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# Effects of Rhizoma Drynariae Cataplasm on Fracture Healing in a Rat Model of Osteoporosis

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**Background:** Osteoporosis is an increasingly prevalent disease characterized by decreased bone mass and deterioration of the bone microstructure, which contribute to increased fragility and subsequent fragility fractures, especially in elderly individuals. Rhizoma Drynariae (DRE) is among the most frequently used herbal medicines for the treatment of osteoporosis. Transdermal delivery is a proven novel pathway for drug treatment and has several advantages over traditional drug delivery routes.

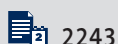
**Material/Methods:** Female Sprague-Dawley osteoporotic fracture model rats were divided into 3 groups: the control group, the DRE (90 mg/kg/day) group and the DRE cataplasm (containing 30 mg DRE, administered at right femur site daily) group. At 3 and 6 weeks after operation, we performed x-ray, histological, and biomechanical analyses, and evaluated bone marrow density of the femur.

**Results:** Treatment with DRE increased callus formation and bone union compared with the control group. Moreover, DRE enhanced bone strength at the femoral diaphysis in the osteoporotic fractures in rats by increasing the ultimate load and stiffness compared with the control group. Furthermore, DRE restored the trabecular bone mineral density in the femur compared with the control group. DRE cataplasm application further enhanced the therapeutic effects against osteoporotic fracture in this rat model.

**Conclusions:** DRE cataplasm application might be useful against osteoporotic fracture.

**MeSH Keywords:** **Drugs, Chinese Herbal • Femoral Fractures • Osteoporosis • Pinellia**

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## Background

Osteoporosis is an increasingly prevalent disease characterized by decreased bone mass and deteriorated bone microstructure, which contribute to increased fragility and subsequent fragility fractures, especially in elderly individuals [1]. A recent study suggested that half of women and 20% of men (age >50 years) will experience osteoporosis-related fractures, such as hip and spine fractures, resulting in disability, mortality, and economic burden [2].

Post-menopausal women are generally accepted to have higher risks of osteoporosis and consequent fractures due to estrogen deficiency-induced bone catabolism. The acceleration of bone loss during menopause contributes to increases in the incidence rates of spine, hip, and wrist fractures [3,4]. Traditional treatment aims to upregulate the estrogen level via exogenous intake of estrogen in post-menopausal women. In addition, estrogen therapy may reduce the risks of perimenopausal symptoms and osteoporosis [5,6]. The Women's Health Initiative study demonstrated that estrogen treatment markedly decreased the rate of fracture among post-menopausal women [7,8]. However, estrogen treatment is contraindicated in pregnant women and in patients with liver diseases, cancer, and cerebral ischemic diseases. Estrogen treatment for fracture prevention is no longer advised for post-menopausal women due to its side effects; thus, a novel therapeutic strategy is required.

Most treatments for osteoporosis prevention are based on drug interventions [9,10]. Despite their effectiveness, however, drug interventions may cause the development of side effects and are compromised by poor longterm patient adherence [11]. Traditional Chinese medicine has been applied widely for the prevention and treatment of osteoporosis and related bone diseases for thousands of years [12]. Rhizoma Drynariae (DRE) is among the most frequently used herbal medicines for the treatment of osteoporosis in post-menopausal women. DRE has been confirmed to trigger osteogenic differentiation of bone-marrow mesenchymal stem cells by increasing the level of Foxc2 expression, and it has been shown to have greater binding affinity to estrogen receptor (ER)- $\alpha$  than to ER- $\beta$ , leading to osteogenesis [13–15]. In addition, our previous study suggested that a combination of DRE and alendronate has beneficial effects on fracture healing and callus formation in the rat model of osteoporotic fracture [16].

Despite its effectiveness, DRE may induce gastrointestinal side effects [17], and a strategy to avoid these effects is required. Transdermal delivery is a proven pathway for drug treatment [18]. Transdermal drug delivery systems have several advantages over traditional drug delivery routes. They have sustained effects, while minimizing first-pass metabolism related

to gastrointestinal drug administration [19,20]. Furthermore, relative to oral administration, they are more convenient and promote better patient compliance. Therefore, the transdermal delivery of conventional medicines has been used for the treatment of various diseases. Here, we established a rat model of osteoporotic fracture and assessed the differences between oral DRE and DRE cataplasm – a cloth for external application containing a traditional Chinese medicine extract or drug mixed with a suitable hydrophilic matrix and appropriate amounts of excipients – in treating osteoporotic fracture.

