Upregulation of the angiotensin-converting enzyme 2-angiotensin-(1-7)-Mas receptor axis by a combination of Yinyanghuo (Herba Epimedi Brevicornus) and Cheqianzi (Semen Plantaginis) improves erectile function in spontaneously hypertensive rats

Zhang Hongguan, Liu Yude, Rao Lian, Cen Yanyou, Cheng Kaili

Abstract

OBJECTIVE: To evaluate the effects of a combination of Yinyanghuo (Herba Epimedi Brevicornus) (HEB) and Cheqianzi (Semen Plantaginis) (SP) on erectile dysfunction caused by essential hypertension and its association with macrovascular compliance in spontaneously hypertensive rats (SHRs), and to elucidate the role of the angiotensin-converting enzyme 2-angiotensin-(1-7)-Mas receptor (ACE2/Ang (1-7)/Mas receptor) axis in this process.

METHODS: A total of 24 SHRs were randomly assigned to three groups: SHR-control, low-dose (12.5 g/kg) and high-dose (25 g/kg) HEB + SP (HEBSP). Eight Wistar-Kyoto rats were used as normal controls. HEBSP was administered by oral gavage for 28 d. Erectile function was measured once a week using the Heaton test. After 4 weeks of treatment, the corpus cavernosum was harvested from each rat to measure nitric oxide (NO), nitric oxide synthase (eNOS) and Ang (1-7) levels, as well as ACE2, Mas receptor and neuronal nitric oxide synthase (nNOS) protein expression.

RESULTS: After 4 weeks of treatment, HEBSP significantly increased erectile function in the treated group compared with SHR-control group (P < 0.01). Additionally, HEBSP treatment significantly increased cavernosal levels of Ang (1-7), eNOS and NO. Moreover, HEBSP significantly elevated the expression levels of ACE2, Mas receptor and nNOS. These beneficial effects were elevated in the high-dose HEBSP group.

CONCLUSION: HEBSP improved erectile function in SHRs by upregulating the ACE2/Ang (1-7)/Mas receptor axis, eNOS and nNOS pathways.

INTRODUCTION

Studies have shown that erectile dysfunction (ED) is more prevalent in hypertensive patients than in normotensive subjects, and that more than 40% of men with ED concurrently share a diagnosis of hypertension. Moreover, a large number of epidemiological studies have shown that ED is associated with hypertension, and the association includes macrovascular compliance changes, microvascular endothelial damage, and abnormal connections between cavernous smooth muscle cells or different signal transduction systems. Erectile dysfunction is a clinical complication of essential hypertension (EH), and can be a sign of systemic vascular disease, which may in turn be an early warning sign of hypertension. Erectile dysfunction is also known to
be a marker of vascular aging, and chronic hypertension can also cause chronic peripheral arterial insufficiency and reduced dynamic penile peak systolic velocity (D-PSV). Both ED and chronic arterial disease may occur or worsen with persistent hypertension. The corpus cavernosum has its own renin-angiotensin-aldo-sterone system (RAS), which influences erection and softening of the penis. Furthermore, the angiotensin-converting enzyme 2-angiotensin-(1-7)-Mas receptor axis [ACE2-Ang (1-7)-Mas axis] participates in the erection process. Combined with the Mas receptor, Ang (1-7) promotes nitric oxide (NO) release from the penile vasculature and corpus cavernosum, penile vascular dilatation and relaxation of the corpus cavernosum smooth muscles, and antagonizes the excitatory effect of angiotensin II (Ang II) on the sympathetic nerve and penile fibrosis induced by Ang II. Therefore, abnormalities of the ACE2-Ang (1-7)-Mas axis can contribute to ED.

Nitric oxide is the major non-cholinergic non-adrenergic neurotransmitter involved in the process of penile erection. Nitric oxide synthase (nNOS) is the major source of NO in the penis and is found mainly in the central and peripheral nerves. Changes in nNOS levels in the pudendal nerve and corpus cavernosum are directly related to erectile function. nNOS is also present in the pudendal blood vessels and involved in the release of NO. Past studies have shown that Ang (1-7) enables upregulation of nNOS levels in SHRs.

