

The Effects of *Leucaena leucocephala* (Imk) De Wit Seeds on Blood Sugar Levels: An Experimental Study

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Abstract: *Leucaena leucocephala* (Imk) De Wit is known as traditional medicine to treat degenerated diseases such as diabetic, liver degeneration, kidney failure etc. The purpose of this study is to assess the effects of *L. leucocephala* extract in diabetic rat, which is induced by streptozotocin. Blood glucose, regeneration of pancreatic islets, serum lipids in streptozotocin-induced diabetic rats were assessed at day 0, 3, 7 and 14 after injection of streptozotocin as diabetic induces. The results indicated that the extract could inhibit the elevated blood glucose and lipids levels and could increase the number of pancreatic islets per unit area significantly ($p < 0,05$) for 14 days. It can be concluded that the extract of *L. leucocephala* seed acts as hypoglycaemic agent by a selective regeneration of beta-cells of streptozotocin-damaged pancreas. Beside that β cell of pancreas is also protected from necrotic effect of streptozotocin.

Keyword: diabetes mellitus; streptozotocin; rats; blood glucose; *Leucaena leucocephala*.

1. Introduction

Diabetes mellitus is a syndrome characterized by chronic hyperglycaemic and disturbance of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and / or insulin action (De Fronzo, 1997). Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter *et al.*, 1982). Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and triglycerides. With diabetes conditions, a profound alteration in the concentration and

composition of lipid occurs (Sochor *et al.*, 1985). One component in management of the diabetes especially for the Non-insulin dependent diabetes (NIDDM) is oral hypoglycaemic agents. In Indonesia, traditional medicines are fairly popular among the people at large. Many Indonesian medicinal plants are reported to be useful in diabetes. One of the alternatives to oral hypoglycaemic agents, which is used traditional plants, is called lamtoro (*Leucaena leucocephala* (Imk) De Wit).

In Indonesia, an aqueous extract derived from boiling the seeds of *L. leucocephala* is taken orally to treat type-2 (NIDDM) diabetes and is claimed to be

efficacious. *Leucaena leucocephala*, popularly known as *Lamtoro* or in Indonesia, belongs to the Leguminosae family and is one of the fastest-growing leguminous trees (Dalimarta., 2006).

The purpose of this study is to assess the effects of *L. leucocephala* extract in diabetic rat, which is induced by streptozotocin. Using laboratory test results from different groups of rats, we have evaluated *L. leucocephala* as a potential source of a specific oral hypoglycaemic agent. We have prepared an aqueous extract derived from a traditional preparation of *L. leucocephala* seeds which demonstrated significant hypoglycaemic, hypolipidemic effects in streptozotocin diabetic rats.

2. Materials and methods

Preparation of L. leucocephala seed extracts

The main source of *L. leucocephala* seeds were collected from BALITRO (Balai Penelitian Obat dan Rempah) Bogor, West Java. Identification for the species of the Lamtoro was conducted in the Herbarium Bogoriense, Bogor. The seeds were washed, ground into powder and boiled and refluxed the aqueous extract and used in all experiments.

Experimental procedure

Adult, male Wistar rats weighing 250-300g were used. The randomly divide into six groups of six rats per group and control.

- Group 1 : Normal untreated rats
- Group 2: Diabetic rats controlled with 1 ml aqueous solution daily using an intragastric tube for 14 days.
- Group 3: Diabetic rats given with *L. leucocephala* seed extract amounting 0, 25 g/kg bw per day using an intragastric tube for 14 days.
- Group 4 : Diabetic rats given with *L. leucocephala* seed extract amounting 0, 5 g/kg bw per day using an intragastric tube for 14 days.
- Group 5: Diabetic rats given with *L. leucocephala* seed extract amounting 1 g/kg bw per day using an intragastric tube for 14 days.

- Group 6: Diabetic rats given with chlorpropamide 200 mg/kg bw per day using an intragastric tube for 14 days.

