ORIGINAL ARTICLE

PHOTOPROTECTIVE ACTIVITY OF A CREAM CONTAINING LYOPHILIZED AQUEOUS EXTRACT OF *Lepidium meyenii* (MACA) AGAINST ULTRAVIOLET IRRADIATION ON MOUSE SKIN

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This study is part of the thesis: Castañeda-Alarcón M Evaluación de la actividad fotoprotectora in vitro y efecto fotoprotector in vivo de una formulación a base de extracto acuoso liofilizado de *Lepidium meyenii* (MACA) [master's thesis]. Lima: Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos; 2019.

ABSTRACT

Objectives: To evaluate the photoprotective activity of a cream with lyophilized aqueous extract of maca (LEM) against ultraviolet (UV) irradiation in the skin of mice. **Materials and methods**: An experimental study was carried out on 35 BALB/c mice. Treatment was applied topically on the dorsum of the animals, which were subsequently irradiated with ultraviolet B rays, and then we measured the thickness in microns (μm) of histological samples of the skin of the mice. Seven groups were assigned, divided into non-irradiated: Blank (G1) and irradiated with UV light: no treatment (G2); with commercial sunscreen with sun protection factor (SPF) 30 (G3); cream (placebo) (G4); LEM at 15% in water (G5); LEM cream at 5% (G6); and LEM cream at 15% (G7). *In vitro* SPF was determined using the Mansur method. Absorbance readings were taken in an ultraviolet- visible spectrophotometer (UV-VIS) and SPFs were determined for the following formulations: LEM cream at 5%, benzophenone-4 (BZF-4) and commercial sunscreen SPF 30. **Results**: Mouse skin thickness in microns (μm) was 27.28 in G2; 18.31 in G3; 27.33 in G4; 19.51 in G5 and 18.04 in G6. There was no significant difference between the group not exposed to radiation (G1) and the 15% LEM cream group (G7), both had the lowest thicknesses (12.76 and 14.20 μm, respectively). The SPF of LEM cream at 15% was 5.480 ± 0.020. **Conclusions**: The formulation with LEM cream showed photoprotective activity against UV irradiation, alkaloids were the phytochemical components mostly found and the formulation was compatible with the active principle (LEM).

Keywords: Radiation Effects; *Lepidium*; Sun Protection Factor; Sunscreening Agents; Phytotherapy (source: MeSH NLM).

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INTRODUCTION

Worldwide, the increase in ultraviolet (UV) radiation has been identified as an immediate consequence of the ozone layer depletion [1,2], which constitutes a threat to human beings due to the high carcinogenic potential caused by its exposure [3]. Increased cumulative UV radiation and the degree of sensitivity according to skin type are associated with non-melanoma cancer and cutaneous melanoma, respectively [4,5].

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Climatic and environmental changes are responsible for the ozone layer depletion, leading to greater exposure to solar radiation [6]. The National Center for Epidemiology, Disease Prevention and Control indicated through hospital reports that skin cancer (10.8%) ranks third in frequency after cervical cancer (18.6%) and stomach cancer (11.1%), in relation to the total number of registered cancers [7].

An alternative way of protection is the use of photoprotective formulas with UV filters, which directly interfere with solar radiation through absorption, reflection or dispersion of energy [2]. These filters are classified as chemical (organic) filters that absorb UV rays, and physical (inorganic) filters, which are based on the reflection and dispersion of UV light [8]. Some chemical filters are known to be absorbed systemically; others, such as oxybenzone, are known to damage reef corals [9]; or may be photo-unstable, such as avobenzone [10].

Certain extracts made from plant metabolites can be used as sunscreens, thanks to their ability to absorb different radiations [11], in combination with other synthetic components or mineral pigments to optimize photo-filtering efficacy [10,12].

Plant extracts used for solar filtration must meet specific characteristics: they must absorb radiation of a wavelength range between 290-400 nm [13], be tested under normal conditions of use and manufacture, be compatible with the excipients and with the packaging material of the products used in the solar lines and, finally, be non-toxic [2].

