Blackwell Publishing

Asian J Androl 2005; 7 (1): 71–76
DOI: 10.1111/j.1745-7262.2005.00006.x

AJA

·*Original Article*·

Lepidium meyenii (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice

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Abstract

Aim: To observe the effect of the aqueous extract of hypocotyls of the plant *Lepidium meyenii* (Maca) on spermatogenic damage induced by the organophosphate insecticide malathion in mice. **Methods:** Mice were treated with 80 mg·kg^{-1} of malathion in the presence or absence of an aqueous extract of Maca, which was orally administered 7, 14 or 21 days after injection of the malathion. Stages of the seminiferous epithelium were assessed by transillumination on days 0, 7, 14 and 21. **Results:** The administration of Maca increased significantly the length of stage VIII on days 7, 14 and 21 of treatment compared with the controls. An increase in the length of stage IX occurred on day 14 of treatment. Malathion affected spermatogenesis by reducing the lengths of stage IX on day 7, stages VII and IX–XI on day 14 and a recovery of stages IX–XII on day 21. The magnitude of alteration in the length of stage IX produced by malathion was significantly reduced by Maca on days 7 and 14. The length of stage VIII was increased when Maca was administered to mice treated with malathion. Assessment of the relative length of stages of the seminiferous epithelium showed that Maca treatment resulted in rapid recovery of the effect of malathion. **Conclusion:** Maca enhances spermatogenesis following spermatogenic damage caused by the organophosphorous pesticide. *(Asian J Androl 2005 Mar; 7: 71–76)*

Keywords: malathion; spermatogenesis; *Lepidium meyenii* (Maca); mice; seminiferous epithelium stages

1 Introduction

Malathion [S-1, 2-bis (ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate] is a commonly used organophosphorus insecticide. Administration of malathion to experimental animals resulted in sperm abnormalities [1–3]. Malathion in doses of 2–10 mg·kg^{-1} in mice did not affect sperm count, although sperm abnormalities and genotoxicity were observed [1]. The administration of a single dose of malathion [240 mg·kg^{-1} (1/12 LD_{50})] resulted in seminiferous tubule atrophy on day 8 post injection (one seminiferous epithelial cycle in mice) measured as epithelial height and tubular diameter [3]. However, the epididymal sperm count was not affected by the administration of 2–240 mg·kg^{-1} malathion [1, 3]. The effects of lower doses of malathion on spermatogenesis have not been reported.

Recently, the effect of drugs on spermatogenesis has been studied in male rats using a transillumination technique to determine the lengths of stages of the seminiferous tubule epithelium [4, 5]. The wave of the seminiferous epithelium can be visualized by transillumination of freshly unstained specimens [6] and it is possible to detect changes in the lengths of stages induced by different pharmacological treatments that inhibit [4] or improve

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Received 2004-06-15 Accepted 2004-10-12

spermatogenesis [5]. This technique has been demonstrated to correlate with histological data [4].

The aqueous extract of hypocotyls of *Lepidium meyenii* (Maca), a plant of the Brassicaceae family growing in the Andean region of Peru (at an altitude of over 3000 meters) has been demonstrated to improve spermatogenesis in normal rats [5].

In the present study we applied this method to assess the lengths of seminiferous epithelium stages in mice. We attempted to determine the lengths of the stages of the seminiferous epithelium cycle after a single injection of 80 mg·kg^{-1} malathion in adult mice in the presence or absence of the aqueous extract of hypocotyls of *Lepidium meyenii* (Maca).

2 Materials and methods

2.1 Animals

Forty-eight male Asnell mice (10–12 weeks old) obtained from the Faculty of Medicine, the University of Chile, were used. Animals were separated at random into three groups: one receiving Maca, the second receiving Maca plus malathion and the third receiving only malathion (80 mg·kg^{-1}). Each treatment group was subdivided into four groups of four animals each (Days 0, 7, 14 and 21). Animals were housed under standard conditions (12 h light/12 h night, 22 °C) and provided with food and tap water *ad libitum*. At the end of the experiments animals were killed by cervical dislocation.

