

## Effects of xanthoxylin on melanogenesis

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### Abstract

The important role of melanin is skin protection against UV radiation from sunlight which induces DNA damage and skin cancer. The purposes of this study were to determine the effects of xanthoxylin on melanin content by measure melanin content at 405 nm, mRNA expression of regulatory melanogenesis proteins by RT-PCR and dendricity in mouse B16F10 melanoma cells by photograph the pictures under microscope. This study found that xanthoxylin increased melanin content and mRNA expression of regulatory melanogenesis proteins (tyrosinase and Mitf). In addition, xanthoxylin also increased dendrites of mouse B16F10 melanoma cells and no effect on viability of mouse B16F10 melanoma cells. It could be concluded that xanthoxylin induced melanogenesis, increased dendrites and activated tyrosinase and Mitf expressions in mouse B16F10 melanoma cells.

**Keywords:** xanthoxylin, melanogenesis, melanin, tyrosinase

### Introduction

Melanin from melanocytes in epidermis plays an important role in skin protection against UV radiation from sunlight which induces DNA damage and skin cancer [1]. In melanosomes of melanocytes, tyrosinase is a rate-limiting step enzyme that converts tyrosine to dopachrome, while tyrosinase-related proteins (TRP-2 and TRP-1) convert dopachrome to melanin [2]. Expression of these proteins is regulated by microphthalmia-associated transcription factor (MITF). Melanin-containing melanosomes are transferred from dendrites of melanocytes to keratinocytes. Melanin is an excellent photoprotectant that absorbs the harmful energy of UV radiation (UVR) and helps prevent the UVR from damaging skin cells and penetrating deeper into the tissues. Agents that increase melanin production or melanogenesis have the potential to reduce both photodamage and skin cancer incidence [3].

Xanthoxylin (2'-hydroxy-4', 6' dimethoxyacetophenone) is a common phenolic compound in Rutaceae family. Its several pharmacological activities have been reported including neurotransmitter-mediated contractions in nonvascular smooth muscles [4], antifungal [5], antispasmodic [6], antioedema [7] and inhibitor of prostaglandin synthetase and 5-lipoxygenase [8]. Many phenolic compounds have been investigated for their activities on melanogenesis. This study aimed to elucidate the effects of xanthoxylin purified from *Zanthoxylum piperitum* on melanogenesis and dendricity as well as on proteins involving melanin synthesis in mouse B16F10 melanoma cells.

### Methods

Mouse B16F10 melanoma cells (from ATCC), at the density of  $1 \times 10^4$  cells/well in melanin content assay and mRNA expression or  $1 \times 10^3$  cells/well in melanocyte dendricity assay, were treated with 6.25-25  $\mu$ M xanthoxylin for 72 hours. Untreated cells and 10 nM  $\alpha$ -

MSH-treated cells were used as the negative and positive control, respectively. The treated cells were used for the following objectives;

1. Determination of melanin content: The treated cells were undergone lysis by 2 M NaOH. Melanin in the cells was dissolved by heating at 60 °C for 5 min and its content was measured at 405 nm.

2. Determination of melanocyte dendricity: The numbers of dendrites of the treated cells were observed under a light microscope at 40X amplification.

3. Determination of mRNA expression of proteins involving in melanogenesis: Total RNA was isolated from the treated cells using TRIzol<sup>®</sup> reagent and then reversing to cDNA using Improme II<sup>™</sup> reverse transcription system reagent. The cDNA was PCR amplified with the specific primers of tyrosinase, MITF, TRP-1 and TRP-2 genes. The PCR products were run on 1.5% agarose gel electrophoresis and semiquantitative determined by a gel documentation.

All assays were performed in 3 independent experiments (n=3). The data were presented in mean  $\pm$  S.E. One-way ANOVA was used to determine the statistical analysis and *p*-value < 0.05 was considered statistically significant.

## Results

### Effect of xanthoxylin on melanogenesis

Xanthoxylin induced melanogenesis in B16F10 cells, in a concentration dependent manner (table 1 and Fig. 1). It significantly increased melanin content at the concentration of 12.5 and 25  $\mu$ g/ml without increasing in cell proliferation or cell toxicity (data not shown).