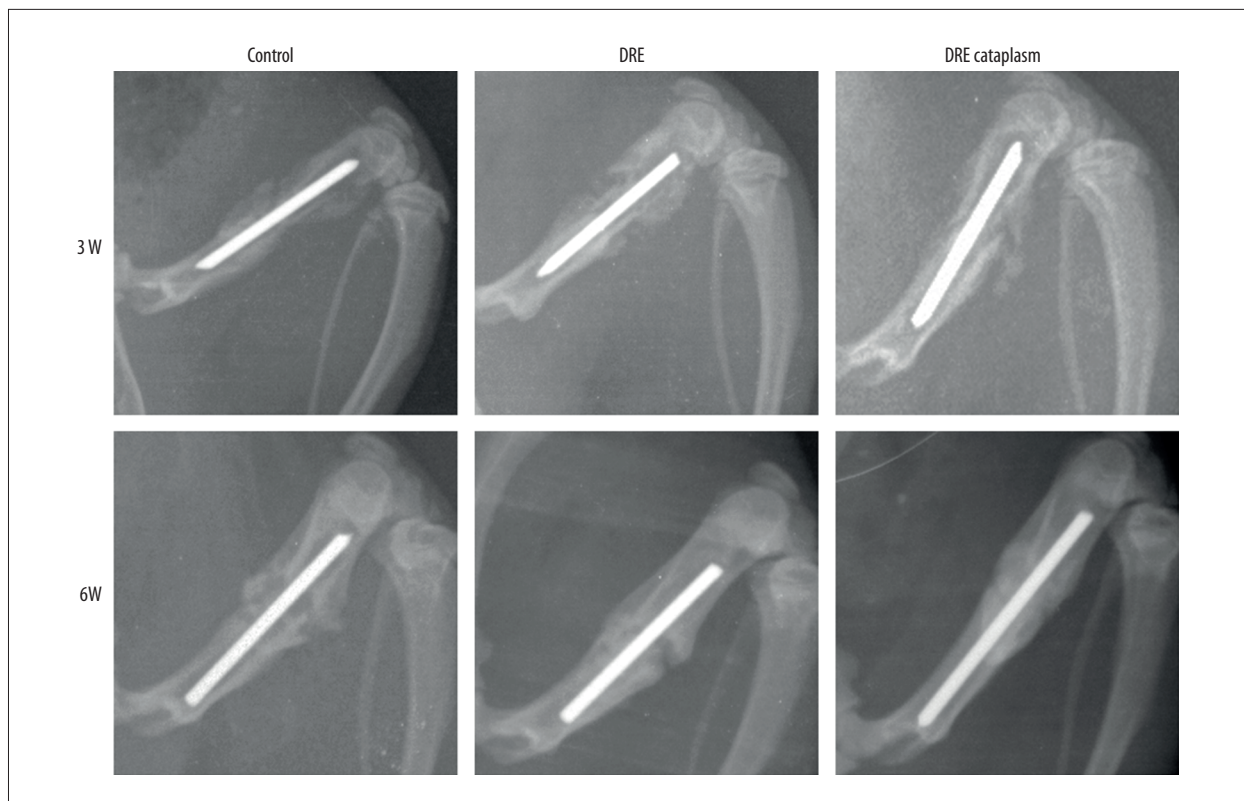
## Material and Methods

### Animal model and pharmaceutical treatment

Female Sprague-Dawley rats (5 months old, 280–320 g) were acquired from the Animal Center of the Chinese Academy of Sciences in Shanghai, China. All rat experimental procedures were conducted in accordance with the guideline of the Animal Care and Use Committee of Wenzhou Medical University. All animals were kept in a temperature-controlled room (25°C) with constant humidity (50%) and given a standard diet supplemented with 1.56% calcium, 0.8% phosphorus, and 800 IU/kg vitamin D. The experimental rats were administered retinoic acid (80 mg/kg/day) intragastrically for 14 days to establish the osteoporotic model [16]. The bone mineral density (BMD) of the tibial metaphysis was tested to assess the establishment of osteoporosis [21]. Then, unilateral transverse osteotomy at the middle of the right femur and fixation by intramedullary nailing (diameter, 1 mm; Wego Medical Systems Co., Shenzhen, China) were performed in osteoporotic model rats. After surgery, all rats were divided randomly into one of three groups, and the experimental interventions commenced from the first post-operative day according to the following arrangements: control group, DRE group (DRE, 90 mg/kg/day; Beijing Pharmaceutical, Qihuang, China), and DRE cataplasm group (cataplasm containing 30 mg DRE applied daily to the right femur site). The DRE group received DRE in the drinking water at a dose of approximately 100 mg/kg/day [16]. The rats in control group were given an equivalent amount of water. All surgical procedures and post-operative experimental procedures were approved by the Animal Care and Use Committee of Wenzhou Medical University.

