



Tianchang Capsule prevents ovariectomy induced osteoporosis in rats

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Abstract

This article performed a model of osteoporosis rats using the method of ovariectomy and observed efficacy of Tianchang Capsule on treatment of osteoporosis rats. Comparing the effect with positive drug to explore the mechanism of Tianchang Capsule in postmenopausal osteoporosis. Female SD rats were randomly assigned to 6 groups (10 in each group): control (sham-treated), OVX (by ovariectomy), OVX+AL (by ovariectomy, received alendronate sodium), OVX+LTC (by ovariectomy, received low dosage Tianchang Capsule), OVX+MTC (by ovariectomy, received middle dosage Tianchang Capsule), OVX+HTC (by ovariectomy, received high dosage Tianchang Capsule). After ovariectomy operation, alendronate or Tian Chang capsule were given for 12 weeks. Bone metabolism was measured by bone mineral density (BMD). With 12-week treatment, BMD reduced in OVX group, in bone formation and resorption parameters increased however, pathological sections indicated destruction of trabecular bone structure in the meanwhile ($P < 0.05$ or $P < 0.01$). In alendronate sodium and Tianchang Capsule treatments groups, comparing with control, both treatments significantly increased BMD level and improved the trabecular bone tissue damage, also inhibit excessive actively bone resorption and bone formation ($P < 0.05$ or $P < 0.01$). Tianchang Capsule were used as good natural drugs for the treatment of ovariectomy induced osteoporosis rats, by increasing bone mineral density, bone formation and bone resorption.

Keywords: postmenopausal osteoporosis; Tianchang Capsule; alendronate sodium; bone resorption; bone formation.

Practical Application: Prevention of Tianchang Capsule from ovariectomy induced osteoporosis in rats.

1 Background

Osteoporosis is characterized by a progressive decrease bone mass and density, result in the increase of bone fragility which lead a high risk for fragility fracture (Rachner et al., 2011). The pathological core of osteoporosis is reducing of bone mass. The 2010 epidemic surveying date of EU shows 22 million female and 5.5 million male patients have a osteoporosis, 3.5 million of them have a fragility fracture, including hip fractures 18%, vertebral fracture 15%, forearm fracture 16%, and other types of fractures 51%. Excepted the date will increase by 25% in 20 years (Hernlund et al., 2013). In the United States in 2010, about eight million women and one to two million men had osteoporosis (Wade et al., 2014; Willson et al., 2015). White and Asian people are at greater risk (Chunlin et al., 2014). With aging of the population in worldwide, the prevalence of osteoporosis increases rapidly and it will be a huge financial burden to all the countries. So it's necessary to prevent and treat the osteoporosis, medication, exercise and additional treatments are commonly used in course of osteoporosis treatment (Milat & Ebeling, 2016; Henriksen et al., 2016; Lee et al., 2020; Eor et al., 2020). Plenty of clinical experience has been accumulated in the therapy of osteoporosis with Chinese medicine, which has many advantages. Therefore, this study will focus on the effect of Chinese medicine on postmenopausal osteoporosis, which

belongs to the range of primary osteoporosis (Chinese Medical Association of Osteoporosis and Mineral Disease, 2017).

The growth, development, metabolism and aging of bone tissue are characterized by the increase or decrease of bone mass, and morphology of bone metabolism are bone modeling and bone remodeling two ways (Kanis et al., 2008). The cyclic metabolism of bone completed by bone remodeling, because bone modeling stopped after skeletally mature (Ismail et al., 2020; Özden & Kiliç et al., 2020). Bone remodeling is a metabolic process of bone, which is a dynamic balance process of bone formation, resorption and motionless in order to maintain the relative stable state of the bone mass. Bone resorption is the process by osteoclasts remove bone matrix and minerals, which is generally evaluated by the number of osteoclasts. Bone formation refers to the process of new bone formation and maturation. Unmineralized osteoid is first appeared in the process of bone formation, followed by calcified (mineralization) frontiers. Osteoblasts are arranged on the osteoid, and the number of osteoid can be reacted to the activity of osteocyte. Osteoclast cells are derived from hematopoietic stem cells (HSC), osteoclasts have the characteristics of mononuclear/macrophage lineage cells. Osteoblast cells come from mesenchymal stem cells (MSC),

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osteoblasts and osteoclasts control the bone transformation efficiency by their interrelated, restricted and promoted (Rankin et al., 2012; Calvi et al., 2003; Greenbaum et al., 2013; Zhang et al., 2003; Mediero & Cronstein, 2013).

