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Antioxidant activity of the cruciferous vegetable Maca (*Lepidium meyenii*)

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Abstract

Maca (*Lepidium meyenii*) is a plant from the Andes of Peru. Maca is used as a food for its nutritional value and ethnomedicinal properties linked to fertility and vitality. The purpose of this study was to evaluate the antioxidant activity of Maca. For all experiments an aqueous extract of Maca was used. The antioxidant activity of Maca was assessed by the inhibition of peroxynitrite, 1,1-diphenyl-2-picrylhydrazyl (DPPH), peroxyls and deoxyribose degradation. The cytoprotection capacity of Maca was assessed using macrophages (RAW 264.7) treated with peroxynitrite or hydrogen peroxide (H_2O_2). Catechins were quantified by reversed-phase HPLC. Addition of Maca extract (0.3–1 mg/ml) to peroxynitrite (300 μM) decreased peroxynitrite concentration by 15 and 41%, respectively ($P<0.01$). The IC_{50} for scavenging DPPH and peroxyls was 0.61 and 0.43 mg/ml, respectively. Deoxyribose protection by Maca (1–3 mg/ml) against hydroxyl radicals was in the order of 57 and 74%. Maca (1 mg/ml) protected RAW 264.7 cells against peroxynitrite-induced apoptosis ($P<0.01$), and increased ATP production in cells treated with H_2O_2 (1 mM). The concentration of catechins in Maca was lower than in green tea (2.5 mg/g vs 145 mg/g). Collectively, our results indicate that Maca has the capacity to scavenge free radicals and protect cells against oxidative stress.

1. Introduction

Maca *Lepidium meyenii* Walp is the only cruciferous vegetable native to the Americas that grows in the suni and puna ecosystems of Peru (altitude > 3500 m above sea level). The tuber of the plant is used for human consumption because of its nutritional value, and phytochemical content (Dini, Migliuolo, Rastrelli, Saturnino, & Schettino, 1994). Maca has been claimed to help alleviate altitude-related compromises in fertility, enhance sexual drive of domestic animals, and promote vitality for humans (Obregon, 1998). Peruvian researchers have been investigating Maca for its fertility-enhancer capacity, especially in livestock raised at high altitudes where fertility is compromised. Results from these experiments, indicate that Maca helps to improve fertility performance of sheep and guinea pigs, through actions on both the male and female reproductive systems (Obregon, 1998).

During the past 5 years several developed countries from North America, Europe and Asia have shown interest for Maca, particularly for its aphrodisiac effects. Zheng et al. (2000) has reported that oral administration of a lipid extraction of Maca increased the sexual function of mice and rats. The researchers assessed number of complete intromissions, number of sperm-positive females in normal mice, and decrease in the latent period of erection in male rats with erectile dysfunction.

Recently, Maca has been introduced to developed societies and is available in several processing forms, such as micropulverized (powder, tablets), freeze-dried and hydro-alcoholic extracts. It is claimed by the nutraceutical industry that Maca has the ability to improve energy and modulate the response against oxidative stress but these assertions have not been scientifically substantiated. In our continuous effort to study medicinal plants from the Andes we decided to investigate Maca. There is a wide interest to know if Maca consumption could promote health, especially in developing countries where the emerging of chronic diseases due to nutrition transition and oxidative stress is prevalent (Tucker & Buranapin, 2001).

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The purpose of this study was to evaluate the antioxidant activity of Maca (*Lepidium meyenii*) assessed by its capacity to inhibit free radicals and to protect cells against oxidative stress.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were reagent grade and were purchased from Sigma Chemical Co (St. Louis, MO).

2.2. Plant material

Farmers from Cerro de Pasco, Peru, cultivated Maca (*L. meyenii*) for this study. Cerro de Pasco (>4200 m above sea level), is the major producing area in the Andes of Peru. Maca is harvested after 8–9 months of planting the crop, and was provided by Rainforest Phytoceuticals (Delmar, NY). The samples (tuber) were thoroughly washed (3×) with water containing sodium hypochlorite (NaOCl, 1%) then dried in convection ovens at 60 °C for 16 h. The samples were ground in a stainless steel Wiley Cutting mill (Fisher Scientific, Pittsburgh, PA), and sifted with a mesh (200 μm). The micropulverized sample was hermetically stored in plastic bags to avoid microbial contamination.

