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Promotion Effects of Yeast Hydrolysates and a Mixture of Safflower Seed and *Gasiogapi* Extract on Longitudinal Bone, Proximal Epiphysis, and Growth Hormone in Rats

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Abstract

This study examined the growth effects of yeast hydrolysate (YH) and a traditional Korean herbal mixture (HM, a mixture of safflower seed and *gasiogapi* extract). Three-week old male SD rats were divided into the following five groups: negative control (saline), positive control (foremilk 0.5 g/kg/day), YH (YH 0.5 g/kg/day), HM (HM 0.2 g/kg/day), and YH+HM (YH 0.5 g/kg/day and HM 0.2 g/kg/day). Tibia bone length was 9.22 mm in the normal control rats, while both the YH and YH+HM groups had significantly longer tibia bones than the control rats (9.75 mm and 10.46 mm, respectively). The proximal epiphyses of YH, HM, and YH+HM measured 0.75, 0.70, and 0.75 mm, respectively, while the length in the control group was 0.50 mm. Plasma insulin growth factor-1 (IGF-1) level was slightly higher in the YH group (1.36 mg/mL) than in the control rats (1.29 mg/mL), but the difference was not significant. Plasma IGF-1 level was significantly increased in the HM (1.49 mg/mL) and YH+HM (1.53 mg/mL) groups compared to the control group (1.29 mg/mL). Growth hormone (GH) levels in YH (17.45 ng/mL), HM (15.49 ng/mL), and YH+HM (16.07 ng/mL) were significantly different compared to the control group (3.63 ng/mL).

Key words: yeast hydrolysate, herbal mixture, tibia, proximal epiphysis, growth hormone

INTRODUCTION

Herbal medicine has been widely used in clinical practice to for thousands of years treat growth-related diseases. It is the foremost alternative medical system available for growth therapy in the modern world, and will undoubtedly continue to be used as a cost-effective alternative therapy to commercial pharmaceutical products by traditional users in Asian countries, including China, Japan, and Korea (1). To promote potency, which is perceived to be caused by multiple factors, herbal formulations containing standard plant ingredients are employed (2). Multi-herb recipes are aimed at collectively exerting therapeutic actions and modulating the ingredients of the constituent herbs. The benefits of consuming an herbal mixture (HM) may be due to its synergistic effects (3). In formulating traditional HM recipes, special herb pairs, which are claimed to be unique combinations of traditionally defined medicinal herbal properties (4), are frequently used to achieve mutual enhancement, mutual assistance, mutual restraint, mutual suppression, or mutual antagonism (5).

In Korea, safflower seed extracts have traditionally been used for the treatment of blood stasis, the promotion of bone formation, and the prevention of osteoporosis (6). Safflower seeds might also have potential uses in drugs for bone regeneration (7), Ob mineralization (3), and regeneration of periodontal defects (8). In addition, safflower seeds have protective effects against bone loss caused by estrogen deficiency without a substantial effect on the uterus (9). Yang et al. (10) reported that a growth-stimulating material (GSM) containing *Eleutherococcus senticosus* promoted proliferation zones and IGF-1 mRNA expression in rat growth plates, IGF-1 mRNA expression in MG-63 osteoblasts and Hep-G2 hepatocytes, and growth of mouse tibia bones. Various herbal medicines have also been recommended for children, such as *Panax ginseng*, *Astragalus membranaceus*, *Atractylodes macrocephala*, *Glycyrrhiza glabra*, *Angelica sinensis*, *Poria cocos*, and *Eleutherococcus senticosus* (11).

Yeast, which is composed of many peptides and amino acids, is another dietary supplement that humans have used for growth therapy for a long time. The relationship between humans and yeast dates back many thousands of years. Yeast has various physiological functions. Yeast hydrolysate (YH), which is acquired by protein hydrolysis enzyme treatment, has shown effectiveness in reducing the emotional, physical, and behavioral symptoms of premenstrual syndrome (12). It has also dis-

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played physiological effects on reproductive function (13) and body fat reduction (14), as well as anti-stress and immunopotentiating activities (15). In our previous study, YH administered to rats showed a potent effect on bone growth (16).

