



Study of acupuncture stimulation on experimental osteopenia

Shigeyuki Kanai^{1,*}, Norimasa Taniguchi^{1,2}, Kayo Kanda¹ and Hideaki Higashino³

¹Kansai college of Oriental Medicine, 2-11-1 Wakaba Kumatori-cho, Sennan-gun, Osaka 590-0482, Japan;

²Department of Science PIP-FUJIMOTO Co., Ltd, 1-36 Noninbashi 2-choume, Chuo-ku, Osaka 540-0011, Japan;

³Department of Pharmacology Medicine Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

SUMMARY

In order to study the effect of acupuncture stimulation on bone mineral density (BMD), using the ovariectomized (OVX) rat model, we assessed the degree of osteopenia by dual-energy X-ray absorptiometry, measured the level of locomotor activity using a metabolism measuring system, and performed histological studies of bone tissue. Twenty-four female Wistar rats (8 weeks old, 160 - 180 g) were divided into three groups. Rats in the OVX-A group underwent ovariectomy followed by acupuncture stimulation. The OVX rats in the Vehicle control group were not treated with acupuncture as a control. The rats in the control group received neither ovariectomy nor acupuncture. Acupuncture stimulation for 12 weeks in the OVX-A group inhibited the reduction in BMD of the femoral bones caused by ovariectomy. Moreover, in the two OVX groups, there was no clear difference in the level of locomotor activity between the active and resting phases prior to acupuncture stimulation in each rat, and the pattern of locomotor activity was irregular. After acupuncture stimulation of the OVX-A rats, the pattern of locomotor activity became diphasic with clear active and resting phases, as was observed in the Control group. On histological studies, the continuity of trabecular bone was maintained more favorably and bone mass was higher in the OVX-A group than in the vehicle control group. These results suggest that the increased locomotor activity that had been induced by acupuncture stimulation increased the BMD.

Key words: Acupuncture; Ovariectomized rat; Thermography; Locomotor activity

INTRODUCTION

The biological responses to acupuncture stimulation have been widely studied from the perspective of possible health effects. It has been reported that acupuncture stimulation has beneficial effects on chronic pain. Acupuncture has been administered to provide pain relief from neck and shoulder pain, as well as relief from pain due to ischemic conditions

of blood microcirculation (Kuo *et al.*, 2004; Stener-Victorin *et al.*, 2004). Osteoporosis patients with climacteric disturbance are frequently encountered in Oriental medicine clinics, but their osteoporosis is rarely treated. The ovariectomized (OVX) rat has been usually used as a model of osteopenia to study climacteric disturbance (Harada and Nakata, 1995). After menopause or ovariectomy, women tend to develop osteopenia and so-called menopausal symptoms including hot flashes, abnormal feelings, palpitations, and insomnia (Zhao *et al.*, 2000). In particular, premenopausal women who undergo ovariectomy develop osteoporosis and severe

*Correspondence: Shigeyuki Kanai, Kansai college of Oriental Medicine, 2-11-1 Wakaba Kumatori-cho, Sennan-gun, Osaka 590-0482, Japan. Tel: +81-724-53-8251; Fax: +81-724-53-0276; E-mail: kanai@kansai.ac.jp

menopausal symptoms due to sudden estrogen deficiency. Moxibutim situation is useful for the treatment of climacteric disturbance (Zhang *et al.*, 2004). In a previous study, we found Traditional Chinese medicine alleviated climacteric disturbance and inhibited the decrease in bone mineral density (BMD) observed in post-menopausal women (Jo *et al.*, 2003). Many studies have recently reported that acupuncture stimulation inhibited the reduction in BMD in postmenopausal women (Eastell *et al.*, 1997; Kanai *et al.*, 1998). In the present study, we examined the effects of acupuncture stimulation on ovariectomized rats as an experimental osteopenia model.

MATERIALS AND METHODS

Study animals and environmental conditions

This study was approved by the Animal Committee of Kansai College. Female Wistar rats (age, 7 weeks; body weight, about 150 g) were purchased from Japan Crea Co., Ltd. (Shizuoka, Japan). The animals were housed individually in cages and kept in a room maintained at a temperature of $23 \pm 1^\circ\text{C}$ with a relative humidity (RH) of $55 \pm 5\%$ under a 12 h / 12 h light-dark cycle (light: 9:00 AM to 9:00 PM). Solid rodent chow and tap water were given *ad libitum*. After 1 week of acclimation under these conditions, animals showing favorable growth were selected and used for further studies.

