2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(2): 421-433.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj42
PII: S232245682500042-15

Effects of Javanese Cardamom and Turmeric on the Prevention of Colibacillosis and Its Impact on Broiler Chickens' Hearts

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ORIGINAL ARTICLE
Received: March 24, 2025
Revised: April 25, 2025
Accepted: May 22, 2025
Published: June 30, 2025

ABSTRACT

Colibacillosis represents a major threat to vital organs, particularly the heart, in broiler chickens. Concerns over rising antibiotic resistance have prompted interest in alternative therapies utilizing active compounds such as 1,8-cineole from Javanese cardamom essential oil (JCEO) and curcumin from turmeric ethanol extract (TEE), both known for their antibacterial and anti-inflammatory properties. This study aimed to evaluate the histopathological effects of JCEO and TEE and to determine the optimal dosage for reducing heart tissue damage caused by colibacillosis in broiler chickens. A total of 72 Cobb-strain day-old chicks (DOCs) were randomly allocated into eight groups (three chickens per group, three replications). The groups included a negative control (no *E. coli* infection or treatment), a positive control (*E. coli* infection without treatment), and six treatment groups including *E. coli* + JCEO (0.06 ml/kg BW) + TEE (400 mg/kg feed), *E. coli* + JCEO (0.1 ml/kg BW) + TEE (400 mg/kg feed), *E. coli* + JCEO (0.06 ml/kg BW), *E. coli* + JCEO (0.1 ml/kg BW), *E. coli* + TEE (400 mg/kg feed), and *E. coli* + ciprofloxacin (10 mg/kg BW). Colibacillosis was induced via intraperitoneal injection of *E. coli* strain O78 at four weeks of age, and herbal treatments were administered orally via drinking water from day 7 to week 5. Histopathological evaluation of heart tissues was conducted, scoring lesions as mild, moderate, or severe. The positive control group exhibited the highest total lesion score, indicating extensive heart damage, while the group treated with JCEO (0.1 ml/kg BW) + TEE (400 mg/kg feed) showed the lowest lesion score, suggesting strong protective effects. Severe lesions were notably observed in the ciprofloxacin and TEE-only groups. The combination of JCEO (0.1 ml/kg BW) + TEE (400 mg/kg feed) proved most effective in minimizing heart tissue damage, outperforming both single-agent treatments and ciprofloxacin, likely due to synergistic antibacterial and anti-inflammatory actions.

Keywords: 1,8-cineole, Antibacterial, Anti-inflammatory, Colibacillosis, Curcumin

INTRODUCTION

Developing broiler chickens requires intensive management, particularly in ensuring their health. Broiler chickens are highly susceptible to colibacillosis, a condition caused by infection with *Escherichia coli* (*E. coli*). In mammals, *E. coli* infections commonly manifest as primary diseases affecting the gastrointestinal or urinary tracts. Conversely, in poultry, colibacillosis typically presents as a secondary local or systemic disease when the immune system is compromised by virulent strains of *E. coli* (Jahantigh and Dizaji, 2015). Colibacillosis in broiler chickens can spread through the bloodstream, so it is often known as colisepticemia. Colisepticemia can attack different organs, especially the heart organ which plays a vital role as a pump to circulate blood throughout the body, supplying the necessary oxygen and nutrients. The primary indicators of colisepticemia impacting the heart include pericarditis and myocarditis. During the initial stages of infection, fluid and a soft, pale exudate begin to accumulate within the pericardial sac, subsequently leading to the development of fibrinous exudate (Nolan et al., 2013). As the disease progresses, the exudate increases, and the inflamed pericardial sac undergoes fibrosis, resulting in constrictive pericarditis of the heart (Panth, 2019). Constrictive pericarditis reduces pericardial elasticity, which causes restriction of ventricular filling. This restriction of ventricular filling leads to a decrease in end-diastolic volume and cardiac output, which ultimately contributes to heart failure (Panth, 2019; Yadav and Siddique, 2021).

Colibacillosis is generally treated with antibiotics. However, the use of a high-dose antibiotic in chickens creates a major concern, which can lead to increased antibiotic resistance (Jahantigh et al., 2020). The rise of antibiotic resistance complicates the treatment of colibacillosis in broiler chickens (Nawaz et al., 2024). Biopharmaceutical plants can be used as an alternative antibiotic for anti-colibacillosis in broiler chickens. Javanese cardamom (*Amomum compactum* Soland. Ex Maton) and turmeric (*Curcuma domestica* Valeton) are biopharmaceutical plants with the potential for natural colibacillosis treatments (Wientarsih et al., 2013; Masood et al., 2021). Javanese cardamom contains alkaloids, flavonoids, terpenoids, and tannins that can inhibit bacterial growth (Komala and Maulana, 2020). In addition, Javanese

To cite this paper: Hartady T, Sugandi SD, Septiyani, Hiroyuki A, and Goenawan H (2025). Prevention of Colibacillosis and Its Impact on Broiler Chickens' Heart with Javanese Cardamom and Turmeric. *World Vet. J.*, 15(2): 421-433. DOI: https://dx.doi.org/10.54203/scil.2025.wvj42

cardamom contains the essential oil in the form of 1,8-cineole, which can inhibit the growth of *Salmonella typhimurium*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and Methicillin-resistant *Staphylococcus aureus* (Ujilestari et al., 2019). Curcumin can act as an anti-inflammatory pleiotropic molecule that interacts with various targets in inflammatory reactions, such as TNFα and ILs in mice (Aggarwal et al., 2013). Curcumin inhibits bacteria by preventing the polymerization of Filamenting temperature sensitive mutant Z (FtsZ) is an important protein needed when *E. coli* divides, forming a contractile ring structure (Z-ring) at the site of cell division (Kaur et al., 2010). With the presence of curcumin, the formation of FtsZ can be inhibited. The present study aimed to determine the histopathological effects on the hearts of broiler chickens and to indicate the formulation and the optimal dose of Javanese cardamom essential oil and curcumin.

MATERIALS AND METHODS

Ethical approval

This study has received approval from the Padjadjaran University Research Ethics Commission, number 1048/UN6.KEP/EC/2024. This study was conducted from September to October 2024 at the Subang Veterinary Center, Padjadjaran University Faculty of Medicine Laboratory, and Padjadjaran University Central Laboratory in Indonesia.

