Evaluation of Protective Effect of a Water-In-Oil Microemulsion Incorporating Quercetin Against UVB-Induced Damage in Hairless Mice Skin

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ABSTRACT – **Purpose.** Histological aspects were considered in order to evaluate the *in vivo* photoprotective effect of a w/o microemulsion containing quercetin against UVB irradiation-induced dermal damages. The toxicity in cell culture and the potential skin irritation resulting from topical application of this formulation were investigated. **Methods.** Mouse dorsal surfaces were treated topically with 300 mg of the unloaded and quercetin-loaded (0.3%, w/w) microemulsions before and after exposure to UVB (2.87 J/cm^2) irradiation. The untreated control groups irradiated and non-irradiated were also evaluated. UVB-induced histopathological changes as well as the photoprotective effect of this formulation were evaluated considering the parameters of infiltration of inflammatory cells, epidermis thickening (basale and spinosum layers) and collagen and elastic fiber contents. The cytotoxicity of the reported formulation was evaluated in L929 mice fibroblasts by MTT assay and the skin irritation was investigated after topical application of both unloaded and quercetin-loaded microemulsions once a day for 15 days. **Results.** The results demonstrated that the w/o microemulsion containing quercetin reduced the incidence of histological skin alterations, mainly the connective-tissue damage, induced by exposure to UVB irradiation. This suggests that protective effects of this formulation against UV-induced responses are not secondary to the interference of UV transmission (i.e., blocking the UVB radiation from being absorbed by the skin), as is usually implied with UVB absorbers and sunscreens, but is instead due to different biological effects of this flavonoid. Furthermore, by evaluating the cytotoxic effect on L929 cells and histological aspects such as infiltration of inflammatory cells and epidermis thickness of hairless mice, the present study also demonstrated the lack of toxicity of the proposed system. **Conclusion.** Based on these mice models, a detailed characterization of the w/o microemulsion incorporating quercetin effects as a photochemoprotective agent on human skin is presented.

INTRODUCTION

The UV components of sunlight are now recognized as major environmental factors deleterious to human health. Photodamage, the specific damage produced in skin tissue by single or repeated (cumulative) exposure to UV light (290-400 nm), is considered to be an initiating step of photocarcinogenesis. UV irradiation-induced injury to the skin can be subdivided into acute and chronic photodamage (1). Acute exposure to UV irradiation from the sun is harmful to skin, causing sunburn, immune suppression, DNA damage and connective tissue degradation. Accumulated damage, resulting from chronic sun exposure, causes skin cancer and premature skin aging (photoaging) (2, 3).

UV irradiation is known to alter cellular function *via* DNA damage and inflammatory responses. In addition, damage to the extracellular matrix integrity in skin tissues is an important event entailing skin wrinkle and blister formation, hallmarks of photoaging (4).

Skin connective tissue (dermis) provides structural support for the skin's vasculature, appendages and epidermis, which are vital to the function of the skin. Structural integrity and function of the dermis are primarily dependent on its extracellular matrix, which is primarily composed of type I collagen fibrils.

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Type I collagen is the most abundant structural protein in skin, and fragmented collagen fibrils are prominent, characteristic features of aged human skin *in vivo*. This fragmentation seriously impairs both the mechanical properties of skin and the functions of cells that reside within the dermis (5). Dermal damage induced by UV irradiation is principally manifested histologically as the disorganization of collagen fibrils and the accumulation of abnormal elastin-containing material. Biochemical evidence of connective-tissue alteration in photoaged skin includes reduced levels of types I and III collagen precursors and cross-links, an increased ratio of type III to type I collagen and an increased level of elastin (6).

In principle, conscientious use of sunscreens/blocks or protective clothing should prevent UVB exposure of the skin. Unfortunately, practical considerations encompassing the use of inadequate amounts of sunscreen and the popularity of tanning appear to limit the success of approaches aimed at reducing skin sun exposure (7). Thus, considering that the use of sunscreen and sun block are not completely effective in preventing UV-induced skin cancer, new targeted chemopreventive approaches need to be identified.

Strategies to prevent early pathological events induced by UV light may be useful for providing efficient and adequate photoprotection. One approach to ameliorate adverse effects of UVB on the skin is the use of antioxidants scavenging and quenching reactive oxygen species (ROS) (4). The therapeutic application of antioxidants in photoprotection is based on the photooxidative stress hypothesis. If this hypothesis is correct, generation of ROS, depletion of antioxidants, damage of the type caused by ROS and activation of redox sensitive signal transduction pathways can be expected in irradiated skin. Manipulation of skin antioxidants would affect both these molecular changes and subsequent biological and clinical sequela (3).

