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EFFECT OF A LIPIDIC EXTRACT FROM *Lepidium meyenii* ON SEXUAL BEHAVIOR IN MICE AND RATS

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ABSTRACT

Objectives. To determine the effect of oral administration of a purified lipidic extract from *Lepidium meyenii* (MacaPure M-01 and M-02) on the number of complete intromissions and mating in normal mice, and on the latent period of erection (LPE) in rats with erectile dysfunction.

Methods. Mice and rats were randomly divided into several experimental and control groups. A 10% ethanol suspension of M-01 and M-02 was orally administered for 22 days to the experimental groups according to the dosage specified by the experimental design. On day 22, 30 minutes after the dose was administered to the male mice, 2 virgin female mice were placed with 1 male mouse. The number of complete intromissions of each male mouse in 3 hours was recorded. In an assessment of 1 day of mating, each male mouse was cohabited with 5 estrous female mice overnight. The number of sperm-positive females was recorded. The LPE was measured to assess the sexual function in rats with erectile dysfunction. By using a YSD-4G multifunction instrument, an electric pulse at 20 V was applied to stimulate the rat's penis, and the duration from the start of the stimulus to full erection was measured in seconds as the LPE.

Results. In the normal male mice, the number of complete intromissions during the 3-hour period was 16.33 ± 1.78, 46.67 ± 2.39, and 67.01 ± 2.55 for the control group, M-01 group, and M-02 group, respectively. In the assessment of mating, the number of sperm-positive females increased from 0.6 ± 0.7 in the control group to 1.5 ± 0.5 in the M-01 experimental group. The LPE of male rats with erectile dysfunction was 112 ± 13 seconds with a regular diet (control group). The oral administration of M-01 at a dose of 180 or 1800 mg/kg body weight and M-02 at a dose of 45, 180, or 1800 mg/kg body weight reduced the LPE to 54 ± 12 seconds, 54 ± 13 seconds, 71 ± 12 seconds, 73 ± 12 seconds, and 41 ± 13 seconds, respectively. The LPE of the surgical rats treated with M-01 at the lowest dose (45 mg/kg) was 121 ± 12 seconds; thus, the change was not significant.

Conclusions. Oral administration of M-01 and M-02 enhanced the sexual function of the mice and rats, as evidenced by an increase in the number of complete intromissions and the number of sperm-positive females in normal mice, and a decrease in the LPE in male rats with erectile dysfunction. The present study reveals for the first time an aphrodisiac activity of *L. meyenii*, an Andean Mountain herb. UROLOGY **55:** 598–602, 2000. © 2000, Elsevier Science Inc.

The plant *Lepidium meyenii* Walp, with the common name of maca, belongs to the plant family of Solanaceae. It was domesticated at least 2000 years ago in the Andean Mountains at an altitude of more than 10,000 feet.[1] Although it is a less known domesticated plant of Peru, maca roots were used by Andean Indians as food and as a folk medicine. It has been used to enhance the fertility and sexual performance of men and women and to treat women with menopausal symptoms.

There is no reliable scientific report to confirm a positive effect of maca on an increase in energy or sexual function. In 1961, some studies were conducted to determine the effect of maca in rats; however, the results were far from satisfactory.[1,2] Therefore, a systematic approach to provide scientific evidence to confirm the claims of the folk medicine is greatly needed.

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MATERIAL AND METHODS

The dried maca roots were collected from Peru in 1998. A voucher specimen was deposited in the Herbrio de Museo de Historia Natural 'J. Prado' Un. H. S. Lima, Peru. Primary extraction was carried out using methanol or ethanol and water as a solvent by way of a proprietary extraction process. The alcohol extract was further purified through a series of chromatographic processes.[3] The resulting lipidic fractions were formulated with an excipient, such as maltodextrins or tricalcium phosphate, and were dried to a powdered extract. M-01 and M-02 were two formulas standardized in the content of macaene and macamide, novel multiunsaturated fatty acids and their amides.

