International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.5, pp 1703-1711, Sept-Oct 2014

Antidiabetic Effect of Roselle Calyces Extract (*Hibiscus Sabdariffa* L.) in Streptozotocin Induced Mice

Rosemary[1]*, Rosidah[1], Ginda Haro[1]

[1]Faculty of Pharmacy, University of Sumatera Utara, Medan, North Sumatera, Indonesia.

***Corres.author: rosemary191053@gmail.com**

Abstract: Diabetes mellitus is a symptom that can be characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, fats and proteins associated with the lack of insulin secretion or insulin action both in absolute and relative terms. Treatment with drugs distributed on the market either relatively more expensive because of its use in the long term or have considerable side effects. Previous studies show that roselle calyces have a potent antidiabetic activity. Therefore the research of antidiabetic activity of n-hexane, ethyl acetate and ethanol extracts of roselle calyces (*Hibiscus sabdariffa* L.) has been conducted in order to improve the utilization of herbs and natural antidiabetic and the information can be useful in extracting roselle calyces in order to obtain the optimal antidiabetic effect. Roselle calyces extract was made by means of stratified percolation using n-hexane as solvent followed by ethyl acetate, and the last solvent is ethanol, then the macerate respectively was concentrated by rotary vacuum evaporator. Then the test of Antidiabetic activity was conducted consisting of 11 treatment groups, ie groups of CMC suspension 0.5% as a negative control; glibenclamide 0.65 mg/kg as a positive control; n-hexane extracts of roselle calyces dose of 200 mg / kg, 400 mg / kg and 600 mg / kg; ethyl acetate extracts of roselle calyces dose of 200 mg / kg, 400 mg / kg and 600 mg / kg; and ethanol extracts of roselle calyces dose of 200 mg / kg, 400 mg / kg and 600 mg / kg, which is respectively administered orally every day. Blood glucose levels were measured by glucometer Accu Chek every seven days, the data were analyzed by analysis of variance followed by Duncan's method. The results showed that the ethanol extract of roselle calyces proven to reduce the blood glucose levels on diabetic mice, while the n hexane and ethyl acetate extract were not proven to reduce the blood glucose levels on diabetic mice. The results of the statistical test ($\alpha = 0.05$) showed that the extracts of n-hexane and ethyl acetate perform a real mean of difference on glibenclamide, whereas the ethanol extract did not perform significant differences with glibenclamide in lowering blood glucose levels on mice, which means that the ethanol extract can lower blood glucose levels of diabetic mice.

Keywords: antidiabetic, streptozotocin, blood glucose levels, roselle calyces (*Hibiscus sabdariffa* L.).

Introduction

Diabetes mellitus 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 relative[1]. Insulin therapy and oral hypoglycemic medications offer effective glycemic control, but insulin therapy has drawbacks such as the ineffectiveness of oral administration, the short half-life, the need for direct cooling, and hypoglycemia may occur in cases of fatal overdose[2]. Therefore need search a compound having antidiabetic activity taken orally. The use of oral antidiabetic limited because of adverse side effects including haematological reactions, cutaneous and gastrointestinal, coma, hypoglycemic and impaired liver and kidney function. These substances are also not appropriate to use during pregnancy[3].

Many herbs in traditional herbal medicine which is believed to have hypoglycemic activity. Available literature indicates that there are more than 800 species of plants that showed hypoglycemic activity[4]. Previous studies have shown that chemical compounds isolated from plants have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure[5].

Roselle (*Hibiscus sabdariffa* L.) grown in all parts of the world and has been used as a health drink in many countries such as Australia, India, Myanmar, Thailand, Senegal, France, Gambia, Nigeria, Greece, Saudi Arabia, Sudan, Latin America, Panama, Indonesia, Malaysia, China, and others. Chemical constituents that are antioxidative in *Hibiscus sabdariffa* L. anthocyanins are very high, such as: flavonoids and polyphenols have a cardioprotective effect, reducing Low Density Lipoprotein oxidation in vitro and reduce blood serum cholesterol levels of mice and rabbits[6], has the effect hypocholesterolemic[7] as well as the effect of anti-oxidative and hepatoprotective[8]. High levels of antioxidants in roselle calyces can inhibit the damaging effects of free radicals. Some chronic diseases are often found today largely caused by exposure to excessive free radicals, such as kidney damage, diabetes mellitus, coronary heart disease, to cancer[9].

