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Histopathological Profile of Therapeutic Doses of Methanolic Extract Combination of Tea and Mango Mistletoes in Cardiac of Preventive Hypertensive Rats (DOCA-salt) Model

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Abstract

Tea and mango mistletoes have to lower blood pressure by improving oxidative stress and endothelial dysfunction. The flavonoid compounds found in these two mistletoes act as antihypertensives. Hypertension is a risk factors that influences the incidence of cardiac disease. This study examines the histopathological of the cardiac of hypertensive rats (preventive model) after treatment of methanolic extract combination of tea and mango mistletoes. This study used a completely randomized experimental design with 25 Wistar rats in total for five treatments, namely negative control (-), positive control (+), treatment 1 (dose of 50 mg/kg BW), treatment 2 (doses of 100 mg/kg BW), and treatment 3 (doses of 200 mg/kg BW). In this study, Deoxycorticosterone acetate (DOCA) were used to induce hyperttension in experimental animals. Data were analysed with one-way ANOVA test using JAMOVI (version 1.1.9.0) and posteriori. There was a significant difference between all groups ($p<0.05$). Administration of a methanolic extract combination of tea and mango mistletoes as the preventive model can significantly reduce hypertrophy of cardiac muscle cells. This effect occured with a dose of 50 mg/kg BW, the optimal dose to reduce hypertrophy of cardiac muscle cells in Wistar rats male.

Keywords

Hypertrophy, Hypertension, Mango mistletoe, Muscle Cells Cardiac, Tea Mistletoe.

INTRODUCTION

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITIES) stated that the use of medical materials uses approximately 60,000 plant species worldwide for both traditional and modern medicines (1). Mistletoes tea (*Escurrula atropurpurea*) and mango mistletoes (*Dendrophthoe pentandra* L.) Miq is a plant that has benefits for maintaining health and fitness (2,3). Tea mistletoes have been shown to reduce contractility of the tail arteries of hypertensive rats in vitro. While in vivo, tea mistletoes reduce blood pressure by improving oxidative stress and endothelial dysfunction, lowering MDA (Malondialdehyde) levels, and increasing NO (Nitrix Oxygen) levels (2,4,5,6). In addition, the flavonoid and tannin compounds in the *Dendrophthoe pentandra* L. act as antihypertensives.

Hypertension, known as the silent killer, is a condition of increased systolic of more than 140 mmHg and diastolic blood pressure of more than 90 mmHg (7,8). In the incidence of cardiac and blood vessels (vascular system) diseases, hypertension is one of the most influential risk factors (9,10,11,12,13). People with hypertension have a narrow blood vessel, known as vasoconstriction, which increases the cardiac work to pump blood throughout the body. This condition can weaken the cardiac muscle and cause hypertrophy.

Traditional medications can be an alternative to prevent and treat hypertension. Therefore, on this occasion, the authors examinethe histopathological profile of the cardiac in male Wistar rats with a preventive model of hypertension after administration of a methanolic extract combination of tea and mango mistletoes.

MATERIALS AND METHODS

This study used a completely randomized experimental design with 25 Wistar rats in total for five treatments of administration of the extract combination methanolic tea leaf mistletoe and mango mistletoe, namely negative control (-), positive control (+), P1 with a dose of 50 mg/kg BW, P2 with a dose of 100 mg/kg BW and P3 with a dose of 200 mg/kg BW. A methanolic extract combination of tea and mango mistletoes was exposed to *Rattus novergicus* (male Wistar rats) for 28 days and followed by the administration of Deoxycorticosterone acetate (DOCA)-salt in the last 14 days as a preventive model.

The research was carried out in March – May 2021 in the animal house Faculty of Dentistry, Laboratory of Anatomical Histopathology, Faculty of Medicine Universitas Brawijaya Malang, Laboratory of the Faculty of Medicine

Islamic University of Malang, and Laboratory of Balai Materia Medica Batu, East Java. All experiments were performed with ethical approval from the Islamic University Malang Faculty of Medicine Health Research Ethics Commission (No. 006/LE.001/IV/03/2020).

Preparation Methanolic Extract Combination of Mistletoe Tea Leaves and Mistletoe of Mango Mistletoe

The methanolic extract combination of tea and mango mistletoes is produced using Simplicia derived from mashed dried mistletoe leaves. The maceration process includes immersing the material with a solvent that is by following per under the active compound to be extracted (14).

