

Progress of Research on the Prevention and Treatment of Osteoporosis by the Traditional Chinese Medicine Cow's Knee

Li Dan^{1,a}, Qiao Feng^{2,b,*}

¹Integration of Traditional Chinese and Western Medicine, Shaanxi University of Chinese Medicine, Xiayang, 712000, China

²Department of Orthopedics, Honghui Hospital, Xi'an Jiaotong University, Xi'an, 710054, China
^a2521409921@qq.com, ^b18317577811@163.com

*Corresponding author

Abstract: Osteoporosis (OP) is a pervasive disease of bone metabolism characterized by decreased bone density and degeneration of bone cells, leading to fractures. This disease poses a serious threat to human health. At present, the treatment of OP is mainly to inhibit bone resorption and promote bone formation, although the drug has a clear target and definite efficacy, the treatment method is relatively limited and has certain side effects. In traditional Chinese medicine, osteoporosis is called "osteoporosis", and with the deepening of the research on traditional Chinese medicine, it has been found that the anti-osteoporosis effect of traditional Chinese medicine oxknee and its active ingredients is significant, and the relevant formulas are widely used in the clinical treatment of osteoporosis. This article summarized the research on the main anti-osteoporotic components in ox knee, such as oxknee sterone and oxknee saponins, and further revealed the mechanism of action of oxknee in the prevention and treatment of OP, in order to explore the unique advantages of traditional Chinese medicine in the prevention and treatment of OP.

Keywords: *Achyranthes bidentata*, Osteoporosis, *Hyssophora steroid ketone*, *Hyssop saponin*, *Achyranthes bidentata* formulas

1. Introduction

Osteoporosis (OP) is a widespread and serious disease whose main symptoms include decreased bone strength, increased risk of fracture, and easy bone fragility^[1]. The disease is most common in middle-aged and elderly patients and postmenopausal women^[2] and can have a significant impact on the health of patients^[3]. The common feature of this patient population is under-diagnosis and under-treatment, which can be extremely harmful, leading to bone loss in postmenopausal women, increased susceptibility to hip, spine, and wrist fractures in middle-aged and elderly patients, and a tendency to be disabling and, in severe cases, life-threatening^[4, 5]. According to global data^[6, 7], the number of osteoporosis patients will exceed 1.36 billion by 2030. Therefore, osteoporosis is a major and growing public health problem^[8, 9] that needs to be addressed urgently.

In recent years, Chinese medicine has made certain achievements in the prevention and treatment of osteoporosis. It has shown remarkable results in increasing bone density and improving bone metabolism, giving full play to the advantages of traditional Chinese medicine and serving as an integrative therapy^[10]. Niu Knee, also known as Baibei and Huai Niu Knee, is the dried root of an amaranth plant, first recorded in the ancient book "Shen Nong's Classic of the Materia Medica", with its main production area in Henan Province, which is one of the traditional Chinese medicines in China. It has the effect of tonifying the liver and kidney, strengthening the muscles and bones, and is often used to treat weakness of the muscles and bones, lumbar and knee pain, menstrual obstruction and obstruction in the abdomen^[11]. Pharmacological studies have shown that hyssop has pharmacological effects such as anti-inflammatory, analgesic, immune modulation, and aging retardation^[12]. In the clinic, it is widely used in the treatment of cardiovascular diseases^[13], asthma, abortion^[14], and osteoporosis and primary dysmenorrhea^[15], especially in the treatment of OP. Osteoporosis in Chinese medicine is categorized as "bone dryness, bone impotence, bone paralysis". According to Bie Lu (《supplementary book》), "Niu Knee benefits the essence, fills the bone marrow, and removes pain in the lumbar and spinal regions." Since the kidney is in the body and integrates with the bones, and is the

main bone marrow producer, it is thought that cow's knee can achieve anti-osteoporosis effects by tonifying the kidney ^[16]. The studies on the active ingredients of hyssop and its hyssop-related formulas for the prevention and treatment of OP are summarized, with a view to providing certain reference for the clinical prevention and treatment of OP.

