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Effect of Raspberry on Osteoporosis Model in Ovariectomized Rats

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ABSTRACT

To investigate the effects of raspberry on various indexes of serum and urine andbone mineral density in rats with osteoporosis

Methods: *Female ovariectomized osteoporosis animal model was established by ovariectomy. Ratswere completely removed the left ovary, the right removal of 80%, after careful breeding, intramuscular injection of penicillin 800000 u / kg (each 0.1mL) to prevent infection, continuous 3d, 1 day. After that, except for the model blank group, the rats in each group were given gavage for 60 days. The rats in the blank group were given the same amount of normal saline. The rats were fasted on the 58th day, Urine was taken 2h after the last administration on the 60th day. Blood was taken from the eye and the serum was separated. Serum calcium (Ca), phosphorus (P), serum BGP, urinary calcium, phosphorus, creatinine (Cr) and hydroxyproline (HYP) were measured. The tibias on one side of femur on both sides of the rats were used for bone mineral density, pathological examination and biomechanical measurement.*

Results: *Compared with the blank group, serum Ca in the model group decreased significantly. High-dose raspberry group and Xianlinggubao group could significantly reduce the level of serum Ca, and middle-dose raspberry group also decreased serum Ca.*

Conclusion: *The raspberry high, medium and low dose groups have a good intervention onosteoporosis model in ovariectomized rats.*

Keywords: *Osteoporosis; Mouse; Raspberry;*

INTRODUCE

The raspberry is the dry fruit of east China raspberry. Raspberry, taste sweet, sour. It's mild. Raspberry liver, kidney, bladder by. It has the benefits of kidney, solid essence, urine reduction, and nourishing liver. This drug can be used to treat spermatorrhea, enuresis, urinary frequency, impotence, premature ejaculation, head dark dim and other symptoms. Osteoporosis is a common and frequently occurring disease that occurs in post-menopausal women, the elderly and chronic diseases. The main cause of osteoporosis is the decreased bone mass and the deterioration of the fine bone structure, resulting in increased bone fragility and fracture risk. In recent years, the incidence of osteoporosis gradually increased, about 80% of elderly people over the age of 60 in our country with varying degrees of osteoporosis, mainly female patients. At present, there are mainly estrogen receptor modulators, bisphosphonates, calcitonin, and fluoride preparations clinically used for the prevention and treatment of osteoporosis. Although some curative effects have been obtained, the long-term medication adverse reactions are large, And expensive. Therefore, to find a safe and effective drug for the prevention and treatment of osteoporosis has become the current research hotspot. In this study, ovariectomized animal models of ovariectomized osteoporosis in ovariectomized rats were established to investigate the effects of raspberry on the osteoporosis model in ovariectomized rats and to provide theoretical and experimental basis for the clinical breakthrough of osteoporosis.

MATERIALS

Experimental Equipment

FA (N) / JA (N) series of electronic balances, Shanghai Minqiao Precision Instrument Co., Ltd .; HWS12 electric heated water bath, Shanghai Yiheng Scientific Instrument Co., Ltd .; KDC-160HR high-speed refrigerated centrifuge, HKUST Innovation Co., Ltd. Good branch; 680 microplate reader, the United States BIO-RAD company; adjustable pipette, Shanghai Leibo Analytical Instruments Co., Ltd.

Experimental Reagents

Raspberry; usage dosage 6-12g, kidney, solid fine, urea.

Positive drugs: Xianling Gubao capsule, Guizhou Tongjitang Pharmaceutical Co., Ltd. Z20025337 ,; saline, Henan double crane Huali Pharmaceutical Co., Ltd. Production.

Experimental Animals

Laboratory Animal Name: 220-240g Rat Female GermLine: Wistar Grade: SPF Shandong Experimental Animal Center Quality Certificate: NO.37009200002630.

