Journal of Ethnopharmacology 176 (2015) 79–89

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Effects of 6 weeks oral administration of *Phyllanthus acidus* leaf water extract on the vascular functions of middle-aged male rats

Watchara Chongsa [a], Kanyanatt Kanokwiroon [b,e], Chaweewan Jansakul [c,d,*]

[a] Department of Physiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Thailand
[b] Department of Biomedical Sciences, Faculty of medicine, Prince of Songkla University, Hat-Yai, Thailand
[c] Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat-Yai 90112, Thailand
[d] Natural Research Center of Excellence, Prince of Songkla University, Hat-Yai, Thailand
[e] The Excellent Research Laboratory of Cancer Molecular Biology, Prince of Songkla University, Hat-Yai, Thailand

ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form
6 October 2015
Accepted 20 October 2015
Available online 22 October 2015

Keywords:
Phyllanthus acidus
Thoracic aorta
Mesenteric artery
Nitric oxide
H_2S
Middle-aged rat

Chemical compounds studied in this article:
acetylcholine chloride (Pubchem CID: 6060)
N^G-nitro-l-arginine (Pubchem CID: 440005)
phenylephrine hydrochloride (Pubchem CID: 5284443)
dl-propagylglycine (Pubchem CID: 95575)
tetraethylammonium (Pubchem CID: 5413)
glybenclamide (Pubchem CID: 3488)
glyceryl trinitrate (Pubchem CID: 4510)
nitric oxide (Pubchem CID: 145068)
Hydrogen sulfide (Pubchem CID: 402)
Kaempferol (Pubchem CID: 5280863)

ABSTRACT

Ethnopharmacological relevance: Leaves of *Phyllanthus acidus* (PA) have been used in Thai traditional medicine for the treatment of hypertension. We have previously shown that chronic treatment of a PA water extract to middle-aged male rats caused a lowering of the body and serum lipids, two of the parameters that are implicated in cardiovascular disease.
Aim of the study: To investigate if chronic treatment of middle-aged male rats with a PA water extract affected the perivascular (aortic) adipose tissue (PVAT) and/or their vascular functions
Materials and methods: Fresh leaves of PA were extracted with water and orally gavaged to the middle-aged male rats for 6 weeks. Vascular functions were studied *in vitro* using isolated thoracic aorta with and without PVAT, and mesenteric rings in Krebs Heinseleit solution with results recorded with a Polygraph or a Myograph system. The amount of blood vessel eNOS and CSE (cystathionine-γ-lyase) expression was measured by Western blotting.
Results: PA treatment caused a lower maximal contractile response to phenylephrine (Phe) of the endothelium-intact aortic ring than that of the control group. This effect was abolished by N^G-nitro-L-arginine (L-NA) or by denudation of the endothelium. DL-propargylglycine (PAG, H_2S inhibitor) and TEA (Ca^{2+}-activated K^+ channel blocker), but not glybenclamide (ATP-activated K^+ channel blocker), caused a similar increase in the baseline of the endothelium-intact aortic ring in the presence of L-NA in both the PA-treated and control aortic rings. This effect sequentially resulted in a greater contractile response of the aortic rings of both groups to Phe. Glybenclamide also caused a similar increase in the maximal contraction of the endothelium-intact blood vessels with L-NA to both groups. PAG, TEA or glybenclamide did not modify the phenylephrine *C–R* curves for either group of the PVAT-endothelium-intact aortic rings preincubated with L-NA. The CSE levels of the thoracic aorta and at the PVAT were not different between the PA-treated and the control group. Relaxation of the Phe-precontracted thoracic aortic ring to acetylcholine, but not to glyceryl trinitrate, was higher for the PA-treated than for the control aortic rings and this effect was abolished by L-NA. The mesenteric rings of the PA treated group showed a lower sensitivity on the contractile response to Phe than that of the control group, and this effect was abolished by L-NA. Vasodilatation to acetylcholine, but not to glyceryl trinitrate, of the PA treated-mesenteric ring was more sensitive than that of the control group and this effect was abolished by L-NA. The expression of eNOS by the PA treated thoracic aorta and the mesenteric arteries was higher than the control group. These results demonstrated that chronic treatment with a PA water extract to middle-aged rats affected their vascular functions by increasing the nitric oxide production from the endothelial cells and also modulated the responsiveness of the thoracic aortic- and mesenteric rings to phenylephrine and acetylcholine.

