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Effect of *Ipomoea hederacea* Jacq. methanolic extract on blood pressure and relaxation of rat thoracic aorta: Evidence indicated the release of NO and H_2S

Chaweewan Jansakul[1,2], Pilaipan Chairuk[1,2], M. Zia-Ul-Haq[3], Imran Imran[4]

[1]Faculty of Traditional Thai Medicine, Prince of Songkla University, Songkhla 90112, Thailand, [2]Natural Product Research Centre of excellence, Prince of Songkla University, Songkhla 90112, Thailand, [3]Office of Research, Innovation and Commercialization, Lahore College for Women University, Lahore 54600, Pakistan, [4]Department of Pharmacy, Bahauddin Zakariya University, Multan 60800, Pakistan

Corresponding Author:
Chaweewan Jansakul, Faculty of Traditional Thai Medicine,Prince of Songkla University, Songkhla, Thailand.
E-mail: chaweewan.j@psu.ac.th

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ABSTRACT

Introduction: Seeds of *Ipomoea hederacea* Jacq**.** (IH) are used in traditional medicine to manage ailments pertaining to the vasculature, circulatory system, and related disorders. However, scientific investigations to confirm these therapeutic claims are not yet available. **Purpose of the Study:** The present study was conducted to investigate the plant seed methanolic extract (IH extract) on blood pressure (BP) in an anesthetized rat and to elucidate the possible mechanisms on isolated thoracic aorta. The IH extract led to a decrease in BP and a relaxation of isolated thoracic aortic ring precontracted with phenylephrine. **Results and Conclusion:** The relaxant effect was significantly inhibited by N^G-nitro-L-arginine, ODQ, or removal of the endothelium but not by glibenclamide or tetraethylammonium. The IH extract potentiated the relaxant effect of glyceryl trinitrate in a similar way to that of sildenafil with a further increase in maximal relaxation, the effect of which was significantly inhibited by DL-propargylglycine. An IH extract or Y-27632, a Rho-kinase inhibitor, each suppressed the concentration-response (C-R) curve of the phenylephrine on thoracic aorta, and when both were added, this resulted in a total suppression. In normal Krebs solution, nifedipine caused no further inhibition of the phenylephrine C-R curve on thoracic aortic rings in the presence of the IH extract. These results indicate that the IH extract causes a relaxation of the thoracic aortic ring by stimulating the release of nitric oxide and H_2S and might also act as an inhibitor of phosphodiesterase-5 and a voltage-gated Ca^{2+} channel, which then results in a lowering of the BP in anesthetized rats.

INTRODUCTION

Ipomoea hederacea (IH) Jacq**.** (*Convolvulaceae*) is an important medicinal plant that is used as a purgative, diuretic, abortifacient, and tonic to promote general health and well-being.[1,2] The plant seeds are used as antispasmodic, antidepressant, aphrodisiac, diuretic, and blood purifier and to relieve headache.[3] However, scientific investigations to confirm these therapeutic claims are not readily available. Previous studies showed that IH extract possess antioxidant and anticancer potential,[4-6] hepatoprotective,[7] and analgesic activity.[8] So far, only one publication relates to vasculature. It was found that oral administration of non-lethal doses of IH seed extract to male rats increased *libido*, as indicated by increased mounting frequency on female rats in estrous.[9] This might be due partly to dilatation of the penile blood vessels, the corpora cavernosa, resulting in penile erection.[10] The traditional use of the seeds of the IH is as a reducer of high pulse pressure, aphrodisiac, blood purifier and to relieve headache. Therefore, in the present study, we aimed to provide scientific evidence for this possibility by exploring

the pharmacological potential of components of a methanolic extract from the seeds on the basal BP of anesthetized rats, and the mechanism of the action was further investigated on isolated thoracic aortic rings.

MATERIALS AND METHODS

Drugs and Chemical Reagents

The following drugs were used. Acetylcholine chloride, nifedipine, N^G-nitro-L-arginine (L-NA), phenylephrine hydrochloride, tetraethylammonium (TEA), and DL-propargylglycine (PAG, a cystathione-γ-lyase inhibitor, or H_2S inhibitor) were purchased from Sigma, St. Louis, MO, USA. 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ) and trans-4-[(1R)-1-aminoethyl]-N-4-pyridinylcyclohexanecarboxamide dihydrochloride (Y-27632) were obtained from Trocis, UK. Sildenafil citrate (Viagra) was from Pfizer. Glyceryl trinitrate (GTN) was from Mycomed, Denmark. L-NA, GTN, Y-27632, and sildenafil were dissolved in distilled water, nifedipine was dissolved in dimethyl sulfoxide (20%), and the other chemicals were dissolved in a solution (1 L) containing NaCl (9 g), NaH_2PO_4 (0.19 g) and ascorbic acid (0.03 g).

