ฤทธิ์ลดน้ำตาลในเลือดของยาสมุนไพรไทยสามตำรับในหนูแรทเบาหวาน

Hypoglycemic Activity of Three Thai Traditional Medicine Regimens in Streptozotocin-Induced Diabetic Rats

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บทคัดย่อ

ประเทศไทยมีประวัติการรักษาโรคด้วยตำรับยาสมุนไพรมายาวนาน แม้ในปัจจุบันก็ยังมีประชาชนจำนวนไม่น้อยใช้สมุนไพรเพื่อรักษาเบาหวาน อย่างไรก็ตาม ยังขาดการทดลองเพื่อยืนยันฤทธิ์ในการรักษา การศึกษาครั้งนี้จึงได้ทำการตรวจสอบฤทธิ์ลดน้ำตาลในเลือดของยาสมุนไพรรักษาเบาหวานจำนวนสามตำรับ ในหนูแรทที่ถูกเหนี่ยวนำให้เอยู่ในภาวะเบาหวานชนิดที่ 1 และ ชนิดที่ 2 ด้วยสาร streptozotocin ผลการทดลองพบว่า ยาสมุนไพรตำรับที่ 1 และ 3 ในขนาด 1 กรัมต่อน้ำหนักตัวหนึ่งกิโลกรัม มีฤทธิ์ลดน้ำตาลในเลือดของหนูแรทเบาหวานชนิดที่ 2 ได้ร้อยละ 20.67±4.86 and 33.11±7.61 ตามลำดับ ซึ่งลดลงอย่างมีนัยสำคัญทางสถิติ แต่ยาสมุนไพรตำรับที่ 2 นั้นไม่มีฤทธิ์ดังกล่าว ยาสมุนไพรตำรับที่ 1 ประกอบด้วย ขมิ้นเครือ ชะเอมไทย มวกแดง โมกหลวง มะแว้งเครือ ลำเจียก คนทา และแส้ม้าทะลาย ส่วนยาสมุนไพรตำรับที่ 3 ประกอบด้วย บอระเพ็ด เหงือกปลาหมอดอกขาว หญ้าใต้ใบ และขมิ้นเครือ เป็นที่น่าเสียดายที่ไม่พบฤทธิ์ลดน้ำตาลในเลือดของยาทั้งสามตำรับในหนูแรทเบาหวานชนิดที่ 1.

Abstract

Thailand has a long history of utilization of traditional medicine and until now not a few Thai people still use medicinal plants for diabetic treatment. However, there is no scientific evidence ascertaining the hypoglycemic activity of any of them. This study investigated the hypoglycemic activities of three Thai traditional antidiabetic regimens (I, II and III) in both streptozotocin-induced type 1 and type 2 diabetic rat models. It was found that the ethanolic extract of Regimen I and III at doses of 1 g/kg bw, but not Regimen II, decreased the blood glucose of type 2 diabetic rats significantly by 20.67±4.86 and 33.11±7.61% respectively. Regimen I consisted of *Arcangelisia flava* Merr., *Albizia myriophylla* Benth., *Xylinbaria minutiflora* Pierre., *Holarrhena pubescens* Wall., *Solanum trilobatum* Linn., *Pandanus tectorius* Sol., *Harrisonia perforata* Merr. and *Capparis micracantha* DC.. The components of Regimen III were *Tinospora crispa* Miers., *Acanthus ebracteatus* Vahl., *Phyllanthus amarus* Schum. & Thonn., and *Arcangelisia flava* Merr. Unfortunately, none of the three tested Regimens had hypoglycemic actvitity in type 1 diabetic rats. In conclusion, the Regimen I and III showed antidiabetic activities in streptozotocin-induced type 2 diabetic rats.

