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Effect Of Roselle Petal Extract On Decreased Levels Of MDA In Rats With Type 2 Diabetes

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A B S T R A C T

Diabetes mellitus type 2 is a metabolic disorder, namely a decrease in peripheral tissues' response to insulin. Free radicals increase, which results in the forming of Malondialdehyde (MDA). Roselle flower petals is an antioxidant to reduce free radical damage. This study aims to analyze the effect of roselle petal extract on decreased MDA levels in mice induced by diabetes. The rats were divided into four treatments group as follow: 1) control negative, 2) control positive, 3) treatment dose I (administering roselle petal extract 195 mg/200 gram body weight), 4) treatment dose II (giving roselle petal extract 260mg/ 200 gram body weight). MDA levels were measured using Thiobarbituric acid (TBA) assay. There was an effect of rosella petal extract in the group with 260 mg/200 of rosella petal extract (p=0.005) and 195 mg/200 of rosella petal extract (p=0.041) compared with the positive control group. Rosella petal extract significantly reduces MDA levels in rats induced by type 2 diabetes.

INTRODUCTION

Diabetes type 2 is the most common type of diabetes. The pathogenesis of this diabetes type 2 is characterized by a metabolic disorder that leads to decreased peripheral tissue response to insulin (Kumawat *et al*., 2009). The increased levels of free radicals in the body damage the insulin receptor or the glucose transporter found in cell membranes of peripheral tissue. Lipid peroxidation is caused by excessive free radicals that will oxidize and attack the cell membrane's lipid components (Jusman and Halim, 2010). Along with the increase in free radicals, the lipid peroxidation of cell membranes also increases, resulting in the final product in the form of Malondialdehyde (MDA). To, the body needs antioxidants to reduce the damage caused by free radicals. Antioxidants are compounds that can neutralize free radicals by complementing the lack of electrons that free radicals have so that they become stable and inhibit the chain reaction of forming new free radicals (Erejuwa, Sulaiman and Wahab, 2011). Xanton, phenols, and flavonoids in rosella petals extract can work as an anti-oxidation capable of reducing negative impacts in the form of damage in the host body due to free radical compounds *Reactive Oxygen Species (ROS)*. Rosella petals contain calcium, vitamins C, D, B1, B2, magnesium, omega-3, beta-carotene, and 18 essential amino acids for the body. Rosella petals have anti-cancer, antihypertensive, and antidiabetic properties (Mardiah, Ashadi and Rahayu, 2009). High levels of antioxidants in roselle petals can inhibit free radicals. The active substances in roselle petals include

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gossypetin, anthocyanins, and glucoside hibiscus. Excessive exposure to free radicals, including kidney disease and diabetes mellitus, results in several chronic diseases (Hamzah, Ismail and Sandi, 2014). Roselle can prevent the development of atherosclerosis and cardiovascular complications due to diabetes (Maria, 2009).

Previous research by (Ulilalbab and Maskanah, 2018) reported an effect of treatment rosella on MDA levels. Other studies showed an effect of rosella extract in prediabetic patients, but its effectiveness was less optimal because of the lack of dosage (Mayasari *et al.*, 2018). Therefore, the researchers want to prove roselle petal extract's effect on reducing MDA levels in white rats induced by diabetes type 2. This study used different doses and times of experiment to reduce MDA levels more effectively.

METHOD

The in vivo stage used is the true experimental laboratory, a post-test control – treatment design randomized (CRD). The sample consisted of 24 male rats selected by random sampling. There were four groups: one negative control group (KN), one positive (KP), and two treatments (P1 and P2). The researchers gave roselle petal extract on two treatment groups (P1 and P2). The rats' treatment group provided roselle petal extract dose 195 mg / 200 g BW in P1 and 260 mg / 200 g BW in P2.

This study's tools and materials were: roselle petal extract, experimental mice, rat food (BR-1 Comfeed chicken pellets), TBA solution, TCA solution, chloroform, distilled water, injection syringe, non-EDTA tubes, capillary tube microhematocrit, gastric swabs, Eppendorf tubes, microtube, and mouse cages.

The authors obtained roselle petals from Joho Village, Semen District, Kediri Regency, East Java. The roselle petals extract used the brewing method with water solvent at 70 ° C while stirring for 15 minutes. Rosella petal extract was administered using gastric swabs and carried out for 21 days with each dose, namely dose 195 mg / 200 g BW in group P1, dose 260 mg / 200 g BW in group P2. The dose of rosella petals used was 3-4 roselle petals. So the dose for mice, namely: (10 x 1000 mg x 0.018 x 50/70) / 200 g BW = 128.6 mg / 200 g BW, equivalent to 130 mg / 200 g BW. The first dose, 1.5 x 13 mg / 200 g BW = 195 mg / 200 g BW. The second dose, which is 2 x 130 mg / 200 g BW = 260 mg / 200 g BW.

We took 0.5 ml of blood from the hearts of rats for the determination of biochemical parameters. Then put into a tube, centrifuged at 3000 rpm for 15 minutes at a temperature of 20^0C. We checked MDA levels by the TBA method from the serum of red blood cells. The wavelength of spectrophotometric was 532 nm with maximum adsorption. The Ethics Committee of the Faculty of Medicine, the University of Hangtuah Surabaya, approved this study with registration E/024/UHT.KEPK.03/IV/2020 dated 18 April 2020. The animal protocol followed an ethical review. Data analysis took three weeks because the time and dose were different. We did this research at the Laboratory of the Faculty of Medicine, Airlangga

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University, Surabaya. Normality analysis of the data used the Shapiro-Wilk test and homogeneity test with Levene's Test. Data were analyzed by one-way ANOVA using SPSS 16 software.

