



UNIVERSITÀ DEGLI STUDI DI MILANO  
FACOLTÀ DI AGRARIA



## **Studio *in vitro* per valutare il ruolo di antociani e metaboliti nella modulazione del processo infiammatorio ed aterogenico**

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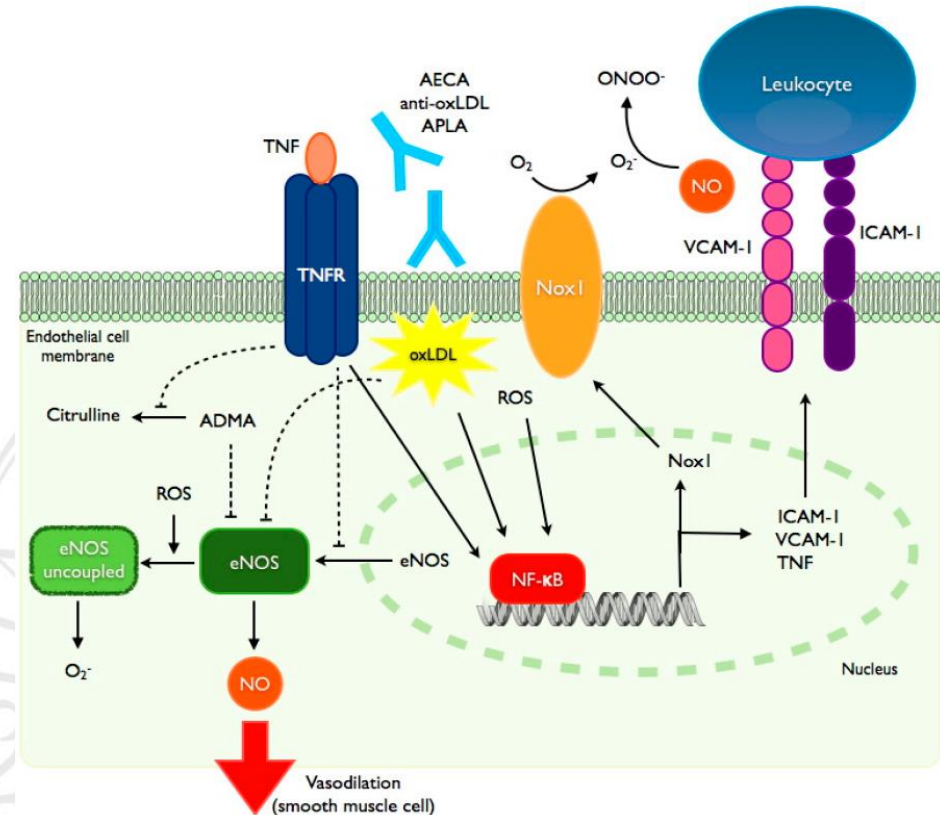
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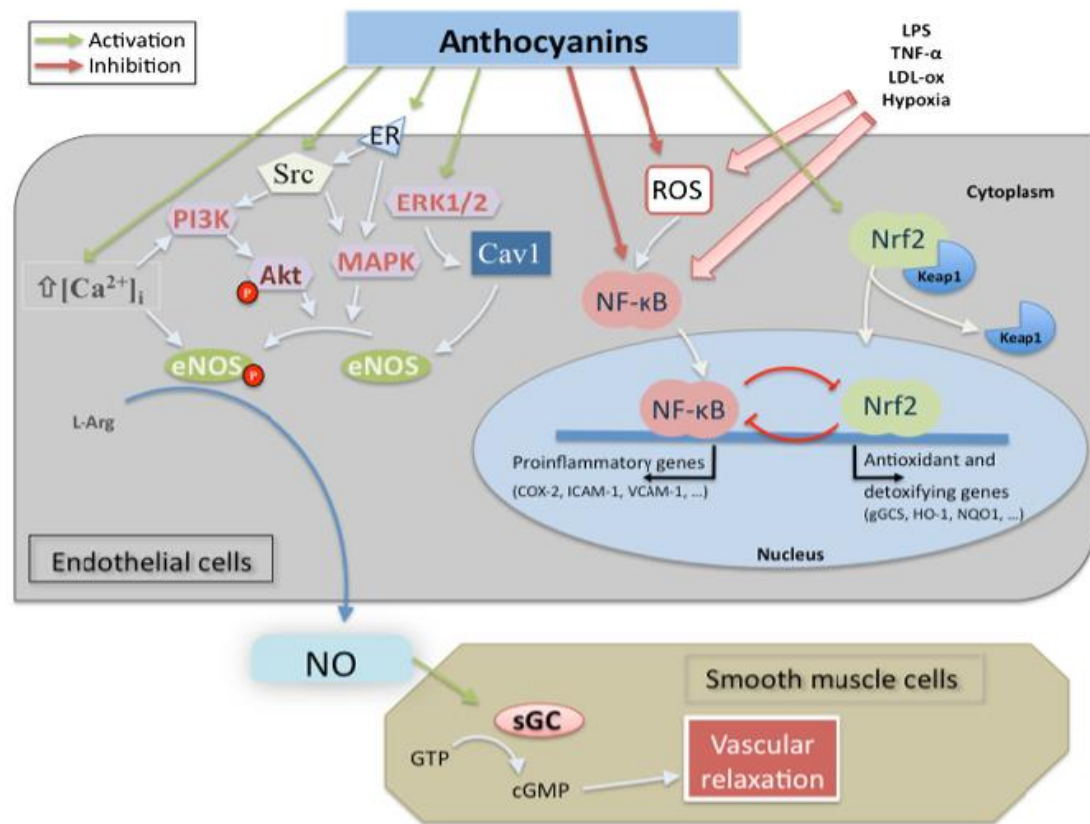
# BACKGROUND