คำสำคัญ: แพทย์แผนไทย ยารักษาเบาหวาน สมุนไพร

Keywords: Thai traditional medicine, Antidiabetic drug, Medicinal plant

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Introduction

Diabetes is a chronic metabolic disorder that continues to be a major health problem. The number of people with diabetes worldwide is expected to rise to over 200 million by 2010 (Zimmet and Alberti, 2001). In particular, the prevalence of type 2 diabetes is increasing at an alarming rate, regardless of the population studied. It is also well documented that chronic hyperglycemia is associated with long-term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Brownlee, 2001). The pharmacological treatment of diabetes mellitus is based on oral hypoglycemic agents and insulin. It is notable that Thailand has a long history of utilization of traditional medicine and until now a substantial proportion of Thai people still use medicinal plants to treat their illnesses. Especially for the diabetes, several polyherbal formulations are documented. However, there is very little experimental evidence revealing their hypoglycemic activity. For the health benefit of people who could not access to safe and efficacious modern drugs as well as for the development of novel drugs, it is worthwhile investigating the a ntidiabetic activity of some Thai antidiabetic herbal regimens. In this study, we tested three regimens; the first one was recommended by a folk medicine man, the second was the regimen that is published in a general textbook of Thai medicinal herb and the third one was passed down verbally among native Thai people in Khon Kaen province in the northeast of Thailand.

Materials and Methods

Plant materials and preparation of extract

All the dried plants were obtained from the same traditional medicine shop in Bangkok, Thailand. The composition of each Regimen is shown in Table 1. For extraction, the ingredients of each regimen were minced and mixed together, soaked in 50 % ethanol for 3 days, and then centrifuged at 1500 rpm for 15 min. Ethanol in the supernatant was removed by rotary evaporator under reduced pressure. The remaining solution was dried by lyophilization and finally, dry powder was obtained. The yields of Regimen I, II and III were 6.86, 15.24 and 9.77 % respectively (w/w in terms of dried starting material).

Induction of diabetes

Diabetes simulating insulin dependence (type 1 diabetes) was induced by intraperitoneal injection of streptozotocin (STZ, 55 mg/kg body weight, Sigma, USA) to adult male Sprague-Dawley rats. After two weeks, rats with a nonfasting blood glucose over 200 mg/dl were included in the experiment (Rakeiten et al., 1963). Non insulin dependent diabetes (type 2 diabetes) was induced by intraperitoneal injection of STZ 90 mg/kg body weight to 48-hour old pups of Sprague-Dawley rats (Bonner-Weir et al., 1981). At 8 weeks old, the rats with nonfasting blood glucose more than 200 mg/dl were used in the study. The rats used in this experiment were obtained from the Experimental Animal Unit of the Faculty of Medicine, Khon Kaen University. All animal experiments were conducted in accordance with the general guidelines for the care and use of laboratory animals.

Effect of the antidiabetic regimen extracts on nonfasting blood glucose level of STZ induced diabetic rats

The extracts from each regimen were dissolved in 1% tragacanth solution and were fed through intragastric tube to STZ-induced diabetic rats twice daily at doses of 0.5 g/kg/day for three days and followed by escalating dose to 1.0 g/kg/day for another three days. Then the treatment was withdrawn. Control groups were given 1% tragacanth solution. For the positive control groups of type-1 and type-2 diabetic rats, the animals were injected subcutaneously with insulin (10 iu/kg/day, Mixtard®, Novo Nordisk, Denmark) and fed orally with tolbutamide (100 mg/kg/day, Sigma, USA), respectively. The nonfasting blood glucose levels were examined at 9-10.00 am on the day before and the last day of treatments, and blood was collected from tail vein to examine the blood glucose by glucose oxidase method using Automate analyzer (Technicon RA 100, USA).

Data and statistical analysis

The number of animals in each group was 4-5. Data were expressed as mean±S.E.M. Statistical comparisons within the same group were performed by Student's paired *t*-test. $P<0.05$ were considered significant.

Results

The hypoglycemic effect of the three Thai traditional antidiabetic regimens

In STZ-induced type 1 diabetic rats

None of the tested regimens decreased the blood glucose of type 1 diabetic rats (Table 2). However, subcutaneous injection of insulin 10 iu/kg significantly reduced the blood glucose of these type 1 diabetic rats (Table 2).

