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Dietary flavan-3-ol metabolites modulate proinflammatory human fibroblast activation *in vitro*: potential perspectives in the prevention of CVD

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BACKGROUND

Consumption of food rich in flavan-3-ols, like tea, dark chocolate, red wine, grapes, has been associated with a protective effect against chronic diseases characterized by systemic inflammation, such as CVD. Specifically in atherosclerosis, it is known how the deposition and oxidation of LDL particles in the intima of the artery triggers an inflammatory process that becomes chronic and unresolved. This process involves macrophages and fibroblasts, which secrete proinflammatory cytokines such as IL-6 and IL-8 to resolve this damage (Fig.1). Currently, FANS and glucocorticoids are use against the inflammation. These class of drugs are responsible of various side effects. For this reason, there is an increasing research for new molecules characterized by fewer adverse effects.

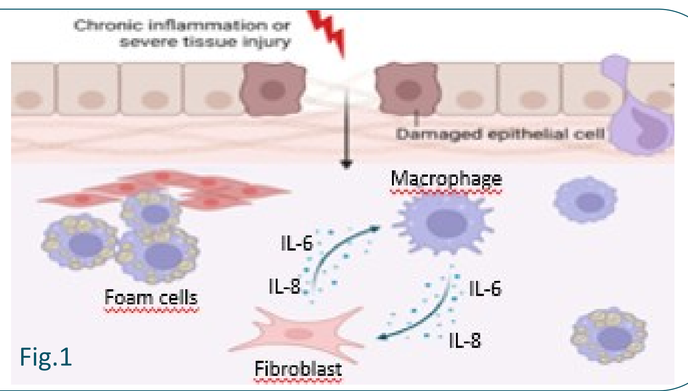

Fig.1

AIM

This study aims to evaluate the in vitro anti-inflammatory effect of colonic metabolites of flavan-3-ols as OH-PVL, as the most representative molecules present in plasma after the ingestion of flavan-3-ol-containing food.

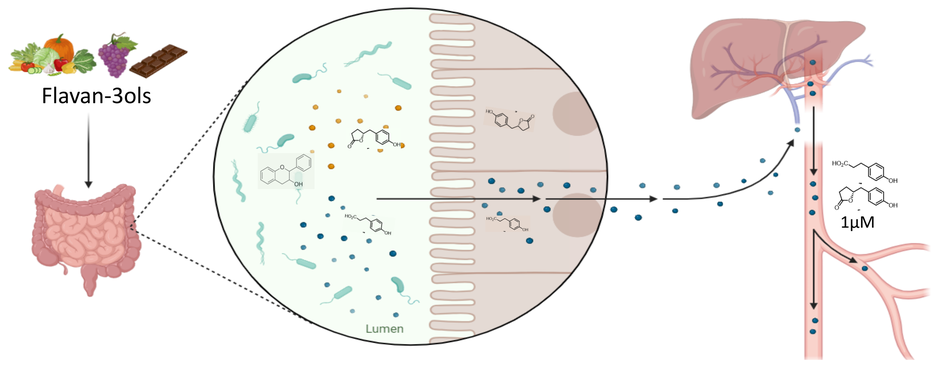

Fig.2 Colonic metabolism and absorption process of flavan-3ols.

MATERIALS

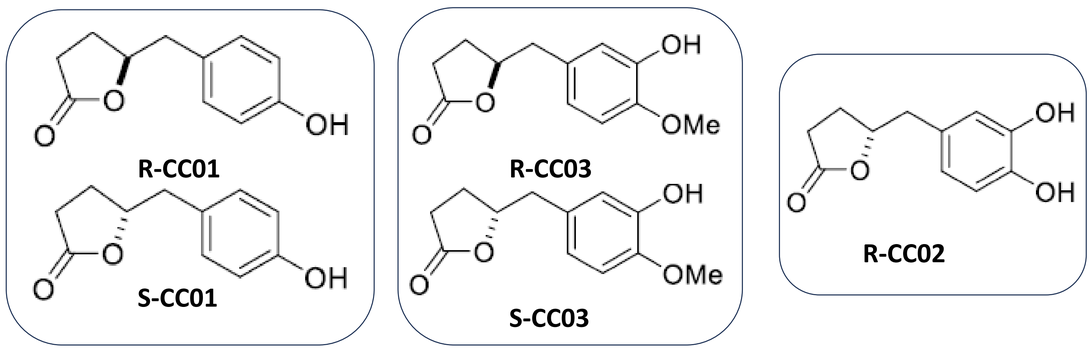

Fig.3 *In vitro* tested γ–valerolactones, colonic metabolites isolated in metabolomics studies, chemically synthesized.

