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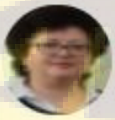

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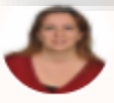

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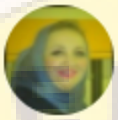

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RESEARCH ARTICLE

Antidiabetic and Antidyslipidemic activity of Secang (*Caesalpinia sappan* L.) Wood extract on Diabetic Rat

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ABSTRACT:
Diabetes mellitus is a syndrome due to disorders of carbohydrate, lipid, and protein metabolism due to decreased insulin secretion or reduced insulin sensitivity. The number of people with diabetes mellitus is increasing every year. However, diabetes mellitus is a major cause of cardiovascular disease, blindness, kidney failure, and amputation due to gangrene. Patients with diabetes mellitus have a possibility of 2-3 times higher cardiovascular disease than non diabetic. Sappan wood containing brazilin that have antioxidant activity and had a potential activity to lower the incidence of type 2 diabetes mellitus. Objective of this research was to determine the activity of secang wood extract as an antidiabetic and antidyslipidemic on diabetic rat. Diabetic rat induced by alloxan and given extract once daily for 14 days. At 15th day, blood glucose level, lipid profile was determine, pancreas was harvested and processed to hystopathological examination. Secang wood extract decreased blood glucose, cholesterol, triglyceride, and LDL level, increase HDL level, and repair the histology of pancreas on diabetic rat after 14 days treatment. Based on the result, secang wood extract had antidiabetic and antidyslipidemic activity on diabetic rat.

KEYWORDS: Diabetes mellitus, Secang wood extract, Diabetic, Dyslipidemic.

INTRODUCTION:

Diabetes mellitus is a syndrome due to disorders of carbohydrate, lipid, and protein metabolism due to decreased insulin secretion or reduced insulin sensitivity[1]. Diabetes mellitus is classified into four types, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes mellitus, and other specific types of diabetes mellitus. Type 1 diabetes mellitus occurs due to β-pancreatic cell damage due to autoimmune, and this causes the body to lack the hormone insulin. Type 2 diabetes mellitus is caused by the progressive deficiency of insulin production by the pancreas and decreased receptor sensitivity to insulin. Gestational type diabetes mellitus is suffered by women who are experiencing pregnancy during the third semester. Other types of diabetes mellitus are caused by genetic abnormalities of the hormone insulin, infections, endocrinopathy, and drugs that cause decreased work and secretion of the hormone insulin[2].

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The number of people with diabetes mellitus is increasing every year. According to data from the International Diabetes Federation (IDF) in 2017, more than 425 million people worldwide suffer from diabetes mellitus. This number increased compared to the data in 2015 and is expected to increase to 629 million in 2045[3]. Data from the Basic Health Research (RISKESDAS) in 2018 showed the prevalence of diabetes diagnosed by a doctor with an over 15 years of 8.5 % increased by 1.6 % compared to the known 2013[4]. In 2017 around 4 million people aged 20-79 years are estimated to die from complications of diabetes mellitus. The condition of hyperglycemia that occurs continuously can cause interference or damage to the pancreatic Langerhans islands, especially in beta cells. This is caused by excess reactive oxygen species (ROS), resulting in oxidative stress on beta cells. This situation resulted in pancreatic beta cells are damaged, and decreased function of insulin secretion[5].

Diabetes mellitus is a metabolic disorder that does not cause death directly. However, diabetes mellitus is a major cause of cardiovascular disease, blindness, kidney failure, and amputation due to gangrene. Patients with

diabetes mellitus have a possibility of 2-3 times higher cardiovascular disease, higher blood glucose levels persistently cause damage to blood vessels that can affect heart, kidneys, and nerves[3]. There were 14.9% of 187 diabetes mellitus patients experiencing cardiovascular complications, and as many as 57.7% had died due to cardiovascular complications in diabetes mellitus patients[6]. The cardiovascular complication in diabetics is associated with hyperlipidemia, obesity, and pressure blood high[7].