- Inflammation plays an important role in the aetiology and pathogenesis of atherosclerosis and plaque formation.
- Tumour necrosis factor-alpha (TNF- $\alpha$ ) is a mediator of systemic inflammation and implicated in the pathogenesis of atherogenesis/atherosclerosis.
- TNF- $\alpha$  induces the transcription factor NF- $\kappa$ B leading to enhanced expression of intercellular adhesion molecules, vascular endothelial and fibroblast growth factors, chemokines that promote the recruitment and the adhesion of monocytes to inflamed luminal endothelium triggering the atherogenic process.

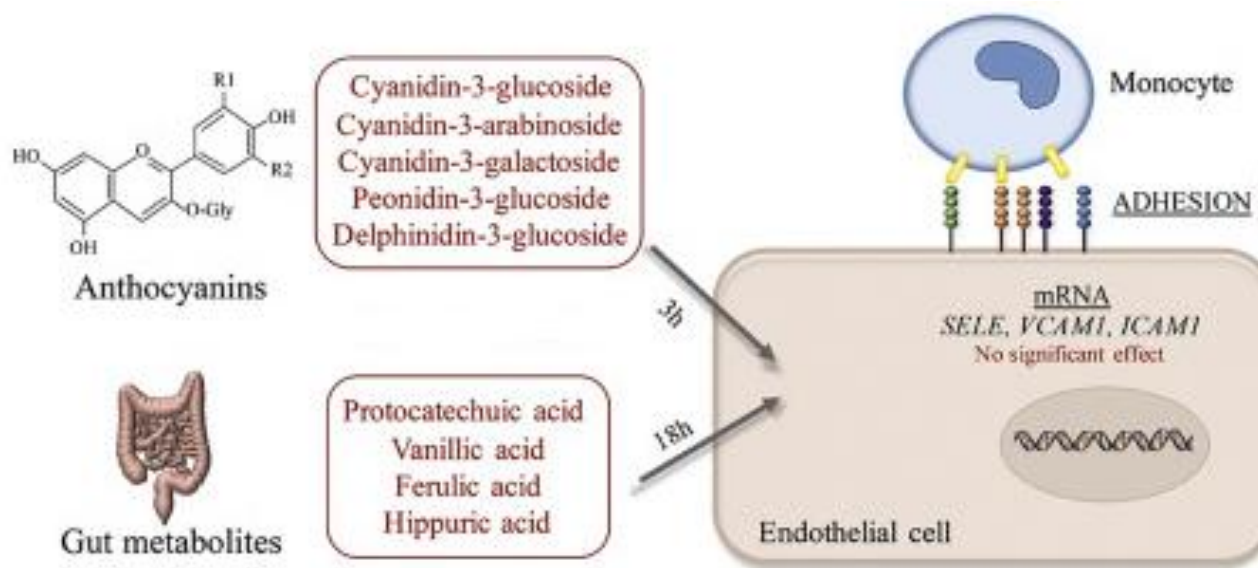


Steyers et al., 2014; Int. J. Mol. Sci. 15:11324-49.

- Anthocyanins (ACNs) are a group of abundant and widely consumed flavonoids providing the red, blue, violet colors at many fruit- and vegetable-based food products.
- ACNs may prevent endothelial cells dysfunction thanks to their capacity to modulate the expression and activity of several enzymes involved in nitric oxide (NO) metabolism by influencing NO levels.
- Furthermore, ACNs can down-regulate the expression of adhesion molecules and prevent the adhesion of monocytes to endothelial cells challenged by pro-inflammatory agents.



