THE EFFECT OF SAPPAN WOOD (*Caesalpinia sappan* L.) EXTRACT ON BLOOD GLUCOSE LEVEL IN WHITE RATS

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ABSTRACT

Sappan wood or kayu secang (*Caesalpinia sappan* L.) was reported of having medicinal properties, such as natural antioxidant, relieve vomiting of blood, and mix of ingredients for malaria drugs. The research was conducted to study the influence of ethanol extract from sappan wood on blood glucose level of white rats. The study of the blood glucose level in rats was carried out by using glucose tolerance method. It was measured by Refloluxs (Accutrend GC) with Chloropropamide 50 mg/200 g BW (Body weight) as positive control. The ethanol extracts were used in various concentrations 10, 20, 30, 40 and 50 mg/200 g BW per-oral and was observed every hour, beginning one hour before to 7 hours after the extract being administered. The results showed that treatment of ethanol extract of sappan wood by administer doses gave remarkable effect on the blood glucose level in white rat. It reduced the glucose level in the blood compared to the negative and positive control. Treatment of dose 30 mg/200 g BW gave similar effect to positive controls, while a dose of 50 mg/200 g BW gave lower blood glucose level (93 mg/dl) than the positive controls.

Keywords: Sappan wood, ethanol extract, blood glucose level, white rat

ABSTRAK

Kayu Sappan atau kayu secang (*Caesalpinia sappan* L.) dilaporkan memiliki banyak manfaat sebagai tanaman obat, misalnya untuk antioksidan alami, meredakan muntah darah, dan bahan-bahan campuran untuk obat malaria. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak etanol dari kayu sappan pada kadar glukosa darah tikus putih. Tingkat glukosa darah pada tikus putih dilakukan dengan menggunakan metode toleransi glukosa. Ini diukur dengan Refloluxs (Accutrend GC) dengan kloropropamida 50 mg/200 g BB (berat badan) sebagai kontrol positif. Ekstrak etanol yang digunakan dalam berbagai konsentrasi 10, 20, 30, 40 dan 50 mg/200 g BB per-oral dan diamati setiap satu jam dan dimulai satu jam sebelum sampai 7 jam setelah ekstrak diberikan. Pemberian ekstrak 30 mg / kg BB tidak berbeda nyata dengan kontrol positip, sedang pemberian ekstrak 50 mg/200gBW menurunkan kadar glukosa darah (93 mg/dl) dibandingkan dengan kontrol positif.

Kata kunci: Kayu secang, ekstrak etanol, kadar glukosa darah, tikus putih

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I. INTRODUCTION

Indonesian forests provide more than 9,606 species of medicinal plants (Pranoto, 1999). Most of them are native to Indonesia and many of the plants are endemic (Walujo, 2008). The high diversity of the plant species also has a great potential for the discovery of bioactive compounds they contain. Several studies have successfully revealed the antioxidant potential of some Indonesian plants (Hakim et al., 2008; Rohman et al., 2006; Amrun and Umiyah, 2005; Praptiwi et al., 2006). Three criteria must be fulfilled when extracting plants to medicine materials, i.e. quality, safety and efficacy. Advanced research must be done until the discovery of effective and simple drugs (Chairul, 2003).

Sappan wood or kayu secang (*Caesalpinia sappan* L.) is one of the traditional medicine materials. This species is a type of flowering tree in the group of the Fabaceae legume family. Sappan wood is native to Southeast Asia and the Malay archipelago. Common names of sappan are patanga-chekke sappanga (Kanada name) and sumu (Japanese). Sappan belongs to the same genus or synonym with *Caesalpinia echinata* or braziel wood (*C. echinata*), and was originally called "brezel wood" in Europe (Anonymous, 1998; Xu and Lee, 2004). Furthermore, this wood was a major trading goods during the 17th century, when it was exported from Southeast Asian nations (especially Siam) to Japan.