Study design

This study employed an experimental method to assess the effects of pathogenic *E. coli* strain O78 on broiler chickens (day-old chick, strain Cobb), following a modified protocol by Song et al. (2022). The *E. coli* strain was obtained from the Subang Veterinary Center, Indonesia. A total of 72-day-old chicks (DOCs), comprising males and females, were randomly allocated into 8 treatment groups, each containing 3 birds and 3 replications. The treatment group namely negative control, positive control, *E. coli* + 0.06 ml/kgBW JCEO + 400 mg/kg feed TEE, *E. coli* + 0.1 ml/kgBW JCEO + 400 mg/kg feed TEE, *E. coli* + 0.06 ml/kgBW JCEO, *E. coli* + 0.1 ml/kgBW JCEO, *E. coli* + 400 mg/kg feed TEE, and *E. coli* + 1 g/2L of drinking water ciprofloxacin. All treatment groups, including the positive control, were infected with *E. coli* strain O78, except for the negative control group, which received no *E. coli* injection or treatment. The positive control group was injected with *E. coli* but received no additional interventions. In contrast, the group treated with ciprofloxacin received the antibiotic as a therapeutic intervention following *E. coli* infection. The selected dosages of JCEO (0.06 ml/kgBW and 0.1 ml/kgBW) and TEE (400 mg/kg feed) were based on prior studies and preliminary research (Osborne, 2007; Godbole et al., 2018). The plants used for Javanese cardamom oil (JCEO) and turmeric ethanol extract (TEE) were obtained from the Bumi Herbal in Bandung, Indonesia.

The chickens were housed in the Animal Husbandry Laboratory of Padjadjaran University for five weeks. Each treatment group was kept in separate cages, with each cage having a total area of 1.08 m² (9 chickens per group), and the entire experimental area totaled 8.64 m². The cages were constructed using galvanized steel, plastic, and water-resistant wood, with side ventilation to ensure adequate airflow. The temperature was maintained between 24–28°C using fans, and lighting was provided by LED lamps with an intensity of 20-30 lux. To prevent contamination or disease transmission, access to the cages was restricted. Personnel were required to wear personal protective equipment (PPE) and follow sanitation and disinfection protocols before entering the facility.

Herbal treatments, including Javanese cardamom oil (JCEO) and turmeric ethanol extract (TEE), were administered through drinking water starting from the age of seven days and continued until five weeks. At four weeks of age, all treatment groups were intraperitoneally infected with E. coli strain O78, while the negative control group was excluded from the infection. Clinical signs were observed daily for one week post-infection. At the end of the intervention period (five weeks), all chickens from the treatment groups were slaughtered, and their heart samples were aseptically isolated for histopathological analysis.

Sample collection

After reaching the age of five weeks, the chickens from all treatment groups were terminated, and their heart organs were separated aseptically to make histopathology preparations at the Subang Veterinary Center. The preparation of histopathology slides of heart tissues followed the standard histopathology protocol as described by Slaoui et al. (2017). The heart tissue was fixed in 10% formalin solution (Thermo Fisher Scientific) for 24 hours at room temperature (20–25°C) and then immersed in paraffin to become a paraffin block. The paraffin block was cut with a microtome (Slee Mainz-Cut6062, Germany) with a thickness of five micrometers. Tissue staining was performed using hematoxylin and eosin (Merck, Germany).

Javanese Cardamom Essential Oil (JCEO) production

The production of JCEO was carried out following the method of Raissa et al. (2020). The extraction process utilized seeds of Javanese cardamom (*Amomum compactum*) that were cleaned before extraction. Steam distillation was conducted for approximately six hours, starting from the first drop of distillate. The oil obtained, which was mixed with water, was separated using petroleum ether extraction. To remove residual water from the oil, anhydrous sodium sulfate was added in sufficient quantities until all water was completely absorbed. The results of this process finally produce JCEO with a calculated density of 0.98 g/ml (Sani et al., 2014).

Turmeric Ethanol Extract (TEE) production

The production of TEE was carried out following the method of Malahayati et al. (2021). The extraction method of turmeric was carried out by drying it in an oven for 24 hours and a temperature of 70℃. After that, the turmeric was dissolved in 70% ethanol and put into a 500 ml Erlenmeyer tube at room temperature (approximately 25°C). The mixture of turmeric powder and solvent was homogenized two times for five minutes each and subsequently macerated for 48 hours (2 x 24 hours). After the maceration process, the solution was filtered with filter paper with a pore size of 11 µm and a diameter of 12.5 cm. The resulting filtrate was evaporated at 60°C using a rotary vacuum evaporator to remove the ethanol. This extraction process yielded 20.17 grams of TEE.

Data analysis

Histopathological data of the heart samples were analyzed quantitatively using a structured illumination microscope (Zeiss Apotome2, Germany) and the ImageJ application (v1.54d; NIH, USA) to assess the level of tissue damage with a scoring method modified by Krishnegowda et al. (2020; Table 1). For each treatment group, histopathological images were analyzed from 10 fields of view per sample, ensuring consistency across groups. The area of each identified lesion (categorized as mild, moderate, or severe) was measured and quantified using the ImageJ application. These measurements were then used to assign lesion scores according to the following criteria:

To determine the total lesion score for each group, the lesion severity assessment results from ImageJ were converted into corresponding scores: 1 for mild, 2 for moderate, and 3 for severe. The identified lesions were categorized accordingly, and the total score was obtained by summing all lesion scores within a group. The final score was derived from three lesion severity categories (mild, moderate, severe) multiplied by 10 lesion descriptions, resulting in a total of 30 lesion assessments per group (Schafer et al., 2018).

The total lesion scores for each group were calculated by summing the scores of all analyzed fields of view using the GraphPad application. Statistical analysis was performed using two-way ANOVA to identify significant differences between groups, followed by Tukey's post hoc test ($p < 0.05$ was considered statistically significant). The scoring method was modified by standardizing the number of fields of view (10 fields per group) and employing quantitative area measurements to improve accuracy and reduce observer bias.

Table 1. Modified cardiac histopathology lesion parameter indicator

Indicator	Score	Lesion description
Mild	1	Congestion of blood vessels
		Thrombosis of blood vessels
		Vasculitis
		Hemorrhage
Moderate	2	Infiltration of inflammatory cells
		Damage to the nucleus (pyknosis, karyorrhexis, and karyolysis)
		Vacuolization
Severe	3	Formation of granulation tissue in fibrin exudate
		Proliferation of fibroblast cells
		Myocardium damage by granulation tissue

RESULTS AND DISCUSSION

The results of the total accumulation assessment of histopathology-scoring lesions (combining mild, moderate, and severe categories) in each treatment group have been presented in Table 2 and Figure 1. The lesion scores were derived from the histopathological evaluation of heart tissue, which included observations of mild lesion indicators (congestion of blood vessels, thrombosis of blood vessels, vasculitis, and hemorrhage), moderate lesion indicators (infiltration of inflammatory cells, damage of the nucleus, and vacuolization), and severe lesion indicators (formation of granulation tissue in fibrin exudate, proliferation of fibroblast cells, and myocardium damage by granulation tissue). These lesions were totaled for each group to allow comparison. Statistical analysis showed significant differences in lesion scores across treatment groups, highlighting the efficacy of different treatments in minimizing heart tissue damage caused by colibacillosis ($p < 0.05$).