Maca (*Lepidium meyenii* or *Lepidium peruvianum Chacon*) is a plant native to the central Andes of Peru, resistant to hailstorms, frost and prolonged drought [14]; and has been cultivated since the Inca period at altitudes between 3,800 and 4,500 meters above sea level [15]. Evidence shows that the increase in alkaloid production is related to altitude and exposure to solar radiation, as described for *Meconopsis quintuplinervia*, a plant native to China [16], and for *Arnica montana* which, at high altitude, increased its caffeic acid derivatives [17]. It is likely that organisms living at these altitudes develop protective mechanisms against overexposure to UV radiation. Experimental studies report that topical application of maca extract provides some protection against UV radiation [12,18].

Photoprotective formulations are required to prevent the harmful effects of UV radiation on the skin, these formulations must have stable and innocuous active ingredients or serve as adjuvants to optimize the action of chemical filters. There is previous evidence of the use of photoprotective plant extracts, so it is possible to propose the use of the maca extract for photoprotective purposes so that it can be used in phytotherapeutic formulations.

KEY MESSAGES

Motivation for the study: The effect of ultraviolet (UV) radiation produces skin pathologies that can cause cancer. It is necessary to look for alternatives in phytotherapy that contribute to photoprotection. Maca is a plant that grows at high altitudes under increased radiation, therefore it could contain metabolites that provide protection against UV radiation.

Main findings: Topical use of a cream formulated with *Lepidium meyenni* (maca) showed photoprotective activity and formulation compatibility.

I**mplications:** To promote the use of maca as a complementary alternative for photoprotection, probably due to the presence of alkaloids, and continue searching for natural and stable filter alternatives.

For this reason, this study aimed to evaluate the photoprotective activity of a topical formulation of maca, by using UV irradiation on the skin of mice; as well as to determine the sun protection factor (SPF) *in vitro*.

MATERIALS AND METHODS

Design

An experimental study was conducted using lyophilized aqueous extract of *Lepidium meyenii* "maca" (LEM) applied topically on the skin of mice. The preparation of the aqueous extract and the phytochemical screening were carried out at the Laboratory of Analytical Chemistry and Pharmacognosy of the Faculty of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos (UNMSM). Lyophilization and bioassay were conducted at the Microbiology and Virology Laboratory of the Universidad Peruana Cayetano Heredia (UPCH). Formulation, SPF evaluation and physicochemical evaluation were carried out at the Physicochemical Laboratory of the National Quality Control Center (CNCC) of the Instituto Nacional de Salud (INS). Histological evaluation was conducted at the Institute of Pathology, Faculty of Medicine, UNMSM.

Animals

We used thirty-five male BALB/c strain mice weighing an average of 26 ± 2 g, approximately 10 weeks of age and acquired from the UPCH vivarium. The mice were placed in seven cages, five per cage. They were placed in a quarantine

period of five days, before starting the trial, for physiological and behavioral adaptation purposes. The test conditions were as follows: temperature of 20-25 °C, humidity of 50-70%, and photoperiod of 12 hours of light and 12 hours of darkness. The animals consumed balanced food from the Universidad Nacional Agraria La Molina and water *ad libitum*. We followed all the guidelines described in the Guide for the care and use of Laboratory animals [19] and the "Guide for the handling and care of laboratory animals: mouse" [20].

Preparation of the lyophilized aqueous extract of *Lepidium meyenii*

The roots of the yellow variety of *Lepidium meyenii* were collected from the Andean area of Pachacayo (3,600 meters above sea level), Canchaylla district, Jauja province, Junin region, in May 2014. Taxonomic identification was carried out at the Museum of Natural History, UNMSM (Record No. 57-USM-2014). To prepare the LEM, 500 g of maca roots (previously dried and pulverized) were weighed and boiled with seven liters of deionized water for 60 minutes. The filtrate was stored at -20 °C and subsequently freeze-dried.

Phytochemical screening

Phytochemical screening was carried out to determine phytochemical compounds by staining or precipitation reactions [12].

Formulation

Solubility was determined using the following solvents: methanol, ethyl alcohol and water, listed from lowest to highest polarity. The LEM was added to oil/water (O/W) emulsions at two concentrations of 5% and 15% [21].

