Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES
April 2023, Volume 17, Number 2

Research Paper

Kleinhovia Hospita Leaf Extract Protects the Heart Against Infarction by Isoproterenol

Fitriani W. Alani[1], YuliaYusrini Djabir[2*], M. Aryadi Arsyad[2]

1. Graduate School Biomedical Science Program, Hasanuddin University, Makassar, Indonesia.
2. Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia.

Use your device to scan and read the article online

How to cite this paper Alani FW, Djabir YY, & Arsyad MA. *Kleinhovia Hospita* Leaf Extract Protects the Heart Against Infarction by Isoproterenol. Iranian Journal of Toxicology. 2023; 17(2):87-94. http://dx.doi.org/10.32598/IJT.17.2.854.4

http://dx.doi.org/10.32598/IJT.17.2.854.4

Article info:
Received: 14 Jan 2023
Accepted: 21 Jan 2023
Online Published: 01 Apr 2023

*** Corresponding author:**
Yulia Yusrini Djabir, PhD.
Address: *Department of Pharmacy, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia.*
E-mail: *yulia.yusrini@unhas.ac.id*

ABSTRACT

Background: Isoproterenol, a β-adrenergic agonist, may induce myocardial infarction when used in high dosage in rats. This study was conducted to evaluate the effect of the ethanol extract of *Kleinhovia hospita* leaves on cardiac biomarkers and myocardial structures of rats induced by isoproterenol.

Methods: Male rats (n=30) were assigned as a normal controls or treatment groups. The treatment groups received pretreatments, either placebo or the extract at doses of 250, 500, or 750 mg/kg for 14 days, followed by two isoproterenol injections at 100 mg/kg. After 24 hours, blood samples were taken and the hearts dissected. The tested cardiac biomarkers were creatinine kinase myocardial band (CKMB) and lactate dehydrogenase (LDH). Heart histopathology analysis was performed followed by staining of samples with hematoxylin and eosin.

Results: The isoproterenol injections significantly increased CKMB and LDH levels in the placebo group compared to those in normal controls. Pretreatment with the extract at doses of 500 and 750 mg/kg significantly reduced the serum CKMB and LDH levels compared to those of the placebo. The histopathological examinations showed the presence of diffused necrosis and severe inflammation in the placebo group. Pretreatment with the extract at 500 or 750 mg/kg significantly reduced the myocardial tissue damages in rats.

Conclusion: The *K. hospita* extract at doses of 500 or 750 mg/kg significantly reduced the infarctions in the rats' heart tissue, shown by significantly low levels of CKMB and LDH, and reduced necrotic lesions and inflammation in the rat heart tissue samples induced by isoproterenol pretreatment.

Keywords: Cardiac biomarkers, CKMB, LDH; Isoproterenol, *Kleinhovia hospita* L, Myocardial infarction

Introduction

Cardiovascular disease is one of the leading causes of death, which accounts for 32% of the mortality around the world [1]. Myocardial Infarction (MI) is still one of the most fatal cardiovascular diseases, even though the mortality rate is declining over the past two decades due to advancements in the healthcare [2]. This condition mainly occurs due to blockage of the coronary arteries, causing continuous deprivation of oxygen and nutrition to areas of the myocardium. As a result, oxidative stress, lipid membrane peroxidation, and damages occur in the cellular lipid membranes.

These events inevitably lead to pathological alterations in myocardial metabolism, and consequently terminate in cellular death or necrosis [3]. In an effort to search for potential treatments, it is important to use experimental models that mimic the pathogenesis of myocardial infarction in clinical settings. Isoproterenol is a β-1 and β-2 adrenergic receptor agonist. This drug over-stimulates the receptors when used in high dosage, thereby, it is often used to induce myocardial infarction in animal models [4, 5].