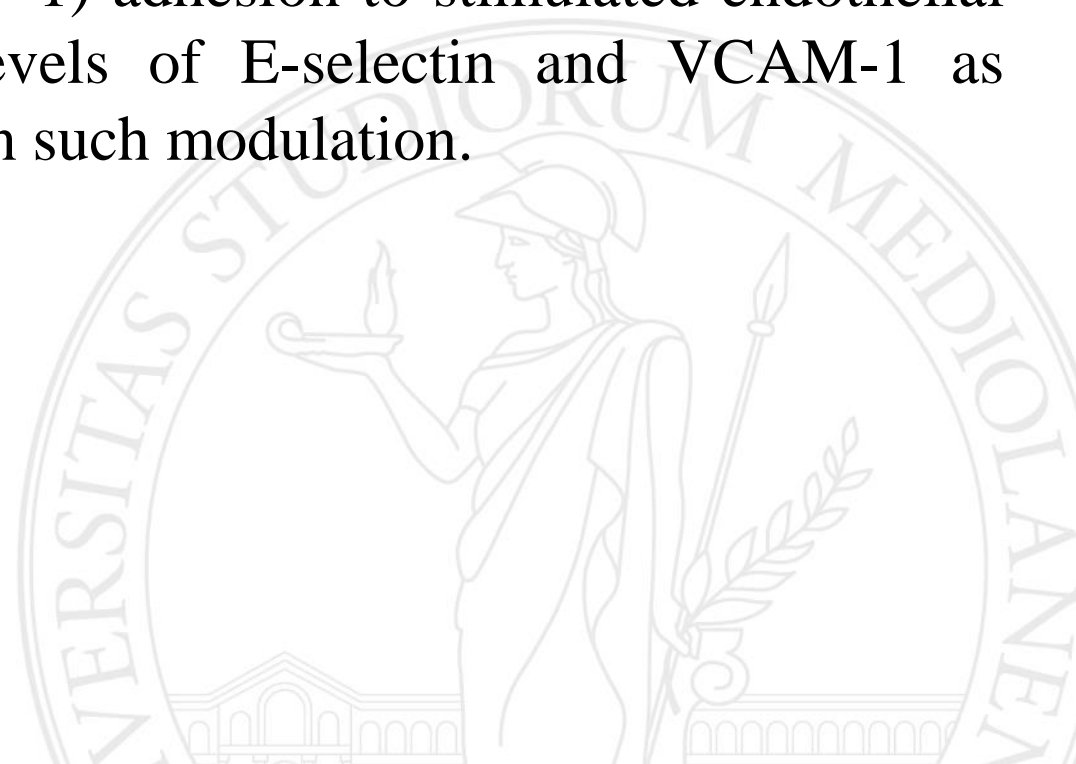
- The link between atherogenesis and health promoting effect of ACNs has not been deeply clarified and some studies reported a different effect depending on the compound tested.
- Most of ACNs are rapidly transformed by human gut in metabolic products, reaching a plasmatic concentration much higher than that of parental ACNs, and their contribution in the biological activity observed should be considered.



Karga et al., 2016 Arch. Biochem. Biophys. 599:51-9.

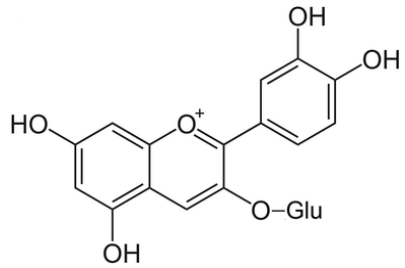
# AIM OF THE STUDY

To investigate the capacity of anthocyanins and related metabolites to counteract inflammation and atherogenic process through the evaluation of monocytes (THP-1) adhesion to stimulated endothelial cells (HUVECs), and the levels of E-selectin and VCAM-1 as potential molecules involved in such modulation.