Preparation of Plant Extract

Seeds of IH Jacq. were procured from local market, Lahore, Pakistan, in July 2007 and identified by Dr. Saima Shahzadi, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, and a voucher specimen was submitted in the herbarium of the same institute. Extracts of seeds were prepared as described by our research group previously.[9,11] The chemical profile of extract was established by high-performance liquid chromatography (HPLC). The extract was analyzed using HP 1100 system (Agilent) comprising of a photodiode array detector and C18 column (Waters) with gradient of methanol:water containing $C_2HF_3O_2$ 0.05% (5:95→100:0) with 1 ml/min flow rate and ultraviolet (UV)-DAD spectra were recorded (200–600 nm).

Pharmacological Studies

Determination of BP in anesthetized rat

Adult female Wistar rats (220–250 g) were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. The animal methods employed and the experimental protocols followed in the study were approved by Ethical Committee of Prince of Songkla University (Ref. 37/2014). The investigation conformed to the Guide for the care and use of laboratory animals. Pentobarbital (60 mg/kg, i.p.) was used to anesthetize the rats. A cannula connected with a polyethylene tube was used to facilitate the spontaneous respiration. Pressure transducer (P23ID, Gold Statham Instrument, Puerto Rico) was used to record the systemic BP from the right common carotid artery, while heart rate was recorded by a tachograph connected with a polygraph (Model 7D, Grass Instrument, MA). The dose-response relationships to IH extract were measured by intravenous injection (volume 0.1 ml for each dose) into the left jugular vein and flushed with saline (0.1 ml). Changes in systolic or diastolic BP were measured between the steady basal state before and after injection of IH extract. Statistical comparisons were performed using two-tailed unpaired Student's *t*-test.

Preparation of thoracic aortic rings

Adult female Wistar rats (220–250 g) were killed by cervical dislocation, and their thoracic aortic rings were prepared in our laboratory as described previously by Jansakul *et al*.[12] The rings were mounted in organ bath containing Krebs solution (mM: NaCl 118.3, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4$ $7H_2O$ 0.45, KH_2PO_4 1.18, glucose 11.66, Na_2EDTA 0.024, $NaHCO_3$ 25.0, and ascorbic acid 0.09) maintained at 37°C and continuously bubbled with O_2 and CO_2 mixture (95%:5%). The isometric tension generated by aortic rings was recorded using force-displacement transducers connected to a polygraph. Before the addition of sample, tissues were equilibrated for 1 h under a resting tension (1g) and the bath solution was replaced with oxygenated Krebs solution (37°C) every 15 min. Before testing, a functional endothelium of intact thoracic aortic rings was verified in every preparation by pre-contracting the rings with phenylephrine (3 μM) until a plateau was observed (5–8 min) followed by addition of acetylcholine (30 μM). The preparations were then washed with Krebs solution many times and allowed to relax for 45 min before the start of experiment.

Each thoracic aortic ring was obtained from a different rat. Drug-induced relaxation was measured as the decline from the maximal steady tension produced by phenylephrine (0.3 μM for the endothelium-denuded- and 3 μM for the endothelium-intact thoracic aortic rings). The steady decline achieved at each drug concentration was expressed as a percentage of the initial maximum produced by phenylephrine (0.3 or 3 μM). The contractile cumulative C-R curves to phenylephrine and the steady increase achieved at each phenylephrine concentration were expressed as a percentage of the Emax obtained from their control group. Statistical comparisons were performed using the statistical differences between 2 measurements as determined by the two-tailed unpaired Student's *t*-test and among the group were determined by one-way ANOVA and *post hoc* analysis was performed with a Tukey's range test.

