

Research article

Cardioprotective effect of ethanolic extract of mengkudu (*Morinda citrifolia*) on rats induced by doxorubicin

Lyvia Lovin, I. Nyoman Ehrich Lister*, Edy Fachrial, Sukirman Lie

Faculty of Medicine, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia.

Key words: Cardioprotective, Creatinin kinase - MB, Lactate dehydrogenase.

***Corresponding Author: I. Nyoman Ehrich Lister**, Faculty of Medicine, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia.

Email: jennysim2904@gmail.com

Vol. 4 (4), 06-11, Oct-Dec, 2019.

Abstract

Doxorubicin is an effective drug used in cancer therapy but produces reactive oxygen species (ROS) that are toxic to heart cells. The purpose of this study was to determine the cardioprotective activity of Mengkudu fruit ethanol extract against the heart induced by doxorubicin. Mengkudu fruit ethanol extract was obtained by maceration. Cardioprotective activity test is done by measuring blood LDH and CK-MB levels as well as cardiac histology. Animals were induced with DOX 5 mg/kg BW on day 1,7,14 and 20th. Administration of Mengkudu extract 100 mg / kg bw, 300 mg / kg bw, and 500 mg / kg BW given from day 1 to day 20 and on the 21st day cardiac serum levels of CK-MB normal group had a significant difference ($p < 0.05$) with a negative control treatment group, treatment group I, treatment group II, and treatment group III and did not have a significant difference ($P > 0.05$) with a positive control group with vitamin e supplementation and serum LDH levels the normal group had a significant difference ($p < 0.05$) with the negative control group, the treatment group I, the treatment group II, and the treatment group II and did not have a significant difference ($P > 0.05$) with the positive control group with vitamin e supplementation. Cardiology histology of the Mengkudu extract 100 mg/kg bw + DOX and the Mengkudu extract 300 mg/kg bw + DOX, and the negative control group showed bleeding, fragmentation and myocytolysis, in the treatment of group III, the group normal, and the positive control group did not show heart muscle damage. Based on the description above it can be concluded that the ethanolic extract of Mengkudu fruit containing flavonoids has cardioprotective activity by inhibiting the formation of ROS. The higher the dose of an extract, the greater the decrease in LDH and CKMB levels and increase protection against heart damage.

Introduction

Cardiovascular disease (CVD) and cancer are the leading causes of death worldwide. Risk factors that cannot be modified, including age, sex, race/ethnicity, are uncontrollable features that affect the incidence of cardiovascular disease and cancer. According to WHO (World health organization) data, as many as 17.8 million people die from heart disease and more than 80 percent of heart disease sufferers are in low and middle-income countries [1].

Cardiotoxicity associated with cancer treatment is the leading cause of treatment-related mortality and morbidity in cancer sufferers. Age, previous cardiac dysfunction, heart disease, hypertension, smoking, and obesity are risk factors that have been described as potential cardiotoxicity associated with anthracyclines as well as several new agents. With the increasing number of patients receiving chemotherapy, there is no good tool to distinguish the development of underlying heart disease from the immediate cardiotoxic effects of chemotherapy agents.

Echocardiography used to assess cardiac function before the start of chemotherapy and to monitor the development of cardiac toxicity during therapy is also not perfect in this role because it is relatively insensitive for detecting cardiac toxicity during treatment. This is because the normal heart has very good reserve capacity, so even loss of left ventricular function is low even though it is an indication of significant cardiovascular damage. These problems due to the high cost of repetitive imaging make the discovery and utilization of guided biomarker approach a very appropriate choice [2].

Doxorubicin is a class of anthracyclines, as a chemotherapy agent that is widely used and is now part of a standard therapy regimen for various types of cancer, such as hematopoietic malignancies and solid tumors in the breast, ovaries, thyroid and bone malignancies. However, potentially fatal and dose-dependent cardiotoxicity that appears within a short time after treatment limits the use of doxorubicin in cancer patients. Although the mechanism of

cardiotoxicity induced by free radicals and excessive production of reactive oxygen species is a major driver of its toxicity [3].