Our previous investigation demonstrated that the combination of Yin yang huo (Herba Epimedi Brevicornus) (HEB) and Cheqianzian (Semen Plantaginis) (SP) can improve ED outcomes in hypertensive patients, improve erectile function in spontaneously hypertensive rats (SHRs), raise testosterone plasma levels of two-kidney, one clip hypertensive rats, correct the imbalance of estradiol and testosterone and improve hemorhology in hypertensive rats. Furthermore, combined HEB and SP (HEBSP) exerts a hypotensive effect on SHRs and essential hypertensive patients, and increases the plasma level of Ang (1-7) in SHRs. However, the mechanism underpinning the specific actions of this drug remain unclear. The present work investigated the influence of HEBSP upon erectile function in SHRs, and its effects on the expression of ACE2, Ang (1-7), Mas and nNOS in the corpus cavernosum of SHRs.

MATERIALS AND METHODS

Drug and reagents
HEBSP tablets, provided by the First Affiliated Hospital of Guangzhou University of Chinese Medicine, were composed of HEB and SP at a ratio of 1:1. Each tablet was equivalent to 5 g of the crude drug and the adult dose was 75 g per day. Anti-ACE2 and anti-nNOS antibodies were purchased from Abcam (New England, MA, USA), anti-Mas antibody from Bios (Beijing, China), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody from KangChen Bio-tech Inc. (Shanghai, China). The horseradish peroxidase-labeled secondary antibody was provided by Southern Biotech Co. (Birmingham, AL, USA), and the immunoprecipitation assay lysis kit was supplied by Beyotime Biotechnology. The bicinechinonic acid protein assay kit was purchased from Nanjing KeyGen Biotech. Co., Ltd. (Nanjing, China). The Ang (1-7) enzyme-linked immunosorbent assay (ELISA) kit was from Kamiya Biomedical (Seattle, WA, USA), the eNOS ELISA kit from Yuanmu BioTechnology Co., Ltd. (Shanghai China) and the NO reagent kit from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Animals and experimental groups
Twenty-four male SHRs (10-11 weeks of age; 160-180 g body weight) and eight age-matched Wistar-Kyoto (WKY) rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All rats were housed in an environmentally-controlled room, with temperature maintained at 20-22 °C and humidity maintained at 50%-60%. The rats were exposed to a 12-h dark-light cycle and fed a standard rodent diet with access to fresh tap water ad libitum. After 10 d of adaptation, SHRs were randomly assigned to the following three experimental groups (n = 8/group): SHR-control, SHR + low-dose HEBSP (12.5 g/kg) and SHR + high-dose HEBSP (25 g/kg). Another eight WKY rats were selected as normal controls; SHR-control and WKY rats received normal saline in place of the HEBSP treatment. Rats were treated via intra-gastric administration for 4 successive weeks. The current study was approved by the Ethics Committee (TCMF-2016005) of Animal Research at Guangzhou University of Chinese Medicine and all experimental procedures followed the international guidelines for the use and care of experimental animals.

Erectile function test
Erectile function was determined using a technique previously described by Heaton. In this experiment, rats were placed in an observation cage to adapt to their surroundings for 10 min, and the room ambience was quiet and calm. Each rat received 100 mg/kg of apomorphine (APO), prepared with 100 mg/kg ascorbic acid dissolved in physiological saline, via subcutaneous injection (1 mL/kg) into the back of the neck. After injection, the rats were immediately placed back into an observation cage and video monitoring equipment was activated. Erectile responses induced by APO were recorded at intervals of 5 min for a total of 30 min once a week for a total experimental period of 4 weeks. We defined an erection when an engorged glans penis and distal shaft were fully exposed.
Sample collection and preparation
Following the last erectile function test, rats were anesthetized by an intraperitoneal injection of 10 mL/kg chloral hydrate. The corpus cavernosum was then separated and removed from the penis; harvested tissues were collected under sterile conditions and frozen in liquid nitrogen until further analyses.

Determination of Ang (1-7), eNOS and NO concentrations
Ang (1-7) levels in the corpus cavernosum of each specimen were determined by enzyme-linked immunosorbent assay (ELISA), following the manufacturer’s instructions. Cavernosal eNOS levels were determined by ELISA, while NO levels were determined by measurement of nitrite (NO₃⁻) in accordance with the manufacturers’ instructions.

