

Eleutherine Palmifolia Tuber Extract As An Antioxidant In Lowering Malondialdehyde Level Of Liver Tissue

Risqia Damayanti

Student of Veterinary Medicine Faculty, Airlangga
University
Surabaya, Indonesia
risqia_damayanti@yahoo.com

Anwar Ma'ruf

Postgraduate School Universitas Airlangga and
Departement of Veterinary Medicine. Airlangga University
Surabaya, Indonesia
anwarunair@gmail.com

Abstract—*The purpose of this study is to find out the influence of the extracted tubers of dayak onions (Eleutherine palmifolia) orally in lowering the malondialdehyde (mda) level of liver tissue in male white rats (Rattus norvegicus) induced by alloxan. The examined animals were 24 white rats of the Wistar strain divided into 6 groups. 1) negative control group K (-) were given aquabidest and CMC-Na 1% during therapy, 2) positive control group K (+), 3) K (0) oral drug metformin therapy 9 mg/200 gbw, 4) P (1) therapy by tuber dayak onion extract with an oral dose of 100 mg/kgbw, 5) P (2) therapy by tuber dayak onion extract oral dose is 200 mg/kgbw and 6) P (3) therapy per tuber dayak onion extract with an oral dose of 400 mg/kgbw. A group 2 to 6 alloxan were induced with a dose of 110 mg/kgbw in intraperitoneal. The therapy was done over 14 days, then the rats were euthanised with ketamine and examined for the mda levels in the liver tissue using the thiobarbituric acid (TBA) method. The data analysis results by oneway ANOVA (Analysis of Variance) test shows that there is a significant difference between group K(-) and group K(+). In addition, there is also an insignificant difference between group K(-) and groups P(1), P(2) and P(3). The results of this study have concluded that tubers of the dayak onion can lower liver MDA levels but an increase in the therapeutic dose did not have a significant effects.*

Keywords—*Eleutherine palmifolia; Antioxidant; Liver; Malondialdehyde*

I. INTRODUCTION

The liver is the largest gland in the human body. It is important for life because the liver has various biochemical and metabolic functions, including purging the body of substances that will spoil if left to accumulate. One of the diseases that has an effect on liver is diabetes mellitus. The latest data shows that the highest increase in the number of diabetes mellitus cases has actually happened in Southeast Asia. Diabetes mellitus also occurs in animals. Diabetics mellitus in animals chiefly occurs in dogs and cats by a prevalence of 0.4 – 1.2 % (1).

Diabetes Mellitus (DM) is a chronic disorder characterised by the impaired metabolism of glucose and

lipids due to defects in insulin secretion (beta cell dysfunction) or action (insulin resistance). The characteristic properties of diabetes mellitus are chronic hyperglycemia, and microvascular as well as macrovascular pathologies (2). Hyperglycemia-driven oxidative stress is a major contributor, enhanced by advanced glycation end product (AGE) formation and polyol pathway activation. There are several polymorphous pathways that lead to oxidative stress in the peripheral nervous system in chronic hyperglycemia (3). It is believed that oxidative stress plays an important role in the development of vascular complications in diabetes, particularly type 2 diabetes. ROS level elevation in diabetes may be due to the decrease in destruction and/or increase in production by catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) antioxidants. The variation in the levels of the enzymes makes the tissues susceptible to oxidative stress, leading to the development of diabetic complications. A critical biomarker of oxidative stress is lipid peroxidation, which is the most explored area of research when it comes to ROS. Malondialdehyde (MDA) is formed as a result of lipid peroxidation that can be used to measure lipid peroxides after making it react with thiobarbituric acid (4).

The purpose of this study was to find out the influence of the extracted tubers of the dayak onion (*Eleutherine palmifolia*) orally in lowering the malondialdehyde level of liver tissue in male Wistar white rats (*Rattus norvegicus*) induced by alloxan.

II. RESEARCH METHODOLOGY

A. The Examined Rats Preparation and Sampling

The white mice (*Rattus norvegicus*) were placed into mice cage made of a plastic tub and given a cage cover that was made of wire. The cage was placed in one place with normal temperature and humidity. The rats were kept and adapted for one week in a cage and were given feed in the

form of pellets and drink the *ad libitum* way. The rats that had adapted for one week were then given certain treatments.

The rats were divided into 6 groups. 1) negative control group K (-) were given aquadest and CMC-Na 1% during therapy, 2) positive control group K (+), 3) K (O) oral drug metformin therapy 9 mg/200 g bw, 4) P (1) therapy by dayak onion extract by an oral dose of 100 mg/kgbw, 5) P (2) therapy by dayak onion extract with an oral dose of 200 mg/kgbw and 6) P (3) therapy per dayak onion extract with an oral dose of 400 mg/kgbw. A group of 2 to 6 alloxan were induced with a dose of 110 mg/kgbw intraperitoneal.

B. The Alloxan Doses Determination

The diabetic condition of the examined rats was induced with an injection compound of alloxan monohydrate intraperitoneal of dissolved aquadestilata in a pro-injection solution with a dose of 110 mg/kgBB. Alloxan was injected with a dose of 22 mg/ 200 gBB/ 0.5 ml.

C. Measurements Of Blood Glucose Level

Before being checked, the animal test fasted first in order to avoid the effect of the feed when the blood glucose measurement was done. The amount of glucose in the blood in the fasted rats was ensured to be within normal limitations which was 50-109 mg/dl. The measurement of glucose in the blood in the therapy phase was done at day-11 and day-25. The amount of blood glucose was taken from tail-snipping, and measured using a glucometer.

D. The Extraction Of *Eleuthehrine palmifolia* Tuber

The clean and dry onion was sliced thin to about 3 mm slices. The next stage was drying in order to produce the dayak onion tuber powder.

