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RESEARCH ARTICLE

Cardiac Histopathology Alterations Induced by Subchronic Mangosteen Rind Extract in Wistar Rats

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Abstract

Mangosteen (*Garcinia mangostana* L.) is renowned for its potent antioxidant characteristics, including free radical scavenging, and its antibacterial, antifungal, anti-inflammatory, and antidiabetic properties. This study aims to assess the subchronic toxicity effects of ethanol extract from mangosteen rind on the cardiac histopathology of male and female Wistar rats. This research was a posttest-only control group design. Forty biological specimens from 40 Wistar rats were divided into four groups, a control group, and three treatment groups, which were given ethanol extract of mangosteen rind with doses of 250 mg/kg, 500 mg/kg, and 1,000 mg/kg for 28 days. Cardiac specimens were prepared and examined using hematoxylin-eosin (HE) staining at the Anatomical Pathology Laboratory of Universitas Jenderal Achmad Yani in December 2021. The findings indicated that prolonged ingestion of large amounts of ethanol extract from mangosteen rind can cause toxic effects characterized by an inflammatory response in cardiac tissue. No fibrosis or hypertrophy was detected; however, inflammatory changes such as the presence of inflammatory cells, vacuolar changes, and neovascularization were observed. The inflammation observed might be due to excessive antioxidant administration leading to oxidative stress. Inflammatory cells may trigger fibrotic remodeling in the heart. The difference in the quantity of inflammatory cells between male and female rats suggests that gender influences the inflammatory response. Overall, administration of ethanol extract from mangosteen rind at doses of 250 mg/kg, 500 mg/kg, and 1,000 mg/kg cause subchronic toxicity effects on the heart histopathology of Wistar rats, marked by inflammation.

Keywords: Cardiac histopathology, ethanol extract, mangosteen, subchronic toxicity, Wistar rats

Introduction

Herbal medicines are pharmaceuticals derived from plants that have undergone extraction to be transformed into liquids, powders, or pills without including chemical compounds.[1] The advantages of using plants as medicinal components are that they are preferred over synthetic substances because the general public believes that herbal medicines have fewer side effects than synthetic medicinal ingredients.[2–3] Indonesia is a lucrative market with significant potential for herbal medicines and phytopharmaceuticals.4 Indonesia has utilized different varieties of plants as medicinal components, including the mangosteen fruit (*Garcinia mangostana* L.).[4–5]

Alpha mangostin and xanthone 5, 10, 20 mg/BW showed antidiabetic effects on fasting blood glucose level, insulin plasma, and Langerhans islet.[9] Alpha mangostin 5, 10, 20 mg/BW, xanthone 10, and 20 mg/BW showed potential effects on preventing tolerance and increasing peroxisome proliferator-activated receptor γ (PPARγ) expression on adipocytes.[10] GLUT-4 expressions also increased in adipocytes treated with 3.125 mM, 6.25 mM, and 25 mM α–mangostin, equivalent to pioglitazone. GLUT-4 expressions in mice cardiac-muscle cells that were treated with α-mangosteen, xanthone, glibenclamide, and metformin significantly increased when compared to the positive control group, except in the group treated with xanthone 5 mg/kgBW.[11]

To ensure the safety of utilizing natural ingredients as medicinal substances, it is imperative to undergo multiple phases of testing, including the assessment of toxicity.[12] A toxicity test is used to identify a harmful impact on a

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substance within a living system and gather data on the typical relationship between dosage and response.[13] The LD50 values were determined at 625 mg/kg, 1,250 mg/kg, 2,500 mg/kg, and 5,000 mg/kg. The results were classified as a non-toxic, practical category.[14]

The oral subchronic toxicity test is used to identify any toxic effects that may occur after repeated administration of the test preparation.[14] Other studies discovered that administering ethanol extract of mangosteen rind at doses of 250 mg/kgBW, 500 mg/kg BW and 1,000 mg/kgBW did not result in any subchronic toxicity effects on liver damage. This conclusion was reached by evaluating the serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels in rats. The administration of the mangosteen rind ethanol extract at a dose of 250 mg/kg BW resulted in elevated SGPT levels in male rats. Similarly, female rats also experienced an increase in SGOT levels after receiving the same extract dose. However, these increases did not reach toxic levels.[15]

