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

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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).

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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

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).

This technique has been demonstrated to correlate with histological study (Gonzales and Del Valle, 1995). The length of the tubule was measured using a micrometer plate below the Petri plate in which tubules are placed. We have calibrated the methods through coefficient of variations for each stage.

2.7. Epididymal sperm count and motility

For sperm count, after being extracted from the rat, cleaned of fat and weighed the right epididymis was separated in caput/corpus, and cauda epididymides. The each part was cut in small pieces and homogenized with a Polytron homogenizer. Sperm count was done as described by Gonzales et al. (2004).

For sperm motility, a cut to the distal end of the cauda epididymis was made. Sperm with epididymal fluid was diluted with phosphate buffered saline at room temperature (PBS) and forward sperm motility immediately assessed using a compound microscope at 40×. One-hundred spermatozoa were counted. Data are referred as percent of motile sperm (motile sperm/total sperm × 100). Motility was defined as sperm with forward sperm motility. Forward sperm motility was assessed as described previously by Srikanth et al. (1999).

2.8. Spermatid count, daily sperm production (DSP) and sperm transit rate

One testis per rat was homogenized in 10 ml of 0.9% saline-0.05% (v/v) Triton X-100 solution for 1 min with 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) was 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 (Takahashi and Oishi, 2003; Kubota et al., 2003). The epididymal sperm transit rate was calculated by dividing the cauda epididymal sperm number by the daily sperm production (Dalsenter et al., 2003).

2.9. Serum testosterone levels

Serum testosterone levels were determined by RIA using ^{125}I-testosterone as the radioactive marker. The assay was performed using a commercial kit (Diagnostic Products Co., Los Angeles, CA, USA). All samples were run in the same assay period. The within-assay variation was 5.5% and sensitivity was 4.0 ng/dl.

2.10. Statistical analysis

Data were analyzed using statistical package STATA (version 8.0) for personal computer (STATA Corporation, College Station, TX, USA). Data are presented as means ± SEM. Homogeneity of variances was assessed by the Bartlett test. If variances were homogeneous, differences between groups were assessed by analysis of variance (ANOVA). The differences between pair of means were assessed by the Scheffé test. When variances were not homogeneous, the differences between groups were assessed by the Mann–Whitney-U test. Correlation analysis between serum testosterone levels and stages VII–VIII and IX–X were done. A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Reproductive organ weights

Table 1 shows the effect of Maca on absolute reproductive organ weights in male rats treated with three different doses of LA with or without Maca.

Rats treated with lead acetate at 8 and 16 mg/kg showed lower absolute testis weight than the control group ($P < 0.05$). No difference with control group was observed when rats were treated with lead acetate at 24 mg/kg (P:NS: not significant). Maca treatment reversed the deleterious effect of lead acetate on testis weight. In fact, absolute testicular weights from groups with lead acetate plus Maca were higher ($P < 0.05$) than testis weights in rats treated with lead acetate alone. No differences were observed in the testis weight between rats treated with vehicle and rats treated with lead acetate plus Maca (P:NS). Absolute epididymal weight was not modified by lead acetate treatment (P:NS). Maca also does not affect epididymal weight in rats treated with lead acetate (P:NS). Absolute seminal vesicles weight was lower in rats treated with 16 mg/kg of LA. This effect was not reversed with Maca. Absolute ventral prostate weight was lower in rats treated with lead acetate at the three doses used but without a dose–response effect. This reduction was also observed in the rats treated with LA plus Maca ($P < 0.05$). No differences in ventral prostate weights were found between rats treated with LA and LA plus Maca (Table 1).

