Blackwell Publishing

Asian J Androl 2007; 9 (2): 245–251
DOI: 10.1111/j.1745-7262.2007.00228.x

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·*Complementary Medicine*·

Effect of two different extracts of red maca in male rats with testosterone-induced prostatic hyperplasia

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Abstract

Aim: To determine the effect of two different extracts of red maca in male rats. **Methods:** Prostatic hyperplasia was induced in male rats with testosterone enanthate (TE). The study comprised six groups: one control group (group 1), one group treated with TE (group 2), two groups treated with TE and aqueous extract of red maca (groups 3 and 4), one group treated with hydroalcoholic extract of red maca (group 5) and one group treated with finasteride (0.1 mg, group 6). Differences in the aqueous extract dependent on the length of time of boiling, whether for 2 or 3 hours, for groups 3 and 4 was assessed. Extracts of red maca contained 0.1 mg of benzylglucosinolate. Thereafter, a dose-response effect of different doses of benzylglucosinolates (0.02–0.08 mg) in red maca extracts was assessed. **Results:** Prostate weight was similar in rats treated with freeze-dried aqueous extract of red maca prepared after 2 and 3 hours of boiling. Freeze-dried aqueous extract of red maca, hydroalcoholic extract of red maca and finasteride reduced prostate weight in rats with prostatic hyperplasia. No difference was observed between the data obtained from aqueous extract or hydroalcoholic extract of red maca. A dose dependent reduction of prostate weight was observed with the increase of the dose of benzylglucosinolates in red maca extracts. **Conclusion:** The present study showed that hydroalcoholic or aqueous extract of red maca containing 0.1 mg of benzylglucosinolate can reduce prostate size in male rats in which prostatic hyperplasia had been induced by TE. *(Asian J Androl 2007 Mar; 9: 245–251)*

Keywords: red maca; *Lepidium meyenii*; freeze-dried aqueous extract; hydroalcoholic extract; prostatic hyperplasia; prostate weight; benzyl glucosinolates

1 Introduction

Lepidium meyenii (maca) is a plant that grows exclusively above 4 000 m in the Central Andes of Peru. The hypocotyls of this plant are edible which has been described since the 17th century by a chronicler of the conquest of Peru who stated that maca, the only plant growing in such an environment, was used by natives as a nutrient as well as to improve fertility [1]. Recent biological studies on different varieties of maca have shown that for prostate size, red maca is effective, yellow maca has an intermediate effect and black maca has no effect [2, 3]. However, black maca had a strong effect on testicular and epididymal sperm count, whereas red maca had no [3]. The effect of red maca on prostate size is a

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Received 2006-04-20 Accepted 2006-07-30

novel finding since it has not been traditionally reported, but it could be an important alternative for the treatment of benign prostatic hyperplasia (BHP). It has been suggested that benzyl glucosinolates are responsible for the effect of red maca on prostate size [2], but it has never been assessed.

Maca, after being harvested, is dried naturally [1] and can be stored for many years [4]. Before being consumed, maca is processed by boiling the hypocotyls in water to obtain a soft product which is then taken as juice [1]. The effect of temperature on plants might affect the availability of several of their secondary metabolites. For example, quercetine is sensitive to temperature. Similarly, the content of glucosinolates is sensitive to heating [5]. Cooking red cabbage will cause more thermal degradation to indole glucosinolates (38%) as compared with aliphatic glucosinolates (8%) [5]. However, other metabolites are also increased. Heating decreases epithiospecifier protein activity and increases sulforaphane formation, a derivative of isothiocyanates in broccoli [6]. After 2, 15, and 30 min of heating at 88ºC, the vitamin C content of raw tomato dropped significantly. However, the content of translycopene/g of tomato increased [7]. Furthermore, antioxidant activity of tomatoes also increased after heating tomatoes [7].

Most biological studies on maca have been carried out with the freeze-dried aqueous extract of the plant [1]. However, other preparations, as hydroalcoholic extracts, are also available as nutraceutical products in the market. A study on *Uncaria tomentosa* (uña degato) showed that hydroalcoholic extract had a better anti-inflammatory activity than the aqueous extract [8].

The present study attempted to determine the effect of the length of boiling time on the biological activity of red maca and to compare the biological activity of red maca prepared as an aqueous extract and as a hydroalcoholic extract after controlling the amount of benzyl glucosinolates. Red maca has been shown to reduce prostate size in rats in which hyperplasia of the prostate has been induced by testosterone enanthate (TE) [2].

