

# The Effect of Different Fractions of Chinese Herb Extract (Shuang Gu Tang) on BMP-3 Gene expression Level of Mouse Bone Marrow Cell

Pingsheng Tu<sup>1</sup>, Xin Xie<sup>2</sup>, Mingchiu Fung<sup>2</sup>, Yanxin Liu<sup>1</sup>, Yuesen Zhi<sup>1</sup>, and Ying-Ying Lee<sup>2</sup>

<sup>1</sup> Orthopedics and Traumatology of Panyu Hospital Of Traditional Chinese Medicine Guangzhou 511400, China

<sup>2</sup> The School of Life Sciences, The Chinese University of Hong Kong, Shatin, New Territory, Hong Kong SAR, China

<sup>3</sup> Department of Biochemistry, La Trobe Institute of Molecular Science, La Trobe University, Bundoora, VIC, Australia

Email: tps59@sina.com; mingchiufung@cuhk.edu.hk

**Abstract—Aims:** Osteoporosis is a skeletal disease characterized by low bone mass and structural deterioration of bone tissue, with bone fragility and an increased risk of fractures. Bone is subject to constant remodeling mediated by two principal cells, the osteoclast for bone resorption and the osteoblast for bone formation. After the menopause in human and mice, the loss of bone mass is often coupled with diminished osteoblast production and function. It has been shown that the deficiency of estrogen and high level of expression of BMP-3 were linked to the accelerated bone loss after menopause. In this study, the effect of Chinese herb Shuang Gu Tang on the *BMP-3* expression of bone marrow cells of ovariectomized mice was investigated. **Methods:** The ovary of mouse was surgically removed to mimic the estrogen deficiency at menopause. The bone marrow cells of ovariectomized mice were collected in cell culture. The bone marrow cell culture was treated with different fractions of the Chinese herb extracts (Shuang Gu Tang) and the *BMP-3* expression level of each treatment was analyzed by quantitative real-time PCR. **Results:** Among the bone marrow cells of validated ovariectomized mice, the crude extracts and 40% ethanol fraction showed consistent effect on inhibiting the up-regulation of *BMP-3* gene. Compared to untreated bone marrow cells, crude extract and 40% ethanol fraction reduced the expression of *BMP-3* level by 41% and 45% respectively. Our results showed the inhibitory effect of Chinese herb (Shuang Gu Tang) on the up-regulation of *BMP-3* in cultured bone marrow cells from ovariectomized mice. The reduction of *BMP-3* expression may increase the differentiation of osteoblast and account for the anti-osteoporosis effect.

**Index Terms—**Shuang Gu Tang, osteoporosis, BMP-3, bone marrow cells

## I. INTRODUCTION

Osteoporosis is a disease, which is characterized by low bone mass and structural deterioration of bone tissue, and leads to bone fragility and an increased risk of fractures of the hip, spine, and wrist [1]-[3]. In vertebrate, in a healthy state, the bone is constantly remodeled. The resorption of preexisting bone by osteoclasts and formation of new bone by osteoblasts are strictly coordi-

nated to maintain bone mass [3], [4]. The functions of osteoclasts and osteoblasts are regulated by hormones such as bone morphogenetic proteins (BMPs), estrogen and others by binding to various hormonal receptors on these cell surfaces [5], [6].

BMPs which belong to the transforming growth factor b (TGF-b) superfamily are multifunctional growth factors involved in a wide range of biological processes. Both BMPs and other TGF-b members were associated with the regulation of the differentiation and activity of osteoblast and osteoclast [7], [8]. Previous studies have shown that the up-regulation of BMP-2, 4 and 6 could induce osteoblast differentiation in both mouse and human bone marrow cell population, hence enhancing bone formation [9]-[11]. Genetic study in mouse with *BMP-2* and *BMP-4* gene knock out demonstrated that critical level of BMP-2 and BMP-4 are required for osteoblast differentiation [12]. Mice lacking BMP-2 showed post-natal defects in bone mineral density, resulting in frequent fractures that failed to heal [13]. It has been reported that estrogen receptor was expressed in osteoblast and the activation of the BMP-2 expression in mouse mesenchymal stem cells was through the binding of estrogen to high affinity receptor on osteoblasts [14], [15].

In contrast to the well-characterized osteogenic effect of BMP-2, BMP-3 functions as an inhibitory regulator of bone formation. BMP-3 antagonized the osteogenic effects of BMP-2 by blocking the osteoblast differentiation in primary bone marrow stromal cell [16]. BMP-3-null mice displayed an over-osteogenic phenotype in adult, including the increase in bone mineral density and in trabecular bone volume [17], [18]. The inhibitory effect of BMP-3 on osteoblast differentiation was mediated through the activation of signaling in TGF-b/activin-pathway, which counteracts the osteogenic signaling initiated by BMP-2 and -4 [18]-[20].