The data presented in Figure 1 show the total lesion scores for all treatment groups. Group 2, which served as the positive control (*E. coli* infection without any treatment), exhibited the highest average total score, indicating severe tissue damage due to colibacillosis. In contrast, Groups 3 and 4, which were treated with a combination of JCEO (0.06 ml/kgBW or 0.1 ml/kgBW) and TEE (400 mg/kg feed), achieved the lowest average total scores. These results suggest that the combination treatment effectively reduced the severity of heart tissue lesions compared to the positive control. Group 5 (*E. coli* + JCEO 0.06 ml/kgBW), Group 6 (*E. coli* + JCEO 0.1 ml/kgBW), and Group 7 (*E. coli* + TEE 400 mg/kg feed) also demonstrated lower lesion scores compared to the positive control, though their reductions were less pronounced than those observed in Groups 3 and 4. Group 8, which received ciprofloxacin, showed a moderate reduction

in lesion scores but was not as effective as the herbal combination treatments. These findings highlight the potential of JCEO and TEE, particularly in combination, to mitigate heart tissue damage caused by colibacillosis in broiler chickens. In mild lesion indicators, lesions of vasculitis and hemorrhage were observed in all groups (Figure 2). Vasculitis can cause an infiltration of inflammatory cells, leading to histopathological observations that reveal the presence of these cells and vacuolation of the tunica. This inflammatory process can cause thickening of the tunica media and adventitia in blood vessels, caused by infiltration of fibrinogen tissue as an effort to replace endothelial cells. Vasculitis was observed in almost all groups (Carbone et al., 2013). The oral administration of all treatment herbal groups had a lengthy pharmacokinetics process before it reached the target organ. Although *E. coli* can rapidly disseminate through the bloodstream, resulting in infection, the oral administration of the extract takes longer to reach the target organ due to its pharmacokinetics (Butterweck et al., 2004). In the present study, *E. coli* was injected peritoneally, which facilitated a quicker hematogenous spread and infection. This dissemination of the bacteria led to the development of vascular lesions, such as hemorrhage, which were observed across all herbal treatments (groups 3, 4, 5, 6, and 7, as shown in Figure 2) and some congestion in groups 5 and 6 (Figure 3). In addition, vasculitis can trigger the activation of coagulation factors in blood vessels, leading to thrombus formation in the lumen of blood vessels. In Group 6, thrombosis was found in the lumen of vessels. The finding of thrombosis was only found in group 6 and not in group 2, most likely because the level of vessel damage in group 2 was higher than in group 6, so the process of vessel damage occurred quickly without any activation of the coagulation system (Figure 4).

Table 2. Accumulation of total histopathology scoring assessment for treatment groups of broiler chickens

Group	Mean ± SD [*]	Total lesion
Negative control [1]	0.0015 ± 0.00093	1.72/30
Positive control [2]	0.014 ± 0.0034	9.2/30
E. coli+ JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed	0.0066 ± 0.0004	4.9/30
E. coli + JCEO[4] 0.1 ml/kgBW + TEE[5] 400 mg/kg feed	0.0063 ± 0.001	4.5 /30
E. coli + JCEO 0.06 ml/kgBW	0.007 ± 0.002	5.7/30
E. coli + JCEO 0.1 ml/kgBW	0.007 ± 0.0015	5.5/30
E. coli + TEE 400 mg/kg feed	0.0099 ± 0.0015	7.0/30
Ciprofloxacin [3]	0.0096 ± 0.0013	6.8/30

[1] Negative control: Chickens that did not receive *E. coli* infection or treatment. [2] Positive control: Chickens that were infected with E. coli but did not receive any treatment. [3] Ciprofloxacin: Chickens that were treated with ciprofloxacin after *E. coli* infection. [4] JCEO: Javanese cardamom essential oil. [5] TEE: Turmeric ethanol extract. [*] Mean ± SD: The average lesion score ± standard deviation, calculated from three replicates per treatment group.

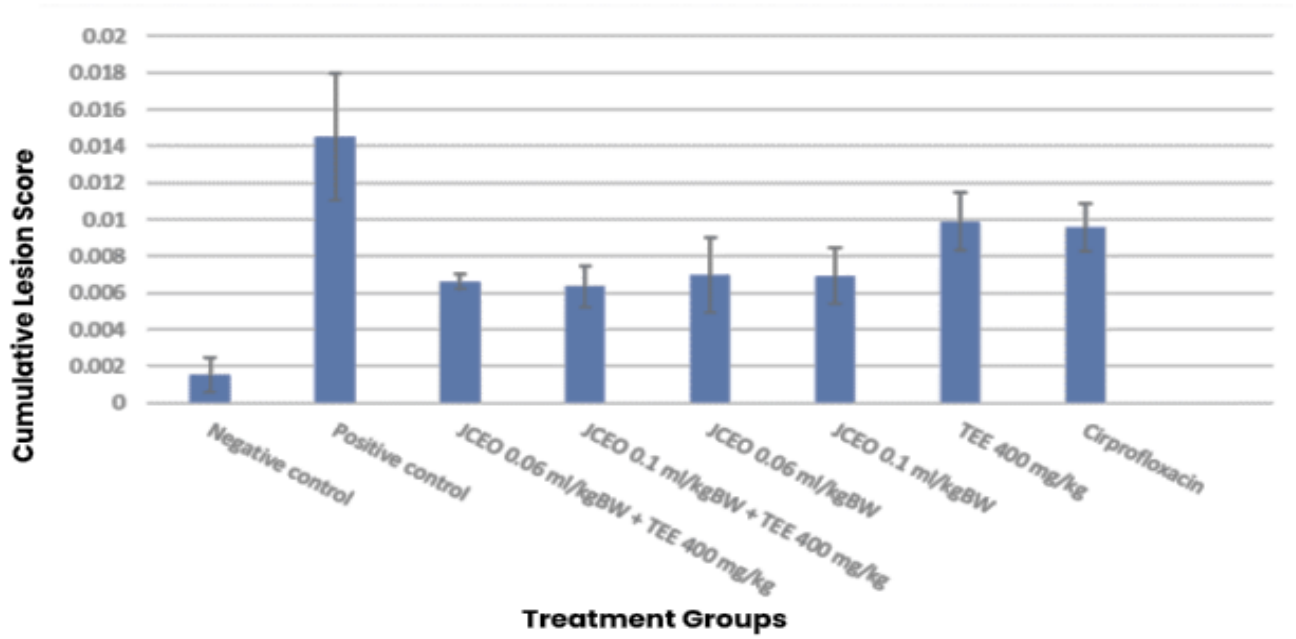

Figure 1. Total lesion scores (mild, moderate, and severe) for each treatment group. The Y-axis represents the cumulative lesion score, and the X-axis denotes the treatment groups: Group 1 (negative control), group 2 (positive control), group 3 (*E. coli* + 0.06 ml/kgBW JCEO + 400 mg/kg feed TEE), group 4 (*E. coli* + 0.1 ml/kgBW JCEO + 400 mg/kg feed TEE), group 5 (*E. coli* + 0.06 ml/kgBW JCEO), group 6 (*E. coli* + 0.1 ml/kgBW JCEO), group 7 (*E. coli* + 400 mg/kg feed TEE), and group 8 (*E. coli* + ciprofloxacin). JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

