Antihypertensive and Antioxidant Activity of Herbal Medicine (Jamu) B on Rats Induced by 10% Fructose

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ABSTRACT

Prolonged fructose intake can activate the renin-angiotensin-aldosterone system and enhance oxidative stress conditions, causing hypertension and hyperuricemia. This study aimed to determine the antihypertensive and antioxidant activity of Jamu B on the activity of superoxide dismutase (SOD), level of glutathione (GSH), and malondialdehyde (MDA) in the kidneys of Sprague Dawley rats induced by 10% fructose. Thirty rats were divided into six groups. Groups B1-B3 were Jamu groups at a dose of 0.12 g/kg BW (B1), 0.24 g/kg BW (B2), and 0.36 g/kg BW (B3); group C (positive control) was given captopril 0.1 g/kg BW, group D (negative control) was given only 10% fructose, and group E (normal control) was healthy rats. The 10% fructose was induced for eight weeks and the treatment was given on the week 9th and 10th. Jamu B reduced systolic and diastolic blood pressure at all doses, but was not significantly different from the normal control group (p>0.05). The SOD activity in groups B1, B2, B3, and C was significantly different from the negative control group (p<0.05). However, GSH levels only showed a significant difference between B2, B3, C, and the negative control group (p<0.05). The MDA levels only showed a significant difference between B3 and the negative control group (p<0.05). Jamu B at the dose of 0.12 g/kg BW, 0.24 g/kg BW, and 0.36 g/kg BW have antihypertensive and antioxidant activity in the kidneys of rats induced by 10% fructose. Nevertheless, Jamu B at a dose of 0.36 g/kg BW was the most effective as an antihypertensive and antioxidant agent.

Keywords: fructose; jamu; kidney; glutathione; malondialdehyde; superoxide dismutase

INTRODUCTION

Kidneys play an essential role in excreting the body’s metabolic wastes. These organs are susceptible to damage due to excessive free radicals or oxidative stress (Dornas et al., 2017). A recent study has shown that continuous fructose administration in rats can increase oxidative stress, uric acid production, and inflammation that induce glomerular hypertension and kidney damage (Nakagawa et al., 2020). Excessive fructose consumption causes hyperuricemia, hyperinsulinemia, and hypertension, leading to impaired kidney function. Fructose also increases sympathetic nerve activity. It also produces vasoconstrictors, like endothelin-1, angiotensin II, and prostanooids, and decreases nitric oxide (NO) levels, contributing to endothelium dysfunction (Sánchez-lozada et al., 2012). Several in-vivo studies in rats have shown that an increase in free radicals, including superoxide anions and nitric oxide, can cause lipid and protein oxidation which was observed in cases of kidney damage. Thus, overconsumption of fructose would raise free radicals production and subsequently impacts the development of kidney disease by decreasing the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and selenium (Dennis & Witting, 2017). The antioxidant compound is necessary to protect the body from oxidative stress and stabilize free radicals by donating one electron to free radical compounds. The human body has its defense mechanism to fight free radicals and oxidative stress through endogenous antioxidants, SOD, and glutathione (GSH) enzymes, yet under oxidative stress conditions, the amount is insufficient to overcome the excess of free radicals. Therefore, adding exogenous antioxidants, such as herbal medicines, is required to impede oxidative stress activity (Dennis & Witting, 2017; Azab et al., 2017).

Jamu B, a herbal medicine, contained Curcuma xanthorrhiza rhizome (Temulawak), Andrographis paniculata herb (Sambiloto), Tinospora crispa caulis (Brootwali), Ortosiphon stamineus folium (Kumis Kucing), and Phyllanthus niruri folium (Meniran), is indicated as antihypertensive. Interestingly, a previous in vitro study on Jamu B has shown antioxidant activity with an IC<sub>50</sub> value of 11.4 ppm (Eff, Hurit, et al., 2020; Eff, Rahayu, et al., 2020). The study conducted by Eff, Rahayu, et al. (2020) also showed that Jamu B has an antihypertensive effect through its mechanism as an ACE inhibitor with an IC<sub>50</sub> value of 103.75 µg/ml. Jamu B extracts contain flavonoid compounds, alkaloids, tannins, saponins, steroids, and triterpenoids (Eff, Rahayu, et al., 2020). The flavonoid and polyphenolic...
metabolites in Jamu can improve kidney function by reducing oxidative damage and increasing the activity of antioxidant enzymes, including SOD and GSH (Zeng et al., 2019).