The macaene and macamide of the purified standardized product, M-01 and M-02, were determined by high-performance liquid chromatography, and included three new compounds, *N*-benzyl octanamide, *N*-benzyl-16-hydroxy-9-oxo-10*E*, 12*E*,14*E*-octadecatrieneamide, and *N*-benzyl-9,16-dioxo-10*E*,12*E*,14*E*-octadecatrieneamide and 17 other analogues of macaene and macamide. The product also contained 3.72% free fatty acids, which included 0.14% caprylic acid, 0.13% capric acids, 0.97% lauric acid, 0.38% myristic acid, 0.67% palmitic acid, 0.92% palmitoleic acid, 0.17% stearic acid, 0.21% oleic acid, 0.69% linoleic acid, and 0.33% linolenic acid. Other minor constituents were 0.03% to 0.04% sterols (campesterol, stigmasterol, beta-sitosterol) and 0.10% to 0.15% benzyl isothiocyanate. All the abovementioned constituents were determined by gas chromatography-mass spectrometry.

For the study of the effect of maca on mounting behavior in mice, a total of 45 male and 90 virgin female mice (a strain of Shenyang, grade II) were obtained from the Experimental Animal Center of China Medical University. The study groups included control, M-01, and M-02 groups; 15 males and 30 females were randomly placed in each group. The age range of the mice at the start of the experiment was 8 to 10 weeks; the body weight was 25 ± 1 g. Each animal was identified by ear tags or color codes. The control group received a common granulated feed in 10% ethanol suspension for 22 days. A 10% ethanol suspension of M-01 and M-02 was administered twice daily by gavage to the animals in the experimental groups at a daily dosage of 40 mg/g body weight for 22 days. On day 22, 30 minutes after the dose was administered to the male mice, 2 female mice were placed with 1 male in a cage kept in darkness. The male began to copulate immediately. After a sequence of precopulatory behavior, the male mounted the female from the rear and clasped his forelegs about her laterolumbar region. While clasping the female, the male palpated her side with rapid movements of his forelimbs, and simultaneously his pelvic region moved in rapid piston-like thrusts. After a final and unusually forceful thrust, the male lunged backward. This backward lunge was indicative of intromission or complete copulation. The number of complete intromissions within 3 hours was recorded manually by an observer.[4]

For the assessment of 1 day of mating, a strain of Beijing mice (grade II) with a body weight of 24 ± 1 g was used. Twenty male mice were randomly divided into two groups of 10. One group served as the control and received a common granulated feed. The experimental group received oral M-01 at dose of 4 g/kg body weight for 1 day only. On the same day at 5 PM, each male mouse was placed in a separate cage. After 1 hour, 5 females were admitted into each cage. The female mice were brought into estrus with a single subcutaneous dose of estradiol benzoate and progesterone. The next morning (7:00 to 8:00 AM), a vaginal smear from each female mouse was examined under a microscope for the presence of sperm. The number of sperm-positive females in each cage was recorded. The average number of sperm-positive females was calculated for the control and experimental groups.

To study the effect of maca on erectile function in rats with erectile dysfunction, 90 grade III Wistar male rats were obtained from the China Medicinal and Biological Institute. Ten rats per test article (M-01 and M-02) at three different dose levels and 10 rats each in three different vehicle control groups were used. All male rats, except the normal and testosterone-treated rats in the control group, were weighed and anesthetized using 4.5 mg/g body weight of 0.6% pentobarbital subcutaneously injected for the surgical removal of the testes. The body weight of the rats at the start of the experiment was 200 ± 20 g. After surgical removal, sodium penicillin (2000 U/kg body weight) was injected for 3 days (one injection daily). The control group consisted of three subgroups: normal rats, testes-removed rats, and testosterone-treated rats. The rats in the control group received a regular oral diet by gavage. The test articles were administered by gavage to the male rats at a dose of 45, 180, or 1800 mg/kg body weight for 20 days. The testosterone propionate control group consisted of normal rats injected subcutaneously with 20 mg/kg body weight of testosterone propionate (one injection daily for 3 days before the experiment). On day 20, 30 minutes after dose administration, each male rat was placed in a restraining device, and an electrode of the YSD-4G multifunction instrument gave an electric pulse at 20 V to stimulate the rat's penis. The latent period of erection (LPE), the time to reach a full erection from start of the stimulation, was then recorded.

Pairwise statistical comparisons between the control and treated groups were done with Student's *t* test. Mean differences were considered statistically significant if $P < 0.05$.

