ABSTRACT—Leonuri herba (I-mu-ts’ao, the Chinese motherwort) is an ancient Chinese traditional herb. Although the pharmacological effects of extracts of Leonuri herba have been shown in platelets and uteri, the effect on the vascular system has not been determined. In the present study, we investigated the effects of extracts of Leonuri herba on the contraction of the isolated rat aorta. Although the H₂O-extract (0.3 – 3 mg/ml) by itself showed a limited effect, the extract enhanced phenylephrine-induced contraction of the aorta with endothelium, but not without endothelium. The H₂O-extract, like N⁴-nitro-L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase), significantly inhibited the relaxation induced by acetylcholine in the aorta with endothelium. The inhibitory effect of H₂O-extract on the relaxation decreased by co-addition with 1 mM L-arginine. The vasoconstrictive effect of H₂O-extract was not due to leonurine, which is a constituent in Leonuri herba and shows uterotonic activity. Intravenous injection of the H₂O-extract (1.5 mg/kg) to rats caused an increase in blood pressure for 5 min, like L-NAME (1.35 mg/kg). These findings suggest that there is a component(s) in Leonuri herba, which shows a vasoconstrictive activity in rat aorta in vitro and in vivo and has similar pharmacological profile to that of L-NAME.

Keywords: Vasoconstriction, Nitric oxide, Endothelium, Leonuri herba, Rat aorta

The therapeutic value of Chinese herbal medicine has been attracting much attention. We previously examined the pharmacological effects of several medicinal plants and identified chemicals having a Ca²⁺-channel blocking effect in Uncaria genus (1) and an opioid-like effect in Mitragyna speciosa (2). Leonuri herba (I-mu-ts’aao, the Chinese motherwort) is an ancient Chinese traditional herb. The extract from Leonuri herba has been known to have regulating effects on blood circulation, relief in blood stasis or menstruation, and thus, produces mainly sedative, anti-hypertensive and uterotonic activities (for review, see Refs. 3 and 4). In China, the extract of Leonuri herba has been examined for its use in the treatment of myocardial ischemia (5). The aqueous extract of Leonuri herba can reduce blood viscosity and inhibit platelet aggregation (4). In rat uteri, leonurine (4-guanidino-n-butyl syringic acid), which is present in H₂O-extract of Leonuri herba (6), stimulated isometric contraction in vitro (7). However, the effects of Leonuri herba and leonurine on aorta and blood pressure have not been determined.

In the present study, we investigated the effects of the extracts of Leonuri herba and leonurine on the contraction of rat aorta. Because the contraction of aorta is regulated by nitric oxide (NO) that is produced by NO synthase in the endothelium, we examined the effects of Leonuri herba on rat aorta with and without endothelium. Also, the effect of the H₂O-extract of Leonuri herba on vasoconstrictive activity was compared with that of N⁴-nitro-L-arginine methyl ester (L-NAME, an inhibitor of NO synthase) and that of identified constituents in Leonuri herba.

MATERIALS AND METHODS

Animals

Male Wistar rats (300 – 400 g) were obtained from Takasugi Exp. Animals Co. (Kusakabe). They were housed under conditions of controlled temperature (24 ± 2°C) and a 12-h light cycle (starting at 7 a.m.) for at least 1 week. Food and tap water were available ad libitum. All animal
experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the animal care and use committee of Chiba University.