Table 1
Effect of Maca on reproductive organs weight in male rats treated with three different doses of lead acetate

	Control (NaCl 0.9%)	8 mg/kg LA	16 mg/kg LA	24 mg/kg LA	8 mg/kg LA + Maca	16 mg/kg LA + Maca	24 mg/kg LA + Maca
Left testis	1.62 ± 0.05	1.46 ± 0.06[*]	1.43 ± 0.07[*]	1.55 ± 0.04	1.69 ± 0.04[a]	1.70 ± 0.07[b]	1.75 ± 0.07[c]
Left epididymis	0.53 ± 0.02	0.48 ± 0.01	0.47 ± 0.03	0.49 ± 0.01	0.55 ± 0.02	0.49 ± 0.02	0.52 ± 0.02
Seminal vesicle	1.22 ± 0.05	1.14 ± 0.13	0.97 ± 0.14	0.77 ± 0.08[*,a]	1.15 ± 0.10	0.79 ± 0.14[*,d]	0.66 ± 0.21[*,d]
Ventral prostate	0.54 ± 0.04	0.40 ± 0.03[*]	0.39 ± 0.04[*]	0.42 ± 0.03[*]	0.38 ± 0.03[*]	0.37 ± 0.05[*]	0.27 ± .04[*]

Data are presented as mean ± SEM.
[*] $P < 0.05$ respect to Control group.
[a] $P < 0.05$ respect to rats treated with 8 mg/kg of LA.
[b] $P < 0.05$ respect to rats treated with 16 mg/kg of LA.
[c] $P < 0.05$ respect to rats treated with 24 mg/kg of LA.
[d] $P < 0.05$ respect to Maca-treated rats with 8 mg/kg of LA.

