

Experimental Study on the Treatment of Osteoporosis Rats After Castration with Liuwei Dihuang Decoction*

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Abstract: This study observes the preventive and therapeutic effect of Liuwei Dihuang decoction on bilateral excision of ovary from osteoporosis-induced rat. The results show that, Liuwei Dihuang decoction can reduce urine calcium and urinary hydroxyproline excretion, increase osteocalcin content in serum, femur minerals, as well as calcium and phosphorus contents in bone, and enable uterus, spleen and thymus to bear a load. It's prompted that Liuwei Dihuang decoction can oppose bone loss, inhibit bone resorption, promote bone formation, and effectively prevent from osteoporosis.

Key Words: Liuwei Dihuang decoction; osteoporosis; bone metabolism

Osteoporosis is of changes in bone metabolism, which features in reduction of bone mass in unit volume. Chinese medicine practitioners consider the occurrence of this disease is closely related with the kidney. Kidney deficiency induced osteoporosis has been confirmed by many physicians.^[1,2] Liuwei Dihuang pill is of a popular formula of nourishing Yin and invigorating the kidney. It was originally used to treat non-closure of fontanels induced by renal deficiency and delay of skeleton growth and development. Modern research found that the formula can increase bone calcium and phosphorus storage, maintain the normal metabolism of calcium and phosphorus^[3]. This study explores the curative effects of Liuwei Dihuang decoction on ovariectomized female rat osteoporosis, reported as follows.

1. Data and Methods

- 1.1. **Experimental Drugs:** By the original formula ratio of Liuwei Dihuang pill (rehmanniae vaporata: dogwood: yam: cortex moutan alismatuckahoe = 8:4: 4: 3: 3: 3), purchase herbal medicines from Medicinal Materials Corporation of Shanxi Province, and it is verified that such medicines are all genuine. According to the conventional decoction methods, decoct twice, filter, blend evenly, and concentrate to crude drug content of 1.35g/lid, place it into box at 4°C and seal for storage. The whole process is LWD for short. Nilestriol, provided by Shanghai No.12 Pharmaceutical Plant, Lot: 920908.
- 1.2. **Animal:** adult female Wistar rat purchased from Shanxi Medical College, with body weight 208~232g(215~16g)
- 1.3. **Method:** These rats are randomly divided into 4 groups after conventional feeding for a week, with 12 rats for each group. Rats in Group A accept pseudo-operation, with their dorsal shields sheared after the anesthesia by intraperitoneal injection of 2.8% pentobarbital sodium, and part of adipose tissue removed from the incision. Group B is the model group, Group C is the group treated with Liuwei Dihuang decoction, and Group D is the group treated with nilestriol. The three groups of rats have ovariectomized osteoporosis (OP) models^[4,5]

copied by corresponding methods quoted in the references. They have their dorsal shields sheared for the anesthesia by the method mentioned above. Under aseptic conditions, respectively excise bilateral ovarian from the incisional wounds on both sides of lumbar, stop bleeding and suture, and do anti-infectious treatment. Within one week after the operation, start intragastric administration: distilled water (5ml/kg), Liuwei Dihuang decoction with a daily dosage of 5ml/kg (6.75g/kg) and once a day, nilestriol suspension with a dosage of 6ml/kg (1mg/kg), once a week and for 90 consecutive days. Rats in each group are fed under the same conditions, and freely drink and eat.

On the day before the end of the experiment, respectively collect 24-hour urine with metabolic cages, and measure the contents of urine calcium and urinary hydroxyproline. Upon the completion of the experiment, get blood sample from the rat under the etherization, and separate serum by the centrifugal separation. Remove spleen, thymus and uterus, and respectively weigh. Remove femur from the right side and tibia from the right side, and eliminate all the soft tissues attached to bone. Separately measure the contents of calcium, phosphorus and alkaline phosphatase in serum. According to the instructions of reagent kit, measure the contents of serum estradiol and osteocalcin by using radioimmunoassay. Weigh thymus, spleen and uterus, and calculate the indexes of thymus, spleen and uterus. Weigh wet weight of femur. Measure the volume of femur by Archimedes 'law, and the wet weight of femur per unit volume is also the specific weight. Weigh dry weight after drying. Defat three times and dry. Weigh fat-free dry weight. Incinerate femur, and the weight of ash content (ash weight) is the weight of inorganic substance. Measure bone calcium and bone phosphorus in ash content. Tibiae is made into bone slices after decalcification, dehydration and imbedding. Observe the morphological changes, and observe the number of multinucleated osteoclasts with high power lens.