Effect of nitric oxide, guanylate cyclase, and K^+ channels on relaxation of thoracic aorta by IH extract

To assess the role of IH extract as a stimulator of nitric oxide, guanylate cyclase, and/or K^+channels, the thoracic aortic rings were precontracted with 3 μM phenylephrine for 10 min (establishment of plateau), and the cumulative relaxation concentration-response (C-R) relationships of the rings to IH extract (0.01–0.03 mg/ml) were measured. Following several washings followed by re-equilibration of aortic rings for 1 h, the rings were incubated with N^G-nitro-L-arginine (L-NA, 0.3 mM, a nitric oxide synthase inhibitor) for 1 h or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ, 10 μM, a guanylate cyclase inhibitor), glibenclamide (10 μM, an ATP-sensitive K^+-channel inhibitor), or TEA, 1 mM, a Ca^{2+}-sensitive K^+-channel inhibitor for 20 min. The impact of IH extract on C-R relationship was determined on the phenylephrine-precontracted thoracic rings in the presence of each drug.

Phosphodiesterase (PDE) inhibition and H_2S stimulation

To assess the role of IH extract as a PDE inhibitor, a cumulative relaxation C-R curve to glyceryl trinitrate (GTN) on the denuded-thoracic aortic rings precontracted with phenylephrine (0.3 μM) was performed. Following several washings, the aortic ring was incubated with IH extract (0.1 mg/ml) or sildenafil citrate (0.1 μM) for 20 min, and the relaxation C-R relationship to GTN on the phenylephrine-precontracted aortic ring was obtained in the presence of the incubating drugs. After washing and re-equilibration, the aortic ring was incubated with IH extract or together with DL-propargylglycine (PAG, 10 mM) for 20 min, then the third C-R curve to GTN on the phenylephrine-precontracted aortic ring was observed.

Rho-kinase inhibitor

To assess the potential role of IH extract as Rho-kinase inhibitor, after equilibration, a cumulative C-R relationship to phenylephrine on the denuded thoracic aortic ring was obtained. The ring was incubated with the IH extract (0.1 mg/ml) and/or Y-27632 (30 μM, a Rho-kinase inhibitor) for 20 min, and the C-R relationship to phenylephrine was obtained in the presence of the IH extract and/or Y-27632.

Inhibition of voltage-dependent Ca^{2+}channels

To determine whether the IH extract plays any role as a voltage-dependent Ca^{2+}channel blocker, the C-R relationship to phenylephrine of the endothelium-denuded thoracic aortic rings was studied in normal Krebs solution. The thoracic aortic rings were incubated with IH extract (0.1 mg/ml) or nifedipine (3 μM) for 20 min after which the cumulative phenylephrine C-R relationship was studied in the presence of each drug. The same procure was repeated in the presence of nifedipine with the IH extract.

Statistical Analysis

Results are expressed as a mean ± SEM where n indicates a number of rats or thoracic aortic rings used. A $p \leq 0.05$ was considered to indicate a statistically significant difference between values.

RESULTS

Chemical Analysis of the IH Extract

The HPLC chromatogram of IH extract was shown in Figure 1. There are 9 HPLC major peaks could be detected at the wavelength of 210 nm at the retention time (min) of 1.46, 2.10, 6.30, 7.36, 8.63, 11.27, 12.92, 14.38, and 16.34 for the HPLC peak 1–9, respectively. On UV spectral analysis, HPLC peak 1 found no peak of UV spectrum. HPLC-peak 2 found 2 peaks of UV spectra. HPLC peak 3, 4, and 5 showed 3 peaks of UV spectra. HPLC peak 6–9 showed 4 peaks of UV spectra.

Effects of IH Extract on BP in Anesthetized Rat

The original trace recording from a polygraph of BP before and after injection of different dosages of the IH extract (2.5–40 mg/kg) is shown in Figure 2a. Basal mean systolic and diastolic BP of anesthetized rats were 167 ± 5.0 and 122 ± 5.0 mmHg, respectively. The IH extract caused a decrease in both the systolic and diastolic BP of the anesthetized rat in a dose-dependent manner [Figure 2b and c]. In addition, at the highest dosage of the IH extract (40 mg/kg), there was a more than 2 h long decrease in BP.