The cardiac system serves as a pump that circulates blood throughout the body. The cardiac organ is composed of cylindrical cardiac muscle fibers located within the walls and septum of the heart.[16] Any harm inflicted upon this organ will disturb the functioning system within the human body. The cardiac organ is a vulnerable organ that different types of chemicals can harm. These toxic substances can affect the myocardium via neural or vascular pathways and circulate throughout the body.[17] Possible pathologies that may arise include cellular hypertrophy and fibrosis, which can be attributed to tissue damage.[18,19] Oxidative stress can lead to tissue damage. The most frequently observed histopathological findings in the cardiac are cardiomyocyte abnormalities, including hypertrophy and disorganized myocytes, as well as interstitial fibrosis. Hypertrophic cardiopathy is one of the diseases associated with these abnormalities.[18]

This study aims to investigate the harmful impacts of 28 days of orally administering ethanol extract from mangosteen rind at doses of 250 mg/kgBW, 500 mg/kgBW, and 1,000 mg/kgBW on the cardiac histopathology of Wistar rats that may cause histopathological changes like hypertrophy, inflammation, and fibrosis, while other parameters of subchronic toxicity test will not be reported in this study.

Methods

This study utilized male and female Wistar rats that met the following criteria: age range of 6–8 weeks, weight range of 150–200 g, and acclimatization period of seven days before treatment. AcclimatizationAcclimatization was conducted under standard room temperature conditions, with a light-dark cycle of 12 hours each. The animals were provided with ad libitum access to water and food and then randomly assigned to either the control or experimental groups.

The dosage used is determined following the guidelines provided by the National Agency of Drug and Food Control and the Organization for Economic Co-operation and Development (OECD). The experimental animals are divided into four groups, each comprising ten rats, with five rats of female sex and five rats of male sex. The control group received only water and feed. In contrast, the treatment group was administered water, feed, and a dose of mangosteen rind ethanol extract orally in varying amounts of 250 mg/kgBW (low dose), 500 mg/kgBW (medium dose), and 1,000 mg/kgBW (high dose), respectively. On the 29th day, animals were euthanized using the cervical luxation procedure after being anesthetized with ketamine. Cardiac specimens were prepared and examined at the Anatomical Pathology Laboratory of Universitas Jenderal Achmad Yani.

The experimental protocols adhered to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and were approved by the Health Research Ethics Committee of the Faculty of Medicine Universitas Padjajaran Bandung, under ethical approval number 686/UN6.KEP/EC/2021.

The organs were immersed in a 10% neutral buffered formalin (NBF) solution for fixation. Subsequently, the tissue undergoes a trimming procedure, precisely cut and placed into a cassette. The cassette is then subjected to dehydration, during which it is impregnated with paraffin and cleared using graded alcohol. The subsequent step involves the embedding of samples for printing and the slicing of tissues.[20,21]

In this study, the dye hematoxylin-eosin (HE) was utilized to enhance visibility under a

microscope and facilitate differentiation of the examined tissue components. Hematoxylin is a dye that stains explicitly the cell nucleus, while eosin acts as a contrasting dye. The preparations were submerged in a container filled with 100% xylol for 12 minutes to remove the paraffin. Perform dehydration using absolute ethanol I and II for 5 minutes, followed by the addition of 70%, 80%, and 96% alcohol for another 5 minutes. The slides underwent a 12-minute washing process with running water, followed by a 1-minute immersion in Mayer's hematoxylin. They were rewashed with running water and stained with eosin for 5 minutes. The preparations were rinsed twice with a solution of 75% alcohol. The preparations underwent a process of dehydration by being immersed in 70% alcohol eight times. Subsequently, they were exposed to 80% alcohol, 96% alcohol, and absolute ethanol I and II for 10 minutes each. The preparations were immersed in xylol for 12 minutes. Moreover, the slide is affixed by placing a cover glass over it.[20–22]

The ultimate phase of the procedure involves examining the tissue that has undergone the attachment process using an Olympus CX-23 microscope. The microscope employed was an optical microscope with a magnification of 400×, observing five fields of view for each microscopic sample. The preparatory reading method employs visual examination by the researcher to observe the damage to cardiac cells. This method involves straightforward and uncomplicated observations, which an anatomical pathology specialist subsequently validates.