Although HM and YH have been accepted for their efficacies on growth for a long time, very few studies have examined them. In this study, we evaluated whether YH and HM could improve longitudinal bone and proximal epiphysis growth, as well as the releasing properties of insulin like growth factor 1 (IGF-1) and growth hormone (GH) in male rats, in order to assess their possible uses as nutrition supplements with growth-promoting effects.

MATERIALS AND METHODS

Preparation of herbal mixture (HM)

Safflower seed (*Carthamus tinctorius*) and *gasiogapi* (*Eleutherococcus senticosus*) were purchased from a local commercial market (Kyungdong Herb-Market, Seoul, Korea). The air-dried raw materials (300 g) [safflower seed (150 g) and *gasiogapi* (150 g)] were extracted with water (2 L, repeated 3 times) in a percolator at 100°C for 4 hr. The extract was then filtered using a filter (200 mesh) and concentrated by a vacuum falling filter evaporator (Artisan 100SF, Machinery & Equipment Inc., San Francisco, CA, USA). The concentrate was dried by a spray-drier (Filtermat, GEA Process Engineering Inc., Columbia, MD, USA) using dextrin as the stabilizer.

Yeast hydrolysate (YH)

Saccharomyces cerevisiae IFO 2346 was incubated in medium containing 2% molasses, 0.6% $(NH_4)_2SO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.2% KH_2PO_4, 0.03% K_2HPO_4, and 0.1% NaCl for 3 days at 30°C. To acquire the cells, the culture was centrifuged at 10,000×*g* for 20 min. The cells were suspended in 20 mM phosphate buffer (pH 7.0) and were hydrolyzed with 1,000 units of bromelain at 30°C for 4 hr. Then, the hydrolysate was centrifuged at 10,000×*g* for 20 min to remove the residues. The supernatant was passed through a 30 kDa molecular weight cut-off membrane (Satocon Cassette; Sartorius, Gőttingen, Germany) and the filtrate was re-passed through a 10 kDa molecular weight cut-off membrane. The permeated solution was removed immediately, and the supernatant fractions containing the 10～30 kDa molecular weight peptides were lyophilized and used as the YH sample.

Animals and treatments

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee. A total of 36 3-week old male Sprague Dawley (SD) rats (Central Lab. Animal Inc., Seoul, Korea) were used. They were individually housed in plastic cages with grated stainless steel floors. The colony room was maintained at 24±1°C with 60% atmospheric humidity, and a 12-hr light/dark cycle. Before the experiment, the rats had *ad libitum* access to water and to a commercial diet (Samyang Co., Seoul, Korea) containing the following (g/kg of diet): moisture: 80, protein: 230, fat: 35, fiber: 50, carbohydrate: 600, and water. After an adaptation period, the rats were divided into 5 groups (6 rats/group): negative control (control) group: saline, positive control (P-control) group: foremilk 0.5 g/kg/day, YH group: YH 0.5 g/kg/day, HM group: HM 0.2 g/kg/day, YH+HM group: YH 0.5 g/kg/day and HM 0.2 g/kg/day. In preliminary experiments the administered doses were determined (data not shown). Each experimental material in saline was administered orally in the same volume every day for 3 weeks, respectively. Each group was fed the commercial diet *ad libitum* during the experiment.

Nutritional and biochemical analyses

Body weight and food intake were monitored every other day for 3 weeks. The food was removed for 12 hr at the end of the experimental term, and the rats were anesthetized with ethyl ether and dissected. Blood was collected from the heart with a heparinized syringe. The plasma was separated by centrifugation at 3,000×*g* for 15 min at 4°C and was then stored at -70°C until analysis. Plasma glucose, triacylglycerol (TG), total cholesterol (TC), and HDL cholesterol were measured using enzymatic kits (Wako Chemical Co., Osaka, Japan). LDL cholesterol levels were calculated according to the method of Friedewald et al. (17) as follows:

$$\text{LDL cholesterol} = \text{TC} - \text{HDL cholesterol} - (\text{TG}/5)$$

Measurements of tibial and proximal epiphysis lengths

Radiographs of tibia and proximal epiphysis lengths were obtained on day 0 before the experiment, and then obtained every week thereafter. After 3 weeks, the tibial and proximal epiphysis lengths were assessed radiographically on dorsoventral films. The vertical distance from the X-ray tube to the platform was always 25 cm. The radiographs were taken at 25 kV and 15 msec. Measurements were made directly on the radiographs using a microfilm reader (model 605-0070 837; NCR, Dayton, OH, USA). Total tibial length was measured as the long axis of the bone between the proximal articular line and the distal articular line, and proximal epiphysis length was defined by the rounded end of a long bone.