2 Materials and methods

2.1 Animals

Adult Holtzman rats aged 3 months and weighing 300 g in average obtained from the animal house at Universidad Peruana Cayetano Heredia and from the National Institute of Health (Lima, Peru) were used for the present study. The rats were maintained, four to six per cage, at environmental temperature (22ºC) with a 12 h :12 h light/dark cycle. Rats were provided with Purina laboratory chow and tap water *ad libitum*. All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health of USA [9]. The Institutional Review Board of the Scientific Research Office from the Universidad Peruana Cayetano Heredia approved the study.

2.2 Preparation of Lepidium meyenii

The dried hypocotyls from the red variety of *Lepidium meyenii* were obtained from Carhuamayo, Junin at 4 000 m altitude. All hypocotyls were purchased at the same time. Dr Irma Fernandez, a botanist in the Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia, authenticated the identity of the plant by visual inspection.

2.2.1 Preparation of aqueous extract

In brief, 200 g of the dried hypocotyls of red maca were pulverized and placed in two containers with a bag containing 100 g of pulverized maca each. To each container was added 700 mL of water, and boiled for 2 h or 3 h like an infusion, respectively.

The preparation was left standing to cool and then the bag was removed and the infusion filtered. The filtrate was frozen at –20ºC, then at –70ºC and finally lyophilized.

Maca recovered after the freeze-dried procedure was 23.37% and 25.59% for the boiling time of 2 h and 3 h, respectively. The freeze-dried extract was dissolved with water before the experiments.

2.2.2 Preparation of spray dried hydroalcoholic extract

The spray dried hydroalcoholic extract of red maca was provided by Eng. Alfonso Higa (Agroindustrial Chanchamayo, Lima, Peru). Maca recovered after hydroalcoholic extraction was dissolved in water at the time of the experiments.

2.3 Experimental design

2.3.1 Groups and treatment

Rats were injected (i.m.) with 0.1 mL (25 mg) of TE on day 1 and day 7. Control rats received 0.1 mL oil (i.m.)

at day 1 and day 7. Rats received TE treatment for 14 days were divided into three groups: 1) freeze-dried aqueous extract of red maca obtained after 2-h of boiling; 2) freeze-dried aqueous extract of red maca obtained after 3-h boiling; or 3) spray-dried hydroalcoholic extract of red maca. Control rats received vehicle by oral route for 14 days. Oral treatment (maca or vehicle) and intramuscular treatment (TE or vehicle) both started on day 1.

The present experiment included six groups: 1) control (six rats treated with vehicle); 2) TE control (15 rats treated with only TE); 3) TE + red maca for 2-h boiling (five rats); 4) TE + red maca for 3-h boiling (five rats); 5) TE + hydroalcoholic extract of red maca (10 rats); and 6) positive control treated with TE + 0.1 mg finasteride (six rats).

Maca (freeze-dried aqueous extract or spray-dried hydroalcoholic extract), finasteride or aqueous vehicle were administered by gavage in 1 mL. The 1 mL maca (freeze dried aqueous extract or spray dried hydroalcoholic extract) given daily to the rats contained 0.1 mg benzyl glucosinolates. Animals were killed on day 15.

2.3.2 Dose-response effect of different dose of benzyl glucosinolates in extracts of red maca on prostate weight

Rats in which prostate hyperplasia was induced with TE as described above received different doses of benzyl glucosinolates in the red maca extract (0.02, 0.04, 0.06 and 0.08 mg/mL) for 14 days. Control animals were used as dose 0. Each group included six rats. Animals were killed on day 15.

2.3.2 Organ weights

After the experiments, the animals were killed by decapitation. The testes, epididymis, seminal vesicles, ventral prostate and liver were carefully dissected out, cleaned of the adhering connective tissues and accurately weighed.

