



**EFFECT OF *LEPIDIUM MEYENII* (MACA) EXTRACTS ON SEXUAL PERFORMANCE IN MICE, AND THE SEMINAL VESICLE WEIGHT AND SERUM TESTOSTERONE LEVELS IN CASTRATED MICE**

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**ABSTRACT**

This study aims to evaluate the effect of oral administration of aether, ethanolic and aqueous extract of Maca on sexual performance in healthy male mice, and the sexual gland weight and serum testosterone levels in castrated male mice. Healthy male mice or mice with orchidectomy were randomly divided into several experimental and control groups, the experimental groups were oral administrated with aether, ethanolic or aqueous extract of Maca, both sham and castrated control received vehicle, the intromission latency (IL) and the number of intromissions in 1 hour were evaluated in healthy mice, the seminal vesicle weight and serum testosterone levels were measured in castrated mice. It was observed that Maca extracts significantly decreased IL (except for ethanol extract) and increased the number of intromissions ( $P < 0.01$ ) as compared to controls, and the most effective fraction was aether extract. Higher dose of Maca aether, ethanol and aqueous extract remarkably increase weight of seminal vesicle in castrated mice ( $P < 0.05$ ), but no measurable difference was observed in serum testosterone levels compared with control group. Our results indicated that Maca extracts improved sexual performance of healthy male mice, the ether fraction was most effective, and increase the sexual gland weight in castrated male mice, but this effect was not due to the changes in serum testosterone levels.

**KEYWORDS:** *Lepidium meyenii*, Maca, sexual performance, seminal vesicle weight, serum testosterone.

**INTRODUCTION**

Maca (*Lepidium meyenii*) is a perennial plant from the Andes of Peru, which belongs to the Brassicaceae family and grows in the suni and puna ecosystems at altitudes between 11,000 to 14,000 feet, where the land is exposed to intense sunlight, violent winds and a rough climate below freezing temperatures. The tuber of Maca are both used as food and folk medicine for its nutritional value and ethnoedicinal properties<sup>[1,2]</sup>, it has been also used to enhance fertility and sexual performance in humans and animals and to improve women menopausal symptoms.<sup>[3,4]</sup> Furthermore, osteoprotective effect of *L. meyenii* have been demonstrated in ovariectomized rats.<sup>[5]</sup> A previous study report showed that oral administration of a lipidic extract of Maca increases the number of complete intromissions and of spermpositive females in normal mice and decreases the latent period of erection in male rats with erectile dysfunction.<sup>[6]</sup> Studies in 2001 reported a beneficial effect of Maca on male sperm production in rats and improvement of sperm count and motility in healthy adult men.<sup>[7,8]</sup>

In order to determine which fractions of Maca were more effective on sexual performance in mice, the present study was conducted to investigate the effect of aether, ethanolic and aqueous extract of Maca on sexual performance in healthy male mice, as well as the weight of seminal vesicle and serum testosterone levels in emasculated mice.

**MATERIALS AND METHODS**

***Extracts of Lepidium meyenii preparation***

The dried Maca pulverised root (standardised 0.6% macamides and macaenes), offered by MAYA bio-engineering Ltd.Co (Hubei, China), was collected from Peru. The plant was authenticated by Irma Fernandez, Botanist, Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia. The voucher specimen (PA.247) was placed in School of Life Science and Technology, Huazhong University of Science and Technology.

The powdered tuber was extracted successively with aether, ethanol and distilled water. In brief, 500g of the dried pulverized hypocotyls was extracted with 1500 ml

of aether at room temperature for 48h, the extractive solution was filtered and concentrated under reduced pressure at 45°C with rotary evaporator, giving 6.5g, the residual was extracted with 1500ml of ethanol for 2 days, the ethanol fraction was evaporated using a rotary evaporator and obtaining 95.0g extract, the residual was boiled with 1500ml water for 2 hours, the aqueous extract was evaporated and the final dry extract weighed 178g.

The above extracts were to be used in following experiments. before use, the extracts were further diluted with distilled water to obtain different concentrations and kept at 4°C for 24 h.

