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Research Article

Effect of Mistletoe (Tea and Mango) Extract Combination on Histopathological Profile of Brain in Hypertensive Rats

Nimas Ajeng Oktaviana[1], Nour Athiroh Abdoes Sjakoer[2], Nurul Jadid Mubarakati[3]
[1,2,3]Universitas Islam Malang, East Java, Indonesia

Corresponding author: nour.athiroh@unisma.ac.id

Abstract

The purpose of this study was to analyze the brain histopathological profile in hypertensive rats (DOCA-Salt) treated to Tea and Mango extract combination (Mistletoe). Samples were obtained using a true experimental design completely randomized on 25 rats with three treatments (TI, TII, TIII), negative control, and positive control with replication five times. Ratio of extract for treatment was 3 : 1 made up 50 mg/kg BW, 100mg/kg BW, 200 mg/kg BW. Data analysis using one-way analysis of variance (ANOVA) with the JAMOVI application. The study results obtained Mistletoe (Tea and Mango) extract combination in hypertensive rats (DOCA-Salt) can significantly reduce the necrosis of brain cells in the white matter area. The combination of Mistletoe extract can reduce the number of brain cell necrosis in hypertensive rats optimally obtained at a dose of 50 mg/kg BW

Keywords: DOCA-Salt, Hypertensive rats, Mistletoe, Necrosis, White Matter

1. Introduction

Indonesia is the second-largest biodiversity globally after Brazil, consisting of tropical plants and marine biota. There are around 30,000 plants, and 7,000 have medicinal properties, but only 2,500 have been used as medicinal plants (Roberson, 2008; Sofowora et al., 2013). Indonesia is very rich in various kinds of medicinal plants (Sholikhah, 2016). Currently, the traditional medicinal plant industry has overgrown in Indonesia.

One of the potential medicinal plants is tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans) and mango mistletoe (*Dendrphthoe Pentandra*), which belong to the Loranthaceae family (Adesina et al., 2013; Muttaqin et al., 2016). This plant is a parasitic plant or a pest that lives on the tea and mango plants. Apart from their parasitic nature, these two plants contain flavonoid compounds that are very useful and can potentially be an alternative to *phytopharmaca* preparations or herbal medicines. The Loranthaceae contains several secondary metabolite compounds such as flavonoids, saponins, tannins, alkaloids, glycosides, and inulin. These active substances have been reported to have a role in hypertension and act as antioxidants to prevent free radicals (Athiroh et al., 2014). And the antioxidants may be an alternative to prevent endothelial dysfunction seen in hypertensive patients.

The bioactive content of tea mistletoe (*Scurrula atropurpurea* Bl. Dans) has been known through in vitro and in-vivo tests. The *Scurrula atropurpurea* extract can reduce

the contractility of the rat tail arteries apart through the role of the endothelium (Athiroh, 2009). In other studies, it is stated that mango mistletoe is used to treat hypertension, coughs, diabetes, cancer, diuretics, gastric ulcers, smallpox, and infections in the skin (Endharti et al., 2016; Mustarichie, 2015). In previous research, administration of a methanolic extract combination of mistletoe tea (*Scurrula atropurpurea* (Bl.) Dans) and mango mistletoe (*Dendrphthoe pentandra*) did not cause toxic properties in the kidney and lipid profiles (Lestari et al., 2020; Sukandar & Sheba, 2019). Another study stated that giving EMSA to female rats was not toxic, and the most effective dose to preventbrain cell damage was a dose of 500 mg/kg BW (Mihmidati, 2017).

The DOCA-salt model of hypertension incorporates the activation of mineralocorticoid receptors and high salt intake, which have played critical roles in developing hypertension and inflammation (Young & Rickard, 2012). DOCA induction increases systolic blood pressure (Lee et al., 2016). DOCA-salt induction significantly increased blood pressure in male and female rats. The DOCA-salt mouse model is excellent for studying hypertensive injuries (Belanger et al., 2020).

