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Note

Antihypertensive Effect of an Extract of *Passiflora edulis* Rind in Spontaneously Hypertensive Rats

Toshiaki ICHIMURA,[1,†] Akiko YAMANAKA,[1] Toshio ICHIBA,[2] Tetsuya TOYOKAWA,[2] Yasuhiro KAMADA,[2] Takako TAMAMURA,[2] and Susumu MARUYAMA[1]

[1]*Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), AIST Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*
[2]*Okinawa Industrial Technology Center, 12-2 Suzaki, Gushikawa, Okinawa 904-2234, Japan*

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Orally administered methanol extract of *Passiflora edulis* rind (10 mg/kg or 50 mg/kg) or luteolin (50 mg/kg), which is one of consistent polyphenols of the extract, significantly lowered systolic blood pressure in spontaneously hypertensive rats (SHRs). Quantitative analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) showed that the extract contained 20 μg/g dry weight of luteolin and 41 μg/g dry weight of luteolin-6-*C*-glucoside. It also contained γ-aminobutyric acid (GABA, 2.4 mg/g dry weight by LC-MS/MS or 4.4 mg/g dry weight by amino acid analysis) which has been reported to be an antihypertensive material. Since the extract contained a relatively high concentration of GABA, the antihypertensive effect of the extract in SHRs might be due mostly to the GABA-induced antihypertensive effect and partially to the vasodilatory effect of polyphenols including luteolin.

Key words: *Passiflora edulis*; γ-aminobutyric acid; luteolin; spontaneously hypertensive rat

Extracts of the family *Passifloraceae* are known to have several important physiological effects on mammals. For example, the methanol extract of leaves of *Passiflora incarnata* has been confirmed to possess anxiolytic and anti-tussive properties.[1,2] Luteolin-6-*C*-chinovoside and luteolin-6-*C*-fucoside have been isolated from leaves of *Passiflora edulis*.[3] Flavonoids exhibit diverse biological effects, including inhibition of protein kinase C, inhibition of cyclic nucleotide phosphodiesterase, decrease in Ca^{2+} uptake, and vasodilatory actions.[4,5] For the purpose of using *Passiflora edulis* rind, which remains as an unused resource after squeezing juice from the fruits, as a functional food, we examined the antihypertensive effect of *Passiflora edulis* rind extract in SHRs.

The *Passiflora edulis* used in this experiment was harvested from farms in Okinawa Prefecture, Japan. One hundred and fifty grams of the rind of *Passiflora edulis* was crushed with a food mixer, then 200 ml of methanol was added and stirred for 60 min. The methanol solution was filtered through a filter paper and evaporated to dryness under reduced pressure. Finally, 3.6 g of purple powder was obtained.

Blood pressure was measured by the tail-cuff method using a blood pressure monitor for rats (Model MK-2000, Muromachi Kikai, Tokyo). The study was done in accordance with the guidelines for the care and use of laboratory animals of the National Institute of Advanced Industrial Science and Technology of Japan. Eighteen 14 week-old male SHRs (Charles River Japan, Yokohama, Japan) having systolic blood pressures of 195 mmHg or higher were divided into three groups. Single oral administration of 10 mg/kg ($n = 6$) and 50 mg/kg ($n = 6$) of *Passiflora edulis* rind extract, dissolved in distilled water at a concentration of 10 mg/ml and 50 mg/ml respectively, was performed. A control group ($n = 6$) was given the corresponding volume of distilled water. As shown in Fig. 1A, 50 mg/kg of extract administered orally significantly decreased the blood pressure of SHRs after 1 to 7 h. Blood pressure reached the maximum decrease of approximately 28 mmHg 1 h after administration and then gradually returned to control levels. Ten mg/kg of *Passiflora edulis* rind extract significantly decreased blood pressure after 1 h and showed a tendency to decrease blood pressure after 3 to 7 h. On the other hand, 50 mg/kg of *Passiflora edulis* rind extract administered orally showed little effect on the blood pressure of normotensive Wistar-Kyoto (WKY) rats (Japan SLC, Shizuoka, Japan) (Fig. 1B).