The study examined the histopathological changes in each preparation, which were categorized according to the dosage of each treatment. The histopathological variables, including normal hypertrophy, inflammation, and fibrosis, will be summarized descriptively. Normal is characterized by the presence of cardiomyocytes and connective tissue of topical size and shape. Hypertrophy is characterized by abnormalities in the cardiomyocytes, such as hypertrophy (enlargement) and irregularly shaped cells. The presence of scar tissue characterizes fibrosis.[17,18,20–23]

Results

Figure 1 shows the histopathology images of each study group, male and female Wistar rat. The cardiac muscle cells in the negative control group exhibited no cellular abnormalities in the images. There are no structural alterations, such as an increase in muscle mass and enlargement of myocytes.

The image indicates that the majority of the myocytes exhibit a normal size. Subsequent to necrosis, fibrosis, characterized by a noticeable augmentation in collagen fibers, was not developed. No structural changes in hypertrophy were observed in the cardiac structure of male subjects in test group 1. The microscopic image continues to display myocytes of typical size and shape. No fibrosis is present in the image. However, inflammatory cells are present, suggesting an inflammatory process occurring in the cardiac muscle cells. No structural changes related to hypertrophy or fibrosis were observed in the cardiac structure of males in test group 2. The histopathological examination reveals myocytes of normal size. No fibrosis was detected in the image. However, the presence of inflammatory cells is evident.

No structural changes indicative of hypertrophy or fibrosis were observed in the cardiac structure of the male subjects in test group 3. The microscopic image depicts myocytes of typical dimensions. No fibrosis is present in this image. However, there is evidence of vacuolar degeneration in the cells. Microscopic examination of test group 3 reveals the presence of neovascularization. It demonstrates the presence of discernible histopathological distinctions among test groups 1, 2, and 3 compared to the control group, manifested as indications of inflammation.

Microscopic readings of the female cardiac organs test group 1 of the female subjects continued to exhibit myocytes that were both normal in size and regular in shape (Figure 2). No evidence of fibrosis is present in the image. No structural changes, such as hypertrophy or fibrosis, were observed in the cardiac structure of females in test group 2. The histopathological examination reveals myocytes of normal size. No fibrosis was detected in the image. However, there is evidence of an inflammatory process characterized by vacuolar and inflammatory cells. The examination of the three females did not uncover microscopic alterations in the form of hypertrophy or fibrosis. The microscopic image displays myocytes of regular dimensions.

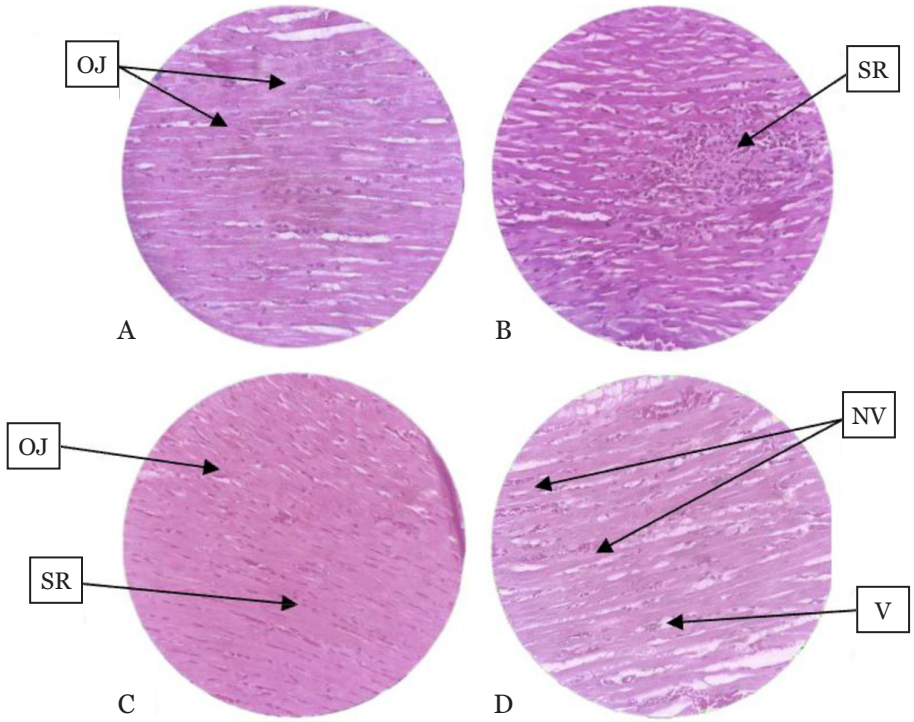

Figure 1 Histopathological Picture of 4 Male Test Groups with HE Staining (400×)