Research proved that roselle (*Hibiscus sabdariffa* L.) can lower blood glucose levels of rabbits with streptozotocin-induced diabetes which increases the activity of the enzyme catalase roselle and glutathione. In histological experiments, roselle cause osmotic diuresis in the proximal renal tubule diabetic animals [10], [11]. Research on the activity of the water extract and ethanol extract of roselle calyces are very much done, only a few studies have examined the n-hexane extract and very rarely examine the ethyl acetate extract of roselle calyces. Therefore, researchers are interested in this opportunity examining the content of n-hexane extract, ethyl acetate, and ethanol extracts obtained through percolation extraction technique in which the content of the terraced flower calyces are separated according to their solubility roselle, then testing the activity of each extract in lowering blood glucose levels in diabetic mice that had been induced with streptozotocin (STZ) in various doses of extract and also compare the activity of the three extracts with glibenclamide. It is expected that this study can be useful in extracting information roselle calyces in order to obtain optimal antidiabetic effects.

Materials And Methods

This is an experimental study that tested the effects of antidiabetic extract obtained from n-hexane, ethyl acetate, and ethanol roselle calyces (*Hibiscus sabdariffa* L.) against streptozotocin induced mice. In this study there are two variables that n-hexane, ethyl acetate, ethanol extracts obtained from roselle calyces (*Hibiscus sabdariffa* L.) and glibenclamide as an independent variable and mice blood glucose levels (mg / dl) as the dependent variable. This study consists of several stages and involves taking a sample processing, which includes examining phytochemical screening of alkaloids, flavonoids, saponins, steroids, and tannins, making of extracts, animal preparation, and testing of antidiabetic effect. This research was conducted at the Laboratory of Pharmacognosy and Pharmacology Laboratory, Foundation TP Arjuna, Laguboti, North Sumatra.

Materials

The tools used in this study is a glass equipment, water heaters, aluminum foil, blender, and glukotest glukometer strips (Accu chek), filter paper, mortars and stamfer, the balance of the animal, a rough balance, balance of electrical, oral sonde , rotary vacuum evaporator, and syringe. Plant materials used are roselle calyces (*Hibiscus sabdariffa* L.), streptozotocin were obtained from Merck. All chemicals were used unless otherwise stated is pro - analysis quality is CMC (Carboxy Methyl Cellulose), 96% ethanol, n-hexane, ethyl acetate, sodium citrate, acetic acid, ammonia, hydrochloric acid, dragendorf reagent, mayer reagent, sulphuric acid, feric chloride, magnesium powder, chloroform, liebermen bouchardat reagent, anhydrous acetic acid, glibenclamide (PT. Indofarma), 0.9% sodium chloride solution.

Animal Experiments

This study used a test animal of male mice strains BALB / C 3-4 months old and weighs between 25-35 grams. Before the first experiment mice were maintained for 2 weeks in a good enclosure to adapt to its environment.

Preparation of Extract

The sample used in this study is roselle calyces (*Hibiscus sabdariffa* L.) were still fresh red and dark enough that I obtained from the Market Village Tapanuli, District Percut Sei Tuan, Deli Serdang regency. Samples roselle calyces are still fresh collected, cleaned (be sorted), washed with water, then drained and spread. Then the calyces dried by air dried until the calyces dry and brittle. Then stored in a place protected from sunlight. Roselle calyces (*Hibiscus sabdariffa* L.) extract conducted by stratified percolation. The procedure of making extracts was 1 kg of crude drug powder wetted with n-hexane and left for 3 hours. Then put in a percolator tool, then poured liquid n-hexane until all botanicals are submerged and fluid layer on top, mouth percolator tubes covered with aluminum foil and left for 24 hours, then the valve is opened and allowed to flow at the speed of droplets extract set 1 ml / min, extract collected. Percolation extract droplets suspended until clear (colorless), then concentrated by means of rotary evaporator. The pulp or residue is dried and then extracted using solvents successively with ethyl acetate and ethanol same procedure as above.

Examination of Alkaloids

A total of 1 gram of n-hexane extract was added 1 ml of 2N hydrochloric acid and 9 ml of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate was used to test alkaloids as follows:

a. 3 drops of filtrate was added with 2 drops of meyer reagent, will form a white or yellow precipitate.
b. 3 drops of filtrate was added with 2 drops of bouchardat reagent, will form a brown to blackish precipitate.
c. 3 drops of filtrate was added with 2 drops of dragendorff reagent, will form a red or orange precipitate.

