ANTI-DIABETIC EFFECT OF *THUNBERGIA LAURIFOLIA* LINN. AQUEOUS EXTRACT

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Abstract. Several kinds of medicinal plants have been used to treat diabetes mellitus. In the present study, *Thunbergia laurifolia* Linn. (purple flower strain) were tested for hypoglycemic activity in rats. The effects on the reproductive system and the histology of the pancreas were also investigated. The hypoglycemic properties of the aqueous extract from the leaves (60 mg/ml/day) were evaluated in normoglycemic and alloxan-induced diabetic rats. The results showed that high blood glucose levels of diabetic rats were associated with severe destruction of β-cells (insulin-secreting cells) in the islet of Langerhans. The 15-day-treatment of *T. laurifolia* extract decreased levels of blood glucose in diabetic rats. The recovery of some β-cells was found in diabetic rats treated with the extract, although not completely normal. Whether *T. laurifolia* leaf contains insulin-like substance(s) which directly act as hypoglycemic agents, or contains substances that induce the regenerative process of β-cells remains to be further investigated. Alteration of the reproductive system was also observed in diabetic rats, but it was not improved by treatment with *T. laurifolia* extract.

INTRODUCTION

Diabetes mellitus is generally acknowledged to be due to insulin deficiency (Type I) which results from pathologic changes in pancreatic β-cells, or due to insulin insensitivity (Type II). Elevation of glucose blood level and a greatly increased risk of reproductive malfunction are common symptoms in diabetic patients (Jiang, 1996). At present, a large number of Thai people is affected by diabetes mellitus. Since the cost of imported anti-diabetic drugs is relatively high, traditional herbal medicine has become an alternative, affordable choice for diabetes treatment. Many plant species have been recommended for curing diabetes and some of them have proven hypoglycemic activity, for instance; *Enicostemma littorale* Blume (Maroo *et al*, 2002), *Gymnema inodorum, Stevia rebaudiana* and *Tinospora crispa*, (Saenphet *et al*, 2002; Pongchaidecha *et al*, 2000; Anulukanapakorn *et al*, 1999). In Thailand, various kinds of medicinal plants have been used widely, as recommended by traditional physicians. Nevertheless, most plants have been used without scientific evidence to support their safety and effectiveness. The use of medicinal plants for diabetes treatment has also been popular among Thai people. Due to the high cost of synthetic drugs for curing diabetes, the attempt to develop Thai medicinal plants into modern herbal products with reliable quality and efficacy is of interest.

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Thunbergia laurifolia Linn. has also been recommended by Thai traditional healers for treating diabetes. It is a climbing plant with smooth opposed leaves along the stem. The leaves are 8-10 cm long and 4-5 cm broad, broad-based, narrowing to a pointed tip, usually with scalloped lobes towards the base. The purple flowers are trumpet-shaped and produced during November-January. The seed pod is cone-shaped, 1 cm long, with a round base. In Thailand, it is called Rang Juad, Yaw Kaew, Kob Sha Nang, Gum Lung Chang Puak, or Krua Nan Nae in the North. Generally, this plant could be used for various purposes, including anti-toxicity, anti-inflammatory, and in the treatment of pneumonia (Utogapachn, 1976). According to Thai traditional medicine, a mixture of the root or leaf of *T. laurifolia* with water rinsed from rice can be effectively used as a detoxifying agent. The antidotal activity of *T. laurifolia* against insecticides has been confirmed by scientific research (Tejasen and Thongthapp, 1980). Nevertheless, reports of its anti-diabetic activity are still insufficient.

Due to its popular use as a detoxifying and anti-diabetic herb, *T. laurifolia* was selected in the present study for investigation of its hypoglycemic effect. A single dose of aqueous extract from dried leaves was administered to alloxan-induced diabetic rats. At the end of the treatment period, the levels of blood glucose and reproductive alteration of the rats were examined. The results of this primary research would provide valuable information for intensive studies of the effective concentration of the extract and the toxicity of this plant, to develop effective natural products for treating diabetes.

