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# 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



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## ABSTRACT

**Aim:** The research was to study the effect of Motherwort total alkaloids on the prostate hyperplasia mice model of pathological changes of related tissue morphology.

**Results:** Compared with the model group(MG), Motherwort total alkaloid high, medium dose group(HD, MD) could significantly reduced the pathological changes of the prostate ( $P < 0.01$ ); Finasteride(FG) and Motherwort total alkaloid low dose group(LD) could significantly reduce the pathological changes of the prostate ( $P < 0.05$ ); Longbishu capsules(LG), Finasteride, Motherwort total alkaloid medium dose group could significantly reduce the pathological changes of the kidney ( $P < 0.01$ ); Motherwort total alkaloid low dose group could significantly reduce the pathological changes of the kidney ( $P < 0.05$ ); Motherwort total alkaloids could improve the pathological changes of the thymus and spleen.

**Conclusion:** Motherwort total alkaloid can improve the pathological changes of prostatic hyperplasia in mice.

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## 1. Introduction

Traditional Chinese medicine regards the body as in a whole, if an organ has problems, it will involves other related organs, so does the whole body. Western medicine believes in the main hormone endocrine theory, growth factors, and cell apoptosis theory. Traditional Chinese Medicine lists it as “Longbi”, “cream card” category (Iftakhar et al., 2015; Xiao and Miao, 2014), common pathogenesis, blood stasis and gas depression, spleen, kidney deficiency etc. It often uses the treatment principles of activating blood to resolve stasis, inducing diuresis for treating stranguria, spleen and kidney deficiency and so on. Motherwort has the effect of activating blood to resolve stasis, inducing diuresis for treating stranguria, clearing away heat and toxic materials. It aims at the main pathogenesis of prostate hyperplasia, improving the pathological changes of related tissues (Zhao and Ashraf, 2016; Huang et al.,

2015; Sarfraz et al., 2016). This paper researches the effect of Motherwort total alkaloids on the prostate hyperplasia mice model of pathological changes of related tissue morphology.

## 2. Material and methods

### 2.1. Experimental animals

KM Mice, male, Weight 25–30 g, was supplied by the Experimental Animal Center of Henan Medical, with Animal Permit Number: 0003912.

### 2.2. Experimental drugs and reagents

Motherwort total alkaloids were supplied by Baoji Guokang Biological Technology Co., Ltd., content >80%, Batch No.: 20090615; Longbishu Capsules, Shijiazhuang Cody Pharmaceutical Co., Ltd., Batch No.: 090106; Finasteride capsules, Jiangsu Yabang Johnson Pharmaceutical Co., Ltd., Batch No.: 080714; Formaldehyde, Zhengzhou Paini Chemical Reagent Factory, Batch No.: 20090401 Penicillin Sodium for Injection, North China Pharmaceutical Co., Ltd., Batch No.: Y0903319.

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### 2.3. Experimental instrument

Olympus X51 microscope, from Olympus Company; Image pro plus 6.0, from Media Cybernetics Company.

### 2.4. Experimental methods

We selected 70 7-week-old KM mice, male, weight 25–30 g. They were randomly and uniformly divided into seven groups—one group was the blank control group (BG), with sham surgery; the other six groups were modeled, respectively.

The fetal urogenital sinus was prepared: take clean level of sexual mature mice, weight 25–30 g, with the ratio of male and female (2:1) in a cage. Check vaginal suppository every morning, and use tweezers open vulva, when the semen was in the vaginal solidified into a white emboli, and then block it in the vagina. It indicated that it had mated. As the first day of pregnancy when vaginal plug appeared, kill 16 d pregnant female mice, and remove the 16 d fetal mice. Place the fetal urogenital sinus in a glass flat dish containing saline, and reserve it.

