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Histopathological Profile of Therapeutic Doses of Methanolic Extract Combination 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. It is believed that the flavonoid compounds found in these two mistletoes act as antihypertensives. Hypertension is one of the risk factors that influence the incidence of cardiac disease. This study to examine the histopathological of the cardiac of hypertensive rats (preventive model) after **treatment** of methanolic extract combination tea and mango mistletoes. This study used complete random design with a true experimental design in 25 wistar rats, each treatment had 5 replications, divided into 5 treatments, namely negative control (-), positive control (+), treatment 1 dose of 50 mg / KgBW, treatment 2 doses of 100 mg / KgBW, and treatment 3 doses of 200 mg / KgBW. Data analysis using JAMOVI with version 1.1.9.0, the one-way ANOVA test and *a posteriori* namely,. The results showed $p < 0.05$, that there was a significant difference between all groups. So that the administration of a methanolic extract combination tea and mango mistletoes as the preventive model can significantly reduce hypertrophy of cardiac muscle cells. This effect was controlled with a dose of 50 mg / KgBW, which is the optimal dose to reduce hypertrophy of cardiac muscle cells in Wistar rats male.

Keywords: *Hypertension; Hypertrophy; Muscle Cells Cardiac; Mango mistletoe; 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 in the world for both traditional and modern medicines [1]. Mistletoes tea (*Escurrella atropurpurea*) and mango mistletoes (*Dendrophthoe pentandra* L.) Miq is a plant that has benefits for maintaining health and fitness [2,3]. Tea mistletoes in vitro have been shown to reduce contractility of the tail arteries of hypertensive rats, whereas in vivo in hypertensive rats, tea mistletoes reduce blood pressure by improving oxidative stress and endothelial dysfunction, lowering MDA (Malondialdehyde) levels, and increasing NO (Nitric Oxygen) levels [2,4,5,6]. Meanwhile, the flavonoid and tannin compounds contained in the *Dendrophthoe pentandra* L. are thought to 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 diseases of the cardiac and blood vessels (vascular system) hypertension is one of the most influential risk factors [9,10,11,12,13,]. People who have hypertension experience a narrowing of the vessel's blood, also known as *vasoconstriction*. This has an impact on the increase of the work of the cardiac to pump blood throughout the body. This increase can the cardiac muscle to weaken and hypertrophies. Hypertension can be prevented by reducing risk factors and the use of traditional medications such as prevention and treatment of hypertension. Therefore, on this occasion, the authors examine the histopathological profile of the cardiac in male wistar rats with a preventive model of hypertension after administration of a methanolic extract combination tea and mango mistletoes.

MATERIALS AND METHODS

This research is true experimental design with a completely custom design randomized with 5 treatments and 5 repetitions with the 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 / KgBW and P3 with a dose of 200 mg / KgBW. The study was carried out after obtaining approval from the Islamic University Malang Faculty of Medicine Health

Research Ethics Commission (ethical clearance) Number: 006/LE.001/IV/03/2020.

A methanolic extract combination tea and mango mistletoes was exposed to *Rattus norvegicus* (male wistar rats) for 28 days and followed by administration of DOCA-Sal 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.

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

The manufacture of methanolic extract combination tea and mango mistletoes using simplicia derived from dried mistletoe leaves that have been mashed. Then the extraction process is carried out using the maceration method. The maceration process includes immersing the material with a solvent that is by following per under in the active compound to be extracted [14].

Prepared powder or Simplicia leaf mistletoe tea and mango mistletoe each 100 grams and then put into a plastic bottle measuring 1.5 litres. Then put 1 litre of 90% methanol solution in each bottle and shake until the solution is homogeneous for 1 hour. After 1 hour, the homogeneous solution was allowed to stand and precipitated for 24 hours. When equilibrium has been reached between the concentration of the compound in the solvent and the concentration in the cell this process is stopped [15]. The result of the deposition process for 24 hours will form a bottom layer in the form of a *natant* and the top layer is called *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 in room temperature $\pm 24^{\circ}\text{C}$ with humidity of approximately 50-60% protected from smoke, industry, motor vehicle fumes, and other pollutants as well as being fed and drinking.

- **Test Animal Care**

Weigh 25 test animals before acclimatization and at the end of acclimatization (pre-treatment) and 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 replaced regularly once every 2 days. Feeding was carried out every morning and evening with a feed weight of 7 grams per rat.

- **Treatment Methanolic Extrac Combination Tea And Mango Mistletoes**

This study used a dose of methanolic extrac combination 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 / KgBW, and the third dose of 200 mg/Kg BW. The administration of the methanolic extrac combination tea and mango mistletoes was carried out for four weeks by disorder and carried out five times in one week.

