Hypoglycemic effect of *Aemotoxylon campechanum* on streptozotocin (STZ)-induced diabetic rats

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Abstract

Hypoglycemic activity of *Aemotoxylon campechanum* was examined in STZ-induced diabetic rats. The water extract of *A. campechanum* lowered fasting blood glucose level of STZ-induced diabetic rats significantly and dose-dependently. The water extract further divided into MeOH and H_2O-soluble fractions. The MeOH-soluble fraction showed the strongest hypoglycemic effect, which lowered fasting blood glucose level by 37% at a dose of 100 mg/kg (*i.p.*). The water extract and the MeOH-soluble fraction were found to be more effective in lowering the blood glucose level of diabetic rats than the mixtures of tolbutamide (200 mg/kg) and buformin (1 mg/kg) used as positive control, which lowered blood glucose level by 35.1%. The active fraction led to isolation of five guainane-type sesquiterpenes and a coumarin derivative.

Key words *Aematoxylon campechanum*, hypoglycemic effect, streptozotocin, guainane-type sesquiterpene, coumarin.

Introduction

Diabetes mellitus is a metabolic disorder affecting the metabolism of carbohydrate, fat and protein. It is a leading cause for human death and has been reported to affect 10% of the world population.[1,2] There is still a lack of reliable drugs for the treatment of diabetes patients except for insulin or some hypoglycemic agents with various side effects.[1] On the other hand, there are several medicinal plants reported to have been used traditionally for the treatment of diabetes patients in different ethnic societies of Asia, Africa and South America. Even in the developed countries of Europe, North America and Japan, several plant products are used for the treatment of diabetics under the name of herbal drugs. *Aematoxylon campechanum* L. (Leguminosae) is one of such plants widely distributed in South America. It is commonly known as "Palo Azul" in Paraguay, and its aerial parts are used traditionally for the treatment of various diseases such as diarrhea, anemia, diuretic, tuberculosis and diabetes.[3] In the present study, we examined the hypoglycemic activity of the water extract of *A. campechanum* in STZ-induced diabetic rats.

Materials and Methods

Chemicals: Streptozotocin (STZ) and tolbutamide were purchased from Sigma Chemicals (St. Louis, USA). Glucose detecting kit (Glucose CII Test Wako) was from Wako Pure Chemicals Industry (Osaka, Japan). Buformin was from Japan Galen (Saitama, Japan). Column chromatography was performed on silica gel 60 (Nacalai Tesque, Kyoto, Japan) and thin layer chromatography (TLC) was carried out on percolated Merck Kieselgel 60 F_{254} or RP-18 F_{254} plates (0.25 or 0.50 mm thickness). UV measurements were done on a Shimadzu UV-160A UV-Visible spectrophotometer.

Plant material: The aerial parts of *A. campechanum* were collected from Paraguay in 2000. A voucher sample (TMPW 21964) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan, as a reference.

Extraction and isolation: Air dried aerial parts of *A. campechanum* (500 g) were extracted with refluxing H_2O

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(3 l, 3 h×2). The filtrate was evaporated under reduced pressure and lyophilized to yield an H_2O extract (97.2 g; 19.4%). A part of the extract was then subjected for hypoglycemic activity and the remaining part (80 g) was dissolved in MeOH (500 ml×2) to give a MeOH-soluble fraction (42.2 g). The MeOH-insoluble portion was dissolved in H_2O (500 ml×2) to yield an H_2O-soluble fraction (35.5 g) (Fig. 1). These fractions were also tested for their hypoglycemic activity. The MeOH extract was then separated by a combination of Sephadex LH-20 and silica gel column chromatography and preparative TLC to give five guainane-type sesquiterpenes and a coumarin; *i.e.*, desacylcynaropicrin (**1**, 22.2 mg),[4] 11,13-dihydrodesacylcynaropicrin (**2**, 12.8 mg),[5] aglycone of ixerin E (**3**, 1.7 mg),[6] crepiside E (**4**, 27.6 mg),[7] 11,13-dihydrodesacylcynaropicrin-3β-*O*-glucoside (**5**, 11.1 mg)[8] and 6,7-dihydroxy-8-methoxy-cumarin (**6**, 40.8 mg).[9] Their structures were confirmed by comparing the spectral data with those in literature.