For many years, the use of natural resources as traditional medicines has become a growing demand because they are considered safer, inexpensive, and reasonably effective [6]. Natural resources contain numerous bioactive compounds, which may be useful in the treatment of myocardial infarction [7]. *Kleinhovia hospita (K. hospita)* is a plant that is commonly found in Indonesia and has been used for its therapeutic effects. This plant contains phytochemicals, such as flavonoids, tannins, and saponins [8]. Also, most of the bioactive compounds from *K. hospita* plant have demonstrated potent antioxidant properties by earlier studies [9-12]. In addition, the *K. hospita* leaves are rich flavonoid glycosides with potent anti-inflammatory properties [12].

The extract of *K. hospita* leaves have been extensively studied in preclinical settings since it has anti-inflammatory and antioxidant activities [13-15]. Moreover, it has been reported that the extract is not only effective in protecting against hepatic injuries, but also reduces doxorubicin-induced myocardial damages [16]. Therefore, the present study aimed to investigate the effects of *K. hospita* extract against cardiac biomarkers elevation and myocardial damages induced by isoproterenol in rats.

Materials and Methods

Chemicals and drugs: Isoproterenol was purchased from Sigma Chemical Co. (Singapore). Other laboratory chemicals, such as CK-MB NAC activated and LDH SCE mod. Liquid UV were purchased from HUMAN Biochemical and Diagnostic GmbH (Wiesbaden, Germany).

Plant materials and preparation of extract: Fresh samples of *K. hospita* leaves were collected in Makassar, Indonesia. They were dried and cut into small pieces then softened for three days in 70% ethanol. The liquid extract was concentrated using a rotary evaporator, and stored in a desiccator. The thick extract material was suspended in 1% sodium carboxymethyl cellulose (Na-CMC) and prepared at three concentrations of 250, 500 and 750 mg/kg. Each of the extract preparations was dissolved in distilled water to make up a volume of 1ml/100 g of the body weight and administered to the rats orally.

Experimental animals: In this study, thirty male Wistar rats, weighing 170-200 g, were used as the experimental animals. The rats were cared for in a laboratory with standard facilities under 12-hour of light and dark cycles at 25°C temperature and 50-70% humidity. All study protocols were designed and adhered to the standards of care for animal experiments and were approved by the Institutional Ethics Committee, Hasanuddin University, Makassar, Indonesia (Code: 751/UN4.6.4.35.1/PP36/2022).

Experimental design: The rats were divided into five groups of six:

Group 1: Normal controls; rats received no treatment.

Group 2: Placebo; rats received 1% sodium carboxymethyl cellulose.

Group 3: Rats received the extract at 250 mg/kg.

Group 4: Rats received the extract at 500 mg/kg.

Group 5: Rats received the extract at 750 mg/kg.

The treatments in the placebo and extract groups (groups 2-5) were administered daily for 14 consecutive days. On days fourteenth and fifteenth, isoproterenol (ISO) was injected at 100 mg/kg into the animals subcutaneously. After 24 hours of administration of the last ISO dose, the animals were anesthetized with 0.5-1 mL ether through inhalation. Subsequently, a blood sample was taken from each rat through the retro-orbital veins and collected into a vacutainer tube, containing anticoagulant. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain the sera, which were stored at -20°C until they were analyzed for their biomarkers' contents.

Serum Biomarker Analyses: The levels of creatinine kinase, myocardial band (CK-MB) and lactate dehydrogenase (LDH) were determined with Humalyzer 3500 (HUMAN; Wiesbaden, Germany), using the CK-MB NAC and LDH SCE reagents, based on the supplier's instructions.

Histopathological examinations: After the blood samples collection, the rats were euthanized and the hearts were dissected carefully and fixed in 10% formalin for 48 hours. Next, the hearts were sectioned at 1-cm thickness before being placed into a tissue processing machine for 12 hours. The sample tissues were embedded in paraffin wax blocks and cut into 4 µm sections on a microtome. The tissue sections were placed onto glass slides and dried on a heating plate for 2 hours before staining with hematoxylin and eosin. Each slide was covered with a glass cover and allowed to dry. Finally, the slides were examined under light microscopy at 200x magnifications and photographed with a digital camera.

