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Effect of the extract combinations of Curcuma zedoaria and Aloe vera in retarding melanin synthesis in murine melanoma cells

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Abstract:

The objective of the study was to determine the effect of role of combination of extracts of Curcuma zedoaria and Aloe vera in reducing melanin synthesis. Varying concentrations of the extracts of the plants were tested for melanogenesis and tyrosinase activity in murine melanoma cells. Extract combinations at a concentration of 1-5µl showed 50- 150% reduction in melanogenesis without altering the cell proliferation. Tyrosinase activity was very low in extract treated cells when compared to control.

Introduction

Tyrosinase enzyme plays a major role in melanogenesis process. This rate limiting enzyme oxidizes the amino acid tyrosine to DOPA and then to melanin [1]. Retarding the tyrosinase enzyme activity is considered to be the key approach for achieving skin lightening effects with most of the cosmetic skin lightening/ whitening/ fairness creams in the market [2,3]. Further, this method of inhibiting the melanogenesis process is reversible in nature hence does not produce any permanent pigmentory problem to the skin.

In the systems of Indian medicine, several plants have been recognized to have effect in modulating the skin pigmentation. The glabradin, isolated from licorice, arbutin from mulberry and some other compounds of natural origin have been widely used in several skin lightening preparations all over the world. Curcuma zedoaria and Aloe vera, although used extensively in various cosmetic and drug preparations currently, the effect of the combinations of these extracts in inhibiting melanin

synthesis is not studied in detail. In the present investigation, we discuss the role of the combination of extracts of Curcuma zedoaria and Aloe vera in retarding the melanogenesis through tyrosinase inhibition.

Materials and Methods

The extracts of Curcuma zedoaria and Aloe vera were extracted separately in propylene glycol: water at 1:1 ratio. The solid to liquid ratio was maintained at 1:100. The extracts were combined at 1:1 ratio. The mixture of the extract at varying concentration was used for testing the activity.

Cell Culture

B16F10 murine melanoma cells were cultured in Eagles minimal essential medium supplemented with 10% heat inactivated fetal bovine serum and 2mM L-glutamine at 37?C in a humified atmosphere containing 5% $CO2$. Different concentrations of the extract ranging from 1-5 ul were added to the culture after the cells being seeded. The cells were incubated for 24, 48 or 72 hrs and cell numbers (determined by counting in a haemocytometer chamber), melanin contents and tyrosinase activities were determined in triplicate for each treatment as detailed below.

Melanin Measurement

Melanin content was measured as per the method described as follows. Approximately 107 cells were pelleted by centrifugation at 1000 g for 5 minutes and then washed twice with phosphate buffered saline. After further centrifugation, the supernatant was decanted, the precipitated cells were re- suspended in 200 µl of distilled water, and 1 ml of ethanol-ether 1:1 was added to remove opaque substances other than melanin. The mixture was stored and suspended at room temperature for 15 minutes. After further centrifugation at 3000 g for 5 minutes, the precipitate was solubilized by treatment with

1 ml 1N NaOH/10% dimethyl sulfoxide at 80°C for 30 minutes in a capped tube. The absorbance was measured at 470 nm and the melanin content per cell was calculated and expressed as percentage of control (=100%).

Tyrosinase Assay

Tyrosinase activity was assayed as DOPA oxidase activity. Approximately107 cells were pelleted and then washed twice with phosphate buffered saline. After centrifugation, the supernatant was decanted. The cell pellet was dissolved in 1.0 ml of 0.5% sodium deoxycholate in distilled water and allowed to stand at 0°C for 15 minutes. Tyrosinase activity was assayed spectrophotometrically by following the oxidation of DOPA to DOPA chrome at 475 nm. The reaction mixture consisting of 3 ml of 0.1% L-DOPA in 0.1 M phosphate buffer pH 6.8 was mixed with cell lysate. Assay was performed at 37°C in a spectrophotometer. The rate was measured during the first 10 minutes of the reaction while it was linear. Corrections for auto oxidation of L- DOPA in controls were made. Specific activity was defined as the amount of

DOPAchrome formed per 10 min per cell, and is expressed as percentage control (=100).

Results

Visible reduction in melanin content was observed in cell pellets incubated with the extract. However, the growth rate of B16F10 murine cells was not significantly altered by the extract treatment during 72 hr incubation period. This clearly indicates that the melanogenesis modulation occurs in the cells without affecting the cell proliferation. The level of decrease of melanin synthesis in relation to the concentration of the extracts was 50% to 190% for 1-5 µl of the extract combinations respectively.

The tyrosinase activity was recorded to be very low in cells treated by the extract combination when compared to control.

Optimum concentration of the extract that showed very high activity in decreasing the melanin synthesis was 4µl. A concentration higher than this level of the extract did not significantly retard the melanogenesis in the murine melanoma cells.

The extract up to a concentration of 50µl did not show cytotoxic activity when tested by MTT.

Discussion

The present investigation clearly suggests that the combination of the extracts of Curcuma zedoaria and Aloe vera is very effective in decreasing the melanin synthesis when tested in murine melanoma cells. The key mechanism of action the extract combination was found to be through tyrosinase blockage. Tyrosinase, being the primary enzyme, that play a major role in the oxidization of tyrosine to melanin, most of the approaches for achieving the skin lightening effects through various cosmetic preparations were by inactivation of the above enzyme. Further, the above approach is reversible, which is very safe and does not cause any permanent damage.

Curcuma, being a very old traditional plant used in India for skin care is also known to have antiseptic properties [4,5,6]. Similarly, Aloe vera, the other widely used medicinal plant for various cosmetic and drug preparations all over the world. This plant is also known to have sun screening effect and hence the use of this plant extract in skin creams offers sun protection benefits [7]. The potent skin lightening effect what we established for the combination of the extracts of Curcuma zedoaria and Aloe vera assumes great importance as both plants are widely used for various skin benefits.

Retarding the melanogenesis known to make the skin relatively more vulnerable to UV damage, and that is why most of the skin lightening creams contains sun screeners. Use of the combination of the above extracts offers dual benefit of skin lightening effect as well as sun protection to the skin. This approach also would

provide the advantage of eliminating the chemical sun screeners in the product, thereby can ensure total benefit come purely from herbal source.

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