2.3.3 Quantification of benzyl glucosinolate in red maca extract

The glucosinolate content was measured by high-performance liquid chromatography (HPLC) in freeze-dried and in spray-dried hydroalcoholic red maca extracts. Then, 1 g maca extract sample was leached with 45 mL of 70% ethanol (JT Baker, Phillipsburg, NJ, USA) and stirred for 30 min at 40ºC, then centrifuged at 1 000 × *g* for 10 min and decanted. This procedure was repeated once again and the combined extractions were diluted to a final volume of 100 mL with 70% ethanol. A standard of benzyl glucosinolate (glucotropaeolin) (30 μg/mL) was also diluted with 70% ethanol. All samples and standard were filtered through a membrane filter (0.45 μm). Maca extracts and standard were analyzed using an automatic Hewlett Packard HPLC series 1100 (Hewlett Packard, Waldbronn, Germany), with an RP-C(18) column at 235 nm wavelength. Samples and standard injection volume were 100 μL. The mobile phase consisted of a mixture of 1 006 mg tetraoctylammonium bromide (Fluka, Buchs, Switzerland) in 600 mL of methanol (Fisher Scientific, Fair Lawn, NJ, USA) and 1 137 mg of disodium hydrogen phosphate anhydrous (Mallinckrodt-Baker, Edo de Mexico, Mexico) in 400 mL of water, adjusted to pH 7.0 with phosphoric acid (Mallinckrodt-Baker, Edo de Mexico, Mexico) (flow rate 1.0 mL/min). The running program consisted of a flow constant of mobile phase and column temperature of 30ºC. The quantification was carried out by comparing the peak area of the samples with the mean peak area of the standard.

2.3.4 Measurement of infrared spectra

Infrared (IR) spectra of freeze-dried aqueous extracts and spray-dried hydroalcoholic extracts of red maca were measured from 4 000 cm-1 to 650 cm-1 with an FT-IR spectrophotometer (SPECTRUM ONE, Perkin Elmer, Beaconsfield, UK). An overhead-attenuated total refraction (ATR) accessory was equipped as the sample stage for solid samples. All spectral measurements were carried out at 1 cm-1 resolution. Data are presented as transmittance units (%). Each peak represents the presence of a functional chemical group. Differences in the height of transmittance peaks reflect differences in amount of functional groups. The lower the transmittance peak, the highest the amount of particular chemical functional group.

2.4 Statistical analysis

Data were analyzed using the statistical package STATA version 8.0 for personal computer (Stata Corporation, College Station, TX, USA). Data are presented as mean ± SEM. Homogeneity of variances was assessed by the Bartlett test. As 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 a pair of means were assessed by Scheffé test. $P < 0.05$ was considered statistically significant.

3 Results

Analysis of the infrared profile of freeze-dried extracts of red maca showed that peaks of transmittance were not affected by boiling time (Figure 1). However, a lower peak was observed with spray dried hydroalcoholic extract of red maca (Figure 2).

Quantification of benzyl glucosinolates showed that spray-dried hydroalcoholic extracts of red maca had the higher content of benzyl glucosinolates than freeze-dried extracts of red maca (Table 1). After correction per 100 g dry hypocotyls of red maca, the qualtity of benzyl glucosinolates were closer but were still higher in the spray-dried hydroalcoholic extracts (Table 1). Testosterone enanthate significantly increased the prostate weight ($P < 0.01$) from 280.00 ± 20.00 mg (mean ± SEM) in the control group (vehicle) to 746.50 ± 26.45 mg after two injections of TE. Similarly, TE significantly increased seminal vesicles weight ($P < 0.01$) from 1 040.00 ± 200.00 mg in the control group (vehicle) to

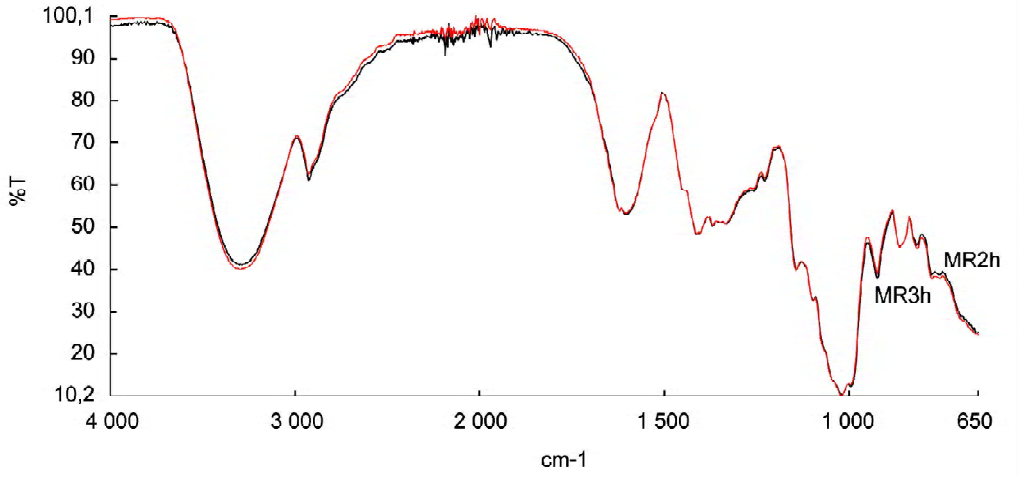

Figure 1. Infrared spectra of freeze-dried aqueous extract of red maca obtained alter boiling for 2 h (MR2h) or 3 h (MR3h). T, transmitance.