Vitamin E is an antioxidant that can reduce the effects of tissue damage caused by free radicals so that vitamin E is useful in the treatment of neurological, cardiovascular and malignant diseases such as tumors and cancer [4]. The cardioprotective effect of Vitamin E is by protecting cells from free radicals or oxidative stress [5]. Tocotrienol is a component of natural Vitamin E besides tocopherol is a fat-soluble antioxidant that protects cell membranes from oxidative damage. Tocotrienol and tocopherol are combination ingredients to reduce the effects of free radicals, inhibit cancer growth, cardioprotective, and premature aging [6]. Mengkudu (*Morinda citrifolia*) is a plant that is widely spread in subtropical and tropical regions such as China, India, Indonesia, and the Malay Peninsula and is used in many traditional treatments such as Chinese medicine, Tibetan Medicine and Ayuverdic medicine [7]. Mengkudu fruit with a high content of flavonoid compounds has been reported to have antioxidant effects, hypolipidemia [8], and hypoglycemic activity [9], and work as important compounds in several hepatoprotective formulas [10]. Mengkudu has also been known as an antimicrobial agent [11], antitumor [12] or anti-inflammatory agent [13].

Based on the background of providing cancer therapy with doxorubicin, it often causes organ toxicity, especially cardiotoxicity, and Mengkudu fruit containing rich phenols and flavonoids that are strong anti-free radicals, thus encouraging researchers to test the cardioprotective activity of Mengkudu fruit ethanol extract against doxorubicin-induced experimental animals by measuring the biochemical parameters of CK-MB (Creatine kinase-MB) and LDH (Lactate dehydrogenase) as biomarkers and conducting histopathological examination of heart tissue.

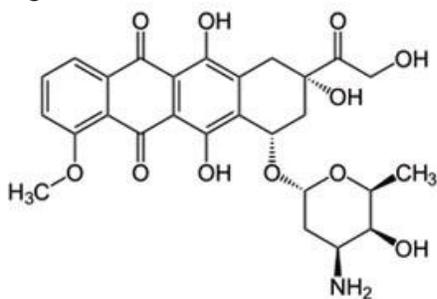


Figure 1. Structure of doxorubicin.

Materials and method

Material

Microplate Reader, pH meter (OHAUS Starter300 Portable) Beaker glass (IWAKI CTE33), Multiskan Go Reader (Thermo Fisher Scientific 1510), analytic measure, Eppendorf tube, vial 1 ml, Spatula, Micropipet (1-10 μ L, 50-200 μ L, 100-1000 μ L) (Eppendorf), Termometer, automated

plate washer, Extract ethanol of *Morinda citrifolia*, Ketamine (Sigma P-4417), Doxorubicin (Merck 109057), CMC-Na (Sigma P-4417).

Animals

Animals used in research are rat (*Rattus norvegicus*) Wistar male 150 – 200 g. Before the study began, animal test adjusted for one week with the condition of the room temperature (22-25 $^{\circ}$ C), under the cycle of 12 hours light/dark, given the food and the drinking water ad libitum. Ethics Commission from health and science commission, University of Sumatera Utara.

Preparation of ethanol extract of Mengkudu

Air-dried leaves of Mengkudu (*Morinda citrifolia*) (800g) were extracted with 90% ethanol (12L) three times (2h each) using a soxhlet under reflux. The ethanol extract was concentrated under vacuum to give a crude extract (150g).

Phytochemical screening of ethanol extract of Mengkudu

Phytochemical screening of extract ethanol Mengkudu method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin and alkaloids.

In vivo test of cardioprotective effect of ethanolic extract of mengkudu

In vivo tested in an experiment by using 24 Wistar rats (*Rattus norvegicus*) male and weight 150 g - 200 g, as many as 24 and divided into 6 groups and each group consisted of 4 rats:

Normal: Suspension Na-CMC.

Negative control: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw.

Positif control: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + Vitamin E 1%.

Group 1: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 100 mg/kgbw.

Group II: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 300 mg/kgbw.

Group III: Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 500 mg/kgbw.