METHOD

Take a weight of 220-240g female wistar rats made castration osteoporosis model. Rats were housed in animal centers for 3-4 days. On the fifth day, 12 rats were randomly selected as the most blank group. The remaining rats in each group were weighed and injected intraperitoneal with 10% chloral hydrate (0.3mL / 100g) Bit fixed, and then from the back of the rat under the ribs, about 2cm from the spine at the intersection of shear, disinfection, cut the skin and back muscles, cut about 1cm, incision field of vision can be seen a milky white cellulite gently tweezers Catch the cellulite pull out the incision, the separation of cellulite, you can see a group of thin linear irregular, yellow-red tissue, that is, the ovary. First cut the ovary fallopian tubes (including fat) with a thin line ligation, complete removal of the left ovary, the right removal of 80%. After the homeopathic tissue back into the abdominal cavity, suture muscles and skin osteoporosis model. Postoperative intensive care, intramuscular injection of penicillin 800000 u / kg (each 0.1mL) to prevent infection, continuous 3d, day 1, after the mice were awakened and fed 1-2 days after weighing were randomly divided into model group, positive group The dosage of Xianlinggubao group was 0.5g / kg, which was equivalent to 10 times of the clinical dose, the volume of the drug was 2mL / 100g, the dosage was 0.025g / mL), and the high, medium and low dose raspberry The doses were 4g / kg, 2g / kg, 1g / kg, the dosage volume was 2mL / 100g, the dosage was 0.2g / mL, 0.1g / mL and 0.05g / Times, 10 times, 5 times). The remaining groups in the model group and the blank group were administered with the corresponding drugs daily for 60 consecutive days. The rats were weighed once every two weeks, the food intake was observed during the intragastric administration, and the activity of the rats was observed. On the 58th night, fasting could not help but water. The rats were put into metabolic cages to collect the urine of rats on the next day. Blood was taken from the eyeballs and serum was separated 2h after the last administration on the 60th day. Both sides of the rat femur, one side placed in 10% formalin, for pathological observation. The other side of the femur was immersed in saline gauze wrapped, refrigerated at minus 40 ℃, for bone mineral density determination. In addition, the left tibia of rats was taken, wrapped in gauze soaked in saline and refrigerated at minus 40 ℃ for biomechanical measurement. Main outcome measures; Levels of serum Ca, P, BGP, urinary calcium, phosphorus, urine creatinine (Cr), urinary hydroxyproline (HYP).

Table1. *Raspberry on the impact of body weight*

Group	N	1w	3w	5w	7w	9w
Blank group	12	222.50±10.81	245.83±12.71	254±13.02	263.83±9.34**	273.20±15.11**
Model group	12	220.04±10.99	253.83±18.35	265±28.95	294.38±19.16	306.46±21.54
Positive group	12	221.46±10.50	250.67±13.29	258.42±15.86	267.92±9.10**	276.10±16.38**
High-dose raspberry group	12	227.75±15.56	254.79±13.47	257.25±18.62	270.63±19.37*	278.33±23.92**
Medium-dose raspberry group	12	231.88±8.49	253.67±11.74	255.5±12.32	271.79±18.29**	281.33±19.48**
Low-dose raspberry group	12	225.63±9.95	256.20±17.81	257.29±17.70	277.46±17.80*	283.17±18.80**

*Note: * indicates p <0.05 and ** indicates p <0.01*

Rat Femur BMD (BMD)

Rat biomechanical determination of tibia; tibia 10% formalin injection, to be routine light microscopy.

RESULTS

Body Weight

As can be seen from the above table, compared with the blank group, the model group at week 7, the body weight was significantly increased

(p <0.01), showing successful modeling, compared with the model group, raspberry high and medium dose groups and cents In the seventh week, the Linggu capsule could significantly reduce the body weight (p <0.01), and the low dose raspberry group also could reduce the body weight (p <0.05). In the ninth week, the raspberry high, medium and low dose groups and Xianlinggubao group can significantly reduce body weight (p <0.01).

