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ORIGINAL ARTICLE

The Effect of Leonuri Herba alkaloids on Senile BPH (male and female hormone induced) model rats

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KEYWORDS

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BPH;
bFGF;
Urine flow dynamics

Abstract *Objective:* To investigate the pharmacological effects of Leonuri Herba alkaloids (LHA) on prostate hyperplasia in older rats and the effect mechanism. *Methods:* Remove bilateral testes from BPH model rats, and conduct subcutaneous injection of testosterone and estradiol. At the same time, feed corresponding drugs to the rats by gastric perfusion for 30d. In the first 27d, conduct bladder fistula surgery. Three days after feeding, carry out the detection of the urine flow dynamics. Eyeball blood taking, determination of serum E2 levels, and quickly remove the prostate, thymus gland, spleen, kidney, lung, and bladder. 1/3 prostate homogenate, determine the level of PACP, T, DHT. 1/3 prostate was determined by mRNA expression in bFGF. The remaining 1/3 prostate was observed by light microscopy. *Results:* LHA could significantly decrease the animal prostate index, level of DHT, T, PACP, and elevate levels of E2 in the serum. It could also significantly reduce the maximum voiding pressure, intercontraction interval, and bladder resting pressure. *Conclusion:* LHA has good therapeutic effect on prostatic hyperplasia model rats induced by male and female hormone.

1. Introduction

LHA (*Leonurus japonicus* Houtt.) is also known as winds, Vallisneri. Research findings have shown that motherwort contains effective components of total alkaloids (total alkaloids) (Miao et al., 2016). LHA mainly includes Leonurine and stachydrine hydrochloride and other ingredients, according to TCM treatment of uroschesis "certificate" of the main treatment. LHA has the effect of detoxification, blood circulation, and diuresis. Pharmacological studies show that LHA can improve blood rheology and microcirculation and has the functions such as anti-inflammatory and analgesic, anti oxygen free radical, potassium sparing diuretic, enhance immunity and protection of myocardial.

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2. Material

2.1. Animal

Rat, SD, male (270–300 g), male (540–630 g), provided by the experimental animal center of Shandong Province, Qualification No.: 0019881; Laboratory Certificate No.: SYXK (Lu) 2013001.

2.2. Drugs and reagents

LHA, Baoji Guokang Biological Technology Co. Ltd., concentration >80%, Production Batch No.: 20090615; Longbishu capsule, Shijiazhuang Kedi Pharmaceutical Co. Ltd., production Batch No.: 130706; Finasteride Capsules, Wuhan Humanwell Pharmaceutical LLC, Production Batch No.:20140202; Testosterone Propionate Injection, Shanghai GM Pharmaceutical Holdings C., Ltd, Production Batch No.: 130614; Estradiol Benzoate Injection, Harbin Three Beast Horse Industry Co., Ltd, Production Batch No.: 20131001; Benzylpenicillin Sodium for Injection, North China Pharmaceutical Co., ltd, specifications: 400 units, Production Batch No.: C1406807; Dihydrotestosterone (DHT) kit, Estradiol (E2) kit, Testosterone (T) kit, Prostatic acid phosphatase (PACP) kit, American R&D company, Production Batch No.: 20140702B.

2.3. Instrument

Type 680 eliasa, America BIO-RAD; UV1000 type UV VIS spectrophotometer, manufacturer: Shanghai Techcomp Instrument Co. Ltd.; KDC-160HR type high speed refrigerated centrifuge, manufacturer: Zhongjia Branch of KDCX Holdings Co., Ltd.

3. Method

3.1. Experimental grouping

Take 70 clean grade male SD rats aged 18 months, and take another 10 young rats (250–270 g) as a young control group. The eighteen-month old rats were randomly divided into 7 groups (10 in each group), and one of the groups was the control group, the other 6 groups of aged BPH model rats were divided into the model group, the Longbishu control group, the Finasteride group, and the small, middle, and large dose LHA group.

3.2. Modeling and administration

The model rats should be removed of bilateral testes, after intraperitoneal injection of 10% hydrate (0.3 ml/100 g), suture skin, and intramuscular inject of penicillin 200 thousand μ/kg, 1 time a day, 7 times a day, which continued for 30d (Liu et al., 2011). On the first day of surgery, for the large, middle small dose LHA (50 mg/kg, 25 mg/kg, 12.5 mg/kg), Longbishu suspension (dose equivalent to 10 times the clinical dosage), Finasteride (dose equivalent to 10 times the clinical dosage), feed the rats equal volume of distilled water.

