Antidiabetic Effectiveness Test of Ethanol Extract of Brown Leaves (Theobroma Cacao L.) Against Mice (Mus Musculus)

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\textbf{Article Info}

\textbf{ABSTRACT}

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both. DM sufferers are increasing from year to year, accompanied by increasing costs of therapy, therefore, as an alternative to controlling blood glucose levels, use natural ingredients such as cocoa leaves. The aim of this research was to determine the effectiveness of ethanol extract of cocoa leaves (\textit{Theobroma cacao} L.) in reducing blood glucose levels in mice that had been induced by Aloxan. The research method used was experimental research. The research stages were sample collection, sample identification, making simpilicia, examining the characteristics of simpilicia, making extracts using maceration using 96\% ethanol solvent, and testing the antidiabetic effect of EEDC in mice induced by alloxan at a dose of 175 mg/kg bw intraperitoneally. Diabetic mice were divided into 5 groups, each group consisting of 5 mice that were given the test material 50 mg/KgBB; 100 mg/KgBB; and 200 mg/KgBB, Metformin 65 mg/KgBB, and CMC 0.5\%. Next, blood glucose levels were measured on days 3, 6, 9, 12, and 15, the percentage reduction in blood glucose levels was calculated, then the data was analyzed using the One-Way ANOVA method and Duncan's test, to see real differences between treatments. The research results showed that simplicia, the ethanol extract of brown leaves contains flavonoids, alkaloids, tannins, steroids/triterpenoids, saponins and glycosides. Cocoa leaf ethanol extract has effectiveness in lowering glucose levels, at the 5th and 6th hours it shows good effectiveness at a dose of 200 mg/KgBW of \(72.16 \pm 0.1.51\)\% and \(82.80 \pm 0.73\)\%, not significantly different from metformin 65 mg/KgBB of \(74.45 \pm 1.51\)\% and \(83.29 \pm 1.01\).  

\textbf{Keywords:} alloxan, antidiabetic, chocolate, leaves, mice diabetes mellitus

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1. \textbf{INTRODUCTION}

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both. According to WHO data, Indonesia ranks 4th in terms of the number of diabetes mellitus sufferers in the world and in 2000 it was estimated that there were 4 million diabetes mellitus sufferers in Indonesia. One alternative is to use traditional medicine to treat a disease, because the use of traditional medicine is considered safer and minimizes side effects on the body [1].
Traditional medicine is a treatment medium that uses ingredients from animals, minerals and plants containing natural ingredients as raw materials. Since ancient times, Indonesian people have known and used plants as medicine to treat several diseases. Knowledge about medicinal plants is a national culture that has been passed down from generation to generation. Some people prefer treatment with medicinal plants rather than chemical drugs. People believe that medicinal plants are safer to consume and cause less undesirable side effects, so they choose to use traditional medicine to cure diseases [2].

About 800 plants have antidiabetic potential showing antidiabetic activity. which contains bioactive compounds in the form of phenolic compounds, flavonoids, and has an anti-diabetic role. One of the plants that is widely available in Indonesia and has been widely used as a traditional anti-diabetic medicine is cocoa leaves, but this has not been scientifically proven. Chocolate leaves (*Theobroma cacao* L.) contain theobromine, caffeine, anthocyanin, leukoanthocyanin, and catechol, the amounts of which vary depending on the age of the leaves and the age of the plant [3].

Based on research, the ethanol extract of brown leaves shows that there is central nervous system stimulation activity by administering a dose of 500 mg/kgBB. Ethanol extract of brown leaves [4]. From the results of research conducted by Osman et al, it is known that brown leaves have almost the same antioxidant activity as green tea. Methanol extract of cocoa leaves contains catechin-polyphenols such as epicatechin, epigallocatechin gallate, epigallocatechin, epicatechin gallate as a highly reactive hydroxy radical scavenger so that it can prevent diabetogenic action. In patients suffering from diabetes mellitus [5]. Based on this, researchers are interested in examining the effectiveness of ethanol extract of cocoa leaves (*Theobroma cacao* L.) as an antidiabetic in mice induced by alloxan.

2. METHOD

The research was carried out using laboratory experimental methods, using a research design in the form of the Duncan Test. The total number of samples used in the research was 1 sample; grouped randomly into 5 groups, each group consisting of 5 mice and given treatment orally. Results of identification of the type of brown leaf, namely *Theobroma cacao* L., obtained from Gang Rahmat, Sidahanon Village, Batang Toru District, South Tapanuli Regency, North Sumatra. Fresh brown leaf material was collected, washed thoroughly under running water, drained, and weighed (5,000 g) [6]. The chocolate leaves are then dried in a drying cupboard at a temperature of 40-50°C, until dry, discard any foreign objects that remain on the simplicia during drying (dry sorting), after sorting the dried simplicia, then grind them using a blender, the simplicia powder is stored in a plastic container. tightly closed to prevent moisture and other influences. 5 L of 96% ethanol was added as a solvent [7]. The maceration vessel was tightly closed and left for 5 days protected from light and stirred every few hours. The maserate is separated by filtration, then the dregs are dried in a drying cupboard. After drying, they are re-dissolved in 5 L of 96% ethanol solvent and then left for 2 days. All the macerate is collected, then the macerate obtained is concentrated using a rotary evaporator. Then concentrate in a water bath for approximately 24 hours. The thick extract is then thickened using a water bath to obtain a thick ethanol extract [8].