Quercetin, one of the most common flavonoid in nature, is reported to have antioxidant properties associated with antithrombic, antihypertensive, anticarcinogenic and anti-inflammatory effects (8). The protective effects of quercetin have been attributed to the inhibition of key signaling enzymes (e.g. protein kinase C, tyrosine kinase and phosphoinositide 3-kinase) involved in the regulation of cell proliferation, angiogenesis and apoptosis, as well as to antioxidant properties as scavengers of ROS (9).

In our previous study, the potential of a w/o microemulsion as a topical carrier system for delivery of the antioxidant quercetin was demonstrated (10). In the present study, histological aspects were considered in order to evaluate the *in vivo* photoprotective effect of this formulation against UVB irradiation-induced dermal damages.

MATERIAL AND METHODS

Materials

Quercetin dihydrate 99% ($C_{15}H_{10}O_7.2H_2O$, M_w = 338.26) was purchased from Acros Organics (New Jersey, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and antibiotic solution containing 5 mg of penicillin, 5 mg of streptomycin and 10 mg of neomycin per ml were purchased from Gibco (Grand Island, NY, USA). Propylene glycol and polyoxyethylene (80) sorbitan monolaurate (Tween 80®) were from Synth (Diadema, SP, Brazil), sorbitan monolaurate (Span 80®), Sirius red and glutaraldehyde 25% were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and orceine was purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and were used without further purification.

Microemulsion containing quercetin
Microemulsion was prepared by adding the following components to the final stated percentages (w/w): 15% of a mixture of propylene glycol and water (3:1) as water phase, 46.75% of a mixture of Span 80® and Tween 80® (3:1) as the surfactant/co-surfactant system and 38.25% of canola oil as the external phase. Quercetin (0.3%, w/w) was incorporated into this system as previously described (10).

Protective effect of a microemulsion containing quercetin against UVB irradiation- induced skin damages

Animals and experimental protocol
In vivo experiments were performed on 3-month-old sex matched hairless mice obtained from the colony of the Faculty of Pharmaceutical Science of Ribeirao Preto; They were housed in a temperature-

controlled room, with access to water and food *ad libitum* until use. All experiments were conducted in accordance with National Institutes of Health guidelines for the welfare of experimental animals and with the approval of the Ethics Committee of the Faculty of Pharmaceutical Science of Ribeirao Preto (University of Sao Paulo, Ribeirao Preto, SP, Brazil).

Randomly chosen animals were divided into groups of 3-5 and topically treated on the dorsal surface with 300 mg of the unloaded (quercetin free microemulsion - ME) or quercetin-loaded (0.3%, w/w) (ME+Q) microemulsions. The formulations were applied 1 h and 5 min before irradiation of the dorsal surface and also immediately after irradiation as previously described (11). The untreated control groups, irradiated and non-irradiated, were included in the experiments.

Irradiation

The UVB source of irradiation consisted of a Philips TL40W/12 RS lamp **(Medical-Eindhoven, Holand)** i.e. city, country?), mounted 20 cm above the table on which the mice were placed, and which emitted a continuous light spectrum between 270 and 400 nm with a peak emission at 313 nm. UVB output (80% of the total UV irradiation) was measured using a model IL-1700 Research Radiometer (International Light, USA; calibrated by IL service staff) with a radiometer sensor for UV (SED005) and UVB (SED240). The UVB irradiation rate was 0.27 mW/cm^2 and the dose used was 2.87 J/ cm^2.

Erythema formation

The increase in skin erythema, after irradiation (3 h) and before animal sacrifice (6 h), was measured using the Chroma Meter CR 200 **(Minolta Instrument Systems, Milton Keynes, UK)**, and readings on the a* scale, which measures the intensity of redness of skin were recorded. The increase in this redness was expressed as the difference between the basal a* (before irradiation) and irradiated skin values.

Histopathological changes

UVB-induced histopathological changes were evaluated considering the parameters of infiltration of inflammatory cells, epidermis thickening (basale and spinosum layers) and collagen and elastic fiber contents.

The animals were sacrificed with an overdose of carbon dioxide 6 h after the start of UVB exposure, and the skin treated area was dissected and fixed by immersion into 2.5% glutaraldehyde in cacodylate buffer solution at room temperature for 24 h. Following dehydration and inclusion in paraffin wax, 5 μm thick sections were cut and stained with hematoxylin and eosin (H&E), Sirius red and orceine.

In the H&E stained sections, a test-system composed of points and cycloid arcs was overlaid on the captured images, and thicknesses of the basale and spinosum layers of epidermis were measured by stereological analysis counting the points on the epithelium and the intersections with the interface of layers (12). Sirius red and orceine staining were used to determine the collagen and elastic fiber contents, respectively. Stained sections were examined with light microscopy, and the images were captured at 100X magnification. The collagen and elastic fiber contents were evaluated using the ImageJ Program (NIH-National Institute of Health, Bethesda, Maryland, USA) software.