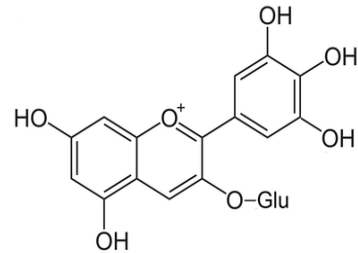




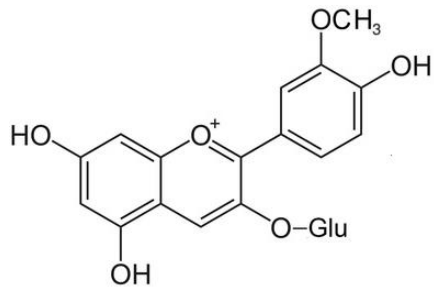
# ACNs



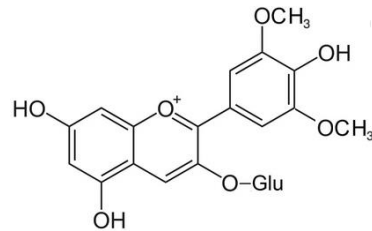
**Cyanidin 3-glucoside (Cy 3-glc)**



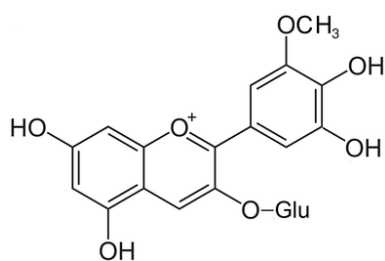
**Delphinidin 3-glucoside (Dp 3-glc)**



**Peonidin 3-glucoside (Peo 3-glc)**

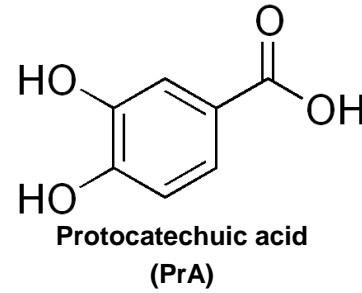


**Malvidin 3-glucoside (Mv 3-glc)**

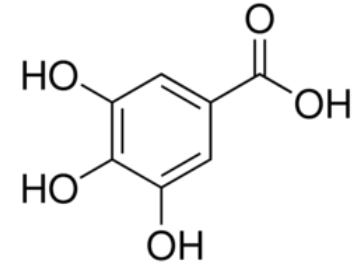


**Petunidin 3-glucoside (Pet 3-glc)**

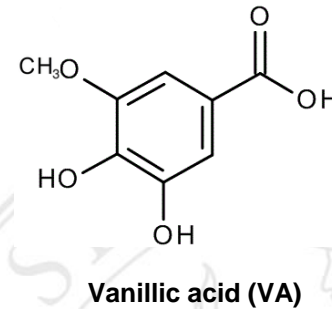
# METABOLITES



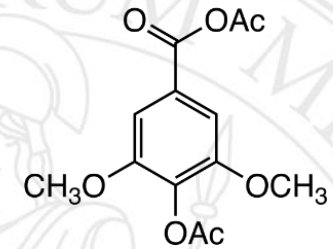
**Protocatechuic acid (PrA)**



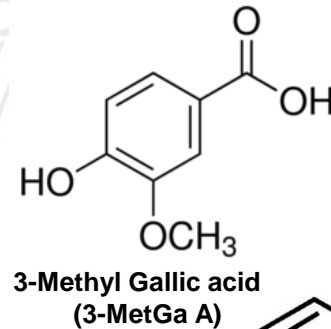
**Gallic acid (Ga)**



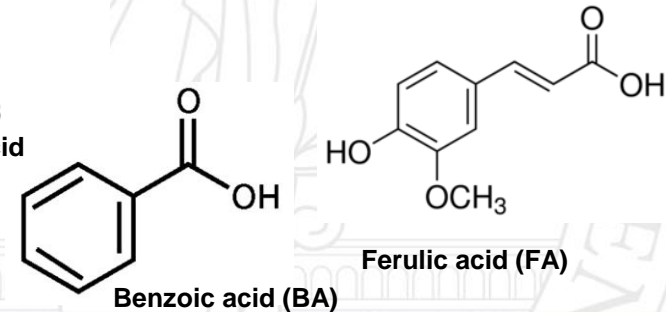
**Vanillic acid (VA)**



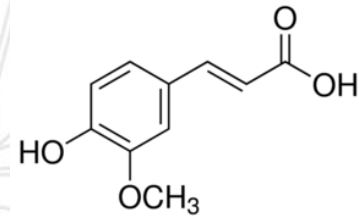
**Syringic acid (SA)**



**3-Methyl Gallic acid (3-MetGa A)**



**Benzoic acid (BA)**



**Ferulic acid (FA)**



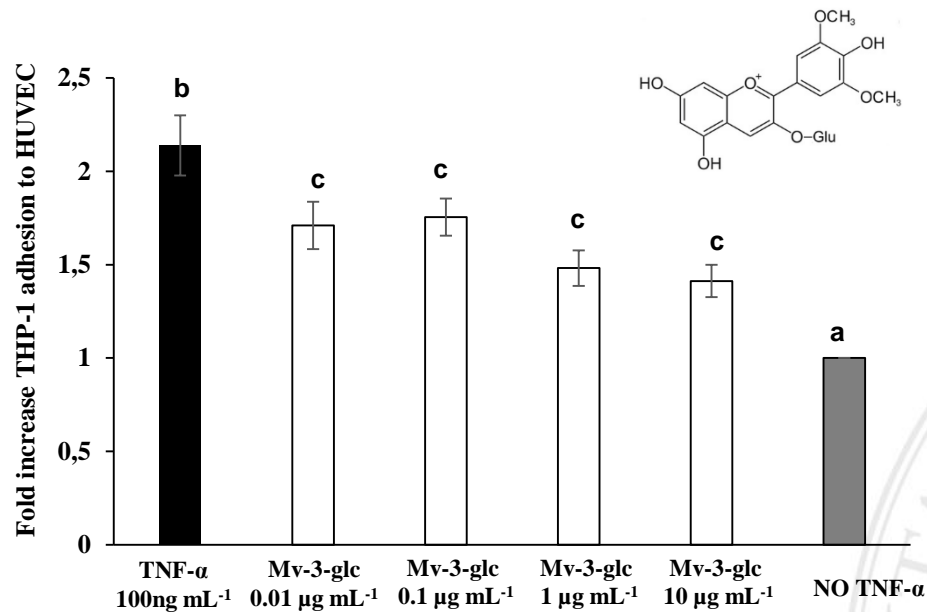
## METHODS: PROTOCOL TO STUDY THE ANTI-INFLAMMATORY AND ANTIATHEROGENIC ACTIVITY OF ACNs AND METABOLITES

- Day 1-Preparation of 96 wells plate ( $2 \times 10^4$  HUVEC per well);
- Day 2- Labelling of THP-1 cells with CellTracker™ Green CMFDA, addition of THP-1 ( $2 \times 10^5$  cells/well) and TNF- $\alpha$  ( $100 \text{ ng mL}^{-1}$ ) to HUVEC, and incubation for 24h;
- Day 3-Incubation with ACNs or meabolites at different concentrations (from 0.01 till  $10 \mu\text{g mL}^{-1}$ );
- Day 4- Reading of the fluorecence (excitation: 485 nm, emission: 538 nm, mod. F200 Infinite, TECAN Milan, Italy)

Supernatants were collected for the evaluation of markers of inflammation and vascular function (e.g. E-selectin, VCAM-1, VEGF) by ELISA kits