### X-ray analysis

At 3 and 6 weeks after the operation, anteroposterior x-rays of the rat femora were obtained to evaluate bone formation and fracture union using a small animal imaging system (12 s, 26 kV, MX20; Faxitron, Lincolnshire, IL, USA).



**Figure 1.** X-rays of the healing fractures in each group at 3 and 6 weeks after operation.

### Histology

At 3 and 6 weeks after surgery, the specimens were fixed in 4% paraformaldehyde for 1 day at 48°C. After decalcification for 2 days, the tissues were dehydrated through an ethanol series, and then embedded in paraffin. Sagittal sections (5- $\mu$ m thick) were cut for the hematoxylin and eosin (H&E) staining. Histological images were captured under light microscopy and quantified using the Image Pro Plus Software (Media Cybernetics, Silver Spring, MD, USA). Several histological parameters, including the total sagittal cross-sectional area, callus area, and callus thickness, were evaluated [22].

### Biomechanical examination

The biomechanical properties of the healing fracture were measured using the 3-point bending test (Instron 4302; Instron, Norwood, MA, USA) at 3 and 6 weeks after the operation. Briefly, 2 loading bars spaced 18 mm apart were used to mount the femur, and a mobile head was used to compress the central part of the callus until fracture occurred. The compressive velocity was 2 mm/min. Stiffness (N/mm) and ultimate load (N) were measured from the load-deformation curve using the material testing machine [23].

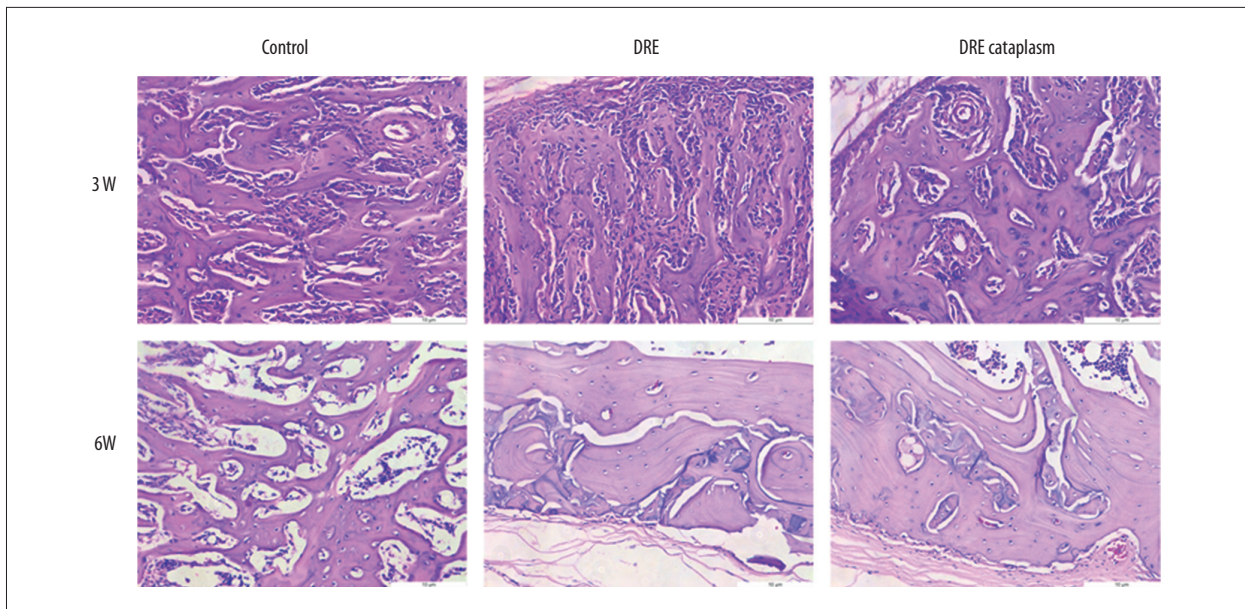
### Statistical analysis

Data from at least 3 times independent experiments are presented as means  $\pm$  standard deviations (SD), and were analyzed by the SPSS software (version 19.0; SPSS, Chicago, IL, USA). One-way analysis of variance and Tukey's *post hoc* test were applied to examine difference between each group. In all analyses,  $P < 0.05$  was taken to suggest statistical significance.

