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Research Article

The Effect of Jujube Leaf Extract in Preventing Memory Scores Deficit in White Wistar Rat Hypertension Model

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Abstract.
Memory impairment is one of the effects caused by hypertension. Jujube leaves (*Ziziphus mauritiana Lam*) have active ingredients which are believed to control hypertension and, therefore, prevent memory deficit, especially spatial memory in white rats. The aim of the study was to determine the effect of jujube leaves extract (*Ziziphus mauritiana Lam*) on the memory score of white rats with hypertension induced by NaCl and Prednisone. The study was a true experimental with a post-test-only control group design. Rats were divided into five groups consisting of two control groups and three groups with jujube leaves extract: 200mg/KgBW/day, 400mg/KgBW/Day, and 800mg/KgBW/Day. Memory functions were measured by Radial Arm Maze to measure spatial memory function. Data were analyzed with One-Way ANOVA and then Post-hoc Bonferroni. All groups were found to be significant (p = 0.00) in One-Way ANOVA test. In a Post hoc test, only group 3 with a dose of 800mg/KgBW/day was found to be significant (p = 0.00) to prevent memory score deficit in white hypertensive Wistar rats induced by NaCl and Prednisone. Jujube leaves extract (*Ziziphus mauritiana Lam*) can prevent memory score deficit in white Wistar rats with hypertension.

Keywords: hypertension, memory function, jujube leaves

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1. Introduction

Memory impairment is the inability to learn newly acquired information and recall previously acquired information. This disorder can appear in people with acute hypertension. In America, a survey by the third National Health and Nutrition Examination Survey reported an association between decreased memory function and patients with blood pressure higher than 140 mmHg [1].

In a previous study, it was found that rats with acute hypertension had decreased memory function and learning ability after being induced with DeoxyCorticosterone Acetate (DOCA) for 1 week. Another study also showed memory deficits in hypertensive wistar rats in the second week [2], [3]. The cause of decreased memory function due to

acute hypertension is related to the possibility of a metabolic imbalance, atherogenesis, demyelination or white matter microinfarcts in hypertensive patients [4]. In addition, the buildup of β amyloid protein due to acute hypertension causes neurovascular dysfunction so that blood flow to the brain is reduced [5].

One of the inductions that can increase blood pressure is using NaCl and Prednisone. Administration of NaCl and prednisone for 2 weeks will increase SBP up to 192.3 mmHg, causing hypertension. This administration will increase Angiotensin II, causing an increase in aldosterone and ROS. The increase in aldosterone will trigger and maintain an increase in blood pressure for a long time. Meanwhile, an increase in ROS will inhibit the release of NO so that the ability to vasodilate decreases and it leads to increased blood pressure [6]. This continuous change will cause vascular damage and buildup of Aβ protein. This will later cause inflammation of the cerebral blood vessels, resulting in ischemia and decreased cerebral neurogenesis and a gradual deficit in memory function [1], [7].

To prevent memory impairment due to hypertension is to treat hypertension first which can be done by administering anti-hypertensive drugs [1]. One of the natural ingredients believed to have active ingredients in reducing hypertension is the jujube plant. Ziziphus mauritiana or jujube is an edible fruit and is believed to be one of the medicinal plants in various Asian countries such as Pakistan, India and Africa [3]. In Indonesia, this plant can be found in the area of Sumbawa, Bali, East Java and has been cultivated in Central Java [8]. Jujube plants contain active ingredients such as saponins, tannins, alkaloids and flavonoids which have an anti-hypertensive effect by inhibiting the formation of Angiotensin II or ACE Inhibitors [9]. Based on the description above, the researcher wanted to know the effect of jujube leaf extract in preventing memory score deficit in white wistar rats (Rattus novergicus) hypertension model induced by NaCl and prednisone.

2. Materials And Methods

This research was true experimental with post test only control group design. The sample of this study were 25 male rats (Rattus novergicus strain wistar) aged 2-3 months, weighing 150-200 grams and healthy. First, the mice were adapted for 7 days, then 25 white male rats were divided into 5 groups; positive control (without any induction), negative control (2% NaCl + Prednisone 1.5mg/KgBW for 28 days) and three treatment groups (2% NaCl induction + 1.5mg/KgBW/day Prednisone for 28 days then they were

induced by jujube leaves extract at a dose of 200 mg/kgBW/day, 400 mg/kgBW/day, 800 mg/kgBW/day for 14 days).