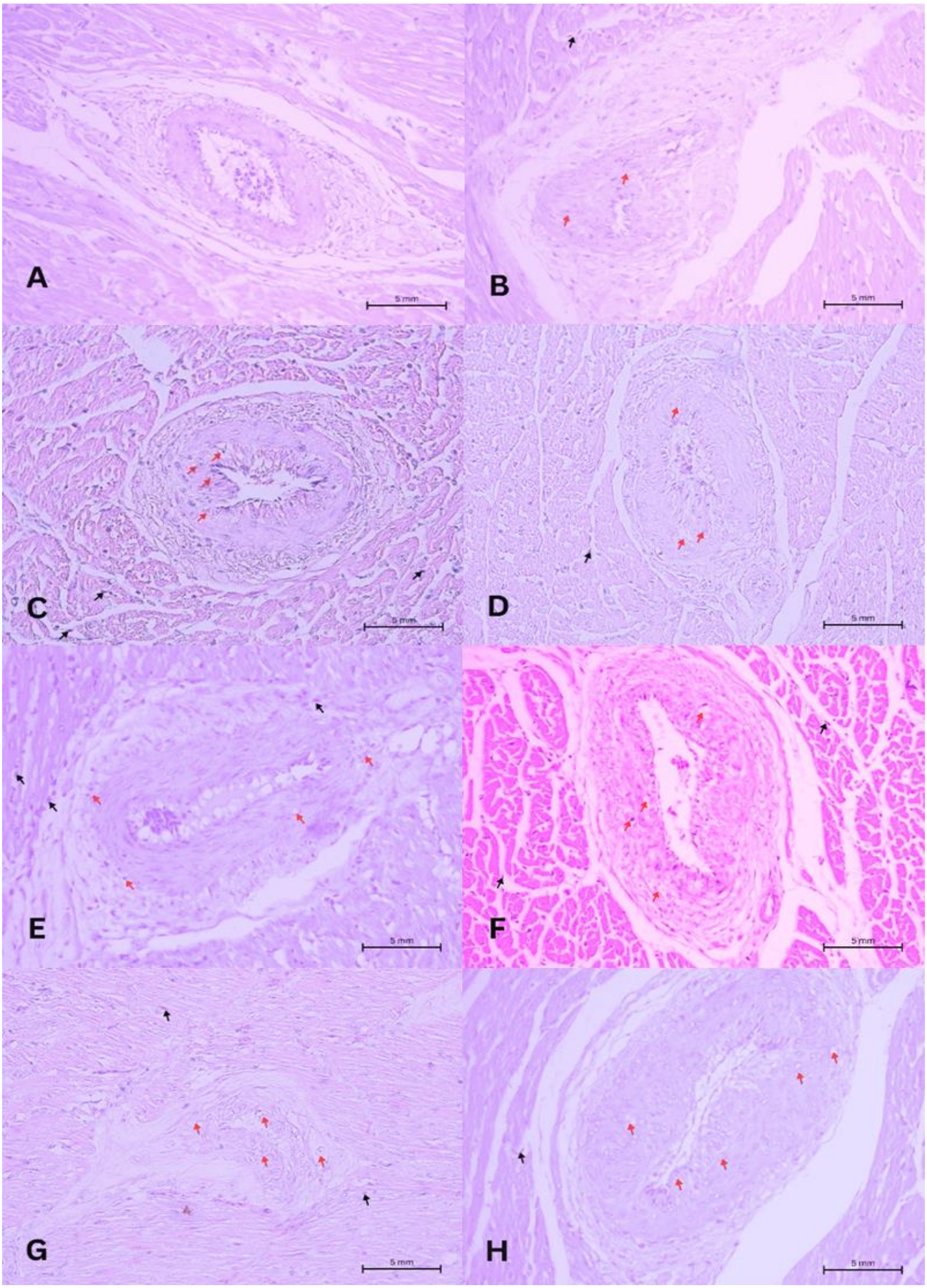

Figure 2. Histopathological examination of heart tissue in broiler chickens showing vasculitis (red arrows) and hemorrhage (black arrows) in blood vessels at magnification 40x. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