Hypertension itself is a disease that causes significant death in humans related to cardiovascular disease. About 80% of deaths occur in developing countries. In 2012, hypertension was the cause of death for the world's population of 9.4 million in one year (Mills et al., 2016). Patients with hypertension are challenging to detect because there are no symptoms, but it can be indicated by systolic blood pressure> 140 mmHg and diastolic blood pressure >90 mmHg (Norman M. Kaplan, 2014).

Hypertension is one of the non-communicable diseases with high prevalence and a significant risk factor for cardiovascular disease and other complications, along with technological advances in developing countries such as Indonesia, which tend to adopt unhealthy lifestyles (Singh et al., 2017). Hypertension sets to become a vital factor in health worldwide since it causes an increase in death rate and disability among people in many countries. Hypertension is identified as a concomitant risk for cardiovascular disease. A well-recognized risk of cardiovascular-related death is left ventricular hypertrophy (LVH). The mechanism driving hypertensive LVH reveals various key factors: hemodynamic load, endothelial, neuro-humoral, and oxidative stress (Cramariuc & Gerdts, 2016). LVH is a sensitive indicator of early alteration in the heart because of pressure overload in hypertension (Epstein et al., 2017). Oxidative stress is caused by an imbalance between antioxidants and the production of oxidants, including ROS (Fitria et al., 2019).

One of the organs that play a role in blood pressure regulation is the brain. Blockage of the arteries (atherosclerosis) in the brain will cause endothelial(pro-necrotic) cells. As a result, it causes tissue remodelling around the blood vessels. In the brain, the manifestations can be in the white matter (white substance), so that it will hinder the brain's performance due to obstruction of the flow of oxygen to the brain. The brain is a central part of vital essential functions in humans (Thumb, 2020). Brain disorders due to

hypertension can cause rupture of the main blood vessels in the brain, followed by death in large parts of the brain (Pusparani, 2009).

It is necessary to carry out histopathological examinations on specific organs, one of which is the brain, because it is very susceptible to oxidative stress. If the cell membrane is disturbed, recovery of brain cells is not possible. Brain necrosis is damage to cells that occur in the brain (Mihmidati, 2017). It is necessary to determine the histopathological picture of the brain hypertensive male Wistar rats (*Rattus novergicus*) exposed with the combination of methanolic extract of Belau tea and mango mistletoe.

2. Material and Method

This study was an experimental design using a completely randomized design (CRD), using experimental animals male Wistar strain rats (*Rattus novergicus*) aged 6 - 8 weeks and weighing 100-200 grams totaling 25 individuals. The tested animals are provided from the FAAL Laboratory of the Faculty of Medicine, Brawijaya University Malang. This research has obtained the approval of the Health Research Ethics Commission of the Faculty of Medicine, the Islamic University of Malang, with the number 006/LE.001/IV/03/2020. The experimental animals were divided into five groups, namely: the normal control group (Control-) (normotension), the treatment control+ (hypertension), and the treatment groups that were given a methanolic extract combination of tea mistletoe and mango mistletoe at a dose of 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW, respectively. The research was carried out at the Ecology Laboratory of the Faculty of Mathematics and Natural Sciences, Islamic University of Malang, the Laboratory of the Faculty of Medicine, Islamic University of Malang, the Physiology Laboratory of the Faculty of Medicine, Brawijaya University Malang, the Laboratory of Anatomical Histopathology, the Faculty of Medicine, Brawijaya University Malang and the Laboratory of Balai Materia Medica Batu, East Java.