In human, the accelerated phase of bone loss begins at menopause. Since estrogen acts through high affinity receptor in osteoblasts to restrain bone turnover [14], [15], the dramatic decrease of estrogen level speeds up the overall bone turnover after menopause. Therefore, postmenopausal estrogen deficiency results in increase in bone resorption and decrease in bone formation, leading

Manuscript received July 4, 2013; revised September 5, 2013.

to bone fragility and easy fracture [21], [22]. The postmenopausal bone loss can be prevented by estrogen replacement therapy [23], or by inhibition of BMP-3 expression or its activity [24], [25].

We have previously demonstrated the Chinese herbs used for the preparation of Shuang Gu Tang could induce proliferation and differentiation of bone marrow stromal stem cells [26], [27]. In this study, the effect of Chinese herb medicine extract (Shuang Gu Tang) that was claimed to prevent osteoporosis on the mouse bone marrow cells was investigated. Ovariectomy in female mice could be used as experimental manipulation to mimic the situation of postmenopausal bone loss due to estrogen deficiency [25], [28]. It has also been reported that expression level of *BMP-3* increased in ovariectomized rat compared to normal control [25], which suggests that high level of *BMP-3* may be used as indicator of bone loss after estrogen deprivation. Using bone marrow cells of ovariectomized mice as *in vivo* model, this study aimed to assess the effect of different fractions of the Chinese Herb extracts (Shuang Gu Tang) on *BMP-3* expression level.

## II. MATERIALS AND METHODS

### A. Preparation of Shuang Gu Tang (SGT)

SGT was prepared by boiling 10g of *Epimedium davidii* Franch, 12g of *Rehmannia glutinosa*, 15g of *Radix Astragali* seu *Hedysari*, 12g of *Eucommia ulmoides* Oliv., 12g of *Psoralea corylifolia* L., and 12g of *Drynaria fortunei* (Kunze) J. Sm. in 1.5L of water for 1 hour. SGT was filtered sterilized before used for cell culture experiments.

### B. Fractionation of Xian Gu Tang

C18 Column (HLB 35cc 6g LP Extraction Cartridges, Waters @ Oasis) was used to fractionate the Xian Gu Tang as follow: 20 ml filtered crude Xian Gu Tang was loaded into the column, washed with sterilized distilled water twice (15ml + 30 ml), eluted with 20%, 40%, 60%, and 80% ethanol. Fractions of 20%, 40%, 60% and 80% eluents were subjected to *BMP-3* expression analysis.

### C. Animal and Ovariectomy

BALB/C mice were reared in the animal house of the Chinese University of Hong Kong. The mice have free access to food and water. Ovary removal was performed using 10-months-old female mice. Mice were anesthetized by intraperitoneal injection of thiopental at the dose of 4 mg/kg body weight. Bilateral ovariectomy was performed in 10 mice and the ovariectomized mice were left untreated for 6 months for osteoporosis development. Sham surgery was performed with 5 mice in which the ovaries were exteriorized but remained intact. Another 5 mice were used as control group without any treatment.

### D. In vitro Culture of Bone Marrow Cells and Treatment with Herb Extracts

Bone marrow cells were harvested from tibia and femur bones of hind limbs from three groups of mice (ovariectomized, Sham and control group). The bone

marrow contents in the centre of tibia and femur bones were washed out with PBS buffer. Then red blood cells in the bone marrow were lysed using red blood cell lysis buffer. The remaining bone marrow cells were cultured in RPMI 1640 medium with 10% Fetal Bovine Serum and 1% PSF antibiotics (total  $2 \times 10^6$  cells in 3 ml culture per well) were cultured in 6-well microplate overnight at 37 °C under humidified atmosphere of 5% CO<sub>2</sub>/95% air. The next day, 90µl of each drug fraction including crude extract were added into cultured bone marrow cells (3% concentration, v:v). A negative control was also included which was adding 90µl of fresh RPMI medium into the cell culture. The cell cultures were then incubated at 37°C for 24 hours and ready for RNA extraction.

### E. RNA Extraction and cDNA Synthesis

Total RNA was extracted using RNeasy Mini kit@ Qiagen according to manufacturer's protocol. RNA concentrations were measured spectrophotometrically. First-strand cDNA was synthesized from 0.1µg RNA template using Reverse Transcriptase Polymerase Chain Reaction.

