



Original Article

Antidiabetic and antioxidant activities of seed extract from *Leucaena leucocephala* (Lam.) de WitPichaya Chowtivannakul,^{a,*} Buavaroon Srichaikul,^b Chusri Talubmook^{a,1}^a Department of Biology, Faculty of Science, Maharakham University, Maha Sarakham 44150, Thailand^b Faculty of Public Health, Maharakham University, Maha Sarakham 44150, Thailand

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ABSTRACT

Leucaena leucocephala (Lam.) de Wit has been used for various purposes such as treatment of stomach diseases, facilitation of abortion, contraception and the treatment of diabetes. Scientific information about this plant is limited. Therefore, this study investigated the antidiabetic and antioxidant activities and also the toxicity of seed extract from *L. leucocephala* (LLSE). Antidiabetic activity testing was carried out by giving LLSE at an oral dose of 250 mg/kg body weight to streptozotocin-induced diabetic rats daily for 6 wk. The results revealed that LLSE significantly ($p < 0.05$) reduced the fasting blood glucose and the blood chemistry consisting of: albumin, alkaline phosphatase (ALP) and total protein and red blood cells in the diabetic-treated rats compared to those in diabetic-untreated rats. LLSE slightly increased the serum insulin level in the diabetic-treated rats. Using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay showed that LLSE exhibited relatively low antioxidant activity with the concentration of a sample required for 50% scavenging of the DPPH free radical of $839.56 \pm 37.34 \mu\text{g/mL}$ compared to vitamin C ($1.48 \pm 0.07 \mu\text{g/mL}$). A spectrophotometric technique, based on the Folin-Ciocalteu reagent, revealed that the total phenolic compound contents of LLSE were $37.38 \pm 0.49 \text{ mg GAE/g}$. LLSE at doses of 1000 mg/kg body weight, 1500 mg/kg body weight and 2000 mg/kg body weight did not produce any symptoms of acute toxicity or mortality in the rats.

These results indicated that seed extract from *L. leucocephala* has antidiabetic and antioxidant activities. The antioxidant activity is likely due to the phenolic content. An application of this extract should be considered as it can affect renal function by reducing the levels of albumin, ALP and total protein.

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Introduction

Diabetes mellitus, a metabolic disorder disease, is characterized by hyperglycemia with an increased risk of many complications (Keen and Ng Tang Fui, 1982; Joshi and Mahajan 2003). Medicinal plants have been used as hypoglycemic agents for the treatment of diabetes because they have excellent antioxidant properties and polyphenolic compounds found in plants have been reported to have biological properties including antioxidant activity (Kähkönen et al., 1999).

Leucaena leucocephala (Lam.) de Wit belongs to the Fabaceae family and has been reported to possess medicinal properties that

control stomach diseases, facilitate abortion and provide contraction, and it is often used as an alternative, complementary treatment for diabetes (Salem et al., 2011). Its leaf and seed extracts have antioxidant and antidiabetic activities (Chowtivannakul and Talubmook, 2012). Its leaf extract contains as a principal constituent 2-(H)-benzofuranone-5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl (Salem et al., 2011). The leaves have been reported to contain phenolic compounds and flavonoid quercetin was also isolated from the leaf extract (Adekunle and Aderogba, 2007). An aqueous extract derived from its boiled seeds was taken orally to treat Type-2 diabetes (Jones, 1979). The seed extract from *L. leucocephala* inhibits elevated blood glucose and lipids levels, but increases the number of pancreatic islets (Syamsudin et al., 2006). Moreover, the active fractions from *L. leucocephala* seeds have been reported to have antidiabetic activity (Syamsudin et al., 2010). Unfortunately, its foliage contains mimosine, an amino acid known to be toxic to ruminants (Jones, 1979).

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Although acclaimed traditionally as an antidiabetic, there are no scientific studies regarding the effect of *L. leucocephala* on pharmacological activities and its toxicity. Therefore, the present study was designed to investigate the antidiabetic and antioxidant activities of 80% ethanolic extract of *L. leucocephala* seed. In addition, its toxicity was examined.

Materials and methods

Animals

A sample of 36 male and 36 female albino Wistar rats weighing 250–300 g purchased from the National Laboratory Animal Centre, Mahidol University, Thailand were used. The rats were housed under conditions of $25 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity with a 12 h darkness/light cycle and maintained with *ad libitum* access to water and instant food for rodents (Perfect Companion Co., Ltd; Bangkok, Thailand). The experimental protocol and performance of the rats were approved by the Institutional Ethical Committee for the Purpose of Use and Control, and Supervision on Experiments in Animals, Mahasarakham University, Thailand (License No. 0009/2012).