2.1. Preparation of Methanolic Extract Combination of Mistletoe Tea and Mango Mistletoe

Tea mistletoe leaves and mango mistletoe leaves are obtained from a Sumber Sehat store, Kepanjen. The leaves used are not rotten, separate from the leaf bones, and are clean. The leaves are dried in the oven at a temperature of 50-60°C until the water content is less than 10%, then mashed the tea mistletoe leaves and mango mistletoe leaves using a blender to form simplicia. Then proceed with the maceration process. Simplicia is weighed 100 grams and then put in a 1.5-liter plastic bottle. After that, 1 liter of 90% methanol solution was added and matched for 1 hour until the solution was homogeneous. After 1 hour, then it is allowed to stand for 24 hours. The methanol solvent is hoped that the active substance in the mango mistletoe leaves can be withdrawn. Re-maceration was carried out three times, after which the supernatant obtained was then made into an extract by evaporation using a rotary evaporator

2.2. Maintenance of Experimental Animals

The male Wistar rat (*Rattus norvegicus*) was acclimatized in the Ecology Laboratory of the Faculty of Mathematics and Natural Sciences, the Islamic University of Malang, for seven days with a room temperature of 25°C with a humidity of 50-60%.

2.3. Making Animal Model of Hypertension

Rats were induced by DOCA subcutaneously at a dose of 15 mg/kg BW, which was dissolved in corn oil, and 2% NaCl was given using sonde and drinking water. This DOCA was given twice a week for four weeks.

2.4. Surgery and Histopathological Examination

The rats were given an injection of ketamine, then performed surgery, and brain organs were taken. The separated brain organs were immediately preserved in a 10% formalin solution until all parts of the organs were immersed. Histopathology preparations were made using the organs being cut and multilevel washing using xylol, 70% alcohol, 80% alcohol, and 95% alcohol. Then the organ was compacted with paraffin and cut with a microtome. The organ was attached with the Hematoxylin-Eosin (H&E) staining technique and then observed under a trinocular microscope (Olympus U-TV0.5XC-3, T7 Tokyo, Japan). The width of the white matter area was examined by microscopic observation at 200x magnification, then measured by the *ImageJ* application. The width of the white matter area is expressed in *micrometer* (µm) units.

2.5. Data Analysis

The data obtained were tabulated according to groups, then statistical tests were carried out using the Jamovi application version 1.1.9.0. The mean (mean) and standard deviation (Standard Deviation) for each treatment can be obtained. Significant differences between the means were analyzed using the one-way statistical analysis of variance (ANOVA) method, which compares the mean differences of more than two treatments with a 95% confidence level. Then proceed with the Tukey Post-Hoct Test. The results were significant if $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Cell Damage (Necrosis) on White Matter

The histopathological picture of the brain in the white matter area of male Wistar rats (*Rattus norvegicus*), which had been given EMBTM for 14 days, was assessed based on the amount of cell damage to the white matter of male Wistar rats. The results were tabulated based on the treatment of each group presented in the form figure as follows (Figures 1 and 2).

