Gelatinized and Fermented Powders of *Lepidium meyenii* (Maca) Improve Physical Stamina and Epididymal Sperm Counts in Male Mice

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ABSTRACT

Lepidium meyenii, known as Maca, is traditionally employed in the Andean region for its supposed properties to improve energy and fertility. In the present study, we investigated the effects of gelatinized and fermented Maca on improvement of physical stamina and epididymal sperm counts, and on blood biochemical parameters related to fatigue and tissue injury: creatine phosphokinase, aspartate transaminase, lactate dehydrogenase, blood urea nitrogen, glucose, total cholesterol and total proteins. Adult male mice was divided at random into two main groups (resting and exercise groups). The excercise group was separated into three subgroups (exercise only, exercise with gelatinized Maca and fermented Maca-treatment groups). Gelatinized or fermented Maca (800 mg/kg) were orally administered for 30 days. All animals in exercise groups were subjected to daily 30-min swimming for 28 days 30 min after Maca treatment. Daily exercise decreased the body weight gain, and fermented Maca further attenuated the body weight increase. Gelatinized and fermented Maca significantly increased the maximum swimming time on 14 and 28 days of treatment ($p<0.05$), respectively, suggestive of a long-term stamina-enhancing effect of fermented Maca. Both Maca fully or significantly recovered blood parameters of energy as well as muscular and hepatocytic injuries changed by repeated exercise and maximum swimming performance ($p<0.01$). Moreover, gelatinized and fermented Maca increased epididymal sperm counts 22.0% and 32.0%, respectively. In conclusion, the results indicate potential benefits of Maca for improving both physical stamina by minimizing muscular and hepatic damage and preserving energy during swimming exercise and male reproductive function by increasing epididymal sperm counts.

(Key words : sperm count, stamina, maca, gelatinized, fermented, *Lepidium meyenii*)

INTRODUCTION

Lepidium meyenii, known as Maca, is a plant that belongs to the family Brassicaceae, and grows exclusively between 4,000 and 4,500 m altitude at the central Peruvian Andes (Gonzales *et al.*, 2001b). The hypocotyls of this plant were used by Andean Indians as a nutrient and as a folk medicine. It has been used to enhance the fertility and sexual performance of men and women (Valerio and Gonzales, 2005). The incidence of sexual inadequacy in human males are indirectly indicated by the great number of available treatments (Montorsi *et al.*, 1995). The increasing number of men seeking help for impotence has expanded basic physiological and pharmacological research on sexual performance (Cicero *et al.*, 2001).

Forced swimming test is one of the behavioral performances for rodents, which predict the efficacy of antidepressant treatments (Tejani-Butt *et al.*, 2003; Estrada-Camarena *et al.*, 2002; Griebel *et al.*, 2002). This test induces the development of immobility as a reflection of helplessness when subjected to an incapable situation. After an initial swimming period, the animal exhibits immobility behavior considered a depression-like response (Koo *et al.*, 2004). Recently, the weight-loaded swimming test has also been used as a reference for measuring the fatigue level objectively and quantitatively (Moriura *et al.*, 1996; Kim *et al.*, 2005).

Different biological properties have been observed among different varieties of Maca (Rubio *et al.*, 2006). Recently, it has demonstrated that aqueous, lipidic, alcoholic and hexanic extract of Maca improves spermatogenesis and semen parameters (Chung *et al.*, 2005; Gonzales *et al.*, 2003; Cicero *et al.*, 2002; Zheng *et al.*, 2000). The dried hypocotyls of Maca include the 70~75% carbohydrates, 11~13% proteins, many kinds of minerals and vitamin C. Since Maca contains the high composition of carbohydrates, exposure of Maca to high tempera-

† This work was supported by the research grant of the Chungbuk National University in 2008.

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tures allows unfolding present starches. This process is called gelatinized. When Maca starches are unfolded (gelatinized), they are more quickly absorbed by the organism, resulting in enhanced bioavailability.

