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Anti-diabetic Constituent from the Node of Lotus Rhizome (*Nelumbo nucifera* Gaertn)

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Abstract – To investigate anti-diabetic component from the node of lotus rhizome (*Nelumbo nucifera*; Nymphaeaceae), activity guided isolation was conducted. One amino acid was isolated from active fraction of the aqueous methanolic extract. The structure of this compound was identified as tryptophan (**1**) by the analysis of spectroscopic evidences and comparisons with the data of authentic samples. Tryptophan reduced the blood glucose level significantly in glucose-fed hyperglycemic mice compared with glucose-treated group and exhibited 44.3% of activity compared with tolbutamide,.
Key words – *Nelumbo nucifera*, anti-diabetic activity, tryptophan.

Introduction

The node of lotus rhizome (*Nelumbo nucifera*) has been used for the remedy of bleeding, blood stagnancy and thirstiness as a traditional medicine in Korea (Bensky and Gamble, 1993). It was reported that lotus rhizome exhibited anti-diabetic (Mukherjee *et al.*, 1995 and 1997), antipyretic (Sinha *et al.*, 2000) and hyperlipidaemia activity (La Cour *et al.*, 1996) in extract level. To investigate anti-diabetic constituent from the node of lotus rhizome, activity guided isolation and structure elucidation were conducted.

Experimental

General – ^{1}H- and ^{13}C-NMR spectra were recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR), respectively. Chemical shift are given in δ (ppm) scale with TMS as internal standard. EIMS spectrum was obtained with GC-EI Mass, JMS-AX505WA, HP5890 Series II spectrophotometer. Column chromatography was carried out on Sephadex LH-20 (25-10 μm, Pharmacia), MCI-gel CHP 20P(75-150 μm, Mitsubishi) and YMC-gel ODS-A (230/70 and 500/400 mesh, YMC Co.). TLC was conducted on precoated silica gel 60 F_{254} plate (Merck). Spots were detected under UV and by spraying with dil. H_2SO_4, followed by heating.

Plant materials – Plant materials used in this study were purchased from Kyung-Dong Herbal Market in Seoul. A sample of these materials was deposited at the Medicinal Plants Herbarium of Chung-Ang University.

Extraction and isolation – Powdered nodes (3 kg) were extracted with 80% aqueous MeOH (4 L) at room temperature for 3 times. After removal of MeOH *in vacuo*, the aq. soln was filtered. The filtrate (fr. A) showing anti-diabetic activity was applied to a Sephadex LH-20 column chromatography using increasing proportions of MeOH which afforded 2 subfractions, A-1 (200 g) and A-2 (10 g). Repeated column chromatography of A-2, which showed potent anti-diabetic activity, on low-pressure liquid chromatography using YMC-gel ODS-A with a H_2O-MeOH gradient yielded tryptophan (**1**) (600 mg).

Tryptophan (1): brown amorphous powder. $[\alpha]_D^{20}$: –86.6° (c = 0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3407 (NH_2), 1660 (C = O, N-H), 1591, 1458, 1415 (aromatic C=C) cm^{-1}. EI MS: *m/z* 204 $[M]^+$. ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 10.88 (1H, s, N-H), 7.58 (1H, d, *J*=7.8 Hz, H-4), 7.34 (1H, d, *J*=8.1 Hz, H-7), 7.20 (1H, s, H-2), 7.07 (1H, m, H-6), 6.97 (1H, m, H-5), 3.44 (2H, m, N-H_2), 3.43 (1H, m, H-2'), 3.31 (1H, dd, *J*=15.1, 3.7 Hz, H-1'eq), 2.96 (1H, dd, *J*=15.1, 9.1 Hz, H-1'ax). ^{13}C-NMR (DMSO-d_6, 125MHz): δ 169.8 (C-3'), 136.3 (C-8), 127.3 (C-9), 124.0 (C-2), 120.9 (C-6), 118.4 (C-4), 118.3 (C-5), 111.3 (C-7), 109.6 (C-3), 54.8 (C-2'), 27.1(C-1').

