Pule'an tablet (Conprosta) preclinical research report

1、pharmacy research summarize

1.1 formulas

Rape seed pollen	500g	（base）
Carboxymethylstach Sodium	50g	（disintegrating agent）
Sodium Carboxymethyl Cellulose	appropriate amount	（adhesion agent）
silicon dioxide（silica gel micro powder）	10 g	（fluidizer）
Be made into	1000 tablet	

1.2 specifications

Each tablet weight 0.57g（include rape seed pollen 0.5g）

1.3 manufacturing technique

Put rape seed pollen to recycled hot-wind drying cabinet for sterilization、grinding。Grab grinding rape seed pollen of the unit formula to mix with half quantity Carboxy methylstach Sodium of unit formula then mix evenly in wet type granulator，add appropriate adhesion agent（1.8% Sodium Carboxymethyl Cellulose ethanol water liquid）granulate、dry，pelletization，add rest Carboxymethylstach Sodium with silicon dioxide，tablet compressing、coating、tablet pick、bottling、seal，labeling、flask，thermal shrinkage、outer packing。

1.4 quality standard

operation details refer to YBZ01262007。

Test character、identification、disintegration time limited、assay、hygienic inspection and other items of 3 batches of pilot scale experiment sample，all conform to the standard。

1.5 stability studies

Grab 3 batches packed for selling samples，experiment in temperature40℃±2℃、relative humidity75%±5% artificial climate chest。Sampling respectively during experiment according to pule'an quality standard in first、second、third、sixth，check character、identification、disintegration time limited、assay、microbial limit and so on，every index of three bathes sample within 6 month conform to the standard，demonstrate quality stable。So expiry date is within 24 months。

2、Pharmacology and toxicology

2.1 Pharmacodynamics

2.1.1 The impact of rape pollen on castration and testosterone propionate inducing rats' prostatic hyperplasia model

This experiment adopts the castration and testosterone propionate inducing rat's prostatic hyperplasia model to observe impact of Pule an' s raw material--- rape pollen on castration and testosterone propionate inducing rat's prostatic hyperplasia.

2...1.1.1 Group and dose

Divide the rats randomly into the following 6 groups by their weights, with 10 in one group, that is, sham operation control group; Model group; Qianlieshule group(1.68g/kg/d， corresponds to 20 times of the clinical dosage); Rape pollen large, medium and dosage groups(2 g/kg/d, 1g/kg/d, 0.5 g/kg/d, corresponds respectively to 20, 10 and 5 times of the clinical dosage). Mix it with distilled water into appropriate concentration in experiment, and feed each group with the above-mentioned drugs, while the model group with distilled water.

2.1.1.2 Experiment method

Grab 280～300g male rat，adaptable feed one week then intraperitoneal injection anaesthesia with 0.3％ pentobarbital sodium，1mL/100g，fix rat，ligature and cut both side testicle in sterile condition，leave10 rats as sham-operation compare group，only cut、peel，not ligature and cut both side testicle，immediately sew up after surgical operation，sterilize surgical area。One week after surgery，divide ligatured rats into groups randomly，except sham-operation compare group，all rats have hypodermic injection testosterone propionate 0.3mg/each，one time a day，continue 30days，during inject testosterone propionate lavage the same dosage by testosterone propionate， one time a day， continue30days， dosage volume0.4mL/100g。sham-operation compare group and model compare group feed respectively same volume distilled water，adjust dosage volume according to weight each week。After dosage 30 days intraperitoneal injection anaesthesia with 0.3％pentobarbital sodium，weigh，celiotomize，peel prostate and seminal vesicle，remove adipose tissue，weight wet weigh by electrical scale，count volume of prostate by volumetric method。Fix prostate by 4％methylene oxide，embed ceresin wax，ordinary strip method，HE dye，observe pathological change of prostate under light microscope，measure diameter of glandular cavity and height of epithelium。

calculate prostate ratio、prostate volume weight compare、glandular cavity ratio as following formula。

Prostate ratio(g/100g)=（prostate weight/body weight）×100

glandular cavity ration(g/100g)=（glandular cavity weight/body weight）×100

prostate volume weight compare ratio(ml/kg)=)=（prostate volume/body weight）×1000

2.1.1.3data statistical analysis

Use mean ± standard deviation（$\bar{X}$ ±s），use SPSS 11.0 statistical software to carry out statistical analysis, one-way analysis of variance between groups，then use LSD method to compare between two sample。Put P＜0.05 as statistical meaning。

