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Dose–response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats

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Abstract

Lepidium meyenii (Brassicaceae) known as Maca grows exclusively between 4000 and 4500 m over the sea level in the Peruvian central Andes. The dried hypocotyls of Maca are traditionally used as food and for its supposed fertility-enhancing properties.

A dose–response study was performed to determine the effect of 7 days oral administration of an aqueous lyophilized extract of Maca at 0.01–5 g/kg (corresponding to 0.022–11 g dry hypocotyls of Maca/kg) on body and different organ weights, stages of the seminiferous tubules, epididymal sperm count and motility, and serum testosterone and estradiol levels in rats. In doses up to 5 g extract/kg, no toxicity was observed. Almost all organ weights were similar in controls and in the Maca extract-treated groups. Seminal vesicles weight was significantly reduced at 0.01 and 0.10 g extract/kg. Maca increased in length of stages VII–VIII of the seminiferous tubules in a dose–response fashion, with highest response at 1.0 g/kg, while caput/corpus epididymal sperm count increased at the 1.0 g dose.

Cauda epididymal sperm count, sperm motility, and serum estradiol level were not affected at any of the doses studied. Serum testosterone was lower at 0.10 g extract/kg. Low-seminal vesicle weights correlated with low-serum testosterone levels ($R^2 = 0.33$; $P < 0.0001$) and low-testosterone/estradiol ratio ($R^2 = 0.35$; $P < 0.0001$). Increase in epididymal sperm count was related to lengths of stages VII–VIII. Highest effect on stages VII–VIII of the seminiferous tubules was observed at 1.0 g Maca aqueous extract/kg.

The present study demonstrated that Maca extract in doses up to 5 g/kg (equivalent to the intake of 770 g hypocotyls in a man of 70 kg) was safe and that higher effect on reproductive parameters was elicited with a dose of 1 g extract/kg corresponding to 2.2 g dry Maca hypocotyls/kg.

Keywords: Spermatogenesis; Maca; *Lepidium meyenii*; Testosterone; Estradiol

1. Introduction

Maca (*Lepidium meyenii* Walp) from the Brassicaceae family grows exclusively between 4000 and 4500 m above sea level at the Peruvian central Andes (Gonzales et al., 2001b). According to traditional beliefs reported by Spaniard chronicles in the XVII century (Cobo, 1956), Maca is a plant that enhances fertility in humans and domestic animals, which tends to be reduced after acute exposure to high altitudes.

The first evidence that Maca improved spermatogenesis was reported in male rats (Gonzales et al., 2001b). Thereafter, Gonzales et al. (2001a) demonstrated that Maca (1.5 or 3.0 g) also improved sperm count and sperm motility in normal men without affecting serum testosterone and estradiol levels.

We have studied the effect of aqueous extract of dried Maca hypocotyls in doses up to 2 g/kg body weight (Gonzales et al., 2001b, 2004). It is important to know what for effect are elicited with higher doses and how safe is consuming high amounts of Maca. A dose–response study was undertaken to determine the effect of an aqueous Maca extract at doses up

Abbreviations: BW, body weight; dl, deciliter; E_2, estradiol; ng, nanogram; R^2, coefficient of determination; T, testosterone

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to 5 g/kg body weight in male rats, corresponding to 11 g dry Maca hypocotyls/kg body weight.

2. Material and methods

2.1. Animals

Three-month-old male rats from the Holtzman strain obtained at the animal house of the Universidad Peruana Cayetano Heredia were used. Rats were allotted randomly into five groups according to the extract dose administered.

Rats were housed 4–6 per cage and maintained at 22 °C with a 12-h light:12-h dark cycle. Rats were provided with Purina Laboratory chow and tap water ad libitum.

Animals were managed and experiments were performed in accordance with the internationally accepted principles for laboratory animal use and care. The Institutional Review Board from the Universidad Peruana Cayetano Heredia approved the study.

Male rats per group received vehicle, 0.01, 0.1, 1 or 5 g/kg of lyophilized aqueous Maca extract daily during 7 days. These doses represent 0, 0.022, 0.22, 2.2, and 11.0 g of dry Maca hypocotyls/kg. The lyophilized extract or vehicle was administered by gavage with an intubation no. 18 needle (Fisher Scientific, Pittsburgh, PA). Rats were sacrificed at day 8 by decapitation and blood was collected.