Photoprotective activity - bioassay

To determine the photoprotective activity of the LEM formulation, we used the biological model [22] of the incidence of UV irradiation in mouse skin [18,23]. The mice were divided into seven groups, five per group, and were treated topically. The first group (G1) was the only one not exposed to UV irradiation. The other groups were exposed and were classified as follows: no treatment (G2); commercial sunscreen with SPF 30 (G3); placebo (formulation without LEM) (G4); 15% LEM in water (G5); 5% LEM in cream (G6); and 15% LEM in cream (G7). A circle of approximately 2 cm in diameter was drawn on the dorsal surface of each animal and then depilated 24 hours before the start of UV irradiation. The mice were left to rest in order to observe any alteration at the dermal level (irritation, erythema or edema). The volume applied for each treatment was 100 uL for each exposed depilated area.

Mice were anesthetized with ketamine (40 mg/kg) and xylazine (15 mg/kg) minutes before irradiation in the exposure chamber. Each mouse was placed 15 cm from the UV radiation source for 30 min. Mice were irradiated once a day for three consecutive days (equipment: Spectroline, LongLife filter no. 1575586, NY, USA), with 3.3 mW/cm^2 being the amount of UV energy applied on the skin and measured with a radiometer (UDT 371 optical power meter instruments, model # 268 UVC; Thorlabs, Newton, New Jersey, USA).

For histological evaluation, the animals were sacrificed by anesthetic overdose, two hours after the last exposure. For each animal, a 2 cm diameter skin sample was sectioned and placed in 4% paraffin for histological analysis. The skin samples were processed by the paraffin embedding technique and stained with hematoxylin and eosin for histopathological study. A Nikon eclipse Ci optical microscope (Japan) was used to carry out section measurements between the stratum granulosum and the dermoepidermal junction [18].

Determination of the sun protection factor

We weighed 1 g of the 15% LEM formulation and diluted it to a final concentration of 0.2 mg/mL. The same procedure was followed for the formulation with benzophenanone-4 (BZF-4) at 1.5% and for the commercial sunscreen.

The absorbance of the solutions was determined using a Jasco V-650 UV spectrophotometer (Japan), in the range of 290 to 320 nm (Table 1), with 5 nm intervals using a 1 cm quartz cuvette.

$$\textit{Spectrophotometric}\ \text{SPF} = \text{CF.}\ {}^{320}\Sigma_{290}\ \text{x EE}\ (\lambda)\ \text{x I}\ (\lambda)\ \text{x ABS}\ (\lambda)$$

Where SPF: sun protection factor; CF: 10 (correction factor); EE(λ): ABS effect of the radiation of wavelength λ; I

Table 1. Relationship between erythemogenic effect (EE) versus radiation intensity (I) according to constant wavelength (λ) determined by Sayre *et al.*

Wavelength λ (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

EE: Erythemogenic effect of wavelength radiation; I: sun intensity at wavelength. Source: Sayre *et al* [30].

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(λ): intensity of the sun at wavelength λ; ABS (λ): absorbance of the solution at wavelength λ. The ratio between the erythemogenic effect and the intensity of radiation of each wavelength (EE (λ) x I (λ)) is a constant determined by Sayre *et al.* [30] (Table 1).

The analyses were carried out in triplicate and the SPF was calculated according to the mathematical equation developed by Mansur *et al.* [24].

Evaluation of the physicochemical characteristics

We evaluated the appearance, color and odor of the 15% and 5% LEM formulations by sensory, visual and olfactory inspection on samples stored at room temperature, avoiding contact with sunlight. For pH determination, we used a Metrohm potentiometer 691 pH Meter (Switzerland), a Metrohm combined glass electrode 6.0228.00 (Switzerland), Chapter <791>pH, USP 38 and buffer solutions (pH 4.00 and pH 7.00). For determining phase separation, samples were centrifuged in a Hettich EVA21 Zentrifugen (Germany) at 3000 rpm for 30 min [25]. For the viscosity test, we used a Brokfiels DV-II+Viscosimeter (MA 02346 United States), which was measured at 25 °C temperature. Evaluations were carried out at 24 hours, 90, 180 and 240 days.