Hence, this study aimed to determine the antihypertensive and antioxidant activity of Jamu B; with the composition of Curcuma xanthorrhiza rhizome 1500 mg, Andrographis paniculata herb 1200 mg, Tinospora crispa caulis 1600 mg, Orthosiphon stamineus folium 750 mg, and Phyllanthus niruri folium 750 mg; in 10% fructose-induced white rats Sprague Dawley. Systolic (SBP) and diastolic blood pressure (DBP) were measured weekly non-invasively using IITC life science. While the activity of SOD, level of GSH, and malondialdehyde (MDA) in the kidneys of rats measuring by spectrophotometer.

**MATERIALS**

Jamu B was purchased from herbal medicine shops in North Jakarta. Thirty white rats Sprague Dawley (two months old, weight 175-300 grams, healthy, and not disabled) were obtained from the Faculty of Veterinary Bogor Agricultural Institute, surgical instrument (minor set), beaker glass, drinking bottle, stirring rod, centrifuge, container box, freezer temperature -20°C (minor set), beaker glass, stirring rod, Nalgene, beaker glass, stir bar, Eppendorf tube (Sartorius®), glass pot, mouse probe, spectrophotometer (Genesys), Sartorius®, Eppendorf tube (Sartorius®), plastic clip, and vortex. Fructose (Merck), physiological NaCl, PBS solution pH 7.4, trichloroacetic acid (TCA), thiobarbituric acid (TBA), Ransod kit (Randox Laboratories Ltd), dithiobisnitrobenzoate (DTNB), sodium pentobarbital.

**METHODS**

Sample Preparations

Jamu B, which indicates lower blood pressure and blood glucose levels, is available in the market in 6 g sealed packets. The Jamu solution was made as stated in the instructions by brewing into hot water to roughly 100 mL and consumed two times on the same day. Experimental dosages for this study were selected based on the packet content of 6 g per human being. The average weight of a human being is taken is 50 kg, and the standard weight of a rat is 250 g. The various doses for experimental were 0.12, 0.24, and 0.36 g/kg BW. The solution was given orally to rats based on group division using a feeding tube (Nahar et al., 2013).

Experimental Design

This research has received approval from the Universitas Esa Unggul health research ethics committee with approval number 0161-20.152/DPKEKEP/FINAL-ENUEUN/2020. Rats were acclimatized and divided into six groups; Jamu groups at a dose of 0.12 g/kg BW (B1), 0.24 g/kg BW (B2), 0.36 g/kg BW (B3), the captopril control group (C) with a dose of 0.0005 g/kg BW, negative control group (D) (only 10% fructose), and normal control group (E) (a healthy rats). 500 mL of 10% fructose solution was given in a drink bottle every day for each group except for group E. At the 9th and 10th weeks, the rats were given treatment according to the group division. Blood pressure and body weight were measured every week (Eff et al., 2021). At the beginning of the 11th week, the rats were anesthetized with sodium pentobarbital at a 50 mg/kg BW dose, then sacrificed, and the kidneys were removed. The kidneys were homogenized using PBS solution pH 7.4, then vortexed and centrifuged at 3000 rpm for 10 minutes. The supernatant was separated and stored in a freezer at -20 °C before measuring SOD activity, GSH levels, and MDA levels (Kum et al., 2007).

**SOD Activity Measurement**

SOD activity was measured using a Ransod kit and read with a spectrophotometer (Genesys) at a wavelength of 505 nm. The initial absorbance (A1) was read after 30 seconds, and the final absorbance (A2) was read after 3 minutes. The percentages inhibited by standards and samples were determined using the RANSOD kit’s superoxide dismutase instructions, and an inhibition percent curve was created. The specific activity is calculated from the standard curve (U/mL) divided by protein concentration (mg/mL) (Farahnak et al., 2013).

