Antidiabetic Drug Ethyl Acetate Fraction of *Leucaena leucocephala* Seed Extract in Wistar Aloxan Induced

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Abstract

Diabetes mellitus (DM) is a disease *with high prevalence in Indonesia. According to data of Republic Indonesia Healthy Ministry Department in 2008, the prevalence of DM in Indonesia is 5.7 % and WHO also predicts in 2025 Indonesia will be the fifth biggest country for diabetics in the world.* Leucaena leucocephala *is used empirically as antidiabetes, anticholesterol and intestinal worms. Flavonoid compounds in the seed were thought to reduce insulin production. The aim of this research was to determine the effect of ethyl acetate fraction from* Leucaena leucocephala *extract to reduce blood sugar levels in alloxan-monohydrate-induced hyperglycaemic wistar rats. The extract was prepared by maceration of dried and powdered seeds of* L. leucocephala *using ethanol 70 % and it was then fractionated with ethyl acetate. The experiment was carried out by controlled group post-test method using 24 male wistar rats, divided into 6 groups, i.e. A = normal control, B = negative control (alloxan), C = positive control (alloxan+glibenclamide), D = (alloxan+ethyl acetate fraction of* L. leucocephala *extract 100mg/kgBW), E = (alloxan+ethyl acetate fraction of* L. leucocephala *extract 250mg/kgBW) and F = (alloxan+ethyl acetate fraction of* L. leucocephala *extract 500mg/kgBW). The results were analyzed with ANOVA and post hoc test statistical assay. The results of the research showed that the ethyl acetate fraction of* Leucaena leucocephala *seed extract had an effect to decrease the rats glucose blood level in the 100 mg/kg BW dosage and 500 mg/kg BW can decrease the glucose blood level (P<0,05) more than the negative control is (235.00 ± 54.903) and (240.28 ± 34.794) mg/dL.*

Keywords: *Alloxan,* Leucaena leucocephala*, Antidiabetic, in vivo assay*

INTRODUCTION

Diabetes is a chronic disease. It occurs when pancreas cannot produce insulin or the body cannot effectively use the insulin. Diabetes mellitus (DM) is a disease with high prevalence in Indonesia. According to data of Republic Indonesia Healthy Ministry Department in 2008, the prevalence of DM in Indonesia is 5.7 % and WHO also predicts that in 2025 Indonesia will be the fifth biggest country for diabetics. Diabetes mellitus type 2 is reported to be 90% in all of the diabetes case in the world. Bad lifestyle and habit in food consumption may result in diabetes. (Maulana, 2008).

One of the herbal medicines is *Leucaena leucochepala* seed. Some people empirically use it as antidiabetes (Nurhasanah, 2005). *Leucaena leucochepala* seed contains active compounds like alkaloid, saponin, flavonoid, mimosin, leukanin, protein, fat, calcium, phosphor, iron, vitamin A and vitamin B (Anonym, 2004). *Leucaena leucochepala* is known for its ability to repair diabetes mellitus type 2. So, we have hypothesis that there is

an active compound especially flavonoid as a therapy medicine for DM type 2.

Based on some researches *Leucaena leucochepala* has pharmacological effects for antiinflammation, anthelmintic and antioxidation (Nurhasanah, 2005). Super-oxide dismutase enzyme (SOD) increases in the rat injected with *Leucaena leucochepala* seeds extract. The increase of SOD enzyme and the decrease of malondoaldehid (MDA) plasma is indicative of an antioxidant activity. Increasing the MDA plasma as a product of fat to the β-pancreas is related to abnormal insulin secretion. It makes hyperglycaemia. This mechanism is related to the chemical compound in *Leucaena leucochepala* seed as an antidiabetes. (Nurhasanah, 2005).

MATERIAL AND METHODS

Plant Material

Leucaena leucochepala seeds were obtained in February in Ngebel, Bantul. The taxonomic determination of the species was done in Pharmacy Faculty Gajah Mada University. The seeds were dried in oven at 50°C. The dried seeds were powdered with a blender. The extraction method used was maceration with ethanol 70% for 7 days followed by evaporation using rotary evaporator. The thick extract was fractionated with ethyl acetate. The fractions of *Leucaena leucochepala* seed was thickened using rotary evaporator. The thick fraction was saved at 10°C.