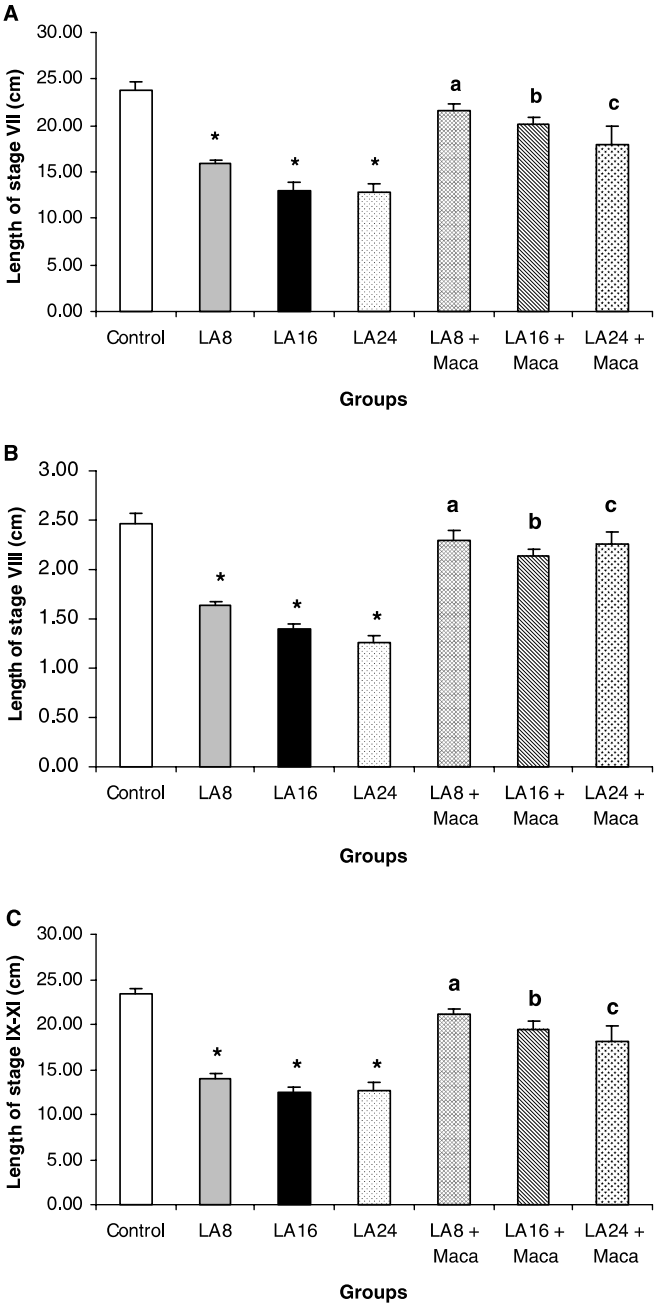

Fig. 1. Effect of Maca on stages VII (A), VIII (B) and IX–XI (C) of spermatogenesis in rats treated with 8, 16 and 24 mg/kg of LA. Data are presented as mean ± SEM. $^{*}P < 0.05$ respect to Control group; $^{a}P < 0.05$ respect to rats treated with 8 mg/kg of LA; $^{b}P < 0.05$ respect to rats treated with 16 mg/kg of LA; $^{c}P < 0.05$ respect to rats treated with 16 mg/kg of LA.

3.2. Stages of spermatogenic cycle

Fig. 1 shows data related to the effects of LA alone and when administered with (Maca) on lengths of stages VII, VIII and IX–XI of the rat seminiferous tubules.

Lead acetate reduced the lengths of stages VII, VIII (spermiation) and IX–XI (onset of mitosis) ($P < 0.05$) as compared with the control group. Lengths of stages VII+ VIII decreased in a dose dependent fashion ($R^2 = 0.71$; $P < 0.05$). Also, stages IX–XI decreased in a dose–response manner ($R^2 = 0.58$; $P < 0.05$). As stages VII–XI were reduced, relatively the other stages were increased ($P < 0.05$). When lengths of stages II–VI were grouped, a dose–response effect was also observed ($R^2 = 0.64$; $P < 0.05$). Length of stage I was not affected by any dose of LA administered.

Treatment with LA at 8 mg/kg or 16 mg/kg plus Maca resulted in improvement in lengths of stages VII, VIII and IX–XI with respect to rats treated with 8 mg/kg or 16 mg/kg respectively of LA alone ($P < 0.05$). Compared to rats treated with 24 mg/kg of LA alone, an increase in lengths of stages I, VII, VIII and IX–XI were observed ($P < 0.05$) in rats treated with LA plus Maca. Relatively, lengths of stages IV–V and XII ($P < 0.05$); lengths of stages IV–V, VI and XII ($P < 0.05$) and lengths of stages II–III, IV–V, VI and XII ($P < 0.05$) were decreased in rats treated with 8, 16 or 24 mg/kg of LA plus Maca respectively ($P < 0.05$).

3.3. Epididymal sperm count and motility

Table 2 shows the effect of Maca on epididymal sperm count and motility in rats treated with different doses of LA for 35 days.

Sperm counts in caput/corpus epididymis were lower in rats treated with 8, 16 or 24 mg/kg of lead acetate with respect to the control group ($P < 0.05$). Sperm counts in male rats treated with 8 and 16 mg/kg of LA plus Maca showed higher values than rats treated with 8 and 16 mg/kg of LA alone ($P < 0.05$). Rats treated with 24 mg/kg of LA plus Maca did not differ from rats in control group (P:NS).

In cauda epididymis, lower sperm numbers were observed in the group treated with lead acetate at doses of 8 and 16 mg/kg when compared with the control group ($P < 0.05$). Rats treated with 8 and 16 mg/kg of lead acetate plus Maca showed higher sperm count than rats treated with 8 and 16 mg/kg of lead acetate alone ($P < 0.05$). Rats treated with 24 mg/kg of LA plus Maca did not differ from rats in the control group (P:NS).

Lead acetate administration did not modify sperm motility as values were similar to control group (P:NS). Similarly, rats treated with lead acetate plus Maca showed similar values for sperm motility than the control group (Table 2).

