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ORIGINAL ARTICLE

Effects of verbenalin on prostatitis mouse model

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KEYWORDS

Verbenalin;
Prostatitis;
Mouse model

Abstract The aim of this study was to observe the treatment characteristics of verbenalin on a prostatitis mouse model. Give Xiaozhiling injection in the prostate locally to make a prostatitis mouse model. High, medium and low doses of verbenalin were each given to different mouse groups. The amount of water was determined in 14th, 28th. The number of white cells and lecithin corpuscle density in prostatic fluid were determined. Morphological changes in the prostate, testis, epididymis and kidney were detected. Compared with the model control group, the mice treated with high, medium and low doses of verbenalin had significantly increased amounts of water, and prostate white blood cell count and prostate volume density (Vv) were decreased significantly, the density of lecithin corpuscle score increased, and pathologic prostatitis changes were significantly reduced. Pathological change in the testis was significantly reduced and the change in the epididymis was obviously reduced. The thymic cortex thickness and the number of lymphocytes increased significantly and could reduce the renal pathological changes in potential. Verbenalin has a good therapeutic effect on the prostatitis mouse model.

1. Introduction

Verbena, belonging to verbena family Verbena officinalis L., has the property to activate blood circulation to dissipate blood stasis, prevent attack (or recurrence) of malaria, and has application in amenorrhea, dysmenorrhea, edema, infection, etc. (NPC, 2010). Pregnant women shouldn't use it (NUCM, 2006). Yang et al. (2013) noted that modern research proves, Verbena whole plant contains Verbena glycoside (verbenalin), chemical composition of tannin, volatile oil and so on. And Guo and Miao (2014) mentioned that it has anti-inflammatory, anti fungal anti-virus pharmacological action. Gao and Li (2013) indicated that prostatitis is a common disease in adult males, about 25–30% resulting in urinary surgery, mostly occurring in 20–40 year old young adults, mainly because of pain or discomfort in the pelvic region and various voiding symptoms in a group of diseases. This disease lingers and seriously affects the patient's mental health and quality of life. Therefore, the research on prostatitis is particularly important. Verbena had a good effect on chronic prostatitis, hematuria and other male diseases (Yang et al., 2013), but the basic research data are only about Verbena's antibacterial, anti-inflammatory, and other pharmacological effects. This paper reports the verbenalin effects on a prostatitis mouse model.

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Table 1 Effect of verbenalin on the volume of water drunk in the prostatitis mouse model (ml).

Group	*n*	14th	The percentage compared with model group (%)	28th	The percentage compared with model group (%)
BC	10	48.3	5.0	49.5	19.8
MC	10	46.0		41.3	
PC	10	47.2	2.6	46.2	11.9
Verbenalin-HD	10	47.3	2.8	49.2	19.1
Verbenalin-MD	10	47.1	2.4	47.4	14.8
Verbenalin-LD	10	46.2	0.4	44.5	7.7

2. Materials and methods

2.1. Animals

SPF grade KM mice (weight, 25.0 ± 1.0 g) were supplied by Hebei Laboratory Animal Center (animal permit number, 9040134).

2.2. Medicines and reagents

Verbenalin was provided by Nanjing Zelang Pharmaceutical Technology Co., Ltd., whose content was 50.13%, detected by the HPLC; Qianliekang Pian was provided by Zhejiang Conba Pharmaceutical Co., Ltd. (batch number: 20090122); Xiaozhiling injection was provided by Beijing China Resources Hi-Tech Nature Pharmaceutical Co., Ltd. (batch number: 20070405); Sodium pentobarbital was provided by China National Pharmaceutical Group Corporation Shanghai Chemical Reagent Co., Ltd. (batch number: F20060715); Penicillin sodium for injection was provided by North China Pharmaceutical Group Corporation (batch number: X0901038); Medical alcohol was provided by Luoyang Jiekang Disinfectant Factory (batch number: 20080003); Physiological saline was provided by Zhengzhou Yonghe Pharmaceutical Co., Ltd. (batch number: 090217221); Glacial acetic acid was provided by Kaifeng Chemical Reagent Factory (batch number: 061208); Methanal was provided by Lai Yang of China Shuangshuang Chemical Co., Ltd. (batch number: 20070152).

2.3. Instruments

FA-N/JA-N Series was purchased from Shanghai Minqiao Precise Science Instrument Co., Ltd.; BL-2000 Medical Image Analysis System was purchased from Chengdu TME Technology Co., Ltd.

2.4. Methods

60 KM male mice (weight, 24–26 g) were randomly divided into blank control (BC), model control (MC), positive control (PC) and verbenalin high dose (verbenalin-HD), medium dose (verbenalin-MD) and low dose (verbenalin-LD) groups. The blank control group underwent sham operation, and the rest of the groups operation. Respectively model mice were weighed, and then intraperitoneal injection of Sodium

Table 3 Effect of verbenalin on prostate in the prostatitis mouse model.

Group	*n*	–	+	++	+++
BC	10	10	0	0	0
MC	10	0	0	7	3
PC	10		2	3	5
Verbenalin-HD	10	4	6	0	0
Verbenalin-MD	10	2	8	0	0
Verbenalin-LD	10	0	2	5	3

"–" The prostate gland, glandular epithelium and stroma were normal; " + " Prostate glands were few expansion, glandular epithelium was flat, glands around less fiber hyperplasia and a small amount of inflammatory cells infiltration; "++" Hyperplasia of the prostate gland significantly and gland cavity expansion and glandular epithelium flat, interstitial hyperplasia of fiber and a small amount of inflammatory cell infiltration; "+++" Hyperplasia of prostate gland significantly and gland cavity expansion and glandular epithelium flat, interstitial has obvious fiber hyperplasia and inflammatory cell infiltration.