- **Provision DOCA-Salt as A Preventive Hypertension Models**

DOCA is a precursor of the mineralocorticoid hormone of the adrenal cortex which has an effect analogous to the mineralocorticoid aldosterone, which causes retention of Na⁺ and H₂O 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 extrac combination tea and mango mistletoes. DOCA was administered by subcutaneous injection in the positive control (+), the first treatment (P1), (P2), and (P3). Before and after administration of DOCA, blood pressure measurements were carried out in rats. DOCA given to rats was first homogenized with sesame oil and according to the dose.

- **Surgery**

Surgery on the test animals was carried out after 28 days of treatment, by taking organs for histopathological examination. The organ observed is the cardiac. In the surgical process, the Wistar rats were anaesthetized with ketamine first by injection. 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 cardiac taken was used as a microscopic preparation which was then processed following the standard histological method with Hematoxylin and Eosin (HE) staining.

- **Histopathological Examination**

Histopathological examination was carried out on the cardiac. Histopathological observations of cardiac organs were carried out after staining using Hematoxylin and Eosin (HE) by taking 400x and 200x magnification photos with 5 fields of view under a trinocular microscope Olympus U-TV0.5XC-3, T7 Tokyo, Japan. The photos obtained at 400x magnification were then 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) and follow-up tests was carried out, namely Post Hoc Test.

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, histopathological observations of the cardiac were carried out 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 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, the results of measuring the diameter of cells in the cardiac muscle of male wistar rats, and have been tested using the test One Way ANOVA. From the results of this test, the results have been tabulated in the form of table 1 as follows.

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

Treatment	Average \pm SD	Notation
Negative Control	15.5 \pm 0.918	a
Positive Control (DOCA-Salt)	26.2 \pm 2.489	b
P1 (Dose 50 mg/KgBB + DOCA-Salt)	15.6 \pm 0.982	a
P2 (Dose 100 mg/KgBB + DOCA-Salt)	15.1 \pm 0.691	a
P3 (Dose 200 mg/KgBB + DOCA-Salt)	14.4 \pm 0.357	a

Description: The different letters in the same column indicate a significant difference at $p < 0.05$.

Based on Table 1. the data on the mean cell diameter of the cardiac muscle of Wistar rats were obtained. In the positive control (K +), rats induced by DOCA-Salt without the administration of a methanolic extract combination tea and mango mistletoes showed the highest mean when compared to the negative control (K -) or the group without DOCA-Salt induction and the treatment group (P1, P2, and P3). This shows that there is a very significant difference between the negative control group (-), treatment 1,2, and 3 and the positive control group (+) .

Observations were made by measuring the diameter of cardiac muscle cells in the myocardium layer. The results of the normality test, a value of 0.112 (<0.05) was obtained, which means this data is normally distributed. Then the next analysis uses the One Way Anova test. The results of the One Way Anova test obtained a p-value of $<.001$ which means statistically (<0.05) then H_0 rejected and H_a received. Where it shows the administration of a methanolic extract combination tea (*Scurrula atropurpurea* B1. Dans) and mango (*Dendrophthoe petandra*) mistletoes effect on cardiac histopathology in hypertensive male Wistar rats (preventive model).

Test post Hoc The next step is to see the differences in each treatment group. From the test results, *Post Hoc* The comparison between the positive control (K+) induced by DOCA-Salt and the negative control (K-) without DOCA-Salt induction showed a very significant difference (significant), while in the P1, P2, and P3 (treatment group) the not significant (difference was not significant) with the negative control (K-) and

significantly (significantly different) with the positive control group (+). This indicates that the methanolic extract combination tea and mango mistletoes can prevent an increase in the diameter of cardiac muscle cells. The three variations of the dose used did not show a significant difference in the size of the diameter of the cardiac muscle cells, which means that these three doses have the same potential in preventing the increase in the diameter of the muscle cells in the cardiac.

- **Histopathological Observation**

Observations obtained photos of cardiac muscle cells both at 200x and 400x magnification are as follows.

Figure 2 is a microscopic visualization of myocardial tissue in *Cross-section* 200x magnification of five study groups that had previously been stained with Hematoxylin and Eosin (H&E). Figure 3 is also a microscopic visualization of myocardial tissue, but *ventricular section* 200x magnification of the five study groups. While Figures 4 and 5 are microscopic observations *cross* and *ventricular section* 400x magnification of the myocardium of the five groups in this study. From this microscopic observation, it was found that the positive (+) control mice were significantly different from the negative control mice (-) and the treated mice (P1, P2, and P3). While the negative control rats (-) and P1, P2, and P3 (treatment group) were not significantly different. This shows that hypertensive rats have the potential to experience hypertrophy in the cardiac organ, while from this treatment rats indicate that the administration of a methanolic extract combination tea and mango mistletoes can reduce hypertrophy in the cardiac of rats as a preventive measure for hypertension.

