Honey Inhibits Elevated Levels of Matrix Metalloproteinase-9 (MMP-9) *in vitro* and *in vivo*: a New Strategy for Reduction of Wound Inflammation ?

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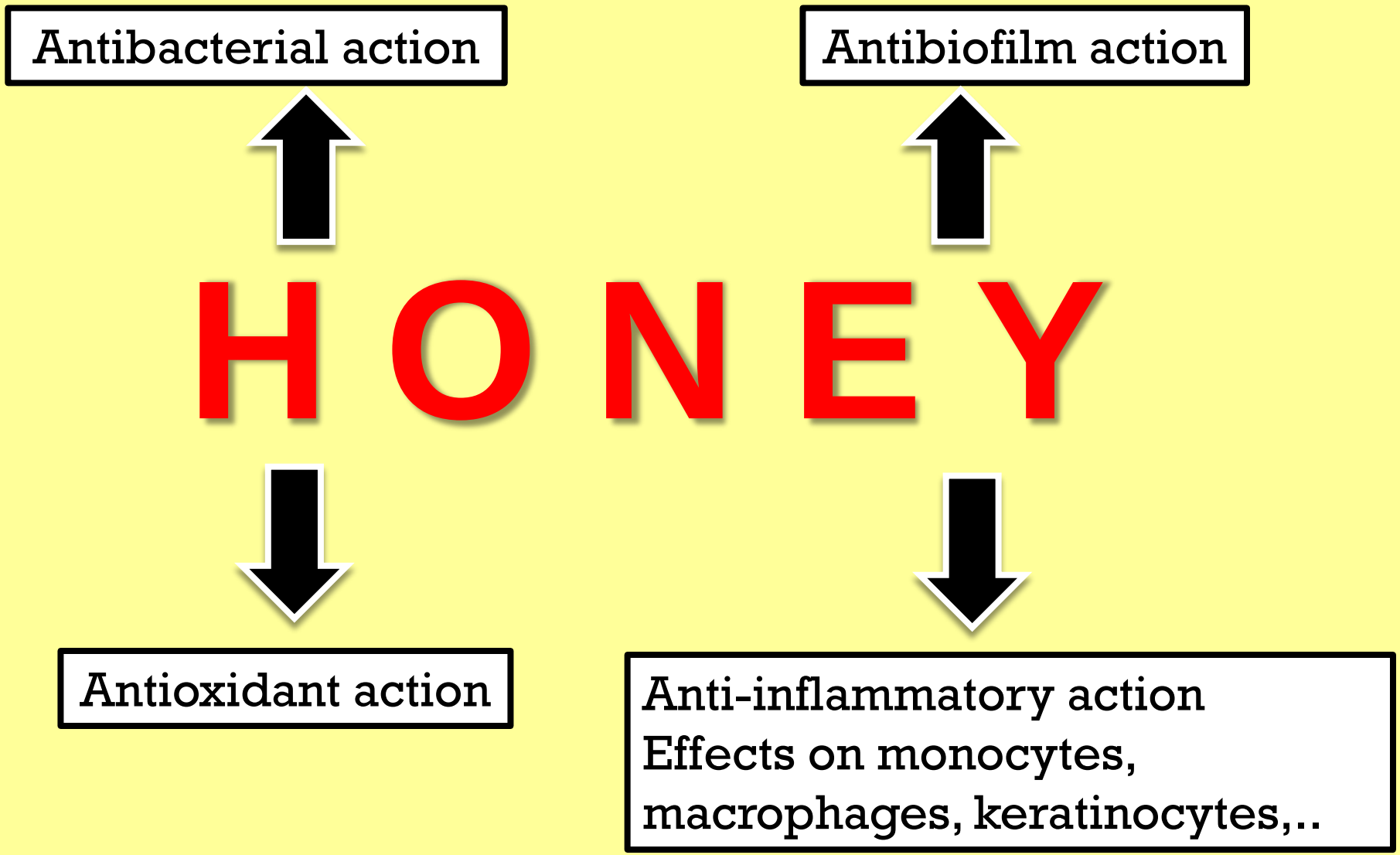
Honey – a natural remedy