2.3. Sample preparation

The sample was prepared only with water to resemble the usual preparation of Maca for consumption as part of the diet. We know that all phenolic compounds present in Maca are not extracted with this form of preparation. There are alcoholic preparations with 44% alcohol that are available commercially; however, for ethical reasons we do not encourage this form of Maca. An aqueous extract was made by boiling in water the micropulverized Maca (50 mg/ml) for 10 min. For in vitro chemical reactions, the aqueous extract was filtered at 20 μm with a Whatman paper. All assays were carried out with a fresh solution of Maca. To compare Maca with another source of natural antioxidants we collected tea (*Camellia sinensis*) from Jardines de Te S.A. (Tingo Maria, Peru). Green tea was manufactured in our laboratory following a proprietary methodology. Stock solutions of green tea (50 mg/ml) were prepared for HPLC analysis.

2.4. Peroxynitrite synthesis

Peroxynitrite was synthesized by modifying a previous methodology. Briefly, solutions of (a) 0.6 M $NaNO_2$, 3% H_2O_2 and (b) 0.6 M HCl, were pumped using a syringe infusion pump (Harvard Apparatus, South Natick, MA) at 24 ml/min, into a Y junction and mixed in a 2-mm diameter silicone tubing. The acid catalyzed reaction was quenched in 1.5 M KOH solution. The prepared stock solution contained 40 mM peroxynitrite as determined by absorbance at 302 nm (Sandoval-Chacón et al., 1998). The stock peroxynitrite solution was purified with MnO_2 to remove excess of unreacted H_2O_2, then filtered at 0.2 μm.

2.5. DPPH assay

The 1,1-diphenyl-2-picrylhydarazyl (DPPH) radical scavenging method previously reported by (Yamaguchi, Takamura, Matoba, & Terao, 1998) was modified as follows. The aqueous extract of Maca (50 mg/ml) was dissolved in ethanol (1:4 vol/vol) prior to the reaction with a methanolic solution of DPPH (100 μM). An aliquot (500 μl) of Maca was placed in a cuvette and DPPH (500 μl) was added to obtain a final vol 1 ml. The decrease in absorbance at 515 nm was determined continuously with data capturing at 30-s intervals with a Beckman Coulter DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA). All determinations were performed in triplicate. The DPPH scavenging capacity of Maca was expressed as percentage inhibition (% inhibition), and was determined by the following expression: % inhibition $=[(A_{control}-A_{sample})/A_{control}]\times 100$ where $A_{control}$ is the absorbance at time = 0, and A_{sample} is the absorbance of the sample at time = 15 min.

2.6. Peroxyl radical scavenging

Peroxyl radicals can be generated by several methods, and their reaction with antioxidants studied. We selected to use the total (peroxyl) radical-trapping antioxidant parameter (TRAP) assay (Bartosz, Janaszewska, Ertel, & Bartosz, 1998; van Overveld, Haenen, Rhemrev, Vermeiden, & Bast, 2000), which is used to study the antioxidants in biological fluids. Peroxyl radicals were generated by 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH). In this method, the peroxyl radical has a green color and is quantified spectrophotometrically at 414 nm. For our experiments, Maca at different concentrations was added to evaluate the rate of peroxidation determined by the decrease in absorbance. The original method was modified as follows. A stock solution containing: 225 μM, 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) and 2 mM, AAPH in PBS buffer (50 mM phosphate, 0.9% NaCl, pH 7.4) was prepared and incubated at 70 °C for 20 min then cooled on ice. Inhibition of peroxyl radicals was determined by adding Maca (10 μl) to the reaction mixture in a final volume of 1 ml at 37 °C. The decrease in absorbance at 414 nm was determined continuously with data capturing at 30-s intervals for 6 min with a

DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA). Inhibition of peroxyl radicals was calculated by the following expression: % inhibition $=[(A_{control}-A_{sample}) / A_{control}]\times 100$ where $A_{control}$ is the absorbance at time = 0, and A_{sample} is the absorbance of the sample at time = 6 min.

