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Report

Photoprotection against the UVB-induced oxidative stress and epidermal damage in mice using leaves of three different varieties of *Lepidium meyenii* (maca)

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Abstract

Background Skin exposure to ultraviolet (UV) B radiation leads to epidermal damage and generation of reactive oxygen species. The photoprotective effect of extracts of three varieties of leaves (red, yellow, and black) from maca (*Lepidium meyenii*), a plant from the Peruvian highlands, was assessed in mouse skin exposed to UVB radiation.

Materials and methods The hydroalcoholic extracts of three varieties of maca leaves were applied topically to the dorsal skin of young–adult male mice prior to exposition to UVB radiation.

Results The three varieties had UVA/UVB absorptive properties and presented antioxidant activity, being highest with red maca, followed by black and yellow maca. The three varieties of maca leaves prevented the development of sunburn cells, epidermal hyperplasia, leukocytic infiltration, and other alterations produced by UVB radiation. Mice treated with black maca showed the highest superoxide dismutase levels, and mice treated with black and yellow maca showed higher catalase levels in skin, whereas red maca protected the skin and liver against significant increases in the lipid peroxidation activity observed in the unprotected animals.

Conclusion The presence of significant antioxidant activity and the inhibition of lipid peroxidation suggest that the observed protection could be partly attributable to this mechanism.

Introduction

Ultraviolet (UV) B radiation constitutes over 10% of the UV radiation that reaches the earth surface and is the most biologically active.[1] It can act directly by generating inflammation and proliferation in human and animal skin, or indirectly by producing reactive oxygen species (ROS) and by depleting the antioxidants in the skin.[2,3] These processes are implicated in several of the biological effects caused by ultraviolet radiation (UVR), such as sunburn, hyperpigmentation, photoimmunosuppression, photoaging, and photocarcinogenesis.[4,5]

UV-induced oxidative stress is countered in the body by endogenous antioxidants that neutralize the ROS before they can produce oxidative changes in the tissues.[5,6] These endogenous antioxidants include the superoxide dismutase (SOD), catalase, and other molecules, such as glutathione.[7] However, several factors, including age[8] or chronic exposure to UVR, can generate an imbalance in the oxidant/antioxidant activity, which reduces the ability of the skin to repair damage due to ROS.[9] There have been several attempts to develop topically administered compounds to protect the skin against UVR.[10,11] Recently, several studies have been published that demonstrate a protective effect of medicinal plant extracts on skin exposed to UVR.[12–15]

Lepidium meyenii, also known as maca, is a plant that grows exclusively over 4000 m above sea level in the Peruvian Central Andes, in a zone where the UVR intensity is higher than at sea level.[16] To survive in this hostile environment, maca has developed adaptations that include protection against UVR, and aqueous extracts of yellow maca hypocotyls have been shown to prevent UV-induced epidermal hyperplasia in the skin of rats.[12] Because hypocotyls grow below the ground, whereas the leaves are directly exposed to solar radiation, then it is possible that leaves could also develop mechanisms for protection against UVR. Maca exists in three color varieties,[17] each

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with different biological properties.[18–20] However, it is not known if maca leaf extracts from different color varieties also protect against the effects of UVR.

The aim of this study was to evaluate the photoprotective effect of the leaves of the three different varieties of maca (yellow, black, and red) against the UVB-induced oxidative stress and epidermal damage in the skin of mice.

Materials and Methods

Experimental animals

Three-month-old, male Swiss mice were used for the UVR experiments. Mice were obtained from Universidad Peruana Cayetano Heredia animal house and housed under a 12-hour light:dark cycle in cages in a controlled environment at 20–22 °C and 60–70% humidity. Mice were given a commercial mice diet and water *ad libitum*. The study included six experimental groups of six animals per group. This research study complied with the guidelines described in the Guide for the Care and Use of Laboratory Animals[21] and was approved by the Institutional Review Board of the Scientific Research Office of the Universidad Peruana Cayetano Heredia.

Maca extract preparation

Dried leaves of the three different varieties of maca (yellow, red, and black) were pulverized, boiled twice for 60 minutes in 50% EtOH, and the final volume of the ethanolic extract was allowed to cool, and then filtered, frozen at −70 °C, and freeze dried. Maca was boiled, as this plant is traditionally used after a boiling process.[12] The recovery was about 10.56–11.68% for the three different extracts. The lyophilized extract was diluted with distilled water for use in the different assays, and the value of total polyphenols (TP) was used as a marker to standardize the assays.

TP assay

The TP content of the three extracts was determined using the Folin–Ciocalteau method,[22] with several modifications: 0.3 ml of the sample (maca leaves extract, 0.3 mg/ml) was added to a flask with 1.5 ml of the Folin–Ciocalteau reagent (1/5 dilution). After one minute, 1.2 ml of sodium carbonate (7.5 g/100 ml) was added. The contents of the tubes were mixed and stored in darkness for 30 minutes. Absorbance was measured at 760 nm using a spectrophotometer.

A calibration curve was made using pyrogallol (50 μg/ml) as standard, and the results were expressed as pyrogallol (g)/100 g of sample. All samples were analyzed in triplicate.

