Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 103 (2006) 448–454
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (MACA) on spermatogenesis in rats

Carla Gonzales, Julio Rubio *, Manuel Gasco, Jessica Nieto, Sandra Yucra, Gustavo F. Gonzales

Department of Biological and Physiological Sciences, Faculty of Sciences and Philosophy, Instituto de Investigaciones de la Altura, Universidad Peruana Cayetano Heredia, P.O. Box 1843, Lima, Peru

Received 6 July 2005; received in revised form 16 August 2005; accepted 18 August 2005
Available online 19 September 2005

Abstract

Lepidium meyenii (Brassicaceae), known as Maca, is a Peruvian hypocotyl that grows exclusively between 4000 and 4500 m above sea level in the central Andes. Maca is traditionally employed in the Andean region for its supposed fertility-enhancing properties. The study aimed to test the hypothesis that different ecotypes of Maca (Red, Yellow and Black) after short-term (7 days) and long-term (42 days) treatment affects differentially spermatogenesis adult rats. After 7 days of treatment with Yellow and Red Maca, the length of stage VIII was increased ($P < 0.05$), whereas with Black Maca stages II–VI and VIII were increased ($P < 0.05$). Daily sperm production (DSP) was increased in the group treated with Black Maca compared with control values ($P < 0.05$). Red or Yellow Maca did not alter DSP and epididymal sperm motility was not affected by treatment with any ecotype of Maca. After 42 days of treatment, Black Maca was the only ecotype that enhanced DSP ($P < 0.05$). Moreover, Black Maca was the only that increased epididymal sperm motility ($P < 0.05$). In relation to the control group, Red Maca did not affect testicular and epididymal weight nor epididymal sperm motility and sperm count; however, prostate weight was reduced ($P < 0.05$). Black or Yellow Maca did not affect prostate weight. In conclusion, there were differences in the biological response of the three ecotypes of Maca (Yellow, Red and Black). Black Maca appeared to have more beneficial effect on sperm counts and epididymal sperm motility.

Keywords: Spermatogenesis; Maca; Ecotypes; *Lepidium meyenii*

1. Introduction

Lepidium meyenii (Brassicaceae), known as Maca, is a Peruvian hypocotyl that grows exclusively between 4000 and 4500 m altitude at the central Peruvian Andes, particularly in Carhuamayo, Junin and is used traditionally to enhance fertility. A Chronicler of the Spaniard conquest to Peru, Father Bernabe Cobo referred in the first half of the seventeenth century the first description of the enhancing-fertility property of Maca (Cobo, 1956). We found that oral administration of an aqueous extract from the hypocotyls of Yellow *Lepidium meyenii* (Yellow Maca) during 7 days (about 2 g/kg BW) increased length of spermiation stage (stage VIII) (Gonzales et al., 2004), whereas when the treatment was for 14 days, it increases lengths of stages in which first mitosis occurs (stages IX–XI) (Gonzales et al., 2001). Furthermore, we demonstrated that Maca also enhances sperm count and sperm motility in normal men without affecting serum testosterone, luteinizing hormone (LH) or follicle stimulating hormone (FSH) levels (Gonzales et al., 2001a).

Most of the experimental studies have been performed in rats administering Maca in periods of 7–21 days (Gonzales et al., 2001, 2004). The duration of the seminiferous cycle in rat is 12.5 days (Aslam et al., 1999). This means that studies did not cover two spermatogenetic cycles. After interruption of spermatogenesis by gonadotropin-releasing hormone antagonist (GnRH-A) treatment in the adult rat, the restoration of advanced spermatids (steps 17–19) occurred 42 days after termination of GnRH-A treatment (Hikim and Swerdloff, 1994). For this is necessary at least 42 days of treatment with Maca.

Moreover, it was shown that Yellow Maca restores spermatogenesis in models when spermatogenesis was diminished. For instance, oral administration of aqueous extract of Yellow Maca prevented disruption of spermatogenesis in rats exposed to high altitude (Gonzales et al., 2004). Furthermore, Yellow Maca prevented the deleterious effect of administration of Malathion on spermatogenesis in mice (Bustos-Obregón et al., 2005).

* Corresponding author. Tel.: +51 98412004; fax: +51 14821195.
E-mail address: 09008@upch.edu.pe (J. Rubio).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.08.035

Maca is a cultivated plant and different ecotypes are described according to the color of its hypocotyls. In the Department of Junin (Carhuamayo), 13 ecotypes of Maca ranging from White to Black have been described. The most frequent ecotype of Maca found in the region was the Yellow color (47.8%), the most commercially preferred (Tello et al., 1992). Yellow, Red and Black Maca obtained from Carhuamayo, Junin have different nutritional components when compared (Yllescas, 1994). For instance, Red Maca has a higher content of pure protein and potassium, and lower content of soluble direct reducing sugars, riboflavin, and iron than Black Maca, whereas Yellow Maca has intermediate values for these compounds (Yllescas, 1994). Thus, it is possible that different biological activities may be observed when different ecotypes are used.