**GSH Levels Measurement**

50 L of kidney homogenate was added to 200 L of 5% TCA, then vortexed. After that, it was centrifuged and added 25 L of DTNB and 1750 L of phosphate buffer pH 7.4, then allowed to stand for 1 hour. The reaction between DTNB and glutathione will produce 5’-thio-2-nitrobenzoic acid (TNB) yellow with glutathione disulfide (GSSG). By interacting with GSH to generate GSGG, glutathione peroxide catalyzes the reduction of H₂O₂ to H₂O. The absorbance was measured using a spectrophotometer at a wavelength of 412 nm and compared with the absorbance of the standard glutathione solution. The enzyme activity was expressed as units/mg of protein (Abarikwu et al., 2017).

**MDA Level Measurement**

200 µL of homogenate was added to 1800 L of distilled water and 1 mL of 20% TCA, then vortexed and centrifuged at 3000 rpm for 10 minutes. The supernatant formed was taken and added 2 mL of 0.67% TBA. Then it was vortexed again and heated at 100 °C for 10 minutes. Color absorption was measured using a spectrophotometer at a wavelength of 530 nm. MDA levels were calculated using the MDA standard curve (Abarikwu et al., 2017).
Data Analysis
The measurements of body weight, SOD activity, MDA levels, and GSH were analyzed statistically using the ANOVA test and multiple comparisons to determine differences between treatment groups. Data analysis was carried out using SPSS software version 26. The p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION
The results of the mean body weight measurement (Figure 1) showed that the average body weight in the first week was 207.5 grams and increased to 266.5 grams in the eighth week. Weight gain occurred by 28.5% in all treatment groups, while in the normal control group, there was an increase of 18% from the first week to the eighth week after administration of fructose 10%. All the treatment groups were statistically significant compared to the normal control group in terms of body weight, indicating that fructose affected weight gain. This is similar to the study conducted by Sandeva et al. in rats induced by 20% fructose for eight weeks that showed an increase in body weight, length, and body fat. Köseler et al. also showed that the administration of fructose 10% and 30% in rats for six weeks had gained more weight than in the group given glucose 10%. Uncontrolled fructose consumption triggers weight gain and disrupts the metabolic profiles, which contributes to the high risk of obesity and metabolic syndrome (Köseler et al., 2018). Weight gain is accompanied by increased fat accumulation and circulating triglyceride levels (Köseler et al., 2018). Moreover, high fructose intake will interfere with the secretion and production of neuropeptides, such as ghrelin and leptin, affecting appetite control (Sandeva et al., 2015).

Furthermore, excessive fructose consumption induces hyperglycemia and hypertension (Yanti et al., 2014) (Yanti et al., 2015). The mechanism by which fructose causes these metabolic syndromes is influenced by renin-angiotensin-aldosterone (RAS) activity (Kim et al., 2020). RAS is a significant regulator of blood pressure; electrolyte balance; and endocrine functions, such as controlling the nervous system, kidneys, and cardiovascular system. In this study, we measured the systolic and diastolic blood pressure to evaluate the antihypertensive activity of Jamu B (Figures 2a and 2b). The results of blood pressure measurements in all treatment groups and the normal control group showed an increase in blood pressure, both systolic and diastolic, starting from the third week of fructose administration, except in the normal control group. Induction of 10% fructose in rats for eight weeks showed an increase in blood pressure in the treatment groups, and the administration of Jamu B for two weeks caused a decrease in blood pressure. In the negative control group (D), systolic blood pressure increased until fructose administration stopped, but the diastolic blood pressure remained the same. Interestingly, the administration of Jamu B (B1, B2, and B3) and captopril have shown a decrease in systolic blood pressure, which was not significantly different from the normal control group. Our results were in line with the previous research by Eff et al., by which 10% fructose administration in rats for eight weeks has increased blood pressure and was reduced following the consumption of antihypertensive therapy in the first and second week of treatment (Eff et al., 2021). Another study also showed that the administration of 66% fructose for two weeks could increase blood pressure from 124 mmHg to 145 mmHg (Hwang et al., 1987).
Figure 2. (a) Mean of systolic blood pressure (mmHg) within ten weeks for each treatment group. (b) Mean of diastolic blood pressure (mmHg) within ten weeks for each treatment group. B1: Jamu group at a dose of 0.12 g/kg BW; B2: Jamu group at a dose of 0.24 g/kg BW; B3: Jamu group at a dose of 0.36 g/kg BW; C: captopril group at a dose of 0.1 g/kg BW; D: negative control group; E: normal control group.