Experimental groups

Twenty-four rats aged 8 weeks were divided into 3 groups of 8 rats each. Bilateral ovariectomy was performed in the rats in the OVX-A and Vehicle control groups under anesthesia with ether. Acupuncture was administered to the rats in the OVX-A group. The rats in the Vehicle control group were not treated with acupuncture after ovariectomy. The animals in the Control group did not undergo ovariectomy nor receive acupuncture.

Experimental schedule

After a postoperative recovery period of 3 weeks,

acupuncture stimulation was performed on the OVX-A rats under ether anesthesia twice a week for a total of 24 times. The rats in the Vehicle control and Control groups were only anesthetized with ether twice a week for a total of 24 times and they did not receive acupuncture stimulation.

In the OVX-A group, the rat was mildly anesthetized with ether prior to acupuncture stimulation. The *Guanyuan* [(CV4) 5 mm below umbilicus] was punctured with a stainless steel acupuncture needle (0.24 mm in diameter) that had been sterilized with ethylene oxide gas, and the needle was left inserted for 10 min. We determined the sites of acupuncture stimulation by consulting the rat meridian points defined by Kitaichi *et al.* (1994).

OBSERVATIONS

Measurements of body weight, serum total cholesterol (T-chol) level and urinary deoxypyridinoline (Dpd) level before and after acupuncture stimulation for 12 weeks

Measurements of body weight, serum T-chol level and urinary Dpd level before and after 12 weeks of acupuncture stimulation were carried out. Blood samples (each 1.5 ml) were collected from the cervical vein under ether anesthesia, and the serum T-chol level was measured using the Wako Serum Test Kit (Wako Pure Chemicals Industry Osaka, Japan). Urine samples were collected by pooling for 24 h using rat metabolic cages, and the urinary level of Dpd was measured by the ELISA method (Villanueva and Mehr, 1977).

Measurements of tail surface temperature before and after acupuncture stimulation for 12 weeks

To avoid the influence of the haircoat, the tail temperature, which is used as an indicator of peripheral circulation, was measured. The tail surface temperature was measured using a thermograph (Dong *et al.*, 2000) (TVS-2300 MkII_{ST}, Tokyo, Japan Abionics Co., Ltd.) before and after 12 weeks of acupuncture stimulation. After 15 min of acclimation

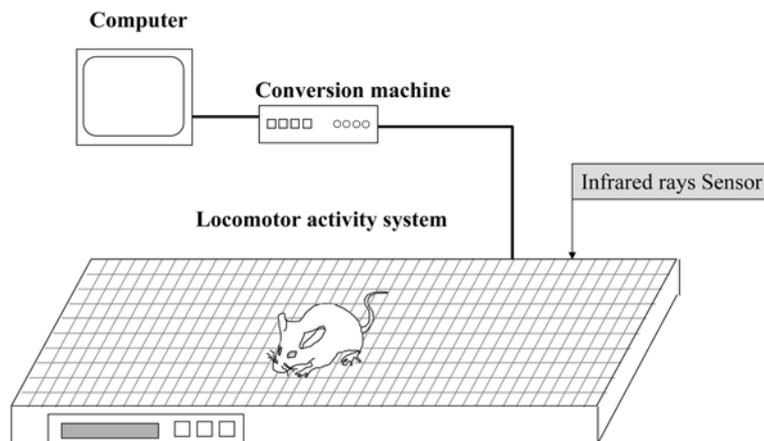


Fig. 1. The level of locomotor activity was measured using a metabolism measuring system (SCANET MV-10; MELQUEST Co., Ltd., Toyama, Japan). In the metabolism measuring system, infrared rays were spread horizontally (lengthwise and crosswise) at 5-mm intervals, and the number of infrared rays blocked by the animal were counted at 30-s intervals. The total locomotor activity over 24 h was measured as a daily locomotor activity.

to the environment, the tail surface temperature was measured in conscious animals in a windless room maintained at a temperature of $15 \pm 1^\circ\text{C}$ and a relative humidity of $55 \pm 5\%$. The temperature of the rat tails was measured at a distance of 1 m from the thermography device.