Alkaloids positive if there is a precipitate or turbidity at least two of the three experiments above. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Examination of Flavonoids

A total of 10 g of n-hexane extract was dissolved in 10 ml of hot water, boil for 5 minutes and filtered in hot conditions, into 5 ml of the filtrate was added 0.1 g of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Flavonoids positive case of red or yellow or orange in the lining of amyl alcohol. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Examination of Saponins

A total of 0.5 grams of n-hexane extract incorporated in a test tube, add 10 ml of hot water, cooled and then shaken vigorously for 10 seconds, if formed stable froth as high as 1 to 10 cm in a stable of no less than 10 minutes and not missing with the addition of 1 drop of 2 N hydrochloric acid showed a saponin. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Examination of Steroids / Triterpenoids

A total of 1 gram of n-hexane extract macerated with 20 ml of ether for 2 hours, filtered, the filtrate evaporated in the evaporating dish and the rest was added 20 drops of acetic acid anhydride and 1 drop of concentrated sulfuric acid (liebermann bouchardat reagent). The emergence of blue or blue-green color indicates the presence of steroids, while the color red, pink, or purple indicates triterpenoids. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Examination of Tannins

A total of 0.5 grams of n-hexane extract dissolved with 10 ml of distilled water and then filtered, the filtrate was diluted with water until colorless. 2 ml solution was taken and added 1 to 2 drops of reagent iron (III) chloride 1%. If there is a blue or black color indicates the presence of tannins. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Examination of Glycosides

A total of 3 grams of n-hexane extract dissolved with 30 ml of 95% ethanol mixture with water (7:3) and 10 ml of 2 N hydrochloric acid, refluxed for 2 hours, cooled and filtered. Taken 20 ml of the filtrate was added

25 ml of distilled water and 25 ml of lead (II) acetate 0.4 M, shaken, allowed to stand 5 minutes and then filtered. Dissolved premises filtrate with 20 ml of isopropanol and chloroform mixture (2:3), repeated 3 times. Water extract was collected and evaporated at a temperature of not more than 50°C. The rest was dissolved in 2 ml of methanol. The remaining solution is used for the following experiments: 0.1 ml of solution in a test tube experiments included and evaporated over a water bath. In the remainder was added 2 ml of water and 5 drops of mollish reagent. Then slowly add 2 ml of concentrated sulfuric acid through the tube wall, the formation of a purple ring at the boundaries of the two fluids showed glycosides. The same experiment performed on extracts of ethyl acetate and ethanol extracts.

Preparation of 0.5% CMC Suspension

A total of 500 mg of CMC, sown in a porcelain dish containing 10 ml of hot distilled water. Allowed to stand for 30 minutes to obtain a transparent mass. After that crushed while diluted with distilled water. Then put into calibrated erlenmeyer 100 ml, make volume with distilled water to 100 ml.

Preparation of Extract Suspension

Each roselle (*Hibiscus sabdariffa* L.) extract obtained from n-hexane, ethyl acetate and ethanol, weighing 600 mg of extract of flower calyces, crushed in a mortar, CMC suspension was added gradually while crushed until homogeneous, then put in to 10 ml volumetric flask and adjust the volume until the mark line. The suspension of the extract was made as the mother liquor, which is then diluted to obtain a concentration suspensions of different extracts.

In this study the dose of extract given to diabetic mice in three groups, namely:

GROUP	NAME	DOSE
I	Low Dose	200 mg / kg
II	Moderate Dose	400 mg / kg
III	High Dose	600 mg / kg

Preparation of Glibenclamide Suspension

Weighed as much as 0.65 mg glibenclamide, crushed in a mortar, CMC suspension was added gradually while crushed until homogeneous, then put in to 10 ml volumetric flask and adjust the volume until the mark line. Dose of glibenclamide therapy in humans is 5 mg. Dose conversion from human to mouse dose, ie: 0.0026 × 5 mg = 0.013 mg × 1000 / 20 = 0.65 mg / kg

Preparation of Streptozotocin in 0.9% Sodium Chloride Solution

A total of 55 mg of streptozotocin put in to 10 mL volumetric flask and adjust the volume until the mark line. Streptozotocin is a compound produced from Streptomyces acromogenes which is a glucose analogue nitroso compound urea. Streptozotocin easily soluble in water, slightly soluble in alcohols and ketones. In a study used as inducer of diabetes in experimental animals. This drug has a high specificity towards β cells. intraperitoneal injection in doses of 40-60 mg / kg, single dose will cause hyperglycemia after 2 - 4 days.