RESULTS

The maca extracts M-01 and M-02 were orally administered to the normal male mice experimental groups at a preliminary dose of 40 mg/g body weight for 22 days. The number of complete intromissions during the 3-hour study period (after a 22-day feeding) increased to 46.67 ± 2.39 times ($P < 0.01$) in the M-01 experimental group and to 67.01 ± 2.55 times ($P < 0.01$) in the M-02 experimental group compared with 16.33 ± 1.78 times in the control group (Fig. 1 and Table I).

In the assessment of 1 day of mating, the oral administration of M-01 at a dose of 4 g/kg body weight increased the number of sperm-positive females from 0.6 ± 0.7 in the control group to 1.5 ± 0.5 ($P < 0.01$) in the M-01 experimental group (Fig. 2 and Table II).

In the third study, three control groups were used to evaluate the LPEs of the experimental groups. The LPE of the surgical control group was 112 ± 13 seconds. The LPE of the normal control group and testosterone-treated control group was 78 ± 13 seconds and 50 ± 12 seconds ($P < 0.05$ compared with the surgical group). As predicted, the surgical rats demonstrated the weakest sexual ability, as exhibited by the longest LPE values, and the testosterone-treated rats demonstrated the strongest sexual ability, as shown by the shortest LPE values. The LPE of the surgical group was reduced to 121 ± 12 seconds, 54 ± 12 seconds,

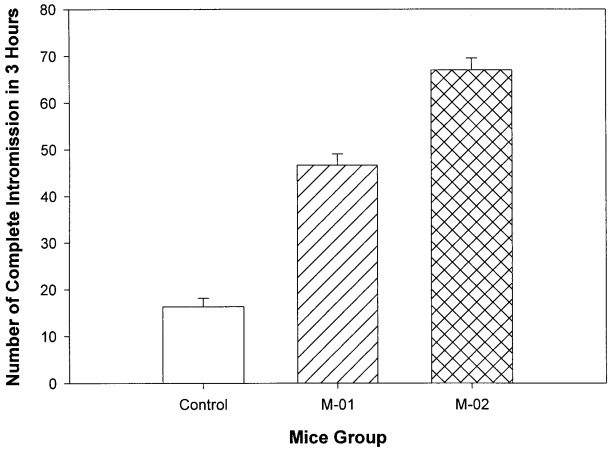

FIGURE 1. *Effect of purified powdered extract of* L. meyenii *(MacaPure M-01 and M-02) on the number of complete intromissions in normal male mice during a 3-hour period. Values presented as the mean ± SD; n = 15.* P *<0.01 for M-01 and M-02 data. The control group received a common granulated feed in a 10% ethanol solution. M-01 and M-02 were administered to normal male mice at a daily dosage of 40 mg/g body weight for 22 days.*

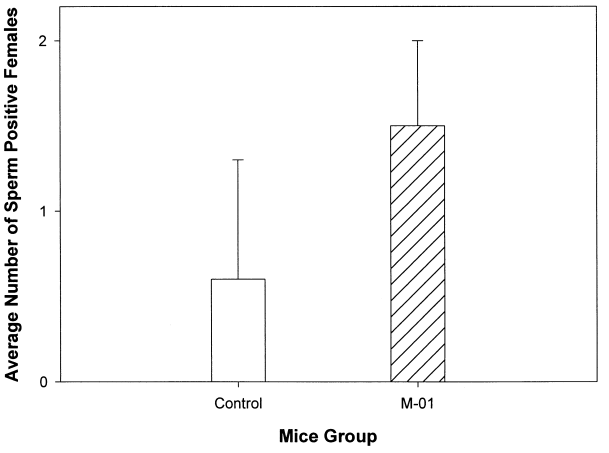

FIGURE 2. *Effect of purified powdered extract of* L. meyenii*, MacaPure M-01, on the mating performance of normal male mice. The mice in the control group received a regular feed in a 10% ethanol/water solution; mice in the M-01 group received a one-time oral dose of M-01 of 4 g/kg body weight. One hour after oral administration, each male was cohabitated with 5 estrous females overnight. The number of sperm-positive females was recorded in each case.* P *<0.01 for M-01 compared with the control group.*

TABLE I. ***Study results of mounting behavior in normal mice***

Group	No. of Complete Intromissions
Control (n = 15)	16.33 ± 1.78
M-01 (n = 15)	46.67 ± 2.39
M-02 (n = 15)	67.01 ± 2.55

Data presented as mean ± SD.

