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Effects of Cervi Penis Wine on Bone Mineral Density and Lipid of Ovariectomized Female Rats

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Abstract

Objective To explore the effects of Cervi penis wine on bone mineral density and lipid of ovariectomized female Sprague Dawley (SD) rats. Methods Fifty female SD rats were randomly divided into five groups: normal control group, osteoporosis model group, low dose of Cervi penis wine intervention group, medium dose of Cervi penis wine intervention group and high dose of Cervi penis wine intervention group. Normal control group and osteoporosis model group were given saline with the dose of 4ml/d. The doses of Cervi penis wine were 2， 4 and 6ml/d respectively in low, medium and high dose of Cervi penis wine intervention group. After the feeding of Cervi penis wine for 30 days, the bone mineral density and lipid of rats were assessed. Results The difference in bone mineral density among the three doses of Cervi penis wine intervention groups was significant ($P < 0.05$); the most obvious improvement of one mineral density was found in low and medium dose of Cervi penis wine groups ($P < 0.05$). The TG、TC and LDL-C significantly decreased, and HDL-C increased significantly in Cervi penis wine group compared with those in the osteoporosis model group ($P < 0.05$). The most obvious improvement was found in low and medium dose of Cervi penis wine groups ($P < 0.05$). Conclusions Tree different doses of Cervi penis wine could prevent BMD reducing and the osteoporosis， and Cervi penis wine could improve the lipid metabolism. The effects of low and medium dose of groups were more obvious.

Keywords: Cervi Penis Wine; Osteoporosis; Bone Mineral Density; Lipid Metabolism

Abbreviations: SD: Sprague Dawley, PMOP: Postmenopausal Osteoporosis

Introduction

Postmenopausal osteoporosis (PMOP) is a metabolic bone disease characterized by decreased bone mass, decreased bone mineral density, and increased fracture risk caused by decreased levels of estrogen secreted by the ovaries after Menopause **[1]**. At the same time, postmenopausal

osteoporosis often triggers significant dyslipidemia **[2]**.

Postmenopausal osteoporosis patients have an increased risk of fracture, which can easily lead to disability or even death, causing a heavy burden on society and families **[3]**. According to statistics, the prevalence of OP in women over 50 years old is 32.1%, and the prevalence of OP in women over 65 years old reaches 51.6% **[4]**. In recent years, with the aging of the population, the incidence and disability rate of osteoporosis have been increasing year by year, and it has become one of the biggest global problems.

Deer whips are also known as deer kidney and deer penis in classical Chinese medicine writings, which are the penis and testicles of sika deer or horse deer **[5]**, and the deer penis in "Beiji Qianjin Yaofang. Deer penis is not only as a tonic for the kidneys and aphrodisiacs by generations of Chinese medicine practitioners, but also use its nourishing and strengthening, regulating the function of the body, since ancient times used in the treatment of diseases and daily health care. Modern studies have shown that both aqueous and alcoholic extracts of deer penis have good anti-fatigue and stamina-enhancing effects **[6-8]**. Currently, there are not many clinical studies on deer penis in the prevention and treatment of osteoporosis and the improvement of blood lipid function. In this study, we analysed the effects of different doses of deer whip wine on bone mineral density and lipid metabolism in depopulated female Sprague Dawley (SD) rats to provide a new basis for the prevention and treatment of osteoporosis and dyslipidaemia.

Materials and Methods

1 Materials: This study was conducted after approval by the Ethics Committee of West China Hospital, Sichuan University (Grant No.2008(1150)).

1.1 Experimental Animals: 50 SPF grade 2-month-old SD female rats, body mass 170~230 g, purchased from Chengdu Dashuo Biotechnology Co., Ltd. in Sichuan Province, with the license number of XK(Sichuan) 2008-24, standard feeding, ambient temperature of 20-25℃, and the room is well ventilated. The experiment was reviewed by the Animal Ethics Committee and met the relevant ethical requirements.

1.2 Reagents and Instruments: penicillin, 1% sodium pentobarbital, 10% formaldehyde. The main ingredients of deer whip wine were deer whip, wolfberry, lotus seed, Euryale, Yizhiren rock candy, 39% alcohol, and the concentration of deer whip was 0.115 uL/kg. Deer whip wine was responsible for the experimental study by Sichuan University and was supplied by Sichuan Jinhuang Leshuang Deer Industry Co. Ltd. and sodium citrate 1:9 anticoagulant blood collection tubes (Hebei Kangweishi). Bone densitometer Lunar iDXA (US GE Healthcare); medical freezing centrifuge LDZ522 (Beijing Medical Centrifuge Factory); automatic biochemical analyzer COBAS C501 (Roche, Switzerland).

2 Animal grouping and modeling

2.1 Grouping: After rats were acclimatized and fed for 1 week, 50 test rats were randomly assigned to the normal group, the PMOP model group, and the low-dose (2 ml), medium-dose (4 ml), and high-dose (6 ml) Deer Whip Wine intervention groups, 10 rats each, by applying the method of randomized numerical table.