Tianchang Capsule (TC Capsule) is a traditional Chinese medicine (TCM) preparation for Gu-Song-Tang (GST), which is a widely used in clinical experience prescription, and previous findings showed that GST could kidney-reinforcing and bone-strengthening, spleen and nourishing Qi. Tianchang capsule is composed of astragalus, radix rehmanniae preparata, poria cocos, concha ostreae, psoralen, epimedium, and all the other thirteen drugs, which focus on regulating liver to regulating the Qi and Blood of the inter organs (Zu et al., 2016). The project preliminary study directed against diabetic osteoporosis shows that GST has good effect on treating diabetic osteoporosis. A clinical trial have found GST treatment (basic treatment and GST) could reduce TCM symptom integral of patients and PINP, increase bone mineral density (BMD) and IGF-1, moreover the stage for decreased in TCM symptom integral of patients is faster than in basic treatment (Caltrate and alendronate sodium). GST could improve the condition of osteoporosis and decrease the incidence rate of fracture may be decrease the differentiation and proliferation of osteoblastic, suppressing hyperactivity bone transformation (Liu, 2013). GST could not only improve the condition of osteoporosis and decrease the incidence rate of fracture, but also had particular advantages in improving clinical symptom. The study on the primary osteoporosis is inconclusive, so our aim is to investigate efficacy of Tianchang Capsule in postmenopausal osteoporosis and its mechanism of action.

2 Materials and methods

2.1 Drugs

Tianchang Capsule is provided by pharmacy department of our hospital. Alendronate sodium (10 mg) is provided by CSPC Pharmaceutical Group Limited.

2.2 Animals

60 adult female Sprague-Dawley rats aged eight weeks weighing 264.7-338.4 g were routinely feed up until twelve weeks.

2.3 Grouping and modeling

After routinely feeding up until twelve weeks, rats would be undergone bilateral ovariectomy. For the model group, rats were narcotized by intraperitoneal injection of 15 ml/kg of 3% pentobarbital sodium, 75% ethanol was used to disinfect the skin. 1~2 cm longitudinal skin incision in the back, and the abdominal cavity was opened in the thinner muscle layer of the posterior wall of the lateral abdomen to expose the milk white banded fat mass of rats. Removed the fatty group in ovaries, ligated the surrounding blood vessels with the zero suture line. Afterwards placed the uterus and fat backed to the abdominal cavity, next sutured each layer and sterilized the surgical wound with Iodophor. The sham-treated group performed similar surgery, but without removing the ovary.

3 days' post operation, rats were randomly assigned into 6 groups (10 in each): control (sham-treated), OVX (by

ovariectomy), OVX+AL (by ovariectomy, received alendronate sodium), OVX+LTC (by ovariectomy, received low dosage Tianchang Capsule), OVX+MTC (by ovariectomy, received middle dosage Tianchang Capsule), OVX+HTC (by ovariectomy, received high dosage Tianchang Capsule). After grouping, the OVX+AL group was given 0.00167 g/kg (10 times of human clinical dosage (10 mg/d)) alendronate sodium solution for lavage administration, and purified with purified water as the solution of alendronate sodium. The OVX+LTC group was administered with 1.66 g/kg of Tianchang Capsule solution, which dosage was 5 times of clinical dosage. The OVX+MTC group was given to 3.33 g/kg in 10 times of clinical dosage. The OVX+HTC group was given to 6.66 g/kg in 20 times of clinical dosage. Sham-treated group and OVX group were given with 0.5%-CMC-Na, 20 ml/kg. Lavage administration was administered once a day for 12 weeks.

2.4 The general condition

During the experiment, growth, survival, size, hair, skin, feces, gait, muscle tension, mental breathing were observed once a day. Measured weight once a week.