The powder or simplicia leaves of tea parasite and mango parasite was measured at 100 grams were put into five 1.5 liter plastic bottles. Then, 1 litre of 90% methanol solution was put into each bottle and shaken until the solution was homogeneous for 1 hour. After 1 hour, the homogeneous solution was allowed to stand and precipitated for 24 hours. This process was stopped when equilibrium was reached (14). The result of the deposition process for 24 hours will form a bottom layer in the form of *a natant*, while the top layer is called a *supernatant*. Part *supernatant* will be accommodated and proceed to the extraction stage using a *rotary evaporator* to produce leaf extract of tea mistletoe and mango mistletoe in the form of a paste.

Test Animal Acclimatization Process

Wistar rats (*Rattus norvegicus*) 2-3 months old with a bodyweight of 150-250 were acclimatized at the Animal House, Faculty of Dentistry, Universitas Brawijaya Malang for five days at room temperature ± 24°C with humidity of approximately 50-60% protected from the smoke of industry, motor vehicle fumes, and other pollutants as well as being fed and drinking.

Test Animal Care

The test animals were weighed before and after the acclimatization (pre-treatment) once a week for 28 days of treatment. Weekly monitoring of body weight is required to determine the dose amount. Wistar rats use a cage with a cover in the form of woven wire and wood shavings given as a base for mice. The husks are regularly replaced once every two days. Feeding was performed every morning and evening with a feed weight of 7 grams per rat.

Treatment Methanolic Extract Combination Tea and Mango Mistletoes

This study used a dose of methanolic extract combination of tea and mango mistletoes in a ratio of 3:1 with the first

dose of 50 mg/kg BW, the second dose of 100 mg/kg BW, and the third dose of 200 mg/kg BW. The methanolic extract combination of tea and mango mistletoes was administered for four weeks (5 times/week).

Provision DOCA-salt as A Preventive Hypertension Model

DOCA is a precursor of the adrenal cortex mineralocorticoid hormone, which has an analogous effect to the mineralocorticoid aldosterone, which causes retention of Na^+ and H_2O in the distal tubule. DOCA-salt is one model of secondary hypertension due to endocrine (hormonal) influences. DOCA was administered twice a week during the last two weeks of treatment which was also at the same time as the administration of a methanolic extract combination of tea and mango mistletoes. DOCA was administered by subcutaneous injection in the positive control (+), the first treatment (P1), (P2), and (P3). Blood pressure readings were taken in rats before and after DOCA treatment. DOCA given to rats was first homogenized with sesame oil and according to the dose.

Surgery

Surgery on the test animals was performed after 28 days of treatment by taking organs for histopathological examination. The organ observed is cardiac. After 3 hours of fasting, the rats were given either intramuscular ketamine injections of anesthesia. Then dissection was performed after the rat was unconscious. Then the cardiac organ was taken and put in an organ tube with a 10% formalin solution. The organ taken was used as a microscopic preparation and then processed following the standard histological method with Hematoxylin and Eosin (HE) staining.

Histopathological Examination

Histopathological observations of cardiac organs were performed after staining using Hematoxylin and Eosin by taking 400x and 200x magnification photos with five fields of view under a trinocular microscope Olympus U-TV0.5XC-3, T7 Tokyo, Japan. The photos with 400x magnification were followed by observations with the ImageJ application to measure the diameter of cardiac muscle cells, while the photos with 200x magnification were used as visualizations.

Data Analysis

The data obtained were statistically tested using the JAMOVI application version 1.1.9. One-way analysis of variance (ANOVA). A post-hoc test was conducted after additional tests. We employed ANOVA with post hoc testing to examine differences between means while reducing the family error rate.

RESULTS

The Measure of Cardiac Muscle Cell Diameter

Microscopic measurement of the diameter of the cells in the cardiac muscle was performed using the ImageJ application. Previously, previously, cardiac histopathology was observed under a trinocular microscope with an objective magnification of 400x in 5 fields of view. Histopathological features of the cardiac in male Wistar rats were observed after administration of a methanolic extract combination of tea and mango mistletoes for 28 days and followed by injection of DOCA-salt as a model of hypertension in rats in the last two weeks. The results were obtained based on the treatment in each group (Table 1).