METHODS AND RESULTS

STEP 1: Evaluation of cytotoxicity

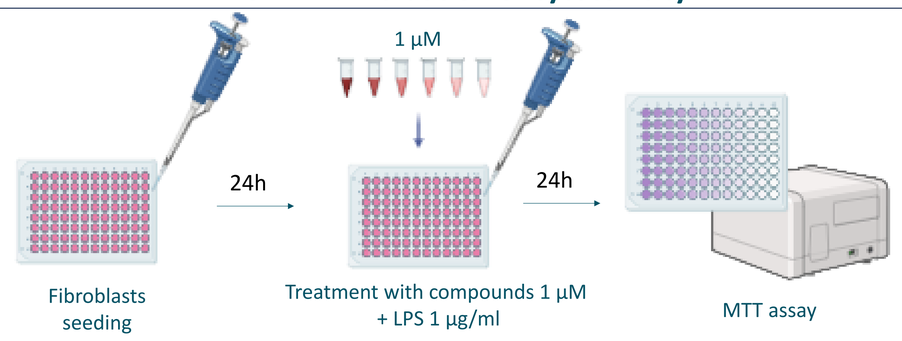

Fig. 4 The cytotoxicity of all compounds at 1µM was evaluated in human fibroblast by MTT assay.

	LP	Basal	R-CC01	S-CCO1	R-CC03	S-CC03	R-CC02
Viability cells % ± s.d.	100 ± 6,90	92 ± 9,62	77 ± 0,61	73 ± 3,03	63 ± 0,91	58 ± 2,05	85 ± 0,08
p-Value	-	ns	0,0158	0,0031	0,0004	0,0091	0,0002

Table 1. **Fibroblasts viability after the treatment with LP + compounds at 1µM**. The MTT results show increased cell viability in the LP vs basal condition, while all compounds slightly reduce cell viability vs LP condition. However, from microscopic observation the cells were morphologically unaltered and non-suffering compared to the basal condition. *p*-value ≤ 0,05 vs LP condition. The statistical analysis was conducted by one-way ANOVA and post-hoc analysis by Dunnett's test.

STEP 2: Evaluation of anti-inflammatory activity

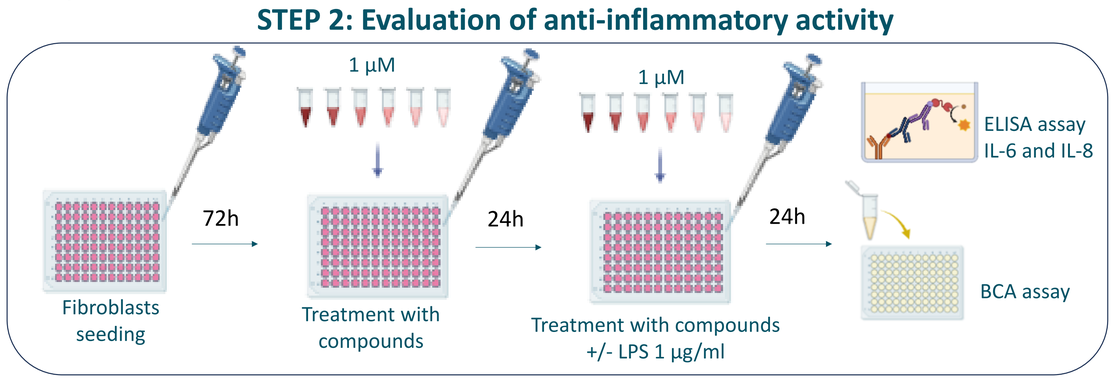

Fig. 5 The efficacy of all compounds at 1µM in inhibiting lipopolysaccharide (1µg/ml) LPS-induced IL-6 and IL-8 secretion was evaluated in human fibroblast by ELISA kit. Supernatant IL-6 and IL-8 concentrations were normalized against the cell extract protein content, assessed by BCA assay.

