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Effects of Fermented *Pueraria radix* by *Lactobacillus acidophilus* on Lipid and Bone Metabolism in Ovariectomized Rats

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ABSTRACT

This study was performed to determine the effects of fermented *Pueraria radix* on lipid and bone metabolism in ovariectomized (OVX) rats. Sprague-Dawley rats were divided into 4 groups: A sham operated control group, an OVX control group, an OVX group, treated *Pueraria radix* and OVX group treated fermented *Pueraria radix*. Rats were fed experimental diets with Ca and P free for 8 weeks after ovariectomy. The femur weight was significantly increased in the treatment groups, compared with that of the control group. Abdominal fat mass and serum alanine aminotransferase and aspartate aminotransferase activities and serum triglyceride, total cholesterol, LDL-cholesterol and osteocalcin levels in the treatment groups were significantly decreased compared with the control group. Serum estradiol level in the treatment groups was significantly increased compared with the control group. Bone mineral density of the femur in the fermented *Pueraria radix* treatment groups was significantly increased than the control group. Further, the result of histopathologic observation was shown in that the treatment of *Pueraria radix* and fermented *Pueraria radix* prevented trabecular loss. These results suggest that a *P. radix* powdered supplement as a source of isoflavones might ameliorate serum cholesterol and estradiol levels and protect against bone loss. Furthermore, fermented *P. radix* might be more effective than non-fermented *P. radix* and can be useful as a functional food.

Key words: *Pueraria radix*, *Lactobacillus acidophilus*, fermentation, ovariectomized rat

INTRODUCTION

Estrogen deficiency during menopause is the primary cause of osteoporosis or metabolic disorders including obesity and dyslipidemia (Babaei *et al.*, 2010; Cho *et al.*, 2012). Synthetic isoflavones such as estradiol-17β (E2), ipriflavone and raloxifene have been used to treat osteoporosis and menopausal symptoms (Wang *et al.*, 1995; Saloniemi *et al.*, 1995). They are believed to serve as an Estrogen Receptor (ER) beta agonists due to structural similarities with estrogens (Suetsugi *et al.*, 2003). However, synthetic chemicals have some adverse effects and various complications (Wang *et al.*, 1995; Suetsugi *et al.*, 2003).

The root of *Pueraria lobata* called kudzu (*Pueraria radix*), is a traditional medicine used for fever, hypertension, dyspepsia and liver injury in some Asian countries (Zeng *et al.*, 1982).

Furthermore, commercial kudzu beverages have been introduced in Korea as a functional food for their useful ingredients as well as dietary fiber based on scientific data. Several isoflavones such as daidzin, daidzein, genistin and genistein contained in soybean are also found in kudzu (Guerra *et al.*, 2000). However, kudzu has puerarin as the main isoflavone glycoside (8-C-glucoside of daidzein). Boue *et al.* (2003) reported that the estrogenic activity of isoflavones in kudzu is higher than that in soybean in a cellular proliferation assay. They also showed that isoflavones have strong binding activity to ER-alpha and beta rather than those of legume extracts of other types. Bebrevska *et al.* (2010) reported that oral administration of a kudzu extract at 500 mg kg^{-1} b.wt. (including 10% puerarin) to diabetic rats for 3 weeks restores the pro-oxidative/antioxidative balance in plasma without signs of toxicity. In fact, puerarin does not cause adverse effects, even when used in an intravenous drip because of its low toxicity (Wang *et al.*, 2006).

