei was observed. Myocardial interstitial edema, inflammatory cells, dilation, or vein congestion were not detected. In another study, the histopathological results of cardiomyocytes indicated near-normal conditions in studies involving pre-treatment with *C. asiatica* before induction by cardiotoxic agents. Pre-treatment with *C. asiatica* extract reduced mitochondrial dysfunction by minimizing the increase in cardiac calcium levels caused by cardiotoxic agents. The antioxidant properties of *C. asiatica* were demonstrated to safeguard mitochondria in a laboratory experiment of rat on myocardial ischemia-reperfusion injury following pre-treatment with *C. asiatica* extract [38,39].

Histopathological examination of K2, given azithromycin at a single oral dose of 15 mg/kg BW daily for an interval of fourteen consecutive days, showed significant changes. Cardiomyocytes damage occurred in the majority of rats in this group. Myocardial interstitial edema, inflammatory cells, vein dilation, and congestion were observed at moderate-to-severe levels. Cardiomyocytes damage has also been confirmed in other studies. The results of studies on groups receiving only azithromycin showed findings consistent with this study. Routine H&E staining revealed distortion, fragmentation, and atrophy of cardiac muscle fibers, mononuclear cell infiltration in areas of focal myocyte destruction, protein degradation in cardiac muscle fibers, interstitial edema, and vascular congestion. The nuclei appeared shrunken due to damage (pyknosis), with vacuolation in the cytoplasm and hypereosinophilia, which are indicative of myocardial necrosis. Fibrosis was also evident with Masson's trichrome staining, characterized by an increased amount of collagen fibers deposited around the congested blood vessels. The cardiotoxicity induced by azithromycin is attributed to excessive production of ROS, which damages cellular components, leading to dysfunction and cell damage [1,26].

Despite significant advancements in healthcare, the mortality rate attributed to cardiovascular diseases (CVD), the predominant cause of death globally, continues to rise substantially. CVD not only affects the heart but also impacts blood vessels, making this group of disorders closely associated with hypertension, heart failure, stroke, coronary heart disease, and peripheral vascular diseases [15]. Recently many natural agents have been studied as treatments for CVD due to their abundance of beneficial active compounds [40]. Antioxidant medications are presently required as cardioprotective strategies for cardiac cells to safeguard against oxidative stress caused by azithromycin. Although synthetic antioxidants are commonly used, natural antioxidants are preferred in this context, as synthetic antioxidants have been shown to possess carcinogenic properties. Therefore, natural antioxidants with proven efficacy and safety are essential. One natural plant well-known for its high antioxidant properties is *C. asiatica* (L) urban was historically employed in Ayurveda and TCM. For the treatment of neurological and digestive ailments, *C. asiatica* has demonstrated efficacy in wound healing, anti-cancer, and anti-inflammatory capabilities. Due to its capabilities as an antifibrotic, antiapoptotic, and antioxidant agent [16,41-43]. *C. asiatica* has proven its efficacy as a cardioprotective agent for cardiomyocytes [38,44,45].

The antioxidant activity of *C. asiatica* originates from various phytochemicals, which have been identified as active compounds over the years. These active compounds include isoprenoids, such as plant-derived sterols, sesquiterpenes, saponins, and pentacyclic triterpenoids, as well as derivatives of phenylpropanoid, including caffeoylquinic acid, eugenol, and flavonoids [15,46-49]. Pentacyclic triterpenoids, comprising asiaticoside, asiatic acid, madecassoside, and madecassic

acid, constitute the most vital components in *C. asiatica* [15,39,50-52]. These compounds exhibit antioxidant properties due to their ability to terminate radical chain reactions, inhibit free radical formation, and scavenge ROS [49,53]. In addition to triterpenoids, *C. asiatica* also comprises other phenolic substances, including flavonoids (kaempferol and quercetin), phytosterols (campesterol, sitosterol, and stigmasterol), and various acids such as glucuronic acid, ferulic acid, and chlorogenic acid. Nonetheless, although this plant is abundant in many active chemicals, its nutritional benefits and therapeutic capabilities are largely ascribed to triterpenes as the principal active elements [15,51]. As mentioned earlier, *C. asiatica* has proven its efficacy in managing cardiovascular disorders, which has led to increased interest in researching *C. asiatica* as a cardioprotective agent in recent years, particularly regarding the active compounds it contains.

