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Topical formulation containing *Ilex Paraguariensis* extract increases metalloproteinases and myeloperoxidase activities in mice exposed to UVB radiation

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ABSTRACT

Ultraviolet B radiation represents 10% of the total UV radiation that reaches the Earth's surface, being the primary responsible for the biological effects related to skin cancer and photoaging. *Ilex Paraguariensis* A. St. Hil., known as Yerba mate (YM), is a native tree of South America whose polyphenols in its leaves are described to exhibit photochemoprotective effect and are employed in the treatment of cancer. Additionally, the polyphenols are used to prevent lipid peroxidation and reduce the UV-induced damage, which ultimately decreases the oxidative stress. Thus, the present study aimed to characterize a new YM extract, evaluate the extract cytotoxicity and develop a formulation containing YM extract to prevent UVB-induced damage in mice skin. The YM extract showed high levels of polyphenols, flavonoids, and tannins and exhibited excellent antioxidant activity. Its main components were suggested as chlorogenic acid (1.92%) and caffeic acid (0.41%). Besides, YM extract did not exhibit cytotoxicity in fibroblasts and decreased the activity of myeloperoxidase and metalloproteinase-2 after acute UVB exposure. As a result, the formulation containing the YM extract showed a potential photochemoprotective.

1. Introduction

There are different exogenous sources of oxidative stress and among them is the ultraviolet (UV) radiation, which can cause harmful effects on the cells [1]. The acute UV exposure of the human skin can lead to several diseases and morphological changes, such as inflammation, erythema, edema, hyperplasia and sunburn cell formation. Besides, chronic exposure to UV radiation may disrupt the connective tissue and decrease the level of collagen, leading to skin aging [2].

The most abundant protein in the skin connective tissue is type I collagen, which is secreted by dermal fibroblasts as type I procollagen [3]. The UV-induced decrease in type I collagen is one of the leading cause of photoaging. The UV radiation decreases the collagen content by inhibiting its production and increasing matrix metalloproteinases (MMPs) expression. The UV radiation increases reactive oxygen species (ROS) formation, which activates complex signaling pathways and induces proinflammatory cytokines release from epidermal keratinocytes and dermal fibroblasts [4,5]. Neutrophils are also activated and stimulate the activity of myeloperoxidase (MPO), which is an enzyme that generates ROS [2,6].

It is well established in the literature that single UVB exposure can increase the activity of MMP and MPO significantly, besides decreasing

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the collagen content in skin cells [7,8]. The development of topical formulations containing active ingredients capable of decreasing the oxidative stress and inhibiting the activity of MPO and MMP's is an effective alternative to delay skin aging and prevent the UV-induced skin photodamage, which primarily includes the risk of skin cancer [9].

One approach to reduce the UV-induced oxidative stress and ultimately to reduce the occurrence of skin cancer is through "Photochemoprotection". Photochemoprotective agents are capable of ameliorating the adverse effects of the UV radiation on the skin, and botanical antioxidants are promising photochemoprotective agents [8,10].

Also, many countries have rich biodiversity, which stimulates the use of plant extracts and their chemical components for therapeutic purposes. Innovative researches have been employing plant extracts with known antioxidant activity to assess the possible effects and mechanisms involved in preventing the UV-induced damage.

Ilex Paraguariensis A. St.-Hil. (Yerba mate, YM) is a native shrub to South America that is grown in Argentina, Brazil, Uruguay, and Paraguay. Through their leaves is prepared a traditional energy tea, which has been shown to exhibit antioxidant, vaso-dilating and lipid reduction properties, besides antimutagenic and anti-glycation effects. The beverage is known as maté, té del Paraguay, chimarrão, and tereré [11]. Previous investigations showed many bioactive compounds such as xanthines, phenolic acids (chlorogenic and caffeic acid), flavonoids and triterpenoid saponins [12,13].

The polyphenols present in the leaves of *I. paraguariensis* have many biological activities such as chemoprevention and therapeutic activity in the cancer treatment; prevention of lipid peroxidation in mammals and prevention of adverse effects of UV radiation, reducing the oxidative damage [14].

Mate tea reduces oxidative stress, inflammatory cell influx, cellular metabolic activity, and MMP-9 and TNF-α expression [15]. Other research tested the effects of topical application of ferulic acid (FA), a phenolic compound present in various medicinal plants and similar compound to caffeic acid (present in *I. paraguariensis*) on UVB-induced increase in MMP-2 and -9 activities in mouse skin. The skin treated with FA showed a significant reduction in chronic UVB radiation-induced levels of MMP-2 and -9, showing its possible long-term beneficial effects against the deleterious effects of UVB radiation [16].