Isoflavone aglycones are hydrolyzed from glycosides, either by microbial β-glucosidase formed during fermentation or by native β-glucosidase action (Kaya *et al.*, 2008). Izumi *et al.* (2000) reported that the absorption rate of aglycones is higher than that of their corresponding glycosides due to high lipophilicity and small molecular size. Additionally, Okabe *et al.* (2011) reported that isoflavones from fermented soybean are absorbed faster than those of non-fermented soybean in postmenopausal women. In our previous study, the soy isoflavone aglycone was obtained in high yield through fermentation using *Lactobacillus acidophilus* (Hong *et al.*, 2012a). However, other microbes such as *Bacillus subtilis* do not have as effective fermentation ability compared to that of *L. acidophilus*. Furthermore, fermenting the pulp of soybean or black soybean is more effective for preventing osteoporosis than the non-fermented pulp in ovariectomized (OVX) rats (Hong *et al.*, 2009, 2012b). Moreover, it has been suggested that intestinal bacteria are involved in the bioconversion of puerarin to daidzein (Kim *et al.*, 1998). Cho *et al.* (2012) reported that a high dose of kudzu extract (500 mg day^{-1} kg^{-1} b.wt.) decreases plasma triglycerides and increases Bone Mineral Density (BMD) in OVX mice. However, little information is available on the effect of fermented kudzu on lipid and bone metabolism in OVX rats.

We hypothesized that kudzu fermented by *L. acidophilus* would be more effective than non-fermented kudzu for lowering serum lipid levels and inhibiting bone loss by estrogen deficiency in OVX rats.

MATERIALS AND METHODS
This study was carried out during the period, November 2011 to May 2012.

Bacteria and fermentation: Powdered *P. radix* (Korean cultivar) was purchased from an Oriental medicine market in Seoul, Korea. Puerarin, daidzin, daidzein, genistin and genistein standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Lactobacillus acidophilus (ATCC 4356) was obtained from the Korean Collection for Type Culture (Daejeon, Korea) and anaerobically cultured in MRS broth (Oxoid LTD., Basingstoke, Hampshire, UK) at 37°C for 24 h. The activated culture was inoculated into MRS broth at 37°C for 24 h. For fermentation, pre-activation media was inoculated into 1 L flasks with *P. radix* powder (5%, v/v) and incubated at 37°C with *L. acidophilus* for 48 h. The final cell concentration was 3.6×10^{-7} CFU mL^{-1}. After freeze-drying, the samples were stored at -80°C until the experiment.

Determination of aglycone and micronutrient contents: Aglycone contents were measured to calculate the amount to add to feed using liquid chromatography/tandem mass spectroscopy (LC/MS-MS). The LC/MS-MS analysis was performed with an Agilent 1200 Series G1367D

Table 1: Compositions of the experimental diets

Ingredient (g kg⁻¹ diet)	AIN-93M	KP[1]	F-KP[2]
Casein	140.000	139.990	140.000
Sucrose	100.000	100.000	100.000
Dextrose	155.000	155.000	155.000
Corn starch	465.692	463.992	465.192
Cellulose	50.000	49.770	49.930
Soybean oil	40.000	39.980	39.950
Pueraria radix	-	1.970	-
Fermented *Pueraria radix*	-	-	0.580
TBQH[3]	0.008	0.008	0.008
Minerals (Ca, P free)	35.000	35.000	35.000
Vitamin mix	10.000	10.000	10.000
L-Cysteine	1.800	1.800	1.800
Choline bitartrate	2.500	2.500	2.500

[1]*Pueraria radix* added to the AIN-93M diet without Ca or P. [2]Fermented *Pueraria radix* by *L. acidophilus* was added to the AIN-93M diet without Ca or P. [3]tert-Butylhydroquinone.

(Agilent Technologies, Palo Alto, CA, USA) autosampler combined with an Agilent 6410 Triple-Quadrupole using a modified method described by Holder *et al*. (1999). The High Performance Liquid Chromatography (HPLC) column was a Hypersil BDS-C18 (4.0×100 mm, 3 µm, Agilent Technologies), flow rate was 0.5 mL min⁻¹ and injection volume was 10 µL. The HPLC solvent A mobile phase was 0.1% formic acid and solvent B was acetonitrile set as an isocratic elution (0.1% formic acid acetonitrile, 65/35, v/v). Ionization was performed in the positive electrospray ionization mode and ion detection was conducted in multiple reaction monitoring mode. The nebulizing gas (N_2) temperature was 320°C, gas flow was 12 mL min⁻¹ and capillary voltage was 4000 V. Each isoflavone ion's optimum condition was determined using the Agilent optimizer program.