Statistical analysis

For evaluating mouse skin thickness in microns, we used the Shapiro Wilk normality test for each treatment group including positive and negative controls; for the intergroup analysis, we applied the Kruskal Wallis nonparametric test because the intergroup variances were unequal (Bartlett's test p=0.010). Dunn's test was used to make simultaneous pairwise inferences. Likewise, the results of the sun protection factor were expressed with the mean and standard deviation. A significance level of 0.05 was assumed for all statistical tests. All analyses were carried out with the statistical program Stata 15.

Ethical Aspects

This study was approved by the Institutional Research Ethics Committee of the Instituto Nacional de Salud (CIEI-INS) (Code No. OT-047-14) and by the Ethics Committee for the Use of Animals in Research of the Instituto Nacional de Salud (CIEA-INS). We used the euthanasia method of anesthetic overdose, an acceptable method for rodents described in the Guide for the care and use of laboratory animals [19].

Table 2. Compounds found in lyophilized aqueous extract of *Lepidium meyenni* (LEM).

Metabolite group	Used reactive	LEM
Tannins	Gelatin	-
Proteins (amino acids)	Ninhydrin	-
Phenolic compounds	Iron trichloride	+
Alkaloids or nitrogen compounds	Dragendorff	++
Alkaloids	Mayer	++
Quinones	Borntrager	-
Sterols	Lieberman	-
Sugars	Molish	+
Flavonoids	Shinoda	-

(-) absence; (+) small quantity; (++) regular quantity.
Qualitative evaluation, according to the color reaction or precipitate.

RESULTS

Mainly alkaloids or nitrogenous compounds were found during the phytochemical study of LEM, and to a lesser extent sugars and phenolic compounds; tannins, quinones, sterols and flavonoids were not found (Table 2). LEM was totally soluble in water, partially soluble in ethyl alcohol and insoluble in methanol.

In the evaluation of the photoprotective activity of maca in mice, the groups in which no treatment was used (G2) or in which cream without LEM was used (G4) had higher median skin thickness (27.28 µm and 27.33 µm, respectively) compared to the group that was not exposed to radiation (G1) or to the group that used a commercial sunscreen (G3) and to those that used LEM in the formulations. Among the intervention groups, the lowest median thickness was found in the one using 15% LEM cream (G7) (14.2 IR: 10.42-19.27) (Table 3, Figure 1).

Table 3. Distribution of mice skin thickness according to group.

	Group	Skin thickness (µm)	
		Median	Interquartile Range
G1	Not exposed to radiation	12.76	9.9-15.47
G2	No treatment	27.28	22.84-33.04
G3	SFP 30 commercial sunscreen	18.31	18.26-18.42
G4	Cream without LEM*	27.33	22.47-29.69
G5	Water + LEM 15%	19.51	14.59-26.61
G6	Cream + LEM 5%	18.04	15.32-22.31
G7	Cream + LEM 15%	14.2	10.42-19.27

LEM: Lyophilized aqueous extract of maca

For pairwise inferences, statistically significant differences were found in the group not exposed to radiation (G1) compared to the groups exposed to radiation without treatment (G2) ($p < 0.001$), with SPF 30 sunscreen ($p = 0.041$), LEM cream ($p < 0.001$), water with LEM 15% ($p = 0.005$) and 5% LEM cream ($p = 0.044$) (Table 4).

For G3 only a significant difference was found with G4 ($p = 0.050$). In the other cases, all formulas offered photoprotection with no significant detectable variations in skin thickness. In the case of G4, a significant difference was found with G6 ($p = 0.047$) and G7 ($p < 0.001$), and no significant difference was found with G5 ($p = 0.088$). G5 had a significant difference with (G7) ($p = 0.011$). Finally, for G6 no significant difference was found with G7, which indicates that in relation to skin thickness, the photoprotection of the formula and the concentration at 5% and 15% would not have a significant difference (Table 4). The *in vitro* SPF in nanometers (nm) for the cream formulation with LEM at 15% was 5.480 ± 0.020, for BZF-4 at 1.5% it was 6.854 ± 0.001 and for the commercial sunscreen it was 11.504 ± 0.027.