Measurements of IGF-1 and GH

At the end of 3 weeks, plasma IGF-1 was measured

using a mouse/rat IGF-1 kit (DSL, Webster, TX, USA) after acid ethanol extraction, according to the manufacturer's recommendations (18). The assay included quality controls provided by the manufacturer, and the standard curve of the assay was made in accordance to the manufacturer's provided samples. The average coefficient of variance was 5.96%, in keeping with the manufacturer's reported intra-assay variability (3.8～5.9 %). Plasma GH was determined by the enzyme-linked immunosorbent assay (ELISA) method using the protocol provided in the kit (Amersham Pharmacia Biotech., Little Chalfont, UK) and previously described (19). Each sample was assayed in duplicate.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS Inc., Chicago, IL, USA). The differences among groups were evaluated by one-way analysis of variance (ANOVA) and Duncan's multiple range tests. All data were two-sided at the 5% significance level and are reported as means±standard deviations (SD).

RESULTS

Body weight, food intake, and food efficiency ratio (FER)

The changes in body weight gain, food intake, and FER are shown in Tables 1 and 2. The dietary supplement groups (YH, HM, and YH+HM) tended to exhibit increased body weight gain compared to the control groups (control and P-control), but these differences were not significant. As shown in Table 2, the body weights of all rats were not statistically different until after 10 days of the experimental period. The rats given YH (111.9 g/day) gained significantly ($p<0.05$) more weight than the rats in the control group (100.3 g/day) after 14 days. Furthermore, the cumulative body weight gains of YH+HM (133.1 g/day) as well as the YH group (131.0 g/day) were significantly ($p<0.05$) higher compared to the control group (116.5 g/day) after 17 days, although a significant difference in body weight gain was not observed after 21 days.

There were no differences in amount of food intake among the 5 groups. FER was slightly higher in the rats administrated foremilk, YH, HM or YM and HM together than in the control rats administered saline, but these differences were not significant ($p>0.05$).

Relative organ weights and plasma lipids

Table 3 presents the relative internal organ and epididymal fat tissue weights. The rats in the experimental groups tended to exhibit decreased relative liver, spleen, kidney, and epididymal fat weights compared to the rats administered saline. In particular, relative liver weight was significantly ($p<0.05$) lower in the P-control group (3.5 g) than in the control group (5.3 g).

Plasma glucose and lipid levels are summarized in Table 4. YH and/or HM treatment had a tendency to reduce plasma glucose level, although there were no significant differences compared to the control group. Plasma cholesterol levels (TC, HDL, and LDL cholesterol) in rats administered foremilk, YH, HM or YH+HM did not differ from those observed in rats administered only saline. The P-control (32.4 mg/dL), YH (42.2 mg/

Table 1. Body weight gain, food intake, and food efficiency ratio (FER) of rats fed experimental diets for 3 weeks

Parameter	Control	P-control	YH	HM	YH+HM
Body weight gain (g/day)	5.2±2.2	6.0±0.6	6.4±0.5	6.0±0.6	6.4±0.3
Food intake (g/day)	20.2±2.4	19.3±2.7	19.9±2.4	21.1±4.0	20.4±2.8
FER	0.3±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0

FER: body weight gain/food intake. Values are the mean±SD for 6 rats. Means with different superscript letters within a row are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

Table 2. Changes in body weight of rats fed experimental diets for 3 weeks

Body weight (g/day)	Control	P-control	YH	HM	YH+HM
3 days	16.8±2.1	19.6±5.1	21.4±3.9	17.7±1.2	20.8±4.2
7 days	51.3±4.1	55.9±7.2	56.2±4.6	52.9±4.0	58.2±6.4
10 days	72.7±4.5	73.6±7.4	79.2±6.8	73.2±6.8	78.6±6.0
14 days	100.3±4.5^{a}	102.0±11.1^{ab}	111.9±8.4^{b}	96.8±8.8^{a}	105.5±6.4^{ab}
17 days	116.5±7.8^{a}	121.8±11.3^{ab}	131.0±6.7^{b}	123.7±10.8^{ab}	133.1±5.6^{b}
21 days	127.8±2.6	132.0±8.5	135.3±9.9	134.4±7.8	134.9±6.7