Protein, lipid, dietary fiber and ash contents in the *P. radix* powder and fermented *P. radix* powder were determined by AOAC (2005). Moisture was regarded as 0% because of freeze-drying. Carbohydrate was regarded as the remains excluding the aforementioned items. The micronutrient contents of the non-fermented kudzu powder were (%): Crude fat, 28.5; crude protein, 12.9; crude ash, 5.5; crude fiber, 14.3 and carbohydrate, 38.9. The micronutrient contents of the fermented kudzu powder were (%): Crude fat, 31.9; crude protein, 16.3; crude ash, 5.2; crude fiber, 11.2 and carbohydrate, 35.4.

Animals and diets: All animal experiments were conducted according to the guidelines provided by the Institutional Animal Care and Use Committee of Konkuk University. Twenty-four 8-week-old, sexually mature female Sprague-Dawley rats were purchased from the Central Lab. Animal Inc. (Seoul, Korea). Six rats were randomly assigned for laparotomy as Sham operated control (SHAM) and the remaining 18 rats for bilateral OVX. One week after surgery, the OVX rats were divided into three groups: Normal diet group (Con), normal diet plus non-fermented kudzu powder group (KP) and normal diet plus Fermented Kudzu Powder group (F-KP). All animals were housed individually in stainless cages environmentally controlled laboratory (23±2°C and 55±5% relative humidity, 12 h light/dark cycle).

The compositions of the experimental diets are shown in Table 1. All animals were fed Ca and P deficient purified rodent diet (Dyets, Bethlehem, PA, USA) with water *ad libitum*. The Con and SHAM groups were fed a purified control diet and the KP and F-KP groups were fed the same

control diet with lyophilized *P. radix* and fermented *P. radix* added at 1.97 and 0.58 g kg^{-1} diet, respectively, based on isoflavone aglycone concentration. The KP and F-KP groups were supplied the isoflavone aglycone at a rate of 0.5 mg kg^{-1} diet, which is similar to the level in soybean pulp (Hong *et al.*, 2009). Total isoflavone aglycone contents in *P. radix* and fermented *P. radix* were approximately 253 and 857 mg kg^{-1} powder, respectively. Food intake was recorded daily and body weight was measured weekly. At the end of the 8 week treatment period, the rats were fasted overnight and anesthetized under appropriate conditions. Blood was collected from the abdominal aorta and serum was separated by centrifugation (3000 rpm, 20 min). Liver, kidney, spleen and abdominal fat were immediately removed, weighed and stored at -80°C for further analysis. Muscle, adipose and ligament tissues were removed entirely from both sides of the femur, which were then weighed and fixed in 10% formalin before analysis.

Serum biochemical parameters: Total cholesterol, High-density Lipoprotein (HDL) cholesterol, Low-density Lipoprotein (LDL) cholesterol and triglyceride (TG) concentrations, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured by commercial kits (Biotoxtech Co., Chungwon, Korea).

Osteocalcin level was analyzed using a Rat Osteocalcin EIA Kit (Biomedical Technologies Inc., Stoughton, MA, USA) using the sandwich enzyme-linked immunosorbent assay (ELISA) method. Experiments were carried out in duplicate and results are presented as averages. Absorbance was measured by a microplate spectrophotometer (Biotek Inc., Winooski, VT, USA) at 450 nm within 15 min after adding the stock solution.

E2 concentrations were analyzed using a commercial ELISA kit (Calbiotech Inc., Spring Valley, CA, USA) at 450 nm. A five-stage E2 standard curve was prepared from 3-300 pg mL^{-1}. The analysis was performed in duplicate and the results are presented as averages.

BMD: To measure BMD due to OVX and treatment, a dual energy X-ray absorptiometry densitometer (Fisher Biomedical Inc., Venice, FL, USA) was used on the right femurs. Scan information was as follows: Resolution 1.0×1.0 mm, speed 20 mm sec^{-1}, width 11.4 cm, host/scanner 3.9.4/1.1.0 and analysis revision 3.9.4.

