Original article

Effect of stachydrine hydrochloride to the prostate hyperplasia model in mice

Shuo Tian, Mingsan Miao *, Ming Bai, Yanyi Wu, Jianlian Gao, Lin Guo

Henan University of Chinese Medicine, Zhengzhou 450046, China

1. Introduction

Prostatic hyperplasia is a progressive disease that is an inevitable disease of men with an increase in age. It occurs in middle-aged and older men and can cause symptoms of lower urinary tract obstruction in severe cases (Roehrborn et al., 2005). According to epidemiological statistics, the rate of 10 per cent increase in morbidity increases with age by an average of 10 years, with an incidence of approximately 80 per cent over 80 years of age (Sarma and Wei, 2012; Stroup et al., 2012). The pathogenesis of prostatic hyperplasia is a very complicated pathological process, involving many factors (Zhu et al., 2013), mainly due to obstruction of the bladder outlet, and symptoms such as urinary frequency, urgency of micturition, urine pain, increased number of night urine, difficulty in urination, urinary retention and other symptoms (Wei, 2007; Li and Xing, 2011). Due to the complexity of prostate tissue, the treatment of prostate hyperplasia is also quite difficult. Modern medicine is a treatment for disease, has no obvious effect on the etiology, and Western medicine has many adverse reactions, unstable curative effects, and high costs, which limits clinical application.

Prostatic hyperplasia belongs to the category of “Uroschesis”, “certificate” in Chinese medicine. The most commonly witnessed clinical forms are damp-heat accumulation and blood stasis obstructing collaterals type (Xiao et al., 2009). Treatment often uses drugs such as activate blood and resolve stasis, inducing diuresis for treating strangurta, expelling toxin cooling, etc., Traditional Chinese medicine can treat the causes of the disease, and can also improve the pathological changes of related organs. Therefore, the development of Chinese medicine for the treatment of prostatic hyperplasia has a greater advantage.

2. Materials and methods

2.1. Experimental animal

KM male mice (25 ~ 30 g), were purchased from Henan Medical Laboratory Animal Center. Animal permit number::0003912. the lab certificate No. SYXK (Henan) 2010-001.
2.2. Drugs and reagents

Stachydrine hydrochloride, Sichuan Institute of natural active ingredients, the content is more than 90%, Batch number: 20091212; Finasteride Capsules, Jiangsu Yabang Johnson Pharmaceutical Co Ltd, batch number: 080714; formaldehyde, Zhengzhou painl chemical reagent factory, Batch number: 20090401; Sodium Chloride Injection, Zheng Zhouyong and pharmaceutical Limited by Share Ltd, Batch number:20091210; Benzylpenicillin sodium injection, North China Pharmaceutical Limited by Share Ltd, Batch number: Y0903319; dihydrotestosterone (DHT), RD, batch number:20091216; total acid phosphatase (ACP), nonspecific acid phosphatase (non PACP), prostatic acid phosphatase (PACP), Nanjing Jiancheng Biological Engineering Institute, Batch number:20091228; bFGF, TGF-, EGF, beta 1 I IGF- kit were purchased from Wuhan boster Biological Engineering Co Ltd.

2.3. Experimental Instrument

FA (N)/JA (N) series electronic balance, Shanghai Minqiao Precision Instrument Co Ltd; TGL-168 centrifuge, Shanghai Anting scientific instrument factory; UV-2000 UV VIS spectrophotometer, unique (Shanghai) Instrument Co Ltd.; Type 680 enzyme mark instrument, United States BIO-RAD company; X51 Microscope, Olympus; Image Pro plus 6.0, Media Cyparts.

2.4. Experimental methods

Taken 60 old and male KM mice (25 ~ 30 g) which were 7 weeks, and they were randomly divided into 6 groups. 10 mice was randomly selected as the blank group (BG), and they were all did the false operation. And the remaining mice were used to the prostatic hyperplasia mice, they respectively were the model group (MG), Finasteride group (FG), stachydrine hydrochloride high, medium and low doses group (SHH, SHM, SHL).