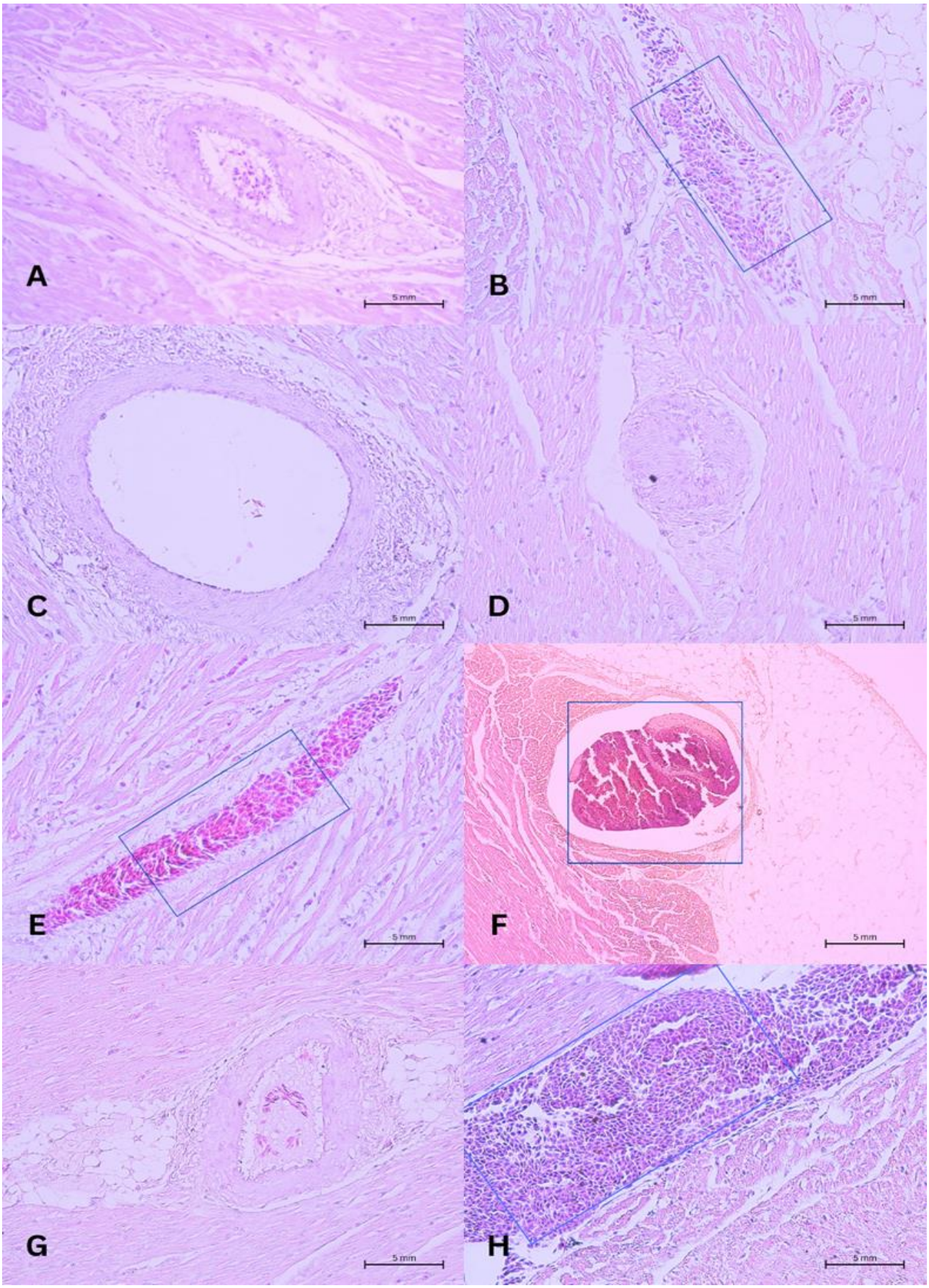

Figure 3. Histopathological examination of heart tissue in broiler chickens showing congested blood vessels (blue box) at magnification 40x. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

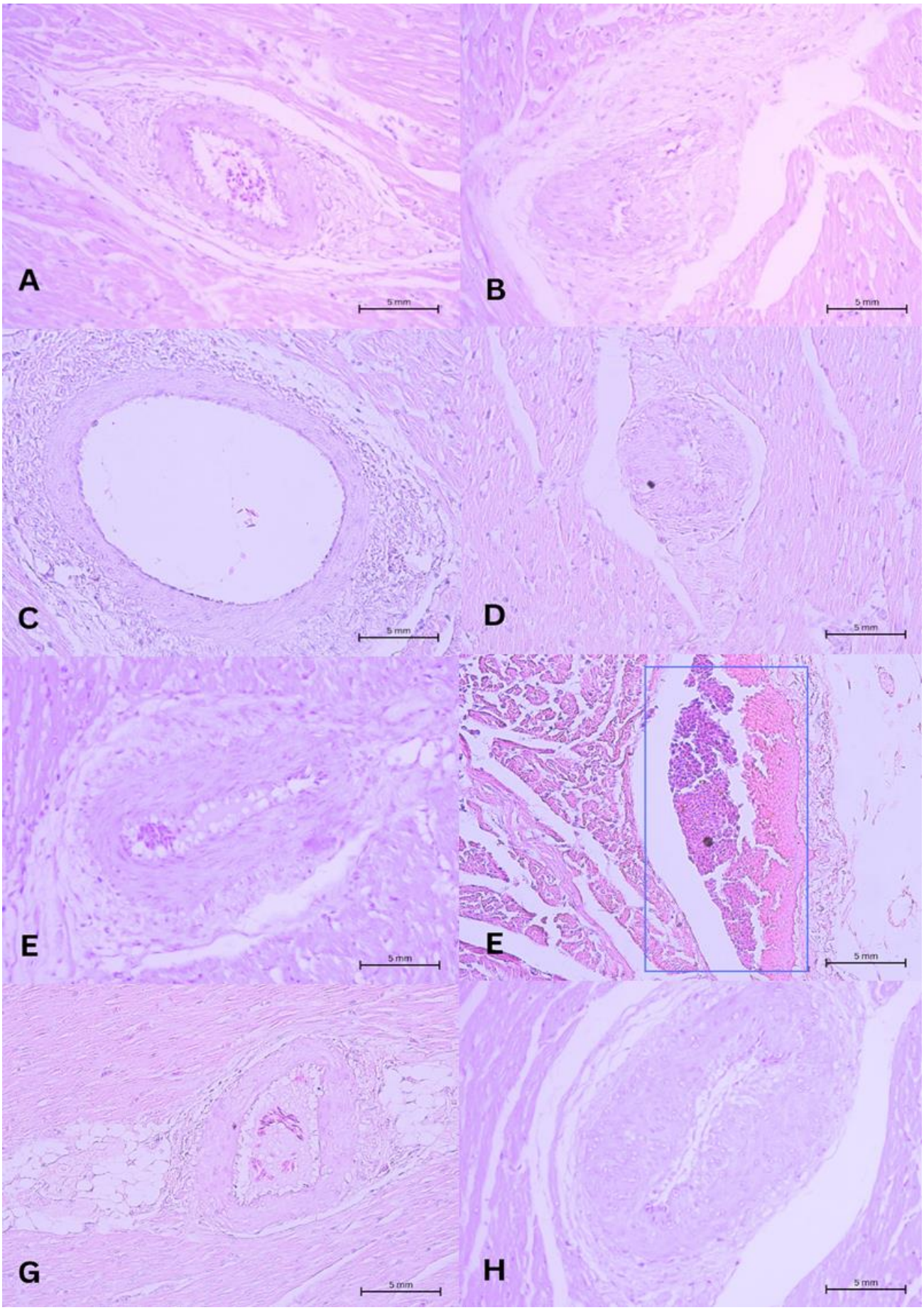

Figure 4. Histopathological examination of heart tissue in broiler chickens showing thrombosed blood vessels (blue box) at 40x magnification. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

In the moderate damage category, inflammatory cell infiltration lesions, nuclear damage, and vacuolization were found in the myocardium in all groups (Figure 5). Once *E. coli* penetrated the blood vessels and reached the heart tissue, toxins in the form of *ibeA*, *vat*, *tsh*, *ace4*, and *ompT* would release which disrupt cellular processes and resulted in cell death (Wang et al., 2015; Kathayat et al., 2021). This cell death would release Damage-Associated Molecular Patterns (DAMPs) signals that can be detected by a subset of immune cells in the heart called neutrophils. In addition to the role of neutrophils in phagocytizing bacteria and debris cells, neutrophils can also release proteolytic enzyme compounds in the form of Reactive Oxygen Species (ROS), which can cause heart tissue damage (Carbone et al., 2013).