Raspberry on Serum and Urine Biochemical Indicators

As can be seen from the above table, compared with the blank group, the model group serum Ca, decreased significantly (p <0.01), indicating successful modeling, compared with the model group, high-dose raspberry group and Xianlinggubao group Serum Ca levels were significantly lower (p <0.01), and the middle-dose raspberry group also decreased serum Ca levels (p <0.05). Compared with the blank group, serum P was significantly increased in the model group (p <0.01), indicating successful modeling. Compared with the model group, high-dose raspberry group and Xianlinggubao group could significantly reduce serum P level <0.01). The middle-dose raspberry group also decreased serum P level (p <0.05). Compared with the blank group, the model group serum osteocalcin was significantly higher (p <0.01), indicating successful modeling, compared with the model group raspberry high, medium and low dose groups and Xianlinggubao group can significantly reduce the serum Osteocalcin levels (p <0.01). Compared with the blank group, the levels of urinary Ca and urinary P in the model group were significantly increased (p <0.01), indicating that the model was successful. Both raspberry height and Xianlinggubao group could significantly reduce urinary P levels (p <0.01), and raspberry middle dose group also reduce urinary P levels (p <0.05). Compared with the blank group, urinary hydroxyproline content in the model group was significantly increased, indicating successful modeling, compared with the model group, raspberry high, medium and low dose groups and Xianlinggubao group can significantly reduce the large Urine hydroxyproline content (p <0.01). Compared with the blank group, urinary creatinine in the model group was significantly lower, indicating that the model was successful. Compared with the model group, Xianlinggubao group could significantly increase urinary creatinine (p <0.01). High-dose raspberry group also increased urine creatinine in rats (p <0.05).

Table2. *Effect of raspberry on serum biochemical indexes in rats*

Group	N	Blood Ca（mmol/L）	Blood P（mmol/L）	Blood osteocalcin (ng/mL)
Blank group	12	2.47±0.11**	1.32±0.16**	7.06±0.38**
Model group	12	1.72±0.26	1.75±0.22	10.69±1.09
Positive group	12	2.24±0.33**	1.43±0.18**	8.00±0.84**
High-dose raspberry group	12	2.12±0.35**	1.49±0.20**	8.11±0.55**
Medium-dose raspberry group	12	1.98±0.23*	1.54±0.09*	7.90±0.58**
Low-dose raspberry group	12	1.64±0.34	1.63±0.14	8.09±0.67**

Table3. *Raspberry on rat urine biochemical indicators*

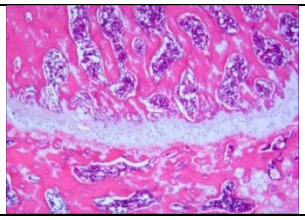
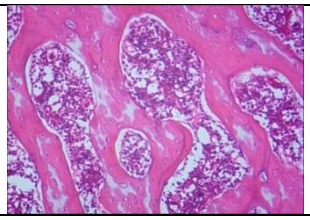
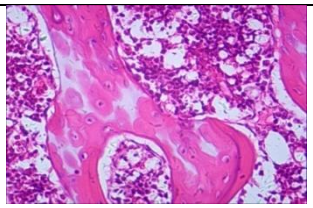
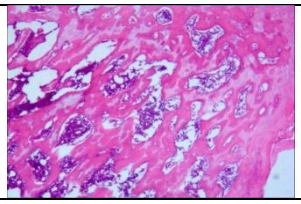
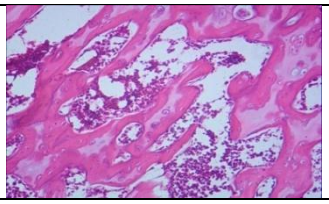
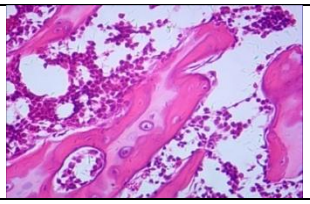
Group	N	Urine Ca（mmol/L）	Urine P（mmol/L）	Hydroxyproline (ng/mL)	Urinary creatinine(umol/L)
Blank group	12	3.20±0.20**	7.16±0.43**	810.60±83.86**	26.63±2.10**
Model group	12	4.56±0.55	11.70±0.16	1389.30±259.44	22.26±2.69
Positive group	12	3.76±0.31*	9.02±0.44**	945.55±81.33**	26.16±1.46**
High-dose raspberry group	12	3.95±0.25	9.35±0.48**	889.07±70.79**	25.78±1.78*
Medium-dose raspberry group	12	3.97±0.32	10.09±0.65*	1015.00±126.00 **	24.73±3.12
Low-dose raspberry group	12	4.13±0.37	10.38±0.93	1016.85±80.45**	24.19±4.81

