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Lepidium meyenii (Maca) reversed the lead acetate induced—Damage on reproductive function in male rats

Julio Rubio [a,b,*], Marissa I. Riqueros [a], Manuel Gasco [a,b], Sandra Yucra [a,b], Sara Miranda [a], Gustavo F. Gonzales [a,b]

[a] *Department of Biological and Physiological Sciences, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru*
[b] *Instituto de Investigaciones de la Altura, Universidad Peruana Cayetano Heredia, Lima, Perú*

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Abstract

Rats were treated with 0, 8, 16 and 24 mg/kg of lead acetate (LA) (i.p.) for 35 days with or without Maca. Maca was co-administrated orally from day 18 to day 35. The lengths of stages of the seminiferous epithelium were assessed by transillumination. Also, sex organ weights, testicular and epididymal sperm count, sperm motility, daily sperm production, sperm transit rate and serum testosterone levels were measured. Lead acetate treatment resulted in a dose–response reduction of lengths of stages VIII and IX–XI, and serum testosterone levels. However, rats treated with 8 and 16 mg/kg but not 24 mg/kg of lead acetate showed a low number of testicular spermatids, low daily sperm production (DSP) and low epididymal sperm count. Administration of Maca to rats treated with lead acetate resulted in higher lengths of stages VIII and IX–XI with respect to lead a cetate-treated rats. Moreover, treatment with Maca to lead acetate-treated rats resulted in lengths of stages VIII and IX–XI similar to the control group. Maca administration also reduced the deleterious effect on DSP caused by lead acetate treatment. Maca prevented LA-induced spermatogenic disruption in rats and it may become in a potential treatment of male infertility associated with lead exposure.

Keywords: Lead acetate; *Lepidium meyenii*; Maca; Spermatogenesis; Daily sperm production

1. Introduction

Lead is a heavy metal, crystalline and with a slightly sweet flavor, water soluble and toxic. Lead is used as an additive in the manufacture of several commercial products such as paintings, dyers, plastics, and some types of gasoline (Srianujata, 1997; Sanín et al., 1998; Tong et al., 2000).

Several studies indicate that reproductive function can be damaged by lead exposure (Gustafson et al., 1989; McGregor and Mason, 1990; Lerda, 1992; Pinon-Lataillade et al., 1995; Alexander et al., 1996; Apostoli et al., 1998; Telisman et al., 2000; Bonde et al., 2002; Eibensteiner et al., 2005). Also, lead administration to adult male rats (Sokol et al., 1985; Sokol, 1987, 1989, 1990; Nathan et al., 1992; Murthy et al., 1995; Piasecka et al., 1996; Gorbel et al., 2002; Batra et al., 2004) and mice (Godowicz and Galas, 1992; Wadi and Ahmad, 1999; Graca et al., 2004) adversely affect male reproductive function.

Some of the effects of lead in the organism have been suggested to be related to the generation of reactive oxygen species (Hsu et al., 1997, 1998a; Gurer and Ercal, 2000; Aykin-Burns et al., 2003; Marchlewicz et al., 2004; Ni et al., 2004), and the treatment with antioxidant compounds may be useful to counteract the deleterious effect of lead on different systems (Hsu et al., 1998b; Kowalczyk et al., 2003; Dipti et al., 2003; Acharya et al., 2003; Mishra and Acharya, 2004; Shalan et al., 2005).

* Corresponding author. Address: Department of Biological and Physiological Sciences, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, Lima, Peru. Fax: +00 51 1 4821195.
E-mail address: 09008@upch.edu.pe (J. Rubio).

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Maca (*Lepidium meyenii* Walp) which belongs to the Brassicaceae family grows exclusively between 4000 and 4500-m above sea level at the Peruvian central Andes (Gonzales et al., 2001b). Spaniard chronicles in century XVII (Cobo, 1956) described the use of Maca by natives from the Central Peruvian Andes to enhance fertility in humans and domestic animals. This plant is extensively used in the Peruvian Central Andes because its nutritive property (Valerio and Gonzales, 2005). The biological activity of the plant is located in the hypocotyls that are consumed by natives after natural drying. Traditionally, the dried hypocotyls of Maca are boiled and served as juice (Valerio and Gonzales, 2005).

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

Moreover, it was shown that Maca restore spermatogenesis in models when spermatogenesis was diminished. For instance, oral administration of aqueous extract of Maca prevented disruption of spermatogenesis in rats exposed to high altitude (Gonzales et al., 2004). Furthermore, Maca (2 g/kg BW) prevented the deleterious effect of administration of Malathion (80 mg/kg) on spermatogenesis in mice (Bustos-Obregón et al., 2005).