**Chemical materials**

Phenylephrine, L-NAME, L-arginine and sodium nitroprusside were obtained from Sigma (St. Louis, MO, USA). Acetylcholine (Ovisol®) was purchased from Daiichi Pharm. Co. (Tokyo). The H₂O₂, 70% acetone/H₂O₂, 70% methanol/H₂O₂, 100% ethanol-extract from *Leonuri herba* and leonurine were prepared by Dr. Uchida’s laboratory (Meiji Milk Co., Odawara). Briefly, *Leonuri herba* was finely powdered and extracted with H₂O, 70% methanol/H₂O or 70% ethanol/H₂O at 100°C for 1 h or with 70% acetone/H₂O at room temperature for 15 min. After removal of the insoluble portion by filtration, the filtrates were concentrated under reduced pressure and then lyophilized to yield residues. The yield of the extract was 2.8 – 8.5% by weight of the original material. The estimation was based on the method of the Japanese Industrial Standard. The extracts were dissolved in water or dimethyl sulfoxide (100 mg/ml) and were further diluted with buffer in each experiment. Syringic acid was obtained from Kanto Chemical (Tokyo). CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxy-carbonylaminomethyl]-1-ethylpyridinium chloride), an antagonist of platelet activating factor (PAF) receptor, was obtained from Biomol Research Lab. (Plymouth Meeting, PA, USA).

**Measurement of contraction in isolated rat aorta rings**

Contraction in isolated rat aorta rings was determined as described previously (1) with minor modifications. Briefly, the thoracic aortas were dissected and cut into rings of 5 mm. The rings were mounted in an organ bath containing 5 ml of Krebs-Henseleit solution (118.1 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 11.1 mM glucose), gassed with 5% CO₂ in oxygen (pH 7.4) and kept at 37°C. The upper end of the rings was connected by a thread to the lever of a force-displacement transducer (model T7-8-240; Toyo Baldwin, Tokyo) with an amplifier (model AS2102; NEC San-ei, Tokyo), and the contraction was isometrically recorded on a recorder (model 056; Hitachi, Tokyo) via an amplifier. After an initial load of 1 g was applied to the rings, the rings were allowed to equilibrate for 60 min. The contraction of rat aorta by phenylephrine was similar as that by previous reports (8 – 10), and the contraction was presented as percentage of the 10 μM phenylephrine-induced contraction. The endothelium of the aorta was denuded by gently rubbing with absorbent cotton. The presence or absence of functional endothelium was assessed by the ability of 10 μM acetylcholine to induce the relaxation of the rings that were pre-contracted with 3 μM phenylephrine. The preparations showing more than 70% relaxation were used as aorta with endothelium, and the preparations showing less than 5% relaxation were used as aorta without endothelium.

**Pressure response to the H₂O-extract of Leonuri herba in vivo**

In the in vivo studies, the rats were anesthetized with urethane (1.35 g · kg⁻¹· 2 ml⁻¹, i.p.). Arterial blood pressure was measured at the right carotid artery using a pressure transducer (DTX/Plus™; Spectramed Inc., Oxnard, CA, USA). A cannula was also inserted into left femoral vein for the administration of the drugs. Following surgery, a period of at least 30 min was allowed for stabilization of preparations. The H₂O-extract of *Leonuri herba* (1.5 – 3 mg/kg) and L-NAME (1.35 mg/kg) were intravenously applied with a bolus injection over a period of 3 s and then flushed with saline solution into 5 rats.

**Statistical analyses**

Values are expressed as means ± S.E.M. of 3 – 5 independent experiments from different animals. Values were analyzed by the paired t-test. P values <0.05 were considered to be significant.
Vasoconstrictive Effect in *Leonuri herba*

The effect of the H$_2$O-extract by itself on basal tension of the aorta was variable, not concentration-dependent and not observed in some experiments (Table 1). Thus, we investigated the effect of the H$_2$O-extract on phenylephrine-induced contraction of rat aorta in the following experiments.

Addition of phenylephrine induced the contraction of rat aorta without endothelium (Fig. 2). The ED$_{50}$ value of phenylephrine in rat aorta without endothelium was 93 ± 23 nM (n = 5), which was significantly smaller compared with that with endothelium (270 ± 62 nM, n = 5). The maximal contraction induced by 10 μM phenylephrine in rat aorta without endothelium was 110 ± 10% (n = 5), which was similar to that with endothelium (100%). Interestingly, the stimulatory effect of the H$_2$O-extract was not observed in rat aorta without endothelium (Fig. 2B).