Microemulsion cytotoxicity and skin irritation

Determination of cytotoxicity
Cell culture and treatment

L929 mice fibroblast from the Rio de Janeiro cell Bank, University of Rio de Janeiro, Rio de Janeiro, Brazil, were routinely grown in 150 cm^2 tissue culture flasks in DMEM supplemented with 1% (v/v) of an antibiotic solution containing 5 mg of penicillin, 5 mg of streptomycin and 10 mg of neomycin per ml, as well as with 7.5% (v/v) heat-inactivated FBS, at 37 °C under 5% CO$_2$. Quercetin (raw material) was dissolved in dimethyl sulfoxide (DMSO, final concentration below 0.1%) and diluted using phosphate buffer solution (PBS), and ME and ME+Q were dissolved in PBS. The final quercetin concentrations ranged from 50 – 400 μg/mL. Then, 20 μL of each dilution was used in the MTT assay.

MTT assay
The sensitivity of cells to quercetin activity was determined by a standard spectrophotometric 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (13). Cells were seeded at a cell density of 10^5 cells/well into 96 well plates and

incubated for 24 h at 37°C in an atmosphere of 95% air and 5% CO_2. Then, 20 µL of quercetin (raw material), ME and ME+Q, at different concentrations in PBS, were added to culture plates for 24 h. After treatment, cells were rinsed twice with PBS, and the serum free culture medium without phenol red was replaced in all wells. Cells were then incubated for 4 h with the MTT solution (5 mg/mL). The yellow tetrazolium salt was metabolized by viable cells to purple crystals of formazan. The crystals were solubilized overnight in a mixture consisting of 20% sodium dodecyl sulfate (SDS) in HCl (0.01 M). The product was quantified spectrophotometrically by absorbance measurement at a 570 nm wavelength using a microplate reader (µQuant^{TM}, BioTek Instruments Inc., Vermount, USA). The cellular viability was expressed as the percentage of viable cells compared to the control group.

Determination of skin irritation

ME and ME+Q (300 mg) were applied topically and non-occlusively on a limited area of the dorsal skin of hairless mice once a day for 15 days, and the animals were sacrificed on the sixteenth day with an overdose of carbon dioxide. The skins of untreated animals were used as controls. The histological analyses were conducted as described above, and skin irritation was evaluated according to the established endpoints of infiltration of inflammatory cells and epidermis thickening (basale and spinosum layers).

Statistical analyses

Data were statistically analyzed by one-way ANOVA, followed by Bonferroni's multiple comparisons t-test. The evaluations were made using the GraphPad Prism® (GraphPad Software,

San Diego, California, USA) and results considered significantly different when $p < 0.05$ was obtained.

RESULTS

Protective effect of a microemulsion containing quercetin against UVB irradiation- induced skin damages

Erythema formation

A comparison of the UV-induced skin reddening of ME- and ME+Q- treated skin to untreated skin was performed. Neither unloaded nor quercetin-loaded microemulsion pretreatment conferred protection against UV-induced skin reddening (Table 1).

Histopathological changes

Histological examination of hairless mice skin exposed to UVB irradiation revealed an exulcerated epidermis with bandlike inflammatory infiltrates in the dermal connective tissue and also, as demonstrated by arrows in Figure 1, the formation of sunburn cells (apoptotic keratinocytes). However, the topical treatment with both ME and ME+Q decreased the severity of the UVB-induced histological effects, reducing the extent of formation of sunburn cells by about 66% (data not shown).

The preventive effects of the w/o microemulsion containing quercetin on the UVB-induced skin thickening were evaluated by directly measuring the thickness of the basale and spinosum layers of epidermis. The UVB induced epidermal hyperproliferation (skin fold thickness increased by 30%) was significantly reduced by the treatment with both ME and ME+Q (Figure 2 and Table 2).

Table 1. Effect of unloaded and quercetin-loaded microemulsions treatment on UVB-induced erythema formation in hairless mice skin 3 and 6 hours after the beginning of UV exposure.

Treatment	Skin reddening (Δa*)	
	3 h	6 h
Control	0.07±0.02	0.13±0.00
Irradiated	7.83±0.13	7.68±0.12
ME	7.78±0.05	7.63±0.11
ME+Q	7.72±0.10	7.69±0.11

