International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.622.629

Research Article
Effects of Gelatinized Maca on Andropause Symptoms in Aged Male Mice

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Abstract

Background and Objective: Although, Maca (*Lepidium meyenii*) has gained worldwide attention as a powerful energizer that improves physical health and mental well-being and increases reproductive capability, the effects of Maca on andropause symptoms in experimental animals remain poorly understood. This study aimed to investigate the potential effects of gelatinized Maca on andropause symptoms in aged mice. **Materials and Methods:** Maca was administered orally to aged rats at dose levels of 0, 500 and 1,000 mg/kg/day for 4 weeks and its effects on clinical signs, body weight, reproductive organs, oxidative stress, testosterone content, lipid metabolism and muscle mass were examined. All statistical comparisons were made using a one-way analysis of variance followed by Dunnett's multiple comparison test and $p<0.05$ indicated significance. **Results:** The 4 week repeated oral administration of Maca to aged male mice resulted in a decrease in retroperitoneal fat and an increase in antioxidant enzyme SOD activity at doses of $\geq$500 mg/kg/day. Furthermore, there was an increase in the vastus lateralis muscle at a dose of 1,000 mg/kg/day. Notably, Maca treatment did not result in any adverse effects on general symptoms, body weight, reproductive organ weight and histopathology, oxidation-antioxidant balance, testosterone content, lipid metabolism and muscle mass at any of the tested doses. **Conclusion:** The results of this study suggested that gelatinized Maca is a safe natural substance that can help alleviate andropause symptoms. It may serve as a promising alternative therapy for individuals who cannot receive testosterone treatment due to its side effects.

Key words: *Lepidium meyenii*, andropause, retroperitoneal fat, vastus lateralis muscle, superoxide dismutase

Citation: Kim, W.I., J.S. Oh, S.W. Pak, S.J. Lee and C. Moon *et al.*, 2024. Effects of gelatinized maca on andropause symptoms in aged male mice. Int. J. Pharmacol., 20: 622-629.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maca (*Lepidium meyenii*) is a biennial herbaceous plant that belongs to the Brassica family and grows almost exclusively in a restricted area in the Peruvian Central Andes[1]. The Andes mountains are known for their harsh conditions, making it difficult for organisms to thrive. However, various substances produced by plants for survival in this region have been found to have beneficial effects on human health. Maca is not only consumed as food but also used as a traditional medicine[2]. Maca, also known as "Peruvian ginseng", has long been used in the highlands of Peru to improve sexual function and promote reproduction in humans and animals[3].

Maca has high nutritional quality, containing carbohydrates, proteins, lipids, fiber, amino acids, sterols, fatty acids and minerals[4,5]. It is a popular food supplement known for its various biological effects including antioxidant, neuroprotective, bacteriostatic, antifungal, antihypertensive, antidiabetic, antifatigue, antiviral, anticancer, hepatoprotective, antiarthritic and immunomodulatory properties[6-8]. Maca has also gained global attention as a powerful energizer that improves both physical and mental conditions and enhances reproductive capability[9,10]. Numerous studies have demonstrated that Maca improves fertility in human and experimental animals[11-16].

Andropause, also known as late-onset hypogonadism (LOH), is a condition experienced by men as a result of an age-related decrease in testosterone production. The main causes of andropause are a decrease in male hormones due to aging of the brain and testes, metabolic diseases such as diabetes, high blood pressure and obesity and environmental factors such as drinking, smoking and stress[17,18]. Common symptoms of male menopause include reduced libido and sexual function, muscle weakness, increased body fat, fatigue and lethargy, memory loss and decreased bone density[19]. Testosterone administration is widely used to treat male menopause; however, owing to the side effects of hormone therapy, there is a growing interest in alternative treatments using safer and more effective natural products[20,21]. Recent studies on Maca have suggested its effectiveness in treating various conditions in addition to sexual dysfunction and menopausal symptoms. However, these studies have limitations such as small sample sizes, methodological quality, number of trials and inconsistent results, indicating a need for more rigorous studies[22-26]. Furthermore, the effects of Maca on andropause symptoms in experimental animals have not yet been extensively studied.

This study aimed to investigate whether repeated oral administration of Maca for 4 weeks improves the effects of andropause on clinical signs, body weight, reproductive organs, oxidative stress, testosterone content, lipid metabolism and muscle mass in aged mice.