In STZ-induced type 2 diabetic rats

The blood glucose of diabetic rats receiving 1% tragacanth were at high levels throughout the experimental period (Figure 1) indicating that the solvent of antidiabetic regimen extract had no effect on blood glucose level. As expected, in this experimental diabetic rat model, the oral hypoglycemic drug tolbutamide (100 mg/kg) significantly reduced the blood glucose level from 290.47±37.47 to 210.12±41.29 mg/dl (Figure 2). However after 3 days of tolbutamide withdrawal, the blood glucose was back to the high level again (278.42±32.41 mg/dl). This indicated that the animals were in a hyperglycemic condition all over the period of the experiment.

Interestingly, the oral administrations of Regimen I and III extracts significantly decreased the blood glucose levels in the type 2 diabetic rats (Figures 3 and 5). Regimen I extract (1 g/kg) reduced the blood glucose by 20.67±4.86 % (from 300.84±44.60 to 238.90±43.37 mg/dl) and by 33.11±7.61 % in the group receiving 1 g/kg of Regimen III extract (from 293.46±46.40 to 183.44±11.54 mg/dl). However, the Regimen II extract showed a tendency to diminish the blood glucose level but non-significantly (Figure 4). The blood glucose levels of all diabetic rats returned to the high levels again 3 days after stoping the extract administration (withdrawal period).

Discussion

The present study demonstrated the hypoglycemic effect of two Thai traditional antidiabetic regimens in STZ-induced type-2 but not type-1 diabetic rats.

STZ, the most widely used agent, induced severe insulin deficient diabetes in rats and other rodents when given as a single high dose (50-100 mg/kg in rats). At this dosage, STZ caused pancreatic beta-cell necrosis and insulin typically fell to 10-30 % of normal leading to hyperglycemia with in 1-2 days. This imitated type-1 diabetes (Ozturk et al., 1996). When STZ was injected to adult rats, no evidence of beta-cell function recovery was detected (Bonner-Weir et al., 1981). Unfortunately, in this study, none of the Thai traditional antidiabetic regimens decreased the blood glucose level of the insulin dependent or type-1 diabetic rats.

The clinically used tolbutamide (a sulfonylurea drug) is known to lower the blood glucose level by stimulating beta-cells to release insulin. In this experiment, tolbutamide significantly reduced the blood glucose level of the STZ-induced type-2 diabetic rats. The injection of STZ into 2-day old neonatal rats imitates the condition of type-2 diabetes mellitus. During 6-8 weeks of age, the blood insulin levels of the STZ animals were 30-50% of control and blood glucose levels were in a hyperglycemic condition (Bonner-Weir et al., 1981). This implies that there are still some functional beta-cells remaining, but insufficient to normalize blood glucose level. Subsensitivity to endogenous insulin was observed in neonatal STZ-injected rats (Levy et al., 1884, Portha et al., 1989). This is not a severe diabetic state, as no insulin treatment is required (Bonner-Weir et al., 1981). The extracts of Regimen I and III reduced the blood glucose of the animals in this condition. Therefore, one possible mechanism among others for their hypoglycemic action may be the stimulation of insulin release as tolbutamide does. In addition, other mechanisms that may also be involved include (1) enhancing the action of insulin, (2) increasing the peripheral glucose utilization, (3) increasing the number of glucose transporters and (4) inhibiting the gluconeogenesis.

In general, the Thai traditional medicine regimens are always composed of many kinds of plants with the aims of (1) making good taste and smell, (2) reducing adverse effects, or (3) enhancing activity of the main active constituents. The common component of Regimen I and III is *Arcangelisia flava* Merr. Unfortunately, the scientific evidence showing hypoglycemic activity of each components of Regimen I is very limited. However, the components of Regimen III, *Tinospora crispa* Miers and *Phyllanthus amarus* Schum.& Thonn. have been reported to have hypoglycemic activities (Noor and Ashcroft, 1989; 1998; Srividya and Periwal, 1995, Moshi et al., 2001, Raphael et al., 2002). It is interesting that over the last two decades, several comprehensive reviews have been written on the evidence that higher plants are of use in the treatment of diabetes (Oubre et al., 1997).

