



MODULATION OF TAU AGGREGATION WITH NATURAL COFFEE COMPOUNDS



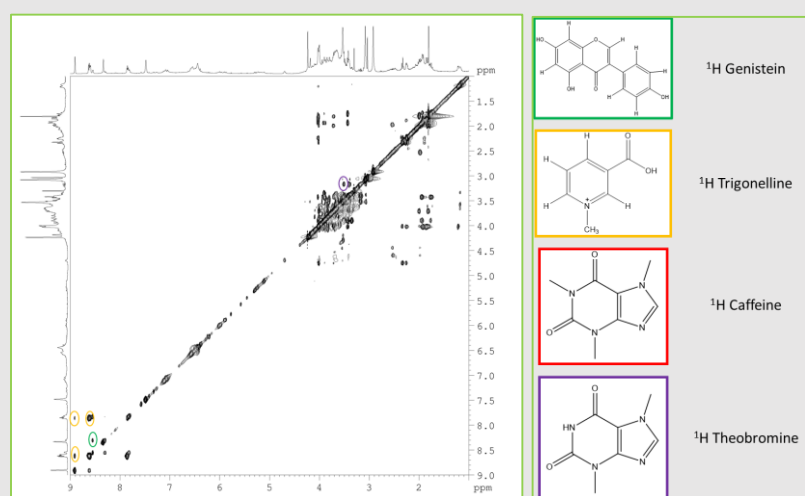
R. Tira,‡ G. Viola, ‡ C. G. Barracchia, ‡ F. Parolini, ‡ F. Munari, ‡ M. Assfalg, ‡ M. D'Onofrio.‡

‡Department of Biotechnology, University of Verona, Italy
E-mail: roberto.tira@univr.it

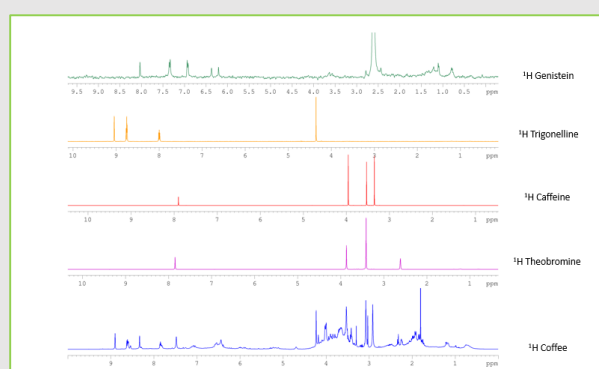
BACKGROUND

Coffee and coffee compounds are attracting interest in the field of neuro-inflammation and neuro-protection against oxidative-stress thanks to their bioavailability and ability to cross the Blood Brain Barrier [1]. Recent works demonstrate that brewed-coffee and some of these molecules, such as phenylindanes and other flavonoids, have the additional ability to inhibit A β and Tau protein aggregation [2]. We investigate the effects of Italian espresso coffee and a selection of coffee-derived bioactive molecules towards mitigation of Tau aggregation, using NMR spectroscopy, fluorimetry, CD and TEM spectroscopy.

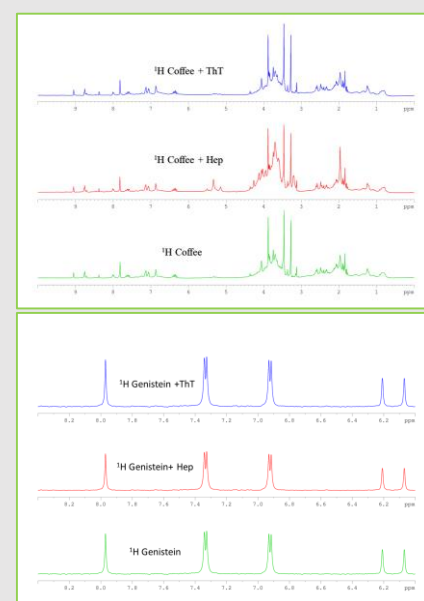
NMR



1. ^1H - ^1H COSY spectrum of Italian espresso coffee. Each identificative spectrum of selected molecules are highlighted in the ^1H , ^1H -COSY spectrum



2. ^1H mono-dimensional spectra of Italian espresso coffee and selected molecules. The mono-dimensional spectrum of coffee includes the peaks of the selected molecules