2.1.1.4 Result

⑴ Influence to normal situation of hyperplasia of prostate gland model rat

Model group rat after molding for 7days，become hair matt、activity decreased、emaciated and curliness，molding for 30days，weight increase speed lower distinctively，obvious difference with sham-operation rats。Extent of phenomenon of hair matt and activity decreased of all groups lightened，though compare weight with model group that extent leavened，no obvious difference（refer to table1）。

Table 1、rape seed pollen to weight of prostate of hyperplasia of prostate gland model rats（ $\bar{X}$ ±s；n=10 ）

group	dose (g crude drug/kg)	weight (g)	Wet weight of prostate (g)	Prostate ratio (g/100g)	Prostate volume (ml)	Volume weight ratio (ml/kg)
Sham operation group		472.8±15.3	0.736±0.219	0.156±0.047	0.637±0.120	1.35±0.24
Model group		416.7±17.0##	1.175±0.340##	0.282±0.077##	1.112±0.140##	2.67±0.32##
Qianlie Shule group	1.68	429.3±20.4	0.905±0.283*	0.211±0.065*	0.843±0.281**	1.97±0.67**
Rape seed pollen large dose group	2	430.3±22.0	0.749±0.252**	0.176±0.069**	0.744±0.159**	1.74±0.42**
Rape seed pollen medium dose group	1	423.5±17.9	0.917±0.239*	0.216±0.055*	0.890±0.167**	2.10±0.37**
Rape seed pollen small dose group	0.5	419.0±15.7	1.037±0.380	0.249±0.096	1.017±0.268	2.42±0.63

notes：compare to sham operation group：## P<0.01；compare to model group：* P<0.05；**P<0.01。

⑵ influence to weight 、volume and seminal vesicle weight of hyperplasia of prostate gland rats

Compare model group with sham operation group，weight of seminal vesicle and prostate increase obviously，volume of prostate increased，has distinctive difference。Positive and rape seed pollen different dosage groups compare to model group，weight and ratio of seminal vesicle and prostate all lowered different extent respectively。Obvious difference of prostate ratio of rape seed pollen large and medium dose group compare to model group；Obvious difference of prostate volume of rape seed pollen large and medium dose group compare to model group，effect of small dose was not obvious；Obvious difference of prostate wet weight and seminal vesicle ratio of rape seed pollen large and medium dose group compare to model group。Different dose group of rape seed pollen show dose effect connection。

（refer to table 1、2）。

Table 2、influence of rape seed pollen to seminal vesicle weight of hyperplasia of prostate gland rats（ $\bar{x}$ ±s；n=10 ）

group	dose (g crude drug /kg)	Wet weight of prostate (g)	Prostate ratio (g/100g)
Sham operation group		1.18±0.15	0.250±0.033
Model group		1.48±0.33##	0.357±0.095##
Qianlie Shule group	1.68	1.27±0.24*	0.297±0.057*
Rape seed pollen large dose group	2	1.15±0.12**	0.269±0.040**
Rape seed pollen medium dose group	1	1.21±0.23**	0.287±0.053**
Rape seed pollen small dose group	0.5	1.26±0.18*	0.301±0.047*

notes：compare to sham operation group：## P<0.01；compare to model group：* P<0.05；**P<0.01。

2.1.1.5 Conclusion

Result indication：rape seed pollen to testosterone propionate induced emasculated rats establish hyperplasia of prostate gland have distinctive restrain effect, show rape seed pollen anti hyperplasia of prostate gland effect to rats。

2.1.2 Influence of rape seed pollen to prostatitis rat rats model

The experiment adopt croton oil－methylene oxide induced prostatitis rats model，observe influence of raw material of pule'an---rape seed pollen to croton oil－methylene oxide induced prostatitis rats。

2.1.2.1division and dosage

Divide rat to 6 groups according to weight of rats，10 rats per group，as sham operation contrast group；model group；Qianlie Shule group（1.68g/kg/d，dosage amount equal to 20 times of clinical amount）；rape seed pollen large、medium、small dose group（2 g/kg/d、1g/kg/d、0.5 g/kg/d，equal to clinical amount 20、10、5times）。Blend with distilled water during experiment，all group drench all kinds of drug，sham operation and model groups drench distilled water。