#### **Animals**

One hundred and forty male and two hundred and ten female mice (a strain of Kunming, grade II) with a body weight of 25±1g were obtained from Tongji Medical College of Huazhong University of Sci. & Tech. They were housed in Plexiglas cages, males and females separately, in climatized colony rooms (20±2°C; 65% humidity) on a 12h light/dark cycle, with lights off at 07:00 h. All of them were screened for copulatory ability 1 week after arrival. All experimental designs and procedures were approved by the Animal Ethics Committee for Animal Care and Use at Huazhong University of Science and Technology.

#### **Experimental Methods**

For the study of the effect of Maca extracts on sexual behavior in healthy male mice, the 70 male mice were randomly divided into 7 groups, including the control, groups of aether, ethanolic and aqueous extract of Maca, with all mice continuously administered Maca extracts at a dose of 4mg/g or 0.8mg/g body weight or 0.9% saline solution for 6 days through gastric tube. On the sixth day, 1 hour after the dose was administered to the male mice, 3 female mice were placed with 1 male mouse in a cage kept in darkness (2 days before, all female mice were injected 5µg benzestrol subcutaneously), then the number of intromissions in 1 hour and the intromission latency (IL) were recorded.

For the test of the effect of Maca on the weight of seminal vesicle and serum testosterone levels in emasculated male mice, seventy sexually active male mice were bilaterally castrated or sham-operated under ether anaesthesia (ten mice/group). A longitudinal midscotal incision was made and the testes were tied off and removed with a cut distal to the ligature, in sham operations the gonad was exposed but not removed. Each

subject was received a subcutaneous injection of penicillin G sodium immediately after surgery, 3 days after which, all emasculated mice were randomly assigned to Maca extract groups, positive medicine(methyltestosterone) or 0.9% saline group. Then, each mouse orally received high or low dose Maca extracts, methyltestosterone (MT, 10mg/kg) or 0.9% saline continuously for 22 days. Half an hour after the last administration, all animals were sacrificed, trunk blood was collected into centrifuge tubes and serum was prepared and stored frozen for testosterone determination and seminal vesicles were removed and weighed. Serum testosterone levels was determined by magnetic solid phase immunoassay.

#### **Statistical analysis**

The results were reported as means±S.E.M, pairwise statistical comparisons between the control and Maca-treated groups were done with *t*-test and one-way ANOVA. Mean differences were considered statistically significant if  $P < 0.05$ .

### **RESULTS**

#### ***Effect of L.Meyenii on sexual performance in male mice***

The ether, ethanol and aqueous extract of Maca were orally administered to the healthy male mice for 6 days. The number of complete intromissions during a hour remarkably increased and IL decreased (except for ethanol fraction) compared with the control group ( $P < 0.01$  or  $P < 0.05$ ), there was no significant difference of number of complete intromissions and IL between high and low dose groups. The results were presented in Table 1.

#### ***Effect of L.Meyenii on seminal vesicle weight and serum testosterone levels in castrated mice***

In the assessment of the effect of Maca extracts on weight of seminal vesicles and serum testosterone levels in emasculated mice, the seminal vesicles weight of castrated mice were remarkably decreased, while the mice treated with Maca aether, ethanol and aqueous extract at high-dose or MT at 10mg/kg for 22 days resulted in significant increase in weight of seminal vesicles compared with castrated control group ( $P < 0.05$ ), there were no significant differences in the body weight, thymus and spleen weight (not shown in data). Serum testosterone levels were very low in the castrated mice and no measurable difference was found in the groups treated with Maca extracts and in those treated with 0.9% saline in emasculated mice(table 2).

**Table 1. Effect of different Maca extracts on the number of intromissions and IL in healthy male mice (n=10 per group).**

Group	Number of intromissions	IL(s)
Saline	5.02±1.84	607±196
<b>Aether extract</b>		
High-dose	15.51±2.04**	305±131**
Low-dose	13.89±1.83**	320±122**

<b>Ethanol extract</b>		
High-dose	10.76±2.16**	485±167
Low-dose	9.12±1.97**	496±154
<b>Aqueous extract</b>		
High-dose	12.83±1.96**	417±153*
Low-dose	10.96±1.74**	430±136*

\* $P<0.05$ , \*\*  $P<0.01$  vs saline treated mice.

