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The Effect of Dayak Onion Bulb-Stem (*Eleutherine Palmifolia (L.,) Merr.*) Extract on Blood Glucose Levels of Mouse Suffered Diabetes Mellitus

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ABSTRACT

Dayak onions (*Eleutherine Palmifolia (L.) Merr.*) bulb stem contains phtyochemical contents, which act as antidiabetic compounds, such as eleutherol, eleuthocide A, and eleutherinoside B, as well as antioxidant compounds, which include triterpenoid, poliphenol, and flavonoid. Dayak onions was able to be used as the antidiabetic, since it had the ability to lower the blood glucose level and prevent from the free radicals, thus supressing the oxidative stress condition. This research had purpose to analyze the effect of Dayak onions bulb-stem as antioxidant and anti diabetic drugs. The research used experimental method with the population in this research was 25 male white mice strain wistar. The concentration of Dayak onions bulb-stem extracts were 300mg.kgBW^{-1}, 400mg.kgBW^{-1}, and 500mg.kgBW^{-1}. Data analysis used Tukey HSD Test with 95% of significance degree and was continued using manova test (average group ratio test). The result showed that. the extract of Dayak onions bulb-stem had the antidiabetical and antioxidant activity, which could lower the blood glucose levels and malondialdehid on the male white mice strain Wistar with the optimum effective doze of 500 mg.kgBW^{-1}.

Keywords: Dayak onions bulb-stem, Blood glucose level, Malondialdehid (MDA)

INTRODUCTION

Indonesia is located on the 7th position in the world among ten countries with the highest incidence of Diabetes mellitus after China, India, United States of America, Brazil, Russia, and Mexico, which reached 7.6 millions at the age of 20-76 years old in 2012. In 2000, the population of Diabetes Mellitus case diasease was 8.4 millions and estimated to reach 21.3 millions in 2030[(1)]. Most of diabetes mellitus consequence is macrovascular amd microvascular complications, such as heart attack and cataracs. Those disease caused death threat among the populations for 2-4 times greater than the non diabetic caused disease[(2)].

Herbal medicine has been passed down through generations due to the effective treatment with minimum side effects and cost relatively cheaper, although has yet been proved to cure any disease as it had not been acclaimed before by scientists. Nowadays, people are interested towards alternative and herbal medicine to cure wide range of degeneratibe disease as well as increasing the scientist interest in conducting research on plants and animals secondary metabolite that have medicinal properties. It was reported that about 800 plants had the potential of antidiabetic medicine.

Wardhani (2016) revealed there were useful metabolites of eleuthocide A, B and C on the liquid extracts of dayak onion, which also contained eleutherol, eleutherin, and isoeleutherin in methanol extracts of dayak onion (*Eleutherine palmifolia (l.) Merr.*)[(3)]. Li *et al.* (2009) also reported there were 15 types of naphthalene found in dayak onion that served as Wnt/b signal on the formation of the tumor inhibitor[(4)]. Ieyama et al. (2011) revealed the active the active compounds from dayak onion extract was eleutherinoside A, eleuthoside B, and eleutherol. These three active substances that serve as antidiabetical by way of inhibiting the alpha-glucoside formation[(5)].

Dayak onions also had some other active metabolites include triterpenoid, quinon, phenolate[6], flavonoids, and polyphenols[7]. According to Bettuzi *et al*. (2006), a compound of the polyphenols had strong antioxidant activity. This antioxidant of activity of polyphenol component was characterized by the relatively high activity as hydrogen or electron donor, as well as the ability of radical inhibited polyphenols to stabilize and move the electron pair (as the chain termination) and also the ability to inhibit the metal transition[8].

The dayak onion-bulb stem juice stew was traditionally believed to have a wide range of benefits, such as a cure for breast cancer, hypertension, diabetes mellitus, hyper cholesterol, and ulcers inflammation[9],[10]. Dayak onions as potential medicinal plants are very largely belived, but in the scientific studies regarding to the efficacy of dayak onion bulb stem are still unknown. This research aimed to analyze the effect of dayak onion bulb stem extract as antidiabetical and antioxidant drug from various dozes to decrease the blood glucose content on mice induced alloxan.

METHODS

This research was conducted for 2 months, started from August until September 2017. This research was held in the Animal Sampled Test Faculty of Animal Medicine Laboratory Universitas Airlangga for materials preparation, treatments, and animal sampling test, and blood samples taking, Balai Penelitian dan Konsultasi Ilmiah (BPPKI) Surabaya for dayak onion bulb-stem extraction, and Biochemistry laboratory of Universitas Hangtuah for MDA content analysis.