- treatment of chronic wounds, burns and secondary skin infections
- treatment of eye infections
- treatment of stomach ulcers
- increases overall body's immunity and suppresses inflammation

Honey in wound healing



Infected chronic wounds

- **expensive treatment:** per episode is € 6, 650 for leg ulcers and € 10, 000 for foot ulcers (Gottrup et al, 2010)

- **prolonged uncontrolled inflammation**

- **↑ bacteria count**
- **↑ pro-inflammatory cytokines (TNF-α,...**
- **↑ matrix metalloproteinases (MMP-9,...**

MMPs in chronic wounds

MMPs

- a dominant protease group in chronic wounds
- a large family of zinc-dependent endopeptidases capable of degrading extracellular matrix (ECM) components
- MMP-9 – a major protease degradating of matrix and growth-promoting agents in wound fluid.

Treatment options for chronic wounds

- reduction of bacterial burden in wounds

- reduction of elevated levels of pro-inflammatory cytokines

- reduction of MMP-9 protease

Aim of the study

Investigation:

- the effects of honey on reduction of TNF-α-induced MMP-9 production from human keratinocytes (HaCaT cells)
- antiprotease (MMP-9) anti-inflammatory capacity of honey *in vivo* in patient with infected post-operative surgical wound

Honeydew honey

- honeydew honey produced in Cergov mountains (*Abies alba* Mill) has pronounced antibacterial activity
- it is more effective than manuka honey UMF 15+
- kills multidrug-resistant bacteria such as *Stenotrophomonas maltophilia* and wound pathogens

Majtan J, Majtanova L, Bohova J and Majtan V. (2011) Honeydew honey as a potent antibacterial agent in eradication of multi-drug resistant *Stenotrophomonas maltophilia* isolates from cancer patients. *Phytotherapy research* 25: 584-587

Honeydew honey

- honeydew honey inhibits biofilm formation and disrupts established biofilm of wound pathogens

- contains a high level of polyphenols

- without or extremely few pollen proteins – less risk of allergy

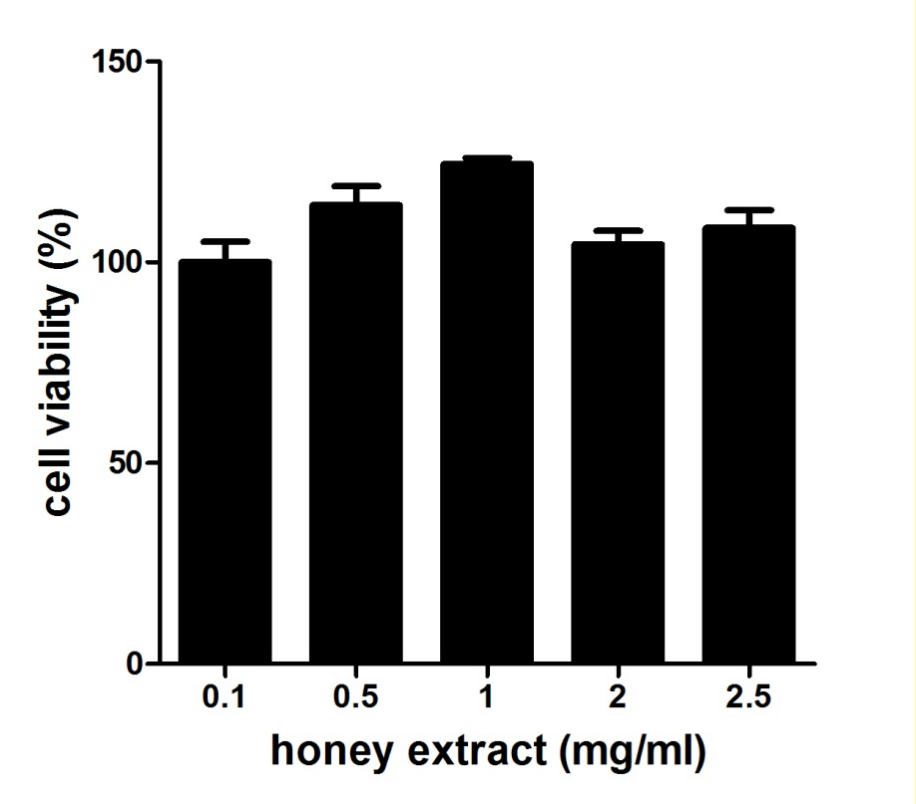
Honeydew honey extract

Honeydew honey was dissolved in distilled water (adjusted to pH 2) until completely fluid.