Urogenital sinus preparation: taken SPF level sexually mature mice (25 ~ 30 g), they were raised by the pattern of male and female 2:1 in cage, and they were checked vaginal suppository every morning, the vaginal door were opened with small tweezers, the semen coagulation were saw in the vagina after mated into a white embolus which blocked in the vagina, it indicated that the mated success. The date on which the Yin plug appears as the first day of pregnancy, the mother mice who was pregnant for 16 days was executed, 16 day old fetal mice were taken out, and the urinary reproductive sinus was placed in a glass plane dish containing saline.

The preparation method of prostatic hyperplasia animal model in mice: After the mice were successfully anesthetized by 10% chloral hydrate (30 ml/kg) intraperitoneal injection, the abdomen opened by the sterile operation, the abdominal leaves of the prostate were carefully separated, and under the body vision microscope, the urinary reproductive sinus tissue of three 16-day-old identical feral mice were implanted into the abdominal leaves of the prostate. The mice of BG only used needles to probe the abdominal prostate 3 times, and immediately sewn and intramuscular penicillin to prevent infection. For 3 consecutive days, once a day, after 3 days. The prostatic hyperplasia mice respectively were divided MG, FG, SHH, SHM, SHL.

Method of administration: At the third day of model, SHH, SHM, SHL were given the stachydrine hydrochloride suspensions (90 mg/kg, 45 mg/kg, 22.5 mg/kg, equivalent to the clinical dose of 30, 15, 7.5 times) by 0.2 ml/10 g, FG were given the Finasteride suspensions (15 times of clinical dosage dose equivalent) by 0.2 ml/10 g. BG and MG were given equal volume of distilled water, one time a day, for 3 consecutive weeks.

Testing indicators: Mices in each group after the last administration of 2 h (fasting for 12 h), abdominal anesthesia, the blood was taken from the abdominal aorta, the serum and plasma were separated (4 °C, 3500 rpm, 10 min), stored in a refrigerator at −80 °C. The levels of DHT in serum measured by enzyme immunoassay. The levels of ACP, non PACP measured by colorimetry, the specific method of operation was performed according to instructions. After taken blood, the mice were sacrificed, taken the prostate quickly and weigh, calculate the average of the wet weight of the prostate in each group and the prostate index(prostate weight /the body weight of mice(g)). The prostate was fixed with 4% polyformaldehyde, and the expression of bFGF, EGF, IGF-I, and TGF-β1 in the prostate tissue of the model animal was determined by immunohistochemical method. The average optical density (MOD) of each growth factor was determined by the Image Pro plus 6.0 software, 5 fields were taken under each slice of light mirror, MOD = cumulative optical density (IOD)/its integral area(area); Taken the prostate, kidneys, spleen, and thymus and fix it with 10% formaldehyde to observe the histopathological changes.

2.5. Statistical processing method

Data analysis uses the SPSS21.0 medical statistical package to perform statistical processing of data, and the metrological data are expressed as mean ± standard deviation(−x ± s), the comparison between the groups uses a single factor variance analysis. The LSD method was used for the variance test homogeneity, the Games-Howell method was used for the variance test uneven, and the Ridit test was used for the grade data.

3. Results

3.1. Effects of wet weight of prostate, prostate index and serum DHT in the prostate hyperplasia mice model

It can be seen from Fig. 1, compared with the BG, wet weight of prostate and prostate index were significantly increased in model mice (P < 0.01), the content of DHT in serum of model mice was significantly increased (P < 0.01), it shows that the model copied successfully. Compared with the MG, FG could significantly reduced the prostate wet weight (P < 0.05), the prostate index and the level of DHT in serum (P < 0.01) in prostatic hyperplasia model mice. SHH, SHM, SHL could not significantly affect on the wet weight of prostate. SHL could significantly reduced the prostate index (P < 0.01) and the level of DHT in serum (P < 0.01), SHM could reduced the prostate index (P < 0.05). The experimental results show that, FG, SHM, SHL has the effect of inhibited prostatic hyperplasia on urogenital sinus implantation in an prostate hyperplasiamice model.