Materials used for this research were 24 male mice from Wistar strain weighed 175 – 200 g, alloxan monohydrate (*Aldrich cat no* : 7413-10G), dayak onion bulb-stem extract, metformin 500 mg.kgBW^{-1} of metphormine generic drug, sterilized water for injections Otsu-WI® as the alloxan diluter, chicken feed starter CP511, clear water, and blood mice serum.

Tools used during this research were animal sampled test cage made from plastic container with iron wired lid, the drinking bottle, 1 ml and 3 ml of terumo syringe® syringe for alloxan induction, sonde lambung euqipments for dayak onion extraction process, glucometer Easy Touch® GCU, glucose strip test Easy Touch®, Camry digital scale, and vacutainer tube for keeping the blood sample.

Dayak onion were retrieved from Palangkaraya, Central Kalimantan. Dayak onions were collected in dried and cleaned form. The extraction process was done using maceration method with ethanol 96% as the solvent. Dayak onion were grinded until becoming a powder and put into an Erlenemeyer tube added with a solvent, then soaked for 2-3 hours. The soaked onions were covered with alluminum foil. The extraction process was done approximately 24 hours during maceration with screening and obtaining the filtrate produced. The filtrate was obtained and evaporated along with the solvent using rotary evaporator at a temperature of 50°C until the extract was obtained which was free from the condensed solvent. The extract were freeze-dried and turned into powder.

Determining the doze of bulb-stem extract was based on the research of Febrinda *et al*. (2014) and Putri (2016), who used a doze of 100 mg.kgBW^{-1} and 400 mg.kgBW^{-1} as the optimum doze to the mice as the sampled animals[7],[11]. Other dosage variations was required to know the effectiveness of antidiabetic compound found in the dayak onion bulb-stem extract, thus this research used 300, 400, and 500 mg.kgBW^{-1} as the treatments dozes. The extract were given to the sampled mice perorally for 14 days in every treatments, which were composed of a negative control group (K0) without induction of alloxan and extract, a positive control group DM (K1) with induction of alloxan and solvent extract treatment, a positive control (K2) with induction of alloxan and Metphormine treatment, the first treatment group (P1) with induction of alloxan and 300 mg.kgBW^{-1} per day of dayak onion bulb-stem extract, the scond treatment group (P2) with induction of alloxan and 400 mg.kgBW^{-1} per day of dayak onion bulb-stem extract, and the third treatment group (P3) with induction of alloxan and 500 mg.kgBW^{-1} per day of dayak onion bulb-stem extract.

The alloxan dosage used was based on the research conducted by Febrienda *et al*. (2014) on the mice as the sampled animal who used the single doze of 120 mg.kgBW^{-1}. The metphormine dosage used for humans was 500 mgper 50 kg of BW or 12.6 mg per 200 g of BW in mouse after conversion[7].

The sampled mice were exhibited on the sampled animal cage and adapted for 1 week. The cages were placed on the normal temperature and humidity with ambient light. The mice were given feed and water ad libitum. A week after adaptation the mice were induced alloxan with the single doze of 120 mg.kgBW^{-1} with intraperitoneal method[7].

The blood glucose in the sampled mice were examined using the digital glucometer. The examined mice blood glucose was obtained in the blood vascular by tail dissection method. The hyperglycemic measurement was conducted 4 days afetr the alloxan induced. The blood glucose examination was done on 7 and 14 days after treatment given. Mice with hyperglycemic condition would have fasting blood glucose >135 mg.dL^{-1} [12].

This research used experimental laboratories method. All populations were assumed having the same criteria and characteristic. The initial measurement was not conducted due to the group treatments came from the same population. The data collected was the blood glucose level of the sampled mice. The statistical analysis for

the data collected was determined using IBM SPSS 16.0.The data normality was determined using Kosmolgorov-Smirnov One Sample Test continued with homogenity test used Levene Test. The data were analyzed using Tukey HSD test with 95% degree of significance to determine the difference between treatments and continued using Manova Test for determining the average comparation in every treatment groups ($\alpha<0.05$).

RESULTS

Dayak Onion Bulb-Stem Extract Contents

The content of dayak onion bulbu-stem extract was investigated before given to the sampled animal in Balai Penelitian dan Konsultasi Industri Surabaya. The result of dayak onion bulb-stem can be seen on Figure 1.