Table 2 Effect of verbenalin on the number of white blood cells in the prostate tissue and the influence of the lecithin corpuscle density in the prostatitis mouse model ($\bar{x} \pm s$).

Group	*n*	No. of WBCs ($\times 10^9$/L)	Density of lecithin corpuscles
BC	10	1.58 ± 0.69**	3.36 ± 0.58**
MC	10	6.62 ± 1.80	1.55 ± 0.50
PC	10	2.10 ± 0.57**	3.00 ± 0.40**
Verbenalin-HD	10	1.47 ± 0.38**	3.36 ± 0.58**
Verbenalin-MD	10	2.05 ± 0.89**	3.20 ± 0.80**
Verbenalin-LD	10	2.19 ± 0.67**	2.82 ± 0.59**

** Compared with MC group, $P < 0.01$.

Table 4 Effect of verbenalin on the Vv of the prostate gland in the prostatitis mouse model ($\bar{x} \pm s$).

Group	*n*	Dosage (mg/kg)	Vv (%)
BC	10		2.3 ± 0.4**
MC	10		6.2 ± 0.6
PC	10	1500	4.6 ± 0.5**
Verbenalin-HD	10	200	0.3 ± 0.1**
Verbenalin-MD	10	100	0.5 ± 0.1**
Verbenalin-LD	10	50	3.4 ± 0.2**

** Compared with MC group, $P < 0.01$.

Table 5 Effect of verbenalin on kidney tissue in the prostatitis mouse model.

Group	*n*	–	+	++	+++
BC	10	10	0	0	0
MC	10	8	2	0	0
PC	10	5	3	2	0
Verbenalin-HD	10	10	0	0	0
Verbenalin-MD	10	10		0	0
Verbenalin-LD	10	9	1	0	0

"–" Glomerular, renal capsule, renal tubules and epithelial cells were normal; " + " Glomerular, renal capsule, renal tubules and epithelial cells were about 25% in bubble; "++" Glomerular, renal capsule, renal tubules and epithelial cells were about 50% in bubble; "+++" Glomerular, renal capsule, renal tubules and epithelial cells were about 75% in bubble.

Table 6 Effect of verbenalin on testicular tissue in the prostatitis mouse model.

Group	*n*	–	+	++	+++
BC	10	10	0	0	0
MC	10	0	3	5	2
PC	10	2	8	0	0
Verbenalin-HD	10	4	6	0	0
Verbenalin-MD	10	3	5	2	0
Verbenalin-LD	10	0	2	2	6

"–" The testis seminiferous tubules spermatogonia had no eosinophilic change; " + " About 25% of the testis seminiferous tubules spermatogonia had eosinophilic change; "++" About 50% of the testis seminiferous tubules spermatogonia had eosinophilic change; "+++" About 75% of the testis seminiferous tubules spermatogonia had eosinophilic change.

Table 7 Effect of verbenalin on epididymis tissue of prostatitis mouse model.

Group	*n*	–	+	++	+++
BC	10	8	2	0	0
MC	10	0	2	7	1
PC	10	0	2	4	4
Verbenalin-HD	10	8	2	0	0
Verbenalin-MD	10	4	6	2	0
Verbenalin-LD	10	0	4	6	0

"–" Epididymis glands around without fiber hyperplasia and inflammatory cell infiltration were normal; " + " Epididymis glands around appeared a few fiber hyperplasia and inflammatory cell infiltration; "++" Epididymis glands appeared obvious fiber hyperplasia and inflammatory cells infiltration around; "+++" Epididymis glands around appeared a lot of fiber hyperplasia and inflammatory cell infiltration.

pentobarbital was given, 35 mg/kg dose, after the success of the anesthesia, alcohol was used to disinfect the skin, and operation was performed. Incision from the ventral midline to the abdominal cavity was made, bladder and seminal vesicles on both sides of the bladder were pulled out, then dorsal prostate was exposed, 0.02 ml 25% Xiaozhiling was injected with a microsyringe, then the viscera was put back, lamination muscle and skin oversewn, alcohol was used to disinfect operation wound, when the mice were awake they were placed back in the cage of conventional breeding. Postoperative intramuscular injection of penicillin 200,000 u/kg, for 3 days, was administered in order to prevent infection. Postoperative day 8, mice were drenched with 0.2 ml/10 g drug solutions, for 21 d. BC and MC mice were given distilled water, while PC mice were given Qianliekang Pian (1.5 g/kg). Verbenalin-HD, MD and LD mice respectively received 200 mg/kg, 100 mg/kg and 50 mg/kg of verbenalin solutions. The day after modeling 14 days and 28 days, respectively the volume of water drunk by mice in 24 h in each group was measured. 24 h after the last administration, mice were weighed, then killed by cervical dislocation, quickly pulling out the part of the prostate tissue, precision weighing, the number of white cells and lecithin corpuscle density in prostatic fluid was determined according to the methods in the literature (Xu et al., 2015). Take a part of the prostate tissue and testis and epididymis, kidney, thymus and rinse clean with Physiological saline, fixs in 10% formalin solution, paraffin embedding, conventional dehydration, section, HE stain.

Table 8 Effect of verbenalin on the thymus cortex thickness and the number of lymphocytes in the prostatitis mouse model ($\bar{x} \pm s$).