Hyperlipidemia is a heterogeneous disorder characterized by increased levels of lipids in the systemic circulation such as cholesterol, cholesterol esters, phospholipids, and triglycerides[8]. Insulin is a hormone of glucose metabolism also has a relationship with lipid metabolism. Insulin works as an inhibitor of Lipase Sensitive Hormone (LSH)[9]. LSH has the function to break down the triglyceride deposits in adipose tissue into free fatty acids and glycerol[10]. Free fatty acids will be channeled into the systemic channel and then will be taken by the liver for the formation of liver triglycerides. Patients with diabetes mellitus accompanied by hyperlipidemia have decreased insulin secretion so that LSH becomes very active, which causes an increase in the movement of fatty acids from adipose cells to the systemic circulation. This led to increased production of triglycerides (TG) and secretion very-low-density lipoprotein (VLDL) by the liver. VLDL containing apolipoprotein C-III, which can inhibit the action of lipoprotein lipase from making the process of lipolysis of VLDL TG to be stored in the adipose tissue. This will inhibit the storage of fatty acids from the liver into adipose tissue[11].

Diabetic patients with hyperlipidemia generally use more than one pharmacological therapy agent[12]. This causes the high cost of therapy that must be incurred. The cost of treating diabetes mellitus with cardiovascular complications borne by governments around the world reaches USD 1.3 T or 1.8% of global GDP (Gross Domestic Product)[13]. An alternative therapy that can overcome the blood glucose levels at once at the same cardiovascular risk in diabetes continues to be developed to get treatment more effective. One of them is using natural materials such as plants.

One of the potential as medicinal plants is secang (*Caesalpinia sappan* L,) wood, which containing brazilin as a compound that has antioxidant activity. The antioxidant activity of brazilin is higher than vitamin C and vitamin E and can increase the SAT (Body Antioxidant Unit) by 5.19mmol/L[14]. Flavonoid compounds had a potential activity to lower the incidence of type 2 diabetes mellitus by inhibiting the mechanism of damage to the cell β pancreas caused by free radicals[15].

MATERIAL AND METHODS:

Chemicals and Reagen:

Secang wood (*Caesalpinia sappan* L.) from Sumenep (Madura), NaCl 0.9%, alloxan, CMC N a 1%, glibenclamide, aquabidest, ethanol (50%, 70%, 95% and absolute), Fluitest and *neutral buffered formalin* (NBF) reagents, paraffin, xylene, *hematoxylin eosin* dyes. Reagent Fluitest® CHOL, Fluitest® GLU reagent, Fluitest® reagent TG, Fluitest® reagent LDL, Fluitest® reagent HDL, quercetin, methanol and distilled water.

Extract preparation:

Secang wood was collected in Sumenep, Madura, Indonesia. This plant has been identified by Plants Laboratory, Jember State Polytechnic, with register number: 65/PL17.3.1.02/LL/2018. Secang wood was washed and drier indirectly from the sun. the sample was grinded and then extracted by maceration. A total of 500 g of Simplicia was soaked in 5 L ethanol 96% for 48 hours. The filtrate is filtered using a Buchner funnel and then concentrated with a rotary evaporator at 50°C until a concentrated extract is obtained, and the remaining solvent is evaporated with an oven at 40°C until the weights of the secang wood extract (SWE) are constant.

Determination of total flavonoid levels:

A sample of 100mg extract was dissolved using 10mL methanol. Then filtered and dissolved samples added AlCl 3 2% (w/v) of 5mL. Samples that had been added with AlCl 3 2% were vortexed and incubated at room temperature for 10 minutes. Samples were measured using a UV-VIS spectrophotometer with a wavelength of 443nm. The standard curve used to determine total flavonoid levels in sappan wood is quercetin with series concentrations of 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg/mL, 7 µg/mL and 8 µg/mL [16].