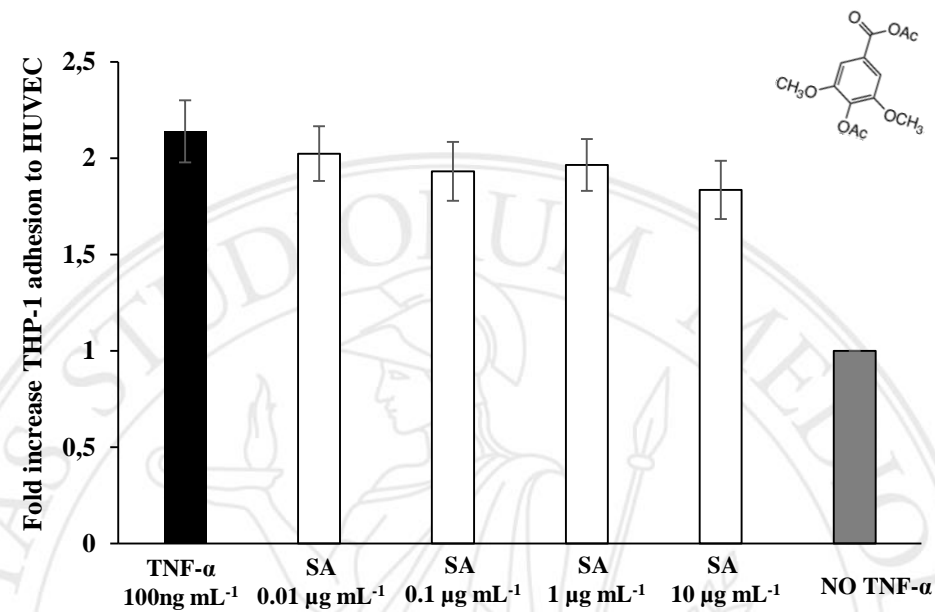
# RESULTS: Effect of Mv-3-glc and Syringic acid on THP-1 adhesion to HUVECs

## Malvidin-3-glucoside



Significant reduction of THP-1 adhesion to HUVEC at all concentrations tested with respect to the control treatment with TNF- $\alpha$ . Maximum reduction at 10  $\mu$ g/mL<sup>-1</sup> (-33.9%;  $p < 0.001$ ).

## Syringic acid



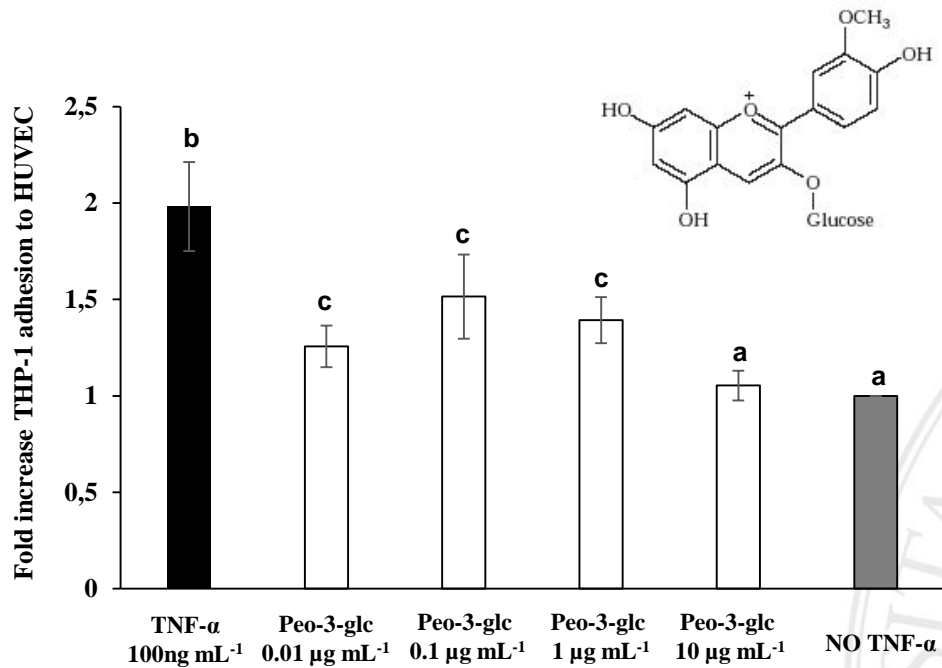
No significant effect after SA supplementation



