The Effect Of The Methanol Extract Of Cemba's Leaves (*Acacia pennata*) In Lowering Blood Glucose Levels Of Male Mice (*Mus musculus*)

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Abstract: The plants Cemba (*Acacia pennata*) included is family Leguminoceae, this is plants specific in Enrekang, South Sulawesi. The research to knowing the effect of the methanol extract Cemba to decrease blood glucose levels in mices male ICR strain induced by alloxan. Cemba's leaf are extracted using 96% methanol by maceration. This research used of randomized design (CRD) that Consist of five treatments and five repetition. The extract was dissolved using Carboxy Methyl Cellulose (CMC) 0.5% and given orally in mice that had been injected previously alloxan 120 mg / kg intraperitoneally. The extract is given different doses: 125, 250 and 500 mg / kg. Data were analyzed using Analysis of Variance (ANOVA) using SPSS Program is 2.0 and Followed by Duncan test (α 0:05), as control drugs using glibenclamide 5 mg / kg and normal control using CMC 0.5%. The results showed the methanol extract of leaves with a dose of 250 mg / kg had a most effective to decrease blood glucose levels for 14 days cemba leaf extract. Reviews these results were significantly different with normal control (105.6 mg / dl) and drug control (105.80 mg / dl), as well as groups of mice that were given the leaf extract cemba 150 and 500 mg / mm. Cemba's leaf extract shows the effect of the decrease in blood glucose levels in mices male.

Keywords: Cemba leaf extract, family Leguminoceae, glucose level, district Enrekang

INTRODUCTION

The pattern of less healthy lives is one of a variety of diseases in the community. One of the diseases that the number of cases has increased significantly in the last ten years and the sixth leading cause of death around the world is diabetes mellitus (Smeltzer et al., 2009). World Health Organization (WHO) states that Indonesia ranks sixth in the world as a country with a number of his patients with diabetes mellitus after India, China, the Soviet Union, Japan, and Brazil. Recorded in 1995, the number of diabetics in Indonesia reaches 5 million with an increase of 230,000 patients with diabetes per year, so that by 2005 is expected to reach 12 million people. (Soegondo, 1999). Diabetes mellitus can be treated with antidiabetic drugs either in oral dosage form or injection. The drugs available have side effects, but could not finish the diabetes perfectly with the workplace in accordance with the mechanism of diabetes type2, because the mechanism itself is very complicated (Kurniawati, 2011).

Treatment is not cheap for diabetes mellitus because the patients should take the drug in the long term. Whereas medicinal chemical synthesis (conventional medicine) are consumed and circulated in the market are quite expensive. Good with oral hypoglycemic agents or insulin was possible to control diabetes mellitus, some medicinal plants can also be used empirically and both a pharmacological treatment (aquilar et al., 2006). WHO recommends the use of traditional medicine, including herbs in the maintenance of public health, prevention and treatment of disease, especially for chronic diseases, degenerative diseases and cancer. WHO also supports efforts to increase the safety and efficacy of traditional medicine (WHO, 2003).

One of the traditional medicines that is growing toward ***fitofarmaka*** are antidiabetic drugs (Moningkey, 2000). According to the Ethnobotanical report, there are 800 species of plants as a potential anti-diabetic (Singh, 2010). Some of them that leave paliasa (*Kleinhovia hospita L.)* (Yuliana, et.al, 2013), the leaves of Moringa (*Moringa oleifera*,) (Aini, et.al .2015), meniran (*Phyllanthus niruri L.)* (Fahri, et. al., 2005). Cemba plant (*Acacia pennata*) is a typical plant Enrekang the leaves are used as a flavor enhancer in food by people who are often referred to nasu cemba. Traditionally, Acacia pennata has been used as a medicinal plant to treat coughs, headaches, rheumatism and fever in certain areas of Myanmar (Kim, et.al, 2015). Based on the description above, it is necessary to study the utilization of methanol extract of leaves cemba as medicine antI-hiperglikemia. This research is expected to lower glucose levels of male mice with administration of methanol extract of cemba leaves.

RESEARCH METHODS

This study is an experiment conducted in April - June 2016, research conducted in the laboratory of Biology and Chemistry Laboratory UNM.

A. Animal Testing

Test animals used were ICR male mice strains, healthy, and normal activities are kept in the Laboratory of Biological Science UNM. 2 months old mice as much as 25 animals were divided into 5 groups, each consisting of 5 mice in one cage. Body weights of mice ranges from 20-30 grams were divided into 5 groups.