Group	*n*	Thymus cortex thickness (nm)	Number of lymphocytes
BC	10	35.27 ± 6.25**	48.26 ± 9.38**
MC	10	12.36 ± 3.18	19.24 ± 4.20
PC	10	18.12 ± 4.37**	24.61 ± 5.20**
Verbenalin-HD	10	32.35 ± 5.22**	40.38 ± 5.34**
Verbenalin-MD	10	41.25 ± 7.43**	62.27 ± 9.32**
Verbenalin-LD	10	23.65 ± 6.21**	31.24 ± 6.25**

** Compared with MC group, $P < 0.01$.

2.5. Statistical method

The SPSS 13.0 statistical software for Windows was used for data analysis. The measurement results were expressed as "mean ± standard deviation ($\bar{x} \pm s$)". Compared with single factor analysis of variance between groups, count the Ridit analysis data.

3. Results and discussion

3.1. Effect on the volume of water drunk

14th and 28th days after modeling, respectively measure the volume of water drunk by mice in 24 h in each group, the result is shown in Table 1.

According to Table 1, the volume of water drunk by the MC group was lower than that by the BC group, the decrease percentage was respectively 5.0% and 19.8%, it was found that

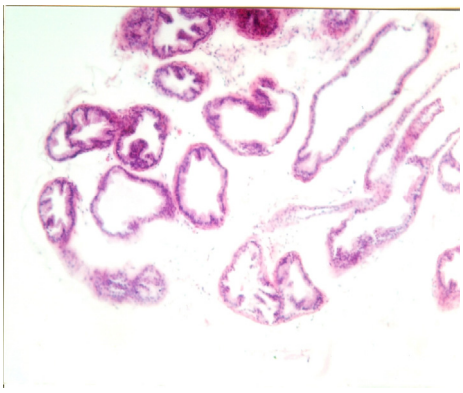

Figure 1 BC group HE×100

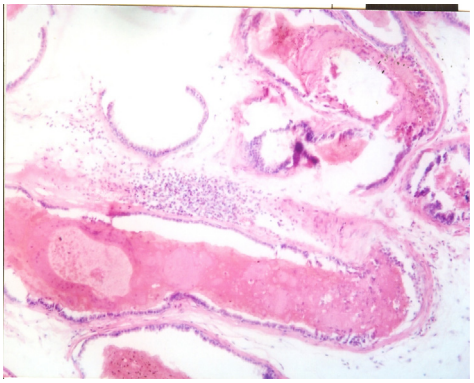

Figure 2 MC group HE×100

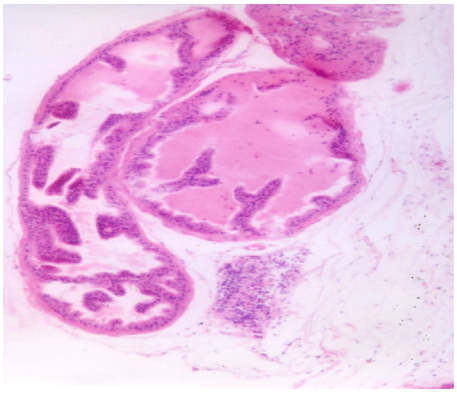

Figure 3 PC group HE×100

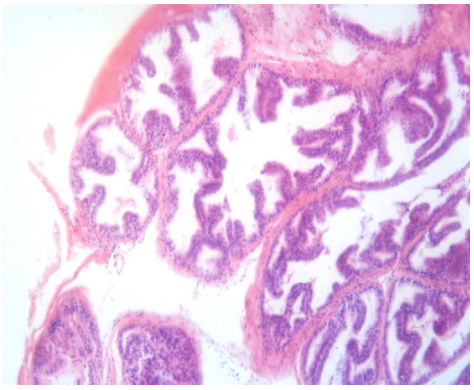

Figure 4 Verbenalin-HD group HE×100

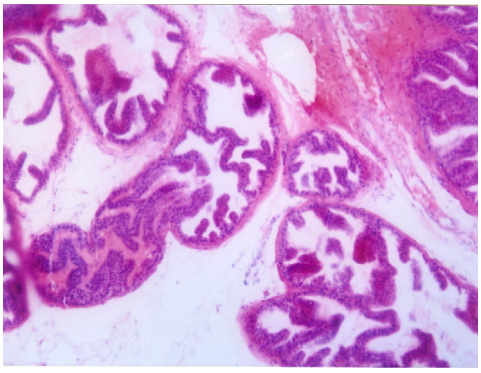

Figure 5 Verbenalin-MD group HE×100

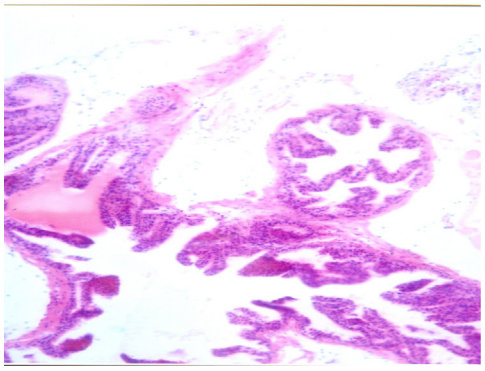

Figure 6 Verbenalin-LD group HE×100

Figure 1 Prostate tissue pathology in mice.

in the model group, mouse prostate inflammatory was aggravating day by day. 14 days and 28 days after dosing, the volume of water drunk by verbenalin groups with high, middle and low dosages group was respectively larger 2.8%, 19.1%, 2.4%, 14.8%, 0.4%, 7.7% than that in the MC group, it was found that prostate inflammation was significantly decreased over the dosing period (Zhiwei et al., 2015).

3.2. Effect on number of white blood cells and the lecithin corpuscle density

According to the corresponding experiment method and standard, the total number of white blood cells in the prostate tissue and lecithin corpuscle density score were observed and recorded, the results are shown in Table 2.

