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Hypotensive effect and toxicology of the extract from *Coscinium fenestratum* (Gaertn.) Colebr.

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Abstract

The water extract from *Coscinium fenestratum* (Gaertn.) Colebr. (CF extract) was tested for hypotensive and vasorelaxant effects. Acute and subchronic toxicity as well as motor activity of CF extract were also evaluated. The present study demonstrates that CF extract is effective in reducing blood pressure in anesthetized normotensive rats. This effect is shown to be dose-related and rapid in onset. The extract showed an endothelium-dependent and independent vasorelaxant activity in isolated aortic rings precontracted with phenylephrine (1 μM) and KCl (60 mM). The capacity of L-NAME (100 μM), an inhibitor of nitric oxide synthase, to reduce the vasorelaxant action of the extract indicates the involvement of nitric oxide. In the acute toxicity test, an oral dose of 5000 mg/kg of the CF extract did not produce mortality or significant changes of the general behavior of animals and gross appearance of internal organs of rats. Similarly, in the subchronic toxicity test, an oral dose of 2500 mg/kg/day of the CF extract given to rats for 90 days did not cause any significant change of any of the parameters observed when compared with those of the control animals. Moreover, the CF extract did not produce any effect on the central nervous system when spontaneous motor activity in rats was assessed. However, because some average hematological and blood chemistry values were found to be statistically different, further studies, including chronic toxicity test, should be done to confirm the safety of this plant when it is used over a long period of time.

Keywords: *Coscinium fenestratum* (Gaertn.) Colebr.; Hypotensive; Blood pressure; Vasorelaxant effect; Toxicity

1. Introduction

Coscinium fenestratum (Gaertn.) Colebr., family Menispermaceae, is a woody climber found in Southeast Asia, and is widely used as a medicinal plant (Siwon et al., 1980; Pinho et al., 1992). It is a large liana with yellow wood and sap, known in Thai as "Hamm" or "Khamin khruea". Various parts of this plant have been used for a variety of diseases such as fever, muscle pain, stomach pain, malaria, diarrhea, ulcers and infection of the eyes (Siwon et al., 1980; Jittaprasatsin et al., 2005). Moreover, it is widely used as traditional medicine in the North-Eastern part of Thailand, particularly along the border of the Lao People's Democratic Republic for many purposes including the treatment of high blood cholesterol, hyperglycemia as well as hypertension (Jittaprasatsin et al., 2005). For those purposes, the water extract of the dried stem is used. In phytochemical studies, *Coscinium fenestratum* has afforded berberine as the main alkaloidal constituent and a smaller amount of protoberberine (Malhotra et al., 1989; Pinho et al., 1992; Rojsanga et al., 2006).

Recently, *Coscinium fenestratum* has been reported to possess various pharmacological actions such as antioxidant (Venukumar and Latha, 2002), antiproliferative (Ueda et al., 2002), antidiabetic (Jittaprasatsin et al., 2005; Punitha et al., 2005; Shirwaikar et al., 2005), antiplasmodial (Tran et al., 2003) and antibacterial (Nair et al., 2005) activities. The hypotensive action of the 50% ethanol extract of *Coscinium fenestratum* in anesthetized dogs, rats and guinea pigs was also documented (Singh et al., 1990), however the mechanism of action has not

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; HCT, hematocrit; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell

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yet been clearly verified. In Thai folklore, the hot water extract of the dried stem of *Coscinium fenestratum* is used for controlling blood pressure. Therefore the crude water extract of this plant was used in this study to assess the hypotensive and vasorelaxant effects. Subchronic and acute toxicity tests were also performed to establish the safety of the extract.

2. Material and methods

2.1. Animals

Sprague-Dawley rats of both sexes, 8 and 10–12 weeks of age (weighing 180–200 g and 250–300 g) were purchased from the National Laboratory Animal Center (NLAC), Salaya, Mahidol University, Nakorn Pathoms. All animals were kept in a room maintained under automatically controlled conditions of 24 ± 1 °C and 12 h light–12 h dark cycle. They were fed a standard laboratory diet (Pokphan Animal Feed Co. Ltd., Bangkok, Thailand) and water, and were acclimatized at least 1 week before starting the experiment. The experimental protocol was approved by the Animal Ethics Committees in accordance with the guidelines for the care and use of laboratory animals set by the Faculty of Medicine, Chiang Mai University, Thailand.