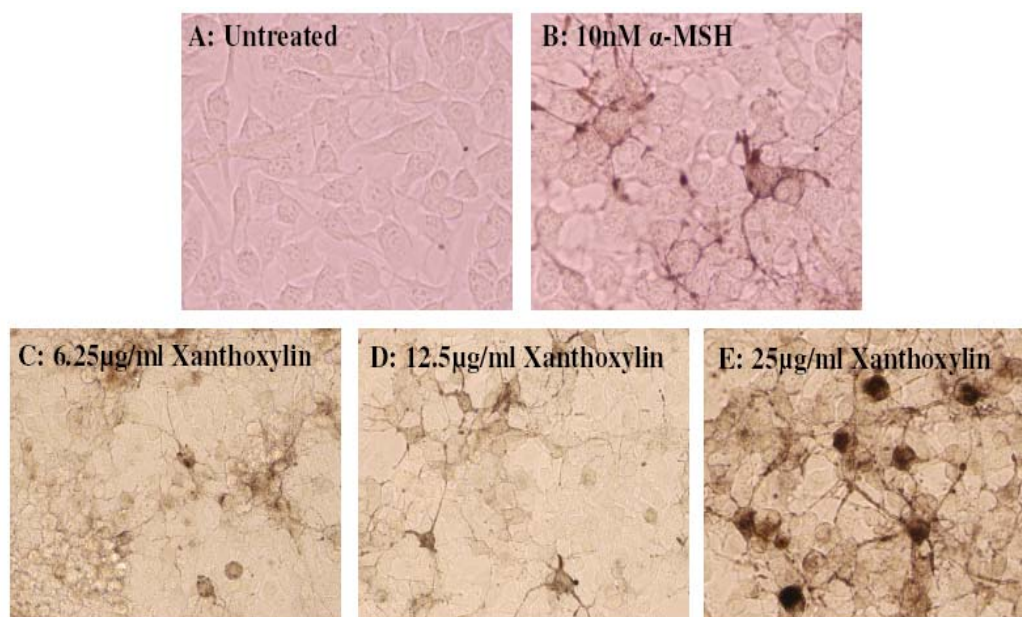
**Table 1:** Effect of xanthoxylin on melanigenesis.

Compounds	% Stimulation of melanin content
Untreated control	100 $\pm$ 0.0008
10nM $\alpha$ -MSH(positive control)	307.6 $\pm$ 0.0020*
6.25 $\mu$ g/ml Xanthoxylin	153.3 $\pm$ 0.0067
12.5 $\mu$ g/ml Xanthoxylin	268.8 $\pm$ 0.0075*
25 $\mu$ g/ml Xanthoxylin	650.7 $\pm$ 0.0056*#

B16F10 cells were treated with 6.25, 12.5, 25  $\mu$ g/ml xanthoxylin for 72 h. Ten nM  $\alpha$ -MSH was used as the positive control. Melanin content in the treated cells was determined and compared to the untreated control (n=3). \* *P*<0.01, significantly when compared to untreated control. # *P*<0.01, significantly when compared to 12.5  $\mu$ g/ml xanthoxylin.

### Effect of xanthoxylin on melanocyte dendricity

Xanthoxylin induced the increase in the number of dendrites in B16F10 cells, in a concentration-dependent manner (Fig. 1)



**Figure 1:** A representative result of the effect of xanthoxylin on melanin content and dendricity. B16F10 cells were treated with 6.25, 12.5, 25  $\mu\text{g/ml}$  xanthoxylin for 72 h. Ten nM  $\alpha\text{-MSH}$  was used as the positive control. (n=3)