Δa* corresponds to the difference between basal a* (skin color before irradiation) and irradiated skin values
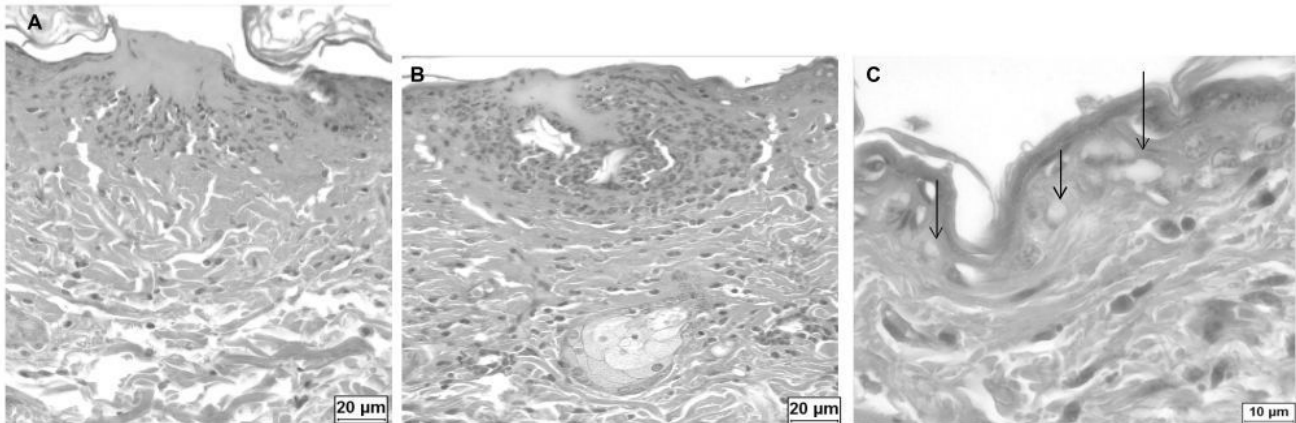Results are represented by means ± S.D. (n=3).

Figure 1. UVB-induced histopathological changes in hairless mice skin. Representative photomicrographs of samples derived from untreated-irradiated animals. Sections (A) and (B) were analyzed through a 40X objective and section (C) through a 100X objective and stained by hematoxylin and eosin (H&E).
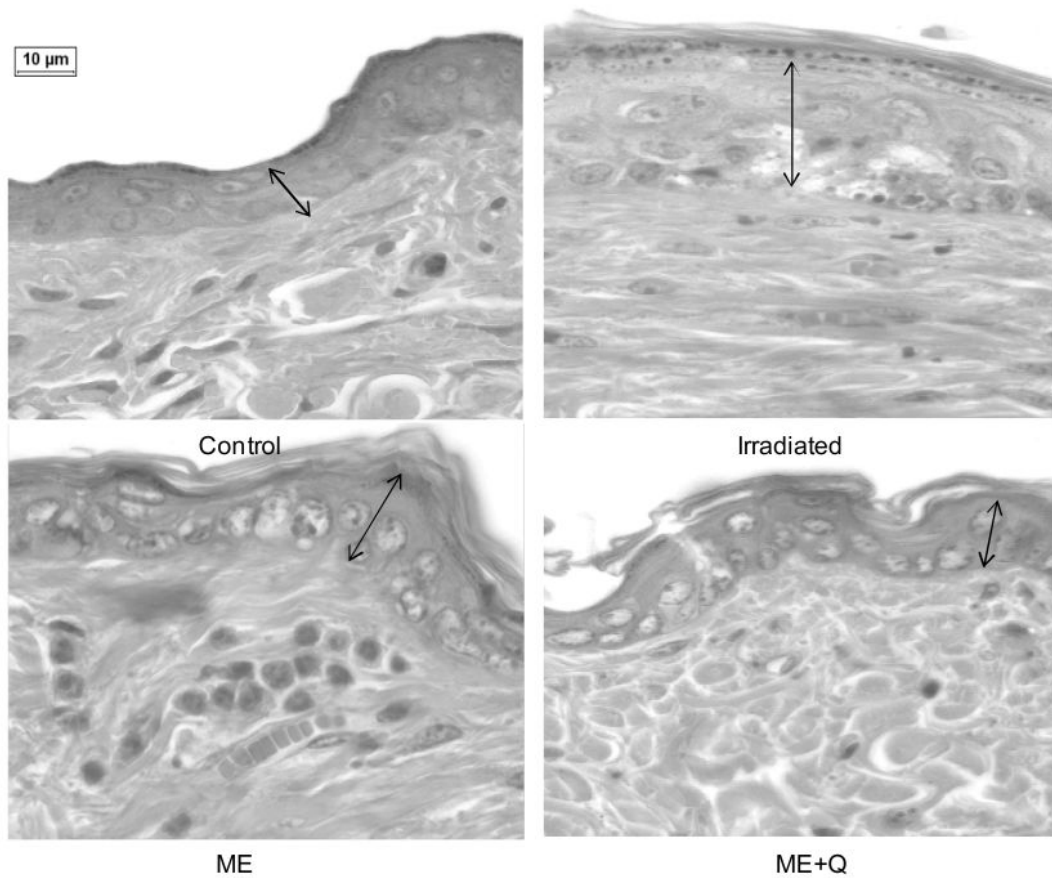

Figure 2. Effect of a w/o microemulsion on UVB-induced histopathological alterations. Representative photomicrographs of samples derived from untreated-unexposed skin (control), untreated-irradiated skin (irradiated), ME and ME+Q treated skins. Sections were visualized by conventional light microscopy through a 100X objective and stained by hematoxylin and eosin (H&E).