Uncontrolled fructose intake has also been reported to cause chronic kidney disease characterized by renal hypertrophy with tubular cell proliferation and tubulointerstitial injury, resulting in renal dysfunction (Choi et al., 2011). The research conducted by Bratoeva et al. showed an increase in kidney weight of 26% in Wistar rats induced by 35% fructose for 16 weeks (Bratoeva et al., 2017). Similarly, we found an increase of 2.8% in kidney weight in the negative control group, indicating hypertrophy or increased cell size in the kidney organs due to the induction of fructose. We suggest that the difference in kidney weight was influenced by the animal species, the concentration of fructose used, and the duration of fructose consumption. Interestingly, we discovered that the Jamu B treatment at all doses was considerably reduced kidney weight compared with the negative control group. The percentage of the reduction in kidney weights for B1, B2, B3, and captopril were 21.46%, 20.54%, 26.06%, and 9.13%, respectively (Table 1) where B3 showed a significant reduction even compared with captopril (p=0.015).

The effectiveness of Jamu B treatments in reducing kidney damage was associated with the ability of its antioxidant activity. Several studies have shown that fructose consumption could increase lipid peroxidation levels and decrease antioxidant enzyme activity in the kidneys (Choi et al., 2011; Bratoeva et al., 2017). We confirmed this towards an increase in MDA levels (9.79 ± 1.73 nmol/mL), a decrease in SOD activity (88.10 ± 6.43 U/mL), and GSH levels (0.19 ± 0.065 nmol/mg protein) in the kidneys only in the negative control group (Table 1). Under normal conditions, free radicals can be formed slowly. When the amount endogenous antioxidants is insufficient to overcome the excess of free radicals, oxidative stress occurs, leading to lipid peroxidation. MDA is a product of lipid peroxidation (Ayala et al., 2014). The study conducted by Bulboacă et al. showed that the induction of 10% fructose for 12 weeks increased MDA levels in the non-treated group (Bulboacă et al. 2016). Giving Jamu B treatments at all doses caused a decrease in MDA levels. Surprisingly, the treatment dose of 0.36 g/kg BW (B3) reduced the highest MDA level
The renal GSH levels showed that the negative control group had the lowest GSH levels, around 0.19 ± 0.065 nmol/mg protein. The decrease in GSH levels indicates that the induction of 10% fructose increases free radicals in the kidney, thereby depleting endogenous antioxidant defenses. The study conducted by Bratoeva et al. showed a decrease in GSH levels in rats after being given 35% fructose for 16 weeks compared to the control group (Bratoeva et al., 2017). Choi et al. also proved that fructose induction for ten weeks reduced GSH levels (Choi et al., 2011). The statistical tests on GSH levels showed no significant difference between B2 and B3 with the captopril group and the normal control group (p=0.05).

The measurement of SOD activity in kidneys showed that the captopril group had the highest SOD activity (221.84 ± 23.26 U/mL) compared to Jamu B groups at all doses and the negative control group. These results indicate that the administration of captopril effectively maintained and increased the activity of the SOD when oxidative stress occurs. Captopril reduces lipid peroxidation and free radicals by reducing NADPH oxidase and expanding the regulation of antioxidant enzymes (Karimani et al., 2018). Nevertheless, giving Jamu B treatment with a dose of 0.36 g/kg BW (B3) (175.63 ± 8.49 U/mL) had the lowest close value of SOD activity to the captopril value (221.84 ± 23.26 U/mL) and normal control group (215.55 ± 10.55 U/mL) compared to the lower doses (B1 108.03 ± 6.06 U/mL) and B2 (129.19 ± 6.32 U/mL). Although the value was still lower than the captopril group, the results of this study showed that the administration of Jamu B was able to ward off free radicals by increasing SOD activity. The statistical tests on SOD activity showed a significant difference between the captopril group and Jamu B groups with a negative control group and the normal control group (p<0.05). However, there was no significant difference between the captopril and the normal control group (p=0.4).

