Short communication

Effect of *Andrographis paniculata* Nees extract on the malondialdehyde (MDA) levels of male Wistar rats hypercholesterolemia-induced model

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(Received: September 2022 Revised: January 2023 Accepted: February 2023)

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ABSTRACT

Introduction and Aim: Globally, cardiovascular diseases are now the leading cause of death. A plant that has anti-inflammatory and antioxidant effects is *Andrographis paniculata* Nees. The aim of the study was to determine whether *A. paniculata* Nees extract could increase Malondialdehyde (MDA) concentrations in the serum of rats with hypercholesterolemia.

Materials and Methods: The study used only a post-test control group with Thirty-six Wistar rats. The control group (K1) was not given both *A. paniculata* Nees and a high cholesterol diet, group 2 (K2) was given atorvastatin 5 mg/kg BW (positive control) and groups 4,5 and 6 were given *A. paniculata* Nees, respectively, 400, 800 mg/kg BW, following a high cholesterol diet. The concentration of MDA was checked on the 36th day using the TBARS method.

Results: Between treatment groups 1, 2, and 3, all three had a significant drop in total cholesterol levels after being administered the *A. paniculata* Nees extract for two weeks, however, the differences were not statistically significant (p=0.868).

Conclusion: The results of the present study showed no effect of *A. paniculata* Nees extract on the levels of malondialdehyde (MDA) in male Wistar rats with a hypercholesterolemia model.

Keywords: Hypercholesterolemia; coronary heart disease; MDA; malondialdehyde.

INTRODUCTION

The world's population health problems are plagued by cardiovascular disease. Heart disease is one of the leading causes of death worldwide. As per WHO data, 56.5 million deaths were caused by non-communicable diseases, and 31%, or around 17.9 deaths, were caused by cardiovascular disease (1). This number is expected to grow rapidly and is estimated to reach 23.3 million in 2030. The most common cardiovascular disease-causing deaths worldwide are coronary heart disease (CHD), which affects 7.4 million people and is on the rise. According to the American Heart Association (AHA), 1 in 7 deaths in the United States is caused by coronary heart disease (2). Mortality rates related to coronary heart disease have increased in developing countries, including Indonesia (3).

In 2013, the Ministry of Health of Indonesia reported that CHD was the most predominant cardiovascular disease with a prevalence of 1.5%. In addition, it was reported that the prevalence of coronary heart disease increases with age, with the highest in the 65-74 age group (4). The highest rate was in East Nusa Tenggara (4%) and the lowest was in Riau (0.3%). In addition, it was reported that there was an increase in the prevalence of CHD with increasing age, where it was the highest in the age group of 65-74 years and decreased slightly at the age of ≥ 75 years. The prevalence of the disease is higher in women (0.5%) than in men (0.4%) (5).

According to the AHA, CHD is a heart attack due to atherosclerosis, which is a disease of the blood vessels in the arteries of the heart due to the buildup of cholesterol and residual metabolic substances. If this continues, there will be atheromatous accumulation in the walls of the arteries that supply blood to the heart muscle. This causes the blood vessels to thicken and narrow, which causes a reduction in the supply of blood and oxygen to the heart. Cholesterol buildup in the blood vessels occurs due to increased cholesterol levels in the blood above the average value of 200 mg/dL, called hypercholesterolemia (6).

Various factors can lead to hypercholesterolemia, including aging, obesity, genetics, smoking, low physical activity, stress, hypertension, and poor sleep quality. These conditions can cause the formation of lipid peroxidation. The end product of lipid peroxidation in the body is malondialdehyde (MDA), a dialdehyde compound. A high concentration of MDA describes the oxidation of cell membranes that can damage the cell membrane. The increase in oxidative stress and lipid peroxidation that occurs due to hypercholesterolemia can be countered by consuming antioxidants (7, 8).

Andrographis paniculata Nees, a very easy-to-grow plant in Indonesia, is a great source of antioxidants. *In vitro* and *in vivo* studies of *A. paniculata* have shown that the methanol extract of *A. paniculata* inhibits the formation of free radicals such as superoxide (32%), hydroxyl radicals (80%), lipid peroxidation (80%),

and nitric oxide (42.8%). *A. paniculata* Nees extracts also inhibited phorbol myristate acetate (PMA)-induced superoxide formation (32.4%) as well as nitrite oxidation (65.3%). When diabetic rats are given *A. paniculata* Nees for 28 days, total cholesterol, HDL, and LDL levels are significantly reduced (9-11).

MATERIALS AND METHODS

A post-test-only control group design was used in both the treatment and control groups. The experimental animals were male Wistar rats obtained from the Faculty of Veterinary Medicine Laboratory, Indiana, five weeks old and weighing 100-150 grams. The rats were divided into five groups of five each. Prior to starting the study, the researcher sought ethical clearance from the Undana Medical Faculty Ethics Commission. The Wistar rats which were obtained based on the criteria were housed in cages for seven days before they were weighed.

The treatment was conducted on experimental male Wistar rats, specifically the standard control group (K0); male Wistar rats were fed standard diets for 36 days. The negative control group (K1) of hypercholesterolemic male Wistar rats were fed standard feed for 36 days. Positive control (K2) hypercholesterolemic male Wistar rats at 08.00 hours were administered atorvastatin with a 5 mg/kg/BW probe per day for 14 days from day 21 to day 36. Treatment group 1 (K3) male Wistar rats hypercholesterolemia was treated with *A. paniculata* Nees with a dose of 200 mg/kg/BW per day for 14 days from day 21 to day 36. Treatment group 2 (K4) of hypercholesterolemic male Wistar rats at 08.00 hours were given *A. paniculata* Nees 400 mg/kg/BW per day for 14 days - from day 21 to day 36. At 08.00 hours, hypercholesterolemic male Wistar rats in group 3 (K5) were given *A. paniculata* Nees at 800 mg/kg/BW per day for 14 days from day 21 to day 36. The rats were anesthetized with ketamine and diazepam subcutaneously on day 36, 10 hours after the last dose, and then fixed. Specimens of blood and tissue were collected.