Rat induced by doxorubicin with an accumulative dose of 15 mg/kgbw over for 21 days, with the dose of administered 5 mg/kgbw once a week. Before treatment, the rats adapted for 14 days and then continued with the administered of doxorubicin and the treatment of rats for 21 days administered by the extract with a dose of 100 mg/kgbw, 300 mg/kgbw, 500 mg/kgbw. On the last day of treatment the rats fasting 18 hours before performed surgery on the animal test. Rats administered by ketamine 70 mg/kgbw an intraperitoneal then continued to the surgery. Thoracic dissected and the blood was taken as much as 3 ml. The

blood transferred into a microtube. Then the blood is centrifuged for 10 minutes at a speed so that the generated 3000-4000 rpm until it divided into 2 layers as serum and supernatant. A layer of serum is taken 1 ml and put into microtubes and stored in the refrigerator at a temperature of 4 °C. Blood serum used to determine CK-MB, and LDH.

Determination of CK-MB and LDH

Measurement of the levels of CK-MB and LDH is performed by following the method described by Adeyemi, *et al.* [5]. As many as 50 µl samples and 500 µl of CK-MB reagent/LDH mixed in a test tube. Then the initial absorbance read after 1 minute at a wavelength of 340 nm. Next absorbance was measured again after 1, 2, and 3 minutes [5].

Statistical analysis

Test analysis was carried out by using one-way analysis of variance (ANOVA) followed by Post Hoc Test using the Tukey HSD test. $P < 0.05$ was considered as statistical significance and also use IBM SPSS 20.

Result and discussion

Authentication of plant

The results of the identification of plants carried out by Livia lovin (2019) at the Medanese Herbarium (MEDA) the University of North Sumatra, the fruit used in this study was Mengkudu (*Morinda citrifolia*) Kingdom: Plantae, Subkingdom: Tracheobionta, Super Division : Spermatophyta, Division : Magnoliophyta, Kelas : Magnoliopsida, Subkelas : Asteridae, Ordo : Rubiales, Family : Rubiaceae, Genus : Morinda, Species : *Morinda citrifolia* L.

Phytochemical tests

The results of phytochemical screening qualitatively in Mengkudu extract are shown in the table 1.

Table 1. Results of phytochemical screening of mengkudu extract.

Chemical Component	Result
Tanin	+
Saponin	+
Flavonoid	+
Steroid	+
Glikosida	+
Alkoloid	+

Phytochemical screening of ethanol extract of mengkudu showed the positive result of flavonoids, tannins, saponins, glycosides, alkaloid, and steroids.

Chemical content in mengkudu includes organic acids, phenolic compounds, alkaloids, glycosides, polysaccharides, lignans, fatty acid esters, vitamins and

minerals isolated from mengkudu fruit, roots, and leaves. Phenolic compounds found in mengkudu are tannin, flavonoids, alizarin, anthraquinone, damnacanthol, morindone, morindin, acubin, asperuloside, routine and scopoletin. Noni fruit contains 90% water and the main dry component consisting of dissolved solids, fiber and protein. The main minerals found in mengkudu are potassium, sulfur, calcium, phosphorus and selenium. Mengkudu also contains vitamins, especially ascorbic acid (25-158mg / 100g dry matter) and pro-vitamin A. The main volatile compounds have been identified in ripe noni fruit which include organic acids (caproic, caprylic, octanoic, and hexanoic acid), alcohols (3 methyl 3-buten-1-ol), esters (methyl octanoate, methyl decanoate), ketones (2-heptanone) and lactone (E-6-dodeceno-lactone) [18].

CK-MB level

In this research, conducted an examination of CK-MB from the blood of rats. Results of serum CK-MB obtained can be seen in table 2.

Table 2. CK-MB level.

No.	Doses	Mean CK-MB ± SD (U/L)
1.	Normal	328.5 ± 8.5
2.	Negative control	809 ± 14
3.	Positive control	399 ± 3
4.	Group I	657.5 ± 54.5
5.	Group II	493.5 ± 24.5
6.	Group III	437.5 ± 6.5

The data presented in the form of Mean ± SD. Data obtained results based on the results of the statistical tests, the levels of serum CK-MB normal have a significant difference ($p < 0.05$) with the negative control, positive control, treatment group I, II, III.