In conclusion, Regimen I and III showed hypoglycemic activities in STZ-induced type-2 but not in type-1 diabetic rats. The hypoglycemic effect may be mediated by the stimulation of insulin release. Further investigation is essential to elucidate their mechanisms of action as well as their toxicity.

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Table 1. Composition of the three Thai traditional antidiabetic regimens.

	Botanical names	Part used	Dry weight (g)
Regimen I			
	Arcangelisia flava Merr	Climbing stem	30
	Albizia myriophylla Benth.	Stem	30
	Xylinbaria minutiflora Pierre.	Climbing stem	30
	Holarrhena pubescens Wall.	Root	30
	Solanum trilobatum Linn.	Root	30
	Pandanus tectorius Sol.	Root	30
	Harrisonia perforata Merr.	Root	30
	Capparis micracantha DC.	Root	30
Regimen II			
	Smilax china L.	Rhizome	70
	Smilax glabra Wall.	Rhizome	70
	Phyllanthus amarus Schum. & Thonn.	Whole plant	70
Regimen III			
	Tinospora crispa Miers	Climbing stem	40
	Acanthus ebracteatus Vahl.	Leaves	50
	Phyllanthus amarus Schum. & Thonn.	Whole plant	40
	Arcangelisia flava Merr.	Climbing stem	50

Table 2. Effects of Thai traditional medicine regimens on the nonfasting blood glucose of type 1 diabetic rats.

Groups	**Nonfasting Blood Glucose (mg/dl)**	
	Before	**After**
1% Tragacanth	390.48±18.26	330.73±10.72
Insulin 10 iu/kg	483.56±20.63	257.32±85.56*
Regimen I		
0.5 g/kg	457.36±35.69	423.44±31.03
1.0 g/kg		435.88±26.00
Regimen II		
0.5 g/kg	362.60±24.64	359.83±27.23
1.0 g/kg		329.38±65.48
Regimen III		
0.5 g/kg	438.12±24.66	409.14±22.35
1.0 g/kg		412.78±21.75

*: $P < 0.05$ as compared to before insulin injection, Number of animals in each group=5

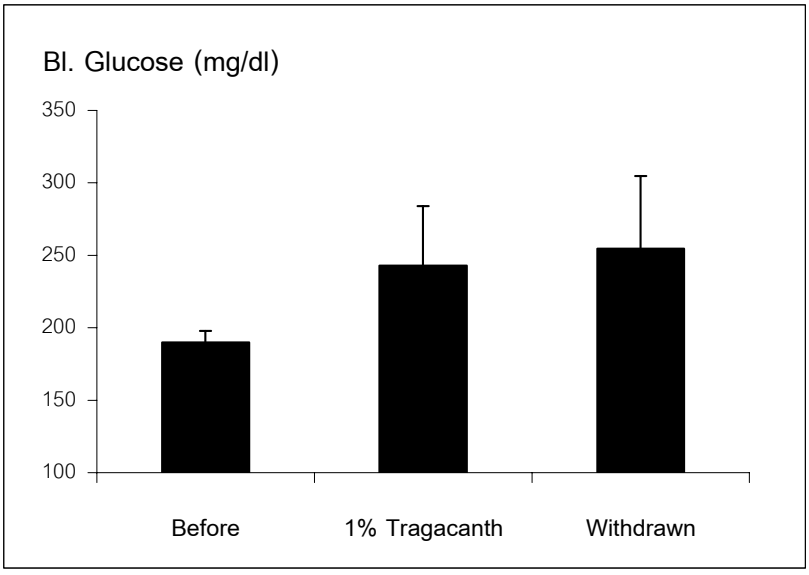

Figure 1. The nonfasting blood glucose level of STZ-induced type 2 diabetic rats receiving 1 % tragacanth for 3 days.
number of animals = 5
withdrawn: the blood glucose 3 days after the withdrawal of 1% tragacanth