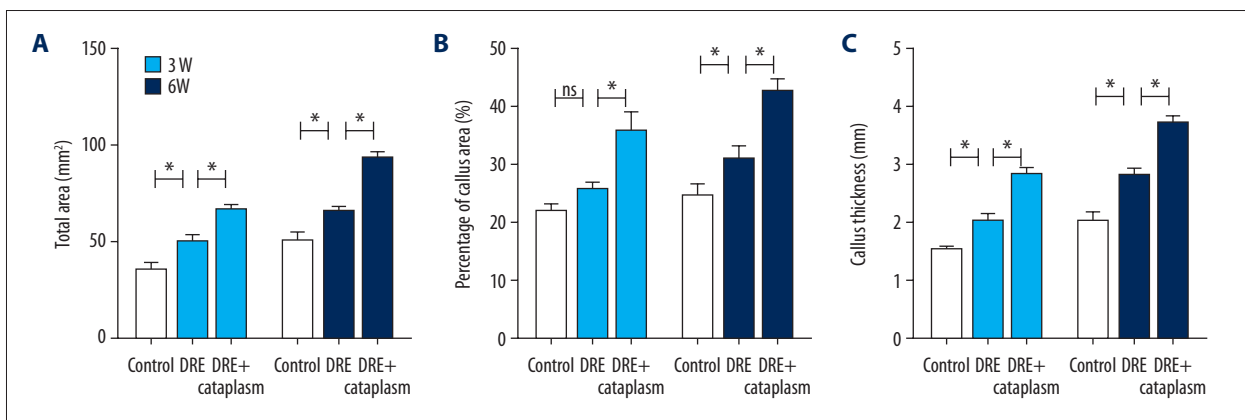
## Results

### X-ray findings

The effects of DRE on callus formation and bone union were evaluated by x-ray assays at 3 and 6 weeks after the operation. Compared with the control group, the DRE and DRE cataplasm groups showed wider translucent zones at the fracture end of the right femur at 3 weeks after intramedullary nail fixation. At 6 weeks, callus formation and bone union were noted in the DRE group, whereas the control group showed only partial callus formation with no complete bone union. The DRE cataplasm further enhanced the bone remodeling effects (Figure 1).



**Figure 2.** Hematoxylin and eosin (H&E) staining was performed at 3 and 6 weeks after the surgery in each group to evaluate bone histology (H&E 100× magnification).



**Figure 3.** (A–C) Quantification analysis of the fracture calluses at 3 and 6 weeks according to hematoxylin and eosin (H&E) staining. Data are expressed as mean ± standard deviation, significant differences among different groups are presented as \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ,  $n = 5$ .

### Histological findings

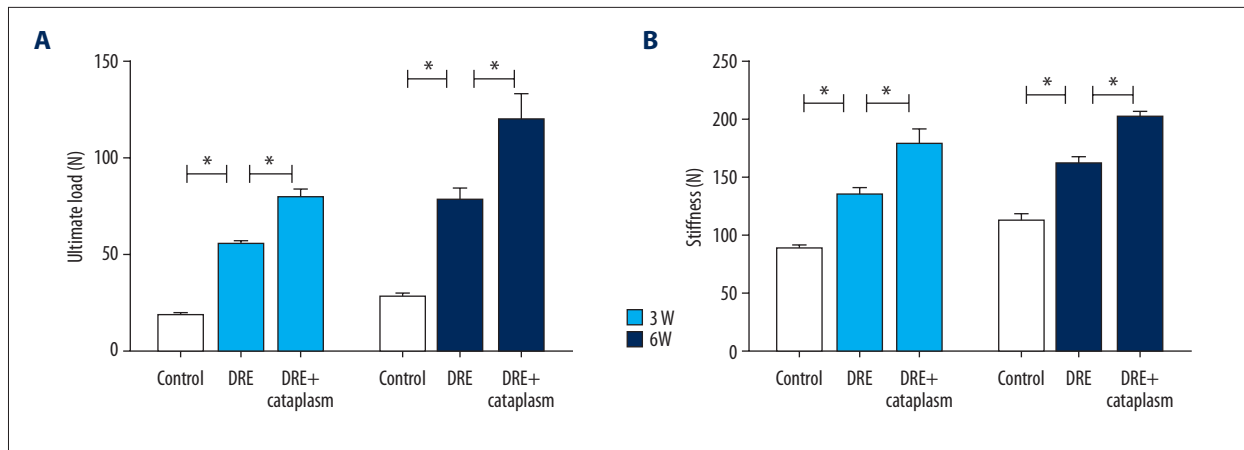
Histological analysis of bone callus formation was performed with H&E staining at 3 and 6 weeks after the operation (Figure 2). Compared with the control group, the DRE and DRE cataplasm groups exhibited active bone cell differentiation and proliferation, and regular arrangement of collagen fibers and bone trabeculae at 3 weeks after the operation. At 6 weeks after surgery, all bone fractures had healed almost completely. Tissue samples from the DRE group showed thicker and more mature bone trabeculae compared with the control group. Abundant cement lines were also noted in the DRE group, and were absent in controls. Cytoplasm enhanced all of these DRE effects.