Measurements of locomotor activity before and after acupuncture stimulation for 12 weeks

Locomotor activity was measured using a metabolism measuring system (SCANET MV-10; MELQUEST Co., Ltd., Toyama, Japan) (Fig. 1). Over a 24 h period (1 day) prior to acupuncture stimulation and after acupuncture stimulation for 12 weeks. In the metabolism measuring system, infrared rays were spread horizontally (lengthwise and crosswise) at 5-mm intervals, and the number of infrared rays blocked by the animal was counted (Sandberg *et al.*, 2000). The level of total locomotor activity in every 30-min interval was shown graphically over a 24 h period and this was used as the daily behavioral pattern. The total locomotor activity over 24 h was defined as the daily locomotor activity. The level of locomotor activity in the day-time (9:00 AM - 9:00 PM) and night-time (9:00 PM - 9:00 AM) were measured.

Measurements of BMD and histological examination of bone tissue

After acupuncture stimulation for 12 weeks, the femoral bones were removed from each rat under ether anesthesia. The BMD was measured by DEXA, and was analyzed using the DCR - 600R Dichroma scan (Aloca Co., Ltd., Tokyo, Japan). Histological examination of the femoral bones was performed by the bone stain method described by Villanuava (Yamaguchi *et al.*, 1997), and morphological measurements were made.

Statistical analysis

The data obtained in each group are expressed as mean \pm standard error. The significance of time-related differences between the groups was assessed by the Wilcoxon sum rank test, and the level of significance was set at $P < 0.05$.

RESULTS

Changes in body weight, serum T-chol level and urinary Dpd level before and after acupuncture stimulation for 12 weeks

The body weight and serum T-chol level were

Table 1. Change of measurement of body weight, serum cholesterol level and urinary Dpd level before and after acupuncture stimulation for 12 weeks

	Body Weight (g)		Urinary-Dpd (nmol/mmolCre)		Serum T-Chol (mg/dl)		Tail Surface Temperature (°C)		Locomotor Activity (count)	
	Before	After	Before	After	Before	After	Before	After	Before	After
OVX-A	187.2 ± 5.1**	357.1 ± 3.9**	162.8 ± 12.3*	116.2 ± 14.8**	102.6 ± 3.7**	110.0 ± 11.4**	14.93 ± 0.26*	16.06 ± 0.26	110492 ± 10126*	125781 ± 11021
Vehicle control	189.5 ± 4.7**	361.0 ± 7.1**	152.4 ± 10.7*	139.6 ± 13.6**	104.6 ± 5.1**	114.2 ± 16.0**	15.18 ± 0.17*	15.37 ± 0.30*	106411 ± 11717*	100012 ± 9830**
Control	167.7 ± 2.9	310.5 ± 4.3	112.5 ± 11.7	82.4 ± 6.0	81.5 ± 3.7	94.9 ± 2.6	16.14 ± 0.19	16.34 ± 0.46	149140 ± 16125	154683 ± 19719

Results are shown as Mean ± S.E.M. * $P < 0.05$; significantly different between Control and OVX-A, Vehicle control. # $P < 0.05$; significantly different between OVX-A and Vehicle control.

higher in the OVX-A and Vehicle control groups than in the Control group before and after acupuncture stimulation ($P < 0.01$), suggesting that the rats in the OVX-A and Vehicle control groups tended to be obese. The urinary Dpd level was higher in the OVX-A and Vehicle control groups than in the Control group before and after acupuncture stimulation ($P < 0.05$). After acupuncture stimulation for 12 weeks, the urinary Dpd level tended to be lower in the OVX-A group than in the Vehicle control group ($P < 0.01$) (Table 1).

Changes in tail surface temperature before and after acupuncture stimulation for 12 weeks

Twelve weeks after ovariectomy and before acupuncture stimulation, the tail surface temperature was significantly lower in the OVX-A and Vehicle control groups than in the Control group ($P < 0.05$). After acupuncture stimulation, there were no significant differences (does'nt match; $P < 0.05$) in the tail surface temperature between the OVX-A group and Control group nor between the Vehicle control group and Control group ($P < 0.05$). (Table 1).

Changes in locomotor activity before and after acupuncture stimulation for 12 weeks

In the Control group, the pattern of locomotor activity was regular with a diphasic pattern. In the OVX groups, there was no clear difference in the level of locomotor activity between the active and resting phases before acupuncture stimulation,

and the pattern of locomotor activity was irregular. After acupuncture stimulation for 12 weeks in the OVX-A group, the pattern of locomotor activity had become diphasic with clear active and resting phases, as was observed in the Control group (Fig. 2).

