ETHANOL EXTRACT GUAVA LEAVES (PSIDIUM GUAJAVA) GIVE ANTIDIABETIC EFFECT ON MALE MICE WITH ALLOXAN INDUCTION

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ARTICLE INFO
Received:
Revised:
Approved:

ABSTRACT

Diabetes mellitus is a chronic condition caused by increasing glucose level in blood. Long term use of antidiabetic drugs can cause various unwanted side effects, which makes people with diabetes try to use natural materials to control blood glucose levels. The aim of this study was to determine the antidiabetic activity of ethanol extract guava leaves (Psidium guajava) on male mice with alloxan induction. Mice were induced intravenously by alloxan dose of 160 mg/kg bw. After 7 days, mice were diabetic (blood glucose levels > 175 mg/dL) and were divided into 5 groups: negative control group (only treated with CMC-Na) ,positive control group (glibenclamide 0.65 mg/kg bw) and the 3 other groups were given ethanol extract guava leaves (Psidium guajava) at doses of 150 ,300 and 600 mg/kg bw. Dosage administered for 15 days and blood glucose levels was checked every 3 days using a glucotest. Data were analyzed by LSD (Least Significant Difference). Ethanol extract guava leaves (Psidium guajava) at doses of 150 ,300 and 600 mg/kg bw can lower blood sugar levels from day 3 to day 15 and was significantly different compared to the negative control group (p<0,05). Ethanol extract guava leaves (Psidium guajava) at doses of 150 ,300 and 600 mg/kg bw showed antidiabetic activity on male mice with alloxan induction.

KEYWORDS: Antidiabetic, Psidium guajava, Ethanol extract, Alloxane, In Vivo

INTRODUCTION

Diabetes can happened because increased glucose levels continuously because of insulin deficiency. According to International Diabetes Federation (IDF) data, people with diabetes in 2021 has reached 537 million and most happened to patients with an age range of 20-79 years. This amount is expected to increase to 643 million in 2030 and 783 million in 2045. Indonesia is in fifth place, with amount of people with diabetes is 19.47 million and population is 179.72 million, this thing can be interpreted that the prevalence of diabetes in Indonesia is 10.6% (IDF, 2021). One of the antidiabetic drugs which can be used in the treatment of diabetes is glibenclamide. This drug works by increasing insulin secretion. However, glibenclamide has some side effects such as allergic skin reactions, hypoglycemia, cholestasis, aplastic anemia and hemolytic anemia. Many side effects which caused by these, make people with diabetes start looking for other alternatives such as using natural materials to lower blood sugar levels, one of them is guava leaves *(Psidium guajava)*. Secondary metabolites contained in guava leaves (*Psidium guajava*) are tannins that can lower blood glucose levels (Maharani et al, 2013). This study was further strengthened by the research of Deguchi & Miyazaki (2010) which reported that the tannin content in guava leaves can reduce the activity of the α-glucosidase enzyme. α-glucosidase enzymes such as α-amylase, maltase and sucrase digest carbohydrates into glucose which is absorbed through the intestine. Guava leaves extract (*Psidium guajava*) was able to inhibit the activity of maltase, sucrase and α-amylase by in vivo or in vitro which depends on the dose. According to research by Maharani et al, in 2013 about the effect of giving boiled water guava leaves (*Psidium guajava*) on blood glucose levels in patients with type 2 diabetes mellitus in Leyangan Village, East Ungaran District, Semarang Regency (Buheli & Ratnawati, 2021). Guava leaves (*Psidium guajava*) can be developed for treatment as medicine antidiabetic. Therefore, a study was conducted on the antidiabetic effect of ethanol extract guava leaves (*Psidium guajava*) on male mice with alloxan induction

RESEARCH METHOD

Collection of plant material :

The sample used in this study is fresh guava leaves (*Psidium guajava*) were collected from the local area of Nganjuk, Jawa Timur, Indonesia and authenticated by Ahmad Dahlan University Yogyakarta.

Preparation of extract :

The leaves of guava (*Psidium guajava*) were shade dried and powdered in a grinder. The air dried powder extracted with Ethanol 96% in maceration method. The extract was concentrated under reduced pressure using rotary evaporator at 60°C, then continued the drying process using a water bath until a thick extract is obtained.

Phytochemical analysis :

Ethanol extract was tested to know the different constituents present in it by the standard procedures. The extract was tested for alkaloids, saponins, flavonoids, steroids and tannins.