Animals: Male Wistar rats, 5 to 6-weeks-old (150-250 g) were used for the present study. All the animals were purchased from Sankyo Labo Service (Tokyo, Japan) and were maintained on a 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed with a laboratory pellet chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* unless anything else was specified during the experiment. This study was conducted in accordance with the standards outlined in the Guidelines for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

Diabetic model and treatment of extract and fractions: Rats were made diabetic by a little modification of the previously described method.[10] In brief, diabetes was introduced by a single intraperitonial dose (55 mg/kg) of STZ dissolved in citrate buffer (pH 4.5) into 16h-fasted rats. Five days after STZ-injection, the rats were fasted 6h and blood was taken from the tail vein of anesthetized rats using a capillary tube. By centrifuging for 10 min (3000 rpm) plasma was separated from blood and then the glucose level was measured by an enzymatic method (Glucose-Test kit, Wako, Japan). Rats were considered diabetic when their fasting glycemia was > 250 mg/dl and the diabetic rats were divided randomly into different groups consisting of five animals each and treated with extract and fraction of *A. campechanum* intraperitonally (*i.p.*) at various doses. The group treated with a mixture of tolbutamide (200 mg/kg) and buformin (2 mg/kg) was taken as positive control.[2,11] The group treated with an equal volume of physiological saline was taken as negative control. A group of rats without treatment of STZ was taken as normal, which were also treated with an equal volume of physiological saline. The blood glucose lowering effects were measured after administering the extracts and fractions at around 9:00 and 16:00 on the first and second days and at 9:00 on the third day and fasting. The blood sample was collected at 15:00 from the tail vein. The decrease of fasting blood glucose level was calculated as follows: % decrease in glucose = $(G_o - G_x)/G_o \times 100$, where G_o and G_x are the values of initial glycemia and the glycemia after treatment of extract and fractions. The data are shown as mean ± S.E. and statistical significance was evaluated by Student's t-test.

Results and Discussion

The aerial part of *A. campechanum* was extracted with water under reflux for three hours to give a water extract (19.4%). The water extract was then administered in diabetic rats for five times at a dose of 100 and 200 mg/kg (*i. p.*). The fasting blood glucose levels of both groups were lowered significantly compared to that of the negative control group treated with an equal volume of physiological saline in diabetic rats (Fig. 2). The blood glucose level of 100 mg/kg group was 327.1 ± 11.2 mg/dl before administration of the extract, it was lowered to 252.2 mg/dl (lowered by 22.8%) after treatment of the water extract for five times. Moreover, the lowering rate of fasting blood glucose at 200 mg/kg dose