2.5 Sample collection

The rats were anesthetized by intraperitoneal injection of pentobarbital on the second day after the last lavage administration. The rats were killed after taking blood from abdominal aorta. Room temperature for about 40 min, centrifuged at 3000 rpm, 10 min, supernate was taken. Both sides of the femur were taken and the muscles attached to the bone were removed with gauze. The left femur was immediately used for detecting the bone density, and the right femur was soaked with 4% formaldehyde solution, histopathological examination were performed subsequently.

2.6 Measurement of BMD

After 12 weeks, BS224S electronic balance suites were used to determine the bone mineral density (BMD) of the left femur samples.

2.7 Biochemical analysis of serum

According to manufacture's protocol, serum bone alkaline phosphatase (BALP), osteocalcin (OT), tartrate resistant acid phosphatase (TRACP) and human deoxyypyridinoline crosslinks (DPD) were measured with ELISA kits (Cusabio., Ltd., Wuhan, China).

2.8 Histomorphological analysis

Right femur was fixed with 4% formaldehyde and then followed up with decalcification, embed in paraffin and serially cut. Sections were stained with hematoxylin and eosin (H&E). Histomorphological examination was performed by BX43 optical microscope (OLYMPUS, Tokyo, Japan).

2.9 Statistical analysis

The values were expressed as mean \pm SEM. Comparisons between different groups were carried out with analysis of variance and Student's *T*-test. Statistical analyses were performed

using SPSS 22.0 (SPSS, Chicago, Illinois). The difference with $P < 0.05$ was considered to be significant.

3 Results

3.1 The general condition

After lavage administration 70 days, one of OVX+MTC rat died of gavage operation. The rats of OVX+HTC had appeared the symptoms of exhaustion, exercise slightly reduced, and restored automatically after 2-3 h. The rest of the groups of animals did not see abnormal sign.

3.2 BMD in each group

The BMD of the femur were presented in Figure 1. BMD in the OVX group significantly decreased compared with sham-treated group ($P < 0.01$). Compared with the OVX group, BMD were increased in OVX+AL group and OVX+LTC group as well as OVX+MTC group ($P < 0.05$) but not the OVX+HTC.

3.3 Serum biochemical parameters

Bone formation parameters

As seen in and Figure 2, the bone formation parameters were bone alkaline phosphatase (BALP) and osteocalcin (OT) of OVX group appeared to a significantly increase compared with Sham-treated group ($P < 0.01$), which considered the ovariectomized led to a dramatically dropping of estrogen levels. Compared with OVX group, the BALP level of OVX+AL group have a decline ($P < 0.05$). BALP level in the group of using TC capsule decreased compared with OVX group, but there's no statistical significance. Furthermore, we could see a statistical significance between OT level in OVX+LTC and OVX group ($P < 0.05$), but showed no statistical significance in other groups.

Bone resorption parameters

In treatment groups (OVX+AL, OVX+TC), hyperactive bone resorption was inhibited as showed in Figure 3. The deoxypyridinoline crosslinks (DPD) and tartrate resistant acid phosphatase (TRACP) level in OVX group have an obviously increase compared with sham-treated group ($P < 0.01$, $P < 0.05$). Positive control alendronate sodium have a good effective with restrain the absorption of bone in the current study compared with OVX group ($P < 0.01$, $P < 0.05$). Each dose groups TC capsule all have a good effects on inhibiting the TRACP level compared with OVX groups ($P < 0.05$). OVX+LTC had a significantly inhibit effects in DPD level ($P < 0.01$), all the other dose of TC capsule had an effect on decrease DPD levels, but there were no statistical significance compared with OVX group ($P > 0.05$).

3.4 Histomorphological results

As seen in Figure 4, OVX group were evident decreased in the number of trabecular bone and became thinner, loosen and fracture in trabecular bone. These evidences could prove the OVX group successfully duplicate the osteoporosis models in rats. Trabecular bone structure in OVX+AL and OVX+LTC group marked improvement compared with OVX group,