Moreover, before acupuncture stimulation, the daily level of locomotor activity was significantly higher in the Control group than in the OVX-A and Vehicle control groups ($P < 0.01$). After acupuncture stimulation for 12 weeks, the daily level of locomotor activity in the OVX-A group was significantly higher than that in the Vehicle control group ($P < 0.05$) (Table 1).

Measurement of BMD and histological study of bone tissue

In the OVX-A and Vehicle control groups, the continuity of the trabecular bone was lost, the bone mass was significantly lower than that of the Control group, and histological findings of osteoporosis were observed. However, the continuity of the trabecular bone was maintained more favorably and the bone mass was higher in the OVX-A group than in the Vehicle control group.

The BMD was significantly lower in the OVX-A and Vehicle control groups than in the Control group ($P < 0.05$). However, in the OVX-A group, its decrease was obviously inhibited after acupuncture stimulation for 12 weeks compared with that in the Vehicle control group ($P < 0.05$) (Fig. 3).

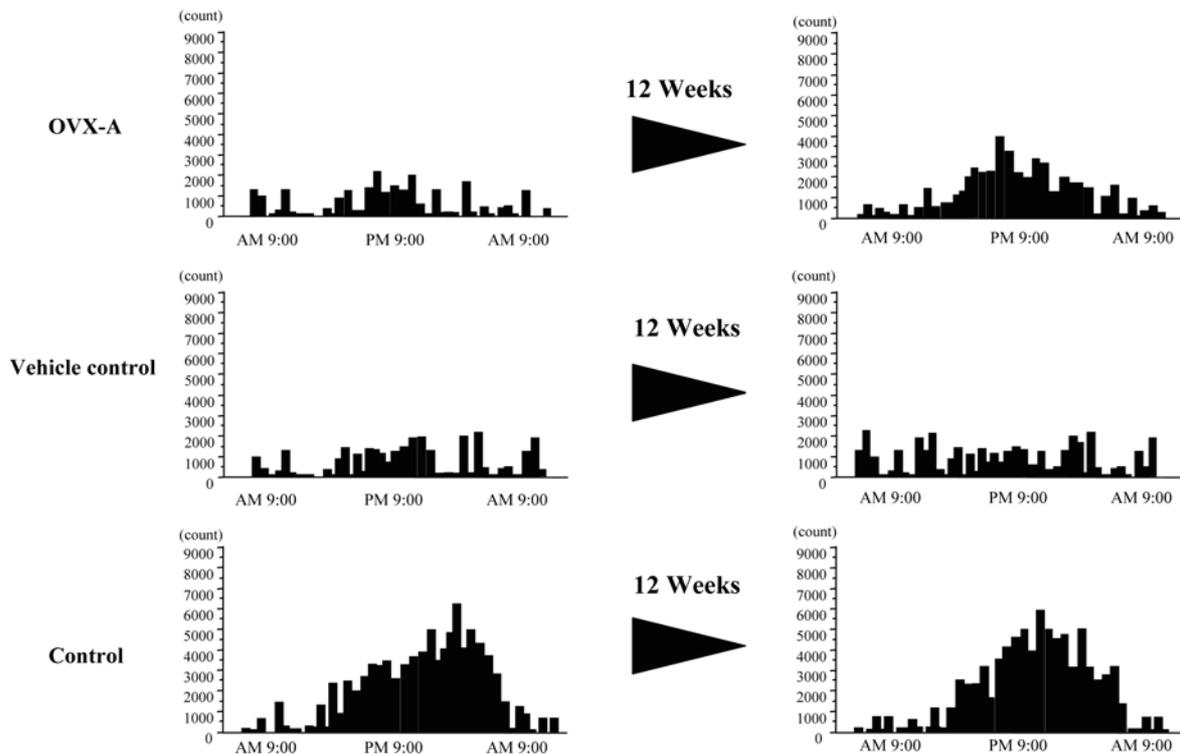


Fig. 2. Level of locomotor activity of the rats in the OVX-A, Vehicle control and Control groups before and after acupuncture stimulation for 12 weeks. The OVX-A and Vehicle control rats underwent ovariectomy (Typical case).