### F. Quantitative Real-Time PCR

Quantification of relative gene expression levels was determined by an absolute standard curve method. Normalization of the data with a single internal reference gene, 18s rRNA was designed to facilitate the comparison of expression levels of the target gene. RT-qPCR amplifications were conducted on BioRad (Hercules, California, USA), using SYBR greenER (Invitrogen) as fluorescent label. Triplicate reactions were performed for the cDNA template and their corresponding negative controls, both for target and reference genes, from 10 ovariectomized mice, 5 untreated mice and 5 mice from sham group. The primer used for *BMP-3* is: forward 5' TACGCCAACGATGCTGCCATTT 3'; reverse: 5' GCCTTGCCTTCTTCAGGGTCT 3'. The primer for 18s rRNA is: forward 5' TGAACAACACGGGAAACC3'; reverse 5' TCGCTCCACCAACTAGAA C 3'.

### G. Statistical Analysis

Each experiment was repeated for consistency of the result. The results were expressed as mean ± standard deviations (SD). Student T-test was applied for analyzing the significance of different between each group using Graph Pad Prism 5.0 software (San Diego, CA, USA). The results with  $P < 0.05$  were considered statistically significant.

## III. RESULTS

### A. Experimental Flow of the Study

10 months-old female BALB/C mice were used as experimental models. The workflow is showed in Fig. 1. Surgical ovariectomy was performed in ovariectomized group (OVX) of ten mice. The uterus of 5 sham mice was surgically opened while the ovary remained intact. Another 5 mice were included as control group without any treatment. After six months' feeding, all the mice survived and the mice were sacrificed for bone marrow

cells collection. As the ovary removal could up-regulate the *BMP-3* expression which is related to osteoporosis onset [25], the total mRNA of bone marrow cells were collected and *BMP-3* expression level was analyzed by quantitative real-time PCR to validate the success of ovary removal among the OVX mice.

The bone marrow cells of OVX mice that showed *BMP-3* expression increase was selected as the validated OVX samples. The validated bone marrow cells were treated with each herb fraction including crude extract for 24 hours. The *BMP-3* expression level was determined and then compared to the untreated samples to study the effect of each fraction of the herb extracts.

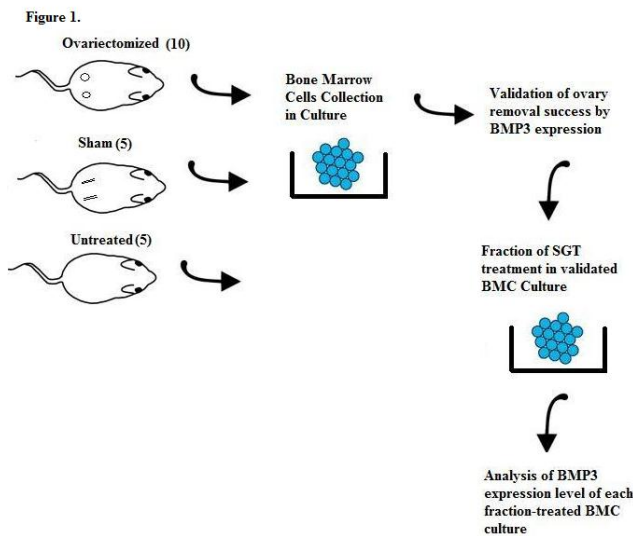


Figure 1. Experimental workflow of studying the effect of herb extracts on *BMP-3* expression using bone marrow cells of ovariectomized mice. Ovariectomized mice (OVX), sham mice and control groups were fed for 6 months before the collection of bone marrow cells. The effect of successful ovary removal was validated by checking the up-regulation of *BMP-3* gene level in each OVX individual. The validated bone marrow cells were treated with fractions of herb extract and then subject to *BMP-3* expression analysis.

**B. Qualification of the Ovariectomization**

Ovariectomization was performed in OVX mice group to accelerate osteoporosis, as shown by previous studies [25], [26]. Bone marrow cells were prepared from BALB/c mice oviectomized for 6 months, the sham group mice and the control ones. The expression level of *BMP-3* in bone marrow cells of each group was determined by quantitative real-time PCR. As shown in Fig. 2, no significant change was found between sham and control group. Compared to the mean expression level of control and sham group, not all the oviectomized mice showed an up-regulation of *BMP-3* gene. Only mice (OVX#2, 4, 5, 6 7 and 9) showed an up-regulation of *BMP-3*. As the *BMP-3* is a negative regulator of osteogenesis and is up-regulated in ovariectomized mice [16]-[18], [25], the significant increase of *BMP-3* could be used as an indicator of successful ovary removal and estrogen deprivation. Therefore, the bone marrow cells of mice OVX#2, 4, 5, 6 7 and 9 were selected as validated samples for further herb extract treatment.

