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Effects of distribution ratio of different groups between motherwort total alkaloids and astragalus total saponins on benign prostate hyperplasia modles in mices

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ABSTRACT

Objective:Explored effects of Distribution ratio of different groups between Motherwort total alkaloids (MTAs)and Astragalus Total saponins (ATS) on Benign Prostate Hyperplasia(BPH)modles in mices, screened optimal ratio of active ingredient of prevention and treatment of benign prostatic hyperplasia. Methods: Benign prostatic hyperplasia models induced by testosterone propionate in mices,measured wet weight of the prostate, prostate index, measured levels of Dihydrotestosterone(DHT) and pathological changes of prostate tissue morphology .Results: 7: 3,6:4,5:5 group of distribution ratio of different groups between MTAs and ATS significantly reduced wet weight,index of prostatic on benign prostatic hyperplasia in mice ($P<0.01$); 6:4,5:5 group of distribution ratio of different groups between MTAs and ATS significantly reduced levers of serum DHT ($P <0.01$); 6:4,5:5 group of distribution ratio of different groups between MTAs and ATS Pathological changes of benign prostatic hyperplasia（$P<0.01$,$P<0.05$.. Conclusion: 6:4,5:5 distribution ratio of different groups between MTAs and ATS has better treatment on Benign Prostate Hyperplasia modles in mices.

KEYWORDS

MTAs and ATS; Different proportions proportion; Prostatic hyperplasia model; Mice.

INTRODUCTION

Due to the special nature of the organizational structure of the prostate, causing the drug is difficult to reach lesions, caused considerable difficulties to the treatment, Resently, Treatment of chemotherapy drugs focused on α-adrenergic receptor blockers, growth factor inhibitors, 5α- reductase inhibitors, etc. However, due to the long period of treatment, the efficacy of the drug's chemical instability, adverse reactions, higher costs brought many restrictions for the treatment of benign prostatic hyperplasia[1]. Our previous studies had shown that motherwort alkaloids exact effects on prostate hyperplasia[2]. The main active site of motherwort is MTAs, the main active site of Astragalus is ATS. The experiment reported effects of Distribution ratio of different groups between MTAs and ATS on BPH modles in mices.

EXPERIMENTAL SECTION

Animal: Kunming mices,weighing18~22g,half male and half female, provided by medical experimental animal center of Henan Province, the certificate number: 0008376; Laboratory Certificate of Conformity SYXK (Henan) 2010-001

Instrument: FA(N)/JA(N) series electronic balance, Shanghai Minqiao Precision Instrument Co., ltd.; OLYMPUS BX61 motorized microscope, Japan OLYMPUS company; 680 microplate reader, BIO-RAD US company.

Drugs and reagents : MTAs, ATS provideded by chemistry lab of Henan university of TCM, Content >58%,batch:120710;Content>50%,batch:111008,Longbishu capsules,Cody Shijiazhuang Pharmaceutical Co, batch:120706;Injection of testosterone propionate, Shanghai GM Pharmaceutical Co.,Ltd.production,batch:110702; Sodium for injection of penicillin, North China Pharmaceutical Co, batch:c1107702; Chloral Hydrate, Guangfu 5th Chemical Research Institute, Tianjin, batch:20120606; AR, Shuangshuang Chemical Co,Yantai, batch:20120701.