This solution was applied onto an activated Sep-Pack C18 cartridge. The bound compounds were eluted with 80% (v/v) methanol, lyophilisated and re-dissolved in distilled water.

RESULTS

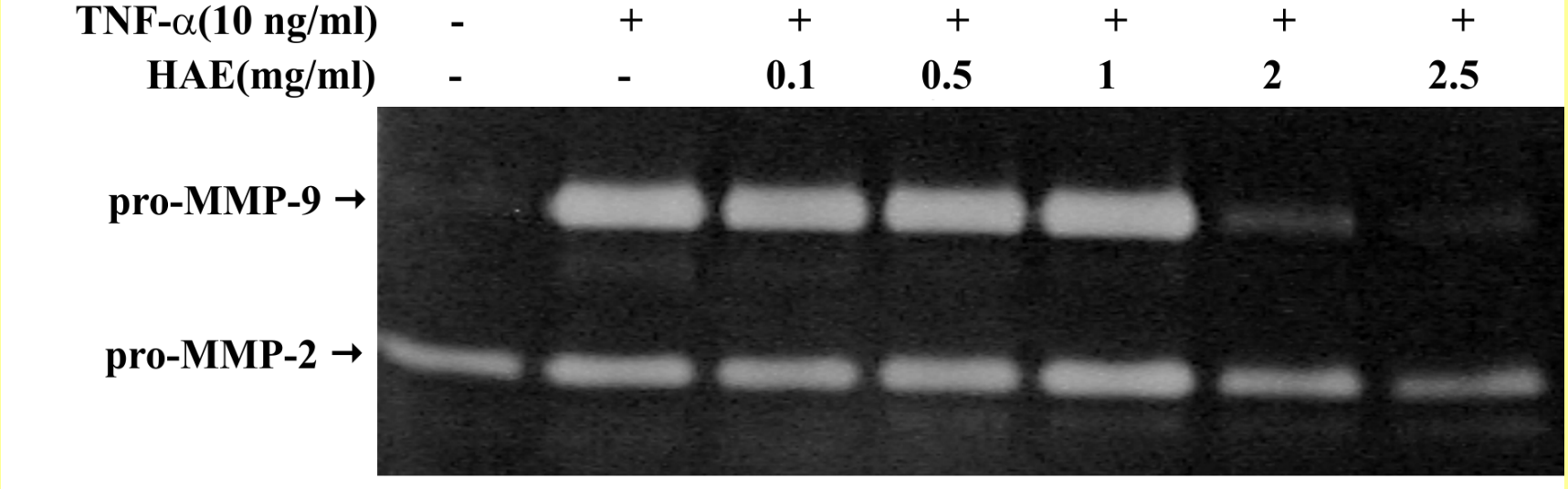
Toxicity of honey extract to human keratinocytes (HaCaT)