Detection of ACE2 and Mas receptor protein expression
Expression of ACE2 and Mas receptor was determined using immunohistochemistry. Briefly, sections of corpus cavernosum were de-paraffinized with xylene then rehydrated in a series of descending alcohol concentrations. For antigen retrieval, sections were treated with boiling citrate buffer in a microwave oven. Endogenous peroxidases were then de-activated by incubation with 3% hydrogen peroxide for 10 min. Sections were then incubated with anti-ACE2 antibody (1:5000 dilution) or anti-Mas receptor antibody (1:1000 dilution) overnight at 4 ℃. The next morning, sections were washed three times with PBS and incubated with biotin-conjugated secondary antibody (1:2000 dilution) for 30 min at room temperature. Finally, sections were incubated with 0.01% dianisobenzidine, counterstained with hematoxylin, and photographed under light microscopy at a magnification of 400 x. Negative controls were incubated with normal serum instead of the primary antibody.

Determination of ACE2, Mas receptor and nNOS protein levels
Western blot analysis was used to determine protein levels of ACE2, Mas receptor and nNOS in the corpus cavernosum. Briefly, total protein was extracted by homogenizing samples of corpus cavernosum in ice-cold immunoprecipitation assay lysis buffer. Protein levels in the extractions were measured using a bicinchoninic acid protein assay kit following the manufacturer’s instructions. Protein samples (30 μg) were loaded onto a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis system and transferred electrophoretically onto a polyvinylidene fluoride membrane. After transfer, membranes were blocked with 5% skimmed milk and then incubated with primary antibodies: anti-ACE2 (1:5000 dilution), anti-Mas receptor (1:1000 dilution), anti-nNOS (1:1000 dilution), or anti-GAPDH (1:10000 dilution). Sections were incubated with antibodies overnight at 4 ℃. GAPDH was used as an internal control. Following incubation, membranes were incubated with the secondary antibody (1:1000 dilution) at a temperature of 37 ℃ for 60 min and then examined using a fluorescent luminescent substrate. Protein band intensity was determined using ImagePro plus 5.0 software (Media Cybernetics, Rockville, MD, USA). Finally, protein levels of ACE2, Mas receptor and nNOS were calculated relative to the intensities of the GAPDH bands.

Data analysis
All values are shown as mean ± standard deviation (x ± s). One-way analysis of variance with least squares difference post-hoc analysis and the paired t test were applied to the data. Statistical analyses were conducted using SPSS 20.0 software (SPSS, Chicago, IL, USA). A P value < 0.05 was considered statistically significant.

RESULTS
Erectile function
Table 1 shows changes in erectile function in the three groups of rats throughout the 28-day study period. By the end of the experiment, erectile events were markedly fewer in the SHR-control rats compared with normal WKY rats (P < 0.01). A marked increase in erectile function was found in the HEBSP rats after 4 weeks of treatment compared with the SHR-control group (P < 0.01). High-dose HEBSP treatment was associated with a more pronounced improvement in erectile function than the low-dose treatment (P < 0.01).

Table 1| Observation of sexual activity (erectile function) induced by apomorphine in rats (frequency, x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>2.9±0.6</td>
<td>2.3±0.5</td>
<td>2.8±0.7</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.6±0.5</td>
<td>0.8±0.5</td>
<td>0.5±0.5</td>
</tr>
<tr>
<td>Low dose HESP</td>
<td>8</td>
<td>0.8±0.7</td>
<td>1.8±0.7</td>
<td>2.0±0.5</td>
</tr>
<tr>
<td>High dose HESP</td>
<td>8</td>
<td>0.8±0.7</td>
<td>1.8±0.7</td>
<td>2.5±0.5</td>
</tr>
</tbody>
</table>