Values are the mean±SD for 6 rats. Means with different superscript letters within a row are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

Table 3. Relative liver, spleen, kidney, and epidymal fat weights of rats fed experimental diets for 3 weeks

Organ (g/100 g of BW)	Control	P-control	YH	HM	YH+HM
Liver	5.3±1.8[b]	3.5±0.2[a]	3.7±0.3[ab]	3.8±0.1[ab]	4.0±0.1[ab]
Kidney	1.2±0.4	0.9±0.0	0.9±0.0	0.9±0.1	0.9±0.0
Spleen	0.3±0.1	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0
Epididymal fat	0.9±0.2	0.8±0.1	0.8±0.2	0.9±0.2	0.8±0.0

Values are the mean±SD for 6 rats. Means with different superscript letters within a row are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

Table 4. Plasma glucose, triacylglycerol, and cholesterol level of rats fed experimental diets for 3 weeks

Parameter (mg/dL)	Control	P-control	YH	HM	YH+HM
Glucose	160.6±28.3	122.8±29.5	137.8±45.5	120.3±26.8	117.0±33.9
Total cholesterol	78.6±17.4	63.8±14.8	77.2±13.0	65.0±21.3	74.5±14.7
Triacylglycerol	57.4±6.4[b]	32.4±6.3[a]	43.2±13.7[a]	32.0±11.2[a]	36.5±8.9[a]
HDL-cholesterol	21.6±17.1	19.0±9.8	30.8±9.7	16.0±13.7	19.0±12.8
LDL-cholesterol	45.5±7.4	38.3±7.3	37.8±4.8	42.6±8.0	48.2±26.6

Values are the mean±SD for 6 rats. Means with different superscript letters within a row are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

dL), HM (32.0 mg/dL), and YH+HM (36.5 mg/dL) groups showed significant ($p<0.05$) decreases in plasma TG level compared to the control group (57.4 mg/dL).

Tibia and proximal epiphysis lengths

Fig. 1 shows the increases in tibia bone and proximal epiphysis lengths. The tibia bone length of the normal control rats was 9.22 mm, and both the YH (9.75 mm) and YH+HM (10.46 mm) groups had longer tibia bones than the control rats. However, the HM administered group (9.32 mm) was not significantly different than the control group.

The proximal epiphysis, also known as the growth plate, is the expanded articular end of a long bone that develops from a secondary ossification center, which, during the growth period, is either entirely cartilaginous or is separated from the shaft by a cartilaginous disk (20). The proximal epiphyses of the YH, HM, and YH+HM groups showed lengths of 0.75, 0.70 and 0.75 mm, respectively; however that of the control group was 0.50 mm. The YH, HM, and YH+HM groups showed significant differences for increased proximal epiphyses lengths compared to the control group ($p<0.05$). Overall, the results indicate that both the YH and HM treatments promoted proximal epiphysis and the YH promoted tibia bone growth in rats.

Plasma IGF-1 and growth hormone

Linear bone growth is mediated by a complex series of events that occur within the epiphyseal growth plate. Longitudinal bone growth is exquisitely sensitive to regulation by growth hormone (GH), which may produce alterations of several folds in adult stature. Insulin-like growth factor 1 (IGF-1, also known as somatomedin C) is an important mediator of GH's effects on longitudinal growth, although its mode of action remains controversial (21,22).