Fermentation of organic materials decomposes their structure in the absence of air (oxygen). Some of these products (for example, alcohol and lactic acid) are of importance to humans, and fermentation has therefore been used for their manufacture on an industrial scale. These processes are performed by lactic acid bacteria, such as *Bifidobacterium* sp. and *Lactobacillus* sp., and some molds including *Saccharomyces* sp. (Hur *et al.*, 2006; Kim and Ji, 2006; Nyman, 1995; Steinkraus, 1983). These microbes transform some components of foods as well as convert sugars to alcohol and lactic acid.

Maca contains carbohydrates and proteins. When Maca is fermented by *Saccharomyces* sp., proteins is transformed to macamides, which is a representative constituent in fermented Maca. In recent studies, it has been demonstrated that many kinds of Maca improved sexual behaviour and epididymal sperm counts (Gasco *et al.*, 2007; Gonzales *et al.*, 2006; Rubio *et al.*, 2006; Chung *et al.*, 2005; Gonzales *et al.*, 2004; Gonzales *et al.*, 2003: Gonzales *et al.*, 2001a; Zheng *et al.*, 2000) as well as stamina (Rubio *et al.*, 2006). However, the effect of gelatinized and fermented Maca on improvement of sexual behaviour, epididymal sperm counts and stamina has not been assessed. Therefore, the aim of the present study is to determine the effect of the gelatinized and fermented Maca on epididymal sperm counts and stamina. Especially, we analyzed not only the maximum swimming distance, but also the blood parameters of muscular and hepatic injuries as well as energy metabolism following forced swimming.

MATERIALS AND METHODS

1. Animals

Eight week-old male ICR mice (30~33 g body weight; $n = 24$) were purchased from the Daehan Laboratory Animal Center (Eumseong, Korea). The animals were maintained at a constant temperature (22 ± 1℃), relative humidity of 55 ± 10% and 12-h light/dark cycle. The animals were fed with standard rodent chow and purified water *ad libitum*. All experimental procedures were approved and carried out in accordance with the Institutional Animal Care and Use Committee of Laboratory Animal Research Center at Chungbuk National University, Korea.

2. Experimental Protocol

For adaptation on swimming pool, all male mice were swarm for 30 min 4 days before the experiment. After 4 days of adaptation period, the adult male mice divided at random into two main groups: resting and exercise groups. Exercise group was separated into three subgroups; control, gelatinized and fermented Maca groups. Resting and control groups received 5 ml/kg of the vehicle (water) daily during 30 days by gavage. Gelatinized and fermented Maca groups received freeze-dried gelatinized and fermented Maca, respectively, in a single daily dose of 800 mg/kg. All mice in exercise group were swarm for 30 min daily 30 min after treatment during the experimental period except the day of swimming test.

3. Preparations of Maca Powder

Gelatinized Maca: Gelatinized Maca powder was purchased from Cabex company (Lima, Peru). For gelatinization, the whole Maca root was exposed to the sun light as a natural dehydration process. Then, the hypocotyls of a high quality were selected, and subjected to appropriate disinfection processes such as washing and soaking. Finally, it was gelatinized by drying at high temperature for short time, reducing to the minimum any possibility of contamination of microorganisms in the final product. Special care has been had in the selection of the cooking and drying method with parameters completely controlled to assure a lower content of final humidity (6%).

Fermented Maca: Fermented Maca powder was offered by Easter B&F (Seoul, Korea). For fermentation, the gelatinized Maca powder was dissolved in a fermentation tank with 10 volumes of water, and added *Aspergillus*. Then, this mixed materials were incubated at 50℃ for 2~3 hrs. After sterilization at fermentation tank, *Saccharomyces* sp. was inoculated in this mixture. The digested Maca was fermented at 25~38℃ for 4~5 days. Each preparation was left standing to sterilize (at 121℃ for 30 min), freeze-dried and milled. This fermented Maca was placed in small vials and kept in a refrigerator at 4℃ until use.