2.7. Deoxyribose assay

To further evaluate the antioxidant activity of Maca we assessed deoxyribose protection against hydroxyl radicals (•OH) generated by reacting Fe^{3+}-EDTA, ascorbic acid and H_2O_2 (Halliwell, Gutteridge, & Aruoma, 1987). Briefly, the reaction mixtures contained, in a final volume of 1 ml, the following reagents: deoxyribose (2 mM), KH_2PO_4–KOH buffer, pH 7.4 (20 mM), $FeCl_3$ (100 μM), ascorbate (100 μM), and variable concentrations of Maca. Formation of malonaldehyde (MDA) was quantified at 532 nm with a Beckman Coulter DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA).

2.8. ATP production

Experiments were conducted to evaluate whether Maca had the ability to maintain the intracellular levels of adenosine 5′-triphosphate (ATP) in macrophages (RAW 264.7) exposed to conditions of oxidative stress. RAW 264.7 cells were either treated with H_2O_2 (1 mM) or pretreated with Maca (1 mg/ml) for 1 h, then cells were fed medium containing H_2O_2 (1 mM) and incubated for 4 h. The concentration of ATP was quantified following a previous methodology (Hinshaw, Burger, Delius, & Hyslop, 1990) using a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA). A commercial standard of ATP was used to establish the calibration curve for this assay.

2.9. Apoptosis

RAW 264.7 cells were treated with peroxynitrite (300 μM) and/or supplemented with Maca (1 mg/ml), and incubated for 4 h. Apoptosis was quantified using a Vmax Kinetic microplate reader (Molecular Devices, Sunnyvale, CA) with a cell death detection ELISA kit (Roche Molecular Biochemicals, Nutley, NJ) as described previously (Sandoval-Chacón et al., 1998).

2.10. HPLC and chromatographic conditions

The HPLC system consisted of a GBC LC 1150 series pump, a GBC 1650 advanced autosampler (GBC Scientific Equipment Pty Ltd, Dandenong, Australia), a 25 cm×4.6 mm I.D. Discovery® C18, 5 μm analytical column (SUPELCO, Bellefonte, PA), and a Waters 490E programmable multi wavelength detector (Waters, Milford, MA). To determine the content of catechins in Maca and green tea, aqueous extracts were prepared (50 mg/ml) in hot water. Then the extract was centrifuged at 4000×***g*** and filtered at 0.2 μm prior to HPLC analysis. For HPLC calibration, we used the standards Catechin (CAT), epigallocatechin gallate (EGCG), and Polyphenon 60 (Sigma Chemical, St. Louis, MO). Stock solutions of the standards were prepared by dissolving weighed quantities of standards in HPLC-grade methanol. Aliquots were stored at −20 °C to preserve its stability. All separations were performed at ambient temperature by reversed-phase HPLC using gradient elution. Flavanols were detected at 210 nm (Bronner & Beecher, 1998), using a methanol-aqueous acetate buffer (1 mM acetic acid, 1 mM sodium acetate in water, pH 4.5) programmed linearly from 30 to 50% methanol (0–40 min) at a flow rate of 0.5 ml/min.

2.11. Statistical analysis

Each experiment was performed at least three times and results are presented as the mean ± S.E. Statistical analyses were performed using *t*-test and one-way ANOVA. Post hoc comparison of means was done by Least Significant Difference test and unpaired *t*-test. A probability value of <0.05 was considered significant.