Table 1. Histopathological Observations on the Mean Cell Diameter in Cardiac Muscle (µm) Male Wistar Rats After Treatment 28 day.

Treatment	Mean Cell Diameter in Cardiac Muscle (µm)
Negative Control	15.5 ± 0.918[a]
Positive Control (DOCA-salt)	26.2 ± 2.489[b]
P1 (Dose 50 mg/KgBB + DOCA-salt)	15.6 ± 0.982[a]
P2 (Dose 100 mg/KgBB + DOCA-salt)	15.1 ± 0.691[a]
P3 (Dose 200 mg/KgBB + DOCA-salt)	14.4 ± 0.357[a]

The different letters in the same column indicate α significant difference at p<0.05. Data are presented as mean ± SD

Based on Table 1, the positive control (K+) group had the widest cell diameter. The result also showed there was a significant difference between treatment groups.

Observations were done by measuring the diameter of cardiac muscle cells in the myocardium layer. The normality test showed that the data were normally distributed (p<0.05). The results of the One Way ANOVA test obtained a p-value of <0.001, in which there was a significant difference statistically.

The *post Hoc* test was done by comparing the positive control (K+) induced by DOCA-salt, the negative control (K-) without DOCA-salt induction, P1, P2 and P3. The result showed a statistically significant difference between positive control (K+) induced by DOCA-salt and the negative control (K-) without DOCA-salt induction. In contrast, there was no significant difference in the P1, P2, and P3 (treatment group).

Histopathological Observation

Observations obtained photos of cardiac muscle cells both at 200x and 400x magnification (Figure 1-4). From this microscopic observation, the positive control mice were significantly different from the negative control mice (-) and the treated mice (P1, P2, and P3). On the contrary, there was no significant

difference between the negative control and treatment groups (P1, P2, and P3).

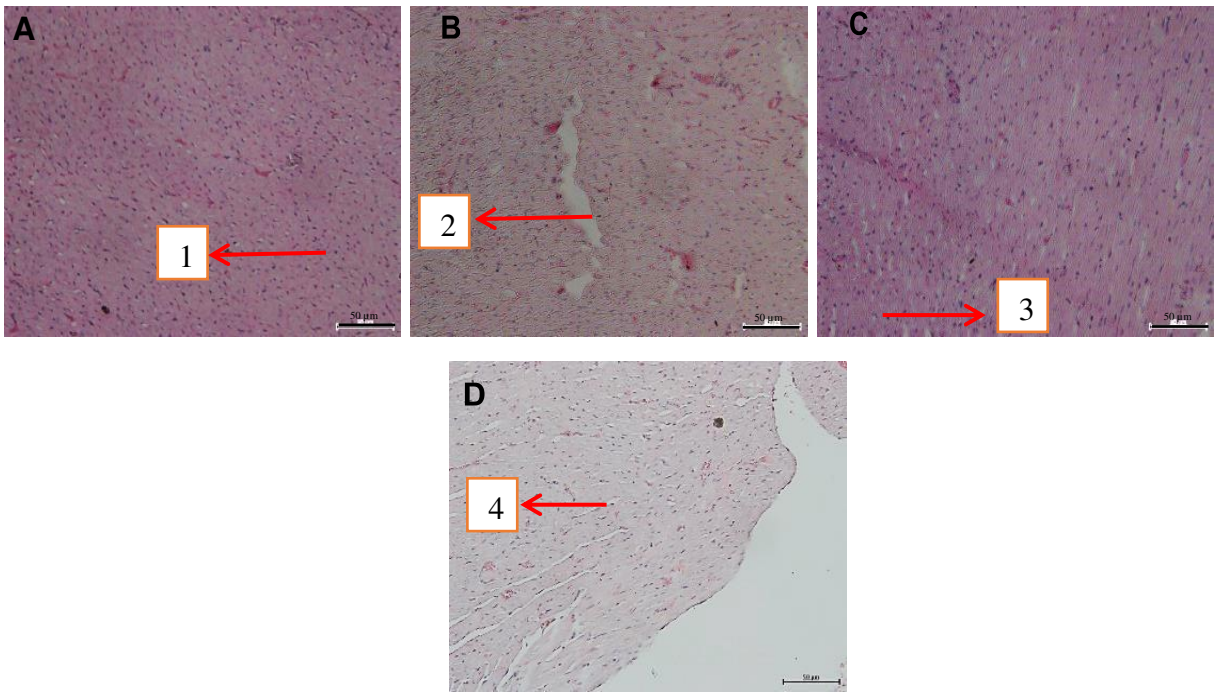

Figure 1. Microscopic observations (*cross section*: 200x) Myocardial tissue. (A) Control Group (-), (B) Control Group (+), (C) Group P1 (50 mg / KgBW), (D) Group P3 (200 mg / KgBW). Description: 1,2,3,4 : cell nucleus

