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Effect of red maca (*Lepidium meyenii*) on prostate zinc levels in rats with testosterone-induced prostatic hyperplasia

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Summary

Lepidium meyenii (maca) is a plant that grows exclusively above 4000 m in the Peruvian central Andes. Red maca (RM) extract significantly reduced prostate size in rats with benign prostatic hyperplasia (BPH) induced by testosterone enanthate (TE). Zinc is an important regulator of prostate function. This study aimed to determine the effect of RM on prostate zinc levels in rats with BPH induced by TE. Also, the study attempted to determine the best marker for the effect of RM on sex accessory glands. Rats treated with RM extract from day 1 to day 14 reversed the effect of TE administration on prostate weight and zinc levels. However, RM administered from day 7 to day 14 did not reduce the effect of TE on all studied variables. Finasteride (FN) reduced prostate, seminal vesicle and preputial gland weights in rats treated with TE. Although RM and FN reduced prostate zinc levels, the greatest effect was observed in TE-treated rats with RM from day 1 to day 14. In addition, prostate weight and zinc levels showed the higher diagnosis values than preputial and seminal vesicle weights. In conclusion, RM administered from day 1 to day 14 reduced prostate size and zinc levels in rats where prostatic hyperplasia was induced with TE. Also, this experimental model could be used as accurately assay to determine the effect of maca obtained under different conditions and/or the effect of different products based on maca.

Introduction

The growth of prostate gland is an androgen-dependent process in which testosterone (T) is converted, by 5α-reductase action, to dyhydrotestosterone (DHT) that is the most active androgen involved in the regulation of prostate gland (Campbell *et al.*, 2006). Benign prostatic hyperplasia (BPH) is a progressive and androgen-dependent disease characterized by a dysregulation of 5α-reductase action (Bauman *et al.*, 2006) which leads to higher prostatic levels of DHT and the increase in size of the prostate gland (Martinez Caballero *et al.*, 2004; Petraki & Sfikas, 2007).

Previous studies have demonstrated that zinc is a marker of prostate function (Wong *et al.*, 2000; Yucra *et al.*, 2006, 2008). In this gland, higher values of zinc inhibit citrate oxidation in the Krebs cycle, allowing its accumulation as an end-product of metabolism rather than an utilizable intermediate of Krebs cycle (Costello *et al.*, 2004; Costello & Franklin, 2006). In BPH, increased levels of prostatic zinc are responsible for the impaired citrate oxidation observed in this pathology (Habib *et al.*, 1979; Costello & Franklin, 2006; Sing *et al.*, 2006). Also, it was reported that zinc regulates androgen metabolism (Abbasi *et al.*, 1980; Wong *et al.*, 2000) where high prostatic zinc levels observed in BPH were associated with DHT/T ratio > 1 (Habib *et al.*, 1979).

Finasteride (FN), a 5α-reductase inhibitor, is one of the most used drugs to induce androgen suppression in patients with BPH (Gao *et al.*, 2004). 5α-reductase blocks the conversion of T to DHT (Bartsch *et al.*, 2002). However, the cost burden associated with FN is substantial. For instance, its survival benefit is small and the effect is

only realized after a long time period (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).

There is an association between the high glucosinolates content in cruciferous vegetables and its role in cancer prevention including different prostate pathologies (Talalay & Fahey, 2001; Keum *et al.*, 2005; Ray, 2005; Steinbrecher *et al.*, 2009; Hwang & Lee, 2010). Isothiocyanates, the biologically active breakdown products of glucosinolates, present chemoprotective activities (Chiao *et al.*, 2004; Hwang & Lee, 2010). In fact, plants from the genus *Lepidium* have been demonstrated to reduce prostate size and volume in castrated rats with prostatic hyperplasia induced by exogenous steroids (Martinez Caballero *et al.*, 2004).