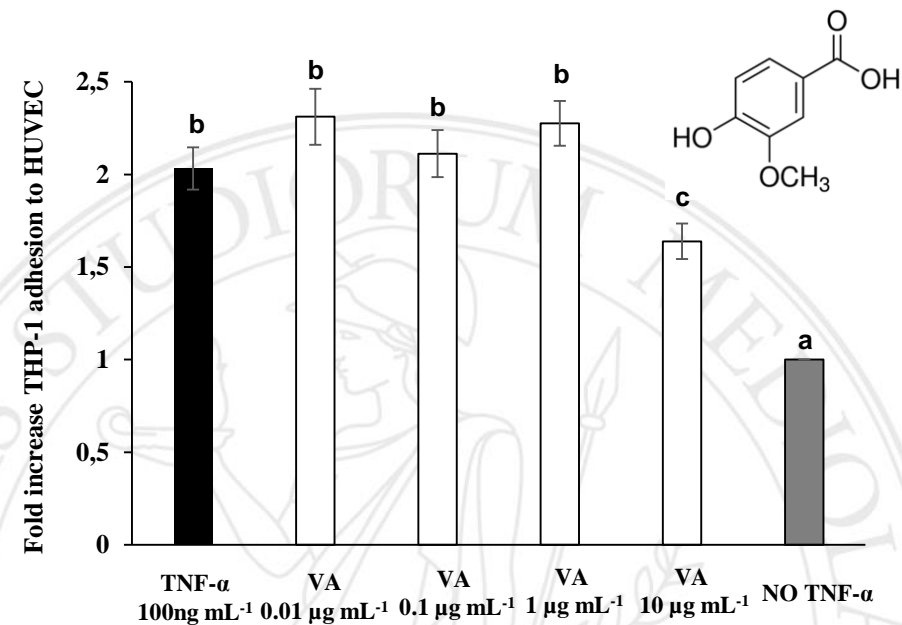


# RESULTS: Effect of Peo-3-glc and Vanillic acid on THP-1 adhesion to HUVECs

## Peonidin-3-glucoside



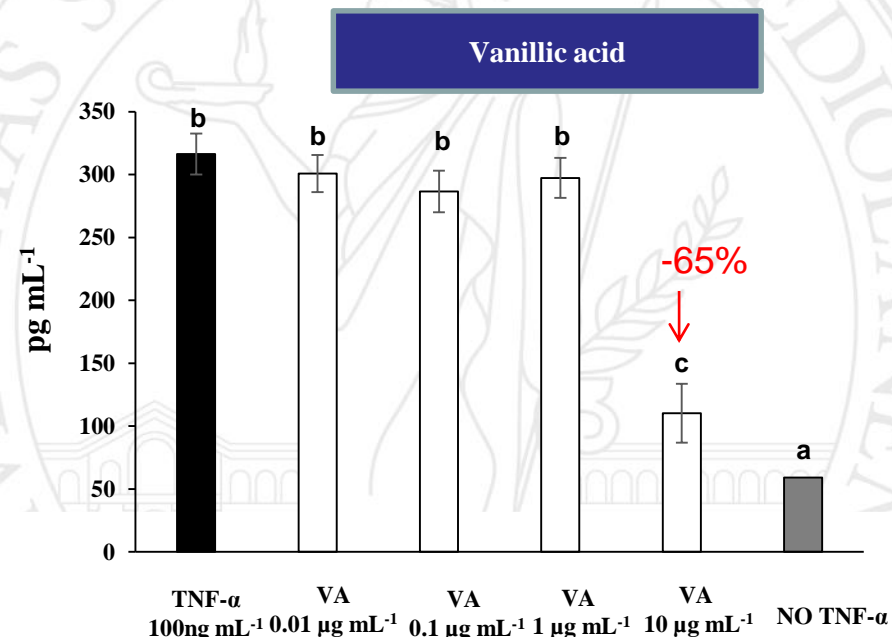