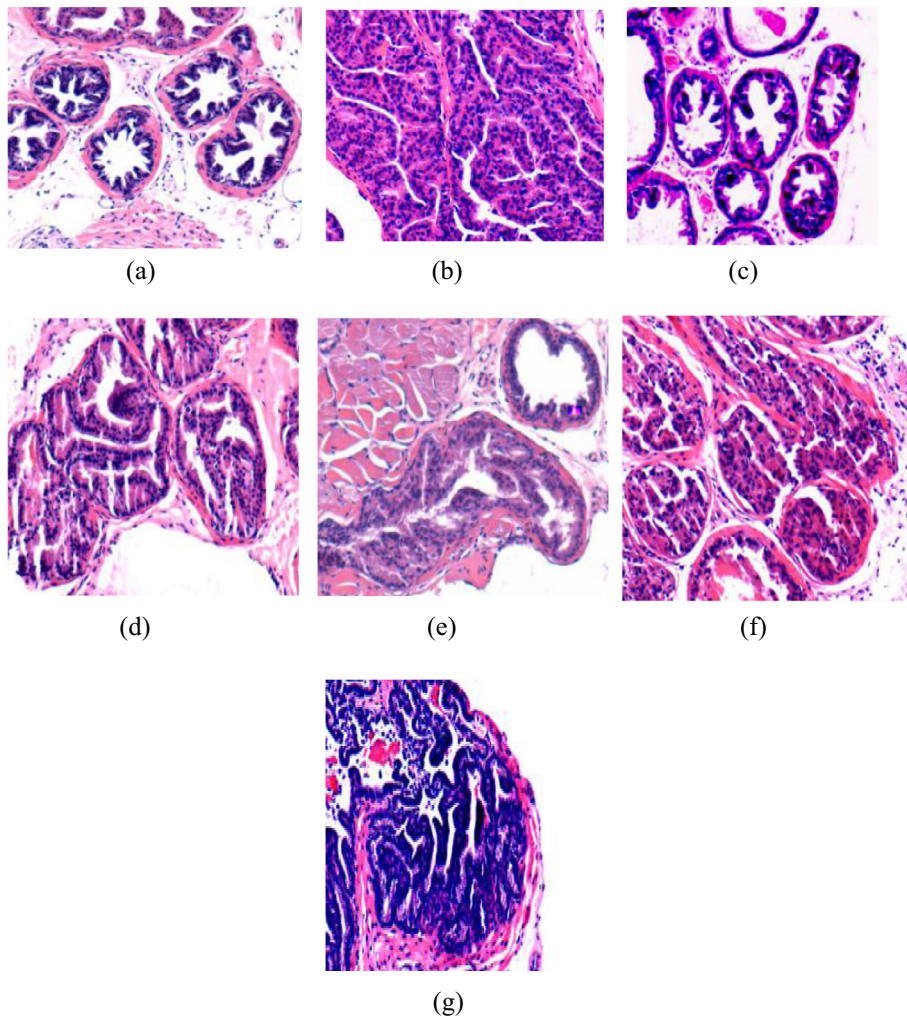
After successful anaesthetizing that model mice by using 10% chloral hydrate (30 ml/kg) to intraperitoneal injection, cut and open the abdomen. Carefully separate the ventral prostate, and take three fetal urogenital sinus tissues from 16d gestational age with the strains of fetal rats. Then implant it into ventral prostate under the stereomicroscope. The mice in the blank control group

were only probed thorn ventral prostate 3 times, and then immediately suture and intramuscular injection of penicillin to prevent infection, 1 times a day for 3 days. After that, divide the model mice random into five groups (10 rats in each group)—model group, positive control group, Motherwort total alkaloids of high, medium and low dose groups. Based on built model, according to the 0.2 ml/10 g, we obtained HD, MD, and LD suspension (75 mg/kg, 37.5 mg/kg, 19 mg/kg, equivalent to 30, 15, and 7.5 times of clinical dose), LG and FG (equivalent to 15 times of clinical dose). The blank group and the model group were given equal volume of distilled water, 1 times a day for 3 weeks.