Based on the table it is known that the average serum CK-MB in the largest treatment group is 657.5 U / L in the treatment group I. And the average serum CK-MB in the smallest treatment group is 437.5 U / L in the treatment group III. In addition, it can be seen in figure 2.

LDH level

In this research, conducted an examination of LDH from the blood of rats. Results of serum LDH obtained can be seen in table 3.

Table 3. LDH level.

No.	Doses	Mean LDH ± SD (U/L)
1.	Normal	634.6 ± 4.7
2.	Negative control	2153.66 ± 4.02
3.	Positive control	784 ± 8.3
4.	Group I	1245 ± 10.5
5.	Group II	916.33 ± 10.9
6.	Group III	839.33 ± 4.1

The data presented in the form of Mean ± SD. data obtained results based on the results of the statistical tests, the levels of serum CK-MB normal have a significant difference ($p < 0.05$) with the negative control, positive control, treatment group I, II, III.

Based on the table it is known that the average serum LDH value for the normal group is still within the normal range of 634.6 U/L. The positive control group (doxorubicin induction) had an average serum LDH of 2153.66 U/L. The treatment group I had a serum LDH

value of 1245 U/L. Treatment group II had a serum LDH value of 916.33 U/L. The treatment group III had a serum value of 839.33 U/L and the positive control group using Vitamin e as a comparison had an average serum LDH of 784 U/L. Based on the table, it is known that the average serum LDH in the largest treatment group is 1245 U/L in the treatment group I. And the average serum LDH in the smallest treatment group is 839.33 U/L in the treatment group III. In addition, it can be seen in figure 3.

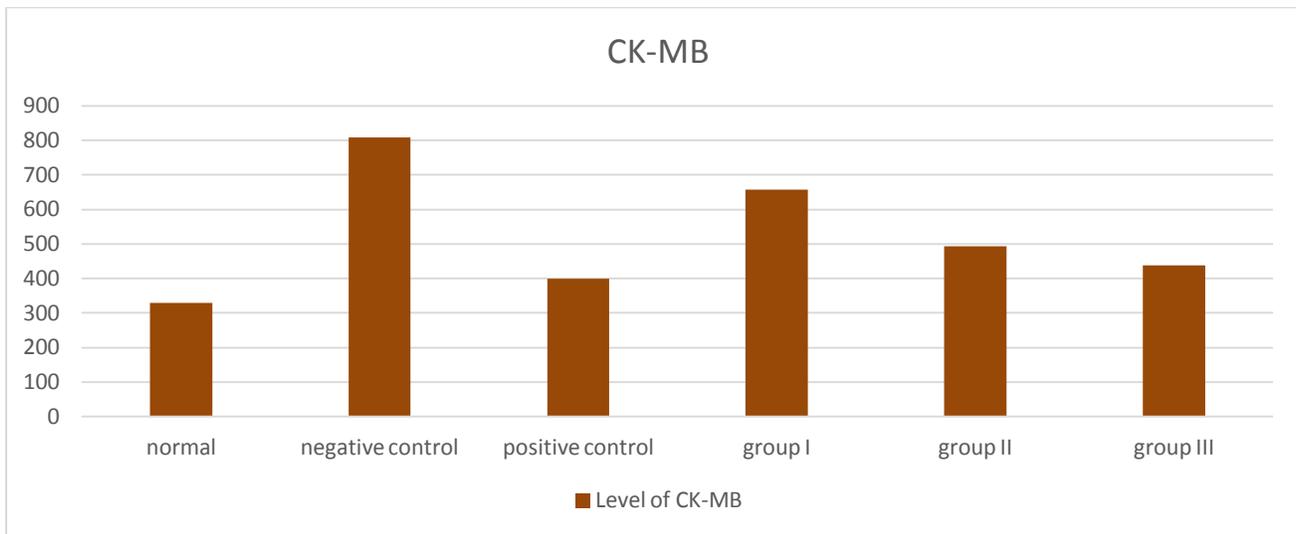


Figure 2. CK-MB level.