In all the experiments, the TP content of the maca leaf extracts was standardized against a concentration of 1 mg pyrogallol/ml.

Absorption spectrum of maca leaves extract

An absorption analysis was performed for each of the three varieties of maca leaves (1 mg pyrogallol/ml). Samples were diluted 1:10 and filtered (Millex; Millipore, Farnaceutico Responsavel, Brasil). Absorbances were measured in a spectrophotometer (Perkin Elmer Spectrum One FT-IR Spectrometer, Norton, OH, USA) using a standard quartz cuvette with 1-cm path length.

UVR treatment

Animals, including those in the control group, were shaved on the dorsal surface 48 hours prior to exposition to UVB radiation. On the day of UVB exposition, the animals were anesthetized with pentobarbital (30 mg/kg) in 0.9% saline solution before irradiation. Once anesthetized, the treatment was administered topically to the dorsal shaved skin surface. The following treatments were placed topically on the dorsal surface of the animal: black maca leaves (BM); yellow maca leaves (YM); red maca leaves (RM); and a commercial sunscreen SPF 60 used as positive control. A broad spectrum sunscreen that protects the skin throughout the UVA/UVB spectrum was used; the sunscreen contains active ingredients, such as octocrylene, octyl methoxicinnamate, titanium dioxide, benzophenone-3, and octyl salicylate, that provide physical and chemical protection.

The volume used was 200 μl per treatment, at a concentration of 1 mg pyrogalol/ml. Dilutions were made on distilled water. Animals that were exposed to UVR and left untreated [irradiated control (IC)], and animals that were left untreated and unexposed [non-irradiated control (NIC)], were used as controls. The total number of groups was six.

Mice were then placed, according to their group, in the radiation chamber to be irradiated on the shaved dorsum. The radiation chamber consisted of a cage coupled to a UVB lamp (Spectroline Longlife Filter, Model: ENB-260C; Spectronics Corporation, Westbury, NY, USA). The animals were placed at a distance of 15 cm from the source of radiation. Exposure to UVB radiation was for 60 minutes daily, for five consecutive days.

Animals were humanely killed two hours after the last UVB radiation by cervical dislocation. Liver and skin were dissected, cleaned, and weighted. Skin biopsies were divided in two: one fixed in 10% neutral-buffer formalin for histological analysis and the other placed in phosphate buffer (pH 7.4, 1:20 proportion) for biochemistry analysis. The tissue samples for biochemical analysis were then homogenized once for four minutes and then centrifuged for 10 minutes at 10,000 ***g***. The supernatant was stored at −20 °C for protein and biochemical assays.

The liver was homogenized in phosphate–potassium chloride buffer (1:9) and centrifuged for 10 minutes at 800 ***g***. The supernatant was stored at −20 °C for protein and thiobarbituric acid-reactive substances (TBARS) assay. The Lowry protein assay[23] was assessed to determine the protein content in the homogenized tissues.

Histopathological evaluation

Skin biopsies fixed in 10% neutral-buffer formalin were embedded in paraffin, sectioned at 5 μm, and routinely stained

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using hematoxylin and eosin (H–E). Histological evaluations were carried out using an optical microscope (Leica DM 1000; Wetzlar, Germany) at 40× magnification. Measurements were carried out using the computer software Leica Application Suite for Windows (Leica). The histopathological examinations were done by a pathologist. The samples were blinded previous to examinations.

To determine the epidermal hyperplasia, the mean distance (30 measurements/section) between the stratum granulosum and the dermo-epidermal junction was calculated.[24]

The number of sunburned cells (SBC) was determined according to the method described by Reefman.[25] SBC are apoptotic keratinocytes characterized for having a pyknotic nucleus and hyaline eosinophilic cytoplasm. The epidermis area was measured by drawing a line around the epidermal area and calculating the total surface (square millimeter). The number of pyknotic nuclei or its fragments present in the area were counted. The total number of SBC was determined by dividing the number of pyknotic nucleus counted by area of epidermal surface.

The presence of apoptotic keratinocytes was also evaluated semiquantitatively in the epidermis, according to a 0–3 scale,[26] where: 0, absent; 1, mild (1–3 solar cell damage present); 2, moderate (four or more solar cell damage present); and 3, severe (extended epidermal necrosis).

Leukocyte infiltration was evaluated semiquantitatively by observing the presence of leukocytes in the different sections of the epidermis and dermis. The system of evaluation used a 0–4 scale,[26] where: 0, absent; 1, mild (<5 leukocytes in a 40× section); 2, moderate (between 5 and 10 leukocytes in a 40× section); 3, severe (more than 10 leukocytes in a 40× section); and 4, extensive inflammation with darkening of the dermo-epidermal junction.

Atypia was characterized according to the cells that seem abnormal (nuclear size and shape, staining characteristics, or relative position to other cells of the same type). Evaluation was made using a 0–3 scale, where: 0, absent; 1, focal; 2, multifocal; and 3, diffuse. In the presence of atypia (score >1), the severity of atypia was evaluated by the following scale: 1, minimum; 2, mild; 3, moderate; and 4, severe.