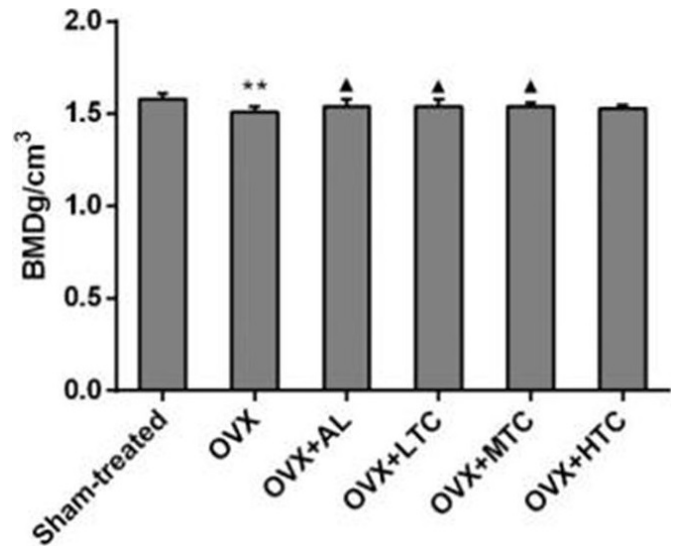


Figure 1. Comparison of BMD in different groups. Compared with the sham-treated group, ** $P < 0.01$. Compared with the OVX group, ^ $P < 0.01$.

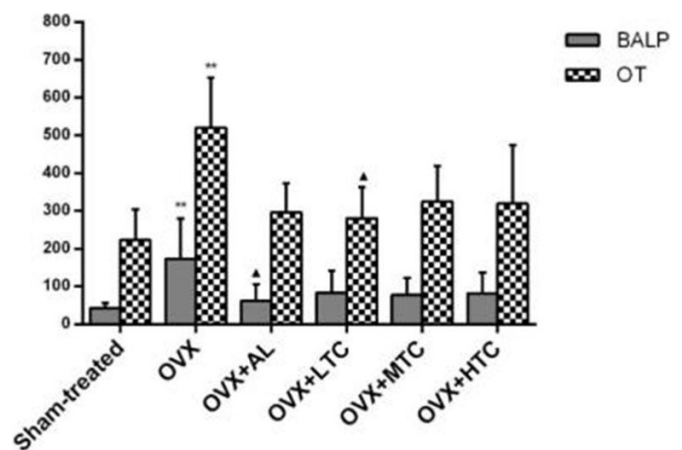


Figure 2. Comparison of the bone biochemical index in different groups. Compared with the sham-treated group, ** $P < 0.01$. Compared with the OVX group, ^ $P < 0.01$.

OVX+MTC and OVX+HTC improved slightly. The results mentioned above could also reflect in femur pathological scores in Table 1. The scoring standards of the femur pathological based on increasing the evaluation method of bone density function (draft for comments) and revised description (2012) of methods of bone histopathological examination.

4 Discussion

Osteoporosis in elderly patients can cause bone pain, shorter height, deformity, decreased mobility, increased fracture risk and mortality, and seriously affect the quality of life of patients (Kawate et al., 2010; Iwamoto et al., 2011). Postmenopausal women due to its estrogen levels and ovarian function decline, estrogen levels, resulting in increased risk of osteoporosis. Since postmenopausal osteoporosis is mostly caused by calcium

