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The Effect of Colchicine on St. Thomas II Cardioplegia on Ischemia-Reperfusion Injury: A Histomorphological Study in Rabbits

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Abstract. *The maternal mortality rate in South Sumatra Province was recorded at 128 cases in 2020 and increased Cardioplegia is an important method for protecting the heart from ischemia-reperfusion (I/R) injury. However, myocardial damage from reperfusion can't be completely prevented by standard cardioplegia solutions alone. The St. Thomas II cardioplegia solution, with a lower potassium concentration, has been shown to have a cardioprotective effect, while colchicine, as an anti-inflammatory agent, works by inhibiting NLRP3 inflammasome activation and neutrophil modulation. This experimental study used a randomized controlled trial (RCT) post-test-only design on 12 New Zealand white rabbits, which were randomly divided into two groups: a control group (St. Thomas II) and a treatment group (St. Thomas II + colchicine). The primary outcome was the degree of myocardial histomorphological damage at 200× magnification. The results showed that five out of six samples from the control group had severe damage (score 3), while five out of six samples from the treatment group had no damage (score 0). A Mann-Whitney test yielded a p-value of 0.0152, indicating a significant difference. The addition of colchicine to St. Thomas II was shown to significantly reduce myocardial histomorphological damage. These findings suggest that colchicine could be considered as a cardioplegia adjuvant to enhance myocardial protection during cardiac surgery.*

Keywords: *Cardioplegia Adjuvant; Colchicine; Ischemia-Reperfusion Injury; Myocardial Histomorphology; Myocardial Protection.*

1. INTRODUCTION

The application of an aortic cross-clamp during cardiac surgery can induce myocardial damage due to ischemia-reperfusion injury (Beyersdorf, 2009). To minimize this risk, cardioplegic solutions are used to temporarily arrest cardiac activity, preserve metabolism, and protect the myocardium. Various types of cardioplegia solutions have been developed, one of which is the St. Thomas solution, widely used in clinical practice for its effectiveness and ease of use. St. Thomas II is known to have a lower potassium concentration than its previous formulation and has been proven to provide a cardioprotective effect, although myocardial damage from reperfusion cannot be entirely avoided (Beyersdorf, 2009; Nidorf et al., 2020). In the context of protecting the myocardium and mitigating cardiovascular risk, various pharmacological approaches are being explored (Bonaventura & Abbate, 2023).

Colchicine is an anti-inflammatory drug that has long been used for the treatment of gouty arthritis and is now increasingly utilized in the cardiovascular field (Fischesser et al., 2021). Several studies report that low-dose colchicine is effective in reducing the risk of major adverse cardiovascular events, including myocardial infarction, stroke, and cardiovascular death. Its primary mechanism of action is related to the modulation of the inflammatory response by

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inhibiting the activation of the NLRP3 inflammasome, which plays a crucial role in tissue damage during ischemia-reperfusion injury (Beyersdorf et al., 2021).

Inflammation following cardiac reperfusion is known to exacerbate tissue damage by triggering the release of inflammatory mediators, including interleukin-1β (IL-1β), which acts as an initial trigger for inflammation (Bakhta et al., 2018). Experimental research indicates that colchicine can reduce NLRP3 inflammasome activation, suppress IL-1β release, and decrease myocardial infarct size. This cardioprotective effect is thought to work by reducing oxidative stress and improving cardiac remodeling in the early phase of reperfusion Robertson et al., 2016).

Considering that the St. Thomas II solution does not fully protect the myocardium from reperfusion injury, the administration of colchicine has the potential to enhance its cardioprotective effect during cardiac surgery procedures. Therefore, this study was conducted to evaluate the influence of combining St. Thomas II and colchicine on the degree of myocardial tissue damage in a rabbit heart model, aiming to identify the potential of colchicine as an adjuvant in myocardial protection strategies.

2. LITERATURE REVIEW

Mature myocardial cells (myocytes) can reach up to 25 µm in diameter and 100 µm in length, featuring one or two central nuclei surrounded by a capillary-rich connective tissue, distinguishing them from multinucleated skeletal muscle (Oberman et al., 2023). The primary contractile unit of these cells is the sarcomere, composed of actin and myosin protein filaments whose interaction produces muscle contraction, with sarcomere length varying between 1.5–2.2 µm during the cardiac cycle (Lilly, 2016). The cell membrane, or sarcolemma, has specialized structures called intercalated discs that maintain structural and electrical continuity between cells, and a T-tubule system that facilitates the transmission of electrical impulses and ion movement for synchronized excitation and contraction (Lilly, 2016). The sarcoplasmic reticulum, a calcium reservoir, is critical for the excitation-contraction coupling mechanism, while abundant mitochondria, occupying about 35% of the cell volume, support the high energy demands of the myocardium. Cardiac electrical activity is controlled by three cell types: pacemaker cells (SA and AV nodes), Purkinje fibers, and ventricular and atrial muscle cells, which function through differences in sodium, potassium, and calcium ions across the sarcolemma to generate action potentials and rhythmic contractions (Lilly, 2016).

The application of an aortic cross-clamp in open-heart surgery interrupts coronary blood flow, causing myocardial ischemia that risks inducing ischemia-reperfusion injury (Minamino, 2012). Myocardial damage is characterized by cell membrane disruption, intracellular edema, sarcomere disorganization, myofibril fragmentation, neutrophil infiltration, and the release of reactive oxygen species (ROS), which trigger apoptosis and necrosis of cardiomyocytes (Perrelli et al., 2011). Various strategies, such as ischemic preconditioning, have been investigated to understand and mitigate the mechanisms underlying this damage (Yang et al., 2010). An increase in biochemical biomarkers such as troponin I (TnI) reflects necrosis, while malondialdehyde (MDA) is used as a marker for oxidative stress from lipid peroxidation (Wardoyo et al., 2025). Longer cross-clamp duration correlates with increased levels of MDA, 8-isoprostane, and nitrite/nitrate, thereby worsening myocardial damage (García et al., 2013). Myocardial protection strategies such as cold blood potassium cardioplegia and continuous retrograde warm blood cardioplegia have been proven effective in reducing perioperative mortality, inotropic support needs (Catinella et al., 1982), levels of damage biomarkers, and hospital length of stay (Bar-El et al., 1999), even in cases with clamping durations exceeding two hours.