2.2 Preparation of aqueous extract of Lepidium meyenii

Lepidium meyenii is a cultivated plant in the Peruvian Central Andes. The hypocotyls of *Lepidium meyenii* were obtained from Carhuamayo, Junin at 4000 meters altitude (Junin, Peru). The identity of the plant was authenticated by Irma Fernandez, a botanist from the Universidad Peruana Cayetano Heredia (Lima, Peru). An aqueous extract of the hypocotyls was prepared according to a traditional method used by natives in Carhuamayo. In brief, the dried hypocotyls were pulverized and boiled for 30 min. The preparation was left standing to cool and then filtered. The filtrate was prepared to obtain a concentration of 333 mg·mL^{-1} and was placed in small vials and kept in a refrigerator at 4 ºC until use.

2.3 Treatments

Commercial malathion (96 % w/v in corn oil; ANASAC, Santiago, Chile) was used. Appropriate dilution was performed with phosphate buffered saline solution (PBS). Animals were killed on days 0, 8, 15 and 22 of the experiment. The dose of 80 mg·kg^{-1} was selected from our previous study, in which 80 mg·kg^{-1} clearly demonstrated an effect on spermatogenesis [7].

Using intubation needle No. 18 (Fisher Scientific, Pittsburgh, USA) for gastric feeding, mice received orally 0.2 mL (66.6 mg of Maca) daily for 7, 14 or 21 days. This dose (about 2 g·kg^{-1} BW) is similar to that used previously in male rats [5]. Malathion was injected intraperitoneally (approximately 200 mL). A group of mice received Maca alone, another group received Maca plus malathion (80 mg·kg^{-1}) on day 0 and a third group received only malathion (80 mg·kg^{-1}) on day 0. Mice treated with malathion alone, also received orally the corresponding vehicle used to administer Maca. All animals were sacrificed one day after the last oral treatment (Days 8, 15 or 22). According to our previous studies, there were no differences in the length of stages of the seminiferous epithelium found in the control groups on days 0, 7, 14 and 42 [7]. For that reason, in the present studies the mice were killed on day 0 as control group for each treatment.

2.4 Assessment of the stages of the mouse seminiferous cycle

After killing the animals, their testes were removed and decapsulated. The seminiferous tubules were dissected free from interstitial tissue using fine forceps. Assessment of the length of stages was made by transillumination under an inverted stereomicroscope at 40× magnification. For each mouse, a 600-mm seminiferous tubule was assessed. The length of each stage in each 600-mm tubule was recorded.

There are 12 stages in a mouse's spermatogenic cycle. Spermatogenesis lasts 33 days and each spermatogenic cycle 8.45 days [8]. Stages were classified as I, II–III, IV–V, VI, VII, VIII, IX, X–XI, and XII. At stage VIII, when the dark homogeneous central absorption abruptly ceases, spermiation occurs. Stages IX–XI were characterized by pale absorption and stage XII by weak spots in the central part of the seminiferous tubules. Definite spotty configuration is observed at stage I. During stages II to V there is an increase in spot density, including an extension of the spots towards the tubular periphery.

Stage VI is a transition between the spot- and continuous darkness patterns. Stage VII is recognized as a

continuous strong absorption in the center of the tubule [9]. On this basis, four main absorption zones may be recognized: pale (stages IX–XI), weak spot (stages XII–I), strong spots (stages II–VI) and a zone of strong homogeneous central absorption (stages VII–VIII) [9].

2.5 Data analysis

Data obtained from the experimental groups are presented as relative values with respect to values obtained in the control group of animals killed on day 0 and are expressed as mean ± SEM in the tables. To calculate relative values, the mean control value at each stage was defined as 1 [4].

Data from each stage in each treatment were compared with the corresponding control value. When variances were homogeneous, one-way analysis of variance was used in the statistical assessment of these comparisons. In this case, the Scheffee test was used to detect differences between pairs of means. When data from a given stage was compared between the malathion group and the malathion plus Maca group, the *t*-test was used. $P < 0.05$ was considered statistically significant.