Note: (A) control group; (B) test group 1 ethanol extract of mangosteen rind 250 mg/kgBW; (C) ethanol extract of mangosteen rind 500 mg/kgBW; (D) test group 3 ethanol extract of mangosteen rind 1,000 mg/kgBW; OJ: cardiac muscle cell; SR: inflammatory cells; V: vacuolar; NV: neovascularization

No fibrosis is present in this image; however, neovascularization indicates an inflammatory process in the cardiac muscle cells. There are discernible histopathological disparities in inflammatory indicators among test groups 1, 2, and 3 compared to the control group.

Discussion

Following histopathological examination, no evidence of fibrosis or hypertrophy was observed. However, an inflammatory process was detected. It pertains to administering doses of mangosteen rind ethanol extract, categorized into three levels: 250 mg/kgBW, 500 mg/kgBW, and 1,000 mg/kgBW. Mangosteen contains various antioxidants, such as neutralizing free radicals, fighting against bacteria and fungi, reducing inflammation, and acting as an antihistamine.[8,24] Overconsumption of antioxidants can induce oxidative stress, harming organ cells.[19]

Cardiac hypertrophy can develop when the heart adjusts to a higher workload, producing additional sarcomeres and enlargement of the myocytes.[14] The study did not observe any signs of hypertrophy, indicating that the dose of mangosteen rind ethanol extract had no impact on hypertrophy in the cardiac organs of Wistar rats. The inflammatory process is a stage that is associated with fibrosis. The cell types that contribute to fibrotic remodeling of the heart include macrophages, mast cells, lymphocytes, cardiomyocytes, and vascular cells. These cells either directly produce matrix proteins, leading to fibrosis, or indirectly secrete fibrogenic mediators.[25]

This study did not observe any fibrosis, but it found an inflammatory process characterized by inflammatory cells, vacuolar changes, and increased vascularity. These findings suggest that the dose of ethanol from mangosteen rind can induce the release of inflammatory cell mediators in the cardiac organs of Wistar strain rats, potentially leading to fibrosis. Myocarditis is an inflammatory condition that affects the heart muscle cells, potentially leading to tissue

