International Journal of the Physical Sciences Vol. 6(1), pp. 112-115, 4 January, 2011
Available online at http://www.academicjournals.org/IJPS
DOI: 10.5897/IJPS10.604
ISSN 1992 - 1950 ©2011 Academic Journals

Full Length Research Paper

Protective effect of *Morinda officinalis* polysaccharides on bone degeneration in the aged rats

Zong-Bao Wang[1], Qing-You Lu[2]*, Hai-Yan Lu[1], Wei-Ming Liao[3], Zhuo-Peng Wu[1], Guang-Zhi Kuang[1] and Hua-Jie Feng[1]

[1]Science Research Base of Kaiping Central Hospital, Postdoctoral Mobile Station of Sun Yat-sen University, Kaiping 529300, China.
[2]Department of Traumatic Surgery, Dongfang Hospital Affiliated to Tongji University, Shanghai 200120, China.
[3]Department of Orthopedic, First Hospital Affiliated to Tongji University, Guangzhou 510700, China.

Accepted 22 December, 2010

In the current study, we examined the effect of *Morinda officinalis* polysaccharides (MOP) on bone quality in aged rats. Rats were given orally by daily gavage at 200 and 400 mg/kg/day for 3 months, respectively. The results showed that there was a significant decrease in serum alkaline phosphatase (ALP), Interleukin 6 (IL-6) content and a significant increase in density and strength of femur and lumbar vertebrae in aged rats 3 months after *Morinda officinalis* polysaccharides treatment. In conclusion, these data suggest that *Morinda officinalis* polysaccharides can protect the age-induced bone degeneration.

Key words: *Morinda officinalis* polysaccharides, bone, osteoporosis, ALP.

INTRODUCTION

Osteoporosis is characterized by low bone mass and enhanced fragility of the bones, making the bones susceptible to fractures from minor trauma, and it is often associated with aging. Until recently, the focus of age-related bone loss has been on postmenopausal women mainly because women start loosing bone earlier than men and the bone loss proceeds more rapidly in women than in men (Ferrari, 2008; International Osteoporosis Foundation, 2006). In addition to perfusion, systemic metabolism and the influence of medication, age plays an important role as a biological factor. Animal experiments in rats as well as clinical studies in humans show a delayed course of bone healing with increasing age (Meyer et al., 2001; Skak and Jensen, 1988). As reasons for this, a reduced number of osteogenic stem cells, their reduced proliferation and differentiation potential, and reduced systemic or local blood flow have been discussed (Battmann et al., 1997; Bloomfield et al., 2002; Quarto, et al., 1995; Silbermann et al., 1987). Chinese traditional tonic herbal medicine BaJiTian (*Morinda officinalis*) has been extensively used in China for about two thousand years, for tonifying kidney, strengthening Yang-qi, relieving rheumatism and so on. It is used in Chinese medicine to nourish “Kidney-Yang” and strengthen the “Bone and Muscle” indicated for impotence, emission, enuresis, infertility, and to treat Cold and Dampness syndromes such as rheumatism, pain and fatigue (Zhang et al., 2002). Recently, studies on *M. officinalis* including chemical ingredients and relevant pharmacological properties have been performed. Iridoids isolated from *M. officinalis* have displayed obvious antinociceptive and anti-inflammatory effects (Choi et al., 2005), oligosaccharides have been shown to possess antidepressant effects and its anthraquinones have been isolated (Li et al., 2004). Polysaccharides have also been determined and recently were shown to have protective effect against bone loss (Wu et al., 2005). The objective of the present study was to evaluate the protective effect of *M. officinalis* polysaccharides on bone degeneration in the aged rats.

*Corresponding author E-mail: qingyou266@sina.com

MATERIALS AND METHODS

Materials

M. officinalis was obtained from Changsheng herb shop, China. The material (No. 2009-0004) was identified by Professor C.Y Wang, College of Pharmacy, Our University, China.

Extraction of *M. officinalis* polysaccharides

M. officinalis samples were grind into fine powder and defatted with ethanol for 10 h and extracted three times with boiling water (100°C), each time for 2 h. The extracts were then filtered and concentrated under reduced pressure. The crude polysaccharide fraction, termed MOP, was obtained by ethanol precipitation at the final concentrations of 80% of ethanol.

Animals and treatments

Thirty-two male Wistar rats were provided from our institute (Guangzhou city; China). They were kept in a temperature-controlled environment (20±1°C) with a 12:12-h light–dark cycle, and fed standard chow, for at least 1 week before any manipulation. Rats were kept and treated in accordance with the China regulations concerning animal care, in an approved environment.