The plasma IGF-1 and GH concentrations of the treat-

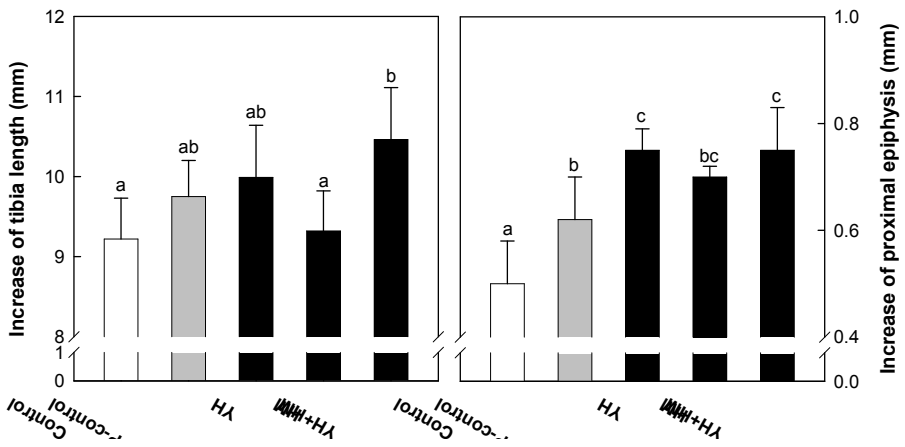

Fig. 1. Increases in tibia bone and proximal epiphysis lengths of rats fed experimental diets for 3 weeks. Values are the mean±SD for 6 rats. Means with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

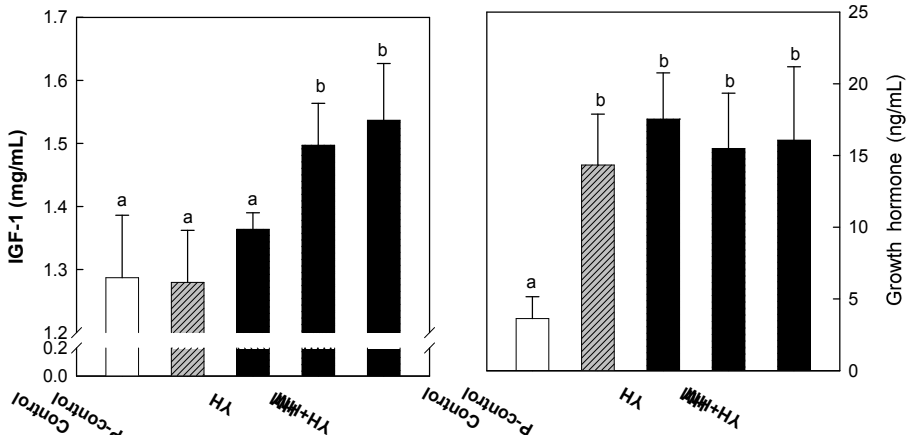

Fig. 2. Plasma insulin-like growth factor-1 (IGF-1) and growth hormone concentrations of rats fed experimental diets for 3 weeks. Values are the mean ±SD for 6 rats. Means with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple range tests. Control: saline, P-control: foremilk 0.5 g/kg/day, YH: YH 0.5 g/kg/day, HM: HM 0.2 g/kg/day, YH+HM: YH 0.5 g/kg/day and HM 0.2 g/kg/day.

ed groups are presented in Fig. 2. Plasma IGF-1 levels were slightly higher in the rats administered YH (1.36 mg/mL) than in the control rats (1.29 mg/mL), but the difference was not significant. The plasma IGF-1 levels of HM (1.49 mg/mL) and YH+HM (1.53 mg/mL) were significantly ($p<0.05$) increased compared to the control group (1.29 mg/mL). The GH levels of YH (17.45 ng/mL), HM (15.49 ng/mL), and YH+HM (16.07 ng/mL) were significantly different compared to the control group (3.63 ng/mL).

DISCUSSION

Traditional herbs have been used in the Korean population for the treatment of bone diseases and to promote bone growth for many years. In recent years, there have been many interests in natural or herbal mixtures, partly because of the recognition that medicine is not able to provide a panacea against human diseases and partly because the presence of unwanted side-effects. Interestingly, natural compounds contained in the herb mixture can act in a synergistic manner within the human body, and can provide therapeutic effectiveness with minimal or no unwanted side-effects (23).

In order to promote their potency, which is perceived to be caused by multiple factors, herbal formulations containing standard plant ingredients are employed. Despite the accepted efficacy of these remedies, few experimental studies have examined their clinical use as nutritional supplements for modifying bone mineral density in prepubescent children.