3.4. Spermatid count, daily sperm production (DSP) and sperm transit

In rats treated with lead acetate with doses of 8 and 16 mg/kg, both the spermatid count and the DSP per testis decreased as dose increased ($P < 0.05$). However, spermatid count and DSP per testis in rats treated with 24 mg/kg of lead acetate were significantly higher than rats treated with 8 and 16 mg/kg of lead acetate ($P < 0.05$) and with values similar to rats in the control group (P:NS). When

Table 2
Effect of *Lepidium meyenii* (Maca) on epididymal sperm count ($\times 10^6$) and motility (%) in rats treated with three different doses of lead acetate

	Control (NaCl 0.9%)	8 mg/kg LA	16 mg/kg LA	24 mg/kg LA	8 mg/kg LA + Maca	16 mg/kg LA + Maca	24 mg/kg LA + Maca
Caput/corpus epididymal sperm count	80.28 ± 2.95	45.00 ± 4.17*	41.67 ± 6.72*	57.07 ± 4.33*	72.03 ± 6.19[a]	61.63 ± 8.35[b]	74.15 ± 7.36
Cauda epididymal sperm count	82.37 ± 7.38	51.93 ± 4.78*	46.81 ± 5.91*	69.14 ± 5.92	72.76 ± 4.40[a]	68.28 ± 9.20[b]	61.05 ± 12.85
Total epididymal sperm count	162.50 ± 8.82	96.93 ± 8.33*	88.48 ± 12.29*	126.21 ± 9.54*	144.79 ± 9.97[a]	129.90 ± 16.73[b]	135.20 ± 18.00
Sperm motility	55.83 ± 3.59	47.71 ± 5.32	50.20 ± 5.34	55.71 ± 3.66	61.43 ± 10.35	59.36 ± 4.90	50.50 ± 3.59

Data are presented as mean ± SEM.
* $P < 0.05$ respect to Control group.
[a] $P < 0.05$ respect to rats treated with 8 mg/kg of LA.
[b] $P < 0.05$ respect to rats treated with 16 mg/kg of LA.

Table 3
Effect of *Lepidium meyenii* (Maca) on testicular spermatid count, DSP and sperm transit in rats treated with three different doses of lead acetate

	Control (NaCl 0.9%)	8 mg/kg LA	16 mg/kg LA	24 mg/kg LA	8 mg/kg LA + Maca	16 mg/kg LA + Maca	24 mg/kg LA + Maca
Spermatid count ($\times 10^7$/testis)	12.62 ± 0.77	10.31 ± 0.90*	8.73 ± 0.46*	12.30 ± 0.55[b]	12.29 ± 1.18[a]	11.15 ± 1.24[b]	9.50 ± 1.19
DSP ($\times 10^7$/testis)	2.01 ± 0.12	1.64 ± 0.14*	1.38 ± 0.07*	1.95 ± 0.09[b]	1.95 ± 0.19[a]	1.77 ± 0.20[b]	1.51 ± 0.19
Sperm transit (days)	4.18 ± 0.40	3.35 ± 0.43	3.35 ± 0.40	3.52 ± 0.24	3.98 ± 0.53	4.10 ± 0.53	4.67 ± 1.68

Data are presented as mean ± SEM.
* $P < 0.05$ respect to Control group.
[a] $P < 0.05$ respect to rats treated with 8 mg/kg of LA.
[b] $P < 0.05$ respect to rats treated with 16 mg/kg of LA.

Maca was added to the group treated with 8 and 16 mg/kg of lead acetate, spermatid count and DSP were significantly higher than the groups treated with lead acetate alone ($P < 0.05$).

Sperm transit rate did not differ in all groups of treatment with lead acetate when compared with the control group (P:NS). Similarly, sperm transit rate was not affected by treatment with Maca (Table 3).

3.5. Serum testosterone levels

Fig. 2 shows the effect of LA on rats treated with or without Maca on serum testosterone levels. Testosterone levels decreased in LA-treated rats in a dose–response fashion ($R^2 = 0.55$; $P < 0.05$) with respect to control group ($P < 0.05$). Also, rats treated with LA plus Maca showed lower values than control group ($P < 0.05$).