2. Study on Anti-osteoporosis of Hyssop Extracts

Some researchers ^[17] found that the use of *Boswellia serrata* extract to intervene in rats with osteoporosis model showed that the extract improved bone mineral density, bone trabecular microstructure, and bone biomechanical quality, slowed down bone loss, and exhibited anti-osteoporotic effects by detecting bone conversion indexes such as ALP, OC, and DPD. In addition, He et al ^[18] utilized dual-energy X-ray absorptiometry (DXA) to determine the bone density of the lumbar vertebrae (L2 -4) of rats before and after administration of the drug, and the results showed that the n-BuOH-soluble fraction of the root of *Hippophae rhamnoides* effectively prevented bone loss, and possessed a great potential for the treatment of osteoporosis. Bovine extract intervened in antimycin A-induced MC3T3-E1 cells, and then measured RANKL, TNF- α , 1L-6, and ALP activities, which showed a significant increase in ALP activity of osteoblasts, a significant increase in osteocalcin secretion, and an increase in the activity of TNF- α , and 1L-6 osteoblasts after the intervention. This suggests that *Boswellia serrata* extract induces osteogenic differentiation and attenuates osteoblast dysfunction ^[19].

In addition, recent studies have shown that hyssop alcohol extract is able to promote type I collagen synthesis in bone tissue by increasing the level of type I collagen expression in osteoblasts, thereby promoting bone formation ^[20]. Li JX et al ^[21] gavaged OVX osteoporosis model aged rats with the n-butanol site in the methanol extract of hyssop, and measured the bone mineral density of the second to fourth lumbar vertebrae of the rats using dual-energy X-ray absorptiometry (DXA). The results showed that n-butanol site could inhibit the reduction of bone density and had a significant effect against OVX-induced osteoporosis. Hua et al ^[22] showed that hyssop could promote osteoblast proliferation and enhance miRNA expression in Runx2 by activating the ERK signaling pathway through in vitro osteoblast experiments following the action of hyssop extract. Gao Changkun ^[23] measured the changes in blood free calcium ion concentration, blood phosphate ion concentration and alkaline phosphatase level in osteoporotic rats by gavage of retinoic acid-induced OP rats with bovine knee decoction and found that bovine knee decoction significantly reduced the activity of alkaline phosphatase (ALP) in the blood, which could promote the healing of the diseased bone. Therefore, it is believed that a variety of alcoholic extracts and aqueous extracts of the active ingredients in hyssop have significant anti-osteoporosis effects.

3. Study on the Anti-osteoporosis of the Active Ingredients of Cow's Knee

The main chemical constituents of Cow's knee include polysaccharides, saponins, phytosterones, flavonoids, cyclic dipeptides, sterols, volatile oils, amino acids, and 5-carboxymethylfurfural ^[24, 25], among which it is the Cow's knee sterones and saponins that play a major role in anti-osteoporosis.

3.1 Anti-osteoporosis Studies of the Oxysterones

Dong Qunwei et al ^[26] By treating osteoporosis model rats with oxalis desmethylsterone, it can reduce serum alkaline phosphatase (ALP) levels and significantly improve bone morphology and biomechanical indexes as well as BMP levels, which has a positive effect on the prevention and treatment of osteoporosis. Wei Yuanji ^[27] et al intervened in dexamethasone-induced apoptosis of MLO-Y4 osteoblast-like cells using cowhide-ecdysterone and found that cowhide-ecdysterone could promote the expression of Bad and caspase9 proteins, and intervene in dexamethasone-induced apoptosis of osteoblast-like cells by activating the PI3K/Akt pathway. Another researcher ^[28] found that oxysterone was able to increase the autophagy level of osteoblasts and promote their differentiation, showing the feasibility of oxysterone in the treatment of osteoporosis. Study found that cupressus amaranthus stigmaterone in oxalis extract activated the migration of rat BMSCs in vitro. This migration may be related to the upregulation of CXCR4 protein expression. In addition, cowslip extract was found to inhibit osteoblastic differentiation and promote osteogenic differentiation ^[29, 30]. Gao et al ^[31] found that cowrie ecdysterone substantially stimulated osteogenic differentiation of bone marrow mesenchymal stem cells and osteoblasts in a dose-dependent manner, thus cowrie ecdysterone slowed down the progression of osteoporosis.