YH was tested both alone and in the presence of HM (a mixture of safflower seed and *gasiogapi* extract) to examine effects on bone growth in male rats in order to assess their possible uses as nutritional supplements for growth promoting effects. According to Table 2, there were no differences in amount of food intake or FER among the 5 groups. However, the YH, HM, and YH+HM groups showed significant decreases in plasma TG levels compared to the control group ($p<0.05$) (Table 4). Kim et al. (14) reported that the TG levels of a control and yeast hydrolysate group were lower compared to a high fat fed group. They concluded that yeast hydrolysate with a high protein concentration could positively influence plasma lipid levels in rats. A recent study evaluated the antioxidant activities of *Eleutherococcus senticosus* in rats (3,7). It seems that partial improvements in serum lipid profiles would likely occur via roles played by the herb's functional components (8). The present study shows that the study animals had significant decreases in serum LDL levels and LDL/HDL ratios after *Eleutherococcus senticosus* supplementation. Hepatic TG content was also significantly lowered by the supplementation of safflower seed ethanol extract and safflower seed water extract compared to the control group. However, safflower seed water extract supplementation was more potent than safflower seed powder in lowering hepatic cholesterol and TG levels (safflower seed extract lowers plasma and hepatic lipids in rats fed a high-cholesterol diet). The supplementation of safflower seed extracts prepared with ethanol or water resulted in lower plasma and hepatic total-C, plasma TG, and atherogenic index values in high-cholesterol fed rats with a simultaneous increase in hepatic HMG-CoA reductase activity. More studies are needed to explain the decrease in TG content by HY and/or HM.

Several clinical studies have examined the effects of traditional HM for growth (24,25). These studies show that some HMs improve growth rate after several months of treatment as compared to standard growth rates. In an animal study, it was reported that an HM increased GH levels and bone mineral density in pigs (26). Leem et al. (27) reported that an HM increased the rate of bone formation in the tibia and proximal epiphysis lengths in rats during the developmental period. Lee et al. (3) studied the efficacy of an HM nutritional supple-

ment on height and bone mineral density development in prepubescent children. The results revealed that the HM enhanced height and the rate of bone mineral density development in both girls and boys compared to standard rates. In addition, we previously demonstrated that an HM showed potential growth promoting capacity in rats, including longitudinal bone growth and GH releasing properties (11). The results of this study are consistent with the growth effects demonstrated in previous studies of HM treatment.

The HM components in the present study were safflower seed (*Carthamus tinctorius*) and *gasiogapi* (*Eleutherococcus senticosus*). In Korea, safflower seed extracts have traditionally been used for the treatment of blood stasis, the promotion of bone formation, and the prevention of osteoporosis (6). Safflower seeds might also have potential uses in drugs for bone regeneration (7), osteoblast mineralization (3), and regeneration of periodontal defects (8). Furthermore, Lee et al. (28) reported that rats selected for 8 weeks of oral treatment of safflower seed methanolic extract had significantly increased osteoblast markers in serum such as osteocalcin content (4～8 weeks), bone-specific alkaline phosphatase activity (1～2 weeks), and IGF-I levels (1 week), along with increases in growth parameters such as femur (2～8 weeks) and tibia (4 weeks) lengths. They concluded that the effect of safflower seeds on bone formation appears to be mediated by IGF-I at the early stage of treatment (28).

Some researchers have reported the active substances and pharmacological effects of *Eleutherococcus senticosus* (29). Nishibe et al. (30) reported that the stem bark of *Eleutherococcus senticosus* prolonged swimming time of rats in a forced swimming test. Furthermore, it was reported that the root bark and stem bark of *Eleutherococcus senticosus* possess immuno-stimulating action (31). Lupu et al. (22) reported the effects of a growth-stimulating material containing *Eleutherococcus senticosus* on longitudinal bone growth. The material significantly increased the proliferative zone of the growth plate of the proximal tibia, and IGF-1 mRNA expression in the growth plate was also increased. They concluded that *Eleutherococcus senticosus* had activity toward bone growth promotion by increasing the expression of IGF-1, a major bone growth factor.

Bowers et al. (32) reported that small synthetic peptides selectively stimulated GH secretion at the pituitary and hypothalamic levels. Then, following their administration in rats, pigs, and humans, these GH releasing peptides were found to be very potent, selective, and efficacious GH secretagogues (33,34). When parenterally administered, GH secretagogues (His-D-Try-Ala-Trp-D-Phe-Lys-NH_2) are highly potent in several animal species, including humans (35,36). YH is produced from *Saccharomyces cerevisiae* and is considered a generally recognized as safe material (12). YH may be a type of peptide mixture, which is composed of many peptides and amino acids. Similar to the abovementioned reports, in our previous study (16) and present study, YH accelerated longitudinal bone and proximal epiphysis lengths, and also stimulated GH secretion in adolescent rats.

