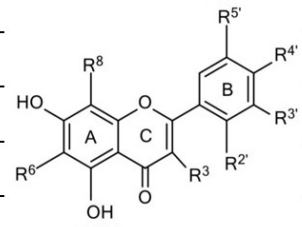
Atlas of Science
another view on science
https://atlasofscience.org

# Intestinal inflammation mediated by silver nanoparticles is prevented by dietary flavonoids

The interest in silver nanoparticles (AgNP) has been growing exponentially around the world, due to their unique physical, chemical and biological properties, as evidenced by their wide application in numerous products of our daily lives. In fact, AgNP are currently the most widely produced and marketed type of nanoparticles worldwide, being present in about half of the products that contain nanoparticles in their composition. AgNP assume special relevance in food industry, by improving food quality, safety and nutritional attributes. However, as AgNP are absorbed in the small intestine, the increased oral exposure to AgNP may result in an extensive contact of the intestinal cells, which may result in local adverse effects and intestinal inflammation. Hence, it becomes imperative to thoroughly evaluate the potential pro-inflammatory effects of AgNP, especially at the intestinal level.

| Compound | Structure | $R^{2'}$ | $R^{3'}$ | $R^{4'}$ | $R^{5'}$ | $R^{3}$ | $R^{6}$ | $R^{8}$ | $IC_{50}$ µM (Mean ± SEM) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | AgNP 4 nm | AgNP 19 nm |
| Diosmetin |  | H | OH | OMe | H | H | H | H | 37 ± 3 * | 37 ± 7 * |
| Luteolin | | H | OH | OH | H | H | H | H | 1.3 ± 0.2 | 1.3 ± 0.2 |
| Quercetin | | H | OH | OH | H | OH | H | H | 0.94 ± 0.09 | 3.1 ± 0.6 |
| Myricetin | | H | OH | OH | OH | OH | H | H | 2.3 ± 0.1 | 3.1 ± 0.2 |
| Morin | | OH | H | OH | H | OH | H | H | 1.4 ± 0.4 | 1.5 ± 0.4 |
| Quercetagetin | | H | OH | OH | H | OH | OH | H | 0.53 ± 0.06 | 3.0 ± 0.2 |
| Gossypetin | | H | OH | OH | H | OH | H | OH | 2.1 ± 0.3 | 4.5 ± 1.2 |



* Percentage of inhibition at the highest tested concentration, 25 µM.

Tab. 1. Structures and protective role of the studied flavonoids against AgNP (4 and 19 nm)-induced reactive pro-oxidant species production (IC50 µM, mean ± SEM).

Flavonoids are the most common group of plant polyphenols that are present in our daily diet, being recognized by their antioxidant and anti-inflammatory activities. Despite the lack of information in the literature about the protective role of flavonoids at the intestinal level, it can be hypothesized that flavonoids may probably be able to exert a protective effect against deleterious effects of AgNP in the intestine.

To evaluate the potential of AgNP to induce pro-inflammatory effects in the intestine, we used the Caco-2 cell line, that mimics the morphological and functional features of intestine, and neutrophils, the first line defense cells of the immune system against xenobiotics. Following the demonstration of AgNP-induced deleterious effects in these cell models, we evaluated the protective effect of a panel of structurally related flavonoids

[diosmetin, luteolin, quercetin, myricetin, morin, quercetagetin, gossypetin (Tab. 1)]. In this context, cells were exposed to two sizes (4 and 19 nm) of polyethylenimine (PEI)-coated AgNP, alone or concomitantly with the flavonoids under study, and the effects on cell viability and generation of reactive pro-oxidant species were assessed.

As expected, PEI-coated AgNP (4 and 19 nm) significantly increased cell death by apoptosis and the production of ˙NO. Concerning the protective role of flavonoids against AgNP-induced cytotoxicity in Caco-2 cells, quercetin notably reduced the percentage of AgNP (4 and 19 nm)-induced apoptosis to values that were not significantly different to those of untreated cells (Fig. 1). Nonetheless, regarding the production of ˙NO, none of the tested flavonoids were able to revert this production.

