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Effect of leaf extract Haramonting (*Rhodomyrtus tomentosa*) to lower blood sugar levels in mice induced by alloxan

Rosmidah Hasibuan[1], Syafruddin Ilyas[2], Saleha Hanum[3],

[1]College of Teacher Training and Education, Labuhan Batu – Rantauprapat, Indonesia
[2,3]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatra Utara. Jl. Bioteknologi I Kampus USU Medan 20155, Indonesia.

Abstract: Oral antidiabetic drugs mostly provide undesirable side effects, it is necessary to the development of a system of traditional medicine for diabetes mellitus is relatively safe. This study uses haramonting leaf extract to lower blood sugar levels in mice induced by alloxan. The research was conducted with pure experimental method with 5 groups and 5 replications. Group-I, normal mice without treatment, as the base line. Group-II, alloxan-induced diabetic mice + standard diet. Group-III, diabetic mice (given suspensions of the aqueous extract of haramonting leaf with dose of 50 mg/kg). Group-IV, diabetic mice (given suspensions of the aqueous extract of haramonting leaf with dose of 100 mg/kg). Group-V, diabetic mice (given suspensions of the aqueous extract of haramonting leaf with dose of 200 mg/kg). The suspension is given for 11 consecutive days orally. Then measured the blood sugar levels of mice on day 3rd, 5th, 7th and 11th after administration of the test material. On the 11thday of test animals dissected and parts used to test the pancreas organ histology. Results of the study found that aqueous extract of haramonting leaf have an activity to lower blood sugar levels alloxan-induced diabetic mice. Aqueous extract of haramonting leaf at a dose of 100 mg/kg is the dose that gives the best effect in lowering blood sugar levels in mice.

Introduction

Diabetes mellitus (DM) is a symptom that can be characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, fats and proteins associated with the deficiency of insulin secretion or insulin action both in absolute and relatif[1]. This is caused by the pancreas as the insulin manufacturers do not produce insulin in sufficient quantities larger than needed by the body, so that the burning and the use of carbohydrates is incimplete[2]. Tan & Kirana[3], disclose, diabetes mellitus is characterized by high levels of glucose in the blood. This disease requires attention and medical treatment in good time to prevent complications and pain treatment. Nothing diabetes mellitus is a disease caused by genetic or heredity is called diabetes mellitus type-1 and the resulting lifestyle called diabetes mellitus type-2.

Diabetes mellitus is a disease that is very difficult to treat. Treatment of the disease is mostly done by way of insulin therapy. By Anuradha *et al.*[4], insulin therapy and oral hypoglycemic drugs offer effective glycemic control, but insulin therapy has drawbacks such as the ineffectiveness of the oral administration, a short half-life, the need for direct cooling, and fatal hypoglycemia can occur in cases of overdose. The use of oral antidiabetic have adverse side effects including haematological reactions, cutaneous and gastrointestinal, coma, hypoglycemic and impaired liver function and kidney[5]. Because oral antidiabetic drugs most side effects

are undesirable, then the experts developed a system of traditional medicine for diabetes mellitus is relatively safe[6]. One way is to use traditional medication grown-plants[7].

Previous studies have shown that the chemical constituents isolated from plants have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and higher blood pressure[8]. Indonesia is one country that many use as a traditional medicinal plants. Lately usage is increasing in line with the advancement of knowledge about the properties of grown-plant[9]. Plants that have potential as a drug is haramonting. But the plant has not been widely used by the community.

Patil[10] said plant haramonting (*Rhodomyrtus tomentosa*) containing compounds triterpenoids/steroids, alkaloids and flavonoids. Alkaloids and flavonoids is one of the content of secondary metabolites that are widely used as a drug, among others, to treat skin disorders, diabetes, menstrual disorders, malaria and anti-inflammatory. While the compounds triterpenoids/steroid saponin is widely used as a raw material for the manufacture of steroid hormones, and until now the search for plants producing more and more done sapogenin[11,12].