The activity of the dermo-epidermal junction was characterized by degeneration and disintegration of its junction. This was evaluated using a 1–3 scale,[26] where: 1, mild (small vacuolization, with no disintegration of the dermo-epidermal junction); 2, moderate (vacuolization with minimal disintegration); and 3, severe (large disintegration with cleft formation).

Lipid peroxidation assay in irradiated animals

In order to determine the amount of lipid peroxidation generated from UVB radiation, a TBARS assay was used. The skin or liver homogenate was added to a mixture that contained 0.1 N HCl, 10% phosphotungstic acid, and 0.7% TBA. malondialdehyde (MDA) (5, 10, and 20 μl; 131.7 μM) was used as control. The mixture was heated in boiling water for 60 minutes, and further extracted in 5 ml *n*-butanol and centrifuged for 10 minutes at 10,000 ***g***; fluorescence of the butanolic phase was recorded at 515 nm (excitation) and 553 nm (emission). Values were expressed as pmol MDA/mg protein.

Antioxidant enzyme activity in skin homogenates of irradiated animals

Catalase activity was determined spectrophotometrically by the disappearance of hydrogen peroxide (H_2O_2). The results were expressed as picomoles of catalase per mg of protein. SOD activity was determined spectrophotometrically following the rate of adenocrome formation at 480 nm in a medium with 50 mM glycine–NaOH, pH 10.2, and with a final concentration of 1 mM epinephrine. The SOD activity was expressed as a unit of SOD (USOD)/mg of protein.

Antioxidant activity *in vitro*

DPPH radical-scavenging assay

The radical-scavenging activity of the three maca leaf extracts against DPPH radical was assessed as described previously,[27] with a slight modification. Briefly, different concentrations (0.01, 0.025, 0.05, 0.075, and 0.1 mg/ml) of the extracts were mixed with DPPH (6.5×10^{-5} mol/l, in MeOH). Ascorbic acid was used as control and distilled water as vehicle. The solution was incubated at room temperature for 30 minutes in darkness, and the changes in the absorbance at 515 nm due to the scavenging of DPPH radical were measured with a spectrophotometer.

The antioxidant activity (%) was calculated using the following equation:

$$\text{Antioxidant activity}(\%) = (A_{\text{vehicle}} - A_{\text{sample}})/A_{\text{vehicle}} \times 100$$

where, A_{vehicle} is the absorbance of the vehicle mixed with DPPH solution, and A_{sample} is the absorbance of each extract or ascorbic acid mixed with DPPH.

Statistical analysis

Mean values and standard deviations were calculated from the different replications for each treatment. A Bartlett test was used to determine if the data were distributed normally. For normal distribution, the differences among means were analyzed for the different experiments and treatments using ANOVA. The Scheffé test was used to determine the difference between the pair of means.

Results

Maca extracts activity

Red maca leaves showed significantly greater TP content (13.46 ± 0.14) in the hydroalcoholic extracts ($P < 0.005$)

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than the other two extracts, but no difference was observed between the BM (9.47 ± 0.39) and YM (10.44 ± 0.21) leaves ($P > 0.05$).

Figure 1 shows the UVA–UVB–UVC spectrogram of all maca species. The three different varieties of maca leaf extracts absorbed the UV portion of the spectra, from 200 to 320 nm. When the segments were evaluated individually, in the UVB wavelength (290–320 nm), RM had the greatest absorbance, whereas BM and YM had a similar absorbance. In the UVA wavelength (320–350 nm), the absorbance values were similar to those observed with the UVB wavelength for the three different extracts. Over 360 nm, the absorbances started to diminish for the three varieties. In the UVC wavelength (200–290 nm), the absorbance could not be determined as interference was observed.

Macroscopic analysis

Mice that received radiation with no treatment presented with erythemas and skin burns. However, those that did not receive any kind of radiation showed a normal non-pigmented skin. Normal skin was also observed when a sunscreen SPF 60 was used. Prior treatment with the three varieties of maca leaf extract (RM, YM, BM) had a similar effect to those observed with the sunscreen and a comparable skin pattern to those of non-irradiated mice.

Histopathological analysis

Sunburn cells (Fig. 2a) and atypical keratinocytes (Fig. 2b) were observed in the untreated UVB-exposed animals. Mice that were not treated prior to irradiation also show destruction of the epidermis, as evidenced by necrosis of almost all the tissue (Fig. 2c) compared with a normal non-irradiated skin (Fig. 2d). UVB also produced extensive inflammation, characterized by leukocyte infiltration (Fig. 3) and vacuolization and disintegration of the dermo-epidermal junction (Fig. 4), which was not observed in the non-irradiated controls. These effects were mild or absent in the photoprotected animals.

The epidermal hyperplasia observed in untreated irradiated animals was prevented by the previous application of YM, RM, or BM extract ($P < 0.001$). These treated animals had a similar epidermal thickness as the NIC and those that were previously treated with a sunscreen (Fig. 5). Mice treated with RM and YM extracts had the lowest epidermal thickness ($P < 0.001$) compared with those treated with BM extract or sunscreen ($P < 0.001$). There was no significant difference between the YM and RM treatment (Fig. 6).

The number of SBC increased in the absence of treatment prior to the UVB exposition. However, when the animals were previously treated with the maca extracts or with the sunscreen, the production of SBC was significantly lower (Fig. 7).