Histopathological scoring: The scores of myocardial damages were determined by an anatomy pathologist, who was blinded to the treatment groups. The damages were scored based on valid signs of inflammation, necrosis, and/or hemorrhage. A score of 0 meant no pathological alterations found in the heart tissues, and considered as being healthy. A score of one meant slight to minimal damages affecting <25% of the observed areas. A score of two meant moderate damages found in 25-50% of the observed areas. A score of three meant severe damages affecting 50-75% of the areas, and a score of four meant heavy or massive damages observed in 75-100% of the examined histological areas [4].

Statistical analysis: All experimental data were presented as the Means±SE of the means, and the data normality was validated, using a Saphiro-Wilk test. All data representing cardiac biomarkers were normally distributed and statistically analyzed, using one-way ANOVA followed by a post hoc Tukey's HSD test. The differences among and between the groups were considered statistically significant at P≤0.05. The categorical data were analyzed, using a Kruskal-Wallis analysis method followed by a Mann-Whitney test to obtain the statistical significance between pairs of the groups.

Results

Cardiac biomarkers analyses: The levels of rats' cardiac biomarkers are presented in Figure 1. The subcutaneous injection of ISO for two days (days 14 and 15) significantly increased CK-MB (P<0.001) and LDH levels (P<0.05) compared to the normal controls. The administration of the extract at doses of 500 and 750 mg/kg before ISO injection significantly reduced the CK-MB and LDH levels in the sera compared to the placebo group (P<0.05).

Histopathological examinations: Figure 2 illustrates the microscopic images of the myocardial tissue samples of the rats. It is shown that in the normal control, the heart tissue did not show marked histopathological changes, and the cardiac myocytes' architecture was

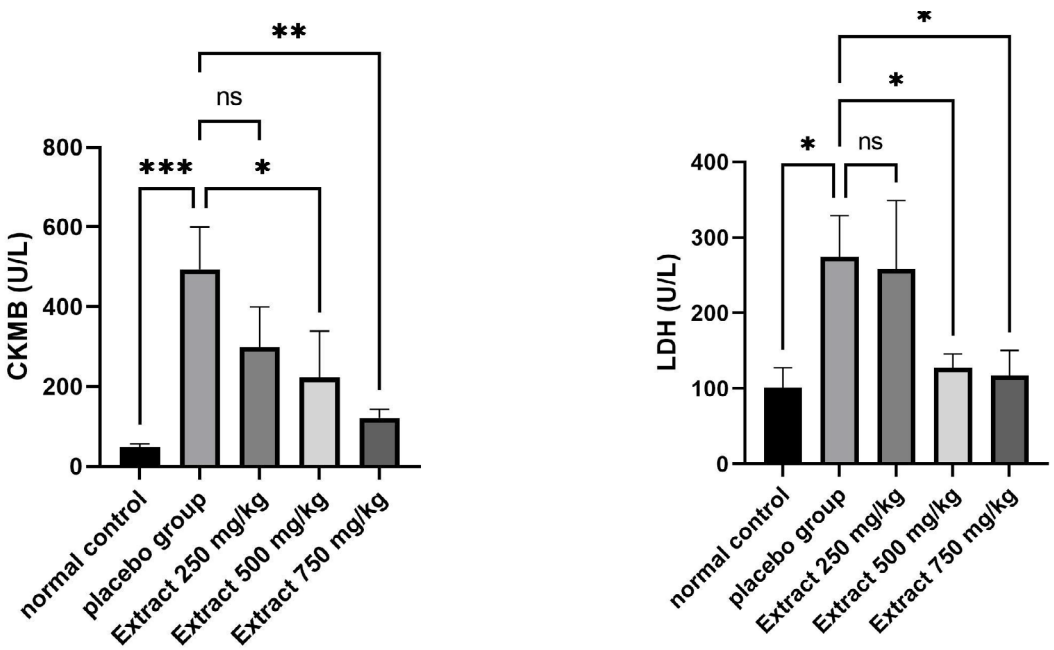

Figure 1. The levels of creatine kinase myocardial band (CKMB) and lactate dehydrogenase (LDH) in various treatment groups

*P<0.05, **P<0.01, ***P<0.001 compared to the placebo group.

