Chinese topical herbal medicine gives additive effect on pharmaceutical agent on fracture healing

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**Abstract**

**OBJECTIVE:** To investigate the efficacy on the combination of oral strontium ranelate (SrR) with a topical Chinese herbal paste on facilitation of fracture healing.

**METHODS:** An open fracture was created at the mid-shaft of the right tibia of rat. A herbal paste called CDR containing Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati) was prepared. The rats were treated with either CDR topically on the fracture site, or SrR orally, or their combinations. Bone turnover biochemical markers in serum were measured. Microarchitecture of the fracture was analyzed using micro-CT after 14 and 28 d, followed by histomorphometrical analysis.

**RESULTS:** Micro-computed tomography analysis revealed that the combined treatment of CDR with 600 mg/g SrR significantly increased the total callus density, mineralized callus volume fraction, mineralized callus mineral content and mineralized callus density of the callus after 28 d of treatment. This result was consistent with the histomorphometrical analysis on the osteoid volume. Analysis of biochemical markers showed that the combined treatments reduced the bone resorption that occurs temporarily after fracture.

**CONCLUSION:** This study demonstrated that the combined treatment of oral SrR and topical CDR is effective to promote fracture healing by their additive effect on promoting bone formation and retarding bone resorption.

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**Keywords:** Fracture healing; Osteogenesis; Bone resorption; Medicine, Chinese Traditional; Integrative medicine; Strontium ranelate
ture for the fractures at lower extremities cause over one billion US dollars annually in United States. Patients with bone fracture also require a long hospitalization time and many rehabilitation services. Considering that fractures are more common in the elders, the number of fracture cases is expected to increase persistently in the coming years due to the rising aging population. Consequently, fractures are increasing the social-economic burden.

Orthopaedic manoeuvres are undoubtedly effective in fracture fixation. However, the healing process after fixation is seldom attended except for the pain management and complications. Although clinical investigations on the development of cost-effective means to improve fracture healing, especially for difficult fractures, have started, their application remains controversial. For instances, the off-label use of anti-osteoporotic agents in complicated fractures and non-unions has been reported. One of these agents is the strontium ranelate (SrR). SrR not only increases deposition of new bone by osteoblasts but also reduces the resorption of bone by osteoclasts. Nonetheless, it has been recommended restricting the use for the treatment of osteoporosis because of its cardiovascular risks.

In Traditional Chinese Medicine (TCM), a specific sector is devoted to fracture healing through the topical application of herbal pastes. Notwithstanding the long history of its application, the diversities of the formulae of the herbal medicines and the lack of evidence-based scientific support have failed to maintain its historical value. In view of this, we have conducted preclinical studies to re-look of the efficacy of topical herbal pastes on facilitation of fracture healing: topical application of a 6-herb paste elevated the plasma bone specific alkaline phosphatase activities and increased the callus size in a rabbit tibial fracture model; a simplified 4-herb paste resulted in a higher mechanical strength and bearing than the control in a drill-hole defects of rat tibia. Recently, a clinical trial on the efficacy of a 3-herb formula herbal paste named CDR, containing Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati), in the treatment of the fifth metatarsal fracture has shown good effects on pain control, swelling reduction and foot and ankle functional scores. Importantly, the radiological examinations revealed effective fracture unions. Because of the positive effect of the CDR herbal paste on fracture healing, it is hypothesized to have additive or even synergistic effects if it is applied simultaneously with the oral medication of a bone forming pharmaceutical agent. SrR was selected in the current study because of its bone forming ability as well as clinical reports of fracture healing under conditions of osteoporosis. Its effective dosage could be reduced so that its adverse effects could be minimized if synergistic effect is observed on its combination with CDR.

This is a preclinical study aims to verify the efficacy of the combined topical use of CDR paste with oral SrR on fracture healing. Evidence-based scientific data could be achieved to support for its clinical application.