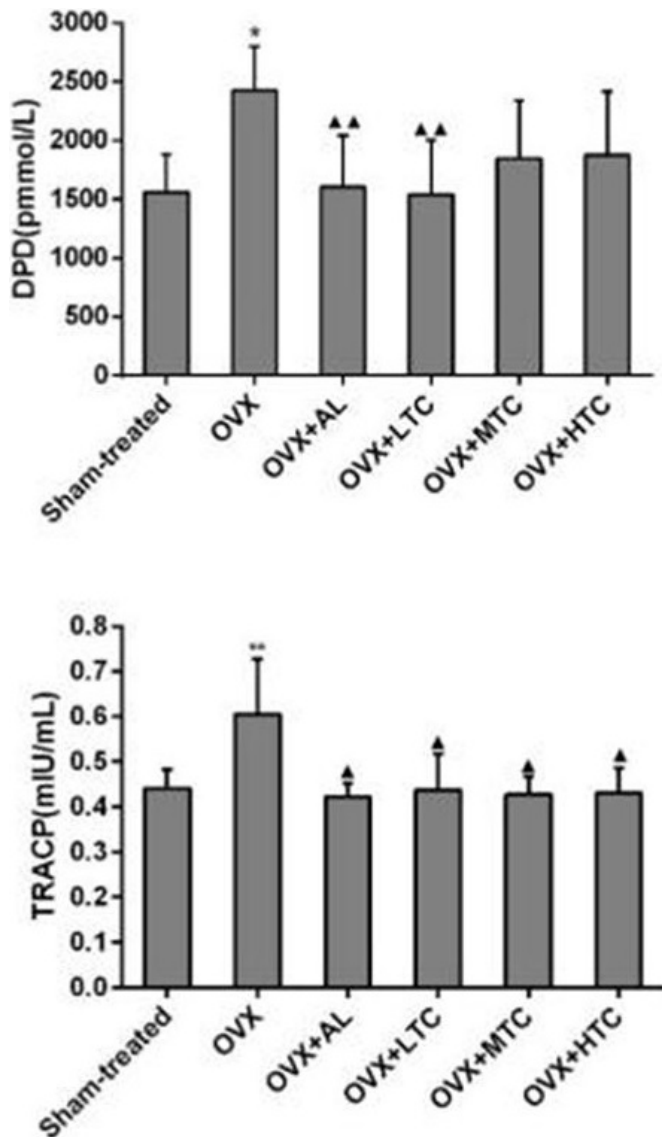


Figure 3. Comparison of bone resorption index in different groups. Compared with the sham-treated group, * $P < 0.01$, ** $P < 0.05$. Compared with the OVX group, $\blacktriangle\blacktriangle P < 0.01$, $\blacktriangle P < 0.05$.

Table 1. Femur pathological score in each groups.

Group	Score
Sham-treated	1.1 ± 1.1
OVX	6.0 ± 2.0*
OVX+AL	3.8 ± 2.1 \blacktriangle
OVX+LTC	4.7 ± 1.6 \blacktriangle
OVX+MTC	4.8 ± 2.3
OVX+HTC	5.0 ± 1.3

*Compared with the sham-treated group, $P < 0.05$; \blacktriangle Compared with the ovx group, $P < 0.05$.

deposition and loss, calcium supplementation alone cannot produce good preventive and therapeutic effects (No authors listed, 2010). In order to achieve the best clinical effect, the treatment of postmenopausal women with osteoporosis should consider

the main causes of osteoporosis and avoid the adverse reactions caused by blind hormone replacement therapy. The ultimate goal of osteoporosis treatment is to alleviate the bone pain, enhance bone strength and reduce the occurrence of fractures.

The model we used was similar to that of postmenopausal osteoporosis. The decrease of estrogen level after menopause weakened the function of non-nuclear receptors, which greatly increased the number of osteoclasts and osteoblasts compensatory increase. The rate of bone remodeling also increased. Furthermore, due to the lack of estrogen, the apoptosis of osteoblasts and osteocyte were also observed. Therefore, bone resorption and bone formation were imbalanced, which resulted in bone loss (Clarke & Khosla., 2010). Tianchang Capsule is mainly based on sovereign drug astragalu and radix codonopsis to nourish qi and spleen. Minister drug radix rehmanniae preparata, angelica sinensis, and psoralen are used to nourish blood and essence as supplementary. Parasitic, epimedium, eucommia ulmoides are used to tonify liver and kidney, strengthening muscles and bones. The combination of various drugs supplement insufficient kidney essence and deficiency of liver and blood, while taking into account the functions of acquired spleen and stomach, starting from the whole treatment of viscera's qi and blood to treat bone arthralgia.

The ovariectomized osteoporosis rat model which was suitable for the study of traditional Chinese medicine effect was selected in this study (Guo et al., 2015). The results in OVX group of bone mineral density, bone formation, bone resorption index and pathological section at 12 weeks after the model establishment showed that bone mineral density decreased, while bone transformation rate increased, indicated that the model was successful duplicated the postmenopausal osteoporosis. Previous study showed that the function of "kidney" in TCM is similar to that of "hypothalamus-pituitary-gonad" axis, dysfunction of this axis can be considered as kidney deficiency syndrome described in TCM (Lou et al., 2001; Ren et al., 2006). Therefore ovariectomy can be regarded as a postmenopausal osteoporosis model of kidney deficiency syndrome. The rats model in our study could be regarded as the postmenopausal osteoporosis rat model with kidney deficiency syndrome, which conformed to compel indication for Tianchang Capsule.