Effect of Nitric Oxide, Guanylate Cyclase, and K^+ Channels on Relaxation of Thoracic Aorta by IH Extract

The IH extract caused a relaxation of the endothelium-intact thoracic aortic rings (Endo) that had been precontracted with phenylephrine in a concentration-dependent manner. Removal of the vascular endothelium (endothelium-denuded thoracic aortic rings, No endo), L-NA (a nitric oxide synthase inhibitor), or ODQ (a guanylate cyclase inhibitor) significantly inhibited the C-R curve of the IH extract [Figure 3a-c], and no further inhibition was found by ODQ (a guanylate cyclase inhibitor) in the presence of L-NA [Figure 3d]. Glibenclamide or TEA

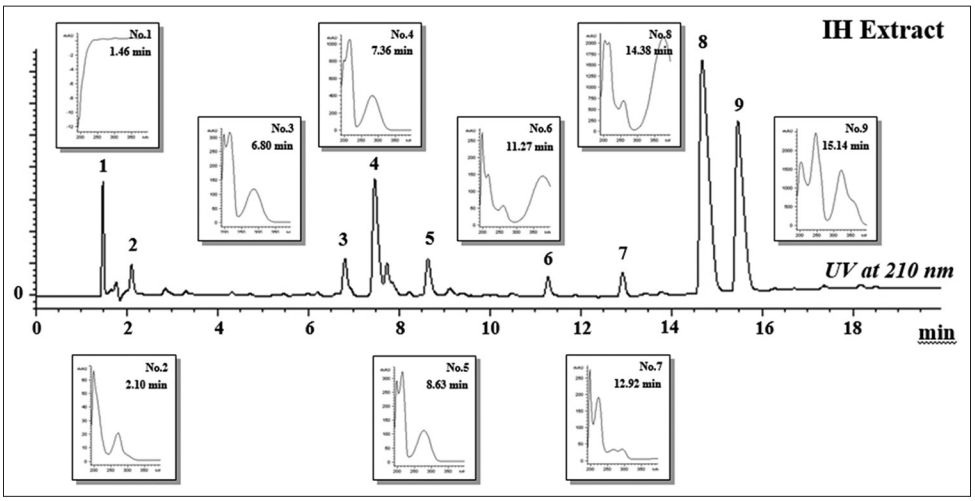

Figure 1: High pressure liquid chromatography-chromatogram of IH extract, the column eluant from the IH extract (9 peaks) was scanned at the wavelength of 210 nm. Retention time in minute of each peak is shown as a miniature of each peak of its UV spectra

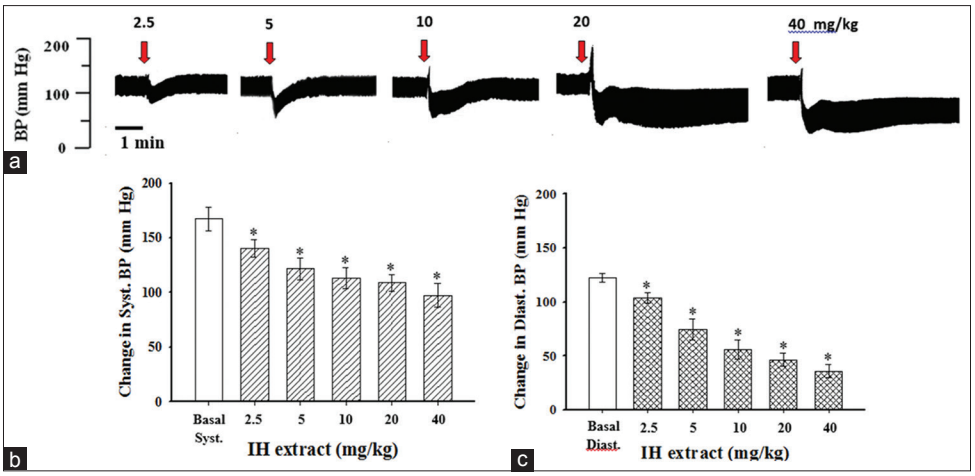

Figure 2: An original trace recording systolic and diastolic blood pressure (BP) of an anesthetized rat from a polygraph is shown in (a), the arrow indicates time for the IH extract injection. Effects of IH extract on BP, specifically the decrease in systolic (b) and diastolic BP (c) in anesthetized rats. Each point represents a mean ± SEM of 6 different rats (n=6). *Significantly lower than their corresponding basal pressure, $P < 0.05$