Histopathological analysis: The femurs were collected and fixed in 10% neutral buffered formalin solution for histopathological examination after the surrounding muscle and connective tissue were removed. After decalcification in a nitrogen solution, the femur was trimmed and processed for paraffin embedding and 2-3 μm sections of femur tissue were cut. Hematoxylin and eosin staining was performed and the slides were examined microscopically (Olympus BX43, Olympus Optical Co., Tokyo, Japan) and photographed with an Olympus DP70 (20x, Olympus Optical Co.).

Statistical analysis: Results are presented as Mean±Standard deviation and analyzed using SAS ver. 8.2. software (SAS Institute, Cary, NC, USA). Duncan's multiple range test was used to determine which means were significantly different at p<0.05.

RESULTS AND DISCUSSION
Isoflavone aglycone content: Kim *et al.* (1998) reported that intestinal bacteria are related to the bioconversion of puerarin to daidzein. In this study, the levels of puerarin, daidzin and genistin

Table 2: Body weight, feed efficiency and organ weights of rats fed for 8 weeks

Parameters	Con	SHAM	KP	F-KP
Initial body weight (g)	225±8[a]	207±9[b]	225±6[a]	225±7[a]
Final body weight (g)	335±17[a]	258±13[b]	334±20[a]	325±21[a]
Body weight gain (g)	111±17[a]	51±6[b]	109±19[a]	100±22[a]
Food intake (g/8 week)	1020±23[b]	982±20[c]	1063±13[a]	955±9[d]
Feed efficiency (%)	10.8±0.7[a]	5.2±0.2[b]	10.2±1.2[a]	10.5±0.7[a]
Kidney (g)	1.71±0.08[a]	1.52±0.16[b]	1.57±0.05[b]	1.55±0.05[b]
Spleen (g)	0.65±0.03[a]	0.49±0.06[c]	0.60±0.04[ab]	0.56±0.03[b]
Liver (g)	7.49±0.33[a]	6.94±0.70[ab]	7.12±0.27[ab]	6.75±0.39[b]
Femur (g)	1.75±0.09[c]	2.09±0.09[a]	1.96±0.02[b]	2.04±0.12[ab]
Abdominal fat (g)	19.6±2.9[a]	8.82±1.60[c]	17.2±1.8[ab]	14.9±2.7[b]

Con: Control, ovariectomized, SHAM: Sham-operated, KP: Ovariectomized, *Pueraria radix*, F-KP: Ovariectomized, fermented *P. radix*. Values are Mean±Standard deviation of six rats in each group for 8 weeks. Values with different superscripts within a row are significantly different (p<0.05), Food efficiency ratio was calculated as body weight gain per total food intake

decreased significantly and the levels of aglycones such as daidzein and genistein increased significantly during fermentation (p<0.05). Puerarin concentration decreased (p<0.05) from 7,252-6,514 mg kg^{-1} after 48 h. Daidzin concentration decreased sharply (p<0.05) from 1,253-222 mg kg^{-1} after fermentation. Genistin also decreased significantly from 121-19.8 mg kg^{-1} at 48 h of fermentation. Daidzein, which is an isoflavone aglycone of fermented kudzu powder, increased from 239.4-790.7 mg kg^{-1} at 48 h. Additionally, genistein content of the fermented kudzu powder increased from 14.1-67.1 mg kg^{-1} at 48 h of fermentation. The total isoflavone and its aglycone contents in non-kudzu powder were 8,878.6 and 253.4 mg kg^{-1}, respectively. During fermentation, total isoflavones in the fermented sample were 7,613.2 mg kg^{-1} and aglycone was 857.8 mg kg^{-1} at 48 h. According to these results, puerarin, the most abundant isoflavone in kudzu, was converted to daidzein during fermentation. Converted daidzein is metabolized to equol by gut microflora in the gastrointestinal tract (Atkinson *et al.*, 2005). As both daidzein and its metabolites are structurally similar to E2, they can bind to the ERs, specifically to ER-β (Kinjo *et al.*, 2004). As mentioned earlier, the isoflavone aglycone form is more effectively absorbed in the gastrointestinal tract than the glycoside form (Izumi *et al.*, 2000). Our previous study also showed similar results that fermented black soybean pulp (0.5 mg kg^{-1} diet) is more effective than non-fermented soybean on lipid and bone metabolism in OVX rats (Hong *et al.*, 2012b).