2.2. Preparation of aqueous extract of Lepidium meyenii

The hypocotyls of *Lepidium meyenii*, which is cultivated by farmers, were obtained from Carhuamayo at 4000 m above sea level in Junin (Central Andes of Peru) in January 2003. The identity of the plant was authenticated by Irma Fernandez, Botanist, Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia. The hypocotyls correspond to the yellow ecotype. The voucher number IFV 1885 was deposited at the Department.

An aqueous extract of the hypocotyls was prepared according to the traditional method. In brief, 500 g of the dried pulverized hypocotyls was placed in a container with 1500 ml of water, and boiled for 120 min. The preparation was left standing to cool, filtered, and lyophilized. One gram of dried Maca hypocotyls produced 0.46 g of lyophilized Maca. The lyophilized extract was further diluted to obtain different concentrations in 1 ml. Solutions were placed in small vials and kept at 4 °C until use.

After animals were sacrificed selected organs (testes, epididymis, seminal vesicles, liver, kidney, spleen, and lungs) were carefully dissected out, cleaned of the adhering connective tissues, and accurately weighed.

2.3. Assessment of stages of rat seminiferous cycle

Assessment of the stage length was made by transillumination under an inverted stereomicroscope at 40× magnification as previously described (Gonzales et al., 2001b). For each rat, a total length of 1000 mm of seminiferous tubule was assessed. The stages assessed as described by Parvinen (1982) were pooled as stages VII–VIII, I–VI, and IX–XIV.

2.4. Epididymal sperm count and motility

Homogenization-resistant epididymal sperm from non-perfused rats were counted as described previously (Gonzales et al., 2004) with some modifications. Modifications included measurements in caput/corpus and cauda epididymides. Homogenization was performed in 5 ml saline (NaCl 0.9%). Homogenates were kept refrigerated at 4 °C for 24 h to allow sperm to be released from the walls. Then, 5 ml of eosine (2%) was added and vortexed. One milliliter of this mixture is diluted with 2 ml eosine (2%) and a sample is placed in a Neubauer chamber and head sperm counted in 25 squares. Data are referred as sperm per caput/corpus or cauda epididymis.

In the contra-lateral epididymis, a cut was done to the cauda and a drop of fluid is obtained and diluted with saline (0.9%). A drop is putted in a slide and after covered with another slide observed in a microscope at 400×. One hundred spermatozoa were counted.

2.5. Measurements of serum estradiol and testosterone

Serum estradiol and testosterone were measured by radioimmunoassay using commercial kits (Diagnostic Products Co., Los Angeles, USA). Samples were run in the same assay to avoid between assay variations. The within assay variation was 6.42% for estradiol and 5.5% for testosterone. Sensitivity of testosterone assay was 40 ng/dl and that for estradiol assay was 8 pg/ml.

2.6. Statistical analysis

Data were analyzed using the statistical package STATA (Version 8.0) for personal computer (Stata Corporation, 702 University Drive East, College Station, TX, USA).

Data are presented as mean ± standard error of the mean (S.E.M.). Homogeneity of variances was assessed by Bartlett test. If variances were homogeneous, differences between groups were assessed by two-way analysis of variance. Differences between pair of means were assessed by the Scheffé test. When variance was not homogeneous a non-parametric analysis was performed. A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of Maca on body and organ weights in male rats

After 7 days of treatments, all groups, except that treated with 5 g extract/kg increased body weight. Final

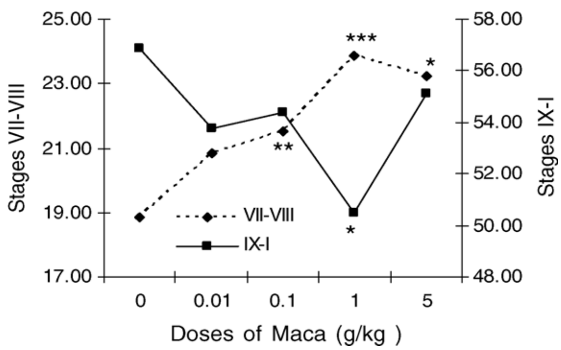

Fig. 1. Effect of different doses of Maca on lengths stage of the seminiferous epithelium in male rats after 7 days of treatment. Data show means of lengths (cm) of stages VII–VIII and IX–I. $^{*}P < 0.05$; $^{**}P < 0.01$, and $^{***}P < 0.001$ with respect to control group (0 g/kg).