Determination of total phenolic content:

Total phenolic content of the extract was evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent. 150µL aliquots of 2, 4, 8, 10 and 15µg/mL methanolic gallic acid solutions were mixed with 750µL Folin–Ciocalteu reagent (diluted ten-fold) and 600µL (7.5%) sodium carbonate and then incubate it for 45 min in the dark place, absorbance was measured at 760nm against reagent blank using the UV-Vis Spectrophotometer. Total phenolic content was expressed as mg gallic acid equivalent/gusing the following equation based on the calibration curve: y=0,103x - 0,00735, r = 0.9999, where x was the absorbance and y was the gallic acid equivalent (mg/g). All determinations were performed in triplicate. Total phenolic content was expressed as

milligrams of gallic acid equivalent (GAE) per g of extract.

Experimental design:

This experimental design approved by "The Ethical Committee of Medical Research Faculty of Dentistry Universitas Jember" with certificate number 455/UN25.8/KEPK/DL/2019. Rats were adapted to laboratory conditions for seven days to adapt to their environment. Then rats were induced intraperitoneally with alloxan at a dose of 135mg/kg body weight against five groups of animals to develop diabetes mellitus except for the normal group. Rats were given food and drink as usual. On the 3rd day after induction, rat blood glucose level was measured using a photometer. If blood glucose levels are ≥ 200mg/dL, rats can be used for further experiments[17]. Animals are divided into six groups, as follows:

1. Normal group: given a 1% CMC Na suspension orally
2. Diabetes group: given a 1% CMC Na suspension orally
3. Positive control group: given a suspension glibenclamide dose 0.9mg/kg bb orally
4. SWE group I: given a secang wood ethanol extract dose of 50mg/kg orally
5. SWE group II: given a secang wood ethanol extract dose of 100/kgBB orally
6. SWE group III: given a secang wood ethanol extract dose 4mg/kg orally

Rats were treated once daily for 14 days. Measurement of glucose, total cholesterol, triglyceride, LDL, and HDL levels were carried out on day 0 taken from the eye (sinus orbitals) and day 15 after administration of extracts, taken through the heart. The blood was collected using a microtube, then allowed to stand for 30 minutes, the blood was centrifuged for 10 minutes at 4,000rpm. Blood glucose, total cholesterol, triglyceride, LDL, and HDL were measured using a photometer.

Histopathology:

All rats were dissected, and their pancreatic organs were taken on the 15th day for histopathology. The tissue was stored in neutral buffered formalin (NBF) 10% for one night under conditions of pH 7.4 and at room temperature, then the sample was dehydrated using 70% alcohol, 80%, 90%, 95%, and absolute alcohol. After that, it was moved into xylol I and xylol II for 1 hour to make the tissue clear. Network nodes included in the liquid paraffin which is then carried casting (blocking) so that a block of preparations that can be cut with a microtome to 5µm and placed on an object-glass. Before staining hematoxylin-eosin, was deparaffinized with xylol I, xylol II and continued with rehydration of alcohol absolut, alcohol 95%, 80%, and 70%. Then staining hematoxylin-eosin and dehydration with 70% alcohol, 80%, 95%, and absolute alcohol. Before the repairs are glued with silicone and covered with a glass cover, the sections are put in liquid xylol I and xylol II. Analysis of section using a microscope magnification 100 to 400 times.

Statistical analysis:

Data were presented as mean±SD. The mean difference in blood glucose, total cholesterol, triglyceride, LDL, and HDL were analyzed using One-Way ANOVA followed by Least Significantly Different (LSD) with a 95% confidence level. The data were considered significant at $p<0.05$. Data histopathology were analyzed descriptively.

RESULT:

Secang wood was extracted in ethanol 96% by maceration method and total yield was 16.2% b/b of dry powder. The extract compound analysed quantitatively by total phenols and flavonoids. The amount of total phenol was 120mgGAE/g extract and flavonoid was 9.59 mgQE/g extract.

This study demonstrate that blood glucose increased to above 200mg/dL after being induced by alloxan. The administration of secang wood extract dose 50, 100, and 400mg/kgBW for 14 days resulted in significant ($p<0.05$) reduction in plasma glucose level, with the maximum reduction being 62.14% for extract dose 100 mg/kgBW. However, the standard drug, glibenclamide exhibit 62.74% reduction of glucose plasma, comparable with extract dose 100mg/kgBW (Table 1).