During the physicochemical evaluation of the formulation, we observed compatibility between the LEM and the cream (base), resulting in a preparation of uniform appearance, without changes in texture, color and odor; no flocculation, coagulation, precipitation, coalescence or granulation was observed. The creams with LEM had a particular odor, which is typical of the species. No phase separation or texture changes of the product were observed when centrifuged. The pH was within the acceptable range for topical use (Table 5).

DISCUSSION

In our study, we observed that the 15% LEM cream showed photoprotective activity in the skin of mice irradiated with UV rays, the formulation also showed *in vitro* SPF and it was determined that the main components of LEM were alkaloids.

The biological model provides information on the damage caused by UV radiation on irradiated mouse skin [22]. Exposure to unprotected UV radiation produces an increase in epidermal thickness (hyperplasia) and an increase in the number of sunburn cells [2]. Gonzales-Castañeda [18] found that yellow, black and red maca leaf extracts decreased epidermal thickness and prevented leukocyte infiltration and the formation of atypical keratinocytes in a similar way to the control group. Similar results were observed in our study, since no significant difference was found between the control group (G1) not exposed to radiation and the group (G7) with the 15% LEM cream, this could be explained because the protection provided in the group with the highest concentration of the active principle is quite similar to the non-exposed group and, therefore, there would be no significant changes in the dermoepidermal junction.

Significant differences were found between the group without irradiation treatment (G2) and the group with commercial sunscreen (G3), cream with 5% LEM (G6) and cream with 15% LEM (G7), which can be explained by the fact that the formulas contain the active ingredient of LEM or the sunscreen of the commercial formulation. However, in the groups that used cream without LEM (G4) and water with LEM at 15% (G5) no significant difference was reported; this could be because in G4 there is no active ingredient that provided photoprotection and in G5, despite having LEM at 15%, the vehicle is water, which would not be the most suitable for topical application due to its shorter fixation time on the skin [26], compared to the cream. A significant difference was found in the group that used cream without LEM (G4) compared to the group that used cream with LEM at 5% and cream with LEM at 15%, which could be explained by the existence of a photoprotective effect at both concentrations.

As for using Mansur's method for determining SPF *in vitro*, several studies found an SPF below 3 as reported by Costa *et al.* [13] with an SPF of 2.23 in a formulation with 20% extract of *Marcetia taxifolia*. Alayo *et al.* [21] obtained an SPF of

Table 4. p-values obtained with Dunn's test to compare skin thickness according to groups.

Groups	G1	G2	G3	G4	G5	G6
G2	< 0.001					
G3	0.041	0.034				
G4	< 0.001	0.423	0.050			
G5	0.005	0.059	0.309	0.088		
G6	0.044	0.031	0.488	0.047	0.296	
G7	0.323	< 0.001	0.076	< 0.001	0.011	0.081

LEM: lyophilized aqueous extract of maca; G1: blank (not exposed to radiation); G2: no treatment; G3: commercial sunscreen with sun protection factor 30; G4: cream without LEM (placebo); G5: LEM at 15% in water; G6: LEM at 5% in cream; G7: LEM at 15% in cream.

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Table 5. Physicochemical analysis of the lyophilized aqueous extract of 15% *Lepidium meyenii* cream.

Characteristics	Parameter	Specifications	Results			
			24 hours	90 days	180 days	240 days
Organoleptic	Homogeneity	Homogeneous cream	Homogeneous (Absence of lumps)	Homogeneous (Absence of lumps)	Homogeneous (Absence of lumps)	Homogeneous (Absence of lumps)
	Sensation to the touch	Soft to the touch	Soft to the touch	Soft to the touch	Soft to the touch	Soft to the touch
	Color	Brownish yellow	Brownish yellow	Brownish yellow	Brownish yellow	Brownish yellow
	Odor	Characteristic	Sui generis	Sui generis	Sui generis	Sui generis
Physicochemical	pH (25 °C)	4.5 – 7.00	4.73	4.79	4.85	4.96
	Viscosity (cps)(25 °C)	8,001 – 12,000	10,590	10,562	10,534	10,508

cps: centipoise equivalent to one millipascal second.