The present study was designed to determine if treatment with Maca during 42 days increases sperm production and epididymal sperm count and motility. In addition, the different ecotypes of Maca (Yellow, Red and Black) were also investigated to examine their potential activity on spermatogenesis.

2. Materials and methods

2.1. Animal

Four-month-old male rats from the Holtzman strain obtained at the animal house of the National Institute of Health (Lima, Peru) were used. Rats were divided randomly into four groups of treatment: control (vehicle), Yellow, Red and Black Maca. Rats were housed six per cage. Rats were maintained at environmental temperature (22 °C) with a 12:12 h light/dark cycle in the animal house at the Universidad Peruana Cayetano Heredia. Rats were provided with Purina laboratory chow and tap water ad libitum.

2.2. Experimental protocol

Rats from each group (control, Yellow Maca, Red and Black Maca) were randomly assigned to two further sub-groups: one for the short-term (7 days) study, and the second for the long-term (42 days) study. Each group included six animals. This represents 24 rats in the short-term (7 days) study and 24 rats in the long-term (42 days) study.

An intubation needle no. 18 (Fisher Scientific, Pittsburgh, PA) for nasogastric feeding was used to administer 2 ml water (with or without Maca). The amount of Maca administered daily to each rat was 666.6 mg. This represents in average 1.66 g/kg BW. There were not observable differences in the response of seminiferous tubules when doses of 1 or 2 g/kg of Maca were used (Chung et al., 2005). All animal experiments were conducted in compliance with "Guide of the care and use of laboratory animals" (National Research Council, 1996). The Institutional Review Board of the Scientific Research Office from the Universidad Peruana Cayetano Heredia approved the study. At the end of the 7 or 42 days of treatment, rats were sacrificed by decapitation and a blood sample was obtained from cervical trunk. Blood samples were centrifuged and sera separated, placed in vials and kept frozen until assayed for serum hormones.

2.3. Preparation of aqueous extract of Lepidium meyenii Maca

The dried hypocotyls of *Lepidium meyenii* (Brassicaceae) were obtained from Carhuamayo, Junin at 4000 m altitude. Irma Fernandez, who is a Botanist of the Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia, authenticated the identity of the plant. The voucher number IFV 1885 was deposited at the department. Biological activity of the plant is located in the hypocotyls, that is consumed by natives after be naturally dried. Traditionally, the dried hypocotyls of Maca are boiled and served as juice.

For the present study, the aqueous extract of the hypocotyls was prepared according to the traditional method. In brief, 500 g of the pulverized dried hypocotyls were placed in a container with 1500 ml of water and boiled for 120 min. The preparation was left standing to cool and was then filtered. The filtrate, containing 333 mg root in 1 ml was placed in small vials and kept in 4 °C refrigerator.

2.4. Organ weights

Testes, epididymis, seminal vesicles, ventral prostate, kidney, spleen, lungs and heart were excised and weighed after removal of surrounding adipose tissues. Some testes and epididymis were used for sperm count, whereas others were processed for histological study.

2.5. Assessment of stages of rat seminiferous cycle

Assessment of the seminiferous epithelium tubules stage length was made by transillumination under an inverted stereomicroscope at 40× magnification as previously described (Gonzales et al., 2001). For each rat, a total length of 100 cm was assessed. The stages assessed were as follows: I, II–III, IV–V, VI, VII, VIII, IX–XI, XII, XIII–XIV as described originally by Parvinen (1982). Stage VIII, in which spermiation occurs is easily recognized as an abrupt disappearance of the dark-absorbing center of the tubule. A pale zone follows and is visible during stages IX–XII. Weak spots due to the arrangement of elongated spermatids in dense bundles, concomitant with the condensation of their nuclei, are characteristic of stages XIII–XIV. The density of the spots increases markedly at stages II–V. At stage VI, the bundle arrangement disappears and the late spermatids form a dense layer at the top of the seminiferous epithelium, resulting in a dark, homogeneous, central area in the transillumination seminiferous tubules at stages VII and VIII (Parvinen, 1982). In the present study data were grouped as stages II–VI, VII, VIII and IX–I.

The scoring frequencies of stages of the seminiferous epithelium cycle using a transillumination procedure has been validated with scoring on stained cross sections of rat seminiferous tubules (Gonzales and Del Valle, 1995). There were not differences in the frequencies of the stages of the spermatogenic cycle when measured by transillumination or by histological examination (Gonzales and Del Valle, 1995).