Methods: Clean male mice 144, selected randomly 12 as Blank group, sham surgical treatment. Another 11 groups of mices made benign prostatic hyperplasia modles, 12 in each group, respectively named: Model group, Longbishu group, designed MTAs and ATS different group distribution ratio 10: 0,8: 2,7: 3,6: 4,5: 5,4: 6,3: 7, 2: 8,0: 10 groups based on geometric changes in the baseline. Anesthetized modeling mice by ip10% Chloral Hydrate ($0.03ml.10g^{-1}$), routine disinfection of the skin, Removed scrotal testes, ligated stump, sutured skin, i.p Penicillin for 200 000 $u.kg^{-1}$ under sterile conditions; 3th days after surgery, i.h testosterone propionate for $5mg.kg^{-1}$ (soluble soybean oil), once a day for 3 weeks, i.h Physiological saline for $5mg.kg^{-1}$ to Blank group. Gavaged Longbishu capsule suspension ($450mg.Kg^{-1}$, equivalent to 15 times the clinical dose) to Rongbishu group, different ratio of MATs and ATS ($0.2g.kg^{-1}$, Compatibility MATs : ATS as 10:0,8:2,7:3,6:4,5:5,4:6 3:7,2:8,0:10, dubbed the same concentration of $0.02mg.ml^{-1}$with 0.5% CMC solution, 0.1ml $.10g^{-1}$).Gavaged the same volume($0.1ml.10g^{-1}$) of 0.5% CMC solution to Blank group and Model group. Once a day for 3 weeks.Last administration after 2h (fasted 12h), weighed mice,centrifugated,separated serum, measured serum dihydrotestosterone (DHT) levels;Executeed mice,separated prostate tissue quickly, Weighed prostate wet weight, calculate prostate index (index = prostate prostate wet weight / mouse weight); Fixed prostate in 10% formalin solution, paraffin-embedded sections, HE staining,observed prostate morphological changes in the organization with light microscopy [3].

RESULT AND DISSCUSS

TABLE 1:Effect of different distribution ratio on prostate weight and prostate exponent of (BPH) mice model induced by injection of testosterone propionate (TP).($\bar{x}\pm s$, n=12)

Group	Dose($g.kg^{-1}$)	prostate (mg)	weightprostate ($mg.g^{-1}$)	exponent
Blank	-	88.81±12.39	2.61±0.28	
Model	-	$189.22\pm19.95^{\triangle\triangle}$	$4.95\pm0.38^{\triangle\triangle}$	
Longbishu	0.45	116.49±20.53**	2.92±0.50**	
10:0	MATs：ATS =0.20:0.00	146.90±37.51**	3.62±0.97 **	
8:2	MATs：ATS =0.16:0.04	142.79±36.45**	3.80±1.07**	
7:3	MATs：ATS =0.14:0.06	140.73±34.90**	3.62±1.02**	
6:4	MATs：ATS =0.12:0.08	141.31±19.39**	3.63±0.61**	
5:5	MATs：ATS =0.10:0.10	146.71±16.59**	3.64±0.46**	
4:6	MATs：ATS =0.08:0.12	147.47±33.30**	3.68±0.80**	
3:7	MATs：ATS =0.06:0.14	140.96±38.50**	3.68±1.00**	
2:8	MATs：ATS =0.04:0.16	140.58±31.85**	3.79±0.98**	
0:10	MATs：ATS =0.00:0.20	141.82±29.10**	3.75±0.77**	

Note: comparison with the blank control group ($^{\triangle\triangle}P<0.01$) and comparison with the model control group (** $P<0.01$ and * $P<0.05$)

Table1 shows that,the prostate weight and prostate exponent of mice model group were significantly different with that of the blank group mice ($P<0.01$) .The results indicated the model established successfully. Compared with the model group, the prostate weight and prostate exponent in the Longbishu group and all groups of different distribution ratio of MTAs and ATS showed extremely significant differences ($P<0.01$). The group of 7:3、6:4、5:5 were the best.

TABLE 2:Effect of different distribution ratio on serum DHT of BPH mice model induced by injection of TP.($\bar{x}$ ±s, n=12)

Group	Dose(g.kg^{-1})	DHT （nmol.L^{-1}）
Blank	-	3.78±1.46**
Model	-	11.51±1.61$^{\triangle\triangle}$
Longbishu	0.45	5.08±1.91**
10:0	MATs：ATS =0.20:0.00	7.24±2.45**
8:2	MATs：ATS =0.16:0.04	7.47±2.26**
7:3	MATs：ATS =0.14:0.06	7.34±1.54**
6:4	MATs：ATS =0.12:0.08	5.61±1.80**
5:5	MATs：ATS =0.10:0.10	5.71±1.55**
4:6	MATs：ATS =0.08:0.12	7.05±2.62**
3:7	MATs：ATS =0.06:0.14	8.00±1.93**
2:8	MATs：ATS =0.04:0.16	7.89±2.07**
0:10	MATs：ATS =0.00:0.20	7.76±1.96**

Note: comparison with the blank control group ($^{\triangle\triangle}P<0.01$) and comparison with the model control group ($^{**}P<0.01$ and $^{*}P<0.05$)

Table2 shows that,compared with the blank group ,the serum DHT of mice model group were significantly increase ($P<0.01$) .The results indicated the model was established successfully. Compared with the model group, the serum DHT of Longbishu group and all groups of different distribution ratio of MTAs and ATS were extremely significant reduce ($P<0.01$). The group of 6:4、5:5 were the best.

