

## Dose–response effect of Red Maca (*Lepidium meyenii*) on benign prostatic hyperplasia induced by testosterone enanthate

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### Abstract

The main goal of this study was to determine the effect of a freeze-dried aqueous extract of the red variety of *Lepidium meyenii* (Red Maca) on testosterone-induced benign prostatic hyperplasia (BPH) in adult rats of the Holtzman strain. Rats were treated with freeze-dried aqueous extract of Red Maca at doses of 0, 0.01, 0.05, 0.1, and 0.5 g/kg body wt. A positive control group received Finasteride (0.6 mg/kg body wt.). After treatment, the animals were sacrificed, and the ventral prostate was extracted, and weighed. HPLC was used to determine the presence of glucosinolates in Red Maca. The prostate weight diminished in a dose-dependent fashion in rats treated with Red Maca. The effect of Red Maca was better than that observed with Finasteride. Finasteride, but not Red Maca, reduced seminal vesicles weight. Analysis of the HPLC indicated the presence of benzyl glucosinolate (Glucotropaeolin) with a content of 0.639%. Serum testosterone levels were not affected by Red Maca. Moreover, serum testosterone levels were not related to prostate or seminal vesicles weight in rats treated with vehicle and Red Maca. In conclusion, Red Maca administered orally in rats seems to exert an inhibitory effect at a level post DHT conversion, on the BPH-induced experimentally, although a direct measure of reductase action would still be required.

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**Keywords:** *Lepidium meyenii* (Red Maca); glucosinolates; benign prostatic hyperplasia; rats

### Introduction

It has been suggested that high intakes of Brassica vegetables reduce prostate cancer risk (Kristal and Lampe, 2002). The Brassica vegetable *Lepidium latifolium*, orally administered, reduced prostate size and volume in castrated rats where the hyperplasia was induced by steroid treatment (Martinez Caballero et al.,

2004). *Lepidium meyenii* (Maca) a Peruvian plant that grows exclusively at over 4000 m altitude in the central Andes of Peru (Valerio and Gonzales, 2005) is also a Brassicaceae of the genus *Lepidium*. Recently, we have demonstrated that Red Maca but not Yellow or Black Maca reduced prostatic weight in male rats treated with testosterone enanthate (Gonzales et al., 2005).

Among the many hundreds of Brassica vegetables investigated, all synthesize glucosinolate. These glucosinolates are converted to isothiocyanates before becoming active (Fahey et al., 2001). Almost all of the mammalian chemoprotective activities of these plants are due to the isothiocyanates (Chiao et al., 2004).

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Benign prostate hyperplasia (BPH) and prostate cancer are considered problems of public health (Stoner, 1994). Finasteride, a 5- $\alpha$ -reductase 2 inhibitor, is one of the major drugs used for androgen suppression treatment of BPH (Gao et al., 2004). However, the cost burden associated with Finasteride is substantial, while its survival benefit is small and is only realized many years after initiating treatment (Zeliadt et al., 2005). For such reasons, it would be helpful to find alternative strategies for the treatment of prostate disease. Accordingly, a search for safer, natural products has been undertaken (Talpur et al., 2003).

The present study was designed to determine the dose–response effect of an aqueous extract of Red Maca on prostate size and to determine whether this effect is comparable to the effect of Finasteride, a drug that inhibits 5- $\alpha$ -reductase (Ma et al., 2004).

## Material and methods

### Animals

Male rats from a 3-month-old Holtzman strain were used. Rats were housed 3–6 per cage and maintained at 22 °C with a 12:12 light/dark cycle. Rats were provided with Purina laboratory chow and tap water ad libitum. The animals were treated according to the standards of the National Institutes of Health for the care and use of laboratory animals. The Institutional Review Board of the Scientific Research Office, Universidad Peruana Cayetano Heredia, approved the study.