Animals were divided into four group: Normal control group (NC) (aged 3 months, body weight 137±11 g), Model control group (MC) (aged 18 months, body weight 327±18 g), two MOP-treated group (aged 18 months). Each group included eight animals. In the two MOP-treated groups, *M. officinalis* polysaccharides was given orally by daily gavage at 200 and 400 mg/kg/day for 3 months, respectively, with eight animals per dose. In normal control group (NC), rats was given orally with equal volume of saline for 3 months. In the model control group (MC), rats was given orally with equal volume of saline for 3 months.

At the end of the experiment, all animals were anesthetized via an intraperitoneal injection of Ketamine/Xylazine (Ketamine: 100 mg/ml, 90 mg/kg; Xylazine: 20 mg/ml, 10 mg/kg), and then exsanguinated via cardiac puncture. Blood and tissue samples were removed for further analysis.

Serum biochemical parameters assay

Serum ALP was measured on an automatic analyzer (Ciba-Corning 550, USA) using a diagnostic reagent kit. Serum IL-6 was estimated using an ELISA kit according to the manufacturer's instructions.

Femur and lumbar vertebrae density measurements by peripheral quantitative computed tomography (pQCT)

Femur length was measured with digital calipers (Stoelting, Wood Dale, IL). Isolated femurs and lumbar vertebrae were measured for density using pQCT (Stratec XCT 960 M, Norland Medical Systems, Ft. Atkinson, WI). Femurs and lumbar vertebrae were assessed for cross-sectional and volumetric bone parameters using a multispecimen holder designed for the XCT 960 M. Analysis was carried out by a modification of the previously described method (Beamer et al., 1998). Voxel size was reduced to 0.07 mm for the analysis. The bone scans were analyzed with two different outer threshold settings to separate bone from soft tissue. An outer (and inner) threshold of 630 mg/cm^3 was used to determine bone areas and surfaces. These thresholds were selected to yield area values consistent with histomorphometrically derived values. To determine mineral content, a second analysis was carried out with an outer threshold setting of 230 mg/cm^3. This lower threshold was selected so that mineral from most partial voxels would be included in the analysis. Density values were calculated from the analyzed areas and associated mineral contents. Total volumetric density (vBMD) values were calculated by dividing the total mineral content by the total bone volume (bone and marrow).

Biomechanical strength testing of femurs and lumbar vertebraes

Biomechanical strength testing of right femurs and lumbar vertebrae was performed at the femur or lumbar vertebrae midpoint and femur or lumbar vertebrae neck as previously described (Ward and Fonseca, 2007). Femurs or lumbar vertebrae were soaked in physiological saline (9 g NaCl/L) for 4 h at room temperature prior to testing. Three-point bending at the femur or lumbar vertebrae midpoint and femur or lumbar vertebrae neck fracture was performed using a materials testing system and a specialized software program.

Statistical analyses

Data obtained were expressed as mean ± SD (n = 8). The significance of differences between groups were determined by unpaired student t test using the InStat Statistical software (GraphPad Software Inc, San Diego, Calif). Differences with $P < 0.05$ were considered statistically significant.

RESULTS

Compared with normal control rats, blood ALP and IL-6 levels in model control rats were markedly increased. There was a significant increase in the plasma ALP and IL-6 levels when the MOP was administered to rats (Table 1).

When compared with the normal control rats, femur and lumbar vertebrae density in model control group were significantly reduced. The MOP treatment for 3 months showed that femur and lumbar vertebrae density were significantly lower in the MOP-treated groups than those in model control group. Moreover, the levels increased with increasing dose of MOP (Figure 1).

Compared with normal control rats, maximum load (N) of femur and lumbar vertebrae in model control rats was markedly decreased. Three months after MOP (200 and 400 mg/kg body weight) was orally administered to rats, the maximum load (N) of femur and lumbar vertebrae was significantly enhanced in a dose-dependent manner, as shown in Figure 2.

DISCUSSION

M. officinalis have been widely used for thousands of years to treat fractures and joint diseases. The theory of Chinese medicine is to restore balances at all effective levels. Although the herbal medicines are considered as a cost-effective alternative by their traditional users, their international acceptance as an alternative therapeutic

Table 1. Effect of *Morinda officinalis* polysaccharides on blood ALP and IL-6 level.