3 Results

The relative lengths of stages of the seminiferous tubule epithelium are presented in Tables 1–3. Data related to the effect of Maca on stages of the seminiferous epithelium are presented in Table 1. The administration of Maca resulted in increased lengths of stages IV–V, VII, VIII, X–XI and XII on day 7 of treatment (Table 1) as compared with the controls. On day 14, Maca treatment increased the relative lengths of stages II–III, IV–V, VIII and IX. On day 21, Maca increased the relative lengths of stages II–III, IV–V, VI, VII, VIII and XII. The relative length of stage I at all times and of stage IX on days 7 and 21 was less than the control.

Data related to the effect of a single injection of

Table 1. Relative length of stages of seminiferous tubule epithelium in mice on the day after last treatment with Maca. Length of each stage in control animals killed on day 0 was taken as 1 to calculate the relative length in experimental groups. Maca (66.6 mg·0.2 mL^{-1}) was administered daily by oral route for 7, 14 or 21 days. Data in mean ± SEM. $^bP < 0.05$, $^cP < 0.01$, compared with controls.

Stages	Days of Maca administration		
	7	14	21
I	0.54 ± 0.05^c	0.70 ± 0.05^c	0.73 ± 0.05^c
II–III	1.12 ± 0.05	1.16 ± 0.05^c	1.55 ± 0.04^c
IV–V	1.93 ± 0.10^c	1.38 ± 0.06^c	1.91 ± 0.19^c
VI	1.11± 0.25	0.97 ± 0.05	1.58 ± 0.11^c
VII	1.30 ± 0.12^b	1.14 ± 0.12	1.49 ± 0.07^c
VIII	1.37 ± 0.14^b	1.48 ± 0.05^c	1.57 ± 0.21^b
IX	0.77 ± 0.04^c	1.14 ± 0.04^c	0.71 ± 0.05^c
X–XI	1.71 ± 0.09^c	1.19 ± 0.10	0.74 ± 0.16
XII	2.41 ± 0.34^c	0.98 ± 0.20	1.59 ± 0.24^b

Table 2. Relative length of stages of seminiferous tubule epithelium in mice treated with malathion on day 0 and assessed at 8, 15 and 22 days after single injection. The length of each stage in control animals killed on day 0 was taken as 1 to calculate the relative length in the experimental groups. Malathion was administered i.p. (80 mg·kg^{-1}) at a single dose. Vehicle used for the administration of Maca (See Table 1 and 3), was administered daily by oral route for 7, 14 or 21 days. Data in mean ± SEM. $^bP < 0.05$, $^cP < 0.01$, compared with controls.

Stages	Days of vehicle administration		
	7	14	21
I	1.11 ± 0.04^b	1.46 ± 0.09^c	0.81 ± 0.03^c
II–III	1.21 ± 0.13	1.24 ± 0.14	1.14 ± 0.08
IV–V	1.89 ± 0.08^c	1.33 ± 0.18	1.21 ± 0.35
VI	1.00 ± 0.03	0.63 ± 0.15^c	0.72 ± 0.16
VII	1.05 ± 0.08	0.58 ± 0.16^c	1.13 ± 0.09
VIII	0.94 ± 0.05	0.67± 0.16	0.87 ± 0.05^b
IX	0.53 ± 0.06^c	0.54 ± 0.06^c	0.85 ± 0.10
X–XI	0.90 ± 0.05	0.63 ± 0.11^c	1.48 ± 0.12^c
XII	2.21 ± 0.42	1.50 ± 0.22	2.17 ± 0.31^c

Table 3. Relative length of stages of seminiferous tubule epithelium in mice treated with Maca plus malathion, assessed on the day after last oral treatment. Length of each stage in control animals killed on day 0 was taken as 1 to calculate relative length in the experimental groups. Malathion was administered i.p. (80 mg·kg^{-1}) in a single dose. Maca (66.6 mg·0.2 mL^{-1}) was administered daily by oral route for 7, 14 or 21 days. Data in mean ± SEM. $^bP < 0.05$, $^cP < 0.01$, compared with controls.