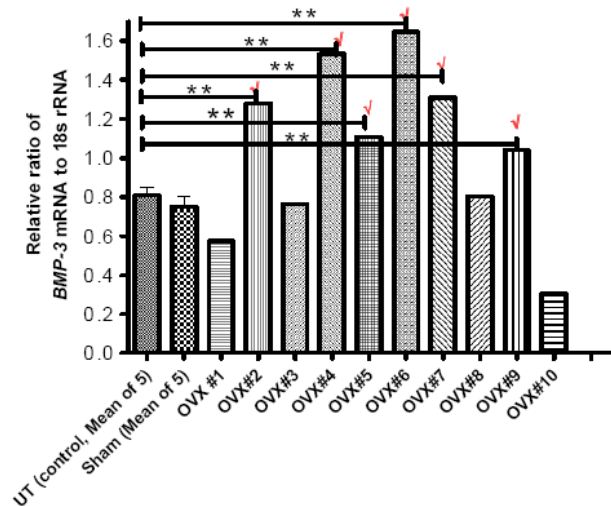


Figure 2. The qualification of ovariectomized (OVX) mice. Bone marrow cells of ten ovariectomized mice (OVX #1 to #10) and 5 untreated (control) mice and 5 sham mice were harvested and cultured at  $2 \times 10^6$  cells/per well. The success of ovary removal and osteoporosis induction were verified by comparing the *BMP-3* RNA levels of bone marrow cells between the untreated group (Mean value of 5 untreated mice), sham group (Mean value of 5 sham mice) and each individual ovariectomized mouse. The 18s rRNA was selected as the reference gene to normalize the gene expression level of *BMP-3* level. Independent one sample t-test for comparing mean of untreated group and the value of each OVX mouse (\* indicates  $p < 0.05$ ; \*\* indicates  $p < 0.01$ ). The OVX mice with normalized *BMP-3* level significantly higher than the mean value of normalized *BMP-3* level of control mice were selected for further analysis, including OVX#2, #4, #5, #6, #7, #9 (with  $\checkmark$  as indication).

**C. *BMP-3* Expression Level of Qualified Ovariectomized Mice Bone Marrow Cells after Treatment with Different Fractions of Shuang Gu Tang (SGT) Extract**

Bone marrow cells were obtained from the selected OVX mice (#2, 4, 5, 6, 7, 9) individually and treated with different fractions of SGT (20%, 40%, 60% or 80%) for 24 hours. Quantitative real-time PCR was used to determine the expression level of *BMP-3*, with 18s rRNA being used for normalization. The expression levels of *BMP-3* in the treated and untreated bone marrow cells of each individual mouse were shown in Fig. 3 and Table I. Different OVX individual showed different response level to the treatment, while there is an overall pattern that 40% ethanol fraction of SGT and the crude SGT could suppress the expression of *BMP-3*. The mean *BMP-3* expression levels of each ethanol fraction taken from 6 qualified OVX mice were shown in Table II and Fig. 4. Crude extract of SGT and 40% ethanol fraction reduced the expression of *BMP-3* level by 41% and 45% respectively. The combined results showed that the 40% ethanol fraction of SGT and the crude SGT could significantly reduce the expression level of *BMP-3* in bone marrow cells of ovariectomized mice (Compared with control mean,  $p < 0.05$  for crude SGT treatment group,  $p < 0.01$  for 40% ethanol fraction treatment group).