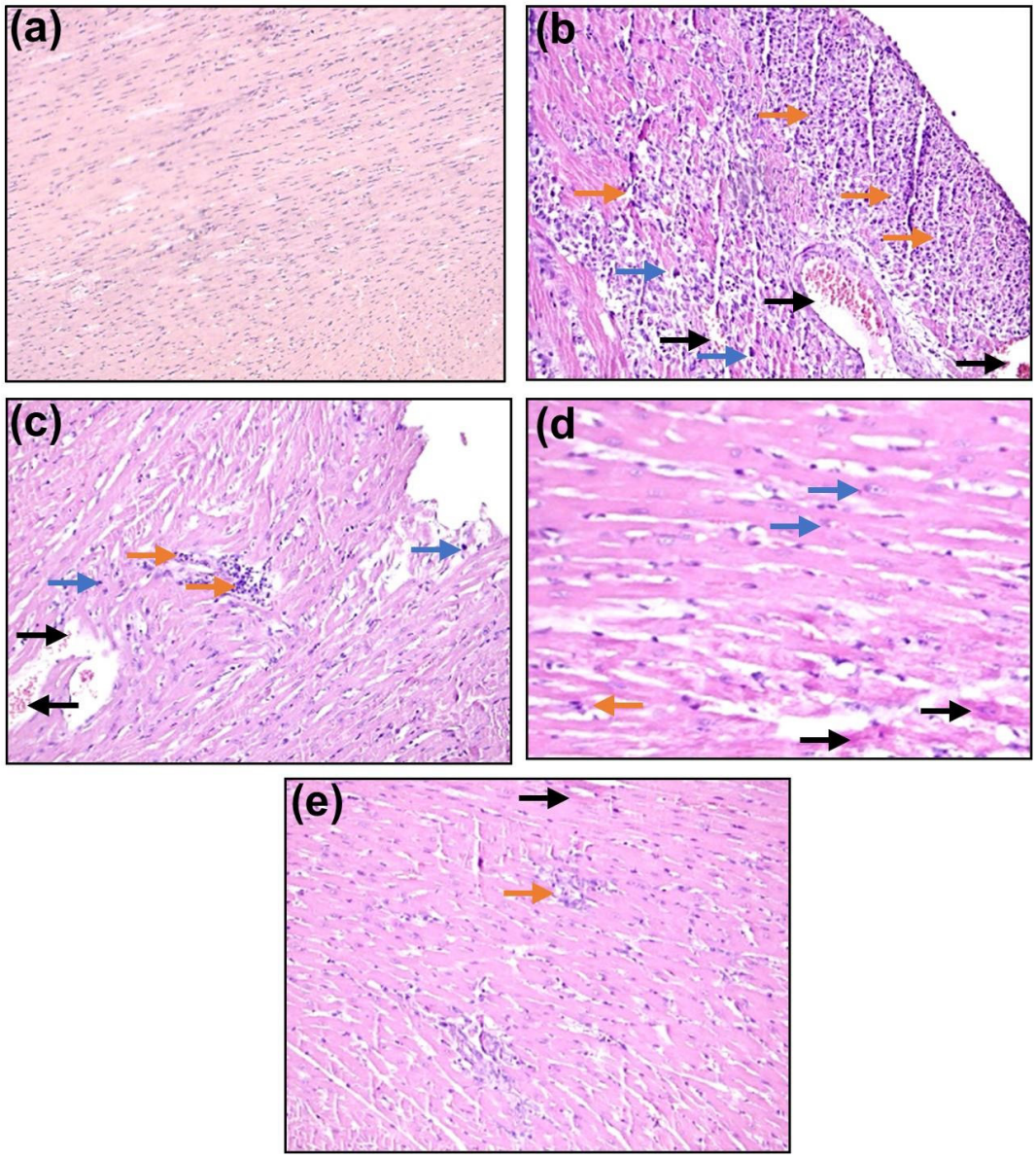

Figure 2. Representative microscopic images of myocardial tissue in normal control group