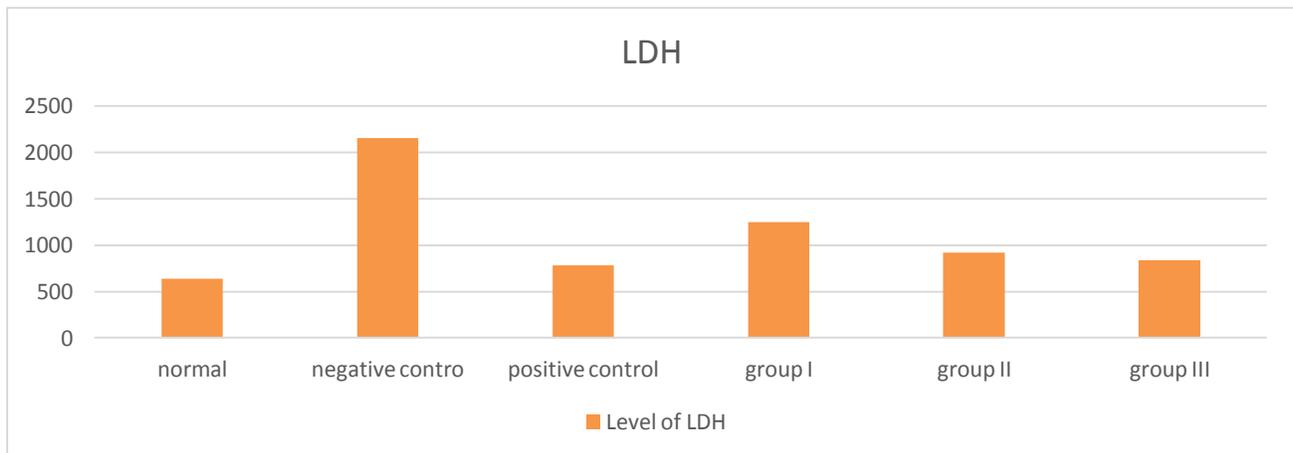


Figure 3. LDH.

The cardiotoxic process is caused by the formation of free radicals in the metabolic process of chemotherapy drugs, impaired adrenergic function, the formation of lipid peroxide, disruption of Ca transport in sarcolemma, and the release of TNF- α and interleukin-2, and cytokines free from tumor tissue. Of all the factors that can cause cardiotoxicity, it is suspected that free radicals or oxidative stress have a large enough role to cause the condition.

Cardiotoxicity conditions due to the use of doxorubicin occur through several stages of the process. First, doxorubicin will bind with iron and form chelate through oxygen bonds. In this process, the reduction reaction occurs by flavin-dependent reductase which changes the form of quinone anthracycline into the form of semiquinone. The form of semiquinone is a form of free radicals. If there is oxygen, semiquinone will provide unpaired electrons to the oxygen molecule to form O₂ superoxide anion [14].

Superoxide anion O_2^- through an enzymatic process by superoxide dismutase will form oxygen and hydrogen peroxide (H_2O_2) molecules. H_2O_2 elimination is an important stage because H_2O_2 can trigger the formation of hydroxyl radicals, an oxidant that is very reactive and destructive. Hydrogen peroxide is inactivated by two enzymes, namely catalase and glutathione peroxidase. Catalase converts hydrogen peroxide to water and oxygen, whereas glutathione peroxidase uses glutathione to reduce hydrogen peroxide to water and oxidized glutathione [15].