TABLE 3:Effect of different distribution ratio on prostate tissue morphology of BPH mice model induced by injection of TP (n=12)

Group	Dose(g.kg^{-1})	-	+	++	+++	*p*
Blank	-	12	0	0	0	
Model	-	0	0	2	10	△△
Longbishu	0.45	0	5	7	0	**
10:0	MATs：ATS =0.20:0.00	0	0	4	8	**
8:2	MATs：ATS =0.16:0.04	0	8	4	0	**
7:3	MATs：ATS =0.14:0.06	0	8	3	1	**
6:4	MATs：ATS =0.12:0.08	2	7	3	0	**
5:5	MATs：ATS =0.10:0.10	1	5	6	0	**
4:6	MATs：ATS =0.08:0.12	0	0	4	8	**
3:7	MATs：ATS =0.06:0.14	0	3	9	0	**
2:8	MATs：ATS =0.04:0.16	0	0	8	4	*
0:10	MATs：ATS =0.00:0.20	0	7	5	0	**

Note: comparison with the blank control group ($^{\triangle\triangle}P<0.01$) and comparison with the model control

Effect of different distribution ratio on prostate tissue morphology of BPH mice model

Determines BPH of mice in each group,results were shown in Histopathology of prostate tissue in each group was studied according to semi quantitative standard and the results were summarized as follows(table3,Picture1).The prostate gland and interstitial tissue of mice in blank group, were almost normal,no hyperplasia(Figure 1).In model group mice,the prostate gland and part of glandular epithelial cells were extremely hyperplasia,and hyperplasia can be seen in interstitial tissue(Figure 2).In Longbishu group mice,the hyperplasia in prostate gland were inhibited,and just part of glandular epithelial

cells were hyperplasia,and interstitial tissue were almost normal(Figure 3).In 10:0 group,the prostate gland were hyperplasia,and glandular epithelial cells were extremely hyperplasia,and interstitial tissue were slight hyperplasia(Figure 4).In 8:2group,the prostate gland were hyperplasia,and the hyperplasia in glandular epithelial cells were inhibited, and interstitial tissue were almost normal(Figure 5).In 7:3 group,the prostate gland were normal,and the glandular epithelial cells were extremely hyperplasia, and interstitial tissue were almost normal(Figure6).In 6:4 group,the prostate gland were slight hyperplasia,and glandular epithelial cells were extremely inhibited,and interstitial tissue were almost normal(Figure7).In 5:5 group,the prostate gland were hyperplasia ,and glandular epithelial cells were inhibited,and interstitial tissue were almost normal(Figure8).In 4:6 group, prostate gland,glandular epithelial cells and interstitial tissue were hyperplasia(Figure9).In 3:7 group,the prostate gland were hyperplasia ,and glandular epithelial cells were inhibited,and interstitial tissue were almost normal(Figure10).In2:8group,the hyperplasia in prostate gland were some inhibited ,and glandular epithelial cells were hyperplasia,and interstitial tissue were slight hyperplasia(Figure11).In0:10group,the prostate gland were hyperplasia,and just a little of glandular epithelial cells were hyperplasia,and interstitial tissue were almost normal(Figure12).