### Quantitative findings for fracture calluses

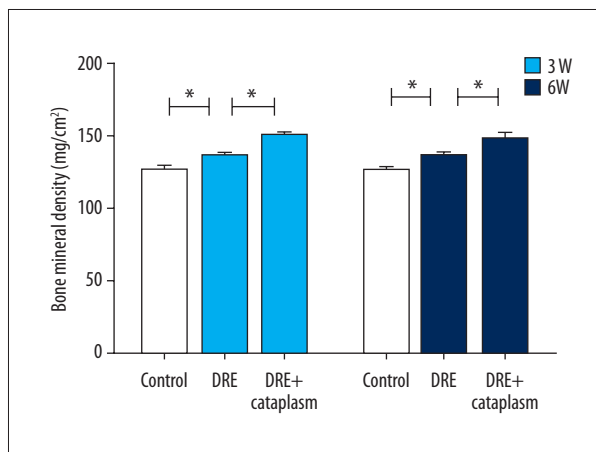
Quantitative analysis demonstrated that the total sagittal cross-sectional areas of the femora were larger in the DRE group compared to control group at 3 and 6 weeks after the operation, and the DRE cataplasm group showed further increases in total area (Figure 3A). The results regarding callus thickness and percentage of callus area were consistent with these observations (Figure 3B, 3C).

### Biomechanical test results

The biomechanical strength of the femoral cortical bone was evaluated by the 3-point bending test. DRE enhanced the



**Figure 4. (A, B)** Biomechanical results at 3 and 6 weeks presented as ultimate load force and stiffness. Data are expressed as mean  $\pm$  standard deviation, significant differences among different groups are presented as \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ,  $n = 5$ .



**Figure 5.** The bone mineral density (BMD) in each group was assessed from the trabecular bone of distal femur of the rats. Data are expressed as mean  $\pm$  standard deviation, significant differences among different groups are presented as \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ,  $n = 5$ .

strength of the femoral diaphysis in osteoporotic fracture rats by increasing the ultimate load and stiffness, compared with the control group at 3 and 6 weeks after operation. The DRE cataplasm group exhibited further increases in biomechanical strength compared with the DRE group (Figure 4).

#### Effects of DRE and DRE cataplasm on BMD

The BMD of the distal femoral trabecular bone was assessed in each group. From 3 to 6 weeks post-fracture, DRE markedly restored the trabecular BMD in the femur compared with the control group. The DRE cataplasm group showed greater femoral BMD recovery compared with the DRE group (Figure 5).

## Discussion

Osteoporosis, which is caused by an imbalance of bone remodeling involving the promotion of bone resorption over bone formation, is characterized by low bone mass and consequent elevated fracture risk [24]. Osteoporosis is associated with high bone fragility due to excessive resorption and reduced bone formation, contributing to the possibility of hip and other bone fractures [25]. During fracture healing, bone tissue is reconstructed, and biomechanical function is restored. However, osteoporosis disturbs this process, resulting in pain and physical disability. Osteoporotic patients tend to lose bone mass in the metaphyseal region, which necessitates symptomatic treatment with osteogenic potential to increase bone mass and decrease bone loss [26]. Post-menopausal osteoporosis is the main type of osteoporosis seen in female patients, and it is known to increase the risk of fracture. As mentioned, estrogen treatment markedly decreases the rate of fracture in post-menopausal women [7,8]. However, this hormone replacement therapy may increase the risks of breast cancer, ovarian cancer, and cardiovascular diseases [27]. Due to the public health concerns associated with the burden of osteoporotic fracture, alternative treatment strategies are required.