Increased oxidative stress by doxorubicin causes mitochondrial damage, increases fat oxidation, and causes damage to heart cells. Heart cell carcinoma is characterized by an increase in cardiac biomarkers including CK-MB and LDH. In this study, the positive control group induced by doxorubicin CK-MB and LDH enzymes had an increase, this was caused by the damage of heart muscle cells by doxorubicin. CK-MB is a biomarker that is widely available in the heart muscle tissue compared to other tissues. An increase in CK-MB indicates the occurrence of heart muscle cell damage caused by the production of reactive oxygenase (ROS) which increases due to doxorubicin, ROS rapidly damages the heart muscle cells. In this study an increase in serum LDH (Lactate dehydrogenase) levels in the positive control group induced by doxorubicin compared with the negative (normal) group and other treatment groups. LDH is an enzyme that is found in many muscle tissues including the heart, kidney, and liver. Increased serum LDH levels indicate damage to muscle tissue and in this study heart muscle cell damage due to doxorubicin, so LDH levels increase [16].

Mengkudu has flavonoids which act as reservoirs of hydroxy and super hydroxyl radicals or slow the onset of cell necrosis but also by increasing vascularity thereby protecting lipid membranes against damaging reactions. Flavonoids can inhibit bleeding. Flavonoids are also known to accelerate the process of wound healing mainly because it has antimicrobial and astringent activity, which has a role in wound shrinkage and an increase in the rate of epithelialization. Flavonoids also function to reduce the production of CK-MB and LDH and Mengkudu also contains 5 flavonol glycosides, namely: quercetin-3-O- β -D-glucopyranoside; kaemperol-3-O- α -L-rhamnopyranosil- (1 \rightarrow 6) - β -D-glucopyranoside; quercetin-3-O- α -L-rhamnopyranosil (1 \rightarrow 6) - β -D-glucopyranoside; quercetin-3-O- β -D-glucopyranosyl- (1 \rightarrow 2) - [α -L-rhamnopyranosil- (1 \rightarrow 6) - β -D-glucopyranoside; and kaemperol-3-O- β -D-glucopyranosyl (1 \rightarrow 2) - [α -L-rhamnopyranosil- (1 \rightarrow 6) β -D-galacopyranoside (Sang *et al.*, 2005). Polyphenol compounds such as flavonoid compounds (including flavonols) can inhibit autocidation through radical scavenging mechanisms by donating one electron from unpaired electrons in free radicals so that the number of

free radicals is reduced [17]. Toxicity research by brett showed Mengkudu seed extract appears to be non-cytotoxic, with an LC50 > 1 mg/mL. It was also non-toxic in the 28-day oral toxicity test in rats. Natural toxicants were not found in the extract nor were any potential antinutrient substances identified, which was further demonstrated by the appropriate weight gain observed in the 28-day test. The primary DNA damage test did not reveal any genotoxic risk associated with the consumption of mengkudu seed extract. These results are consistent with multiple in vivo and in vitro toxicity tests, as well as human clinical trials of mengkudu fruit, seeds, seed oil, and leaves [19].

The I, II and III treatment groups affected reducing serum CK-MB and LDH levels in doxorubicin-induced mice.

Conclusions

Ethanol extract of Mengkudu fruit dose of 100 mg/ kgbw, 300 mg / kgbw, and 500 mg / kgbw had cardioprotective activity by reducing CK-MB levels significantly different ($P < 0.05$) with negative control groups that were only given CMC-Na and doxorubicin.