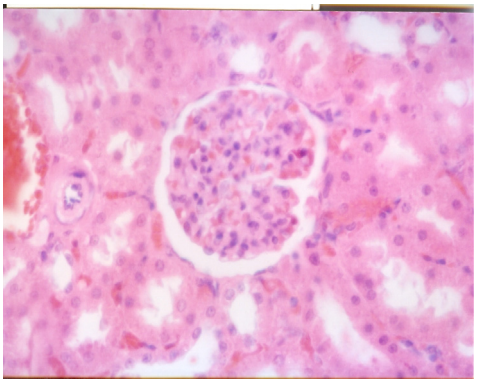

Figure 7 BC group HE×400

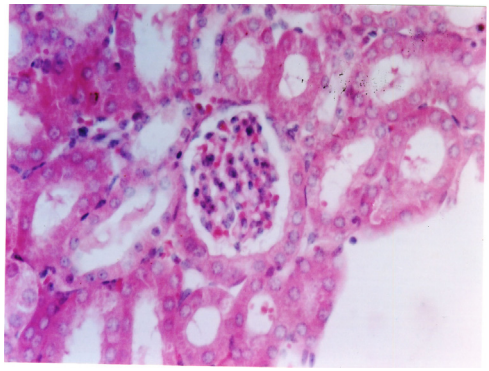

Figure 8 MC group HE×400

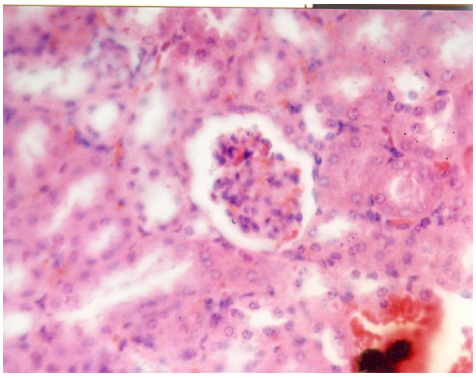

Figure 9 PC group HE×400

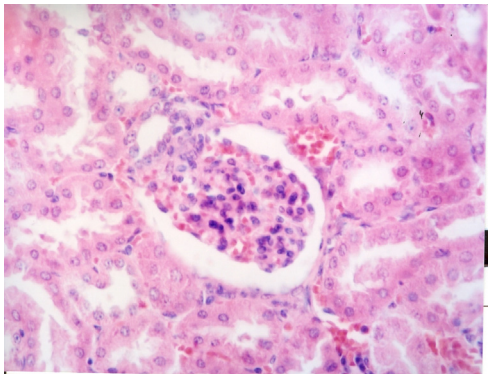

Figure 10 Verbenalin-HD group HE×400

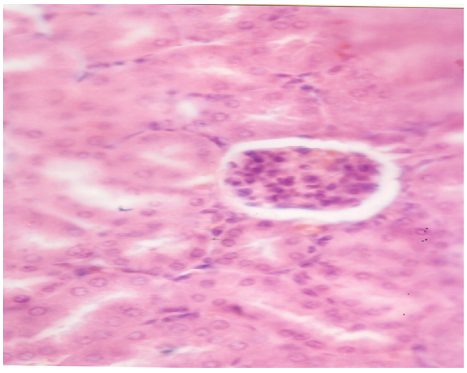

Figure 11 Verbenalin-MD group HE×400

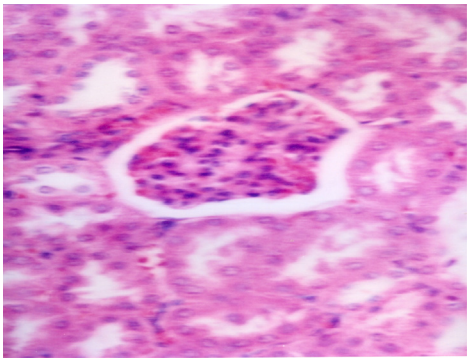

Figure 12 Verbenalin-LD group HE×400

Figure 2 Prostatitis in mouse kidney tissue pathology.

According to Table 2, compared with the BC group, in the MC group, the number of white blood cells in the prostate tissue increased significantly ($P < 0.01$), the lecithin corpuscle density score significantly reduced ($P < 0.01$), prostatitis model copy was a success. Compared with the MC group, verbenalin-HD, MD, LD groups and PC leukocyte count in the prostate was significantly reduced ($P < 0.01$), the lecithin corpuscle density score of the prostate was significantly increased ($P < 0.01$), and verbenalin-HD effect was obvious (Zhang et al., 2014).

3.3. Effect on histomorphology of prostate

Under a light microscope the observation of visible mouse prostate tissue (see Appendix 1 mouse prostate tissue pathological Figs. 1–6) is as follows: BC group normal