In respect to human neutrophils, the AgNP under study did not affect neutrophils viability. Nonetheless, the exposure of these cells to PEI-AgNP (4 and 19 nm) resulted in their activation, with a subsequent production of reactive pro-oxidant species, via NADPH oxidase, which was more pronounced in the case of PEI-AgNP of 19 nm. The tested flavonoids efficiently modulated the oxidative burst induced by 4 nm AgNP, being the most active compounds quercetin and quercetagetin. Nonetheless, regarding the oxidative burst induced by 19 nm AgNP, luteolin and morin were the most potent compounds (Tab. 1).

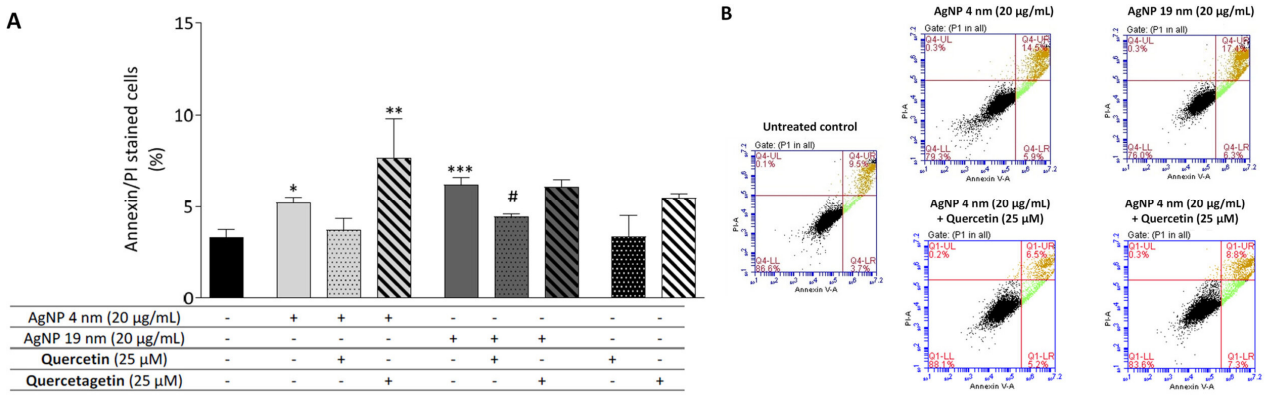



Fig. 1. Effect of AgNPs, 4 and 19 nm (20 µg/mL) on Caco-2 cells in the induction of apoptosis.
*$p < 0.05$, **$p < 0.01$ and ****, $p < 0.001$ when compared with the control (untreated cells). Values are presented as the means ± SEM ($n \geq 5$) (A). Representative flow cytometry plots of Annexin-V/PI binding assay for the apoptotic effects of AgNP (4 nm and 19 nm) in Caco-2 cells and the protective role of quercetin (25 µM) (B).

This study demonstrated that AgNP is deleterious to Caco-2 cells and activates neutrophils, indicating a high potential to induce local intolerance. On the other hand the protective role of the panel of structurally related flavonoids, present in our daily diet, against the harmful effects of AgNP, was also shown. In general, this protective effect was more pronounced in the case of quercetin and quercetagetin, suggesting that the presence of a hydroxy substituent at position 3 of the C ring is important for the function.

***Adelaide Sousa, Ana T. Rufino, Ana Ramalho, Félix Carvalho, Eduarda Fernandes, Marisa Freitas***
*LAQV, REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal*

*UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal*

## Publication

Protective Role of Flavonoids against Intestinal Pro-Inflammatory Effects of Silver Nanoparticles
Ana T. Rufino , Ana Ramalho, Adelaide Sousa, José Miguel P. Ferreira de Oliveira, Paulo Freitas, Manuel A. Gonzalez Gómez, Yolanda Piñeiro-Redondo, José Rivas, Félix Carvalho, Eduarda Fernandes, Marisa Freitas
*Molecules. 2021 Oct 31*