3.2. Effects of total acid phosphatase (ACP), nonspecific acid phosphatase (non PACP), prostatic acid phosphatase (PACP) level of the prostate hyperplasiamice model serum

As shown in Fig. 2, compared with the BG, the level of PACP were significantly increased (P < 0.01), the ACP level were significantly increased (P < 0.05) in serum of MG. The level of non-PACP in each serum of groups had no statistical significance. Compared with the MG, FG, SHM could significantly decreased the level of PACP in serum of model mice (P < 0.01); compared with MG, SHH, SHL has no statistical significance, but it has a tendency to decreased the of PACP in serum. SHM could reduced the level of PACP in serum of prostatic hyperplasia model mice, it is prompted that the mechanism of stachydrine hyperplasia in the model mice may be related to the decrease of prostatic acid phosphatase level.
3.3. Effects of the expression of TGF-β1, EGF, IGF-I in prostate tissue of prostatic hyperplasia model mice

As can be seen from Fig. 3, compared with BG, the expression of TGF-β1, EGF, IGF-I was increased in MG significantly (P < 0.01). Compared with the MG, FG, SHM, SHL could significantly increased the expression of TGF-β1 (P < 0.05); FG could significantly reduced the expression of EGF (P < 0.01); SHH, SHM, SHL could reduced the expression of EGF (P < 0.05); FG, SHM could significantly reduced the expression of IGF-I (P < 0.01); SHH, SHL could reduced the expression of IGF-I (P < 0.05). The experimental results suggested that FG, stachydrine hydrochloride could inhibited the urogenital sinus method cause the prostate hyperplasia mice model, and it prompted that the mechanism of stachydrine hydrochloride in the model mice may be related to the expression of TGF-β1, EGF, IGF-I growth factor.

3.4. Effect of prostate tissue morphology in prostatic hyperplasia model mice

As can be seen from Fig. 4, No hyperplasia, inflammatory cell infiltration and hyperemia were found in the epithelial cells and interstitial tissues of prostate gland of mice in BG. The prostate gland epithelial cells of mice in MG showed obvious hyperplasia, which filled the entire gland cavity with hyperplasia of gland epithelial cells, and the interstitial tissue also showed different degrees of hyperplasia. The hyperplasia of prostate gland epithelial cells was significantly inhibited in FG, only a few glandular cavities were filled with hyperplasia of glandular epithelial cells, and a mild degree of hyperplasia was observed in the interstitial tissue. The hyperplasia of adenoeplithelial cells was obvious in the prostate gland of mice in SHH, which filled part of the glandular cavity with hyperplasia of adenoeplithelial cells, and it had seen with different degrees of hyperplasia in interstitial tissue. The hyperplasia of prostate gland epithelial cells was significantly inhibited in mice in SHM, but only a few glandular lumens were filled with hyperplasia of glandular epithelial cells, and a mild hyperplasia was observed in the interstitial tissue. The hyperplasia of prostate gland epithelial cells of mice in SHL was inhibited in some extent, only a few glandular lumen was filled with hyperplasia of glandular epithelial cells, and the interstitial tissue also showed moderate hyperplasia. The pathological results showed that the urogenital sinus implantation method caused hyperplasia of prostate gland epithelial cells in mice, which filled the cavity, and it had different degrees of hyperplasia in the interstitial tissue. The model was copied successfully, and the stachydrine hydrochloride could inhibit
the hyperplasia of prostate epithelium and interstitial tissue in the model mice.

As can be seen from Table 1, by Ridit test, compared with BG, the pathological changes of prostate hyperplasia were significant in MG \((P < 0.01)\). Compared with MG, SHH, SHM, SHL could reduced the pathological changes prostatic hyperplasia \((P < 0.01)\); FG could obviously reduced the pathological changes of prostate hyperplasia \((P < 0.05)\).