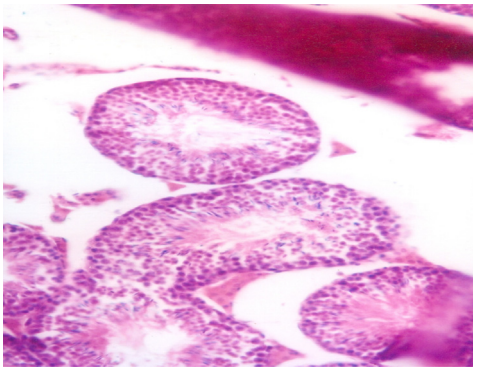

Figure 13 BC group HE×200

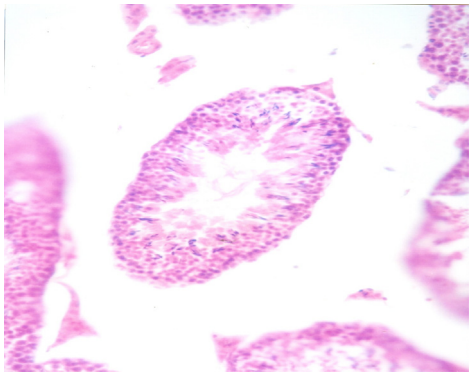

Figure 14 MC group HE×200

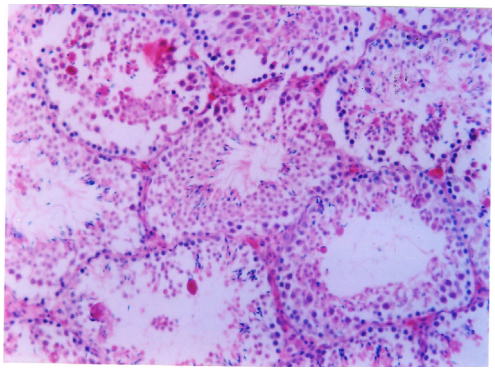

Figure 15 PC group HE×200

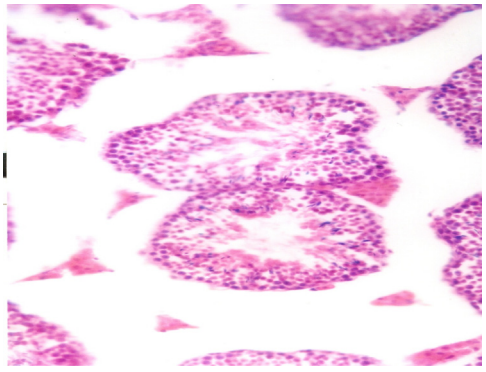

Figure 16 Verbenalin-HD group HE×200

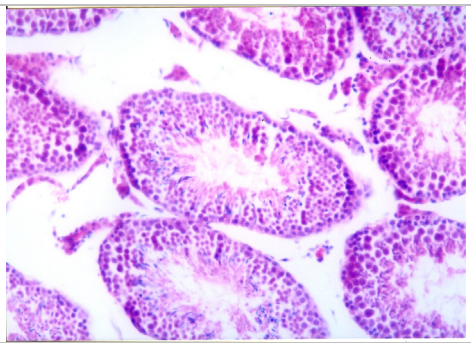

Figure 17 Verbenalin-MD group HE×200

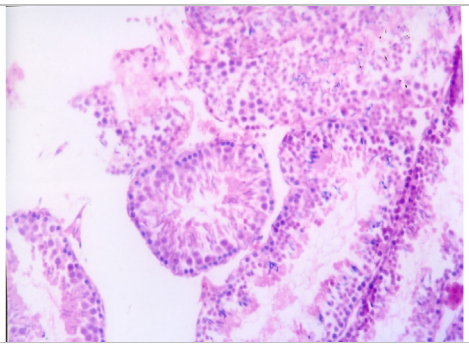

Figure 18 Verbenalin-LD group HE×200

Figure 3 Prostatitis in mouse testicular tissue pathology.

prostate glands, glandular epithelium were arranged in corrugated shape, interstitial inflammatory cell infiltration and fibrous hyperplasia absent; MC group had obvious hyperplasia, prostate gland cavity was expanded, glandular epithelium flattened, interstitial tissue widened, interstitial had obvious inflammatory cells (neutrophils) and fiber hyperplasia; in the PC group prostate gland was enlarged, glandular epithelial cells were arranged in corrugated shape, and glands around had a small amount of fiber hyperplasia and a small amount of inflammatory cells; in the verbenalin-HD group the prostate gland was normal, glandular epithelial cells were arranged in corrugated shape, interstitial inflammatory cell infiltration and fibrous hyperplasia absent; in the verbenalin-MD group the prostate gland cavity basically returned to normal, in glandular epithelial cells most were arranged in corrugated shape, with a small amount of fiber in interstitial hyperplasia and inflammatory cells; verbenalin-LD group had obvious hyperplasia of the prostate, expansion of the glandular cavity and the epithelium.

As in Table 3, using the Ridit test, compared with the BC group, the MC group showed a significant inflammation of the prostate pathological changes ($P < 0.01$), the building model was a success. Compared with the MC group, verbenalin-HD, MD group of prostate inflammation