Lepidium meyenii (maca) is a plant that grows exclusively over 4000 m in the Peruvian central Andes (Valerio & Gonzales, 2005). Maca is naturally present in different varieties which are characterized by the external colour of their hypocotyls (Tello *et al.*, 1992; Yllescas, 1994). From these, red maca (RM) significantly reduced prostate size in rats with prostatic hyperplasia induced by testosterone enanthate (TE) (Gonzales *et al.*, 2005, Gonzales *et al.*, 2007; Gasco *et al.*, 2007). In fact, RM reduced prostate weight in a dose–response manner without any changes in testosterone levels and seminal vesicle weight (Gasco *et al.*, 2007; Gonzales *et al.*, 2007). However, it is still unknown whether RM is acting through prostatic zinc values.

Regarding the secondary metabolites involved in the effect of RM on prostate size, when different doses of benzylglucosinolates in RM extracts were assessed, a dose-dependent reduction in prostate weight was observed, suggesting that these compounds may be responsible for the biological effect of RM (Gonzales *et al.*, 2007). However, it was suggested that other secondary metabolites presented in RM could be also responsible for the effect on prostate size (Gonzales *et al.*, 2007). Consequently, glucosinolates content should not be used as a chemical marker to standardized products based on maca. In fact, others authors found that polyphenols could inhibit prostate size (Ganapathy *et al.*, 2010; Siddiqui *et al.*, 2010). For instance, it was suggested that polyphenols in RM may be related to the reduction in prostate size (Gonzales *et al.*, 2008).

The study aimed to determine the effect of RM on prostate zinc levels in rats with BPH induced by TE. Also, this study attempted to determine the best marker for RM action in the sex accessory glands assessing prostate, seminal vesicles and preputial glands weights as well as prostate zinc levels.

Materials and methods

Animals

Thirty-six male rats from a 3-month-old Holtzman strain (359.32 ± 6.86 g) were obtained from the animal house of the Universidad Peruana Cayetano Heredia (Lima, Peru). Rats were housed 3–6 per cage and maintained at 22 ± 1C with a 12 : 12 light/dark cycle. Rats were provided with Purina laboratory chow and tap water *ad libitum*.

All animal experiments were conducted in compliance with 'Guide of the care and use of laboratory animals' (National Research Council, 1996). The Institutional Review Board of the Scientific Research Office, Universidad Peruana Cayetano Heredia, approved the study.

Design

Figure 1 shows a flow diagram to describe the experiments. In brief, rats were randomly divided in six different groups ($n = 6$) according to the treatment: *Group I*: sesame oil (TE vehicle) + distilled water (RM vehicle) (control); *Group II*: TE + distilled water (TE); *Group III:* TE + RM extract from day 1 to day 14 of treatment (RMd1); *Group IV:* TE + FN from day 1 to day 14 of treatment (FNd1); *Group V:* TE + RM extract from day 7 to day 14 of treatment (RMd7); *Group VI:* TE + FN from day 7 to day 14 of treatment (FNd7).

In groups 2–6, rats were injected with 0.1 ml of TE (25 mg) on day 1 and day 7 of treatment to induce a BPH state as previously reported (Gonzales *et al.*, 2007). Sesame oil was used as a vehicle for TE that was administered in the same way as explained for TE (on days 1 and 7 of treatment).

On the other hand, RM extract (2 g kg^{-1} body weight per day) (Gonzales *et al.*, 2005) or FN (0.1 mg day^{-1}) (Gonzales *et al.*, 2007) was orally administered using an intubation needle No.18 (Fisher Scientific, Pittsburgh, Pennsylvania). For RM and FN treatments, distilled water was used as a vehicle. Before RM and FN treatments from day 7 to day 14, rats in groups 5 (RMd7) and 6 (FNd7) received distilled water.

Acute administration of maca did not show any toxic effect in mice in doses up to 16 g kg^{-1} (Valerio & Gonzales, 2005). In fact, long-term administration of maca did not have adverse effects on different organ weights or histology (Chung *et al.*, 2005; Gonzales *et al.*, 2006; Gasco *et al.*, 2007).