3.2 *Anti-osteoporosis Studies with Hyssop Saponins*

Yu et al [32] examined the effects of different concentrations of triterpene saponins on osteoclast formation by using triterpene saponins from Cow's knee to intervene in PTH-induced osteoclasts. The results showed that triterpene saponins inhibited osteoclast differentiation to varying degrees, thus preventing osteoclast formation. In addition, through cell proliferation and alkaline phosphatase assays, He and other researchers found [33] that oxsaponin (ABS) promoted osteogenic differentiation of bone marrow stromal stem cells (BMSCs) through activation of the ERK signaling pathway, and thus could be used as a preventive and therapeutic approach for osteoporosis. Gan et al [34, 35] intervened on UMR106 cells using carotenoid glycosides contained in *Radix Aurantium Bovis* (RAB) and detected cell proliferation using MTT assay. It was found that carotenoid glycosides in *Boswellia serrata* significantly increased the activity of UMR106 cells, which in turn promoted the proliferation of osteoblasts. At the same time, the researchers also investigated the effects of the extract of *Rauwolfia officinalis* (RAB) on OB-like UMR106 cells and found that *Rauwolfia officinalis* (RAB) may contain compounds that stimulate OB. Ren Xinci et al [36] found that when studying the use of total saponins of hyssop to intervene in a retinoic acid replicated model of osteoporosis in rats, the results showed that administration of total saponins of hyssop resulted in a significant increase in the blood calcium level, ALP activity, and serum OC level, as well as a significant decrease in the urinary level of hydroxyproline. Thus, total saponins of hyssop were able to improve bone metabolism in osteoporotic rats, as well as inhibit osteoclast activity, thereby inhibiting bone resorption.

4. Study on the Anti-osteoporosis Effect of the Formulae of Hyssop

Clinical formulas commonly used in the treatment of osteoporosis contain hyssop, such as Zuo Gui Wan, Tonifying the Kidney and Promoting Blood Formula, Tonifying the Kidney and Strengthening the Bone Soup and other formulas. Therefore, it can be seen that hyssop has a very important role in the fight against osteoporosis.

4.1 *Zuo Gui Wan*

Liu Hui-Wen et al [37] conducted experiments using Zuo Gui Wan suspension to intervene with mouse bone marrow mesenchymal stem cells (BMSCs) and found that Zuo Gui Wan suspension was able to affect the osteogenic differentiation of mouse BMSCs by increasing the expression of ALKBH5, which promotes bone formation and achieves a therapeutic effect on osteoporosis. Zhang et al [38] used Zuo Gui Wan to intervene in mice lacking estrogen-induced bone loss, and found that Zuo Gui Wan could effectively inhibit the cellular function of bone loss, increase the bone mineral density of mice, and play a role in bone protection. This suggests that Zuo Gui Wan has an effect in preventing bone loss caused by estrogen deficiency. In another study [39], Zuo Gui Wan was found to exert osteoprotective effects through the Wnt/ β -catenin and Wnt/Runx 2 pathways, increase BMD, improve bone microstructure, reduce serum BALP and PINP levels, up-regulate the expression of Wnt3a, β -catenin, and Runx 2 proteins in the bone group, promote osteogenic differentiation, and induce osteoprotection, thereby preventing and treating secondary osteoporosis.

4.2 *Formulas for Tonifying the Kidneys and Invigorating Blood*

Jiang Kun et al [40] intervened in ovary removal replica osteoporosis model rats with a kidney tonic and blood activation formula and found that it could increase the level of BMP-2 in femur tissue. It also promotes bone production and repair and inhibits the resorption of bone tissue, resulting in increased bone density.

4.3 *Kidney and Bone Strengthening Formula*

Peng Zhang et al [41] selected a rat model of osteoporosis induced by the use of OVX in their experiments and gave the treatment of Kidney and Bone Strengthening Formula. The results showed that femur weight, bone mineral density, bone biomechanical properties, type I procollagen C-terminal peptide (PICP), 25-hydroxyvitamin D₃(25-(OH)D₃), and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) were improved in rats after treatment. Its treatment also promotes bone cell proliferation, bone calcium and phosphorus metabolism, as well as bone formation and reconstruction, while increasing bone mineral and calcium levels in the body, resulting in an anti-osteoporotic effect.

4.4 Kidney and Bone Strengthening Soup

Kidney and Bone Tonifying Soup is a kidney tonic formula, in which ox knee is an important component. A study^[42, 43] intervened the OVX osteoporosis model rats with Kidney and Bone Tonics and found that it was able to prevent bone loss. Another study^[44] treated primary osteoporosis by tonifying the kidney and strengthening the bone formula and found that the bone density was significantly increased, bone formation was significantly enhanced, and bone resorption was significantly inhibited.