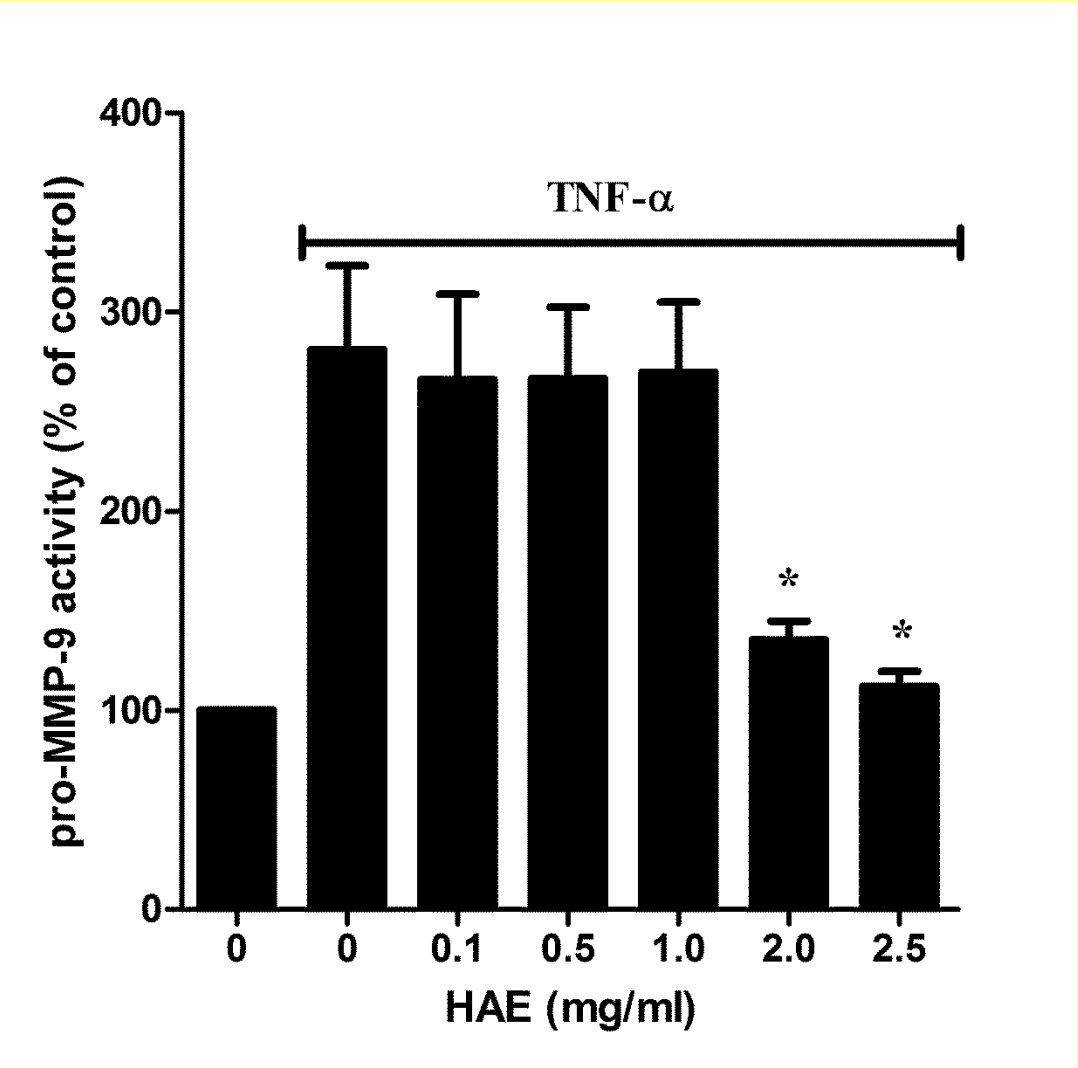
Cytotoxicity – Alamar Blue Assay
3 days incubation

Honey attenuates TNF-α-induced production of MMP-9 in HaCaT cells