2.2 Modeling: The most widely used and most widely used denudation modeling method was used to construct the postmenopausal osteoporosis animal model **[9-11]**. The rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital 0.03g/kg, placed in the prone position, and the skin was prepared and disinfected with a towel. A longitudinal incision was made into the abdominal cavity from 1 cm on either side of the spine and 2 cm above the iliac spine, the ovaries were exposed, the fallopian tubes were ligated, the ovaries were removed, and the incision was sterilized by suturing layer by layer. Penicillin 80,000 units were injected intramuscularly once a day for 3 consecutive days from the day of surgery to prevent postoperative infections.

3 Intervention Method

According to daily drinking habits and related studies **[12-14]**, this experiment calculates the drug dose according to the conversion formula of human and animal body size coefficients **[1,5]**, and the experimental animals were subjected to the equivalent dose conversion, and the intervention doses were as follows: low dose 2 ml

(equivalent to the adult dose of 50 ml), medium dose 4 ml (equivalent to the adult dose of 100 ml) and high dose 6 ml (equivalent to the adult dose of 150 ml). Standardized rearing for 1 month after modeling surgery before intervention. Normal control group: standardized feeding, 4 ml of saline per day; PMOP model group: 4 ml of saline; low-dose deer penis wine intervention group, medium-dose deer penis wine intervention group, high-dose deer penis wine intervention group, respectively, were fed with 2, 4, 6 ml of deer penis wine/d. Once a day, for 30 consecutive days by gavage.

On the 2nd day after the intervention, 1 mL of whole blood from carotid artery of all rats was extracted into anticoagulated blood collection tube, and after standing at room temperature for 30 min, it was put into the centrifuge at 4℃ and centrifuged at 4000 r/min for 15 min, and the supernatant was taken at -80 C for spare, and then the lipid indexes were detected by automatic biochemistry analyzer, including the TC (mmol/L), TG (mmol/L), LDL-C (mmol/L), LDL-C (mmol/L) and HDL-C (mmol/L). The rats were executed,the right femur was isolated, stripped of soft tissues, and the right femur was taken and immersed in 4% formaldehyde. Bone density was scanned using a bone density detector, and small animal detection software was used to analyze the bone density (g/cm2) of the right femur.

4 Statistical methods

The experimental data of this study were statistically analyzed using SPSS 22.0 software. Continuous data were expressed as mean ±standard deviation,and ANOVA was used to analyze the ANOVA for thedata that conformed to normal distribution; if they did not conform tonormal distribution, the rank sum test (Kruskal-Walli's test) was used. The difference was considered statistically significant at $P<0.05$.

Results

1 Comparison of bone mineral density in each group:

The bone density of rats in the osteoporosis model group was significantly reduced ($P<0.05$), indicating that the osteoporosis model modeling was successful. Compared with the osteoporosis model, the bone density of the low, medium and high dose of deer whip wine intervention group were significantly increased ($P<0.05$). Compared with the high-dose deer penis wine intervention group, the bone mineral density was significantly higher in the low- and medium-dose deer penis wine intervention group ($P<0.05$); however, the difference was not statistically significant compared with the low- and medium-dose groups ($P>0.05$). (See Table 1 for details)

2 Comparison of biochemical indexes in each group:

(The results of biochemical indexes of each group are shown in Table 1).

2.1 TG, TC, LDL-C Compared with the normal control group, the osteoporosis model group showed a significant increase in TG, TC and LDL-C ($P < 0.05$); compared with the osteoporosis model group, the low, medium and high dose of deer penis wine intervention group showed a significant decrease in TG, TC and LDL-C ($P < 0.05$), of which the effect of the low and medium dose group was significant ($P < 0.05$), while the difference between the low and medium dose groups was not statistically significant ($P > 0.05$).

2.2 HDL-C Compared with the normal control group, HDL-C in the osteoporosis model group was significantly lower ($P < 0.05$); compared with the osteoporosis model group, HDL-C was significantly higher ($P < 0.05$) in the three doses of deer penis wine intervention groups; comparing the two groups of low-, medium-, and high-dose deer penis wine groups, the low and medium-dose deer penis wine was significantly higher ($P < 0.05$), and the difference between the low- and medium-dose groups was not statistically significant ($P > 0.05$). was not statistically significant ($P > 0.05$).

Discussion

Osteoporosis is a systemic disease characterized by decreased bone mass, destruction of bone structure and increased bone fragility **[16]**. Main pathologic manifestation of osteoporosis is decreased bone mineral density **[17]**. Clinical use of dual energy X-ray bone densitometry provides a rapid and accurate measurement of bone density

[18]. Estrogen inhibits bone resorption and also promotes bone growth. Postmenopausal women are prone to dyslipidemia, which is related to osteoporosis. When the estrogen level of postmenopausal women decreases, the activity of osteoclasts increases, the rate of bone resorption is greater than the rate of bone formation, and the balance between osteoclasts and osteoblasts is disturbed, leading to osteoporosis. Since ancient times, Chinese medicine practitioners have believed that "the kidneys store essence and produce marrow", which is related to the function of the kidneys on the bones. Some studies have found that the kidney plays an important role in the process of bone growth and bone reconstruction, and that the degree of prosperity of the kidney affects bone density **[19]**.