Table 1: Blood glucose level and glycemic change before and after treatment

Treatment group	Blood glucose levels (mean ± SD) (mg/dL) n=6		Glycemic change (%)
	Pre-test	Post-test	
Normal	101.04 ± 6.35	103.84 ± 15	2.77
Diabetic Control	520.15 ± 51.55	643.64 ± 49.83	23.74
Glibenclamide 0.9 mg/kg BW	512.19 ± 15.98	190.83 ± 54.7	-62.74
SWE 50 mg/kg BW	477.93 ± 160.01	271.68 ± 77.08	-43.15
SWE 100 mg/kg BW	535.45 ± 134.49	202.69 ± 118.14	-62.14
SWE 400 mg/kg BW	378.84 ± 117.28	270.55 ± 83.74	-28.58

The administration of alloxan increased the level of total cholesterol, triglyceride, and LDL, and decrease the level of HDL plasma. The treatment with SWE dose 50, 100, and 400mg/kgBW decreased the level of total cholesterol, triglyceride, and LDL, and increase the level of HDL plasma after 14 days. The results are shown in Table 2.

Table 2. Blood lipid parameter after treatment

Treatment group	Blood Lipid Parameter (mean ± SD) (mg/dL) n=6			
	Cholesterol	Triglyceride	LDL	HDL
Normal	28.58 ± 6.90[a]	39.47 ± 9.51[a]	16.60 ± 3.80[a]	27.65 ± 2.22[a]
Diabetic control	106,768 ± 19.15[b]	234.68 ± 46.68[b]	77.87 ± 7.33[b]	8.27 ± 2.05[b]
Glibenclamide 0.9mg/kgBW	82.22 ± 14.56[bc]	62.20 ± 17.06[a]	93.80 ± 5.81[b]	11.83 ± 1.40[b]
SWE 50 mg/kgBW	79.41 ± 23.93[cd]	52.50 ± 9.55[a]	32.89 ± 4.20[a]	36.05 ± 4.31[c]
SWE 100 mg/kgBW	42.54 ± 9.11[a]	61.1 ± 15.14[a]	26.99 ± 0.97[a]	50.61 ±
SWE 400 mg/kgBW	54.68 ± 17.32[ad]	64.18 ± 20.99[a]	31.16 ± 1.29[a]	41.65 ± 7.67[c]

Data are expressed as mean ± SD (n=6)
Different superscript letter in the same row are significantly different by ANOVA followed by LSD test ($p<0.05$)

The administration of alloxan induced diabetes with the alteration in the pancreas. The island of langerhans forms irregularly and showing congestion dan vacuolization of Langerhans cells. The island of Langerhans at SWE dose of 100mg/kgBW group had the lowest damage with normal beta Langerhans cells if compared to the positive control group, SWE dose of 50 mg/kgBW, and SWE dose of 400mg/kgBW. The group got SWE dose 400mg/kgBW had bigger glucose levels and the damage of the island of Langerhans compared to other dose. This results shows that the activity of decreasing the blood glucose level of diabetic rats is not directly proportional to the increasing dose of ethanol extract of secang wood. Basically, an increase in dose will be followed by an increase in the activity of a compound, but if the dose has reached the optimum dose, then the resulting response will decrease[18]. The section of the pancreas are shown in Figure 1.

