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Effectiveness of Sambote Extract on Decreasing Blood Glucose Levels of Male White Rats (*Rattus norvegicus*) Induced with Sucrose

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Abstract: ***The need for very expensive heart drugs has encouraged researchers and the pharmaceutical industry to look for new breakthroughs in hopes of getting new heart drug compounds. The general objective of this study is to obtain new heart drug compounds by utilizing traditional medicinal plants so that sudden death rates can be suppressed. The specific aim is to obtain scientific information about the activities of diabetes plants "Sambote or Pasote". This research will empower the potential of the biodiversity of medicinal plants used by the people in Minahasa tribe, North Sulawesi Indonesia by testing the ethanol extract of the plant Sambote. The research was carried out in stages starting from the introduction and identification of Sambote biologically and then followed by extracting the leaves of Sambote which were dried with ethanol. The extract is varied to determine the exact effect of reducing glucose levels. Then extract is appliedby squeezing the extract through the mouth of the white male rat to analyze the antidiabetic effects of the Sambote extract. The two controls were used, namely positive control and negative control. Measurement of blood glucose levels or antidiabetic is used by the Autocheck Multi-monitoring System. The results obtained were that the measurement of the body weight of the initial 24 rats was around 120 to 220 g with an average of 194 g. The initial blood glucose content of rats is from 65 - 128 mg/dL with an average of 82.5 mg/dL. After statistical data processing that the average body weight of white male rats before treatment or initial control was 194.05 g and blood glucose content was 82.5 mg/dL. After being given sucrose treatment for 48 hours, it weighs around 160-220 g with an average of 184.41 g. Body weight decreased by 4.97%. Then blood glucose levels around 68-228 mg/dL or an average of 76.27 mg/dL. Blood glucose levels increased by around 38.49% after 48 hours of sucrose treatment. After the treatment of the Sambote extract decreased blood glucose levels from an average of 94.33 mg/dL to 88.67 mg/dL or decreased by 5.66 mg/dL after 48 hours of application. Ethanol extract of Sambote (Dizphania ambrosioides) can reduce blood glucose levels in white male rats.***

Keywords: Heart, Antidabetes, Pasote or Sambote, Traditional Medicine, Blood Glucose

1. Introduction

Minahasa Regency from direct observation has a diversity of plants used as medicine. One of them is the "Sambote" designation for the Minahasa Kakas Tribe or "Sambote" for Minahasa Tountemboan subtribe. This plant is often used by parents in the Minahasa tribe who have experienced health problems, especially glucose and cholesterol. From the results of direct interviews that the plant is also used as a complement to Manado porridge ("tinutuan") as a substitute for basil. According to them the pain of glucose and cholesterol recovers after using it regularly. This has become their habit and has been planted around their homes.

The traditional medicinal plant "Sambote or Pasote" is not generally known. The results of direct monitoring in the field that these plants are endemic to North Sulawesi that have not been identified and are a regional asset for the future. Minahasa tribe habits that tend to eat a lot of meat, of course this plant is a cholesterol-lowering solution the main cause of heart disease. It needs to be developed into an herbal medicine for diabetes and cholesterol which is a trigger for heart disease.

The most common heart disease is caused by narrowing of the arteries and swelling of the heart. The causes of both are cholesterol, fat and high glucose. Narrowing of blood vessels can occur in the blood vessels of the heart, brain and other parts. The impact that is quickly visible is that blood pressure rises over time causing heart attacks and strokes. Lately many people have died suddenly, this is usually caused by heart disease that is late to recognize it (Anonim, 2013b). Besides that, at first people did suspect that the backup source of medicinal plant variations was in an unlimited and inexhaustible amount for the needs of present and future generations. But the reserves of plant-based genetic resources at the center of diversity are not always available as we wish. The potential of plants and traditional ingredients, a drug industry or herbal medicine can be opened as its utilization so that the existing potential can be developed and advance the area.