2.6. Daily sperm production

Testes were homogenized in 10 ml of 0.9% saline–0.05% (v/v) Triton X-100 solution for 1 min by a homogenizer (Takahashi and Oishi, 2003). After a dilution 1/10, the number of homogenization-resistant elongated spermatids nuclei per testis was determined with a hemocytometer. Counts for four hemocytometer chambers were averaged. Daily sperm production (DSP) and its efficiency (DSP/g testis) were determined by division of the elongated spermatid count per testis and spermatids per g testis by 6.3 days of spermatogenesis time during steps 17–19 spermatids for Holtzman rats (Kubota et al., 2003; Takahashi and Oishi, 2003). The epididymal sperm transit rate was calculated by dividing the cauda epididymal sperm number by the daily sperm production (Dalsenter et al., 2003).

2.7. Epididymal sperm count

Homogenization-resistant epididymal sperm from nonperfused rats were counted as described previously (Gonzales et al., 2004) with some modifications. Modifications included measurements in caput/corpus, and cauda epididymides. Caput and corpus epididymis were cutted and homogenized separately to the cauda epididymis. Homogenization was performed in 5 ml saline (NaCl 0.9%). Homogenates were kept refrigerated at 4 °C for 24 h to allow sperm be released from the walls. Then, 5 ml of eosine (2%) were 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 sperms were counted in 25 squares. Sperm counts in the 25 squares were multiplied by 0.06 (sperm $\times 10^6$ ml^{-1}) and then by 5 ml (sperm $\times 10^6$ caput^{-1} corpus^{-1} or cauda). Data are referred as sperm per caput/corpus or cauda epididymis.

2.8. Epididymal sperm motility

In the contralateral epididymis a cut was done to the cauda and a drop of fluid is obtained and diluted with phosphate buffered saline (PBS). A sample was observed in a compound microscope at 40×. One-hundred spermatozoa were counted. Data are referred as percent of motile sperm.

2.9. Hormone assays

Serum estradiol and testosterone concentrations were measured by radioimmunoassay using commercial kits (Diagnostic Products Co., Los Angeles, USA) in rats treated for 7 and 42 days with vehicle, Red Maca, Yellow and Black Maca. The hormone labeled with iodine-125 was used as radioactive marker. Samples were run in the same assay to avoid inter-assay variation. The intra-assay variation was 6.42% for estradiol, and 5.5% for testosterone. Sensitivity of testosterone assay was 4 ng/dl and for estradiol assay was 8 pg/ml.

2.10. 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 (SEM). Homogeneity of variances was assessed by the Bartlett test. If variances were homogeneous, differences between groups were assessed by analysis of variance (ANOVA). If F value in the ANOVA test was significant, the differences between pair of means were assessed by the Scheffé test.

When variable were not homogeneous, the Kruskal–Wallis test was used to assess differences between groups. If the result was statistically significant, thus, differences between pair of medians were assessed by the Mann–Whitney-U test.

A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of Red, Yellow and Black Maca on body and organ weights

After short-term (7 days) and long-term (42 days) treatment with the three ecotypes of Maca the body weights did not show differences compared to control group. There were no changes observed in the weight of left testis, left epididymis, seminal vesicles, prostate, left kidney, liver, spleen, lungs and heart after treatment with each ecotype of Maca for 7 days. Treatment with Red Maca for 42 days resulted in a reduction of the prostate weight to 0.34 ± 0.04 g respect to the control value (0.49 ± 0.03; $P < 0.05$). Prostate weight after treatment with Yellow Maca (0.41 ± 0.03 g) or Black Maca (0.48 ± 0.02 g) was similar to value in the control group. In addition, seminal vesicles weights in animals treated with Red Maca (1.37 ± 0.02 g) resulted lower than those observed in rats treated with Black Maca (1.65 ± 0.09; $P < 0.05$). Treatment with Yellow Maca (1.42 ± 0.09 g) or Black Maca did not affect seminal vesicle weight compared with the control group (1.40 ± 0.09 g). After 42 days of treatment, testicular, epididymis, kidney, liver, spleen, lungs and heart weights were similar between Maca-treated groups and control.

3.2. Stages of seminiferous cycle in rats treated with Red, Yellow and Black Maca

Fig. 1 shows the effect of oral administration of Red, Yellow and Black Maca on the lengths of stage VIII of the seminiferous tubule after 7 and 42 days of treatment.

After 7 days of treatment with Red Maca ($P < 0.05$), Yellow Maca ($P < 0.05$) or Black Maca ($P < 0.05$), the lengths of stage VIII were higher than in the control group. Treatment with Black Maca increased also lengths of stages II–VI ($P < 0.05$) and reduced relatively length of stages IX–I ($P < 0.05$) (data not shown).

