Antidiabetic Activity of Coriander (Coriandrum Sativum L) Leaves' Ethanolic Extract

Widhya Aligita*, Elis Susilawati, Harini Septiani, Raihana Atsil

Department of Pharmacology, Bandung School of Pharmacy, Bandung, Indonesia.

ABSTRACT

Diabetes mellitus (DM) is one of the metabolic disorder marked by hyperglycemia caused by carbohydrate, fat, and protein metabolism abnormality. There is a significant increase in diabetes case every year in Indonesia and the long-term use of antidiabetic drugs can cause various side effects. This necessitates the development of a better drug. Coriander has been used as an herbal medicine for diabetes mellitus, particularly the seed. The aim of this research was to evaluate the activity of coriander leaves as an antidiabetic agent, utilizing both in vivo and in vitro method. In in vivo method, the extract was orally administered to the insulin deficiency mice model (aloxan induced at dose of 65 mg/kg bw iv) with dose of 200, 400, and 800 mg/ kg bw for 14 days. At the end of the in vivo study, the pancreas was isolated. While in in vitro method, the α-glucosidase inhibition activity study was performed. The results showed that after 3 days of administrations, the extract at each dose and glibenclamide 0.65 mg/kg bw could lower the glucose blood level significantly, compared to the positive control group ($p<0.05$). The pancreas histology also showed an elevation in pancreatic β cell in the extract and standard drug group. The in vitro study showed that the IC50 of the extract was 32.376 ppm, and 82.272 ppm for acarbose as a standard drug. The conclusion of this study was that the coriander leaves' ethanolic extract has the antidiabetic activity at the dose of 400 mg/kg bw by improving and regenerating the pancreatic β cell and inhibiting the α-glucosidase enzyme activity.

Key Words: Diabetes Mellitus, Insulin Deficiency, Coriandrum Sativum L., Coriander Seed, A-Glucosidase.

eIJPPR 2018; 8(2):59-63

HOW TO CITE THIS ARTICLE: Widhya Aligita, Elis Susilawati, Harini Septiani, Raihana Atsil (2018). "Antidiabetic activity of coriander (Coriandrum Sativum L) leaves' ethanolic extract", International Journal of Pharmaceutical and Phytopharmacological Research, 8(2), pp.59-63.

INTRODUCTION

Diabetes mellitus is a metabolic disorder marked by hyperglycemia caused by the abnormality in carbohydrate, fat, and protein metabolism. This hyperglycemia condition is developed by the decrease in insulin secretion or insulin sensitivity, or both. Diabetes mellitus can lead to some chronic complications, such as microvascular, macrovascular, and neuropati complications [1]. There are several types of diabetes mellitus: type I diabetes, marked by pancreatic β cell disruption; type II diabetes, usually involving excess weight and insulin resistance; gestational diabetes, a temporary metabolic disorder that any previously nondiabetic woman can develop during pregnancy, usually at the third trimester; and secondary diabetes, caused by another condition such as pancreatitis, cystic fibrosis, Down syndrome, hemochromatosis, and medical treatments including corticosteroids, other immunosuppressives, diuretics and pancreatectomy [2].

The incidence rate of diabetes continues to increase every year, especially type 2 diabetes. This is in line with the increasing cases of obesity and changes in lifestyle such as physical inactivity along with a diet that is high in calories, processed carbohydrates and saturated fats and insufficient in fiber-rich whole food. The estimated number of people with diabetes has jumped from 30 million in 1985 to 150 million in 2000 and then to 246 million in 2007, according to the International Diabetes Federation. The number is expected to hit 380 million by 2025 [2].

Diabetics usually require regular monitoring to ensure that the blood glucose level remains within the normal limits so the occurrence of complications can be minimized. Diet and physical activity are imperative factors in managing diabetes, especially in the type II diabetes case. In addition to healthy lifestyle, the antidiabetic drugs like the alpha-

Corresponding author: Widhya Aligita
Address: Department of Pharmacology, Bandung School of Pharmacy, Bandung, Indonesia.
E-mail: ✉ widhya.aligita@stfb.ac.id
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest .
Received: 12 September 2017; **Revised:** 23 February 2018; **Accepted:** 25 March 2018

glucosidase inhibitors, biguanides, meglitinides, sulfonylureas, thiazolidinediones, and a new group called DPP-4 inhibitors is usually taken [2].