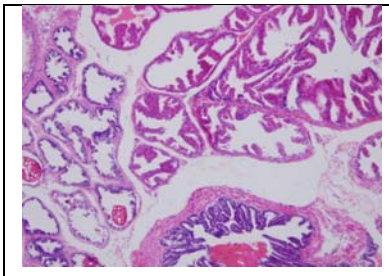

Figure 1 Blank HE×100

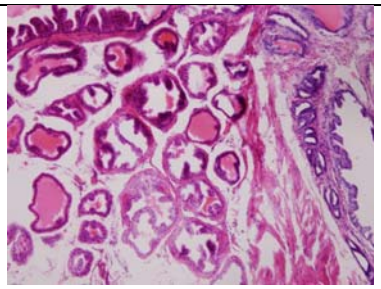

Figure 2 Modle HE×100

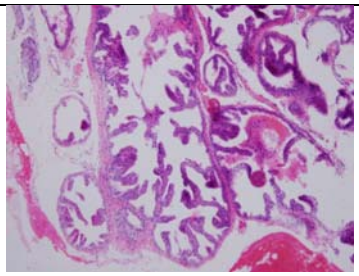

Figure 3 Longbishu HE×100

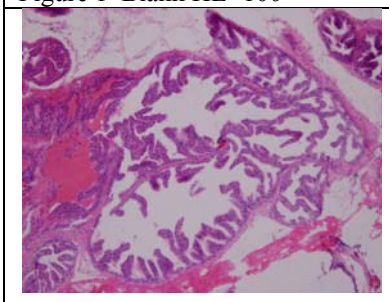

Figure 4 10:0 HE×100

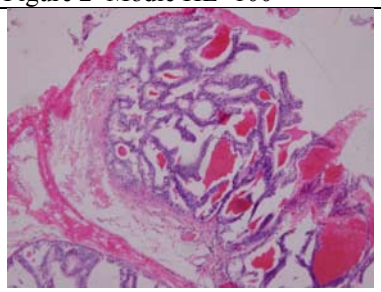

Figure 5 8:2 HE×100

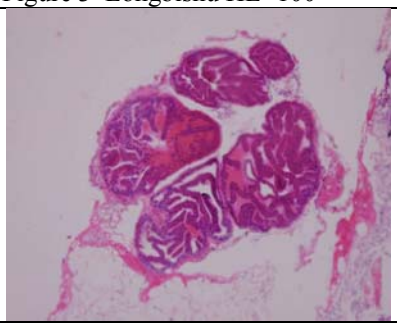

Figure 6 7:3HE×100

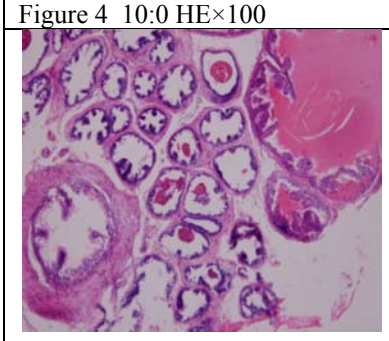

Figure 7 6:4 HE×100

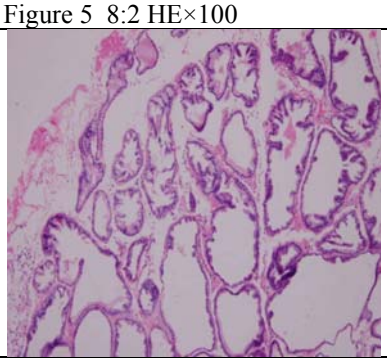

Figure 8 5:5 HE×100

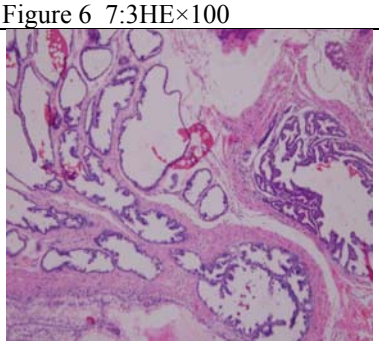

Figure 9 4:6 HE×100

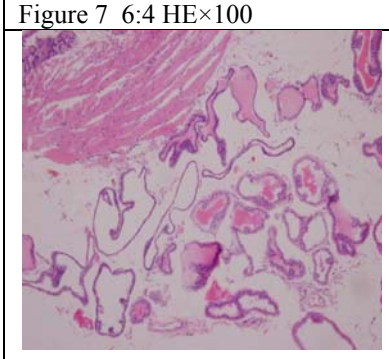

Figure 10 3:7 HE×100

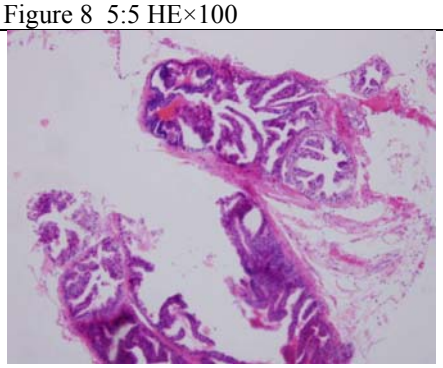

Figure 11 2:8 HE×100

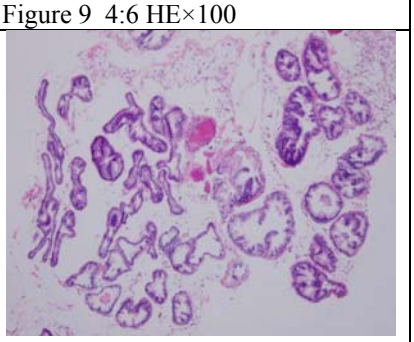

Figure 12 0:10 HE×100

Picture 1: Effect of different distribution ratio on prostate tissue morphology of BPH mice model induced

group (* *P<0.01 and * P<0.05); "-"the prostate gland, glandular epithelial cells and interstitial tissue were normal;"+" the prostate gland, glandular epithelial cells and interstitial tissue were normal,glandular epithelial cells were slight hyperplasia;"++"the prostate gland, glandular epithelial cells were hyperplasia and interstitial tissue were normal;"+++" the prostate gland and interstitial tissue were hyperplasia,glandular epithelial cells were extremely hyperplasia

Table3 shows that,by Ridit inspection,compared with the blank group ,the mice of model group were extremely significant hyperplasia（*P<0.01*）.Compared with the model group, the pathological changes of prostate hyperplasia in MTAs and ATS group of 8:2、7:3、6:4、5:5、3:7、0:10 were extremely significant reduce (*P<0.01*). The pathological changes of prostate hyperplasia in group of 2:8 were significant reduce （*P<0.05*）.The group of 6:4、5:5 were the best.

CONCLUSIONS

