



Evaluation of the *in vitro* biological activity of UrinVita® in a cell model of the bladder

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1. Objectives

The objective of this study is to evaluate the efficacy of a new food supplement, UrinVita®, in the treatment of cystitis.

The secondary objectives are:

- Determine antioxidant activity
- Determine anti-inflammatory activity
- Determine disruptive activity of biofilms

2. Material and methods

***In vitro* bladder cell model**

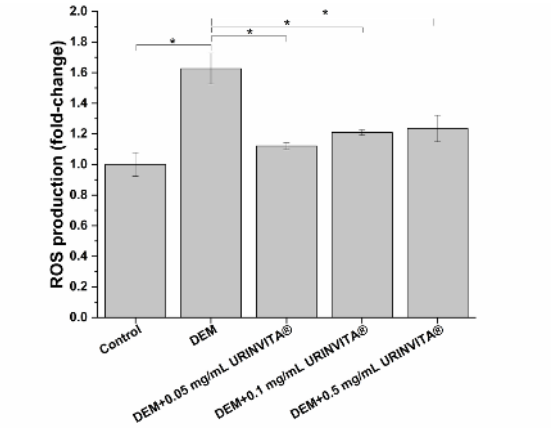
- T24 Cells
- Progressive increase in the concentration of UrinVita
- Exposure time: 6h
- Comparadores: Grupo control positivo y grupo control negativo
- Measurement of the production of reactive oxygen species (ROS)
- Measurement of pro-inflammatory cytokines (IL, TNF)
- Crystal Violet Assay Kit
  (Cell Viability Staphylococcus aereus,)

INGREDIENTES

Pomegranate extract *(Punica granatum L.)*, grape extract *(Vitis vinifera L.)*, cranberry extract *(Vaccinium macrocarpon L.)*, rosemary extract *(Rosmarinus officinalis L.)*, bulking agent (rice protein), vegetable capsule (hydroxypropylmethylcellulose).

3. Antioxidant activity

The model with T24 cells was stimulated with a pro-oxidant substance (DEM) in the presence or absence of UrinVita®. To assess whether the oxidative activity increased, the production of reactive oxygen species (ROS) was measured



Oxidative stress is reduced by 80%, 67% and 62% in the presence of concentrations of 0.05, 0.1 and 0.5 mg/mL of UrinVita®, respectively.

Exclusive use for professionals.
This information must not be disclosed to non-professional users.

4. Anti-inflammatory activity

Levels of IL-1 β, IL-6, and pro-inflammatory cytokines TNF-α were measured with UrinVita® 1mg/ml and diclofenac treatment.