RESULTS

The results showed an overview of the MDA serum levels of strain rats in figure 1.

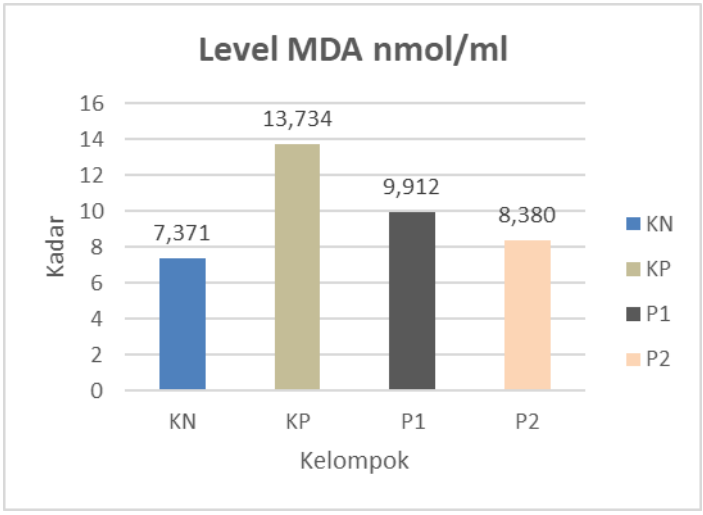

Figure 1. Average MDA Levels by Group

The average MDA levels were 8.380 nmol/ml in the P2 group, 9.912 nmol/ml in the P1 group, 13.734 nmol/ml in the KP group, and 7.371 nmol/ml in the KN group. The highest MDA levels were in the positive control group (KP), while the lowest MDA levels were in the negative control group (KN). In the treatment group, MDA levels close to the negative control group were the P2 group (Figure 1). The results obtained from this study preventively showed that the group treated with roselle petal extract along with alloxan administration showed a decrease in MDA levels compared to the group that was given only alloxan without being given roselle petal extract.

Table 1. Differences in MDA levels in various groups

Group	Unit	Average ± SD
Rosella petal extract dose 260 mg/200 g BB	P2	$8,380^{a}$ ± 2,219
Rosella petal extract dose 195 mg/200 g BB	P1	$9,912^{a}$ ± 2,295
Positive control	KP	$13,734^{b}$ ± 2,850
Negative control	KN	$7,371^{a}$ ± 1,503

The MDA level in the P2 was not significantly different from the P1 group and negative control group, where the significance value of rosella petal extract at a dose of 260 mg / 200 g BB was p=0.685, and the negative control was p=0.882 (p> 0.05). Positive control showed significant differences from all groups. Each of these groups had a significance value of p <0.05. Meanwhile, the P1 group's significance value was p=0.882 (p.>0.05), and in the P2 group was p=0.246. We analyzed the difference in dosage to see the effect between the dose of rosella petal extract and serum MDA levels. The treatment group of rosella petal extract at a dose of 260 mg/kg BW and a dose of 195 mg/kg BW had an average tendency to decrease MDA levels.

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DISCUSSION

There was a significant difference between positive group control (KP) and negative group control (KN) at 7.371 ± 1.503 nmol/ml and 13.734 ± 2.850 nmol/ml. Alloxan treatment increased the MDA levels in the positive control group. The imbalance between the formation of Reactive Oxygen Species (ROS) and antioxidants, where free radicals are higher than antioxidants, produce MDA in the body. Excess hydroxyl and peroxynitrite radicals can attack cell membranes and lipoproteins to produce lipid peroxides and MDA (Akim *et al.*, 2011). MDA is one of the end products where the radicals from lipid peroxidation are toxic to living cells. Besides, MDA is a parameter of free radicals in the body and is considered a biomarker to determine oxidative stress (Pirinccioglu *et al.*, 2010).

Research conducted by Suwandi (2012) reported a 28.1% decrease of MDA levels in the group given roselle petal extract at 250 mg and a 50.2% decrease of MDA levels in the group given roselle petal extract at 500 mg/kg BW. MDA in both groups given treatment in the form of rosella petal extract orally for 14 days. The mean MDA levels in the treatment group with 260mg of roselle petal extract and the treatment group with 195mg of roselle petal extract were lower than the positive control group. Roselle petal extract contains non-enzymatic flavonoid antioxidant compounds, total phenol, and high antioxidant activity (Hassoon, Ussain and Harby, 2018). Flavonoids are phenol group compounds that function as good reducers and inhibit many non-enzymatic oxidation reactions (Selawa, Runtuwene and Citraningtyas, 2013).

Flavonoids can significantly reduce MDA levels. Flavonoids are exogenous antioxidants to prevent oxidative stress. Flavonoids can work as antioxidants directly by donating hydrogen ions to neutralize the toxic effects of free radicals. Moreover, it has an indirect antioxidant impact by increasing endogenous antioxidant genes (Rasyid, Ismiarto and Prasetia, 2012).

CONCLUSIONS

MDA levels differ between the negative control group, the positive control group, and the treatment group. The treatment group given roselle petal extract at 260 mg is more effective in reducing MDA levels in mice with type 2 diabetes. The MDA levels in the positive control group have the highest levels compared to other groups. The group treated with rosella petal extract showed decreased MDA levels than the group without giving roselle petal extract. Further research should conduct additional analysis for the active ingredient components in rosella petal extract.

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