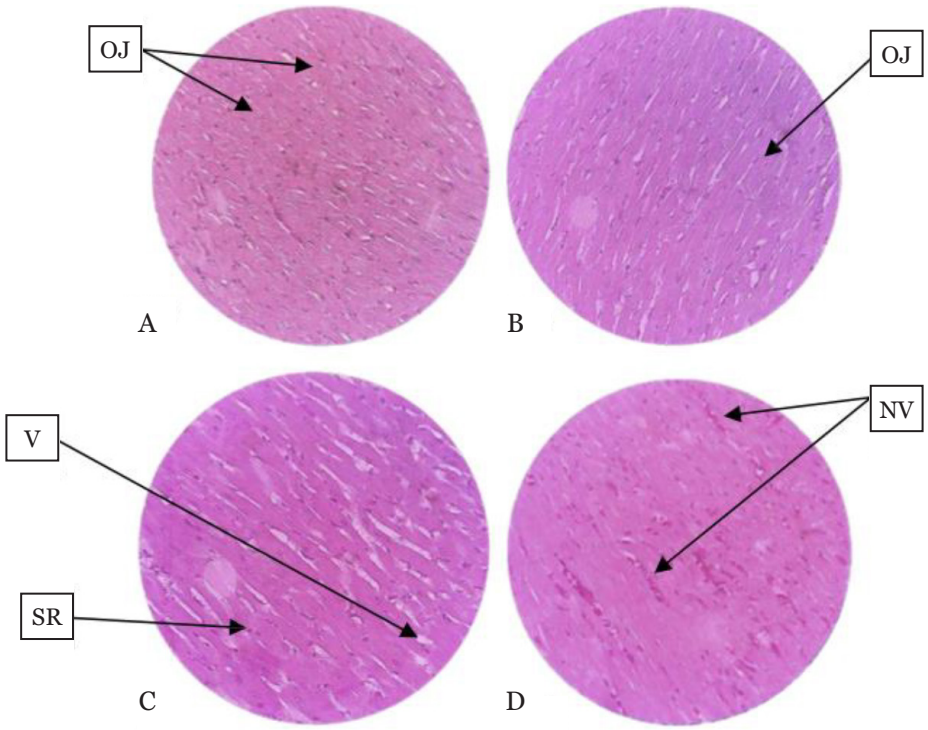

Figure 2 Histopathological Features of 4 Test Groups of HE-stained Females (400×)

Note: (A) control group; (B) test group 1 ethanol extract of mangosteen rind 250 mg/kgBW; (C) ethanol extract of mangosteen rind 500 mg/kgBW; (D) test group 3 ethanol extract of mangosteen rind 1,000 mg/kgBW; OJ: cardiac muscle cell; SR: inflammatory cells; V: vacuolar; NV: neovascularization

degeneration or necrosis.[26] The research findings reveal a disparity in the number of inflammatory cells between females and males, with males exhibiting a higher count. This discrepancy may be attributed to factors that induce inflammation, including gender.

Environmental factors, nutritional status, species, age, and gender can all influence the inflammatory response, causing it to vary between individuals. There may be variations between males and females in their immune system's reactions to foreign substances and their substances and disparities in their natural and acquired immune responses. Genes located on sex chromosomes and sex hormones, such as estrogen, progesterone, and androgen, play a role in the distinct control of immune responses between males and females. Males and females in mammals generally exhibit variations in the number and activity of cells involved in innate and adaptive immune responses, resulting in lower immune responses in males.[27]

Males exhibit a greater abundance of natural killer cells, while females demonstrate higher activity levels in neutrophil and macrophage phagocytes. Male mice exhibit a larger thymus, a higher quantity of thymocytes, and a distinct distribution of thymocyte subsets compared to female mice. The thymus is crucial in forming the immune system by generating a reservoir of peripheral T lymphocyte cells.[28,29] Research limitation in this study was inflammation was not included as a research variable by the researchers.

Conclusions

The administration of ethanol extract of mangosteen rind had a subchronic toxicity effect on the histopathological features of the cardiac tissue in Wistar rats, resulting in inflammatory features. The impact on male and female animal groups reveals a disparity in the number of inflammatory cells, with male rats exhibiting a higher quantity of inflammatory cells compared to female rats.

Conflict of Interest

The authors declared no conflict of interest.