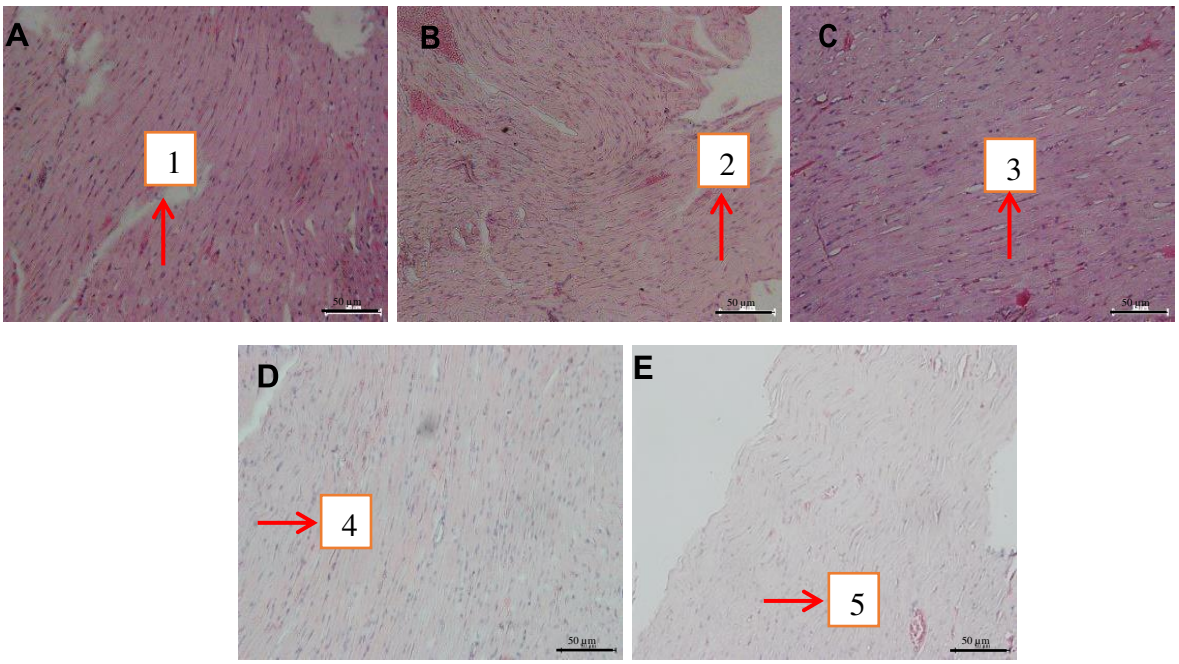

Figure 2. Microscopic observations (*vertical section*: 200x) Myocardial tissue. (A) Control Group (-), (B) Control Group (+), (C) Group P1 (50 mg / KgBW), (D) Group P2 (100 mg / KgBW), (E) Group P3 (200 mg / KgBW). Description: 1,2,3,4,5 : cell nucleus