MATERIALS AND METHODS

Study area and ethical consideration: This study was conducted, in accordance with the Guide to Functional Evaluation of Health Functional Foods of the Ministry of Food and Drug Safety. All experimental procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals from October, 2023 to November, 2023. The Institutional Animal Care and Use Committee of Chonnam National University (Gwangju, Korea) approved the protocols for the animal study (CNU IACUC-YB-2023-133).

Animals and environmental conditions: The male C57BL/6 mice aged 56 weeks were purchased from the Korea Research Institute of Bioscience and Biotechnology (Cheongju, Korea) and were used after a quarantine and acclimatization 1 week. The mice were housed individually in clear polycarbonate cages with stainless steel wire lids and maintained in a room at 23±3°C with a relative humidity of 50±10%. A controlled alternating 12 hrs light-dark cycle was provided. The mice had access to tap water that had been sterilized by ultraviolet irradiation and were fed commercial rodent chow (Purina Korea, Gunsan, Korea) *ad libitum*.

Test article and treatment: Gelatinized Maca acquired from Easter B&F Co., (Seoul, Korea) was used in this study. The test article was suspended in sterilized distilled water and freshly prepared daily before treatment. The prepared test article was daily administered into the stomachs of male mice for 28 days using a sonde and syringe. The application volume (10 mL/kg) was calculated in advance based on the most recently recorded body weights of individual animals. The oral route was selected for administration as it is the clinically intended route for the test article.

Experimental groups and dose selection: In this study, eighteen healthy male mice were assigned to three experimental groups (n = 6). The two treatment groups were administered received repeated oral doses of gelatinized Maca at 500 or 1,000 mg/kg/day, respectively. The control group received distilled water (vehicle) alone. Based on the results of a dose-range finding study, the high dose of 1000 mg/kg/day was chosen to potentially increase sperm motility and sperm count, whereas the low dose of 500 mg/kg/day was set to establish a dose-response correlation by applying a scale factor ×2.

Clinical observation and body weight: Throughout the study period, all animals were observed daily for clinical signs, moribundity and mortality. The body weight of each mouse was measured at the beginning of the treatment, once a week thereafter and at the scheduled end date.

Necropsy and gross pathology: At the scheduled termination, any surviving animals were fasted overnight and euthanized by an intraperitoneal administration of a mixture of Zoletil (40 mg/kg; Virbac) and xylazine (10 mg/kg; Bayer-Korea, Ltd.). Blood was drawn from the posterior vena cava for serum biochemistry tests once anesthesia was verified. The mice were euthanized by completely cutting the posterior vena cava thereafter. All males underwent a thorough detailed gross necropsy, which involved a careful examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities along with their contents. Special attention was given to the reproductive organs.

Organ weight: The absolute and relative weights (organ-to-body weight ratios (%)) of the following reproductive organs were measured: Testes, epididymides, prostates and seminal vesicles.

Visceral fat and muscle mass: To measure the amount of visceral fat, retroperitoneal fat was extracted and washed, moisture was removed with filter paper and then the weight of the fat tissue was measured. To determine hind limb muscle mass, the thigh was incised and the vastus lateralis muscle, which makes up a large part of the quadriceps femoris, was separated and its weight was measured.

Serum lipid content: Approximately 0.7 mL of blood samples was drawn from the posterior vena cava and serum samples were collected by centrifugation at 800 g for 10 min within 1 hr after collection. The samples were stored at 80°C before analysis. To analyze the effect of Maca on lipid metabolism, the cholesterol and triglyceride concentrations in serum were measured using an autoanalyzer (Dri-chem 4000i, Fujifilm Co., Tokyo, Japan).

Testosterone concentration: To analyze the effect of repeated administration of Maca on androgenic hormone, the testosterone content in serum was measured using an ELISA kit (Aviva Systems Biology, San Diego, California, USA).

Determination of lipid peroxidation and antioxidant enzyme activity: To create a 1:9 (w/v) entire homogenate, each frozen right testis was homogenized in a glass-Teflon homogenizer using 50 mM phosphate buffer (pH 7.4). The homogenates were then centrifuged at 11,000 g for 15 min at 4°C to remove any cell debris; the supernatant was assayed using a commercial kit to estimate the concentration of malondialdehyde (MDA; Cayman, Ann Arbor, Michigan, USA) and the activity of the antioxidant enzyme superoxide dismutase (SOD; Cayman). The total protein content was calculated using the technique outlined by Lowry *et al.*[27], using bovine serum albumin as a standard.