2.2. Plant extract

One kilogram of dried stem of *Coscinium fenestratum*, was purchased from a medicinal herbal drug store (FONG KAEWVONGSA), Tambol Kuankao, Amphur Siritorn, Ubonratchthani province. The voucher specimen of *Coscinium fenestratum* (PHCO-CM 028) was authenticated and deposited at the Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Thailand. The dried stem was cut in to thin pieces and boiled in 5 l of distilled water for 30 min and then filtered. The procedure was repeated three times and the water extract was pooled and concentrated using a rotary evaporator at 70 °C. Extracts were pooled and then subsequently lyophilized by a freeze dryer (Leybold–Heraeous, Germany). A yellowish-brown dried powder was obtained (yield 10%) and stored in a desiccator (25 °C) until use.

The water extract solutions of *Coscinium fenestratum* (CF extract) used in the experiment were freshly prepared by dissolving this material in distilled water.

2.3. Drugs

Phenylephrine (PE), acetylcholine (ACh), L-*N*(ω)-nitro-arginine methyl ester (L-NAME), ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Sigma Chemical Company (St. Louis, U.S.A.).

All test drugs were dissolved in distilled water and used for all experiments.

2.4. Experimental protocol

2.4.1. Blood pressure and heart rate of pentobarbital-anesthetized normotensive rats

Male Sprague-Dawley rats 10–12 weeks of age (250–300 g) were anesthetized by an i.p. injection of pentobarbital sodium 40 mg/kg. The trachea was exposed and cannulated to facilitate spontaneous respiration. The femoral vein was cannulated for drug administration, which was standardized to injections of 0.2 ml/100 g body weight over a period of 1 min. Blood pressure was recorded from the femoral artery using a pressure transducer (Statham P 23 Strain Gauge Transducer, Laboratories Inc., Hato Ray, Puerto Rico) connected to a Grass polygraph. Heart rate (HR) was measured by means of a tachograph (Grass model 7P 44C), which was triggered by the pressure pulse and recorded on a separate polygraph channel. Various concentrations of the CF extract were given to the rat and the effect on the blood pressure and the heart rate were recorded. Each rat was tested for only one concentration. The blood pressure was expressed as the mean arterial blood pressure (MABP) according to the following equation:

$$\mathrm{MABP} = P_\mathrm{d} + \frac{1}{3}(P_\mathrm{s} - P_\mathrm{d})\mathrm{mmHg}$$

2.4.2. Isolated rat aortic preparation

The preparations were prepared using the methods modified from those of Bessho et al. (1991) and Hodoglugil et al. (1997). Male Sprague-Dawley rats (250–300 g) were sacrificed. The descending thoracic aorta were rapidly excised, excessive fat and connective tissue were then removed and cut into rings of 3–4 mm in length.

The rings were mounted in an organ-bath containing 20 ml of Krebs' solution (NaCl 119, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1, KH_2PO_4 1.2, and glucose 11 mM), which attached to a force–displacement transducer (Grass FT 03B) and displayed on a Grass model 7D polygraph under a resting tension of 1 g. The bathing fluid was maintained at 37 °C, and continuously aerated with 95% O_2 and 5% CO_2. During the equilibration period of 60 min, the bathing fluid was changed at 15 min-intervals to prevent the accumulation of metabolic products.

In order to elucidate the involvement of the endothelium on the aortic response to the effect of the CF extract, denuded aortic preparations were also used. They were prepared by gently rubbing the internal surface with wire. After equilibration, the integrity of the endothelium was assessed in all preparations by determining the ability of ACh (1 μM) to induce more than 80% relaxation of rings precontracted with phenylephrine (PE, 1 μM); the endothelium was considered to be absent when the relaxation of the ring to ACh was less than 10%.

To study the vasodilating effect of the CF extract, the aortic ring was contracted with PE (1 μM) or KCl (60 mM). When a stable contraction plateau was reached, increasing concentrations of the CF extract (10–160 μg/ml) were added cumulatively. The rings with intact or denuded endothelium were tested in parallel.

To determine the underlying mechanisms of CF-extract-induced vasomotor response in aortic rings with endothelium, the endothelium-intact rings were incubated with L-NAME (100 μM), a specific inhibitor of nitric oxide synthase, for 30 min before administration of PE. Then the response curves of CF extract were assessed. The endothelium-intact rings with or without the treatment of L-NAME were tested in parallel.