Notes: the normal WKY group received normal saline (orally; once a day; 1 mL/dose for 4 weeks); the SHR-control group received normal saline (orally; once a day; 1 mL/dose for 4 weeks); the low-dose HESP group received HESP (orally; once a day; 12.5 mg/kg body weight; 1 mL/dose for 4 weeks); the high-dose HESP group received HESP (orally; once a day; 25 mg/kg body weight, 1 mL/dose for 4 weeks). HESP: Yinyanghuo (Herba Epimedii Brevicornus) and Cheqianzi (Semem Plantaginis); SHR: spontaneously hypertensive rat; WKY: Wistar-Kyoto. P < 0.01 versus the normal group; P < 0.01, versus control group; P < 0.01 versus data from week one.
Immunohistochemical detection of ACE2 and Mas receptor
Figures 1 and 2 show representative images of ACE2-positive and Mas receptor-positive immunohistochemical staining (highlighted in brown). A large number of Mas receptor- and ACE2-positive cells were expressed in the corpus cavernosum. ACE2 and Mas receptor immunostaining was mainly located in the endothelium and smooth muscle cells of the corpus cavernosum. Immunohistochemical staining showed that expression levels of ACE2 and Mas receptor in the corpus cavernosum of rats in the control group were both less than equivalent levels in normal WKY rats. In contrast, HEBSP treatment significantly increased the expression of ACE2 and Mas receptor. Compared with the low-dose group, the high-dose group had increased expression of both ACE2 and Mas receptor.

Structural changes of the corpus cavernosum
Figures 1 and 2 show that the smooth muscle cells in the corpus cavernosum of the normal group were arranged in an orderly manner. Furthermore, the structures of sinusoids, endothelial cells, and interstitial tissue and micro-blood vessels were well-defined, and endothelial cells in the sinusoids were intact. However, in both the control and HEBSP groups, the continuity of endothelial cells in the cavernous body was disrupted. The smooth muscle cells were deformed, swollen and irregularly arranged. The sinusoids and endothelial cells were also disordered. These changes were more obvious in the control group than in the HEBSP group, and there were no significant differences observed between the high-dose HEBSP and the low-dose HEBSP groups.

Concentration of Ang (1-7) in the corpus cavernosum
Figure 3 shows Ang (1-7) concentrations in the corpus cavernosum. After 4 weeks of treatment, the cavernosal Ang (1-7) concentration in the SHR-control group was significantly less than the normal WKY group [(1.26 ± 0.04) vs (0.1 ± 0.01) ng/mg; P < 0.01]. Treatment with HEBSP significantly increased the corpus cavernosum concentration of Ang (1-7) in the low-dose and high-dose groups [(0.37 ± 0.12) and (0.67 ± 0.11) ng/mg, respectively; P < 0.01] compared with the SHR-control group. High-dose HEBSP treatment was associated with higher corpus cavernosum Ang (1-7) concentrations than low-dose treatment (P < 0.01).

Concentration of eNOS in the corpus cavernosum
Figure 4 shows eNOS concentrations in the corpus cavernosum from the experimental groups. After 4 weeks of treatment, cavernosal eNOS concentrations in the SHR-control group were significantly lower than those in the normal WKY group [(6.6 ± 1.8) vs (5.6 ± 2.1) μM/g; P < 0.05]. Treatment with HEBSP significantly increased the concentration of eNOS in the corpus cavernosum in the low-dose and high-dose groups [(8.3 ± 1.7) and (8.7 ± 1.5) μM/g, respectively; P < 0.05] compared with the SHR-control group. High-dose HEBSP treatment was associated with higher corpus cavernosum eNOS concentrations than low-dose treatment (P < 0.05).

Cavernosal NO concentrations
As shown in Figure 5, a significantly lower cavernosal NO concentration was found in the SHR-control group than in normal WKY rats [(168.2 ± 26.3) vs (65.7 ± 0.6) μmol/mL; P < 0.05]. Administration of HEBSP markedly increased the cavernosal NO concentration in the low-dose and high-dose groups [(71.1 ± 1.6) and (93.8 ± 6.3) μmol/mL, respectively; P < 0.05] compared with the SHR-control group. High-dose HEBSP treatment resulted in a significantly higher cava-
ACE2, Mas receptor and nNOS proteins levels

Figure 6 shows representative protein bands of ACE2, Mas receptor and nNOS in the corpus cavernosum. Protein bands for ACE2, Mas and nNOS were weaker in the SHR-control group than those of the normal WKY group and HEBSP-treated groups. Band intensity analyses showed that cavernosal expression levels of ACE2, Mas receptor and nNOS were markedly downregulated in the SHR-control group compared with the normal WKY group ($P < 0.05$). HEBSP treatment upregulated the expression of ACE2, Mas receptor and nNOS proteins compared with the SHR-control group (all $P < 0.05$). High-dose HEBSP treatment resulted in stronger expression of cavernosal ACE2 and Mas receptor proteins than low-dose treatment (all $P < 0.05$).