a) The placebo group; b) The extract, 250 mg/kg treatment group; c) The extract, 500 mg/kg treatment group; d) The extract, 750 mg/kg group; e) Area of necrosis (orange arrow), hemorrhage and edema (black arrow) and inflammatory cells (blue arrow).

normal without the presence of hemorrhage, necrosis, and inflammation. Meanwhile, in the placebo group (group 2), the presence of myocardial infarction was evident, as characterized by diffuse necrosis and severe inflammation. The administration of the extract at doses of 500 or 750 mg/kg before ISO treatment, significantly reduced the cardiac necrosis and inflammatory changes subsequent to the ISO injection ($P<0.05$).

In Table 1, the extents of myocardial damages were scored based on the presence of necrosis, inflammation, and hemorrhage within the observed areas (magnification x200). The rats in the normal control group had no or very minimal damages in the myocardial tissue samples (scored 0). The intensity of inflammation, hemorrhage and the area of necrosis were most severe in the placebo group (group 2). With the extract treatment at 250 mg/kg, the intensity of inflammation was slightly reduced but the necrosis was still prominent (scored 3). With higher doses of the extract treatment at 500 or 750 mg/kg, the severity of inflammation was lowered significantly and only limited cellular necrotic areas were observed.

Table 1. The score of myocardial injuries including inflammation, hemorrhage, and necrosis in rats treated with isoproterenol

Group	Cardiac Histopathological Features		
	Inflammation	Hemorrhage	Necrosis
Normal	0.00±0.00*	0.00±0.00*	0.00±0.00*
Placebo	2.67±0.33	1.00±0.00	3.00±0.00
250 mg/kg	1.67±0.67	1.00±0.00	3.00±0.58
500 mg/kg	1.00±0.00*	0.67±0.33	1.00±0.00*
750 mg/kg	1.00±0.00*	0.67±0.33*	1.00±0.00*

*P<0.05 compared to the negative control group. Extract=*K. hospit*a leaf extract.

Discussion

Myocardial infarction is defined as cellular necrosis due to an imbalance between the demand and supply of oxygen to the myocardium over a long period of time [17]. An increase in oxygen demand in the myocardium may occur due to the increased heart rate and contractility [18]. The decrease in oxygen supply can be caused by obstruction or vasoconstriction that impedes the blood flow to the myocardium, low levels of oxygen in body tissues, anemia, or hypotension [19].

As a β-adrenergic agonist, ISO may instigate severe oxidative stress against the myocardium when administered in high dosage. Such a treatment causes increased contractility and rapid heart rate, forcing the cardiac myocytes to demand high oxygen and ATP supplies. The increased consumption of oxygen and ATP leads to high production of reactive oxygen species, inducing auto-oxidation of catecholamine molecules [20]. Several studies have reported that excess auto-oxidation of catecholamines triggers peroxidation of the membrane phospholipids, mediated by free radicals, and leads to alterations in the myocardial permeability, excessive intracellular calcium, and permanent damages to myocardial cells [21].

In this study, the effect of *K. hospita* extract was examined against the elevation of cardiac biomarkers and histopathological changes in rats' heart tissue induced by ISO. The induced infarction has been widely used to study the cardioprotective effects of phytochemicals; plant extracts, derivatives, analogs; and other herbal or mineral formulations [22]. The injection of ISO subcutaneously at 100 mg/kg resulted in increased release of myocardial enzymes, such as CK-MB and LDH, leading to their elevations in the rats' sera. The administration of *K. hospita* extract at 500 or 750 mg/kg prior to ISO injection was able to significantly reduce both CK-MB and LDH levels in the rats' sera. In addition, the extract at the given doses significantly protected the myocardium against necrosis and inflammation.