Figure 3 shows other types of extracts from *Leonuri herba* (3 mg/ml) on phenylephrine-induced contraction of rat aorta with endothelium. The acetone-extract, but neither methanol- nor ethanol-extract, enhanced the phenylephrine-induced contraction. In rat aorta without endothelium, the acetone-extract did not enhance the phenylephrine-induced contraction (data not shown).

Table 1. Effect of H$_2$O-extract of *Leonuri herba* on the contraction in rat aorta with endothelium

<table>
<thead>
<tr>
<th>Addition</th>
<th>Contraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n=5)</td>
<td>0</td>
</tr>
<tr>
<td>H$_2$O-extract of Leonuri herba</td>
<td></td>
</tr>
<tr>
<td>0.3 mg/ml (n=4)</td>
<td>7.3 ± 1.8</td>
</tr>
<tr>
<td>1 mg/ml (n=5)</td>
<td>7.3 ± 1.4</td>
</tr>
<tr>
<td>3 mg/ml (n=3)</td>
<td>8.3 ± 1.6</td>
</tr>
</tbody>
</table>

Rat aorta with endothelium was incubated with vehicle or the indicated concentrations of the H$_2$O-extract of *Leonuri herba*. After 15 min, the phenylephrine-induced contraction was measured. Values are expressed as percentages of the contraction induced by 3 μM phenylephrine with vehicle. Values are the means ± S.E.M. from 3 – 5 independent experiments. *P<0.05 versus the effect of phenylephrine alone.

Fig. 1. Effect of H$_2$O-extract of *Leonuri herba* on the contraction in rat aorta with endothelium. A) Rat aorta with endothelium was incubated with 3 μM phenylephrine. B) Rat aorta with endothelium was incubated with the H$_2$O-extract of *Leonuri herba* (0.3 mg/ml), and after 6 min, 3 μM phenylephrine was added. Phenylephrine and the H$_2$O-extract was added at the indicated time (square or circle). Values are typical of 3 independent experiments.

Fig. 2. Effect of H$_2$O-extract of *Leonuri herba* on the contraction in rat aorta with and without endothelium. Rat aorta with (A) and without (B) endothelium was incubated for 15 min with vehicle (open circle) or the H$_2$O-extract of *Leonuri herba* (3 mg/ml, closed circle). Then the contraction was measured in the presence of the indicated concentrations of phenylephrine. Values are expressed as percentages of the maximal contraction by 10 μM phenylephrine with vehicle. Values are the means ± S.E.M. from 3 – 5 independent experiments. *P<0.05 versus the effect of phenylephrine alone.
Effect of \(L\)-NAME and \(L\)-arginine on the contraction of rat aorta with endothelium induced by the \(H_2O\)-extract of Leonuri herba

The treatment with 0.1 mM \(L\)-NAME enhanced phenylephrine (3 \(\mu\)M)-induced contraction of rat aorta with endothelium (Fig. 4A). In the co-treatment with 0.1 mM \(L\)-NAME and the \(H_2O\)-extract (3 mg/ml) at the same time, the phenylephrine (3 \(\mu\)M)-induced contraction was 160.7 ± 7.1\% (n = 3), which was almost the same as that in the aorta treated with the \(H_2O\)-extract. Although \(L\)-NAME significantly enhanced phenylephrine-induced contraction in rat aorta without endothelium (Fig. 4B), the effect was limited and the maximal contraction induced by 10 \(\mu\)M phenylephrine was potentiated about 20\% by \(L\)-NAME. Treatment with 1 mM \(L\)-arginine prevented the stimulatory effect of the \(H_2O\)-extract (1 mg/ml, Fig. 5) and \(L\)-NAME (0.1 mM, data not shown) on phenylephrine-induced contraction.