	Basal	R-CC01	S-CCO1	R-CC03	S-CC03	R-CC02
IL-6 secretion % ± s.d.	100 ± 13	12 ± 17	16 ± 18	203 ± 288	129 ± 183	63 ± 14
p-Value	ns	ns	ns	ns	ns	ns

IL-8 secretion % ± s.d.	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
p-Value	ns	ns	ns	ns	ns	ns

Table 2. **The IL-6 and IL-8 secretion in basal condition**. R-CC01 and S-CC01 showed a reduction in IL-6 secretion vs basal condition B (2833 pg/ml/mg protein) in the absence of inflammatory stimulus. All compounds were found not to stimulate IL-8 secretion vs basal condition (0 pg/ml/mg protein). The statistical analysis was conducted by one-way ANOVA and post-hoc analysis by tukey's test *p*-value ≤ 0,05 vs basal condition.

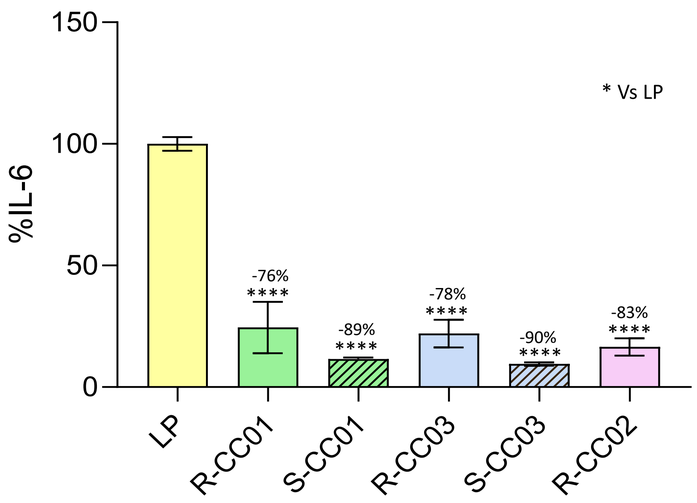

Fig. 6. **The IL-6 secretion in inflammatory condition (LP).** All compounds significantly reduced IL-6 secretion vs LP condition (179680 pg/ml/mg protein). *p*-value ≤ 0,05 vs LP condition.

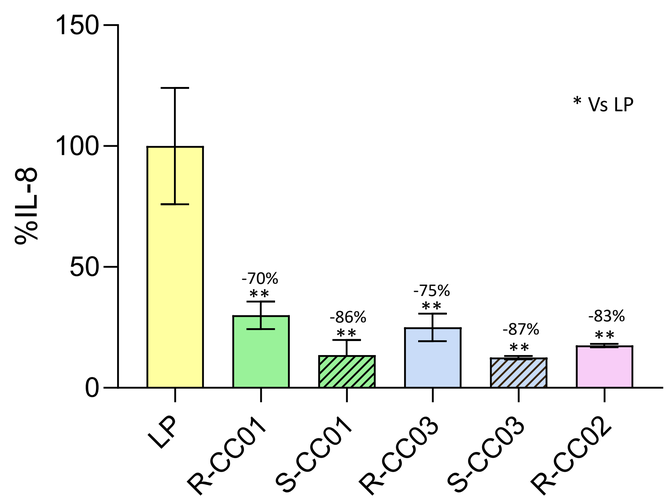

Fig. 7. **The IL-8 secretion in inflammatory condition (LP).** All tested molecules reduced IL-8 secretion vs LP condition (202887 pg/ml/mg protein). The statistical analysis was done by one-way ANOVA and post-hoc analysis by tukey's test. *p*-value ≤ 0,05 vs LP condition.

CONCLUSIONS

Five OH-PLV compounds have shown anti-inflammatory activity in human fibroblasts *in vitro*, with no differences between enantiomers. Our data, pointing to the biological activity of specific colonic metabolites of flavan-3-ols, pave the way to further research on the mechanism of action of these molecules and support the usefulness of *in vivo* studies for the prevention and modulation of CVD.