**Table 2. Effect of different Maca extracts on the weight of seminal vesicles and serum testosterone levels in male emasculated mice(n=10 per group).**

Group	Body weight (g)	Seminal vesicles		Testosterone (ng/ml)
		(mg)	(mg/10g BW)	
<b>Sham control</b>	35.80±2.30	202.65±47.09	53.23±9.60	3.76±0.86
<b>Castrated control</b>	32.92±3.78	9.75±4.28	2.96±1.27	<0.1
<b>Aether extract</b>				
High-dose	33.92±2.87	16.30±6.84*	4.73±1.87*	<0.1
Low-dose	32.25±3.19	10.14±3.54	3.13±1.0	<0.1
<b>Ethanol extract</b>				
High-dose	35.1±3.30	13.9±3.60*	3.93±1.20	<0.1
Low-dose	34.5±4.20	10.6±5.30	3.05±1.53	<0.1
<b>Aqueous extract</b>				
High-dose	34.33±3.11	15.57±6.12*	4.43±1.66*	<0.1
Low-dose	33.33±2.15	12.29±5.31	3.68±1.51	<0.1
<b>MT</b>	35.80±2.44	25.67±10.14**	7.20±2.96**	0.11±0.04

MT= methyltestosterone; \* $P<0.05$ , \*\*  $P<0.01$  vs castrated control group.

## DISCUSSION

During the last few decades, sexual incompetence, including sexual impotence is a problem of increasing concern.<sup>[9,10]</sup> Sexual problems adversely affect mood, well being, and interpersonal functioning.<sup>[11]</sup> There are more than 30% of men aged 40-70 years have been worried by erectile dysfunction.<sup>[12]</sup> Although oral agents and testosterone for erectile dysfunction and hypoactive sexual desire are widely used<sup>[13,14]</sup>, a paucity of evidence-based data is available confirming a positive impact of testosterone supplementation on erectile function, The use of testosterone supplementation in men for the treatment of hypogonadism and erectile dysfunction is questionable.<sup>[15]</sup> Many people in the world prefer the use of nature plants, there are some plants traditionally employed among different cultures to improve sexual performance<sup>[16]</sup>, e.g., Asian ginseng, an essential constituent in traditional Chinese medicine, was extensively used for its supposed property to provoke sexuality, at least six million Americans use the root of this slow-growing perennial to relieve sexual dysfunction.<sup>[17,18]</sup>

Our results demonstrated that oral administration of Maca ether, ethanol and aqueous extracts improved the copulatory performance of sexually inexperienced male mice, the ether fraction was the most effective, so we thought that less polarity fraction of Maca may be more potent in sexual performance. In the second test, the weight of seminal vesicles, an androgen-dependant organ, was significantly higher in the testes-removed mice treated with Maca extracts (high dose), which was another evidence to support Maca's supposed property to

improve sexual performance, however, serum testosterone levels were low with Maca treatment (<0.1 ng/ml), and there were not measurable differences compared with castrated control, which may suggest that either bioavailable testosterone or testosterone receptor binding might be augmented; another possibility was that Maca may act without the participation of androgen mechanism.<sup>[19]</sup> This seemed to be supported by the fact that improvement in sexual desire was observed, but serum testosterone and oestradiol levels didn't vary in men treated with Maca and in those treated with placebo.<sup>[20]</sup>

It may well be that Maca's beneficial effects for sexual function can be explained simply by its high concentration of proteins and vital nutrients. Dried maca root contains about 10% protein—mostly derived from amino acids which transmit signals in the nervous system and play a major role in the process of sexual arousal and physical performance during sex. The amino acid arginine, of which maca is a significant source, is thought to assist in the generation of nitric oxide—which is thought to counteract male impotence (although this is not clinically validated). In addition, chemical research shows that Maca root contains alkaloids and aromatic isothiocyanates, e.g., macaene, macamide and p-methoxybenzyl isothiocyanate, which have reputed aphrodisiac properties. On the basis of the available data, it was difficult to determine which of the numerous Maca biochemical components were active in sexual behavior. Our results indicated that the component of lower chemical polarity, such as alkaloids and aromatic isothiocyanates contributed more positive effect on

sexual function in mice, further molecular biochemistry studies are needed to evaluate pharmacological mechanism of the observed effect on sexual performance.

In conclusion, treatment with Maca extract improved sexual performance parameters in healthy male mice, and increased the seminal vesicles weight of castrated mice independently of changes in serum testosterone levels.

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