Effect of \(H_2O\)-extract of Leonuri herba on the relaxation of rat aorta with endothelium induced by acetylcholine

The contraction by 3 \(\mu\)M phenylephrine in the presence of 10 \(\mu\)M acetylcholine was 10 – 20\% of the contraction without acetylcholine (Figs. 5 and 6). We confirmed that treatment with \(L\)-NAME (0.1 mM) inhibited 10 \(\mu\)M acetylcholine-induced relaxation. Next, we investigated the effect of \(H_2O\)-extract of Leonuri herba on acetylcholine-induced relaxation of rat aorta. Like \(L\)-NAME, treatment with the \(H_2O\)-extract for 15 min markedly inhibited the acetylcholine-induced relaxation of rat aorta with endothelium (Fig. 5), and the effect of \(H_2O\)-extract was concentration-dependent (Fig. 6). Co-treatment with 1 mM \(L\)-arginine, which inhibited the stimulatory effect of \(H_2O\)-extract on
the contraction, recovered the acetylcholine-induced relaxation. In addition, treatment with the H₂O-extract of *Leonuri herba* (1 mg/ml) did not inhibit the sodium nitroprusside (0.1 μM)-induced relaxation (data not shown).

**Effects of the identified chemicals in Leonuri herba on the contraction of rat aorta with endothelium.**

Addition of leonurine (100 μg/ml) and syringic acid (100 μg/ml) induced the relaxation of rat aorta with endothelium slightly (data not shown), and inhibited the phenylephrine-induced contraction in a concentration-dependent manner (Fig. 7). Treatment with CV-6209, an antagonist of PAF receptor (11), did not show vasoconstrictive activity like the H₂O-extract of *Leonuri herba* on the rat aorta; the contraction induced by 10 μM phenylephrine in the presence of 1 μM CV-6209 was 105 ± 5%, which was as same as that in the control (100%).

**Effect of the H₂O-extract of Leonuri herba on blood pressure in vivo.**

Intravenous bolus injection of L-NAME (1.35 mg/kg), but not vehicle, caused a slow-developing increase in blood pressure (Fig. 8). Plateau pressure response to L-NAME was attained 2 – 3 min after injection, and then the response
DISCUSSION

Endothelium-dependent enhancement of contractions in rat aorta by the H₂O-extract of Leonuri herba

In the present study, we found that there was an activity to stimulate the contraction of rat aorta in the H₂O-extract of Leonuri herba. The stimulatory effect of the extract appeared to be derived from an inhibition of NO synthesis in the endothelium, not from a direct stimulation of contraction in rat aortic smooth muscle. The reasons were: 1) the stimulatory effect of the H₂O-extract was dependent on the endothelium (Fig. 2), 2) treatment with the H₂O-extract inhibited the acetylcholine-induced relaxation (Fig. 5), 3) the enhancement of phenylephrine-induced contraction and the inhibition of acetylcholine-induced relaxation of the rat aorta by the H₂O-extract were inhibited by 1 mM L-arginine addition (Figs. 5 and 6). These findings suggested that the H₂O-extract of Leonuri herba probably acts as an inhibitor of NO synthesis similar to L-NAME in the endothelium and, thus, stimulates the contraction in the rat aorta.

Inhibitors of NO synthase such as L-NAME have been shown to interfere with relaxation of various vascular preparations (12, 13). In several species, inhibitors of NO synthase stimulated the contraction of cerebral artery by itself (14, 15). In our conditions, however, treatment with L-NAME enhanced the phenylephrine-stimulated contraction, but not the basal (non-stimulated) contraction, as shown by other reports (16, 17). The addition of NO compounds such as sodium nitroprusside induced the relaxation markedly in phenylephrine-stimulated aorta, but not in the non-stimulated aorta (data not shown). The sensitivity of rat aorta to NO in non-stimulated conditions may be lower compared with that in the phenylephrine-stimulated conditions and/or that of other species.