1. Introduction

Phyllanthus acidus (L.) Skeels (PA) belongs to the Euphorbiaceae family. Its common name is Otaheiti Goose-berry, Star Gooseberry or Mayom in Thai (Van Welzen and Chayamarit, 2007). It is a

* Corresponding author at: Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat-Yai 90112, Thailand. Fax: +66 74 282709.
E-mail address: chaweewan.j@psu.ac.th (C. Jansakul).

http://dx.doi.org/10.1016/j.jep.2015.10.030
0378-8741/

medicinal plant that, since ancient times, has been recommended by the Thai community to be planted in the gardens of their households with the belief that it will help to provide an auspicious environment with good health. In Thai traditional medicine, almost all parts of the plant have been used for the treatment of several ailments (see more details in Leeya et al., 2010), especially its leaves, that have been used to control blood pressure and for the relief of headache due to hypertension (Pongboonrod, 1959; Teingburanathum, 1999). To prove this therapeutic claim, Leeya et al. (2010) were the first group to demonstrate that an n-butanol extract from leaves of the plant had hypotensive activity in anesthetized rats (i.v. injection) and that it resulted from a synergistic effect of at least 5 bioactive compounds: adenosine, hypogallic acid, 5-hydroxy benzoic acid, caffeic acid and kaempferol acting on blood vessels to cause vasodilatation. Later, Chongsa et al. (2014) subjected middle-aged male rats to 6 weeks of chronic oral administration of three types of PA leaf extracts to determine if different leaf preparations affected the extracted drug efficacy. Only the PA water extract from leaves, but not one produced by prolonged heating or extraction of the water extract with n-butanol, affected lipid metabolism. PA water extracts have been shown to cause a decrease of body weight, visceral and subcutaneous fat, liver lipid accumulation, as well as a decrease in the fasting serum cholesterol, HDL- and LDL cholesterol levels, i.e. all the beneficial parameters that had been shown to restore vascular endothelial dysfunction in older adults (Bergholm et al., 2003; Walker et al., 2012).

Therefore, in the present study we aimed to obtain more information about the effect of a PA water extract on the vascular functions of middle-aged male rats which had impaired vascular endothelial nitric oxide production (Yorsin et al., 2014), the early and important state leading to cardiovascular disease such as high blood pressure. We expected to determine whether the chronic oral administration of the PA water extract to middle-aged rats would have some effects on the vascular and/or perivascular adipose tissue (PVAT) functions. It is now well established that PVAT exerts anti-contractile effects on various vascular beds (Chang et al., 2013; Dubrovska et al., 2004; Fang et al., 2009; Gil-Ortega et al., 2010; Lohn et al., 2002; Sun et al., 2013; Verlohren et al., 2004), and that this is impaired in hypertension and aging (Galvez-Prieto et al., 2012; Lu et al., 2011; Melrose et al., 2013; Szasz et al., 2012). We also investigated the effects of PA on the anti-contractile effects of H_2S production from the blood vessels and from PVAT, and of K^+ channel activity. Studies were performed *in vitro* using isolated thoracic aorta, with and without PVAT, and mesenteric arteries incubated in Krebs Henseleit solution using pharmacological techniques and Western blotting. Since cardiovascular disease is one of the major causes of global death and disability and imposes a huge burden on healthcare costs (Yazdanyar and Newman, 2009), our study may provide a basis for improving the use of PA in the treatment of cardiovascular disease.