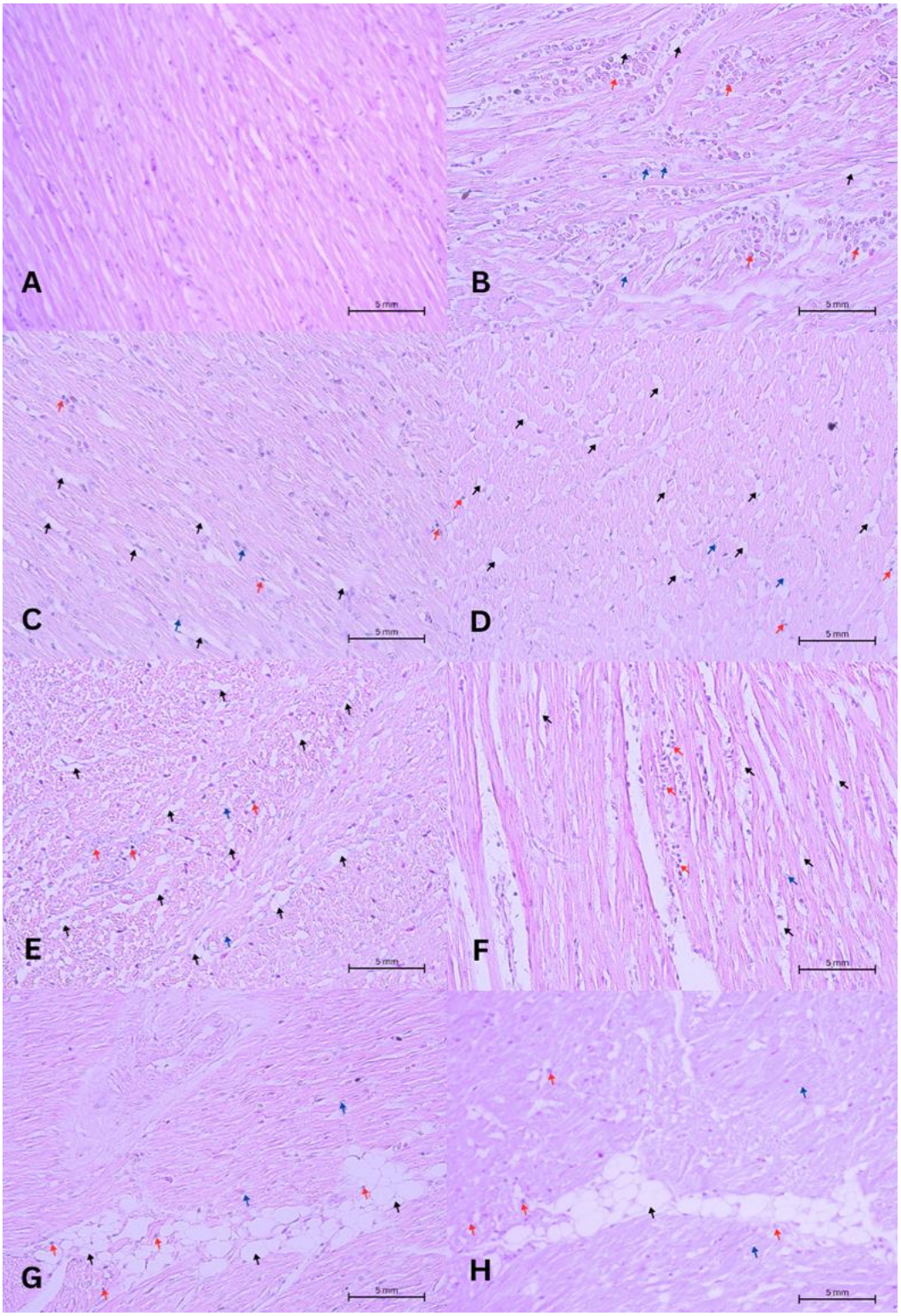

Figure 5. Histopathological examination of heart tissue in broiler chickens showing infiltration of inflammatory cells (red arrows), damage to the nucleus (blue arrows), and vacuolization (black arrows) at 40x magnification. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

In the severe damage category, granulation tissue lesions were demonstrated in fibrin exudate (Figure 6), fibroblast cell proliferation, and damage to the myocardium by granulation tissue (Figure 7) in several groups. Cardiac fibroblasts typically do not exhibit stress fibers under normal conditions. However, upon cardiac injury, these fibroblasts would be activated and transdifferentiated into myofibroblasts that express stress fibers (Hinderer and Schenke-Layland, 2019). These fibroblasts are activated by cytokines and growth factors TGFβ (Hinderer and Schenke-Layland, 2019). Following fibroblast activation, granulation tissue formation commences, which is characterized by the presence of fibroblasts, leukocytes, myofibroblasts, new blood vessels, and Extracellular Matrix (ECM) proteins (Matsui, 2010). Over time, granulation tissue undergoes maturation, evidenced by a decline in fibroblast numbers, increased collagen production, and new blood vessels. This granulation tissue transformed into a denser and stronger fibrotic scar tissue, so that in histopathological appearance it will appear pale pink (Gonciar et al., 2024).