The St. Thomas II cardioplegia solution is a modification of the St. Thomas I solution, developed to lower the potassium concentration and add other components like magnesium and buffers to maintain myocardial cell membrane stability during ischemia, making it more effective in protecting the heart from damage due to prolonged cross-clamping in open-heart surgery (Hearse et al., 1978). Nevertheless, tissue damage from ischemia-reperfusion cannot be entirely prevented by cardioplegia alone, necessitating additional strategies.

Colchicine, an anti-inflammatory drug initially used for gouty arthritis, is now known to have a protective effect on the myocardium by inhibiting NLRP3 inflammasome activation (Sun et al., 2022) and reducing the release of pro-inflammatory cytokines such as IL-1β. Several experimental studies show that colchicine administration can reduce infarct size, decrease oxidative stress, and suppress pyroptosis in models of myocardial injury (Bakhta et al., 2018; Li et al., 2024). Integrating St. Thomas II with an adjuvant strategy like colchicine could potentially offer a more comprehensive protective approach against myocardial damage from ischemia-reperfusion.

3. METHOD

This study was an experimental study with a post-test-only randomized controlled trial (RCT) design. Two study groups were established: a control group receiving St. Thomas II cardioplegia solution without any additional agents and a treatment group receiving St. Thomas II cardioplegia solution with colchicine. The experimental units were physically healthy New Zealand white rabbits, aged 8–12 weeks, and weighing 2500–3000 grams. A total of 12 rabbits met the inclusion criteria and were randomly divided into the control group (n=6) and the treatment group (n=6). Randomization was performed by assigning random identification numbers to their ears, followed by allocation to groups while maintaining uniform cage and treatment conditions to minimize confounding factors. The sample size was determined using the law of diminishing return, with a minimum of 6 rabbits per group to achieve an E value of 10.

The research procedure was conducted at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, from January to April 2025. The animals were administered colchicine at 0.3 mg/kg body weight via oral gavage 24 hours before surgery. Anesthesia was induced with intramuscular ketamine (15–20 mg/kg) combined with diazepam (0.5 mg/kg), and sedation was maintained with ketamine (10 mg/kg). A longitudinal incision was made on the chest to expose the heart after sterilizing the surgical area. Heparin (1500 IU/kg) was given intravenously 15 minutes before cross-clamping, followed by the administration of St. Thomas II cardioplegia through the aorta at a dose of 20 cc/kg, accompanied by external cooling with ice packs. A myocardial sample measuring 0.5×0.5×0.5 cm was taken from the right ventricle at the 30-minute mark. The tissue was fixed, processed into paraffin blocks, and stained with hematoxylin-eosin (H&E). Histomorphological assessment was performed across five fields of view under a light microscope at 100× magnification, using a 0–5 scoring system based on the level of damage, and was evaluated by two independent observers.

The collected data were processed using a computer and analyzed with GraphPad version 10 software. First, a descriptive analysis was performed by calculating measures of central tendency (mean and median) and data spread (standard deviation) for variables by treatment group. A Shapiro-Wilk test was conducted for data normality, and data distribution was visualized. Since the necrosis score is numerical, an Independent T-test was planned for normally distributed data, while the Mann-Whitney U test was used for non-normally distributed data to assess significance.

This research obtained ethical approval with number 2.KEH.172.12.2024 from the Animal Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, on December 4, 2024, adhering to the principles of reduction, refinement, and replacement.

4. RESULTS AND DISCUSSION

A preliminary study was conducted on three rabbits to assess the feasibility of the sternotomy, aortic cross-clamping, and cardioplegia administration procedures up to tissue sampling. Cardiac arrest was achieved within two minutes of cardioplegia administration, after which myocardial samples were taken at the 30-minute mark, fixed in formalin, and examined histomorphologically with H&E staining. The results of this pilot confirmed that the research protocol could be executed as planned and that parameters such as the degree of damage, extent of tissue damage, and inflammatory cell infiltration could be properly assessed at 200× magnification.

The study results showed that normal rabbit myocardium is composed of branched cylindrical cardiomyocytes with central nuclei, homogenous eosinophilic cytoplasm, and intercellular connections via intercalated discs, surrounded by capillary-rich endomysium without signs of inflammation, necrosis, or degeneration. In the St. Thomas II only group (Group A), histological changes were observed, including disorganization of muscle fibers, widening of intercellular spaces indicating interstitial edema, and cellular degeneration with indistinct cell borders, suggesting early necrosis. In contrast, the combined St. Thomas II and colchicine group (Group B) exhibited more orderly muscle fiber structures with narrow intercellular spaces and no signs of degeneration, demonstrating the protective effect of colchicine on myocardial integrity. These findings indicate that colchicine, through its anti-inflammatory mechanism and inhibition of neutrophil activation, can potentially reduce tissue damage from ischemia-reperfusion injury (Figure 1).