Induction of diabetes

To induce diabetes, the rats were injected intraperitoneally with a single dose of 65 mg/kg body weight streptozotocin (STZ; Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) dissolved in 20 mM citrate buffer pH 4.5. After injection, they were given a 2% sucrose solution as their drinking water for 48 h to alleviate the hypoglycemic phase. The rats were confirmed to be in the diabetic stage with a fasting blood glucose higher than 126 mg/dL and were used as diabetic rats in the experiments (Talubmook, 2008).

Preparation of the plant extract

Mature seeds of *L. leucocephala* were purchased from a local market in Sakon Nakhon province, northeastern Thailand. Voucher specimens (BG/LL001) were deposited in Mahasarakham University, Maha Sarakham, Thailand. The plant seeds were air-dried and ground into powder. The plant powder was extracted by macerating in 80% ethanol for 7 d and filtering. The filtrate was evaporated in a rotary evaporator. The obtained extract (LLSE) was stored at 4°C until use.

Antidiabetic activity study

The rats were randomly divided into the following four experimental groups with eight rats in each group: Group I, normal control, rats received 0.5% Tween 80; Group II, normal rats received 250 mg/kg body weight LLSE; Group III, diabetic control, diabetic rats received 0.5% Tween 80; and Group IV, diabetic rats received 250 mg/kg body weight LLSE.

Prior to the administration, LLSE was suspended in 0.5% Tween 80 [(polysorbate 80 or polyoxyethylene (20)) * sorbitan monooleate (emulsifier)]. An amount of 10 mL/kg body weight was administered using an orogastric tube. LLSE and 0.5% Tween 80 were given to the rats orally once a day for 6 wk.

Determination of fasting blood glucose

The rats were fasted overnight and blood samples were taken from the tail vein of the rats. The blood glucose was determined once a week using a glucometer (Roche; Stuttgart, Germany).

Determination of serum insulin, blood chemistry and hematological values

After 6 wk of treatment, the rats were fasted overnight and sacrificed using a cervical dislocation technique. Blood samples were then collected from the rat hearts. The blood samples were put into heparinized and non-heparinized tubes. The blood in the non-heparinized tubes was centrifuged at 3500 rpm for 20 min to separate blood serum. The serum was used for the investigation of insulin and blood chemistry. The serum insulin was estimated using a radio immune assay kit (MP Biomedicals; Orangeburg, NY, USA) and detected using an automatic gamma counter (Wallac 1470 Wizard; Perkin Elmer Instrument; Überlingen, Germany). An automatic blood chemical analyzer (BT 2000 plus, Germany) was used to measure blood chemistry consisting of: total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine, total protein, albumin, and alkaline phosphatase (ALP).

The blood from the heparinized tubes was used for hematological analysis consisting of hematocrit (HCT), hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC), using an anatomic blood chemical analyzer (BT 2000 plus, Germany).

Antioxidant activity study

The antioxidant activity of 80% ethanolic seed extract from *L. leucocephala* and of a standard solution (vitamin C) were investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Vongsak et al., 2013). In total, 750 μL of the extract and standard were added to 750 μL of DPPH in methanol solution. After incubation at 37°C for 20 min, the absorbance of each solution was determined at 517 nm using UV–VIS spectrophotometry. Corresponding blank readings were also taken and the percentage inhibition was then calculated using Equation (1):

$$\% \text{ Inhibition} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \quad (1)$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound (Pothitirat et al., 2010).

The EC_{50} value (the concentration of a sample required for 50% scavenging of the DPPH free radical) was determined from the curve of percentage scavenging plotted against the concentration. Each determination was done in triplicate and the average EC_{50} value was calculated.

Acute oral toxicity study

Acute oral toxicity of LLSE was performed on male and female albino Wistar rats, according to OECD Guideline 423 (Gatne et al., 2015). Five groups of eight rats each were used in the study: Group I, normal rats received 0.5% Tween 80 (normal controls); Group II rats received 500 mg/kg body weight LLSE; Group III, rats received 1000 mg/kg body weight LLSE; Group IV, rats received 1500 mg/kg body weight LLSE; and Group V, rats received 2000 mg/kg body weight LLSE.

