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# Phorbol 12-Myristate 13-Acetate Induced Toxicity Study and the Role of Tangeretin in Abrogating HIF-1 $\alpha$ -NF- $\kappa$ B Crosstalk In Vitro and In Vivo

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**Abstract:** Phorbol 12-myristate 13-acetate (PMA) is a potent tumor promoter and highly inflammatory in nature. Here, we investigated the toxic effects of PMA on different model system. PMA (10  $\mu$ g) caused chromosomal aberrations on the *Allium cepa* root tip and induced mitotic dysfunction. Similarly, PMA caused embryonic and larval deformities and a plummeted survivability rate on zebrafish embryo in a dose-dependent manner. Persistently, PMA treatment on immortalized human keratinocyte human keratinocyte (HaCaT) cells caused massive inflammatory rush at 4 h and a drop in cell survivability at 24 h. Concomitantly, we replicated a cutaneous inflammation similar to human psoriasis induced by PMA. Herein, we used tangeretin (TAN), as an antagonist to counteract the inflammatory response. Results from an in vivo experiment indicated that TAN (10 and 30 mg/kg) significantly inhibited PMA stimulated epidermal hyperplasia and intra-epidermal neutrophilic abscesses. In addition, its treatment effectively neutralized PMA induced elevated reactive oxygen species (ROS) generation on in vitro and in vivo systems, promoting antioxidant response. The association of hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ )-nuclear factor kappa-light-chain-enhancer of activated b cells (NF- $\kappa$ B) crosstalk triggered by PMA enhanced PKC $\alpha$ -ERK1/2-NF- $\kappa$ B pathway; its activation was also significantly counteracted after TAN treatment. Conclusively, we demonstrated TAN inhibited the nuclear translocation of HIF-1 $\alpha$  and NF- $\kappa$ B p65. Collectively, TAN treatment ameliorated PMA incited malignant inflammatory response by remodeling the cutaneous microenvironment.

**Keywords:** phorbol 12-myristate 13-acetate (PMA); *allium cepa* test; zebrafish embryotoxicity test; inflammation; tangeretin (TAN); nuclear factor kappa-light-chain-enhancer of activated b cells (NF- $\kappa$ B); hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ )

## 1. Introduction

Tumor promoters and inflammation inducers such as phorbol esters are naturally occurring compounds referred to as triterpene [1], extracted from numerous plants such as *Jathropa curcas*,