The presence of reactive oxygen species (ROS) in the kidneys due to fructose consumption occurs through the increased formation of uric acid. Fructose is phosphorylated in the liver by fructokinase to form fructose 1-phosphate. During this formation, fructokinase activation requires phosphate and depletes ATP. Furthermore, the enzyme aldolase B will break down fructose 1-phosphate into dihydroxyacetone phosphate (DHAP) and glyceraldehyde. When fructose is consumed in excess, the phosphorylation by fructokinase to form fructose 1-phosphate is fast, but the reaction with aldolase B is slow. This condition causes the accumulation of fructose 1-phosphate and a decrease in intracellular fructose (Pi) and further activates AMP deaminase (AMPD) and promotes the degradation of AMP to inosine monophosphate (IMP). The result is an increased rate of uric acid degradation and formation. Uric acid can react with nitric oxide, causing a decrease in dimucleotide phosphate oxidase (NOX) activation and mitochondrial dysfunction. Uric acid also reacts with radical molecules such as superoxide anion, resulting in oxidative stress (Caliceti et al., 2017).

In addition, the decrease in SOD activity in the negative control group described the abundance of free radicals in the kidneys induced by fructose at 10%, giving an extended time for uric acid levels to rise twice. This increase can reduce nitric oxide, which results in endothelial dysfunction (Bratoeva et al., 2017). Studies with fructose-induced animal models have shown decreased endothelial nitric oxide, increased oxidative stress, and renin-angiotensin-aldosterone activation associated with fructose-induced hypertension (Nakagawa et al., 2020). Moreover, oxidative stress
causes hypertension and increased superoxide anion and hydrogen peroxide free radicals (Tran et al., 2009; Zhang et al., 2017). The research of Choi et al. also showed a decrease in SOD activity in 65% fructose-induced rats for ten weeks (Choi et al., 2011). SOD is one of the first defense enzymatic antioxidants. There are three types of SOD: Cu/Zn-SOD, which is located in the cytoplasm and other cell organelles; Mn-SOD, which is located in the mitochondria; and Fe-SOD, which is found in prokaryotes and plant chloroplasts (Ighodaro & Akinloye, 2018). The SOD enzyme will catalyze the superoxide anion free radicals produced in the formation of hypoxanthine and xanthine by the xanthine oxidase enzyme during uric acid construction. This superoxide anion will be converted into hydrogen peroxide \((H_2O_2)\) and oxygen \((O_2)\) by SOD. ROS that has not been overcome by SOD but has attacked lipids will form MDA, the end product of lipid peroxidation and a marker of oxidative stress. Meanwhile, when GSH reacts with GSHPx, it will reduce \(H_2O_2\) to oxidized glutathione (GSSG) and water \((H_2O)\). Three enzymes that act as antioxidants are catalase, glutathione peroxidase, and SOD. However, the work of these endogenous enzymes is insufficient when there is an excess of free radicals in the body (Santos-Sánchez et al., 2019).

Nevertheless, Jamu B contains various natural substances rich in antioxidants, including Curcuma xanthorrhiza rhizome 1500 mg, Andrographis paniculata herb 1200 mg, Tinospora crispa caulis 1600 mg, Orthosiphon stamineus folium 750 mg, and Phyllanthus niruri folium 750 mg. Hence, this herbal medicine could effectively tackle the abundance of free radicals produced by prolonged fructose 10% in rats. The content of phenolic compounds in flavonoids and tannins in Jamu B has antioxidant abilities to inhibit free radicals in-vitro (Elf, Rahayu, et al., 2020) by donating hydrogen atoms from their hydroxyl groups, chelating metal ions, and inhibiting LDL oxidation (Francenia Santos-Sánchez et al., 2019).

Curcuma xanthorrhiza (Temulawak), containing curcumin compounds and essential oils, has also known to have the potential as antioxidants, antibacterial, anti-inflammatory, anticancer, hepatoprotective, and antidysslipidemic (Rosidi et al., 2016). Research by Bulboacă et al. showed that administration of curcumin in fructose 10%-induced rats lowers blood pressure, improves lipid profile, and works as hepatoprotective (Bulboacă et al., 2016).