Sirius red stained sections showed damage to the collagen in the upper epidermis of untreated irradiated skin. As demonstrated by Figure 3A, these changes were less pronounced in the ME+Q treated group. Finally, as demonstrated by Figure 3B, in non-irradiated skin, fine elastic fibers perpendicular and parallel to the body axis were observed in the lower dermis. On the other hand, in untreated irradiated skin, a significant accumulation of elastic fibers in the lower epidermis was found, with a detected increase of about 54% in the numerical density of the elastic fibers when compared with the control group. In the group of animals pre-treated with both ME and ME+Q, an

increase in the elastic fiber levels of approximately 29% was detected when compared with the control group (untreated-unexposed group), reducing the extent of UVB-induced elastic fiber level increase by 25% when compared with the untreated-irradiated group (data not shown).

Microemulsion cytotoxicity and skin irritation

Cytotoxicity
It is important to mention that because of the interference of redox sensitive molecules which may reduce the MTT irrespective of any cellular mechanism and therefore give false positive results,

Table 2. Epidermis thickness of hairless mice after UVB exposure and treatment with unloaded and quercetin-loaded microemulsions.

Treatment	Epidermis thickness[a] (μm)
Control	8.55 ± 0.87
Irradiated	11.05 ± 1.63*
ME	6.65 ± 1.12
ME+Q	6.23 ± 1.15

[a] Corresponds to the sum of basale and spinosum layers of epidermis. Results are represented by means ± S.D. (n=5). Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test of multiple comparisons. *Significant statistical difference compared to control (untreated-unexposed group) ($p < 0.05$).
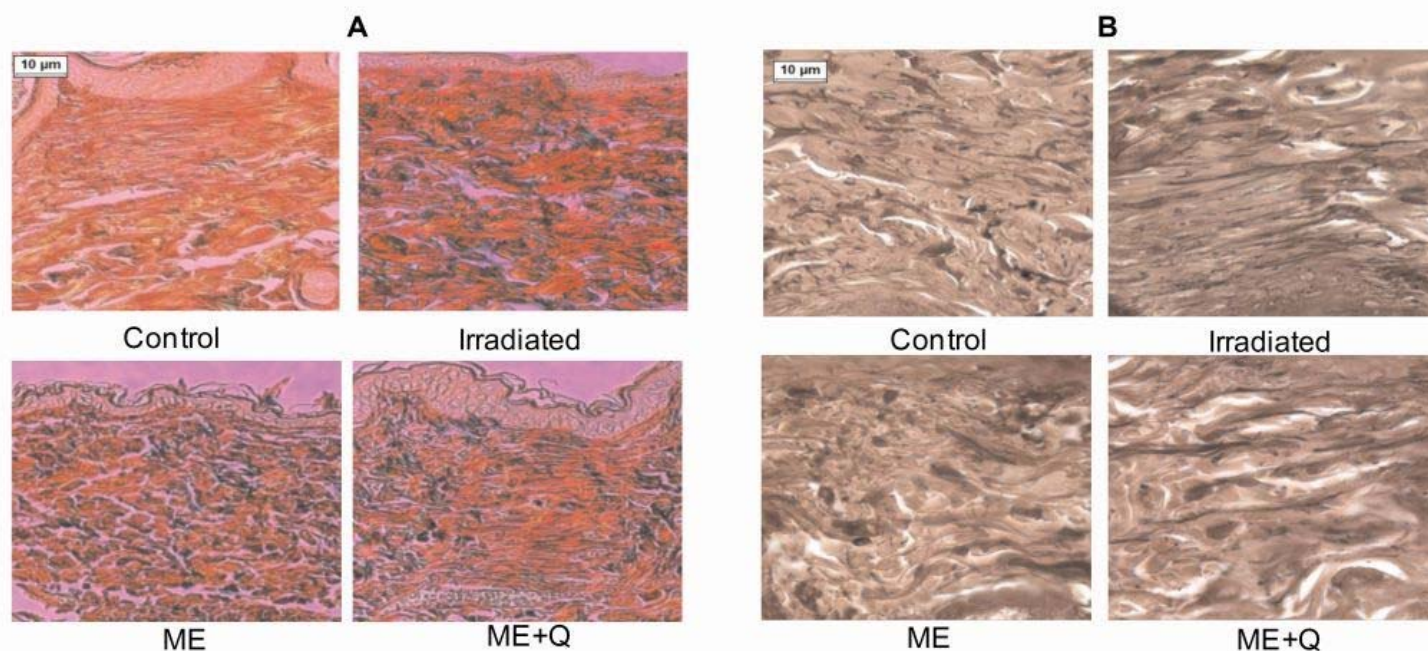