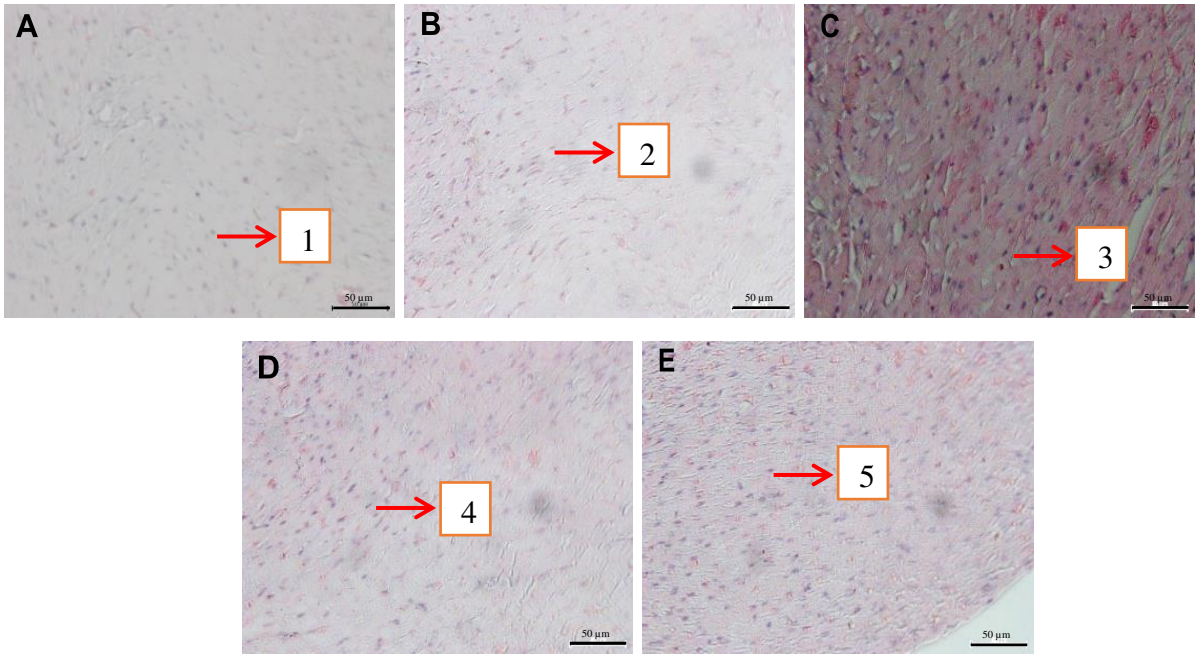

Figure 3. Microscopic observations (*Cross section*: 400x) Myocardial tissue. After 28 (A) Control Group (-), (B) Control Group (+), (C) Group P1 (50 mg / KgBW), (D) Group P2 (100 mg / KgBW), (E) Group P3 (200 mg / KgBW). Description: 1,2,3,4,5: cell nucleus.

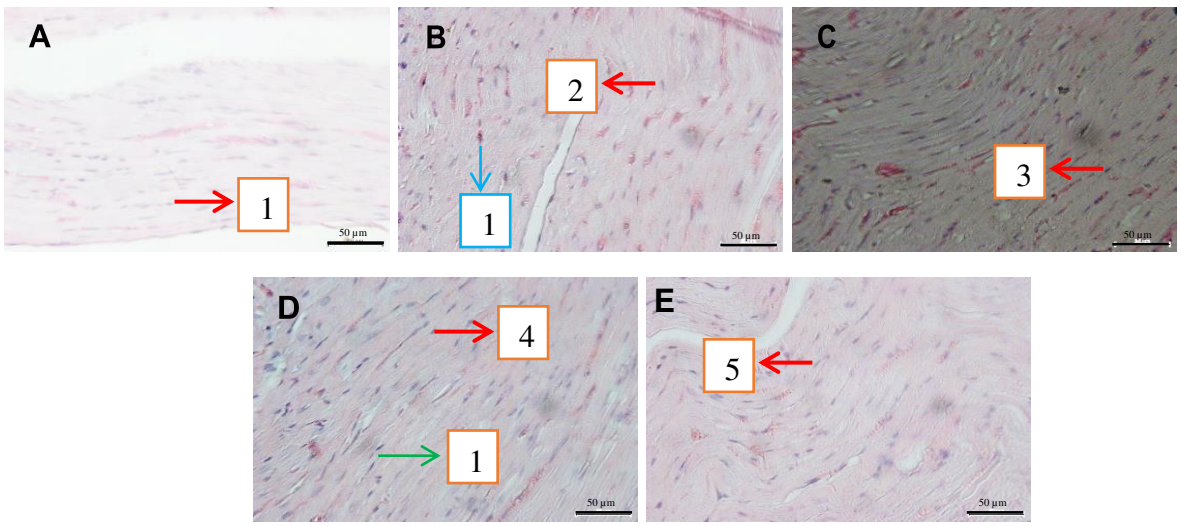

Figure 4. Microscopic observations (*ventricular section*: 400x) Myocardial tissue. (A) Control Group (-), (B) Control Group (+), (C) Group P1 (50 mg / KgBW), (D) Group P2 (100 mg / KgBW), (E) Group P3 (200 mg / KgBW). Description: →1,2,3,4,5 : cell nucleus, →1 : Hypertrophy, → 1 : Intercalated disc