### Experimental protocol

#### Experiment 1: dose–response study of Red Maca (*Lepidium meyenii*) on prostate weight in rats treated with testosterone enanthate

Rats received freeze-dried Red Maca in doses of 0, 0.01, 0.05, 0.1 and 0.5 g/kg body wt. suspended in 2 ml of water. Each group included 6 animals. Red Maca or vehicle was administered orally once daily from day 1 to day 21 using an intubation needle No 18 (Fisher Scientific, Pittsburgh, Pennsylvania). All rats received testosterone enanthate (i.m.) on days 1 and 7.

At the end of each treatment, rats were sacrificed by decapitation. The ventral prostate was carefully dissected out, cleaned of the adhering connective tissues and accurately weighed.

#### Experiment 2: effect of Red Maca (*Lepidium meyenii*) and Finasteride on prostate and seminal vesicle weight in rats treated with testosterone enanthate

Rats were divided into 4 groups of 6 rats each. The first group (Control) received 0.1 ml oil (i.m.) at day 1

and at day 7 plus water (without Red Maca). Rats of the second group were injected with 0.1 ml (25 mg) of TE on day 1 and day 7 plus water (without Red Maca). Rats in the third group received 0.1 ml (25 mg) of TE on day 1 and day 7 plus Red Maca (2 g/kg body wt.). Finally, fourth group were treated with 0.1 ml (25 mg) of TE on day 1 and day 7 plus 0.6 mg/kg body wt. body wt. of Finasteride (positive control). Around 2 ml water (with or without Red Maca) or Finasteride was administered daily during 21 days using an intubation needle No. 18 (Fisher Scientific, Pittsburgh, PA, USA).

One day after the last treatment, rats were sacrificed by decapitation. Blood samples were obtained from the cervical trunk and centrifuged at 1000g. Sera were separated, placed in vials and kept frozen until assayed for serum testosterone levels. Also, ventral prostate and seminal vesicle were carefully dissected out, cleaned of the adhering connective tissues and accurately weighed.

### Preparation of aqueous extract of Red Maca (*Lepidium meyenii*)

The hypocotyls of *Lepidium meyenii*, which is cultivated by farmers, were obtained from Carhuamayo at 4000 m altitude in Junin (Central Andes of Peru). The identity of the plant was authenticated by Irma Fernandez, a botanist from the Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia. The hypocotyls correspond to the red variety. A voucher specimen (IFV 1885) was deposited at the Department.

An aqueous extract of the hypocotyls was prepared. In brief, 100 g of the dried pulverized hypocotyls were placed in a container with 600 ml of water, and boiled for 60 min. The preparation was left standing to cool, filtered, and freeze-dried. After boiling, 1 g of dried hypocotyls of Maca produced 0.34 g of aqueous extract of Maca and 1 g of aqueous extract of Maca produced 0.33 g of freeze-dried Maca. The freeze-dried Maca extract was diluted further to obtain different concentrations in 2 ml. These solutions were placed in small vials and kept under refrigeration at 4 °C until use.

### Quantification of benzyl glucosinolate in freeze-dried Red Maca extract

0.1 g of freeze-dried Red Maca extract was diluted with water at a concentration of 50 mg/ml. Then, 400  $\mu$ l of the extract and of the standard benzyl glucosinolate (glucotropaeolin) (0.72 mg/ml, PhytoLab L071220), were pipetted into an ion-exchange column containing Sephadex DEAE-A25 (Sigma). Then, it was washed three times with a sodium acetate solution (pH 4.0) and water twice and 0.75  $\mu$ l of 0.5% sulfatase (Sigma) was added and kept for 16 h at room temperature. Desulfoglucosinolates were

eluted with 2.5 ml water and analyzed using an automated Hewlett Packard HPLC series 1100, with an ODS column at 230 nm wave length. The sample injection was 20  $\mu$ l. The mobile phase consisted of 20% acetonitrile in water (flow rate 1.5 ml/min). The running program consisted of a constant flow of 1% of mobile phase – 99% water over 1 min, then a linear gradient elution at 99% of mobile phase for 20 min, constant for 10 min and washing of the column with a linear gradient descending from 99% of mobile phase to 1% in 15 min. The identification of the benzyl glucosinolates was performed by using glucotropaeolin as standard. The quantification was carried out by comparing the peak area of the samples with the mean peak area of the standard. Allyl glucosinolate was also used as standard. The % content of benzylglucosinolate in Red Maca was determined as 0.639%.