3.5. Effect of kidney tissue morphology in prostatic hyperplasia model mice

As can be seen from Fig. 5, the pathological changes of glomerulus, renal vesicle, renal tubules and renal interstitial matter in MG were normal. In MG, the proliferation of glomerular cells in mice which caused the disappearance of the renal vesicle, the renal tubules epithelial cells showed mild edema, and the lumen disappeared. In FG, the slight proliferation of cells in the glomerular cell which causes the renal vesicle to become smaller, and some renal tubules have mild edema and lumen narrowing. In SHH, the obvious proliferation of glomerular cells which causes the renal vesicle to disappear obviously, and the edema of renal tubules epithelial cells has recovered to a certain extent. In SHM, the proliferation of glomerular cells significantly reduced the renal vesicle, and the edema of renal tubules epithelial cells disappeared. In SHL, the obvious proliferation of glomerular cells which causes the disappearance of the renal vesicle, and the edema of the renal tubules epithelial cells was disappeared. Pathological results showed that the model mice induced by urinary reproductive sinus implantation had certain effects on the kidneys, which could made glomerular cell hyperplasia, renal tubule edema, and the stachydrine hydrochloride have antagonistic effects on glomerular cell hyperplasia and renal tubule edema.

The pathological changes of hyperplasia and edema of glomerular and tubule epithelial cells in different groups of mice were determined by semi-quantitative criteria. As can be seen from Table 2, by the Ridit test, compared with BG, there were significant pathological changes of the kidney in MG \((P < 0.01)\). Compared with MG, FG could significantly reduced the kidney pathological changes \((P < 0.01)\); stachydrine hydrochloride had no obvious effect on kidney.

3.6. Effect of thymus tissue morphology in prostatic hyperplasia model mice

As can be seen from Fig. 6, in BG, the thymic lobule clear boundaries, dividing the cortex and medulla of the clear, thick dense cortical lymphocytes were normal. In MG, the thymus of mice were significantly atrophy, the lobule was disappeared, the boundaries of cortex and medulla were not clear, the cortex thinning was obvious, the lymphocytes was sparsed. In FG, the thymus lobules were clearly demarcated, the cortex and medulla were not clearly demarcated, the cortex was obviously thinner and the lymphocytes was sparsed; In SHH, the thymus lobules was clearly demarcated, and the cortex and medulla were clearly demarcated, the cortex was thinner and the lymphocytes were more dense; In SHM, the thymus gland was significantly atrophic, the lobules were clearly demarcated, the cortex and medulla were clearly demarcated, the cortex was significantly thinner, and the lymphocytes were sparse; In SHL, the thymus gland was atrophic, the lobules were clearly demarcated, and the cortex and medulla were clearly demarcated, the cortex was thinner and the lymphocytes were more dense. The results of pathological experiments showed that the thymic cortex of mice with prostate hyperplasia induced by urinary reproductive sinus implantation was atrophied, and the thymic cortex atrophied was inhibited to some extent by stachydrine hydrochloride. The results of pathological experiments showed that the effect of urinary reproductive sinus implantation on the atrophy of thymus cortex in mice induced by prostatic hyperplasia and the inhibition of hydrochloric acid on thymus cortex atrophy were observed.

![Fig. 4. Effect on pathological morphology of prostate tissue in mice with benign prostatic hyperplasia (HE × 100).](image-url)
The thymic cortex thickness was measured by micrometers and the number of cells was calculated by measuring the baseline pressure of micrometers on cells. The results of measuring the thickness and lymphocyte in different degrees in each group animals were shown in Fig. 7. As can be seen from Fig. 7, compared with BG, the thickness of cortex and the number of lymphocytes in MG was significantly reduced ($P < 0.01$). Compared with MG, the thickness of each cortex medication and lymphocytes were increased significantly ($P < 0.01$). The hyperplasia model of the prostate caused by uretic sinus implantation resulted in thymic cortical lesions, and the stachydrine hydrochloride could improve the pathological changes of the thymus, it suggested that the stachydrine hydrochloride had the effect of improvement immune.

3.7. Effect of spleen tissue morphology in prostatic hyperplasia model mice

As can be seen from Fig. 8, in BG, the red and white pulp boundaries of the spleen were clear, the splenic knots and sinuses were normal. In MG, the boundary between spleen red pulp and white pulp was clear, the splenic node was significantly reduced, the lymphocytes were sparse, and the spleen sinus was normal. In FG, The boundary between spleen red pulp and white pulp is clear, the splenic node was obviously increased, the lymphocytes were dense, and the spleen sinus was normal. In SHH, the red and white pulp of the spleen were clearly demarcated, the spleen was slightly enlarged, the lymphocytes were dense, and the spleen sinuses were normal. In SHM, the red and white pulp of the spleen were clearly demarcated, the spleen was enlarged, the lymphocytes were dense, and the spleen sinuses were normal. In SHL, the