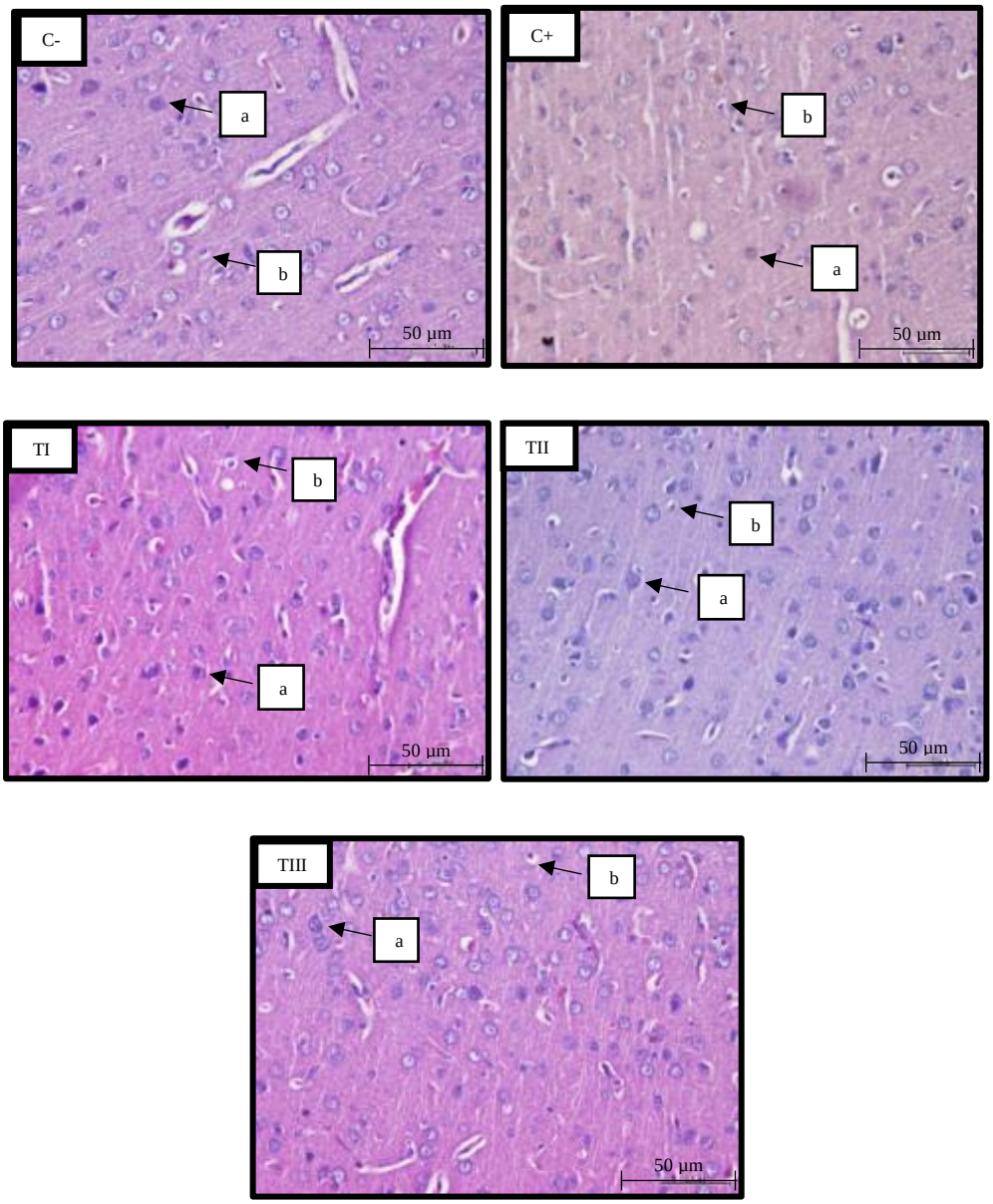

Figure 1. Microanatomy of Necrosis on White Matter After Giving Mistletoe (Tea and Mango) Extract Combination 14 Days (Olympus U-TV0.5XC-3 Trinocular Light Microscope, T7 Tokyo, Japan) 400x Magnification, Hematoxylin-Eosin (H&E) Staining). a. Normal Cell, b. Cell Necrosis