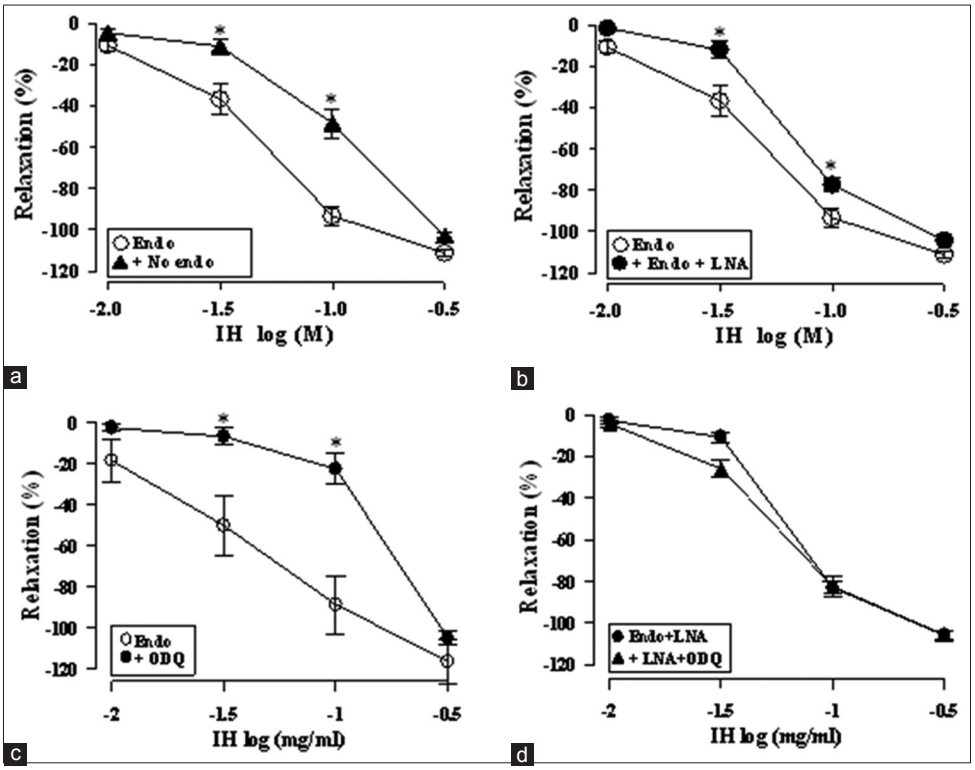

Figure 3: (a) Relaxation activity of IH extract on endothelium-intact (Endo) and endothelium-denuded (No endo) thoracic aortic rings precontracted with phenylephrine. (b) Effects of N^G-nitro-L-arginine (L-NA, 0.3 mM), (c) ODQ (10 μM), (d) L-NA (0.3 mM) + ODQ (10 μM). Phenylephrine concentration 3 μM for the endothelium-intact and 0.3 μM for the endothelium denuded vessels-thoracic and the ones with LNA and/or ODQ. Each point represents a mean ± SEM of 6 thoracic aortic ring each from a different animal (n = 6). *Significantly higher than the control group, $P < 0.05$

did not modulate the relaxant C-R curve of the IH extract [Figure 4].

PDE Inhibitor and H_2S Stimulator

GTN caused a relaxation of the endothelium-denuded thoracic aortic ring precontracted with phenylephrine (0.3 μM) in a concentration-dependent manner and reached a maximal relaxation of about 95%. The vasorelaxation by the GTN was potentiated by incubating the thoracic aortic ring with sildenafil (0.1 μM; Figure 5a) or the IH extract (0.1 mg/ml; Figure 5b). In addition, in the presence of the IH extract, the maximum vasodilatation produced by GTN was higher than the control group and reached its maximum relaxation of about 130%, and the potentiating effect was significantly inhibited by PAG [Figure 5b].