Group	ALP (U/L)	IL-6 (pg/ml)
NC	146.33±7.31	19.78±1.09
MC	199.36±8.04 b	31.47±2.05 b
MOP I	180.84±8.34 d	26.04±1.35 c
MOP II	156.31±7.93 d	20.32±1.47 d

b $p<0.05$, compared with group NC; c $p<0.05$, d $p<0.01$, compared with group MC.

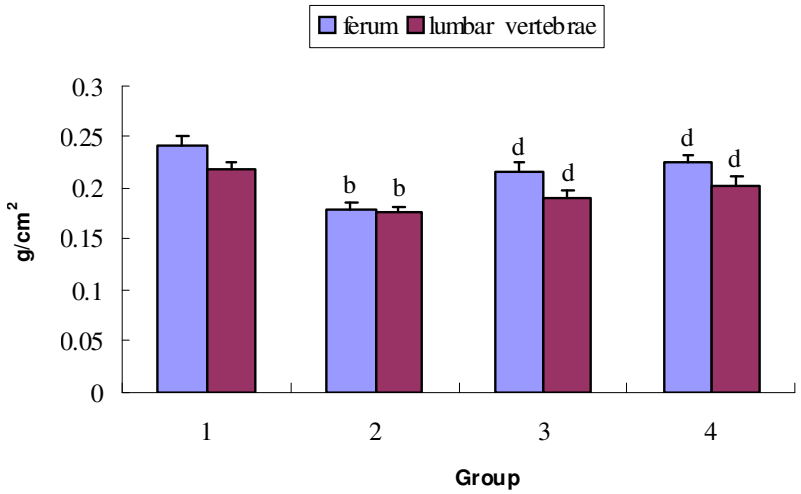

Figure 1. Effect of Morinda officinalis polysaccharides on femur and lumbar vertebrae density (g/cm^2). b $p<0.05$, compared with group NC; d $p<0.05$, compared with group MC.

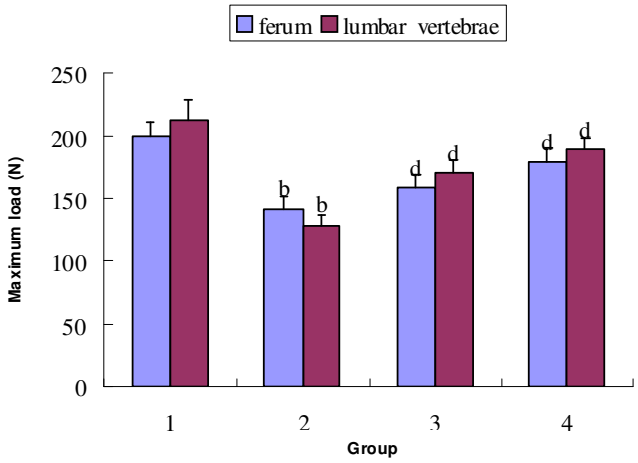

Figure 2. Effect of *Morinda officinalis* polysaccharides on maximum load (N) of femur and lumbar vertebrae. b $p<0.05$, compared with group NC; d $p<0.01$, compared with group MC.

regime for prevention and treatment of osteoporosis will require extensive research using modern science (Thoo et al., 2010).

The reduction of bone mass and predicted bone strength in aged rats was demonstrated in our study. The age-induced increase in bone resorption was confirmed by the increase in the serum level of ALP and IL-6. Alkaline phosphatase (ALP, ALKP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The normal range is 20 to 140 IU/L (Trumble et al., 2008).http://en.wikipedia.org/wiki/Alkaline_phosphatase High ALP levels can show that the bile ducts are blocked (Osathanon et al., 2009). Levels are significantly higher in children and pregnant women. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a by product of osteoblast activity (such as the case in Paget's disease of bone) (Withold et al., 1994). Our study revealed that aging appeared to stimulate the process of osteoclastogenesis in rats like increased serum level of ALP and IL-6. MOP treatment significantly reduced serum level of ALP and IL-6 in rats fed with MOP.

The significant weight increase in aged rats has been reported in other experimental studies. These have noted the increase in body weight as a mechanism to provide an additional stimulus for bone neoformation, serving as a partial protection against the osteopenia which occurs in long bones due to supporting the body weight (Kalu, 1991; Notomi et al., 2003). In the present study, our work confirmed that aging can reduce bone density and bone strength, which was in agreement with above-mentioned reports. Moreover, we demonstrated for the first time that the *M. officinalis* polysaccharides prevent the reduction of bone mass and bone strength induced in aged rats.

