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Anti-Melanogenic Activity of *Foeniculum Vulgare* Extract By Preventing Cellular Tyrosinase Activity- *in Vitro*

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Abstract: ***Foeniculum vulgare methanolic extract has been widely used for various ailments for peoples. Our investigation focussed to identify anti-melanogenic efficacy of Foeniculum vulgare since it has been known to have strong anti-oxidant activities. Anti-melanogenic effect of Foeniculum vulgare extract was analyzed using cultured B16 melanoma cells. α-MSH-induced melanin synthesis was significantly inhibited with dose-dependent manner by treatment of Foeniculum vulgare extract, which was comparable to that of arbutin and ascorbic acid. The extract directly inhibited intracellular tyrosinase activity of B16 melanoma cells. The inhibition of intracellular tyrosinase activity was found to be exerted at the protein expression level when analyzed by spectrometrically. In conclusion, Foeniculum vulgare extract has strong anti-melanogenic activity that is exerted by direct inhibition of tyrosinase enzyme activity The percentage inhibition of cellular tyrosinase activity in B16 cells treated with sample was approximately 18.0- 34.2 %. The sample treated B16 cells inhibited tyrosinase less than those treated with arbutin and L-ascorbic acid. Collectively, data shown in this study strongly suggest that Foeniculum vulgare extract has potential to be used as a novel depigmenting agent for cosmetics.***

Keywords: *Foeniculum vulgare*, Melanogenesis, Melanin, Cellular Tyrosinase, B16 melanoma cell

1. Introduction

Skin-lightening products are commercially available for cosmetic purposes to obtain lighter skin complexion. Clinically, they are also used for treatment of hyper pigmentary disorders such as melasma. All of these target naturally melanin production, and many of the commonly used agents are known as competitive inhibitors of tyrosinase, one of the key enzymes in melanogenesis.

Tyrosinase is a glycoprotein located in the membrane of the melanosome, a minifactorial vesicle inside the melanocyte. It has an inner melanosomal domain that contains the catalytic region (approximately 90% of the protein), followed by a short trans membrane domain and a cytoplasmic domain composed of approximately 30 amino acids [9]. Histidine residues are present in the inner (catalytic) portion of tyrosinase and bind copper ions that are required for tyrosinase activity [5]. Melanogenesis takes place in the melanosomes. Two types of melanin are synthesized within melanosomes: eumelanin and pheomelanin. Eumelanin is a dark brown-black insoluble polymer, whereas pheomelanin is a light red-yellow sulphur-containing soluble polymer [7]. Tyrosinase catalyses the first two steps of melanin production: the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and the subsequent oxidation of this o-diphenol to the corresponding quinone, L-dopaquinone [11-12,14]. Even though L-tyrosine is the building stone for melanin, it can only be transported into the melanosome by facilitated diffusion [4, 1].

Melanin synthesis begins with oxidation of L-tyrosine to L-DOPA (l-3,4-dihydroxyphenylalanine) and then to dopaquinone; both reactions are catalyzed by tyrosinase.[6] The tyrosinase reactions are the rate-limiting step in melanin synthesis; the remainder of the reaction sequence proceeds spontaneously at physiological pH. Although melanin primarily serves a photo protective function, the accumulation of abnormal amounts of melanin in different parts of the skin, which results in pigmented skin patches, can become anesthetic problem. Several studies have focused on inhibition of melanogenesis and the prevention of abnormal pigmentation for cosmetic benefits [5,3].

2. Materials and Methods

2.1 Cellular Tyrosinase Activity Assay

1) B16 Cells were plated in 1 ml of 24 well plate and incubated for 24 hrs.
2) The cells were treated with α- MSH(1 µM) for 72 hrs.
3) They were washed with PBS and lysed in 900µl of 50 mM sodium phosphate buffer (pH 6.8) that contained 1% Triton X-100 and freeze-thawed by incubating them at -800C for 30 min followed by 250C for 25 min and 370 C for 5 min.
4) 100µl of 10mM L-DOPA was then added. The tyrosinase activity in the cell lysates was determined by measuring the oxidation of L-DOPA to depachrome.
5) Following incubation at 370C for 4 h, the absorbance of the agents was measured at 475 nm .

3. Results and Discussion

Foeniculum vulgare dose-dependently decreased cellular tyrosinase activity (Figure 1), the rate-limiting step in melanin biosynthesis, in parallel with the decreased melanin content (Table 1). However, in vitro pre incubation of enzyme with *Foeniculum vulgare* for 25 min at 800°C did not affect the tyrosinase activity. The percentage inhibition of cellular tyrosinase activity in B16 cells treated with sample was approximately 18.0- 34.2 %. The sample treated

B16 cells inhibited tyrosinase less than those treated with arbutin and L-ascorbic acid.

