Antioxidant and anti-inflammatory effect of a flavonoids-rich extract from *Citrus bergamia* juices in both *cell-free* and *cell-based* assays

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It has been reported that oxidant/antioxidant imbalance triggers cell damage, and that flavonoids may be effective in protecting against age-related cognitive and motor decline in both *in vitro* and *in vivo* models. The present study was designed to evaluate the antioxidant effect of a flavonoids-rich extract from *Citrus bergamia* juices (BJe) and its effect against neuroinflammatory processes, such as those observed in Alzheimer's disease.

First, we tested the antioxidant properties of BJe in *cell-free* experimental models by ORAC, DPPH, Folin-Ciocalteu and Reducing Power assays, proving its antioxidant activity. Then, we assayed its ability to prevent the cytotoxic effects induced by H_2O_2 or $Fe_2(SO_4)_3$. Our results provided evidences that BJe reduces cell death, generation of ROS and membrane lipid peroxidation, improve mitochondrial functionality and prevent DNA-oxidative damage in A549 cells incubated with H_2O_2. Moreover, BJe is able to both induce catalase expression and increase its activity. Furthermore, evidences that BJe prevents the cytotoxic effects by $Fe_2(SO_4)_3$, have suggested that this extract could possess chelating properties. This hypothesis was confirmed by measuring the presence of redox-active iron in the cells pre-treated with the extract and then exposed to the metal.

In the light of these observations, we wondered whether BJe may be effective against neuroinflammatory processes. To this aim we used THP-1 monocytes to investigate the mechanisms underlying the beneficial potential of BJe against amyloid-beta$_{1-42}$ (Aβ_{1-42})-mediated inflammation. Exposure of THP-1 cells to Aβ_{1-42} significantly induced the expression and secretion of IL-6 and IL-1β in THP-1 cells and increase the phosphorylation of ERK 1/2 as well as p46 and p54 members of JNK family. Moreover, Aβ_{1-42} raises AP-1 DNA binding activity in THP-1-treated cells. Interestingly, all these effects were reduced in the presence of BJe. Our data indicate that BJe may effectively counteract the pro-inflammatory activation of monocytes/microglial cells exposed to amyloid fibrils.

In conclusion, we provider evidences that the flavonoid-rich extract of bergamot juice may be use in preventing oxidative cell injury suggesting a promising role as natural drug against inflammatory processes.