| Group  | n  |   |   |   |   |
|--------|----|---|---|---|---|
| BG     | 10 | 10| 0 | 0 | 0 |
| MG     | 10 | 0 | 0 | 2 | 8 |
| FG     | 10 | 0 | 0 | 5 | 5 |
| SHH    | 10 | 0 | 0 | 3 | 7 |
| SHM    | 10 | 0 | 0 | 4 | 6 |
| SHL    | 10 | 0 | 0 | 4 | 6 |

Note: “-” There was no hyperplastic edema in the glomerular and tubular epithelial cells; “+” The glomerular cells proliferated and the renal tubules were normal; “++” The epithelial cells of glomeruli and tubules proliferate and edema, and the renal capsule and tube cavity shrink; “+++” Hyperplasia and edema of glomerular and tubular epithelial cells, which lead to the complete disappearance of the renal capsule and tubule lumen.
boundary between spleen red pulp and white pulp was clear, the splenic nodules was significantly reduced, the lymphocytes were sparse, and the splenic sinuses were normal.

The method of microscale were used to measure the size of the spleen, the thickness (thickness = thickness of both sides/2) of the spleen was measured centered on the splenic node artery, and the average number of three splenic nodes was measured which was the thickness of the spleen, and the lymphocyte cell number was calculated by pressing the baseline of the micrometer on the cells. The results of different degrees of thickness and lymphocyte count in each group of animals were measured in Fig. 9. As can be seen from Fig. 9, compared with BG, the cortex thickness and the number of lymphocytes of MG was significantly reduced (P < 0.01). Compared with MG, the cortex thickness of each groups and the number of lymphocytes were increased significantly (P < 0.01). It prompted that the model of prostatic hyperplasia caused by urinary reproductive sinus implantation has the effect of caused splenic nodules lesions, and stachydrine hydrochloride could improved the spleen pathology, it suggested the stachydrine hydrochloride could enhance the immunologic function.

Fig. 7. Effect of the cortical thickness and lymphocytes number in prostatic hyperplasia model mice. (A): Cortical thickness (μm); (B): Lymphocyte count (N). In A, B, “a” represents a significant difference (P < 0.01) between the different administration groups compared with MG, and “b” represents there were significant differences between the different drug groups compared with MG (P < 0.05), n = 10 mices/group.

Fig. 8. Effect of spleen tissue pathological morphology in prostatic hyperplasia mice (HE × 100).

Fig. 9. Effect of cortical thickness and the lymphocytes count in prostatic hyperplasia model mice of splenic nodules. (A): Cortical thickness (μm); (B): Lymphocyte count (N). In A, B, “a” represents a significant difference (P < 0.01) between the different administration groups compared with MG, and “b” represents there were significant differences between the different drug groups compared with MG (P < 0.05), n = 10 mices/group.
4. Discussion

This research adopts the urogenital sinus implantation making the prostatic hyperplasia mice model. This model can reflect the mutual influence between the organization and the environmental hormone in human prostate hyperplasia occurred in the development of the role, it is now recognized as an animal model accords with the process of human disease of prostate hyperplasia (Xu et al., 2011). Prostatic hyperplasia model induced by fetal urogenital sinus lumen catheter, SHH was abundantly expressed in the proximal ductal segment and lumen of the urinary reproductive sinus, which was important for the growth of prostate gland, and it can reflect the mechanism of interstitial epithelial interaction in the pathogenesis. The pathogenesis of prostate hyperplasia is still not completely clear, currently, there are hormone endocrine theory, growth factor (pathway) theory and apoptosis theories (Zhu et al., 2013), and the growth factor theory plays an important position and role in the pathogenesis of prostate hyperplasia (Tang and Miao, 2012), closely associated with prostatic hyperplasia of peptide growth factors mainly include bFGF, TGF-β, EGF, IGF-I, and the bFGF, TGF-β is the major growth factor of interstitial hyperplasia, EGF is the main growth factor of epithelial hyperplasia (Grabowska et al., 2012), IGF-I can stimulate the prostatic epithelial cell growth. PACP is secreted by epithelial cells and synthesis in the prostate gland; when the prostate hyperplasia, the activity of PACP in serum will increased (Liao and Jiang, 2006; Wu et al., 2003). In addition, the concentration of DHT in the prostate can lead to the increase of glandular hyperplasia (Liu et al., 2012). Prostate tissue morphology is key to determining whether the occurrence of benign prostatic hyperplasia. It has been reported that prostatic hyperplasia in mice may involve atrophy of related organs such as thymus and spleen (Zhang et al., 2009).