4.5 Kidney Tonic and Solid Bone Broth

Kang Tianji and his team conducted a study^[45] using Kidney Tonifying and Bone Solidifying Tang to intervene in a rat model of dexamethasone-induced osteoporosis. They found that bone morphology and serum calcium and phosphorus levels were improved in the glucocorticoid osteoporosis model rats, as well as calcitonin as well as sex hormone levels, which improved the rats' osteoporosis.

4.6 Mee Bong Ta Lao Er Pills

In a study^[46], intervention in osteoporosis model rats using the Mibi Daolao Pills showed a significant increase in VDR mRNA expression levels, intestinal calcium uptake and osteoblast activity, as well as a significant increase in bone mineral density in lumbar spine and kidney tissues of the rats. Therefore, this study proves that the Mibu Da Lao Er Pills have some anti-osteoporotic effect.

4.7 Dokpo Parasite Soup

Cow's knee tonifies the liver and kidneys and strengthens the tendons and bones in Dok Ju Parasite Soup. Yang Wu et al^[47] found a significant increase in BMD in patients with osteoporosis after treatment by administering Dokko Parasite Soup to patients with osteoporosis. It has also been studied^[48] that the use of Dokuto Parasite Soup has a significant effect in the treatment of postmenopausal female patients with osteoporosis. The experimental results showed that the soup was able to improve the patients' lumbar spine bone density as well as serum estradiol levels, thus becoming an effective osteoporosis prevention and treatment method.

4.8 Cistanches and Cow's Knee Soup with Flavor Additions

It was found^[49] that combining Cistanches Ox Knee Soup Plus with western medicines in the treatment of osteoporosis could significantly improve bone mineral density and bone metabolism, and it could significantly promote the expression levels of inflammatory factors, such as serum tumor necrosis factor- (TNF-), hypersensitive reactive protein (hs-CRP), and interleukin- (IL-), which would be effective in preventing the onset of osteoporosis.

4.9 Yanghe Tang Plus Reduction

A study^[50] found a significant increase in bone mineral density (BMD) after treatment by giving Yanghe Tang Plus to elderly patients with primary osteoporosis.

4.10 Shouwu Er Zi Wan

Cow's Knee has the effect of tonifying the liver and kidney and strengthening the tendons and bones. Wang Dan et al^[51] found that the use of Shouwu Erzhi Pill combined with herbal acupoint ionization in the treatment of postmenopausal women with osteoporosis was effective in improving the bone mineral density of the patients. As a result, this treatment has proven to be an effective option for the treatment of postmenopausal osteoporosis.

4.11 Cow's Knee - Cortex Eucommia Combination

In traditional Chinese medicine, the combination of hyssop and eucommia is widely used to treat osteoporosis, and Gao Weihui et al^[52] found that in intervening with the combination of hyssop and eucommia in osteoporosis model rats, it could significantly improve the bone mineral density (BMD) of the rats and significantly increase their serum calcium and phosphorus ion concentrations. The

combination is therefore effective in the treatment of postmenopausal osteoporosis. Li et al. study^[53] found that by intervening in glucocorticoid-induced osteoporosis model zebrafish by using a combination extract of hyssop-eucommia, it was found that hyssop-eucommia components had the best preventive and therapeutic effect on glucocorticoid-induced osteoporosis in the zebrafish when used in a ratio of 1:1.

5. Summary and Outlook

Osteoporosis has a huge impact on the health of the nation as the trend of our aging society increases. Drugs used to date for the prevention and treatment of osteoporosis include antiresorptive agents, bone enhancers, and mineralizers, all of which have been associated with a variety of adverse effects, such as liver and kidney damage, gastrointestinal irritation, flu-like symptoms, and an increased risk of malignancy. At the same time, treatment is relatively limited, medication is inconvenient, and patients are restricted from long-term use of Western medications. As the research of Chinese medicine in the prevention and treatment of osteoporosis continues to deepen, the efficacy has been significantly improved, and the treatment of osteoporosis has its unique advantages, i.e., fewer side effects; cheaper; and higher safety and other advantages. The Chinese medicine hyssop and its active ingredients have significant anti-osteoporosis efficacy, and the related formulas are widely used in the clinical treatment of osteoporosis.