Qualitative analysis

The qualitative analysis of flavonoids in ethyl acetate fraction of *L. leucochepala* seed was done using thin layer chromatography method with TLC Silica Gel $60F_{254}$ as the stationary phase and ethyl acetate:methanol:water (10:1:1) as the mobile phase is (Mabry, 1970). The standard flavonoid compounds used were quersetin, rutin and saponin (figure 1). The standard compounds and the ethyl acetate fraction extract *Leucaena leucochepala* seed were spotted on the TLC plate 1 cm apart from each other. The length of the track was 8 cm.

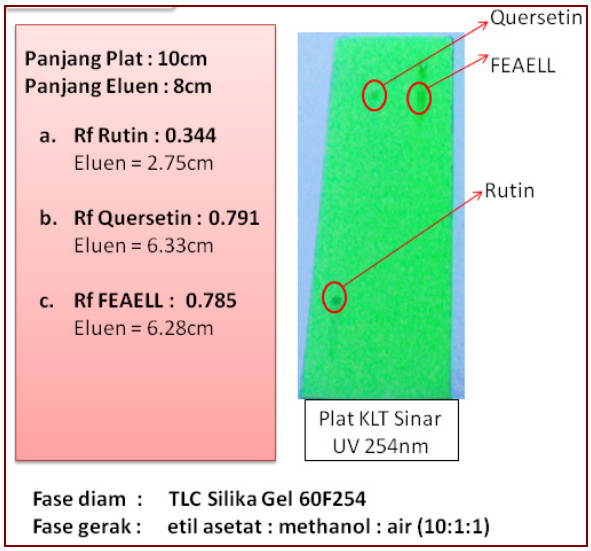

Figure 1. Qualitative analysis of flavonoids in ethyl acetate fraction of *Leucaena leucochepala* seeds using thin layer chromatography method.

Experimental Animals

This research used Wistar rats with body weights 150-185 g. The rats come from UD. Wistar Parangtritis, Yogyakarta. Before the treatment, the rats were acclimatized for 10 days. During treatment, the rats were given pellet AD 2 BRHOILER 80 mg per group and water *ad libitum*.

Induction of diabetes

Induction of diabetes in the rats was done using alloxan (2,4,5,6-tetra oxypyrimidine; 2,4,5,6-pyrimidinetetrone), a toxic glucose analogue that is able to destroy insulin-producing cells in the pancreas of rodents, causing an insulin-dependent diabetes mellitus in the animal (Lenzen, 1998). Alloxan monohydrate was obtained from SIGMA.

Anti-diabetic activity

The experiment animals were made diabetes by induction with alloxan monohydrate in water for injection at 120 mg/kg BW intra peritoneally (i.p.). Five days after the injection, the hyperglycaemia occurred (glucose level >

250 mg/dl) and the rats were then separated in 6 groups (A, B, D, E, and F groups), each consisted of 4 rats. The A group were not induced by alloxan nor treated (normal control). The B group was induced by alloxan monohydrate (negative control) and not treated. The C, D, E, and F groups were induced by alloxan and treated orally with glybenclamide 0.216mg/kgBB, ethyl acetate fraction extract Leucaena leucochepala seed (FEAELL) 100 mg/kg BW, ethyl acetate fraction extract Leucaena leucochepala seed (FEAELL) 250 mg/kg BW, and ethyl acetate fraction extract Leucaena leucochepala seed (FEAELL) 500 mg/kg respectively, starting from 11th day for 10 days. During this period, the rats were given standard chow and the drinking. On the 4th, 7th and 10th days after treatment, the rats were weighed and their blood glucose levels were measured (Kumar, 2010).

RESULT

Qualitative Test

The TLC analysis resulted in Rf values as follows: rutin 0.344 cm, quersetin 0.791 cm, and ethyl acetate fraction extract *Leucaena leucochepala* seed 0.789 cm and saponin in the stationary phase.

Anti-Diabetic Study

The ethyl acetate fraction of *Leucaena leucochepala* extract seeds action in rats that induced aloxan monohydrate (120mg /kgBB i.p.) as an antidiabetic agent. The value of increasing glucose blood as a diabetic control is appropriated on the rat in normal control. The ethyl acetate fraction extracts *Leucaena leucochepala* seed in dose 100 mg/kg BW showed decreasing the glucose blood level significantly. The other dose, that is 250 mg/kg BW and 500 mg/kg BW can decrease the glucose blood level but it is not significant. The antihyperglycemia from ethyl acetate fraction extract *Leucaena leucochepala* seed is not same with the standard medicine, Glibenklamid from Kalbe Farma product. The decreasing glucose blood effect of Glibenclamide is more better in the rats as diabetic control.