The blood samples were obtained from each animal in 0, 3, 7 and 14 days after diabetic injection. Blood samples were collected from the tail vein of each rat for blood glucose analysis. Blood glucose concentration was determined by means of Bayer's Glucometer Elite and Blood Glucose Test Strips. At the end of observation, two ml of blood were collection from all groups prior to and termination of experiment examination cholesterol, triglyceride and HDL was determined using colorimetric assays (Zlatkis *et al.*, 1953). At the day 14, all rats from all groups were sacrificed by ether and decapitated and necropsied for microscopic examination. Expert for pancreas with gross pathological changes they were collected and fixed in 10% buffer formalin. Two to three 5 μ m thick sections were prepared and stained with hematoxylin and eosin and after Gomori's Aldehyde Fuchsin Stain (Cullings., 1963).

Statistical analysis

The results are expressed as mean S.E.M. The significant of various treatments was calculated using ANOVA and were considered statistically when $p < 0,05$.

3. Results and discussion

The effect of the aqueous extracts of *L. leucocephala* seed on blood glucose is shown on table 1. Blood sugar levels were measured prior to and 72 hours after rats received them and the injection of streptozotocin increased blood sugar levels above 313-350 mg/dl. The blood sugar on hyperglycaemic animals treated with extract of *L. leucocephala* seed was given 1 g/kg bw for 14 days after administration of streptozotocin was lowered and returned to normal on the 14th days after streptozotocin. The possible mechanism by which *L. leucocephala* extract brings about its antihyperglycaemic action may be by potentiation of pancreatic secretion of insulin from β -cell of islets. In this context, a number of other plants has antihyperglycaemic and insulin-release stimulatory effect.

As shown in table 2, streptozotocin -induced diabetes resulted in a significant decrease in the number of pancreatic islets per unit area and diameter islets langerhans. The extract of *L. leucocephala* seed

treatment apparently regeneration of these islets and diameter islets langerhans increase to the normal level.

The normal group after induced with streptozotocin decreased diameter islets of langerhans and the number β -cell. The early degeneration of langerhans islets was shown by the presence of vacuolization as indicated of fatty degeneration, this fatty degeneration did not appear in the treatment group. Vesel *et al.* (1981) reported that the herb *Teucrium polium* can cause increased of pancreatic islet in rat induced by streptozotocin (Vesel *et al.*, 1981).

Similar to this study, the effect of *L. leucocephala* can be used for treatment of diabetic rat induced by

streptozotocin. On the other hand Chakravarthy *et al.* (1980) used *Pterocarpus marsupium* for diabetic rat induced by alloxan which can cause regeneration of pancreatic β -cell (Chakravarthy *et al.*, 1980).

During diabetes a profound alteration in the concentration and composition of lipids occurs (Sharma *et al.*, 1996). Hyperglycaemic could result from fat-induced impaired insulin secretion. Indeed theoretically, there is strong rationale for believing that free fatty acids might be responsible for both of these changes. Fatty acids may also lead to hyperglycaemic through effects on the liver in which impaired glycolysis results in more hepatic glucose output from gluconeogenesis and glycogenolysis. Fatty acids metabolism also generates adenosine

Table 1. The restorative effects of *L. leucocephala* seeds extract against the action of β -cells in pancreatic islets of rats.

No.	Group action	β -cell (per islets section)	Diameter islets langerhans (μ m)
1.	Normal group	29.29 ^a \pm 1.08	10.14 ^a \pm 1.30
2.	Diabetic group	3.98 ^b \pm 0.62	5.41 ^b \pm 1.21
3.	Diabetic + Chlorpropamide	23.14 ^c \pm 0.83	7.09 ^c \pm 0.90
4.	Diabetic + <i>Leucaena leucocephala</i> 0,25g/kg	18.96 ^d \pm 2.02	6.92 ^c \pm 0.82
5.	Diabetic + <i>Leucaena leucocephala</i> 0.5g/kg	20.09 ^c \pm 2.12	7.39 ^c \pm 1.01
6.	Diabetic + <i>Leucaena leucocephala</i> 1g/kg	22.90 ^c \pm 2.05	7.58 ^c \pm 0.80

Different superscript indicated of significantly different between treatment (p<0,05)

Table 2: Blood glucose level in different days (0,3,7 and 14) from the treatment in diabetes rats and normal group.