Body weight gain and food efficiency: Body weight gain and food efficiency were calculated during the 8-week treatment period beginning 2 weeks after OVX. Every group showed increased body weight gain compared to that in the SHAM group (Table 2). These results are similar to other reports recording weight gain after OVX in rats (Kalu *et al.*, 1994; Frolik *et al.*, 1996; Lee *et al.*, 2004). In addition, Okazaki *et al.* (2002) reported that estrogen promotes osteoblast differentiation; however, it inhibits adipocyte differentiation and prevents body weight gain.

The organ weights of the experimental animals are shown in Table 2. Kidney weight was significantly higher in the Con group than that in the SHAM and treatment groups (p<0.05). The elevated spleen weight in OVX rats was consistent with that of an enhanced post-surgical immune response. However, spleen weights in the F-KP group did not increase significantly. This result shows that the F-KP treatment inhibited the OVX-induced increase in spleen weight. This result was similar with daidzein preventing weight gain in the spleen following OVX in BALB/c mice

Table 3: Serum biochemical parameters of the rats

Serum biochemicals	Con	SHAM	KP	F-KP
AST (IU L^{-1})	177±30[a]	129±11[b]	140±10[b]	147±16[b]
ALT (IU L^{-1})	65.5±10.3[a]	45.0±3.0[b]	45.2±8.1[b]	43.3±7.1[b]
TC (mg dL^{-1})	91.4±5.0[a]	75.2±2.3[b]	79.6±8.2[b]	75.4±4.2[b]
TG (mg dL^{-1})	17.0±1.6[a]	12.4±2.3[b]	12.2±3.0[b]	10.8±1.5[b]
LDL-C (mg dL^{-1})	6.3±0.5[a]	3.0±0.6[c]	4.9±0.4[b]	5.1±0.5[b]
HDL-C (mg dL^{-1})	23.2±1.1	24.4±1.6	24.5±3.1	25.0±2.1
Atherogenic index[*]	2.5±0.1[a]	2.1±0.3[b]	2.1±0.2[b]	2.1±0.1[b]

Con: Control, ovariectomized, SHAM: Sham-operated, KP: Ovariectomized, *Pueraria radix*, F-KP: Ovariectomized, fermented *P. radix*. Values are the mean±standard deviation of six rats for 8 weeks in each group. Values with different superscripts within a row are significantly different (p<0.05), The atherogenic index was calculated by the Haglund method (1991) as (total cholesterol-HDL-cholesterol)/HDL-cholesterol

(Tyagi *et al.*, 2011). Femur weight was significantly lower in the Con group compared to that in the other groups (p<0.05), indicating that isoflavones in the fermented kudzu supplement had positive effects on preserving the femur. The amount of abdominal fat was significantly (p<0.05) higher in the Con group than that in the SHAM group. The F-KP group had significantly (p<0.05) less abdominal fat than that in the Con group and the KP group tended to be lower in abdominal fat. Babaei *et al.* (2010) reported that estrogen deficiency occurring after OVX increases body weight gain and white adipose tissue in rodents. An isoflavone supplement inhibits body fat accumulation in neutered male and female dogs by 85 and 27%, respectively (Pan, 2007). Genistein can reduce adipose tissue and body fat in ER knockout mice as an action of ER-α (Naaz *et al.*, 2003). In addition, genistein inhibits conversion of acetate and glucose to lipids and increases basal lipolysis (Szkudelska *et al.*, 2000). Therefore, this result indicates that the fermented kudzu supplement supplied isoflavone and inhibited fat accumulation in OVX rats.