Preparation of aqueous extract of red maca

The red variety of the hypocotyls of *L. meyenii* was obtained from Carhuamayo at 4000 m altitude in Junin,

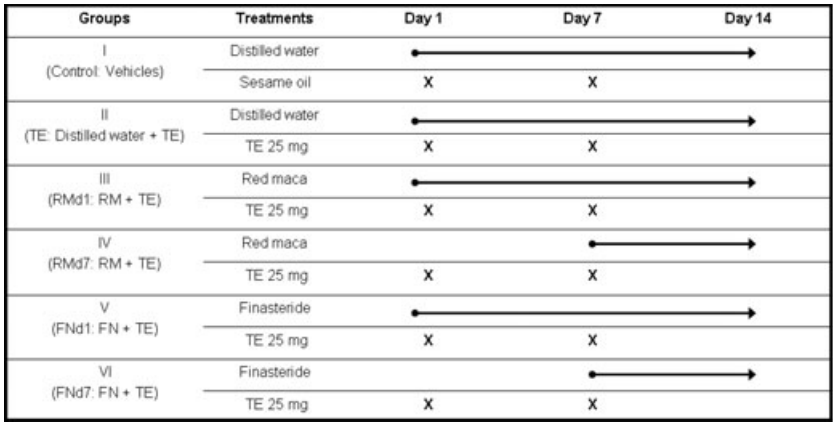


Fig. 1 Diagram of the experimental design in this study. Distilled water and sesame oil are vehicles of red maca (RM) and testosterone enanthate respectively. Finasteride (FN) was also dissolved in distilled water. RM extract from day 1 to day 14 of treatment (RMd1)/FN from day 1 to day 14 of treatment (FNd1), RM (2 g kg^{-1}) or FN (0.1 mg day^{-1}) administered from day 1 to 14; RMd7/FNd7, RM or FN administered from day 7 to 14.

Peru. The identity of the plant was authenticated in the Department of Pharmaceutical Sciences, Universidad Peruana Cayetano Heredia (voucher specimen: IFV 1885).

Thereafter, 100 g of the dried pulverized hypocotyls was 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. From this, 1 g of dried hypocotyls of RM produced 0.34 g of freeze-dried RM. The freeze-dried extract was diluted further to obtain a solution equivalent to a dose of 2 g raw material kg^{-1} body weight (Gonzales *et al.*, 2005).

Organ weights

Twenty-four hours after the last treatment, rats were killed and the following reproductive organs were dissected out, cleaned of adhering connective tissues and accurately weighed: testes, epididymis, seminal vesicle, preputial glands, and ventral prostate.

Prostatic zinc levels

At the end of the experiment, rats were killed and prostates were dissected out. The prostate were homogenized in 5.0 ml of saline (NaCl 0.9%) and centrifuged at 3500 ***g*** for 10 min. The supernatant was obtained for zinc determination by colorimetric assay (Gonzales, 2007). All samples were run in a same assay to avoid between-assay variation.

Statistical analyses

Data were analysed using the statistical package STATA version 8.0 for personal computer (Stata Corporation, College Station, TX, USA). Kruskal–Wallis nonparametric test was used to assess differences between groups. When results were statistically significant, Mann–Whitney *U* test was performed to assess differences between pair of groups. Data are presented as mean ± SEM. The area under receiver operating characteristic (ROC) curve was used to measure the discriminatory ability of the variables assessed (zinc levels in prostate and preputial glands, seminal vesicle, and prostate weights). In addition, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for each variable. In general, $P < 0.05$ was considered statistically significant.