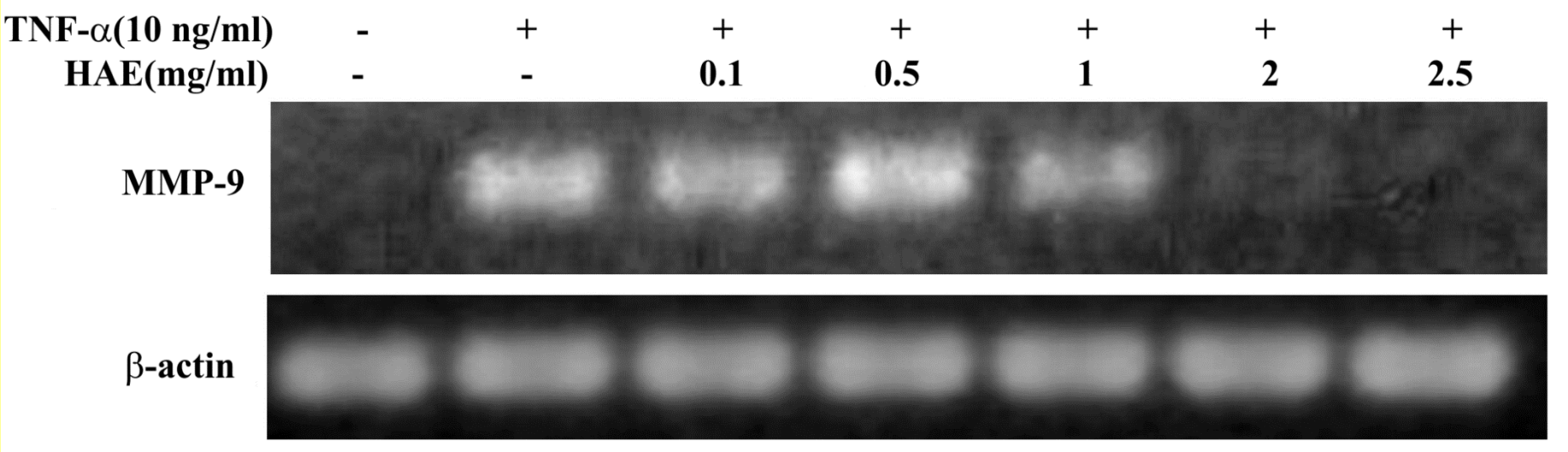
24 h pre-treatment with honey extract
24 h treatment with TNF-α (10 ng/ml)