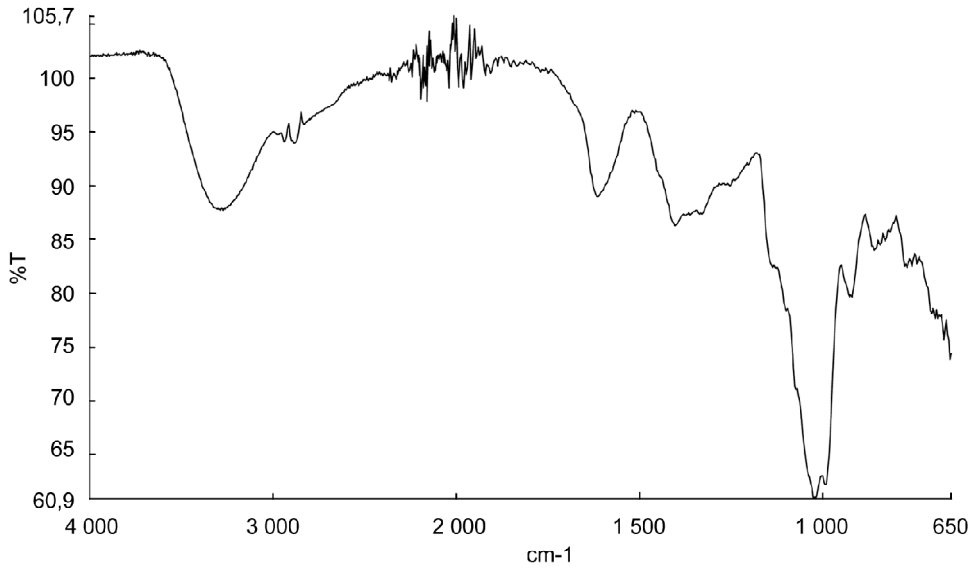

Figure 2. Infrared spectra of spray-dried hydroalcoholic aqueous extract of red maca. T, transmitance.

Table 1. Benzyl glucosinolate content in freeze-dried extract and hydroalcoholic extracts of red maca (*Lepidium meyenii*).

Sample	Time of boiling	Glucosinolate content (mg/100 g extract)	Glucosinolate content (mg/100 g dry hypocotyls)
Freeze-dried extract	2 h	30.0	7.01
	3 h	49.0	12.54
Hydroalcoholic extract	–	204.7	17.50

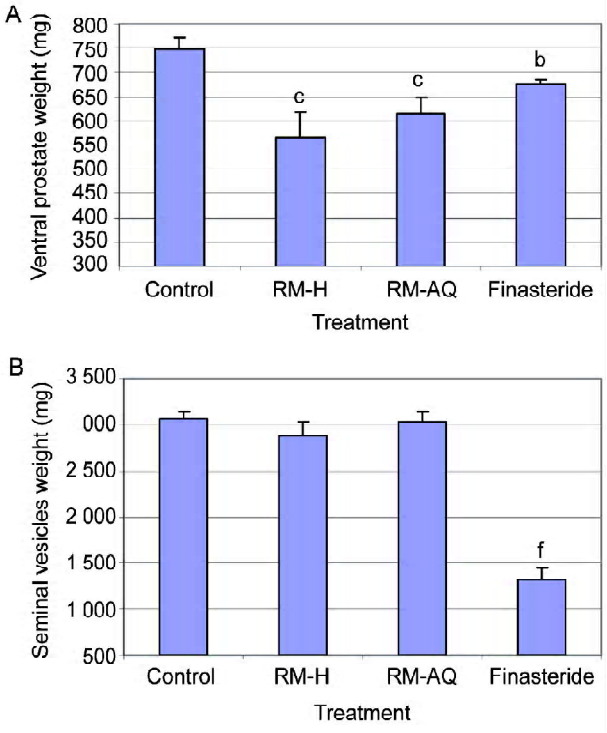

Figure 3. Prostate (A) and seminal vesicles (B) weights in male rats with prostatic hyperplasia treated with vehicle (control, $n = 15$), hydroalcoholic extract of red maca (RM-H, $n = 10$), freeze-dried aqueous extract of red maca (RM-AQ, $n = 10$) or 0.1 mg finasteride ($n = 6$) for 14 days. $^{b}P < 0.05$, $^{c}P < 0.01$, $^{f}P < 0.01$, compared with the control.