The TBARS method was used to determine MDA concentration through colorimetry. A blood sample was taken to check the MDA concentration through the retro-orbital sinus. In a polypropylene tube already containing 0.25 ml of thiobarbituric acid (TBA) solution, 0.75 ml of folic acid was added. To the tube, 0.05 ml of blood serum sample was added, followed by 0.45 ml of water. The mixture was shaken for two minutes. After being heated in a water bath for 60 minutes at 100°C, the mixture was cooled for 1-2 hours so that it reached 30°C. Wash with 5 ml of methanol and water after putting it into Sep-pak C 18. In a cuvette, 4 ml of methanol was added to the mixture. Color density was measured using a spectrophotometer with a wavelength of 532 nm (nmol/mg).

Statistical analysis

Data analysis was performed statistically with ANOVA. The ANOVA analysis showed statistically significant results or significant differences between the groups, with a p-value <0.05.

RESULTS

Total cholesterol data was the average number of each group, namely on day 21 (giving a high cholesterol diet for 14 days). The data obtained during the study can be seen in table 1.

Table 1: Average cholesterol levels of rats in each group

Group of test animals	Average total cholesterol level(mg/dL) Normal value: 10-54 mg/dL		
	Day 7	Day 21	Day 36
Without treatment	48.95	28.23	16.88
Negative Control	48.95	94.18*	73.97*
Positive Control	36.75	84.51*	25.06
Treatment 1	21.03	104.59*	18.39
Treatment 2	52.10	81.11*	39.81
Treatment 3	49.41	81.77*	17.39
Total	42.03	79.07*	31.92

Description: *high value

Five groups of test animals given a high-cholesterol diet for two weeks showed a dramatic increase in blood cholesterol levels. In the negative control group, cholesterol levels rose from 48.95 mg/dL to 94.18 mg/dL then a slight fall to 73.97 mg/dL. Meanwhile, the positive control group which was given atorvastatin showed a sharp decline in total cholesterol level from 84.51 mg/dL to 25.06 mg/dL after two weeks. According to the results between treatment groups 1, 2, and 3, all three had a significant drop in total cholesterol levels after being administered the *A. paniculata* Nees extract for two weeks.

The highest concentration of MDA was seen in the negative control group, which only received a high cholesterol diet (K3), and the lowest was found in the treatment group, which received 800 mg/kg BW (K6). MDA concentrations were normally distributed. Shapiro-Wilk normality test yielded p>0.05.

Additionally, the variance between homogeneous groups was p>0.05 (p=0.924). Using a one-way ANOVA test shows that the conditions for using parametric tests have been met for the analysis of MDA concentrations. A mean and standard error of the mean is shown in table 2 for the examination of MDA concentrations in this study.

Table 2: Average MDA concentration measurement results

	Group	Average ± SEM	*p*
MDA Concentration (nmol/ml)	K1 (No treatment) K2 (Positive control: Atorvastatin 5 mg/kgBB) K3 (negative control) K4 (*A. paniculata Nees extract* 200 mg/kgBB) K5 (*A. paniculata Nees extract* 400 mg/kgBB) K6 (*A. paniculata Nees extract* 800 mg/kgBB)	0.051 ± 0.02 0.049 ± 0.02 0.056 ± 0.01 0.042 ± 0.02 0.044 ± 0.02 0.039 ± 0.02	0.868

**One way* ANOVA: p<0,05. [a] *post-hoc* LSD: p<0,05

Description: normal group (K1), atorvastatin group 5 mg/kgBB + high cholesterol diet (K2), negative control group (K3), *A. paniculata* Nees group 200 mg/kgBB + high cholesterol diet (K4), *A. paniculata* Nees group 400 mg/kgBB + high-cholesterol cholesterol diet (K5), and the *A. paniculata* Nees group of 800 mg/kgBB + high-cholesterol diet (K6) (mean ± SEM).

Analysis with a one-way ANOVA test in all groups found no significant difference between each group with p>0.05. Therefore, post hoc LSD analysis was not carried out.

DISCUSSION

Statistical tests were carried out to see the effect of the *A. paniculata* Nees extract on blood MDA levels of hypercholesterolemic rats. The results of the statistical tests show that p = 0.868. This indicates that there is no significant difference between the groups. Several possibilities can occur, namely, high levels of antioxidant enzymes in the blood of rats and the time of blood sampling. MDA is a compound resulting from lipid peroxidation caused by an imbalance of oxidative compounds against antioxidants. Every living organism has different levels of antioxidant enzymes. In some mammals, including mice, several enzymes and particular mechanism pathways prevent the activity of oxidative compounds. The antioxidant enzyme studied in rats at high levels is GPx4 which has a high activity in neutralizing oxidized hydroperoxides from cholesterol, phospholipids, and lipoproteins. In addition, research has shown that selenium deficiency states (a role in GPx4 enzyme activity) and haploid against GPx4 transcription genes did not affect the low incidence of lipid peroxidation found in rats (11).

CONCLUSION

The results of the present study showed no effect of *A. paniculata* Nees extract on the levels of malondialdehyde (MDA) in male Wistar rats with a hypercholesterolemia model, statistically where p> 0.05.

ACKNOWLEDGMENT

This research was fully funded and supported by the Universitas Nusa Cendana, Indonesia.

CONFLICT OF INTEREST

There is no conflict of interest found during this study.

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