B. Experimental Design

Mice were adapted for 1 week with given commercial feed in the form of standard feed flour AD II and drinking water ad libitum before injecting alloxan to create conditions hyperglycemic. In the second week, mice were grouped according to their group. Groups of mice were determined based on body weight were divided into 5 groups. Treatment as follows:

1. Group I (negative control), namely the Group of male mice are only given standard feed during the trial period
2. Group II (positive Control) as a group of male mice given standard feed during the trial period. Day 7 was injected alloxan 120 mg/kg BB and 5 mg/kg BB glibenklamid.
3. Group III, groups of male mice fed a standard during the trial period. Day 7 was injected alloxan 120 mg / kg BB. Day 9-23 cemba given leaf extract at a dose of 125 mg / kg BB.
4. Group IV, groups of male mice fed a standard during the trial period. Day 7 was injected alloxan 120 mg / kg BB. Day 9-23 cemba given leaf extract at a dose of 250 mg / kg BB.
5. Group V, groups of male mice fed a standard during the trial period. Day 7 was injected alloxan 120 mg / kg BB. Day 9-23 cemba given leaf extract at a dose of 500 mg / kg BB.

C. Tools and Materials

a. Tool
The tools that used in this study is oven, gauges blood sugar levels (NESCO), strips of blood sugar, beaker (Pyrex) 250 ml and 1000 ml Erlenmeyer flask (Pyrex), analytical balance, Ohaus balance, measuring cups (Pyrex), stir bar, scissors, wire rang test, funnel, Buchner funnel, blender, syringe, the drip plate, a pipette, oven, syringe and cage maintenance of test animals.

b. Material
Cemba leaf, 96% methanol, mice (Mus musculus) strain Sprague Dawley 2 months old weigh 20-30 grams, alloxan, feed the mice AD II, alcohol 70%, filter paper wathmen 41, glibenclamide, alloxan and tissue.

D. Sterilization Equipment

Used for the sterilization of the oven. It is intended that the tools used are free of microorganisms.

E. Work Procedures

a. Making Powder of Cemba Leaves
Cemba leaves 2,000 g of washed, dry wind dried up, once dried, blended into a powder. Cemba leaves that will be used are the older leaves are dark green.

b. Sample extraction
Leaf powder cemba 500 grams soaked with 1500 ml of methanol 96% for 24 hours at room temperature, soaking repeated three times. Methanol is used as solvent in this study because methanol is the Universal so it can dissolve polar and non-polar compounds. As according to (Thompson, 1985), methanol can draw alkaloid, steroid, saponins and flavonoids from the plant. Results marinade or maserat filtered using filter paper and then concentrated by rotary vacuum evaporator to obtain a thick extract (Christian et al, 2013; Kusuma et al, 2014; Suratiningsih et al, 2013).

c. Measurement of Blood Glucose
Measurement of blood glucose levels on day 0 as baseline or normal blood glucose levels in mice, day 9 after treatment administration as a data alloxan hyperglycemia, and a day to 23 as the data treatment.

d. Induction process Alloxan
Alloxan was given on the seventh day after the initial measurement of blood glucose levels, in induction by means of intraperitoneal injection at a dose of 120 mg / kg in mice. Induction is considered successful if it occurs within 48 hours of a trend of rising blood glucose levels were measured after the mice were fasted for 10 hours (occurring hyperglycemia). Dosing alloxan referring to research Yuliana et.al (2013) on the granting of the methanol extract of leaves paliasa lowering blood glucose levels of mice hyperglycemic.

e. Leaf Extracts Cemba Processes To Male Mice
Male mice are held and clamped the neck with his fingers. Male mice are conditioned as comfortable as possible so as not to stress. Fill the syringe with cemba leaf extract as much as 1 mL and then orally administered to test animals male mice.

f. Blood Sampling Process In Male Mice
Male mice in 70% alcohol wipe using cotton. Male mice stretched and cut about 1 mm from the tip of the tail with a sterile razor blade or scissors. Blood in blood sugar is contained in the strip as much as 15 mL. Glucose levels were tested using a glucose meter multi check Nesco brand. Male mice rubbing alcohol so that blood does not flow continuously and given antibiotics.

g. Data analysis

The data were determined based on the response of each animal in each treatment trials against cemba leaf methanol extract using ANOVA (Analysis of Variance), followed by Duncan test α of 0.05 using SPSS statics 22.