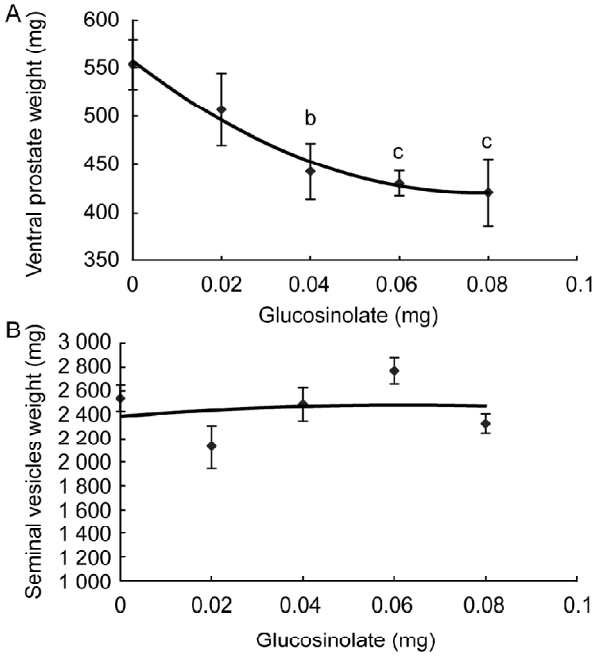

Figure 4. Dose-response effect of different content of benzyl-glucosinolates in red maca on (A) prostate weight and (B) seminal vesicles weights in rats with prostate hyperplasia. $^{b}P < 0.05$, $^{c}P < 0.01$, compared with the baseline value.

3 061.44 ± 75.85 mg.

The present study showed that treatment with aqueous extract of red maca obtained after 2- and 3-h boiling similarly affected prostate weight (570.52 ± 41.11 mg and 603.40 ± 28.66 mg, respectively) ($P > 0.05$). For this reason the data were pooled.

Administration of finasteride for 14 days (0.1 mg, $P < 0.01$), freeze-dried aqueous extract of red maca (0.1 mg glucosinolate, $P < 0.01$) or hydroalcoholic extract of red maca (0.1 mg glucosinolate; $P < 0.05$) significantly reduced ventral prostate weight of male rats with prostatic hyperplasia (Figure 3A). Finasteride also reduced seminal vesicles weight ($P < 0.01$), an effect that was not observed with red maca; aqueous extract ($P > 0.05$) or hydroalcoholic extract ($P > 0.05$) (Figure 3B). Red maca did not affect the weight of liver, testes or epididymis (data not shown).

A dose-dependent reduction in prostate weight was observed with the increasing dose (0.02–0.08 mg) of benzyl glucosinolates in the extracts of red maca (Figure 4A). Seminal vesicles weights were not affected at any dose of benzyl glucosinolate assessed (Figure 4B).

4 Discussion

In the present study, we confirmed that red maca reduced the prostate weight of rats in which hyperplasia had been induced with TE [2]. The study also showed that prostate hyperplasia was prevented by treatment with 0.1 mg finasteride, an 5-alpha reductase type 2 inhibitor, which inhibits conversion of testosterone to dihydrotestosterone [10].

Finasteride also reduced seminal vesicles weight, another androgen-dependent structure. Compared with finasteride, maca significantly reduced the weight of the ventral prostate in rats without affecting the seminal vesicles, suggesting that the effect is at a post-androgen-receptor action level and could be an interesting alternative medical treatment for BPH.

Pharmacotherapy is considered to be the mainstay of treatment for lower urinary tract symptoms (LUTS) caused by BPH. However, they are costly and have side-effects [11]. For this reason, traditional medicines are an attractive alternative for the treatment of BPH.