3. Results and discussion

In the present study we showed that addition of an aqueous extract of Maca (0.3–1 mg/ml) scavenged ($P<0.01$) peroxynitrite (300 μM) by 15.0 ± 0.7 and 41.1 ± 1.2%, respectively (Fig. 1). The decomposition of peroxynitrite was monitored at pH 12 because peroxynitrite has a short-half life at physiological pH. In previous experiments we have shown that antioxidants from botanicals decreased the half-life of peroxynitrite, and by this mechanism they protected DNA against oxidant-induced damage (Sandoval et al., 1997). Our results indicate that Maca contains phytochemicals that have the ability to quench peroxynitrite, which is produced physiologically under chronic inflammation (Beckman & Koppenol, 1996). The antioxidant activity of Maca was also investigated by measuring its capacity to scavenge DPPH and peroxyl radicals. As shown in Table 1, Maca (0.03–3 mg/ml) quenched DPPH (100 μM) in a dose dependent manner ($P<0.01$). The IC_{50} value for DPPH inhibition was 0.61 mg/ml. Results from the peroxyl assays indicated that Maca decreased peroxyl formation ($P<0.01$). The IC_{50} value for peroxyls was 0.43 mg/ml. The formation of peroxyl radicals is a key step in lipid peroxidation (Halliwell & Gutteridge, 1990); our results demonstrate that Maca contains water-soluble scavengers that may contribute to decompose peroxyls produced during inflammatory

states (Dean, Gieseg, & Davies, 1993), hence Maca consumption may afford cytoprotective effects.

In another set of experiments, the ability of Maca to quench hydroxyl radicals was tested in vitro. The results indicated that Maca (1–3 mg/ml) afforded deoxyribose protection ($P<0.01$) against hydroxyl radicals in the range of 57–74%, respectively. As it has been reported, mitochondrial metabolism represents a major source of intracellular ROS, such as superoxide, hydroxyl radicals or hydrogen peroxide (Koufen & Stark, 2000). There is increasing evidence that increased production of reactive oxygen species are involved in various disorders, and also are responsible for cellular damage (Johnson, Ferrans, Lowenstein, & Finkel, 1996).

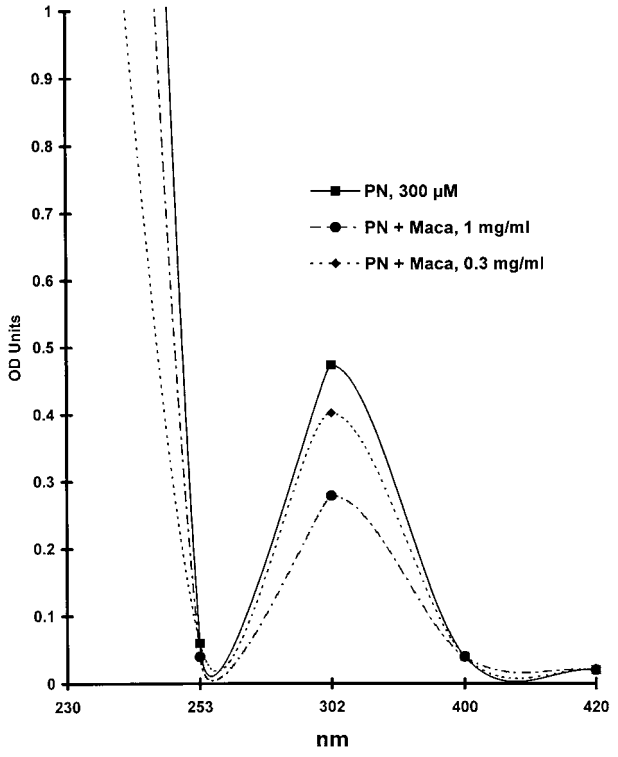

Fig. 1. Antioxidant activity of Maca (*Lepidium meyenii*) determined by its capacity to decompose peroxynitrite. The scavenging of peroxynitrite was quantified spectrophotometrically at 302 nm as described in Section 2. Values are mean±S.E. of three samples. *Significant inhibition ($P<0.01$) compared to control peroxynitrite (PN).