A recent study shows that the protection offered by natural extracts in response to the UV-induced damage may be as high as two-fold as the protection provided by its isolated compounds. The superior effect presented by the extract was suggested to be due to the synergism among several phenolic compounds [8], which highlights the relevance of the use of natural extracts.

Thus, the present study aimed to characterize Yerba mate extract (YME), assess its cytotoxicity and phototoxicity on human skin fibroblasts and evaluate the protector effect of a topical formulation containing the extract on mice exposed to UVB radiation.

2. Experimental

2.1. Chemicals and Reagents

The chlorogenic acid (CGA) and caffeic acid (CA) standards used for HPLC and spectrophotometric assays were provided by Sigma-Aldrich (St. Louis, MO, USA). All solvents used were HPLC grade, and water was purified by deionization in 0.22 µm membrane filtration (Millipore, Billerica, MA, USA). The reagents and equipment used for SDS-PAGE were purchased from Bio-Rad Co. Ltd. (Hercules, CA, USA).

2.2. Plant Material and Extraction Procedure

Leaves of *I. paraguariensis* were collected in Santa Maria, RS, Brazil (29°43′05.3″S 53°43′46.2″W) in September 2014. The plant identification was performed by Prof. Dr. Renato Zachia, and the voucher specimen (SMDB 15.449) was deposited in the Botany Department Herbarium of Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil. The dried leaves of *I. paraguariensis* were extracted by maceration with EtOH:H_2O (40:60, v/v) at room temperature. Then, the extract was concentrated under reduced pressure at 45 °C, following lyophilization.

2.3. Total Phenolic, Flavonoid and Tannins Contents

The determination of total phenolic content (TPC) was carried out using the Folin Ciocalteau reagent, according to the method of Chandra & Mejia [25]. The results were expressed as gallic acid equivalents (mg) per 100 g of dry weight extract (GAE mg/100 g DW).

The total flavonoid content (TFC) was performed according to [42]. The results were expressed as catechin equivalents (mg) per 100 g of dry weight extract (CE mg/100 g DW).

Total tannins content (TTC) was assessed according to Vanillin assay ([43]) with modifications. The results were expressed as catechin equivalents (mg) per 100 g of dry weight extract (CE mg/100 g DW).

All the experiments were performed in triplicate.

2.4. Antioxidant Activity

The antioxidant activity of YME was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and *β*-carotene linoleate assays. The ability of YME to quench the stable free radical DPPH was monitored according to Choi et al. [44]. The test was performed in triplicate and the results were expressed as the concentration required to reduce the DPPH by 50% (IC_{50}). Ascorbic acid was used as the standard antioxidant.

The *β*-carotene linoleate assay was carried out according to Mokbel & Hashinaga [45]. YME was employed in the concentrations 50 µg/mL and BHT was used as standard in the concentration 50 µg/mL. The results were expressed as % inhibition, according to the equation: % inhibition = $[1–(A_{S(0)}–A_{S(120)}/A_{C(0)}–A_{C(120)})] \times 100$, where $A_{S(0)}$ and $A_{S(120)}$ are the absorbance values at 0 and 120 min of the incubation for sample. $A_{C(0)}$ and $A_{C(120)}$ are the absorbances measured in the control at 0 and 120 min of the incubation.

2.5. Identification of the Major Components of YME by Liquid Chromatography MS Detection

The HPLC-UV-DAD comprised a Shimadzu CBM-20A (Kyoto, Japan) system equipped with an LC-20AT pump, an SIL-20A automated sample injector, and an SPD-M20A photodiode array detector controlled by LC Solution software. The analytical column used was Shim-pack CLC-ODS (M), 5 µm particle diameter, dimensions of 4.6 mm × 150 mm. Chromatographic runs were performed at a 0.6 mL/min under gradient conditions following the program: 0–10 min, 31% B; 10–25 min, 31–56% B, 25–33 min, 56% B, 33–45 min, 56–77% B, 45-50 min, 77–56% B and 50–55 min, 56–31% B. CGA and CA standards were employed in the concentrations 30, 50, 100, 150, 200 and 250 µg/mL to elaborate the calibration curves.

The LC-MS/MS was Agilent Technologies 1200 series High-Pressure Liquid Chromatograph interfaced with an Agilent Technologies 6460 Triple Quad LC/MS (San Jose, CA, USA), using negative ion electrospray ionization (ESI). The gas temperature was 300 °C and the dry gas flow was 5 L/min. The nebulizer remained at 45 psi. The voltage of the capillary and shredder was 3500 and 3 eV, respectively. The flow rate of the sample was 0.8 mL/min. Nebulizer and the collision gas utilized was nitrogen. The sample analysis was in negative SCAN mode. Analysis of fragments of selected precursor ions was in the production method, with collision energy of 15 eV.