In conclusion, these studies show the significant beneficial effects of singly used YH or HM on proximal epiphysis and GH releasing properties. Nutrition supplements containing YH and/or HM are thought to be useful for promoting growth. However, singly used YH did not impart an increase in IGF-releasing properties, and singly used HM did not cause an increase in tibia length. In the case of YH + HM, significant beneficial effects were found for all tested aspects. Therefore, we surmise that growth promotion through combinations is the result of synergistic effects. However, these data indicate that vigorous efforts to isolate the active agent or agents should be undertaken along with testing of this preparation in human studies.

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REFERENCES

1. Xu M, Dick IM, Day R, Randall D, Prince RL. 2003. Effects of a herbal extract on the bone density, strength and markers of bone turnover of mature ovariectomized rats. *Am J Chinese Med* 31: 87-101.
2. Lee MS, Park KW, Park JS, Kim HJ, Moon SR. 2005. Effects of nutritional supplement with herbal extract on bone mineral density and height in prepubescent children-a preliminary study. *Phytother Res* 19: 810-811.
3. Lee S, Choi H, Sun K, Song J, Pi S, You H, Shin H. 2005. A study of safflower seed extracts on bone formation *in vitro*. *J Korean Acad Periodontol* 35: 461-474.
4. Seog HM, Jung CH, Kim YS, Park HS. 2005. Phenolic acids and antioxidant activities of wild ginseng (*Panax ginseng* C. A. Meyer) leaves. *Food Sci Biotechnol* 14: 371-374.
5. Chan K. 1995. Progress in traditional Chinese medicine. *Trends Pharmacol Sci* 16: 182-187.
6. Huh J, Kang J, Yoo Y, Kim C, Cho K, Choi S. 2001. The effect of safflower seed fraction extract on periodontal ligament fibroblast and MC3T3-E1 cell in vitro. *J Korean Acad Periodontol* 31: 833-846.
7. Seo J, Kim T, Pi S, Yun G, You H, Shin H. 2000. Effects of safflower seed extracts and bovine bone on regeneration of bone defects in mongrel dogs. *J Korean Acad Periodontol* 30: 553-567.