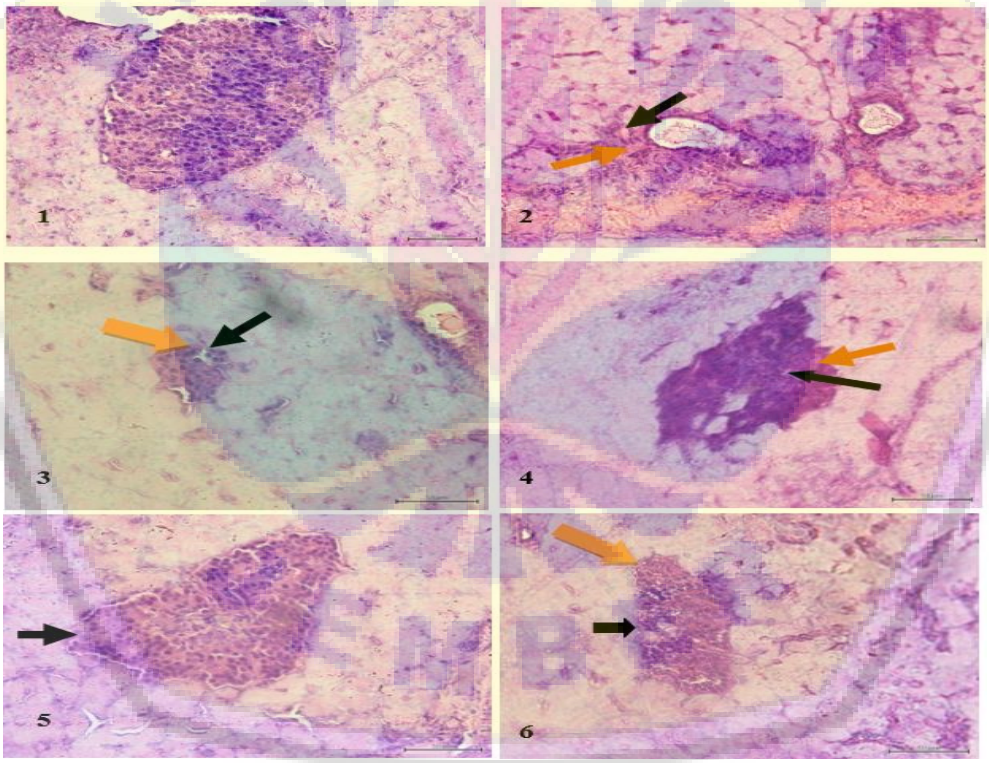

Figure 1. Pacreas histopathology, cross sectional, haematoxylin-eosin staining, magnification 400X. (1) normal; (2) negative control; (3) positive control; (4) SWE dose 50mg/kgBW; (5) SWE dose 100mg/kgBW; (6) SWE dose 400mg/kgBW; ➡ Vacuolization, ➡ Congestion

DISCUSSION:

This study showed that alloxan administration increased blood glucose level. Alloxan plays an important role in the formation of reactive oxygen species (ROS), which can damage pancreatic beta cells. Damage due to the presence of reactive oxygen species (ROS) has similarities with diabetes that occurs in humans[19]. Pancreatic beta-cell damage occurs as a result of the formation of reactive oxygen that begins with the process of reducing alloxan in pancreatic beta cells. The result of the reduction of alloxan is dialuric acid, which then undergoes reoxidation to alloxan to form a redox cycle to produce reactive oxygen species (ROS) and superoxide radicals. This will cause damage to almost all components of pancreatic cells, especially beta cells that exist on the island of Langerhans[20,21].

Reactive oxygen species (ROS) that are formed can also cause pancreatic β cell membrane depolarization and an increase in Ca^{2+}, which can increase cell membrane permeability. This causes acceleration of pancreatic β cell damage, which further impacts on decreased insulin production and can even result in insulin not being reproduced[20]. The other mechanism of pathological action of alloxan is that it can inhibit insulin secretion which is stimulated by glucose through specific inhibition of energy metabolism precisely in the process of glucokinase which is a glucose sensor from pancreatic beta cells. This will result in the diffusion of insulin, which can result in an increase in blood glucose levels[22].

Diabetes mellitus rats have increased blood glucose levels due to damage to the structure of the island Langerhans. This can be seen from the irregular shape of the Langerhans island, vacuolization, and congestion, which can be seen in Figure 4.1. Vacuolization is a stage of necrosis that occurs when cytoplasmic organelles have been digested by enzymes. If congestion occurs continuously, it can lead to the death of parenchymal tissue cells and secondary tissue fibrosis[23]. Damage to the Langerhans islets, especially beta cells, caused by the administration of alloxan. The groups were given ethanol extracts of secang wood at various doses had damage to the pancreas even though it was much smaller when compared to the negative control group. This shows that the ethanol extract of secang wood can repair damaged islands of Langerhans as a result of induction of alloxan.