The cardioprotective effect of the *K. hospita* extract is believed to be linked to the presence of bioactive compounds in the plant's leaves. The study of *K. hospita* compounds revealed the presence of fatty acids with cyclopropene rings [9]. These include kaempferol 3-O-b-D-glucoside and eleuthero, cycloartane triterpenoids, such as gardenolic acid B, and kleinhospitines A, B, C, and D [10]. The *K. hospita* leaves also contain 3-acetyl-12-en-28-oic acid and (R)-N-trans-feruloyl octopamine [9]. More recently, kleinhospitine E and new cycloartane triterpenoids have been isolated from the methanolic extract of *K. hospita* leaves [23].

The potent antioxidant properties of *K. hospita* compounds may potentially contribute to its cardioprotective properties by reducing oxidative stressors in cardiac myocytes. Oxidative stress plays an important role in the development of myocardial infarction, since it triggers a series of events that lead ultimately to cellular death [24]. In addition, oxidative stress also activates the JNK, NF-kB, and NLRP pathways, which further promote the production and release of pro-inflammatory cytokines [25]. The NF-κB is a transcription factor that functions as an important mediator of the inflammatory response. This factor induces the production of various pro-inflammatory genes, including those encoding for such cytokines as TNF-α and IL-6, and IL-1B [26, 27]. These pro-inflammatory cytokines and signaling pathways can activate myocardial cell apoptosis and promote the development of myocardial infarction [28]. Flavonoid glycosides contained in the *K. hospita* extract also have anti-inflammatory activities that suppress the production of tumor necrosis factor-α (TNF-α), interleukins 1 and

6 (IL-1 & IL-6) through inactivation of NF-κB. These compounds are believed to make considerable contributions to the anti-inflammatory effect of *K. hospita* extract, leading to decreased inflammatory areas in the treated rats' myocardium.

Conclusions

This study demonstrated that ISO can induce myocardial infarction. However, the use of the *K. hospita* leaf extract over 14 days prior to ISO exposure reduces the myocardial damages secondary to the effect of this drug. This effect was supported by significant reductions in CK-MB and LDH levels in the rats' sera, and improved myocardial tissue damages, as evident by the findings documented upon histopathological examinations. The cardio-protective effects of the extract are likely to be associated with the bioactive compounds present in the ethanol extract of *K. hospita* leaves.

Ethical Considerations

Compliance with ethical guidelines

All study protocols were designed and adhered to the standards of care for animal experiments and were approved by the Institutional Ethics Committee, Hasanuddin University, Makassar, Indonesia (Code: 751/UN4.6.4.35.1/PP36/2022).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization and data interpretation: M. Aryadi Arsyad; Study design and data analysis: YuliaYusrini Djabir; Experiments conduction: Fitriani W. Alani and YuliaYusrini Djabir; Data collection and experiments conduction: Fitriani W. Alani; Drafting the manuscript: Fitriani W. Alani; Data interpretation and writing review and editting: M. Aryadi Arsyad and Yulia Y. Djabir; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors would like to thank Professor Peter Kabo, Professor Rosdiana Natsir, and Burhanuddin Bahar for their advice provided during the course of conducting this study.