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References

1. Pane MH, Rahman AO, Ayudia EI. Gambaran penggunaan obat herbal pada masyarakat Indonesia dan interaksinya terhadap obat konvensional tahun 2020. JOMS. 2021;1(1):40–62.
2. Krsnik S, Erjavec K. Factors influencing use of medicinal herbs. J Patient Exp. 2024;11:23743735241241181.
3. Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol. 2015;4(1):27–30.
4. Salim Z, Munadi E, editors. Info komoditi tanaman obat. Jakarta: Badan Pengkajian dan Pengembangan Perdagangan Kementerian Perdagangan Republik Indonesia; 2017.
5. Wathoni N, Yuan Shan C, Yi Shan W, Rostinawati T, Indradi RB, Pratiwi R, et al. Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. Heliyon. 2019;5(8):e02299.
6. Ansori ANM, Fadholly A, Hayaza S, Susilo RJK, Inayatillah B, Winarni D, et al. A review on medicinal properties of mangosteen (*Garcinia mangostana* L.). Res J Pharm Technol. 2020;13(2):974–82.
7. Santoso APR, Wulandari DD. Pengaruh pemberian alfa mangostin terhadap kadar glukosa pada tikus yang diinduksi diet tinggi fruktosa. MTPHJ. 2020;4(2):127–33.
8. Abate M, Pagano C, Masullo M, Citro M, Pisanti S, Piacente S, et al. Mangostanin, a xanthone derived from *Garcinia mangostana* fruit, exerts protective and reparative effects on oxidative damage in human keratinocytes. Pharmaceuticals (Basel). 2022;15(1):84.
9. Ratwita W, Sukandar EY, Adnyana IK, Kurniati NF. Alpha mangostin and xanthone activity on fasting blood glucose, insulin and langerhans islet of Langerhans in alloxan induced diabetic mice. Pharmacog J. 2019;11(1):64–8.
10. Ratwita W, Sukandar EY, Kurniati NF, Adnyana IK. Alpha mangostin and xanthone from mangosteen (*Garcinia mangostana* L.) role on insulin tolerance and PPAR-γ in preclinical model diabetes mellitus. J Pharm Nutr Sci. 2018;8:83–90.
11. Ratwita W, Sukandar EY, Adnyana IK, Kurniati NF. Alpha mangostin and xanthone from mangosteen (*Garcinia mangostana* L.) role on glucose tolerance and glucose transporter-4 in diabetes mellitus. IJPPR. 2017;9(9):1206–12.
12. Jelita SF, Setyowati GW, Ferdinand M, Zuhrotun A, Megantara S. Uji toksisitas infusa *Acalypha siamensis* dengan metode brine shrimp lethality test (BSLT). Farmaka. 2020;18(1):14–22.
13. Candra AA, Irwani N. Acute toxicity test of skin mangosteen (*Garcinia mangostana* L.) and histopathologic study of liver. Bangl J Vet Med. 2016;14(2):145–8.
14. Carolina H. Uji toksisitas akut (LD50) ekstrak kulit manggis (*Garcinia Mangostana* L.) pada mencit [undergraduate thesis]. Malang: Universitas Brawijaya; 2015.
15. Ratwita W, Pradini A, Sovia E, Damadika D. Subchronic toxicity effect of mangosteen rind ethanol extract (*Garcinia mangostana* L.) on liver damage in Wistar rats. IMJ. 2022;29(2):7083–9.
16. Eroschenko VP. diFiore's Atlas of Histology with Functional Correlations. 12th edition. Philadelphia: Lippincott Williams & Wilkins; 2013.
17. Hilma N, Nuri N, Puspitasari E, Ningsih IY. Gambaran histopatologi organ jantung tikus putih jantan galur wistar (*Rattus norvegicus*) dalam uji toksisitas akut kombinasi ekstrak daun jati belanda (*Guazuma ulmifolia* Lmk.) dan kelopak bunga rosella (*Hibiscus sabdariffa* L.). JPK. 2018;6(2):240–4.
18. Arackal A, Alsayouri K. Histology, heart. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Feb [cited 2024 March 23]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545143.
19. Salehi B, Martorell M, Arbiser JL, Sureda A, Martins N, Maurya PK, et al. Antioxidants: positive or negative actors? Biomolecules.

2018;8(4):124.
20. Rahmawanti A, Setyowati DN, Mukhlis A. Histopathological of brain, eye, liver, spleen organs of grouper suspected VNN in Penyambuan village, North Lombok. J Biol Tropis. 2021;21(1):140–8.
21. Pratiwi HC, Manan A. Teknik dasar histologi pada ikan gurami (*Osphronemus gouramy*). J Ilm Perikan Kelaut. 2015;7(2):153–8.
22. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 7th edition. Oxford: Elsevier Limited; 2013.
23. Galati G, Leone O, Pasquale F, Olivotto I, Biagini E, Grigioni F, et al. Histological and histometric characterization of myocardial fibrosis in end-stage hypertrophic cardiomyopathy: a clinical-pathological study of 30 explanted hearts. Circ Heart Fail. 2016;9(9):e003090.
24. Ovalle-Magallanes B, Eugenio-Pérez D, Pedraza-Chaverri J. Medicinal properties of mangosteen (*Garcinia mangostana* L.): a comprehensive update. Food Chem Toxicol. 2017;109(Pt 1):102–2.
25. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci. 2014;71(4):549–74.
26. Shakuntala P, Sumitra V. Acute myocarditis. Indian J Pract Pediatr. 2021;10(2):65–73.
27. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626–38.
28. Thapa P, Farber DL. The role of the thymus in the immune response. Thorac Surg Clin. 2019;29(2):123–31.
29. Li YR, Zúñiga-Pflücker JC. Thymus aging and immune reconstitution, progresses and challenges. Semin Immunol. 2023;70:101837.