RESULTS AND DISCUSSION

A. Research Result

Table 1 Average glucose levels in mice male at the first measurement, the second measurement and the third measurement.

No	Treatment	The average blood glucose levels (mg / dl) of male mice			The percentage reduction in glucose levels (mg / dL) of male mice
		phase I	phase II	phase III	
1	Normal control	107,600[a]	103,800[a]	105,60[ab]	-0,51 %
2	positive control	108,00[a]	228,00[b]	105,80[ab]	35,09 %
3	EDC(Ap) 125 mg/kg BB	105,80[a]	176,20[ab]	123,40[b]	15,16 %
4	EDC(Ap) 250 mg/kg BB	104,20[a]	186,80[ab]	88,00[a]	28,37 %
5	EDC(Ap) 500 mg/kg BB	101,80[a]	179,80[ab]	103,60[ab]	21,88 %

Description: The letters in the same column shows "no significant". Different letters in one column showed "significantly different". Different letters between columns one with another column showing "very different real" EDC (Ap) (*Cemba Leaf Extract* (*Acacia pennata*) (Duncan HSD ($\alpha = 0.05$).

On average glucose levels total (mg / dL) of mice (Mus musculus) male on the first measurement between all treatment groups (normal control group, the control group of drugs, the group give the leaf extract cemba (Acacia pennata) at a dose of successively 125 mg / kg, 250 mg / kg, and 500 mg / kg body weight of mice (Mus musculus) males showed no significant results and within are normal limits, ie 70-110 mg / dL. At the beginning of the measurement of glucose levels on average total glucose levels (mg / dL) of mice (Mus musculus) the highest male produced by the treatment of drug control group and normal control group, amounting to 108.00 mg / dL and 107.600. As for the average total glucose levels (mg / dL) the lowest in the treatment group EDC (Ap) 500 mg / kg, in the amount of 101.80 mg / dL.Measurements after induced alloxan produces an average total levels glokosa blood mg / dL were significantly different between the normal control group with a control group of drugs and the group given the leaf extract cemba (Acacia pennata) dose of 125 mg / kg, 250 mg / kg and 500 mg / kg body weight of mice (Mus musculus) male. Average rat highest level of glucose produced by the treatment of drug control and EDC (Ap) 250 mg / kg, in the amount of 228.00 mg / dL and 186.80 mg / dL. For the measurement of low glucose levels produced by normal control treatment, which amounted to 103.800 mg / dL is still within the limits of normal glucose levels.

Measurements after being given the leaf extract cemba produce an average glucose levels total (mg / dL) of mice (Mus musculus) males showed different results are not apparent between the normal control group, drug control and EDC (Ap) 500 mg / kg but shows significantly different results between permberian EDC (Ap) 125 mg / kg and EDC (Ap) 250 mg / kg. Total glucose levels (mg / dL) were the highest in the group of EDC (Ap) 125 mg / kg, in the amount of 123.40 mg / dL and the average total glucose levels (mg / dL) were the lowest in the group given EDC (Ap) a dose of 250 mg / kg in mice (Mus musculus) amounted to 88.00 mg / dL.

Decreased levels of total glucose (mg / dl) mice (Mus musculus) males in each treatment after administration cemba leaf extract (Acacia pennata) dissolved with CMC (Carboxy Methyl Cellulose) 0.5% is administered orally in the control group showed normal decreased levels of total glucose in mice (Mus musculus) male but on the contrary there was an increase in total glucose levels of 0.51% mg / dL in the control group but the drug, EDC (Ap) 125 mg / kg, EDC (Ap) 250 mg / kg, and EDC (Ap) 500 mg / kg, reflecting the decrease in total blood glucose levels in mice (Mus musculus) males each successive 35.09%, 15.16%, 28.37% and 21.88%.

B. Discussion

Compounds alloxan diabetogenic is one of the substances those are toxic, especially against pancreatic beta cells, and when administered to experimental animals such as mice, it can lead to the experimental animals became hyperglycemic mice. Alloxan is a chemical used to induce diabetes in laboratory animals. Diabetogenic antagonistic effect with glutathione (Setiawan, 2010). Alloxan reacts by damaging substances essential in the cell β-pancreas causing reduced granule granule carrier of insulin in the cell β-pancreatic thus causing hyperglycemia. According Suastuti, et. al (2015), hyperglycemia caused by abnormal secretion of insulin, or a work interruption of insulin.