The sappan plant is being used worldwide for a large number of traditional medicinal purposes. This plant produces brazilin that is found to be responsible for several of its biological activities (Badami et al., 2004). Modern day research confirmed it's cytotoxic from heartwood (Badami et al., 2003), antitumor from part of stem and heartwood (Dhawon et al., 1980; Itokawa et al., 1990 *in* Badami et al., 2004), anti-inflammatory from heartwood (Hikino et al., 1977 *in* Badami et al., 2004), anti-coagulant properties (Takaoka and Tagakaki, 1995), and blood vomiting cure and drug treatment after child birth (Aulia, 2002). According to Aviratnant and Pongpan (1983) and Yadava et al. (1978) in Badami et al. (2004), the essential oil obtained from the leaves, the 95% ethanol and water extracts of the wood showed strong antibacterian activity againts *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhosa* and *Escherichia coli*. Prawirosujanto (1977) and Sugati (1981) said that the bark of this plant had been used for folk medicine as anti-diarrhea, anti-microbial, expectorant, anti-pyretic, cataract, and tonic. Xu and Lee (2004) reported that brazilin from *C. sappan* is antibacterial and it has the potential to be developed into an antibiotic.

This paper studies the potential use of sappan wood for reducing blood glucose level in white rats. The analysis was carried out to see the influence of ethanol extract from sappan wood on blood glucose level of white rats.

II. MATERIAL AND METHOD

A. Plant Materials and Experimental Animals

1. Sappan wood (*Caesalpinia sappan* L.) was collected from Kemangkon village, Purbalingga, Central Java and was identified at the Herbarium Bogoriense, Research Center for Biology, Cibinong. Authentic specimen was deposited at the same Institution.
2. The experimental animals used were 2.5 to 3 months male white rats (*Ratus ratus*) Winstar strain of 200 – 300 g weight. Before being tested the animals were fed for 14 days to get the expected weight (Malole and Purnomo, 1989).

B. Sample Preparation

The preparation of samples and the testing procedures in the experiment used the Taylor Method (Chairul, 2003).

1. The sappan wood extract

Preparation of extract : 1 kg powder of air dried sappan wood was macerated by 95% ethanol for 24 hours, until the solvent covered the surface of the plant material.

After 24 hours, the filtrate was concentrated under vacuum rotary evaporator. This work was repeated 2 or 3 times until the colorless solvent was obtained. Filtrate was combined and concentrated. Then, the extracts were dried by freeze dryer to get dry sappan extracts.

2. The glucose suspension

2.1. 1% CMC suspension

1 g CMC was balanced on watch glass, developed in mortar by hot water and grinded until it was homogenous and 100 ml purified water was added.

2.2. 1% glucose stock solution

1 g glucose stock solution anhydrate was put into a 100 ml volumetric flask, 50 ml aquadest was added, it was shaken and 100 ml aquadest was added, and than it was shaken well until the glucose solved. The glucose solution from the 100 ml volumetric flask was removed to a 150 ml beaker glass, 2% active carbon was added, then shaken well and heated for 30 minutes on water bath, then filtered and kept in the infuse bottle.

2.3. Standard glucose solution

1% glucose stock solution (2.2) was pipetted and put into a 100 ml volumetric flasks, and 100 ml aquadest purified water was added to each volumetric flask, shaken well and than homogenized. This was done to obtain glucose concentrations of 50, 100, 200 and 400 mg/dl in each of the four 100 ml vials respectively.

2.4. 100% glucose injection solution

100 g glucose monohydrate was put in a 100 ml volumetric flask, 50 ml aquadest was added and shaken well to become homogenous and then 100 ml aquadest was added. Filtered and removed to 200 ml vial and sterilized in autoclave at 120°C for 20 minutes.

C. Treatment Schedule

1. Testing extracts

The ethanol extract was treated with various concentrations i.e. 10, 20, 30, 40 and 50 mg/200 g BW (Body Weight).

2. Preliminary testing

Preliminary testing was aimed to get the normal glucose level in the blood of the rat when suffering hyperglycemic condition, after administering a 100 % glucose solution and various concentrations of sappan extracts i.e. 50, 100, 200 and 400 mg/dl by intravenous injection in the tail literal vena (Table 1).

3. Glucose tolerance testing

Glucose tolerance testing had been carried out by administrating 100% glucose solution with a dose of 0.1 g/200 g BW, which was added orally. Each group consisted of six testing animals (rats), The extract of sappan wood was administered by various doses: 10, 20, 30, 40 and 50 mg/200 g BW, respectively and destilated water was used as the negative control (K -), while chlorpropamide 50 mg/200 g BW as the positive control (K +). Blood glucose level in rats was measured 1 hour before to seven hours after the treatment.