Histopathological examination: Histopathological examination was performed on the testis and epididymis collected from the animals of the vehicle control and treatment groups as described by researchers[28,29]. A portion of the testis and epididymis was dissected and fixed in 10% neutral buffered formalin solution for 2 weeks. The fixed organs were routinely processed, embedded in paraffin wax and sectioned at 4-5 µm. The sections were stained with Hematoxylin and Eosin stain for microscopic examination. All observations were made manually using a light microscope with 5, 10, 20 and 40× objective lenses and a 100× oil immersion lens in a fully blinded manner.

Statistical analysis: The data are presented as the mean value and Standard Deviation (SD). All statistical comparisons were made using a one-way analysis of variance followed by Dunnett's multiple comparison test. Statistical analysis was performed by comparing the treatment groups to the control group using GraphPad InStat v. 3.0 (GraphPad Software, Inc., La Jolla, California, USA). Statistical significance was attributed to p-values that reached a significance level of 0.05.

RESULTS

Clinical observation and body weight: No treatment-related clinical symptoms or changes in body weight were observed throughout the study period at any of the tested doses (Table 1).

Gross pathology: Macroscopic pathological findings associated with the administration of Maca were not observed in any of the animals in any test group at the scheduled necropsy on the trial end date (data not shown).

Table 1: Body weights of aged male mice treated with Maca for 4 weeks

	Maca (mg/kg/day)		
Item	0	500	1000
Number of mice examined	6	6	6
Day 0	33.0±2.00[a]	34.0±2.98	33.5±2.18
Day 7	33.7±2.67	33.6±3.04	33.4±1.55
Day 14	33.3±2.96	33.2±3.27	32.2±2.43
Day 21	33.1±3.25	32.6±2.75	32.6±1.48
Day 28	33.4±3.36	33.4±3.11	32.1±1.59

[a]Values are expressed as Mean±SD (g)

Table 2: Relative weights (organ-to-body weight ratios) of reproductive organs, retroperitoneal fat and vastus lateralis of aged male mice treated with Maca for 4 weeks

	Maca (mg/kg/day)		
Item	0	500	1000
Number of mice examined	6	6	6
Body weight at term (g)	33.4±3.36[a]	33.4±3.11	32.1±1.59
Testis: Left (%)	0.32±0.045	0.33±0.031	0.34±0.006
Testis: Right (%)	0.31±0.040	0.32±0.041	0.33±0.019
Epididymis: Left (%)	0.13±0.016	0.13±0.028	0.14±0.019
Epididymis: Right (%)	0.13±0.024	0.14±0.009	0.13±0.010
Seminal vesicles (%)	1.22±0.136	1.11±0.224	1.21±0.166
Prostate glands (%)	0.36±0.089	0.37±0.053	0.36±0.041
Retroperitoneal fat (%)	0.99±0.246	0.61±0.204*	0.68±0.111*
Vastus lateralis (%)	0.85±0.081	0.91±0.048	1.02±0.087**

[a]Values are expressed as Mean±SD, *p<0.05 vs control group and **p<0.01 vs control group, respectively

Table 3: Serum total cholesterol, triglyceride and testosterone levels of aged male mice treated with Maca for 4 weeks

	Maca (mg/kg/day)		
Item	0	500	1000
Number of mice examined	6	6	6
Total cholesterol (mg/dL)	186.0±33.86[a]	152.0±28.65	163.7±25.65
Triglyceride (mg/dL)	102.0±26.31	84.2±27.32	83.5±23.86
Testosterone (ng/mL)	2.37±0.042	2.39±0.074	2.47±0.198

[a]Values are expressed as Mean±SD

Relative weights of reproductive organs, visceral fat and muscle mass: To investigate the effect of Maca treatment on visceral fat, the weight of retroperitoneal fat was measured (Table 2). The relative weight of retroperitoneal fat in the 500 and 1,000 mg/kg groups was statistically significantly lower than that in the control group. Conversely, the relative weight of the vastus lateralis muscle in the 1,000 mg/kg group showed a statistically significant increase compared to the control group. However, no statistically significant difference was observed in the relative weights of the testes, epididymides, prostates and seminal vesicles between the control and treatment groups.

Serum lipid content: The concentration of total cholesterol and triglycerides in serum was measured (Table 3). Although, no statistically significant difference was observed compared to the control group, the concentrations of total cholesterol (81.7 and 88.0% compared to the control group) and triglyceride (82.5 and 81.9% compared to the control group) tended to decrease in the 500 and 1,000 mg/kg groups, respectively.