Testing Blood Glucose Levels Normal Mice

Before being given treatment, blood glucose levels were measured prior mice, mice that were fasted for 18 hours. Weighted then measured fasting blood glucose levels by taking blood through the tail vein using a syringe. Blood coming out touched on Glukostrip installed on Glukotest. Tool will work automatically, the numbers that appear on the screen as a means of recorded blood glucose levels (mg / dl).

Determination of Blood Glucose Levels

Before the experiments conducted, mice were fasted (not eating but still drinking) for 18 hours, then weighted. Then each mouse was measured fasting blood glucose levels. Mice tail were cleaned with alcohol, and then have blood drawn through the tail section of vein is punctured with a syringe. Blood coming out touched on glukotest strip that has been installed on the appliance and let glukometer measuring blood glucose levels automatically. Number that appear on the screen as a means of recorded blood glucose levels (mg / dl).

Inducing Diabetes

Induced diabetic mice were fasted for 18 hours (water is still given), were injected intraperitoneally with streptozotocin solution at a dose of 55 mg / kg. On the 3rd day determined blood glucose levels of mice, are considered diabetic if their blood glucose levels above 200 mg / dl.

Test Activity of Extract

Antidiabetic test performed on each extract which n-hexane, ethyl acetate, and ethanol extracts are made with 3 doses of 200 mg, 400 mg and 600 mg of extract, each group consisted of 6 mice, namely:

Group	Name	Treatment
I	Negative Control	Given 0.5% CMC Suspension
II	Positive Control	Given Glibenclamide 0.65 mg / kg
III	Low Dose N-Hexane Extract	Given N-Hexane Extract 200 mg / kg
IV	Moderate Dose N-Hexane Extract	Given N-Hexane Extract 400 mg / kg
V	High Dose N-Hexane Extract	Given N-Hexane Extract 600 mg / kg
VI	Low Dose Ethyl Acetate Extract	Given Ethyl Acetate Extract 200 mg / kg
VII	Moderate Dose Ethyl Acetate Extract	Given Ethyl Acetate Extract 400 mg / kg
VIII	High Dose Ethyl Acetate Extract	Given Ethyl Acetate Extract 600 mg / kg
IX	Low Dose Ethanol Extract	Given Ethanol Extract 200 mg / kg
X	Moderate Dose Ethanol Extract	Given Ethanol Extract 400 mg / kg
XI	High Dose Ethanol Extract	Given Ethanol Extract 600 mg / kg

Provision of treatment started after a positive test animals with diabetes, which is done every day. Every interval of seven days is held measuring blood glucose levels. Testing was stopped until one of the test group had normal blood glucose levels.

Analysis of Data

The data were analyzed using Analysis of Variance (ANOVA) at the 95% confidence level. The statistical analysis using Statiscal Product and Service Solutions (SPSS) Version 16.

Results And Discussions

Extraction roselle calyces done using a stratified percolation solvent n-hexane, ethyl acetate, and ethanol, with the intention that the chemical constituents contained in roselle calyces can be separated based on their solubility in the solvent liquid. Results of 1000 g of crude drug powder extract obtained a total of 74.2 g (7.42%) in which the n-hexane extracts were obtained as 28.4 g, the extract is dark green; 12.6 g of ethyl acetate extract, the extract is green; and 33.2 g of ethanol extract, the extract is dark red.

Chemical constituents of roselle calyces are separated based on their solubility using stratified percolation technique. Extraction stage begins by using a non-polar solvent n-hexane is then followed by using a semi-polar solvent is ethyl acetate, last by using a polar solvent is ethanol. Chemical constituents contained in each roselle calyces extract shown in Table 1**.** below.

Table 1. Chemical constituents contained in each roselle calyces extract.

Phytochemical Constituents	n-Hexane	Ethyl acetate	Ethanol
Alkaloids	–	+	+
Steroids	+	–	–
Saponins	–	–	+
Tannins	+	+	+
Glycosides	–	+	+
Flavonoids	–	+	+

N-hexane extract positive for steroids and tannins; ethyl acetate extracts positive for alkaloids, tannins, glycosides, and flavonoids; and ethanol extract positive for alkaloids, saponins, tannins, glycosides and

flavonoids. Bioflavonoids content in the plant extract has effects that mimic insulin. Routine oral administration of bioflavonoids to diabetic animals can lower blood glucose levels, increase insulin levels, stimulates insulin secretion and stimulates the formation of glycogen in muscle cells[12].