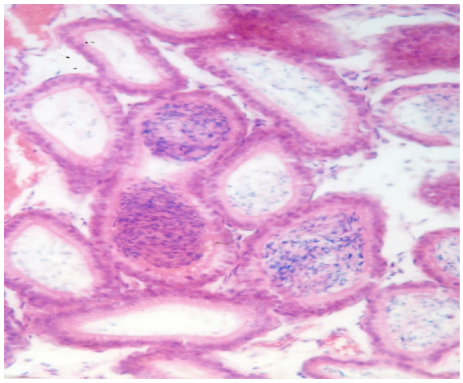

Figure 19 BC group HE×200

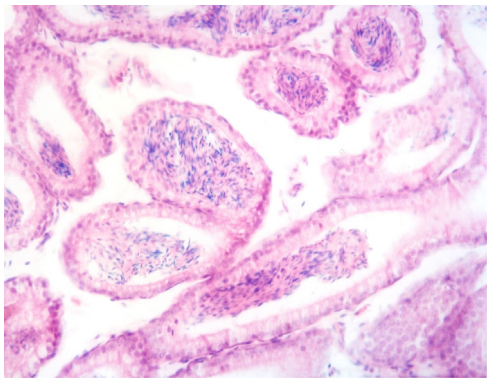

Figure 20 MC group HE×200

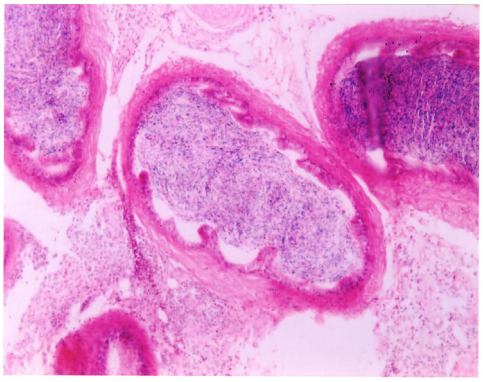

Figure 21 PC group HE×200

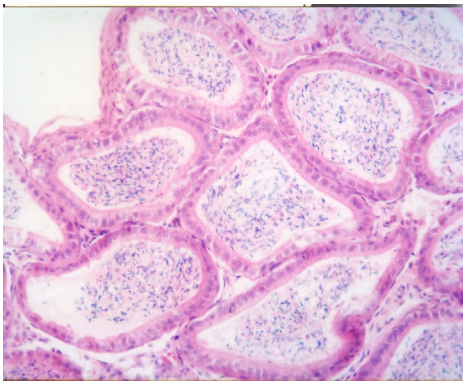

Figure 22 Verbenalin-HD group HE×200

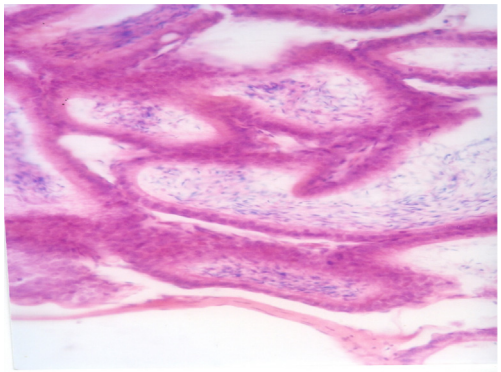

Figure 23 Verbenalin-MD group HE×200

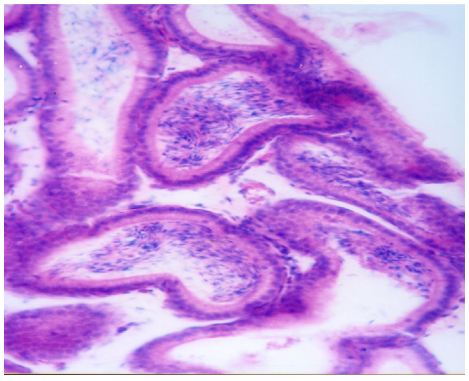

Figure 24 Verbenalin-LD group HE×200

Figure 4 Prostatitis epididymis tissue pathology in mice.

pathological changes significantly reduced ($P < 0.01$), the verbenalin-LD group of pathological changes only alleviated the trend.

According to the different pathological changes in the experimental groups of prostate cells stereo metrology method, test lattice notation was used to test and calculate the volume density (Vv) of inflammatory cells in the inflammatory area. Differences in the pathological change in the prostate in the different Experimental groups are shown in Table 4:

$$\text{Formula}: \text{Vv} = P\sum n/\text{reference system}\sum n \times 100\%$$

According to Table 4, compared with the BC group, in the MC group Vv increased significantly ($P < 0.01$). Compared with the MC group, in verbenalin-HD, MD, LD groups and the PC group Vv was significantly decreased ($P < 0.01$).

Through the analysis of the test results of experimental groups of mouse gland stereo metrology, it can be seen that prostatitis animal models in mice's main stereoscopic metrology change for prostate gland was volume density

