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Therapeutic Effects of Safflower (*Carthamus tinctorius* L.) Seed Powder on Osteoporosis

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홍화 (*Carthamus tinctorius* L.)씨 분말의 골다공증 치료효과

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ABSTRACT

The therapeutic effect of safflower seed powder on experimental osteoporosis in the rat induced by ovariectomy was evaluated. Thirty Sprague Dawley rats were ovariectomized at the age of 12 weeks. Seven weeks postovariectomy, rats were divided into two groups: control and safflower seed powder treated group. Five animals from each group were sacrificed at the following time points: 1, 3, and 5 weeks. Scanning electron microscopic observation and morphometric analysis of the tibiae epiphysis showed that the administration of safflower seed powder significantly prevented reduction of cortical bone width and bone volume compared with the control group. In conclusion, safflower seed powder contains something that prevent bone loss due to estrogen deficiency, and was effective in preventing the osteoporotic decrease of bone mass.

Key words : Ovariectomy, Safflower seed powder, Morphometric analysis, Bone mass

INTRODUCTION

Osteoporosis has been defined as a group of conditions in which the mass and structure of the skeleton are altered in such a way that the risk of fracture is increased (Kulak & Bilezikian, 1998). Osteoporosis can be categorized according to etiology as follows: Primary osteoporosis refers to the condition of reduced bone mass and increased susceptibility to fracture found in

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postmenopausal women, or in older men and women, in which the osteoporotic process cannot be attributed to another identifiable disease. Secondary osteoporosis refers to bone loss resulting from specific, identifiable clinical disorders (Gennari et al., 1998).

There are three major components of an effective osteoporosis preventive strategy. The first is to ensure that optimal peak bone mass is achieved during childhood, adolescence and early adulthood. The second aspect to prevention is maintaining bone mass that has been acquired and the third is counteracting the process of age-related bone loss that occurs after 40-45 years of age (Kulak & Bilezikian, 1998). Treatment of osteoporosis include estrogen replacement, calcium and vitamin D supplementation, bisphosphonates, calcitonin, growth hormone, selective estrogen receptor modulators (Kenny & Prestwood, 2000; Miller, 2000; Wuster, 1998).

Safflower (*Carthamus tinctorius* L.), a thistle-like annual plant, was originally grown in the Middle East and south Asia for the red/orange pigment in the flower petals which was used for coloring rice and bread, and for dyeing cloth. After synthetic aniline dyes took over this market in the 1800's the crop was grown as an oilseed. Traditionally, the oil has mainly been sold in the health food market because it is unsaturated having high linoleic and oleic acid levels (Lee & Choi, 1998). With increased health consciousness in recent years, the oil quality has become a more general health issue to a large sector of our population.

According to previous studies, linoleic acid possess anti-inflammatory activity in bone by moderating prostanoid formation (Watkins & Seifert, 2000), correct bone loss due to ovariectomy (Schlemmer, 1999), lower cholesterol synthesis (Cox et al., 1998), alter biliary lipid secretion (Ohshima et al., 1996), increase intestinal calcium absorption (Claassen, 1995; Kruger, 1998), stimulate hematopoiesis (Young, 1987), has a protective and beneficial effect on cerebral ischemia (Kuang et al., 1983), prevent atherosclerosis (McCullagh, 1976), and according to our laboratory's previous experiment (Bae et al., 2001) safflower seed powder was effective in preventing osteoporotic process caused by bilateral ovariectomy in rat model.

The aim of this study was to evaluate the therapeutic effects of safflower seed powder on established osteoporosis.

MATERIALS AND METHODS

Thirty 12-week-old female Sprague-Dawley rats with a mean initial body weight of 230 g were used for the experiment (Dae Han Laboratory Animal Research Center, Seoul, Korea). The rats were fed a commercial diet containing 0.8% calcium and 0.4% phosphate. Animals were allowed free cage activity and food and water were supplied *ad libitum*. The rats were exposed to electric light from 9 a.m. to 9 p.m.

Rats were anesthetized intraperitoneally using ketamine hydrochloride (15 mg/100 g). Ovariectomies were performed using two incisions: one placed on each flank in a dorsolateral position to the rib cage. The fascia and muscle were separated and the ovary was secured by grasping the adipose tissue in which it was embedded. The ovary was ligated with a single suture and excised. This procedures was then repeated on the animal's other side (Bae, 1999).

Harvested safflower seeds were washed with water and dried at the air. Dried seeds were fried for fifteen minutes at 150~170°C and then pulverized into fine powder (Bae et al., 2001).

Seven weeks after ovariectomy, rats were divided into control (CON) and safflower seed powder treated group (SAF), and safflower seed powder was orally administered 0.3 g/day during the experimental period (Bae et al., 2001).