Biological assays

Experimental animals – The experimental animals used were male ICR mice weighing 25 g and each

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group was composed of five or seven animals. Mice were housed in a room with a controlled 12 hr/12 hr light/dark cycle, with lights on between 07:00 and 19:00 hr, a temperature of 24±1°C and at a humidity of 55±5%. Experiments were performed between 13:00 and 17:00 hr. And animals were fasted for 18 hr before the experiment.

Glucose-fed hyperglycemic mice – Glucose (1 g/kg) was freshly dissolved in saline and administered orally. And 1 hr later, the experimental animals elicited significant hyperglycemia at a dosage of 1 g/kg.

Drugs and treatment – The extracts and fraction A were administrated at 400 mg/kg and Sub-fractions A-1 and A-2 were administrated at 300 mg/kg. Tryptophan (**1**) and positive control (tolbutamide) were administrated at 100 mg/kg. Control mice received the saline. All drugs were given at a volume of 0.1 ml/10 g body weight.

Determination of blood glucose – Blood samples from mice were collected from the post cava under light ether anesthesia. Plasma glucose levels were determined by glucose oxidase method. And levels were expressed in mg/100 ml of blood.

Statistical analysis – Results were expressed as means±S.E.M. The significance of the differences between the means of tests and control studies were established by student *t*-test for independent samples with one tail. *P*-values less than 0.05 were considered to be significant.

Results and Discussion

Activity guided isolation and structure elucidation were conducted to investigate anti-diabetic constituent from the node of lotus rhizome. In glucose-fed hyperglycemic mice, the node extracts showed anti-diabetic activities compared with control group (See Table 1). Then, the subfractions A-1 and A-2 which were obtained by column chromatography also showed hypoglycemic activities compared with control (See Table 2). Compound **1** was isolated from fraction A-2 which showed more potent hypoglycemic activity than A-1 and reduced the blood glucose level significantly compared with control. Compound **1** exhibited 44.3% of activity compared with tolbutamide in glucose-fed hyperglycemic mice (See Table 3). Structure of **1** was identified as tryptophan by analysis of instrumental data and comparison with authentic sample. It has been also reported that tryptamine which is metabolite of tryptophan elicited dose-dependent hypoglycemia and hyperinsulinemia in intact mice by the activation of central 5-HT receptor (Sugimoto *et al.*, 1991) and could act as a 5-HT receptor agonist and enhance serotonergic activity and/or regulate insulin release (Jamnicky *et al.*, 1993; Atienza *et al.*, 1995). This result showed that tryptophan has potential anti-diabetic activity and the node of *Nelumbo nucifera*, a rich source of tryptophan, could be developed as an antidiabetic agent.

Table 1. Effect of MeOH extract on blood glucose level in glucose-fed hyperglycemic mice (Medisense®, glucose electrode used)

Study	n	Dose (mg/kg)	Blood glucose (mg/dl, mean±S.E.D.)
Saline treated	7	–	171 ± 9
Glucose	7	1000	276 ± 13*
Glucose + MeOH Extract (fr. A)	7	1000 + 400	198 ± 9**

*p<0.01 vs. saline-treated group, **p<0.01 vs. glucose-treated group.

Table 2. Effect of sub-fractions on blood glucose level in glucose-fed hyperglycemic mice

Study	n	Dose (mg/kg)	Blood glucose (mg/dl, mean±S.E.D.)
Saline-treated	5	–	98 ± 5
Glucose	5	1000	146 ± 5*
Glucose + fr. A-1	5	1000 + 300	116 ± 5***
Glucose + fr. A-2	5	1000 + 300	95 ± 10**

*p<0.01 vs. saline-treated group, **p<0.01 vs. glucose-treated group, ***p<0.05 vs. glucose-treated group.

Table 3. Effect of Compound **1** on blood glucose level in glucose-fed hyperglycemic mice

Study	N	Dose (mg/kg)	Blood glucose (mg/dl, mean±S.E.D.)
Saline-treated	5	–	96 ± 5
Glucose	5	1000	140 ± 5*
Glucose + Tolbutamide	5	1000 + 100	94 ± 3**
Glucose + Compound **1**	5	1000 + 100	123 ± 6***

*p<0.01 vs. saline-treated group, **p<0.01 vs. glucose-treated group, ***p<0.05 vs. glucose-treated group

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