The semi-quantitative histopathological evaluation showed that the UVB-induced skin damage (solar cell damage, atypical keratinocytes, tissue necrosis, leukocytic infiltration, and disintegration of the dermo-epidermal junction) was attenuated when mice were given prior treatment with extracts of maca leaves. These effects were similar to those observed when animals were treated with a sunscreen. When comparing the three varieties of maca leaves, all were equally effective against solar damage ($P > 0.05$) and the dermo-epidermal junction. However, YM and BM were more effective than RM at reducing the leukocytic infiltration. YM was the most effective in preventing development of atypical keratinocytes (Table 1).

Lipid peroxidation analysis in skin and liver

Thiobarbituric acid-reactive substances production was quantified both in liver and skin of the different groups. In skin, the TBARS production in the irradiated group was increased compared with the NIC ($P < 0.001$). This effect was not observed in those animals previously treated with the RM, BM, and YM extracts prior to irradiation ($P > 0.05$). RM and YM extracts gave similar values to the non-irradiated group ($P > 0.05$) and to mice treated with sunscreen (Fig. 8a).

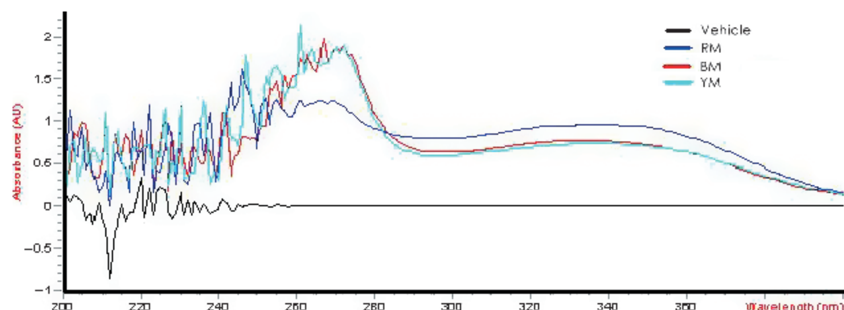

Figure 1 UV (200–320 nm) absorption spectrum of three different varieties of maca leaves. BM, black maca leaves; H_2O_d, distilled water (vehicle); RM, red maca leaves; YM, yellow maca leaves

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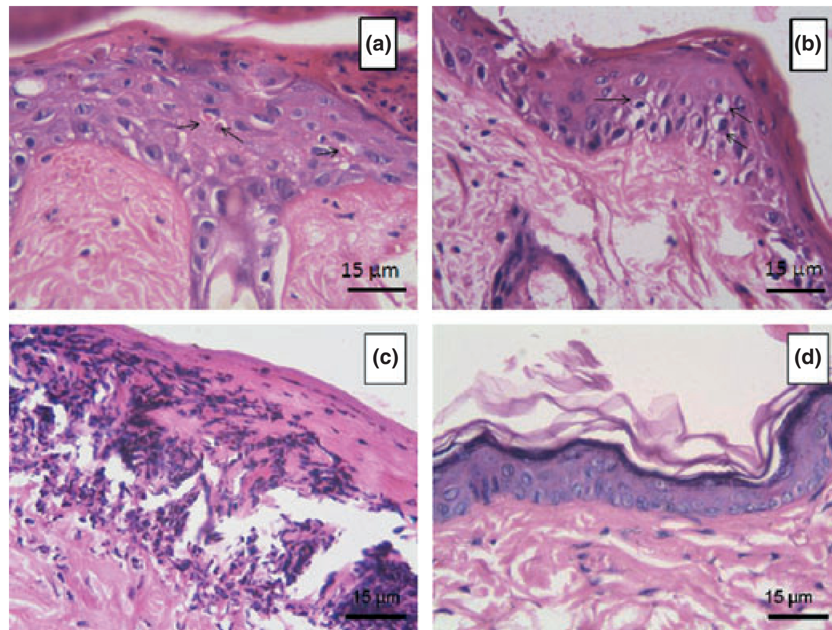

Figure 2 Epidermal damage caused by UVB radiation. (a) SBC (arrow); (b) atypical keratinocytes (arrowhead); (c) tissue necrosis; (d) NIC. Hematoxylin-eosin ×40. Scale bar: 15 µm

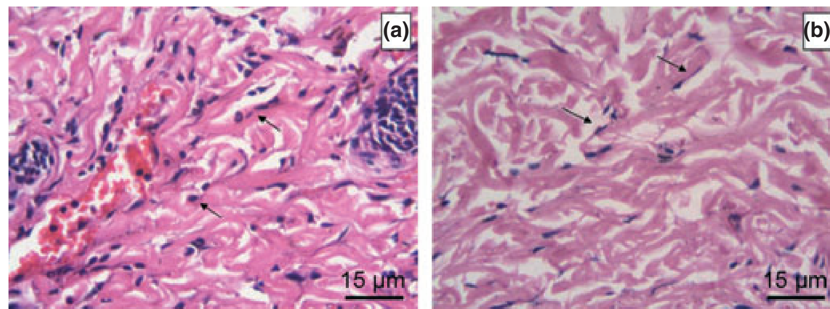

Figure 3 Leukocytic infiltration (arrows) in animals. (a) Irradiated without previous treatment; (b) non-irradiated. Hematoxylin-eosin ×40. Scale bar: 15 µm