Rats were sacrificed at the following time points: 1, 3, and 5 weeks, under ketamine hydrochloride anesthesia (15 mg/100 g) by cardiac puncture. The tibiae were

carefully dissected free of soft tissue, then immediately fixed in 0.1 M cacodylate buffered 2.5% glutaraldehyde and 2% paraformaldehyde solution for 4 hours followed by decalcification in 10% nitric acid for 12 hours. Decalcified tibiae were postfixed in 1% osmium tetroxide for 2 hours then dehydrated in an ascending series of ethyl alcohol and dried using hexamethyldisilazane (HMDS) in air. Next, the tibiae epiphysis were affixed to scanning electron microscopy mounts, coated with gold inside a vacuum evaporator (IB-5, Eiko), examined in a scanning electron microscope (S-450, Hitachi) with an accelerating voltage of 20 kV and photographed (Bae, 1999).

To evaluate the bone loss rate, quantitative bone morphometric analysis was performed on the tibiae epiphysis from each rat. The printed images were captured by Sony XC-77 CCD camera using NIH-image freeware (developed at the U.S. National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The captured screens were saved as tiff file according to the method of Rasband & Bright (1995). Initially, scale was set as micrometer and segmentation of bone and empty space area was done with free drawing tools and measured.

Statistical differences at $p < 0.05$ were determined by student's t-test.

RESULTS

In the scanning electron microscopic examination, there was no significant differences in cancellous bone volume and cortical bone width of tibial epiphysis between safflower seed powder treated group and control group at one week (Fig. 1a, b) and three weeks (Fig. 2a, b). At five weeks, cancellous bone volume and cortical bone width of tibial epiphysis of control group were significantly decreased (Fig. 3b), but those of safflower seed powder treated group were remained relatively constant (Fig. 3a).

In the morphometric analysis, the tibial epiphysis bone volume in the ovariectomized control group was significantly decreased to about 63% of the safflower seed powder treated group level at five weeks ($p < 0.05$).

DISCUSSION

The etiology of human osteoporosis is multifactorial. Osteoporosis can result from such conditions as senility, postmenopause, calcium deficiency and immobilization as well as endocrinological and nutritional changes (Gennari et al., 1998). Increased bone loss in postmenopausal osteoporosis is suggested to result from estrogen deficiency (Ohta et al., 1992). Estrogen deficiency and calcium deficiency are reported to be additive factors in the genesis of osteoporosis in rats (Hodgkinson et al, 1978). Wronski et al. demonstrated by using histomophrmetric methods that the tibial metaphysis of ovariectomized rats had reduced bone volume and increased erosion and formation surfaces (Wronski et al., 1989). The experimental osteoporosis examined here was induced by bilateral ovariectomy (Bae, 1999; Bae et al., 1999, 2001; Bagi, 1992; Peng, 1997).

Previous studies have shown that safflower seed oil has high linoleic acid level (Lee & Choi, 1998), which possess anti-inflammatory activity in bone by moderating prostanoid formation (Watkins & Seifert, 2000), correct bone loss due to ovariectomy (Schlemmer et al., 1999), and increase intestinal calcium absorption (Claassen et al., 1995; Kruger et al., 1998).

According to our laboratory's previous study, safflower seed powder contains many minerals especially calcium, magnesium and potassium, and was effective in preventing the osteoporotic process caused by bilateral ovariectomy in rat model (Bae et al., 2001).

In this study, safflower seed powder effectively prevented reduction of cortical bone width and bone loss in the established osteoporotic rat resulting from estrogen deficiency. This result is consistent with that of Schlemmer et al., estrogen and essential fatty acid supple-

mentation corrected bone loss due to ovariectomy in the female Sprague Dawley rat (Schlemmer et al., 1999). The ovariectomized control group had significantly reduced cortical bone width and bone mass compared with the safflower seed powder treated group at 5 weeks.

In conclusion, the current study demonstrated that the safflower seed powder effectively inhibited bone loss associated with estrogen deficiency in rats.

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<국문초록>

홍화씨는 한방 및 민간에서 뼈에 우수한 작용이 있는 것으로 알려져 오랫동안 복용되어 왔다. 최근 고령화 사회를 맞이하면서 골다공증 문제는 사회적인 문제로 인식되고 있으며 저자들이 기존에 실시한 실험결과에 의하면 홍화씨 분말은 난소를 적출한 rat에서 골다공증의 예방효과를 나타내었다. 그러나 아직까지 골다공증이 유발된 rat에서 홍화씨 분말이 미치는 효과에 관한 실험적 자료가 제시된 것은 별로 없다. 이에 저자들은 홍화씨 분말이 골다공증의 치료에 미치는 효과를 알아보기 위하여 본 실험을 실시하였다.

실험동물은 체중 230 g의 12주령의 Sprague Dawley Rats를 사용하였으며, 양쪽 난소제거 후 7주부터 홍화씨 분말을 매일 0.3 g씩 복용시키면서 1, 3 및 5주 후에 경골을 채취하여 관찰하였다. 채취된 조직은 통상적인 주사전자현미경 시료제작법으로 고정과정을 거친 후 10% 질산으로 12시간 탈회하여 뼈의 단면을 노출시키고 탈수, 건조 및 금도금 과정을 거쳐 주사전자현미경(Hitachi, S 450)으로 관찰하여 촬영하였다.