Alpha-glucosidase enzyme is an enzyme responsible for the conversion of carbohydrates into glucose. The α-glucosidase enzymes include maltase, isomaltase, sucrase, lactase, and α-dextrinase [3]. Carbohydrates in the diet will be digested by enzymes in the mouth and intestines into a simpler sugar which will then be absorbed into the body and increases the blood sugar level. The carbohydrate digestion process causes the pancreas to release the enzyme α-glucosidase into the intestine which will digest carbohydrates into oligosaccharides which will then be converted into glucose by the α-glucosidase enzyme released by the small intestine cells which will then be absorbed into the body [4]. The α-glucosidase enzyme hydrolyzes the glycosidic alpha (α) bond located between the sugar residues [5]. Inhibition of α-glucosidase enzyme activity causes decreased monosaccharide absorption and decreased postprandial glucose level [3]. The use of alpha glucosidase inhibitors in diabetics had been shown to decrease blood glucose level, body weight, and insulin secretion [6].

Since a long time ago, plants have been used as traditional medicines to treat various diseases, and this includes diabetes. Coriander (Coriandrum sativum L.), which belongs to the Umbeliferae family, is one of the many plants used as traditional medicines. All parts of this plant are edible, but the most commonly used parts are the leaves and the seeds. In Indonesia, coriander seeds are traditionally used to treat cold, thrush, gastric inflammatory, dizziness, nausea, and irregular menstruation [7]. Previous study had shown that the ethanol extract of coriander seed (Coriadrum sativum L.) could lower blood sugar level in diabetic animal [8, 9]. Another study also shows that coriander leaves methanol extract had antidiabetic activity in alloxan-induced animals [10]. Major active constituents of Coriandrum sativum L. are essential oils and fatty oil, with linalool as a major constituent [11]. The aim of this research was to evaluate the antidiabetic activity and its mechanism of action of coriander leaves' ethanol extract.

MATERIALS AND METHODS

Identification and authentication of plant material:

The Coriander (Coriandrum sativum L.) leaves we used were obtained from Manoko, Lembang, West Java, Indonesia. Fresh plants were dried at 60-70°C heat and then grinded into small pieces. The plant identification and authentication was performed by Biological Department, Padjajaran University.

Preparation of Coriandrum sativum L. leaves ethanolic extract:

1 kg dried leaves of Coriandrum sativum L. was macerated with 10 L ethanol, twice. The mixture was filtered using a filter paper and the filtrate was concentrated using rotary vacuum evaporator at 60°C [12].

Animals

Male Swiss–Webster mice 2-3 months old weighing 20-30 g were kept at standard laboratory conditions at 24-26°C, humidity 70-75%, and 12 hours light/dark cycle. Animals were fed with standard chow and water ad libitum. The methods in this study were performed in accordance with ethics and guide for animals care and used.

Inhibition of α-glucosidase enzyme activity of coriander leaves' ethanolic extract

Evaluation of inhibiting activity of α-glucosidase enzyme was performed by using standard method [13]. Measurement of α-glucosidase enzyme inhibiting activity was performed on blank solution, control, standard drug (acarbose), and coriander leaf extract with different concentrations of 1000, 500, 250, 125, 62.5, and 31.25 ppm. A 60 µl of sample solution was added with 50 µl phosphate buffer solution (pH 6.8) containing α-glucosidase (0.2 U / mL) solution and incubated at 96 well plates at 37 °C for 20 min. After pre-incubation, 50 µl of 5 mM p-nitrofenil α-D-glukopiranosida (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 °C for another 20 min. After 20 minutes of incubation, an addition of 160 µl of $NaCO_3$ 0.2 M solution was introduced into each well to stop the reaction, and the absorbance was measured at a wavelength of 405 nm. The α-glucosidase enzyme inhibitory activity is calculated as the percentage of inhibition by using the following formula:

$$\%\ inhibition = \frac{Aco - At}{Aco} \times 100\%$$

Aco is the absorbance of the control solution and at is the absorbance of the sample solution.