Serum biochemical examination: The results of serum biochemical parameters are shown in Table 3. As AST and ALT are detected in blood by exocytic release when the liver is damaged, serum AST and ALT activities have been used as markers of liver damage. As shown in Table 3, serum AST and ALT activities in the SHAM, KP and F-KP groups were significantly (p<0.05) lower than those in the Con group. This result shows that the fermented kudzu powder had a protective effect on the liver. The Con group also had a significantly (p<0.05) higher level of total cholesterol than that in the SHAM group. However, the KP and F-KP groups were significantly (p<0.05) lower than that in the Con group. LDL-cholesterol levels in the KP and F-KP groups were significantly (p<0.05) higher than that in the SHAM group but they were significantly (p<0.05) lower than that in the Con group. HDL-cholesterol levels were not significantly different among the groups. Serum TG concentrations in all treatment groups were significantly (p<0.05) lower than that in the Con group. Estrogen prevents arteriosclerosis and cardiovascular disease by decreasing LDL-cholesterol and increasing HDL-cholesterol (Bush and Barrett-Connor, 1985). The atherogenic index of the KP and F-KP groups was lower than that in the Con group and it was the same in the F-KP group as that in the SHAM group. This result indicates that fermented kudzu has a positive effect on lipid metabolism in the blood. Taku *et al.* (2007) reported that isoflavones may ameliorate hypolipidemic menopausal disorders. Keung and Vallee (1993) reported that daidzin potently and selectively inhibits human liver mitochondrial aldehyde dehydrogenase related with alcoholic liver disease. In this study, *P. radix* and fermented *P. radix* had a protective effect on liver damage and reduced
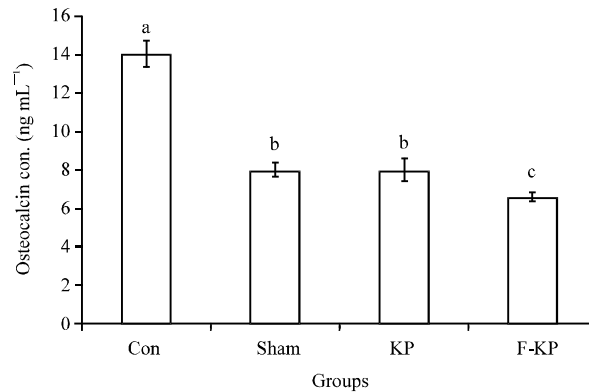
Fig. 1: Effect of isoflavone supplement derived Pueraria radix on serum osteocalcin concentration. Con (control, ovariectomized), Sham (sham-operated), KP (ovariectomized, pueria radix) and F-KP (ovariectomized, fermented pueria radix). Values with different superscripts are significantly different (p<0.05)

serum LDL-cholesterol and TG levels. This result was the same as a report that investigated isoflavone from *P. lobata*, which has various metabolic benefits in OVX mice, such as decreased serum TG level and no apparent liver toxicity (Cho *et al.*, 2012).

Serum osteocalcin level was measured as an indicator of bone formation. Serum ALP activity, usually used as a bone formation marker, has less sensitivity for fine bone changes because it is also synthesized in the liver and kidney (Lian and Gundberg, 1988). Osteocalcin, a small noncollagenous protein secreted by osteoblasts, contains gamma carboxyglutamic acid, the only molecule that can bind with calcium and exists specifically in bone dentin. Moreover, it combines with the bone extracellular matrix and some of these complexes can be isolated in the blood (Sitara *et al.*, 2004). Osteocalcin increases in serum during metabolic bone disease and bone reformation by increased osteoblast activity, as during rapid bone turnover (Son, 2006). In the results shown in Fig. 1, the osteocalcin levels in the Con group (14.0 ± 0.7 ng mL^{-1}) increased significantly (p<0.05) compared to those in the SHAM group (8.0 ± 0.4 ng mL^{-1}) for oophorectomy. The osteocalcin levels in the KP and F-KP groups were significantly (p<0.05) lower than those in the Con and SHAM groups. This was an apparent effect of the isoflavones in the KP and F-KP groups.