Heartbeaters are the number one killer that is very frightening for many people, especially for areas where the tradition of meat eaters "B2" and "RW". Meals eating habits trigger an increase in cholesterol, fat, uric acid and glucose in the blood. Both diseases are triggers for narrowing of the coronary arteries. It is not surprising that many die suddenly, because they do not know that they have had heart disease. This research is very urgent to be carried out so that standardization of traditional medicines that are ready to be used is typical in North Sulawesi Indonesia

The purpose of this study was to determine how much blood glucose levels decreased, from Sambote or pasote, which would be promoted as a heart drug. The benefits that can be obtained from this research are as an effort to preserve the excellent medicinal knowledge of the Minahasa tribe people for national and international health development, using Sambote plants or sambote as unpublished drugs.

2. Research Methods

This research was conducted in February - July 2018, at the Pharmacology Laboratory of FMIPA Sam Ratulangi University Manado Indonesia. The research was carried out in stages including:

2.1 Plant Collection (Sampling)

The collection technique was followed (Kandou and Pandiangan, 2006). In order for the sample to be good then complete collection of plant parts is carried out including, roots, stems, leaves, flowers, fruits and seeds if all are present. In this study the most commonly obtained are Sambote seeds, so the sample from this study is a mixture of leaves and Sambote seeds (Figure 1). Samples of Sambote are completely removed or cut off in the stem and then washed in water when close to the ground. Then dried air in the room one night, then the Sambote plant is dried in an open space in the sun all day then when the leaves are fragile when squeezed then the leaves are pureed by squeezing it first, then blending to smooth it out.

Figure 1: Sambote (*Dizphania ambrosioides*) morphology (A) Sambote appearance in the field at North Sulawesi Indonesia, (B). Appearance of samples with seed is dried in the sun

2.2 Extraction and Maseration of Samples

The sample was weighed as much as 500 grams (wet weight) and then chopped. The leaf pieces are dried using an oven at 45ºC. Dry Sambote leaf then weighed (dry weight) and calculated the yield of dry simplicia. After that the simplicia is mashed up with a blender to reduce the surface of the particles so that the contact between the material and the solution is larger, then sifted using a 65mesh sieve.

Extracting Sambote leaf simplicia using 95% ethanol. The making of Sambote leaf extract was done by remaseration method, namely Sambote leaves that had been sifted, weighed as much as 150 g then extracted using 900 mL of 95% ethanol by maceration for 5 days (every day shaken). The extract was then filtered using filter paper (filtrate 1) and the rest was extracted again for 2 days using 95% ethanol as much as 600 mL then filtered (filtrate 2). Then filtrate 1 and 2 were collected, evaporated with vacuum evaporator at 70ºC until the volume became dry from the initial volume, and continued with drying in the oven at 40ºC until it became thick extract. Get thick extract. Thick extract is ready to be used in extract effectiveness test.

2.3 Extract Effect Test on Decreasing Rat Blood Glucose Levels

Test animals were divided into 3 groups. Before being given treatment, all rats were fasted for 24 hours (drinking is still given). All rats that were fasted were weighed, then examined fasting blood glucose levels, after which all sucrose-induced rats were 5,625 g/KgBW. After 30 minutes, all rats were examined for blood glucose levels after sucrose induction. Furthermore, all rats were given oral preparations, for the negative control group (K-) were only given 0.5% CMC, for the treatment group (KP) were given extracts at a dose of 150 mg/kgBW, and for the positive control group (K +) were given glibenclamide with a dose of 0.45 mg/KgBW, then the blood glucose levels of Rats were examined at 15, 30, 60, and 120 minutes after treatment. All blood samples were taken from rat veins and blood glucose levels were measured by glucometer Autocheck Multi-Monitoring System.

Sucrose doses were calculated based on sucrose doses in rabbits which were 3 g/KgBW orally (Widyastuti and Suarsana, 2011), then the calculation of sucrose doses for Rats was 1.5 x 3 x 0.25 = 5.625 g/KgBW. (0.25 is a rabbit dose conversion factor to Rats according to Harmita and Radji (2006). The sucrose dose to be used is calculated based on the body weight of each mouse, then dissolved in distilled water as much as 2.5 mL and drunk on each rat 0.5 g of CMC is sprinkled in a mortar containing ± 30 mL of hot distilled water. Let stand for 15 minutes until a transparent mass is obtained, then crushed to homogeneous, diluted with distilled water and put into a 100 mL volumetric flask, the volume is sufficient with distilled water to the limit of the anchor mark.