4. Forced Swimming Performance

Mice were subjected to a weight-loaded forced swimming on 14th and 28th days 30 min after treatment as described previously with some modifications (Moriura *et al.*, 1996). The test was induced by forcing animals to swim until exhaustion. The mice were loaded with lead rings that weighed 5% of their body weight to the tail, and were then placed in the

swimming tank filled with fresh water. Water temperature was maintained at 25±0.5℃. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 7 sec. This 7-sec criterion was considered to correlate with exhaustion, and was used as an indication of maximum swimming capacity of the animal. Mice were removed at this point, before drowning.

5. Organ Weights

After 30 days of treatment with Maca with 2-day recovery period following maximum swimming test on 28th day, the mice were fasted for 4 hrs, and sacrificed under ether anesthesia. The following organs, which are susceptible or related to exhaustion and reproduction, were removed and weighed: liver, spleen, thymus, adrenal glands, testes and muscle.

6. Blood Biochemistry

Just before sacrifice, blood was collected from the abdominal aorta for the blood biochemistry. Blood was centrifuged at 1,500 g for 10 min to obtain sera. The concentrations of creatine phosphokinase (CPK), aspartate transaminase (AST), lactate dehydrogenase (LDH), alanine transaminase (ALT), urea nitrogen (BUN), glucose, total cholesterol (TC) and total proteins (TP) were determined using an autoanalyzer (Hitachi-747, Hitachi Medical Co., Ltd, Japan).

7. Epididymal Sperm Counts

The cauda epididymides of each animal were homogenized for 2 min by tissue tearer (Biospec Product Inc., Germany), and sonicated at 4℃ for 3 min to obtain homogenization-resistant sperm heads. Homogenates were kept refrigerated at 4℃ for 24 hrs to allow sperm to released from the walls. The number of sperm heads was counted using a hemacytometer.

8. Statistical Analysis

The results were expressed as the mean ± SD. The significance of the mean difference was determined by Duncan's multiple-range test after one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

1. Clinical Signs and Body Weights

The mice orally administered with gelatinized or fermented Maca (800 mg/kg) did not show any abnormal signs. As shown in Fig. 1, daily swimming exercise for 30 min tended to reduce the body weight gain. Both gelatinized and fermented Maca further decreased the body weight gain of exercised mice, especially, showing a significant reduction the animals treated with fermented Maca ($p<0.05$). Therefore, it was confirmed that intake of Maca during exercise synergistically reduce the body weight gain. Several researchers could not observe significant differences in the body weight gain between control and Maca-treated groups in resting condition (Gasco *et al.*, 2007; Gonzales *et al.*, 2006; Rubio *et al.*, 2006; Chung *et al.*, 2005; Cicero *et al.*, 2001). However, we demonstrated that Maca, especially fermented Maca, facilitated the effect of exercise on body weights, which was similar to the decreased body weights in sea level observed by Gonzales *et al.* (2004). However, the exact action mechanisms of Maca in the body weight-lowering effects in sea level and during exercise remain to be clarified.

2. Maximum Swimming Time

A weight-loaded swimming test was considered to be a model for evaluating of extent of behavioral despair and fatigue (Wu *et al.*, 2007). The mice loaded with 5% of their body weight were placed to swim in the water at room temperature (25 ± 0.5℃). Fig. 2 shows the effect of Maca on the maximum swimming time at 14 and 28 days in mice treated with gelatinized or fermented Maca. After 14 days of treatment, the