After the last administration of 24 h, kill the mices, and quickly take prostate, kidney, thymus, and spleen tissue, fixed by 4% paraformaldehyde. Then, observe the pathological changes of the prostate, kidney, thymus, spleen tissue morphology.

### 2.5. Statistical analysis

Data was analyzed by using SPSS 17 for windows statistical package for statistical data, and the measurement data use the average add and subtract the standard deviation, Comparison between the groups use the analysis of variance; If the variance test was together, it will use the smallest used least significant difference (LSD) method. If the variance was not together, it will used with the Games-Howell test methods, and the date for the grade data was used the Ridit test.



**Fig. 1.** Pathological observation results of prostate tissue in each group mice (HE\*100).

**Table 1**  
Effect of the prostate tissue in each experimental group mice (units: a).

Group	n	–	+	++	+++
BG	10	10	0	0	0
MG	10	0	0	3	7
LG	10	8	2	0	0
FG	10	0	1	7	2
HD	10	0	6	4	0
MD	10	0	5	5	0
LD	10	0	2	5	3

Note: “–” glandular epithelial cells and interstitial tissue without hyperplasia; “+” few glandular epithelial cells appear with hyperplasia; “++” part of glandular epithelial cells appear with hyperplasia, and interstitial tissue appears with mild hyperplasia; “+++” most glandular epithelial cells appear with hyperplasia, and interstitial tissue appear with medium hyperplasia.

### 3. Results

#### 3.1. Effect of the prostate tissue morphology in prostate hyperplasia mice

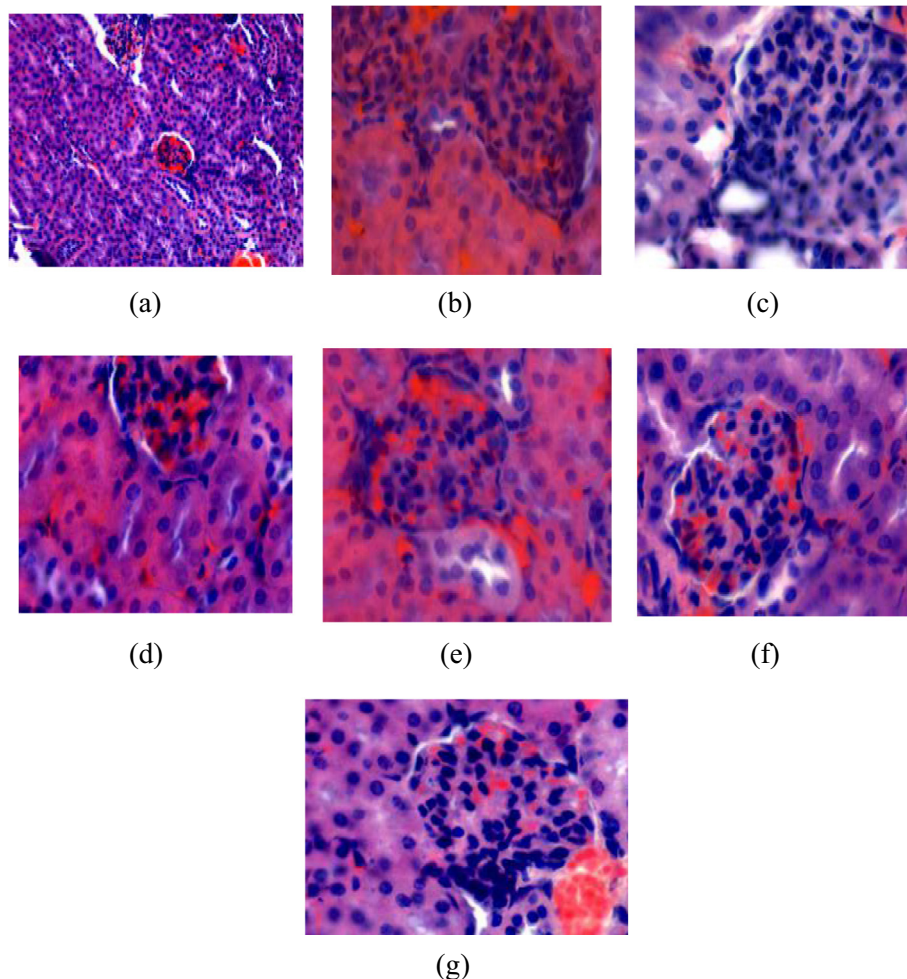
The BG mice are normal (see Fig. 1(a)). In MG mice the glandular epithelial cells obviously appear with hyperplasia phenomenon, and interstitial tissue also shows different degrees of hyperplasia (see Fig. 1(b)). In the LG mice the glandular epithelial cells has significantly inhibited, and interstitial tissue also shows mild degree