TABLE II. ***Study results of 1 day of mating in normal mice***

Group	No. of Sperm-Positive Females*
Control	0.6 ± 0.7
M-01	1.5 ± 0.5

** The control and M-01 groups each contained 10 subgroups; each subgroup consisted of 1 male and 5 female mice.*

and 53 ± 13 seconds when treated with M-01 at a dose of 45, 180, and 1800 mg/kg body weight, respectively. The LPE of the surgical group was reduced to 71 ± 12 seconds, 73 ± 12 seconds, and 41 ± 13 seconds when treated with M-02 at the same dose levels (Fig. 3 and Table III).

COMMENT

Maca was widely used during the precolonial and colonial periods of Peru under the Spaniards. Vásquez de Espinoza, who visited Peru around 1598, and Cobo, who was in Peru from 1603 to 1629, gave descriptions of the plant and its uses.[5,6] H. Ruiz of the Royal Spanish Botanical Expeditions in 1777 to 1778 found it in cultivation close to Lake Junin and briefly described its use. In modern works, it is not mentioned in most of the ethnobotanical publications. Recently in Peru, Chacón[2] and Pulgar[8] were interested in its medicinal properties.[7]

It was first observed by the Spaniards that in the Andean highlands, their domestic animals, cattle, sheep, chickens, and even their men had a reproduction rate markedly inferior to that in Spain. The chronicles frequently referred to this phenomenon and to the problems created by the lack of young animals. It was stated that Andeans recommended feeding maca to the animals and that the Spaniards noticed the positive effects of this feed. Today, maca is used by both Andean and white women who desire pregnancy.

Two Andean mountain herbs, anu and maca, are both cultivated by the Andeans for their edible underground tubers, and both carry the reputation as having putative effects on human reproductive potential. In his study of the two plants, Johns[9] demonstrated that although anu and maca belong to two different plant families, each has similarities in chemical composition—both contain glucosinolates as their major secondary metabolite. Further analysis revealed that M-01 and M-02 contain benzyl isothiocyanate as the major isothiocyanate and

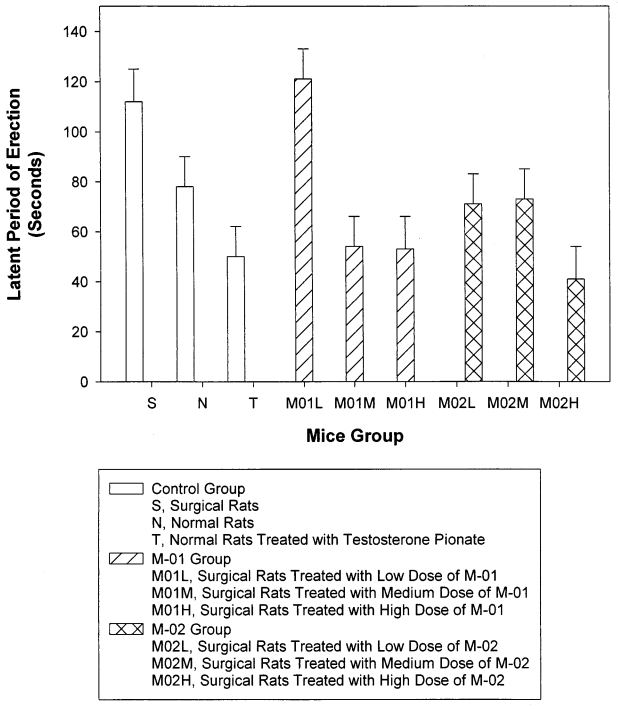

FIGURE 3. *Effect of purified powdered extract of* L. meyenii, *MacaPure M-01 and M-02, on the erectile function of male rats measured by the LPE. The control groups consisted of normal rats fed a regular diet for 20 days, surgical rats (testes surgically removed) fed a regular diet for 20 days, and normal rats treated with testosterone propionate (20 mg/kg body weight per injection, one injection daily for 3 days before the experiment). The M-01 and M-02 experimental groups were surgical rats that received twice daily oral doses of M-01 or M-02, respectively, at low (45 mg/kg body weight), medium (180 mg/kg body weight), or high (1800 mg/kg body weight) doses for 20 days. Values presented as mean ± SEM; n = 10 per group, except M01H and M02H.* P *<0.05 for all data, except the normal group and M01L group, by pairwise comparison with the surgical control group.*

