# Effect of alcoholic extract of *Lepidium meyenii* (Maca) on testicular function in male rats

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## Abstract

**Aim:** To evaluate the effect of the alcoholic extract of *Lepidium meyenii* (Maca) on the spermatogenesis in male rats. **Methods**: In Holtzman rats, Maca alcoholic extract (5 %) was given by oral route at doses of 48 mg/day or 96 mg/day for 7 days, 14 days and 21 days. Testicular function was assessed by measurements of lengths of different stages of seminiferous epithelia and by epididymal sperm count. **Results:** Ethanolic extract of Maca increased the length of stages IX-XI of seminiferous epithelium at treatment day 7, day 14 and day 21. Progression of spermatogenesis was evident only after day 21 when lengths of stages XII-XIV of seminiferous epithelium were increased; at day 7 and day 14, no important change in spermatogenesis was observed. Epididymal sperm count was increased with 48 mg/day at all times. With 96 mg/day an increase in sperm count was observed at day 7, but it was reduced at day 14 and day 21 of treatment. Serum testosterone levels were not affected. **Conclusion:** The alcoholic extract of Maca activates onset ant progression of spermatogenesis at 48 mg/day or 96 mg/day in rats.

## 1 Introduction

*Lepidium meyenii* (Maca) is a perennial plant cultivated in the Andean mountains over 4,000 m altitude. The root traditionally has been used for its fertility-enhancing properties as described in the Spaniard conquest Chronicles of Peru in 1653 [1]. Probably Maca was domesticated in San Blas, Junin, 1300 to 2000 years ago [2]. Recently, it has been demonstrated that aqueous extract of Maca improves spermatogenesis and semen parameters [3, 4]. In the present study, the effect of the alcoholic extract of Maca on spermatogenesis and epididymal sperm count was assessed.

## 2 Materials and methods

### *2.1 Plant material*

*Lepidium meyenii* was obtained from Carhuamayo, Junin at the Central Andes of Peru at 4,000 m altitude and authenticated by Irma Fernandez, Assistant Professor and botanist of the Department of Biochemistry, Molecular Biology and Pharmacology, Universidad Peruana Cayetano Heredia (Peru).

### *2.2 Extraction*

The fat of the dried and powdered roots of *Lepidium meyenii* (750 g) was removed with soaking in hexane at room temperature (20 ) for 3 days. The residue obtained (14.5 g) was extracted with 1,500 mL absolute ethanol for 72 h at room temperature. The filtrate was concentrated under reduced pressure at 40 with Rotovapor (Bchi, Water bath B-480, Switzerland) and the final dry extract weighed 53.1 g. The dry extract, 2.4 g and 4.8 g, were dissolved separately in 100 mL of 5 % ethanol with resultant solutions of 24 mg/mL (solution A) and 48 mg/mL (solution B).

### *2.3 Animals*

Three-month-old male Holtzman rats, weighing 295.4 g 7.8 g (SD), from the animal house of the Instituto de Investigaciones de la Altura, Universidad Peruana Cayetano Heredia were used. Animals were housed under standard conditions (12 h light/12 h dark, 22 ). Rats were fed Purina laboratory chow (Agribrands Purina Peru S.A, Lima, Peru) and tap water *ad libitum.* Purina is a standard laboratory food containing protein 18 %, carbohydrates 50 %, fat 3.5 %, fibre 6 %, calcium 0.8 %, phosphorus 0.8 %, vitamins (A, D, B12, K, E, riboflavine, niacin, panthotenic acid, choline chloride, piridoxine, thiamine, biotin, folic acid) and minerals (copper, Manganese, zinc, iodine and selenium).

### *2.4 Study protocol*

Male rats were divided at random into 3 groups of 18 animals each. Group 1 (Controls) received 2 mL/day of 5 % ethanol. Group 2 (low Maca treated) received solution A 2 mL/day (48 mg/day). Group 3 (high Maca treated) received solution B 2 mL/day (96 mg/day).

Each group was equally divided into 3 subgroups and they were treated for 7 days, 14 days, and 21 days, respectively. One day after cessation of treatment, the rats were sacrificed. The testes and epididymides were removed and cleared off the attached fat and connective tissue. The seminiferous tubules were prepared for transillumination assessment and the epididymis for sperm count.