To assess the cytoprotective capacity of Maca against peroxynitrite-induced apoptosis we used macrophages (RAW 264.7). Results from these experiments demonstrated the ability of Maca (1 mg/ml) to protect against DNA damage induced by peroxynitrite (Table 2). The formation of peroxynitrite from nitric oxide (NO) and superoxide has been proposed as a mechanism to explain the cytotoxic effects of NO and superoxide (Beckman & Koppenol, 1996). Peroxynitrite has been shown to induce apoptosis in several cell lines (Sandoval et al., 1997), inactivate the DNA repair enzyme poly ADP-ribose polymerase (Zingarelli, O'Conner, Wong, Salzman, & Szabó, 1996), and oxidize intracellular proteins (Grune, Klolz, Gieche, Rudeck, & Sies, 2001). The observed cytoprotective effects of Maca may be due in part to its capacity to diminish the deleterious effects of excessive production of reactive oxygen species. This is the first report, to our knowledge, of the cytoprotective effect of Maca against cell death induction by peroxynitrite.

To investigate whether Maca may contribute to the maintenance of intracellular ATP production in conditions of oxidative stress generated by H_2O_2, we conducted experiments with RAW 264.7 cells. The results indicated that H_2O_2 (1 mM) decreased ATP production by 42.3% ($P<0.001$) compared to the control group (100%). On the other hand, pretreatment with Maca (1 mg/ml) for 1 h increased ATP production by 48.2% compared to the H_2O_2 treated cells ($P<0.01$). As it has been reported, hydrogen peroxide exerts deleterious effects on cell function influencing molecular and biochemical processes, which in turn decrease ATP production observed during oxidative stress (Allen & Tresini, 2000). To counteract the cytotoxic effect of

Table 1
Free radical scavenging capacity of Maca (*Lepidium meyenii*)[a]

Maca (mg/ml)	Free radical inhibition (%)	
	DPPH	Peroxyl
0.03	21.64±0.02	11.3±1.3
0.10	34.79±0.05	21.8±1.5
0.30	44.35±0.10	46.7±1.4
1.00	55.40±0.19	93.3±0.4
3.00	71.38±0.31	ND

[a] An aqueous extract of Maca was used for these experiments. The absorbance inhibition for DPPH and peroxyls was monitored at 515 and 414 nm, respectively. Values represent mean±S.E. of three different reactions as described in Section 2. Significant ($P<0.01$) inhibition of DPPH as the dose of Maca increased.

Table 2
Effect of Maca (*Lepidium meyenii*) on peroxynitrite-induced apoptosis in RAW 264.7 cells[a]

Treatment	Apoptosis OD $_{490/650}$
Control	1.366±0.08
Peroxynitrite, 300 μM	2.834±0.09[b]
Maca, 1 mg/ml	1.413±0.13
PN + Maca	1.962±0.05[c]

[a] An aqueous extract of Maca was used for this experiment. Cells were seeded at a density of 1×10^6 cells/well. Details of the experiment are described in Section 2. Values represent mean±S.E. of three experiments, each with three samples.

[b] Significant increase in apoptosis ($P<0.01$) compared to control and Maca.

[c] Significant decrease in apoptosis ($P<0.01$) compared to peroxynitrite (PN).

H_2O_2, catalase is required for the dismutation of H_2O_2 into water and molecular oxygen (Halliwell & Gutteridge, 1990). During oxidative stress the production of ROS is exacerbated, less ATP is produced, and more catalase may be required for keeping intracellular H_2O_2 concentrations at a steady-state levels (Tan et al., 2000). The in vitro results indicated that Maca reacted with oxidants and free radicals, and protected cells against peroxynitrite and H_2O_2. Hence, Maca may help to maintain a balance between oxidants and antioxidants.