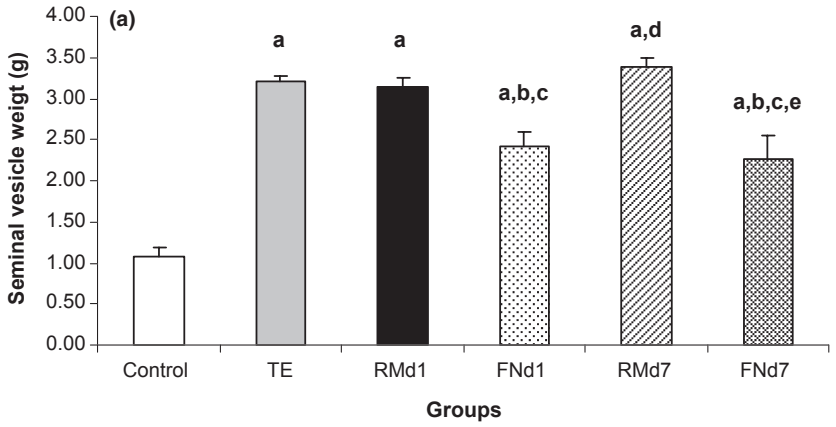
Results

Reproductive organ weights

No significance differences in testis and epididymis weights between groups (Data not shown).

Figures 2(a,b) and 3 showed the effect of RM on seminal vesicle, preputial gland and prostate weights in rats treated with TE to induce prostatic hyperplasia. TE-treated rats showed an increase in seminal vesicle (3.10 ± 0.07 g, media ± SEM), preputial gland (0.30 ± 0.03 g) and prostate (0.80 ± 0.09 g) weights when compared with control group (1.07 ± 0.11, 0.10 ± 0.01 and 0.43 ± 0.03 g respectively; $P < 0.05$).

In rats treated with TE, RM administered from day 7 (RMd7) was not able to reduce seminal vesicle (3.39 ± 0.11 g), preputial gland (0.26 ± 0.04 g) and prostate (0.90 ± 0.05) weights when compared with rats that received only TE ($P > 0.05$). In fact, rats from RMd7 group showed higher values than those in control group ($P < 0.05$). Seminal vesicle and preputial gland weights in



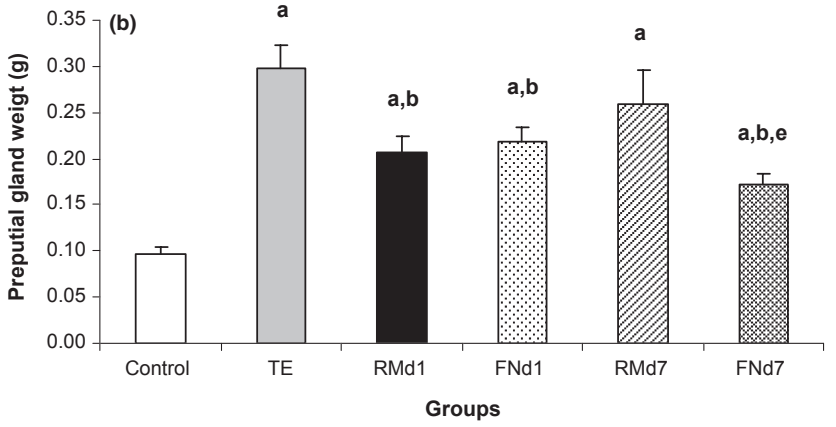


Fig. 2 Effect of red maca (RM) on seminal vesicle (a) and preputial gland (b) weights in male rats with prostatic hyperplasia induced with testosterone enanthate (TE). Data are expressed in mean ± SEM. [a]$P<0.05$, [b]$P<0.05$, [c]$P<0.05$, [d]$P<0.05$ and [e]$P<0.05$ versus control, TE, RM extract from day 1 to day 14 of treatment (RMd1), Finasteride from day 1 to day 14 of treatment (FNd1) and RM administered from day 7 to 14 (RMd7) groups respectively.