E2 is the main female sex hormone secreted by follicles and controls the female reproductive cycle. E2 is approximately 10 times more potent than estrone and approximately 80 times more potent than estriol in its estrogenic effects. E2 not only has a critical impact on reproductive and sexual functioning but also affects other organs, including bone. Serum E2 levels decreased rapidly after OVX. Figure 2 shows serum E2 levels after 8 weeks of treatment. Serum E2 levels decreased following OVX in the Con group. E2 levels in the KP and F-KP groups were significantly (p<0.05) higher than that in the Con group, which was similar to the SHAM data (6.19 ± 1.21 pg mL^{-1}). This result shows that kudzu had an ameliorative effect on menopausal symptoms caused by estrogen deficiency.

BMD: The BMD values for the right femurs of the rats are shown in Fig. 3. The BMD values in all OVX groups were significantly (p<0.05) lower than that in the SHAM group. The BMD value
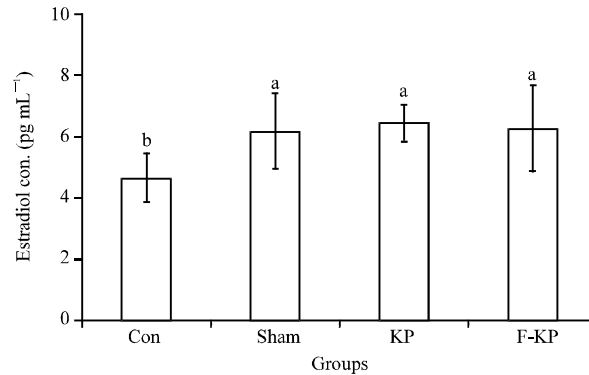
Fig. 2: Effect of isoflavone supplement derived Pueraria radix on serum estradiol (E2) concentration. Con (control, ovariectomized), SHAM (sham-operated), KP (ovariectomized, pueria radix) and F-KP (ovariectomized, fermented pueria radix). Values with different superscripts are significantly different (p<0.05)
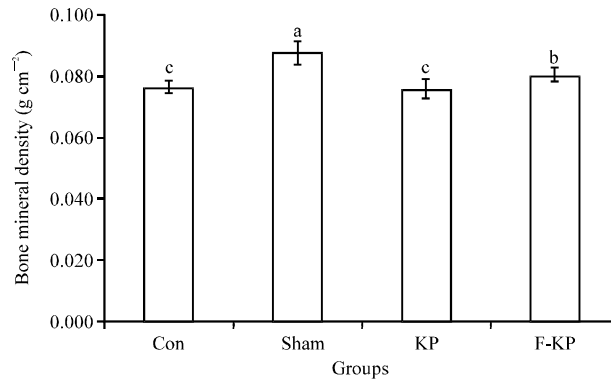

Fig. 3: Effects of isoflavone supplement derived Pueraria radix on bone mineral density. Con (control, ovariectomized), SHAM (sham-operated), KP (ovariectomized, pueria radix) and F-KP (ovariectomized, fermented pueria radix). Values with different superscripts are significantly different (p<0.05)

