Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2016, **14**(11): 0835−0843
Chinese Journal of Natural Medicines

Prophylactic effects of alkaloids from *Ba lotus* seeds on *L*-NNA-induced hypertension in mice

SUN Peng [1, 2, 3, 4, 5Δ], ZHU Kai [1Δ], WANG Cun [1, 3], LIU Wei-Wei [6], PENG De-Guang [2*], ZHAO Xin [1, 2, 3, 4, 5*]

[1] *Chongqing Collaborative Innovation Center for Functional Food, Chongqing University of Education, Chongqing 400067, China;*
[2] *Chongqing Enterprise Engineering Research Center of Ba-lotus Breeding and Deep Processing, Chongqing 400041, China;*
[3] *Department of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, China;*
[4] *Chongqing Engineering Research Center of Functional Food, Chongqing University of Education, Chongqing 400067, China;*
[5] *Chongqing Engineering Laboratory for Research and Development of Functional Food, Chongqing University of Education, Chongqing 400067, China;*
[6] *School of Public Health and Management, Chongqing Medical University, Chongqing 400016, China*

Available online 20 Nov., 2016

[ABSTRACT] Alkaloids from *Ba lotus* seeds (ABLS) are a kind of important functional compounds in lotus seeds. The present study was designed to determine its hypertension prophylactic effects in the *L*-NNA-induced mouse hypertension model. The mice were treated with ABLS, the serum and tissues levels of NO, MDA, ET-1, VEGF, and CGRP were determined using the experimental kits, the mRNA levels of various genes in the heart muscle and blood vessel tissues were further determined by RT-PCR assay. ABLS could reduce the systolic blood pressure (SBP), mean blood pressure (MBP), and diastolic blood pressure (DBP), compared to that of the model control group. After ABLS treatment, the NO (nitric oxide) contents in serum, heart, liver, kidney and stomach of the mice were higher than that of the control mice, but the MDA (malonaldehyde) contents were lower than that of the control mice. The serum levels of ET-1 (endothelin-1), VEGF (vascular endothelial growth factor) were decreased after ABLS treatment, but CGRP (calcium gene related peptide) level was increased. The ABLS treated mice had higher mRNA expressions of HO-1, nNOS, and eNOS and lower expressions of ADM, RAMP2, IL-1β, TNF-α, and iNOS than the control mice. Higher concentration of ABLS had greater prophylactic effects, which were close to that of the hypertension drug captopril. These results indicated the hypertension prophylactic effects of ABLS could be further explored as novel medicine or functional food in the future.

KEY WORDS] *Ba lotus* seed; Hypertension; Alkaloids; *L*-NNA; Mice

CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2016)11-0835-09

Introduction

Nymphaea L. is a kind of perennial aquatic herbaceous plants, and lotus seeds can be used as food or medicine. The lotus seeds are very important in traditional Chinese medicine and have effects of sedation, hemostasis, and improving heart and renal functions [1]. *Ba lotus* is one of the nymphaeaceae plants. Because the main producing area is in Chongqing, China, it is also called *Ba lotus*. The seeds can also be used as Chinese medicine or health care products, full of efficacy. The lotus seeds contain a number of ingredients, including carbohydrates, proteins, vitamins, trace elements, fatty acids, and alkaloid compounds [2-3]. One of the most important ingredients is alkaloids. NO (nitric oxide) could regulate blood vessel tension, reducing blood pressure. Nobel prize laureate Ignarro has found that NO is a key material for prevention of cardiovascular and cerebrovascular diseases,

[Received on] 17-Feb.-2016
[Research funding] This work was supported by Chongqing Engineering Research Center for Functional Food (No. cstc2015yfpt_gcjsyjzx0027) and the Program for Innovation and Team Building at the Chongqing Institute of Higher Education (No. CXTDX 201601040).
[*Corresponding author] E-mail: pengdeguang@163.com (PENG De-Guang); foods@live.cn (ZHAO Xin).
[Δ]These authors contributed to the work equally.
These authors have no conflict of interest to declare.

such as hypertension [4]. *L*-NNA is a kind of NOS inhibitor, which can inhibit NO (nitric oxide) generation, causing high blood pressure [5]. It is often used in the experimental high blood pressure models. NOS inhibitor can inhibit endothelium-dependent relaxation induced by various vasodilatation factors *in vitro* [6]. Massive NOS inhibitor can strongly inhibit the generation of NO (nitric oxide) in mouse vascular endothelia in a short time, which promotes the proliferation of vascular smooth muscle cells and obviously changes the structure of vascular tissues [7]. This can result in endothelial function damage and elevated arterial blood pressure, which may further damage the synthetic ability of NO (nitric oxide) in endothelial cells to maintain the high blood pressure [8]. Angiotensin II is also used for developing experimental hypertension model [9-10]. It could cause over-activation of the RAAS (rennin-angiotensin-aldosterone system), inducing hypertension [11], and NO could prevent different kinds of hypertension. It has been suggested that *L*-NNA could be used for evaluation of traditional Chinese medicines as well.