DISCUSSION

Tea and mango mistletoes are parasitic plants but have also proven to be efficacious as herbs. These two plants contain flavonoids, compounds in herbal medicines or alternative phytopharmacology preparations. The research conducted by Atiroh et al., (2,3) found that mistletoe tea reduced the contractility of the artery blood vessels on

the rat tail and blood pressure by improving oxidative stress and endothelial dysfunction.

The parameters observed in this study were cardiac histopathology, precisely by measuring the diameter of cardiac muscle cells. The cardiac diameter muscle cell chose because of the cardiac function as a blood pump throughout the body (15). If cardiac function decreases because of cardiac muscle infection, this can affect body oxygen (O_2) and nutrient demand (16). In the same way as the adaptation mechanism of hypertension, cell hypertrophy will occur in the cardiac muscles that receive an additional workload.

Cardio output increases because it experiences vasoconstriction then the cardiac is forced to work extra to meet the blood supply for the body. As a result, the cardiac muscle is forced to work hard while the cardiac muscle is not strong enough then the cells develop hypertrophy.

The administration of the methanolic extract of the combination of tea mistletoe and mango mistletoe showed a significant difference with a p-value <0.001 ($p<0.05$) between all treatment groups. There are differences in each treatment group. The longest mean cell diameter of cardiac muscle was found in the positive control group (K+), which was 26.2 µm. This positive control group is a group of hypertensive rats that received DOCA-salt treatment.

Meanwhile, treatment groups 1, 2 and 3 showed the results of the p-value calculation obtained from the three treatments >0.05 to the negative control group (K-). This means that exposure to the methanolic extract combination of tea and mango mistletoe can prevent the increase in the diameter of cardiac muscle cells. The average of the three treatment groups (P1, P2, P3) has a value close to the control group negative (K-). This indicates that the administration of dose variations in each treatment group has been able to prevent the increase in the diameter of cardiac muscle cells. The P1 group (50 mg/Kg BW) had an average of 15.6 µm and was able to reduce hypertrophy. Tea mistletoe and mango mistletoe have antioxidants and can protect the body against damage caused by ROS (reactive oxygen species) (4).

From various studies, tea and mango mistletoes contain flavonoid compounds. Flavonoids can act as natural antioxidants that protect biological systems and inhibit cell oxidation by reducing and capturing active oxygen and free radicals, especially superoxide. One of the efficacious flavonoids is quercetin. Quercetin is found in abundance both in tea mistletoe and in mango mistletoe. The activity of this compound as an antioxidant is by releasing

or donating hydrogen ions to peroxyl free radicals to become stable. In addition, flavonoid compounds also have antithrombosis and anti-inflammatory effects that can reduce the risk of cardiac disease (17).

From the ANOVA test, the standard deviation (SD) value was also obtained, namely in the negative control group (K-) of 0.918, positive control (K+) of 2.488, P1 of 0.982, P2 of 0.691, and P3 of 0.357. The results of the analysis data obtained show that the standard deviation value in all groups has a value smaller than the average value. The data obtained in this study shows that the overall average of the data obtained is representative.

The cell data obtain from cells that are not tangential or cells that have a ratio. Histopathological examination results showed that the P1, P2, and P3 groups improved precisely in the diameter of cardiac muscle cells. Those groups had a wider diameter size of the cardiac muscle or myocardium than the average size (10µm-20µm). Meanwhile, in the group with DOCA-salt injection or the group with hypertension, the diameter of the cardiac muscle cells exceeded the normal size (hypertrophy).