The presence of flavanols in Maca was determined by reversed-phase HPLC (Fig. 2), and the catechin content was compared with green tea (Table 3). Maca has a lower content of catechins than green tea (2.5 mg/g vs 145 mg/g). It has been reported that polyphenols in green tea contribute to its potent antioxidant capacity (Trevisanato & Kim, 2000; Salah, Miller, Paganga, Tijburg, & Rice-Evans, 1995; Singh, Ravindranath, & Singh, 1999). Since greater concentrations of Maca were required for inhibiting oxidants and free radicals than

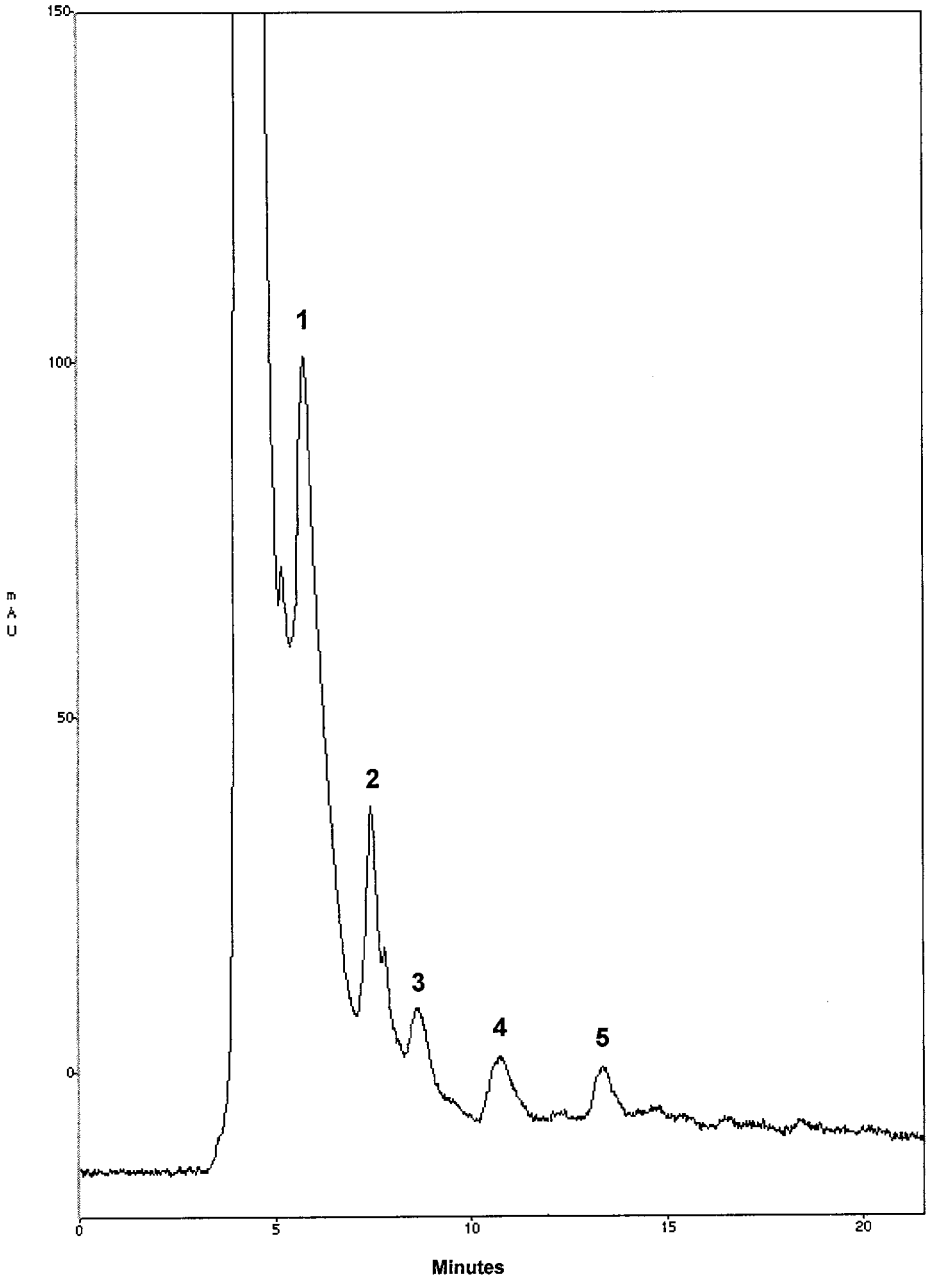

Fig. 2. HPLC chromatogram of an aqueous extract of Maca (*Lepidium meyenii*). Calibration curves were obtained from commercial flavanol standards. Epigallocatechin gallate **(1)**, Epigallocatechin **(2)**, Catechin **(3)**, Epicatechin gallate **(4)**, and Epicatechin **(5)**. A linear relationship between peak size and concentration was observed. For quantification of the compounds **(1–5)**, the area under the curve was used. Chromatographic conditions are described in Section 2.