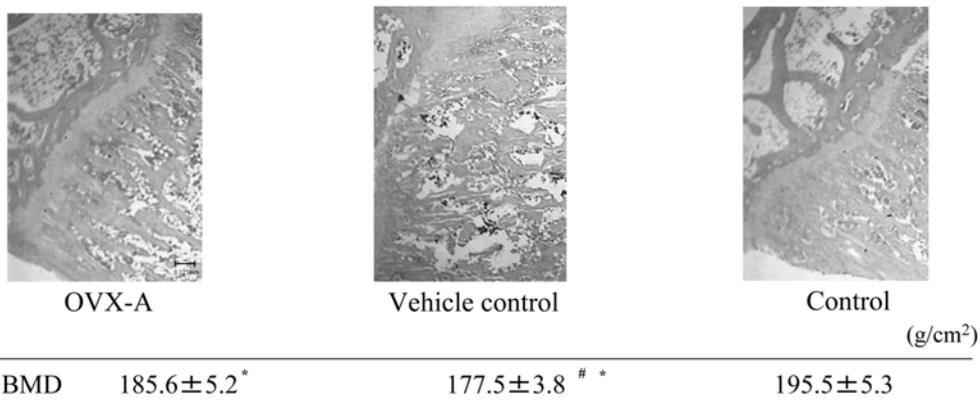


Fig. 3. Histological views of the femoral bones of OVX-A, Vehicle control and Control rats. * $P < 0.05$; significantly different between Control and OVX-A, Vehicle control. # $P < 0.05$; significantly different between OVX-A and Vehicle control.

DISCUSSION

Postmenopausal women with osteoporosis are frequently encountered by acupuncturists, but the effects of acupuncture on climacteric disturbance

symptoms have rarely been investigated (Ettinger *et al.*, 1987; Hidaka *et al.*, 1997). On the other hand, the OVX rat has been widely studied and used as a model of osteopenia and climacteric disturbance since the secretion of estrogens is reduced by

ovariectomy. Estrogen is known to induce the growth of reproductive organs, proliferation of mammary glands, facilitate Ca^{2+} deposition in bones, and reduce the serum T-chol level. Therefore, a variety of symptoms are induced in the state of estrogen deficiency (Bollen and Eyred, 1994).

In the present study, our finding that the serum level of T-chol was increased in the OVX-A and Vehicle control groups after ovariectomy may be taken as a sign of decreased secretion of estrogens (Bollen and Eyred, 1994). The urinary Dpd level is a good index that directly reflects bone resorption for the following reasons: Dpd is released in association with the decomposition of type I collagen during bone resorption; Dpd is not generated at the time of bone formation; the serum Dpd level is not affected by meals; and Dpd is excreted into the urine without being metabolized (Kanai, 1998; Kanai et al., 2000). Because the urinary Dpd level was increased in the two OVX groups compared with the Control group before acupuncture stimulation, it was confirmed that ovariectomy enhanced bone resorption.

Since the tail surface temperature of OVX rats clearly increased after acupuncture stimulation for 12 weeks, acupuncture stimulation may improve the peripheral circulation. Furthermore, using a metabolism measuring system, it was confirmed that acupuncture stimulation for 12 weeks increased the level of locomotor activity. It was reported in a clinical study that acupuncture stimulation therapy relieved the pain of patients with frozen shoulder and low back pain. Therefore, the increase in locomotor activity in our OVX-A rats was presumably ascribable to the removal of blood circulation rather than the induction of stress by acupuncture stimulation (Hayashi, 1998).

We previously reported that administration of oriental medicine to postmenopausal women improved their menopausal symptoms and inhibited the reduction in BMD (Suzuki et al., 1998). In the present study, after acupuncture stimulation (CV_4 treated patients with climacteric disturbance), the

pattern of irregular locomotor activity in the ovariectomized rats changed to a night-day diphasic pattern as was observed in the Control rats, and the overall locomotor activity in the OVX-A rats was significantly increased.

Physical therapy including therapeutic exercise of bone was reported to be very effective for osteoporosis. Briefly, since the dynamic exercise of bone is considered to improve the bone structure and increase BMD, physical therapy is generally preferable to drug therapy, which may cause side effects, for the treatment of osteoporosis in women. In Japanese individuals, it was confirmed by the quantitative ultrasound method that the BMD among people who habitually exercised was significantly higher than that among people who ate much nutritional food but did not exercise. This suggests that the increased BMD in the OVX-A rats that underwent acupuncture stimulation for 12 weeks, was partially ascribable to the increased locomotor activity.