2. Results

2.1 For the effects of LWD on OP rat urine calcium (u-Ca) and urinary hydroxyproline (u-HoP), see Table 1. The outputs of urine calcium and urinary hydroxyproline of the model group are significantly higher than those of the control group. The administrations of Liuwei Dihuang

decoction and nilestriol enable lower both of the outputs declined. Note: Liuwei Dihuang decoction can oppose the loss of calcium and increase of bone matrix collagen protein decomposition like nilestriol, thereby preventing bone calcium and bone matrix reduction.

Table 1 Effects of LWD on Rats' U-Ca, U-Hop, Uterus, Thymus and Spleen Indicators ($\bar{x} \pm s$)

Category	n	U-Ca (μg/24h)	U-Hop (μg/24h)	Uterine (mg/100g)	Thymus (mg/g)	Spleen (mg/g)
Comparison	12	13.44 ± 3.31**	281.43 ± 42.38**	189.27 ± 22.12**	1.36 ± 0.04**	2.46 ± 0.17*
Model	10	27.21 ± 6.89	359.37 ± 39.22	65.78 ± 17.93	0.98 ± 0.12	1.33 ± 0.05
Liuwei Dihuang Decoction	11	20.48 ± 5.43**	294.59 ± 41.13**	163.26 ± 18.11**	1.23 ± 0.09**	2.31 ± 0.16*
Nilestriol	12	19.36 ± 5.94**	285.77 ± 42.74**	170.83 ± 19.86**	1.02 ± 0.11	1.62 ± 0.24*

Compared with the Model Group, * P < 0.05, ** P < 0.01 (the same as below)

2.2 Effects of LWD on Rats' Femur Specific Weight, Fat-Free Dry Weight, Ash Weight, Bone Calcium and Bone Phosphate: As Table 2 shows, the five indexes of the Model Group are significantly lower than those of the Control Group. The two groups administrated can improve the above-mentioned reductions. Liuwei Dihuang

decoction can not only improve femur specific weight, fat-free dry weight and weight of inorganic substances in bone but also increase the contents of calcium and phosphorus in femur. Note: Liuwei Dihuang decoction has a facilitating effect on the accumulation of calcium and phosphorus contents in bone and the increase of bone minerals.

Table 2 Effects of LWD on Rats' Femur Weight, Fat-Free Dry Weight, Ash Weight, Bone Calcium and Bone Phosphorus ($\bar{x} \pm s$)

Category	n	Specific Weight (mg/cm ³)	Fat-Free Dry Weight (mg/cm ³)	Ash Weight (mg/cm ³)	Bone Calcium (mg/cm ³)	Bone Phosphate (mg/cm ³)
Comparison	12	1672 ± 18**	898.7 ± 17.3**	618.5 ± 9.2**	244.4 ± 10.3**	92.82 ± 6.44**
Model	10	1279 ± 22	797.6 ± 18.1	563.7 ± 8.1	162.2 ± 8.2	60.09 ± 7.03
Liuwei Dihuang Decoction	11	1594 ± 36**	860.6 ± 19.2**	598.5 ± 8.8**	207.3 ± 9.9**	80.88 ± 6.72**
Nilestriol	12	1668 ± 20**	882.4 ± 18.0**	595.3 ± 9.1**	198.5 ± 9.4**	69.97 ± 7.92**

Effects of LWD on Rats' Calcium Serum (Ca), Phosphorus (P), Alkaline Phosphatase (AKP), Estradiol (E2) and Bone Gla Protein (BGP): As Table 3 shows, the three groups have no significant difference in the concentration of calcium blood and serum inorganic phosphorus. The alkaline phosphatase content in rats of the model group increases, but the osteocalcin

content decreases, and the estrogen content is out of the detection scope. Both Liuwei Dihuang decoction and nilestriol can lower SAKP level and improve bone gla protein level. Note: Liuwei Dihuang decoction can inhibit the rise of alkaline phosphatase content, and increase the osteocalcin content.