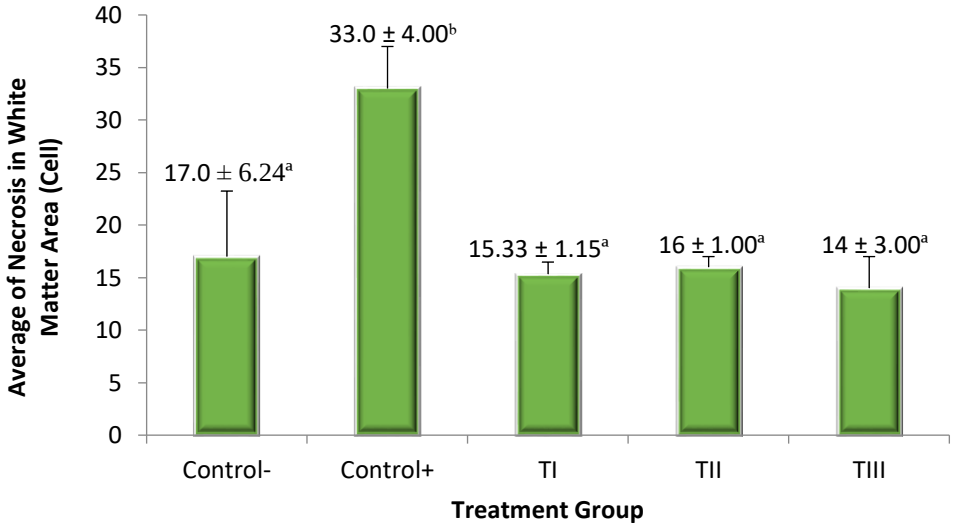

Figure 2 Mean of Cell Damage (Necrosis) in Male Wistar Rats in White Matter Area after Giving Methanolic Extract Combination of Tea Mistletoe and Mango Mistletoe Mistletoe (Tea and Mango) Extract Combination for 14 Days

Note :

Control-	:	The normal group without any treatment (DOCA-Salt and Mistletoe (tea and mango) extract combination)
Control+	:	The treated group was induced with DOCA-Salt without Mistletoe (tea and mango) extract combination
TI	:	The group was treated with DOCA-salt induction and exposed to Mistletoe (tea and mango) extract combination at a dose of 50 mg/kg BW.
TII	:	The group was treated with DOCA-Salt induction and exposed to Mistletoe (tea and mango) extract combination at a dose of 100 mg/kg BW.
TIII	:	The group was treated with DOCA-Salt induction and exposed to Mistletoe (tea and mango) extract combination at a dose of 200 mg/kg BW

Figure 1 showed that the mean cell damage (necrosis) in the brain in the white matter area rats without treatment showed significantly different results from the treatment group (positive control). DOCAsalt-induced rats without Extract Combination Mistletoe (Tea and Mango) showed the highest mean compared to the negative control group (Control-) or normal rats without DOCA-salt induction. It indicates that DOCA-salt induction canincrease cell damage (necrosis) in the white matter area. The DOCA-salt-treated group was given 50 mg/KgBW extract mistletoe; the mean number of necrosis cells decreased by 15.3. The average number was close to the average number in the normal rat group (Control-), which is 17.0. It shows that Mistletoe at 50, 100, and 200 mg/kg BW can reduce cell necrosis. The three-dose variations have the same potential, namely reducing cell necrosis in the white matter area of the brain.

Image Analysis with *ImageJ*

The width of the white matter area was examined by microscopic observation at 200x magnification, then measured by the ImageJ application. The width of the white matter area is expressed in micrometer (µm) units, and the results are tabulated based on the treatment of each group presented in the following figure (Figures 3 and 4).