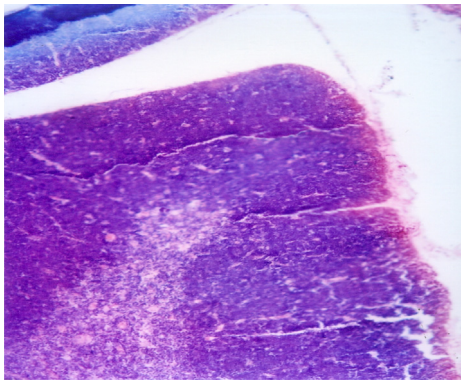

Figure 25 BC group HE×100

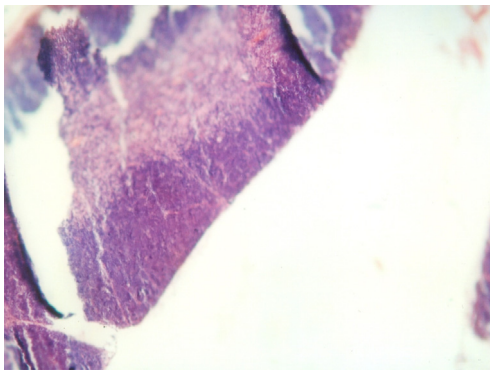

Figure 26 MC group HE×100

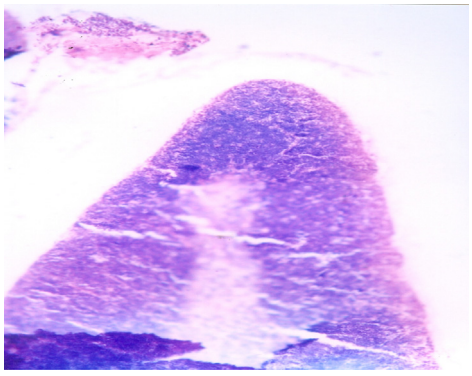

Figure 27 PC group HE×100

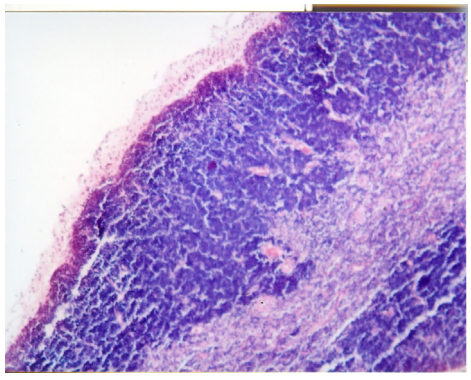

Figure 28 Verbenalin-HD group HE×100

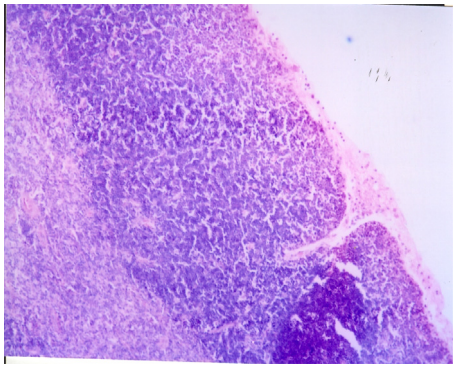

Figure 29 Verbenalin-MD group HE×100

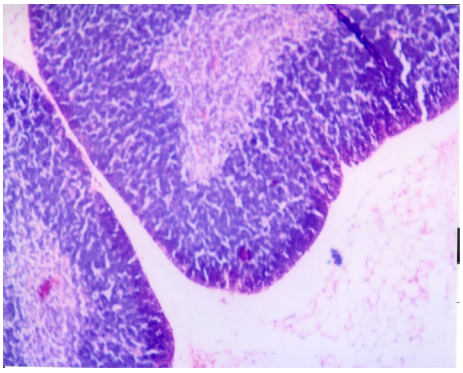

Figure 30 Verbenalin-LD group HE×100

Figure 5 Prostatitis thymus tissue pathology in mice.

(Vv) increased. Qianliekang and verbenalin could reduce body density, inhibit the role of prostatitis, with verbenalin-HD, MD group effect was the best. The main changes in pathological histology were non-expansion of glands, decrease in interstitial inflammatory cells and gland body density.

3.4. Effect on kidney tissue

Observation under a light microscope showed the kidney of mice (see Appendix 1 mouse kidney tissue pathological Figs. 7–12) to be: the BC group glomerular, renal capsule, renal tubules and epithelial cells were normal; MC group glomerular, renal capsule, renal tubular showed no obvious pathological changes; PC group of glomerular basic normal, abnormal renal capsule was not seen, renal tubular epithelial cells in vacuoles were degenerated; verbenalin-HD group glomerular, renal capsule and renal tubular epithelial cells were normal; verbenalin-MD group of glomerular, renal capsule and no obvious pathological changes seen on renal tubule; verbenalin-LD glomerular, renal capsule and low

dose group of renal tubule and epithelial cells were essentially normal.

As in Table 5, using the Ridit test, there were no significant differences between groups, but verbenalin-HD, LD group had a good effect on kidneys and could reduce the potential pathological changes; Qianliekang could aggravate kidney pathological changes.

3.5. Effect on testicular tissue

Observation under a light microscope showed the testis of mice (see Appendix 1 mouse testicular tissue pathological Figs. 13–18) to be: in the BC group, the testis seminiferous tubules spermatogenic cells at all levels, support cells and mesenchymal cells were normal; in the MC group, in seminiferous tubule spermatogenic cells were normal, and after sperm, there are many eosinophilic spermatogonia; in the PC group, seminiferous tubules spermatogonia showed an acidophilic change; in verbenalin-HD group, the seminiferous tubules spermatogenic cells at all levels were normal; in the verbenalin-MD group, seminiferous tubules 1/3 spermatogonia showed an acidophilic change; in the verbenalin-LD group, seminiferous tubules most spermatogonia showed an acidophilic change (Safi et al., 2015a).

As in Table 6, using the Ridit test, compared with the BC group, the MC group had obvious testicular pathological changes ($P < 0.05$), compared with the MC group, verbenalin-HD, MD, LD group could significantly relieve the pathological changes in the testis ($P < 0.01$).

3.6. Effect on epididymis tissue

Observation under a light microscope showed the epididymis tissue of mice (see Appendix 1 mouse epididymis pathology Figs. 19–24) to be: epididymis of BC group, epididymal duct and stroma were normal; epididymis of the MC group, in the epididymal duct, sperm was rich, around the lumen there appeared obvious fibrous proliferation and inflammatory cell infiltration; epididymis of the PC group, the epididymal duct, sperm was rich, around the lumen there appeared many fibrous proliferation and inflammatory cell infiltration; in the verbenalin-HD group, epididymal duct and stroma were normal; in the verbenalin-MD group, the surrounding fibrous hyperplasia in the epididymis was significantly thinner and reduced, inflammatory cells decreased significantly; in the verbenalin-HD group, fibrous hyperplasia and a few inflammatory cells were obviously around the epididymis.

As in Table 7, using the Ridit test, compared with the BC group, the MC group had significant epididymis pathological changes ($P < 0.01$), compared with the MC group, verbenalin-HD group had significantly relieved the pathological changes of the epididymis ($P < 0.01$), the verbenalin-MD group was obviously reduced ($P < 0.05$), the verbenalin-LD group could not reduce the epididymis pathology change obviously.