The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 d. Body weights were recorded once weekly. On day 14, animals were sacrificed. The internal organs consisting of the liver, kidneys, heart and lungs were removed for gross pathological examination.

Estimation of total phenolic content

The total phenolic content (TPC) was determined using the spectrophotometric method (Aiubi et al., 2015). In brief, a 1 mL sample (1 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 10 mL of a 7% Na₂CO₃ solution was added to the mixture followed by the addition of 13 mL of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of the calibration curve, which was made by preparing a gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample.

Statistical analysis

All data were expressed as mean \pm SE. Statistical analysis was carried out using an F test (one-way ANOVA) followed by Duncan's new multiple range test. The criterion for statistical significance was at a *p*-value less than 0.05. Data were analyzed using SPSS for Windows (SPSS Inc; Chicago, IL, USA).

Results

Antidiabetic activity

Fasting blood glucose levels

The initial fasting blood glucose level of the diabetic controls was significantly higher than that of the normal controls. At the end of experiments, the fasting blood glucose of the LLSE-treated diabetic rats was significantly reduced compared to the fasting blood glucose in the diabetic controls. However, the fasting blood glucose in the LLSE-treated diabetic rats was still significantly higher than in the normal controls. LLSE had no effect on the fasting blood glucose level in normal rats as the fasting blood glucose level in normal rats treated with LLSE was not different from that in the normal controls (Table 1).

Body weight

The initial body weight of all rats was similar. However, at the end of the experiments, the body weight of the normal control rats was significantly higher than that of the diabetic controls. LLSE significantly decreased the body weight of the normal rats but had no effect on the body weight of the diabetic-treated rats (Table 2).

Serum insulin

Serum insulin was significantly decreased in the diabetic control rats compared with the normal controls. LLSE increased the serum insulin in the diabetic LLSE-treated rats. The levels of serum insulin were not significantly different between the diabetic and diabetic-LLSE treatments. However, there was a tendency for a higher

Table 1

Fasting blood glucose levels in normal and streptozotocin-induced diabetic rats with and without seed extract from *Leucaena leucocephala* (Lam.) de Wit (LLSE).

Group	Fasting blood glucose levels (mg/dl)	
	Initial	Final
Normal controls	85.12 \pm 5.20 ^a	71.25 \pm 10.97 ^a
Normal rats + LLSE	98.60 \pm 11.25 ^a	68.25 \pm 3.69 ^a
Diabetic controls	310.12 \pm 9.93 ^c	340.37 \pm 7.89 ^c
Diabetic rats + LLSE	281.25 \pm 11.15 ^b	182.87 \pm 4.66 ^b

* = values of mean \pm SE followed by different lowercase superscript letters are significantly different at *p* < 0.05 according to Duncan's multiple range test.

Table 2

Body weight in normal and streptozotocin-induced diabetic rats with and without seed extract from *Leucaena leucocephala* (Lam.) de Wit (LLSE).

Group	Body weight (g)	
	Initial	Final
Normal controls	296.66 \pm 11.11 ^a	365.5 \pm 12.50 ^b
Normal rats + LLSE	286.59 \pm 12.12 ^a	371.87 \pm 7.55 ^b
Diabetic controls	291 \pm 12.23 ^a	262.50 \pm 8.81 ^a
Diabetic rats + LLSE	289.31 \pm 11.15 ^a	272.50 \pm 14.36 ^a

* = values of mean \pm SE followed by different lowercase superscript letters are significantly different at *p* < 0.05 according to Duncan's multiple range test.

insulin level in the diabetic LLSE-treated group. Furthermore, LLSE did not alter the serum insulin level in the normal rats (Fig. 1).

Blood chemistry

The study on the effect of LLSE on blood chemistry revealed that the levels of BUN and creatinine in the diabetic controls were significantly higher than those in the normal controls. The levels of BUN and creatinine in the LLSE-treated diabetic rats did not differ from the levels in the diabetic controls. The level of BUN, but not of creatinine, in normal rats that received LLSE did not differ from the normal controls. However, the levels of BUN and creatinine in the LLSE-treated diabetic rats were significantly higher than those in the normal controls. Albumin was significantly decreased when the total protein was significantly increased in the diabetic rats compared to those in normal controls. LLSE significantly decreased the levels of albumin and total protein in the diabetic-treated rats compared to those in the diabetic controls. In normal rats, LLSE significantly increased the total protein level in the normal-treated rats but did not alter the albumin level in the normal-treated rats. The ALP level in the diabetic controls was significantly higher than that in normal controls. LLSE slightly decreased the level of ALP in the diabetic-treated rats compared to that of diabetic controls. However, LLSE did not alter the ALP level in the normal-treated rats compared to that in normal controls (Table 3).