Animals:

All the experiments on animal were conducted according to protocols that were approved by Health Research Ethics Committee Faculty of Medicine Universitas Muhammadiyah Surakarta. The animals used were male white mice with a weight of about 20-30 grams. The mice used must be healthy and have never experienced any treatment. Before conducting the research, the test animals to be used must be adapted for a week to the same environmental conditions, food and drink. The test animals were fasted for 12-18 hours.

Alloxan-induced diabetes:

Fasted mice, weighed for each, then determined fasting blood sugar levels. Mice were induced intravenously by alloxan dose of 160 mg/kg bw. Mice were fed and drank as usual and their blood glucose levels were measured on 3rd day to 7th day. Mice are considered diabetes when blood glucose level >175 mg/dl and can be used for testing. They were divided into five groups into 5 groups: negative control group (only treated with CMC-Na), positive control group (glibenclamide 0,65 mg/kg bw) and the 3 other groups were given ethanol extract guava leaves (*Psidium guajava*) at doses of 150 ,300 and 600 mg/kg bw. Dosage administered for 15 days and blood glucose levels was checked every 3 days using a glucotest. Blood samples from the experimental mice were collected by the tail using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl. The data was represented as standard error of mean (SEM) of blood glucose level and were calculated.

Statistical analysis:

Data were expressed as mean ± SEM and performed on the Area Under Curve (AUC) value. Statistical analysis was carried out by one-way ANOVA to determine the average difference between groups, with a Least Significant Difference (LSD) test to determine the significantly different treatment. $p<0.05$, $p<0.01$ and $p<0.001$ were considered to be significant.

RESULT AND DISCUSSION

In phytochemical analysis indicated that, the ethanol extract of Guava leaves (Psidium guajava) contain alkaloids, saponins, flavonoids, steroids and tannins. The positive results with the color reaction and the precipitate reaction from each test using the tube method are in accordance with the reference. Positive result of the alkaloid test with Mayer's reagent producing a white precipitate, positive result of the tannin test producing a blue-black color, positive result of the saponin test producing a stable foam, positive result of the steroid test producing a green color, and positive result of the flavonoid test producing a red color. Phytochemical analysis results using the Thin-layer chromatography (TLC) method also showed good retention factor (Rf) values. The results of the observation of extract spots showed Rf values of 0,2; 0,5 and 0,7. This value has met the requirements of a good Rf value, which is between 0,2 – 0,8 (Rohman, 2009).

In Antidiabetic Effect Test of Alloxan Induction Method showed more higher Percent Decrease in Blood Glucose Leves value then more better antidiabetic effect of the test preparation. Percent Decrease in Blood Glucose Leves value from the highest to the lowest starting from the group glibenclamide 0.65 mg/kg bw which is 84.93%, group of Ethanol Extract of Guava Leaves (*Psidium guajava*) 600 mg/kg bw of 63.06%, Ethanol Extract of Guava Leaves (*Psidium guajava*) 300 mg/kg bw of 49.75% and Ethanol Extract of Guava Leaves (*Psidium guajava*) 150 mg/kg bw of 43.08%. (Table 1.)

Table 1. The Effect of Dosage on Percent Decrease in Blood Glucose Leves Mice with Alloxan Induction

Treatment Group	Percent Decrease in Blood Glucose Leves				
	3 day	6 day	9 day	12 day	15 day
Na CMC 0,5%	-4,06 ± 0,05	-9,53 ± 1,25	-18,57 ± 0,45	-24,29 ± 0,87	-30,12 ± 2,00
Glibenclamide 0,65 mg/kg bw	16,35 ± 0,71	29,35 ± 0,55	44,44 ± 1,00	62,62 ± 0,79	84,93 ± 1,26
Ethanol Extract Guava Leaves 150 mg/kg bw	7,26 ± 0,66	15,31 ± 1,25	26,58 ± 1,64	34,27 ± 1,55	43,08 ± 1,47
Ethanol Extract Guava Leaves 300 mg/kg bw	10,80 ± 0,85	20,05 ± 1,39	28,57 ± 0,91	39,71 ± 1,95	49,75 ± 1,75
Ethanol Extract Guava Leaves 600 mg/kg bw	13,59 ± 0,64	26,05 ± 1,30	36,88 ± 0,92	50,33 ± 0,87	63,06 ± 1,37

Table 2. The Effect of Dosage on Δ Percent Decrease in Blood Glucose Leves Mice with Alloxan Induction