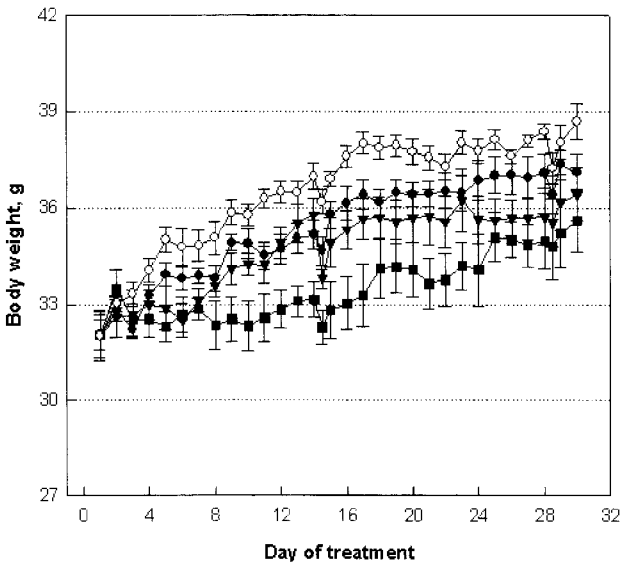

Fig. 1. Change in body weights of mice during treatment with Maca for 30 days. ○, resting control; ●, exercise control; ▼, exercise with gelatinized Maca; ■, exercise with fermented Maca.

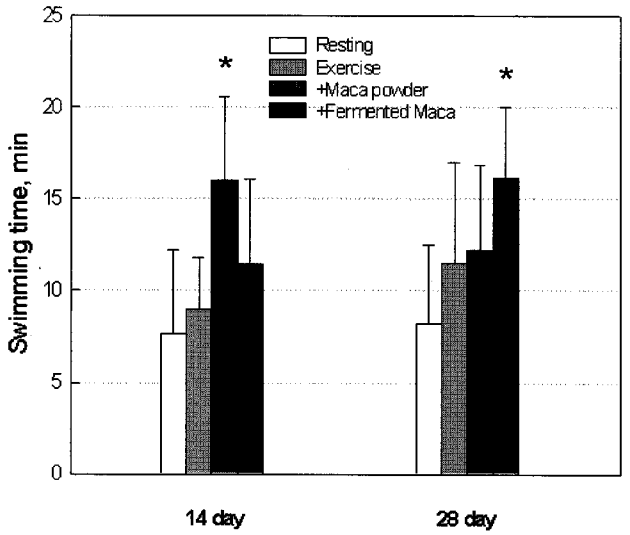

Fig. 2. The maximum swimming time of mice (n=6) treated with gelatinized or fermented Maca (800 mg/kg) for 14 and 28 days. Values are the mean±SD. *Superscript indicates significant difference from the resting group (p<0.05).

maximum swimming times of resting control, exercise control, gelatinized Maca and fermented Maca groups were 7.62 ± 4.6, 9.01 ± 2.78, 16.01 ± 4.56 and 11.15 ± 4.60 min, respectively. By comparison, on 28 days, the maximum swimming times were 8.22 ± 4.28, 11.52 ± 5.45, 12.24 ± 4.61 and 16.15 ± 3.88 min, respectively. It was found that repeated exercise for 14 and 28 days increased the stamina (maximum swimming time) compared to the resting control. After 14 days of treatment, the maximum swimming time of gelatinized Maca group was longer than that of the other groups (p<0.05). However, after 28 days of treatment, fermented Maca was superior to gelatinized Maca in increasing the stamina (p<0.05). Notably, gelatinized Maca exhibited a relatively short-term effect, in comparison with a long-term efficacy of fermented Maca in spite of its lowering activity on body weight gain (Fig. 1). It was reported that Maca hypocotyls contain phytoestrogens such as anthocyanins and quercetin (Lee *et al.*, 2004; Anjaneyulu *et al.*, 2003). However, previous studies demonstrated that estradiol concentration was not affected by treatment with Maca (Gonzales *et al.*, 2003; Oshima *et al.*, 2003). Therefore, the effect of improvement of stamina may be due to the energy-boosting and/or anti-depressant activities of Maca (Rubio *et al.*, 2006).