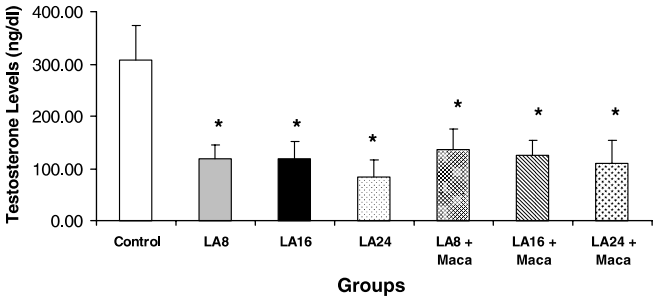

Fig. 2. Effect of Maca on serum testosterone levels in male rats treated with three different doses of lead acetate (LA). Data are presented as mean ± SEM. *$P < 0.05$ respect to Control group.

In LA-treated rats, correlation analysis showed that length of stages VII–VIII ($R^2 = 0.47$; $P < 0.05$) and IX–XI ($R^2 = 0.65$; $P < 0.05$) were related to testosterone levels (Fig. 3).

4. Discussion

Several studies found that spermatogenesis is affected by lead exposure (Chowdhury et al., 1984; Sokol et al., 1985; Sokol, 1989; Chowdhury et al., 1984; Murthy et al., 1995; Batra et al., 2001). Also, epididymal sperm count and motility (Hsu et al., 1998a,b), sperm status of capacitation and acrosome reaction (Hsu et al., 1997) were negatively affected by lead administration.

In this study, administration of lead acetate decreased lengths of stages VII–VIII and IX–XI. Final maturation of spermatids occurs from stages I to VII (Kangasniemi et al., 1990) suggesting that lead acetate arrest spermatogenesis at stages VII and VIII (spermiation) and relatively increasing lengths of stages II–VI.

Data from the present study shows that length of stages VIII and IX–XI in rats treated with Maca plus LA presented similar values to control group. This suggests that Maca reversed the effects of LA administration on spermatogenesis by protecting onset of mitosis (stages IX–XI) and spermiation (stage VIII).