However, the possibility that acupuncture stimulation directly affects the bone cannot be ruled out. Acupuncture stimulation may increase BMD to some extent. Therefore, we strongly recommend to provide acupuncture therapy in combination with Western medicine or exercise therapy for the treatment of osteoporosis.

REFERENCES

- Bollen AM, Eyred DR. (1994) Bone resorption rats in children monitored by the urinary assay of collagen type I cross-linked peptides. *Bone* **15**, 31-34.
- Dong H, Ludicke F, Comte I, Campana A, Graff P, Bischof P. (2000) An exploratory pilot study of acupuncture on the quality of life and reproductive hormone secretion in menopausal women. *J. Altern. Complement. Med.* **7**, 651-658.
- Eastell R, Colwell A, Hampton L, Reeve J. (1997) Biochemical markers of bone resorption compared with estimates of bone resorption from radiotracer kinetic studies in osteoporosis. *J. bone Miner. Res.* **12**, 59-65.

- Ettinger B, Genant HK, Cann CE. (1987) Postmenopausal bone loss is prevented by treatment with low-dosage estrogen with calcium. *Ann. Intern. Med.* **106**, 40-45.
- Harada K, Nakata Y. (1995) Effects of kampo therapy on serum estrogen levels and bone mineral content in climacteric disorder. *Jpn. J. Orient. Med.* 521-527.
- Hayashi Y. (1998) Physical training. *Jpn. J. Clin. Med.* **56**, 1551-1556.
- Hidaka S, Okamoto Y, Nakajima K, Suekawa M, Liu SY. (1997) Preventive effects of traditional Chinese (Kampo) medicines on experimental osteoporosis induced by ovariectomy in rats. *Calcif. Tissue Int.* **61**, 239-246.
- Jo ban *et al.* (2003) Acupuncture experimentable. *Acupunct. Moxibustion* **2**, 94-96.
- Kanai S, Susuki R, Taniguchi N, Okano H. (1998) Clinical study of adjuvant arthritis rats monitored by thermography. *Orthop. Traumatol.* **47**, 450-453.
- Kanai S, Taka A, Taniguchi N. (2000) Study of moxibustion for experimental arthritis model rat. *Orient. Med. Pain Clin.* **30**, 16-21.
- Kanai S. (1998) The effect of Kami-kihi-to on the maintenance of bone mass in patients with osteoporosis. *J. Orient. Med.* **49**, 9-66.
- Kitaichi K, Yamada K, Hasegawa T, Furukawa H, Nabeshima T. (1994) Effects of risperidone phencyclidine-induced Behaviors Comparison with Haloperidol and Ritanserin. *Jpn. J. Pharmacol.* **66**, 181-189.
- Kuo TC, Lin CW, Ho FM. (2004) The soreness and numbness effect of acupuncture on skin blood flow. *Am. J. Chin. Med.* **32**, 117-129.
- Sandberg M, Wijma K, Wyon Y, Nedstrand E, Hammar M. (2000) Effects of electro-acupuncture on psychological distress in postmenopausal women. *Complement. Ther. Med.* **10**, 161-169.
- Stener-Victorin E, Kobayashi R, Kurosawa M. (2004) Ovarian blood flow responses to electro-acupuncture stimulation at different frequencies and intensities in anaesthetized rats. *Reprod. Biol. Endocrinol.* **26**, 2-16.
- Suzuki Y, Uehara R, Ide M, Chikawa Y. (1998) Osteoporosis in rheumatoid arthritis. *J. Clin. Med.* **56**, 1598-1603.
- Villanueva AR, Mehr LA. (1977) Modifications of the Goldner and Gomori one step trichrome stain for Plastic-embedded thin sections of bone. *Am. J. Med. Technol.* **43**, 536-538.
- Yamaguchi K, Yashita T, Kato M. (1997) Pharmacological effects of Three products of Chinese herbal remedies on experimental osteoporosis induced by ovariectomy in rats. *Oyo Yakuri* **57**, 13-30.
- Zhang W, Kanehara M, Ishida T, Guo Y, Wang X, Li G, Zhang B, Kondo H, Tachi S. (2004) Preventive and therapeutic effects of acupuncture on bone mass in osteopenic ovariectomized rats. *Am. J. Chin. Med.* **32**, 427-443.
- Zhao Ying-xia, Yan Zhen-guo, Wang Cai Yuan. (2000) The effects of Acupuncture and moxibustion to BMD of rats after ovariectomy. *Orient. Med. Pain Clin.* **28**, 107-110.