Since luteolin glycosides have been isolated from the leaves of *Passiflora edulis*,[3] we examined the antihypertensive effect of luteolin. Single oral administration of 10 mg/kg ($n = 6$) and 50 mg/kg ($n = 6$) of luteolin (Extrasynthese, Lyon, France), dissolved in 0.5% methyl cellulose solution at a concentration of 10 mg/ml and 50 mg/ml respectively, was performed. As shown in

† To whom correspondence should be addressed. Fax: +81-29-861-6047; E-mail: ichimura@ni.aist.go.jp

Abbreviations: SHR, spontaneously hypertensive rat; GABA, γ-aminobutyric acid; LC-MS/MS, liquid chromatography tandem mass spectrometry

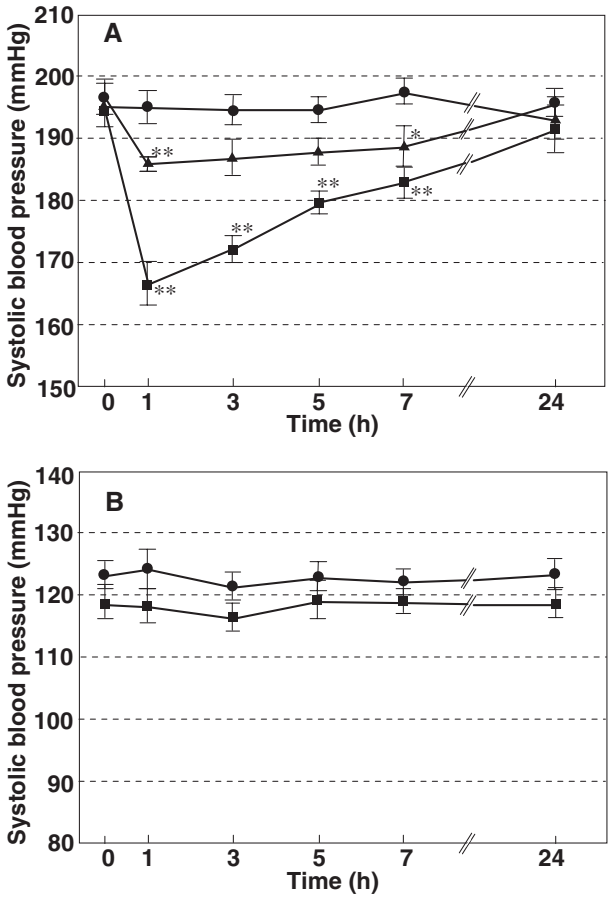

Fig. 1. Effect of a Single Oral Administration of Extract of *Passiflora edulis* Rind on Systolic Blood Pressure in SHRs (A) and WKY Rats (B).

Values are the mean ± SE (n = 6). The asterisks represent a significant difference between control and extract administered SHRs ($^{*}P < 0.05$, $^{**}P < 0.01$, Student's t-test). ●, control (distilled water); ▲, extract of *Passiflora edulis* rind (10 mg/kg); ■, extract of *Passiflora edulis* rind (50 mg/kg).

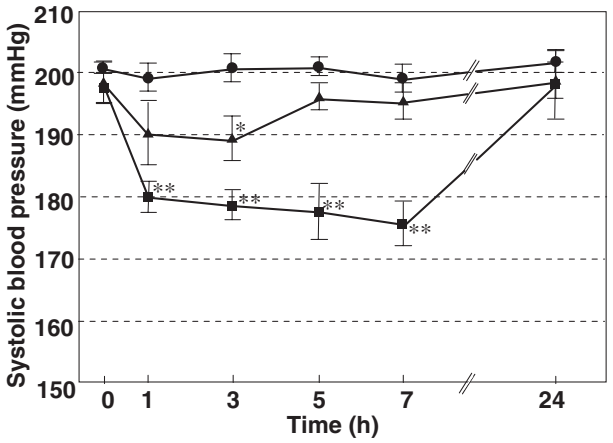

Fig. 2. Effect of a Single Oral Administration of Luteolin on Systolic Blood Pressure in SHRs.