In this study, we found that the extracts of hyssop, hyssop steroids, hyssop saponins, and hyssop group formula could effectively prevent and control OP, which mainly increased femoral and lumbar BMD and up-regulated the expression of some markers promoting osteogenesis, such as ALKBH5, Runx2, BMP2, Wnt3a, and β -catenin. Down-regulation of bone resorption-promoting markers such as TNF- α , IL-6, ALP, etc., acts as a traditional Chinese medicine, hyssop has been widely used in the treatment of osteoporosis. Although basic experiments have shown that it has significant osteoprotective effects and clinical trials have demonstrated good therapeutic effects, the current research on the treatment of osteoporosis with hyssop is still based on basic experiments, and the diversity of samples is still insufficient to a certain extent. In addition, the lack of multicenter controlled clinical studies makes the clinical application of hyssop in the treatment of osteoporosis still has some limitations. In order to better promote the application of Cow's Knee in the treatment of osteoporosis, we should pay more attention to clinical and mechanistic studies in order to understand its therapeutic mechanism more comprehensively. Clinical studies should adopt a multicenter controlled trial design to increase the diversity of samples, thus improving the reliability and generalizability of the study. Meanwhile, mechanistic studies should deeply explore the mechanism of action of hyssop in the treatment of osteoporosis, including its effect on osteoblast proliferation and differentiation, and the mechanism of regulating bone metabolism. This will not only effectively guide the rational application of oxalis, but also help to discover the therapeutic potential of other Chinese medicines. Through the in-depth exploration of clinical and mechanistic studies, we expect to reveal the specific mechanism of cow's knee in the treatment of osteoporosis and provide a reference for the development of other Chinese medicines. This will help provide safer and more effective treatment options for osteoporosis patients and improve their quality of life. Therefore, it is of great significance to strengthen the clinical and mechanistic studies on the treatment of osteoporosis with ox knee and deserves further in-depth exploration. Herbal extracts have been shown to be effective in the prevention and treatment of OP, but the composition is complex and variable, and the effectiveness of single or multi-component interventions is not clear. It is worth noting that most trials of anti-osteoporosis mechanisms are based on a single signaling pathway, whereas the mechanism of action of TCM is not a single pathway, but rather multi-rooted and multi-targeted. Moreover, in the absence of any robust medication-related clinical information data, it is difficult for kidney tonic herbs to be widely used and their anti-osteoporotic effects to be verified. Therefore, subsequent studies can propose a pharmacological activity-oriented mapping analysis strategy to characterize the chemical components of kidney tonic herbs in multiple directions and levels by using bioaffinity chromatography and multiple histological techniques. Although the anti-osteoporotic effects of renal tonic Chinese herbal medicines have been studied more, more relevant clinical trials are needed for further research, not only through the metabolic process of each component in the body to explore the anti-osteoporotic mechanism of renal tonic Chinese herbal medicines. At the same time, large-scale clinical control studies will be conducted on Chinese medicines with a long history of use and proven clinical efficacy, in conjunction with the dialectical thinking of Chinese medicine, the holistic view of the use of medicines and the efficacy studies of modern medicine.

I believe that the Chinese herb hyssop, compared with a single compound, includes many active

ingredients and is more likely to have a synergistic effect on the process of bone reconstruction osteoblasts are characterized by a focus on holistic regulation and dialectical treatment, which can be used as an ideal alternative to drugs for the treatment of osteoporosis.