Raspberry on Femoral Bone Mineral Density and Tibial Mechanical Strength

From the above table, compared with the blank group, the model group bone mineral density was significantly lower (p <0.01), modeling success, compared with the model group raspberry high, medium and low dose groups and positive group can significantly increase the rat femur Bone mineral density was significantly lower (p

<0.01), modeling success, compared with the model group raspberry high, medium and low dose groups and positive group can significantly increase the rat femur Bone mineral density values (p <0.01). By comparing the strength of bone mechanics can be seen in the model group, the maximum strength of the tibia was significantly less than the blank group (p <0.01), indicating successful modeling, compared with the model group raspberry high dose group and the positive group can significantly increase the rat tibia (P <0.01). The low-dose raspberry group also significantly increased the maximum tolerance of the tibia (p <0.05).

Attachment1. *Raspberry on rat femur pathology*

		
Blank group×10	Blank group×20	Blank group×40
		
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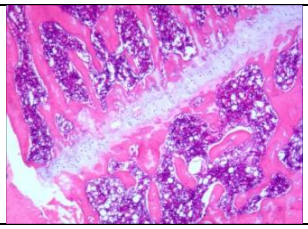
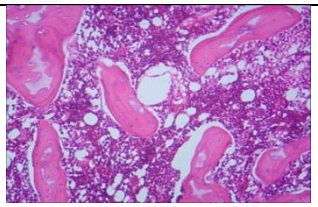
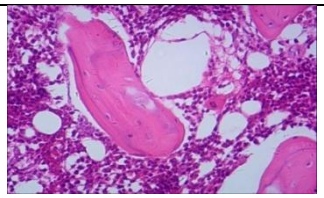
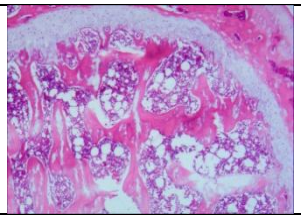
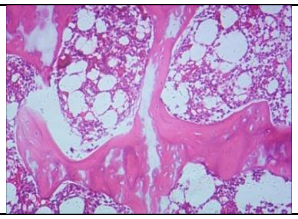
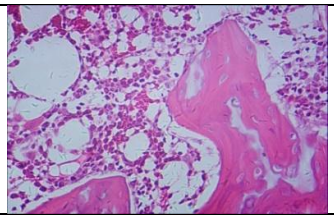
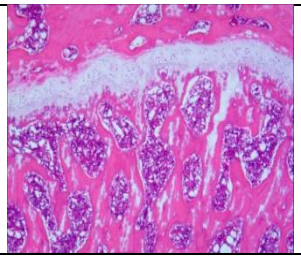
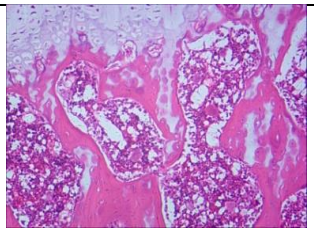
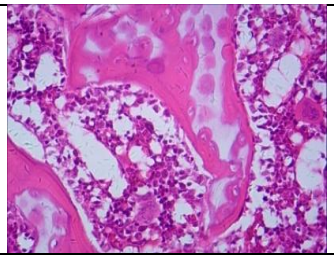
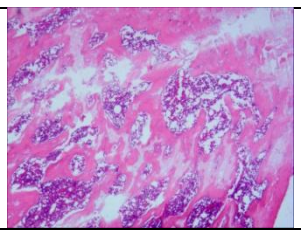
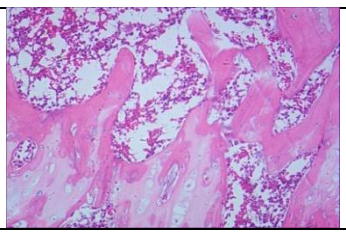
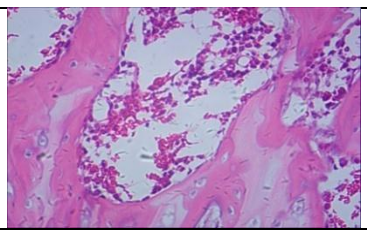
		
Positive group×10	Positive group×20	Positive group×40
		
High-dose raspberrygroup×10	High-dose raspberrygroup×20	High-dose raspberrygroup×40
		