MATERIALS AND METHODS

Plant materials

The leaves of *T. laurifolia* were collected, washed, and sliced. The slices were dried at 30˚-40˚C for 2 hours. The extract, at a concentration of 60 mg/ml, was prepared by extracting a 6-g-portion of the slices in a 1 liter beaker containing 100 ml of distilled water. After extraction at 30˚-40˚C for 2 hours, the filtrate of 60 mg/ml extract was obtained.

Animal preparation

Male Wistar rats (*Rattus norvegicus*) weighing between 100-150 g were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. They were allowed to acclimate in the departmental animal facility for at least one week prior to the day of the experiment. They had access to water and a standard pellet diet (C.P. No.082). The study room was maintained at approximately 25˚ ± 2˚C. The photoperiod was 12-hour light and dark.

Diabetes was induced by intraperitoneal injection of alloxan (40 mg/ml) once daily, for 2 days. Seven days post-injection, blood glucose concentrations were assayed by enzymatic (glucose oxidase) determination using a commercial test kit (Sigma, No. 510). Rats with blood glucose concentrations of 250 mg/ml or higher were counted as diabetic rats.

Experimental design

Rats were randomized into 4 groups (10 each); 1) normal rats that received distilled water 1 ml/day, 2) normal rats that received 60 mg/ ml/day of *T. laurifolia* extract, 3) diabetic rats that received distilled water 1 ml/day, 4) diabetic rats that received 60 mg/ ml/day of *T. laurifolia* extract. They were treated for 15 days. At the end of the treatment period, the rats were sacrificed to measure blood glucose, the weights of the testes and seminal vesicle, and the density of the sperm in the epididymis. The histology of the pancreas and testes was also investigated.

Histology

Tissue sections (5 μm) of pancreas and testes were routinely processed for paraffin-embedding. The sections of testes were stained with hematoxylin, while those of the pancreas were stained by Gomori method (Grimstone and Skaer, 1972). Morphological examination was conducted under a light microscope. Pancreatic β-cells were stained light blue, while α-cells were stained red.

Statistical methods

Means and standard deviations were calculated. The significant difference was analyzed by Student's *t* -test.

RESULTS

Blood glucose levels and histology of pancreas

As shown in Table 1, the blood glucose levels of the control group of normoglycemic rats were relatively low (38.31-60.03 mg/100ml.). There was a trend of decreased blood glucose levels in the normoglycemic rats that received *T. laurifolia* extract (60 mg/ml/day) for 15 days, although significant differences were not found between pre- and post-treatment periods, or between groups (Table 1, Fig 1). The blood glucose levels of the diabetic rats were markedly higher than the normoglycemic rats. The diabetic rats that had received *T. laurifolia* extract had significantly lower blood glucose levels compared with those that received distilled water. Nevertheless, the blood glucose levels of this group were not as low as those of the normoglycemic rats (Table 1, Fig 1).

Histological study of pancreas revealed that endocrine tissues in both groups of normoglycemic rats formed the islets of Langerhans, which were scattered throughout the pancreatic tissues. They accounted for about 10% of all pancreatic tissues. β-cells (insulin-secreting cells) were found in the central region of the islets, while α–cells (glucagon-secreting cells) were found at the peripheral region (Fig 2 A and B). Severe destruction of β-cells was found in the diabetic rats. Only a small area of islets of Langerhans were present and both β- and α–cells could rarely be seen. Pyknotic nuclei were detected in some β-cells (Fig 2C). The islets of Langerhans of the diabetic rats treated with *T. laurifolia* extract were larger than the untreated group and more β-cells were also found (Fig 2 D). The numbers of islets of Langerhans in the normoglycemic groups were, however, higher than those of treated and untreated diabetic groups.

Reproductive system

Significant differences in testicular weights were not found between groups (Table 1, Fig 3). Nevertheless, the diabetic rats in both groups showed a significantly lower weight of seminal vesicle and lower density of sperm compared with the rats in the normoglycemic groups. Treatment of the diabetic rats with *T. laurifolia* extract did not give better results for sperm density or weight of seminal vesicle than the untreated diabetic rats (Table 1, Fig 4 and 5).