Table 3
Content of flavanols in Maca (mg/g) (*Lepidium meyenii*) and green tea (*Camellia sinensis*) determined by HPLC[a]

Flavanol	Maca	Green tea
Catechin	0.32±0.001	30.39±1.9
Epicatechin	0.17±0.009	13.74±0.9
Epicatechin gallate	0.37±0.003	2.15±0.7
Epigallocatechin	0.66±0.001	47.84±1.1
Epigallocatechin gallate	0.92±0.002	54.01±1.7

[a] Aqueous extracts of Maca and green tea were used for these experiments. The content of flavanols was determined by reversed-phase HPLC as described in Section 2. Values (mg/g dry matter) represent mean±S.E. of three injections.

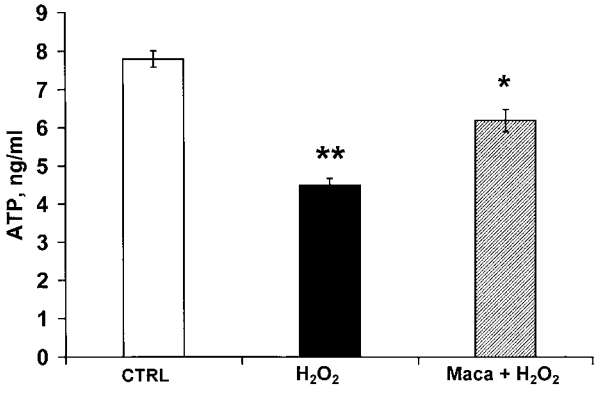

Fig. 3. Effect of Maca (*Lepidium meyenii*) on ATP production in macrophages (RAW 264.7). Cells were seeded at 1×10^6 cells/well. Bars represent ATP production of cells treated with hydrogen peroxide and Maca. The concentration of ATP in the medium was quantified using a luminometer as described in Section 2. Values are mean±S.E. of three samples. **Significant inhibition ($P<0.001$) compared to control; *significant increase in ATP production compared to hydrogen peroxide ($P<0.01$).

green tea and *Uncaria tomentosa* (Erba et al., 1999; Sandoval et al., 2000), it is possible that its polyphenols content explains the observed difference of action for quenching oxidants. The pattern of consumption of both sources may explain the health benefit of consuming Maca (approximately 75 g/day) in conditions of oxidative stress compared to green tea intake (1 g/cup). Another potential benefit for Maca may be its isothiocyanates content. Isothiocyanates have been shown to have antioxidant activities (Fahey & Talay, 1999), and anticarcinogenic properties as assessed by the ability to inhibit phase I enzymes, and induce phase II enzymes (Rose et al., 2000).

The recent growth in knowledge of reactive oxygen and nitrogen species (ROS/RNS) and antioxidants in biology is generating interest on natural products that may contribute to cell protection or improve health conditions (Hardy, 2000). The most notorious ROS/RNS (superoxide, hydroxyl, peroxyl, H_2O_2 and peroxynitrite) have been implicated in the etiology of degenerative diseases including cardiovascular disease, diabetes, cancer, neurodegenerative disorders, and aging (Allen & Tresini, 2000). There are few studies evaluating the mechanisms for the beneficial effects of Maca. Here, we demonstrated that Maca degraded free radicals, ameliorated peroxynitrite induced-cell death similar to what we described for other plant extracts, and protected cells against hydrogen peroxide by maintaining the intracellular production of ATP. Collectively, our results indicate that Maca has the ability to scavenge free radicals, and provides cytoprotection during oxidative stress conditions (Fig. 3).

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