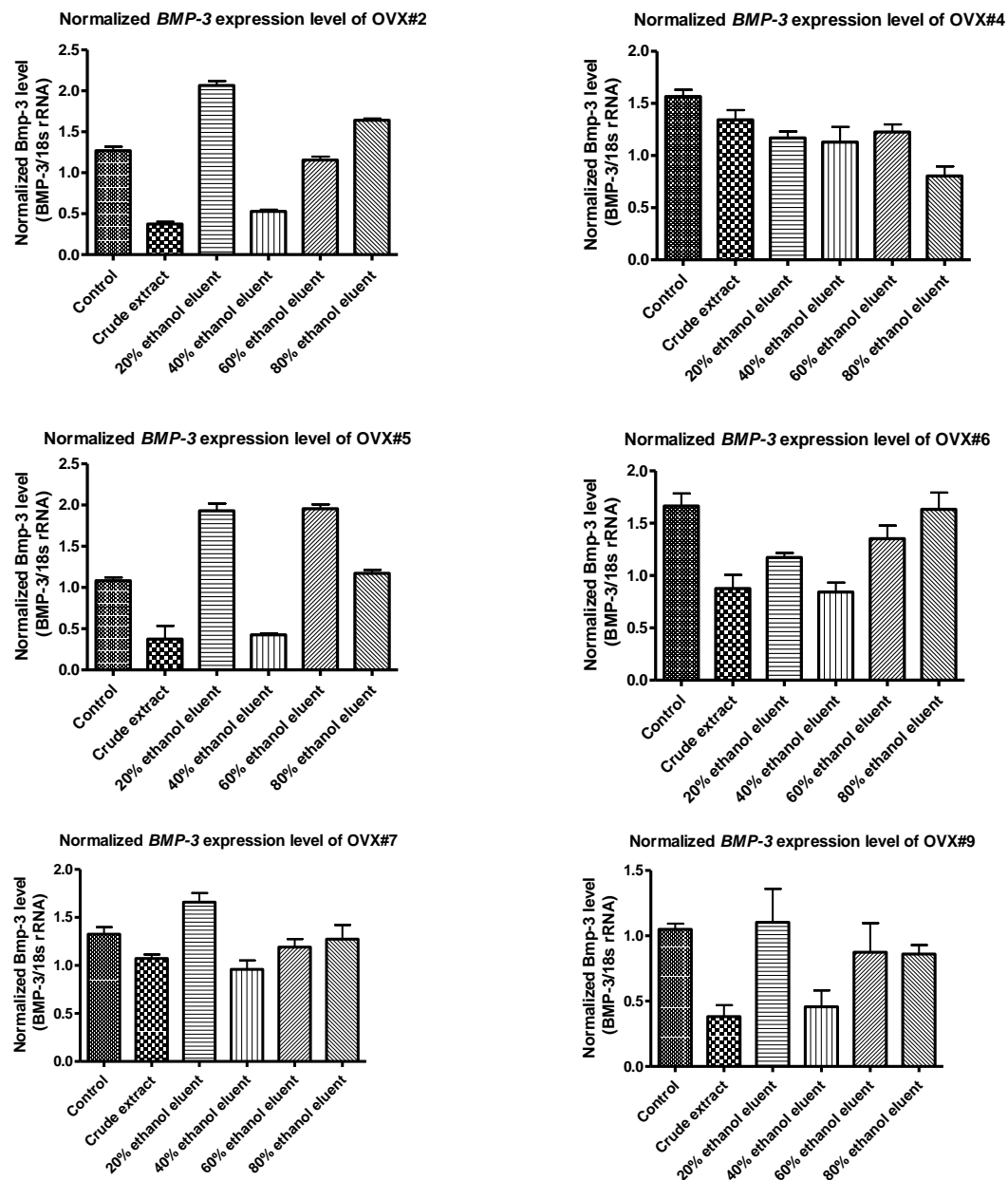


Figure 3. The normalized *BMP-3* expression levels of BMCs after treatment with different herb extract fractions. Bone marrow cells ( $2 \times 10^6$  cells/per well in 6-well plate) from each OVX mouse were treated with crude extract, 20%, 40%, 60% and 80% ethanol eluents from C18 column at 3% concentration (v:v) for 24 hrs. The total RNA was extracted and converted to cDNA by RT-PCR. Each sample was triplicated and quantitative real time PCR was applied using 18s rRNA as the reference gene.

TABLE I. SUMMARY OF NORMALIZED *BMP-3* EXPRESSION LEVEL OF SIX QUALIFIED OVARIETOMIZED MICE.

Mouse No. \ Treatment	Control	Crude extract	20% ethanol eluent	40% ethanol eluent	60% ethanol eluent	80% ethanol eluent
OVX#2	1.28±0.08	0.38±0.04	2.05±0.09	0.52±0.03	1.14±0.06	1.65±0.03
OVX#4	1.54±0.16	1.37±0.17	1.16±0.14	1.15±0.20	1.21±0.14	0.76±0.16
OVX#5	1.11±0.08	0.57±0.21	1.91±0.04	0.42±0.01	1.94±0.08	1.19±0.05
OVX#6	1.65±0.13	0.88±0.11	1.20±0.06	0.89±0.09	1.33±0.12	1.61±0.14
OVX#7	1.31±0.09	1.10±0.06	1.69±0.10	0.98±0.11	1.18±0.11	1.22±0.13
OVX#9	1.04±0.06	0.37±0.14	1.05±0.29	0.44±0.19	0.87±0.21	0.86±0.09

Data are presented as mean normalized *Bmp-3* expression level ± standard deviation.