Stages	Days of Maca administration		
	7	14	21
I	1.01± 0.09	0.96 ± 0.06	0.73 ± 0.12
II–III	1.45 ± 0.16^c	1.82 ± 0.06^b	1.13 ± 0.18
IV–V	1.67 ± 0.16^b	1.29 ± 0.31	1.14 ± 0.22
VI	1.17 ± 0.25	0.70 ± 0.09^b	0.88 ± 0.20
VII	1.20 ± 0.14	0.67 ± 0.06^b	1.90 ± 0.28^b
VIII	1.13 ± 0.03^b	0.83 ± 0.16	1.58 ± 0.19^c
IX	0.71 ± 0.04^b	0.82 ± 0.04^b	1.04 ± 0.16
X–XI	0.75 ± 0.17	0.71 ± 0.03^b	1.06 ± 0.25
XII	0.96 ± 0.14	1.29 ± 0.19	1.43 ± 0.43

malathion are presented in Table 2. On day 7, malathion reduced the relative length of stage IX to 53 % of the control value. This was accompanied by a relative increase in the lengths of stages I and IV–V. On day 14, the relative lengths of stages VI, VII, IX and X–XI were reduced, while stage I was relatively increased. On day 21, stages X–XI and XII were higher than the control; while stages I and VIII were lower than the control and the relative lengths of stages VII and IX were similar to the control.

On day 7, malathion plus Maca treatment (Table 3) resulted in higher relative lengths of stages II–III, IV–V and VIII, whereas the relative length of stage IX was reduced. On day 14, length of stages II–III was higher and stages VI, VII, IX and X–XI were reduced. On day 21, stages VII and VIII were significantly increased, whereas relative lengths of other stages were not different from the controls.

The negative effect of malathion on the stages of the seminiferous epithelium were significantly reduced by treatment with Maca. Alterations in length of stage IX produced by malathion was significantly reduced by Maca on days 7 and 14 ($P < 0.05$). The relative length of stages VII and VIII was also higher ($P < 0.01$) in the Maca plus malathion group than that in the group with malathion alone (Tables 2 and 3).

4 Discussion

Malathion is a pesticide widely used by farmers in many parts of the world; it is also a potential health risk. Previously it has been demonstrated that a dose of 240 $mg \cdot kg^{-1}$ of malathion to mice resulted in the atrophy of seminiferous tubules on day 8 post-injection, although recovery was observed thereafter [3]. In the present study we have used another approach in order to determine the effect of drugs on spermatogenesis: the transillumination technique. Using this method we were able to demonstrate that a single dose of malathion (80 $mg \cdot kg^{-1}$) inhibited spermatogenesis. In fact, reductions in the length of stage VII on day 14, stage VIII (spermiation) on day 21 and stage IX on days 7 and 14 were observed. Stages I and IV–V were relatively increased on day 7 as a consequence of the reduction in stage IX. On day 14 a more dramatic effect is observed. Certainly, stages VI, VII, IX and X–XI were reduced. These reductions were accompanied with a relative increase in stage I. The pattern of reduction in a determined stage associated with an increase in the preceding stage has been previously described [4], suggesting an arrest in spermatogenesis in the stage where reduction in length was observed.