2.29 in an O/W type photoprotective cream with hydroalcoholic extract of *Piper aduncum* leaves. Inocente *et al.* [27] reported a SPF of 2.667 ± 0.044 in a formulation with 15% camu camu extract and a SPF of 0.589 ± 0.057 for the placebo lotion. These results could be explained by the fact that the low SPF values are a consequence of the low concentration of molecules with the ability to absorb UV radiation (chromophores) [27]. They could also be considered in the low SPF category, as indicated by the 2019 Australian Regulatory Guidelines for Sunscreens [28].

This study found a SPF of 5.480 ± 0.020 in the formulation (cream with 15% LEM). These results are different from those reported by Prudencio Quiroz and Bustamante Arroyo [12], who found a SPF of 8.354 ± 0.003 in their formulation with *Lepidium meyenii* hydroglycolic extract (base + sunscreen 5% + maca 10%), it is worth mentioning that their formulation used a sunscreen (benzophenone-3) at 5% with a SPF of 4.960 ± 0.001. The results between the two studies differed due to the different types of maca extracts used.

Regarding the extract concentration in the formulations, Soares *et al.* [11] reported a SPF of 5.05 in a formulation with 20% and 40% propolis extract; in the latter, they found SPF values above 10. It can be inferred that, to reach SPFs above 10, it is necessary to use concentrations higher than 20% of the plant extracts that act as sunscreen.

Zhou *et al.* [29] found mainly alkaloids, glucosinolates and macaenes in *Lepidium meyenii* extracts, similar to our study, in which we found that major components were also alkaloids. Also, previous studies have isolated three types of alkaloids from maca roots: imidazole alkaloids of Lepidiline A and B, as well as Macaridine (benzylated derivative1,2-di-di- hydro-N-hydroxypyridine) [15]. Yang *et al.* [16] reported that the total alkaloid content of *Meconopis uintuplinervia* increases with altitude. Spitaler *et al.* [17] indicated that the secondary metabolites of *Arnica montana* were related to altitude, their results are discussed because of the radical uptake of phenolic compounds and their importance for plant life in environments with high UV radiation, this could indicate that the alkaloids are responsible for the photoprotective activity.

Regarding the quality control of the formulations with LEM at 15%, we observed that the macroscopic properties showed an adequate interaction between the components, a high compatibility between the LEM and all the excipients of the formulation. The base of our formulation was an O/W emulsion, a base that has a greater proportion of water. There is previous data that states that the alkaloids in maca combine with acids resulting in the formation of alkaloid salts, these are crystallizable and soluble in water [15], which could explain why the active ingredient of LEM was easily solubilized when added to the emulsion (O/W), coinciding with the solubility results we reported, where LEM is totally soluble in water.

pH evaluation is one of the parameters used to monitor changes in the formulation structure (hydrolysis and oxidation reactions or changes resulting from the manufacturing process, such as bacterial contamination) that are sometimes not visually detectable and can affect the quality, efficacy and safety of the final product [10,26]. This study found the pH to be within the acceptable range, 4.0 to 6.5, for use on skin. The observed color was beige, a color very similar to human skin, so this characteristic could have a quite acceptable sensory application in dermatology, because, compared to physical filters that leave the skin somewhat whitish, this formulation provides a more natural appearance.

A limitation of the study is that Mansur's method uses only UV radiation values from 290 to 320 nm to calculate SPF, without including the entire UV range, i.e., from 200 to 400 nm [24].