2.1.2.2experiment method

Grab 60 weight 280～300g male rats，intraperitoneal injection anaesthesia with 0.3％pentobarbital sodium，1mL/100g，fix，laparotomy，expose urinary bladder，pull out prostate behind back neck lightly，inject 20μL 3.3mol/L croton oil－methylene oxide (8:2) mix inflammation induced liquid into prostate by

sterile micro scale sample injector，randomly pick 10 rats to inject 20μL physiological saline into prostate as sham operation group，reset urinary bladder，close abdomen。Drench drug as former dose after randomly division，drench distilled water to sham operation and model group，dose volume all 0.4mL/100g，one time a day，continue 10 days。1 hour after last dosage，weigh rats，intraperitoneal injection anaesthesia with 0.3％pentobarbital sodium and kill，paunch，cut prostate，leave adipose tissue and weigh wet weight。Get prostate liquid 10μl，add into 190μl leukocyte dilution to count number of leukocyte。Then grab prostate liquid smear，score density of LLZXT under microscope，score standard：within 40 times object glass，total field of view LLZXT（46～60 个）score 4，LLZXT 3/4 field of view score 3，LLZXT 1/2 field of view score 2，LLZXT 1/4 field of view score 1。count number of LLZXT and leukocyte by 5 field of view under 40 times light microscope，get average value。Fix prostate by 4％ methylene oxide，embed in ceresin wax，pick normal section of right side of prostate，HE dye，observe pathology change of visceral organ under light microscope。

Prostate ratio calculation formula：prostate ratio (g/100g)=（prostate weight/body weight）×100

2.1.2.3 Data statistical analysis

measurement data use mean± standard deviation（$\bar{X}$ ±s），adopt SPSS 11.0 census software to statistical analysis，groups compare adopt one-way analysis of variance，then comparison by LSD method。Put P＜0.05 as statistical meaning。Grade material count by SPSS software as classification，compare difference between groups。

2.1.2.4 Result

⑴ Influence to prostate weight of big rats of prostatitis model

Model group compare to sham operation group，wet weight of prostate and ratio increased distinctively，difference show significance；all medication groups compare to model group，no significant weight difference，positive drug and rape seed pollen large、medium dose group the wet weight of prostate and ratio decreased significantly，difference show significant meaning，small dose group show no significant difference of wet weight of prostate（refer to table 3）。

Table 3、influence to prostate weight of big rats of prostatitis model（ $\bar{X}$±SD；n=10 ）

Group	Dose (g crude drug/kg)	Weight (g)	Prostate weight (g)	Prostate ratio (g/100g)
Sham operation group		347.64±24.99	0.644±0.128	0.185±0.034
Model group		334.62±26.86	1.178±0.401##	0.350±0.108##
Qianlie Shule group	1.68	340.62±16.57	0. 796±0.175**	0.233±0.050**
Rape seed pollen large dose group	2	338.42±15.69	0.711±0.125**	0.212±0.045**

Rape seed pollen medium dose group	1	336.94±22.22	0.820±0.298**	0.243±0.087**
Rape seed pollen small dose group	0.5	332.81±20.53	0.960±0.394	0.291±0.122

notes：compare to sham operation group：## P<0.01；compare to model group： * P <0.05；** P <0.01。

(2) influence to Leukocyte number and LLZXT density

Leukocyte number and LLZXT density result refer to table 4。Result of table 4 indicate，in model contrast model Leukocyte number in prostate liquid increased obviously，in middle dose and positive contrast group Leukocyte number in prostate liquid decreased（P>0.05），large dose group Leukocyte number in prostate liquid decreased distinctively（P<0.01）。rank-sum test result of LLZXT density indicate，in medium、large dose group the LLZXT density in prostate liquid higher distinctively than model contrast group（P<0.01），in small dose group the LLZXT density not increase obviously，in positive contrast group the LLZXT density much more than model contrast group（P<0.05）。