The treatment procedure for experimental animals begins with alloxan induction carried out on day 7 after adaptation of the experimental animals. The alloxan dose for mice is 175 mg/kgBB. Then alloxan is injected interperitoneal and waited for 72 hours. Before measuring blood glucose levels, mice were fasted from food for 8-12 hours (only water was provided). If there is an increase in the blood glucose levels of mice exceeding 200 mg/dl, then the mice have diabetes.

Measurement of blood glucose levels (pre test) was measured by disinfecting the tip of the mouse's tail with alcohol, then wounding the tip of the tail, then touching the dripping blood to a glucometer test strip (Autocheck™). After the mice were in a diabetic state, they were treated with
ethanol extract of brown leaves at a dose of 175 mg/kg BB for 21 days. Blood glucose levels on the 21st day after treatment (post-test), mice were first anesthetized using ether. Next, the mice were dissected and their blood was taken through the heart. Blood drops are placed on a test strip that has been attached to a glucometer (Autocheck™). Then the results are read on the screen within 10 seconds. The value shown is the blood glucose concentration value in mg/dl. Data analysis was carried out using the Duncan test.

3. RESULTS AND DISCUSSION

Data from measurements of blood glucose levels were analyzed statistically using the Duncan test. The results of the Duncan test on and 15th showed that the treatment group of mice given Metformin 65mg/KgBB and the group given EEDC 200mg/KgBB showed that the effectiveness of reducing blood glucose levels in mice was not significantly different, while the group given EEDC 100 mg/KgBB, and EEDC 50 mg/KgBB showed that the effectiveness of reducing blood glucose in mice was significantly different from each other so that EEDC 200mg/KgBB could be given as an antidiabetic [10].

In this study, mice were made to develop diabetes by alloxan induction. Alloxan compounds can cause damage to the pancreatic β cell membrane by increasing its permeability [11]. Damage to the membrane can facilitate damage to pancreatic β cells so that insulin decreases. The mechanism of action of alloxan in vitro, seen from its effect through increasing permeability, shows that alloxan will induce the release of calcium ions from mitochondrial organelles which will then result in the oxidation process of cells, tissues and organs being disrupted. Calcium ions that leave the mitochondria will disrupt homeostasis which is the beginning of cell death [12]. Therefore, administration of alloxan produces experimental diabetic conditions (hyperglycemia) in experimental animals.

Tannins can increase glycogenesis, namely the breakdown of glucose into glycogen so that glucose deposits in the blood can be avoided, function as an astringent or chelator which can contract the epithelial membrane of the small intestine thereby reducing the absorption of food essence, inhibiting sugar intake so that the rate of increase in blood sugar is not too high [13]) . Tannins are also able to increase antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) which protect tissue from free radicals and capture these free radicals and reduce the increase in oxidative stress in DM sufferers so they can control blood glucose levels [14].

Saponin is able to regenerate the pancreas which causes an increase in the number of pancreatic β cells and islets of Langerhans so that insulin secretion will increase and help reduce KGD [15]. Overall, the test results can be concluded that the ethanol extract of brown leaves is effective in reducing blood sugar levels in mice induced by alloxan, and the best dose is 200mg/KgBB, not significantly different from administering Metformin 65mg/KgBB on Day 12 and 15 after administering the test material [16].

From research that has been conducted, extracts of polyphenols and polysaccharides from cocoa leaves (Theobroma cacao L.) have antidiabetic potential. Because the mechanism of polyphenolic compounds has antioxidant properties which can stabilize free radicals by completing the lack of electrons that alloxan free radicals have and inhibiting the chain reaction of the formation of alloxan free radicals which can cause oxidative stress [17,18]. So that the increase in blood glucose levels in diabetic conditions by alloxan induction can be prevented.

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The mechanism of action of metformin is by not increasing plasma insulin levels and increasing insulin sensitivity through the effect of increasing peripheral glucose uptake. In addition, metformin is an ADO drug which can reduce blood glucose levels by inhibiting liver glucose production (gluconeogenesis) [22,23,24]. The results of this study showed that the blood glucose levels of diabetic mice after being given metformin were still higher than those of diabetic mice given ethanol extract of brown leaves. This is thought to be because administering metformin alone is often not optimal and is usually given together with glibenclamide so that treatment targets are achieved [25].

4. CONCLUSION

Based on the research results, it can be concluded that cocoa leaves (Theobroma cacao L.) contain secondary metabolite compounds, namely alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids and glycosides. The ethanol extract of cocoa leaves (Theobroma cacao L.) is antidiabetic in mice induced by Aloxan and Ethanol extract of cocoa leaves (Theobroma cacao L.) at a dose of 200 mg/kgBB has the best effectiveness in lowering blood glucose levels, not significantly different from that given with Metformin 65 mg/KgBB.

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REFERENCE