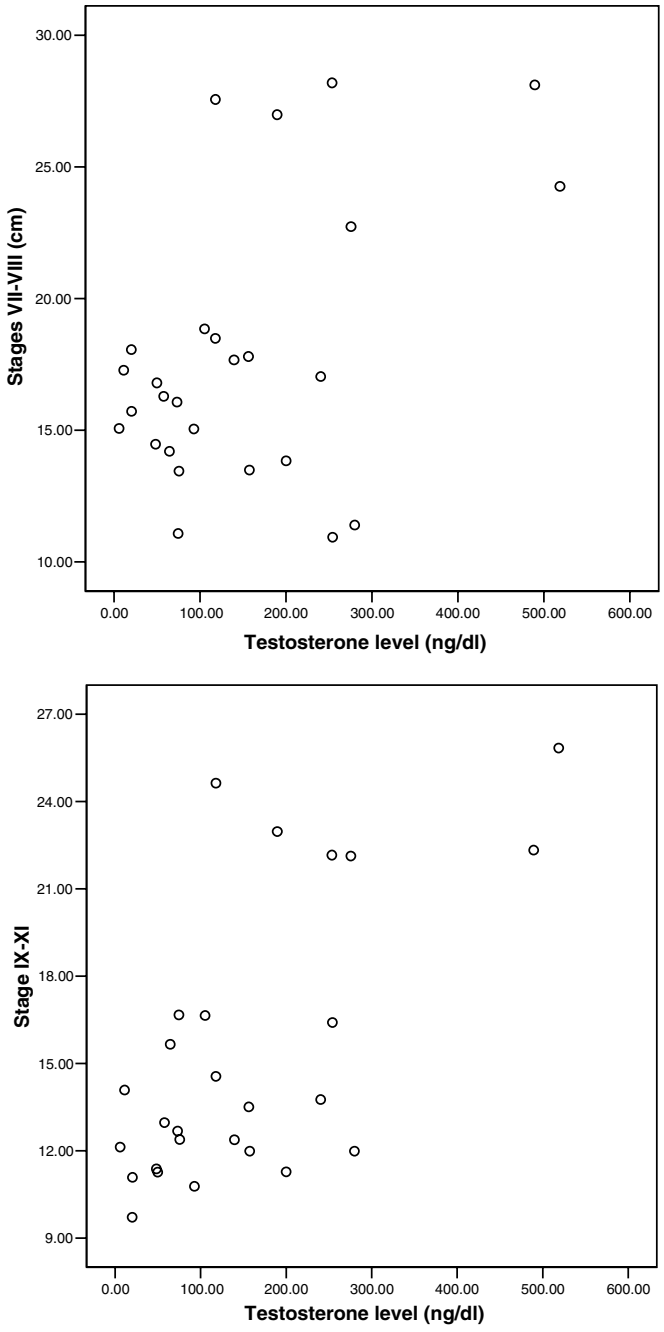

Fig. 3. Correlation analysis between serum testosterone level and stages VII–VIII ($R^2 = 0.47$; $P < 0.05$) and IX–XI ($R^2 = 0.65$; $P < 0.05$).

Previous studies found a correlation between the level of lead exposure and intratesticular sperm count in rats treated with lead in drinking water (Sokol et al., 1985; Sokol, 1989). Data obtained in the present study confirmed this finding at doses of 8 and 16 mg/kg of lead acetate. Other researchers described the effect of a mixture of persistent contaminants including lead chloride and they observed no effects over DSP and its efficiency in rats (Wade et al., 2002). To sum up, it was observed no changes over DSP in rabbits exposed to a mixture of common chemical contaminants in drinking water which include lead acetate as one of the components (Veeramachaneni et al., 2001).

Rats treated with 8 and 16 mg/kg of LA plus Maca showed higher values of spermatids count and DSP/testis with respect to LA-treated rats reaching similar values to control group. The fact that Maca increases onset of spermatogenesis (stages IX–XI) (Gonzales et al., 2004) may explain the recovery in spermatids number and DSP/testis in rats treated with LA plus Maca. As lengths of stages VIII were increased when Maca was administered, it is possible that Maca is preventing apoptosis of the developing germ cells such that more germ cells progress through the spermatogenesis. We have observed relatively per 1000 mm high amounts of stages VIII after treatment with Maca. Under light microscopy we observed a higher amount of sperms in the lumen of the seminiferous tubules from rats treated with Maca (Gonzales et al., 2006).

Rats treated with 8 and 16 mg/kg of lead acetate showed a reduction in cauda epididymis sperm count but in caput–corpus epididymis the reduction is observed with all doses of lead used. The latter makes that total epididymal sperm count diminished with 8, 16 and 24 mg/kg of lead acetate in respect to control group. Previously, it was demonstrated that lead produces more oxidative stress in caput epididymis than in testis or cauda of epididymis, in that order (Marchlewicz et al., 2004). This may explain the differences in sperm counts produced by lead acetate between caput and cauda epididymis.

As we have previously demonstrated that transillumination technique reflects very well data from histology (Gonzales and Del Valle, 1995), the assessment of stages of the cycle of the seminiferous epithelium by transillumination was used in this study. Moreover, the end-points in spermatogenesis are the values of DSP and epididymal sperm counts since they reflect what are happening on testis. Results showed that lead at two doses reduced DSP and epididymal sperm count whereas Maca returned to control values.