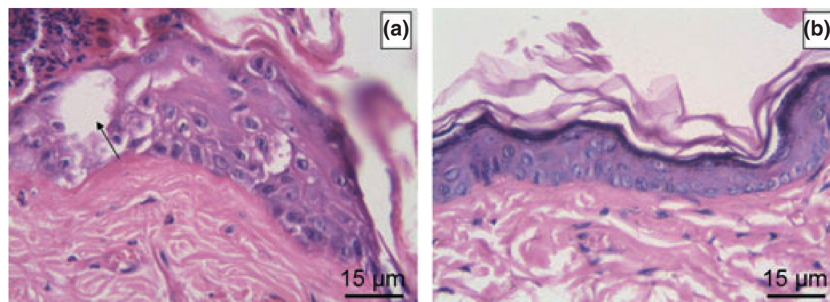

Figure 4 Dermo-epidermal integrity in animals irradiated without previous treatment (left) compared with those non-irradiated (right). Arrow shows vacuolization produced by the UVR exposure. Hematoxylin-eosin ×40. Scale bar: 15 µm

As with skin, UVB radiation produced a significant increase in the TBARS values in liver compared with the NIC ($P < 0.05$). No increase in TBARS was observed after treatment with RM extract ($P < 0.01$) or sunscreen ($P < 0.05$). There were no significant effects observed with YM or RM (Fig. 8b).

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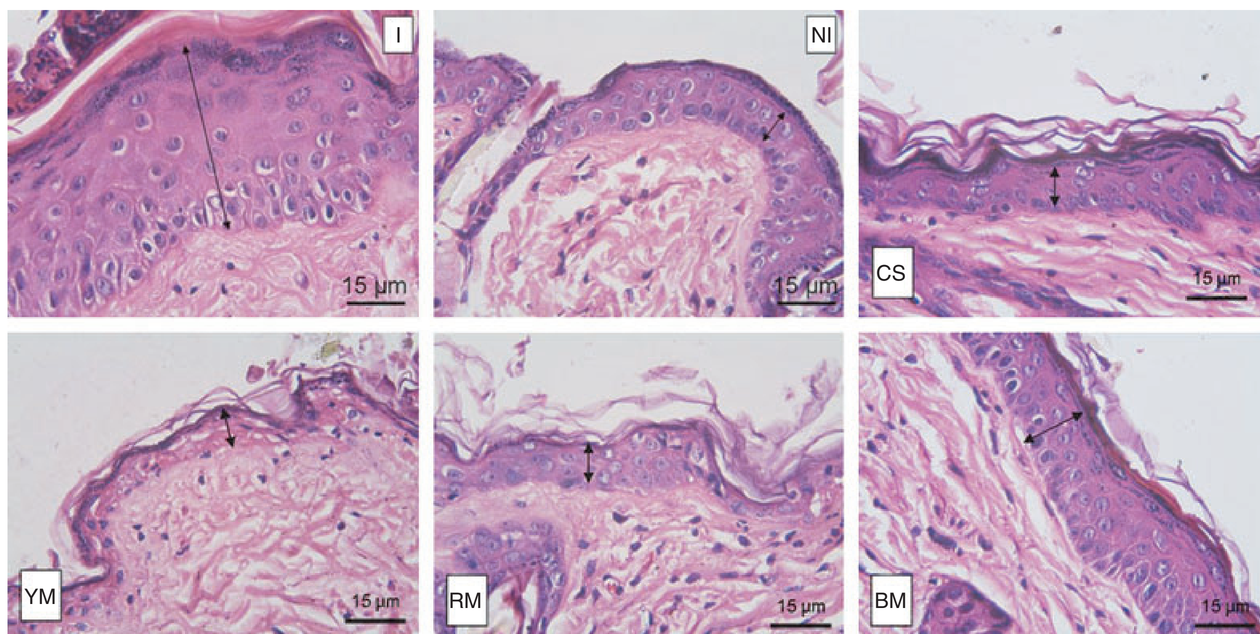

Figure 5 Microphotography of mice exposed to UVB radiation. BM, black maca; I, irradiated control; NI, non-irradiated control; RM, red maca; CS, commercial sunscreen; YM, yellow maca. Arrows show the epidermal height evaluated. Hematoxylin-eosin ×40. Scale bar: 15 µm

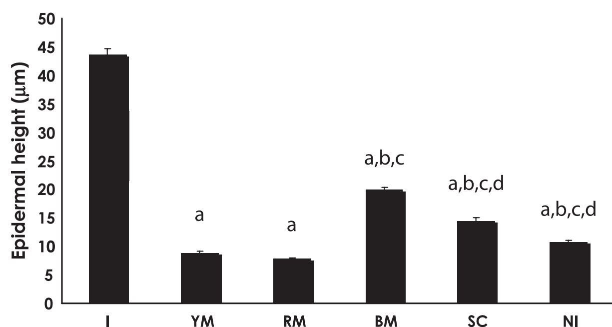

Figure 6 Epidermal height evaluation of mice exposed to UVB radiation. BM, black maca; I, irradiated control; NI, non-irradiated control; RM, red maca; CS, commercial sunscreen; YM, yellow maca. Each mouse received 200 µl of the hydroalcoholic extract previous to exposure to UVR. Data presented as mean ± SEM. One-hundred and fifty measurements were performed per treatment. [a]$P < 0.001$ vs. I; [b]$P < 0.001$ vs. YM; [c]$P < 0.001$ vs. RM; [d]$P < 0.001$ vs. BM; [e]$P < 0.001$ vs. CS