After 42 days of treatment with Red Maca, stage lengths of the seminiferous tubule epithelium were similar to the control

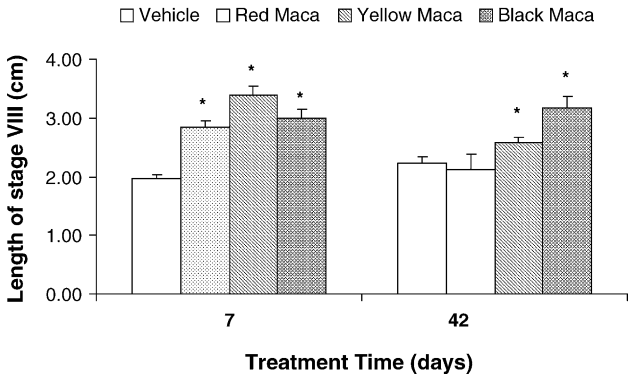

Fig. 1. Length of stage VIII (spermiation) in rats treated with three different ecotypes of Maca (1.66 g/kg BW) (Red, Yellow and Black) for 7 and 42 days. Data are mean ± SEM. $^{*}P < 0.05$ with respect to control value. Number of animals was six per group. White bars = vehicle (control group); dotted bars = Red Maca; hatched bars = Yellow Maca; cross-hatched bars = Black Maca.

group. Treatment with Yellow Maca ($P < 0.05$) or Black Maca ($P < 0.05$) both increased length of stage VIII.

3.3. Yellow and Black Maca but not Red Maca increased epididymal sperm number plus spermatids in testis

Fig. 2 shows the effect of Red, Yellow and Black Maca on epididymal sperm number plus spermatids in testis after 7 and 42 days of treatment.

In rats treated with Yellow and Black Maca during 7 days, a significant increased ($P < 0.05$) in the epididymal sperm number plus spermatids in testis was observed respect to control group. Red Maca did not have effect at this point. Also, rats treated during 42 days with Yellow and Black Maca produced high number of spermatids in testis plus epididymal sperm count compared with the control group ($P < 0.05$). Again, treatment with Red Maca did not affect the testicular spermatic count plus epididymal sperm count.

Fig. 3 shows the epididymal sperm count in rats treated with Red, Yellow and Black Maca during 7 and 42 days.

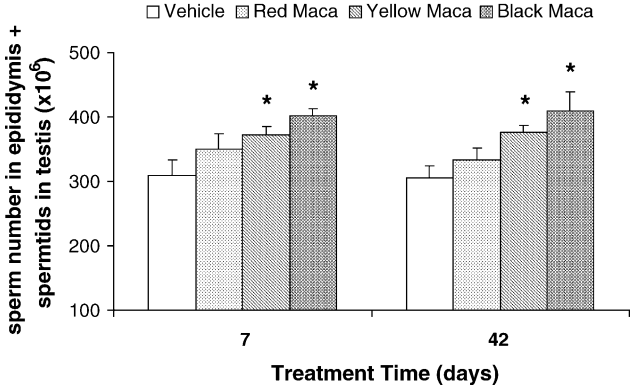

Fig. 2. Effect of short-term (7 days) and long-term (42 days) treatment with Red, Yellow and Black Maca (1.66 g/kg BW) on epididymal sperm number plus spermatids in testis in rats. Data are mean ± SEM. $^{*}P < 0.05$ with respect to control value. Number of animals was six per group. White bars = vehicle (control group); dotted bars = Red Maca; hatched bars = Yellow Maca; cross-hatched bars = Black Maca.

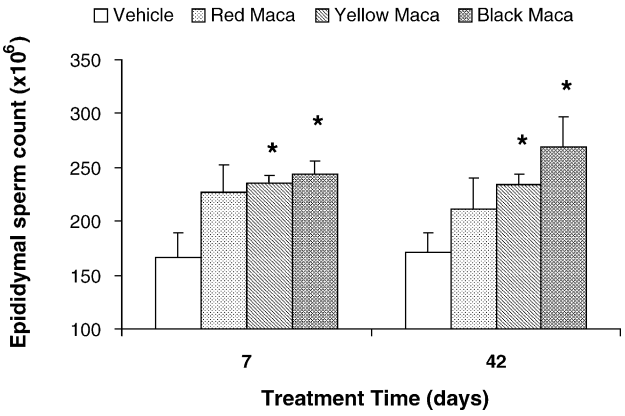

Fig. 3. Effect of short-term (7 days) and long-term (42 days) treatment with Red, Yellow and Black Maca (1.66 g/kg BW) on epididymal sperm count. Data are mean ± SEM. $^{*}P < 0.05$ with respect to control value. Number of animals was six per group. White bars = vehicle (control group); dotted bars = Red Maca; hatched bars = Yellow Maca; cross-hatched bars = Black Maca.

Epididymal sperm count in rats treated Yellow and Black Maca for 7 and 42 days increased significantly with respect to control groups ($P < 0.05$). This effect was not observed with Red Maca at any time of treatment.

3.4. Effect of Red, Yellow and Black Maca on daily sperm production, its efficiency and sperm transit rate

Fig. 4 shows the effect of three different ecotypes of Maca (Red, Yellow and Black) on DSP (Fig. 4A) and its efficiency (DSP/g testis) (Fig. 4B) in rats.