References

[1] World Health Organization. Cardiovascular Diseases. Geneva: World Health Organization; 2021. [Link]

[2] Camacho X, Nedkoff L, Wright FL, Nghiem N, Buajitti E, Goldacre R, et al. Relative contribution of trends in myocardial infarction event rates and case fatality to declines in mortality: an international comparative study of 1·95 million events in 80·4 million people in four countries. The Lancet Public Health. 2022; 7(3):e229-39. [DOI:10.1016/S2468-2667(22)00006-8] [PMID]

[3] Zhan Z, Bi X, Yang J, Zhao T. Research progress on the effect of calcium overload on myocardial ischemia-reperfusion injury. Frontiers of Computing and Intelligent Systems. 2022; 1(2):74-8. [DOI:10.54097/fcis.v1i2.1883]

[4] Mehdizadeh R, Parizadeh MR, Khooei AR, Mehri S, Hosseinzadeh H. Cardioprotective effect of saffron extract and safranal in isoproterenol-induced myocardial infarction in wistar rats. Iranian Journal of Basic Medical Science. 2013; 16(1):56-63. [PMID]

[5] Ghasi SI, Umana IK, Ogbonna AO, Nwokike MO, Ufelle S. Cardioprotective effects of animal grade piperazine citrate on isoproterenol induced myocardial infarction in wistar rats: Biochemical and histopathological evaluation. African Journal of Pharmacy and Pharmacology. 2020; 14(8):285-93. [DOI:10.5897/AJPP2020.5164]

[6] Ho JW, Jie M. Pharmacological activity of cardiovascular agents from herbal medicine. Cardiovascular and Hematological Agents in Medicinal Chemistry. 2007; 5(4):273-7. [DOI:10.2174/187152507782109854] [PMID]

[7] Bandyopadhyay D, Chattopadhyay A, Ghosh G, Datta AG. Oxidative stress-induced ischemic heart disease: Protection by antioxidants. Current Medicinal Chemistry. 2004; 11(3):369-87. [DOI:10.2174/0929867043456016] [PMID]

[8] Hanum F, Maesen LJG. Plant resources of South-East Asia. Leiden: Backhuys Publisher; 1997. [Link]

[9] Ramesh P. Flavonoids of Kleinhovia hospita. Journal of Medical Education Research. 1984; 10:76-7. [Link]

[10] Arung ET, Kusuma IW, Kim, YU, Shimizu K, Kondo R. Antioxidative compounds from leaves of Tahongai (Kleinhovia hospita). Journal of Wood Science. 2012; 58:77-80. [DOI:10.1007/s10086-011-1217-7]

[11] Mo JX, Bai Y, Liu B, Zhou CX, Zou L, Gan LS. Two new cycloartane triterpenoids from Kleinhovia hospita. Helvetica Chimica Acta. 2014; 97(6):887-94. [DOI:10.1002/hlca.201300331]

[12] Soromouo LW, Chen N, Jiang L, Huo M, Wei, M, Chu X, et al. Astragalin attenuates lipopolysaccharide-induced inflammatory responses by down-regulating NF-κB signaling pathway. Biochemical and Biophysical Research Communications. 2012; 419(2):256-61. [DOI:10.1016/j.bbrc.2012.02.005] [PMID]

[13] Djabir YY, Arsyad A, Murdifin M, Tayeb R, Amir MN, Kamaruddin FA, et al. Kleinhovia hospita extract alleviates experimental hepatic and renal toxicities induced by a combination of anti-tuberculosis drugs. Journal of Herbmed Pharmacology. 2020; 10(1):102-8. [DOI:10.34172/jhp.2021.10]

[14] Lyrawati D, Muslimah AG, Laksmita D, Santoso DI, Poernomo EL, Larasati K, et al. Hepatoprotective and hepatoregenerative, therapeutic effects of polyherbal medicine on Rattus norvegicus Wistar with liver fibrosis. Thai Journal of Pharmaceutical Sciences. 2017; 41(4):123-9. [Link]

[15] Tayeb R, Alam G, Pakki E, Djabir YY. Paliasa (Kleinhovia hospita L.) Hepatoprotector Tea Bag preparation as supporting therapy in the use of fixed-dose combination of antituberculosis drugs. Journal of Physics: Conference Series. 2019; 1341(7):1-7. [DOI:10.1088/1742-6596/1341/7/072016]