BALP (bone alkaline phosphatase) is a polysaccharide protein attached to the surface of osteoblasts, released into the blood under the action of hydrolase. In the process of osteogenesis, BALP can improve the content of local phosphoric acid for providing necessary raw materials for bone matrix mineralization, by hydrolyzing phosphate esters, wherefore BALP is an important indicator of osteoblast activity and its action process (Li, 2008).

OT (osteocalcin) is a biochemical index reflecting bone formation secreted by osteoblasts and is stable and do not affect by bone resorption. The functions of OT are both locally in bone and as a hormone depends on a novel post-translational mechanism that alters OT's affinity for the bone matrix and bioavailability (Zoch et al., 2016). More importantly is that serum OT is a specific index for differentiating high bone transfer which can reflect osteogenesis activity, the rate of bone formation and evaluate bone mineralization (Chopin et al., 2012; Ersoy et al., 2015). Serum TRACP (tartrate resistant acid phosphatase) is

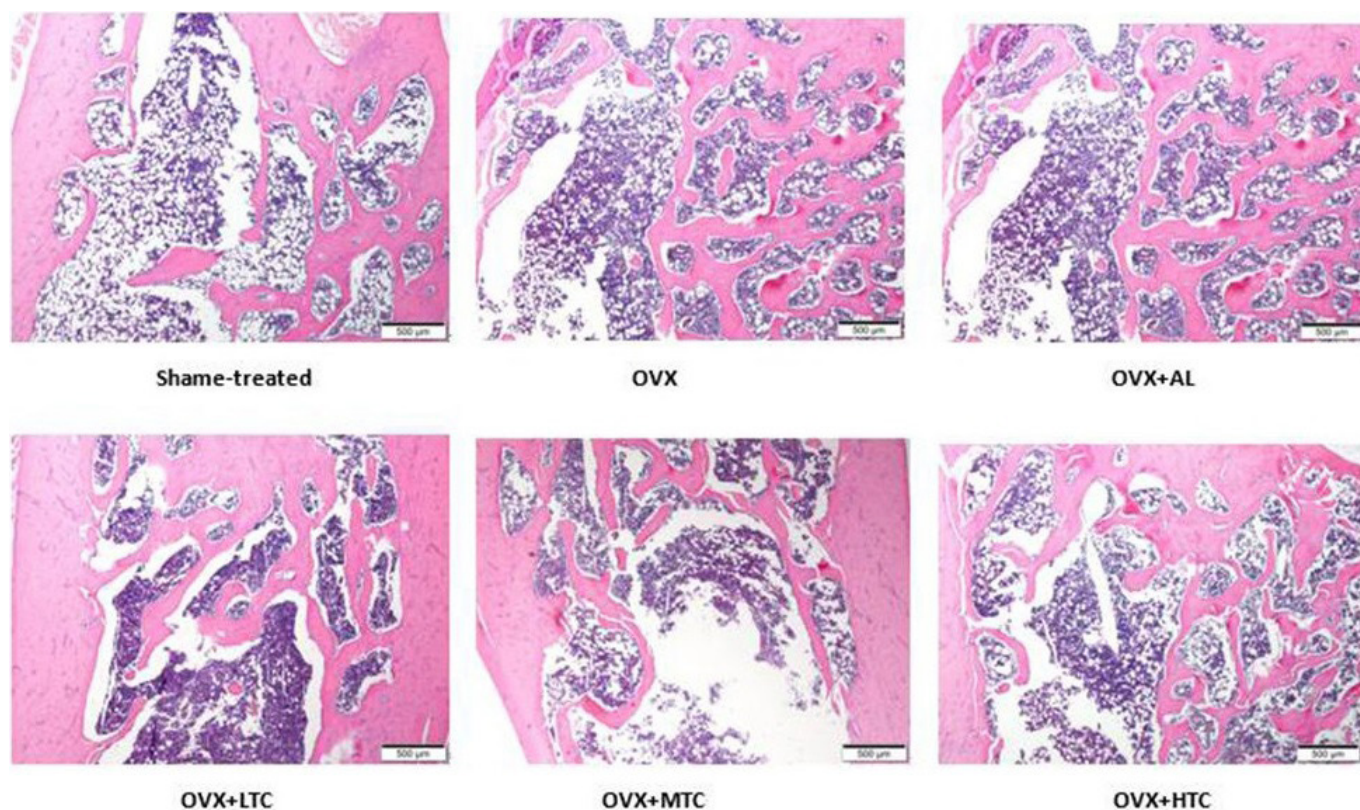


Figure 4. Bone histomorphological photomicrographs in each group (HE stain $\times 40$).