Figure 3. Effect of a w/o microemulsion on collagen and elastic fibers content in the skin of hairless mice exposed to UVB irradiation. Representative photomicrographs of (A) collagen and (B) elastic fibers in the skin. Sections were visualized by conventional light microscopy through a 100X objective and stained by sirius red for collagen and by orceine for elastic fibers analysis.

some cautions during the cytotoxicity experiments were taken, as suggested by Bruggisser, R. et al. (14). Firstly, a prescreening in cell-free systems was performed to examine the potential reductive activity of quercetin before the performance of any cell culture experiment. The results demonstrated that only the highest concentration of quercetin (400 μg/mL) employed in the present study showed some interference. Subsequently, washing procedures were implemented to properly remove the potentially interfering agent before the addition of MTT.

The effects on cell proliferation of quercetin as well as the unloaded and quercetin-loaded microemulsions were evaluated. The incubation with quercetin (raw material) for 24 h induced a concentration-dependent inhibition of cell proliferation as determined by MTT assay (Table 3). The inhibition was 18.28, 23.34 and 32.50% at 100, 200 and 400 μg/mL of quercetin, respectively. The evaluation of unloaded and quercetin-loaded microemulsion cytotoxicity demonstrated that, until the concentration corresponded to 200 μg/mL of quercetin, the formulations protected against quercetin cytotoxicity since no inhibition of cell proliferation was observed. Nevertheless, for the highest concentration studied, the reduction in cell viability was about 65% for ME+Q, due to the sum

of the cytotoxic effects of both quercetin and unloaded ME.

Skin irritation

Previous work by our group demonstrated that the daily application of ME+Q for up to 2 days did not cause skin irritation (10). However, a deeper investigation should be conducted in order to evaluate the effect of this formulation when applied over a greater period of time. Therefore, in the present work, the histopathological changes in the skin of hairless mice were evaluated after administration (for 15 consecutive days) of the proposed formulation with and without quercetin. The formulations were applied topically and non-occlusively on a limited area of the dorsal skin of hairless mice once a day for 15 days, and the animals were sacrificed on the sixteenth day and the skin analyzed.

No histopathological alterations in the skin of animals treated with ME and ME+Q were seen by light microscopy, as compared to the untreated animals (Figure 4A). Furthermore, no statistical difference was observed in the thicknesses of the basale (Figure 4B) and spinosum (Figure 4C) layers of epidermis in topically ME-treated and ME+Q-treated groups when compared to untreated animals.

Table 3. Effect of quercetin (raw material), unloaded and quercetin-loaded microemulsions on cellular viability (as % of control) of L929 mice fibroblasts measured by MTT assay.

Quercetin concentration (μg/mL)	Cellular viability (% of control)		
	Quercetin (raw material)	ME	ME+Q
50	103.26 ± 6.77	105.64 ± 7.11	98.98 ± 8.02
100	81.72 ± 9.95	116.03 ± 8.39	114.10 ± 9.10
200	76.66 ± 14.37	91.99 ± 17.33	118.33 ± 6.39
400	67.50 ± 5.67	63.12 ± 25.38	35.19 ± 10.95
Results are represented by means ± S.D. (n=6).			

DISCUSSION

Chronic exposure of skin to solar UV radiation markedly alters the composition and architecture of the dermal extracellular matrix, resulting in both clinical and histologic changes in human skin. Both UVB (290–320 nm) and UVA (320–400 nm) portions of the terrestrial solar UV spectrum are believed to contribute to the processes that initiate these responses. UVB is almost entirely absorbed

by cellular components in the epidermis, suggesting that dermal remodeling initiated by this waveband involves mediators that diffuse from the epidermis into the dermis, where they stimulate fibroblast production of elastin and glycosaminoglycans (15).