관찰결과 대조군은 골수강에서 피질골까지의 두께의 감소와 골수강의 확장이 심하였으나, 홍화씨 분말을 투여한 실험군에서는 1주에서 5주까지 거의 같은 소견을 나타내었다.

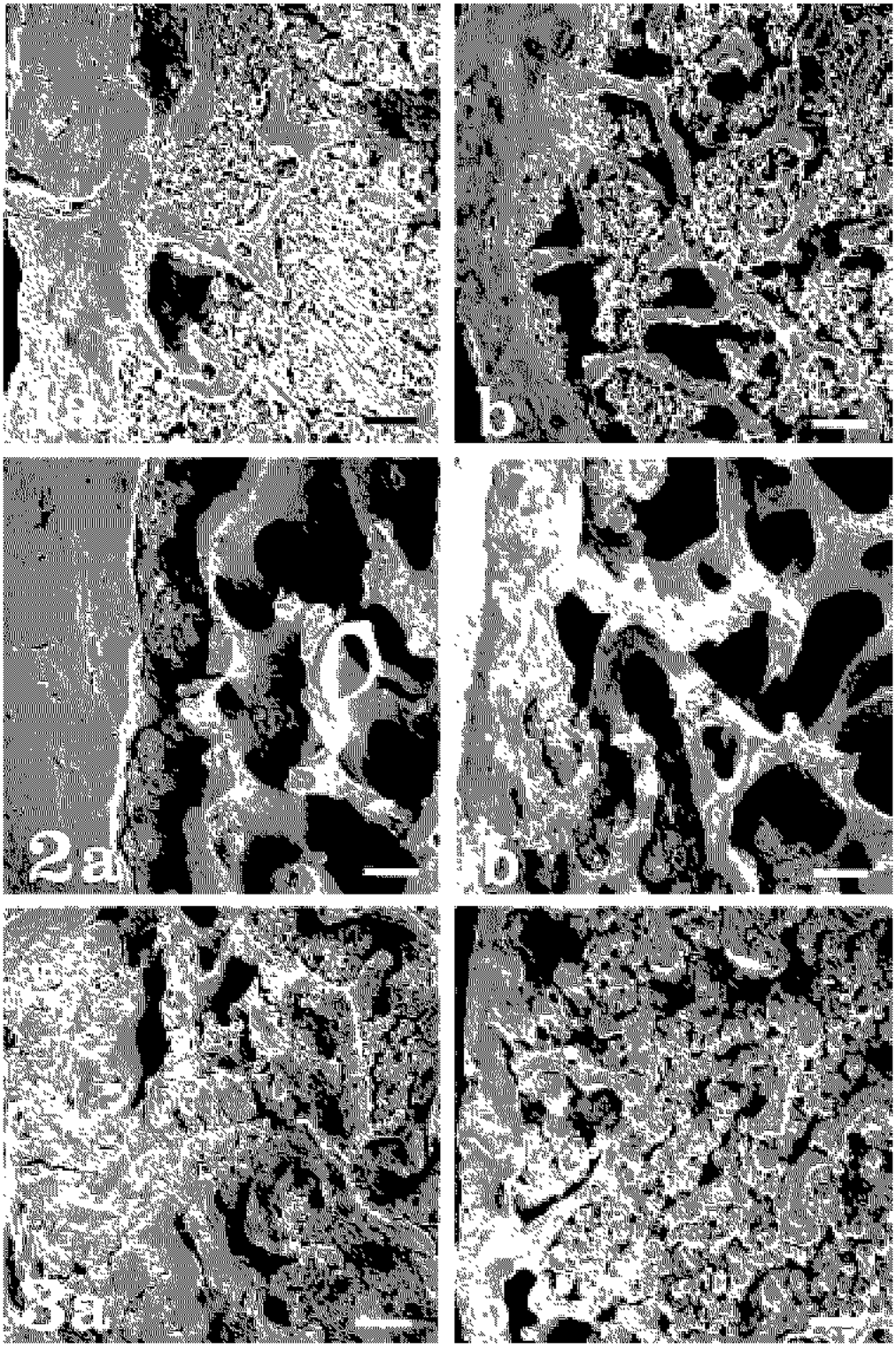
이상의 결과를 종합해보면 홍화씨 분말은 여성호르몬 결핍으로 인한 골다공증의 치료에 효과가 있는 것으로 사료된다.

FIGURE LEGENDS

Fig. 1. Scanning electron micrographs of epiphysis of rat tibia administered safflower seed (a) and control (b) at 1 week (scale bar = 110 μm).

Fig. 2. Scanning electron micrographs of epiphysis of rat tibia administered safflower seed (a) and control (b) at 3 weeks (scale bar = 110 μm).

Fig. 3. Scanning electron micrographs of epiphysis of rat tibia administered safflower seed (a) and control (b) at 5 weeks (scale bar = 110 μm).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