In the present conditions, L-NAME enhanced phenylephrine-stimulated contraction of rat aorta without endothelium, although the effect was limited and not greater than 20% (Fig. 4B). The H₂O-extract of Leonuri herba also showed a similar tendency (Fig. 2B). Moritoki et al. (10) reported that L-arginine induced relaxation of rat aorta via formation of NO by an endothelium-independent mechanism. Recently, it was reported that a constitutive type of NO synthase was present in rat vascular smooth muscle cells (18). It was probable that L-NAME inhibited NO synthesis and, thus, enhanced the contraction in rat aorta without endothelium. Otherwise, the effect of L-NAME on the contraction may be independent of the ability to inhibit NO synthase. L-NAME and other alkyl ester analogs of L-arginine are muscarinic acetylcholine receptor antagonists (19). El Mabrouk et al. (20) reported that L-NAME, but not other NO synthase inhibitors such as N⁵-monomethyl-L-arginine, reduced serum-stimulated DNA synthesis of rat vascular smooth muscle cells. Since the effect of H₂O-extract of Leonuri herba on the phenylephrine-induced contraction in rat aorta without endothelium was not significant (Fig. 2B), the chemical(s) in the H₂O-extract appeared to show vasoconstrictive activity in an endothelium-dependent manner selectively.

Inhibitors of NO synthase such as L-NAME caused an increase in blood pressure, which was antagonized by the NO donors and by L-arginine (8, 21–25). In the present conditions, we confirmed the effect of L-NAME and found that i.v. injection of the H₂O-extract of Leonuri herba showed an increase in blood pressure in rats in vivo (Fig. 8). The vasoconstrictive effect of the H₂O-extract of Leonuri herba may contribute to the therapeutic value of this herb such as relief in blood stasis or menstruation (4).

Probable chemicals in the H₂O-extract of Leonuri herba

There are many constituents in Leonuri herba; alkaloids,
induced relaxation in rat aorta, probably via the inhibition of stimulated contraction and inhibited the acetylcholine- and ethanol-extract of vasoconstrictive activity was not observed in the methanol-extract of Leonuri herba. It was reported that the H$_2$O-extract of Leonuri herba did not show any vasoconstrictive effect on rat aorta. CV-6209, a highly potent antagonist of platelet activating factor (PAF) receptor. This compound from Leonuri herba (CV-6209) did not show any vasoconstrictive effect on rat aorta. Syringic acid is known to have several pharmacological effects. Tawa et al. (27) found that syringic acid, a derivative of leonurine, inhibited the contraction of the rat aorta. Syringic acid is known to have several pharmacological effects. Tawa et al. (27) reported that the metal complex of syringic acid, like the complex of etoposide, breaks in double-strand DNA. Syringic acid generates reactive oxygen species in the presence of Cu$^{2+}$ (28). The mechanisms for inhibition of contraction in rat aorta by leonurine and syringic acid should be determined in future studies.

Lee et al. (29) isolated a novel diterpene, prehispanolone, from Leonuri herba and showed that this diterpene acted as a specific antagonist for PAF receptor. This compound probably inhibited platelet aggregation and reduced blood viscosity (4). PAF is known to stimulate NO production in the endothelium and, thus, induce relaxation in the rat aorta (30). Thus, it is probable that prehispanolone in Leonuri herba stimulates the contraction by inhibition of relaxation induced by endogenous PAF. However, the vasoconstrictive activity was not observed in the methanol- and ethanol-extract of Leonuri herba containing oils and diterpenoids (Fig. 3), and an antagonist of the PAF receptor (CV-6209) did not show any vasoconstrictive effect on rat aorta. It was reported that the H$_2$O-extract of Leonuri herba enhanced the enzymatic activity of the partially purified prostaglandin E 9-ketoreductase from pig kidney (31). These findings and those of the present study suggest that there are new chemicals that have biological activities, and/or identified compounds that have pharmacological effects that have not been demonstrated, in Leonuri herba. The isolation and identification of the effective components showing vasoconstrictive activity in the H$_2$O-extract of Leonuri herba are in progress in this laboratory.

In conclusion, the present study demonstrated that the H$_2$O-extract of Leonuri herba enhanced the phenylephrine-stimulated contraction and inhibited the acetylcholine-induced relaxation in rat aorta, probably via the inhibition of NO synthesis in endothelium. The present findings may be the first report showing the pharmacological effect of Leonuri herba on the vascular system.

REFERENCES