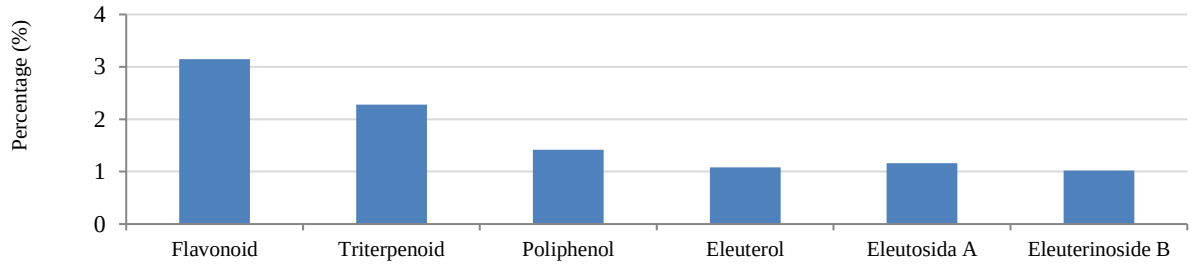

Figure 1. Dayak onion *(E. palmifolia L., Merry)* extract contents
Source: Balai Penelitian dan Konsultasi Industri Surabaya, 2017

Figure 1 showed that the dayak onion bulb-stem extract had the antioxidant types, such as flavonoid (3.15%), Triterpenoid (2.28%), Poliphenol (1.42%), and the antidiabetic types, such as Eleutherol (1.08%), Eleutocide A (1.16%), and Eleuterine B (1.08%)

Blood Glucose Levels

The average level of the blood glucose in the sampled animal before and after alloxan induced, as well as after 14 days of research conducted in each group treatments can be seen on table 1.

Tabel 1. Average levels of initial blood lucose and average levels of final blood glucose on 7 days (GDP Pre) and 14 days (GDP Post) of research conducted after the treatment given.

Treatment Groups	Initial Blood Glucose Level ($mg.dL^{-1}$)	GDP Pre ($mg.dL^{-1}$)	GDP Post ($mg.dL^{-1}$)
K0	86.75	99.00 ± 2.944	87.00 ± 2.000^{a}
K1	85.50	301.00 ± 102.326	270.25 ± 97.794^{b}
K2	88.00	200.25 ± 14.796	91.00 ± 14.900^{a}
P1	80.50	189.50 ± 23.331	118.25 ± 32.999^{a}
P2	88.75	210.50 ± 19.757	136.25 ± 30.977^{a}
P3	74.75	205.50 ± 15.984	82.25 ± 3.202^{a}

Note:
K0: Normal (No treatments given); K1: Alloxan + CMC-Na Solvent; K2: Alloxan + Metphormine; P1: Alloxan + 300mg.kgBW^{-1} dosage extract; P2: Alloxan + 400mg.kgBW^{-1} dosage extract; P3: Alloxan + 500mg.kgBW^{-1} dosage extract; Superscript shows significant difference ($p<\alpha$) based on Tukey Test using $\alpha = 0.05$

Based on table 1, it can be seen that the average of blood glucose level after alloxan induced increased significantly than expected (> 126 $mg.dL^{-1}$) after 7 days of research. The highest blood glucose level was shown on K1 treatment, which was 301.00 ± 102.326 $mg.dL^{-1}$. Table 1 also shows the decrease level of blood glucose after the dayak onion bulb-stem treatment with the lowest level obtained from the treatment P3.

Based on Figure 2, it shows that the final average blood levels on treatmen K1 was significantly different with the average blood glucose levels taken from K0, K2, P1, P2, and P3 treatments. Based on the general linier model manova test which was analyzed using Wilks' Lambda, the significance value was $p = 0.000$ ($p < 0.05$), which means that there was a difference between each of the average blood glucose levels in the treatment groups after given the dayak onion bulb-stem extract.

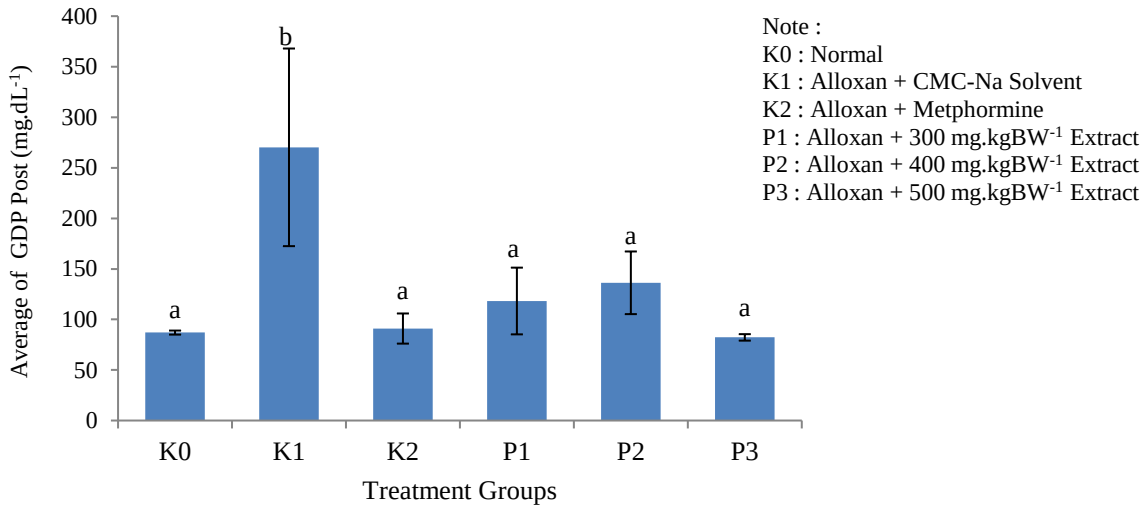

Figure 2. The Average Blood Glucose Levels after 14th days of research (Post GDP) after given dayak onion bulb-stem extract. (Different letters on the above diagram shows significant difference between treatments based on Tukey Test with $\alpha = 0.05$)