References

- [1] ZHANG N-D, HAN T, HUANG B-K, et al. Traditional Chinese medicine formulas for the treatment of osteoporosis: implication for antiosteoporotic drug discovery [J]. *Journal of ethnopharmacology*, 2016, 189: 61-80.
- [2] CURTIS E M, MOON R J, DENNISON E M, et al. Recent advances in the pathogenesis and treatment of osteoporosis [J]. *Clin Med (Lond)*, 2016, 16(4): 360.
- [3] VAN DEN BERGH J P, VAN GEEL T A, GEUSENS PPJNRR. Osteoporosis, frailty and fracture: implications for case finding and therapy [J]. *Nat Rev Rheumatol*, 2012, 8(3): 163-72.
- [4] VIDAL M, THIBODAUX R J, NEIRA L F V, et al. Osteoporosis: a clinical and pharmacological update [J]. *Clin Rheumatol*, 2019, 38(2): 385-95.
- [5] HARVEY N, DENNISON E, COOPER C J N R R. Osteoporosis: impact on health and economics [J]. *Nat Rev Rheumatol*, 2010, 6(2): 99-105.
- [6] CENTER J R, NGUYEN T V, SCHNEIDER D, et al. Mortality after all major types of osteoporotic fracture in men and women: an observational study [J]. *The Lancet*, 1999, 353(9156): 878-82.
- [7] CAULEY J A J J O G S A B S, SCIENCES M. Public health impact of osteoporosis [J]. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 2013, 68(10): 1243-51.
- [8] CHAN C K, MASON A, COOPER C, et al. Novel advances in the treatment of osteoporosis [J]. *British medical bulletin*, 2016, 119(1): 129-42.
- [9] ANTHAMATTEN A, PARISH A J J O M, HEALTH W S. Clinical update on osteoporosis [J]. *Journal of midwifery & women's health*, 2019, 64(3): 265-75.
- [10] GUO Qiangqiang, ZHANG Xiaogang, CAO Panju. Current status of experimental research on the prevention and treatment of osteoporosis by traditional Chinese medicine [J]. *Clinical Journal of Traditional Chinese Medicine*, 2020, 32(03): 577-580.
- [11] National Pharmacopoeia Commission. Instructions for clinical use in the Pharmacopoeia of the People's Republic of China: 2010 edition, chemical drugs and biological products [M]. Instructions for clinical use in the Pharmacopoeia of the People's Republic of China: 2010 edition, chemical drugs and biological products, 2011.
- [12] TIAN Shuo. Discussion on the chemical, pharmacological and application characteristics of Radix Aconiti [J]. *Journal of Traditional Chinese Medicine*, 2014, 29(8): 1186-8.
- [13] YU Jiawen, ZHOU Daxing. Experimental study on the anti-acute myocardial ischemia effect of total saponins of hyssop in rats [J]. *Journal of Zhejiang University of Traditional Chinese Medicine* 2009, 33(06): 870-871.
- [14] He X, Wang X, Fang J, et al. The genus *Achyranthes*: A review on traditional uses, phytochemistry, and pharmacological activities [J]. *Journal of ethnopharmacology*, 2017, 203: 260-278.
- [15] Li J. Research progress of Huai Niu Knee [J]. *Chinese Medicine Guide*, 2013, 11(10): 462-463.
- [16] Ju D, Liu M, Zhao H, et al. Mechanisms of "kidney governing bones" theory in traditional Chinese medicine [J]. *Frontiers of medicine*, 2014, 8: 389-393.
- [17] ZHANG R, HU S-J, LI C, et al. *Achyranthes bidentata* root extract prevent OVX-induced osteoporosis in rats [J]. *Journal of Ethnopharmacology*, 2012, 139(1): 12-8.
- [18] HE C-C, HUI R-R, TEZUKA Y, et al. Osteoprotective effect of extract from *Achyranthes bidentata* in ovariectomized rats [J]. *Journal of ethnopharmacology*, 2010, 127(2): 229-34.
- [19] SUH K S, LEE Y S, CHOI E M J C. The protective effects of *Achyranthes bidentata* root extract on the antimycin A induced damage of osteoblastic MC3T3-E1 cells [J]. *Cytotechnology*, 2014, 66: 925-35.
- [20] YANG Guofu, SONG Guosheng, ZHANG Tao, et al. The effects of Cow's knee extract on bone mineral density, bone turnover and type I collagen expression in ovariectomised rats [J]. *Chinese Journal of Osteoporosis*, 2011, 17(02): 109-112.
- [21] Li Jianxin, Kadota Shigenori. Anti-bone resorption active constituents of Cow's Knee [J]. *Asia-Pacific Traditional Medicine*, 2006, (01): 77-78.
- [22] HUA S, ZHANG X J E, MEDICINE T. Effects of *Achyranthes bidentata* alcohol on proliferation capacity of osteoblasts and miRNA in Runx2 [J]. *Experimental and Therapeutic Medicine*, 2019, 18(3): 1545-50.
- [23] Gao J. Experimental study on the preventive and curative effects of Hua Niu Knee on retinoic acid-induced osteoporosis in rats [J]. *Journal of Basic Chinese Medicine*, 2001, 15(2): 9-11.
- [24] YANG Y, SHEN L, WANG P, et al. Anti-osteoporosis bioactivity evaluation in zebrafish model of raw and salt-processed *Achyranthes bidentata* followed by liquid chromatography-mass spectrometry