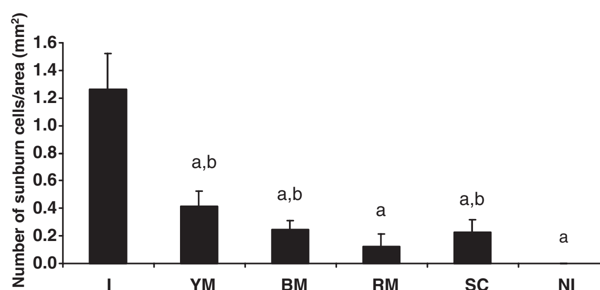

Figure 7 Number of sunburn cells (SBC) per epidermis area evaluated (square millimeter). BM, black maca; I, irradiated control; NI, non-irradiated control; RM, red maca; CS, commercial sunscreen; YM, yellow maca. Data presented as mean ± SEM. Fifteen counts were performed per treatment. [a]$P < 0.05$ vs. I; [b]$P < 0.01$ vs. NI

Antioxidant enzyme activity

Ultraviolet B radiation exposition without treatment reduced the SOD and catalase levels, compared with those animals that were not irradiated. Previous treatment with maca extracts showed higher SOD levels, and this varied according to the maca leaf extract used, being higher when BM was used ($P < 0.01$). This value was similar to that for commercial sunscreen and the NIC. There was no difference between the yellow and red varieties (Fig. 9a). As with commercial sunscreen, previous treatment with BM and YM extract slightly increased the catalase levels in the irradiated animals. On the other hand, treatment with RM resulted in significantly lower catalase levels than for the commercial sunscreen ($P < 0.01$; Fig. 9b).

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Table 1 Mean values of the histopathological evaluation of mice skin (mean ± SEM)

Group	Solar cell damage (MS = 3)	Leukocyte infiltration (MS = 4)	D-E activity (MS = 3)	Atypical keratinocytes (MS = 3)	Severity of atypia (MS = 4)
I	2.72 ± 0.2	3.83 ± 0.1	2.11 ± 0.2	2.83 ± 0.1	3.78 ± 0.1
YM	0.8 ± 0.2*	1.2 ± 0.1*,**	0.7 ± 0.2*	0.5 ± 0.2*,**	0.4 ± 0.1*,**
RM	0.8 ± 0.2*	1.7 ± 0.2*	0.7 ± 0.1*	1.1 ± 0.2*	0.9 ± 0.2*
BM	0.87 ± 0.3	1.47 ± 0.2*	0.4 ± 0.2*	0.87 ± 0.3*	1.0 ± 0.3*
CS	0.0 ± 0.0*	1.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*
NI	0.0 ± 0.0*	1.2 ± 0.0*	0.0 ± 0.0*	0.9 ± 0.2*	0.7 ± 0.1*

BM, black maca; CS, commercial sunscreen; D-E, dermo-epidermic; I, irradiated control; MS, maximal score; NI, non-irradiated control; RM, red maca; YM, yellow maca.
*P < 0.01 vs. I; **P < 0.05 vs. RM.

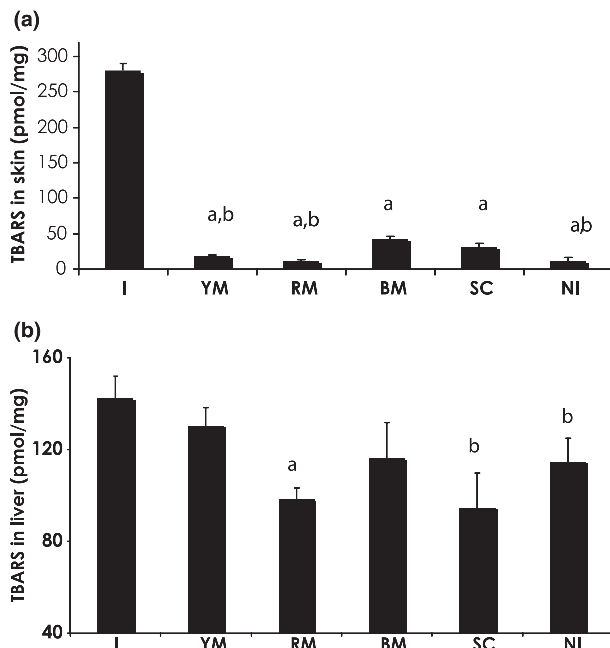

Figure 8 (a) TBARS production in skin of animals irradiated with UVB. BM, black maca; I, irradiated control; NI, non-irradiated control; RM, red maca; CS, commercial sunscreen; YM, yellow maca. Data presented as mean ± SEM. ^{a}P < 0.01 vs. I; ^{b}P < 0.05 vs. I. (b) TBARS production in liver of animals irradiated with UVB. Data presented as mean ± SEM. ^{a}P < 0.001 vs. I; ^{b}P < 0.05 vs. BM