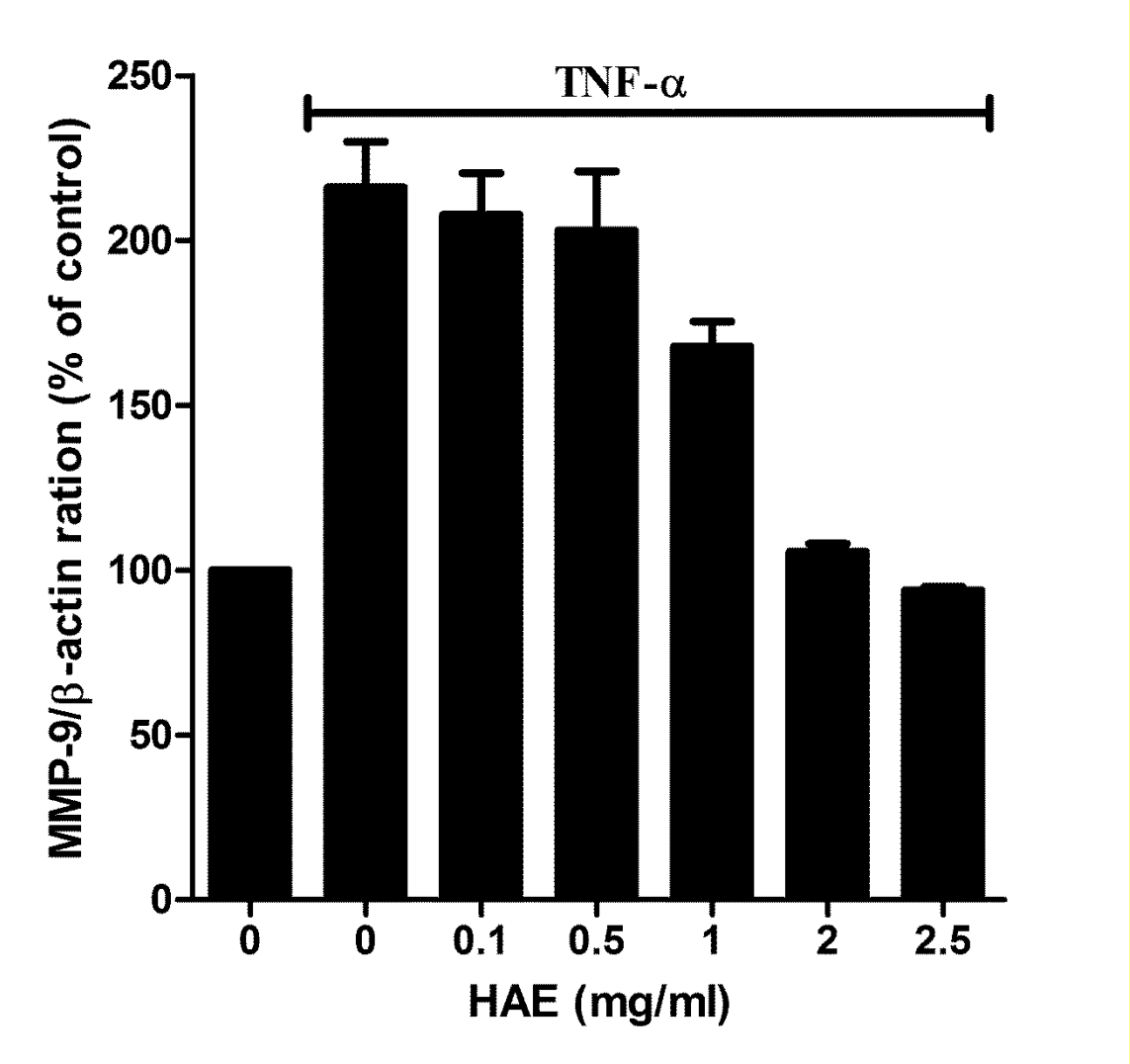
Densitometric analysis of zymography gels