Haramonting can be used as an herbal remedy. Chuakul[13]; Sutomo *et al*[14]; Lai *et al*.[15], that the people of Kalimantan and South Thailand utilize this plant as an anti-diabetic medications, diarrhea, burns, and abdominal pain. Haramonting leaf extract contains methanol which can lower blood glucose in blood[16,17]. Flavonoid content results in leaves and fruit haramonting may be free-radical scavengers (anti-oxidants) for human[18,19,20].

Extract is a collection of compounds from various groups dissolved in a suitable solvent, including active compounds or are not aktif[21,22]. Selection of solvent is very important in the extraction process so that nutritious ingredient that may be taken to be drawn perfectly. The Department of Health recommends aqueous extract extracts of raw materials for traditional medicinal purposes[23]. Related research has been done several times haramonting of which do Sulistyo *et al*[17], which tested the activity of methanol extract of leaves haramonting and provide a significant effect on blood sugar levels drop test animals at doses of 200 mg/kg.

Eperimental

Animal Experiments

The study sample consisted of 40 mice (*Mus musculus* L.) DDW strain, aged 8 -11 weeks, weighing 22-38 grams were obtained from the Laboratory of Animal Physiology of Faculty of Matermatics and Natural Sciences - University of Sumatera Utara) Medan. Mice were kept in plastic cages the size of 30x20x10 cm^3 and covered with wire gauze with a basic enclosure coated rice husk 0.51 to 1 cm and replaced every 3 days. The cage is set to 12 hours of light and 12 hours of dark and room temperature. Feed (CP 551) of Pockphan-Tanjung Morawa and drinking (tap water) provided each day at *adlibitum*. Maintenance of laboratory animal experiments are placed in Life Sciences USU Medan. This study was initiated after approval Research Ethics Committee of the Faculty of Mathematics and Natural Sciences USU Medan.

Collection, Manufacture and Production Crude Extracts-Aqueous

Haramonting leaf sampling (*Rodhomyrtus tomentosa*) undertaken purposively fresh green and was obtained from District of Gunung Tua - North Padang-Lawas. Simplicia made by drying the leaves Karamunting in conditions protected from direct sunlight in a greenhouse. Samples were left for a week. After drying, the leaves are crushed to powder Karamunting using a smoothing machine (grinder) to measure a certain subtlety. Manufacture of extracts made by soaking the bulbs for 24 hours with 100 grams of crude drug composition in 1 liter of water solution (maceration). Then do the solvent by evaporation to separate the extraction results. The selected temperature in the evaporation process is 60-70°C (Aqueous Extract of Haramonting Leaf/AEHL)[24].

Phytochemical analysis of leaves Haramonting[25]

Alkaloids testing using a reagent Dagendorf, Meyer, and Wagner. Test method saponin foam on boiling water that has been cooled. Flavonoids and Phenolic test, using NaOH 10% (w/v) or concentrated H_2SO_4. Test Triterpenoid and Steroids with Liebermen Buchard reagent test by adding $FeCl_3$ Tanin 1% (w/v).

Action Procedure of the Test Anti Diabetes in Mice with Induction of Alloxan

Animals were induced alloxan, then fattened prior alloxan injected intra-peritoneal (ip). Test antidiabetic based methods Tanquilut *et al*[26]. Experimental animals were fasted for about 18 hours. Then the weight was weighed and measured fasting blood sugar levels by means of Accutrend GCT (Roche). Alloxan solution of 200 mg/kg administered intraperitoneal (ip)[27]. Then measured the blood sugar levels of mice on days 3 and 7. On 7th day, animals that had higher levels of blood sugar (blood sugar) is higher than 200 mg/dL is separated and used as test animals. Animals that have a lower blood sugar level of 200 mg/dL induced back. If the test animals at 7thday showed blood sugar levels over 200 mg/dL, then the animal can already be given the test material. Blood sampling performed a total of 1 drop through mice. Mice were randomized into 5 groups, each consisting of 5 mice are then given oral treatment.