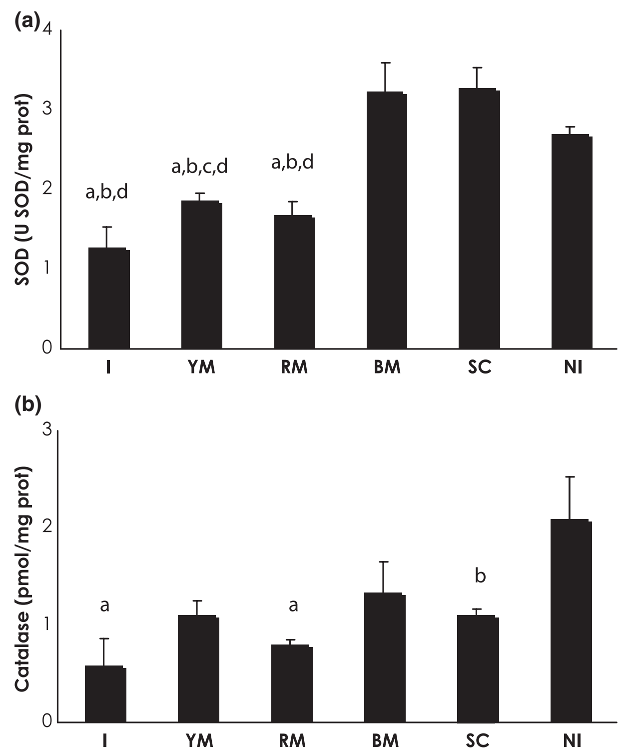

Figure 9 (a) Superoxide dismutase (SOD) levels in the skin of animals exposed to UVB radiation. BM, black maca; I, irradiated control; NI, non-irradiated control; RM, red maca; CS, commercial sunscreen; YM, yellow maca. Data presented as mean ± SEM. ^{a}P < 0.01 vs. NI; ^{b}P < 0.01 vs. BM; ^{c}P < 0.05 vs. I; ^{d}P < 0.01 vs. CS. (b) Catalase levels in the skin of animals exposed to UVB radiation. Data presented as mean ± SEM. ^{a}P < 0.05 vs. NI; ^{b}P < 0.01 vs. RM

Antioxidant activity *in vitro*

DPPH assay

Red maca leaves extract produced a higher antioxidant activity *in vitro* than either the BM or YM extracts (P < 0.01) in a concentration-dependent manner. RM had the highest percentage of antioxidant activity, with almost 90% of activity at a concentration of 0.075 mg/ml. In contrast, BM reached 80% of activity with a concentration of 0.1 mg/ml, and YM had a maximum activity of 70% at a concentration of 0.075 mg/ml and maintained that percentage with a concentration of 0.1 mg/ml. IC_{50} values showed that YM required a concentration of 0.021 mg/ml to reach 50% of the antioxidant activity, whereas RM required 0.026 mg/ml and BM 0.034 mg/ml (data not shown).

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Discussion

As a defense mechanism against the deleterious effect of solar radiation, plants have developed systems of production of different relatively stable compounds that function as light screens.[28] The purpose of these compounds is to reduce the fraction of absorbed radiation and, with that, diminish the damage induced by the UVR. One of the problems with commercial sunscreens is that not all of them act protecting against both types of radiation that reach the earth's surface: UVA and UVB.[29] In the present study, we have used a model of skin damage due to UVB radiation in order to evaluate the photoprotective effect of the hydroalcoholic extracts of maca leaves of three different varieties (red, yellow, and black).

The three extracts of maca leaves presented the ability to absorb the UVR. However, it was difficult to make an analysis of the spectra obtained on the UVC (200–290 nm) range as there was interference. In the UVB (290–320 nm) portion of the spectra, the extracts of RM leaves showed a greater absorbance, followed by BM and YM leaves. In the UVA (320–400 nm) portion of the spectra, the extracts of RM leaves had the greatest capacity to absorb in the range from 320 to 350 nm.

The presence of erythemas and pigmentation are two of the most prominent and known acute responses against UVR.[30] Due to its great reproducibility and simplicity, erythemas and pigmentation induced by UVR constitute an excellent model of inflammation to characterize the Pathophysiology of the skin and to evaluate the efficacy of different agents.[31] In the present study, although there was no quantitative measurement of erythema or hyperpigmentation, it was clinically apparent, as we observed the erythema and skin burns on the dorsum of the animals when they were exposed to UVB radiation without treatment. The previous application of the maca extracts prevented these effects.