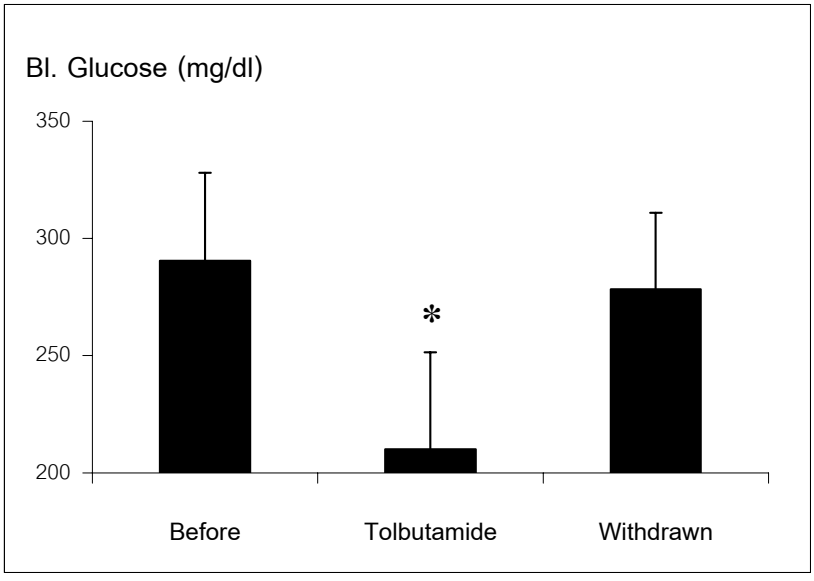

Figure 2. The effect of tolbutamide administration at dose of 100 mg/kg/day for 3 days on the nonfasting blood glucose level of STZ-induced type 2 diabetic rats.
*: $P< 0.05$ as compared to before tolbutamide administration
number of animals = 4
withdrawn: the nonfasting blood glucose level 3 days after the withdrawal of tolbutamide

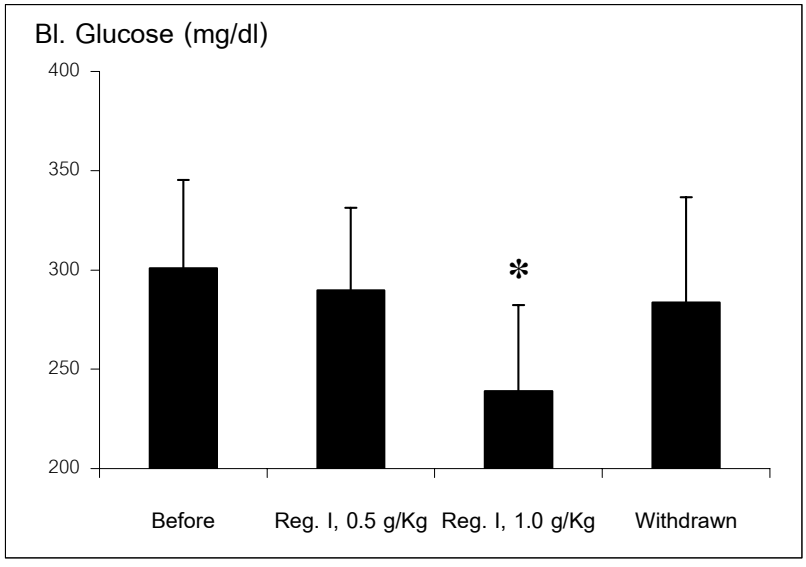

Figure 3. The effect of Regimen I administrations at doses of 0.5 and 1.0 g/kg/day for 3 days on the nonfasting blood glucose levels of STZ-induced type 2 diabetic rats.
*: $P < 0.05$ as compared to before Regimen I administration,
Reg I: Regimen I
number of animals = 5
withdrawn: the blood glucose 3 days after the withdrawal of 1% tragacanth