In antidiabetic testing used streptozotocin as antidiabetic inducer, is due streptozotocin have effects to destruction of the beta cells of langerhans in pancreas which producing insulin in mammals. Streptozotocin injection refers to the clinically symptoms of diabetes evident in mice in 3 days with a injection dose 55 mg / kg intraperitoneally. After adapting for two weeks, the mice were given feed with the same type and number of food, mice were fasted for 18 hours, measured normal blood glucose levels. On the same day mice induced by streptozotocin, the third day after inducing, mice were fasted for 18 hours, measured blood glucose levels. The result can be seen in Table 2**.** below.

Table 2. Data results for normal blood glucose levels and blood glucose levels after inducing of streptozotocin

Treatment	**Blood Glucose Levels (mg / dl)** (Mean ± Standard Deviation)	
	After Induction	Before Induction
Given 0.5% CMC Suspension	78,33 ± 4,84	326,50 ± 24,44
Given Glibenclamide 0.65 mg / kg	71,67 ± 3,67	314,00 ± 18,56
Given N-Hexane Extract 200 mg / kg	78,50 ± 4,51	324,17 ± 20,72
Given N-Hexane Extract 400 mg / kg	75,17 ± 7,00	326,50 ± 49,28
Given N-Hexane Extract 600 mg / kg	75,67 ± 6,28	304,83 ± 15,82
Given Ethyl Acetate Extract 200 mg / kg	78,67 ± 5,75	320,83 ± 33,87
Given Ethyl Acetate Extract 400 mg / kg	79,83 ± 10,80	324,33 ± 20,49
Given Ethyl Acetate Extract 600 mg / kg	76,67 ± 3,72	315,67 ± 14,83
Given Ethanol Extract 200 mg / kg	74,33 ± 6,56	309,33 ± 19,05
Given Ethanol Extract 400 mg / kg	74,00 ± 8,15	303,00 ± 24,33
Given Ethanol Extract 600 mg / kg	76,17 ± 4,62	303,33 ± 14,14

Different doses of streptozotocin produce different types of diabetes. Streptozotocin with dose 70-250 mg / kg is very damaging to the five cells of the pancreas in 24 hours of administration, resulting in mice with type 1 diabetes mellitus. Providing low dose streptozotocin in the few days resulted in mice with type 2 diabetes mellitus and will be developed to type 1 diabetes mellitus.

Each group of diabetic mice treated with the administration of a group with 0.5% CMC suspension as a negative control, a group with administration of glibenclamide as a positive control, a group with n-hexane extract with dose 200 mg / kg, a group with n-hexane extract with dose 400 mg / kg, a group with n-hexane extract with dose 600 mg / kg, a group with ethyl acetate extract with dose 200 mg / kg, a group with ethyl acetate extract with dose 400 mg / kg, a group with ethyl acetate extract with dose 600 mg / kg, a group with ethanol extract with dose 200 mg / kg, a group with ethanol extract with dose 400 mg / kg, a group with ethanol extract with dose 600 mg / kg. Mice blood glucose levels measurement results are shown in Table 3., Figure 1. and Figure 2. below.

Table 3. Mice blood glucose levels measurement results after administration of n-hexane, ethyl acetate and ethanol extract

Treatment	**Blood Glucose Levels (mg / dl) (Mean)**						
	Normal	Diabetes	Week I	Week II	Week III	Week IV	Week V
Group I	78,33	326,50	326,83	329,17	332,33	334,17	336,33
Group II	71,67	314,00	267,50	213,17	137,50	110,00	76,17
Group III	78,50	324,17	325,50	324,67	318,83	312,33	306,17
Group IV	75,17	326,50	343,83	342,33	340,00	336,67	316,17
Group V	75,67	304,83	303,83	297,17	294,33	287,83	282,17
Group VI	78,67	320,83	322,67	324,33	325,67	327,67	329,00
Group VII	79,83	324,33	326,17	327,33	329,50	331,67	333,67
Group VIII	76,67	315,67	317,50	320,00	321,67	324,17	326,33