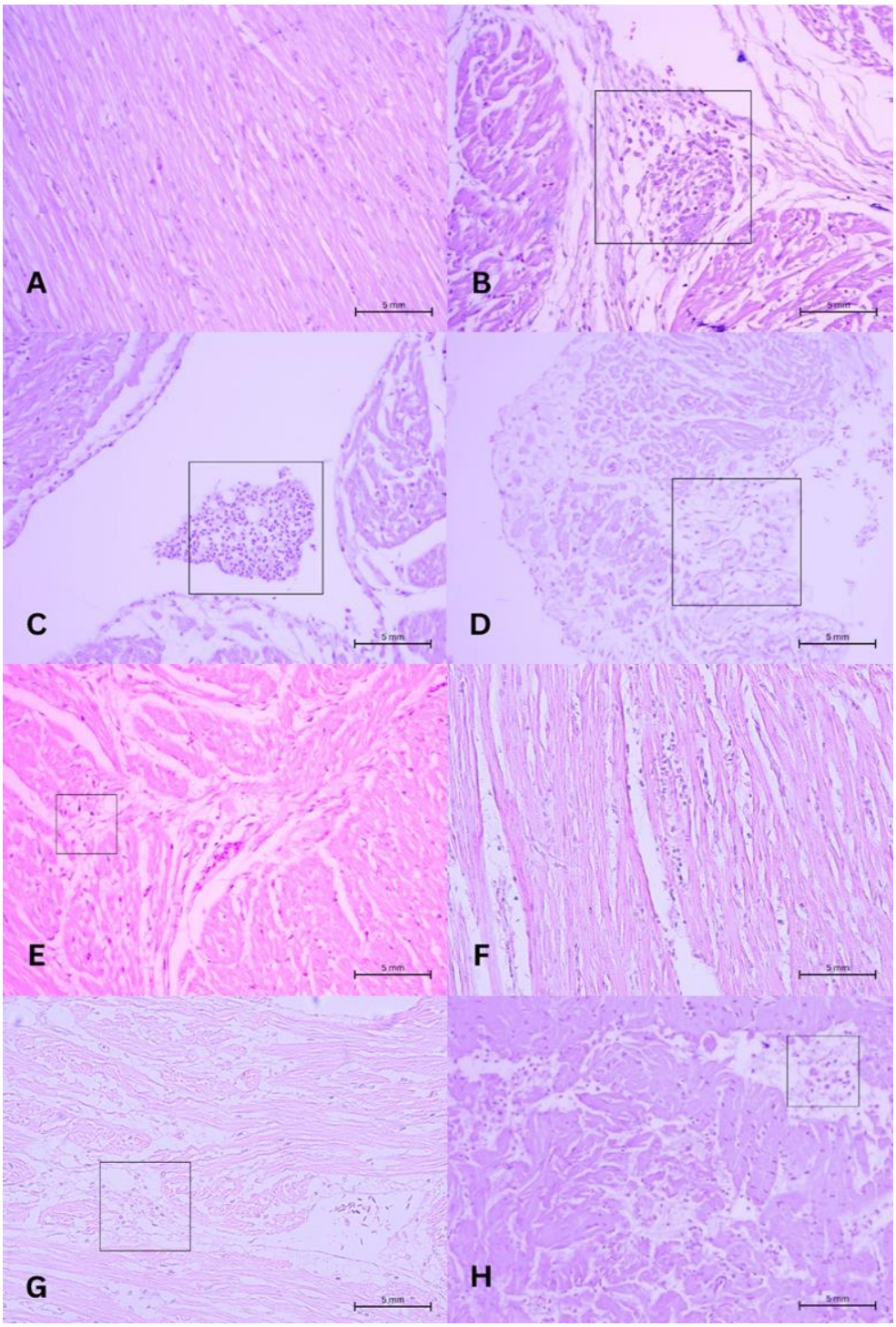

Figure 6. Histopathological examination of heart tissue in broiler chickens showing formation of granulation tissue in fibrin exudate (black box) at 40x magnification. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

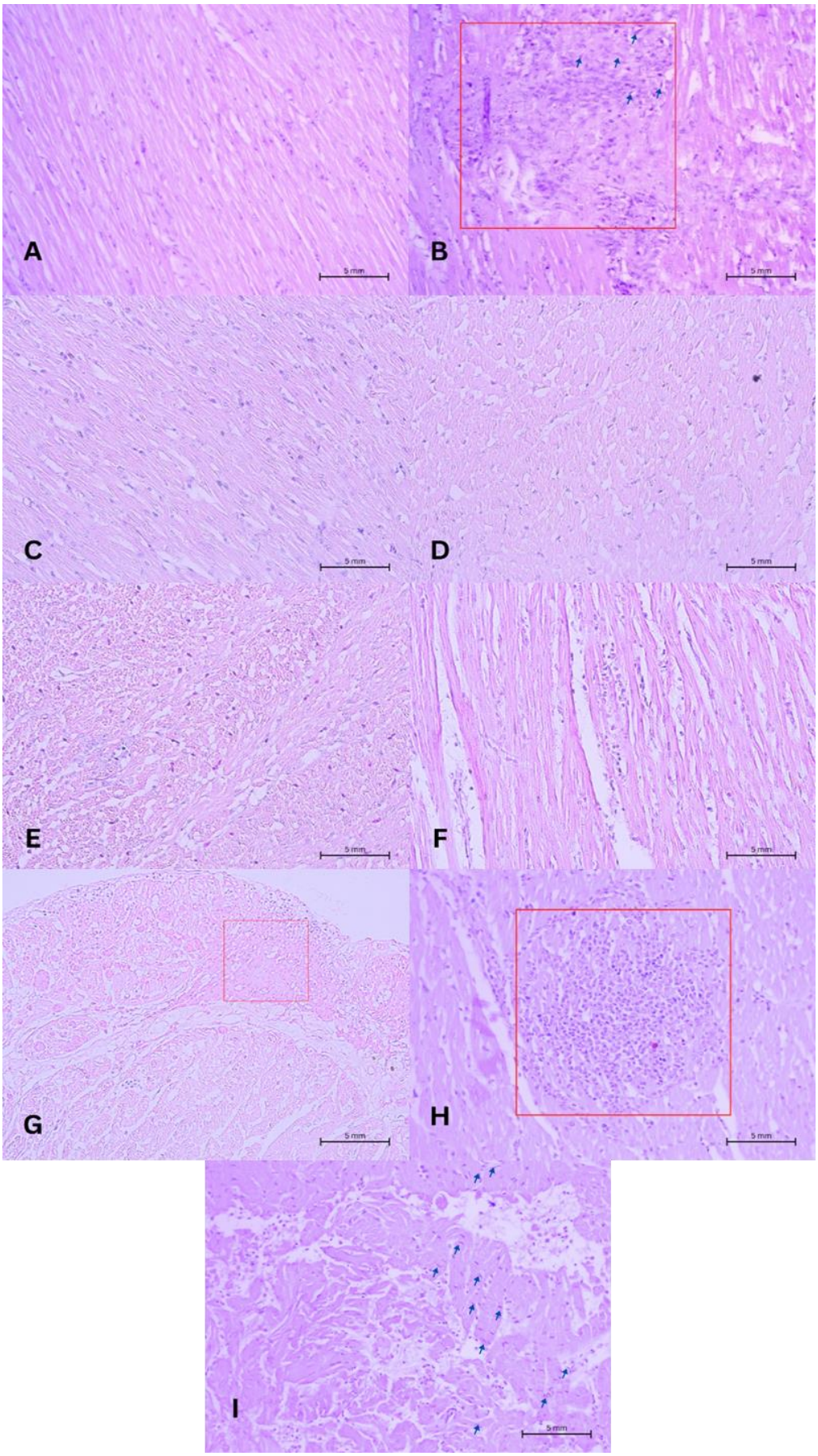

Figure 7. Histopathological examination of heart tissue in broiler chickens showing proliferation of fibroblast cells (blue arrows) and myocardium damage by granulation tissue (red box) at 40x magnification. **A**: Negative control, **B**: Positive control, **C**: *E. coli* + JCEO 0.06 ml/kgBW + TEE 400 mg/kg feed, **D**: *E. coli* + JCEO 0.1 ml/kgBW + TEE 400 mg/kg feed, **E**: *E. coli* + JCEO 0.06 ml/kgBW, **F**: *E. coli* + JCEO 0.1 ml/kgBW, **G**: *E. coli* + TEE 400 mg/kg feed, **H** and **I**: *E. coli* + ciprofloxacin. JCEO: Javanese cardamom essential oil. TEE: Turmeric ethanol extract.