of hyperplasia phenomenon (see Fig. 1(c)). In the FG mice the glandular epithelial cells have significantly inhibited, and interstitial tissue also shows mild degree of hyperplasia phenomenon (see Fig. 1(d)). In the HD mice the glandular epithelial cells have inhibited to certain degree, and interstitial tissue hyperplasia is significantly inhibited (see Fig. 1(e)). In the MD mice the glandular epithelial cells have inhibited, and interstitial tissue also has mild degree of hyperplasia phenomenon (see Fig. 1(f)). In the LD mice the glandular epithelial cells have hyperplasia, and interstitial tissue also shows different degrees of hyperplasia (see Fig. 1(g)).

According to semi-quantitative criteria measuring the prostatic hyperplasia of each experiment group mice, Table 1 shows the result. Compared with the BG, the MG mice appear with significant pathological change of the prostatic hyperplasia ( $P < 0.01$ ). Compared with the MG, HD, MD, and LD, it can significantly alleviate the prostate hyperplasia of pathological change ( $P < 0.01$ ); FG and LD can significantly decrease the prostate hyperplasia of pathological changes ( $P < 0.05$ ).

#### 3.2. Effect of the kidney tissue morphology in prostate hyperplasia mice

The BG mice are normal (see Fig. 2(a)). Mice in MG the renal cysts disappear, and renal tubular epithelial cells appear with mild edema, obliteration of the lumen (see Fig. 2(b)). LG mice in the renal cysts disappear, and the renal tubular is normal (see Fig. 2



**Fig. 2.** Pathological observation results of kidney tissue in each group mice (HE\*400).

**Table 2**  
Effect of experimental groups on prostate hyperplasia model of mice kidney.

Group	n	–	+	++	+++
BG	10	10	0	0	0
MG	10	0	0	2	8
LG	10	0	8	2	0
FG	10	0	2	8	0
HD	10	0	0	4	6
MD	10	0	3	7	0
LD	10	0	0	6	4

Note: “–” indicates glomerular and renal tubular epithelial cell hyperplasia goiter without water; “+” glomerular cell proliferation and normal renal tubules; “++” the renal capsule and the tubular lumen narrowing; “+++” the kidney vesicle and tubule completely disappear.

(c). FG mice in the renal cysts are slightly smaller, with mild edema lumens narrowing part of renal tubular epithelial cells (see Fig. 2(d)). HD mice in the renal cysts disappear, and so is the renal tubular epithelial cell edema, (see Fig. 2(e)). MD mice in the renal cysts is narrowed, with the renal tubular epithelial cell edema recovered (see Fig. 2(f)). LD group mice in the renal cysts are narrowed, and the renal tubular epithelial cell edema has a certain extent recovery (see Fig. 2(g)).

According to the semi-quantitative criteria determination, Table 2 shows the result of varying degrees of glomerular and tubular epithelial cells accumulate edema pathology.

Compare with the BG, the renal pathological of the MG is significantly changes ( $P < 0.01$ ). Compare with the MG, LG, FG and MD can significantly reduce the renal pathological changes ( $P < 0.01$ ).