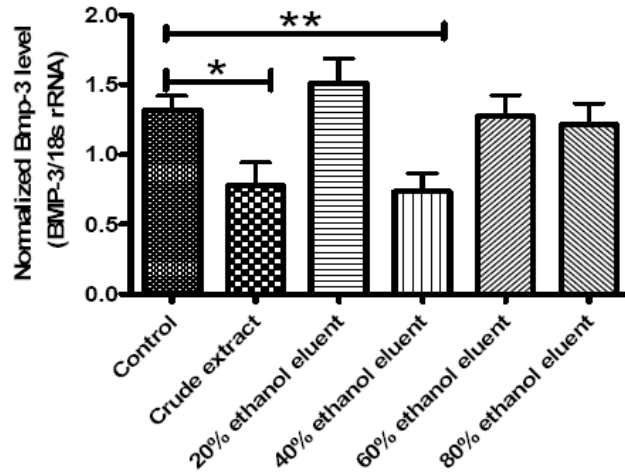


Figure 4. Summary of BMC *BMP-3* expression level of 6 qualified OVX mice for each herb fraction treatment. The data were presented as the mean *BMP-3* expression level of qualified OVX mice (n=6) ± standard deviation. \* indicates p<0.05, \*\* indicates p<0.01.

TABLE II. SUMMARY OF THE MEAN *BMP-3* EXPRESSION LEVEL OF 6 QUALIFIED OVX MICE.

Treatment	Mean Normalized <i>BMP-3</i> level (Mean ± SD)	Percentage change: (Mean of treatment group - Control Mean/Control Mean)
Control	1.32 ± 0.237	-
Crude extract	0.78 ± 0.408	-41%
20% ethanol eluent	1.51 ± 0.428	+14%
40% ethanol eluent	0.73 ± 0.313	-45%
60% ethanol eluent	1.28 ± 0.358	-3%
80% ethanol eluent	1.22 ± 0.368	-8%

The mean value of normalized *BMP-3* expression level in bone marrow cells of each qualified OVX mouse is summarized in this table. The percentage change of *BMP-3* expression level in each fraction treatment was compared to the control, calculated as: (Mean of treatment group – Control Mean/Control Mean).

#### IV. DISCUSSIONS

The ovariectomization was performed to stop the estrogen production and release by ovary, simulating the situation of mammal female menopause when estrogen level drops dramatically [26]. As estrogen has been correlated with the activation of osteoblasts [14], [15], estrogen deficiency in ovariectomized mice could accelerate the osteoporosis. However, the ovary removal may not be successful or complete due to technical problem, like the ovaries are embedded in adipose tissues which cannot be easily distinguished. The qualification or verification of successful ovary removal is necessary. Since *BMP-3* can antagonize *BMP-2*-induced osteoblastic differentiation in bone marrow stromal cell lines [16], and it has been reported that expression level of *BMP-3* increased in ovariectomized rat compared to normal control [25]. The success of ovariectomization could be assessed by comparing the *BMP-3* expression levels in bone marrow cells between the untreated group, sham group and each individual ovariectomized (OVX) mouse in Fig. 1. The mean *BMP-3* expression levels of untreated group and sham group showed no significant difference. The OVX mice with *BMP-3* expression level significantly higher than that of untreated group and sham

group were selected as the OVX mice with successful ovary removal for further analysis.

The bone marrows cells of verified 6 OVX mice were treated respectively with crude extract, 20%, 40%, 60% and 80% ethanol eluents of the herb extract through affinity chromatography column. In Fig. 2 and Table I, individual difference in responses to herb fraction treatment was observed, reflected by the difference change in *BMP-3* expression level in each kind of treatment. This individual variation requires the summarization of all verified mice to get the overall change pattern in response to different herb extract fraction. In Table II and figure 3, when compared to control group, the mean values of six verified OVX mice indicates both crude extract (p<0.05) and 40% ethanol eluent (p<0.01) can significantly reduce the *BMP-3* expression level in bone marrow cells. As *BMP-3* is a negative regulator of bone formation and the inhibition of *BMP-3* expression can be a curable method for osteoporosis [24]-[26], this herb crude extract can be used as an anti-osteoporosis agent. In addition, the 40% ethanol eluent though affinity chromatography column contains the most effective component which can suppress *BMP-3* expression, thus attenuating the osteoporosis induced by *BMP-3* expression increase after menopause.

## V. CONCLUSION

To conclude, the Chinese herb (Shuang Gu Tang) extracts showed inhibitory effect on the up-regulation of BMP-3 in bone marrow cells of ovariectomized mice, indicating its potential anti-osteoporosis benefit for postmenopausal female. Since the 40% ethanol eluent through affinity chromatography column has been identified as the most effective fraction, further study can be preformed to identify the chemical composition of the herb extract and investigate the mechanism involved in BMP-3 suppression.