Before administering cemba leaf extract in mice (Mus musculus) is conditioned by way of inducting alloxan hyperglycemia via intraperitoneal injection at a dose of 120 mg / kg in mice. Glucose levels of mice (Mus musculus) males in the third measurement, ie after the methanol extract of leaves cemba in the control group of drugs and the treatment group the extract of leaves cemba (Acacia pennata) doses respectively 125 mg / kg in mice, 250 mg / kg BB mice and 500 mg / kg body weight of mice show decreased glucose levels. Provision of Carboxy Methyl Cellulose (CMC) of 0.5% was not able to lower blood glucose levels in the normal group, whereas there was an increase in blood glucose levels but within normal limits. While the provision of glibenkamid as antihiperglikemia drug capable of lowering blood glucose levels in mice that have hyperglycemia. The same effect was also shown by the methanol extract of leaves cemba (Acacia pennata) the dose of 125 mg / kg bw, 250 mg / kg bw, 500 mg / kg bw able to lower blood glucose levels in mice were experiencing hyperglycemia in which a dose of 250 mg / kg bw more both in lowering blood glucose levels.

Increasing the dose of medication should be improving response proportional to the dose increased, but the response will eventually decline, as already accomplished doses that were not able to improve the response again. This often occurs in natural medicines produced from extracts of natural materials, because they contain no components of a single compound but rather consists of a wide variety of chemical compounds, in which these components work together to effect. But with increasing doses, the number of chemical compounds which contained more and more, so that the interaction causes a decrease in adverse effects (Bourne and Zastrow, 2001).

Based on the results of this study each treatment the methanol extract of leaves (Acacia pennata) cemba, namely EDC (Ap) dose of 125 mg / kg, EDC (Ap) a dose of 250 mg / kg, EDC (Ap) dose of 500 mg / kg could potentially in lowering glucose levels in total mice (Mus musculus) male. This is due to secondary metabolites, such as alkaloids, saponins, triterpenes, flavonoids, and tannins contained in cemba leaf extract (Acacia pennata) that act as antioxidants.

Antioxidants can reduce the level of oxidative stress thereby slowing premature aging and complications of various diseases and alkaloids generally have physiological activity of a prominent, so that human alkaloids are often used for treatment. Ethnobotany study based on local knowledge in Enrekang that cemba leaves are often used as a treatment (Husain, 2015). Some of the Acacia genus is reported to contain secondary metabolites such as alkaloids, saponins, triterpenes, flavonoids, and tannins (Siegler, 2003). From some of the compounds contained in extracts of the genus Acacia (tannins, alkaloids and flavonoids), alkaloids and flavonoids are the active compounds of natural ingredients that have been studied have a hypoglycemic activity while the tannins act as an antioxidant and inhibitor of tumor growth.

Tannins also serves as an astringent or chelating which can be screwed epithelial membrane of the small intestine, thereby reducing the absorption of nutrients and consequently inhibit the intake of sugar and the rate of increase in blood sugar level is too high (Daliamartha, 2005). Alkaloid

works by stimulating the hypothalamus to increase the secretion of Growth Hormone Releasing Hormone (GHRH), so that the secretion of growth hormone (GH) on the pituitary increases. GH levels are high will stimulate the liver to secrete Insulin-like Growth Factor-1 (IGF-1). IGF-1 has the effect of inducing hypoglycemia and decrease gluconeogenesis that blood glucose levels and insulin demand decreases. Flavonoids can prevent complications or progression of diabetes mellitus by ridding the excessive free radicals, break the chain reaction of free radicals (Soewanto, 2001), metal ion binding (chelating), and blocked the polyol pathway by inhibiting the enzyme aldose reductase (Milss and K. Bone, 2002).

Flavonoids also have an inhibitory effect on the enzyme alpha hydroxylation gukosidase through bonds and the substitution on the ring β. The principle is similar to the inhibition of acarbose which has been used as a drug for the treatment of diabetes mellitus, by generating delays and disaccharide carbohydrate hydrolysis and the absorption of glucose and inhibits the metabolism of sucrose into glucose and fructose (Ho and TM Bray, 1999). The results showed that the methanol extract of leaves cemba (Acacia pennata) can lower blood glucose levels in mice (Mus musculus) males who have hyperglycemia, potentially as a herbal medicine for lowering blood glucose levels.

CONCLUSION

Based on research done that, cemba leaf extract (Acacia pennata) can lower total blood glucose levels in mice (Mus musculus) males with better reduction in the dose of 250 mg / kg BB.

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