3.7. Effect on thymus tissue

Observation under a light microscope showed the thymus tissue of mice (see Appendix 1 mouse thymus tissue pathologic Figs. 25–30) to be: in the BC group, thymic lobule boundary was clear, clear demarcation of the cortex and medulla was seen, cortex cells were more intensive; in the MC group, cortical became thin and lymphocytes became sparse; in the PC group, the thymic cortex was thick and lymphocyte dense; in the verbenalin-HD, MD group, the thymic cortex ws obviously thick and lymphocyte dense; in the verbenalin-LD group, the thymic cortex was thick and lymphocyte dense.

The widest point and the narrowest place of thymus cortex were determined by a micrometer, the average of the two was the cortical thickness (Safi et al., 2015b). The number of lymphocytes in the baseline pressure was calculated, and the average number of lymphocytes was counted. Results are shown in Table 8.

As in Table 8, using the Ridit test, compared with the BC group, in the MC group, mouse thymus cortex significantly thinned, the number of lymphocytes was significantly reduced ($P < 0.01$), suggesting a decline in the cellular immune function. In the PC, verbenalin-HD, MD, LD group mouse thymus cortex markedly thickened ($P < 0.01$), the number of lymphocytes increased significantly ($P < 0.01$), suggesting verbenalin and Qianliekang could significantly enhance the immunity of the prostatitis model mice.

4. Conclusions

The experimental results showed that, the amount of water drunk by mice in each dose group of verbenalin increased, the lecithin corpuscle density score significantly increased, the number of white blood cells in the prostate tissue were significantly decreased, Vv significantly reduced, the pathological changes of the prostate significantly reduced. To verify the therapeutic effect of verbenalin of prostatitis model mice, at the same time, verbenalin in each dose could reduce the pathological changes of the testis, epididymis, and had a beneficial effect on the kidneys, could improve its potential pathological changes. It was proved that verbenalin intervention in inflammatory symptoms of prostatitis mouse model was effective, could improve the model and improve the pathological changes in mice as secondary disease caused by the prostatitis model. In addition, the experimental results showed that, verbenalin in each dose could significantly increase the thymus thickness and the number of lymphocytes, that verbenalin could enhance immune function of the prostatitis animal model, by enhancing immune function to reverse the pathological physiology process, which had an effect on the treatment of chronic prostatitis. The experimental results showed that, verbenalin could not only improve the pathological changes of the prostate gland of the mouse model, but also had better protection function on the animal testicles subsidiary organs and immune organs.

This experiment used Xiaozhiling injection induced prostatitis mouse model, pathological changes were more close to clinical prostatitis. One of the clinical manifestations of prostatitis was the tongue taste (Hu et al., 2015). Therefore, the changes in the volume of water were the most direct indicators of prostatitis clinical manifestations. Changes in the prostate tissue in white blood cells and the density of lecithin corpuscle were the direct signs of inflammation, and to observe the pathological changes of the prostate tissue, could more directly reflect the severity of chronic prostatitis. The pathological

features of chronic prostatitis were mainly interstitial cell infiltration, fibroblast hyperplasia, glandular tube obstruction, lumen of gland secretion was reduced, so the observation and calculation of the prostate gland on three-dimensional dosimetry were intuitive judgment index of prostatitis, and also was an effective drug standard (Miao et al., 2014). At the same time, because prostatitis was a chronic disease, through the observation of the pathological changes of the testis and epididymis, kidney and immune organ, could further explore the impact of prostate on prostatitis appendages and immune function. The experimental results show that the verbenalin of prostatitis mouse model has better protective effect, but still needs to further the molecular mechanism and target, to perfect the mechanism.

In the traditional Chinese medicine, no organ was named prostate, there is no prostatitis, prostatitis belonged to ''pouring muddy'', ''jingzhuo'', etc., in traditional Chinese medicine category. Wet, heat, blood stasis, deficiency were the basic pathogenesis of prostatitis, and damp heat and blood stasis were throughout the course of disease, and were the main pathogenesis characteristics of prostatitis. Activating blood circulation to dissipate blood stasis and tonify Qi of the kidney was the principle of traditional Chinese medicine dialectical therapy in the treatment of prostatitis. Verbena had the functions of promoting blood circulation for removing blood stasis and inducing diuresis for removing edema, and in traditional Chinese medicine, these functions were consistent with the basic pathogenesis of prostatitis disease and were the main treatment of ''certificate''. Verbena had a good effect on chronic prostatitis, hematuria and other male diseases in modern clinical application (Yang et al., 2013). At present, there are a large number of clinical observations about Verbena, but the basic research data were less, and the observation only studies the antibacterial, anti-inflammatory, analgesic and other pharmacological effects, the choice of animal model with the characteristic of chronic prostatitis disease research, had important significance to observe the therapeutic effects and the further study of the treatment mechanism.

In recent years, the age at onset of chronic prostatitis in advance, juvenile chronic bacterial prostatitis was increased (Yuan and Zheng, 2014). Verbena was a kind of a natural drug, the toxic side effects were lesser, and was the double anastomosis of the main treatment and drug selection. At the same time, research on prostatitis didn't observe the pathological changes of the relevant organ. The experimental results showed that the prostatitis of the testis, epididymis and kidney had certain influence, also impact on animal model immune organ, and verbenalin can improve the pathological changes. The experimental results showed that the verbenalin had good effects on treating prostatitis, and provided experimental support for verbenalin having clinical treatment on prostatitis, also provided new ideas and methods for the prevention and treatment of prostatitis.

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Appendix 1

See Figs. 1–5.

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