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Research Article

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Effect of *Foeniculum vulgare* on melanogenesis in B16 melanoma cells

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ABSTRACT

Over expression of tyrosinase can cause excessive production of melanin and lead to hyper pigmentation disorders, including melasma and freckles. Recently, agents obtained from plants are being used as alternative medicines to down regulate tyrosinase synthesis and decrease melanin production. Ayurveda and various traditional systems of medicines claim that foeniculum vulgare (Umbelliferacacae) is also highly recommended for diabetes, bronchitis and chronic cough, for the treatment of kidney stones, and is considered to have diuretic, stomachic and galactogogue properties. The anti melanogenesis activity and molecular biological mechanism underlying the activity of the methanolic extract of Foeniculum vulgare have not been investigated to date. This study aimed to determine the effect on melanin production and biological mechanisms underlying anti melanogenesis of methanolic extract of Foeniculum vulgare inB16 cells. Confluent monolayer of B16 cells were taken α-MSH was prepared, Reverse transcriptase PCR was carried out and the PCR product was examined using agarose gel electrophoresis. RT-PCR (Reverse Transcriptase) was carried out to study the expression of MITF and TRP-2. The results showed a significant time dependent decrease gradually, especially the decrease was observed at 72 hrs. The control showed no change with the incubation time. The result confirms that the sample down- regulated the gene expression.

Key words: MITF, microphthalmia-associated transcription factor; PKG, protein kinase G; TRP, tyrosinase-related protein; *Foeniculum vulgare*, α-MSH; α-melanocyte stimulating hormone

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INTRODUCTION

Melanin is a unique pigmented biopolymer synthesized by melanocytes, dendritic cells that exist in the dermal-epidermal border of the skin. Melanin has a number of important functions, including the determination of phenotypic appearance, protective coloration, balance and auditory processing, absorption of toxic drugs and chemicals, and neurologic development during embryogenesis [2] Melanogenesis itself is a complex process, with at least 125 distinct genes involved in the regulation of melanogenesis either directly or indirectly [11].Mutations of these genes are associated with different pigmentary diseases, including various forms of ocular syndromes.2].

The tyrosinase gene family plays a pivotal role in the regulation of melanogenesis [9]. The tyrosinase gene family consists of tyrosinase, tyrosinase-related protein 1(TRP-1), and tyrosinase-related protein 2 (TRP-2) [3]. Tyrosinase is a bi functional enzyme that modulates melanin production, first by catalyzing the hydroxylation of tyrosine to DOPA and secondly by catalyzing the oxidation of DOPA to DOPA quinone [4]. TRP-2, which functions as a DOPA chrome tautomerase, catalyzes the rearrangement of DOPA chrome to5,6-dihydroxyindole-2-carboxylic acid (DHICA) [12] and TRP-1 oxidizes DHICA to a carboxylated indole-quinone [5]. Microphthalmia-associated transcription factor (MITF) is a master regulator of melanocyte developmentand melanogenesis [6]. It is a melanocyte-specific transcription factor, regulates the transcription of three major pigmentation enzymes: tyrosinase, TRP-1, and TRP-2.

Inhibiting Tyrosinase Gene Expression via MITF

MITF is the master regulator of melanogenesis in melanocytes via binding to the M box of a promoter region and regulating the gene expression of tyrosinase, TRP-1, and TRP-2. The down-regulation of MITF activity depresses the expression of the related enzymes, thereby inhibiting melanogenesis. In recent years, many melanogenesis inhibitors, acting through the down-regulation of MITF activity, have been discovered. The mechanism linked to one of the three MITF-related pathways of melanogenesis regulation, shown in Figure 3, has been studied in detail. These inhibitors will be discussed in the following subsections. On the other hand, some studies have shown only the results of the down-regulation of the MITF protein by either a western blotting method or a reverse-transcription polymerase chain reaction (RT-PCR) method, and have not produced a clear explanation of MITF inhibition.[7]

EXPERIMENTAL SECTION

i) PROCEDURE FOR GENE EXPRESSION ANALYSIS:

1. Confluent monolayers of B16 cells were taken .
2. The cells were sub cultured and seeded at a concentration of one lakh cells /ml in a 24 well plate and incubated at 37^oC at 5 % CO2 atmosphere for 24 Hrs of time
3. 30 µg/ml of sample and 1µM α-MSH was prepared and added in to the wells and the plates were incubated for 24, 48 and 72 Hrs of time at 37^o C with 5 % CO2 atmosphere.
4. After incubation cells were harvested and RNA was isolated using standard procedure
5. Complementary DNA was synthesized using research kit (Life Teck Kit code :)
6. Reverse transcriptase PCR was carried out with the following PCR conditions
7. Complementary DNA was synthesized from 1µg of total RNA using 100ng of primer and 200ng reverse transcriptase in a 25µl solution containing 200µmol/l each of all four dNTPs , 80 U of RNAse inhibitor, 50mm /l Tris HCL(pH-8.3),75mm/l KCl, 10mm/l Dithiotheritol(DTT), 3mm/l $MgCl_2$. The reaction was allowed to proceed for 60 min at 37^0C(Sugimoto *et al*.,2014)

For the reverse transcriptase PCR 25µl of a solution containing 25 µmol/l of dNTPs , 100ng of Primer

For MITF (F- 5' – CCGTCTCTCACTGGATTGGTG 3'R-5' – CGTGAATGTGTGTTCATGCCTGG -3')
For TRP-2 (F- 5' – TACCATCTGTTGTGGCTGGA-3'R 5' – TGGGTCATCTTGTCTTGCTG -3')

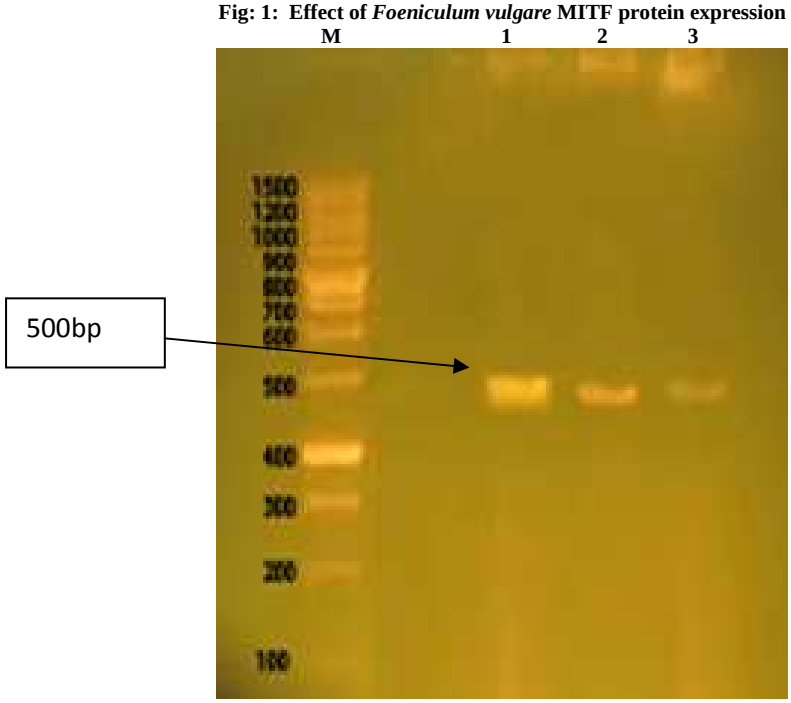
8. 10mm/l Tris HCl(pH:8.3), 50mm/l KCl and 3U of Taq Polymerase were added. PCR was performed for 25 cycles of 94^0 C for 1 min, 50^0C for 1min and 72^0C for 2min.