Blood was taken via tail venous, centrifuged

Table 1. Preliminary testing of extract in white rats

Control	Glucose level in blood after treatment							
+	+	+	+	+	+	+	+	+
-1	0	1	2	3	4	5	6	7

Note:
-1 = Glucose level in blood when fasting
0 = Glucose level in blood in treatment [glucose, extracts, Negative control (-), positive control (+)]
1 to 7 = Glucose level in blood after treatment

and one drop of blood serum was dropped on glucose strip test and it was let for drying for one minute. Measuring the glucose level was done by Reflolux S (Accutrend GC). The data of blood glucose level was calculated by statistical analysis (ANOVA) by making the correlation curve of glucose level versus period (time). From the curve the "Area Under the Curve 0-7 or AUC0-7 " could be calculated with accuracy for each testing groups of animals (P= <0.05) (Sudjana, 1982).

III. RESULT AND DISCUSSION

The blood glucose level of each testing animal after administering glucose solution (100%) with various doses, i.e. 50, 100, 200, and 400 mg/dl via tail literal venous on hyperglycemic condition were measured and calculated. The results showed that the blood glucose level in testing white rat animals increased with the increase of the doses. The blood glucose level on testing animals increased to 49; 103.83; 196.50 and 374.70 mg/dl, respectively (Table 2). Hyperglycemia conditions of the blood of white rats was most striking when given 100% glucose solution at a dose of 400 mg/dl, the average increase in glucose level was 7 times higher than with 50 mg/dl.

The results of the determination of the time interval of hyperglycemic condition in rat (mg/dl) showed the different blood glucose level in testing animals. The average blood glucose level was 145 mg/dl. The hyperglycemic condition was reached in 3 hours after the treatment (Table 3).

It appears glucose tolerance by administering 100% glucose solution at a dose of 0.1 g/200 g BW intravenously. Rats increased blood glucose levels and hyperglycemic conditions occur. Blood glucose tolerance also occured after treatment of the extract that is 10, 20, 30, 40, and 50 mg/200 g BW. Observations during the 4-6 hours after treatment, the rats were in hyperglycemic conditions, but the average blood glucose adapt to the normal condition.

Differences were seen in the rats between control (-1) and fasting conditions given aquadest added with chlorpropamide drugs 50mg/200 gr body weight (Table 3). The time interval required was 2-3 hours for adjustment after the food was absorbed (ingestion) by administering 100% glucose solution. After that time the blood glucose levels rose from an average of (114-117) mg/dl when fasting to (137-152) mg/dl at the time of hyperglycemic. The condition of blood glucose after extract treatment and when anti-diabetic drugs

Table 2. Preliminary recovery of glucose level in blood of testing animals by Reflolux

No	Glucose level (mg/dl)			
	50	100	200	400
1	47	100	208	387
2	57	104	166	362
3	50	114	199	368
4	48	99	203	373
5	49	103	189	376
6	47	106	194	382
Average	49	103.83	196.50	374.70
SD	2.08	5.55	7.67	8.32
Recovery (%)	98.00	103.83	98.25	93.68
CV (5)	5.70	5.34	3.90	2.22

Note : SD = Standard deviation, CV = Coeficient of variation, N= 3

Table 3. Determination of the time interval of hyperglycemic condition in rat (mg/dl)

Periods (hours)	Groups			Average
	1	2	3	
-1	114	117	117	116a
0	124	137	129	130ab
1	130	140	135	135b
2	137	144	142	141bc
3	138	152	145	145c
4	121	132	128	127ab
5	113	127	120	120a
6	108	120	114	114a
7	105	115	110	110a

Note: The average value followed by the same letters were not significantly different

chlorpropamide was added, dropped to an average of (124-137) mg/dl

The results of the average blood glucose level in testing animals after treatment (in mg/dl) gave different levels it depended on the doses of the extracts. There was a difference in the blood glucose level in hyperglycemic conditions between control groups and fasting groups (-1) and extract treatment groups. Negative control groups showed an average blood glucose level of 145 mg/dl three hours after treatment and groups II to VI (extract 10-50 mg/200 g BW) showed a decrease of the blood glucose level to 100- 137 mg/dl, while the level of the positive control was 102 mg/dl. Those results showed that treatment of ethanol extract of sappan wood by administer doses gave a remarkable effect on blood glucose level in rat and also reduced the glucose level in blood compared to negative control and positive control. Treatment with a dose of 30 mg/200 g BW (103 mg/dl) gave a similar effect as the positive control (102 mg/dl), while a dose of 50 mg/200 g BW gave lower blood glucose level (93 mg/dl) than the positive control.