body weight was similar in control rats and rats-treated with 0.01–1.0 g extract/kg. There was no evidence of toxicity after oral administration of Maca extract in doses up to 5 g extract/kg. Regarding organ weight, only seminal vesicle weight was significantly reduced at doses of 0.01 g extract/kg (from 1.01 ± 0.04 to 0.45 ± 0.15; $P < 0.01$) and 0.10 g extract/kg (from 1.01 ± 0.04 to 0.57 ± 0.11; $P < 0.01$). Regression analysis showed that low-seminal vesicles weight was related with low-serum testosterone levels ($R^2 = 0.33$; $P < 0.0001$) or low-serum testosterone/estradiol ratio ($R^2 = 0.35$; $P < 0.0001$), but not serum estradiol levels ($R^2 = 0.002$; P: NS).

3.2. *Effect of Maca on spermatogenesis assessed by transillumination*

Maca extract increased in a dose–response fashion the lengths of stages VII–VIII of the seminiferous tubules. The highest value was obtained with a dose of 1.0 g extract/kg. Thereafter, a plateau was observed. Relatively, lengths of stages IX–I were reduced (Fig. 1).

3.3. *Effect of Maca on epididymal sperm count and sperm motility*

Epididymal sperm count significantly increased in animals-treated with 1.0 g/kg of Maca extract (from 87.85 ± 6.63 to 115.26 ± 13.44; $P < 0.01$). This increase was at the level of the caput/corpus of the epididymis (from 42.60 ± 3.61 to 60.54 ± 7.30; $P < 0.05$). Sperm motility was not affected by treatment with Maca. Multiple regression analysis showed that lengths of stages VII–VIII (3.24 ± 1.44; coefficient of regression ± S.E.M.; $P < 0.033$) were related to sperm count.

3.4. *Effect of Maca on serum testosterone and estradiol levels*

Serum testosterone levels and serum testosterone/estradiol ratio were significantly reduced with 0.10 g extract/kg but not with other doses (Table 1). Serum estradiol levels were similar at different doses of Maca with respect to the control group.

4. Discussion

Herbal medicine is used by up to 80% of the population in the developing countries (Hilaly et al., 2004). In the United States, approximately one-quarter of adults reported use of herbs to treat a medical illness within the past year (Bent and Ko, 2004). Despite the widespread use, few scientific studies have been undertaken to ascertain the safety and efficacy of traditional remedies (Hilaly et al., 2004).

In the present study, Maca, a cultivated plant from highlands of Peru, administered to rats in doses up to 5 g aqueous extract/kg for 7 days was not toxic. This amount corresponds to 11 g of dry hypocotyls of Maca. The lack in toxicity was also verified when organ weights were assessed.

Although Maca is used as food, there are few studies related to its nutritional properties. In one study, mice feed cooked Maca showed a best growth curve than those fed raw Maca or control (Canales et al., 2000). In rats aged 75 days, aqueous Maca extract at doses of corresponding to 0.2 and 2 g dry hypocotyls/kg increased body weight after 7 days of treatment (Gonzales et al., 2004). In the present study using 90-day-old rats, the final body weights were similar in control and Maca-treated animals. It is possible that effect of Maca is more noticeable in growing animals.

The present investigation also shows that a lyophilized aqueous extract of Maca produced a dose–response effect on stages of the seminiferous tubules cycle particularly at stages VII–VIII. Spermiation or sperm release to the lumen

Table 1
Dose–response effect of 7 days oral administration of lyophilized aqueous extract of *Lepidium meyenii* (Maca) on serum testosterone, estradiol, and testosterone/estradiol ratio of adult male rats

Maca extract g/kg	Testosterone (ng/dL)	Estradiol (pg/ml)	Testosterone/estradiol ratio
0 ($n = 9$)	170.00 (44.00–580.00)	15.00 (8.56–21.50)	11.33 (3.33–34.12)
0.01 ($n = 6$)	59.50 (4.00–750.00)	16.50 (8.00–19.50)	4.59 (0.18–38.46)
0.10 ($n = 6$)	13.50** (4.00–76.00)	17.75 (14.00–21.00)	0.88** (0.19–4.90)
1.00 ($n = 7$)	149.00* (22.00–460.00)	17.00 (16.00–18.00)	8.28* (1.38–25.26)
5.00 ($n = 6$)	142.50 (6.90–410.00)	17.00 (10.00–19.00)	9.29* (0.38–22.78)