The effect of honey on TNF-α-induced MMP-9 mRNA expression

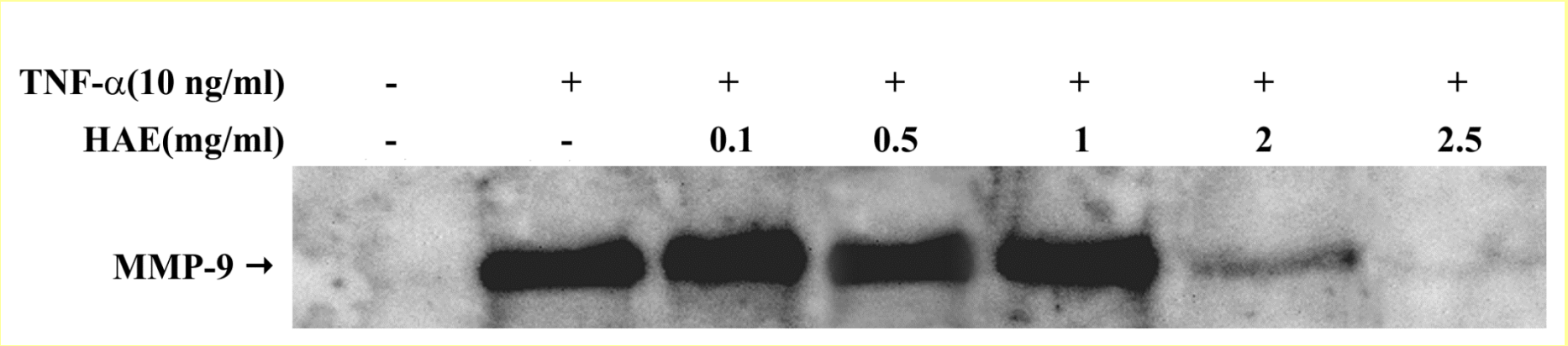


Densitometric analysis of agarose gels

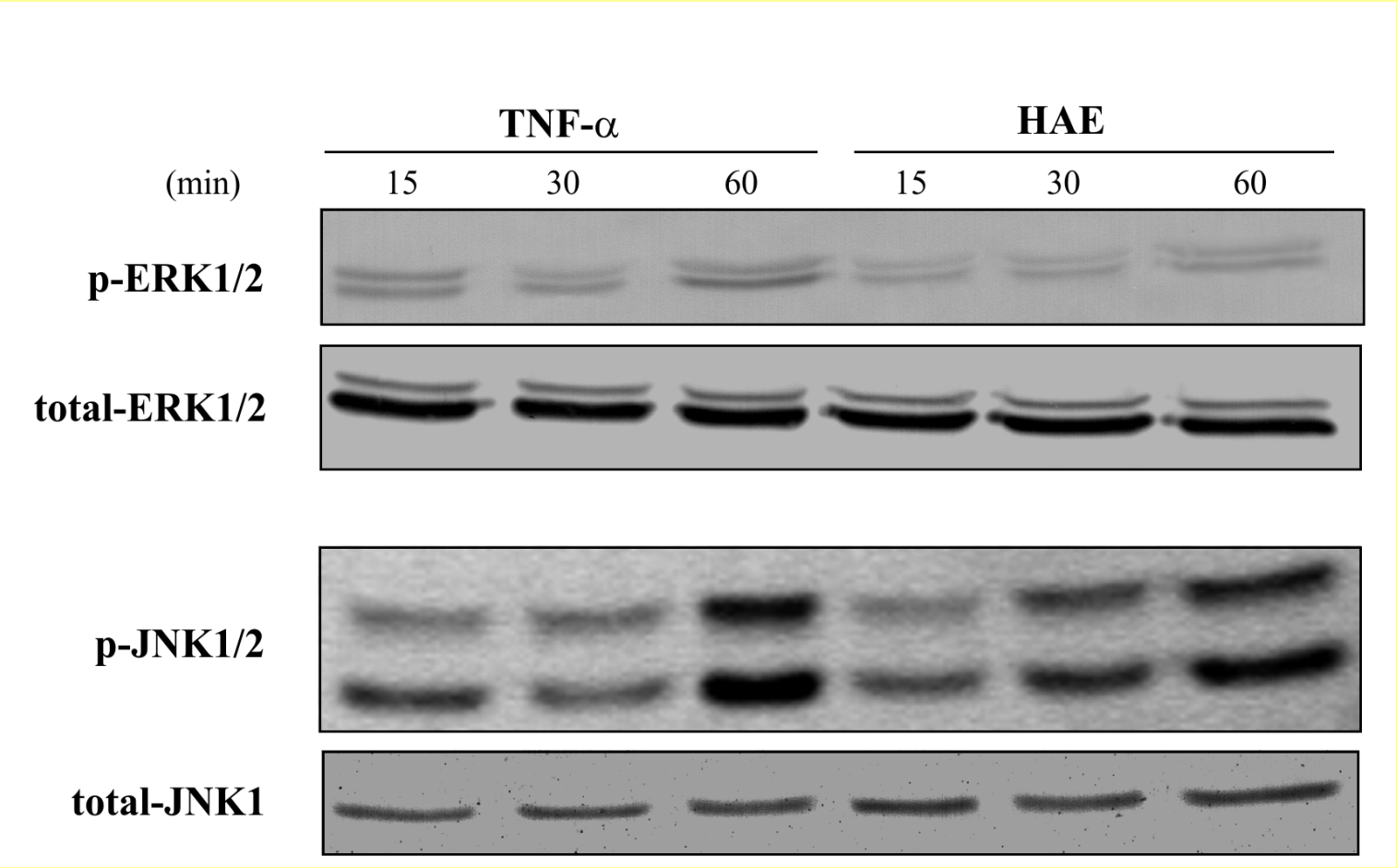


Western blot analysis

- polyclonal antibody against human MMP-9
- detection system based on chemiluminescent substrat