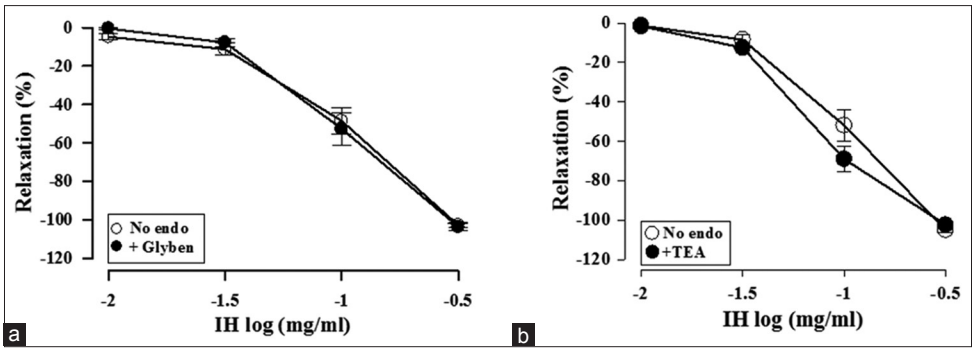

Figure 4: Effects of (a) glybenclamide (Glyben, 10 μM, an ATP-sensitive K^+channel inhibitor) and (b) TEA (1 mM, a Ca^{2+}-sensitive K^+channel inhibitor) on the relaxation of the endothelium-denuded thoracic aortic ring (No endo) precontracted with phenylephrine (0.3 μM) to the IH extract. Each point represents a mean ± SEM of 6 thoracic aortic ring each from a different animal (n = 6)

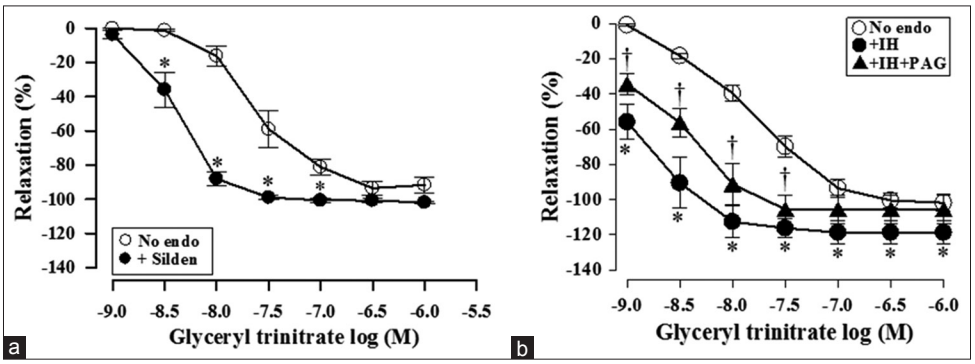

Figure 5: Effects of (a) sildenafil (Silden, 0.1 μM), (b) *Ipomoea hederacea* extract (IH, 0.1 mg/ml) and/or DL-propargylglycine (PAG, 10 mM) on the relaxation of the endothelium-denuded thoracic aortic ring precontracted with phenylephrine (0.3 μM) to GTN. Each point represents a mean ± SEM of 6 thoracic aortic rings each obtained from a different animal (n = 6). *Significantly lower than the control group, †significantly higher than the one with the IH extract and/or lower than the control group, $P < 0.05$

Rho-kinase Inhibitor

Both the IH extract and Y-27632 (a Rho-kinase inhibitor) significantly inhibited the C-R curve of the phenylephrine on the endothelium-denuded thoracic aortic ring. However, when the IH extract was added together with Y-27632, a total suppression of the C-R curve of the phenylephrine on the thoracic aortic ring was obtained [Figure 6].

Blocking of Voltage-dependent Calcium Channels

Nifedipine caused a parallel shift of the phenylephrine C-R curve to the right, with no change in the maximal responsiveness. In contrast, the IH extract significantly inhibited the phenylephrine C-R curve with a decrease in the maximal responsiveness to about 60%. However, when nifedipine was added together with IH extract, no further inhibition by the nifedipine was found when compared to those with IH extract alone [Figure 7].

DISCUSSION

The results indicate that IH extract had a hypotensive effect in the anesthetized rat. The effect might result from its vasodilatory activity, as supported by the finding that the IH extract had a relaxant activity on the isolated thoracic aortic rings. The underlying mechanisms for the relaxation activity were investigated to determine if the IH extract acted by stimulating nitric oxide, guanylate cyclase or H_2S, by opening K^+ channels, by inhibiting PDE-5 or Rho-kinase, or by blocking voltage-dependent calcium channels

Our finding that L-NA, a nitric oxide synthase inhibitor, or ODQ, a guanylate cyclase inhibitor, did inhibit the relaxant activity of the IH extract indicated that the IH extract stimulated the release of nitric oxide that involved the soluble guanylate cyclase pathway. This was confirmed by the finding that ODQ did not further inhibit the C-R curve of the IH extract on the endothelium-intact thoracic aortic ring in the presence of L-NA. The finding that the vasorelaxant activity of the IH extract persisted after the removal of vascular endothelium indicated that the relaxant activity of the IH extract was also endothelium independent. It is unlikely that the IH extract activated the K^+ channel on either the Ca^{2+}sensitive (K_{Ca}) or the ATP-sensitive (K_{ATP}), as TEA or glibenclamide did not modify the IH extract C-R curve on the endothelium-denuded thoracic aortic rings.[13,14]