DISCUSSION

Dayak-Onion Bulb Stem Extract Contents

Dayak onion (*Eleutherine palmifolia l., Merr*) is a typical plant from Borneo Island. The plant has green leaves with white flowers and red colored bulbs that resemble the shape of two shallots tubes. In Indonesia, dayak onion has any other various other names, such as *sabrang* onion, *babawangan beureum siyem* onion, and *lubak* onions. The data results showed that the dayak onion bulb stem contains some metabolites and their derivaives, such as elecanacine naphtoquinones, eleutherine, eleutherol, eleuthernone[(6),(13)]. Naphtoquinones was known as the antimicrobial, antifungal, antiviral, and antiparasitic diseases. Naphtoquinones also had the bioactivity compund as the anticancer and antioxidant found cell vacuole forming in glycosides[(13)].

Dayak onion bulb stem is also be able to be used as the antidiabetic drug. Ieyama *et al*. (2011) revealed the active compounds from dayak onion extract was eleutherinoside A, Eleuthoside B, and eleutherol. These three active substances served as antidiabetic drugs by way of inhibiting the alpha-glucoside formation[(5)]. The results showed that the dayak onion extract contained triterpenoid compounds, quinon, phenolate, and naphtaquinon type, like elecanacine, isoeleutherine, eleutherol, eleutherine, eleuthernone[(6),(13),(14)].

Based on the test results conducted in Balai Penelitian dan Konsultasi Ilmiah (BPPKI) Surabaya, the dayak onion bulb stem extract contained some ontioxidants, i.e. 3.15% of flavonoid, 2.28% of triterpenoid, 1.42 of polyphenol, while the antidiabetic metabolites contained 1.08% of eleutherol, 1.16% of eleuthoside, and 1.02% of Eleuterinoside B.

The Blood Glucose Levels

The blood glucose level examination was determined in the 7th dan 14th day of the research in all treatment groups (K0, K1, K2, P1, P2, P3) after being fasted 8 before and used glucose meters. Blood was drawn through the tail end of the mice. The results showed that P1, P2, and P3 experienced the decreased blood glucose levels and the lowest blood glucose level was occured in the treatment P3, followed with K2 treatment. P1 and P2 also showed the decreased blood glucose contents, however they were still higher than P3. The results also showed that there was a significant difference found in all treatments, which was assumed as the extract of dayak onion bulb stem could lower the blood glucose level on the treatment groups (P1, P2 and P3). This condition was caused due to the different ability of the sampled animal, which affected the given result. The reslut was also caused by the high concentration of antioxidant found in the dayak onion bulb stem extract and if it was consumed on the higher doze as well, it would trigger the free radical compunds inside the body.

This research was in line with the research conducted by Wardani (2016) and Febrinda *et al*. (2014), who stated that alloxan compounds in the body would be reduced to dialuric acid, which produced the alloxan radicals[(3),(7)]. Alloxan radicals could reduce Fe^{3+} ion into Fe^{2+} ion. Fe^{2+} would react with the hydroxyl radical yield in H_2O_2. The hydroxyl radical would damage the pancreas beta cells. The bioactive compounds, such as polyphenols in the dayak onin bulb stem extract had the ability to donate their hydrogen atom to the free radical

compounds, as well as increasing the metal chelation, thus breaking the oxidative chain and helping release the oxidative stress that occured in the pancreatic tissue.

The influence of the dayak onion bulb stem extract through the pancreatic beta cells was statistically the same with the treatment given Metphormine. Metphormine was the commercial antidiabetic drug which was effective to cure the diabetes disease by increasing the hormone insuline secretion. This research also proved that there was any other hypoglycemic mechanism found dayak onion bulb stem extract to inhibit the Alpha-glucosidase formation, which causes the decreased beta cell producing insuline hormone.

CONCLUSION

The extract of Dayak onion Bulb-Stem (*Eleutherine palmifolia l., Merr*.) had the antidiabetical and antioxidant activity, which could lower the blood glucose levels on the male white mice strain Wistar with the most effective doze of 500 $mg.kgBW^{-1}$. It is suggested that to observe further about the effect of dayak onion bulb-stem extract on the hyperglychemic actitivity on the object samples given treatments.

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