Testosterone concentration: The effect of repeated administration of Maca on androgenic hormones was investigated (Table 3). No statistically significant difference in serum testosterone concentration was observed between the control and treatment groups.

Lipid peroxidation and antioxidant enzyme activity: The oxidation-antioxidation balance of testicular tissue was examined (Table 4). No significant difference in the concentration of MDA, an indicator of lipid peroxidation, was observed between the groups. However, the activity of SOD, an antioxidant enzyme, showed a statistically significant increase in the 500 and 1,000 mg/kg groups compared to the control group.

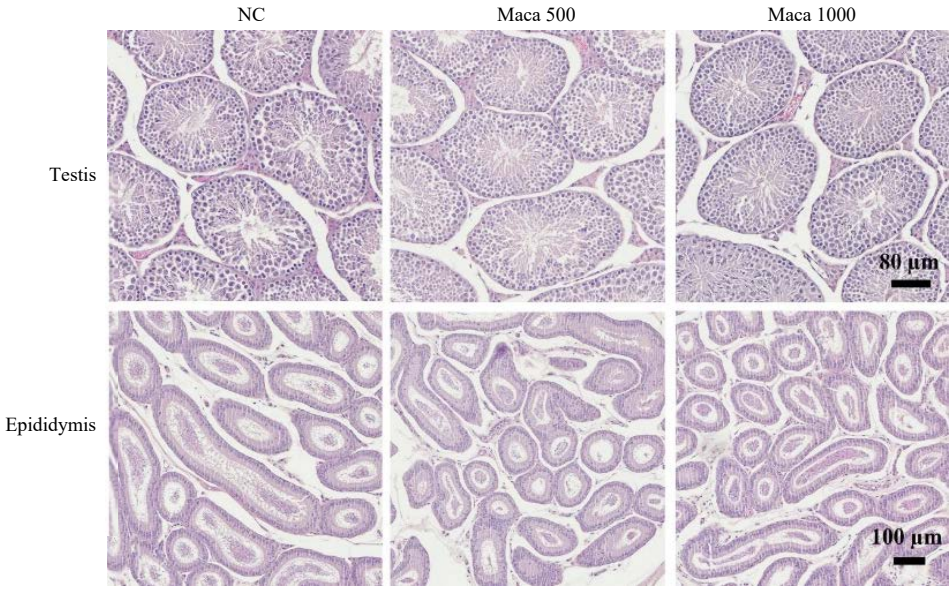

Fig. 1: Representative photographs of testis and epididymis sections treated with Maca for 4 weeks, showing normal architecture
Hematoxylin and Eosin (H&E) stain

Table 4: Oxidative stress analysis of testis from aged male mice treated with Maca for 4 weeks

	Maca (mg/kg/day)		
Item	0	500	1000
Number of mice examined	6	6	6
Malondialdehyde (µmol/ mg protein)	1.84±0.265[a]	1.79±0.221	1.71±0.314
Superoxide dismutase (nmol/min/mL)	0.153±0.0147	0.209±0.0408*	0.234±0.0329**

[a] Values are expressed as Mean±SD, *p<0.05 vs control group and **p<0.01 vs control group, respectively

Histopathological examination: As depicted in Fig. 1, histopathological changes in the testes and epididymis as a result of the administration of Maca were not observed at any of the tested doses.

DISCUSSION

In modern society, the elderly population is increasing significantly owing to the extension of lifespan resulting from developments in life science and medical technology. Andropause symptoms, which are commonly observed in middle-aged or elderly men, are known to be one of the major causes of reduced quality of life[30]. Therefore, modern medicine must develop treatments that safely and effectively relieve or treat andropause symptoms. Maca has traditionally been used to increase physical vitality and endurance, as well as promote mental clarity for various conditions such as male impotence, menstrual irregularities, female hormonal imbalances and chronic fatigue syndrome. The results of this study demonstrate that 4 week repeated oral administration of Maca to aged mice alleviates andropause symptoms by increasing muscle mass, decreasing body fat and increasing antioxidant enzyme activity.