Testicular morphology, including germ cells of normal control rats, were practically normal (Fig 6A). In diabetic rats, most seminiferous tubules showed a loose arrangement due to reduction in diameter and

Table 1
Effect of aqueous extract of *Thunbergia laurifolia* Linn. on blood glucose levels and reproductive systems of diabetic rats.

Group	Blood glucose level prior to treatment (mg/100 ml)	Blood glucose level after treatment (mg/100 ml)	Weight of testes (mg/g BW)	Weight of seminal vesicle (mg/g BW)	Sperm density ($\times 10^5$cells/ml)
Normal control rats (1 ml / day distilled water)	38.31 ± 1.78 [a]	60.03 ± 32.44 [a]	13.18 ± 1.23 [a]	2.00 ± 0.48 [a]	39.07 ± 3.46 [a]
Normal rats (60 mg/ml/day *T. laurifolia* extract)	116.06 ±67.84[a]	92.08 ± 48.13[a]	14.24 ± 5.04[a]	1.67 ± 0.72[a]	25.71 ± 8.74[b]
Diabetic rats (1 ml/ day distilled water)	1,016.83 ± 585.68[b]	772.96 ± 338.67[b]	16.77 ± 4.04[a]	0.64 ± 0.30[b]	10.59 ± 7.68[c]
Diabetic rats (60 mg/ml/day *T. laurifolia* extract)	1,284.14 ± 629.12[b]	494.47 ± 343.25[b]	16.07 ± 4.40[a]	0.68 ± 0.35[b]	5.37 ± 3.81[c]

Results are expressed as means ± SD; a, b, c indicate significant differences between groups ($p < 0.01$)

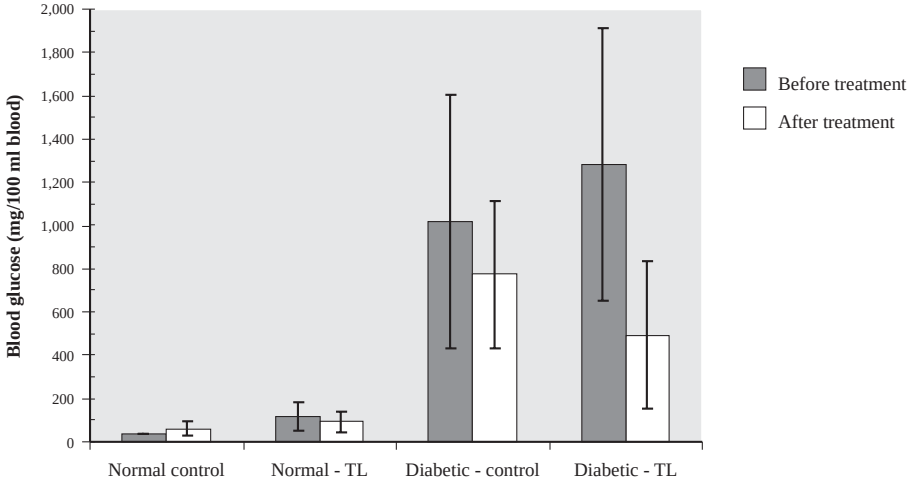

Fig 1- Effect of aqueous extract of *Thunbergia laurifolia* Linn. on blood glucose levels of normal and diabetic rats (means ± SD): TL = *Thunbergia laurifolia* Linn. extract.

atrophy of the tubules (Fig 6B). Spermatogenesis was noticeably disturbed. All kinds of germ cells were markedly degenerated and vacuolization of some germ cells was observed. Sertoli and Leydig cells also showed degeneration. The lumen of some tubules contained sloughed debris (Fig 6B). The alteration of testicular morphology was not improved by treatment with *T. laurifolia* extract. The histological aspects of this group were similar to those of untreated diabetic rats (Fig 6C).