The dose of Glibenclamide in adult humans is 5 mg, then the dose of Glibenklamid for mice is 5 x 0.018 = 0.45 mg/KgBW. Glibenclamide tablets are crushed and taken as much as 15 mg (equivalent to a dose of 0.45 mg/KgBW), put in mortar

and added CMC 0.5% w/v gradually little by little while being grounded homogeneously, the volume is sufficient to 5 mL. Normal fasting blood glucose levels <110 mg/dL.

3. Results and Discussion

Growth of White Male Wistar Rat after treatment of Sambote Extract

The Rat that were obtained by about 24 tails were adapted for 7 days first. After 7 days the standardized and fed whole food was measured by rat body weight called the initial rat weight or before the experiment. Then sorting or selecting Rats from 24 tails was prepared, which previously obtained measurement weight from about 120 g to 220 g (Table 1). The Rats used 17 rats that looked the same size and were healthy and agile. The weight of white male WinstarRats is about 190 to 220 g (Table 1 and Figure 2). After statistical data processing that the average weight of white male rats before treatment or initial control is 194.06 g. But after being given sucrose treatment, the body weight was around 160-220 g with an average of 184.41 g (Table 2). Body weight decreased by 4.97%. The decrease in body weight is thought to be a period of adjustment or environmental adaptation. According to Muray (2013) that the adaptation period should be 10 days. Adaptation carried out still 7 days until the 10th day needs adjustment. However, the weighing of the following day experienced an overall increase (Figure 3). The average change in body weight according to maintenance time is varied but is still at the 10% change threshold (Murphy, 2015). Especially for the treatment of high fat feed, the weight also ranged from 180 to 220 g with an average of 191.67 g to 190.83 g or reduced by about 0.43% during 2 days of fat feeding.

Table 1: Changes in rat body weight (g) during the process of conducting research

Groups of Rats	Body weight	Body weight induction	Body weight treatment with extract Sambote					Average (g)
	Before (7/6)	9/6	11/6	27/6	10/7	28/9	2/10	
I	190	190	220	225	225	310	300	237.14
II	192	170	215	235	225	280	270	226.71
III	192	180	200	225	225	270	260	221.71
IV	190	150	180	200	220	270	250	208.57
V	180	160	200	215	230	mt	mt	197.00
V1	190	190	210	215	240	220	180	206.43
VII	210	190	210	230	240	310	310	242.86
VIII	200	200	220	265	245	300	280	244.29
IX	180	165	220	240	245	300	300	235.71
X	200	180	200	245	250	300	300	239.29
XI	190	190	220	260	255	230	250	227.86
XII	200	200	235	260	255	310	320	254.29
XIII	180	210	200	245	240	360	350	255.00
XIV	190	160	200	210	230	300	300	227.14
XV	190	170	200	mt	mt	Mt	mt	186.67
XVI	205	220	250	170	230	380	350	257.86
XVII	220	210	240	230	240	290	250	240.00
Average	194.06	184.41	212.94	229.38	237.19	295.33	284.67	

Note: Mt = death rat
I-XVII = the group of rats (*Rattus norvegicus L*) test

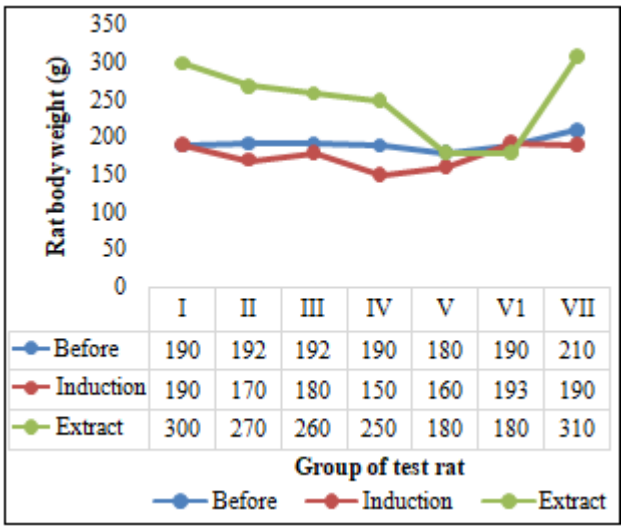

Figure 2: Changes in body weight and growth of mice for 2 months of experimentation with varying time of treatment.