DRE is a well-known kidney-tonifying traditional Chinese medicine, which shows pharmacological effects on osteoporosis, bone fractures, and joint diseases [28]. Our previous study showed that DRE promotes the function of recombinant alendronate to suppress bone resorption by osteoclasts [16]. In addition, the combination of recombinant alendronate and DRE had therapeutic effects on fracture healing and callus formation, contributing to the improvement of biomechanical function. Treatment with DRE accelerated osteoblast differentiation and mineralization in pre-osteoblasts [29] and suppressed bone loss in rodent osteoclasts [30]. DRE treatment has been reported to increase BMD and trabecular number in rodents.

Meanwhile, DRE extracts were shown to promote bone formation on the margins of bone defects in a rabbit bone defect model [13]. Furthermore, several pharmacological studies confirmed that DRE has various biological effects, such as immunoregulatory [31], anti-inflammatory [32], and neuroprotective [33] effects. In the present study, we demonstrated that DRE had beneficial effects on fracture healing and callus formation in a rat model of osteoporotic fracture. In addition, DRE markedly restored trabecular BMD in the femur and improved the biomechanical properties of the bone.

Nevertheless, a strategy is needed for DRE administration to avoid its potential gastrointestinal side effects [17]. Transdermal delivery is a proven pathway for drug treatment [18]. It has been reported that TPGS/LID-NLC showed higher permeation efficiency and anesthetic effects suggesting that TPGS-modified NLC could be a potential drug delivery method for long-acting anesthesia [34]. Transdermal delivery of doxorubicin using hyaluronic acid by transfersomes/microneedle complex improved the therapeutic effects in tumor metastasis treatment [35]. Cataplasm application did not irritate rabbit skin, had no acute toxicity in rabbits and caused no allergic reaction on the skin of guinea pigs, suggesting that this transdermal method is a useful candidate for drug delivery [36]. A cataplasm containing ketoprofen was shown to attenuate adjuvant arthritis by suppressing edematous swelling and bony changes [37]. Furthermore, treatment with a cataplasm containing Yiguanjian was reported to suppress opioid dependence and related withdrawal symptoms [38]. Cataplasm use has been demonstrated to optimize the delivery of drug components and regulate *in vitro* drug release over a specific time period [39].

As previously mentioned, a cataplasm is a cloth for external application infused with a traditional Chinese medicine extract or drug mixed with a suitable hydrophilic matrix and appropriate amounts of excipients. Thus, a cataplasm is a type

of external transdermal patch, which has been used widely around the world and has several advantages. 1) The cataplasm matrix has good compatibility with water-soluble and fat-soluble drugs, and a large amount of matrix loading, and is thus suitable for the application of important multi-component and high-dose medications. 2) Cataplasm contents are usually >50% water, and therefore easily soften the stratum corneum of the skin and are beneficial for percutaneous drug absorption. 3) Cataplasms have superior breathability, skin adhesion, and heat preservation compared with traditional plasters, with less irritation of the skin, and thus are comfortable to use, can be repeatedly peeled off and re-applied, leave no residue on the clothes and are not associated with pain. 4) Compared with oral administration, cataplasm delivery has no hepatic first-pass effect, is unaffected by gastrointestinal degradation and is characterized by high bioavailability; it is convenient and is not associated with pain, unlike injection; and the drug dosage is more accurate than with application in ointment. The absorption area is fixed, the concentration of drugs in tissues is high and stable compared with rubber paste application, and sensitization effects caused by rosin and other tackifiers are avoided. Based on these properties, we examined the effects of DRE cataplasm in the treatment of fracture in a rat model of osteoporosis. Our results indicated that the DRE cataplasm further enhanced the therapeutic effects of DRE by increasing callus formation, restoring the trabecular BMD in the femur, and improving the biomechanical properties of bone in osteoporotic rats.

## Conclusions

Our study evaluated the differences between oral DRE and DRE cataplasm and our results showed the DRE cataplasm might be a novel strategy for the treatment of osteoporotic fractures.

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