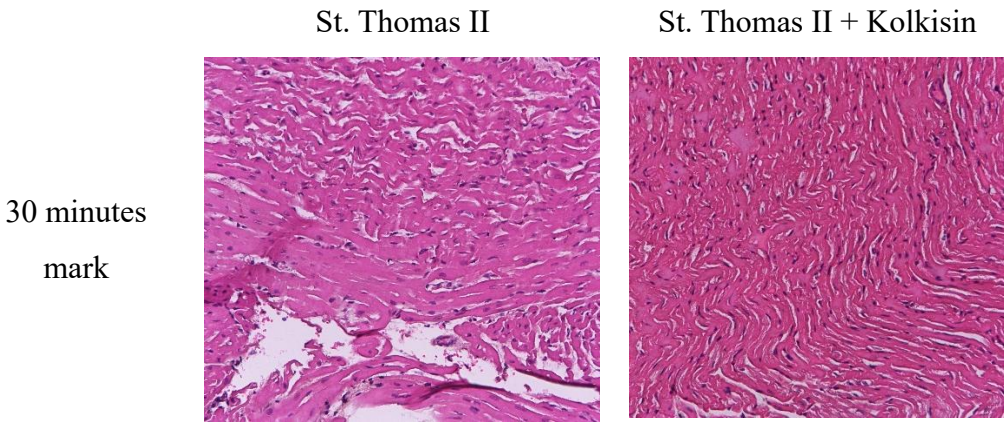

Figure 1. Histomorphological images of H&E stained specimens at 200x magnification at 30 minutes

Histological examination of Group B showed myocardial tissue with a characteristic woven pattern of longitudinally oriented and winding cardiomyocyte fibers, eosinophilic cytoplasm, and oval-shaped central nuclei with uniform morphology. Transverse striations were faint, and intercalated discs were not clearly visible but were presumed to be present based on the cell connection patterns. No histomorphological abnormalities such as necrosis, inflammatory cell infiltration, congestion, or hemorrhage were found, and the presence of small capillaries between muscle fibers indicated that microcirculation was preserved (Figure 2).

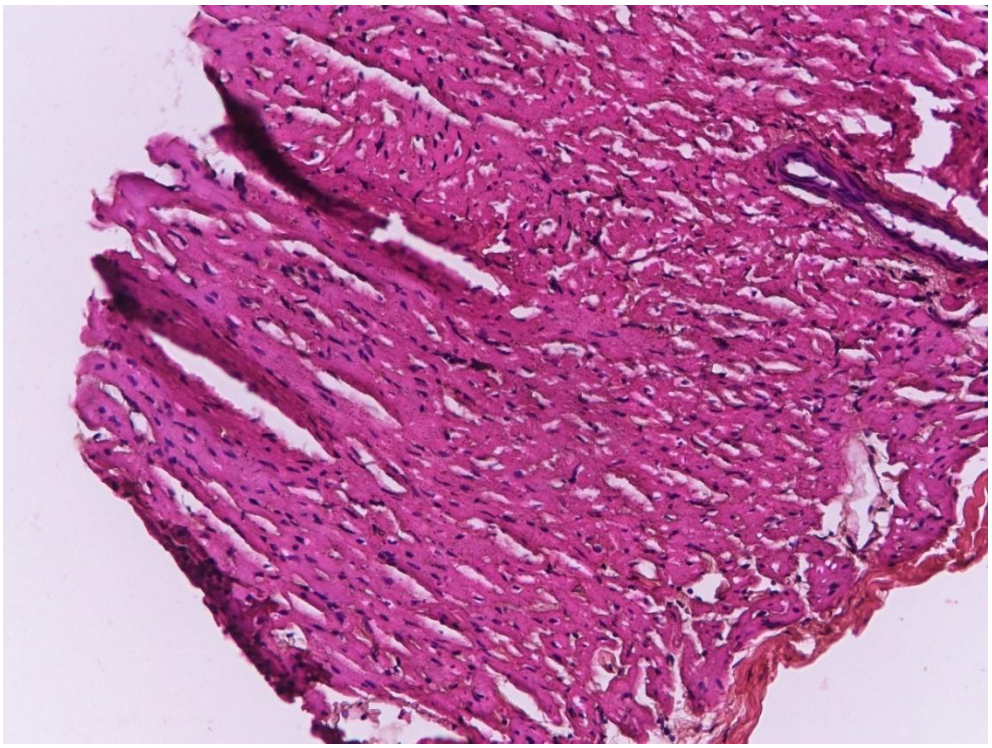

Figure 2. Histological view of the myocardium in a sample from Group B at 30 minutes (200x magnification**)**

Histologically, the myocardial tissue from a Group A sample in Figure 3 shows disorganization of the cardiac muscle fiber structure with a loss of the regular bundle pattern. There is a noticeable widening of the intercellular space and mild infiltration of inflammatory cells. Some cell nuclei show pyknosis, indicating the onset of cellular degeneration. The intercalated discs and transverse striations are not clearly visible, reflecting subcellular structural damage that could potentially affect myocardial contractile function (Figure 3)