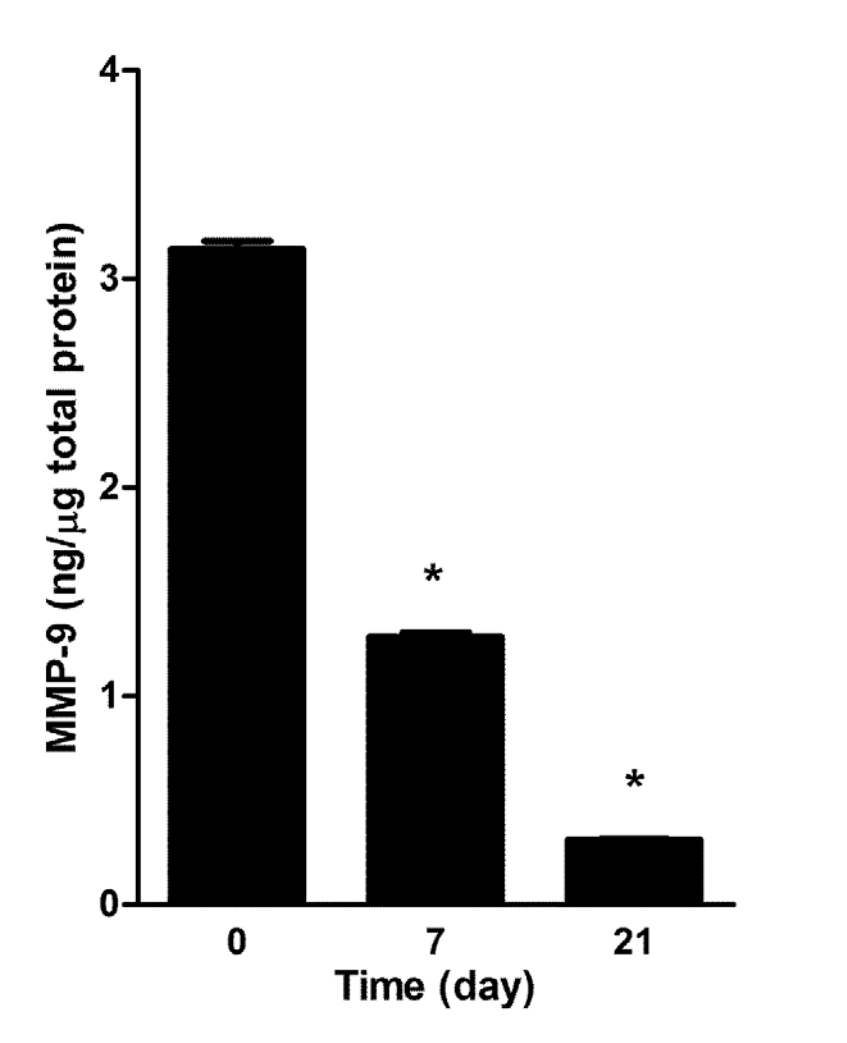


Involvement of JNK and ERK kinases



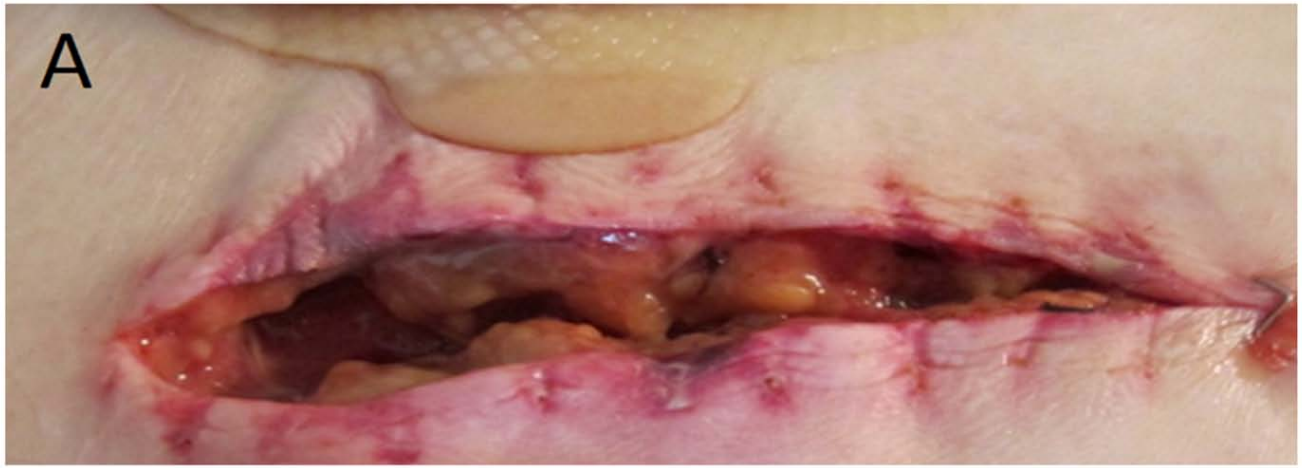
Is honeydew honey effective in vivo?

Honey reduces MMP-9 in wounds



-using ELISA kit against MMP-9
- 3 intervals: 0, 7 and 21 days

Tretament of infected wound with honeydew honey

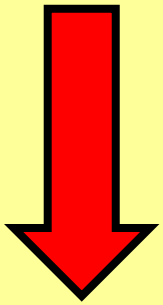


Before

After

Phenolic compounds?

<u>Flavonoids:</u> kaempferol, naringenin and quercetin



dark honeys vs. light honeys

Conclusions

- Honeydew honey extract effective inhibits the production of TNF-α-induced MMP-9 from human keratinocytes

- Honeydew honey reduces the elevated levels of MMP-9 in infected wounds

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