REFERENCES

Battmann A, Battmann A, Jundt G, Schulz A (1997). Endosteal human bone cells (EBC) show age-related activity in vitro, Exp Clin Endocrinol. Diabetes, 105: 98–102.

Beamer WG, Donahue LR, Rosen CJ, Baylink DJ (1998). Genetic variability in adult bone density among inbred strains of mice, Bone, 18: 397–403.

Bloomfield SA, Hogan HA, Delp MD (2002). Decreases in bone blood flow and bone material properties in aging Fischer-344 rats, Clin. Orthop. Relat. Res., 396: 248–257.

Choi J, Lee KT, Choi MY, Nam JH, Jung HJ, Park SK, Park HJ (2005). Antinociceptive anti-inflammatory effect of monotropein isolated from the root of *Morinda officinalis*. Biol. Pharm. Bull., 28: 1915–1918.

Ferrari S (2008). Human genetics of osteoporosis. Best Pract. Res. Clin. Endocrinol. Metab., 22: 723-735.

International Osteoporosis Foundation (2006). Preventing osteoporosis. Women's Health Med., 3: 155-156.

Kalu DN (1991). The ovariectomized rat model of postmenopausal bone loss, Bone Miner., 15: 175–191.

Li YF, Liu YQ, Yang M, Wang HL, Huang WC, Zhao YM, Luo ZP (2004). The cytoprotective effect of inulin-type hexasaccharide extracted from *Morinda officinalis* on PC12 cells against the lesion induced by corticosterone. Life Sci., 75: 1531–1538.

Meyer Jr RA, Tsahakis PJ, Martin DF, Banks DM, Harrow ME, Kiebzak GM (2001). Age and ovariectomy impair both the normalization of mechanical properties and the accretion of mineral by the fracture callus in rats, J. Orthop. Res., 19: 428–435.

Notomi T, Okimoto N, Okazaki Y, Nakamura T, Suzuki M (2003). Tower climbing exercise started 3 months after ovariectomy recovers bone strength of the femur and lumbar vertebrae in aged osteopenic rats, J. Bone Miner. Res., 18: 140–149.

Osathanon T, Giachelli CM, Somerman MJ (2009). Immobilization of alkaline phosphatase on microporous nanofibrous fibrin scaffolds for bone tissue engineering. Biomaterials, 30: 4513-4521.

Quarto R, Thomas D, Liang CT (1995). Bone progenitor cell deficits and the age-associated decline in bone repair capacity, Calcif. Tissue Int., 56: 123–129.

Silbermann M, Weiss A, Reznick AZ, Eilam Y, Szydel N, Gershon D (1987). Age-related trend for osteopenia in femurs of female C57BL/6 mice, Compr. Gerontol., 1: 45–51.

Skak SV, Jensen TT (1988). Femoral shaft fracture in 265 children. Log-normal correlation with age of speed of healing, Acta. Orthop. Scand., 59: 704–707.

Thoo YY, Ho SK, Liang JY, Ho CW, Tan CP (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia). Food Chem., 120: 290-295.

Trumble TN, Brown MP, Merritt KA, Billinghurst RC (2008). Joint dependent concentrations of bone alkaline phosphatase in serum and synovial fluids of horses with osteochondral injury: an analytical and clinical validation. Osteoarthris Cartilage, 16: 779-786.

Ward WE, Fonseca D (2007). Soy isoflavones and fatty acids: effects on bone tissue postovariectomy in mice, Mol. Nutr. Food Res., 51: 824–831.

Withold W, Degenhardt S, Castelli D, Heins M, Grabensee B (1994). Monitoring of osteoblast activity with an immunoradiometric assay for determination of bone alkaline phosphatase mass concentration in patients receiving renal transplants. Clin. Chim. Acta, 225: 137-146.

Wu YJ, Liu J, Wu YM, Liu LE, Zhang HQ (2005). Determination of polysaccharide from Chinese medicine morinda officinalis how and its trace elements analysis. Spectrosc. Spect. Anal., 25: 2076–2078.

Zhang ZQ, Yuan L, Yang M, Luo ZP, Zhao YM (2002). The effect of Morinda officinalis How, a Chinese traditional medicinal plant, on the DRL 72-s schedule in rats and the forced swimming test in mice. Pharmacol. Biochem. Behav., 72: 39-43.