References

1. Abdul, R., Sugeng, R. Antioxidant activity of Mengkudu Fruit (*Morindacitriifolia*, L). UGM 2011; 8:11-15.
2. Aleixo, M., A. Ascensao., A. E. Santos., P. J. Oliveira., P. I. Moreira., J. Magalhaes. The beneficial role of exercise in mitigating doxorubicin-induced mitochondriopathy. *Biochim. Biophys. Acta. Rev. Cancer* 2018; 13: 201-204.
3. Economopoulou, P., A. Kotsakis., I. Kapis., N. Kentepozidis. Cancer therapy and risk of cardiovascular. *CMAR* 2015; (7): 133-143.
4. Armansyah, T.R., Sutriana, A., Aliza, D., Vanda, H., danRahmi, E. Hepatoprotective Activity Ethanol Extract of Cat-cat leaves (*Acalyphaindica* L.) in White Rats (*Rattusnovergicus*) Induced by Paracetamol. 2015; 13(6): 292 – 297.
5. Cardoso, D.A., A. S. B. Moreira., G. M. M. Oliveira., R. R. Luiz., G. Rosa., A coconut extra virgin oil-rich diet increase HDL cholesterol and decreases waist circumference and body mass in coronary artery disease patients. *Nutricion Hospitalaria* 2015; 32(5): 2144-2152.
6. Ewer, S. M. Cardiotoxicity of cancer treatment: What the cardiologist needs to know. *Nature Reviews: Cardiology* 2010; 7: 564-575.
7. Clafshenkel,W.P.,K.L. Tracy., M.P.Kotlarczyk., M,J.Cline.,F.G.Warren., D.L.Vicki.,W.P.A.Enderby. Morinda citrifolia (noni) juice augments mammary gland differentiation and reduces mammary tumor growth in mice expressing the unactivated c-erbB2 transgene. *Evidence-Based Complement. Altern. Med.* 2012; 4(5): 1–15.
8. Djauhariya, Endjo. Mengkudu (*Morindacitriifolia* L.) Potential Medicinal Plants. *Research Institute for Spices and Medicines. Technology Development* 2003; 15(1): 1-16.
9. Gupta, R.K., A. Banerjee., S. Pathak., C. Sharma., N. Singh. Induction of mitochondrial-mediated apoptosis by *Morinda citrifolia* (noni) in human cervical cancer cells. *Asian Pac. J. Cancer Prev.* 2013; 14 (1):237–242.
10. Hill, M.A. Cardiovascular System-Heart Histology. *Embryology Cardiovascular System-Heart Histology*. Retrieved from https://embryology.med.unsw.edu.au/embryology/index.php/Cardiovascular_System_Heart_Histology 2014; 15(10): 45-47.
11. Koene, R.J., A.E. Prizment., S.H. Konety. Shared risk factors in cardiovascular disease and cancer. *Circulation* 2016; 133: 1104-1114.
12. Murli, K. Microscopic anatomy of the liver. *Clinical Liver Disease* 2013; 2(1): 4-6.
13. Nima., Sawpheeayah., Kasiwong., Srirat., Ridditid., Wibool., Thaenmanee, mNiwan., M.Ahattanadul., Sirima. Gastrokinetic activity of *Morinda citrifolia* aqueous fruit extract and its possible mechanism of action in human and rat models. *J. Ethnopharmacol* 2012; 142 (2): 354–361.

14. Renu, K., V.G. Abilash., P.B.T. Pichiah., S. Arunachalam. Molecular mechanism of doxorubicin-induced cardiomyopathy-An update. *European Journal of Pharmacology* 2017; 6(10): 54.
15. Serafini., M.Russo., Santos., R.Correia., Guimaraes., A.Gibara., dosSantos., J.P.Almeida., S.daConceicao., A.Diego., Alves., I.Almeida., Gelain., D.Pens., N.deLima., P.Cesar., J.Quintans., L.Jose., A.deSouza., A.Antunes., Bonjardim., L.Rigoldi. Morinda citrifolia linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. *J. Med. Food* 2012; 14 (10): 1159–1166.
16. Shivananda, N.B., J.R. Marshall., Isitor., Godwin., Adogwa., Andrew. Hypoglycemic and hepatoprotective activity of fermented fruit juice of *Morinda citrifolia* (Noni) in diabetic rats. *Evidence-Based Complement. Altern. Med* 2011; 3(9): 1–5.
17. Thomas, S.A.A. Chemotherapy agents that cause cardiotoxicity. *US Pharm* 2017; 42(9):24-33.
18. Wang, M. Y., West, B. J., Jensen, C. J., Nowicki, D., Su, C., Palu, A. K., & Anderson, G. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. *Acta Pharmacologica Sinica* 2002; 23(12): 1127-1141.
19. West, B. J., Jensen, C. J., Palu, A. K., & Deng, S. Toxicity and antioxidant tests of *Morinda citrifolia* (noni) seed extract. *Advance Journal of Food Science and Technology* 2011; 3(4): 303-307.