9. PCR product was examined using agarose gel electrophoresis.

RESULTS AND DISCUSSION

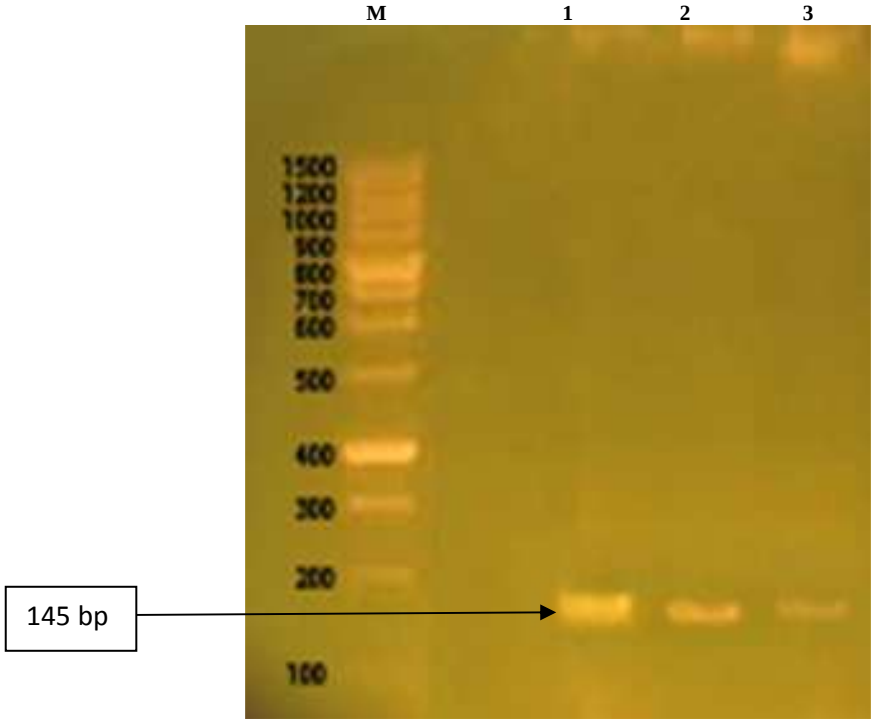
Cellular melanin content was significantly increased in cells treated with 1 µM α-MSH . α-MSH produced by keratinocytes increases adenylate cyclase activity of melanocytes through G proteins [1]. *Foeniculum vulgare* significantly inhibited the melanogenesis induced by both α-MSH (Figure 1), suggesting that *Foeniculum vulgare* regulates the expression of the tyrosinase gene family through a cAMP-dependent pathway. cAMP- mediated activation of PKA induces the expression of MITF, a master transcriptional regulator for melanogenic enzymes[6],and tyrosinase family proteins are important targets of MITF. The presence of *Foeniculum vulgare* significantly decreased the expression of MITF mRNA (Figure 2) expression, suggesting that *Foeniculum vulgare* worked by down-regulating MITF transcription.

Fig: 1: Effect of *Foeniculum vulgare* MITF protein expression



M- 100 bp DNA Ladder
Lane 1: 24 Hrs
Lane 2: 48 Hrs
Lane 3: 72 Hrs

FIG: 2: Effect of *Foeniculum vulgare* on mRNA expression of melanogenesis-related genes-TRP-2 gene



M- 100 bp DNA Ladder
Lane 1: 24 Hrs
Lane 2: 48 Hrs
Lane 3: 72 Hrs

CONCLUSION

Over the last few years, knowledge of melanocyte biology and the processes underlying melanin synthesis have made remarkable progress, opening paths in the identification of new melanogenesis inhibitors. In addition to the direct inhibition of tyrosinase catalytic activity, other approaches to melanogenesis inhibition include the acceleration of tyrosinase degradation and the inhibition of tyrosinase mRNA transcription *via* a reduction of MITF activity and regulation of TRP-2 gene expression. In addition to the roles of protecting skin from harmful solar UV radiation or toxic chemicals, melanin determines racial and phenotypic appearance. The accumulation of melanin in specific parts of the skin as more pigmented patches such as lasma, freckles, ephelides, or senile lentigines might become an aesthetic problem [10].Elucidating the molecular mechanisms underlying hyper pigmentation could lead to technology that allows unwanted pigmentation to be decreased and photo aging to be preserved, as well as the design of tanning products with the potential to reduce the risk of skin cancer. Recently, natural herbal extracts and compounds have gained attention as putative hypo-pigmenting agents [8]. In this study, we showed that *Foeniculum vulgare* could inhibit melanogenesis by inhibiting tyrosinase and related enzyme expression via down-regulating MITF expression, a key regulatory transcription factor of melanogenesis. RT-PCR (Reverse Transcriptase) was carried out to study the expression of MITF and TRP-2. The results showed a significant time dependent decrease gradually, especially the decrease was observed at 72 hrs. In contrast the control showed no change with the incubation time. The results confirm that the sample down- regulated the gene expression.

Since a huge number of melanogenesis inhibitors have been developed, the need to clarify the viability of these inhibitors in terms of their skin-whitening efficiency has become more urgent. In conclusion, more concrete studies of the identified inhibitors from a human clinical point of view are required.

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