Medium-doseraspberrygroup×10	Medium-doseraspberrygroup×20	Medium-doseraspberrygroup×40
		
low-dose raspberry group×10	low-dose raspberry group×20	low-dose raspberry group×40

Table4. *Effect of raspberry on femoral bone mineral density and tibial bone strength in rats*

Group	N	Bone density（g/cm2)	Bone strength(N)
Blank group	12	0.246±0.007**	23.48±1.21**
Model group	12	0.192±0.008	15.34±3.05
Positive group	12	0.237±0.008**	18.72±1.84**
High-dose raspberry group	12	0.237±0.007**	18.33±2.38**
Medium-dose raspberry group	12	0.233±0.006**	18.16±2.08**
Low-dose raspberry group	12	0.217±0.011**	17.74±2.37*

Note: ** indicates p <0.05 and ** indicates p <0.01*

Group	n	-	+	++	+++
Blank group	12	12	0	0	0
Model group	12	1	11	0	0
Positive group	12	11	1	0	0
High-dose raspberry group	12	10	2	0	0
Medium-dose raspberry group	12	11	1	0	0
Low-dose raspberry group	12	10	2	0	0

Raspberry on Rat Femur Pathology

The number of "+" mild trabecular was decreased, the continuity was poor, the structure was sparse, the arrangement was loose and irregular; the number of moderate trabecular was decreased, the continuity was poor, intermittent, the structure was sparse and the arrangement was loose , Irregular; "+++" severe trabecular number reduction, discontinuous, sparse structure, arranged loose, irregular.

Ridit test showed that compared with the blank group, the model groups showed significant osteoporosis symptoms, the platelets were loosely arranged and the model was established successfully. Compared with the model group, Xianling Gubao capsule group, high, medium and low dose raspberry group Osteoporosis has been significantly improved, the experimental results show that Xianling Gubao capsule and raspberry for ovariectomy established female rat osteoporosis model has a significant improvement.

In this study, ovariectomized animal models of ovariectomized osteoporosis in ovariectomized rats were established to investigate the effects of raspberry on osteoporosis animal models in ovariectomized rats. Fluid indicators and rat femur, tibia bone mineral density and biomechanical strength have a better intervention. Animal into touch, the rats in each group of serum and urine indicators have varying degrees of improvement.

Based on the above analysis, raspberry high, medium and low dose groups have a good intervention on osteoporosis model of ovariectomized rats, providing theoretical basis and experimental basis for clinically clinical breakthrough osteoporosis.

Conclusion

Currently the occurrence and development of osteoporosis and spleen, liver and kidney, evils, lack of exercise, five flavors are closely related. In the present study, osteoporosis model was established by ovariectomy in female rats. The results showed that the change of microstructure of raspberry caused by the reduction of estrogen could prevent the decrease of bone mineral density and bone mass, improve the bone strength and enhance the bone resistance External impact ability to reduce the incidence of fractures. Modern medicine believes that the slow development of osteoporosis, is a chronic metabolic life-long disease, the disease occurs in age, menopause, genetic, diet and lifestyle habits and other factors, the cost of a single factor.

Bone is composed of bone mineral and bone matrix composed of two parts, bone mineral mainly composed of amorphous calcium and phosphorus mixture and calcium apatite crystals, serum calcium levels can indirectly reflect the status of bone metabolism. After ovariectomy in rats, a decrease in Ca content in the model group was observed. The experimental results show that raspberry can be used to treat osteoporosis, and relieve serum Ca levels, to maintain a relatively stable state.

It has been proved through experimental studies that Chinese traditional medicine raspberry has a good therapeutic effect on the model of osteoporosis in ovariectomized rats. However, we must also correctly understand the problems

that modern Chinese medicine needs to be solved in preventing osteoporosis. First, clinical evaluation of traditional Chinese medicine and traditional Chinese medicine for osteoporosis curative effect is not scientific, the lack of clear quantitative indicators and scientific and rational clinical trial design; Second, different batches of drugs will affect the stability of important clinical effects; Third, the long-term use of traditional Chinese medicine will have some side effects. Therefore, we must further improve the scientific, scientific design of clinical trials of traditional Chinese medicine, a reasonable assessment of the safety of traditional Chinese medicine, Chinese medicine treatment of osteoporosis gradually accepted by the international mainstream medicine.

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