3. Organ Weights

Continuous exercise decreased the weights of the liver, spleen and adrenal glands, which might be due to energy-metabolizing and stress effects of exercise (Table 1). On the contrary, exercise increased not only the mass of gastrocnemius muscle, indicative of a fitness effect, but also testes weight. Administration of gelatinized Maca for 30 days to exercised mice, the decreased weights of the liver was somewhat recovered, and the testes weight further increased. In comparison, treatment with fermented Maca for 30 days fully recovered the weights of the liver, thymus and adrenal glands, and increased testes and muscle weights. Although previous investigators did not show differences in organ weights between resting control and Maca-treated animals (Rubio *et al.*, 2006; Gonzales *et al.*, 2004), we showed the recovering effects of gelatinized and fermented Maca on the exercised-induced decrease in metabolism- and stress-related organ weights. Moreover, Maca further increased the muscle and testes weights in exercised mice. Such effects of Maca on organs and muscle may come from its energy-boosting action, thereby enhancing the maximum swimming time.

4. Blood Biochemistry

In order to clarify the mechanisms of Maca's stamina-enhancing effect, we assessed the levels of several blood biochemical parameters 2 days after the 2nd swimming test. Continuous exercise and maximum swimming performance significantly increased the CPK, AST, LDH, ALT and glucose, indicating the muscular and hepatocytic injuries (stresses) and energy depletion (Burr *et al.*, 1997; Coombes and McNaughton, 2000)

Table 1. Relative organ weights (%) of mice treated with Maca for 30 days

Treatment		Liver	Spleen	Thymus	Adrenals	Testes	Muscle
Resting	Vehicle	5.25 ± 0.23	0.414 ± 0.102	0.0934 ± 0.0224	0.0116 ± 0.0038	0.621 ± 0.106	0.917 ± 0.074
Exercise	Vehicle	4.95 ± 0.87	0.261 ± 0.049	0.0904 ± 0.0334	0.0099 ± 0.0035	0.677 ± 0.092	1.023 ± 0.052
	Gelatinized Maca	5.14 ± 0.56	0.251 ± 0.047	0.0980 ± 0.0108	0.0093 ± 0.0038	0.733 ± 0.055	1.016 ± 0.077
	Fermented Maca	5.43 ± 0.57	0.272 ± 0.037	0.1426 ± 0.0243	0.0165 ± 0.0040	0.719 ± 0.082	0.956 ± 0.111

(p<0.05) (Table 2). TC and TP, other energy sources, were also tended to be decreased, while BUN, a nitrogen released from muscles, was markedly increased. Gelatinized or fermented Maca, orally administered fully or significantly recovered the increased injury parameters of the muscle and liver, while increased the energy sources. These results suggest that gelatinized and fermented Maca may play a role in boosting of energy, which might explain the prolonged maximum swimming time, in spite of their body weight-lowering efficacy.

5. Epididymal Sperm Counts

Epididymal sperm counts of the resting control, exercise control, gelatinized Maca and fermented Maca groups were 92.0 ± 12.9 × 10^6/g, 87.1 ± 10.2×10^6/g, 106.0 ± 16.2×10^6/g and 115.3 ± 11.3 × 10^6/g, respectively (Fig. 3). Although the sperm counts slightly lowered following exercise, the numbers more increased by 22.0% and 32.0% in gelatinized and fermented Maca-treated groups, respectively. Such increases in epididymal sperm counts which may enhance the fertility rate have been demonstrated (Gasco *et al.*, 2007; Gonzales *et al.*, 2006; Rubio *et al.*, 2006; Chung *et al.*, 2005; Gonzales *et al.*, 2004; Gonzales *et al.*, 2003). This increased epididymal sperm count was not related to changes in the sperm production in testes and transit rate (Gonzales *et al.*, 2006). Maca is naturally presented in different ecotypes which are characterized by their external color, and had different biological effects according to the ecotypes (Gonzales *et al.*, 2006). Also, Gonzales *et al.* (2006) reported that Black Maca possesses more beneficial effects on sperm counts and epididymal sperm motility than other types of Maca. In the present study, we showed that fermented Maca was superior to gelatinized Maca in increasing epididymal sperm counts, suggestive of an increase in active ingredients during fermentation.