To obtain a better understanding of the PDE-5 inhibitory activity of the IH extract, we studied the effects of the IH extract in comparison with that of sildenafil, a known PDE5 inhibitor,[15] on the relaxant C-R curves produced by GTN, a nitric oxide donor, and on the phenylephrine precontracted

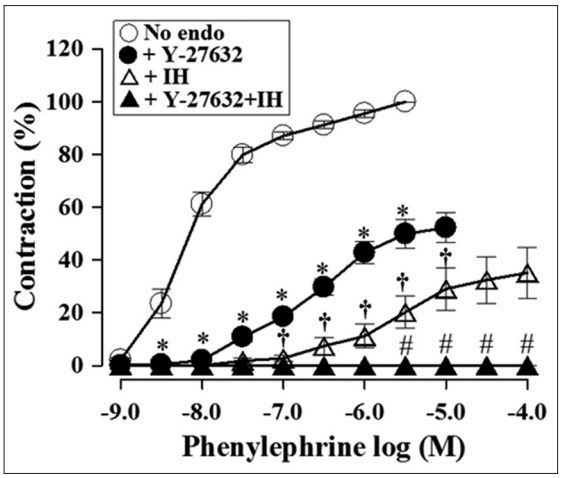

Figure 6: Effects of the IH extract (0.1 mg/ml) and/or Y-27632 (30 μM) on the phenylephrine-induced contractile responses of endothelium-denuded thoracic aortic ring (No endo). Each point represents a mean ± SEM of 6 thoracic aortic rings each from a different animal (n = 6). *Significantly lower than the control group (o), †significantly lower than the control and the one with Y-27632 (●) and #significantly lower than the other groups, $P < 0.05$

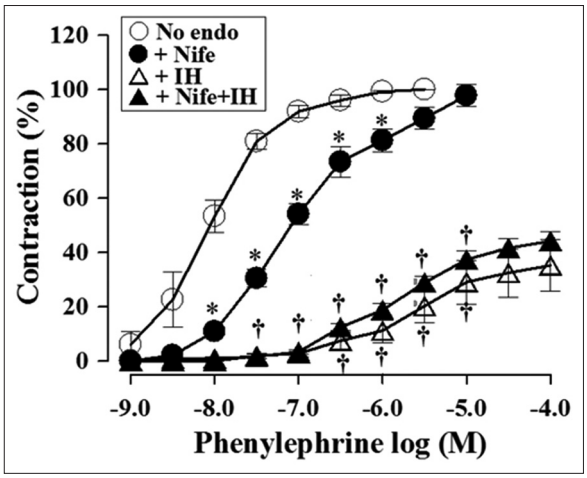

Figure 7: Effects of nifedipine and/or IH extract (0.1 mg/ml) on the contractile responses of the endothelium-denuded thoracic aortic ring (No endo) to phenylephrine. Each point represents a mean ± SEM of 6 aortic rings each from a different animal (n = 6). *Significantly lower than the control group (o) and †significantly lower than control and the one with nifedipine (●), $P < 0.05$

endothelium-denuded thoracic aortic ring. It would be expected that if the IH extract played a role as a PDE-5 inhibitor, the IH extract would modify the relaxant activities of GTN on the phenylephrine precontracted thoracic aortic ring in a similar way to that of sildenafil. However, the IH extract potentiated the relaxant activity of the GTN on the denuded thoracic aortic ring in a similar way to that produced by sildenafil. This result suggested that the IH extract might act as a PDE-5 inhibitor; however, further specific experiments would be needed to clarify this possibility. Furthermore, the maximal relaxation obtained from the thoracic aortic ring using GTN in the presence of the IH extract was higher than the one with sildenafil alone, and this effect was abolished by adding of the PAG, an inhibitor of the cystathionine-γ-lyase, the key enzyme that utilizes L-cysteine as a substrate to form H_2S.[16-18] This indicated that the IH extract also stimulated the release of H_2S, the third gasotransmitter, and vasodilator[19-21] from the vascular smooth muscle of the aortic ring and caused a synergistic relaxation in addition to its effect through inhibition of the PDE-5.