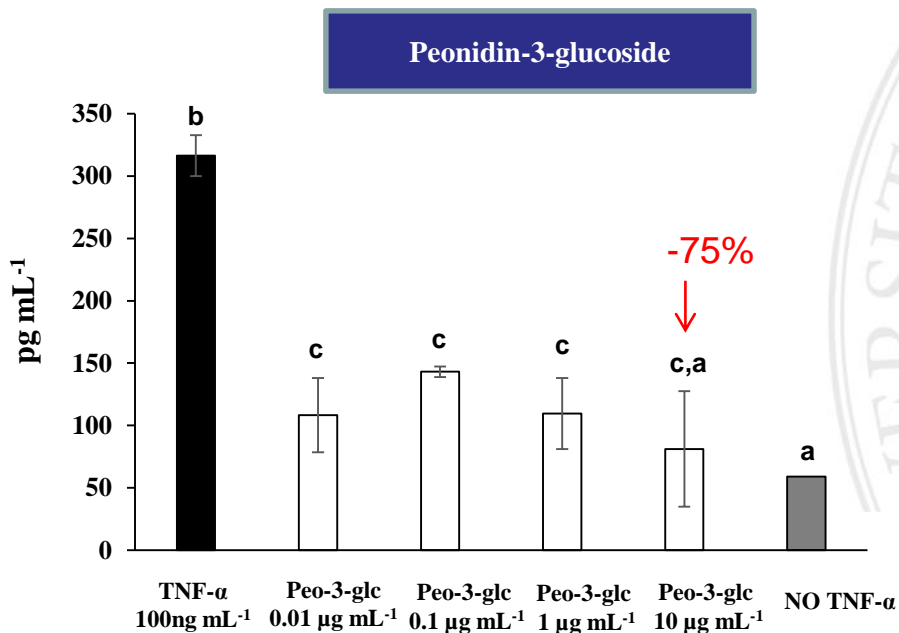
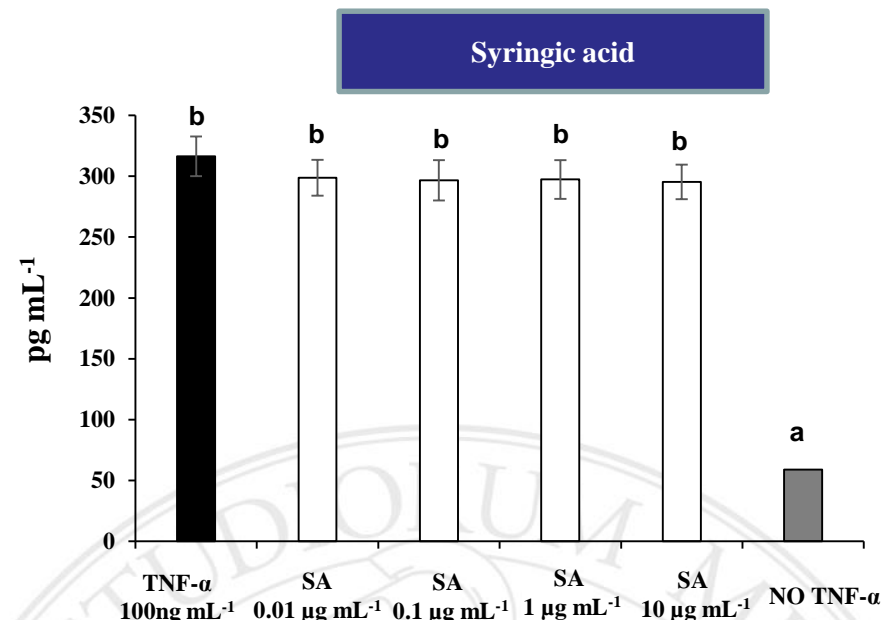
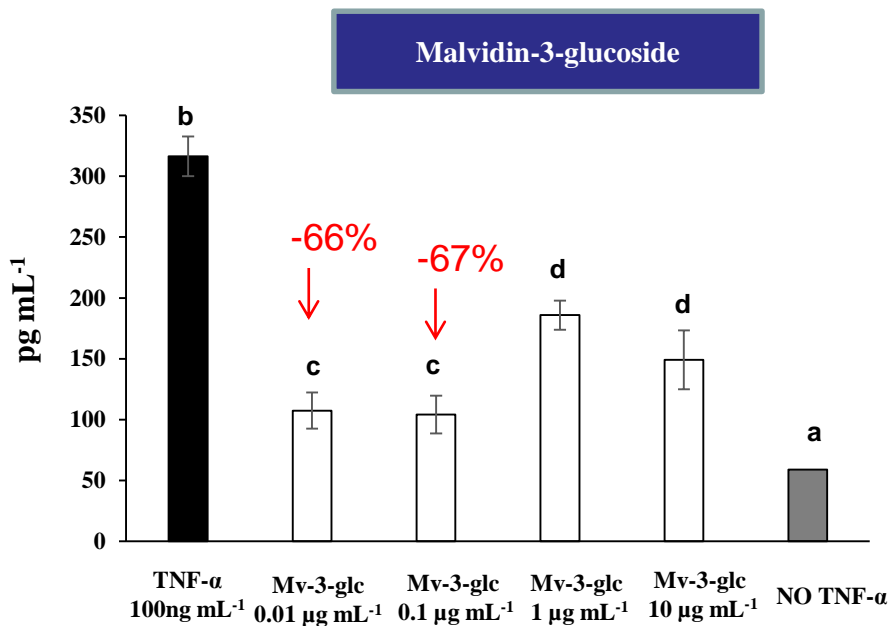
## Vanillic acid