### 2.5 *Seminiferous tubular assessment*

Assessment of the length of stages was made by transillumination under an inverted stereomicroscope at 40? magnification as previously described [5]. For each rat, a total length of 100 cm seminiferous tubules was assessed. The stages assessed included I, II-III, IV-V, VI, VII, VIII, IX-XI, XII and XIII-XIV.

### 2.6 *Epididymal sperm count*

Homogenization-resistant epididymal sperm were counted as described previously [6] with some modifi-cations. Homogenization was performed in 5 mL 0.9 % saline. Modifications included refrigeration of homogenized epididymal preparation at 4 for 24 h to allow sperm be released from the walls. Data are referred as sperm ($10^6$) per epididymis.

### *2.7 Serum testosterone*

Serum testosterone levels were determined by radioimmunoassay (RIA) using commercial kit (Diagnostic Products Co., USA) with $I^{125}$-testosterone as the radioactive marker. All samples were run in one assay period. The within assay variation was 5.5 % and the sensitivity was 4.0 pg/mL.

### *2.8 Statistical analysis*

Data were presented as meanSEM and analyzed using *t*-test and analysis of variance (one way) when more than two groups were compared. $P<0.05$ was considered significant.

## 3 Results

*3.1 Seminiferous tubular length*

Treatment with the ethanolic extract of Maca for 7 days resulted in increase of the lengths of stages VII and IX-XI and a relative reduction in stages XIII-XIV and II-III of the seminiferous tubules. At the higher dose of 96 mg/day, a reduction in stage VIII length was also observed (Table 1). After 14 days of treatment, an increase in lengths of stages VII and a relative reduction in stages II-III were observed (Table 2). The length of stage VII was increased at the dose of 48 mg/day, but not at the higher dose. After 21 days of treatment, there was an increase in stage IX-XI and a relative decrease in stage IV-V with both doses (Table 3). Briefly, Maca treatment increased the lengths of stages IX-XI seminiferous tubules at day 7 , day 14 and day 21. Progression of spermatogenesis was observed after 21 days of treatment as the lengths of stages XII-XIV were increased.

Table 1. Seminiferous tubular lengths (cm) of rats ($n$=6) treated for 7 days. $^{b}P<0.05$; $^{c}P<0.01$, compared with controls.

| Stages | Ethanol-Control | Maca 48 mg/day | Maca 96 mg/day |
|---|---|---|---|
| I | 22.450.70 | 14.880.98$^{b}$ | 20.970.14 |
| II-III | 11.320.58 | 9.220.59$^{c}$ | 7.720.23$^{b}$ |
| IV-V | 9.611.43 | 7.081.26 | 4.890.17$^{b}$ |
| VI | 7.040.61 | 9.772.28 | 7.820.23 |
| VII | 11.901.23 | 20.813.68$^{c}$ | 22.920.15$^{b}$ |
| VIII | 0.670.06 | 0.930.36 | 0.330.06$^{b}$ |
| IX-XI | 15.150.98 | 22.422.02$^{b}$ | 29.230.34$^{b}$ |
| XII | 9.261.44 | 7.131.80 | 3.570.16$^{b}$ |
| XIII-XIV | 12.610.73 | 7.751.66$^{c}$ | 2.550.06$^{b}$ |

Table 2. Seminiferous tubular lengths ( cm) of rats ($n$=6) treated for 14 days. $^{b}P<0.05$; $^{c}P<0.01$, compared with controls.

| Stages | Ethanol-Control | Maca 48 mg/day | Maca 96 mg/day |
|---|---|---|---|
| I | 31.193 .81 | 18.350.55$^{b}$ | 24.870.70 |
| II-III | 21.042.54 | 8.811.46$^{b}$ | 10.700.90$^{b}$ |
| IV-V | 13.962.24 | 8.570.94 | 9.120.24 |
| VI | 2.550.71 | 3.930.50 | 5.780.60$^{b}$ |
| VII | 12.041.55 | 30.621.47$^{b}$ | 22.420.57$^{b}$ |
| VIII | 0.750.17 | 1.670.16$^{b}$ | 1.010.18 |
| IX-XI | 12.292.56 | 17.951.49 | 19.481.73$^{c}$ |
| XII | 3.850.68 | 3.180.75 | 3.190.30 |
| XIII-XIV | 2.33.60 | 6.930.64$^{b}$ | 3.441.01 |

Table 3. Seminiferous tubular lengths (cm) of rats ($n$=6) treated for 21 days. $^{b}P<0.05$; $^{c}P<0.01$, compared with controls.