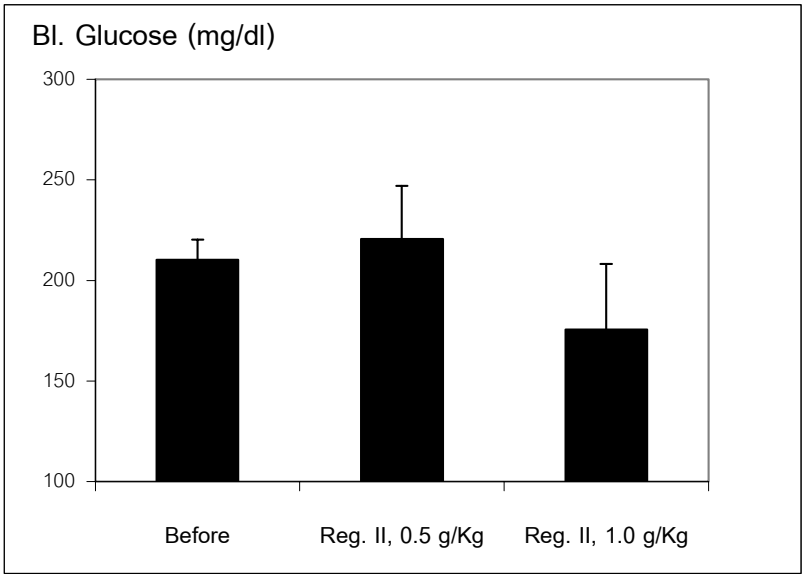

Figure 4. The effect of Regimen II administrations at doses of 0.5 and 1.0 g/kg/day for 3 days on the nonfasting blood glucose level of STZ-induced type 2 diabetic rats.
Reg II: Regimen II
number of animals = 5

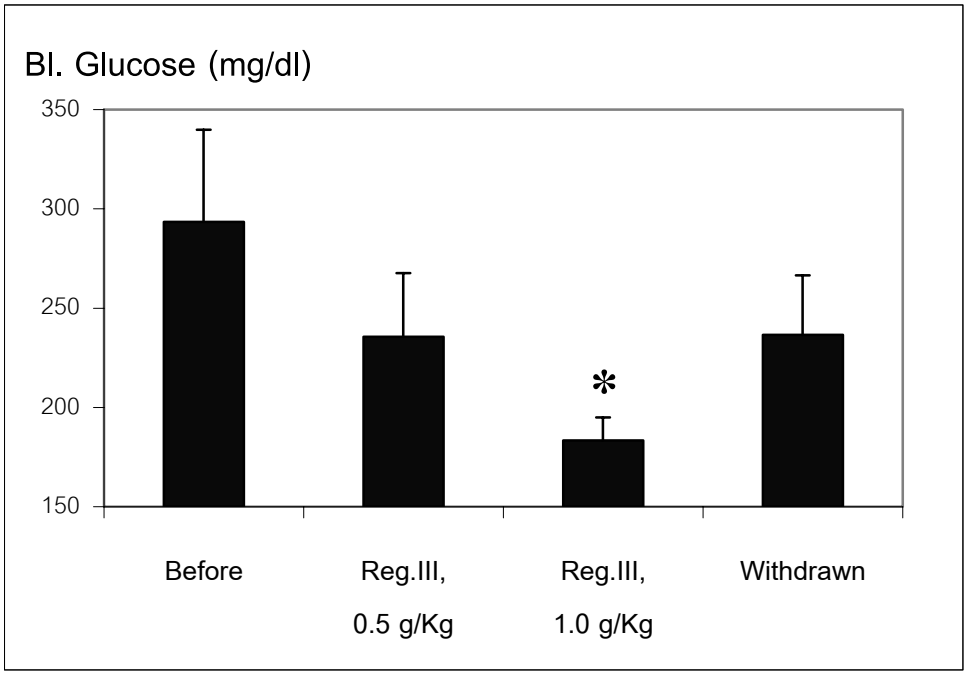

Figure 5. The effect of Regimen III administrations at doses of 0.5 and 1.0 g/kg/day for 3 days on the nonfasting blood glucose of STZ-induced type 2 diabetic rats.

*: $P<0.05$ as compared to before Regimen III administration

Reg III: Regimen III

number of animals = 5

withdrawn: the nonfasting blood glucose level 3 days after the withdrawal of Regimen III