Hematological values

Levels of HCT, Hb and RBC in the diabetic controls were significantly higher than those in the normal controls. LLSE significantly decreased the RBC level in the diabetic-treated rats. However, it did

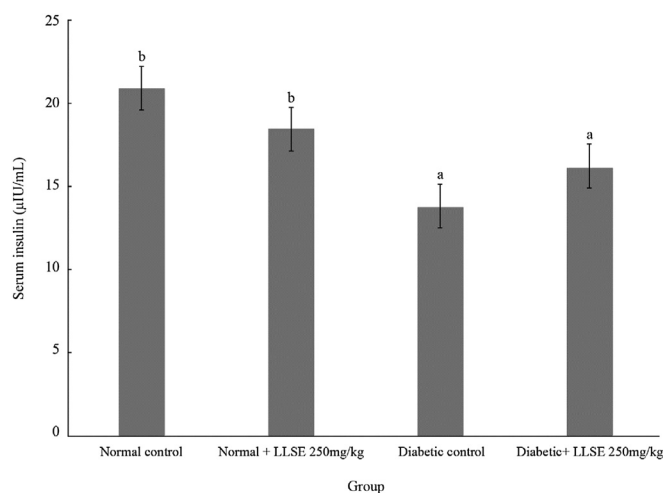


Fig. 1. Effect of seed extract from *Leucaena leucocephala* (Lam.) de Wit (LLSE) on serum insulin levels in normal and diabetic rats (IU = International units; error bars show \pm SE).

Table 3
Blood chemistry values for blood urea nitrogen (BUN), creatinine, albumin, alkaline phosphatase (ALP) and total protein in normal and diabetic rats with and without seed extract from *Leucaena leucocephala* (Lam.) de Wit (LLSE).

Group	Blood chemistry				
	BUN (mg/dL)	Creatinine (mg/dL)	Albumin (mg/dL)	Total protein (mg/dL)	ALP (International units/L)
Normal controls	30.02 ± 1.04 ^{a*}	0.67 ± 0.09 ^a	4.05 ± 0.70 ^b	6.63 ± 0.24 ^b	90.12 ± 10.44 ^a
Normals rats + LLSE	26.75 ± 0.76 ^a	0.83 ± 0.03 ^b	4.15 ± 0.77 ^b	6.86 ± 0.20 ^c	91.00 ± 17.81 ^a
Diabetic controls	41.30 ± 1.86 ^b	0.90 ± 0.04 ^b	3.91 ± 0.05 ^b	6.81 ± 0.15 ^c	242.00 ± 12.15 ^b
Diabetic rats + LLSE	44.46 ± 2.65 ^b	0.88 ± 0.03 ^b	3.60 ± 0.77 ^a	6.05 ± 0.20 ^a	230.75 ± 31.01 ^b

* = values of mean ± SE followed by different lowercase superscript letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

not alter the levels of HCT and Hb in the diabetic-treated rats compared to the diabetic control rats. Interestingly, LLSE did not alter the levels of HCT, Hb and RBC in the normal-treated rats compared to those in the normal controls. The WBC in the diabetic controls significantly decreased compared to the normal controls. LLSE significantly decreased the WBC in normal-treated rats but not in diabetic-treated rats (Table 4).

Antioxidant activity

Using DPPH scavenging assay showed that LLSE possessed antioxidant activity with an EC_{50} of 839.56 ± 21.53 mg/mL, which is less potent than vitamin C (1.48 ± 0.07 µg/mL).

Total phenolic contents

Estimation using Folin-Ciocalteu reagent revealed that the total phenolic contents of LLSE were 37.38 ± 0.49 mg GAE/g.

Acute oral toxicity study

In the acute toxicity study, LLSE-treated animals did not show any change in their behavioral pattern and there were no significant differences in the body weight and food consumption when compared to the vehicle-treated group. Moreover, gross pathological changes were not observed and LLSE could not produce any signs or symptoms of toxicity and no mortality was recorded during the periods of observation (24 h and 14 days). LLSE did not produce any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight in rats; hence the extract was considered to be safe and nontoxic for further pharmacological application.