Group	Days			
	0	3	7	14
1. Normal group	76,0 ^a \pm 1,10	74,4 ^a \pm 1,52	77,0 ^a \pm 2,02	75,8 ^a \pm 2,27
2. Diabetic group	332,8 ^b \pm 20,98	335,0 ^b \pm 14,40	332,2 ^b \pm 15,32	345,2 ^b \pm 13,35
3. Diabetic + Chlorpropamide	271,8 ^c \pm 10,18	218,2 ^f \pm 5,40	127,8 ^h \pm 3,56	104,0 ^k \pm 2,74
4. Diabetic + <i>Leucaena leucocephala</i> 0,25g/kg	292,8 ^d \pm 4,49	256,2 ^e \pm 5,17	176,2 ⁱ \pm 4,09	122,8 ^k \pm 1,48
5. Diabetic + <i>Leucaena leucocephala</i> 0,5g/kg	281,2 ^d \pm 3,11	221,4 ^e \pm 1,14	143,2 ^j \pm 8,32	108,2 ^k \pm 3,70
6. Diabetic + <i>Leucaena leucocephala</i> 1g/kg	243,2 ^e \pm 4,83	192,8 ^g \pm 1,64	119,8 ^k \pm 1,30	77,8 ^a \pm 3,20

Different superscript indicated of significantly different between treatment (p<0,05)

Table 3 : Changes in levels of cholesterol, triglycerides and HDL in serum of normal and experimental animals

Groups	Cholesterol	Triglycerides	HDL
1. Normal group	61,6 ^a ± 1,82	83.4 ^a ± 2.19	47.6 ^a ± 3.65
2. Diabetic group	91,6 ^b ± 2,70	201.4 ^b ± 3.05	24.6 ^b ± 3.36
3. Diabetic + Chlorpropamide	62,2 ^a ± 2,77	133.4 ^c ± 3.13	35.2 ^c ± 2.77
4. Diabetic + <i>Leucaena leucocephala</i> 0,25g/kg	76,0 ^c ± 3,39	114.8 ^d ± 4.44	43.4 ^a ± 3.21
5. Diabetic + <i>Leucaena leucocephala</i> 0,5g/kg	65,4 ^a ± 2,88	93.2 ^c ± 2.59	58.4 ^a ± 3.05
6. Diabetic + <i>Leucaena leucocephala</i> 1g/kg	55,8 ^a ± 3,90	80.4 ^a ± 3.65	90.4 ^c ± 1.82

Different superscript indicated of significantly different between treatment (p<0,05)

triphosphate and the reduced from of nicotinamide adenine dinucleotide which favor gluconeogenesis (Sheperd *et al.*, 1999). The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase the mobilization of free fatty acids from peripheral fat depot since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats (Pushparaj *et al.*, 2000). Repeated administration of *L. leucocephala* extracts had decreased the blood glucose, total cholesterol and triglycerides significantly. Histopathological examination of pancreas showed the recovery of damaged tissues when section of treated groups was compared with diabetic control. Since the mechanism effect of the plants extract still remains speculative, further studies are required to unravel the mechanism of hypoglycaemic, hypocholesterolemic and hypertriglyceridemic action of the herb, and to shed more light on the constituent/s of the plants. Isolation of active principle and its identification is in progress.

In conclusion, *L. leucocephala* aqueous extract showed significant antidiabetic effect in diabetic rats after oral administration. Present efforts are directed to isolate the active constituent/s from *L. leucocephala* seeds extract and elucidation of action mechanism.

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