**MATERIALS AND METHODS**

**Herbal materials and preparation of the herbal paste**

Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati) were purchased from a reputable wholesaler of Traditional Chinese Medicine (Zhixin Limited, Guangzhou, China). The identities of all herbs had been authenticated using thin-layer chromatography with reference to the methods stated in the Chinese Pharmacopoeia. The herbarium voucher specimens of the tested herbs were deposited in the museum of the Institute of Traditional Chinese Medicine, the Chinese University of Hong Kong. The voucher name and numbers were: Honghua (Flos Carthami) (2013-3415); Chuanxuduan (Radix Dipsaci Asperoidis) (2013-3417); Dahuang (Radix Et Rhizoma Rhei Palmati) (2013-3416).

To prepare the CDR herbal paste, 50 g of each herb was boiled with 1 litter distilled water for 1 h. The filtrate was collected and the remaining solid herbal residue was further boiled with 1 litter 95% ethanol for one hour. The aqueous and ethanol extracts were combined and concentrated into paste form. 10 g of the paste of each herb was dried in an oven at 80 °C overnight and the dry weight was measured. After the water content of the paste was determined ((wet weight-dry weight) / wet weight × 100%), the CDR herbal paste was made by mixing the three individual pastes in ratio 1:1:1 (dry weight). 2.0% (w/w) borneol (Alfa Aesar, Shanghai, China) was supplemented additionally to increase the transdermal efficiency. Strontium ranelate (SrR) which is marketed as Protos®, was purchased from a pharmaceutical company (Servier, France).

The chemical composition of the herbal paste was determined by high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS). The main chemical markers for Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati) was Hydroxysafflor yellow A, Asperosaponin VI and Rhein, respectively.

**Animal model and grouping**

Animal ethics approval had been obtained from the Animal and Experimental Ethics Committee of the Chinese University of Hong Kong (CUHK) (14/155/MIS) prior to the start of the study. Sprague-Dawley (SD) rats were supplied by the Laboratory Animal Service Centre (LASEC), CUHK. They received food and water ad libitum and were housed in a constant temperature of 22 °C with a 12-h light-dark cycle throughout the study. The experimental procedures were started after 7 d of acclimatization.
Total 72 male Sprague-Dawley rats with body weight (357 ± 21) g were used. 30 of them were euthanized after 14 d of treatment for microarchitectural analysis using micro-CT. The remains were euthanized after 28 d of treatment. After the rats had been anesthetized using ketamine and xylazine cocktail (im) and followed by buprenorphine (sc) for analgesic purpose, an open fracture was created at their mid-shaft of the right tibia using an electric burr drill (OmniDrill35, World Precision Instrument, Sarasota, FL, USA). A Kirschner-wire with diameter 1.0 mm was then inserted into the intramedullary canal from the anterior-intercondyloid fossa. The incision was closed using suture finally. The left tibia was untreated. After that, the rats were divided into 6 groups randomly (each group with two time points) and treated with different treatment protocols: (C) -fed with distilled water without CDR paste as control; (L) -fed with 200 mg/kg SrR without CDR paste; (M) -fed with 600 mg/kg SrR without CDR paste; (P) -fed with distilled water and CDR paste was applied; (LP) -fed with 200 mg/kg SrR and CDR paste was applied and (MP) -fed with 600 mg/kg SrR and CDR paste was applied. 600 mg·kg⁻¹·d⁻¹ of SrR leads to mean serum strontium concentration in rats close to the median serum strontium concentrations observed in patients administered the normal therapeutic dose of 2 g/d.³ A gavage tube was used for all of the feedings. SrR was dissolved in distilled water and the concentrations were adjusted so that 2 mL of SrR solutions, equivalent to the volume of distilled water, were administered orally. For P, LP and MP, one gram of CDR paste was applied topically onto the anterior part of the mid-shaft of the right tibia using a spatula. The paste was then covered by a piece of gauze and was secured by an adhesive thin plastic sheet (Tegaderm®, 3M, Maplewood, MN, USA). The anterior part of the right tibia of the animals in the groups without CDR paste was also covered with a piece of gauze and secured by the thin plastic sheet. The treatment protocol was 6 d/week, for 2 or 4 weeks. Prior to the surgical procedure and weekly thereafter, the rats were anesthetized using ketamine and xylazine cocktail (im). 1 mL of blood was then collected from the orbital venous plexus prior to the recovery of the animal. Serum was obtained by centrifuging the blood at 3000 rpm at 4 °C for 15 min, and was stored at − 80 °C before the analyses of the biochemical markers. A portion of rats were euthanized at Day 14 and their fractured tibiae were harvested for microarchitectural analysis using micro-computed tomography (micro-CT). For the rest of the rats, calcein and xylene orange (Sigma-Aldrich, St. Louis, MO, USA) respective-ly dissolved in sodium hydrogen carbonate solution were injected (sc) in 5-day interval alternatively. These rats were euthanized at Day 28 and their tibiae were collected for microarchitectural analysis followed by histomorphometrical analysis. The specimens were preserved in 75% ethanol.