On the 27th day, all animals were operated on bladder stoma. PE pipeline with normal saline was fixed to the bladder at the top of the bladder with the method of purse string suture (5–0 silk thread). The other port of PE pipe was fixed on the back of the neck of rats through rat skin. On the 30th day, cut the line on the back of the rat's neck and take out the PE pipe which was fixed on the back of the rat's neck and connect it to the urine flow dynamics instrument. After adaptation for about 1 h, the rats were fed sterile saline solution for 2 h at the rate of 120 μl/min, and 3 micturition peak data were collected. After detection, weigh the rats, take the eyeball blood, determine serum E2 levels, and quickly remove the prostate, thymus gland, spleen, kidney, lung, and bladder. 1/3 prostate homogenate was used for determining the level of PACP, T, DHT. 1/3 prostate was used for determination of mRNA expression in bFGF. The remaining 1/3 prostate was observed by light microscopy.

4. Statistical analysis

SPSS17.0 was used for statistical processing, and measurement data were expressed by ($\bar{x} \pm s$), comparison of the single factor analysis of variance. Homogeneity of variance test was conducted using the LSD method. The variance was tested with Games–Howell method.

5. Result

5.1. Effect of LHA on urine flow dynamics on aged BPH model rats (Male and female hormone induced)

Compared with the blank group of aged rats, the voiding peak pressure, the micturition interval, and the bladder pressure of the rats significantly increased in model group. Compared with the model group, the maximum pressure of micturition, micturition interval, bladder pressure ($p < 0.01$) of the rats in the Finasteride group, the Longbishu group, and the LHA large, small and middle groups significantly decreased (Table 1).

5.2. Effect of LHA on the level of T in the prostate tissue, and E2 in serum on aged BPH model rats (Male and female hormone induced)

Compared with the blank group of aged rats, the level of T and E2 significantly increased in the model group ($p < 0.01$). Compared with the model group, the level of T and E2 ($p < 0.01$) significantly decreased in the Finasteride group, the Longbishu group, and large, middle, small dose groups of LHA (Table 2).

5.3. Effect of LHA on the expression of mRNA in prostate bFGF on aged BPH model rats (Male and female hormone induced)

Compared with the blank group of aged rats, the expression of mRNA in bFGF was significantly higher in the model group ($p < 0.01$). Compared with the model group, the expression of mRNA in bFGF significantly decreased in the Finasteride group, the Longbishu group, and the large dose of LHA group (Table 3).

Table 1 Effect of LHA on urine flow dynamics on aged BPH model rats (Male and female hormone induced).

Group	N	Voiding peak pressure	Urine interval	Urinary bladder resting pressure
Youth blank group	10	32.68 ± 2.14**	9.525 ± 1.05**	504.77 ± 59.69**
Aged blank group	10	56.93 ± 14.06**	9.58 ± 0.99**	506.62 ± 65.26**
Model group	10	90.66 ± 8.14△△	13.94 ± 2.65△△	1455.934 ± 287.80△△
Finasteride group	10	68.82 ± 7.31**	10.21 ± 1.15**	543.76 ± 81.49**
Longbishu group	10	60.27 ± 3.43**	10.34 ± 1.30**	562.64 ± 102.69**
Large dose of LHA group	10	62.560 ± 3.97**	10.92 ± 1.62**	723.35 ± 116.06**
Middle dose of LHA group	10	65.33 ± 8.57**	10.77 ± 0.99**	792.78 ± 138.57**
Small dose of LHA group	10	70.63 ± 6.48**	10.88 ± 0.74**	813.65 ± 139.65**

** Indicate: compared with model group $p < 0.01$.
△△ Indicate: compared with the blank group in the elderly.

Table 2 Effect of LHA on the level of T, E2 in serum on aged BPH model rats (Male and female hormone induced).

Group	N	T (pg/ml)	E2 (pmol/L)
Youth blank group	10	65.61 ± 31.40**	9.98 ± 2.10**
Aged blank group	10	134.90 ± 48.17**	19.68 ± 4.92**
Model group	10	327.14 ± 16.40△△	32.92 ± 3.44△△
Finasteride group	10	200.41 ± 17.65**	26.57 ± 2.72**
Longbishu group	10	203.37 ± 14.32**	26.95 ± 1.33**
Large dose of LHA group	10	215.82 ± 19.84**	27.11 ± 4.43**
Middle dose of LHA group	10	222.24 ± 8.40**	27.35 ± 1.94**
Small dose of LHA group	10	226.94 ± 8.41**	27.73 ± 2.71**

** Indicate: compared with model group p < 0.01.
△△ Indicate: compared with the blank group in the elderly.

Table 3 Effect of LHA to the expression of mRNA in Prostate bFGF on aged BPH model rats (Male and female hormone induced).