The study consisted of five groups. P0: normal mice without treatment, as the base line. P1: alloxan-induced diabetic mice + standard diet. P2: diabetic mice (given suspensions of the aqueous extract of haramonting leaf with dose of 50 mg/kg). P3: diabetic mice (given suspensions of the aqueous extract of haramonting leaf with dose of 100 mg/kg). P4: Mice diabetes (given suspensions of the aqueous extract of haramonting leaf with dose of 200 mg/kg).

The suspension is given for 11 consecutive days orally. Then measured the blood sugar levels of mice on day 3, 5, 7 and 11 after administration of the test material.

Statistical Analysis

Analysis of data using analysis of variance (ANOVA) Completely Randomized Design (CRD) at the 95% confidence level, $\alpha = 0.5$ with Bootstrap analysis. All data were analyzed using SPSS 22.

Result and Discussion

Phytochemical screening Leaves Haramonting

Results of the screening of secondary metabolites of given suspensions of the aqueous extract of haramonting leaf shows that it contains flavonoids, and tannins, phenolic, and terpenes in plants, the result is the same as that in the can by Patil[10], that plant haramonting (*Rhodomyrtustomentosa*) contains phenolic compounds, triterpenoids/steroids, and flavonoids (Table 1).

Table 1 The content of secondary metabolites in plant leaves *R. tomentosa*.

Phenolic/ Reagen	Terpenoid	Alkaloid				Saponin	Flavonoid	Tanin
$FeCl_3$	$CeSO_4$/ TCL	Bouchardat	Wagner	Mayer	Dragen	H_2O (Kocok)	$FeCl_3$	$FeCl_3$
++++	++	-	-	-	-	-	++++	+++

The results also showed that the alkaloids and flavonoids are compounds contained in extracts of leaves haramonting. Compounds alkaloids and flavonoids have an effect on health, therefore this plant can be utilized as a drug ingredient, as did the community Borneo, who use the leaves of this plant as an anti-diabetic drug. The same thing expressed by Sangi*et al*.[29], alkaloids and flavonoids have a function in the health field such as anti-hypertensive and anti-diabetesmellitus. According to Rahman*et al*.[29], function activity alkaloid compounds are anti-bacterial and anti-fungal. Flavonoids also have the effect of preventing bleeding skin.

Based on TLC testing quersetin Flavonoid compounds also contained in a suspensions of the aqueous extract of haramonting leaf. The compound is an active component derived from nature and is a marker or marker compound which is used as one of this parameter the quality of a product of nature. One of the properties is to lower the blood sugar levels of people with diabetes mellitus. Quersetin flavonoids have the ability to lower blood sugar levels of diabetic mice induced streptozotosin[30].

Tannin or phenol were also found in the given suspensions of the aqueous extract of haramonting leaf can lower blood sugar levels in people with diabetes mellitus, through the capture of free radicals and

antioxidants[31]. Because diabetes mellitus is closely associated with necrosis of the cells caused by radical bebas[32].

Steroid compounds contained in the given suspensions of the aqueous extract of haramonting leaf have hypoglycaemic activity. Mahera*et al.*[33], reported that the sterol compounds found in mangrove *Avicennia marina* (Forssk.) has anti-glycation activity or glycosylation of glucose and fructose, which is a degenerative disease process of type 2 diabetes mellitus and other diseases.

Blood sugar levels before and after induction Alloksan

Induction of diabetes mellitus done on day 0 using alloxanintraperitoneally at a dose of 200 mg/kg and the mice were allowed until 3 to 5 days. Mice were fasted before alloxan induced. Changes in blood sugar levels before and after induction can be seen as in Figure 1.