TABLE III. ***Study results of latent period of erection in rats with erectile dysfunction***

Group	n	Mean ± SEM	Model Based SE
Control			
Surgical	8	112 ± 13	13
Normal	10	78 ± 12*	12
Testosterone pionate	10	50 ± 12†	12
M-01			
Low dose, M01L	10	121 ± 12*	12
Medium dose, M01M	10	54 ± 12†	12
High dose, M01H	8	53 ± 13†	13
M-02			
Low dose, M02L	10	71 ± 12†	12
Medium dose, M02M	10	73 ± 12†	12
High dose, M02H	8	41 ± 13†	13

KEY: SE = standard error; L = low dose; M = medium dose; H = high dose.
Low dose, 45 mg/kg; medium dose, 180 mg/kg; high dose, 1800 mg/kg. A statistical method of one-way analysis of variance was used.
Two animals each were considered outliers in the surgical control, M01H, and M02H groups.
* *Probability = not significant.*
† P *<0.05 by pairwise comparison results with the surgical control group.*

p-methoxybenzyl isothiocyanate in a relatively small amount. A correlation of these compounds with fertility still requires confirmation.

In 1961, Chacón[2] conducted a laboratory test to determine the effect of maca in rats. However, the experimental design was far from satisfactory. It failed to demonstrate a positive effect of maca in the increase of reproduction in rats, according to Leon's comments.[1]

The results from the present in vivo study of the effect of the two maca formulas M-01 and M-02 on the mounting behavior in normal mice illustrated that purified maca products significantly enhance the sexual libido and potency in male mice. The dose in this experiment was used as a guideline for a preliminary study. Other dose levels will be studied in the future. In the 1-day mating assessment, the one-time oral administration of M-01 at the dose of 4.0 g/kg significantly enhanced sexual ability. Typically, the dosage used in animal in vivo tests is 10 to 100 times higher than that used for humans.

The results of the in vivo study on the rats with erectile dysfunction indicated that the purified maca products, M-01 and M-02, at doses of 45, 180, and 1800 mg/kg body weight, except for the 45-mg/kg dose of M-01, were all effective in improving the erectile function of the testes-removed rats. As demonstrated by the decrease in LPE, the erectile function of the surgical group treated with the extract was significantly better than that of the surgical control group and was almost identical to that of normal rats. The decrease of LPE in treated rats might be due to the higher concentration of the macaene and macamide, a group of biologically active components. Similar LPE results after the medium and high doses of M-01 and all three doses of M-02 suggest that the concentration of the maximum possible intake by the animal might have been reached. From a perspective of phytochemistry, M-01 and M-02 are both fractionate products from maca and are similar in composition, except that M-01 contains more polysaccharide and less macaene and macamide than M-02. This may account for the smaller degree of improvement in LPE in the surgical rats treated with M-01 at the lowest dosage (45 mg/kg). Further studies to identify the active constituents responsible for the increase in the sexual function in mice and rats and

the mechanism of action are in progress. All animals except two control subgroups, the normal and the testosterone groups, were anesthetized and underwent surgery to remove the testes. It may be necessary to include a sham group to study the effects of surgery without removal of the testes in a further study of the subject.

CONCLUSIONS

Two maca formulas, M-01 and M-02, significantly enhanced the libido and sexual potency in normal mice. The number of complete intromissions during a 3-hour period in normal male mice treated with M-01 and M-02 were 2.9 and 4.1 times higher, respectively, than that of the normal mice in the control group.

Moreover, one-time oral administration of M-01 in a study of 1-day mating showed that the number of sperm-positive female mice in the experimental group was 2.5 times higher than in the control group. The results indicate that the bioavailability of the active ingredients in mice was immediate.

Finally, M-01 and M-02 improved the erectile function in rats with erectile dysfunction. The LPE of testes-removed rats that were treated with different doses of M-01 and M-02 was reduced to that of normal rats, with the exception of the LPE after the low dose of M-01. The present study reveals for the first time an aphrodisiac effect of *L. meyenii*, an Andean Mountain herb from Peru.

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