The lack of effect at the 24 mg dose of lead acetate was not surprising. Several studies have found that effects of lead on reproductive system depend of dose and time of exposure. For instance, treatment with lead acetate to male rats for 15 and 45 days resulted in reduction of absolute and relative weights of testis, epididymis, prostate and seminal vesicles. However, at day 60, these absolute and relative weights returned to control values (Gorbel et al., 2002). It is suggested that the lead exposed animal is able to adapt to the metal's toxic effects. An attenuation of the increase in GnRH mRNA levels with a greater dose of lead exposure without a significant change in the levels of plasma GnRH and LH support the conclusion that the male Sprague–Dawley rat adapts to the toxic effects of lead on the hypothalamus (Sokol et al., 2002). This U-shaped adaptation to lead has been observed in MA-10 mouse Leydig tumor cells (Huang and Liu, 2004). Lead showed inhibitory effects with quadratic curves (U-shaped) on the progesterone production (Huang and Liu, 2004). These authors suggest that MA-10 cells might adapt to the effects of lead on steroidogenesis after treatment of long duration and high concentration. We have also observed

this U-shaped response when testicular weight, DSP and epididymal sperm counts were assessed. The mechanism of this effect needs further investigation.

Maca prevents the deleterious effect of LA on sperm number. In fact, rats treated with 8 and 16 mg/kg of LA plus Maca increased sperm number in epididymis with respect to rats treated with 8 and 16 mg/kg of LA without Maca. Previous studies demonstrated that oral administration of aqueous extract of Maca resulted in higher epididymal sperm counts in rats (Gonzales et al., 2004; Chung et al., 2005). These may suggest that Maca may increase the release of sperm to epididymis and/or storage on epididymis. Also, an increase in epididymal sperm number may be due by the fact that spermiation was enhanced in rats treated with LA plus Maca.

It was demonstrated that intratesticular testosterone levels are decreased in rats treated with lead acetate (Sokol et al., 1985; Sokol, 1989; Thoreux-Manlay et al., 1995; Hsu et al., 1998a), therefore we suggest that decreased lengths of stages VII–VIII were caused by a reduction in intratesticular testosterone levels. Supporting this hypothesis, other researchers concluded that lead alters sperm function by altering the hormonal control of spermatogenesis rather than by direct toxic action on spermatozoa (Sokol et al., 1994). Also, correlation analysis shows that stages VII–VIII (spermiation) and IX–XI (onset of spermatogenesis) were related to testosterone levels in LA-treated rats suggesting that low lengths of stages VIII and IX–XI by effect of lead could be associated to low serum testosterone levels. It was observed that rats injected intraperitoneally with lead acetate (8 mg/kg/day) for 35 days dropped plasma and testicular testosterone by about 80% without changes in germ cells and epididymal function (Thoreux-Manlay et al., 1995). This was not observed in the present study. Also, in the present study we have observed that serum testosterone was related to stages VII and VIII. Maca was unable to affect serum testosterone levels suggesting that lead may affect sperm count through a mechanism not related to a reduction in testosterone levels.

There are not studies on effect of Maca on Leydig cell function. However, several studies showed that Maca does not affect serum testosterone levels (Valerio and Gonzales, 2005). One study showed that intratesticular testosterone was not affected by treatment with Maca (submitted to Andrologia). Moreover, studies from others showed that Maca does not act through androgen receptor (Bogani et al., 2005).

Maca was unable to prevent the reduction in seminal vesicles or prostate weights. This could be to the fact that Maca has been shown to decrease prostate weights (Gonzales et al., 2005). Moreover, Maca did not prevent the effect of LA administration on testosterone levels, this outcome may be due to the fact that previous studies demonstrated that Maca did not affect testosterone levels in rats (Gonzales et al., 2003b, 2005) and humans (Gonzales et al., 2003a).

There is still unknown the active principles for the action of Maca. The only compound that is metabolized after oral administration was glucosinolates which after action of gut mirosinase is converted to isothiocyanates. However, these compounds are anti-proliferatives and the effects in the present study were related to proliferative and/or anti-apoptotic effects (Valerio and Gonzales, 2005). Then, it is probable that active principles were different from isothiocyanates or its metabolites.

5. Conclusions

In conclusion, data obtained from this study confirm the effect of lead in male reproduction. Also, we observed that Maca protect spermatogenesis by increasing lengths of stages VIII and IX–XI and DSP, that result in an increase in epididymal sperm number. Hence, *Lepidium meyenii* (Maca) may become in a potential treatment of male infertility associated with lead exposure.

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