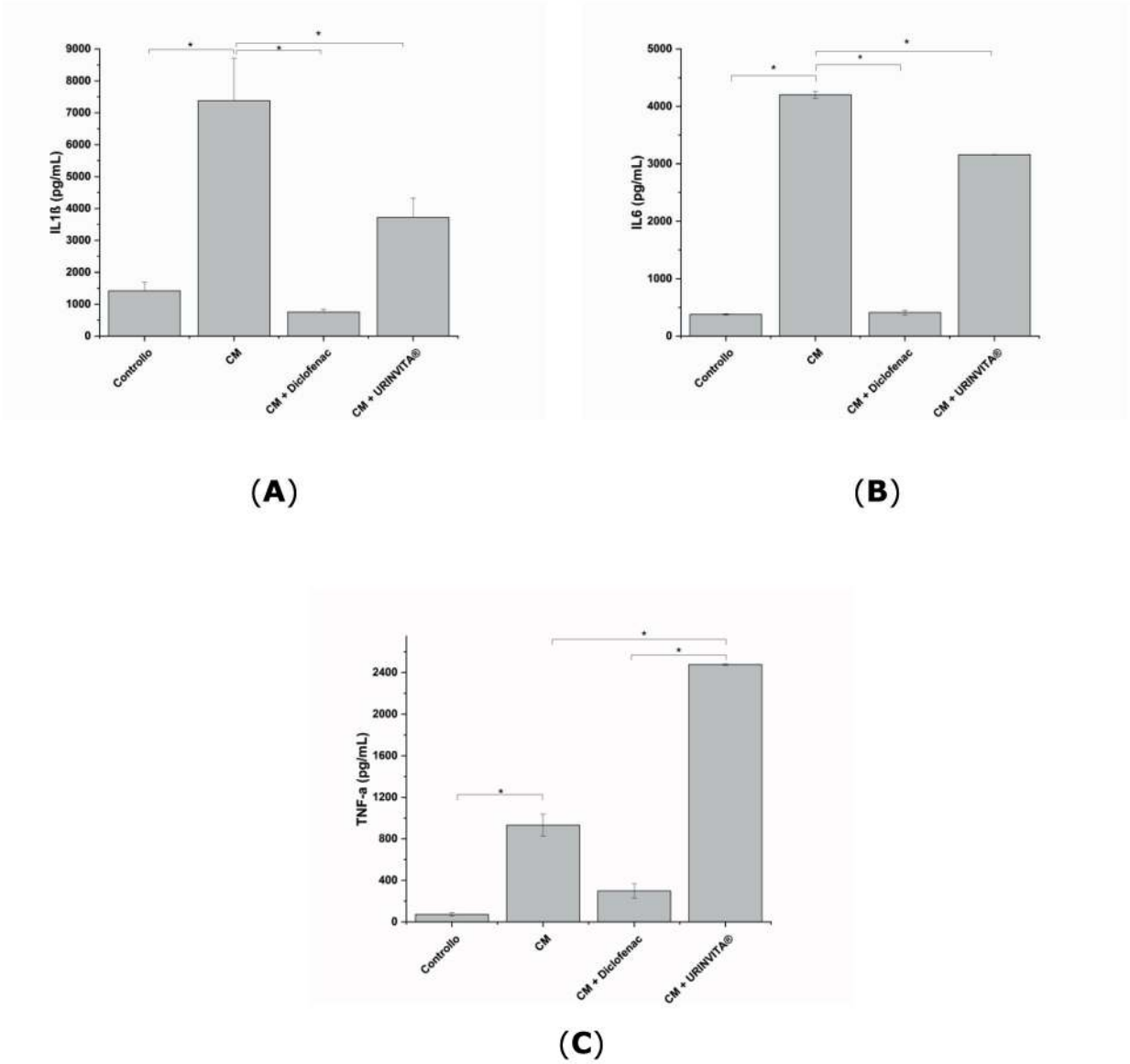
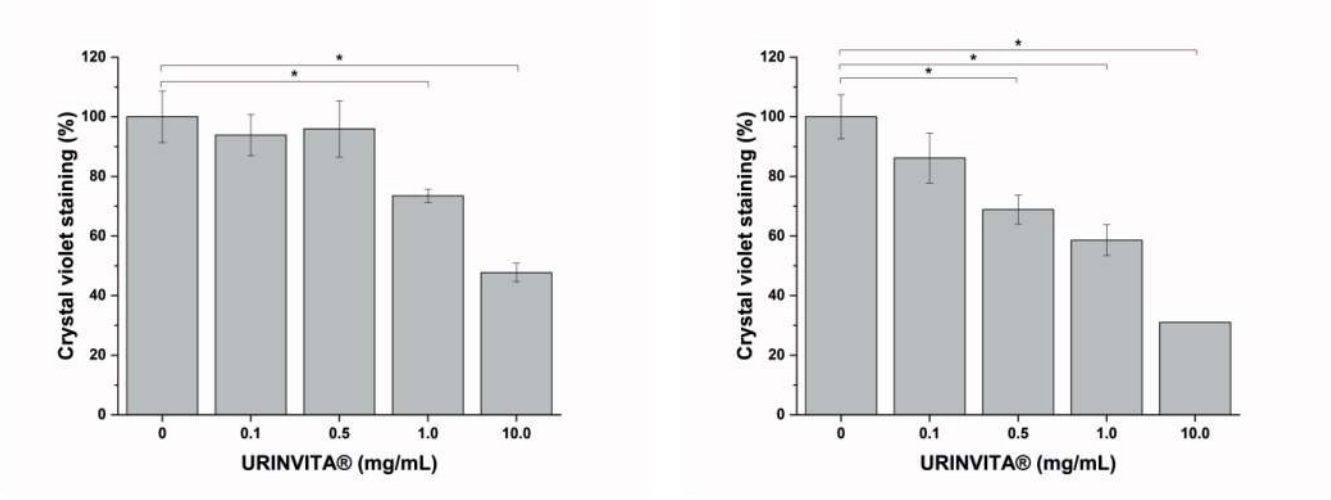