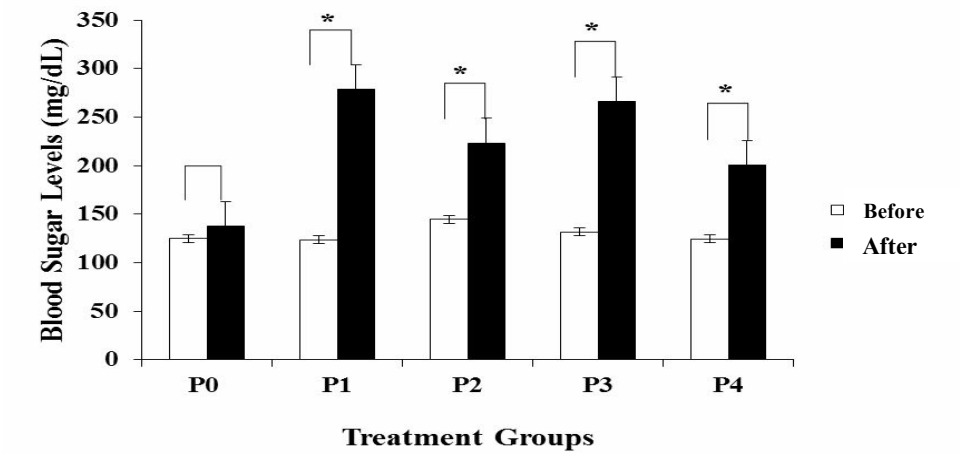

Figure 1. Mean blood sugar levels before and after induction of alloxan 200 mg/kg. The control group and the treatment group showed significant differences ($p<0.05$) is indicated with an asterisk (*), which did not differ significantly not marked (*). Note: P0=alloxan 0 mg/kg Body Weight(BW); P1, P2, P3, and P4=alloxan 200 mg/kg BW.

After induced by alloxan dose of 200 mg/kg, visible response varies from animal experiments. Approximately 80% of the mice had increased blood glucose>200 mg/dL, 17.5% experienced only a slight increase in blood glucose levels or not at all, and even 2.5% of the experimental animals died before 3 days. This situation is caused by the resilience of the experimental animals are different. This result is similar to that obtained Siregar[34] that alloxan inducing a dose of 200 mg/kg intraperitoneally can increase blood sugar levels in mice.

Activities of the aqueous extract of haramonting leafagainst blood sugar levels before and after treatment

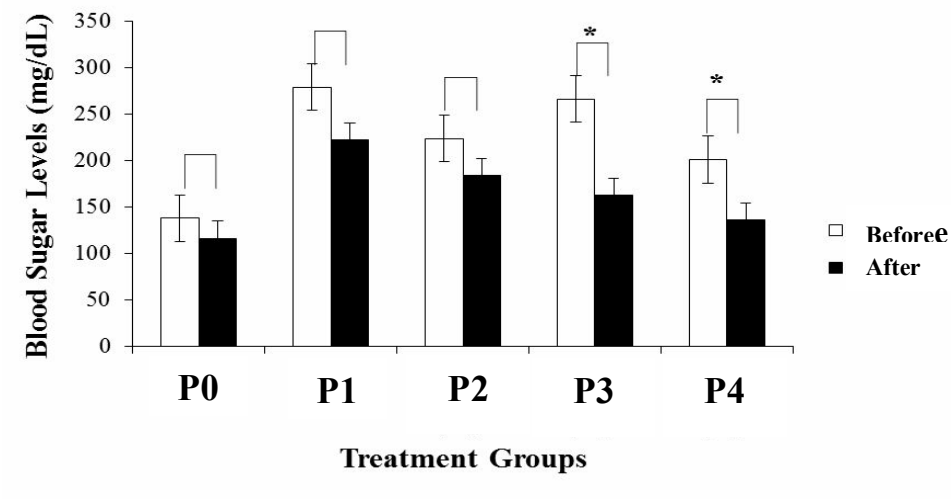

Figure 2.Changes in blood sugar levels in mice before and after the study treatment. Treatment group and the control group showed significant differences (p<0.05) is indicated with an asterisk (*), which was not significantly different (p>0.05) was not marked (*). Note; P0=Control, P1=Alloxan, P2=Alloxan+ WEHL 50mg/kg body weight/BW, P3=Alloxan+ WEHL 100mg/kb BW, P4=Alloxan+ WEHL 200 mg/kg BW).