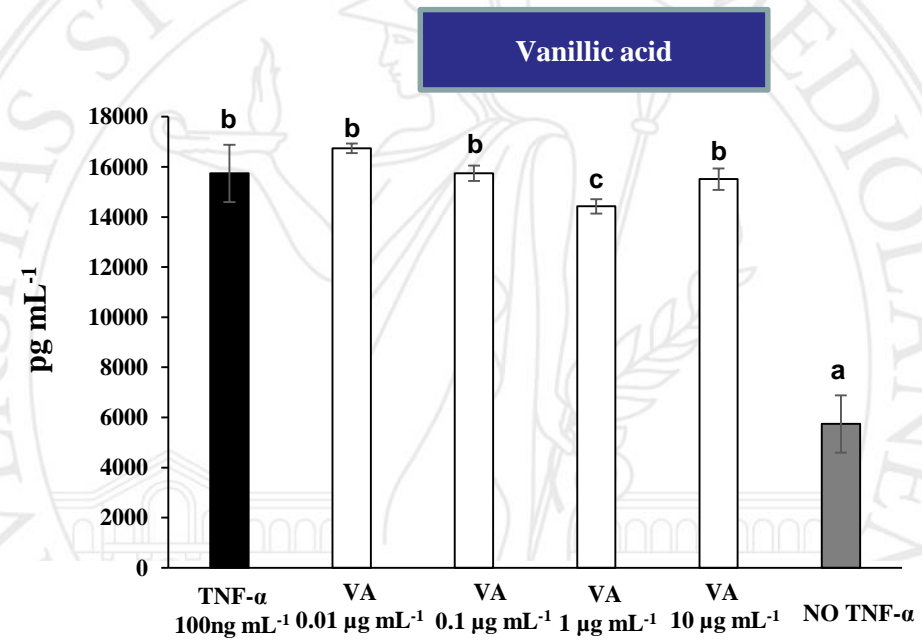
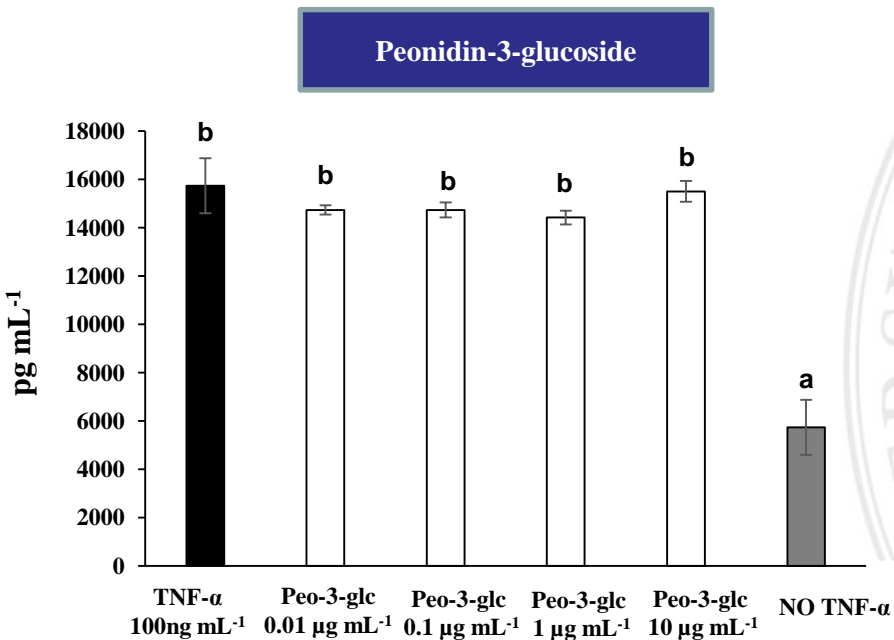
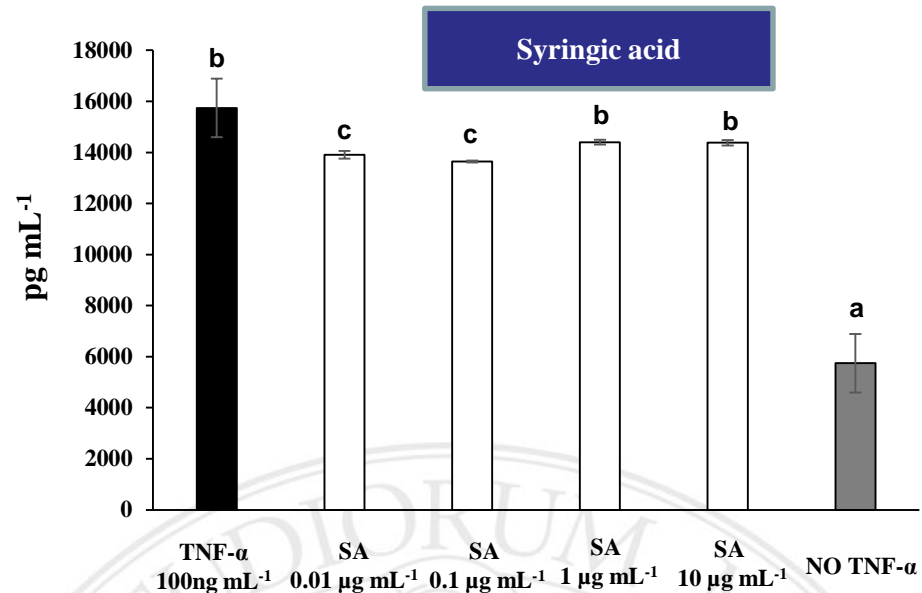
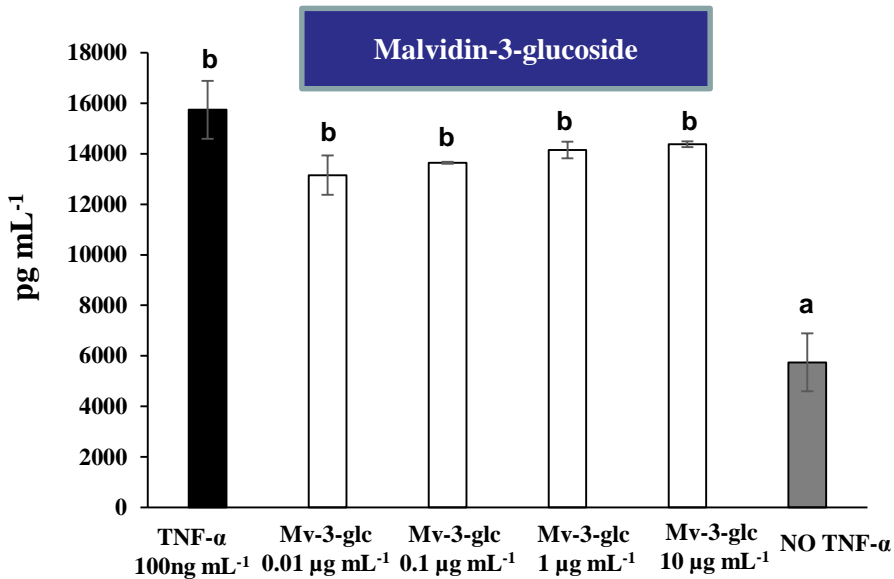
Significant reduction of THP-1 adhesion to HUVEC at all concentrations tested with respect to the control treatment with TNF- $\alpha$ . Maximum reduction at 10  $\mu$ g/mL<sup>-1</sup> (-46.8%;  $p < 0.001$ ).

Significant reduction after VA only at the maximum concentration (-20.8%;  $p < 0.005$ ).

# RESULTS: Effect of ACNs and metabolites on E-selectin concentration



# RESULTS: Effect of ACNs and metabolites on VCAM-1 concentration



# CONCLUSIONS

1-Mv and Peo-3-glc showed to counteract THP-1 adhesion to HUVEC, while the effects of metabolites seem to be compound dependent and only at high doses.

2-ACNs and metabolites seem to reduce the production of E-selectin, but not VCAM-1.

3-Since the effects were observed at the low doses, these results could suggest that the protective effect may be reached also *in vivo* at physiological concentrations.

4-Ongoing experiments are attempting to confirm and clarify the mechanisms of action of each compound involved in the above observations.



THANK YOU FOR YOUR  
ATTENTION!