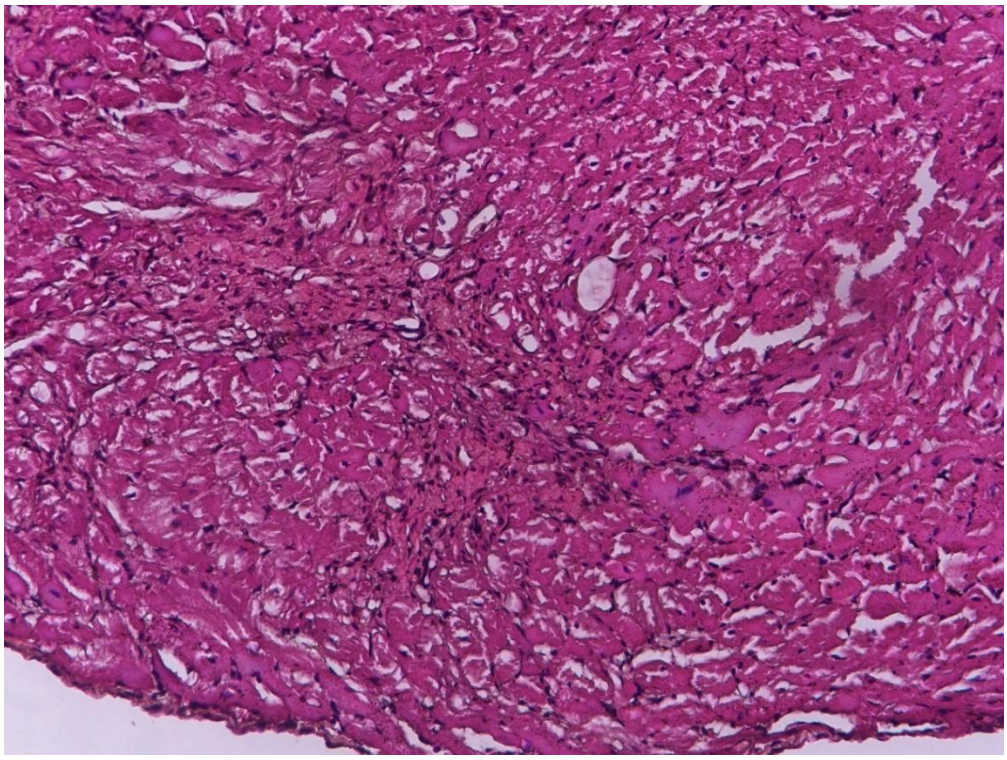

Figure 3. Histological view of the myocardium in a sample from Group A at 30 minutes (200x magnification)

This study involved 12 New Zealand white rabbits randomly divided into two groups of six each. Group A received St. Thomas II cardioplegia solution, while Group B received a combination of St. Thomas II and colchicine. Myocardial tissue samples were taken 30 minutes post-treatment, processed into paraffin preparations, stained with H&E, and observed under a light microscope at 200x magnification. Descriptive analysis showed the median tissue damage score in Group A was 3.00 (mean 2.83 ± 0.41), while Group B had a median of 0.00 (mean 0.50 ± 1.23).

Table.1 Descriptive analysis of the data.

Group	*n*	Minimum	Maximum	Median	*Mean* ± SD
St. Thomas II	6	2	3	3	2,83 ± 0,41
St. Thomas II + Colchicine	6	0	3	0	0,50 ± 1,23

The frequency distribution revealed that in Group A, five of the six samples experienced severe damage (score 3), whereas in Group B, five of the six samples showed no damage (score 0). The Mann-Whitney test yielded a p-value of 0.0152 ($p < 0.05$), indicating a significant difference between the two groups.

Table 1. Difference in the Degree of Damage at 30 Minutes

Group	Damage at 30 Minutes						*p-value*
	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5	
St. Thomas II	0	0	1	5	0	0	0,0152*
St. Thomas II + Colchicine	5	0	0	1	0	0	

*Note: * Significant ($p < 0.05$) on the Mann-Whitney test*

Discussion

St. Thomas II with Colchicine as a Cardioprotective Agent

This study demonstrates that the addition of colchicine to St. Thomas II cardioplegia solution significantly reduces the degree of histomorphological myocardial damage in an animal model (Terkeltaub, 2009). Colchicine acts as an effective anti-inflammatory agent by inhibiting the process of neutrophil polymerization. This mechanism prevents the movement and activity of neutrophils into inflamed areas (Leung et al., 2015). In the context of myocardial ischemia, inflammatory cells exacerbate tissue damage by releasing destructive enzymes like neutrophil elastase and free radicals (Frangogiannis, 2015).

The findings of this study reinforce the hypothesis that inflammatory mechanisms, particularly NLRP3 inflammasome activation and neutrophil infiltration, play a major role in worsening reperfusion injury (Terkeltaub, 2009). By inhibiting neutrophil microtubule polymerization, colchicine suppresses inflammatory cell infiltration and the release of cytotoxic mediators, thereby better preserving the integrity of the myocardial structure. [20,21] Therefore, colchicine's role involves suppressing the inflammatory process and minimizing myocardial damage by reducing the number of affected neutrophils (Leung et al., 2015).

Cytological evaluation of the histomorphological preparations revealed that the myocardial tissue in the combined St. Thomas II and colchicine group showed a more regular muscle fiber architecture and no signs of congestion or hemorrhage. The cellular structure was also better preserved, with homogenous cytoplasm and minimal inflammatory cell infiltration in the combination group compared to the control. This confirms that colchicine has potential as an

adjuvant cardioprotective agent in cardioplegia procedures. This aligns with a study by Defteros et al., which showed that colchicine could reduce inflammation and prevent myocardial damage in an animal model (Deftereos et al., 2014).

Colchicine is also known to inhibit the expression of the NLRP3 inflammasome, which is pivotal in triggering systemic and local inflammatory responses in various pathological conditions, including ischemia-reperfusion injury (Bakhta et al., 2018; Martin-Sanchez et al., 2016). This inhibition prevents the activation of interleukin-1β (IL-1β), a key player in exacerbating myocardial inflammation (Akodad et al., 2020). In a study by Tardif et al., (2019) colchicine was proven to lower levels of IL-1β and TNF-α and reduce the recurrence of myocardial infarction. This study supports experimental findings that colchicine can decrease inflammatory cell infiltration and reduce the degree of myocardial tissue damage.