DSP (for Black Maca), and its efficiency (DSP/g testis) (for both ecotypes) were significantly increased after 7 days of treatment with Yellow or Black Maca ($P < 0.05$). Red Maca had not effect on any of these variables. Sperm transit rate did not differ in any of the ecotypes assessed (Red Maca: 5.43 ± 0.88; Yellow Maca: 5.35 ± 0.29; Black Maca: 4.72 ± 0.26) with respect to control group (4.49 ± 0.63).

After 42 days, DSP and its efficiency (DSP/g testis) were significantly higher after treatment with Black Maca than with controls. Yellow and Red Maca did not affect DSP or DSP/g testis. Sperm transit rate did not differ in any of the ecotypes assessed (Red Maca: $5.73 \pm 0.0.89$; Yellow Maca: $6.36 \pm 0.0.86$; Black Maca: $6.09 \pm 0.0.47$) with respect to control group (5.51 ± 0.89).

3.5. Sperm motility increased in Black Maca-treated rats

Fig. 5 shows the effect of oral administration of Red, Yellow and Black Maca on rat sperm motility after 7 and 42 days of treatment.

Epididymal sperm motility was unchanged after 7 days of treatment with any of the ecotypes of Maca. However, after 42 days of treatment with Black Maca, the sperm motility was significantly higher compared to other groups ($P < 0.05$).

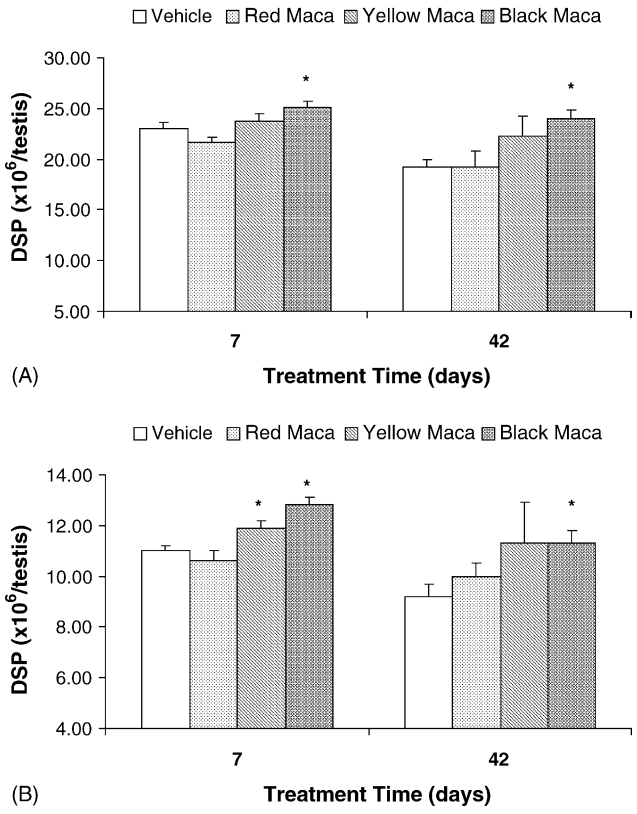

Fig. 4. DSP (A) and DSP/g testis (B) in rats treated for 7 and 42 days with Red, Yellow and Black Maca (1.66 g/kg BW). Data are mean ± SEM. $^{*}P < 0.05$ with respect to control value. Number of animals was six per group. White bars = vehicle (control group); dotted bars = Red Maca; hatched bars = Yellow Maca; cross-hatched bars = Black Maca.

3.6. Ecotypes of Maca and serum hormone levels

After 7 days of treatment, serum testosterone, estradiol, and the ratio testosterone/estradiol levels were similar between control group (197.47 ± 58.96, 10.39 ± 2.39 and 21.11 ± 7.39, respectively) and groups treated with Red (256.37 ± 54.76, 8.28 ± 0.21 and 30.57 ± 6.09, respectively), Yellow (245.14 ± 68.60, 11.20 ± 1.70 and 24.73 ± 5.82, respectively) or Black Maca (152.19 ± 25.35, 10.17 ± 1.21 and 16.52 ± 3.72, respectively). Also, rats treated for 42 days did not showed any change in serum testosterone, estradiol and the ratio testosterone/estradiol levels in groups treated with Red (153.82 ± 90.23, 12.21 ± 1.36 and 10.89 ± 5.41, respectively), Yellow (131.08 ± 40.16, 12.66 ± 1.02 and 10.65 ± 3.03, respectively) or Black Maca (110.01 ± 32.46, 13.18 ± 1.53 and 7.85 ± 1.99, respectively) with respect to control group (116.07 ± 40.54, 9.87 ± 0.85 and 11.62 ± 4.40, respectively).