Determination of dosage based on a study by Dahiru (2008) about the antioxidant activity of jujube leaf extract (Ziziphus mauritiana lam.) In rats that had chronic hepatotoxicity due to ethanol induction, an effective dose against free radicals is obtained, that is 400 mg/kgBW/day. From this data, then the treatment dose was determined using the arithmetic series formula [10].

Measurement of memory function was carried out using the Radial Arm Maze. The test was done for 10 days after the RAM habituation process. The test results were recorded and divided into the time needed by rats to take all bait and the pathway for the mouse to enter the arms. The results of the memory score test were in range of values -1 to 1, with a score of 1 which means perfect and a score of -1 means that all the arms entered by the rats are wrong. At each phase and at each change of rats the Radial Arm Maze was cleaned using 70% alcohol [11], [12]. Data analysis used One Way ANOVA, Post Hoc Bonferroni test, and linear regression.

3. Results

The results of the memory test scores obtained will then be averaged per group then averaged every two days with a score range between -1 to 1, and the results can be seen in the following table.

Table 1: Average Radial Arm Maze memory score.

	Day 26-27	Day 28-29	Day 30-31	Day 32-33	Day 34-35
K-	-0,45	-0,42	-0,46	-0,58	-0,57
K+	0,15	0,31	0,5	0,42	0,25
P1	-0,29	-0,12	-0,33	-0,37	-0,23
P2	-0,31	-0,03	0,16	-0,1	-0,19
P3	-0,25	-0,08	0,15	0,05	0,4

The table above shows that the K- group induced by NaCl and prednisone every day experienced a decrease in memory function compared to the K + group that was not induced by anything. In the P1 group with a dose of 200 mg / KgBW / day of jujube leaf extract, it did not seem that there was any improvement in memory function. In the P2 group with a dose of 400 mg / KgBW / day, the results of the memory score increased on day 5-6 then decreased again on day 32-33 and day 34-35. Whereas in the P3 group who received an exact dose of 800 mg / KgBW / day, it was found that the memory score was clinically quite good so that there was an improvement in

memory function produced by jujube leaves. So with these data, it can be concluded that the treatment group 3, which induced by Nacl 2% + Prednisone 1.5 mg / KgBW for 4 weeks and induction of 800 mg / KgBB jujube leaf extract for 2 weeks showed the best increase in memory scores among all treatment groups.

The data is then processed using SPSS to see normality by Saphiro-Wilk test. In this test, it was found that all data showed a significant value <(0.05), which means that the memory score data was normal and was feasible to proceed to One-Way ANOVA. Furthermore, the homogeneity test was done using the Levene test, which obtained a variant of 0.192 which means that the data variant was homogeneous. Data analysis was continued with the One-Way ANOVA test to see the comparison of memory scores between the administration of jujube leaf extract. The results of data analysis using the ANOVA test showed the value of Significant <0.05, so it can be stated that there is at least a significant difference in the memory score in each treatment. To see a significant difference between treatment groups continued with the Bonferroni Post hoc test.

The Bonferroni Post hoc test aims to see significant differences between treatment groups. The results of this test showed that the comparison with the positive control group and the negative control group had significant results (p <0.05). Significant results were also obtained in the comparison of the positive control group with the treatment group 3 which is the treatment group with a dose of 800 mg / KgBW / day.

Table 2: Bonferroni Post Hoc Test.

Dependendent Variable Memory score		Sig.
K-	K+	0,02
	P1	0,239
	P2	0,072
	P3	0,00

Regression test to find out how strong the relationship between the dosage levels of jujube leaf extract and the memory score test results using the Radial Arm Maze. In this linear regression test, only 1 variable is included, which is the dose of jujube leaf extract. The model of the summary test obtained a value of R2 = 0.816, which means that the percentage role of the dose of jujube leaf extract on the memory score is 81.6%. The ANOVA table resulted in a value of sig = 0.00 (p <0.05) so that the difference in the dose of jujube leaf extract affects the memory score. From these results, it can be found that the regression equation is Y = -0.521 + 0.001 (X) where Y = RAM memory score and X = Jujube leaf extract content. The results obtained a dose coefficient of 0.001, which means that every 1 mg increase in the dose of jujube leaf extract there is an increase in the memory score of 0.001.