Discussion

This study was conducted to confirm the traditional use of seeds and pods from *L. leucocephala* (Lam.) de Wit. as a vegetable and as a traditional medicine in Thailand. Pharmacological activities in animal models in a diabetes and toxicological study of this plant have been poorly documented. Therefore, the present study was carried out to investigate the antidiabetic and antioxidant activities and acute toxicity of the extract from *L. leucocephala* seed to ratify their

traditional use as a treatment for diabetes. A pharmacological property study found that repeated administration of the extract at an oral dose of 250 mg/kg body weight to rats once daily for 6 wk revealed the antidiabetic activity of the extract by significantly decreasing the blood glucose level, but slightly increasing the level of serum insulin in the diabetic-treated rats.

LLSE can increase the level of serum insulin in diabetic-LLSE treated rats. However, the level of serum insulin in the diabetic-treated rats was still significantly lower than in the normal controls, indicating the slightly potent hyperinsulinemia activity of the extracts. Levels of HCT, Hb, RBC and WBC were not different among the rat groups, indicating, the diabetic state and the extracts have no effect on hematological values. The extracts recover the pathology of renal function by lowering the levels of BUN, creatinine and ALP in the diabetic-treated groups compared to those in the diabetic controls. However, LLSE significantly increased the creatinine level in both normal and diabetic rats compared to that in the normal controls. These findings were in line with the study by Syamsudin et al., (2006) who found that the extract could inhibit elevated blood glucose and lipids levels and could increase the number of pancreatic islets per unit area significantly and concluded that the extract of *L. leucocephala* seed acts as a hypoglycaemic agent by the selective regeneration of the beta-cells of the streptozotocin-damaged pancreas. The antioxidant activity study revealed that LLSE has less antioxidant activity than the standard (vitamin C). However, extract of *L. leucocephala* seed (LLSE) exhibited an antioxidant activity (839.56 ± 21.53 µg/mL) less than *L. leucocephala* leaf extract (296.10 ± 16.40 µg/mL) according to Chowtivannakul and Talubmook (2012). The antioxidant activity in plants is mainly attributed to their phenolic contents (Puravankara et al., 2000). The results in the present study revealed that ethanolic seed extract from *L. leucocephala* possessed phenolic content. The antioxidant activity of the seed extract from *L. leucocephala* found in this study is therefore, mainly due to the phenolic content present in the extract.

The acute toxicity study showed that LLSE at a dose of up to 2000 mg/kg body weight did not produce any signs or symptoms of toxicity and mortality in rats throughout the period of observation (24 h and a further period of 14 d), indicating that LLSE has no acute toxicity. Its EC_{50} is, therefore, higher than 2000 mg/kg body weight. LLSE altered the levels of creatinine and TP in the treated rats,

Table 4
Hematological values of hematocrit (HCT), hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) in normal and diabetic rats with and without seed extract from *Leucaena leucocephala* (Lam.) de Wit (LLSE).

Groups	Hematological values			
	HCT (%)	Hb (%)	RBC ($\times 10^6$ cell/mL)	WBC ($\times 10^3$ cell/mL)
Normal controls	41.75 ± 1.89 ^a	14.22 ± 0.58 ^a	7.70 ± 0.18 ^a	4.88 ± 0.84 ^b
Normal rats + LLSE	44.00 ± 2.07 ^{ab}	14.01 ± 0.59 ^a	7.58 ± 0.29 ^a	3.83 ± 0.29 ^a
Diabetic controls	49.00 ± 1.86 ^b	16.77 ± 0.34 ^b	8.45 ± 0.20 ^b	3.23 ± 0.31 ^a
Diabetic rats + LLSE	46.00 ± 1.88 ^{ab}	14.95 ± 0.68 ^{ab}	7.73 ± 0.34 ^a	4.71 ± 0.35 ^{ab}

* = values of mean ± SE followed by different lowercase superscript letters are significantly different at $p < 0.05$ according to Duncan's multiple range test.

indicating LLSE exhibits an adverse effect on renal function. However, it did not alter the ALP level in the treated rats, suggesting that LLSE does not affect hepatic function. The results were in agreement with the studies by Rajendran and Krishnakumar (2010), Poolsil et al. (2011) and Sakuljaitrong et al. (2013).

Conclusion

The seed extract from *L. leucocephala* exhibits antidiabetic and antioxidant activities and can be used for the treatment of diabetes without affecting hepatic function, but there is an impact on renal function.

Conflict of interest

There is no conflict of interest.

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