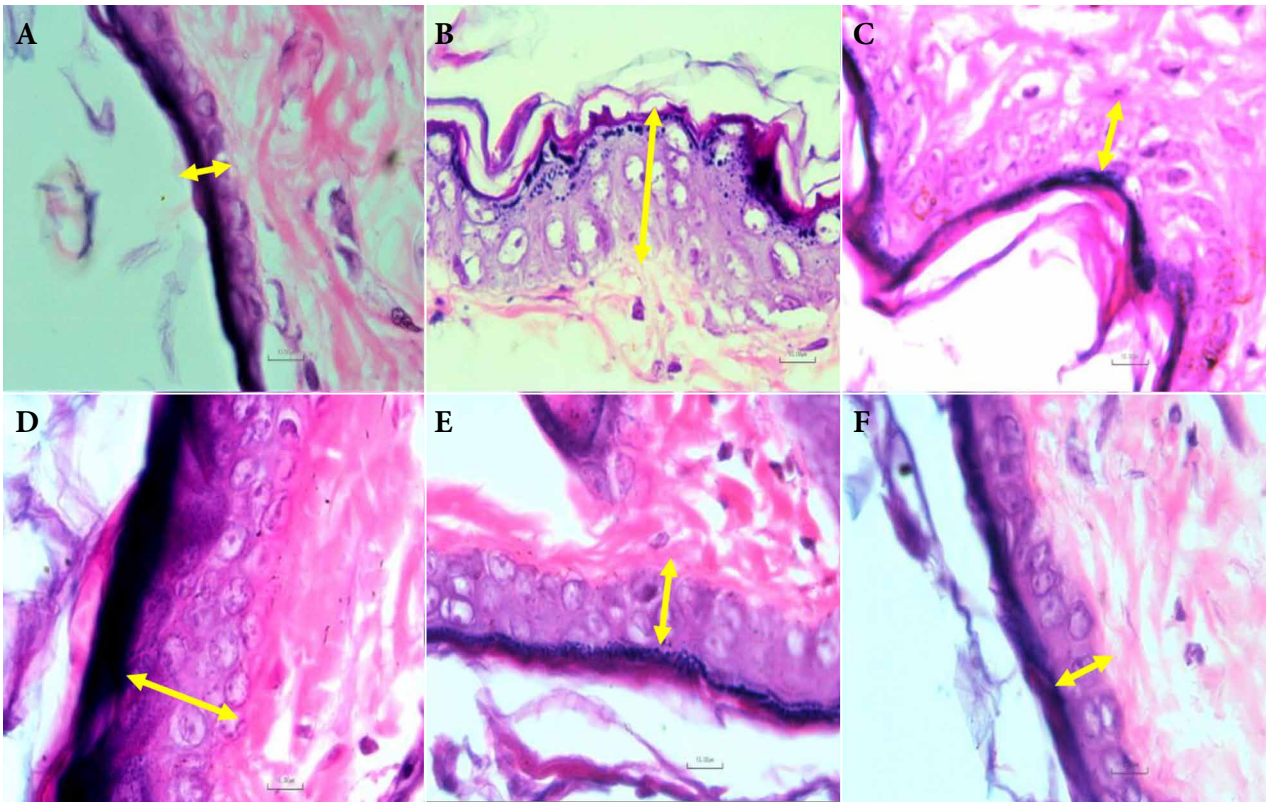

Figure 1. Microphotograph of the skin of mice exposed to UV radiation. The yellow arrows indicate the evaluated epidermal thickness (magnitude 400×). Measured from the dermoepidermal junction to the stratum granulosum, excluding the stratum corneum. Staining technique: eosin-hematoxylin.

A) Blank; without ultraviolet (UV) irradiation and without treatment. Thickness of the dermoepidermal junction is less compared to the control. B) Control; with UV irradiation and without treatment. Epidermal hyperplasia is visible, seen as an increase in dermoepidermal thickness, which is greater than the blank. C) Commercial sunscreen, with UV irradiation. Slight increase in dermoepidermal thickness is visible compared to the blank. D) Cream without lyophilized aqueous extract of maca (LEM), with UV irradiation. Dermoepidermal thickness is similar to the control. E) Cream with 5% LEM, with UV irradiation. The dermoepidermal junction thickness is less than for the 15% LEM cream. F) Cream with 15% LEM, with UV irradiation. Epidermal hyperplasia effect decreased, the dermoepidermal thickness is similar to the blank.

In conclusion, the 15% LEM formulation shows photoprotective activity against UVB radiation *in vivo* and *in vitro*, alkaloids were the major phytochemical components found in the LEM and the cream proved to be chemically compatible with the active ingredient (LEM), thus allowing the possibility of using this formulation as a photoprotective agent on its own or in association with other sunscreens.

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