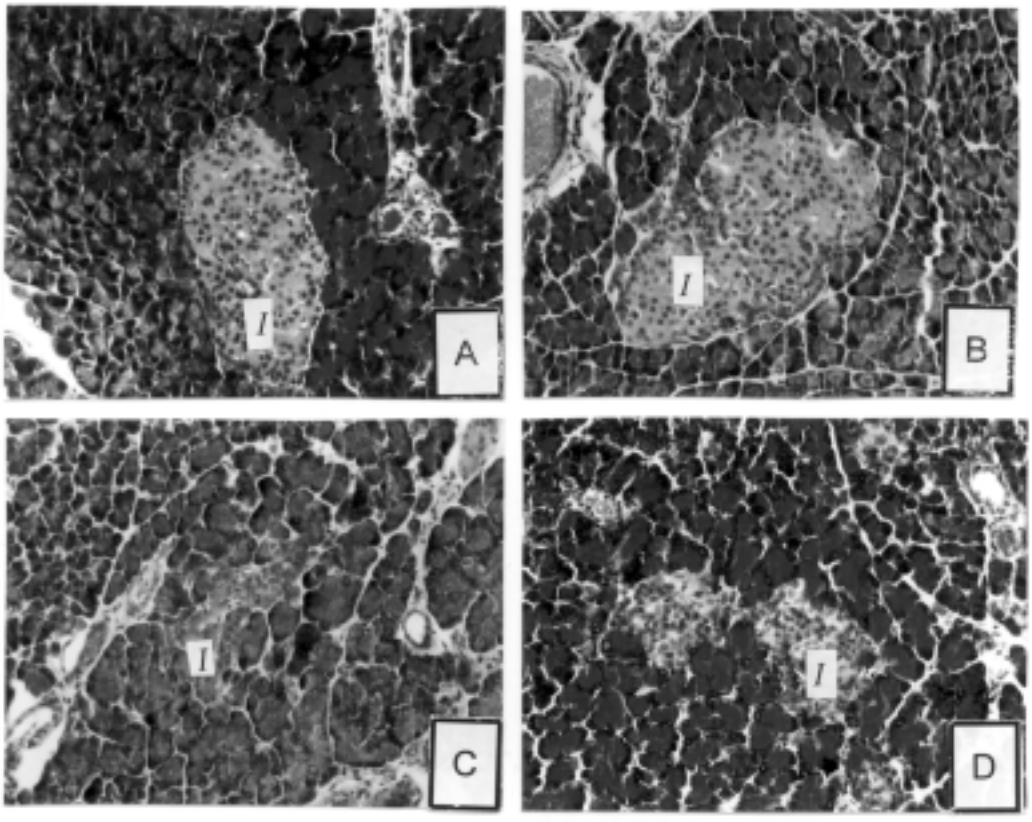

Fig 2- The pancreatic tissue of rat:(x225): **A.** untreated normoglycemic group; **B.** normo-glycemic group treated with *T. laurifolia* Linn. extract. Notice the normal size of islet of Langerhans (I) in both groups; **C.** untreated diabetic group showing degeneration of the islet of Langerhans; **D.** diabetic group treated with *T. laurifolia* Linn. extract. Notice the recovery of islet of Langerhans.

DISCUSSION

The decrease in the blood glucose levels of the diabetic rats treated with *T. laurifolia* extract was significantly higher ($p<0.01$) than the other groups (Table 1). This clearly indicated the hypoglycemic activity of this plant. This result was similar to reports of some medicinal plants that have proven effect in lowering blood glucose, for instance, chloroform extract of garlic (Tewit *et al*, 1996), aqueous extract of *Gymnema inodorum*, *Stevia rebaudiana*, and *Tinospora crispa*, (Anulukanapakorn, 1999; Pongchaidecha *et al*, 2000; Saenphet *et al*, 2002). Extracts from the root of *Helicteres isora* were also found to decrease the levels of glucose, triglycerides, and insulin in mice (Chakrabarti *et al*, 2002).