One of the most notable effects of aging is the involuntary loss of muscle mass, strength and function, known as sarcopenia[31,32]. In particular, in andropause, abdominal fat increases due to the redistribution of body fat and basal metabolic activity decreases as muscle mass decreases, exacerbating fat accumulation[33]. In this study, the decrease in retroperitoneal fat observed in the groups receiving >500 mg/kg of Maca and the increase in vastus lateralis muscle observed in the 1,000 mg/kg group are considered to be pharmacological effects resulting from Maca administration. Although no statistically significant difference was observed compared to the control group, the reduction in serum total cholesterol and triglyceride levels observed in the Maca administered groups was consistent with a significant reduction in visceral fat, suggesting that Maca administration improves lipid metabolism in aged male mice.

Recent reports have also shown that Maca is clinically effective in treating LOH-related symptoms in men with gonadal dysfunction, as evidenced by significant improvement in the Aging Males' Symptoms Scale, International Prostate Symptom Score, International Index of Erectile Function and Androgen Deficiency in the Aging Males positive rates[34]. The results of the aforementioned studies, combined with the findings of current study, clearly demonstrate that Maca administration alleviates andropause symptoms by increasing muscle mass, enhancing lipid metabolism and improving physical activity and reproductive performance.

Testosterone plays a crucial role in muscle growth, bone density, sexual function, mood regulation and overall well-being. Previous studies have shown that Maca consumption can enhance sexual desire in men without affecting their serum testosterone levels[35,36]. In this study, the aged mice treated with Maca showed an increase in muscle mass and an improvement in lipid metabolism without any change in serum testosterone concentration. The results of the aforementioned study, combined with the findings of current study, suggest that Maca consumption can improve symptoms of andropause without causing an increase in testosterone levels. According to a study by Volpi *et al.*[32], nutritional supplements containing amino acids or proteins can stimulate muscle protein synthesis and promote muscle growth by increasing daily calorie intake. Maca has been suggested as a potential alternative to anabolic steroids for athletes because it can enhance physical activity, improve sexual desire and enhance reproductive performance in both experimental animals and men[37-39].

The significant increase in SOD activity observed in the testicular tissue of the 500 and 1,000 mg/kg groups in this study is considered to be an antioxidant effect caused by Maca administration. Peruvian Maca contains significant amounts of antioxidants such as phenols, glucosinolates, alkamides and polysaccharides, which can effectively improve endogenous antioxidant activity and help maintain the balance between oxidants and antioxidants[40,41]. A recent study demonstrated that Maca polysaccharide, the main active ingredient, can improve oxidative stress and energy metabolism, as well as promote mitochondrial respiration by regulating the Keap1-Nrf2 signaling pathway[42]. Another study found that lipids and their derivatives accumulate inside and between muscle cells, leading to mitochondrial dysfunction, interference with β-oxidation of fatty acids, increased production of reactive oxygen species (ROS), lipotoxicity, insulin resistance and enhanced secretion of certain pro inflammatory cytokines[43]. Therefore, the improvement in lipid metabolism observed in the Maca groups in current study is believed to contribute to alleviation of andropause symptoms by suppressing mitochondrial dysfunction, β-oxidation of fatty acids, ROS production and inflammatory cytokine secretion. As antioxidant intake can effectively improve semen parameters in men[44], it is hypothesized that the increase in SOD activity observed in this study may partially contribute to the improvement in sperm motility and sperm count resulting from Maca administration.

CONCLUSION

The results obtained from this study reveal that 4 week repeated oral administration of gelatinized Maca to aged mice alleviates andropause symptoms by increasing muscle mass, decreasing body fat and increasing antioxidant enzyme activity. Under the conditions used in this study, Maca treatment did not show any adverse effects on general symptoms, body weight, reproductive organ weight and histopathology, oxidant-antioxidant balance, testosterone content, lipid metabolism and muscle mass at any of the tested doses. These data suggest that gelatinized Maca may be a promising alternative therapy for individuals who cannot receive testosterone treatment due to its side effects.

SIGNIFICANCE STATEMENT

Andropause is a condition experienced by men due to an age-related decrease in testosterone production. Common symptoms of male menopause include decreased libido and sexual function, muscle weakness, increased body fat, fatigue and lethargy, memory loss and decreased bone density. This study investigated the potential effects of gelatinized Maca on andropause symptoms in aged mice. Current studies suggest that Maca is a safe natural substance that can improve andropause effects and may be a promising alternative therapy for individuals who cannot receive testosterone treatment due to side effects.

ACKNOWLEDGMENTS

This research was supported by 'regional innovation mega project' program through the Korea Innovation Foundation funded by Ministry of Science and ICT (Project Number: 2023-DD-UP-0031). This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIT) (NRF-2021R1A2C2011673).

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