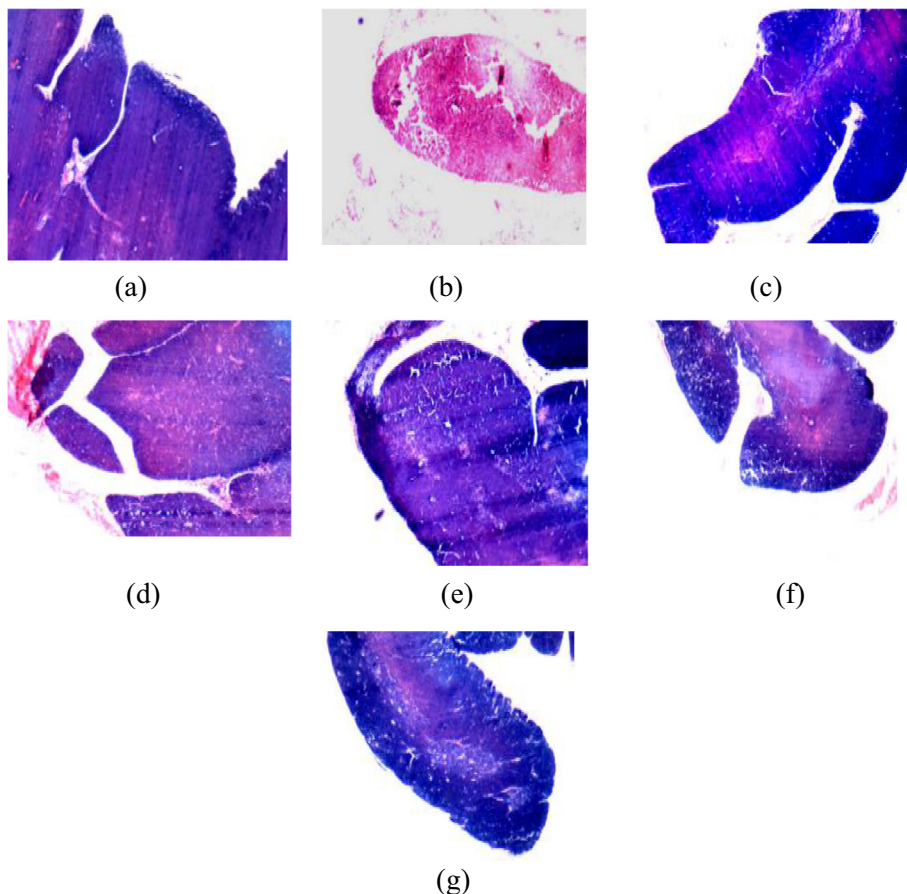
### 3.3. Effect of prostatic hyperplasia model mouse thymus tissue morphology

The BG mice are normal (see Fig. 3(a)). The thymus of mice in MG significantly atrophy, lobule disappear, and it is not clear to divide the cortex and pith. The cortical is thinned, lymphocytes and sparse (see Fig. 3(b)). Thymic lobule cortex and pith of LG mice have clear boundary, and the cortical is significantly thinned, and the lymphocytes are sparse (see Fig. 3(c)). FG mice in the boundary of thymic lobule cortex and pith are not clear, and the cortical is thinned with the sparse lymphocytes (see Fig. 3(d)). The cortex and medulla of HD group mice have clear delineation, and the cortical is thicken, and the lymphocytes are more intensive (see Fig. 3(e)). The cortex and medulla of MD group mice have clear delineation, with the thinner cortex and the intense lymphocyte (see Fig. 3(f)). The thymic lobule LD of group mice has clear boundaries, and the cortex and medulla has clear delineation, with the thinner cortex and the intense lymphocyte (see Fig. 3(g)).

Using micrometer to measure the thymus cortex thickness, calculation the lymphocytes cell number of press the baseline, Table 3 shows the results. Compared with the BG, the cortex thickness and the lymphocyte number are significantly reduced ( $P < 0.01$ ). Compared with the MG, the cortex thickness and the lymphocyte number of each given drug groups are increased significantly ( $P < 0.01$ ).