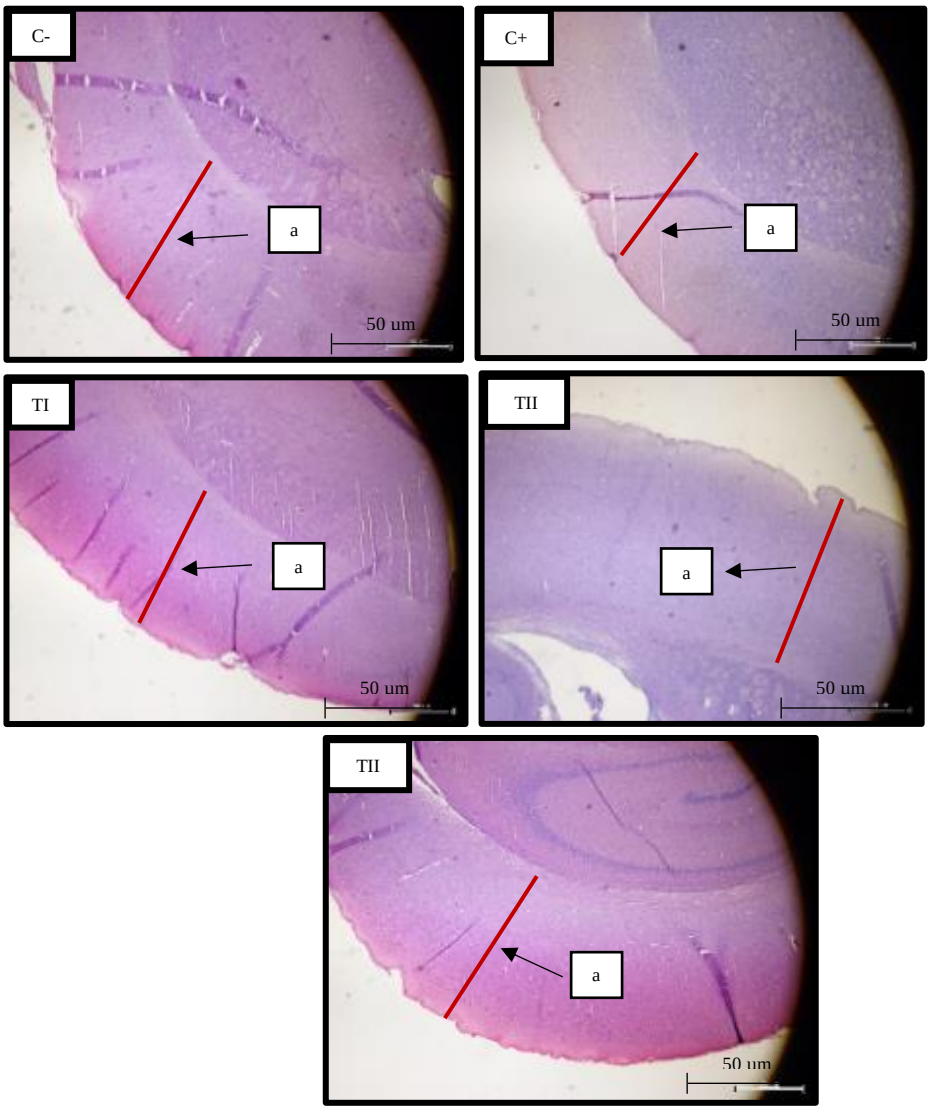

Figure 3. White Matter Area Microanatomy After 14 Days Extract Combination Mistletoe (Tea and Mango) Giving (Olympus U-TV0.5XC-3 Trinocular Light Microscope, T7 Tokyo, Japan) 200x magnification, Hematoxylin-Eosin (H&E) Staining.
Information: a: *White Matter*