Some studies have confirmed that the immune system plays an important role in the occurrence and development of prostate gland. Thymus and spleen, as immune organs of the body, are of great significance to study the morphological changes. This study observed the stachydrine hydrochloride effect on the express of TGF-β1, EGF, IGF-I, which are the direct cause of prostatic hyperplasia, study the growth factor in vivo changes and characteristics of action, study the relevant organizations of prostate tissue, thymus, spleen, kidney and others.

Motherwort has the effects of promoting blood circulation to remove blood stasis, heat clearing and detoxifying, diuresis, which is aim to the main pathogenesis of prostatic hyperplasia, it is the main treatment of Chinese medicine for treating "uroscesis" and "certificate" (Wei and Li, 2009). Previous studies in this laboratory have shown that the drugs commonly used in gynecology to promote blood circulation and remove blood stasis, such as safflower and safflower, which have obvious therapeutic effects on rats, mice models of prostate hyperplasia (Liu et al., 2011). Modern pharmacological research also shows that blood stasis drug can promote the body's blood circulation and organization metabolism, thereby which improving the prostate blood circulation, and promoting the absorption and dissipation of glandular tissue (Hou and Chen, 2003). In addition, Motherwort herb is a moderate potassium sparing diuretic drug (Gu and Gu, 2008), the stachydrine hydrochloride as its main ingredient, in recent years, for the determination containing content of stachydrine hydrochloride in Motherwort formulations, there are many studies (Zhong, 2015; Liu et al., 2014; Wang and Wen, 2014; Miao et al., 2017), and its main component is stachydrine hydrochloride. Motherwort has many clinical reports that it was been putted into the compound for the treatment of prostatic hyperplasia, and the effect is better (Liu et al., 2011).

5. Conclusions

The experimental results show that stachydrine hydrochloride could significantly reduced the levels of DHT and PACP in serum, reduced the PACP, elevated the expression of TGF-β1, significantly reduced the expression of EGF and IGF-1, stachydrine hydrochloride also could significantly reduced the pathological changes of prostate hyperplasia, and significantly increased the cortical thickness and number of lymphocytes of thymus. It can also improved the pathological changes of thymus, significantly increased the cortical thickness and lymphocyte count, and improved the spleen pathological changes. But there is no obvious influence on the kidney pathological changes, its role remains to be further in-depth study. This study provides a reliable basis for the treatment of prostatic hyperplasia of Motherwort, and it provides a new method and idea for the treatment of prostatic hyperplasia. It also promoted the clinical application of Motherwort, and it also promoted the use of traditional Chinese medicine in the treatment of modern diseases.

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Author contributions

Miao Mingsan conceived and designed the experiments the experiments, Tian Shuo, Bai Ming, Gao Jianlian and Guo Lin performed the experiments and analysed the data, Miao Mingsan made the final conclusion, Tian Shuo wrote the paper. Both authors reviewed the manuscript.

Conflicts of interest

There are no conflicts of interest.