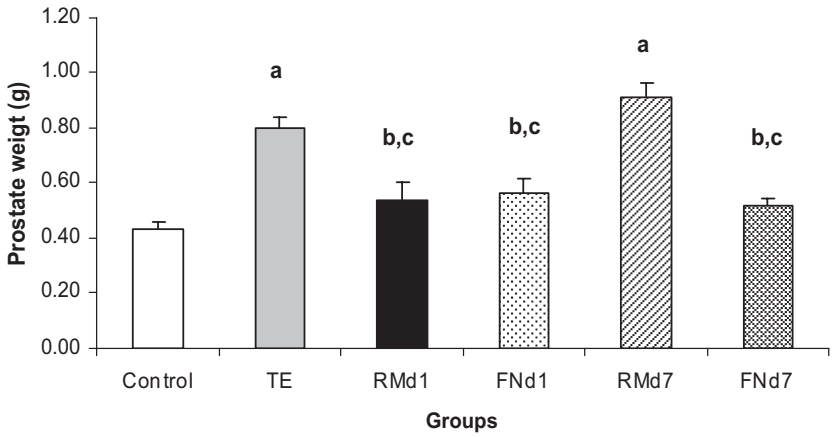


Fig. 3 Effect of red maca (RM) on prostate weight in male rats with prostatic hyperplasia induced with testosterone enanthate (TE). Data are expressed in mean ± SEM. [a]$P<0.05$, [b]$P<0.05$, and [c]$P<0.05$ versus control, TE, and RM administered from day 7 to 14 (RMd7) groups respectively.

FNd7 group (2.26 ± 0.28 g and 0.17 ± 0.01 g respectively) showed intermediate values than rats in control and TE groups ($P < 0.05$). A reduction in prostate weight (0.52 ± 0.03 g) was observed in FNd7 group when compared with TE-treated rats ($P < 0.05$), reaching similar values than control group.

The administration of RM from day 1 (RMd1) reduced prostate weight (0.54 ± 0.06 g, $P < 0.05$) in those rats where prostatic hyperplasia was induced by TE. In addition, the reduction observed in prostate weight reached similar values than those reported in control group. Red maca reversed the effect of TE on preputial gland weight (0.21 ± 0.02 g, $P < 0.05$). Moreover, rats in RMd1 group showed no differences in seminal vesicle weight than those rats treated with TE (3.15 ± 0.10 g, $P > 0.05$). In FNd1 group, a reduction in seminal vesicle (2.42 ± 0.16 g),

preputial gland (0.22 ± 0.02 g), and prostate (0.57 ± 0.05 g) weights was observed with respect to TE-treated rats ($P < 0.05$).

Prostate zinc levels

Testosterone enanthate administration significantly increased prostate zinc levels when compared with control rats (up to 197.3%, $P < 0.05$). In rats from RMd1, a reduction in prostate zinc levels was observed when compared with TE-treated rats (up to 55.8%, $P < 0.05$), reaching similar values than those from control group. Rats treated with FN for 14 days (FNd1) partially reversed the effect of TE showing intermediate values between TE-treated (a reduction through 18.3%, $P < 0.05$) and control (up to 143.8% higher) rats (Fig. 4a). In addition, correlation and regression analysis revealed a positive association between prostate weight and prostatic zinc levels ($r = 0.762$ and $R^2 = 0581$; $P < 0.001$) (Fig. 4b).

Determination of diagnostic efficiency statistics

Because preputial gland, seminal vesicle, prostate weights and prostate zinc levels showed statistical changes among treatments, these parameters were chosen for the diagnostic analysis. Regarding the area under ROC curves, prostate weight (0.80) and prostate zinc levels (0.78) showed higher values than preputial gland (0.50) and seminal vesicle (0.65) weights. Diagnostic test for prostate weight was 62.5% for sensitivity, 90.0% for specificity, 75.0% for PPV and 83.3% for NPV. In prostate zinc levels, diagnostic values were 63.0% for sensitivity, 90.0% for specificity, 84.0% for PPV and 75.2% for NPV.