Table 1: The cellular tyrosinase activity of the sample*(Foeniculum vulgare)* and control samples

Name of the Sample	Concentration (%)		
	10µl	20µl	30µl
Sample	18.0	25.4	34.2
Arbutin	35.2	42.3	49.1
L-Ascorbic acid	28.1	57.6	83.9

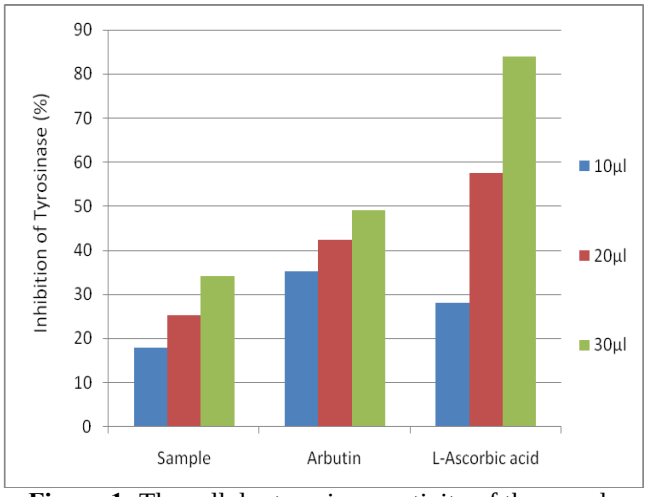

Figure 1: The cellular tyrosinase activity of the sample *(Foeniculum vulgare)* and control samples

4. Summary and Conclusion

Melanin biosynthesis is the primary cause of pigmentation of human skin and diverse factors such as UV light exposure, inflammatory cytokines, stress and genetic factors are known to be involved in regulation of melanogenesis [5, 13]. When UV is irradiated, melanogenesis is initiated by binding of a-MSH to its authentic receptor MC1R, which leads to increase of cAMP level in the melanocytes [10]. Tyrosinase activity in B16 cells was examined by measuring the rate of oxidation of L-DOPA.

There are two different modes of action with respect to inhibition of melanin synthesis in melanocytes. One is direct inhibition of tyrosinase enzyme activity and the other is the suppression of tyrosinase gene expression at the transcription level so that the level of tyrosinase protein is reduced in the cells. Many anti-melanogenic agents such as kojic acid, hydroquinone, and arbutin fall in the first category, direct inhibitor of tyrosinase. On the other hand,some agents exert their anti-melanogenic activities by down-regulating the expression of tyrosinase gene, but they do not have any direct inhibitory effect on tyrosinase in general [2].

Foeniculum vulgare commonly known as fennel is a well-known and important medicinal and aromatic plant widely used to treat carminative, digestive, lactagogue, diuretic, respiratory and gastrointestinal disorders.

Our results clearly showed that *Foeniculum vulgare* extracts have direct inhibition of cellular tyrosinase activity *in vitro*. The results suggest the percentage inhibition of cellular tyrosinase activity in B16 cells treated with sample was approximately 18.0- 34.2 %. The sample treated B16 cells inhibited tyrosinase less than those treated with arbutin and L-ascorbic acid.

5. Conflict of Interest

The authors hereby declare that there is no conflict of interest for the present study.

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References

[1] Chang, T.S.; Lin, M.Y.; Lin, H.J. Identifying 8-hydroxynaringenin as a suicide substrate of mushroom tyrosinase. *J. Cosmet. Sci.* 2010, *61*, 205–210.
[2] Chung SY, Seo YK, Park JM, Seo MJ, Park JK, Kim JW, Park CS Fermented rice bran downregulates MITF expression andleads to inhibition of a-MSH-induced melanogenesis in B16F1melanoma. Biosci Biotechnol Biochem 2009 73(8):1704–1710
[3] Ding, H.Y.; Chang, T.S.; Shen, H.C.; Tai, S.S.K. Murine tyrosinase inhibitors from *Cynanchumbungei* and evaluation of *in vitro* and *in vivo* depigmenting activity. *Exp. Dermatol.* 2011.,
[4] Ding, H.Y.; Lin, H.C.; Chang, T.S. Tyrosinase inhibitors isolated from the roots of *Paeonia suffruticosa*. *J. Cosmet. Sci.* 2009, *60*, 347–352.
[5] Elias PM, Menon G, Wetzel BK, Williams JW Evidence that stress to the epidermal barrier influenced the development of pigmentation in humans. Pigment Cell Melanoma Res 2009,22(4):420–434
[6] Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res 2005, 18(6):393–410
[7] Hearing, V.J. and Jimenez, M. Mammaliantyrosinase-the critical regulatory control point in melanocyte pigmentation. Int. J.Biochem. 1987 19(12), 1141–1147.
[8] Hearing, V.J. Unraveling the melanocyte.Am. J. Hum. Genet. 1993 52(1), 1–7.
[9] Ito, S. and Wakamatsu, K. Quantitativeanalysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res. 2003 16(5), 523–531.
[10] Ito, S., Fujita, K., Takahashi, H. and Jimbow,K. Characterization of melanogenesis in mouse and guinea pig hair by chemicalanalysis of melanins and of free and bound dopa and 5-S-cysteinyldopa. J. Invest. Dermatol 1984 83(1), 12–14 .
[11] Kwon, B.S., Haq, A.K., Pomerantz, S.H.and Halaban, R. Isolation and sequence of cDNA clone for human

tyrosinase that maps at the mouse c-albino locus. Proc. Natl. Acad. Sci. U S A.1987 84(21), 7473–7477.
[12] Lee YS, Park JH, Kim MH, Seo SH, Kim HJ Synthesis of tyrosinase inhibitory kojic acid derivative. Arch Pharm2006 339(3):111–114
[13] Prota, G. The role of peroxidase in melanogenesis revisited. Pigment Cell Res. Suppl. 1992 2,25–31
[14] Strothkamp, K.G., Jolley, R.L. and Mason,H.S. Quaternary structure of mushroom tyrosinase. Biochem. Biophys. Res. Commun.1976 70(2), 519–524 .