#### Effect of xanthoxylin on the mRNA expression of proteins involving in melanogenesis

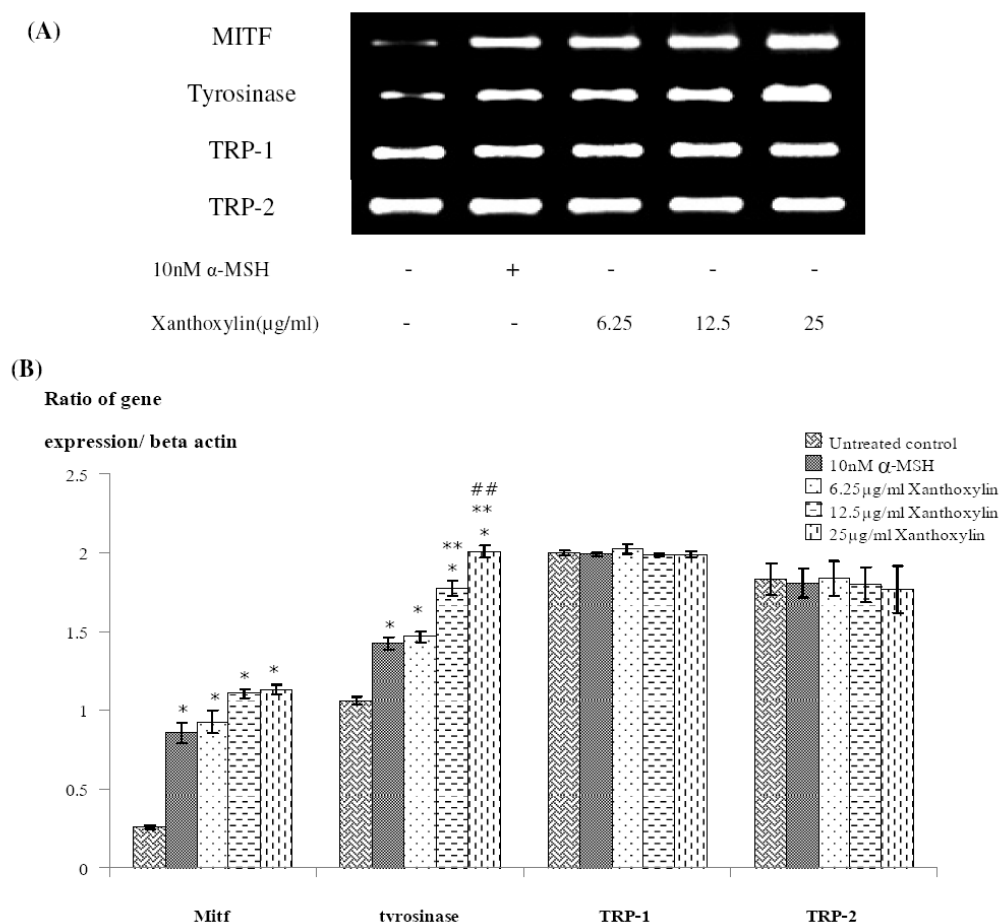
Xanthoxylin, at the concentration of 12.5 and 25  $\mu\text{g/ml}$ , significantly increased the mRNA expression of MITF and tyrosinase when compared to the untreated control (Fig. 2). It didn't induce TRP-1 and TRP-2 expression.

#### Conclusion

The results from this study suggest that xanthoxylin induced melanogenesis by activating the mRNA expression of the transcription factor, MITF, and tyrosinase which are essential for melanin synthesis in melanocytes. It also increased melanocyte dendricity which is important for transferring melanin-containing melanosomes to keratinocytes. The mechanism of xanthoxylin-induced melanogenesis are ongoing investigated.

#### Discussion

It is known that darkly pigmented skin has higher protection against skin cancer than fair skin. Enhancer of skin pigmentation is one of main mechanism to reduce both photodamage and skin cancer incidence. However, some enhancers such as ultraviolet radiation (UVR) and psoralen are associated DNA damage and skin cancer development. Any pigmentation enhancers without DNA damage are still needed. In this study, we demonstrated that xanthoxylin induced melanogenesis and dendricity. It didn't have neither proliferative nor cytotoxic activities. This agent may potentially be an enhancer of skin pigmentation.



**Figure 2:** Effect of xanthoxylin on the mRNA expression of proteins in melanogenesis. B16F10 cells were treated with 6.25, 12.5, 25 μg/ml xanthoxylin for 72 h. Ten nM α-MSH was used as the positive control. Total RNA was isolated from the treated cells and reversing to cDNA. cDNA was amplified by PCR using specific primers for MITF, tyrosinase, TRP-1 and TRP-2 genes (n=3). \* P<0.05, significantly when compared to untreated control. \*\* P<0.05, significantly when compared to 6.25 μg/ml xanthoxylin. ## P<0.05, significantly when compared to 12.5 μg/ml xanthoxylin.

### Acknowledgements

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