References

Grabowska, M.M., Sandhu, B., Day, M.L., 2012. EGF promotes the shedding of soluble E-cadherin in an ADAM10-dependent manner in prostate epithelial cells. Cell Signal 24, 532–538.
Gu, Y.L., Gu, J.L., 2008. Progress of pharmacological research of Herba Leonuri. China Chin. Med. Sci. Technol. 15, 320.
Hou, S.L., Chen, J.R., 2003. Herba Leonuri for the treatment of benign prostate hyperplasia has good effect. China Folk Med. J. 62, 184–185.
Li, H.H., Xing, D., 2011. Suprapubic prostatic resection and transurethral resection of prostate for the treatment of diseases in Department of Urology control research. Modern Med. 17, 15–16.
Liao, Z.Y., Jiang, J.L., 2006. Effect of Qianlie granules on prostatic hyperplasia in rats serum acid phosphatase and T and E2. J. Practical Med. 23, 325–337.
Liu, F.F., Zhao, Z.Q., Pang, L.H., 2014. Determination of stachydrine hydrochloride in lionuri granule by HPLC-ELSD. Asia-Pacific Traditional Med. 10, 56–58.
Liu, S.Y., Cao, S., Miao, M.S., 2011. Analysis of the pharmacological effects of traditional Chinese medicine in the treatment of benign prostatic hyperplasia. J. Clin. Med. 11, 1326–1329.
Liu, S.Y., Zuo, T., Bai, M., Miao, M.S., 2012. Effect of Wuji Baifeng Wan on mouse model of benign prostate hyperplasia. Chin. J. Exp. Traditional Med. Formulae 18, 180–183.
Miao, M.S., Tian, S., Bai, M., Xiang, L.L., Gao, J.L., 2017. Effect of Motherwort total alkaloids on the prostate hyperplasia mice model of pathological changes of related tissue morphology induced by the fetal urogenital sinus implants. Saudi Pharm. J. 25, 601–606.
Roehrborn, C.G., Siami, P., Barkin, J., Damiao, R., Recher, E., Minana, B., Mironé, V., Castro, R., Wilson, T., Montors, F., 2009. The influence of baseline parameters on changes in international prostate symptom score with dutasteride, tamsulosin,
and combination therapy among men with symptomatic benign prostatic hyperplasia and enlarged prostate: 2-year data from the Combat study. Eur Urol 55, 461–471.

Sarma, A.V., Wei, J.T., 2012. Clinical practice Benign prostatic hyperplasia and lower urinary tract symptoms. N. Engl. J. Med. 367, 248–257.

Stroup, S.P., Palazzi-Churas, K., Kopp, R.P., Parsons, J.K., 2012. Trends in adverse events of benign prostatic hyperplasia (BPH) in the USA, 1998 to 2008. BJU Int. 109, 84–87.

Tang, P.P., Miao, M.S., 2012. Methods of screening for prevention and treatment of benign prostatic hyperplasia drugs. J. Tradit. Chin. Med. 27, 594–599.

Wang, M.D., Wen, C., 2014. Determination of Stachydrine Hydrochloride in Fukang Tablets by HPLC. Inner Mongolia Petrochem. Ind. 40, 20–21.

Wei, L.C., Li, Q.J., 2009. Progress of pharmacological and clinical study of Leonurus. Northwest Pharm. J. 24, 133.

Wei, Q., 2007. Treatment of benign prostatic hyperplasia. J. Clin. Treat. 5, 18.

Wu, J.H., Sun, Z.Y., Zhong, E.H., Zhu, Y., Liu, G.M., He, G.L., Cao, L., 2003. The vas deferens pathological canine prostate hyperplasia model changes in Beagle. Chin. J. Pharmacol. Toxicol. 17, 223–226.

Xiao, Y.P., Li, Z.S., Wang, Bo, Zhou, Z.G., 2009. Clinical study of Western medicine combined with Wuhuang decoction by self in the treatment of type III prostatitis. China Pharm. 20, 2383–2384.

Xu, D.H., Jiang, G.J., Mei, X.T., Xu, S.B., 2011. Pharmacological effects of Syngnathus on benign prostatic hyperplasia model of mice induced by fetal urogenital sinus. Chin. J. Mar. Drugs 30, 31–35.

Zhang, Z.M., Miao, M.S., Zhang, Y.L., 2009. Effect of bastard speedwell TFBS on mouse prostate and related organization form. China Modern Appl. Pharm. 26, 112.

Zhong, Y., 2015. Determination of stachydrine hydrochloride content in Yimu Granules by HPLC. China Pharm. 24, 32–34.

Zhu, S.S., Wu, J.H., Sun, Z.Y., 2013. Research progress in the pathogenesis of benign prostatic hyperplasia. J. Toxicol. 27, 387–390.