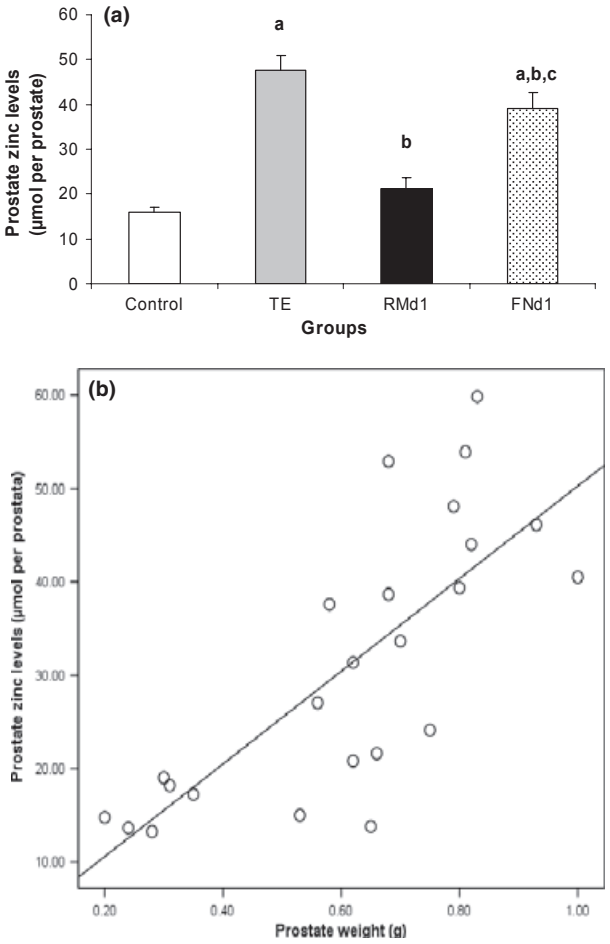


Fig. 4 Effect of red maca (RM) on prostate zinc levels in rats with prostatic hyperplasia induced by testosterone enanthate (a). Correlation analysis between prostatic zinc levels and prostate weight ($r = 0.76$, $P < 0.001$; $R^2 = 0.58$, $P < 0.001$) (b). Data are expressed in mean ± SEM. [a]$P<0.05$ versus control group; [b]$P<0.05$ versus testosterone enanthate group; [c]$P<0.05$ versus RM extract from day 1 to day 14 of treatment (RMd1).

Discussion

Previously, we have demonstrated that RM showed the best beneficial effect, when compared with yellow and black varieties, on prostate weight in normal and TE-treated rats (Gonzales, 2006a; Gonzales *et al.*, 2006). In the present study, TE administration (total dose of 50 mg) resulted in an increase in preputial gland, seminal vesicle and prostate weights. In this study, RM administered from day 1 reversed the effect of TE treatment in prostate weight without affecting seminal vesicle weight; meanwhile, FN showed to reduce both prostate and seminal vesicle weights. These results are in accordance with previous studies (Gasco *et al.*, 2007; Gonzales *et al.*, 2007). Although FN is an elective drug for BPH (Tempany *et al.*, 1993), it is associated with some side effects related to a reduced male and female sexual function (e.g. erectile dysfunction and diminished libido), gynecomastia and depression (Zlotta *et al.*, 2005; Traish *et al.*, 2011). Because of this, RM could become an important alternative for the treatment of BPH.

Prostate zinc levels were increased by TE administration, an experimental model to induce prostatic hyperplasia. The outcomes from the present study revealed that RM was able to reduce zinc levels in TE-treated rats. As mentioned earlier, zinc avoids citrate oxidation to regulate the proliferation of prostatic cells (Duncan, 1984; Vallee, 1988, 1993). Also, other authors reported a relation between the alteration in androgen metabolism and higher zinc levels in patients with BPH (Abbasi *et al.*, 1980; Wong *et al.*, 2000). In the present study, a positive correlation was found between zinc and prostate weight that is in accordance with other studies where higher zinc levels are observed in BPH (Costello & Franklin, 2006;

Sing *et al.*, 2006). The latter makes possible to suggest that RM may become a beneficial alternative treatment to prostatic hyperplasia.