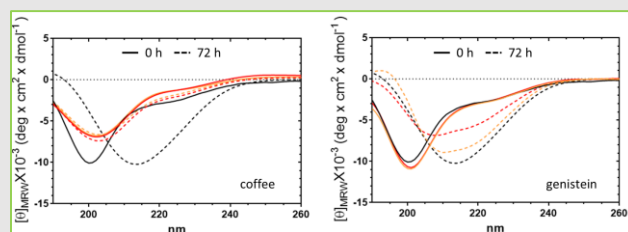


3. ^1H mono-dimensional spectra of coffee and genistein alone (green) and in presence of heparin (red) and ThT (blue)

We acquired ^1H - ^1H COSY spectrum (1) and ^1H mono-dimensional spectrum (2) of an Italian espresso coffee to better characterize the components. Identificative peaks of trigonelline, genistein and theobromine (and caffeine) are observable in both type of spectrum. These molecules were selected for the following experiments.

In presence of heparine and ThT no chemical shift perturbations on ^1H spectra are observed (3). Therefore in the aggregation experimental conditions the only visible effects are due to the presence of the chosen compounds. Only the results of coffee and genistein are shown below.

CIRCULAR DICHROISM

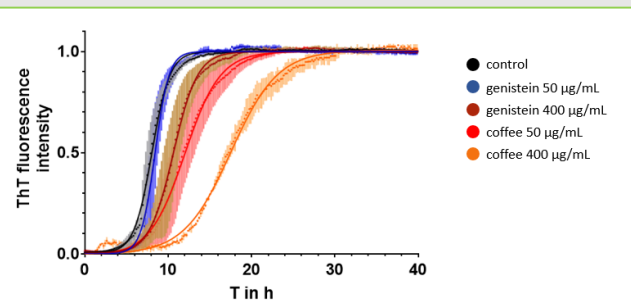


Conformational transitions. Far-UV CD spectra recorded on 6 μM tau4RD (black) in the presence of Italian coffee espresso and genistein at 6 (orange) or 48 (red) $\mu\text{g}/\text{mL}$ concentration. Measurements were performed immediately after sample preparation and after 72 h incubation of a concentrated stock (50 μM protein and 50 or 400 $\mu\text{g}/\text{mL}$ genistein) in quiescent condition.

CD spectra highlight the conformational behavior of Tau4RD.

In presence of Italian coffee extract at different concentration, the protein retains most of its natively unfolded structure even after 72 h. In presence of genistein instead β -sheets are formed after 72 h, but with an ambiguous profile in respect to the control.

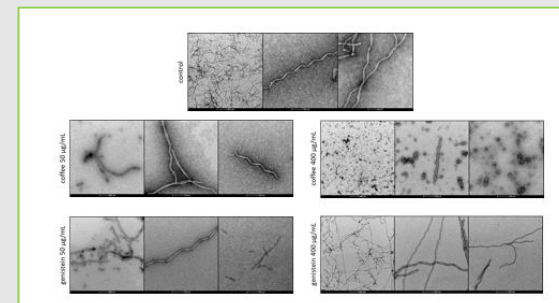
ThT-ASSAY



Kinetics of filament formation. ThT fluorescence-based aggregation kinetics curves measured on 50 μM tau4RD in the presence of Italian coffee espresso (red and orange) and genistein (blue and brown) at 50 or 400 $\mu\text{g}/\text{mL}$ concentration. Measurements were carried out on four replicates and data are reported as mean \pm s.d.. Solid lines correspond to the best fit curves determined using an empirical sigmoid function.

Different concentrations of Italian coffee extract and genistein lengthen the lag phase of the aggregation reaction in a dose dependent manner as displayed by ThT assay. 400 μM of coffee has the major impact.

TEM



TEM morphological analysis. Images acquired on 5 μM of aggregated samples of Tau4RD. Samples were diluted, after 48 h, from a concentrated stock (50 μM) incubated with 50 or 400 $\mu\text{g}/\text{mL}$ of Italian coffee espresso and genistein.

TEM images tell us that in presence of coffee, after 48 h, only shorter fibrils and fragment are formed, with a high presence of spheroidal aggregates. The effect is dose dependent.

In presence of genistein the effect is less evident but short and fragmented fibrils are present.

CONCLUSION

Italian espresso coffee and genistein:

- affect the conformational behaviour of aggregating Tau4RD with a decrease in content of β -sheets
- lengthen the lag phase of the aggregation reaction of different time
- Modify the length and the morphology of fibrils



[1] M. S. Butt et al., «Coffee and its consumption: benefits and risks,» Crit. Rev. Food Sci. Nutr., **51**, 373-383, (2011).

[2] R. Manicini, et al., «Phenylindanes in Brewed Coffee Inhibit Amyloid-Beta and Tau Aggregation,» Front. Neurosci., (2018).