### Serum testosterone levels

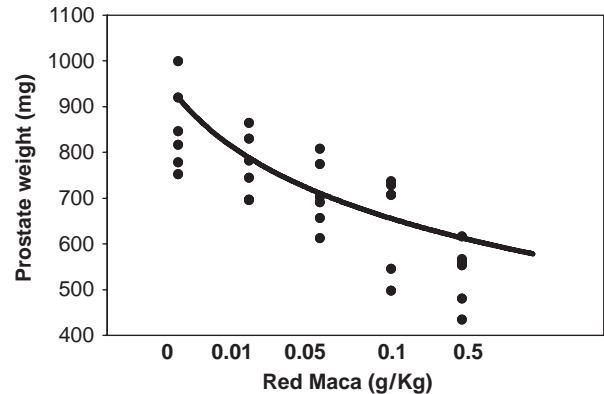
Serum testosterone levels were determined using RIA with  $^{125}$ I-testosterone as the radioactive marker. The assay was performed using a commercial kit (Diagnostic Products Co., Los Angeles, CA, USA). All samples were run in the same assay period. The within-assay variation was 5.5% and sensitivity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

### Statistical analysis

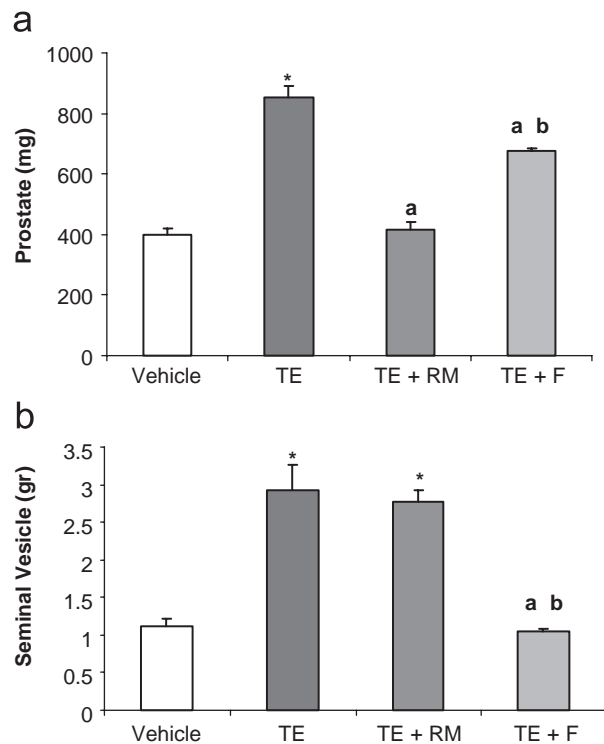
Data were analyzed using the statistical package STATA (version 8.0) for personal computer (Stata Corporation, 702 University Drive East, College Station, TX, USA). Data are presented as mean  $\pm$  standard error of the mean (S.E.M.). Homogeneity of variances was assessed by the Bartlett test. If variances were homogeneous, differences between groups were assessed by two-way analysis of variance. Differences between pairs of means were assessed by the Scheffé test. When variance was not homogeneous, a non-parametric analysis was performed. A value of  $p < 0.05$  was considered to be statistically significant.

### Results

The present study demonstrated that Red Maca reduced prostate weight in adult male rats in which benign prostatic hyperplasia (BPH) has been induced by testosterone enanthate. Reduction in prostate weight was observed in a dose–response fashion. Data showed that reduction in prostate weight was significant from 0.1 g/kg. (Fig. 1). Treatment with Finasteride (0.6 mg/kg body wt.) also resulted in a reduction in prostate weight (mean  $\pm$  S.E.M.) (Fig. 2a). Red Maca at doses of 0.1 g/kg body wt. ( $p < 0.05$ ) and 0.5 g/kg body wt.