The keratinocytes are the principal and most abundant cells in the skin that have a special way of programmed cell death called cornification, which is a process different than the classic apoptosis.[32] This normal process is altered by the UVB radiation, causing an occurrence of SBC or apoptotic keratinocytes. This deregulation of the mechanisms of cell death that is a normal process of the skin could lead to several diseases like cancer and necrolysis. SBC have been described as markers of solar damage, and a compound or substance capable of reducing the number of SBC in an irradiated skin confirms its photoprotective effect.[33]

The qualitative microscopic evaluation revealed that the UVR produced SBC, leukocytic infiltration, thickening of the epidermis, moderate to severe cellular atypia, formation of clefts, and tissue necrosis, which coincides with previous results described by other authors in rats, mice, and guinea pigs.[34–39]

These deleterious effects of the UVB exposition were prevented or reduced by the previous topic administration of the maca leaves extracts. However, the magnitude of the effect seemed to differ among the different maca varieties. These differences in the biological responses to the different varieties of maca have been previously reported in animal models of memory and learning[40] and in the reproductive system.[18,41] The observed protection by RM against UVB-induced SBC may be mostly explained by the absorption in the UVB range.

For instance, YM and BM had a greater effect in preventing the leukocytic infiltration; meanwhile, only YM has a better effect preventing the cellular atypia. However, in the latter, there were no statistical differences among the three varieties and the NIC. The extracts of YM had the lowest capacity to absorb the UVB spectra; however, our results showed that they have good effects reducing the epidermal thickness after UVB exposition. This means that, independently of their ability to absorb UVR, the maca leaves had properties that make them versatile for photoprotection.

The lipid peroxidation is considered the main mechanism of cutaneous photodamage induced by UVR.[42] The UVB radiation increases the lipid peroxidation throughout the production of ROS, generating peroxides from the unsaturated fatty acids.[43] The ROS production in the skin after the UVR leads to an oxidative damage in the DNA and other cellular components[34,35,44] and an increase of over 25 times the TBARS value in skin, indicating a notorious lipoperoxidation.[45] Our results showed that UVB radiation increased the TBARS levels in the skin and that extracts of the three varieties of maca leaves had a direct action on the skin, favoring the anti-lipoperoxidant activity, avoiding the production of TBARS. These levels are also similar to those observed in the animals that did not receive radiation or treatment, which may indicate that these extracts have the ability to protect the skin from the UV-induced lipid peroxidation.

The effect of the UVR is not limited to the exposed skin. Therefore, the TBARS levels in liver are significantly increased after irradiation of the skin. In the present study, those groups that were treated with the RM leaves and with the commercial sunscreen prevented the increase of TBARS in liver as an effect of UVB radiation. It was expected that the topical application of the different treatments would only affect the skin and not other organs; however, this reduction of the TBARS levels found in liver may be due to the fact that the lipid peroxidation found in this organ reflects the lipid peroxidation found in skin. In this way, our results are similar to others.[43,46] These authors demonstrated that irradiation with UVB

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increases the lipoperoxidation in skin the same as in erythrocytes and liver of guinea pigs and that the topical administration of alpha-tocopherol acetate reverts those effects.

The UVB radiation significantly decreased the antioxidant enzymes levels, catalase, and SOD, same as previously described.[47,48] Among the varieties of maca leaves, BM allows the irradiated animals to maintain the activity of SOD similar to the non-irradiated animals. The effect of the maca leaves on catalase activity is much less, but still BM and YM are the most effective.

The property of the maca leaves in reducing oxidative damage *in vivo* leads us to study in more detail the antioxidant properties *in vitro*. The results with the DPPH assay showed that the hydroalcoholic extracts of maca leaves had an antioxidant activity *in vitro*.

The protection observed in our experiments may be due to the phenols content in the maca leaves, as previously described in other experiments.[49] Phenols are produced by all the superior plants to protect them from the different types of biotic and abiotic stress, such as UVR, changes in temperature, infections, wounds, and herbivorous attacks.[50] The phenols in plants have the ability, among other things, to reduce the hyperpigmentation induced by UVB radiation through the reduction of the tyrosinase activity in the melanocytes.[51] The exposition to UVR induces the formation of ROS in the skin;[52] these ROS induce the biosynthesis of melanin and damage to the DNA, which can then lead to proliferation and/or apoptosis of the melanocytes.[51]

It is premature to conclude why there are differences between varieties of maca. Although the phenols may explain the protective effect in maca leaves against UVB radiation in skin, it would not explain the differences among varieties found in those assays where the extract administration was controlled by the phenol content. It is probable that other compounds, not yet determined, might also be participating in the protection against UV exposition. Then, these unknown compounds may be responsible for the different behavior among the different varieties of maca leaves.

Conclusion

The present study demonstrates the effect of the hydroalcoholic extracts of RM, BM, and YM leaves on the skin of animals exposed to UVB radiation. Though RM is the one that showed the greatest antioxidant activity, both YM and RM were the ones that clearly prevented the histological alterations of the skin due to UVR. This suggests that there must be more than one active principle for the action of each of the varieties of maca. Our results suggest that the mechanisms of action of the maca extracts on the different effects observed may be due in part to the ability to absorb the UV radiation and to their antioxidant activity, particularly acting on the lipoperoxidation. This finding is of great importance as the action of UVB radiation on skin is produced directly through skin damage and indirectly through an increase in ROS. Our data would suggest that maca would be protecting the skin from both mechanisms of action of UVB radiation.

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