THERAPEUTIC EFFECT OF COMPLEX GEL CONTAINING HUMULENE IN $PM_{2.5}$-INDUCED SKIN INJURY- A RAT STUDY

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Abstract - $PM_{2.5}$ absorbs toxic substances and easily enters the human circulation through the respiratory system. In addition to affecting the respiratory system, exposure to PM2.5 causes skin acnes and inflammation-related damage to skin. Humulene, a sesquiterpene, exhibit anti-inflammatory properties by reducing inflammatory cytokine levels and pro-inflammatory protein expression. In this study, we prepared a complex gel containing humulene, hydroxypropyl methyl group (HPMC), Sanxianjiao (XG) and hyaluronic acid (HA) to treat $PM_{2.5}$-induced skin injury. The results indicated that the IC50 of $PM_{2.5}$ was approximately 600 μg/mL. The concentration of $PM_{2.5}$ larger than 400 μg/mL significantly induced the inflammatory response (increased IL-6 inflammatory levels). The IC50 of humulene was approximately 0.075 mM. Severe impairment in the epidermis and hair follicles and moderate impairment in the dermis were found exposed with $PM_{2.5}$ and continuous subcutaneous injection of $PM_{2.5}$. Treatment with complex gel containing 0.375 or 0.75 mM humulene repaired the skin injury in epidermis and dermis, restored injured hair follicles. The gel containing 0.75 mM humulene indicate superior repair effect in PM2.5-injured skin. The findings of the current study indicate the therapeutic effects of humulene against various injures caused by $PM_{2.5}$ in the epidermis and dermis of skin.

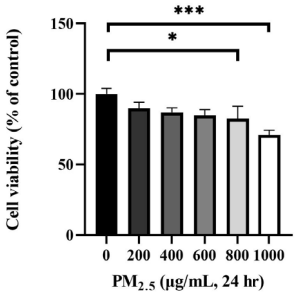
Keywords - anti-inflammatory, humulene, hydroxypropyl methyl group, $PM_{2.5}$

I. INTRODUCTION

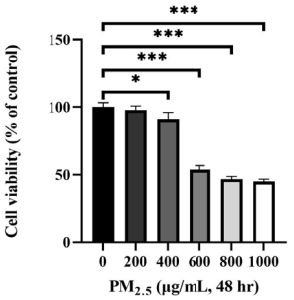
Long-term exposure to an environment containing a large amount of $PM_{2.5}$ can easily cause skin problems such as acne and inflammation. Due to the small size of $PM_{2.5}$, it is easy to invade the skin and cause aging, inflammation, and acne. Humulene is a natural monocyclic alkene, showing a colorless oily substance, with anti-inflammatory and inhibiting TNF-α and IL-1β effects. Gels are often used as drug release materials. HPMC is a semi-synthetic viscoelastic polymer, has good film-forming properties, surface activity, and special gelation properties. Hyaluronic acid (HA), which has the functions of improving dryness, promoting metabolism and nutrient absorption. The present study investigated the deleterious effects of $PM_{2.5}$ on the skin and examined the anti-inflammatory and repair effects of humulene on various skin injuries caused by $PM_{2.5}$.

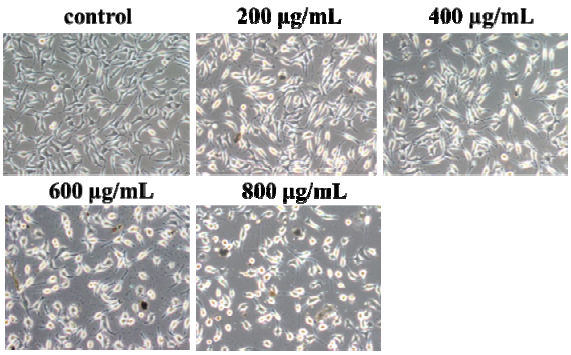
II. RESULTS

(a)

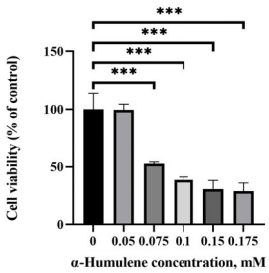


(b)





(c)



(d)

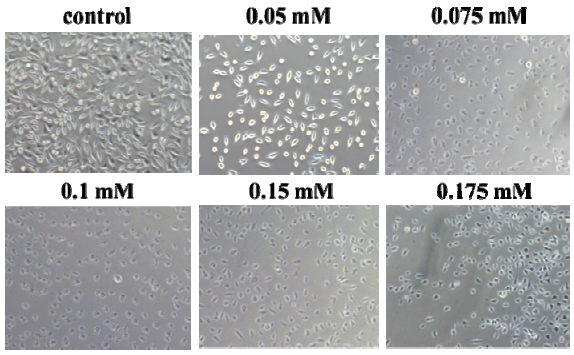


Fig. 1 (a) Cell viability assay and (b) morphology of L929 fibroblasts after treated with various concentrations of $PM_{2.5}$. (c) Cell viability assay and (d) morphology of L929 fibroblasts after treated with various concentrations of humulene. *p<0.05, ***p<0.001 compared with control group.