Statistical analysis of those results gave significant differences in all treatments between blood glucose level of administered extract doses versus period ($P = < 0.05$) (Figure 1). Treatments of doses 20 – 50 mg/200 BW also gave the anti-diuretic effect on testing animals. The research showed that the administered dose of 30 mg/200 g BW of the ethanol extract equals the positive control.

The mechanism of ethanol extract of sappan wood in decreased blood glucose level could be explained as follows:

1. Fructose-2, 6-bisphosphate (F-2, 6-BP), a gluconeogenic intermediate, plays a critical role in hepatic glucose output by regulating gluconeogenesis and glycolysis in the liver. Increased hepatic glucose output is one of the major mechanisms of hyperglycemia in diabetic animal patients.
2. Brazilin, an active component of sappan, decreases blood glucose in diabetic white rat animals

In this study, the effect of brazilin on gluconeogenic intermediate production and enzyme activity were examined to investigate the hypoglycemic mechanism of brazilin. As said by You et al. (2005) brazilin has increased the production of F-2,6-BP in hepatocytes by elevating intracellular levels of fructose-6-phosphate (F-6-P) and hexose-6-phosphate (H-6-P) to enhance insulin receptor function and lower blood sugar.

As reference, Jafri, et.al. (2000) reported that the oral administration of *Punica granatum* L., flower aqueous-ethanolic (50%, v:v) extract led to significant blood glucose lowering effect in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rats. This effect of the extract reached maximum at 400 mg/kg B.W.

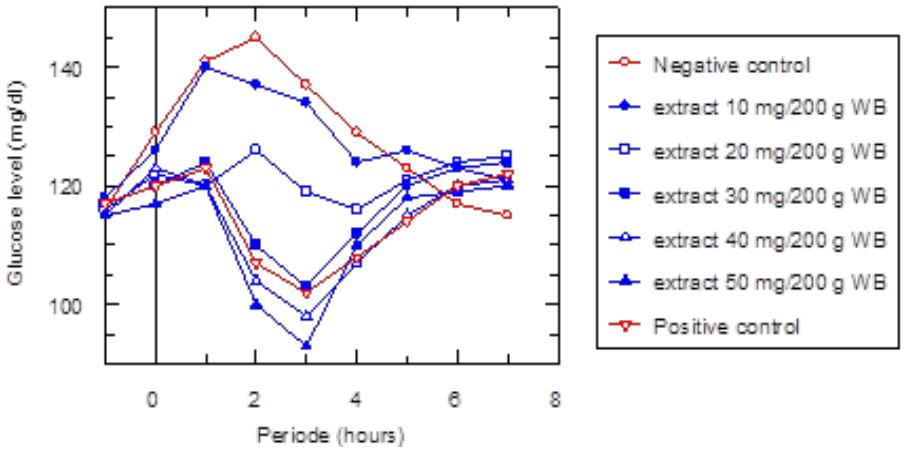

Figure 1. Curve of glucose level in rat blood after treatment

IV. CONCLUSION

This experiment showed that all of the treatment doses decreased the blood glucose level. The treatment of ethanol extract of sappan wood by administering doses gave remarkable effect of blood glucose level in white rats and also reduced glucose level in blood compared to negative control and positive control.

Treatment of dose 30 mg/200 g BW (103 mg/dl) gave similar effect to positive control (102 mg/dl), while dose of 50 mg/200 g BW gave lower blood glucose level (93 mg/dl) than positive control.

As the base material for a standard preparation of fitofarmaka, its effectiveness needs to be advancely tested for quality, safety and efficacy. Further scientific research is still needed to obtain a practical and an effective drug dosage form.

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