It was noted that the destruction accounting for 90% of insulin-secreting β-cells of the islets of Langerhans was caused by alloxan; hence, high blood glucose levels were detected. When compared with the untreated diabetic rats, the higher numbers of islets of Langerhans found in the diabetic rats treated with extract of *T. laurifolia* were associated with lower levels of blood glucose. The extract might aid in the recovery of β-cells to secrete insulin; the blood glucose level, therefore, was lower after treatment. Nevertheless, the recovery of β-cells was marginally observed. High levels of serum insulin and low levels of blood glucose were reported in the diabetic rats that were trained to exercise (Laileard *et al*, 1996). The authors suggest that exercise could induce the recovery of β-cells in diabetic rats. The recovery of β-cells found in this study (Fig 2 C, D), which was associated with low blood glucose levels was likely induced by the extract of *T. laurifolia*. The blood glucose levels of the diabetic rats treated with the extract of *T. laurifolia*, however, was not as low as that of the normoglycemic

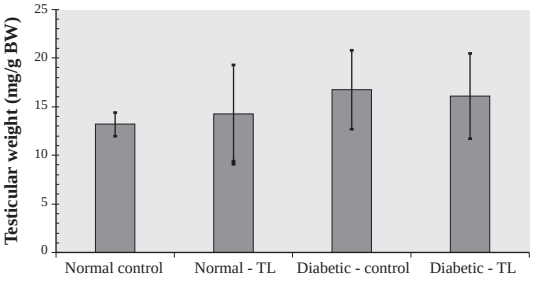

Fig 3- Effect of aqueous extract of *Thunbergia laurifolia* Linn. on testicular weights of normal and diabetic rats (means ± SD): TL = *Thunbergia laurifolia* Linn. extract.

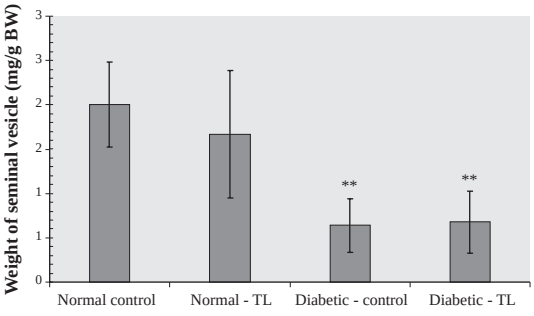

Fig 4- Effect of aqueous extract of *Thunbergia laurifolia* Linn. on the weights of seminal vesicles of normal and diabetic rats (means ± SD): TL = *Thunbergia laurifolia* Linn. extract.

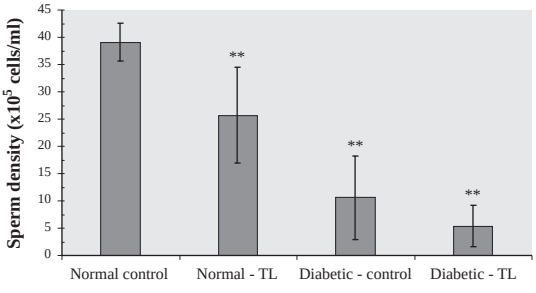

Fig 5- Effect of aqueous extract of *Thunbergia laurifolia* Linn. on epididymal sperm density of normal and diabetic rats (means ± SD): TL = *Thunbergia laurifolia* Linn. extract.

rats. This might be due to the short period of treatment (15 days). Further study with longer periods of treatment might give better results in lowering blood glucose levels. Whether *T. laurifolia* leaf contains insulin-like substance(s), which act directly as hypoglycemic agents or contains substances which could induce the regenerative process of β-cells remains to be further investigated. Our results suggest *T. laurifolia* as a new plant for the treatment of diabetes. It would be interesting and useful to study in detail its hypoglycemic mechanism.