[16] Djabir YY, Arsyad MA, Sartini S, Lallo S. Potential Roles of Kleinhovia hospita L. leaf extract in reducing doxorubicin acute hepatic, cardiac, and renal toxicities in rats. Pharmacognosy Research. 2017; 9(2):168-72. [PMID]

[17] Bęćkowski M. Acute coronary syndromes in young women-The scale of the problem and the associated risks. Kardiochirurgia I Torakochirurgia Polska. 2015; 12(2):134-8. [DOI:10.5114/kitp.2015.52854] [PMID] [PMCID]

[18] Heusch G. Pleiotropic action(s) of the bradycardic agent ivabradine: Cardiovascular protection beyond heart rate reduction. British Journal Pharmacology. 2008; 155(7):970-1. [DOI:10.1038/bjp.2008.347] [PMID] [PMCID]

[19] Sandoval Y, Jaffe AS. Type 2 myocardial infarction: JACC Review Topic of the Week. Journal of American College of Cardiology. 2019; 73(14):1846-60. [DOI:10.1016/j.jacc.2019.02.018] [PMID]

[20] Mert H, Yılmaz H, Irak K, Yıldırım S, Mert N. Investigation of the protective effect of kefir against isoproterenol-induced myocardial infarction in rats. Korean Journal for Food Science of Animal Resources. 2018; 38(2):259-72. [PMID]

[21] Sahoo S, Losordo DW. Exosomes and cardiac repair after myocardial infarction. Circulation Research. 2014; 114(2):333-44. [DOI:10.1161/CIRCRESAHA.114.300639] [PMID]

[22] Mnafgui K, Hajji R, Derbali F, Khlif I, Kraiem F, Ellefi H, et al. Protective Effect of hydroxytyrosol against cardiac remodeling after isoproterenol-induced myocardial infarction in rat. Cardiovascular Toxicology. 2016; 16(2):147-55. [DOI:10.1007/s12012-015-9323-1] [PMID]

[23] Rahim A, Saito Y, Miyake K, Goto M, Chen CH, Alam G, et al. Kleinhospitine E and cycloartane triterpenoids from Kleinhovia hospita. Journal of Natural Products. 2018; 81(7):1619-27 [DOI:10.1021/acs.jnatprod.8b00211] [PMID] [PMCID]

[24] D'Oria R, Schipani R, Leonardini A, Natalicchio A, Perrini S, Cignarelli A, et al. The role of oxidative stress in cardiac disease: from a physiological response to injury factor. Oxidative Medicine Celluler Longevity. 2020; 2020:5732956.[PMID]

[25] Mangali S, Bhat A, Udumula MP, Dhar I, Sriram D, Dhar A. Inhibition of protein kinase R protects against palmitic acid-induced inflammation, oxidative stress, and apoptosis through the JNK/NF-kB/NLRP3 pathway in cultured H9C2 cardiomyocytes. Journal of Cellular Biochemistry. 2019; 120(3):3651-63. [PMID]

[26] Jin JI, Lv RG, Guo J, Liu XH, Liang YW, Wei JR, et al. Improvement of left ventricular remodeling by inhibition of NF-κB in a rat model of myocardial infarction. Heart, Lung and Circulation. 2016; 25(10):1007-12. [DOI:10.1016/j.hlc.2015.11.005] [PMID]

[27] Ma L, Sun P, Zhang JC, Zhang Q, Yao SL. Proinflammatory effects of S100A8/A9 via TLR4 and RAGE signaling pathways in BV-2 microglial cells. International Journal of Molecular Medicine. 2017; 40(1):31-8. [DOI:10.3892/ijmm.2017.2987] [PMID] [PMCID]

[28] Zhang S, Zhang Y. Isoflurane reduces endotoxin-induced oxidative, inflammatory, and apoptotic responses in H9c2 cardiomyocytes. European Review Medical and Pharmacological Sciences. 2018; 22(12):3976-87. [PMID]

This Page Intentionally Left Blank