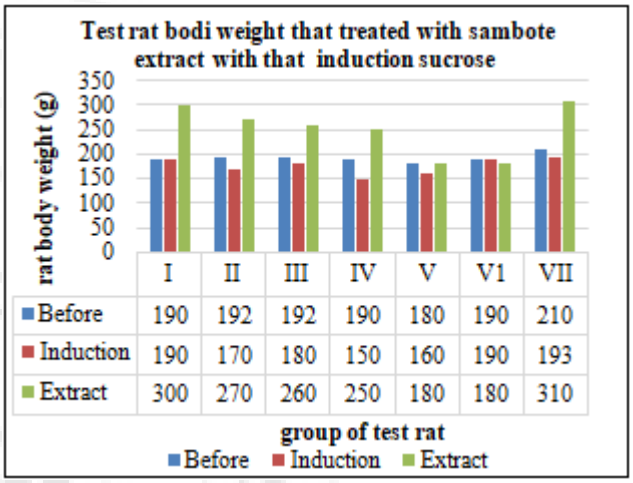

Figure 3: Graph of changes or growth of mice during the experiment. The pattern of growth is relatively the same and homogeneous

Blood Wistar Rats blood measurement results after Sambote extract treatment

The initial blood glucose content of white rats from 65 - 128 mg/dL with an average of 82.5 mg/dL and cholesterol content is low or not detected by the Autocheck Monitoring Multy System. It shows a cholesterol content smaller than 100 mg/mL or 99, 98, 97 mg/dL and so on. After processing data statistically that the blood glucose content of the rats before treatment was 82.5 mg/dL and cholesterol was very low or low less than 99 mg/dL. After being given sucrose treatment, the blood glucose level was around 68-228 mg/dL or an average of 76.27 mg/mL (Figure 4). Blood glucose levels increased by around 38.49% after 48 hours of sucrose treatment (Table 2).

Table 2: The content or blood glucose levels of white rats: aquadest (control rats), sucrose (rats given only sucrose), sucrose + CMC (rats given sucrose and CMC solvents then given glibenclamide) and sucrose + Sambote (after being induced with sucrose then given ethanol extract of Sambote). Measured together before being treated or early, after being given sucrose and after being given Sambote.

Groups of *R. norvegicus*	Before treatment or baseline		Induction by Sucrose		After treatment Sambote extract	
	Evara--ge	STD EV	Evara ge	STD EV	Avera-ge	STED EV
Aquadest	83.33	1.53	97.00	2.65	97.33	1.15
Sucrose	72.33	2.52	108.33	0.58	77.67	1.53
Sucrose+CMC	69.00	5.29	224.33	4.04	112.67	1.15
Sucrose+ Extract	88.67	2.52	94.33	1.53	88.67	0.58
Sucrose+Gliben	82.33	2.08	96.67	3.51	82.33	1.15