Group IX	74,33	309,33	292,00	244,83	199,33	150,50	114,67
Group X	74,00	303,00	276,67	222,83	180,83	127,83	87,67
Group XI	76,17	303,33	248,00	197,00	143,83	91,67	67,67

Description:

Group I : Given 0.5% CMC Suspension
Group II : Given Glibenclamide 0.65 mg / kg
Group III : Given N-Hexane Extract 200 mg / kg
Group IV : Given N-Hexane Extract 400 mg / kg
Group V : Given N-Hexane Extract 600 mg / kg
Group VI : Given Ethyl Acetate Extract 200 mg / kg
Group VII : Given Ethyl Acetate Extract 400 mg / kg
Group VIII : Given Ethyl Acetate Extract 600 mg / kg
Group IX : Given Ethanol Extract 200 mg / kg
Group X : Given Ethanol Extract 400 mg / kg
Group XI : Given Ethanol Extract 600 mg / kg

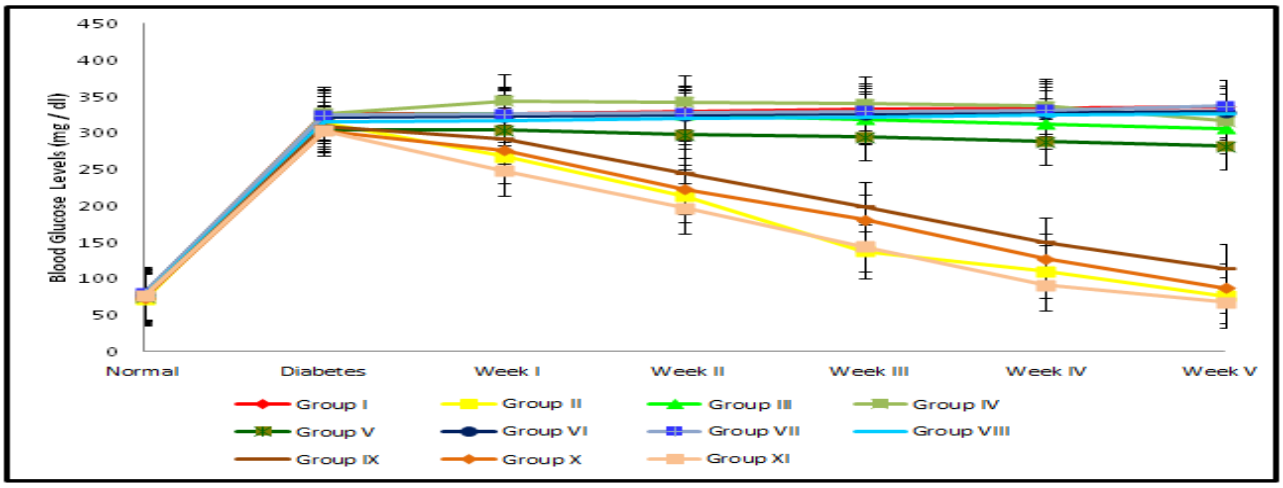

Figure 1. Graphic showing blood glucose levels after administration of n-hexane extract, ethyl acetate extract and ethanol extract