In vivo antidiabetic evaluation of coriander leaves' ethanolic extract

There were two stages in in vivo antidiabetic testing, the first one was the induction phase and the second was the treatment phase. In the first phase, mice were induced with alloxan 50 mg / kgBW intravenously [14]. Mice with hyperglycemia (blood glucose> 200 mg / dL) will be used in the second phase of evaluation. Mice with hyperglycemia were randomly grouped into 5 groups: positive control group, standard drug group (glibenclamide 0.65 mg / kg BW), and ethanol extract group of coriander leaves 200 mg / kg BW, 400 mg / kg BW, and 800 mg / Kg BB. Administration of coriander leaves ethanol extract and glibenclamide as a standard drug were performed for 14

days. Parameters measured were blood glucose level on days 3, 7, 11 and 14 during treatment. Mice blood was taken from the vein part of the tail and measured using EasyTouch® glucometer. On the last day of evaluation, 2 mice from each test group were put to death and a pancreatic organ isolation was performed for histologic observation on β and α cells. The data obtained were then analyzed statistically using ANOVA method.

RESULTS AND DISCUSSION

Effect of coriander leaves ethanol extract on α-glucosidase enzyme inhibition

Parameters generated from in vitro evaluation inhibition of alpha glucosidase enzyme activity are IC_{50} values. IC_{50} or inhibitory concentration 50 is the concentration of the inhibitor needed to inhibit 50% of the activity of the α-glucosidase enzyme. The smaller the value of IC_{50}, the stronger the inhibitor in inhibiting alpha glucosidase enzyme activity.

Based on the experiment, IC_{50} value generated from akarbose as standard drug was 82,272 ppm, while coriander leaves' ethanolic extract was 32,376 ppm. It showed that coriander leaves' extract had stronger activity in inhibiting the enzyme. These result was comparable with another study that showed the coriander leaves had the activity to inhibit the alpha glucosidase enzyme [15].

The mechanism of the alpha glucosidase enzyme inhibitor is by inhibiting the activity of alpha glucosidase enzyme in the small intestine that plays a role in the breakdown of carbohydrates into monosaccharides before being absorbed. With this mechanism, automatically the process of glucose absorption will be inhibited and will reduce postprandial blood glucose level. So the extract that had the activity of inhibiting alpha glucosidase enzyme was potentially used as a drug for diabetes therapy, especially to inhibit the postprandial blood glucose level.

Effect of coriander leaves ethanol extract on insulin deficiency mice model

The elevation of blood glucose level during treatment was shown in Figure 1.

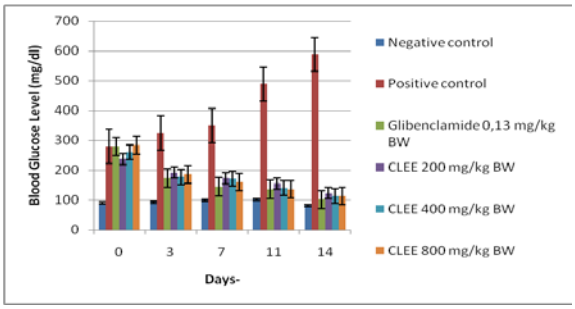

Fig. 1. The elevation of blood glucose level during treatment.

CLEE= coriander leaves' ethanolic extract. $p<0.05$, n = 4 mice/group

Administration of alloxan 50 mg / kg BW induced the hyperglycemia in mice, characterized by elevated blood glucose level (>200 mg / dL) in alloxan-administered groups (positive control, glibenclamide, and extract at dose of 200, 400, 800 mg/kg bw groups) compared to negative control group. Alloxan is a derivate urea compound that causes hyperglycemia conditions by selectively destroying pancreatic β cells that produce insulin. The severity of pancreatic β cell damage is determined by the given alloxan dose [16]. The alloxan toxic mechanism against pancreatic β cells is by oxidizing sulphydryl groups, inhibiting glucokinase enzymes, producing free radicals, and disrupting intracellular calcium homeostasis [17-19]. The selectivity of alloxan to pancreatic β cells is due to the similarity of structures with glucose which resulted in the mechanism of alloxan uptake by pancreatic β cells to be highly efficient [20].

Standard drug administration and extracts were performed for 14 days in hyperglycemic animals. Blood glucose level as a parameter was measured at days 3, 7, 10, and 14 treatment. The blood glucose level and percentage change in blood glucose level was shown in Table 1 and Table 2, respectively.