Previously, it was demonstrated that 240 $mg \cdot kg^{-1}$ malathion did not affect sperm count although the epithelial height and seminiferous tubular diameter were reduced on day 8 [3]. With the administration of a single dose (80 $mg \cdot kg^{-1}$) we have demonstrated that stage IX was significantly affected on day 7 and stages VII, IX and X–XI on day 14. It has been suggested that malathion may deplete the renewing type-A spermatogonia [3]. Our results are in accordance with this hypothesis since the length of stage IX, where mitosis of type-A spermatogonia occurs, was reduced. The reduction in length of stages IX indicates a depletion of seminiferous epithelium that is maintained for up to 14 days. Some mutagenic and/or cytotoxic chemicals have been known to exhibit stage-specific effects on germ cells or on reproductive maturation [13]. The administration of different toxicants such as mono-e-ethylhexyl phtalate (MEHP), adriamycin and N-ethyl-N-nitrosourea (ENU) to mice produced an increase in the number of apoptotic cells in stages IX–XI of the seminiferous tubules [14]. It is assumed that malathion is acting by the same mechanism and as a result the lengths of stages IX–XI will be reduced. A later effect was on spermiation. On day 14, stages VI and VII were reduced such that on day 21 the relative length of stage VIII was significantly lower than the control. Stage VIII seminiferous tubule epithelium corresponds to spermiation [10]. Spermiation is the final step of spermatogenesis and involves the release of mature spermatids from Sertoli cells into the lumen of the seminiferous tubule [11]. This processes is also related to the transfer of sperm to the epididymis [12]. These data suggest that malathion affects spermiation by reducing conversion from stages I–VI to stages VII and VIII.

On the other hand, we have demonstrated that treatment of normal adult mice with the aqueous extract of *Lepidium meyenii* (Maca) improved spermatogenesis. In fact, treatment for periods of 7, 14 or 21 days increased the relative lengths of stages VII–VIII of the seminiferous epithelium. Similar findings have been described for male rats [5, 15]. The present study constitutes the first description of effects on mice. Increases in length of stages VII–VIII suggest an improvement in the late stages of spermatogenesis, prior to spermiation. Protection is

not so efficient in the mid-stages of the cycle.

The main effect of Maca is to increase the lengths of stages VII and VIII. Spermiation may promote the progression of round spermatids through the elongation phase of spermiogenesis [11]. It is possible that spermiation may provide positive signals to the Sertoli cell to continue with spermiogenesis [11]. This effect has been also observed after the administration of Maca aqueous extract to adult male rats [5, 15]. However, when ethanolic extract of Maca was administered to rats, an increase in the length of stages IX–XI, but not the expected increase in stage VIII [16], was observed. This suggests that some active principles that are present in the aqueous extract of Maca are not present in the ethanolic extract.

A second level of the action of Maca was at stage IX where spermatogenesis begins with the first mitosis of spermatogonia A [17]. In fact the length of stage IX increased on days 7 and 14 of treatment with Maca. The positive effect of Maca was observed at most stages after 7 days of treatment (stages IV–V, VII, VIII, X–XI and XII).

The active principles for these activities of Maca are still unknown. Two novel compounds have been recently identified as imidazole alkaloids (lepidine A and lepidine B) [18]. Muhammad *et al.* [19] described a benzylated derivative of 1, 2-dihydro-N-hydroxypyridine, named macaridine, 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. However, the effects of these compounds on spermatogenesis have not been assessed.

Previously it has been demonstrated that Maca may prevent the spermatogenic disturbances induced by high-altitude in male rats [15]. At this time we have demonstrated that Maca may also counteract the damage to spermatogenesis caused by an organophosphorus pesticide, malathion. In fact, the administration of Maca of mice treated with malathion resulted in minor damage to spermatogenesis. In addition, mice treated with malathion and Maca showed a rapid increase in the relative length of stage VIII (spermiation) as observed in the group treated with Maca alone. Similarly, length reduction in stage IX on days 7 and 14 caused by malathion was less prominent when Maca was added. Assessment of the relative lengths of stages of the seminiferous epithelium showed that treatment with the aqueous extract of Maca resulted in the rapid recovery of the negative effect of malathion on spermatogenesis. The use of extract of plants as an alternative treatment for infertile men has recently been emphasized [20]. Maca could be a promising plant for this purpose.

In conclusion, our findings demonstrate that a single administration of 80 $mg \cdot kg^{-1}$ malathion affects spermatogenesis, while Maca improves it. Maca was also able to reduce the deleterious effect of malathion on spermatogenesis.

Acknowledgment

Sandra Yucra was supported by a grant from the Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana (PLACIRH). A research grant from Banco Santander Central Hispano, Madrid, partially financed this work.

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