Maca has been demonstrated to have antioxidant properties in vitro and in vivo (Sandoval et al., 2002; Lee et al., 2005). For such reason, the present study was designed to determine whether the treatment with Maca may be useful for the treatment of the deleterious effect of lead acetate (LA) administration on spermatogenesis in rats.

2. Materials and methods

2.1. Animals

Three-month-old male rats of the Holtzman strain obtained from the animal house of the Universidad Peruana Cayetano Heredia were used for this study. Rats were housed 3–4 per group and maintained in polycarbonated cages at environmental temperature (20–22 °C), humidity between 80% and 85% and a 12:12 h light/dark cycle. 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).

The animals were treated according to the standards of the National Institute of Health for the care and use of laboratory animals (National Research Council, 1996). All experiments were approved by the Institutional Review Board at the Universidad Peruana Cayetano Heredia.

2.2. Lead preparation

Lead acetate (LA) was obtained from SIGMA Laboratories (St Louis, MO, USA). In order to achieve the required dose, the lead acetate was diluted with saline (0.9%). The doses used in the present study were: 8, 16 and 24 mg LA/kg.

2.3. Preparation of aqueous extract of Lepidium meyenii (Maca)

The dried hypocotyls of *Lepidium meyenii* were obtained from Carhuamayo, Junin at 4000 m altitude.

For the present study, the aqueous extract of the hypocotyls was prepared according to the traditional method. First, 500 g of the dried hypocotyls were pulverized and placed in a container with 1500 ml of water, and boiled for 120 min. Next, the preparation was left standing to cool and filtered. Finally, the filtrate containing 333 mg of dry Maca hypocotyls in 1 ml was placed in small vials and kept in 4 °C refrigerator. For this study, a same lot of Maca has been used for the entire study. Therefore, after preparing aqueous extract of Maca it is expected that a similar amount of active principle is present per unit of volume.

2.4. Treatment

The animals were allocated ($n = 7$) in the following groups:

Group A: Control (Saline 0.9%).
Group B: Lead acetate at 8 mg/kg BW.
Group C: Lead Acetate at 16 mg/kg BW.
Group D: Lead Acetate at 24 mg/kg BW.
Group E: Lead Acetate at 8 mg/kg BW + 2 ml Maca (2.2 g/kg).
Group F: Lead acetate at 16 mg/kg BW + 2 ml Maca (2.2 g/kg).
Group G: Lead acetate at 24 mg/kg BW + 2 ml Maca (2.2 g/kg).

Control group (Group A) received saline i.p. during 35 days, and 1 ml water daily from days 18 to 35 by gavage. Groups B, C and D were injected with 8, 16 and 24 mg/kg of lead acetate (LA). Groups E, F and G received both LA and aqueous extract of Maca. In the groups treated with LA, this was injected from day 1 to day 35. Maca was administered by gavage, with an intubation needle No. 18 (Fisher Scientific, Pittsburgh, PA), from day 18 to day 35. In this study, the i.p. route for lead administration was chosen as it is less stressful to rats and blood concentrations reached simulate human levels (Mobarak and P'an, 1984).

We have used a time of treatment with lead of 35 days as previously reported by other authors (Thoreux-Manlay et al., 1995). The aim of the present study was to observe if Maca reversed the effect of lead acetate administration. The idea was to disrupt first reproductive function with lead and at the middle of the period of treatment Maca was administered for 18 days. Eighteen days represents 1.5 spermatogenic cycles. Then we tried to demonstrate that effect of lead during 3 spermatogenic cycles may be reverted after treatment with Maca for 1.5 spermatogenic cycles (18 days).

2.5. Reproductive organs weight

One day after last Maca administration, rats were sacrificed by decapitation for blood collection and the following reproductive organs were removed and weighed: testes, epididymis, seminal vesicles and ventral prostate. Blood was collected from the cervical trunk for testosterone assay.

2.6. Assessment of the stages of the rat seminiferous cycle

Assessment of the length of the stages of the seminiferous tubule epithelium was made by transillumination under an inverted stereomicroscope at 40× magnification as previously described (Gonzales et al., 2001b). A total length of 100 cm was assessed for each rat. The stages assessed were as follows: I, II–III, IV–V, VI, VII, VIII, IX–XI, XII and 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