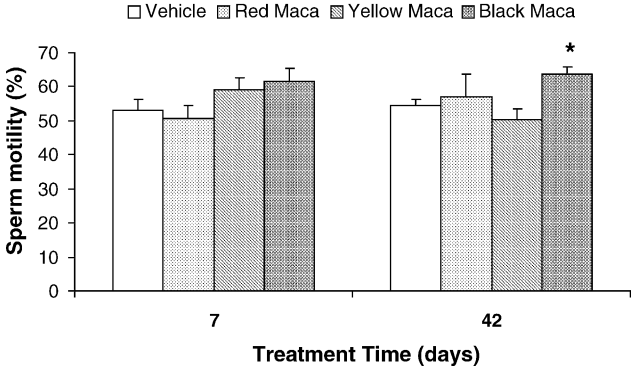

Fig. 5. Sperm motility (%) in rats treated with three different ecotypes of Maca (1.66 g/kg BW) for 7 and 42 days. Data are mean ± SEM. $^{*}P < 0.05$ with respect to control value. Number of animals was six per group. White bars = vehicle (control group); dotted bars = Red Maca; hatched bars = Yellow Maca; cross-hatched bars = Black Maca.

4. Discussion

Maca a traditional food crop from the Peruvian highlands (Balick and Lee, 2002) is used by its supposed libido stimulant effect (Zheng et al., 2000; Cicero et al., 2001, 2002; Balick and Lee, 2002), and its effect on fertility (Cobo, 1956). Maca is naturally presented in different ecotypes which are characterized by their external color (Tello et al., 1992; Yllescas, 1994). In the present study we have assessed the biological effects of three ecotypes: Red, Yellow and Black.

Maca is broadly used in Peru as a nutrient (Canales et al., 2000). However we were unable to find differences in body weight with any of the three ecotypes assessed after 42 days of treatment. Certainly, body weight was not different in the control group or in the Maca-treated groups. Analysis also revealed that different ecotypes of Maca did not affect weight of kidney, liver, spleen, lungs or heart. This suggests that none of the three ecotypes of Maca were toxic in the concentration administered (about 1.66 g/kg).

Red Maca reduced significantly prostate weight. Yellow and Black Maca did not present this effect. This finding confirms previous results in our laboratory in which Red Maca prevented the increase in prostate size induced by testosterone enanthate (Gonzales et al., 2005). Other Brassicas as *Lepidium latifolium* also reduced prostate weight (Martinez Caballero et al., 2004).

Taking into account the effect of Maca on spermatogenesis, the present study showed that Maca increased daily sperm production after short-term (7 days) and long-term (42 days) treatments. However, the effects were not the same for the three ecotypes of Maca. Black Maca produced the highest effect, Yellow Maca an intermediate effect but Red Maca had not any effect on spermatogenesis. The main effect of Maca was on lengths of stage VIII of the seminiferous epithelium tubules. In fact, stage VIII was higher after 7 (Red, Yellow and Black Maca) and 42 (Yellow and Black Maca) days of treatment with Maca. The method of measurement of stage length by transillumination has some limitations. Although this measurement could reveal changes in stage frequency, it could also reflect changes in tubule architecture (such as swelling of the epithelium) or changes in spermatid populations as incorrect chromatin condensation, phagocytosis of spermatids or cell sloughing would all affect the identification of stages by transillumination.

It is not possible for Maca to affect spermatogonia and change sperm count within 7 days. One could speculate that spermiation is enhanced; however quantitative studies on spermiation in Sprague Dawley rats show that at least 97% of spermatids are spermiated in normal adult rats (Saito et al., 2000), meaning

that enhancement of an already successful process would be difficult.

It is unknown the mechanism of action of Maca to increase length of stage VIII. An effect on apoptosis is less probably to occur since in the normal adult rat testis, the germ cells are least at risk of degeneration as they pass through stage VII (Kerr, 1992). Duration of stages I–VII in the rat was 8.43 days (Wen and Yang, 2000), and then treatment with Maca for 7 days should be enough to demonstrate an effect on spermiation. It is possible that Maca may be acting through spermiation. However, this needs to be demonstrated.

In the long-term study (42 days), Yellow and Black Maca also increased spermatid counts in testis and sperm count in epididymis. Red Maca did not have effect on these variables. The present study demonstrated that treatment with Maca during 42 days, period required for a complete restoration of advanced spermatids (steps 17–19) (Hikim and Swerdloff, 1994) resulted in high epididymal sperm count than in rats of the control group. Spermatids at step 19 are observed in stages VII and VIII of the seminiferous epithelium (França et al., 1998). This increased epididymal sperm count was not related to changes in the sperm transit rate.

Daily sperm production (DSP) seems to be a better indicator of sperm output than length of seminiferous tubules or epididymal sperm count. The present study demonstrated that treatment with Black Maca increase DSP after 7 and 42 days of treatment, whereas treatment with Yellow Maca did not increase DSP after 7 or 42 days of treatment. The changes to testicular spermatid numbers are modest at best however more marked changes in epididymal counts are seen.