| Stages | Ethanol-Control | Maca 48 mg/day | Maca 96 mg/day |
|---|---|---|---|
| I | 27.071.90 | 14.940.20$^{b}$ | 19.090.91$^{b}$ |
| II-III | 18.731.97 | 7.690.09$^{b}$ | 10.990.98$^{b}$ |
| IV-V | 21.921.54 | 10.850.29$^{b}$ | 9.290.88$^{b}$ |
| VI | 1.160.23 | 10.270.14$^{b}$ | 8.491.47$^{b}$ |
| VII | 10.561.36 | 8.780.13 | 16.981.88$^{c}$ |
| VIII | 0.840.23 | 1.410.34 | 0.960.20 |
| IX-XI | 11.051.30 | 29.320.36$^{b}$ | 18.711.48$^{b}$ |
| XII | 4.961.09 | 7.760.56$^{c}$ | 7.101.00 |
| XIII-XIV | 3.710.67 | 8.970.09$^{b}$ | 8.371.97 |

*3.1 Epididymal sperm count*

Epididymal sperm count was significantly increased in male rats treated at the dose of 48 mg/day. At the higher dose (96 mg/day) the epididymal sperm count was increased at day 7 of treatment, but thereafter a reduction was observed (Table 4). The increase in epididymal sperm count at day 7 with the higher dose was associated with a reduction in length of stage VIII (Table 1). However increase in sperm count at day 14 and 21 in rats treated with 48 mg/day was associated with an increase in length VIII (Tables 2 and 3).

Table 4. Epididymal sperm counts (106 sperm) in male rats ($n$=6). $^{c}P<0.01$, compared with controls. $^{e}P<0.05$, $^{f}P<0.01$, compared with day 7.

| Treatment days | Ethanol-Control | Maca treated 48 mg/day | Maca treated 96 mg/day |
|---|---|---|---|
| 7 | 513.423.5 | 682.921.9$^{c}$ | 801.36.7$^{c}$ |
| 14 | 867.362.2$^{f}$ | 1108.140.3$^{c, f}$ | 611.79.3$^{c,f}$ |
| 21 | 845.213.7$^{f}$ | 941.09.5$^{c, f}$ | 640.263.4$^{c,e}$ |

*3.2 Serum testosterone*

Serum testosterone levels were not significantly affected after 21 days of treatment with both doses when compared with the Control (1.56 ng/mL0.35 ng/mL, 0.97 ng/mL0.10 ng/mL and 1.69 ng/mL0.81 ng/mL for controls, Maca at 48 mg/day and 96 mg/day, respectively)

**4 Discussion**

We have previously demonstrated that treatment with the aqueous extract of Maca for 7 days increased the length of stage VIII seminiferous tubules and the epididymal sperm count and treatment for 14 days increased the lengths of stage IX-XI, but there was no increase in sperm count; at day 21 a promotion of spermatogenesis was observed as the lengths of stages XIII-XIV were increased [3]. A dose-dependent increase in stage VIII was observed with aqueous extract of Maca at day 7 with the highest response at the dose of 666.6 mg/day [Gonzales GF *et al* Unpublished data ].

As can be seen from the present study using ethanol extract of Maca at two dose levels, 48 mg (equivalent to 666.6 mg aqueous extract) and 96 mg, there were significant differences in their effects, both in regard to the changes in the seminiferous tubular lengths and the epididymal sperm count. The length of stage VIII was not increase any more after 7 days of treatment, while with the higher dose a reduction in length was observed with an increase in epididymal sperm count. This suggest that spermiation was not related with a progression of spermatogenesis from stagesVII to stage VIII. With the aqueous extract at day 7, spermiation was increased, as well as the length of stage VIII.

The epididymal sperm count was increased at all times studied in the 48 mg/day treated group. With the 96 mg/day treated group, after an initial increase in sperm count, a reduction was observed at day 14 and day 21, suggesting a possible down regulation of the factor responsible for stages IX-XI increment.

The authors believe that the aqueous extract has a factor that increases the length of stage VIII, which is not present in the ethanolic extract. The ethanolic extract has a factor that favors spermiation and as a result, the sperm count is constantly elevated after treatment with 7 days, 14 days or 21 days with 48 mg/day. This extract has another factor that favours the onset and progression of spermatogenesis, as stages IX-XI and thereafter XIII-XIV are increased.

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