Table 1. Blood Glucose Level During Treatment

Group	Blood glucose level at day-(%)				
	0	3	7	11	14
Negative control	89±2*	91±4*	100±18*	105±16*	79±12*
Positive control	239±18	263±16	290±46	461±141	585±14
Glibenklamid 0,65 mg/kg BW	298±42	188±15*	156±7*	146±15*	97±39*
CLEE 200 mg/kg BW	242±20	199±21*	178±21*	162±15*	138±38* #
CLEE 400 mg/kg BW	267±60	172±22*	177±20*	138±19*	108±23*
CLEE 800 mg/kg BW	304±46	188±7*	159±16*	134±5*	107±12*

Note: CLEE = Coriander leaves ethanolic extract, data are presented as mean ± SD, n = 4 mice/group, * means significantly different compared to positive control group, # means significantly different compared to glibenclamide group, $p<0.05$.

Based on the blood glucose level measured during treatment, it could be seen that administration of glibenclamide and extract at doses of 200, 400, and 800 mg/kg bw could significantly lower blood glucose level compared with positive control group. The extract group at doses of 200, 400, and 800 mg / kg bw did not show significant activity differences compared with the glibenclamide group on days 3, 7, and 11. It was only on day 14, the extract at dose of 200 mg / kg bw group showed a significant difference compared to the glibenclamide

group. However, the extract at dose of 200 mg / kg bw group showed a decrease in blood glucose level during treatment.

Table 2. The Percentage Change in Blood Glucose Level During Treatment

Group	Percentage change of blood glucose level at day-(%)			
	3	7	11	14
Positive control	10±5	22±16	91±47	146±10
Glibenklamid 0,65 mg/kg BW	-36±12	-49±9	-51±3	-68±11
CLEE 200 mg/kg BW	-18±7	-27±7	-33±4	-43±14
CLEE 400 mg/kg BW	-33±18	-31±19	-47±13	-57±16
CLEE 800 mg/kg BW	-37±7	-47±7	-55±8	-64±7

Note: CLEE = Coriander leaves ethanolic extract, data are presented as mean ± SD, n = 4 mice/group, $p<0.05$

Table 2 showed that the percentage decrease in blood glucose level got higher with the increase of the coriander leaves' extract dose. This data proved that the extract of coriander leaves had antidiabetic activity. These was in line with the results of another studies that showed that the ethanol extract of coriander leaves and stems could lower blood glucose level in alloxan-induced animals [21].

The animals that had been given treatment for 14 days then randomly selected as much as 2 mice to be sacrificed and then pancreatic isolation was performed. The isolated pancreas was then stained using victoria blue and floxin dyes (Gomori staining). The parameters observed from these pancreatic preparations were the average area of the Langerhans islet, the quantity of α cells, and the quantity of β cells in the Langerhans islet. Alpha cells are cells that produce glucagon, whereas β cells are responsible for insulin secretion that decreases blood glucose level. The number of cells α is about 20-40% whereas β cells account for about 75% of the Langerhans islets. The improvement of Langerhans islet condition due to treatment was indicated by the higher amount of β cells and larger Langerhans islet area. The result of histological observation could be seen in Table 3.

Table 3. The Langerhans Islet Conditions After 14 Days Treatment

Group	Wide area of Langer hans islet	Quantity of	
		Alpha cell	Betha cell
Negative control	0,05±0,03	46,50±2,12	292,00±28,28
Positive control	0,02±0,00	83,00±9,89	14,00±5,66
Glibenklamid 0,65 mg/kg BW	0,08±0,03	79,50±17,80	169,00±69,30
CLEE 200 mg/kg BW	0,05±0,04	57,00±29,70	252,00±124,45
CLEE 400 mg/kg BW	0,07±0,02	45,50±16,26	302,00±178,20
CLEE 800 mg/kg BW	0,12±0,03	107,50±54,45	669,50±219,91

Note: CLEE = Coriander leaves ethanolic extract, data were presented as mean ± SD, n = 2 mice/group

From Table 3, it could be seen that the negative control group showed differences in the area of the Langerhans islet, the number of alpha cells, and the number of beta cells compared with the positive control group. These result proved that alloxan could cause hyperglycemia by destroying the Langerhans islet and beta cells. In addition, glibenclamide group, ethanol extract of coriander leaves at doses of 200, 400 and 800 mg / KgBW showed a difference compared with positive control group, ie, increased beta cell quantity and larger Langerhans islet area. From the results of this histology observation, it could be suspected that the mechanism of ethanol extract of coriander leaves in lowering blood glucose level was by improving or regenerating pancreatic beta cells.