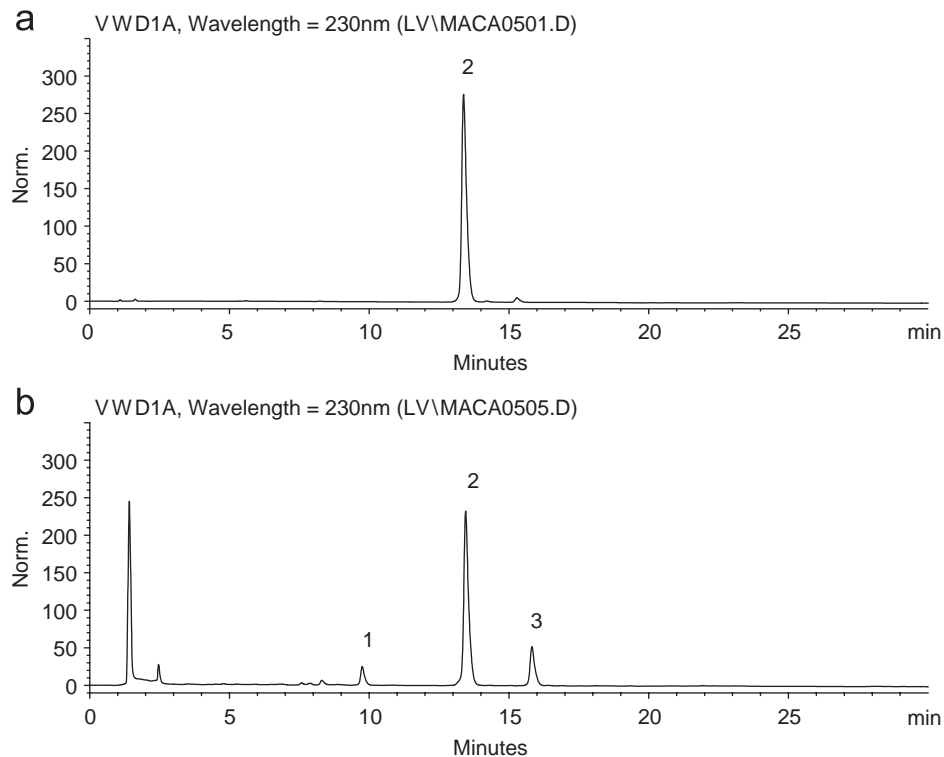


**Fig. 1.** Dose–response effect of Red Maca (*Lepidium meyenii*) on prostate weight (mg) in rats in which prostatic hyperplasia was induced with testosterone enanthate (25 mg, twice).



**Fig. 2.** Effect of Red Maca on prostate (a) and seminal vesicle (b) weight in rats treated with testosterone enanthate. Vehicle: rats treated with 0.1 of oil; TE: rats treated with 0.1 ml (25 mg) of testosterone enanthate at day 1 and 7; TE+RM: rats treated with 0.1 ml (25 mg) of testosterone enanthate at day 1 and 7 plus Red Maca (2 g/kg body wt.); TE+F: rats treated with 0.1 ml (25 mg) of testosterone enanthate at day 1 and 7 plus Finasteride (0.6 mg/kg body wt.). \* $p < 0.01$  with respect to rats treated with vehicle; <sup>a</sup> $p < 0.05$  with respect to TE-treated rats; <sup>b</sup> $p < 0.05$  with respect to rats treated with TE + RM.

( $p < 0.05$ ) resulted in higher reductions in prostate weight than Finasteride. Finasteride, but not Red Maca, reduced seminal vesicles weight (Fig. 2b). Serum testosterone levels in the control group was



**Fig. 3.** HPLC profiles for freeze-dried Red Maca extract of hypocotyls. (a) Standard of benzyl glucosinolate (Glucotropaeolin). (b) Freeze-dried Red Maca extract; 2-benzylglucosinolate.