In the present study, Maca facilitated exercise-induced decrease in body weight gain, in which fermented Maca was more effective than gelatinized one. In spite of their body weight-lowering effects, Maca enhanced maximum swimming time. Interestingly, fermented Maca exerted exhibited long-term (4 weeks) stamina-enhancing effect, in comparison with a relatively sort-term (2 weeks) effectiveness of gelatinized Maca. It was believed that such stamina-enhancing effects of Maca might be due to energy-boosting and anti-stress activities, inferred from the blood biochemical parameters of energy preservation and muscular and hepatic damage. In addition, fermented Maca

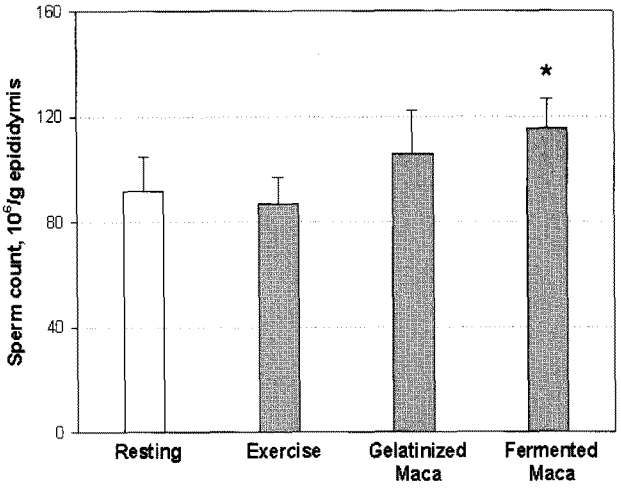

Fig. 3. The epididymal sperm counts of mice (n=6) treated with gelatinized or fermented Maca (800 mg/kg) for 30 days. Values are the mean±SD. *Superscript indicates significant difference from the resting and exercise groups (p<0.05).

Table 2. Blood biochemistry of mice treated with Maca for 30 days

Treatment		CPK (U/l)	AST (U/l)	ALT (U/l)	LDH (U/l)	Glucose (mg/dl)	TC (mg/dl)	TP (g/dl)	BUN (mg/dl)
Resting	Vehicle	165.8 ± 51.0	71.1 ± 13.8	36.6 ± 6.9	806.1 ± 155.1	165.0 ± 47.1	125.0 ± 15.7	5.2 ± 0.4	25.4 ± 4.1
Exercise	Vehicle	264.7 ± 89.6*	133.4 ± 44.4*	60.4 ± 12.2*	1,017.5 ± 345.0*	145.2 ± 49.4*	112.4 ± 12.1	4.6 ± 0.3	38.0 ± 7.1*
	Gelatinized Maca	145.5 ± 102.9**	65.5 ± 20.3**	37.9 ± 11.8**	528.5 ± 197.5**	191.9 ± 24.1**	149.3 ± 21.0**	5.4 ± 0.2**	28.5 ± 4.8**
	Fermented Maca	109.5 ± 22.2**	63.8 ± 2.8**	44.4 ± 18.6**	599.3 ± 88.2**	185.1 ± 29.5***	185.2 ± 39.7**	5.5 ± 0.2**	28.7 ± 3.6**

Values are the mean ± SD (n = 6, respectively). *Superscript indicates significant difference from the resting group (p<0.05). **Superscript indicates significant difference from the exercise control group (p<0.05).

significantly increased epididymal sperm counts. Therefore, the results suggest that fermented Maca could be a promising candidate for the improvement of physical stamina and male reproductive function.

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(접수일: 2008. 11. 16 / 채택일: 2008. 12. 2)