### 3.4. Impact on spleen tissue morphology in the prostatic hyperplasia mice

Fig. 4(a) shows the BG is normal. In the MG mice, the splenic nodules are significantly reduced, with the sparse lymphocytes



**Fig. 3.** Pathological pictures of mice thymus (HE\*40).

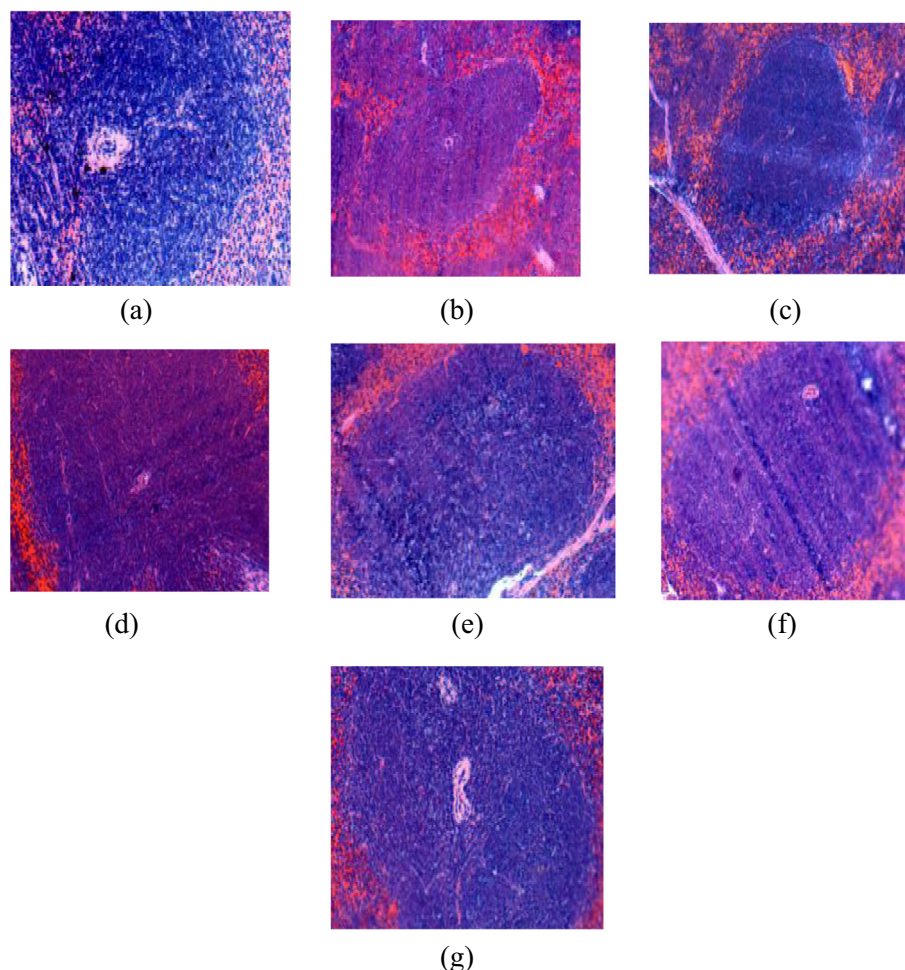


Fig. 4. Pathological pictures of mice spleen (HE\*100).

**Table 3**  
Impact on the thymus cortex thickness and lymphocytes cell number ( $\bar{x} \pm s$ ).

Group	n	Cortical thickness ( $\mu\text{m}$ )	Lymphocyte number (a)
BG	10	12.46 $\pm$ 2.62**	37.58 $\pm$ 11.05**
MG	10	2.52 $\pm$ 1.16	2.18 $\pm$ 1.24
LG	10	10.31 $\pm$ 2.38**	24.46 $\pm$ 4.26**
FG	10	26.27 $\pm$ 3.20**	53.28 $\pm$ 13.48**
HD	10	37.26 $\pm$ 3.52**	68.37 $\pm$ 8.37**
MD	10	6.36 $\pm$ 1.27*	19.26 $\pm$ 3.16**
LD	10	11.28 $\pm$ 2.35**	31.42 $\pm$ 2.37**