4. Discussion

In this research process using RAM which functions to measure the process of working memory or short-term memory in rats with acute hypertension. RAM uses spatial memory in mice to explore the bait-filled labyrinth arm and avoid getting into the arm without the bait. The measurement of memory function uses a score which describes the memory processes that occur in rats. In the experimental group and the negative control group, it was found that a decrease in memory scores on days 30-35 could be due to the condition of the rat that tended to be stressful, which affected the motivation of the rat to find bait on the RAM arm, thus affecting the memory score results. In using this RAM, the motivation and desire of the rat to find bait are the main keys in conducting experiments. In this study, days 26-30 were the acquisition phase and days 31-35 were the asymptomatic phase. The acquisition phase where the rat is still getting used to the RAM process and the asymptomatic phase is the phase where the rat is considered has adapted to and understands the process of RAM [13].

From the research, it was found that the best memory score was in the treatment group 3, which gave 800 mg extract/KgBW/day. In the P1 group with a dose of 200 mg / KgBW / day of jujube leaf extract, it did not seem that there was much improvement in memory scores. In the P2 group with a dose of 400 mg / KgBW / day, the results of the memory score increased on 30-31 days then decreased again on day 32-33 and day 34-35. The process of lowering the memory score can occur due to a lot of vascular damage and decreased blood flow to the cerebral, which causes a decrease in short-term memory work [2]. In acute hypertension, ROS will be active so as to reduce NO production which causes inflammation of blood vessels and accumulation of protein Aβ. This situation will make the brain susceptible to oxidative stress, which can cause an inflammatory cycle, which are an increase in ROS and a continuous decrease in NO [5], [14].

Acute hypertension can also increase Aβ protein production directly by increasing amyloid precursor protein and inducing the amyloidogenesis process. In previous studies, the hypertension condition that increased ANG II, there was an increase in Aβ levels two times higher than normal levels after giving DOCA for 2 weeks[5]. The accumulation of Aβ protein in the intracellular, extracellular, and neurofibrillary tangles will suppress the synthesis and release of ACh thereby disrupting signaling to cholinergic receptors. This cholinergic dysfunction greatly affects the hippocampus area and will later affect the RNA process resulting in loss of dendrites in cortical neurons. The ACh function in the hippocampus in the CA3 area will activate muscarinic receptors, especially the

M1 receptor which will maintain memory signals. In the CA1 area, it is known that acetylcholine will increase potential in the Schaffer collateral pathway by activating nicotinic ACh receptors, namely α7 or non-α7 in pyramidal neurons and GABAergic interneurons [15].

In order to prevent a continuous decline in memory scores, hypertension should first be treated. One way to control hypertension is by administering active ingredients or drugs which can prevent an increase in blood pressure. In previous research on the effect of anti-hypertensive drugs on improving memory in rat, it was stated that a decrease in angiotensin II levels and inhibition of AT1R led to neurogenesis in the cerebellum which was previously inhibited due to increased blood pressure. The administration of jujube leaf extract in this study is expected to have a hypotensive effect which will later help the neurogenesis process and increase cerebral vascularization so that it can help the process of preventing memory score decline in rats. The presence of a hypotensive effect on jujube leaf extract will stimulate the release of NO in the endothelium, especially in the kidneys [16]. This shows that the active substance in jujube leaves has an ACE inhibitor effect and has neuroprotective properties, so it is expected to reduce Aβ levels in the brain and reduce oxidative stress by changing ACE activity and angiotensin II levels in the brain so that it can improve memory function in humans and rat [17].

The active ingredient of jujube leaves which has the function of increasing NO release according to previous studies is flavonoids. The process of increasing NO release in the endothelium will repair blood vessels and in turn will prevent the buildup of Aβ protein. This will help increase cerebral perfusion because it reduces the plaque buildup process in the endothelium so that perfusion to the cerebral is increased and helps the neurogenesis process [8], [14]. Other ingredients in jujube that have benefits are saponins by inhibiting renin activity in RAAS and alkaloids, tannins, flavonoids that can inhibit ACE activity[9], [18]. In this case, jujube leaf extract may improve endothelial function of blood vessels and increase NO production thereby increasing cerebral perfusion and increased activation of choline acetyltransferase which helps acetylcholine process in the brain [16].

5. Conclusion

The conclusion of the study is that there is an effect of jujube leaf extract (Ziziphus mauritiana Lam) in preventing memory score deficit in white rats (Rattus novergicus strain wistar) hypertension model induced by NaCl and prednisone and the dose of

jujube leaf extract (Ziziphus mauritiana Lam) which has the most effect in preventing memory score deficit of white rats (Rattus novergicus strain wistar) is 800mg / KgBW / Day.

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