Values are the mean ± SE (n = 6). The asterisks represent a significant difference between control and luteolin administered SHRs ($^{*}P < 0.05$, $^{**}P < 0.01$, Student's t-test). ●, control (0.5% methyl cellulose solution); ▲, luteolin (10 mg/kg in 0.5% methyl cellulose solution); ■, luteolin (50 mg/kg in 0.5% methyl cellulose solution).

Fig. 2, 50 mg/kg of luteolin administered orally significantly decreased the blood pressure of SHRs after 1 to 7 h, but the maximum decrease was not observed at 1 h. Ten mg/kg of luteolin showed a tendency to lower the blood pressure after 1 to 3 h. Flavonoids have been reported to have vasodilatory effects, and these effects have been assumed to be caused by inhibition of protein kinase C or a decrease in Ca^{2+} uptake.[4,5] The antihypertensive activity of luteolin is consistent with its vasodilatory action.

The luteolin concentration in the extract of *Passiflora edulis* rind was measured by LC-MS/MS using a Quattro micro API tandem mass spectrometer (Waters, Milford, MA) equipped with an Alliance 2695 liquid chromatograph (Waters). Five µl of the authentic luteolin or methanol solution of the extract of *Passiflora edulis* rind was loaded onto a Symmetry C_{18} column (Waters, 2.1 mm × 100 mm) and eluted with a gradient of acetonitrile (10–60%) containing 0.1% formic acid at a flow rate of 310 µl/min. Detection was performed with a tandem mass spectrometer in the multiple reaction monitoring mode using electro spray ionization (ESI), monitoring the transition of the negative precursor ion $(M-H)^-$ for luteolin at m/z 285 to the product ion $(M-H)^-$ at m/z 133. Detection was also performed with a photodiode array detector, type 2996 (Waters), monitoring absorbance at 350 nm. Quantitative analysis showed that the extract contained luteolin 20 µg/g dry weight (Fig. 3A, B). Luteolin-6-*C*-glucoside (41 µg/g dry weight) was also detected by a similar method (Fig. 3C). Since the concentration of luteolin in the extract of *Passiflora edulis* rind was low, we measured the concentration of GABA. GABA, 2.4 mg/g dry weight, was quantified in the extract with LC-MS/MS (Fig. 3D). Amino acid analysis using an amino acid analyzer (Hitachi L-8500A) also showed that the extract contained 4.4 mg/g dry weight of GABA.

It has been proposed that GABA, one of the depressive neurotransmitters in the central nervous system, play an important physiological role in the regulation of cardiovascular function.[6] GABA is present in various kinds of common foods including anaerobically treated tea (gabaron tea) and fermented foods, and antihypertensive activities of GABA and GABA-containing foods have been reported.[7–10] Hayakawa *et al.* reported that a single oral administration of GABA (0.5 mg/kg) significantly lowered systolic blood pressure in SHRs, but not in normotensive rats,[8] and that the antihypertensive activity of GABA was dose-dependent from 0.05 to 5 mg/kg in SHRs.[10] Since 50 mg of *Passiflora edulis* rind extract contains 0.22 mg of GABA, estimated from the data with the amino acid analyzer, we consider that the extract contains a high enough concentration of GABA to decrease blood pressure in SHRs.