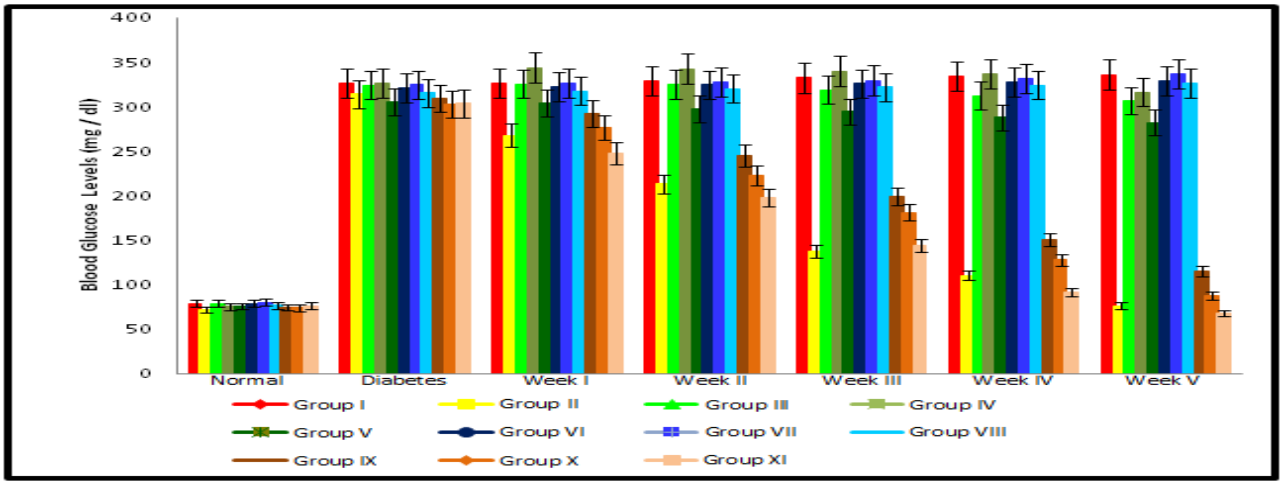

Figure 2. Bar chart that shows the blood glucose levels after administration of n-hexane extract, ethyl acetate extract and ethanol extract

N-hexane extract of roselle flower calyces in various doses showed effects very low compared with the group given glibenclamide, which groups by administering glibenclamide decline blood glucose levels effect is very good. This means that the n-hexane extract of 200 mg, 400 mg, and 600 mg did not have antidiabetic activity. Based on the duncan different test, n-hexane groups were never in the same subset with glibenclamide group since week I.

Ethyl acetate extract of roselle flower calyces in various doses did not show a decrease blood glucose levels, This means that the ethyl acetate extract of 200 mg, 400 mg, and 600 mg did not have antidiabetic activity at all. Administration of glibenclamide showed a decrease blood glucose levels which has significant differences compared to the administration of various doses of ethyl acetate extract.

Ethanol extract of roselle flower calyces in various doses showed a significant decrease in blood glucose levels as the administration by glibenclamide as a positive control. This means that the ethanol extract of 200 mg, 400 mg, and 600 mg have antidiabetic activity. The larger dose of ethanol extract is given, the greater its ability to lower blood glucose levels. Roselle calyces ethanol extract contain bioflavonoids that have proven to lower blood glucose levels in animal models of diabetes.

Ethanol extract dose of 600 mg / kg showed considerable potential in terms of a decrease in the activity of blood glucose levels. In the first week, which means there is no significance in the ethanol extract dose of 600 mg / kg with glibenclamide. At week 4 and 5 it appears that the potential reduction in blood glucose levels by administering a dose of 400 mg / kg ethanol extract did not have statistical significance so it is an indication that the ability of a dose of 400 mg / kg in lowering blood glucose levels did not differ with glibenclamide.

Administration of glibenclamide did not show statistical significance of the group with the ethanol extract of 600 mg / kg and ethanol extract 400 mg / kg. This means that the ability of ethanol extract 400 mg / kg and 600 mg / kg in reducing blood glucose levels very close to effect of decreasing blood glucose levels indicated by glibenclamide. While the ethanol extract 200 mg / kg was not able to give the effect equal to glibenclamide, but a dose of 200 mg / kg had statistically significance of data with a control group given 0.5% CMC.

Listed of variety of content bioflavonoida which have been isolated and tested its bioactivity in terms of lowering blood glucose levels in animal models of diabetes, namely prunin, myrciacitrin, 6 - hydroxyl – flavonoids, 6-hidroksiluteolin, apigenin, and luteolin which have the activity of the enzyme α-glucosidase inhibitor[10]. *Hibiscus sabdariffa* L. contain various compounds that are bioactive, accompanied antioxidative properties, such as protocatechuic acid, catechin, and (-)-epigallocatechin gallate. Mechanism in lowering blood glucose levels is made possible by no potentiation effect on plasma insulin either by increasing the secretion of pancreatic β cells of langerhans[12].

Analyzing the calculated quantity of flavonoids equivalent to quersetin in various extracts of roselle (*Hibiscus sabdariffa* L.) have been analyzed using different spectrophotometric methods[13]. The results showed that the method I (Chang, et al. Method) of measurement of flavonoids in a ethanol solution derived from *Hibiscus sabdariffa* L. obtained 0.0586 g% (g quersetin / 100 g of dry plant material); whereas that the method II (Christ Mullers method) of measurement of flavonoid in a ethyl acetate solution derived from *Hibiscus sabdariffa* L. obtained 0.0243 g% (g quersetin / 100 g of dried plant material). High flavonoid content of a crop gives more potential antidiabetic effects. Flavonoid content of ethanol extract greater than ethyl acetate extract, so that the ethanol extract of antidiabetic effect is greater as well.