## ABBREVIATIONS

BMP: Bone Morphogenic Protein; OVX: Ovariectomized; SGT: Shuang GuTang; TGF-b: transforming growth factor b.

## REFERENCES

- [1] P. A. Hill, "Bone remodeling," *British Journal of Orthodontics*, vol. 25, pp. 101-107, 1998.
- [2] F. Long F, "Building strong bones: Molecular regulation of the osteoblast lineage," *Nature Reviews Molecular Cell Biology*, vol. 13, pp. 27-38, 2012.
- [3] J. C. Gallagher and A. J. Sai, "Molecular biology of bone remodeling: implications for new therapeutic targets for osteoporosis," *Maturitas*, vol. 65, pp. 301-307, 2010.
- [4] P. Ducy, C. Desbois, B. Boyce, G. Pinero, *et al.*, "Increased bone formation in osteocalcin-deficient mice," *Nature*, vol. 382, pp. 448-452, 1996.
- [5] A. Yamaguchi, T. Komori, and T. Suda, "Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1," *Endocrine Reviews*, vol. 21, pp. 393-411, 2000.
- [6] T. Katagiri and N. Takahashi N, "Regulatory mechanisms of osteoblast and osteoclast differentiation," *Oral Diseases*, vol. 8, pp. 147-159, 2002.
- [7] X. Cao X and D. Chen, "The BMP signaling and in vivo bone formation," *Gene*, vol. 357, pp. 1-8, 2005.
- [8] D. Chen, M. Zhao, and G. R. Mundy, "Bone morphogenetic proteins," *Growth factors*, vol. 22, pp. 233-241, 2004.
- [9] D. Chen, M. A. Harris, G. Rossini, C. R. Dunstan, *et al.*, "BMP-2 enhances BMP-3, BMP-4, and bone cell differentiation marker gene expression during the induction of mineralized bone matrix formation in cultures of fetal rat calvarial osteoblasts," *Calcified Tissue International*, vol. 60, pp. 283-290, 1997.
- [10] K. Lavery, P. Swain, D. Falb, and M. H. Alaoui-Ismail, "BMP-2/4 and BMP-6/7 differentially utilize cell surface receptors to induce osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells," *J Biol Chem*, vol. 283, pp. 20948-20958, 2008.
- [11] N. Ghosh-Choudhury, G. G. Choudhury, M. A. Harris, J. Wozney, *et al.*, "Autoregulation of mouse BMP-2 gene transcription is directed by the proximal promoter element," *Biochem Biophys Res Commun*, vol. 286, pp. 101-108, 2001.
- [12] A. Bandyopadhyay, K. Tsuji, K. Cox, B. D. Harfe, *et al.*, "Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis," *PLoS Genetics*, vol. 2, pp. e216, 2006.
- [13] K. Tsuji, A. Bandyopadhyay, B. D. Harfe, K. Cox, *et al.*, "BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing," *Nat Genet*, vol. 38, pp. 1424-1429, 2006.
- [14] E. F. Eriksen, D. S. Colvard, N. J. Berg, M. L. Graham, *et al.*, "Evidence of estrogen receptors in normal human osteoblast-like cells," *Science*, vol. 241, pp. 84-86, 1988.
- [15] S. Zhou, G. Turgeman, S. E. Harris, D. C. Leitman, *et al.*, "Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells," *Molecular Endocrinology*, vol. 17, pp. 56-66, 2003.
- [16] S. Kokabu, L. Gamer, K. Cox, J. Lowery, *et al.*, "BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with Acvr2b," *Molecular Endocrinology*, vol. 26, pp. 87-94, 2012.
- [17] A. Daluiski, T. Engstrand, M. E. Bahamonde, L. W. Gamer, *et al.*, "Bone morphogenetic protein-3 is a negative regulator of bone density," *Nat Genet*, vol. 27, pp. 84-88, 2001.
- [18] M. E. Bahamonde and K. M. Lyons, "BMP3: To be or not to be a BMP," *The Journal of Bone and Joint Surgery American Volume*, vol. 83-A Suppl 1, pp. S56-62, 2001.
- [19] Y. Matsumoto, F. Otsuka, J. Hino, T. Miyoshi, *et al.*, "Bone morphogenetic protein-3b (BMP-3b) inhibits osteoblast differentiation via Smad2/3 pathway by counteracting Smad1/5/8 signaling," *Molecular and Cellular Endocrinology*, vol. 350, pp. 78-86, 2012.
- [20] E. Canalis, A. N. Economides, and E. Gaggero, "Bone morphogenetic proteins, their antagonists, and the skeleton," *Endocrine Reviews*, vol. 24, pp. 218-235, 2003.
- [21] M. N. Weitzmann and R. Pacifici, "Estrogen deficiency and bone loss: an inflammatory tale," *The Journal of Clinical Investigation*, vol. 116, pp. 1186-1194, 2006.
- [22] H. D. Nelson, "Postmenopausal osteoporosis and estrogen," *American Family Physician*, vol. 68, pp. 606, 610, 612, 2003.
- [23] J. G. Roberts, C. E. Webber, and C. A. Woolever, "Estrogen replacement therapy for postmenopausal osteoporosis," *Canadian Family Physician Medecin De Famille Canadien*, vol. 32, pp. 883-891, 1986.
- [24] E. Canalis, "Update in new anabolic therapies for osteoporosis," *The Journal of Clinical Endocrinology and Metabolism*, vol. 95, pp. 1496-1504, 2010.
- [25] P. Simic, J. B. Culej, I. Orlic, L. Grgurevic, *et al.*, "Systemically administered bone morphogenetic protein-6 restores bone in aged ovariectomized rats by increasing bone formation and suppressing bone resorption," *J Biol Chem*, vol. 281, pp. 25509-25521, 2006.
- [26] L. P. Hu, P. S. Tu, F. K. Xie, Z. W. Hung, *et al.*, "Effects of the kidney-tonifying recipe on proliferation and differentiation of bone marrow stromal stem cells from rats" *Chinese Remedies & Clinics*, vol. 7, pp. 329-331, 2007.
- [27] L. P. Hu, J. M. Zhou, P. S. Tu, J. W. Haung, *et al.*, "Tonifying kidney recipes inhibits differentiation of MSCs into adipogenic cells induced by dexamethasone," *Chinese Journal of Osteoporosis*, vol. 13, pp. 576-579, 2007.
- [28] M. L. Bouxsein, K. S. Myers, K. L. Shultz, L. R. Donahue, *et al.*, "Ovariectomy-induced bone loss varies among inbred strains of mice," *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, vol. 20, pp. 1085-1092, 2005.