UV irradiation causes increased synthesis and release of proinflammatory mediators from a variety of skin cells, leading to infiltration and activation of neutrophils and other phagocytic cells into the skin.
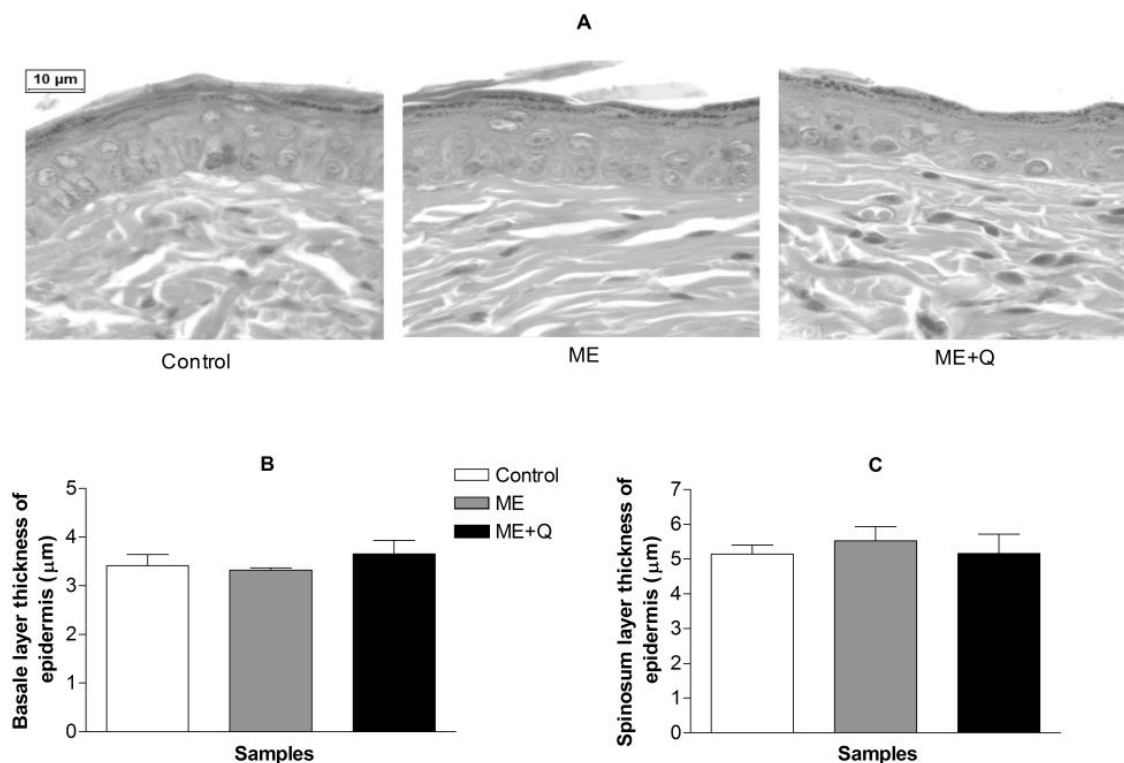
Figure 4. Effect of a w/o microemulsion in the skin of hairless mice. (A) Representative photomicrographs of skin sections of untreated animals and treated with ME and ME+Q. Sections were visualized by conventional light microscopy through a 100X objective and stained by hematoxylin and eosin (H&E). Panels (B) and (C) show the thickness of the basale and spinosum layers of epidermis, respectively, of untreated animals and treated with ME and ME+Q. Results are represented by means ± S.E.M. (n=5 animals per group). No statistical significant difference was detected. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test of multiple comparisons ($p < 0.05$).

Thus, inflammatory mechanisms may accentuate the effect of UV radiation to amplify direct damaging effects on molecules and cells that cause photoaging (4).

In the present work, the acute exposure of hairless mice to a UVB dose of 2.87 J/cm^2 induced an immediate inflammatory response with erythema and leukocyte infiltration. Statistically significant increases were also observed in the epidermis thickness, the collagen and fiber levels and the formation of sunburn cells, a marker of the severity of solar damage (1). These increases in connective tissues may increase skin fold thickness and lead to the formation of skin wrinkles by UV.

It has been suggested that the modulation of redox-sensitive transcription factors, such as nuclear factor (NF)- κB, by ROS is a central and early event in the induction of inflammatory reactions (16). NF-κB is an oxidative stress-sensitive factor that activates multiple target genes involved in the expression of several proinflammatory mediators (17). Quercetin, a member of the flavonoid family, is known to modulate NF-κB activation triggered by various inductors in different cell types (18-26).

The use of antioxidant compounds as photoprotective agents for preventing premature skin ageing and skin disease seems appropriate. In this context, it is important to mention that vitamin E, which is recognized as the most important lipid-soluble, chain-breaking antioxidant in the body, is probably the most widely investigated photoprotective substance besides sunscreens, being indicated as playing a key role in the prevention of skin oxidative damage (27). Therefore, the topical use of the lipid-soluble free-radical scavenger

quercetin may be relevant for a preventive and/or therapeutic approach to photoprotection.

Previous work by our group reported the effectiveness of topical formulations containing quercetin against damage induced by UVB radiation exposure (11). However, incorporation into a w/o microemulsion optimized the effects of the flavonoid, since a dosage approximately 6-fold smaller produced the same *in vivo* results obtained with a non-ionic emulsion containing quercetin (10).

Among the *in vivo* effects, topical treatment with ME+Q inhibited the activity of matrix metalloproteinases (MMPs) in skin exposed to UVB irradiation, probably via MMP inhibition (10). MMPs are a family of proteolytic enzymes that specifically degrade collagens, elastin and other proteins in connective tissue and bone (6).