The use of colchicine added to cardioplegia in this study presents a "dual-action cardioprotection" approach: electrical and metabolic protection from St. Thomas II, coupled with immunological protection from colchicine. This combination could potentially fill a gap in the perioperative management of cardiac surgery patients, which has historically focused more on electrophysiological aspects while paying less attention to the systemic inflammatory response. These results point toward future research directions for integrating immunomodulatory molecules into standard cardioplegia compositions. In the future, cardioplegia solutions may no longer be just a medium for temporary cardiac arrest but also a therapeutic vehicle that actively modulates the biological response to surgical trauma and ischemia.

Assessment of Necrosis and Method Validity

The use of a histopathology scoring system to assess myocardial tissue damage is appropriate as it provides a quantitative standard that allows for comparison between groups. This system has been previously used in other experimental studies to evaluate myocardial necrosis due to ischemia and reperfusion (Bolli et al., 2004). The strength of this method lies in its objectivity, although interobserver variability remains a limitation that should be addressed through blinded assessment to enhance reliability. Furthermore, a histopathology scoring system is considered more representative than purely descriptive assessment because it can classify the degree of tissue damage into measurable categories, such as mild, moderate, or severe (Reimer & Jennings, 1979). Penerapan metode ini memungkinkan analisis statistik yang lebih bermakna sehingga hasil penelitian dapat dibandingkan dengan studi lain secara sistematis.

Despite its utility, the semi-quantitative nature of histological scoring is still influenced by scorer subjectivity, leading some studies to suggest using digital image analysis technology to improve accuracy (McCarty et al., 1985). Technologies such as digital pathology and quantitative image analysis allow for more consistent assessment by leveraging pattern recognition algorithms on histological images (Madabhushi, 2016). While the histopathology scoring system used in this research was adequate, the future development of digital quantification methods could enhance precision, especially in translational studies aimed at clinical application.

Suitability of Hematoxylin and Eosin Staining to Measure Myocardial Tissue Damage

H&E staining is the standard and most widely used method in histopathology for assessing myocardial tissue damage. It can reveal fundamental changes such as coagulative necrosis, loss of cell membrane integrity, nuclear fragility (karyolysis, pyknosis, karyorrhexis), and the presence of inflammatory cell infiltration (Kumar, 2017). Oleh karena itu, pewarnaan HE dapat dianggap cukup untuk tujuan penelitian ini yang berfokus pada penilaian derajat nekrosis.

Therefore, H&E staining is considered sufficient for the purpose of this study, which focused on assessing the degree of necrosis. However, H&E has limitations; it cannot specifically differentiate between necrosis and apoptosis, so the mechanism of cell death cannot be definitively determined. Identifying apoptosis is more accurately done with additional methods like the TUNEL assay or immunohistochemical staining for caspase-3 (Gavrieli et al., 1992). HE sensitivity is also limited in detecting collagen deposition in the late phase, which neccessitates additional staining such as Masson's trichrome or Sirius red to be used for studies targeting long-term remodeling (Toldo & Abbate, 2018).

Several previous experimental studies have also used HE as the primary method for assessing post-ischemia-reperfusion myocardial damage, particularly in animal models. A study by Toldo et al., for example, used only HE to assess acute necrosis before supplementing it with inflammatory markers to deepen the analysis.[34] This fact shows that the use of HE remains valid, especially when the research objective focuses on evaluating initial structural damage. The application of HE in this study is considered adequate because it focuses on assessing the degree of tissue necrosis, although further research targeting deeper pathogenesis should consider additional staining panels or immunohistochemistry techniques to obtain more comprehensive results.

Comparison with Other Studies Using Inflammatory Markers

The results of this study are consistent with other findings showing that inflammation is a major determinant in the pathogenesis of post-ischemia-reperfusion myocardial injury. Classic biomarkers such as troponin I (TnI) and CK-MB have long been recognized as specific indicators of cardiomyocyte necrosis, so that their increase correlates directly with the extent of tissue damage (Reichlin el at., 2009). Malondialdehyde (MDA) as a marker of oxidative stress was also reported to increase in conditions of lipid peroxidation due to longer aortic cross-clamp duration, thereby contributing to oxidative damage to myocardial cell membranes (Djordjević et al., 2020).

A study by Nilsen et al. showed that IL-1β levels increased significantly in patients with acute coronary syndrome and were closely associated with a poorer prognosis, including an increased risk of major cardiovascular events (Nilsen et al., 2025). This indicates a close relationship between systemic inflammation and clinical outcomes. A similar report was presented by Toldo et al., (2018) who found that increased inflammatory cytokines such as IL-1β and TNF-α were associated with enlarged infarct size and left ventricular remodeling.

The methodological differences need to be noted because this study assessed inflammation primarily through a histopathological approach using HE staining, whereas clinical studies generally use serum biomarkers as parameters of inflammation. The relevance remains intact considering that necrosis and inflammatory cell infiltration observed histologically represent the activation of the inflammatory cascade at the tissue level. This study provides additional evidence that strengthens the literature on the role of inflammation and oxidative stress in myocardial damage, and emphasizes the importance of integrating histopathological assessment and inflammatory biomarker analysis to obtain a more comprehensive picture of the mechanisms of myocardial injury after ischemia-reperfusion.

Clinical Implications and Applicability

The findings of this study indicate that the addition of colchicine to cardioplegic solutions has the potential to provide a protective effect against myocardial injury caused by ischemia-reperfusion. This strategy is clinically relevant because reperfusion injury remains a major challenge in cardiac surgery procedures, including coronary artery bypass grafting (CABG) and valve replacement surgery. Therefore, pharmacological approaches that suppress inflammasome activation and reduce the release of proinflammatory cytokines may contribute to improved postoperative outcomes (Ferdinandy et al., 2014).