101.40 ± 51.20 ng/dl (mean ± S.E.M.) and in the group treated with Red Maca (2 g/kg body wt.) was 46.29 ± 17.51 ng/dl (*p*:NS). According to linear regression analysis there was no relationship between serum testosterone levels of normal rats treated with vehicle or Red Maca on prostate weight ( $F = 1.169$ ,  $P = 0.315$ ;  $r = 0.14$ ) and seminal vesicle weight ( $F = 0.011$ ,  $P = 0.921$ ;  $r = 0.04$ ).

In the TE-treated group, the serum testosterone levels (831.85 ± 280.30 ng/dl) were similar to those in the Red Maca (2 g/kg body wt.) + TE treated group (946.40 ± 241.93 ng/dl, mean ± S.E.M.). Data from the HPLC study showed that Red Maca contains benzyl glucosinolate (0.639/100 g Red Maca extract) whereas aliphatic glucosinolate (allyl glucosinolate) content was negligible (Fig. 3).

## Discussion

We have demonstrated previously that administration of 25 mg twice (i.m.) of testosterone enanthate to adult rats resulted in the production of prostatic hyperplasia (Gonzales et al., 2005). The present study showed that Red Maca (2 g/kg body wt.) reduced prostate weight in animals in which the BPH was induced with testosterone enanthate confirming a previous report (Gonzales et al.,

2005). In addition, in the present study, Red Maca reduced prostate weight in a dose-dependent manner. Moreover, doses of freeze-dried Red Maca of 0.1 and 0.5 g/kg body wt. resulted in higher reductions in prostate size than those observed with Finasteride. These doses represent 0.8–4.4 g/kg body wt. from the dried hypocotyls (raw material).

Finasteride is an inhibitor of the enzyme 5- $\alpha$ -reductase 2. This enzyme converts testosterone to dihydrotestosterone (DHT). Finasteride is an elective drug for BPH (Tempany et al., 1993) but is associated with some side-effects (Zlotta et al., 2005). Because of this, Red Maca, a plant that grows exclusively in the highland of the central Andes, could become an important alternative for the treatment of BPH.

DHT is responsible for the proliferative action on prostate and seminal vesicles (Kashiwagi et al., 2005). As shown in the present study, testosterone enanthate administered at a total dose of 50 mg resulted in an increase in both prostate and seminal vesicles weight, suggesting that conversion from *T* to DHT occurred in both glands. Finasteride was able to reduce both, prostate and seminal vesicles weight, whereas Red Maca was specific to prostate weight. These results suggest that a differential effect of Red Maca on prostate and seminal vesicles seems to occur at a level post 5- $\alpha$ -reductase action. More recently, it has been published that Maca has no effect on androgen receptor (Bogani et al., 2006). Our results are in accordance with that

finding, as Red Maca administered orally in rats seems to exert an inhibitory effect at a level post DHT conversion, on the BPH-induced experimentally, although a direct measure of reductase action would be required. Moreover, the linear regression analysis did not show any relationship between the serum testosterone levels in normal rats treated with vehicle or Red Maca on prostate and seminal vesicles weight. This suggests that Red Maca does not affect serum testosterone levels, but reverses androgen action specifically in the prostate organ.

Analysis of the freeze-dried aqueous extract of Red Maca by HPLC showed the presence of benzyl glucosinolate. Metabolites from benzyl glucosinolates have been implicated to arrest proliferation from prostatic cancer cell lines (Nachshon-Kedmi et al., 2003; 2004; Le et al., 2003). Cruciferous plants are recommended to prevent or reduce prostate cancer risk (Kristal and Lampe, 2002). Thus, Red Maca a cruciferous that is unique because only grows in altitudes over 4000 m in the central Andes of Peru, may be an interesting alternative in the treatment of prostatic diseases.

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