in the F-KP group was significantly (p<0.05) higher than those in the Con and KP groups. This result was similar with that of a study on fermented soybean pulp for preventing osteoporosis in OVX rats. Fermented soybean pulp increases BMD significantly compared to that in than OVX control or non-fermented soybean pulp (Hong *et al.*, 2009). In addition, many others have reported that isoflavone supplements have a recovery effect on osteoporosis model rats (Draper *et al.*, 1997). Estrogen deficiency in humans and other animals, including rats, causes a rapid enlargement in the rate of bone resorption and decreased bone formation, resulting in a declining BMD. The BMD value is an indirect indicator of fracture risk and osteopososis (Cummings *et al.*, 2002). The BMD value is a more factual method to diagnose osteoporosis than using biochemical markers (Jeon *et al.*, 2009). Our results indicate that a fermented kudzu supplement could prevent bone loss as a role of phytoestrogen.
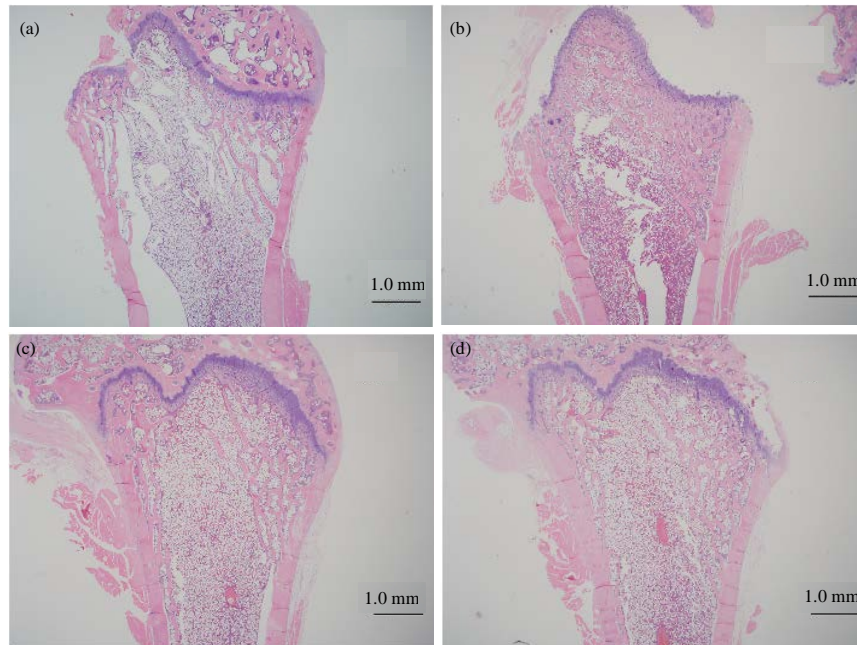
Fig. 4(a-d): Histopathological examination for femur in rats using H and E staining. Magnification is x 20. (a) Con (control, ovariectomized), (b) SHAM (sham-operated), (c) KP (ovariectomized, pueria radix) and (d) F-KP (ovariectomized, fermented pueria radix)

Histopathological examination: Results of the histopathological analysis are shown in Fig. 4. To determine the degree of osteoporosis, bone mass such as the trabecula of the femur was observed. Every group showed more decreased bone mass (trabecula) than that in the SHAM group; although the KP and F-KP groups definitely revealed suppressed bone mass loss compared to that in the Con group. It is very important to prevent osteoporosis because once the trabecula is perforated; it is difficult to recover to its former state (Cummings *et al.*, 2002). Moreover, it has a much greater effect on trabecular perforation to bone strength than trabecular thinning (Silva and Gibson, 1997). Cho *et al.* (2012) reported that *P. lobata* (Willd.) *ohwi* improves femur BMD without elevating plasma AST and ALT levels in OVX mice. Additionally puerarin inhibits the reduction in BMD and bone mineral content and improves femur trabecular bone structure in OVX rats (Wang *et al.*, 2012). Puerarin also stimulates osteoblast differentiation and bone formation through the ER (Wang *et al.*, 2012).

As shown in Fig. 4, the Con group trabecula nearly disappeared compared to that in the SHAM group. The other treatment groups had more trabecula in the femur compared to that in the Con group. Visible changes occurred in the femoral bone mass in rats treated with the kudzu powder. Although the rats were fed a small amount of isoflavone aglycone, among them, the increased puerarin during fermentation might be related with bone strength.

CONCLUSION

The effect of kudzu or fermented kudzu dietary supplements on lipid and bone metabolism was investigated in female OVX rats. Our results suggest that the kudzu and fermented kudzu powder

supplements as a source of isoflavone might ameliorate serum cholesterol and E2 levels and have a protective effect on bone by preventing bone loss. Furthermore, the results suggest that fermented kudzu power might be more effective than normal kudzu power due to the increase in the isoflavone aglycone through bioconversion. Therefore, our results suggest that kudzu might be a possible functional food. However, it is necessary to examine the effects of non-fermented and fermented kudzu powders on lipid metabolism in regular rats. Furthermore, we need to conduct total blood chemistry on these animals to show the safety of both the non-fermented and fermented kudzu powder in both groups of rats.

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