- analysis and correlation analysis [J]. *Biomedical Chromatography*,2023: e5742.
- [25] Shen Shu, Wang Qiong, Strait Pharmacology Li J. Progress in the study of the chemical composition and pharmacological effects of Radix Achyranthis Bidentatae [J]. *pharmacognosy*,2011, 23(11): 1-6.
- [26] DONG Qunwei, CHEN Zhifeng, CHEN Shaoqing, et al. Therapeutic effects of deodorised steroidone from Cow's knee on osteoporosis in de-ovulated rats [J]. *Journal of the Guangdong Pharmaceutical University*,2009, 25(5): 512-5.
- [27] Wei Yuanji, Li Junhao, Wang Libo, et al. β -Ecdysterone, an active constituent of Cow's knee, interferes with dexamethasone-induced osteoblast apoptosis via Akt signalling [J]. *Chinese Journal of Osteoporosis*, 2019, 25(3).
- [28] Jiang T, Shao M, Chen QZ, et al. Osteopontin interferes with the proliferation and differentiation of osteoblasts in SD suckling mice[J]*Chinese Tissue Engineering Research*. 2020, 24(23): 3636-42.
- [29] Chen H.T., Luo Y.W., Chen D.F., et al. Migration and CXCR4 expression of rat bone marrow mesenchymal stem cells in vitro stimulated by cow's knee cup amaranth steroidone [J]. *Chinese Journal of Osteoporosis*,2019, 25(11): 6.
- [30] Ji, Kin-Lung, Kong, Xiang-Dong, Qi, Shan-Hong, et al. Bidirectional action of cup amaranthone inhibiting osteoblastic differentiation and promoting osteogenic differentiation in the treatment of osteoporosis [J]. *Journal of Kunming Medical University/Kunming Yike Daxue Xuebao*,2018, 39(5): 8.
- [31] GAO L, CAI G, SHI X J B, et al. β -Ecdysterone induces osteogenic differentiation in mouse mesenchymal stem cells and relieves osteoporosis [J]. *Biological and Pharmaceutical Bulletin*,2008, 31(12): 2245-9.
- [32] YU D, LV X, SHI L, et al. Inhibitory effect of triterpenoidal saponins from *Achyranthes bidentata* Bl. on osteoclast differentiation [J]. *Chin. J. Tradit. Med. Traumatol. Orthop*,2011, 19: 9-10.
- [33] HE G, GUO W, LOU Z, et al. *Achyranthes bidentata* saponins promote osteogenic differentiation of bone marrow stromal cells through the ERK MAPK signaling pathway [J]. *Cell biochemistry and biophysics*,2014, 70: 467-73.
- [34] XIAOYAN G, DAWEI W, FAMEI L, et al. The effect of the extracts of *Achyranthes bidentata* Bl. on proliferation of osteoblast-like UMR 106 cells [J]. *Journal of Shenyang Pharmaceutical University*,2000, 17(3): 210-3.
- [35] XIAOYAN G, YOUFAN S, FAMEI LJJOCMC. The effect of daucosterol in *achyranthes bidentata* bl. on proliferation of umr106 osteoblast like cells and itsdetermination [J]. *Journal of Chengde Medical College*,2003, 20(1): 1-4.
- [36] Ren Xinci, Xu Xianxiang, Xu Dujuan, et al. Effects of total saponins of hyssop on bone metabolism in retinoic acid-induced osteoporotic rats[J]. *Chinese Journal of Experimental Formulas*. 2011, 17(04): 128-30.
- [37] Liu HW, Liu H, Shang Q, et al. Effects of Zuo Gui Wan up-regulating ALKBH5 on osteogenic differentiation of mouse BMSCs [J].*Chinese Journal of Osteoporosis* , 2023, 29(12): 1744-1749+1762.
- [38] LAI N, ZHANG Z, WANG B, et al. Regulatory effect of traditional Chinese medicinal formula Zuo-Gui-Wan on the Th17/Treg paradigm in mice with bone loss induced by estrogen deficiency [J]. *Journal of Ethnopharmacology*,2015, 166: 228-39.
- [39] CHUNHUI L, LEI Y, LA Z, et al. Bone protection mechanism of Zuogui Pill in an osteoporosis model of breast cancer rats after endocrine therapy [J]. *Journal of Biology and Today's World* ,2023.
- [40] Jiang K, Zhou Q, Shang DY, et al. Experimental study on the effect of kidney tonifying and blood activating formula on bone mineral density and BMP-2 protein expression in rats with osteoporosis model[J]. *Journal of Liaoning University of Traditional Chinese Medicine* , 2016, 18(05): 20-22.
- [41] Zhang Peng. Experimental study on the therapeutic effect of kidney tonifying and bone strengthening formula in treating osteoporosis in rats[J]. *Chinese Traditional Medicine Modern Distance Education*, 2016, 14(24): 145-147.
- [42] SHI L, TEZUKA Y, MIYAHARA T, et al. Inhibitory effect of constituents of Bu-Shen-Jian-Gu-Tang on osteoclast-like cell formation [J]. *Journal of Traditional Medicines*, 2007, 24(1): 19-23.
- [43] SHI L, TEZUKA Y, UEDA J-Y, et al. Inhibitory effect of Kampo medicines on bone resorption in vitro and preventive effect on bone loss in vivo [J]. *Journal of Traditional, treatment of primary osteoporosis in 50 cases*, 2014.
- [44] LIN Shifu, PAN Fuwen,LIU Yingjie et al. Treatment of 50 cases of primary osteoporosis by tonifying kidney and strengthening bone formula[J]. *Chinese Journal of Experimental Formulas*, 2014, 20(018):192-195.
- [45] KANG Tianji, JIANG Aili. Experimental study on the treatment of glucocorticoid osteoporosis by tonifying kidney and solid bone soup[J]. *Journal of Traditional Chinese Medicine*, 2009, 37(4): 32-35.
- [46] Wu, Mishan, Zhao, Suzhi, Ren, Lizhong, et al. Effects of Mibong Taolaoer Wan on vitamin D receptor mRNA expression in lumbar spine and kidney of de-ovulated osteoporotic rats[J]. *Chinese*