Assessment of biochemical markers
For the analyses of the serum biochemical markers, activities of the bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase (TRAP) were analyzed. Activity of BALP was measured using a wheat germ lectin (WGL) (Sigma-Aldrich, St. Louis, MO, USA) precipitation method. Briefly, 15 μL serum was mixed with either 15 μL WGL solution (5 mg/mL) or distilled water in microcentrifuge tubes. Next, the mixture was incubated at 37 °C for 30 min. The microcentrifuge tubes were then centrifuged at 13 000 rpm for 5 min. 20 μL supernatant of each sample was transferred into the wells of a 96-well plate followed by adding 200 μL working ALP Substrate (Stanbio Laboratory, Boerne, TX, USA). The absorbance was measured at 405 nm using a micro-plate reader immediately. Total 16 cycles were measured to ensure a complete kinetic profile of BALP activity could be obtained. The slope of the steepest linear region of the curve was determined. BALP activity was calculated by subtracting the ALP activity of the WGL precipitated serum from that of the serum mixed with distilled water. The result was normalized with the baseline values and expressed in percentage change. Activity of TRAP in serum was measured using a commercial enzyme-linked immuno sorbent assay kit [RatTRAP™ (TRAcP 5b), Immunodiagnostic Systems Limited, East Boldon, UK]. All the measurement procedures recommended by the kit were followed.

Considering that the inter-individual variations of the biochemical markers in the preoperative state were large, comparisons among the groups were reported by expressing all parameters as a percentage of their preoperative values.

Microarchitectural analysis using micro-computed tomography (micro-CT)
The whole tibia was fixed in the holder of the micro-CT (Scanco μCT 40, Scanco Medical AG, Wangen-Brüttisellen, Switzerland) and preserved with 75% ethanol during scanning. The following parameters were used: voltage: 70 kVp, current: 114 μA, integration time: 300 ms. Isotropic resolution of 30 μm was obtained, yielding approximately 330 axial slices per specimen. The following measures of callus structure and composition were evaluated from the μCT image data for each specimen: total callus volume (TV); total callus density (TV Density); mineralized callus volume (BV); mineralized callus volume fraction which is the BV normalized by the TV (BV/TV); mineralized callus density (BV Density); mineralized callus mineral content (BMC, defined as the product of BV and TV Density). Prior to computing the values of each of these outcome measures, an automated contouring approach was applied to delineate the callus. A Gaussian filter (σ = 0.5, support = 1.0) was applied for noise reduction. Threshold value at 233 was used to distinguish the mineralized tissue from unmineralized or
poorly mineralized tissue, including the cartilaginous tissue in the callus. These values were chosen based on visual inspection of the tomograms. Calculation of TV Density, BV Density and BMC was made possible by density calibration data obtained from scans of a hydroxyapatite (HA) phantom provided by the system manufacturer.

**Histomorphometrical analysis**

The specimens after microarchitectural analysis were then dehydrated in ascending graded ethanol, followed by xylene and finally embedded in methyl methacrylate (Sigma-Aldrich, St. Louis, MO, USA). The specimens were sectioned longitudinally using a saw microscope (DM 5500 B, Leica, Wetzlar, Germany) at magnification of X.50 through a microscope (DM 5500 B, Leica, Wetzlar, Germany). The fluorescent signals were captured under a digital research microscope (DM 5500 B, Leica, Wetzlar, Germany) under UV excitation. The mineral apposition rate (MAR) was calculated. The sections were stained with Goldner’s trichrome to evaluate the osteoid volume. It was calculated as the percentage of area stained with orange-red divided by the whole bone area (OV/BV, %) through an image analysis software (ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA). The section showing the most intact structure of the callus and the best staining result of each specimen was selected for the analysis. Four regions of interests in the callus area of each section were captured randomly through a microscope (DM5500B, Leica, Wetzlar, Germany) at magnification of 50X.