Interpretation of these research results still requires caution because the model used is still animal-based, while human biological responses, particularly in postoperative inflammation, are influenced by comorbidities, supportive therapy, and genetic variations that cannot be fully simulated in laboratory animals (Lindsey et al., 2018). Therefore, translational testing in human populations remains necessary before colchicine can be considered as a standard therapy in cardioplegia procedures. In addition, histopathological assessment using HE staining does provide a basic picture of the degree of necrosis and inflammatory infiltration. However, this approach has limited sensitivity in detecting more specific molecular inflammatory activation. Therefore, the use of additional staining techniques such as immunohistochemistry for IL-1β, caspase-1, and oxidative stress markers (e.g., 8-OHdG), as well as measurement of serum biomarkers (such as hs-CRP and TNF-α), can provide a more comprehensive understanding of the protective mechanisms of colchicine (Toldo et al., 2018).

Clinical evidence from previous trials, such as the COLCOT trial (Tardif et al., 2019) and LoDoCo2 trial (Nidorf et al., 2020), shows that low-dose colchicine (0.5 mg/day) reduces the incidence of major cardiovascular events in patients with coronary artery disease, reinforcing the hypothesis regarding the relevance of colchicine's anti-inflammatory effects in clinical practice.[2,26] This strengthens the hypothesis that the anti-inflammatory effects of colchicine have significant clinical relevance. When translated into the context of cardiac surgery, the addition of colchicine to cardioplegic solutions has the potential to improve postoperative recovery and reduce the risk of ischemia-reperfusion complications.

Thus, this study opens up opportunities for further research on the application of colchicine in cardiac surgery. Future research should include translational studies in humans, integration of inflammatory and oxidative biomarkers, and long-term analysis of clinical outcomes to assess the sustainability of colchicine's protective benefits.

Clinical Implications and Further Translational Stages of Study

The results of this study show that the addition of colchicine to St. Thomas II cardioplegia solution significantly reduces the degree of myocardial histomorphological damage caused by ischemia-reperfusion injury in animal models, thus providing a basis for the development of adjuvant strategies in human cardiac surgery practice. The translational potential of these findings still requires further validation through a stepwise approach to ensure efficacy, safety, and ethical and regulatory compliance. Advanced preclinical stages in large animals such as pigs or sheep are necessary because their cardiac physiology more closely resembles that of humans, with assessments covering in vivo cardiac function, colchicine pharmacokinetic/dynamic profiles, and systemic toxicity evaluations. Research on the toxicity

and safety of colchicine use in cardioplegic solutions is also an important step, given its narrow therapeutic range and potential interactions with St. Thomas II electrolyte components that can affect cardiac contractility and electrical conduction.

The next stage involves a phase I clinical trial to assess the safety, tolerability, and pharmacokinetics of local colchicine administration during cardioplegia procedures with a limited number of subjects at specialized cardiac surgery centers. If proven safe, the study will proceed to phases II and III, which will evaluate clinical efficacy based on objective parameters such as postoperative troponin, arrhythmia incidence, postoperative recovery time, and mortality, and compare cardioplegia with and without colchicine through a double-blind randomized design. Strong evidence from these trials could form the basis for registration applications to regulatory bodies such as BPOM, FDA, or EMA to obtain routine use approval, including cost, availability, and feasibility studies for implementation in hospitals.

Clinically, the combination strategy of St. Thomas II cardioplegia with colchicine offers a dual-action cardioprotection approach, namely metabolic-electrophysiological protection by cardioplegia and immunological protection by colchicine. If these findings can be translated to humans, future cardioplegia solutions will not only function to temporarily stop the heart, but also as an active therapeutic medium capable of suppressing inflammation and improving postoperative outcomes. Early-phase clinical trials in cardiac surgery procedures are needed to assess the safety, optimal dosage, and effectiveness of colchicine as a cardioplegia adjuvant.

Study Limitations

This study has several limitations. First, the sample size was relatively small (n=12), so the results should be interpreted with caution. Second, the assessment of tissue damage was based solely on histopathological examination with H&E staining, without confirmation from biochemical biomarkers (e.g., troponin, MDA, or IL-1β) that could strengthen the validity of the findings. Third, the rabbit animal model does not fully reflect the complexity of the human biological response, especially in patients with cardiovascular comorbidities. Therefore, further research with a larger sample size, a more comprehensive panel of biomarkers, and translational studies in larger animals and humans is necessary.

5. CONCLUSION

Based on the results of this study on the effect of administering colchicine with St. Thomas II cardioplegia solution on the degree of myocardial tissue damage in an animal model, it can be concluded that the addition of colchicine results in a histopathologically better reduction in myocardial tissue damage compared to not administering colchicine in the hearts of New

Zealand rabbits. Translational clinical trials are needed to evaluate the safety, effectiveness, and optimal dosage of colchicine as a cardioplegia adjuvant in cardiac surgery. Further research is also recommended to strengthen the evidence supporting colchicine's efficacy as a myocardial protective agent. In addition, future studies should include an evaluation of the left ventricular muscle, which is more relevant to human cardiac pump function.