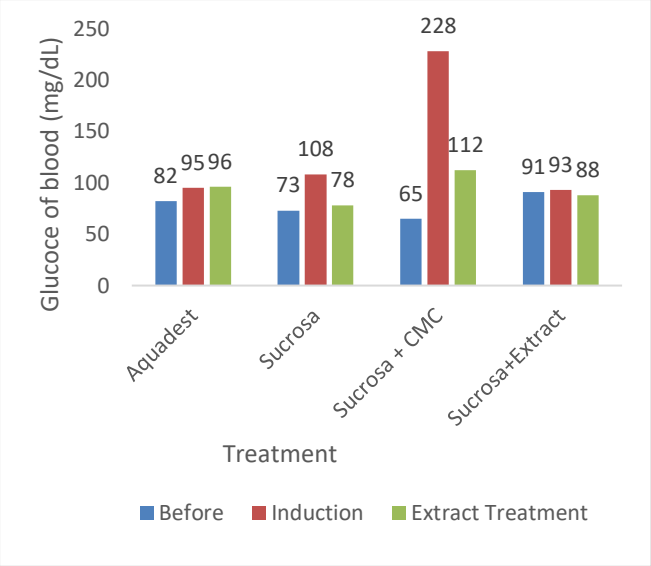

Figure 4: Graph of changes in the blood glucose content of the white wistar rats: aquadest (control rats), sucrose (rats given only sucrose, sucrose + CMC (rats given sucrose and solvents CMC and given glibenclamide) and sucrose + Sambote (after being induced with sucrose and given ethanol extract of Sambote seeds). Measured together before being treated or early, after being given sucrose and after being given Sambote.

To pay attention to how much Sambote extracts reduce blood glucose levels compared to the one in Figure 5. After the treatment of Sambote's ethanol extract 60 mg of ethanol extract dissolved in 2 mL CMC decreased blood glucose levels to an average of 94.33 mg/dL to 88.67 mg/dL or reduced by 5.66 mg/dL.

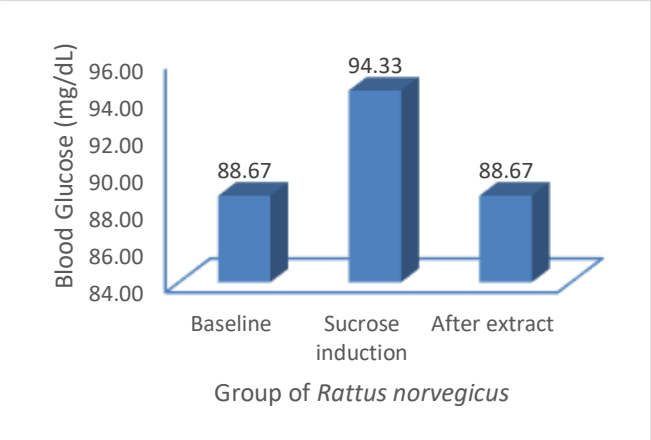

Figure 5: Changes in blood glucose content after being treated with the ethanol extract of Sambote seeds. The decline is around 5.66 mg/dL

Cholesterol content is still low or undetectable after 48 hours after high fat food treatment. Cholesterol levels with only 24 hours of treatment have not been changed or the tool cannot detect, so for the observation of high fat treatment to see the effect of adhesives, high fat feed was carried out for the next 4 months and was being treated until the time this report was made. The effect of the new Sambote extract shows its effect to reduce blood glucose levels in white male rats.

4. Conclusions and Recommendations

The results obtained were that the measurement of 17 body weight of rats beginning around 190 to 220 g. The initial blood glucose content of mice is from 65-128 mg/dL. After statistical data processing that the average body weight of white male rats before treatment or initial control was 194.05 g and blood glucose content was 82.5 mg/dL. After being given sucrose treatment for 48 hours, it weighs around 160-220 g with an average of 184.41 g. Body weight decreased by 4.97%. Then blood glucose levels around 68-228 mg/dL or an average of 76.27 mg / mL. Blood glucose levels increased by around 38.49% after 48 hours of sucrose treatment. The treatment of the Sambote ethanol extract decreased blood glucose levels from an average of 94,33 mg/dL to 88,67mg/dL or decreased by 5.66 mg/dL after 48 hours of application. Ethanol extract of the seeds of "Sambote or Sambote" can reduce blood glucose levels in white male rats. Recommended to be used as a constituent of herbal medicines for people with degenerative diseases.

Suggestions from the results of this study need to be continued with measurements using a spectro with a larger blood volume, which of course will take blood from the heart or other vital parts. Other suggestions need to be continued with the development of phytopharmaca combined with other herbal medicines.

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