Note: Compared with the model group.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

(see Fig. 4(b)). In the LG mice, the splenic nodules are narrowed, with the intensive lymphocytes (see Fig. 4(c)). In the FG mice, the splenic nodules are significantly increased, and the lymphocytes are intensive (see Fig. 4(d)). In the HD group mice, the splenic nodules are more increased, and the lymphocytes are intensive (see Fig. 4(e)). In the MD group mice, the splenic nodules has a litter increased, with intensive lymphocytes (see Fig. 4(f)). In the LD group mice, the splenic nodules are increased, with the intensive lymphocytes (see Fig. 4(g)).

Using micrometer to measure the size of spleen nodules, calculation the lymphocytes cell number of press the baseline, Table 4 shows the results. Compared with the BG, the cortical thickness and the lymphocytes number of the model group are significantly

**Table 4**  
Impact on the spleen nodules thickness and lymphocytes cell number ( $\bar{x} \pm s$ ).

Group	n	Cortical thickness ( $\mu\text{m}$ )	Lymphocyte number (a)
BG	10	11.26 $\pm$ 3.42**	24.18 $\pm$ 7.24**
MG	10	7.46 $\pm$ 2.15	13.27 $\pm$ 4.28
LG	10	9.30 $\pm$ 3.38	19.26 $\pm$ 3.36
FG	10	21.35 $\pm$ 2.48**	68.18 $\pm$ 10.27**
HD	10	18.36 $\pm$ 3.12**	54.17 $\pm$ 12.37**
MD	10	17.14 $\pm$ 3.25**	49.26 $\pm$ 8.26**
LD	10	19.38 $\pm$ 4.35**	56.24 $\pm$ 10.35**

Note: Compared with the model group.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

reduced ( $P < 0.01$ ). Compared with the MG, the cortical thickness and lymphocytes number each given drug groups are increased significantly ( $P < 0.01$ ).

#### 4. Discussion and conclusions

In modern medical research, an effective animal experiment has become a very important means and methods (Tang and Miao, 2012). This research adopted the fetal urogenital sinus implants to establish the prostate hyperplasia mice model, similar to the pathological changes of human prostate. The model is now recognized as an animal model more in line with process of human disease of prostate hyperplasia (Tao et al., 2016; Xu et al., 2011),

which helps to explain the development process of prostate hyperplasia. Furthermore, it provides a new platform for the prevention of prostate hyperplasia, the development of therapeutic drugs and the research on the pathogenesis of the disease (Luo et al., 2016; Mori et al., 2011). The shape of the prostate is the key to determining whether or not to be, the prostate is an immune organ, some studies suggest that the immune response plays an important role in the development of prostate hyperplasia, the prostate hyperplasia can cause changes in the morphology of the prostate (Miao et al., 2014). In addition, it is also reported that the mice of prostate hyperplasia involve the relevant organs such as thymus, spleen atrophy. The long-term hyperplasia can cause pathological changes in renal tissue morphology.

It has proved the activating blood to resolve stasis medicine has the effect of inhibit tissue proliferation, improve the circulation, and inhibit the formation of new blood vessels in the hyperplastic prostate tissue, one of the mechanisms of the activating blood to remove blood stasis drugs for the treatment of prostate hyperplasia (Chen et al., 2016; Liu et al., 2011, 2016). Motherwort contains the effect of potassium sparing diuretic stachydrine and Leonurine. These effects are beneficial to the treatment of prostate hyperplasia. In addition, the previous research has been proved, the common activating blood to remove blood stasis drugs such as safflower, adenophorum has obvious therapeutic effect on prostate hyperplasia.

Motherwort has a good effect on treatment to the fetal urogenital sinus implants, thus establishing the prostate hyperplasia mice model. This study provides an experimental basis for clinical treatment of motherwort prostate hyperplasia, as well as new methods and ideas for the prevention and treatment of prostate hyperplasia.

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