DISCUSSIONS

Tea and mango mistletoes are parasitic plants that are proven to be efficacious as herbs. These two plants contain flavonoid compounds that have the potential as herbal medicines or alternative phytopharmaca preparations. This is evident from the various studies that have been conducted. One of the research conducted by Atiroh *et al* (2000 and 2009) [2, 3] in vitro and in vivo using a rat model of hypertension (DOCA-Salt) mistletoe tea was able to reduce the contractility of the rat tail artery blood vessels and was able to reduce blood pressure by improving oxidative stress and endothelial dysfunction.

The parameters observed were cardiac histopathology, precisely by measuring the diameter of cardiac muscle cells. The choice of this parameter is because the cardiac is one of the vital organs in humans that has a function as a blood pump throughout the body [16]. If cardiac function decreases, such as cardiac muscle infection, this can affect oxygen demand (O_2) and nutrients needed by the whole body [17]. In addition, in the adaptation mechanism for hypertension, one of which causes cell hypertrophy in the cardiac. Hypertrophy itself can be found in various tissues, the most easily observed is in muscles that receive an additional workload. One of them is in the cardiac muscle in response to the excessive workload of the cardiac. Where can you find people who have hypertension? Cardio output increases because it experiences vasoconstriction so the cardiac is forced to work extra to meet the blood supply in the body. As a result, the cardiac muscle is forced to work hard, which in the end the muscle is not strong and the cells develop hypertrophy.

The results of data analysis using the ANOVA test in Figure 1, 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 where the p-count was less than 0.05 ($p < 0.05$) between all treatment groups. This means that there are significant differences in each treatment group. The highest mean (mean) diameter of cardiac muscle cells was found in the positive control group (K+), which was 26.2. This positive control group is a group of hypertensive rats that received DOCA-Salt treatment.

Meanwhile, treatment groups 1, 2, 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 , with a given dose of 50 mg/Kg BW it was able to reduce hypertrophy. This can be seen from the average value of the cell diameter which is the cell diameter in the normal group, which is 15.5 μm . It can be seen that tea mistletoe and mango mistletoe have antioxidants. Antioxidants can protect the body against damage caused by ROS (reactive oxygen species) [18].

From various studies, tea and mango mistletoes contain flavonoid compounds. Flavonoid can act as natural antioxidants that protect biological systems and inhibit cell oxidation by reducing, capturing active oxygen and free radicals, especially superoxide. One of the efficacious flavonoids is quercetin. This 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 peroxy free radicals to become stable. In addition, flavonoid compounds also have antithrombus and anti-inflammatory effects so that they can reduce the risk of cardiac disease [19].

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.

Cell data that taken are cells that are not tangential or cells that have a ratio of From the results of the histopathological examination showed in group P1, P2, and P3 doses of the methanolic extract of the combination of tea mistletoe leaves and mango mistletoe improved precisely in the diameter of cardiac muscle cells. This can be seen from the size the diameter of the cardiac muscle or myocardium which is in the normal range of 10 m - 20 m. Meanwhile, in the group that was injected with DOCA-Salt or the group with hypertension, the diameter of the cardiac muscle cells exceeded the normal cell size. With this, the group with hypertension experienced hypertrophy.

Hypertrophy itself is a progressive disorder in the form of an increase in the volume of cells in a tissue or organ. The increase in the diameter of the muscle cells in the cardiac is an adaptation response to the increased workload of the cardiac due to hypertension. The occurrence of hypertension begins with an increase in intracellular pressure which then causes high blood pressure experiencing shear stress on endothelial cells. Endothelial cells found in the blood vessels of the tunica intima when exposed to high blood pressure will cause shear stress leading to endothelial dysfunction [20]. Because endothelial dysfunction causes vasoconstriction. When there is vasoconstriction or narrowing of blood vessels causing a decrease in O₂ and upgrade growth factor. As a result of endothelial dysfunction and increased growth factor, This means that people who have hypertension usually have no

more elastic arteries. As a result, when pumping blood cardio output cannot be as in normal people. Therefore, the cardiac of hypertensive people will increase its work is to pump blood to meet the amount of CO, eventually, the cardiac muscle is not strong and the cells bloom because they are forced to work harder which causes hypertrophy of the cardiac muscle cells [20].

One of the flavonoid content 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) a result that can cause vasodilation. In addition, quercetin in endothelial cells has the potential to increase the production of NO. This active substance can diffuse directly and synthesize NO in the endothelium and smooth muscle which further stimulates guanylate cyclase to form cGMP resulting in vasodilation [18]. the occurrence of this vasodilation can prevent hypertrophy because the cardiac will not work harder which causes cardiac muscle cells to widen.

CONCLUSION

A methanolic extrac 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 extrac 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 phytopharmaca.

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.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest

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