Deer penis mainly contains progesterone, testosterone, estradiol and cortisol hormones, which have a certain positive effect on the enhancement of human sexual function. In addition, the biogenic amines and phosphatidylcholine contained in deer penis play the role of neurotransmitters and have the physiological functions of promoting thymus development and regulating the neuroendocrine-immune and enzyme systems **[20]**. The research reports proved that the deer whip contains 4 kinds of vitamins, 9 kinds of phospholipids, 22 kinds of minerals, 8 kinds of proteins and polypeptides, 4 kinds of hormones, 17 kinds of hydrolyzed amino acids, 7 kinds of fatty acids, and 3 kinds of prostaglandins **[20]**. This is an important basic condition that deer whip can nourish Yin and Yang, strengthen musculoskeletal and body,

In this experimental study, after 1 month of intervention, compared with the osteoporosis model group, the difference in bone density between the low, medium and high doses of deer penis wine intervention groups were statistically significant ($P < 0.05$), indicating that deer penis wine has the effect of improving the bone density of the rats, Among the low and medium doses of effect of improving the bone density is better.

In this experiment, it was observed that total cholesterol, triglyceride and LDL were significantly elevated in the OP model group of rats, while HDL was significantly reduced ($P < 0.05$), suggesting that decrease in estrogen level did affect the normal regulation of blood lipid levels. Low, medium and high doses of deer penis wine reduced the total cholesterol, triglyceride, LDL and have a tendency to increase HDL to varying degrees in denuded rats, thus improving lipid metabolism. However, this study did not find that the low medium and high doses of deer whip wine intervention groups were compared two by two, and the effect of the low and the medium dose groups was statistically significant ($P < 0.05$).

To summarize, in this experiment, the postmenopausal osteoporosis animal model was constructed by de-emphasis modeling method, and thereafter three different doses of deer penis wine were fed to implement the intervention experiment, and the bone density was detected by dual-energy X-ray bone densitometer, and the blood lipids of SD rats were measured by COBAS C501 biochemistry instrument, and the results showed that the three doses of deer penis wine improved the bone density and positively regulated the blood lipids, and the effects of the low and middle dose groups were significant, with statistical significance. The results showed that all three doses of deer penis wine improved bone density and positively regulated blood lipids. From the perspective of economic cost and possible side effects of drug overdose, low-dose deer penis wine is more advantageous. This experiment provides a certain scientific basis for the improvement of bone density and regulation of dyslipidemia by deer whip wine and provides a research basis for the prevention and treatment of osteoporosis and improvement of lipid metabolism by regulating the body functions of kidney tonic traditional Chinese medicine. However, the sample size of this study is small, the period is short, and there are deficiencies such as insufficient coverage. Subsequently, more in-depth and long-lasting effect observation studies will be conducted if conditions are available.

Table 1 BMD, TG, TC, LDL-C, HDL-C levels in each group (n=10, x±s)

Group	BMD (g/cm^2)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Normal control group	0. 1424±0.0381	0.94± 0. 154	1.387± 0.099	1.067± 0. 114	0.294±0.066
Osteoporosis model group	0.0942±0.0062*	1.31± 0.094*	2.234± 0.210*	0.782± 0.106*	0.519±0.093*
Low-dose deer penis wine intervention group	0. 1300±0.0049#	0.657±0. 164#	1.451± 0. 101#	1. 173± 0.176#	0.264±0.076#
Medium-dose deer penis wine intervention group	0. 1323±0.0057#	0.962± 0.235#	1.53± 0. 17#	1.204± 0.157#	0.305±0.074#
High-dose deer penis wine intervention group	0. 1110±0.0058#▲▽	1.287±0.083#▲▽	1.855± 0. 191#▲	0.935± 0.098#▲▽	0.385±0. 102#▲▽

* P < 0.05 compared with the normal control group

# P < 0.05 compared with the osteoporosis model group

▲ P < 0.05 compared with the low-dose deer penis wine intervention group

▽ P < 0.05 compared with the medium-dose deer penis wine intervention group

Declarations

Acknowledgments: Not applicable

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Availability of data and materials: The datasets supporting the conclusions of this article are included within the article.

Authors' contributions

Dr. Hua Jiang took responsibility for investigation and data curation and drafted the manuscript; Dr. Hongyang Chen revised the manuscript.

All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

Consent for publication: Not applicable.

Ethics approval and consent to participate

All methods were implemented in accordance with relevant guidelines and regulations and passed ethical approval. This study was conducted after approval by the Ethics Committee of West China Hospital, Sichuan University (Grant No.2008(1150)).

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