Table 1. Effect of ethyl acetate fraction extract *Leucaena leucochepala* seed in alloxan induced diabetic rats. Animal: Wistar Rats. Alloxan: 120 mg/kg, i.p.Extract: p.o.

GROUP (treatment)	Fasting blood glucose level (mg/dl)					
	Rat 1	Rat 2	Rat 3	Rat 4	Mean	SD
GROUP A (normal control)	227.1	237	303.9	323.8	272.95	48.091
GROUP B (diabetic control)	270.8	247.5	262	272.6	263.23	11.461
GROUP C (alloxan+glibenklamid 0.216mg/kg BB)	164.7	211.6	242.7	188.6	201.90	33.264
GROUP D (alloxan + FEAELL 100mg/kg BB)	314.1	207.7	227.8	190.4	235.00	54.903
GROUP E (alloxan + FEAELL 250mg/kg BB)	297.5	234.9	318.5	310.2	290.28	37.913
GROUP F (alloxan + FEAELL 500mg/kg BB)	289.5	220.2	239.5	211.9	240.28	34.794

Table 2. One way ANOVA analysing *Leucana leucochephala*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15961,542	3	5320,514	3,144	,065
Within Groups	20306,655	12	1692,221		
Total	36268,197	15			

Table. Post Hoc test of *Leucana leucochephala*

(I) perlakuan	(J) perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
k+ (Ind.+Glibenklamid)	p1	-33,10000	29,08798	,674	-119,4593	53,2593
	p2	-88,37500*	29,08798	,044	-174,7343	-2,0157
	p3	-38,37500	29,08798	,569	-124,7343	47,9843
p1 (Ind. + FEAELL 100mg/kg BW)	k+	33,10000	29,08798	,674	-53,2593	119,4593
	p2	-55,27500	29,08798	,278	-141,6343	31,0843
	p3	-5,27500	29,08798	,998	-91,6343	81,0843
p2 (Ind. + FEAELL 250mg/kg BW)	k+	88,37500*	29,08798	,044	2,0157	174,7343
	p1	55,27500	29,08798	,278	-31,0843	141,6343
	p3	50,00000	29,08798	,356	-36,3593	136,3593
p3 (Ind. + FEAELL 500mg/kg BW)	k+	38,37500	29,08798	,569	-47,9843	124,7343
	p1	5,27500	29,08798	,998	-81,0843	91,6343
	p2	-50,00000	29,08798	,356	-136,3593	36,3593

**. The mean difference is significant at the 0.05 level.*

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose; insulin is secreted from it (Edem, 2009). Alloxan is usually use to induce diabetes. Alloxan has destructive effect in β-pancreas cell (Prince, 2000 & Jelodar, 2003). Alloxan is a strong oxidator which produce free radical in high value and it makes oxidative stress. Oxidative stress is an unbalance condition between the free radical agent (prooxidant) and antioxidant (Gems & Partridge, 2008). This situation damages the β-pancreas cells and results in elevated glucose blood level (hyper-glycaemia).

The result of research showed ethyl acetate fraction extract *Leucaena leucochepala* seed with dose 100 mg/kg BW can decrease the glucose blood level as a mean (P<0,05) more than negative control is 235.00 ± 54.903 and 240.28 ± 34.794. Ethyl acetate fraction extract *Leucaena leucochepala* seed has insignificant hypoglycaemia effect in the positive control (P>0.05) although the glucose blood level test is lower than Glibenclamide. The decreasing effect is depend on antioxidant activity of flavonoid oh it way to inhibit the free radical and take the free radical. *Leucaena leucochepala* has alkaloid, saponin and amino acid (Syamsudinhidayat, 1991). Some of the research showed there is more plant has flavonoid as antioxidant agent (Pari, 2002).

Ethyl acetate fraction extract of *Leucaena leucochepala* seed can decrease hypoglycemia in 250 mg/kg BW, (P<50) than negative control that is 290.28 ± 37.913. This data are supported by statistics that showed the significant more than the positive control with significant value 0.044(P<0.05).

CONCLUSION

The qualitative test in thin layer chromatography get the Rf ethyl acetate fraction extract *Leucaena leucochepala* seed is 0.789cm and Rf quersetin is 0.791cm. it showed that ethyl acetate fraction extract *Leucaena leucochepala* seed has the flavonoid unsure. Ethyl acetate fraction extract *Leucaena leucochepala* seed has effect to decrease the rats glucose blood level in the 100 mg/kg BW dosage and 500 mg/kg BW can decrease the glucose blood level (P<0,05) more than the negative control is (235.00 ± 54.903) and (240.28 ± 34.794) mg/dL.

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