2. Materials and methods

2.1. Plant material

Fresh leaves of *P. acidus* were collected in Songkhla province, Thailand from the same areas as previously reported. Authentication was achieved by comparison with the herbarium specimen in the Department of Biology Herbarium, Faculty of Science, Prince of Songkla University, Thailand, where a voucher specimen (Collecting no. 2548-04) of the plant material has been deposited.

2.2. Extraction

Leaves of *P. acidus* were extracted as previously described (Chongsa et al., 2014). Briefly, fresh leaves of the plant were added to boiling (98 °C) filtered water for 20 min. The clear solution was collected followed by lyophilization when a yellow brown powder of *P. acidus* water extract (PA extract) was obtained that amounted to 5.0% of the fresh leaf weight. The chemical composition profile of the PA extract was analyzed by high performance liquid chromatography (HPLC) using the same method as previously reported (Chongsa et al., 2014). Briefly, the PA water extract (10 mg/ml in 100% methanol) was analyzed on a Symmetrys C18 column (5 μm, 3.9 × 150 mm i.d.; Waters), with a gradient of CH_3OH: H_2O + 0.05% of trifluoroacetic acid (5:95–100:0) which was carried out on an analytical HPLC of the HP1100 system equipped with a photodiode array detector (Agilent Technologies). The flow rate was 1 ml/min; the UV traces were measured at 210 and 254 nm and the UV spectra (DAD) were recorded between 200 and 500 nm. The HPLC chromatograms together with the corresponding UV spectra that measured at 210 and 254 nm for the 5 bioactive compounds: adenosine, hypogallic acid, 5-hydroxybenzoic acid, caffeic acid and kaempferol as well as the other four unknown substances are shown in Fig. 1.

2.3. Pharmacological studies

2.3.1. Animal preparation

Middle aged (12–14 month old) male Wistar rats were purchased from the Southern Laboratory Animal Facility, Faculty of Science, Prince of Songkla University. The animals were housed in controlled environmental conditions at 25 °C on a 10 h dark and 14 h light cycle and allowed access to standard food and tap water *ad libitum*. The experimental methods employed in this study were approved by the Prince of Songkla University Animal Care and Use Committee (Ethic Ref. no.09/53). The investigation conformed to the Guide for the Care and Use of Laboratory Animals.

The rats were prepared as described previously (Chongsa et al., 2014), in brief they were acclimatized in their new environment at the drug treatment room at the Southern Laboratory Animal Facility for 1 week and then each animal was trained to receive oral gavage using distilled water for another week before being gavaged with the vehicle or PA extract with a dosage of 1 g/kg once (9.00 AM) a day for 6 weeks. Records of the body weight of each rat, including a 24 h food intake at day 0 (one day before receiving the oral gavage of the vehicle or PA extract) and then every consecutive 7 days for 6 weeks.

2.3.2. Basal blood pressure measurement

At the end of the chronic treatment by the PA water extract or distilled water, each rat was anesthetized with Nembutal (60 mg/kg, i.p injection). The tracheal tube was cannulated with a polyethylene tube to allow the animal to breathe room air spontaneously. The basal systolic and diastolic blood pressure were measured directly from the right common carotid artery via a polyethylene tubing that had been inserted into the common carotid artery which was connected to a pressure transducer (P23 ID, Gould Statham Instrument, Hato Rey, Puerto Rica) and connected to a Grass Polygraph (model 7D, Grass Instrument, Quincy, MA, U.S.A.). The heart rate was recorded using a tachograph driven by the blood pressure wave. The animals were equilibrated for 40 min and then the basal systolic and diastolic as well as the basal heart rate was measured.

2.3.3. Preparation of thoracic aortic rings and mesenteric artery

After measurement of the basal blood pressure of the anesthetized animal in the Section 2.3.2., the animal was killed by