From data obtained in the present study, Maca had different biological effects according to the ecotype. From the three ecotypes studied only Yellow and Black Maca increased epididymal sperm count after 7 and 42 days of administration. Black Maca increased DSP at 7 and 42 days. Only Red Maca had not effect on spermatogenesis. Highest efficiency was also observed with Black Maca. Since sperm transit rate was not affected with Red, Yellow or Black Maca, it appears that Maca may be affecting epididymal function.

Among the three ecotypes studied, the best reproductive effect was observed with Black Maca. In fact treatment for 42 days with Black Maca resulted in higher DSP/testis, higher DSP/g, high epididymal sperm count, and higher epididymal sperm motility. The use of extract of plants as an alternative for treatment of the infertile man has recently been emphasized (Comhaire and Mahmoud, 2003). Thus, Black Maca may become in a potential treatment of male infertility. Yellow Maca had an intermediate effect, whereas Red Maca had not effect on these parameters. This is not the only biological difference between Maca ecotypes. Recently, it has been demonstrated that Red Maca reduced prostate size in normal rats, and it prevented the increase in prostate size induced by testosterone enanthate (Gonzales et al., 2005).

Previously, it was demonstrated under infrared spectroscopy that absorbance peaks were different between Black, Yellow and Red Maca (Gonzales et al., 2005). The IR spectra of the three ecotypes of Maca present seven peaks in the 3800–650 cm^{-1} region. Highest peak values were observed for Red Maca, intermediate values for Yellow Maca and low values for Black Maca (Gonzales et al., 2005). From this and biological study, it is suggested that Black Maca shares principles with Yellow Maca, and that Yellow Maca shares principles with Red Maca.

It is still unknown the active secondary metabolites present in the plants responsible for the Maca actions. Some novel compounds have been recently identified, as two new imidazole alkaloids (lepidine A and B) (Cui et al., 2003). Also, a benzylated, named macaridine, derivative of 1,2-dihydro-*N*-hydroxypyridine, together with the benzylated alkamides (macamides), *N*-benzyl-5-oxo-6E,8E-octadecadienamide and *N*-benzylhexadecanamide, as well as the acyclic keto acid, 5-oxo-6E,8E-octadecadienoic acid have been described (Muhammad et al., 2002). However, fertility-enhancing properties of these compounds have not been assessed.

5. Conclusions

Indeed, the data presented here show differences biological effects among the three ecotypes of Maca assessed (Red, Yellow and Black), being Black Maca the ecotype that presented most beneficial effects improving spermatogenesis. Hence, it is proposed that Black Maca may become a potential treatment for male infertility.

Acknowledgements

The authors thank Sharon Castillo and Francisco Chung for technical support. This study was supported by the Vicerrectorate of Investigation of the Universidad Peruana Cayetano Heredia. Carla Gonzales received a fellowship grant for a training at the Instituto de Investigaciones de la Altura supported by the Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana (PLACIRH). Julio Rubio is receiving a grant for a fellowship at the Instituto de Investigaciones de la Altura supported by the Training and Research Training in Environmental and Occupational Health from the Fogarty Programme between University of Emory and Universidad Peruana Cayetano Heredia.

References

Aslam, H., Rosiepen, G., Krishnamurthy, H., Arslan, M., Clemen, G., Nieschlag, E., Weinbauer, G.F., 1999. The cycle duration of the seminiferous epithelium remains unalteRed during GnRH antagonist-induced testicular involution in rats and monkeys. Journal of Endocrinology 161, 281–288.

Balick, M.J., Lee, R., 2002. Maca: from traditional food crop to energy and libido stimulant. Alternative and Therapeutic and Health Medicine 8, 96–98.

Bustos-Obregón, E., Yucra, S., Gonzales, G.F., 2005. *Lepidium meyenii* (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice. Asian Journal of Andrology 7, 71–76.

Canales, M., Aguilar, J., Prada, A., Marcelo, A., Huaman, C., Carvajal, L., 2000. Nutritional evaluation of *Lepidium meyenii* (MACA) in albino mice and their descendants. Archivo Latinoamericano de Nutrición 50, 126–133.

Chung, F., Rubio, J., Gonzales, C., Gasco, M., Gonzales, G.F., 2005. Dose–response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats. Journal of Ethnopharmacology 98, 143–147.

Cicero, A.F., Bandieri, E., Arletti, R., 2001. *Lepidium meyenii* Walp. improves sexual behaviour in male rats independently from its action on spontaneous locomotor activity. Journal of Ethnopharmacology 75, 225–229.