The alterations in the reproductive organs of the diabetic rats were not surprising. Most diabetic patients report disturbances in sexual function (Jiang, 1996). Decreased levels of serum lutinizing hormone (LH) and follicle-stimulating hormone (FSH) were detected in the diabetic rats (Feng *et al*, 2001). Since testosterone synthesis is regulated by gonadotropins (Shetty *et al*, 1996), the regression of Ledig cells (testosterone-secreting cells) found in this study was likely due to the deprivation of those gonadotropins. The high blood sugar levels might cause the abnormal homeostatic regulatory systems of the diabetic rats, and the anterior pituitary gland, which produces gonadotropins, might also be a sensitive target. As a consequence of malfunction of the pituitary-gonadal axis, the destruction of germ cells, a decrease in size of the seminiferous tubules and of the seminal vesicles were evident. The lack of alteration in testicular weight, however, is difficult to explain in view of the histologic finding. Since testes are composed of many kinds of tissue, the destruction of some seminiferous tubules might not affect the total weight of the testes. Although *T. laurifolia* extract appeared to have hypoglycemic effect, the function of the reproductive system was not reversible when the extract was given to diabetic rats for 15 days. Appropriate treatment duration with this plant may exert an improvement in the reproductive system deteriorated by diabetes, and further investigation is required.

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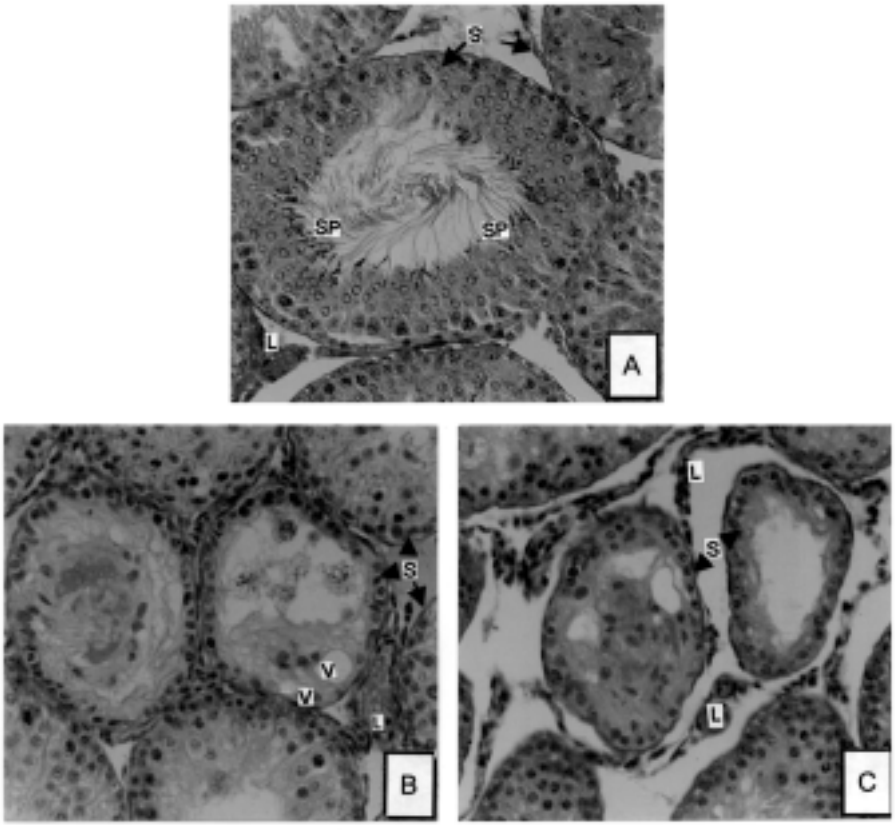

Fig 6- Cross section of rat testis (x200),: **A.** normoglycemic group showing normal histology of the seminiferous tubules (S) with various stages of spermatogenesis. Notice the great number of sperm (SP) and Leydig cells (L); **B.** diabetic group showing degeneration and vacuolization (V) of germ cells. Notice the smaller size of seminiferous tubules (S) compared to **A,** the disappearance of sperm and the regression of Leydig cells (L); **C.** diabetic group treated with aqueous extract of *Thunbergia laurifolia* Linn., the histologic aspects are similar to **B**.

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