Figure 2. Expression of IL-1β (**A**), IL-6 (**B**) and TNF-α (**C**) in inflamed T24 cells (Conditioned medium-CM) exposed to the indicated treatments and in the control (Non-inflamed T24 cells). The results are reported as mean ± S.E.M. * p <0.05

In the presence of UrinVita®,IL-1 β is reduced by 50%, IL-6 is reduced by 25% and the pro-inflammatory cytokine TNF-α shows a higher level in the presence of UrinVita® compared to inflamed T24 cells. The results are not comparable with diclofenac.

5. Biofilm disrupting activity

Preformed biofilms of S. aereus, a pathogen known to develop biofilms on bladder walls, were exposed to different concentrations for 24 and 48 h.



(A) (B)

Figure 4. Relative amount of pre-formed *S. aureus* biofilm (%) following exposure to increasing concentrations (from 0 to 10 mg/mL) of URINVITA® for 24 h (A) and 48 h (B) expressed as % of crystal violet staining. Results are reported as mean ± S.E.M. *p<0.05

With the application of 1mg/ml to 10mg/ml of UrinVita®, a 30-50% reduction of the biofilm is produced in 24 hours.

In 48 hours a reduction of 40-70% is observed using the same product concentrations.

6. Results

- Oxidative stress is reduced by 80%, 67% and 62% in the presence of UrinVita® concentrations of 0.05, 0.1 and 0.5 mg/mL, respectively ($p < 0.05$).
- The induced oxidative stress was not shown to be dependent on the applied concentration.
- In the presence of UrinVita®, IL-1 β is reduced by 50%, IL-6 is reduced by 25%, and the pro-inflammatory cytokine TNF-α shows a higher level in the presence of UrinVita®, compared to inflamed T24 cells. ($p<0.05$).
- With the application of different concentrations of UrinVita® from 1mg/ml to 10mg/ml, a reduction of 30-50% of the biofilm is produced in 24 hours ($p<0.05$).
- In 48 hours there is a reduction of 40-70% using the same product concentrations ($p < 0.05$).

7. Conclusions

- UrinVita® has been shown to have antioxidant activity and controls the expression of pro-oxidant substances (ROS).
- UrinVita® reduces the levels of IL-1 β and IL-6 and shows a good anti-inflammatory capacity.
- The synthesis of TNF-α cytokines behaves differently than expected in the presence of UrinVita®. Additional studies are needed to understand the mechanism of action by which the compound exerts its anti-inflammatory activity
- UrinVita® is effective in breaking down S. aureus biofilms, which together with E. coli are responsible for most urinary pathologies

UrinVita®is a food supplement. Store in a cool, dry place. Keep out of the reach of little kids. Do not exceed the expressly recommended daily dose. Food supplements are not substitutes for a balanced diet. It is important to have a varied and balanced diet and a healthy lifestyle. Consult your doctor or pharmacist in case of pregnancy, if you take any medication or if you suffer from any illness, before starting the supplementation.

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