Conclusions

Ethanol extract from roselle calyces proven to lowering blood glucose levels in diabetic mice whereas the ethyl acetate extract and n-hexane extracts are not shown to lowering blood glucose levels in diabetic mice. Chemical constituents of ethanol extract of roselle calyces are likely efficacious for lowering blood glucose levels in diabetic mice is the chemical content in the form of acid.

Provision of ethanol extract 600 mg / kg, ethanol extract 400 mg / kg and glibenclamide administration did not show significant differences in the lowering blood glucose levels in diabetic mice. This means that the blood glucose levels lowering effect of ethanol extract dose of 400 mg / kg and 600 mg / kg is the same as the effect of glibenclamide administration.

References

1. Schoenfelder, T., Cirimbelli, T.M., and Citadini, Z.V. (2006). Acute effect of *Trema micrantha* on serum glukosa levels in normal and diabetic rats. *J. Ethnopharmacol*. 107(3): 456-459.
2. Anuradha, K., Hota, D., and Pandhi, P. (2001). Investigation of central mechanism of insulin-induced hypoglycemic convulsions in mice. *Indian J Exp Biol*. vol (39): 500-502.

3. Alarcon, F.J., Jimenez, M., Reyes, R., and Romans, R. (2000). Hypoglycemic effect of extracts and fractions from *Psacalium decompositum* in healthy and alloxan diabetic mice. *J. Ethnopharmacol*. 72(2): 21-27.
4. Rajagopal, K., and Sasikala, K. (2008). Antihyperglycaemic and antihyperlipidaemic effects of *Nymphaea stellata* in alloxan-induced diabetic rats. *Singapore Med J*. 49(2): 137-141.
5. Waltner Law, M.E., Wang, X.L., and Law, B.K. (2002). Epigallocatechin gallate, a constituent of green tea, represses hepatic glukosa production. *J. Biol Chem*. 277: 34933-34940.
6. Tzu, L.L., Hui, H.L, Chang, C.C., Ming, C.L., Ming C.C., and Chau, J.W. (2007). *Hibiscus sabdariffa* L. extract reduces serum cholesterol in men and women. *Nutrition Research*. 27: 140-145.
7. Chen, C.C., Hsu, J.D., Wang, S.F., Ching, H.C., Yang, M.Y., and Kao, E.S. (2003). *Hibiscus sabdariffa* L. extracts inhibits the development of atherosclerosis in cholesterol-fed rabbits. *J. Agric. Food Chem*. 51(18): 5472-5477.
8. Dahiru, D., Obi, O.J., and Umaru, H. (2003). Effect of *Hibiscus sabdariffa* L. calyx extract on carbon tetrachloride induced liver damage. *Biokemistri*. 15(1): 27-33.
9. Babalola, S.O., Babalola, A.O., and Aworh, O.C. (2001). Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa* L.). *The Journal of Food and Technology in Africa*. 6(4): 133-134.
10. Wang, S.C., Lee, S.F., Wang, C.J., Lee, C.H., Lee, W.C., and Lee, H.J. (2011). Aqueous Extract from *Hibiscus sabdariffa* Linnaeus Ameliorate Diabetic Nephropathy via Reglukosating Oxidative Status and Akt/Bad/14-3-3γ in an Experimental Animal Model. Hindawi Publishing Corporation: Evidence-Based Complementary and Alternative Medicine. Volume 2011. Artikel ID 938126. Page 9.
11. Omotuyi, I.O., Ologundudu, A., Onwubiko, V.O., Wogu M.D., and Obi, F.O. (2010). *Hibiscus sabdariffa* Linn. anthocyanins alter circulating reproductive hormones in rabbits (*Oryctolagus cuniculus*). *Journal of Diabetes and Endocrinology*. 1(3): 36-45.
12. Brahmachari, G. (2011). Bio-Flavonoids with promising antidiabetic potentials: A critical survey. *Research Signpost India*. 187-212.
13. Istudor, V., and Humadi, S.S. (2008). Quantitative analysis of bio-active compound in *Hibiscus sabdariffa* L. extracts. Note I Quantitative analysis of flavonoids. *Farmacia*. 56(6): 699-708.

*****