The combination of JCEO and TEE illustrated the lowest total lesion score, proving that cineole and curcumin can work synergistically in minimizing heart organ tissue damage due to colibacillosis. The high total lesion score in group 7, treated with a single dose of TEE, was mainly a consequence of curcumin's limited antibacterial activity, resulting from its unstable chemical structure, which impairs its pharmacokinetic and pharmacodynamic properties (Nelson et al., 2017). This is reinforced by the findings of Hussain et al. (2022) that gram-negative bacteria are more resistant to curcumin due to their lipopolysaccharide cell wall structure. Lipopolysaccharides act as the outermost permeability barrier, preventing the entry of lipophilic compounds like curcumin. Consequently, the effectiveness of curcumin in eliminating *E. coli* bacteria is reduced. This led to significant heart tissue damage across mild, moderate, and severe categories in the TEE group.

The 1,8-cineole content in JCEO has higher antibacterial activity compared to curcumin. *E. coli* is known to target blood vessels as its primary entry point to the heart. Cineole works by damaging and changing the structure of *E. coli* biofilm formation, which is composed of an extracellular polymer matrix (Hoch et al., 2023). Its initial bactericidal mechanism involves binding to porin proteins on the outer membrane of the bacterial cell wall. This interaction forms strong polymeric bonds, resulting in porin damage, which reduces the permeability of the bacterial cell wall and ultimately causes bacterial death due to nutrient imbalance and deprivation. This mechanism aligns with the findings of Shah et al. (2019), which demonstrated that cineole exhibits superior efficacy against *E. coli* compared to other Gram-negative bacteria. The study suggested that cineole's effect is enhanced due to its ability to alter the hydrophobicity of the *E. coli* membrane, making its lipopolysaccharides more vulnerable to cineole-induced damage. This increased susceptibility contributes to cineole's ability to inhibit biofilm formation and disrupt bacterial viability more effectively than other Gram-negative pathogens. Furthermore, cineole can interfere with the *quorum sensing* (QS) process or the bacterial cellular communication system through the *LuxS*/AI-2 *QS system*. The inhibition of LuxS transcription prevents the expression of approximately 400 genes associated with bacterial adhesion and the production of virulence factors. Higher concentrations of cineole were associated with the absence of detectable LuxS gene expression, leading to the downregulation of fimbriae-related genes. As a result, the bacteria experienced a significant reduction in motility, ultimately diminishing their pathogenicity (Wang et al., 2022). The findings of the present study support this mechanism by demonstrating that the groups treated with cineole exhibited lower lesion scores compared to the positive control group, indicating reduced bacterial colonization and infection severity. The decreased lesion severity suggests that cineole effectively disrupted biofilm formation and bacterial motility, which are regulated by the LuxS gene. This provides strong evidence that LuxS inhibition plays a crucial role in the antimicrobial activity of cineole against *E. coli*.

The high pathological lesion score in Group 8 requires further investigation. This phenomenon is likely due to the degradation of ciprofloxacin when administered through drinking water, leading to reduced antibiotic efficacy. Additionally, the powder dosage form exhibited slower pharmacodynamic activity compared to the injection form, while *E. coli* was introduced via intraperitoneal injection, allowing for rapid systemic infection before the antibiotic reached effective plasma concentrations. Furthermore, the chickens in Group 8 did not receive additional supplements or specialized nutritional support during the maintenance period, unlike the herbal treatment groups that benefited from bioactive compounds with immunomodulatory effects. The absence of these additional supportive treatments may have contributed to a weakened immune response, making the chickens in the antibiotic group more vulnerable to infection. Another possible explanation for the high lesion scores in the antibiotic group is the presence of antibiotic-resistant *E. coli* strains, which rendered ciprofloxacin ineffective in eliminating the bacteria. Rahmahani et al. (2020) found resistance to the antibiotic ciprofloxacin in 22 isolates out of 58 isolates (38% of isolates had an inhibition zone diameter of less than or equal to 15 mm) from 60 cloacal swabs of native chickens obtained from three traditional markets in Surabaya, Indonesia. The possibility of resistance to ciprofloxacin must be compared with other antibiotics, such as colistin, to observe the resistance pattern of pathogenic *E. coli*.

CONCLUSION

In conclusion, significant differences in histopathological findings were observed among the treatment groups. The positive control group (*E. coli* infection without treatment) exhibited the highest total lesion score, indicating severe heart tissue damage. In contrast, the group receiving JCEO 0.1 ml/kg BW + TEE 400 mg/kg feed had the lowest lesion score, suggesting that this dosage is effective in minimizing heart tissue damage due to colibacillosis in broiler chickens. Further studies are recommended to explore the long-term effects of JCEO and TEE on cardiac health, evaluate their pharmacokinetics and bioavailability, and compare their efficacy with other natural or synthetic antimicrobial agents. Additionally, research on different administration routes and optimal dosages could provide deeper insights into their therapeutic potential.

DECLARATIONS

Funding

This research was funded by an Internal Grant from Universitas Padjadjaran, Indonesia, under the grant No. 1549/UN6.3.1/PT.00/2023.

Availability of data and materials

The initial data used in this study are incorporated within the article. All data is available upon reasonable request to the corresponding author.

Ethical considerations

This manuscript is the original study of the authors and has not been published elsewhere. The authors have verified the text of the article for plagiarism and confirmed that it is based on their original scientific research results.

Authors' contributions

Tyagita Hartady conceived and designed the study. Sarah Darmawan Sugandi contributed to the writing of the manuscript. Septiyani and Andi Hiroyuki designed, monitored, and evaluated the data analysis. Hanna Goenawan contributed to the interpretation of the result. All authors provided critical feedback and helped shape the study, analysis, and writing of the manuscript. The final manuscript was approved by all authors.

Competing interests

The authors declared no competing interests.

Acknowledgments

The authors express their deep gratitude for the resources and support provided by the Faculty of Medicine at Padjadjaran University for this study.

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