abundant in osteoclasts to mark bone resorption, in previous studies on postmenopausal women have shown that as the level of TRACP increases at the same time bone resorption increases (Zhang et al., 2013; Yu et al., 2013). DPD (deoxypyridinoline crosslinks) only exists in type I collagen fibers of bone is a specific marker of bone. DPD is a biochemical marker of early bone resorption, and its change is prior to BMD cause degradation of collagen fibers during bone resorption beginning DPD is released immediately (Legrand et al., 2003). In this study, examination index in OVX group had statistical significance compared with sham-treated group including BMD, BALP, OT, DPD, TRACP, histomorphological outcomes and pathological scores. Experimental results showed that ovariectomy induced female rats succeed copying the postmenopausal osteoporosis model which had a good response to patients bone metabolism. Alendronate sodium is a proven broad-spectrum anti-osteoporosis drug. Its therapeutic effect is mainly exerted by inhibiting bone resorption, we could see bone resorption parameters are restrained DPD and TRACP all decline compared with OVX group. After Tianchang Capsule treatment, compared with OVX group, the level of excessive active bone remodeling decreased and bone mineral density increased, suggesting that the mechanism of Tianchang Capsule in treating postmenopausal osteoporosis may inhibit excessive active bone resorption and reducing the level of bone formation, which made bone formation > bone resorption and increases bone mass. Thus, the effect of increasing bone mineral density was achieved, which was confirmed by pathological section staining of femur. But in OVX+HTC group

we could only observe statistical significance in serum TRACP level, which indicated high dosage Tianchang capsule could only inhibit excessive bone resorption, but no effect in BMD. We considered that results from medications were overdressed. OVX+LTC group could not only improve BMD but also reduce OT, DPD and TRACP, which illustrated low dosage of Tianchang capsule prevent and cured the osteoporosis by suppressing the bone resorption. There was statistical significance in femur pathological score 0.7 ± 1.6 (mean \pm SEM), comparing with OVX group. The results indicated that low dosage of Tianchang capsule had a good effect in the treatment of postmenopausal osteoporosis.

However, there are limitations in the present study. This study only confirms its effect from bone mineral density, pathology and biochemical indicators, lacking a clear mechanism research however. And further research is needed to clarify this point.

5 Conclusion

In summary, our findings show that Tian Chang capsule as a natural drugs have a good result for the treatment of ovariectomy induced osteoporosis rats, its action mechanism may associate with inhibition of bone resorption.

Abbreviations

BMD: Bone mineral density. HSC: Hematopoietic stem cells. MSC: Mesenchymal stem cells. TCM: Traditional Chinese medicine. TC Capsule: Tianchang Capsule.

Ethical approval

This study was approved by The Ethics Committee of Chengdu University of Traditional Chinese Medicine.

Conflict of interest

The author declare that they have no conflict of interest.

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Author contributions

H Chen and Y Zhu carried out the study concepts, study design, definition of intellectual content, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript editing and manuscript review; L Sun performed the study design, definition of intellectual content, clinical studies, experimental studies and manuscript review; X Zhang participated in the study design, literature research, experimental studies, statistical analysis, manuscript editing; L Li was dedicated the definition of intellectual content, clinical studies, data analysis, manuscript preparation and manuscript review; C Hu participated in the definition of intellectual content, clinical studies, experimental studies, data acquisition and manuscript preparation; M Zhou and X Zhao carried out the literature research, data acquisition; S Zhou, X Shi and J Yao were involved in the literature research, data analysis, statistical analysis, manuscript editing; Q Chen participated in its design and manuscript review. All authors read and approved the final manuscript.

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