Andrographis paniculata herb (Sambiloto) has been reported to have pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, antibacterial, and antiviral. Sambiloto contains active compounds andrographolide and flavonoids (Chao & Lin, 2010). The antioxidant activity of Andrographis paniculata works by increasing the enzymes, SOD, catalase, and GSH. This plant extract also can inhibit lipid peroxidation by reducing levels of TBA reactive substances in the kidneys of diabetic rats (Zhang and Tan, 2000). Andrographolide compounds minimize the formation of reactive oxygen species (ROS), thereby reducing oxidative stress (Jayakumar et al., 2013).

Phyllanthus niruri folium (Meniran) contains flavonoids, alkaloids, tannins, essential oils, glycosides, and anthraquinones. This plant can be an antibacterial, antiabetic, and immunomodulator (Da et al., 2016). The presence of quercetin compounds makes the antioxidant activity of Phyllanthus niruri folium extract stronger than vitamin E (Da et al., 2016). P. niruri water extract was reported to have antioxidant activity that can reduce the formation of free radicals and increase the activity of endogenous enzymes in the kidney. Administration of 200 mg/kg BW and 400 mg/kg BW doses increased SOD activity in the kidneys by 43.02 % and 79.93% and increased renal Gpx by 18.94% and 35.78% in rats induced with diabetes, respectively (Giribabu et al., 2014).

Tinospora crispa (brotwali) has pharmacological activities, including anti-inflammatory, immunomodulator, cytotoxic, hypoglycemic, and antioxidant. Brotwali contains chemical compounds of alkaloids, flavonoids, tannins, and triterpenoids (Ahmad et al., 2016). Tinospora crispa aqueous extract showed high antioxidant activity comparable to butylhydroxytoluene (BHT) and vitamin C. The presence of phenolic compounds in this plant can capture free radicals and prevent oxidative stress (Ahmad et al., 2016). Flavonoids catch free radicals by stabilizing ROS by reacting with reactive radical compounds, increasing metal chelating activity, and activating antioxidant enzymes. Flavonoids donate hydrogen atoms, and their antioxidant activity is influenced by the number and position of hydroxyl groups (Warsinah et al., 2020).

Orthosiphon stamineus folium (Cat's whiskers leaf) has been widely used as an alternative therapy for hypertension. This plant extract contains several bioactive compounds, including polyphenols, terpenoids, diterpenoids, triterpenoids. The presence of these polyphenolic compounds shows antioxidant and antihypertensive activity. Administration of Orthosiphon leaf extract at a dose of 250 mg/kg BW for 14 days was able to reduce systolic blood pressure in animal models of hypertension. A dose of 250 mg/kg BW was an optimal antihypertensive dose than 500 mg/kg BW and 1000mg/kg BW. The efficacy of orthosiphon as an antihypertensive is similar to irbesartan 20 mg/kg (Azizan, 2012).
Therefore, with all the abundant antioxidant agents in this Jamu, we observed that administration of Jamu B with doses of 0.12 g/kg BW, 0.24 g/kg BW, and 0.36 g/kg BW could reduce blood pressure, MDA levels, increase SOD activity, and GSH level in rats induced by 10% fructose. The higher dose of 0.36 g/kg BW of Jamu B showed better results in lowering MDA levels, increasing SOD activity, and increasing GSH levels comparable to captopril due to more active metabolite compounds. These results follow the research of Eff et al., which shows that the higher the dose of antihypertensive jamu consists of Phaleria macrocarpa, Gynura procumbens, Imperata cylindrica, Centella asiatica, and Syzygium polyanthum, the lower blood pressure is (Eff et al., 2021). Nevertheless, further studies need to be taken to analyze the subchronic toxicity of this herbal medicine and evaluate renal histopathological examinations for kidney function.

**CONCLUSION**

The administration of Jamu B containing Curcuma xanthorrhiza thizone 1500 mg, Andrographis paniculata herb 1200 mg, Tinospora crispa caulis 1600 mg, Orthosiphon stamineus folium 750 mg, and Phyllanthus niruri folium 750 mg at doses of 0.12 g/kg BW, 0.24 g/kg BW, and 0.36 g/kg BW had antihypertensive and antioxidant effects in 10% fructose-induced rats.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