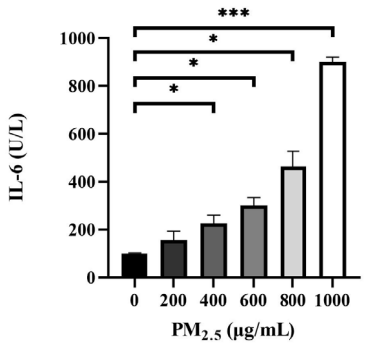


Fig. 2 IL-6 Inflammatory response of L929 fibroblasts after treated with various concentrations of $PM_{2.5}$. *p<0.05, ***p<0.001 compared with $PM_{2.5}$ 0 μg/mL.

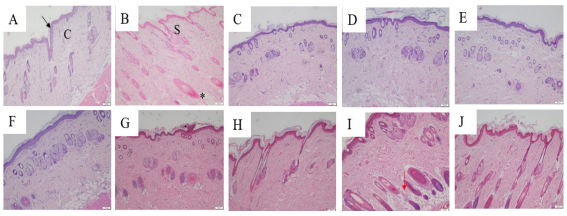
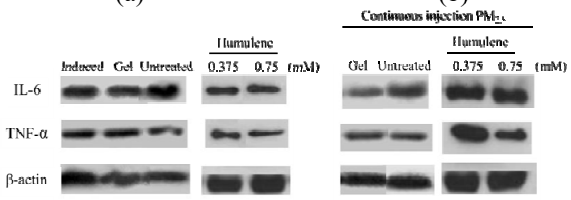
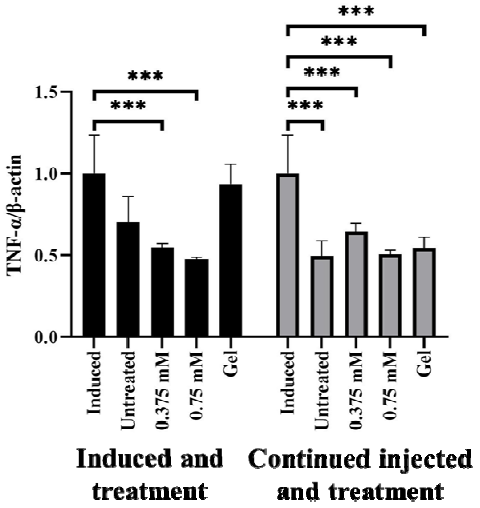


Fig. 3 H&E stains for (A) Normal group (B) Induced group (C) beauty lotion (D) P + beauty lotion (E) Untreated (F) P + Untreated (G) Humulene 0.375 mM (H) P + Humulene 0.375 mM (I) Humulene 0.75 mM (J) P + humulene 0.75 mM. P: Treatment and continue induced to cast $PM_{2.5}$. The stains showed that the epidermis and dermis were impaired, and the hair follicle was injured after exposure to the $PM_{2.5}$. After treatment with complex gel containing humulene, the skin repaired, and the hair follicle also restored. The wound healing in the epidermis and dermis in the rats was summarized in Table 1. Black arrow: epidermis; C: collagenous fiber; S: sebaceous gland; *: inflammatory cells.

(a) (b)



(c)



(d)

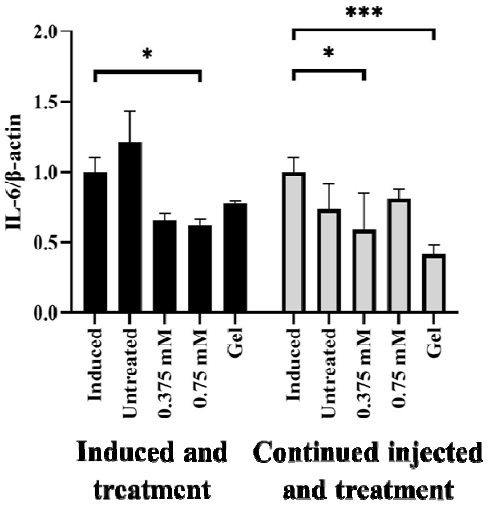


Fig. 4 Western blot analysis of inflammation response on (a) $PM_{2.5}$ induced inflammation and humulene treatment (b) continuous $PM_{2.5}$ induced and humulene treatment. Semiquantitative assay (c) of TNF-α and (d) IL-6 from Western blot by using ImageJ software. Gel: pure HPMC/XG/HA gel, Humulene: HPMC/XG/HA gel containing 0.375 mM or 0.75mM humulene. The results indicated that the complex gel containing humulene could significantly ameliorate the inflammatory response caused by $PM_{2.5}$ as compared to the induced group.

III. SUMMARY

In the current study, we observed that $PM_{2.5}$ induced the production of IL-6 and decreased the viability of skin fibroblasts in vitro. Subcutaneous injection of $PM_{2.5}$ caused severe impairment of skin and hair follicles. Histopathological data showed that treatment with complex gel containing 0.75 mM humulene exerted the superior reparative, regenerative, and anti-inflammatory effects on epidermis, dermis, and hair follicles of rat skin. However, the significant hair-follicle-restorative exerted by complex gel containing humulene was perhaps potential application of the humulene and will be detail examined in future.

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