DISCUSSION

The findings of this study demonstrate that: (a) HEBSP treatment for 28 days significantly decreased ED in...
The SHRs showed notable ED when compared with age-matched WKY rats; the ED represented a complication of EH. A trend towards reduced ED (associated with EH) was generally observed in HEBSP-treated SHRs over the 4-week experimental period, while a pronounced reduction in ED was observed immediately after the treatment period. Notably, the level of improvement of erectile function was more pronounced in the high-dose treatment group.

The HEB component used in this study was from a species in the genus Epimedium of the Berberidaceae family. In China, HEB has been traditionally used to treat skeletal diseases and impotence, as well as dizziness and fatigue. The SP component used in this study was from a species in the genus Plantago of the Plantaginaceae family, and is considered to have diuresis-promoting effects in Traditional Chinese Medicine. The effective component of SP can excite parasympathetic nerves and inhibit sympathetic nerves, resulting in expansion of peripheral blood vessels.

Modern phar-
macology has also demonstrated that the ingredients of HEBSP exhibit powerful antihypertensive properties.15,31 Our earlier studies found that HEBSP had an antihypertensive effect in hypertensive patients and SHRs, and markedly improved erectile dysfunction in an SHR model.15, 20 The RAS family is a principal target for regulating the cardiovascular system. There is a separate RAS in the penis that regulates penile erection and softening. Ang II is able to contract the penile artery and corpus cavernosum smooth muscle and cause vascular endothelial damage through oxidative activation and inflammation, by increasing reactive oxygen species production and promoting fibrosis in the blood vessels and corpus cavernosum. It has also been demonstrated that elevated levels of Ang II can contribute to the development of ED in animals and humans. In contrast, the ACE2-Ang (1-7)-Mas axis appears to mediate penile erection.22 Long-term treatment with Ang (1-7)-CyD reduced penile fibrosis associated with the attenuation of oxidative stress and markedly improved cavernosal endothelial function in hypercholesterolemic mice.31 Intravenous AVE0991, a non-peptide agonist of the angiotensin-(1-7) receptor, was found to strengthen the erectile response in anesthetized WKY rats; however, if the Mas antagonist A-779 was injected at the same time, the action of AVE0991 was inhibited.32 Mas is a specific receptor for Ang (1-7), and previous reports have shown that erectile function in mice with Mas deficiency was significantly reduced and associated with apparent fibrosis of the corpus cavernosum.31 In the present study, ACE2 and Mas receptor were immunohistochemically localized in the corpus cavernosum. Furthermore, Western blot analysis demonstrated approximately 65% lower expression of ACE2 and Mas receptor proteins in the corpus cavernosum of untreated SHRs than in age-matched WKY controls. Moreover, cavernosal Ang (1-7) concentrations were lower in untreated SHRs than in age-matched WKY rats. The HBE PSP treatment markedly increased cavernosal ACE2 and Mas receptor protein expression, and elevated cavernosal Ang (1-7) concentrations in the SHRs. These findings suggest that HEBSP treatment activates the ACE2/Ang (1-7)/Mas receptor axis.

NO is synthesized by a family of NOS, which includes inducible NOS, endothelial NOS, and nNOS. NO is an important non-cholinergic, non-adrenergic neurotransmitter involved in penile erection. nNOS is one of the main substances that induces erection. Previous studies have shown that nNOS inhibitors prevented erectile response induced by electrical stimulation of peripheral and central nerves. These results suggest that nerves are an important source of NO in penile tissue.32 Recent studies have also found that nNOS not only exists in the nervous system, but is also widely distributed in the aorta, coronary artery, radial artery, and other human blood vessels.33 NO, produced by nNOS, plays an important role in relaxing the peripheral vascular system by inhibiting the accumulation of inflammatory molecules.34 A previous study demonstrated that Ang (1-7) induced an increase in cardiac eNOS and nNOS in SHRs.35 Our present study also showed approximately 70% lower expression of cavernosal nNOS protein in SHR-controls than in age-matched normal rats. Accordingly, untreated SHRs showed a significantly lower cavernosal NO concentration than normotensive rats. This study revealed that HEBSP treatment significantly increased the cavernosal NO concentration, suggesting that the effect of HEBSP on ED may be related to increased production or release of NO. Moreover, treatment with HEBSP significantly upregulated the expression of nNOS protein in the corpus cavernosum. This suggests that HEBSP promoted the production or release of NO by modulating the expression of nNOS in the corpus cavernosum.