Glibenclamide can provide histological changes or resemble the normal form of the island of Langerhans within a period of 4 weeks[24]. Regeneration of pancreatic Langerhans, specifically pancreatic beta cells, has a role in insulin secretion. Increased insulin secretion can reduce blood glucose levels in the body. Langerhans is regenerated due to pancreatic beta cells undergoing proliferation or neogenesis of progenitor cells[25].

The antidiabetic activity of secang wood ethanol extract is suspected due to the presence of several bioactive compounds. Research on phytochemical screening and antioxidant testing found that the secang ethanol extract of wood had several classes of compounds including terpenoids, phenols, triterpenoids, alkaloids, saponins, and flavonoids with very high levels in the group[26]. These bioactive compounds are thought to have antidiabetic activity. Flavonoid is a component of a promising compound as antidiabetic because it can increase insulin secretion and repair damaged Langerhans islets. The phenol group has an antioxidant role. Antioxidants can reduce oxidative stress by donating hydrogen atoms from the aromatic hydroxyl group (-OH) to bind free radicals and remove them from the body through the excretion system[27]. In addition, antioxidants can also increase insulin sensitivity[28].

Flavonoid compounds are phenol compounds found in various types of plants. This compound has an antidiabetic action mechanism by regenerating damaged pancreatic beta cells, increasing glucose tolerance, reducing glucose absorption and regulating the activity of the expression of enzymes involved in carbohydrate metabolism[29,30]. The flavonoid of SWE are brazilin, protosappanin, brazilein, and gallic acid compounds[31,32]. Brazilin is the main constituent of compounds in the secang wood core or known as the natural red coloring agent for coloring[31]. Brazilin has a mechanism of action to increase the production of fructose-2,6-bisphosphate (F-2,6-BP) and hexose-6-phosphate (H-6-P)[33]. Fructose-2,6-bisphosphate in the metabolic process has a role in regulating glycolysis and gluconeogenesis in the liver. Increased fructose-2,6-bisphosphate in a state of hyperglycemia will stimulate the process of glucose breakdown (glycolysis) by activating phosphofructokinase-1 so that there can be a decrease in blood glucose levels in the body[34].

Saponin compounds have a mechanism of antidiabetic action by inhibiting glucose transport in the gastrointestinal tract and stimulating insulin secretion in pancreatic beta cells[35,36]. Alkaloids work by regenerating pancreatic beta cells, increasing insulin secretion and sensitivity, and influencing the activity of enzymes related to glucose metabolism[37]. Hydrolyzed condensed tannins and able to lower blood glucose levels by inhibiting α -amylase, and α -glucosidase resulting in carbohydrate digestion and absorption of glucose pending at the time of high blood glucose levels after meals[38,39]. Triterpenoids have the ability to maintain the function of pancreatic beta cells. This will result in biosynthesis, secretion, and insulin sensitivity being controlled. In addition, triterpenoids are also able to inhibit advanced glycation end products (AGEs) so that they can prevent diabetes complications[40].

Treatment with secang wood extract for 14 days reduced plasma total cholesterol, triglyceride, and LDL, and increase HDL in diabetic rats indicating its potent antidyslipidemic activity. The glucose lowering action can be due to the consequence of an improved lipid metabolism apart from the direct interaction with glucose homeostasis. The lipid lowering property of SWE could indirectly contributed by flavonoid. Isoflavonoids can reduce total cholesterol, triglycerides, Apo-B, LDL and VLDL concentrations and increase HDL levels by inhibiting 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase and increasing the activity of LPL, LCAT and LDL receptor expression[41]. Flavonoid compounds can increase the activity of

Lechitin Acyl-CoA Cholesterol Acyl Transferase (LACAT) and lipoprotein lipase (LPL) enzymes, and inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methyl-glutaril-CoA[42].

CONCLUSION:

This study concludes that secang wood extract (*Caesalpinia sappan* L.) has antidiabetic and antidyslipidemic activity that could be useful for future study of this plant as an alternative therapy for diabetes mellitus.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interests in this study.

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