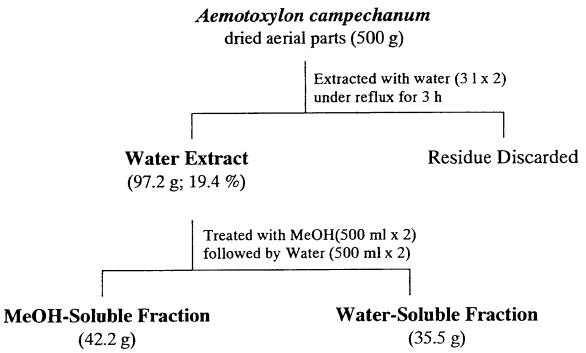

Fig. 1 Extraction and fractionation of *A. campechanum*

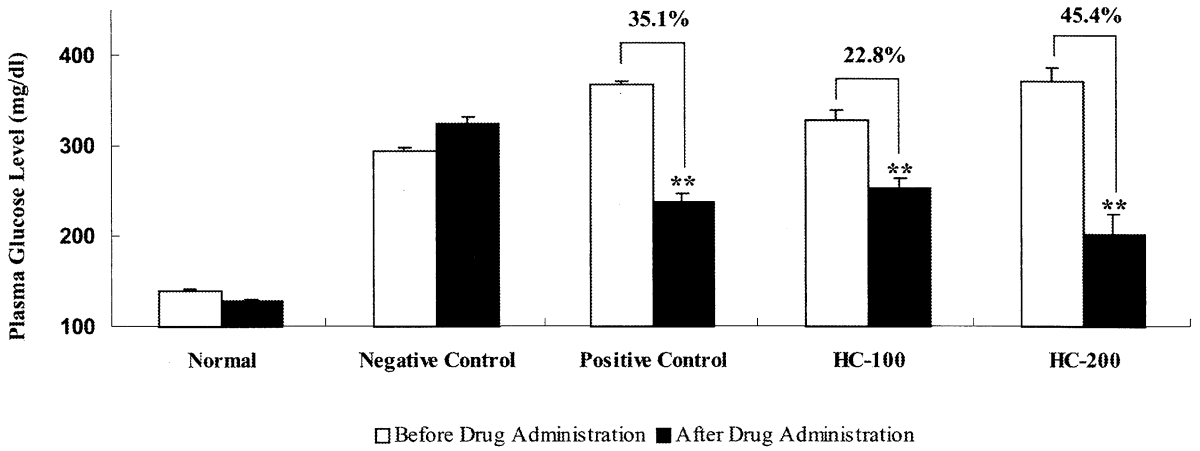

Fig. 2 Hypoglycemic activity of the water extract of *A. campechanum* on STZ-induced diabetic rats. In each group 5 rats were taken $**p<0.01$, $*p<0.05$ significantly different from initial glycemia, *i.e.*, before treating drugs. Normal, without STZ treatment rats with physiological saline treated group; Negative Control, physiological saline treated group; Positive Control, mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg); HC-100, water extract (100 mg/kg) and HC-200, water extract (200 mg/kg).

of the extract was nearly doubled (45.4%), which indicated that the water extract of *A. campechanum* possessed significant and dose-dependent hypoglycemic activity. The water extract at 200 mg/kg showed stronger blood glucose lowering effects than the mixtures of tolbutamide (200 mg/kg) a known sulfonylurea hypoglycemic agent and buformin (1 mg/kg), which was used as positive control lowered blood glucose by 35.1%. The blood glucose level of the rats treated only with STZ (negative control group) remains steady at the same time interval.

The active water extract was further portioned into MeOH-soluble and water-soluble fractions and both of these fractions were tested for their hypoglycemic effect on the same experimental model at a dose of 100 mg/kg (*i.p.*). The MeOH-soluble fraction showed stronger hypoglycemic activity than the water-soluble fraction (Fig. 3). The fasting blood glucose level of diabetic rats was lowered to 208.6 ± 8.8 mg/dl (by 37.0%) by the treatment of the MeOH-soluble fraction, while the water-soluble fraction reduced the blood glucose level of diabetic rats by 22.0%. Then, we performed further chemical examination of the MeOH-soluble fraction because of its higher hypoglycemic potency. The repeated column chromatography and preparative TLC of the MeOH-soluble fraction led us to isolation of five guainane-type sesquiterpenes [desacylcynaropicrin (**1**), 11,13-dihydrodesacylcynaropicrin (**2**), aglycone of ixerin E (**3**), crepiside E (**4**), 11,13-dihydrodesacylcynaropicrin-3β-*O*-glucoside (**5**)] and a coumarin derivative [6,7-dihydroxy-8-methoxy-cumarin (**6**)]. Among the isolated sesquiterpenes, **4** and **5** were glucosides. Unfortunately, due to the small amount obtained, we could not examine the hypoglycemic activity of these compounds.

The mechanism of the hypoglycemic activity and the active components of the water extract of *A. campechanum* are unknown, but it is well established that terpenoids involve to stimulation of pancreatic β-cells and insulin secretion.[12] Moreover, Tommasi *et al.* reported that sesquiterpene glucoside showed hypoglycemic effects by releasing insulin from β-cells.[13] Since STZ is known to destroy pancreatic β-cells, the isolated sesquiterpenes may play an important role in lowering the blood glucose level of diabetic rats by stimulating pancreatic β-cells to secrete insulin. Coumarin was also reported to have hypoglycemic effects,[12] which suggest that the coumarin derivative in the water extract of *A. campechanum* may also contribute to its hypoglycemic activity.

In conclusion, the water extract of the aerial parts of *A. campechanum* possessed potent hypoglycemic effects on STZ-induced diabetic rats. Though further study is needed to confirm the mechanism of the hypoglycemic effects of *A. campechanum*, the results of this experiment would establish the scientific evidence of its traditional uses for treatment of diabetes mellitus in different ethnic societies of Paraguay.