REFERENCES

Akodad, M., Sicard, P., Fauconnier, J., & Roubille, F. (2020). Colchicine and myocardial infarction: A review. *Archives of Cardiovascular Diseases*, *113*(10), 652–659. https://doi.org/10.1016/j.acvd.2020.04.007

Bakhta, O., Blanchard, S., Guihot, A. L., Tamareille, S., Mirebeau-Prunier, D., Jeannin, P., Le Corvoisier, P., & Prunier, F. (2018). Cardioprotective role of colchicine against inflammatory injury in a rat model of acute myocardial infarction. *Journal of Cardiovascular Pharmacology and Therapeutics*, *23*(5), 446–455. https://doi.org/10.1177/1074248418763611

Bar-El, Y., Adler, Z., Kophit, A., Kertzman, V., Sawaed, S., Ross, A., & Pifano, M. (1999). Myocardial protection in operations requiring more than 2 h of aortic cross-clamping. *European Journal of Cardio-Thoracic Surgery*, *15*(3), 271–275. https://doi.org/10.1016/S1010-7940(99)00025-1

Beyersdorf, F. (2009). The use of controlled reperfusion strategies in cardiac surgery to minimize ischaemia/reperfusion damage. *Cardiovascular Research*, *83*(2), 262–268. https://doi.org/10.1093/cvr/cvp110

Beyersdorf, F., Trummer, G., Benk, C., & Pooth, J. S. (2021). Application of cardiac surgery techniques to improve the results of cardiopulmonary resuscitation after cardiac arrest: Controlled automated reperfusion of the whole body. *JTCVS Open*, *8*, 47–52. https://doi.org/10.1016/j.xjon.2021.10.006

Bolli, R., Becker, L., Gross, G., Mentzer, R., Balshaw, D., & Lathrop, D. A. (2004). Myocardial protection at a crossroads: The need for translation into clinical therapy. *Circulation Research*, *95*(2), 125–134. https://doi.org/10.1161/01.RES.0000137171.97172.d7

Bonaventura, A., & Abbate, A. (2023). Colchicine for cardiovascular prevention: The dawn of a new era has finally come. *European Heart Journal*, *44*(35), 3303–3304. https://doi.org/10.1093/eurheartj/ehad453

Catinella, F. P., Cunningham, J. N. J., Adams, P. X., Snively, S. L., Gross, R. I., & Spencer, F. C. (1982). Myocardial protection with cold blood potassium cardioplegia during prolonged aortic cross-clamping. *The Annals of Thoracic Surgery*, *33*(3), 228–233. https://doi.org/10.1016/S0003-4975(10)61916-9

Deftereos, S., Giannopoulos, G., Panagopoulou, V., Bouras, G., Raisakis, K., Kossyvakis, C., Toli, K., Karageorgiou, S., Toutouzas, K., & Pyrgakis, V. (2014). Anti-inflammatory treatment with colchicine instable chronic heart failure. A prospective, randomized study. *JACC. Heart Failure*, *2*(2), 131–137.

Djordjević, A., Kotnik, P., Horvat, D., Knez, Ž., & Antonič, M. (2020). Pharmacodynamics of malondialdehyde as indirect oxidative stress marker after arrested-heart cardiopulmonary bypass surgery. *Biomedicine & Pharmacotherapy*, *132*, 1–5. https://doi.org/10.1016/j.biopha.2020.110877

DWT, N., Leon De La Fuente, R., Gallo, P., Naesgaard, P., Dib Ashur, S., Michelsen, A. E., Omland, T., & Vethe, N. (2025). High admission levels of interleukin-1 receptor antagonist in acute myocardial infarction patients are associated with increased rates of all-cause mortality and cardiac death at five years follow-up. *International Journal of Cardiology*, *439*, Article 133674. https://doi.org/10.1016/j.ijcard.2025.133674

Ferdinandy, P., Hausenloy, D. J., Heusch, G., Baxter, G. F., & Schulz, R. (2014). Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacological Reviews*, *66*(4), 1142–1174. https://doi.org/10.1124/pr.113.008300

Fischesser, D. M., Bo, B., Benton, R. P., Su, H., Jahanpanah, N., & Haworth, K. J. (2021). Controlling reperfusion injury with controlled reperfusion: Historical perspectives and new paradigms. *Journal of Cardiovascular Pharmacology and Therapeutics*, *26*(5), 504–523. https://doi.org/10.1177/10742484211046674

Frangogiannis, N. G. (2015). Pathophysiology of myocardial infarction. *Comprehensive Physiology*, *5*(4), 1841–1875.

García-de-la-Asunción, J., Pastor, E., Perez-Griera, J., Belda, F. J., Moreno, T., García-del-Olmo, E., Boscá, L., & Sastre, J. (2013). Oxidative stress injury after on-pump cardiac surgery: Effects of aortic cross clamp time and type of surgery. *Redox Report*, *18*(5), 193–199. https://doi.org/10.1179/1351000213Y.0000000060

Gavrieli, Y., Sherman, Y., & Ben-Sasson, S. A. (1992). Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *The Journal of Cell Biology*, *119*(3), 493–501. https://doi.org/10.1083/jcb.119.3.493

Hausenloy, D. J., & Yellon, D. M. (2013). Myocardial ischemia-reperfusion injury: A neglected therapeutic target. *The Journal of Clinical Investigation*, *123*(1), 92–100. https://doi.org/10.1172/JCI62874

Hearse, D. J., Stewart, D. A., & Braimbridge, M. V. (1978). Myocardial protection during ischemic cardiac arrest. Possible deleterious effects of glucose and mannitol in coronary infusates. *The Journal of Thoracic and Cardiovascular Surgery*, *76*(1), 16–23. https://doi.org/10.1016/S0022-5223(19)40927-6

Junqueira, L. C., Bignolas, G., & Brentani, R. R. (1979). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochemical Journal*, *11*(4), 447–455. https://link.springer.com/article/10.1007/bf01002772

Kumar, V., Abbas, A. K., & Aster, J. C. (2017). *Robbins basic pathology* (10th ed.). Elsevier - Health Sciences Division.

Leung, Y. Y., Yao Hui, L. L., & Kraus, V. B. (2015). Colchicine—Update on mechanisms of action and therapeutic uses. *Seminars in Arthritis and Rheumatism*, *45*(3), 341–350. https://doi.org/10.1016/j.semarthrit.2015.06.013

Li, H., Yang, H., Qin, Z., Wang, Q., & Li, L. (2024). Colchicine ameliorates myocardial injury induced by coronary microembolization through suppressing pyroptosis via the AMPK/SIRT1/NLRP3 signaling pathway. *BMC Cardiovascular Disorders*, *24*(1), Article 23.