Hypertrophy is a progressive disorder of an increase in the volume of cells in a tissue or organ. The increase in cardiac muscle cell diameter is an adaptation response to the increased workload of the cardiac due to hypertension (18). Hypertension occurs with an increase in intracellular pressure which then causes high blood pressure experiencing shear stress on endothelial cells. The endothelial cells which are found in the tunica intima blood vessel will cause shear stress leading to endothelial dysfunction when exposed to high blood pressure (19,20). Endothelial dysfunction causes vasoconstriction, a decrease in O_2 and an upgrade growth factor. As a result of endothelial dysfunction and increased growth factor, the arteries of people with hypertension usually lose their elasticity. Therefore, the cardio output cannot be the same as in people without hypertension. The cardiac of hypertensive people will increase its work to pump blood to meet the amount of CO. On the contrary, the cardiac muscle is not strong, and the cells swell because they are forced to work, which causes hypertrophy of the cardiac muscle cells (21).

One of the flavonoid contents in tea mistletoe and mango mistletoe is quercetin. In this case, quercetin can act on the smooth muscle of the arteries by stimulating or activating Endothelium Derived Relaxing Factor (EDRF) that can cause vasodilation. Quercetin in endothelial cells has the potential to increase the production of NO. Quercetin

can diffuse directly and synthesize NO in the endothelium and smooth muscle, which stimulates guanylate cyclase to form cGMP resulting in vasodilation (4). The occurrence of vasodilation can prevent hypertrophy because the cardiac will not work harder, which causes cardiac muscle cells to widen.

CONCLUSIONS

A methanolic extract combination tea leaves and mango mistletoes on male wistar rats in a preventive model of hypertension could significantly prevent the enlargement/widening of auto cells cardiac. The three variations of the dose showed that the result data did not show a significant difference. This effect was controlled by a methanolic extract combination tea and mango mistletoes at a dose of 50 mg/KgBW which was the optimum dose in reducing the diameter of cardiac muscle cells in Wistar rats. The methanolic extract of the combination of tea mistletoe leaves and mango mistletoe can be used as herbal ingredients to increase preparations in the field of phytopharmacology.

AUTHOR CONTRIBUTIONS

Durrotul Hasanah: conceptualization, methodology, formal analysis, investigation, writing-original draft. Nour Athiroh AS: supervision, project administration, funding acquisition. Nurul Jadid Mubarokati: data curation, writing-original draft preparation, visualization, and writing-reviewing.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

1. Annisatul H, Athiroh N, Mubarakati NJ. Histopathological profile of therapeutic doses of mango mistletoe methanolic. JSMARTech. 2021;02(02):48–54. DOI: https://doi.org/10.21776/ub.jsmartech.2021.002.02.48
2. Athiroh N, Purnomo Y, Mubarakati NJ. Sub chronic diagnosis of administration with *Scurrula atropurpurea* to blood biochemichal analysis. IOP Conf Ser Mater Sci Eng. 2020;846(1). DOI: 10.1088/1757-899X/846/1/012002

3. Kurniawan A, Yanni M. Pemeriksaan fungsi endotel pada penyakit kardiovaskular. [Examination of endothelial function in cardiovascular disease]. Hum Care J. 2020;5(3):638. DOI: http://dx.doi.org/10.32883/hcj.v5i3.817

4. Ayun AQ, Faridah DN, Yuliana ND, Andriyanto A. Pengujian toksisitas akut LD50 infusa benalu teh (*Scurrula* sp.) dengan menggunakan mencit (*Mus musculus*). [Testing the acute toxicity of LD50 infusion of parasitic tea (*Scurrula* sp.) using mice (*Mus musculus*)]. Acta Vet Indones. 2021;9(1):53–63. DOI: https://doi.org/10.29244/avi.9.1.53-63

5. Athiroh N, Permatasari N, Sargowo D, Widodo MA. Antioxidative and blood pressure-lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate-salt hypertensive rats. Biomarkers Genomic Med. 2014;6(1):32–6. Available from: http://dx.doi.org/10.1016/j.bgm.2014.01.001

6. Athiroh N, Permatasari N, Sargowo D, Widodo MA. Effect of *Scurrula atropurpurea* on nitric oxide, endothelial damage, and endothelial progenitor cells of DOCA-salt hypertensive rats. Iran J Basic Med Sci. 2014;17(8):622–5.