CONCLUSSION

The ethanolic extract of coriander (Coriandrum sativum L.) leaves had the antidiabetic activity at dose of 400 mg/kg bw by improving and regenerating the β cell in pancreas and inhibiting the α-glucosidase enzyme in small intestine

REFERENCES

[1] DiPiro, J.T., Wells, B.G., Schwinghammer, T.L., & DiPiro, C.V. Pharmacotherapy Handbook.7th ed. New York: McGraw-Hill 2009.
[2] Riaz S. Diabetes Mellitus. Scientific Research & Essay 2009; 4 (5): 367-73.
[3] Soumyanath, Amala (Ed.). Traditional Medicines for Modern Times Antidiabetic Plants. Boca Raton: CRC Press 2006.
[4] Bösenberg, L.H. The mechanism of action of oral antidiabetic drugs: a review of recent literature. The Journal of Endocrinology, Metabolism and Diabetes of South Africa 2008; 13(3): 80-8.
[5] Champe, P.C., Harvey, R.A., dan Ferrier, D.R. Lippincott's Illustrated Reviews: Biochemistry. Philadelphia: Lippincott Williams and Wilkins 2005.
[6] Cai X, Han X, Luo Y, Ji L. Comparisons of the Efficacy of Alpha Glucosidase Inhibitors on Type 2 Diabetes Patients between Asian and Caucasian. PLoS ONE 2013; 8(11): e79421. doi:10.1371/journal.pone.0079421

[7] Departemen Kesehatan RI. Inventaris Tanaman Obat Indonesia Jilid 2. Jakarta: Departemen Kesehatan RI 2000.
[8] Sogara, P., dkk. (2014). Pengaruh Ekstrak Etanol Buah Ketumbar (Coriandrum sativum L.) Terhadap Penurunan Kadar Gula Darah Tikus Putih yang Diinduksi Aloksan. ISSN 2302 – 2493.
[9] Abderrahmane A, Soumia ZA, Zafar H. Israili B, Badiaa L. Hypoglycemic and hypolipidemic effects of Coriandrum sativum L. in Meriones shawi rats. J Ethnopharmacol 2011; 137:652– 61.
[10] Mazhar J, Mazumder A. Evaluation of Antidiabetic Activity of Methanolic Leaf Extract of Coriandrum sativum in Alloxan Induced Diabetic Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2013; 4(3): 500-7.
[11] Momin AH, Acharya SS, Gajjar AV. Coriandrum sativum – Review of Advances in Phytopharmacology. International Journal of Pharmaceutical Sciences and Research 2012; 5(3): 1233-9.
[12] Yulinah E, Sukrasno, Fitri MA. Aktivitas Antidiabetika Ekstrak Etanol Herba Sambiloto (Andrographis paniculata Nees (Acanthaceae)). JMS 2001; 6:13-20.
[13] Kakoos, AR. In-vitro α-glucosidase inhibition and antioxidant activity of methanolic extract of Centaurea calcitrapa from Iraq. American Journal of Essential Oils and Natural Products 2013; 1 (1): 122-5.
[14] Srinivasan K, Ramarao P. Animal Models in Type 2 Diabetes Research: An Overview. Indian J Med Res 2007:451-72.
[15] Brindis F, González-Andrade M, González-Trujano ME, Estrada-Soto S, Villalobos-Molina R. Postprandial glycaemia and inhibition of α-glucosidase activity by aqueous extract from Coriandrum sativum. Nat Prod Res 2014; 28(22):2021-5.
[16] Etuk EU. Animals models for studying diabetes mellitus. Agric Biol J N Am 2010; 1:130-4.
[17] Dunn JS, Sheehan HL, Mclethie NGB. Necrosis of islets of Langerhans produced experimentally. Lancet 1943;1;484-7.
[18] Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas Physiol Res 2001; 50:536-46.
[19] Dhanesha N, Joharapurkar A, Shah G, Dhote V, Kshirsagar S, Bahekar R, Jain M. Exendin-4 activates glucokinase. J Diabetes 2012; in press: doi:10.1111/j.1753-0407.2012.00193. x.
[20] Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. Diabetologia 2008; 51:216-26.
[21] Sreelatha S1, Inbavalli R. Antioxidant, antihyperglycemic, and antihyperlipidemic effects of Coriandrum sativum leaf and stem in alloxan-induced diabetic rats. J Food Sci. 2012 Jul;77(7):T119-23.