The extraction was done by soaking the dayak onion tuber powder using 96% ethanol as an extractor. The simplisia powder settled for 3 days and was stirred at certain intervals. The maceration process was done in a room protected from the light. The vacuum pump was used in order to get a clear macerate. A clear macerate was collected in a container, and volatilised by using rotary evaporators at a temperature of 50°C until we obtained a thick extraction free of solvent.

E. The Taking Of Liver Tissue

At day 25, all of the rats were euthanised with ketamine in order to do the dissection processes and to take the liver. The dissection process started by placing the rats on a dissecting tray. The incision was made on the skin and the muscle of the thoracic and stomach area was parted using shears. The use of surgical tool meant that we had to be careful not to cut too deep and we kept the scissors leading upwards in order not hit the other organs. The dark-coloured liver was located under the diaphragm with 4 stamens. We took the liver and washed it using NaCl-Physiology 0.9 %, and after that we put into a Phosphate-buffered saline solution.

F. MDA Examination Levels

The sample preparation began with the liver tissues weighing 100mg being put in mortar and crushed until smooth, then 200 μ l NaCl 0,9 %, 550 μ l *aquadest* was added and homogenised. The homogenate was picked up and moved into a test tube.

The first procedure was done by adding 100 μ l TCA 10 % and homogenising it, then adding 250 μ l HCl 1N, 100 μ l Na-Thio 1 % and re-homogenising the solution. It was heated in a waterbath at 100 °C and set to stand in the room with a normal temperature. After that, it was centrifuged at 500 rpm for 10 minutes, supernatant was taken by 300 μ l, and then its absorbance was measured using a spectrophotometer at a wavelength maximum (λ_{maks} = 532nm).

III. RESULTS AND DISCUSSION

The statistical analysis results showed that there was a significant difference ($p < 0,05$) between the negative control group (K-) having MDA levels of an average 929.75 nmol/g and the positive control group (K+) having MDA levels averaging 1456 nmol/g. The negative control group (K-) compared to the drug control group (KO) had MDA levels averaging 858,50 nmol/g. There was no significant differences ($p > 0,05$). The negative control group was negative (K-) after the treatment. P1, P2, P3 had no significant differences ($p > 0,05$).

There was a significant difference ($p < 0,05$) between the positive control group (K+) and the drug control group (KO). The provision therapy of dayak onion extract tubers for 14 days with doses in the treatment groups P1, P2, P3 resulted in no significant MDA levels differences ($p > 0,05$) compared to the positive control group (K+). Oral drug metformin therapy in the drug control group (KO) had no significant difference compared to the treatment groups P1, P2, P3 on a therapy of dayak onion extract tubers.

MDA levels on average in the treatment groups P1, P2 and P3 did not show significant differences when treated by therapy of dayak onion extract tubers. The liver MDA level average of the three treatment groups was not significantly different to the rat group which was confirmed to be healthy (K-). This was because the extract from the tubers of dayak onions contain a derivative compound polyphenol that can be worked in to an antioxidant namely flavonoid. Flavonoids exhibit antioxidant activity by dual mechanisms; direct and indirect. The former is mediated predominantly via direct scavenging of ROS, the activation of antioxidant enzymes, metal chelating activity and the inhibition of oxidases. The basic structure of flavonoids, i.e., the number, positions and types of substitutions in the flavan nucleus, plays an important role in direct ROS scavenging and chelating activity. For instance, multiple hydroxyl groups are beneficial for antioxidant and chelating activity (5).

(*Rattus norvegicus*) with diabetes induced by alloxan could lower the liver's MDA level. The provision of an increase in the therapeutic dose does not exert an influence on the decrease in liver MDA levels.

TABLE I.

Treatment	MDA Levels \pm SD (nmol/g)
K(-)	929,75^a \pm 222,30
K(+)	1456,50^b \pm 420,11
K(O)	858,50^a \pm 118,59
P1	1028,75^{ab} \pm 463,65
P2	1088,75^{ab} \pm 383,29
P3	1126,25^{ab} \pm 303,84

different superscripts in the same column indicate significant differences

Table 1. The liver MDA Levels Average of white rats (*Rattus norvegicus*) male Wistar 14 Days Treatment.

IV. CONCLUSION

Based on the study, it can be concluded that extract of dayak onions tuber with doses of 100 , 200 and 400 mg/kgBW provided for 14 days in male Wistar white rats

References

- [1] Barrett. K., Barman. S, Boitano. S, Brooks. HL, *Ganong Review's of Medical Physiology*, Mc-Graw Hill Companies Inc, United States of America, 2012.
- [2] B. Monisha and V. Pushpank, "Reactive metabolites and antioxidant gene polymorphisms in Type 2 diabetes mellitus," *Redox Biology*. Elsevier. India, vol. 2. pp 170-177, 2014.
- [3] Mark A. Babizhayev, Igor A. Stokov, Valery V. Nosikov, Ekaterina L. Savel'yeva, Vladimir F. Sitnikov, Yegor E. Yegorov, Vadim Z. Lankin, *Cell Biochemistry and Biophysics*, Vol 71. Pp 1425, 2015.
- [4] Ullah, A. Abad, K. Ismail, K. "Diabetes mellitus and oxidative stress," *Saudi Pharmaceutical Journal*. Pakistan, vol 24, pp 547-553, September 2016.
- [5] M.K. Unnikrishnan, V. Veeresh, N. Yogendra, M. Piya Paul, M. Geetha, *Antidiabetic, Antihyperlipidemic and Antioxidant Effects of the Flavonoids*, Research Gate. India, Chapter 13, 2014.