Both ED and EH are associated with a reduction in vascular compliance.36 Hoffman found that patients with ED were more sensitive to a decline in flow mediated dilation than patients with hypertension.36 Penile erection involves various biological factors, such as the anatomy, blood circulation, innervation, and endocrinology, as well as the coordinated interaction between such factors, and erection also involves psychological and social factors. However, erection is essentially a hemodynamic process, and the degree of erection depends on the dynamic balance between the inflow of arterial blood and the outflow of venous blood. Therefore, RAS plays an important role in the pathophysiological process of ED.

Our previous study found that HEBSP enhanced the levels of Ang (1-7) in SHRs in the corpus cavernosum more strongly than valsartan.38,37 Valsartan and other angiotensin receptor blockers (ARBs) increase the concentration of Ang II by negative feedback while blocking the AT1 receptor and increasing the substrate concentration of ACE2.38,39 Consequently, enhancing the ACE2-Ang (1-7)-Mas axis is an important pharmacological action of ARB drugs. Therefore, HEBSP has similar effects to ARB drugs in promoting the ACE2-Ang (1-7)-Mas axis in the corpus cavernosum of SHRs. We also found that there was no significant difference between HEBSP and valsartan in terms of elevating the levels of plasma Ang (1-7) and downstream NOS in the ACE2-Ang(1-7)-Mas axis, and that HEBSP was superior to valsartan in increasing NO concentrations in SHRs.16,37 Thus, HEBSP provided a therapeutic effect on ED in SHRs and increased levels of ACE2, Ang (1-7), Mas and nNOS in local tissues and plasma, thus representing a common key mechanism. There were some potential limitations in this study. First, icariin, the main effective component of HEB, is known to inhibit all three phosphodiesterase (PDE) isoforms40 and promote erection as a result. High concen-
tations of PDE enzyme have been found in human corpus cavernosum smooth muscle. Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cyclic adenosine monophosphate or cyclic guanosine monophosphate, by inhibiting their degradation by PDE. Thus, changes in erectile function may not represent the role of the ACE2-Ang (1-7)-Mas axis alone. Second, the actions of HEBSP on the ACE2/Ang II/AT1 receptor axis were not evaluated in the current study. Moreover, we did not evaluate changes in inducible nitric oxide synthase (iNOS) and have not investigated whether HEBSP may affect aortic and plasma levels of iNOS. Third, as a Traditional Chinese Medicine, HEBSP has multiple targets and mild effects on the regulation of RAS. Therefore, the induction of the ACE2-Ang (1-7)-Mas axis may represent a manifestation of its regulatory effect, which is different from that of dimazene aceturate, AVE0091 and other drugs that directly activate Mas receptors. At this stage, AVE0091 and other drugs that directly act on the ACE2-Ang (1-7)-Mas axis are not yet available for clinical use. Therefore, there was no positive control available for this study. These issues will be investigated in our future studies.

In conclusion, our results demonstrated that HEBSP can improve erectile function in SHRs by upregulating the ACE2/Ang (1-7)/Mas receptor axis-cNOS and -nNOS pathways. Erectile dysfunction is a common complication of EH and a variety of antihypertensive agents can lead to ED. Therefore, there are favorable prospects for the application of clinical drugs that improve ED at the same time as lowering blood pressure. Traditional Chinese Medicine provides accumulated experience in the treatment of male sexual dysfunction in long-term medical practice, and has achieved therapeutic results. Modern studies show that HEBSP lowers blood pressure while improving erectile function. We propose that these actions could be of great significance for the treatment of patients with EH and associated ED.

ACKNOWLEDGEMENTS

The authors would like to thank all of their colleagues who contributed to this study.

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