The data are expressed as median (minimum–maximum) and analyzed by the Mann–Whitney test.
* $P < 0.05$ with respect to Maca 0.1 g/kg.
** $P < 0.01$ with respect to control group.

of the seminiferous tubules occurs at stage VIII (Harris and Nicholson, 1998). The highest effect was observed with 1.0 g of extract/kg. Thereafter, a plateau is observed. The increase in lengths of stages VII–VIII confirms our previous finding with 2 g of dry Maca hypocotyls/kg in which length of spermiation stage increased after 7 days of treatment in 75-day-old rats (Gonzales et al., 2004).

This increase in length of stages VII–VIII was related with high-sperm count at the dose of 1.0 g/kg of extract. The higher-sperm count was due to high values at caput/corpus epididymis. This suggests that Maca at first stage increases release of sperm to caput/corpus epididymis or storage in epididymis. However, at this time, the transit to cauda epididymis is still not affected. This is in accordance with the absent effect of Maca in cauda sperm count and sperm motility. Our data also demonstrated that higher doses of Maca should be avoided, since dose of 5 g extract/kg had no further effect on epididymal sperm count. People at high altitudes in the central Andes ate about 50–100 g of dry hypocotyls per meal. Five grams of extract/kg represents about 770 g of dry hypocotyls, an amount that is higher than usual. As maximal effect is observed with a dose of 1 g extract/kg and a plateau is achieved with 5 g extract/kg, then it is not necessary to use doses higher than 1 g extract Maca/kg when an effect on sperms is required.

A significant reduction in serum testosterone levels and T/E_2 ratio was observed at 0.10 g extract/kg. Serum estradiol levels were not affected at the doses used. This low value was correlated with low values of seminal vesicles weight. Men-treated with 1.5 g (0.02 g/kg) or 3.0 g (0.04 g/kg) of powdered Maca tablets, the serum levels of testosterone and estradiol were not affected at 1–4 months treatment (Gonzales et al., 2001a, 2003). In male mice, an increase was observed in serum testosterone levels after 30 days of treatment with Maca (Oshima et al., 2003). However, it was difficult to determine the amount of Maca consumed by each mouse since Maca was mixed with food received ad libitum.

Data do not allow an explanation of the low-serum values of testosterone in rats-treated with 0.10 g/kg of Maca extract. Further studies will be required to clarify this effect. Testosterone is converted in estradiol by enzyme aromatase. In the present study, serum estradiol levels did not change after treatment with different doses of Maca.

Seminal vesicles and epididymis are both androgen-dependent (Lund et al., 2004). We have demonstrated that only seminal weight but not epididymal weight was affected. Studies with dietary isoflavones have demonstrated that seminal vesices were the most sensitive organ to testosterone stimulation (Stroheker et al., 2003). The same was observed in men (Gonzales, 2002). This suggests that low doses of Maca should be used if effect on seminal vesicles weight is expected.

The biological activity of Maca may be due to one or more of the phytochemicals present in the extract. Carbohydrate, lipid, protein, fiber, inorganic salt, amino acid, fatty acid, and sterol content of the hypocotyls of yellow Maca has been described (Dini et al., 1994). Some secondary metabolites have been identified as two new imidazole alkaloids (lepidine A and B) (Cui et al., 2003), benzylated derivatives named macamides and macaridine (Muhammad et al., 2002), glucosinolates (Dini et al., 2002; Li et al., 2001; Piacente et al., 2002). However, there is still unknown which particular metabolite has effect on the variables studied.

In conclusion, Maca aqueous extract can be considered safe in doses up to 5 g extract/kg, corresponding to some 11 g dry hypocotyls/kg. The effect on reproductive physiology may be observed at 0.10 g extract/kg of Maca extract that represents 15.4 g of dry hypocotyls for an individual of 70 kg. The highest effect was observed at a dose of 1.0 g extract/kg. Normally, an individual in the Peruvian central Andes ate about 50–100 g of dry Maca hypocotyls (0.71–1.42 g of dry hypocotyls/kg) in a single meal mainly as a juice after boiling the dry hypocotyls.

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