It has been suggested that cruciferous vegetables play an important role in cancer prevention and their chemopreventive effects are the result of high glucosinolate content, which under enzymatic hydrolysis produces bioactive compound isothiocyanates [12, 13]. Maca contains benzyl glucosinolate as the main glucosinolate [14]. After entering the gut, benzyl glucosinolate is transformed to benzyl isothiocyante (BITC) by the enzyme mirosinase [15]. BITC has the potential to induce apoptosis selectively in proliferating precancerous cells through a cell cycle arrest-dependent mechanism [16]. However, there have been no studies related to the effect of benzyl glucosinolates on prostate hyperplasia. Most of the information obtained from the natives of the highlands of the central Peruvian Andes shows that maca should be dried and boiled before being consumed [1]. The boiling time affects the recovery of maca. We have observed that recovery was low with red maca as compared with yellow maca [17]. In previous studies on yellow maca, we have observed that 1 g dried maca hypocotyls after 2-h boiling produced 0.46 g freeze-dried maca [17], a value higher than red maca (approximately 0.25 g).

The present study showed that the boiling process resulted in lower amounts of benzyl glucosinolates per 100 g of dry maca hypocotyls than in the hydroalcoholic extract. One possibility is that heating transforms benzyl glucosinolate to its most potent compound. For instance, sulforaphane, an isothiocyanate from broccoli, is one of the most potent food-derived anticarcinogens. This compound is not present in the intact vegetable, rather it is formed from its glucosinolate precursor, glucoraphanin, by the action of myrosinase. However, a number of studies have shown that the concentration of sulforaphane yield from glucoraphanin is low and that a non-bioactive nitrile analog, sulforaphane nitrile, is the primary hydrolysis product when plant tissue is crushed at room temperature [6].

Heating fresh broccoli florets or broccoli sprouts to 60ºC increases the concentration of sulforaphane and decreases the concentration of sulforaphane nitrile formation. The induction of quinone reductase (QR) in cultured mouse hepatoma Hepa lclc7 cells paralleled increases in concentration of sulforaphane [6]. It is still unknown if any specific compound is produced by the heating of red maca, but this possibility cannot be ruled out. In an attempt to demonstrate the effect of benzyl glucosinolates on BPH in male rats induced by TE, we studied the effect of the administration of maca obtained by different processes of extract preparation, but containing a similar amount of benzylglucosinolate (0.1 mg) per day. The present study showed a similar degree of prostate weight reduction with both spray-dried hydroalcoholic extract and with freeze-dried aqueous extract of red maca. A dose-dependent reduction in prostate weight was also observed in a second experiment in which red maca extracts containing a different dose of benzyl glucosinolates were given to the rats. The first possibility is that this effect is the result of benzyl glucosinolates in red maca, although the activity of other secondary metabolites present in maca cannot be excluded.

The aqueous extract and the hydroalcoholic extract of red maca were able to reduce prostate weight in rats with prostatic hyperplasia induced by TE to the same degree. The differences in the peaks of the infrared spectra could also be associated with the differences in the content of benzyl glucosinolates observed between aqueous and hydroalcoholic extracts. However, more researches are necessary to determine which other compounds might reduce prostate size in this model of hyperplasia in rats.

The similar response of prostate weight to aqueous extract and hydroalcoholic extract of red maca suggested that polar compounds are responsible for the biological

activity of red maca. When a hydroalcoholic mixture is used as an extraction system (hydroalcoholic extract), it is possible to extract secondary metabolites of a relatively wide range of polarities. On rural areas, when water is used, only high polarity compounds are extracted. This has been observed, for instance, when aqueous extract and hydroalcoholic extract of *Uncaria tomentosa* (uña de gato) were assessed for antioxidant activities [8]. The same was also observed when ethanol extract was compared with aqueous extract of *Uncaria tomentosa* [18].

The present study showed the prostate-specific effect of red maca, however, the mechanism(s) underlying its impact on prostate size in response to testosterone is not clear. Further studies are intended to characterize this response, which would constitute an alternative medical treatment for BPH.

In summary, the present study showed that hydroalcoholic extract of red maca reduced prostate size in male rats in which prostatic hyperplasia had been induced by TE, and that effect might be the result of a factor different to benzyl glucosinolates.

Acknowledgment

The present study was supported by funds from PROCOM 2005, a Grant from the Consejo Nacional de Ciencia Tecnología e Innovación (CONCYTEC), Peru. The authors acknowledge Eng. Alfonso Higa from Agroindustrial Chanchamayo for preparing hydroalcoholic extracts of red maca. We acknowledge the technical support from Juan Carlos Valenzuela.

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Edited by Prof. P.P. Mathur