Group	N	mRNA-bFGF
Youth blank group	10	0.0297 ± 0.02068**
Aged blank group	8	0.0176 ± 0.00654**
Model group	3	0.0733 ± 0.01057△△
Finasteride group	8	0.0377 ± 0.01111**
Longbishu group	8	0.0376 ± 0.01756**
Large dose of LHA group	16	0.0354 ± 0.01472**
Middle dose of LHA group	6	0.0571 ± 0.01637
Small dose of LHA group	6	0.0453 ± 0.01674

** Indicate: compared with model group p < 0.01.
△△ Indicate: compared with the blank group in the elderly.

5.4. Effect of LHA on the level of DHT, PACP on aged BPH model rats (Male and female hormone induced)

Compared with the blank group of aged rats, the level of DHT and the content of PACP in model group significantly increased ($p < 0.01$). Compared with the model group, the level of DHT and PACP ($p < 0.01$) in the Finasteride group, the Longbishu group, and the large, middle, small dose of LHA group significantly decreased (Table 4).

6. Discussion

In this experiment, the rat model of BPH was established through subcutaneous injection of testosterone and estradiol in removed bilateral testes of rats (Guo and Miao, 2016). Abnormal micturition is the most common clinical symptoms in patients with BPH (Wang et al., 2006). The normal

Table 4 Effect of LHA to the level of DHT, PACP on aged BPH model rats (Male and female hormone induced).

Group	N	DHT (nmol/L)	PACP (pg/ml)
Youth blank group	10	13.93 ± 1.94**	579.50 ± 184.86**
Aged blank group	10	17.51 ± 1.27**	707.55 ± 84.32**
Model group	10	35.29 ± 3.11△△	1436.00 ± 170.79△△
Finasteride group	10	19.69 ± 1.74**	820.80 ± 32.88**
Longbishu group	10	19.84 ± 1.16**	834.55 ± 46.90**
Large dose of LHA group	10	20.98 ± 1.40**	851.30 ± 66.03**
Middle dose of LHA group	10	21.19 ± 1.81**	895.60 ± 61.08**
Small dose of LHA group	10	17.51 ± 1.29**	926.25 ± 127.53**

** Indicate: compared with model group p < 0.01.
△△ Indicate: compared with the blank group in the elderly.

micturition process involves the bladder and the urethra. With BPH in the elderly forced structure, the change of function of the bladder and urethra, might lead to lower urinary tract symptoms. Therefore, through the determination of rat bladder pressure, the intercontraction interval was alleviated in BPH model of rats. The changes in the clinical index could also be the most intuitive reaction for the improvement of LHA to the BPH model rats. Testosterone (T) is the main androgen in the human body (Kuang et al., 2012). It is on the 5 alpha reductase function into dihydrotestosterone (DHT). The increase in the DHT level in the prostate could lead to BPH. Basic fibroblast growth factor (b-FGF) plays a role in the regulation by DHT and could be called a regulatory growth factor (Miao et al., 2015). It could lead to proliferation of epithelial cells of the prostate gland, causing the cavity to become larger and the secretion to increase, thus generating BPH (Zhang et al., 2011).

Leonuri Herba is a common medicine for activating blood circulation, and it is called "the holy blood family" in the *Compendium of Materia Medica* (Tian and Miao, 2014). It is regarded as the cure for diseases in the department of gynecology. Modern pharmacological studies show that it contains a wide range of active ingredients with a variety of pharmacological effects and is able to be used for treating a variety of diseases. The functions of promoting blood circulation and removing blood stasis, clearing away heat and toxic materials, and diuresis with Leonuri Herba, accord with the treatment mechanism of BPH. The main component of Leonuri Herba is stachydrine and leonurine, and they are the main ingredients used to treat BPH (Xiao and Miao, 2014).

This experiment shows that large, small doses of LHA group could significantly reduce the maximum voiding pressure, the intercontraction interval, the bladder resting pressure, significantly reduce DHT, T, PAC in the prostate homogenate, significantly elevate serum E2 levels, and significantly reduce the expression of mRNA in the prostate of bFGF. The effect of LHA on BPH model rats was very good. This study has provided experimental support for the clinical treatment of LHA to BPH, and also provided new ideas and methods for the prevention and treatment of BPH.

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