Testosterone is primary male hormone in the human body, Conversed dihydrotestosterone (DHT) at reductase,DHT concentrations increaseing leads to the prostate gland hyperplasia, changes in serum DHT concentration response treatment effect; basic fibroblast growth factor leads to prostate epithelial cell hyperplasia by DHT,glandular larger and increased secretions; epidermal growth factor which stimulated prostate epithelial cell growth factor positively correlated with plasma male hormone content, causing prostate hyperplasia, prostate and prostate index increased wet weight reflect the status of benign prostatic hyperplasia, prostate tissue morphology is critical to determine whether proliferation[4-5].

This study was designed to increase or decrease proportionally according to the baseline of MATS and ATS different proportions component, dubbed as 10:0,8:2,7:3,6:4,5:5,3:7,2:8,0:10 groups. Chinese medicine called benign prostatic hyperplasia as "Longbi", "Jingyin", caused by Qi stagnation and disorders,emotional injury, "Yu"is the the most common syndrome type in cline[6-7], the focus on prostate disease syndrome is"Yu", the " Huoxuetongluo " is highlight of treatment[8].

MATS " Huoxuetongluo " ,increase the amount of urine of rats, promote urination, improve internal prostate microcirculation, dredge duct obstruction, has well therapeutic effects on benign prostatic hyperplasia in mice. ATS "BuQi ",expand renal blood vessels, promote prostate cells withered death, improve the symptoms of benign prostatic hyperplasia[9-10]. our prophase studies suggest MATS and ATS have a good role in prevention of benign prostatic hyperplasia.

7: 3,6:4,5:5 was better to reduce wet weight,index of prostatic on benign prostatic hyperplasia in mice; 6:4,5:5 was better to reduce levers of serum DHT; 6:4,5:5 was better to improve pathological changes of benign prostatic hyperplasia.Comprehensive Evaluation of the experiment each index, groups can significantly reduce prostate index mice, significantly reducing DHT levels of mice, effectively improve the pathological changes of prostate tissue morphology mice. Therefore, based on 6:4,5:5 ratio of the group was better in therapy of prostatic hyperplasia.

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