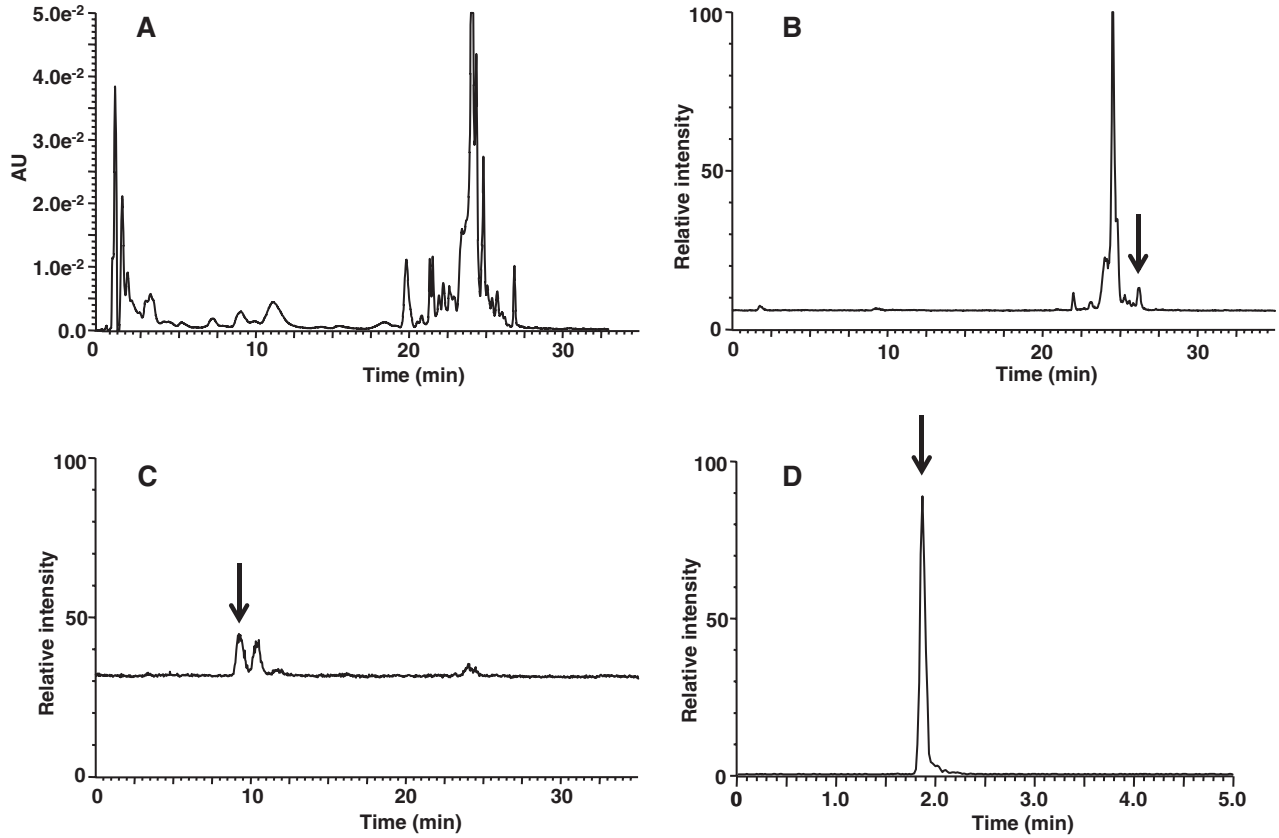

Fig. 3. Reversed Phase Chromatography of Extract of *Passiflora edulis* Rind.

(A) Chromatogram of extract of *Passiflora edulis* rind detected with a photodiode array detector monitoring absorbance at 350 nm. (B) LC-MS/MS chromatogram of extract of *Passiflora edulis* rind, monitoring the transition of the negative precursor ion $(M-H)^-$ for luteolin at m/z 285 to the product ion $(M-H)^-$ at m/z 133. The peak indicated with the arrow is luteolin. (C) LC-MS/MS chromatogram of extract of *Passiflora edulis* rind, monitoring the transition of the positive precursor ion $(M+H)^+$ for luteolin-6-*C*-glucoside at m/z 449 to the product ion $(M+H)^+$ at m/z 329. The peak indicated by the arrow is luteolin-6-*C*-glucoside. (D) LC-MS/MS chromatogram of extract of *Passiflora edulis* rind, monitoring the transition of the positive precursor ion $(M+H)^+$ for GABA at m/z 104 to the product ion $(M+H)^+$ at m/z 87. The peak indicated by the arrow is GABA.

Although the concentration of luteolin and luteolin-6-*C*-glucoside in the *Passiflora edulis* rind was not high, other luteolin glycosides might be contained in the rind because luteolin-6-*C*-chinovoside and luteolin-6-*C*-fucoside have been isolated from the leaves of *Passiflora edulis*.[3] Therefore, the antihypertensive effect of the extract of *Passiflora edulis* rind might be due mostly to the effect of GABA and partially to the effect of polyphenols, which have vasodilatory activity.

Acknowledgment

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