Furthermore, mice treated the aqueous extract of haramonting leaf with different doses, for 8 days. From the data the average blood sugar levels are obtained each of the 5 groups can be seen changes in blood sugar levels before and after each dose for treatment as shown in Figure 2.

This study showed that there was a mean decrease in blood sugar levels in diabetic mice group treated the aqueous extract of haramonting leaf. Where the average reduction in blood sugar levels is highest in the treatment group of the aqueous extract of haramonting leaf with dose of 100 mg/kg BW, with a mean decrease of 103.45 mg/dL. This is slightly different to that reported by Sulistyo et al.[17], that the ethanol extract of leaves haramonting dose of 200 mg/kg was the best dose in lowering blood sugar test animals. This difference is thought to be caused by differences in the manufacture of solvent extract.

While the decline in the average of the lowest blood sugar levels occurred in the treatment group of the aqueous extract of haramonting leaf at a dose of 50 mg/kg, with a mean decrease of 35 mg/dL. Similar results were also obtained Sulistyo et al.[17], haramonting ethanol extract of the leaves of a dose of 50 mg/kg body weight mempu only lowers blood sugar test animals <50 mg/dL. This is presumably due to the low levels of flavonoid compounds contained in the aqueous extract of haramonting leaf with dose of 50 mg/kg. Quesertin flavonoid compounds are compounds that have potential as an anti-diabetic. According to Jadhavet al.[30], one of the properties of the flavonoids quesertin is capable of lowering blood sugar levels in people with diabetes mellitus.

Statistical analysis showed that aqueous extract of haramonting leaf revealed significant effect ($p<0.05$) decrease in blood sugar levels in mice (*Musmusculus* L.). Where the Statistical analysis showed that aqueous extract of haramonting leaf on 3rdand 7th days do not affect blood sugar levels, but the effect on the 10th day and to 16 after administration (Appendix 1). This may imply that the longer time the aqueous extract of haramonting leaf getting better at lowering blood sugar levels in mice with diabetes.

Figure 2 also shows that the activity of the aqueous extract of haramonting leaf in lowering blood sugar levels in mice induced alloksan not in line with the increase in the dose administered. In the aqueous extract of haramonting leaf with dose of 200 mg/kg showed a decrease in blood sugar levels by 65 mg/dL. This is likely due to the amount of steroid compounds/terpenes higher in the aqueous extract of haramonting leaf causing hyperglycemia, as reported by Lansang[35] that, or glucocorticoid steroid compounds may cause hyperglycemia blood. Increased doses of the aqueous extract of haramonting leaf until a dose of 200 mg / kg showed increased

hypoglycemic activity. This indicates that the active components of chemical compounds in the aqueous extract of haramonting leaf have a synergistic effect. Velazquez et al.[36], stating that complementary alternative medicines derived from nature have a synergistic effect in treating a disease.

Brahmachari[37], bioflavonoida review of the mechanisms contained in the plant that produced the effect of a decrease in blood glucose levels. Several mechanisms of bioflavonoida in lowering blood glucose levels is the prevention and improvement of glucose absorption of glucose tolerance, inhibition of α-glucosidase activity of the intestine. Flavonoida also acts as an insulin secretagogue or insulin mimetic, stimulating the use of sugar in peripheral tissues and regulate the activity of enzymes involved in the metabolic pathway of carbohydrates. Flavonoida mechanism that stimulates the synthesis of glycogen in the muscles of mice.

The results obtained that the aqueous extract of haramonting leaf has antidiabetic effects, it is supported by the presence of the chemical constituents contained in the extract are saponins and flavonoids because based on the results of studies suggest that the compounds are useful as antidiabetic compound saponin (Masayuki *et al*.[38], also the presence of other compounds such as flavones (flavonoids)[39].Based on observations during the writing and discussion can be concluded as follows: aqueous extract of haramonting leaf has an activity lowers blood sugar levels alloxan-induced diabetic mice. Aqueous extract of haramonting leaf at a dose of 100 mg/kg is the dose that gives the best effect in lowering blood sugar levels in mice.

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