**Statistical analysis**

Data in was expressed as mean ± standard error of mean. Comparison between groups was done by one-way analysis of variance followed by Dunnett’s multiple comparison test using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA), unless otherwise specified. P<0.05 was considered significant.

**RESULTS**

**Differences in microarchitecture in callus**

Micro-computed tomography images showed the difference of the fracture at Day 28 post-op (Figure 1). Soft callus was still obvious in C, L, P and LP, which resulted in a relatively large total callus volume (TV) (Figure 2A). The fractures of M and MP were healed faster compared with the others. Analysis of the microarchitectural parameters of the callus revealed that there was no significant difference in TV among all the groups at both 14 and 28 d after fracture (Figure 2A). However, the TV Density in C, M and MP increased significantly from Day 14 to 28 by 26.43 (P<0.05), 31.30 (P<0.01) and 28.91% (P<0.01), respectively. At Day 28, the TV Density of MP was the highest (384.3 mg/cm³) and was 22.26%, 21.89% and 21.50% higher than that of C, L and P, respectively (P<0.05 for all) (Figure 2B). After the threshold value of the micro-CT parameter was set higher to evaluate the mineralized tissue in the callus (mineralized callus), the mineralized callus volume (BV) increased in all groups from Day 14 to 28 and significant differences were found in C, LP, M and MP (Figure 2C). Similar outcomes to TV Density were found from the analyses of the BV/TV (Figure 2D) and BMC (Figure 2E), except that there was no statistical difference between Days 14 and 28 in C in the BV/TV. At Day 28, BV of M was significantly larger than that of C by 24.48% (P<0.05); BV/TV of MP was significantly larger than that of C by 40.53% (P<0.05); BMC of M and MP was significantly higher
than that of C by 47.80% \( (P < 0.01) \) and 42.96% \( (P < 0.05) \), respectively. The BV Density of MP was also the highest at Day 28 \( (662.4 \text{ mg/cm}^3) \) among all groups and higher than C, P and LP by 5.38% \( (P < 0.01) \), 6.19% \( (P < 0.01) \) and 4.86% \( (P < 0.05) \), respectively (Figure 2F). BV Density of MP was also higher than that of C at Day 14 by 4.75% \( (P < 0.01) \).

**Differences in histomorphometrical parameters**

In general, there was no statistical difference in the MAR among the 6 groups of rats (Figure 3A). Goldner’s trichrome staining revealed that the osteoid volume (OV/BV, %) of the treatment groups was larger than that of the control in general (Figure 3B). OV/BV of the MP was the largest amount the 6 groups of rats and it was 4.24 times larger than that of C. LP seemed to have additive effect with larger OV/BV than L and P alone. However, the number of sections obtained from using saw microtome was limited and not all the specimens could be grinded and polished successfully for this analysis. The number of sections ready for Goldner’s trichrome staining was therefore reduced. The sample size for C, L, P, LP, M and MP was 4, 5, 5, 4, 4 and 3, respectively. Consequently, all of the comparisons were not significant statistically due to the small sample size.

**Differences of the biochemical markers in serum after treated with CDR and SrR**

The activity of the BALP in the serum of all rats decreased after 1 week of fracture (Figure 4A). It decreased to 77.7%, 80.0%, 54.4%, 83.7%, 79.8% and 63.4% for C, L, P, LP, M and MP, respectively. Significant differences were found in P, LP and MP. After that, the BALP activity of L, P and MP started to increase but similar with other groups, it kept at low levels and could not reach the baseline value. The percentage change of the BALP activity in M and MP was significantly lower than their baseline at Day 28 (63.7% and 69.3% for M and MP, respectively, both \( P < 0.05) \). No difference was observed when the BALP activity among all groups was compared at each of the same time point.