Constriction responses of blood vessels to α-adrenoreceptor agonists including phenylephrine were activated through heterotrimeric G protein-coupled receptors and led not only to increase in intracellular calcium via Gq/11 but also to activation of G12/13 proteins that are coupled to a Rho/Rho-kinase signaling pathway. Rho-kinase activation leads to phosphorylation and thus deactivation of the myosin light chain phosphatase. Y-27632, a Rho-kinase inhibitor,[22-25] induced the relaxation of isolated blood vessels contracted with many different agonists including the α-adrenoceptor agonists. In the present study, we proposed that if the IH extract played a role as a Rho-kinase inhibitor, the phenylephrine C-R curve of the endothelium-denuded thoracic aortic ring would be reduced in the presence of IH extract and/or Y-27632. In this situation, the IH extract or Y-27632 each significantly inhibited the C-R curve of the phenylephrine on the endothelium-denuded thoracic aortic ring. However, when both the IH extract and Y-27632 were added together, a complete suppression of the C-R curve of the phenylephrine on the endothelium-denuded thoracic aortic ring was obtained. These results indicated that the active component of the IH extract might act through other pathway independently synergized the suppression of the contraction. Nevertheless, further specific experiments would need to clarify this possibility.

In vascular smooth muscle, the α_1-adrenoceptor agonist, phenylephrine, induces an initial phasic contraction followed by a tonic contraction. The initial contraction is mediated by the release of intracellular Ca^{2+} from the sarcoplasmic reticulum,[26,27] whereas the sustained tonic contraction results from a Ca^{2+}influx through the voltage-dependent Ca^{2+}channels.[28-31] In the present study, we expected that, if the active component of the IH extract inhibited the extracellular Ca^{2+}entry through the voltage-gated Ca^{2+} channel, the phenylephrine-induced C-R curve would be further shifted rightward in the presence of both nifedipine and the IH extract compared to that with nifedipine alone. We found that nifedipine, a voltage-gated Ca^{2+} channel blocker, caused a parallel shift of the phenylephrine C-R curve to the right with no change in the maximal response. Although the IH extract also shifted the C-R curve of the phenylephrine to the right, the maximal responsiveness was depressed by about 60%. When nifedipine was also added together with the IH extract, no further inhibition by the nifedipine was obtained. These results indicate that the IH extract might in part play a role as a non-competitive Ca^{2+} channel inhibitor through the voltage-gated Ca^{2+} channel and/or it might also act through some other pathways to reduce the maximal responsiveness to the phenylephrine.

So far, there is only one report on phytochemical study on the seed of the IH Jacq. which was found to contain alkaloid lysergol, chanoclavine, penniclavine, isopeniclavin, and elymoclavine.[3] In the present study we used methanolic

extract of the plant seeds. As shown in the result section, IH extract contains at least 9 different compounds [Figure 1], the vasorelaxant activity of the crude IH extract might be due to synergistic effects of several active compounds, each responsible for initiating a different underlying mechanism. Obviously, the dominant chemical species are present in fractions 8 and 9 [Figure 1], but we will first need to carry out tests for activity on blood vessels of all 9 fractions before attempting to further characterize any of them; however, we are now certain that some component(s) in the IH extract do have significant effects on blood vessel. Thus, if the provided preparations from seeds of IH Jacq. are non-toxic, they could be administered by traditional medicine practitioners with the knowledge that they will have significant physiological effects that may alleviate some of the many medical conditions for which they are currently used. Thus, identification of the active chemical constituents is the next critical step.

CONCLUSIONS

The IH extract has a hypotensive activity and exerted relaxant activity on phenylephrine-precontracted isolated thoracic aorta. The results indicate that the active component(s) of the IH extract acted directly on the vascular smooth muscle to stimulate the release of H_2S, and it might be acting as a PDE-5 inhibitor and a voltage-gated Ca^{2+} channel inhibitor that resulted in relaxation. It also acted indirectly through the vascular endothelium to stimulate the release of nitric oxide. It does neither appear to act as an opener of the Ca^{2+}-sensitive K^+ channel nor of an ATP-sensitive K^+ channel or a Rho-kinase inhibitor. This study has confirmed that seeds of IH Jacq. contain bioactive substances that could have therapeutic effects on the anti-vascular spasmodic activity as claimed in traditional medicine. It might also be useful for the treatment of hypertension as it caused a long-lasting effect such as a decrease in BP in anesthetized rat. However, further study would need to identify the active compound(s) of the plant seeds as well as to confirm the mechanisms responsible for the effects.

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