Patent Medicine, 2014, 36(6): 1133-1138.

[47] Yang W, Liu WG. *Clinical study on the treatment of osteoporotic fracture by Dokou parasitic soup [J]. Shaanxi Traditional Chinese Medicine*, 2015, 36(02): 191-192.

[48] LI J, WANG W, FENG G, et al. *Efficacy and safety of Duhuo Jisheng decoction for postmenopausal osteoporosis: a systematic review and meta-analysis [J]. Evidence-Based Complementary and Alternative Medicine*, 2020, 1 (2020): 6957825.

[49] Zhao Yong, Li Ni, Peng Xinyi. *Effect of Cistanches Ox Knee Soup Plus flavour combined with alendronate in the treatment of osteoporosis and its effect on fracture incidence [J]. Journal of Practical Hospital Clinics*, 2022, 19(05): 36-40.

[50] Liu WY. *Treatment of 65 cases of primary osteoporosis in the elderly with Yanghe Tang plus reduction [J]. Chinese Medicine Research*, 2013, 26(01): 35-37.

[51] Wang Dan, Tian Ying. *Shouwu Erzi Pill with Chinese medicine acupoint ion implantation for the treatment of 61 cases of liver and kidney deficiency type postmenopausal osteoporosis [J]. Shaanxi Traditional Chinese Medicine*, 2015, 36(07): 787-788.

[52] Gao Weihui, Xiang Yanhua, Liu Yun, et al. *Experimental study on the intervention of Chinese herbal medicine DUZHONG-NIUHENGXI pairing on osteoporosis in de-ovulated rats [J]. Journal of Hunan University of Traditional Chinese Medicine*, 2016, 36(6): 43-6.

[53] LEE J-H, WEI Y-J, ZHOU Z-Y, et al. *Efficacy of the herbal pair, Radix Achyranthis Bidentatae and Eucommiae Cortex, in preventing glucocorticoid-induced osteoporosis in the zebrafish model [J]. Journal of Integrative Medicine*, 2022, 20(1): 83-90.