Lilly, L. S. (2016). *Pathophysiology of heart disease: A collaborative project of medical students and faculty* (6th ed.). Lippincott Williams & Wilkins.

Lindsey, M. L., Bolli, R., Canty, J. M., Jr., Du, X. J., Frangogiannis, N. G., Frantz, S., Jones, S. P., Lefer, D. J., Mendiz, E. A., & Prunier, F. (2018). Guidelines for experimental models of myocardial ischemia and infarction. *American Journal of Physiology. Heart and Circulatory Physiology*, *314*(4), H812–H838. https://doi.org/10.1152/ajpheart.00335.2017

Madabhushi, A., & Lee, G. (2016). Image analysis and machine learning in digital pathology: Challenges and opportunities. *Medical Image Analysis*, *33*, 170–175. https://doi.org/10.1016/j.media.2016.06.037

Martin-Sanchez, F., Diamond, C., Zeitler, M., Gomez, A. I., Baroja-Mazo, A., Bagnall, J., Bovis, B., Clarke, A., place, M. L., & Latz, E. (2016). Inflammasome-dependent IL-1β release depends upon membrane permeabilisation. *Cell Death and Differentiation*, *23*(7), 1219–1231.

McCarty, K. S., Jr., Miller, L. S., Cox, E. B., Konrath, J., & McCarty, K. S., Sr. (1985). Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Archives of Pathology & Laboratory Medicine*, *109*(8), 716–721.

Minamino, T. (2012). Cardioprotection from ischemia/reperfusion injury: Basic and translational research. *Circulation Journal*, *76*(5), 1074–1082. https://doi.org/10.1253/circj.CJ-12-0132

Nidorf, S. M., Fiolet, A. T. L., Mosterd, A., Eikelboom, J. W., Schut, A., Opstal, T. S. J., The, S. H. K., Feenstra, J., Marais, G. F., & De Smet, B. J. G. L. (2020). Colchicine in patients with chronic coronary disease. *The New England Journal of Medicine*, *383*(19), 1838–1847. https://doi.org/10.1056/NEJMoa2021372

Oberman, R., Shumway, K. R., & Bhardwaj, A. (2023). *Physiology, cardiac*. StatPearls. Retrieved May 17, 2025, from https://www.ncbi.nlm.nih.gov/books/NBK526089/

Perrelli, M. G., Pagliaro, P., & Penna, C. (2011). Ischemia/reperfusion injury and cardioprotective mechanisms: Role of mitochondria and reactive oxygen species. *World Journal of Cardiology*, *3*(6), 186–200. https://doi.org/10.4330/wjc.v3.i6.186

Reichlin, T., Hochholzer, W., Bassetti, S., Steuer, S., Stelzig, C., Hartwiger, S., Biedert, S., Schaub, N., Buergler, D., & Freidank, H. (2009). Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *The New England Journal of Medicine*, *361*(9), 858–867. https://doi.org/10.1056/NEJMoa0900428

Reimer, K. A., & Jennings, R. B. (1979). The changing anatomic reference base of evolving myocardial infarction. Underestimation of myocardial collateral blood flow and overestimation of experimental anatomic infarct size due to tissue edema, hemorrhage and acute inflammation. *Circulation*, *60*(4), 866–876. https://doi.org/10.1161/01.CIR.60.4.866

Robertson, S., Martínez, G. J., Payet, C. A., Barraclough, J. Y., Celermajer, D. S., Bursill, C., & Patel, S. (2016). Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clinical Science*, *130*(15), 1237–1246. https://doi.org/10.1042/CS20160090

Sun, X., Duan, J., Gong, C., Feng, Y., Hu, J., Gu, R., Zhang, M., Hou, J., Zhang, F., & Li, G. (2022). Colchicine ameliorates dilated cardiomyopathy via SIRT2-mediated suppression of NLRP3 inflammasome activation. *Journal of the American Heart Association*, *11*(11), Article e025266. https://doi.org/10.1161/JAHA.122.025266

Tardif, J. C., Kouz, S., Waters, D. D., Bertrand, O. F., Diaz, R., Maggioni, A. P., Schunck, R. J., Pericard, S., Koenig, W., & Pinto, F. J. (2019). Efficacy and safety of low-dose colchicine after myocardial infarction. *The New England Journal of Medicine*, *381*(26), 2497–2505. https://doi.org/10.1056/NEJMoa1912388

Terkeltaub, R. A. (2009). Colchicine update: 2008. *Seminars in Arthritis and Rheumatism*, *38*(6), 411–419. https://doi.org/10.1016/j.semarthrit.2008.08.006

Toldo, S., & Abbate, A. (2018). The NLRP3 inflammasome in acute myocardial infarction. *Nature Reviews. Cardiology*, *15*(4), 203–214.

Toldo, S., Mauro, A. G., Cutter, Z., & Abbate, A. (2018). Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *American Journal of Physiology. Heart and Circulatory Physiology*, *315*(6), H1553–H1568. https://doi.org/10.1152/ajpheart.00158.2018

Wardoyo, S., Djer, M. M., & Busro, P. W. (2025). Evaluation of myocardial injury from use of aortic cross-clamp and cardiopulmonary bypass duration in patients undergoing tetralogy of Fallot corrective surgery. *Paediatrica Indonesiana*, *65*(3), 147–155. https://doi.org/10.14238/pi65.2.2025.147-55

Yang, X., Cohen, M. V., & Downey, J. M. (2010). Mechanism of cardioprotection by early ischemic preconditioning. *Cardiovascular Drugs and Therapy*, *24*(3), 225–234.