Growth of the prostate is a hormone-mediated phenomenon regulated by both androgens and estrogens (Lund *et al.*, 2004). Although RM was able to reverse the effect of TE administration in prostate weight and zinc levels, no effect was observed in seminal vesicle weight, another androgen-dependent organ (Nishino *et al.*, 2004). On the other hand, FN was able to reduce both prostate and seminal vesicle weights but did not completely reduce zinc levels in prostate. Our results may possible to suggest that RM and FN could have different mechanisms of action. In fact, previous studies showed that RM specifically affect prostate size without altering testosterone or estradiol levels either in mice or in rats with prostatic hyperplasia induced by TE (Gonzales *et al.*, 2005, Gonzales *et al.*, 2007, 2008). Also, it has been published that maca has no effect on androgen receptor (Bogani *et al.*, 2006; Brooks *et al.*, 2008). The latter support the hypothesis that the effect RM is at a post-androgen receptor action level (Gonzales *et al.*, 2007) or that RM exerts an inhibitory effect at a level post-DHT conversion (Gasco *et al.*, 2007). More studies related to the mechanisms of action of RM and their relations to its capacity to reduce prostate zinc levels are required.

Red maca administration from day 7 to day 14 was not able to reduce the effect of TE in preputial gland, seminal vesicle and prostate weights. On the other hand, FN reduced the effect of TE administration when administered from day 7 to day 14. Previous studies showed that RM reduced prostate weight in rats treated with TE after 21 and 42 days of treatment (Gonzales *et al.*, 2005; Gasco *et al.*, 2007). These findings may suggest that RM should be administered more than 7 days to show its beneficial effects.

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 as isothiocyanates by the enzyme myrosinase (Keum *et al.*, 2005; Ray, 2005). These compounds have the potential to induce apoptosis selectively in proliferating precancerous cells through a cell cycle arrest-dependent mechanism (Miyoshi *et al.*, 2004). In fact, metabolites from benzyl glucosinolates have been implicated to arrest proliferation from prostatic cancer cell lines (Le *et al.*, 2003; Nachshon-Kedmi *et al.*, 2003, 2004). Also, it has been observed that glucosinolates specifically antagonizes androgen receptor (Le *et al.*, 2003). Thus, cruciferous plants are recommended to prevent or reduce prostate cancer risk (Kristal & Lampe, 2002). It is known that glucosinolates content in maca differ according to the batch, cultivated area, variety, dried time and process (Chung *et al.*, 2005; Gonzales, 2006b; Gonzales *et al.*, 2007). Although many factors could alter the glucosinolates content in RM, the effect of this plant on prostate size has been observed among different studies (Gonzales *et al.*, 2005; Gonzales, 2006a; Gonzales *et al.*, 2006, 2007; Gasco *et al.*, 2007), suggesting that other secondary metabolites presented in RM could be also responsible for the effect of maca on prostate size as suggested by others (Gonzales *et al.*, 2007). Consequently, glucosinolates content should not be used as a chemical marker to standardized products based on maca. In addition, the effect of RM on prostate size could be related to its polyphenols content as previously reported (Gonzales *et al.*, 2008). For instance, polyphenols obtained from green tea has been demonstrated to inhibit 5-α reductase activity (Hiipakka *et al.*, 2002). For this reason, we also determined the diagnostic efficiency statistics of this assay to determine the effect of RM on seminal vesicle, preputial gland and prostate weights and zinc levels. The analysis showed that prostate weight and zinc levels can be useful parameters to determine the effect of RM on rats treated with TE for 14 days. These results should allow us to use this model as a bioassay for quality control to discriminate the effect on prostate weight and/or zinc levels of RM obtained under different conditions and/or different products based on maca.

To sum up, RM may regulate prostatic growth by reducing prostate zinc levels in rats where prostatic hyperplasia was induced with TE. Moreover, the determination of prostate weight and zinc levels can be considered as alternative markers to allow us to use this experimental model to discriminate the effect of RM from different locations, environmental conditions and/or products.

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