MMPs are frequently over expressed by various extracellular stimuli including growth factors, cytokines, tumor promoters and UV, and this increase in MMP-related activities may be involved in the pathogenesis of diseases such as cancer and inflammation, as well as in physiologic processes. It has been reported that up-regulation of some MMPs is responsible for the enhanced degradation of dermal collagen during chronological and UV-induced skin aging (28-30). Therefore, the regulation of MMP activity might be a potential strategy for prevention and/or treatment of UV-induced skin damage (31).

The present study demonstrated that ME+Q was able to reduce the incidence of UV irradiation-induced histological skin alterations, mainly the connective-tissue damage. More recently, changes in collagen metabolism have been brought into focus as a major factor leading to photoaging. Specifically, it has been found that the accumulation of elastic material is accompanied by concomitant degeneration of the surrounding collagenous meshwork, and evidence implicating MMPs as mediators of collagen damage in photoaging has been presented (32). Thus, the capability of ME+Q to inhibit the UVB-induced activity of MMPs resulted in decreased incidence of extracellular matrix damage upon topical application of this formulation in hairless mice. Furthermore, considering that ROS play an important role in the upregulation and activation of extracellular matrix-degrading MMPs and are able to attack and destroy *per se* several extracellular matrix proteins, the antioxidant properties of quercetin may also be responsible for preventing this degradation and, consequently, the deleterious effects of photoaging agents.

The topical treatment of hairless mice with the ME+Q maintained reduced glutathione (GSH) levels close to the non-irradiated control, which demonstrates that this formulation significantly prevented UVB irradiation-induced GSH depletion (10). GSH is an endogenous tripeptide that plays an important role in protection against photooxidative damage. It can act as a direct free radical scavenger by hydrogen atom donation, and the resulting much less reactive thienyl radicals decay bimolecularly or via an oxygen-dependent mechanism, forming glutathione disulfide GSSG (33).

Quercetin may protect against UV-induced GSH depletion either by free radical scavenging activity or by decreasing free radical production through iron chelation since, in addition to its anti-radical activity, quercetin possess structural features that enable it to chelate metal ions, namely the *ortho* dihydroxy (catechol) phenolic structure. Besides this, the protective effects of this flavonoid can be also due to cellularly-mediated effects such as increased enzymatic and non-enzymatic cellular antioxidants, perhaps by stimulating the cytoplasmic machinery involved in the enzymatic resynthesis of GSH. Therefore, the effects of quercetin seem to be due to effects at several levels that include radical scavenging, iron chelation and modulation of cell antioxidant responses (34).

Thus, by evaluating the effects of ME+Q on UV-induced erythema formation and histopathological changes, it is possible to suggest that the protective effects of this formulation on UV-induced responses reported above are not secondary to interference of UV transmission (i.e., blocking the UVB radiation from being absorbed by the skin), as is usually done with UVB absorbers and sunscreens, but is instead due to different biological effects of this flavonoid.

Furthermore, the present study also demonstrated that, although the ME is composed of large amounts of surfactants and oil, the 24 h incubation of L929 cells with the quercetin-loaded microemulsion has no cytotoxic effect until the final concentration reaches 200 µg/mL. Additionally, the daily application of both ME and ME+Q for up to 15 days did not cause skin irritation, as demonstrated by the evaluation of established

endpoints of skin irritation. The irritability of emulsifiers has been demonstrated in a wide variety of experimental models, and it may cause MEs to have an irritative effect on living cells. The interactions between stratum corneum lipids and MEs may cause dehydration and barrier function compromise. In experimental models, these effects cannot always be neatly separated, since barrier compromise will increase irritant penetration and hence increase the irritative effect (35).

As epidemiological studies indicate that the use of sunscreen and sun block are not completely effective in preventing UV-induced skin cancer, new targeted chemopreventive approaches need to be identified (36). Instead of only blocking the UVB radiation from being absorbed by the skin, as is usually done with UVB absorbers and sunscreens, skin could be also protected by preventing the formation of the photooxidants that result in radical damage and cutaneous pathology (37). In this context, the topical application of ME+Q was able to reduce the incidence of histological skin alterations in UV-irradiated hairless mice. Therefore, the present results taken together with the previous data on skin retention and *in vivo* activity (10) suggest that further investigation should be conducted in order to evaluate the effects of ME+Q as a photochemoprotective agent on human skin.

ACKNOWLEDGMENTS

Novelty of the Work: This manuscript evaluates the histological aspects involved in UVB-induced acute skin damages and the protective effect of a w/o microemulsion containing quercetin against these dermal damages. The present results allowed a better understanding about the photoprotective effect of this formulation, suggesting that further studies should be conducted in order to evaluate its effects on human skin.

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