7. Manosroi W, Williams GH. Genetics of human primary hypertension: focus on hormonal mechanisms. Vol. 40, Endocrine Reviews. 2018. 825–856 p. DOI: https://doi.org/10.1210/er.2018-00071

8. Syntya A, Masyarakat FK, Surabaya K. Hypertension and heart disease: literature review. 2021;11:541–50. DOI: https://doi.org/10.32583/pskm.v11i4.1621

9. Lousiana M, Irramah M. Myocardial stunning. Maj Kedokt Andalas. 2017;40(2):100. DOI: http://dx.doi.org/10.22338/mka.v40.i2.p100-110.2017

10. Rossier BC, Bochud M, Devuyst O. The hypertension pandemic: An evolutionary perspective. Physiology. 2017;32(2):112–25. DOI: https://doi.org/10.1152/physiol.00026.2016

11. Kawabe H, Azegami T, Takeda A, Kanda T, Saito I, Saruta T, et al. Features of and preventive measures against hypertension in the young. Hypertens Res [Internet]. 2019;42(7):935–48. DOI: http://dx.doi.org/10.1038/s41440-019-0229-3.

12. Ayu A, Adi M. Perubahan histopatologi otot jantung dan aorta mencit jantan pascapaparan asap rokok elektrik. [Histopathological changes of heart muscle and aorta in male mice after exposure to e-cigarette smoke]. 2019;8(4):541–51. DOI: 10.19087/imv.2019.8.4.541

13. Ediriweera DS, Dilina N, Perera U, Flores F, Samita S. Risk of low birth weight on adulthood hypertension - Evidence from a tertiary care hospital in a South Asian country, Sri Lanka: A retrospective cohort study. BMC Public Health. 2017;17(1):15–20. DOI: 10.1186/s12889-017-4268-x

14. Chairunnisa S, Wartini NM, Suhendra L. Pengaruh Suhu dan waktu maserasi terhadap karakteristik ekstrak daun bidara (*Ziziphus mauritiana* L.) sebagai sumber saponin. [Effect of temperature and maceration time on the characteristics of bidara leaf extract (*Ziziphus mauritiana* L.) as a source of saponins]. J Rekayasa dan Manaj Agroindustri. 2019;7(4):551. DOI: https://doi.org/10.24843/JRMA.2019.v07.i04.p07

15. Pratama APC, Berata IK, Samsuri S, Merdana IM. Pengaruh pemberian vitamin E dan deksametason terhadap gambaran histopatologi jantung tikus putih jantan. [Effect of administration of vitamin E and dexamethasone on the histopathological picture of male white rats heart}. Bul Vet Udayana. 2018;10(2):147. DOI: 10.24843/bulvet.2018.v10.i02.p07

16. Lestari TA, Athiroh N, Mubarakati NJ. Toxicity test of methanolic extract combination of benalu tea leaves and benalu mango leaves against lipid profile of female rats (*Rattus norvegicus*) on 28-day sub-chronic exposure. Known Nat. 2020;3 (1):22–8. DOI:10.33474/j.sa.v3i1.6621

17. Kurniasih F. Sosialisasi bahaya radikal bebas dan fungsi antioksidan alami bagi kesehatan. [Socialization of the dangers of free radicals and the function of natural antioxidants for health]. J Vokasi. 2019;3(1):1. DOI: https://dx.doi.org/10.30811/vokasi.v3i1.960

18. Musyarifah Z, Agus S. Proses fiksasi pada pemeriksaan histopatologik. [Fixation process on histopathological examination]. J Kesehat Andalas. 2018;7(3):443. DOI: https://doi.org/10.25077/jka.v7i3.900

19. Sulistyowati E, Hsu JH, Cheng YB, Chang FR, Chen YF, Yeh JL. Indonesian herbal medicine prevents hypertension-induced left ventricular hypertrophy by diminishing NADPH oxidasedependent oxidative stress. Oncotarget. 2017;8(49):86784–98. DOI: 10.18632/oncotarget.21424

20. Masterclass CMEM. Hypertrophic-cardiomyopathy.indd. 2019;19(1):61–3. DOI: https://doi.org/10.7861/clinmedicine.19-1-61

21. Khansa A, Partiningrum DL. Hubungan antara lama hipertensi dan gambaran. Elektrokardiogram hipertrofi ventrikel kiri dan infark miokard lama. [Relationship between duration of hypertension

and features. Electrocardiogram of left ventricular hypertrophy and old myocardial infarction]. Jurnal Kedokteran Diponegoro. 2018;7(2):1251–65. DOI: https://doi.org/10.14710/dmj.v7i2.21198