In the present study, we adopted a mouse model of NOS inhibitory type hypertension caused by *L*-NNA to observe inhibition effects of alkaloids from *Ba lotus* seeds (ABLS). The blood pressures were determined and several molecular markers in the blood and tissues of the mice were analyzed to explore the underlying mechanisms of action for ABLS. The NO (nitric oxide) and MDA (malonaldehyde) contents in serum, heart, liver, kidney and stomach were determined, and the serum levels of ET-1 (endothelin-1), CGRP (calcium gene related peptide), and VEGF (vascular endothelial growth factor) were also determined. The mRNA expressions of HO-1, ADM, RAMP2, nNOS, eNOS, and iNOS in heart muscle and blood vessel tissues and IL-1β andTNF-α expression in the blood vessel tissues were determined by RT-PCR assays. *Ba lotus* is an important kind of lotus in Sichuan Province and Chongqing City in China, but there are not many literatures on *Ba lotus* seeds. Furthermore, there was no study report on *Ba lotus* seeds in the treatment or prevention of hypertension. The *Ba lotus* seeds have only been used as food or traditional Chinese medicine raw materials. The results for the present study could provide a basis for further drug development based on the alkaloids components in *Ba lotus* seeds.

Materials and Methods

Extraction of alkaloids of Ba lotus seeds (ABLS)

Ba lotus seeds were supplied by Chongqing Enterprise Engineering Research Center of Ba-lotus Breeding and Deep Processing (Chongqing City, China). The samples were verified by Prof. YU Qian (Chongqing University of Education, Chongqing, China) in January, 2015. The dried *Ba lotus* seeds (1 000 g) were knapped and extracted twice with 10 L of 80% ethanol for 1 h each. Then the two extract solutions were conflated and concentrated to 1 000 mL by vacuum concentration. The extract solution was loaded onto the 732 cation exchange resin column (Beijing Zhengguangchuangye Science and Technology Ltd., Beijing, China). After adsorption, the water soluble impurities were washed by distilled water, and the solution was further washed by 80% ethanol. The ethanol elute was collected, concentrated, and dried [12]. The dried residue was washed with 80% ethanol (containing 2% ammonia water) concentrated and dried again to afford ABLS. The ABLS generated in the present study was white powder and I could be dissolved in organic solvents, but difficult to dissolve in water.

Animal experiment

50 male, seven-week-old ICR mice were purchased from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). The mice were housed in the pathogen-free animal experiment room (temperature 25 ± 2 ºC, relative humidity 50% ± 5%; a 12 h/12 h light/dark cycle, SCXK (Yu) 2012-0001, Chongqing, China). These mice were randomly divided into five groups (normal group, model control group, 50 mg·kg^{-1} ABLS group, 100 mg·kg^{-1} ABLS group, and captopril group). The normal mice were fed with normal mice diet and free water access, without drug treatment. The other groups were treated with 700 mg·kg^{-1} of *L*-NNA (N^{ω}-Nitro-*L*-arginine, Shanghai Gold Wheat Biological Technology Co. Ltd., Shanghai, China) by lavage for 3 weeks. From the seventh day, 50 and 100 mg·kg^{-1} of ABLS groups were treated with 50 and 100 mg·kg^{-1} ABLS by lavage, and captopril group (drug control group) was treated with 50 mg·kg^{-1} of captopril (Sigma, St. Louis, MO, USA) by lavage. On every other days, the SBP (systolic blood pressure), MBP (mean blood pressure) and DBP (diastolic blood pressure) of all mice were determined by Tail-cuff method at 45 min after treatment with *L*-NNA. All the mice were sacrificed by CO_2, the heart, liver, kidneys, stomach, and blood vessel tissues and blood samples were collected. These animal experiments followed a protocol approved by the Animal Ethics Committee of Chongqing Medical University (Chongqing, China) [13].

Assays for NO and MDA levels in mouse tissues

The NO (No. A012) and MDA (No. A003-1) contents in heart, liver, kidney and stomach tissues were determined using the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [14].

Assays for serum levels of molecular markers

The serum levels of NO (No. A012), MDA (No. A003-1), ET-1 (No. H093), and CGRP (No. H217) were measured using the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The erum VEGF levels were determined using an ELISA kit (Beijing BLKW Biotechnology Co., Ltd., Beijing, China) [14].

RT-PCR assays for the mRNA expression of various genes in mouse tissues

The RNAzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from the heart muscle and blood vessel tissues. The concentration of the extracted total