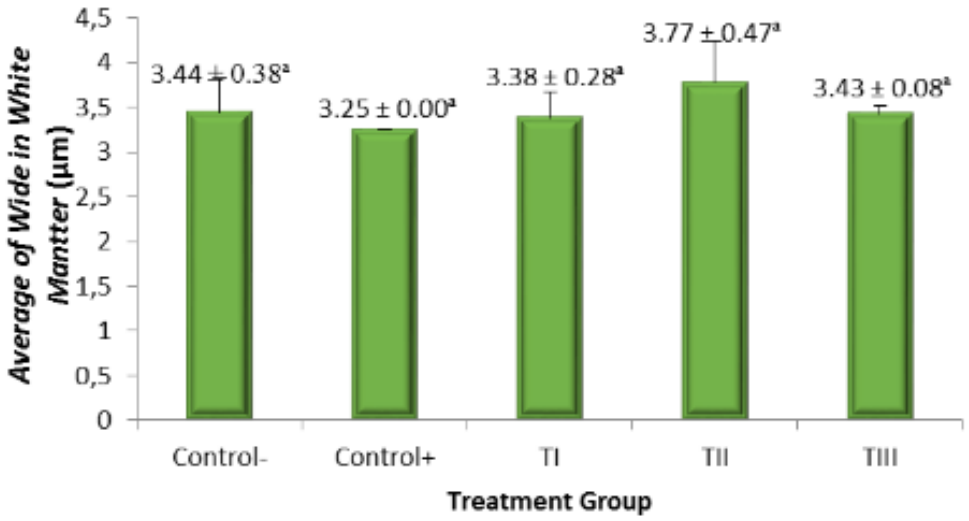

Figure 4. Average of White Matter Width Measurement Results after Giving Methanolic Extract Combination of Tea Mistletoe and Mango Mistletoe for 14 Days

There is no significant difference in the mean width of white matter between each treatment group (Figure 4) because the considerable value or p (value) in all groups is 0.366 (p> 0.05). The treatment of Mistletoe extract combination for 14 days to hypertensive rats at 50, 100, and 200 mg/kg BW has the same potential to increase the width of the white matter area.

Extract combination mistletoe shows that there is a significant difference between all treatment groups with a p-value of 0.001 where the p-value is less than 0.05 (p <0.05) (figure 2). It means that there is a significant difference in each treatment group. In the treated rats has the highest mean number of cell necrosis was 33.0. The administration of DOCA-salt also had no significant effect on the width of the white matter area, where the p-value was more effective than 0.05 (p> 0.05). In the treatment group, the mean width of white matter was smaller than the untreated group, where the positive control had a mean of 3.25 while the negative control had a mean of 3.44. It proves that the DOCA-salt treatment in experimental animals can increase the damage brain cells in the white matter area and affect the width of the white matter.

The provision of DOCA-Salt, according to Athiroh & Permatasari (2011), can increase blood pressure. The increase in blood pressure causes the tension on the walls of the blood vessels to expand and becomes the primary stimulus in the formation of ROS. Aldosterone will bind to *thallocorticoid* receptors, causing oxidative stress through the activation mechanism of NADPH oxidase (NOX) and plays a role in ROS formation in various cardiovascular diseases (Budijanto & Didik, 2015; Iyer et al., 2010). NADPH oxidase (NOX) has an essential role in developing cardiovascular diseases such as atherosclerosis, hypertension, cardiac hypertrophy and remodeling, angiogenesis and collateral formation, stroke, and heart failure. In hypertension, NOX is implicated in

increased oxidative stress by generating reactive oxygen species (ROS). It is well known that hypertension is associated with ROS. Given evidence suggested that redox-dependent pathways significantly contribute to the pathophysiology of hypertension. Hypertension caused SOD activity, and its expression decreased in those tissues. This up-regulation of SOD alleviates oxidative stress in SHRs and prevents further cardiovascular damage.

The parameter observed in this study is the brain because the brain is one of the organs that play a role in the blood pressure regulation system (Purwanti, 2013). Disorders of the brain due to hypertension can cause rupture of the main blood vessels in the brain. Followed by a toxicity test of methanolic extract combination of Benalu tea leaves and Benalu Mango leaves against female rats' lipid profile, the death of most brain cells (Pusparani, 2009). The manifestations can be in the white matter because the white area is one of the areas in the brain that will be affected when hypertension occurs. When blood pressure increases, it will cause ischemic damage to the white matter area (Sedmak & Judaš, 2021; Wycoco et al., 2013).

DOCA-Salt induced over several weeks produces severe hypertension, which is, at least in part, associated with increased production of O2 in the vascular tissue. and this enhancement in vascular O2-exhibition has been shown to play a role in the development and progression of vascular injury, which is one of the most important factors for blood pressure elevations in this hypertensive model. DOCA-Salt induction also causes severe renal dysfunction and tissue injury.