Table 4、prostatitis big rats Leukocyte number and LLZXT density statistic result：

group	Leukocyte measurement （109/L）	LLZXT density（unit）				rank-sum test
		1	2	3	4	P
Sham operation group	10.50±6.64	0	0	4	6	/
Model contrast group	27.63±10.27##	6	3	1	0	0.000##
Small dose group	24.13±8.70	5	4	1	0	0.739
Medium dose group	20.01±10.93	0	6	3	1	0.009**
Large dose group	14.43±6.35**	0	5	4	1	0.005**
Positive contrast group	22.69±5.30	2	3	4	1	0.043*

notes：compare with sham operation group，#P<0.05,##P<0.01，compare to model contrast group，*P<0.05,**P<0.01

2.1.2.4 Conclusion

Result indication：rape seed pollen has distinctive restrain effect to croton oil－methylene oxide induced

prostatitis，indicate anti- prostatitis effect of big rats。

2.1.3 Rape seed pollen influence to granuloma induced by implantation of cotton pellets of big rats

The experiment adopt sterile cotton ball implantation to induce granuloma to establish nonspecific inflammation model，observe influence of pule'an raw material-- rape seed pollen to granuloma induced by implantation of cotton pellets of big rats。

2.1.3.1 Modeling

After all big rats intraperitoneal injection anaesthesia with 0.3％pentobarbital sodium 1ml/100g，cut abdomen on sterile condition，put weight（30mg）sterile cotton ball into both side of groin and under skin，sew incision。

2.1.3.2 Division and medication

Divide randomly into 6 groups after surgery ，each group 10 rats，total 60 rats，as model group（distilled water）；aspirin group（0.1g/kg/d）；Qianlie Shule group（1.68g/kg/d）；rape seed pollen large、medium、small dose group（2. g/kg/d、1 g/kg/d、0.5 g/kg/d）。drench drug on surgery day，medication volume 0.4ml/100g，once a day，model group drench same volume distilled water，continue for 6 days。

2.1.3.3 Index measurement

intraperitoneal injection anaesthesia with 0.3％pentobarbital sodium and kill rats in seventh day，peel and get out Granulation tissue then weigh wet weight of granuloma，put into 90℃ dry oven for 1h，weigh dry weight of granuloma，decrease30mg cotton ball weight，then get granuloma net weight。Measure granuloma net wet weight and net dry weight。Calculate restrain proportion as follow formula：

restrain proportion（%）＝（model group granuloma net dry weight—cure group granuloma net dry weight/model group granuloma net dry weight）×100%

2.1.3.4 Data processing

Data express as（$\bar{X} \pm S$），adopt SPSS 11.0 statistic software to statistical analysis，groups compare adopt one-way analysis of variance，then comparison by LSD method。Put P＜0.05 as statistical meaning。

2.1.3.5 Result

Refer to table 5。Rape seed pollen large、medium dose group、aspirin group、Qianlie Shule group all had obvious difference compared to model group（P＜0.01）；show Rape seed pollen large、medium dose group rape seed pollen has obvious restrain effect to granuloma induced by implantation of cotton pellets of rats，effect connect to dose。

Table 5 rape seed pollen influence to granuloma induced by implantation of cotton pellets of rats

（$\bar{X} \pm S$）

group	n	dose (g crude drug/kg)	Granulation net we weight	Granulation net dry weight	Restrain proportion（%）
Model group	10	—	597.27±40.73	111.47±31.66	
Aspirin group	10	0.1	355.22±55.49**	63.53±14.10**	43.0
Qianlie Shule group	10	1.68	426.05±54.27**	82.93±29.72*	25.6
Rape seed pollen large dose group	10	2.0	398.43±39.36**	74.82±15.49**	32.9
Rape seed pollen medium dose group	10	1.0	422.30±38.93**	83.52±20.71*	25.1
Rape seed pollen small dose group	10	0.5	556.27±48.98	94.99±29.11	14.8

notes：medication group compare with model group* P＜0.05，** P＜0.01。

2.1.3.6 Conclusion

Result demonstrate：rape seed pollen has obvious restrain effect to granuloma induced by implantation of cotton pellets of rats，and anti- nonspecific inflammation effect。

2.1.4 Influence of rape seed pollen to small rats of dimethylbenzene induced ear swelling

The experiment adopt dimethylbenzene induced ear swelling to establish nonspecific inflammation model，observe Influence of pule'an raw material ---rape seed pollen to small rats of dimethylbenzene induced ear swelling。