Meanwhile, the histopathological examination results of K3, treated with azithromycin 15 mg/kg BW combined with *C. asiatica* 500 mg orally every day for 14 days, also showed significant changes. However, these changes reflected an observed improvement in cardiomyocytes. The harm inflicted by azithromycin was mitigated by *C. asiatica*. In the histopathological examination, myocardial interstitial edema, inflammatory cells, vein dilation, and congestion appeared to be at mild levels. This demonstrates that *C. asiatica* is effective as a cardioprotective agent against the cardiotoxic effects of azithromycin. This study aligns with other research that supports the efficacy of *C. asiatica* as a cardioprotective agent. One of the active compounds in *C. asiatica*, asiatic acid, has been proven effective in minimizing damage to cardiomyocytes by reducing the pathological effects of cardiac hypertrophy induced by isoproterenol (ISO). Routine H&E staining demonstrated cellular function improvement by reducing interstitial space widening in cardiomyocytes and inflammatory cell infiltration. Asiatic acid preserves the contour and structure of cardiomyocytes, maintaining their normal state by reducing deformation and enlargement of cardiac muscle cells. Masson's trichrome staining revealed that asiatic acid significantly minimizes fibrosis by reducing Type I collagen deposits caused by ISO. Atrial natriuretic peptide, a marker of cardiac hypertrophy, also significantly decreased. These findings prove the potential of *C. asiatica* and its asiatic acid content as a cardioprotective medication against hypertrophy or cardiomyocyte injury caused by cardiotoxic agents such as ISO [54].

Statistical analyses revealed substantial disparities in the myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins within the histopathological findings. The Kruskal–Wallis test indicated disparities among all treatment groups, aligning with theoretical frameworks in research demonstrating the effectiveness of *C. asiatica* in enhancing the function and structure of cardiomyocytes. The Mann-Whitney test was performed to examine the disparities among the treatment groups. K1 versus K2 demonstrated significant outcomes across all three criteria, substantiating the cardiotoxic effects of azithromycin that inflict considerable harm on cardiomyocytes. K1 versus K3 had significant outcomes across all three parameters, indicating that *C. asiatica* is incapable of completely restoring cardiomyocytes to their normal state. K1 versus K3 had significant outcomes across all three parameters, confirming that exposure to *C. asiatica* serves as an effective cardioprotective agent that markedly diminishes cardiomyocyte damage resulting from the cardiotoxic effects of azithromycin. K2 versus K4 demonstrated significant outcomes across all three parameters, in contrast to the absence of cardiotoxic effects from azithromycin when exposed alone to *C. asiatica*. K3 and K4 had divergent outcomes across the three criteria. The myocardial interstitial edema in K3 compared to K4 exhibits a significant difference, while the notable variations in inflammatory cells and the dilation $ congestion of veins suggest that *C. asiatica* can effectively mitigate the inflammatory response and vascular dilation and congestion, closely approximating a state devoid of azithromycin induction. These findings collectively demonstrate that *C. asiatica* can protect and improve cardiac function in the myocardium of rats exposed to cardiotoxic agents.

CONCLUSION

This study has demonstrated the cardiotoxic effects of azithromycin through histopathological changes observed in heart tissue. Histopathological examination and statistical analyses showed significant differences in myocardial interstitial edema, inflammatory cells, and dilation and congestion of veins in cardiomyocytes treated with *C. asiatica* after azithromycin exposure. The natural agent *C. asiatica* has proven its efficacy as a cardioprotective agent through the antioxidant effects of its active compounds: triterpenes. Therefore, *C. asiatica* has the potential to serve as a cardioprotective agent for patients undergoing azithromycin therapy. Further studies are needed to provide more evidence of the cardioprotective effects of *C. asiatica* in the context of azithromycin therapy, particularly in the areas of cardiac enzymes, inflammatory markers, and immunohistochemistry.

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COMPETING INTEREST

All the authors declare that there are no conflicts of interest.

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UNDERLYING DATA

Derived data supporting the findings of this study are available from the corresponding author on request.

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