8. You KT, Choi KS, Yun GY, Kim EC, You HK, Shin HS. 2000. Healing after implantation of bone substitutes and safflower seeds feeding in rat calvarial defects. *J Kor Acad Periodontol* 30: 91-104.
9. Kim HJ, Bae YC, Park RW, Choi SW, Cho SH, Choi YS, Lee WJ. 2002. Bone-protecting effect of safflower seeds in ovariectomized rats. *Calcified Tissue International* 71: 88-94.
10. Yang DS, Cha MH, Kang BJ, Oh SW, Kim YE, Yoon YS. 2003. A study on the longitudinal bone growth of growth-stimulating material with *Eleutherococcus senticosus*. *Korean J Food Sci Technol* 35: 702-707.
11. Park SS, Oh SH, Bae SH, Kim JM, Chang UJ, Park JM, Kim JM, Suh HJ. 2007. An herbal medicine mixture (HM-10) induces longitudinal bone growth and growth hormone release in rats. *Food Sci Biotechnol* 16: 1046-1050.
12. Yu KW, Kim JM, Oh SH, Chang UJ, Suh HJ. 2002. Physiological effects of yeast hyrdrolysate SCP-20. *Food Res Int* 35: 879-884.
13. Hong J, Kim I, Lee H, Kwon O, Min B, Lee W, Shon K. 2004. The effects of yeast hydrolysate SCP-20 on reproductive function in male mice. *J Korean Soc Food Sci Nutr* 33: 451-454.
14. Kim KM, Chang UJ, Kang DH, Kim JM, Choi YM, Suh HJ. 2004. Yeast hydrolysate reduces body fat of dietary obese rats. *Phytother Res* 18: 950-953.
15. Yu KW, Oh SH, Choi YS, Hwang WJ, Suh HJ. 2001. The reduction effect of yeast hydrolysate SCP-20 on premenstrual syndrome. *J Korean Soc Food Sci Nutr* 30: 1000-1003.
16. Kim JM, Kim SY, Jung EY, Bae SH, Suh HJ. 2009. Yeast hydrolysate induces longitudinal bone growth and growth hormone release in rats. *Phytother Res* 23: 731-736.
17. Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin Chem* 18: 499-502.
18. Lee PDK, Powel D, Baker B, Liu F, Mathew G, Levitsky I, Gutierrez OD, Hintz RL. 1992. Characterization of a direct, nonextraction immunoradiometric assay for free IGF-I. *Endocrinology* 131: 3051-3060.
19. Johansen PB, Nowak J, Skjaerbaek C, Flyvbjerg A, Andreassen TT, Wilken M, Orskov H. 1999. Ipamorelin, a new growth-hormone-releasing peptide, induces longitudinal bone growth in rats. *Growth Horm IGF Res* 9: 106-113.
20. Sokolowska-Pituchowa J, Goszczynska I, Goszczynski M. 1969. Appearance of ossification centers in the proximal epiphysis of both bones of the forearm in the radiological picture and studies on the influence of environment on the rate of maturation of the skeleton of the elbow joint. *Folia Morphol (Warsz)* 28: 45-53.
21. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. 2002. Circulating levels of IGF-1 directly regulate bone growth and density. *J Clin Invest* 110: 771-781.
22. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. 2001. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol* 229: 141-162.
23. Huie CW. 2002. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Anal Bioanal Chem* 373: 23-30.
24. Jeong H, Lee H, Lee J, Kim D. 2001. Clinical study of effect to the height-growth after the administration of Boyangsungjangtang to the prepuberty children. *J Korean Oriental Pediatr* 15: 47-57.
25. Na D. 1999. A clinical inquiry into 200 cases of children coming to the clinic due to the symptoms of growth deficiency. *J Oriental Med Daejun Univ* 7: 609-620.
26. Park K, Han Y, Park K. 2000. Effect of herb-mix supplementation on the growth performance and serum growth hormone in weaned pigs. *Asian-Australas J Anim Sci* 13: 791-794.
27. Leem K, Park SY, Lee DH, Boo YM, Cho KH, Lim J, Jeon H, Park HJ, Chung JH, Kim H. 2003. Effects of Jaoga-Yukmiwon(R), a Korean herbal medicine, on chondrocyte proliferation and longitudinal bone growth in adolescent male rats. *Phytother Res* 17: 1113-1116.
28. Lee YS, Choi CW, Kim JJ, Ganapathi A, Udayakumar R, Kim SC. 2009. Determination of mineral content in methanolic safflower (*Carthamus tinctorius* L.) seed extract and its effect on osteoblast markers. *Int J Mol Sci* 10: 292-305.
29. Davydov M, Krikorian AD. 2000. *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look. *J Ethnopharmacol* 72: 345-393.
30. Nishibe S, Kinoshita H, Takeda H, Okano G. 1990. Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. *Chem Pharm Bull (Tokyo)* 38: 1763-1765.
31. Wagner H, Proksch A, Riess-Maurer I, Vollmar A, Odenthal S, Stuppner H, Jurcic K, Le Turdu M, Fang JN. 1985. Immunostimulating action of polysaccharides (heteroglycans) from higher plants. *Arzneimittelforschung* 35: 1069-1075.
32. Bowers CY, Sartor AO, Reynolds GA, Badger TM. 1991. On the actions of the growth hormone-releasing hexapeptide, GHRP. *Endocrinology* 128: 2027-2035.
33. Chan CB, Fung CK, Fung W, Tse MC, Cheng CH. 2004. Stimulation of growth hormone secretion from seabream pituitary cells in primary culture by growth hormone secretagogues is independent of growth hormone transcription. *Comp Biochem Physiol C Toxicol Pharmacol* 139: 77-85.
34. Raun K, Hansen BS, Johansen NL, Thogersen H, Madsen K, Ankersen M, Andersen PH. 1998. Ipamorelin, the first selective growth hormone secretagogue. *Eur J Endocrinol* 139: 552-561.
35. Jaffe CA, Ho PJ, Demottfriberg R, Bowers CY, Barkan AL. 1993. Effects of a prolonged growth-hormone (Gh)-releasing peptide infusion on pulsatile Gh secretion in normal men. *J Clin Endocr Metab* 77: 1641-1647.
36. Nelson AH, Walker RF, Codd EE, Barone FC. 1991. Intranasal activity of the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in conscious dogs. *Life Sci* 48: 2283-2288.

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