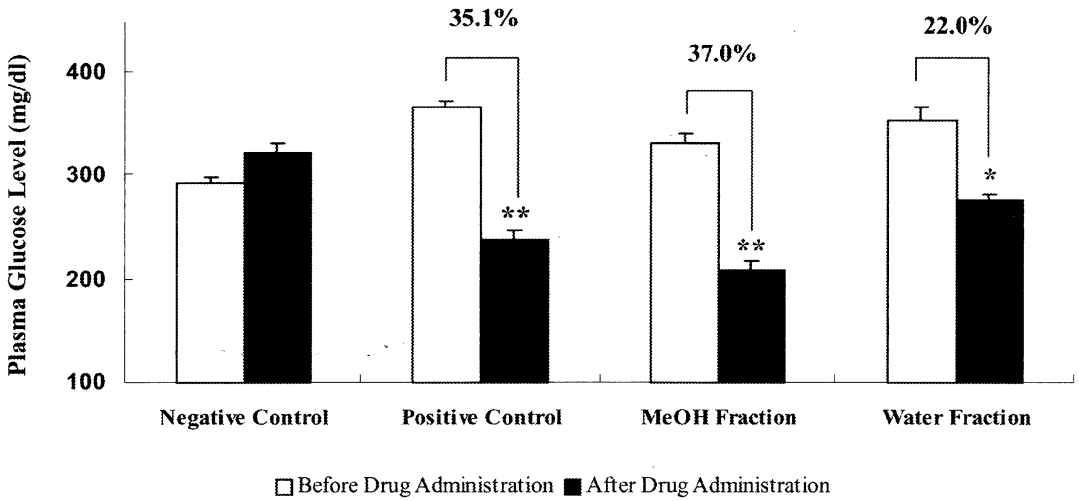

Fig. 3 Hypoglycemic activity of the MeOH- and H_2O-soluble fractions on STZ-induced diabetic rats. In each group 5 rats were taken $^{**}p<0.01$, $^{*}p<0.05$ significantly different from initial glycemia, *i.e.*, before treating drugs. Negative Control, physiological saline treated group; Positive Control, mixture of tolbutamide (200 mg/kg) and buformin (1 mg/kg); MeOH Fraction, MeOH-soluble fraction of the water extract (100 mg/kg) and Water Fraction, the water-soluble fraction of water extract (100 mg/kg).

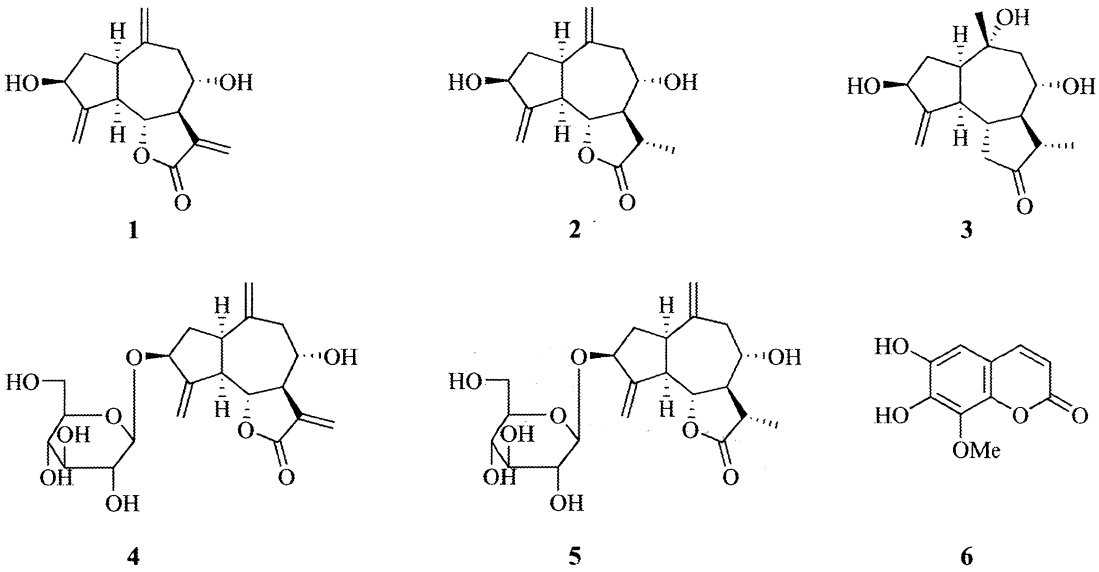

Fig. 4 Structures of compounds isolated from the MeOH-soluble fraction of the water extract of *A. campechanum*

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和文抄録

Aemotoxylon campechanum の血糖降下作用を STZ 誘発糖尿病ラットを用いて検討したところ，水エキスが空腹時血糖を有意かつ用量依存的に低下させた。そこで，水エキスをメタノール可溶性フラクション及び水溶性フラクションに分画したところ，メタノール可溶性フラクションがより強い作用を示し，100 mg/kg の腹腔内投与で空腹時血糖値を 37% 低下させた。また，水エキス 200 mg/kg 及びメタノール可溶部 100 mg/kg は空腹時血糖

値を35.1％低下させた陽性コントロールとして用いたtolbutamide (100 mg/kg) と buformin (1mg/ kg) の混合物よりも強いものであった。最後に，この活性フラクションの分離を行ない，グアイアン型セスキテルペン5種及びクマリン誘導体1種を単離・同定した。

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