Cicero, A.F., Piacente, S., Plaza, A., Sala, E., Arletti, R., Pizza, C., 2002. Hexanic Maca extracts improves rat sexual performance more effectively than methanolic and chloroformic Maca extracts. Andrologia 34, 177–179.

Cobo, B., 1956. History of the New World. Ed. Francisco de Mateos. Ediciones Atlas, Madrid.

Comhaire, F.H., Mahmoud, A., 2003. The role of food supplements in the treatment of the infertile man. Reproductive Biomedicine Online 7, 385–391.

Cui, B., Zheng, B.L., He, K., Zheng, Q.Y., 2003. Imidazole alkaloids from *Lepidium meyenii*. Journal of Natural Products 66, 1101–1103.

Dalsenter, P.R., de Araujo, S.L., de Assis, H.C., Andrade, A.J., Dallegrave, E., 2003. Pre and postnatal exposure to endosulfan in Wistar rats. Human and Experimental Toxicology 22, 171–175.

França, L.R., Ogawa, T., Avarbock, M.R., Brinster, R.L., Russell, L.D., 1998. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biology of Reproduction 59, 1371–1377.

Gonzales, G.F., Del Valle, L., 1995. Adult rat seminiferous tubules secrete a fraction greater than 30 kDa to regulate spermatogenesis. Human Reproduction 10, 1435–1443.

Gonzales, G.F., Ruiz, A., Gonzales, C., Villegas, L., Cordova, A., 2001. Effect of *Lepidium meyenii* (Maca) roots on spermatogenesis of male rats. Asian Journal of Andrology 3, 231–233.

Gonzales, G.F., Córdova, A., Gonzales, C., Chung, A., Vega, K., Villena, A., 2001a. Improved sperm count after administration of *Lepidium meyenii* (Maca) in adult men. Asian Journal of Andrology 3, 301–304.

Gonzales, G.F., Gasco, M., Córdova, A., Chung, A., Rubio, J., Villegas, L., 2004. Effect of *Lepidium meyenii* (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). Journal of Endocrinology 180, 87–95.

Gonzales, G.F., Miranda, S., Nieto, J., Fernandez, G., Yucra, S., Rubio, J., Yi, P., Gasco, M., 2005. Red Maca (*Lepidium meyenii*) reduced prostate size in rats. Reproductive Biology and Endocrinology 3, 5.

Hikim, A.P., Swerdloff, R.S., 1994. Time course of recovery of spermatogenesis and Leydig cell function after cessation of gonadotropin-releasing hormone antagonist treatment in the adult rat. Endocrinology 134, 1627–1634.

Kerr, J.B., 1992. Spontaneous degeneration of germ cells in normal rat testis: assessment of cell types and frequency during the spermatogenic cycle. Journal of Reproduction and Fertility 95, 825–830.

Kubota, K., Ohsako, S., Kurosawa, S., Takeda, K., Qing, W., Sakawe, M., Kawakami, T., Ishimura, R., Tohyama, C., 2003. Effects of vinclozolin administration on sperm production and testosterone biosynthetic pathway in adult male rat. Journal of Reproduction and Development 49, 403–412.

Martinez Caballero, S., Carricajo Fernandez, C., Perez-Fernandez, R., 2004. Effect of an integral suspension of *Lepidium latifolium* on prostate hyperplasia in rats. Fitoterapia 75, 187–191.

Muhammad, I., Zhao, J., Dunbar, D.C., Khan, I.A., 2002. Constituents of *Lepidium meyenii* "Maca". Phytochemistry 59, 105–110.

National Research Council, 1996. Guide of the Care and Use of Laboratory Animals. National Academy Press, Washington, DC, p. 125.

Parvinen, M., 1982. Regulation of the seminiferous epithelium. Endocrine Review 3, 404–417.

Saito, K., O'Donnell, L., McLachlan, R.I., Robertson, D.M., 2000. Spermiation failure is a major contributor to early spermatogenic suppression caused by hormone withdrawal in adult rats. Endocrinology 141, 2779–2785.

Takahashi, O., Oishi, S., 2003. Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. Food and Chemical Toxicology 41, 1035–1044.

Tello, J., Hermann, M., Calderón, A., 1992. La Maca (*Lepidium meyenii* Walp.) cultivo alimenticio potencial para las zonas altoandinas. Boletín de Lima 14, 59–66.

Wen, X.H., Yang, Z.W., 2000. Quantitative (stereological) study on the spermatozoal storage capacity of epididymis in rats and monkeys. Asian Journal of Andrology 2, 73–77.

Yllescas, M., 1994. Estudio químico y Fisicoquímico de tres ecotipos de *Lepidium meyenii* procedentes de Carhuamayo. Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima, Peru.

Zheng, B.L., He, K., Kim, C.H., Rogers, L., Shao, Y., Huang, Z.Y., Lu, Y., Yan, S.J., Qien, L.C., Zheng, Q.Y., 2000. Effect of lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology 55, 598–602.