2.1.4.1 Division and medication

Divide small rats into 6 groups randomly by weight，each group 10 rats，as model group（distilled water）；aspirin group（0.15g/kg/d）；Qianlie Shule group（0.84g/kg/d）；rape seed pollen large、medium、small dose group（2.57 g/kg/d、1.28 g/kg/d、0.64 g/kg/d）。all group drench drug respectively while model group drench distilled water。

2.1.4.2 Experiment method

All medication group drench 3d continuously，medication volume0.3ml/100g，model group drench same volume distilled water，60min after last medication，use 0.1mL sample injector of dimethylbenzene to drip front and back of left ear of small rats，right ear as contrast，intraperitoneal injection anaesthesia with

0.3％pentobarbital sodium and kill rats in 0.5 hour，cut ears along auricle line，cut same area of both ears by 8mm diameter punching bear，weigh by type FA2104 electrical balance，swelling level is calculated by left era weight to decrease right ear weight。And calculate restrain proportion as follow formula：

Restrain proportion（%）＝（model group swelling level-cure group swelling group/model group swelling group）×100%

2.1.4.3 Data processing

Data express as（$\bar{X} \pm S$），adopt SPSS 11.0 statistic software to statistical analysis，groups compare adopt one-way analysis of variance，then comparison by LSD method。Put P＜0.05 as statistical meaning。

2.1.4.4 Result

Refer to table 6。Rape seed pollen large、medium dose group、aspirin group、Qianlie Shule group all had obvious difference compared to model group（P＜0.01）；rape seed pollen all dose groups show effect dose positive correlation，show rape seed pollen distinctive restrain effect to small rats of dimethylbenzene induced ear swelling。

Table 6 influence of rape seed pollen to small rats of dimethylbenzene induced ear swelling（$\bar{x} \pm s$）

group	n	dose（g crude drug/kg）	Swelling level	Restrain proportion（%）
Model group	10	—	11.35±2.17	
Aspirin group	10	0.15	5.25±1.67**	53.7
Qianlie Shule group	10	0.84	8.73±2.45*	23.1
Rape seed pollen large dose group	10	2.57	9.03±2.09*	20.4
Rape seed pollen medium dose group	10	1.28	9.21±3.23*	18.9
Rape seed pollen small dose group	10	0.64	10.92±1.58	3.8

notes：medication group compare to model group* P＜0.05，** P＜0.01。

2.1.4.5 Conclusion

Result indicate：rape seed pollen show distinctive restrain effect to small rats of dimethylbenzene induced ear swelling，and anti- nonspecific inflammation effect。

2.2. Security study

2.2.1 Acute toxicity

Restricted by density of medication，pule ‘an soft capsule extract can not assay LD50 value，adopt largest medication volume、largest medication density to drench small rats，process largest dosage experiment。pule ‘an soft capsule extract medication 2 times a day，4hours between two medication，total medication 128g（crude drug）/Kg，equal to 1280times clinical dose，observe14days continuously after medication，no significant toxic reaction。indication，the LD50 value　more than 128g（crude drug）/Kg。

2.2.2 Big rats’ long-term toxicity test

pule ‘an soft capsule extract large、medium、small dose 10、5、2.5 g（crude drug）/Kg，equal to clinical dose 100、50、25 times，drench big rats continuously for 26 weeks。pule ‘an soft capsule extract medication for 13 weeks，the （Alb）of big rats in large、medium dose groups and （TP）in large dose groups increased significantly；the（RBC）in　large、small dose groups and （HGB）in small dose groups increased significantly，but no significant influence to reticulocyte of all dose groups；medication for 26 weeks，（TP） in large、medium dose group increased significantly，（ALP）in large、medium、small dose group decreased significantly，return to normal station after stop medication for 4 weeks。No significant influence to urinary output of normal big rats，no significant influence connected with drug to main visceral organ and other indices。

Phenomenon of （Alb）、 （TP）、（RBC）、（HGB） increase and （ALP）decrease after medication 13 weeks and 26 weeks are all within normal fluctuation range，with statistic meaning but no biology meaning。The security range not less than 100 times of clinical dose range。