**Ping-Sheng Tu** received his MBBs in Chinese Medical Sciences and M.Phil. from Sun Yat-Sen University.

He is the director of orthopedic surgery department of an orthopedic surgery physician, professor of Guangzhou University of Chinese Medicine, Member of the orthopedic branch of the Chinese Medical Sciences of Guangzhou, and the Guangzhou Medical orthopedic specialty group director. Professor Tu is engaged in clinical trauma, bone and joint disease treatment, and focus on basic research on bone disease and osteoporosis. He has developed a unique Chinese herbal formula Shuang Gu Tang for treating osteoporosis. He is the principle of investigator of a number of provincial and municipal fund for the research of severe orthopedic osteoporosis in the elderly as well as complex cases, and has published more than 20 papers.

**Ming Chiu Fung** received his PhD from the Australian National University in molecular biology in 1985.

He did a postdoc in the Department of Virology of Baylor College of Medicine in Houston and then worked as a research fellow in John Curtin School of Medical Research. He is now a professor of the Chinese University of Hong Kong Hong Kong. He has published more than 70 papers in various journals, such as "Molecular cloning of cDNA for murine interleukin-3" Fung, M.C., Hapel, A.J., Ymer, S., Cohen, D.R., Johnson, R.M., Campbell, H.D. and Young, I.G. (1984). *Nature* 307: 233-237, "Biological properties of molecularly cloned and expressed murine interleukin-3" Hapel, A.J., Fung, M.C., Johnson, R.M., Young, I.G. and Metcalf, D. (1985). *Blood* 65: 1453-1459, "Reversibility of Apoptosis in Cancer Cells" Ho Lam TANG, Ka Leung

YUEN, Ho Man TANG and Ming Chiu FUNG (2009) British Journal of Cancer 100(1): 118-122, and “Cell survival, DNA damage, and oncogenic transformation after a transient and reversible apoptotic response.” Tang HL, Tang HM, Mak KH, Hu S, Wang SS, Wong KM,

Wong CS, Wu HY, Law HT, Liu K, Talbot CC Jr, Lau WK, Montell DJ, Fung MC. Mol Biol Cell. 2012 Jun;23(12):2240-52.

Prof. Fung is a visiting professor of Sun Yat-sen University, adjunct professor of the Soochow University, and the Deputy Chief Editor of the “Journal of Tropical Medicine”.