Treatment Group	Δ Blood Glucose Leves					
	After- Before	3 day - Before	6 day - Before	9 day - Before	12 day - Before	15 day - Before
Na CMC 0,5%	368 ± 15,41	386 ± 15,72	410 ± 12,20	451 ± 16,25	477 ± 15,89	503 ± 11,02
Glibenclamide 0,65 mg/kg bw	408 ± 5,04	327 ± 1,20	262 ± 1,86	187 ± 0,58	98 ± 3,33	-12 ± 1,53
Ethanol Extract Guava Leaves 150 mg/kg bw	419 ± 27,79	381 ± 22,41	339 ± 17,91	291 ± 13,75	239 ± 7,22	191 ± 5,93
Ethanol Extract Guava Leaves 300 mg/kg bw	439 ± 17,03	381 ± 16,51	332 ± 18,85	287 ± 15,94	235 ± 17,67	174 ± 15,70
Ethanol Extract Guava Leaves 600 mg/kg bw	423 ± 14,68	351 ± 9,91	285 ± 7,88	229 ± 10,67	158 ± 10,48	90 ± 14,52

Based on Table 2. can be seen that Ethanol Extract of Guava Leaves (*Psidium guajava*) was able to lower Blood Glucose Leves on Mice with Alloxan Induction. After the LSD statistical test, the results showed that the entire group of Ethanol Extract of Guava Leaves (*Psidium guajava*) have significantly different compared to the Na CMC 0,5% group ($p<0,05$). Viewed from the Blood Glucose Leves value, more lower Δ Percent Decrease in Blood Glucose Leves, then more better antidiabetic effect of the test preparation. The result of 15th day Δ Percent Decrease in Blood Glucose Leves value from the lowest to the highest starting from the group glibenclamide 0.65 mg/kg bw which is -12%, group of Ethanol Extract of Guava Leaves (*Psidium guajava*) 600 mg/kg bw of 90%, Ethanol Extract of Guava Leaves (*Psidium guajava*) 300 mg/kg bw of 174% and Ethanol Extract of Guava Leaves (*Psidium guajava*) 150 mg/kg bw of 191%.

The antidiabetic effect of Extract of Guava Leaves (*Psidium guajava*) on Mice with Alloxan Induction have significantly different between test doses of Extract of Guava Leaves (*Psidium guajava*) compared to the Na CMC 0,5%, this can be seen from the effect of dose on total AUC value of mice with alloxan induction in Table. 3

Table 3. The Effect of Dosage on Total AUC Mice with Alloxan Induction

Treatment Group	Average Total AUC
Na CMC 0,5%	7.73 ± 0,18
Glibenclamide 0,65 mg/kg bw	4,52 ± 0,08
Ethanol Extract Guava Leaves 150 mg/kg bw	6,23 ± 0,40
Ethanol Extract Guava Leaves 300 mg/kg bw	6,05 ± 0,35
Ethanol Extract Guava Leaves 600 mg/kg bw	5,40 ± 0,24

After the statistical test, the results showed that the entire group of Ethanol Extract of Guava Leaves (*Psidium guajava*) have significantly different compared to the Na CMC 0,5% group ($p<0,05$). Viewed from AUC value, more lower AUC value more better antidiabetic effect of the test preparation. Lowering blood sugar levels with ethanol extract of guava leaves (*Psidium guajava*) treatment, may be due to the presence of bioactive compounds contained in the ethanol extract of guava leaves (*Psidium guajava*), including tannins, flavonoids, alkaloids, saponins and steroids. Tannin compounds can increase glycogenesis (the breakdown of glucose into glycogen) so the accumulation of glucose in the blood can be avoided, in addition these compounds also function as chelators that shrink the epithelial membrane of the small intestine, so it can reduce absorption of food essence as a result inhibits sugar intake and the rate of increase in blood sugar is not too high. Alkaloids work by stimulates the hypothalamus which will induce hypoglycemia and reduce gluconeogenesis, therefore decreased blood glucose levels and insulin requirements. Alkaloids are also proven to have the ability to regenerate damaged pancreatic cells (Prameswari and widjanarko, 2017). Flavonoids have antidiabetic effects in several ways among other things inhibits glucose absorption, increases glucose tolerance, stimulates insulin release, increases glucose uptake by peripheral tissues and regulates enzymes that play a role in carbohydrate metabolism (Handayani, 2019). Saponins work by reducing the absorption of glucose in the intestines by damaging cell membranes (Fiana and Oktaria, 2016). Steroids have activity to inhibit α-glucosidase enzyme activity (Isnaniah, 2017). The pathological conditions of the test animals do not fully describe the real physiological conditions in humans. This is due to differences in physiological, pathological conditions, and several models of diabetes mellitus, various diseases and complications of the disease (Nugroho, 2006).

CONCLUSION

Ethanol extract guava leaves (*Psidium guajava*) at doses of 150, 300 and 600 mg/kg bw showed antidiabetic activity on male mice with alloxan induction

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