DOCA-Salt induction can cause oxidative stress where there is an imbalance between free radicals and antioxidants in the body. Giving DOCA accompanied by salt (sodium) will provide additional salt, facilitating the sodium retention process in the body. They increased sodium levels which retention will indirectly increase water retention. Increased sodium and water retention will increase the amount of ECF (extracellular fluid). This increase in ECF will increase blood pressure. Increased blood pressure will cause ROS (reactive oxygen species) through shear stress, causing damage to blood vessels and reducing nitric oxide production. This decrease in nitric oxide can cause vasoconstriction of cerebral blood vessels, resulting in brain ischemia. Additionally, a reduction in NO bioavailability causes endothelial dysfunction, resulting in further deterioration of the pathological condition.

TII and TIII treatment in the TI, the mean value is close to the mean value in the untreated (C-). The mean of TI treatment was 15.3, TII was 16.0, and TIII was 14.0, and the untreated (C-) had a mean of 17.0. It proves that combination administration (EMBTBM) in hypertensive rats can reduce brain cell damage. Brain necrosis is damage to cells that occur in the brain. The mistletoe tea and secondary mistletoe metabolite, namely quercetin from flavonoids containing antioxidants, are thought to inhibit free radicals so that they do not cause damage to cells, one of which is the brain, so that cell damage in the brain can stop. The active compound quercetin can bind free radicals to prevent or reduce the impact of free radicals and quercetin to avoid damage to cells caused by free radicals (Sekar Maya Wijaya Mandrasari, Lisdiana, 2014). Brain cells that

experienced necrosis is then phagocytosed by macrophages and then replaced with new cells so that the histopathological picture of the brain is closer to normal (Mihmidati, LailiAthiroh, 2017).

The content of flavonoid compounds found in tea mistletoe and mango mistletoe can prevent cell damage caused by free radicals. Mango mistletoe (*D. petandra*) leaves are potential anticancer agents and can be developed into phytopharmaceutical products. The main flavonoids found in Mistletoe and function as anticancer are quercetin compounds. Quercetin is a flavonoid derivative compound with antioxidant, anti-inflammatory, and anticancer activity (Ajazuddin et al., 2014; Pietrzak et al., 2017). Flavonoids also have an antioxidant effect so that they can prevent oxidation in the initiation and propagation phases. In the initiation phase, flavonoid compounds, namely quercetin, can stabilize free radicals so that radical compounds cannot damage cell membranes. And in the propagation phase, quercetin prevents autoxidation, preventing the formation of peroxide radicals through tight binding of radical compounds to not bind with oxygen. The increase in free radicals caused by shear stress in hypertension can be stabilized. The flavonoids in tea mistletoe and mango mistletoe can act directly on arterial smooth muscle by stimulating or activating the Endothelium Derived Relaxing Factor (EDRF), causing vasodilation. Flavonoids, namely polyphenols, can increase nitric oxide synthase (NOS) activity in blood vessel endothelial cells. Quercetin has the potential to increase NO production in endothelial cells (Athiroh et al., 2014).

In the treatment of TI, TII and TIII, there was no significant difference. The variations in the dose given, namely 50, 100, and 200 mg/kg BW, have the same potential in reducing the amount of necrosis in brain cells in the white matter. And the optimum dose obtained from the provision of Extract Combination Mistletoe (Tea and Mango) is a dose of 50 mg/kg BW. It is because the dose has been able to reduce the number of cell necrosis. The research results obtained, giving or exposure to Extract Combination Mistletoe (Tea and Mango) for 14 days can reduce brain cell necrosis in the white matter area in hypertensive rats.

Conclusion

Extract Mistletoe inhypertensive rats exposed for 14 days at 50, 100, and 200 mg/kg BW can significantly reduce the necrosis of cells. Cells and affect the width of the white area of the brain. That doses did not show a significant difference. However, the optimum dose of extract Mistletoe in reducing the amount of white matter brain cells necrosis in male Wistarrats was 50 mg/kg BW.

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