On the contrary, the activity of tartrate-resistance acid phosphatase (TRAP) in the serum of the rats in all groups increased after 1 week of fracture (Figure 4B). Significant increase was found in C, L and P (125.7 \( (P < 0.01) \), 127.2% \( (P < 0.05) \) and 130.1% \( (P < 0.01) \). After that, the TRAP activity decreased dramatically and significantly from Day 7 to 14 post-fracture. It decreased continuously to Day 28. The percentage of TRAP activity of all treatment groups, except M, was lower than their baseline value (59.0%, 65.8%, 68.0%, 64.5%, 75.0%, 80.0% for C, L, P, LP, M and MP, respectively). Significant differences were found in P, LP and MP. After that, the TRAP activity of L, P and MP started to increase but similar with other groups, it kept at low levels and could not reach the baseline value.
59.6% and 63.2% for L, P, LP and MP, respectively, \( P < 0.001 \) for all). There was no difference in the TRAP activity among all groups when compared at the same time point.

**DISCUSSION**

Evidence-based scientific studies on topical TCM treatment for fracture healing are not common to be found from international journals. Studies on the integrative use of TCM and pharmaceutical agents to facilitate fracture healing are even less. The results from the microarchitectural analysis using micro-CT showed that MP was the most outstanding treatment to facilitate fracture healing. The TV density, BV/TV and BMC of MP were significantly higher than C, L and P after 28 d of treatment. The BV Density of MP was higher than that of C significantly even after 14 d of treatment. These findings illustrated that both the geometric and material factors of the fracture had been greatly improved by feeding the rats with 600 mg/kg SrR orally and simultaneously applying the CDR paste topically. These two factors may contribute to the mechanical strength of bone. An index derived from the cortical cross-sectional area and cortical BMD has been reported as a good predictor to estimate the bone bending strength.7 Hence, the co-treatment of SrR and CDR improved the geometric and material properties of the fracture callus, thus resulting in stronger bone strength. In fact, this speculation has been proven by the biomechanical 4-point bending test.7 In the bending test, the fractured tibia was harvested after 28 d of treatment. The comparisons in the yield strength and ultimate strength among MP, C, L and P matched well with those in TV density, BV/TV and BMC in the current study.

**Figure 3** Histomorphometrical analysis of the callus after 28 d post-op calculated from the non-decalcified sections: A: mineral appositional rate (MAR) from calculated from the fluorochrome sequential labelling of the non-decalcified tissue sections; B: osteoid volume (percentage of the osteoid volume to the bone volume within the callus (OV/BV, %)) measured from the sections stained with Goldner’s Trichrome; C: an image demonstrating the Goldner's trichrome staining on a MMA section. The image was stitched from 9 images (3×3) captured at 50× under microscope. Osteoid was stained with orange-red in color (arrows). C: control; L: 200 µg/mL SrR; P: CDR paste; LP: co-treatment of 200 µg/mL SrR with CDR paste; M: 600 µg/mL SrR; MP: co-treatment of 600 µg/mL SrR with CDR paste. SrR: strontium ranelate (oral); CDR: Chinese Herbal Paste containing Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati) (topical). Data are expressed as mean ± standard error of mean (error bar).

**Figure 4** Change of serum biochemical markers of rats after fracture: A: bone-specific alkaline phosphatase (BALP); B: tartrate-resistance acid phosphatase (TRAP). C: control; L: 200 µg/mL SrR; P: CDR paste; LP: co-treatment of 200 µg/mL SrR with CDR paste; M: 600 µg/mL SrR; MP: co-treatment of 600 µg/mL SrR with CDR paste. SrR: strontium ranelate (oral); CDR: Chinese Herbal Paste containing Honghua (Flos Carthami), Chuanxuduan (Radix Dipsaci Asperoidis) and Dahuang (Radix Et Rhizoma Rhei Palmati) (topical). Data are expressed as mean of percentage from Day 0 ± standard error of mean (error bar). \( P < 0.05, P < 0.001, P < 0.01 \) compared with Day 0 of its own group.