Table 3 Effects of LWD on Rats' Serum Ca, P, AKP, E2 and BGP ($\bar{x} \pm s$)

Category	n	Ca (mmol/L)	P (mmol/L)	AKP (King Unit)	E2 (pg/ml)	BGP (g/L)
Comparison	12	2.03 ± 0.16	2.44 ± 0.48	6.72 ± 1.97**	162.77 ± 13.29**	2.89 ± 0.32**
Model	10	1.94 ± 0.22	2.37 ± 0.72	9.13 ± 1.82	-	1.74 ± 0.25
Liuwei Dihuang Decoction	11	2.08 ± 0.45	2.47 ± 0.32	6.82 ± 1.27**	-	2.79 ± 0.16**
Nilestriol	12	2.11 ± 0.38	2.39 ± 0.77	6.87 ± 1.42**	-	2.17 ± 0.11**

number of ovariectomized rats' trabeculars significantly

For effects of LWD on the weight of OP rats' uterus, thymus and spleen, see Table 1. Rats' uterine weight significantly decreases after ovariectomy. Both the two groups administrated can increase the uterus weight, and Liuwei Dihuang decoction can significantly increase the weight of thymus and spleen. Note: Liuwei Dihuang decoction can oppose reduction of uterus weight caused by emasculation, with immune organ weight gain. For morphological changes of bone tissue and effects of LWD, see Table 4. Compared with the control group, the

reduces, and their trabecular bones are narrow and thin, with trabecular spacing widened, and cavum medullare ossium expanded. The number of osteoclasts increases under high power lens. After the administration of Liuwei Dihuang decoction, the number of trabecular bones increases, the area of trabecular bone becomes larger, and the number of osteoclasts reduces. Note: Liuwei Dihuang decoction can inhibit the damage to ovariectomized rat's skeleton structure.

Table 4 Changes of the Number of Rats' Trabecular Bones and Osteoclasts of Different Groups ($\bar{x} \pm s$)

Category	n	Number of Trabecular Bones	Number of Osteoclasts
Comparison	12	12.44 ± 1.27**	2.68 ± 0.66**
Model	10	7.26 ± 1.04	5.12 ± 0.83
Liuwei Dihuang Decoction	11	10.35 ± 1.96*	3.05 ± 0.92*
Nylestriol	12	10.93 ± 1.28*	3.78 ± 0.53*

3.

Discussion

In the TCM theory, kidney stores essence, controlling the condition of bone and marrow; and, it has an important role in the growth and development of skeleton, bracing up the whole system and delaying senility. Only when the kidney-essence is rich can the skeleton be fully nourished and become strong. In case of any deficiency of the kidney-essence, the skeleton will be lack of nutrients, damp off, and become fragile. Zhang Jingyue pointed out, the "insufficiency of true-yin" is an important mechanism of the elderly women's aging, and the key of their renal deficiency lies in the loss of bone essence. And, research has found that, the occurrence of women's renal deficiency is positively correlated with their age, and the incidence increases with the age increased [2]. In particular, the bone mineral density of the women after pausimenia continues to decline with their age increase, and bone mass reduces [6]. Therefore, renal deficiency and deficiency of YIN-essence are the important factors for the osteoporosis after pausimenia. It is a permanent solution to treat such symptoms through nourishing the kidney-essence, and Liuwei Dihuang pill focuses on nourishing Yin and invigorating the kidney. This study shows that Liuwei Dihuang decoction has a certain curative effect on female rats with osteoporosis induced after emasculation.

Postmenopausal women has ovarian hypofunction and decay of in vivo estrogen level. Because estrogen can directly acts on bone metabolism via estrogen receptors [7] in osteoblast cell nuclear, intestines and kidney, promote bone formation, and inhibit bone resorption through regulating other hormones and active substances, so, in case of a reduction of estrogen, bone resorption is lager than bone formation, and osteoporosis appears. Clinically, patients' estrogen level often decline due to premenopausal ovariectomy or premature menopause, and higher morbidity of osteoporosis is a better proof. In this experiment, ovariectomized rats' in vivo estrogen level decreases significantly, while their serum alkaline phosphatase level is higher; their output of urine calcium and urinary hydroxyproline increases, and the calcium and phosphorus content in femur and the weight of bone minerals reduce; and, the number of trabecular bone decreases, while that of osteoclasts increases. All of these show that, after model establishment, the bone resorption is enhanced, bone formation is inhibited, and osteoporosis appears. It is more appropriate to apply such model to simulating the primary osteoporosis induced by female rat's decline in estrogen level after pausimenia. Considering the analysis of all experimental results, the mechanism of action of Liuwei Dihuang decoction on such a model of osteoporosis may be related to adjustment of bone metabolism, promotion of bone formation, inhabitation of bone resorption, estrogen-like effect, improvement of endocrine environment in vivo, regulation of bone metabolism, improvement of immunity, reduction of bone resorption, etc.

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