**DISCUSSION**

Evidence-based scientific studies on topical TCM treatment for fracture healing are not common to be found from international journals. Studies on the integrative use of TCM and pharmaceutical agents to facilitate fracture healing are even less. The results from the microarchitectural analysis using micro-CT showed that MP was the most outstanding treatment to facilitate fracture healing. The TV density, BV/TV and BMC of MP were significantly higher than C, L and P after 28 d of treatment. The BV Density of MP was higher than that of C significantly even after 14 d of treatment. These findings illustrated that both the geometric and material factors of the fracture had been greatly improved by feeding the rats with 600 mg/kg SrR orally and simultaneously applying the CDR paste topically. These two factors may contribute to the mechanical strength of bone. An index derived from the cortical cross-sectional area and cortical BMD has been reported as a good predictor to estimate the bone bending strength.7 Hence, the co-treatment of SrR and CDR improved the geometric and material properties of the fracture callus, thus resulting in stronger bone strength. In fact, this speculation has been proven by the biomechanical 4-point bending test.7 In the bending test, the fractured tibia was harvested after 28 d of treatment. The comparisons in the yield strength and ultimate strength among MP, C, L and P matched well with those in TV density, BV/TV and BMC in the current study.
There was no significant difference in total callus volume (TV) from micro-CT among all groups. It might be due to that the TV was defined by free hand contouring of the images of the whole "callus" in the tomodograms of the micro-CT. The space between the tissues, as well as the non-mineralized / poorly mineralized tissues, might have been included. However, other parameters were obtained under specific threshold values. Therefore, the variation of those results would be minimized and they were more objective for comparison.

From the histomorphometrical analysis, the osteoid volume (OV/BV, %) in MP was the largest while that of C was the smallest (although not significant statistically). The increase of osteoid volume in MP might not be caused by the abnormal mineralization in the rats because no difference in the MAR was found amount all 6 groups of rats within the 28-day treatment period. It indicated that the bone remodeling cycle had not been altered by the treatments within the experimental period. Hence, the increase of osteoid volume in MP might properly due to the increase of the non-mineralized bone tissue by MP. According to Dempster, osteoid volume is the percent of a given volume of bone tissue that consists of unmineralized bone. It is the bone matrix that will be, but has not yet, mineralized. Sometimes, it is referred to as pre-bone. The result of OV/BV was consistent with the BV/TV from micro-CT analyses, although BV/TV refers to the specific fraction of the mineralized bone. In addition, the newly formed osteoid in MP increased its overall bone volume. This might contribute to the increase in the biomechanical strength of the callus in MP as its geometrical factor has been increased.

The increase in overall bone volume in MP could be further supported by the results from the analyses of the biochemical markers in serum. Analysis in the change of BALP in the serum could not give conclusive results to differentiate the benefits of different treatment regimens on fracture healing. Generally, BALP dropped 7 d after fracture and then went up gradually thereafter with variable speeds among different groups. On the other hand, a regular pattern was observed in the change of the TRAP. TRAP increased after fracture and then dropped thereafter in all groups of rats. The changes in BALP and TRAP after fracture in this study were in accordance with the reports published by others. The reports illustrated that the sharp increase in bone resorption markers reflected an extensive bone remodeling. Notably, although TRAP in M and MP also boosted up from Day 0 to 7, the increase was not significant statistically. This indicated that SrR at 600 mg·kg⁻¹·d⁻¹ retarded the osteoclastic activity in the rat in the first week after fracture. Interestingly, neither L nor P could retard the osteoclastic activity on Day 7 but LP did. This result revealed that there was an additive effect on anti-osteoclastogenesis when CDR was combined with SrR. This observation was consistent with the results in the in vitro TRAP staining assay of our previous experiment. SrR alone at low concentrations could not reduce the TRAP positive cell number. However, once CDR was supplemented, SrR exhibited the anti-osteoclastogenic effects even at the lowest concentration.

Analyses of the serum biochemical markers consolidated that SrR retarded the osteoclastic activity and its efficacy was strengthened in the presence of CDR. This additive effect on anti-osteoclastogenesis led to net bone gain in MP subsequently.

In conclusion, the present study clearly confirmed the in vivo efficacy of the integrative treatment regimen, which is the combination of topical CDR herbal paste with oral SrR, to facilitate the fracture healing on the rat tibial fracture model. It demonstrated for the first time the integrative intervention of East and West medications on fracture repair. It also provided solid scientific evidences to support this integrative approach as a promising complementary treatment regimen to be considered to facilitate fracture healing.

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