Delphinidin, gallic acid and indirect antioxidant protection

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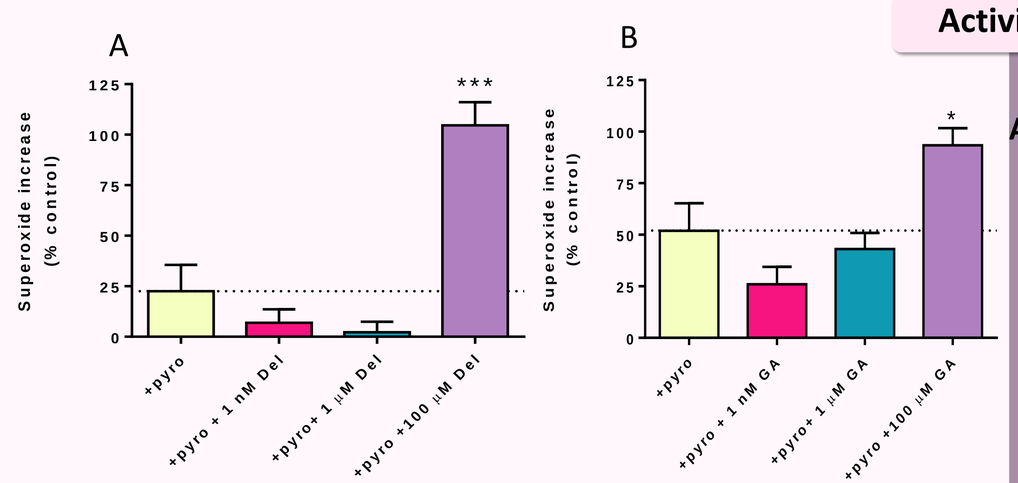
- Oxidative stress underpins key aspects of atherogenesis.
- Antioxidants might help to disrupt the atherogenic process by quenching oxygen-centred radicals.
- Polyphenols are believed to have a wide range of health benefits on account of their ability to scavenge free radicals.
- However, they are poorly absorbed from the diet and are susceptible to rapid degradation and metabolic transformation *in vivo.*

The aim of this study was to test the hypothesis that a polyphenol, delphinidin, present at physiologically attainable concentrations can protect cultured endothelial cells (HUVECs) against chemically induced oxidative stress.

Activity

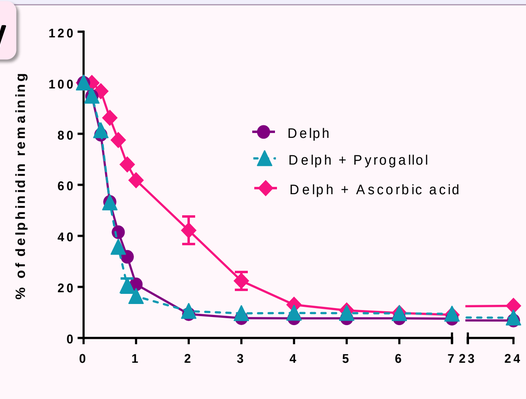
ROS Assay



Delphinidin (A) and gallic acid (B) generated in HUVECs significant amounts of oxygen-centred radicals.

Stability

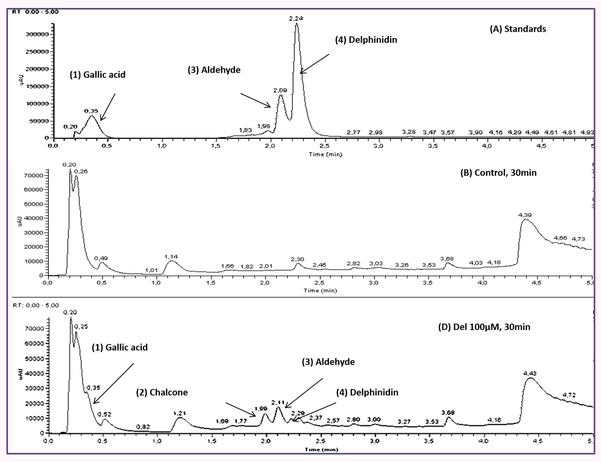
Spectro-metry

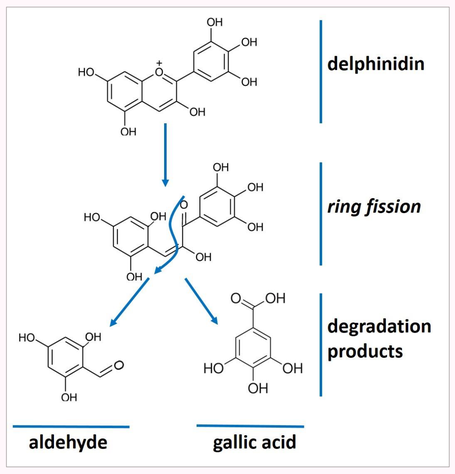


Delphinidin (delph) (200 µM) was not stable under physiological conditions and degraded rapidly in tissue culture media (pH ~ 7.4, 37°C). Half-life was approx. 30min when incubated on its own. ~80% of delphinidin was lost in the first hour. Ascorbic acid offered modest protection against decomposition.

Degradation

LC MS

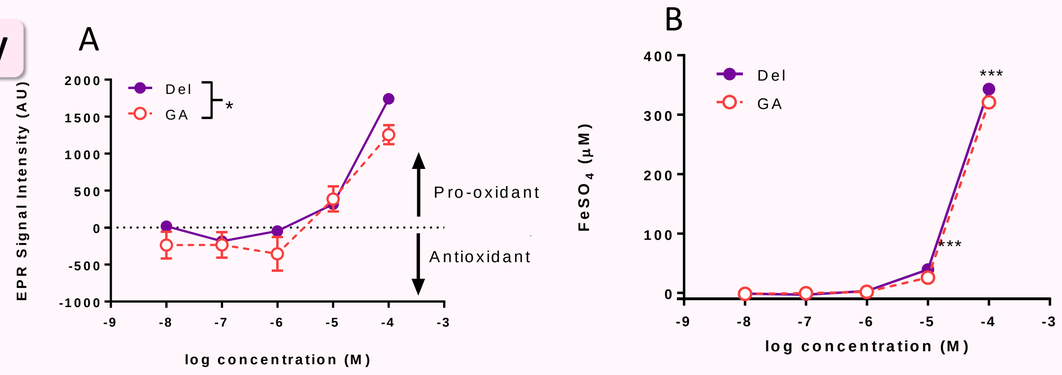




LC MS/MS data indicated that delphinidin degrades spontaneously to gallic acid and phloroglucinol aldehyde under physiologically relevant conditions in tissue culture medium.

Activity

EPR, FRAP

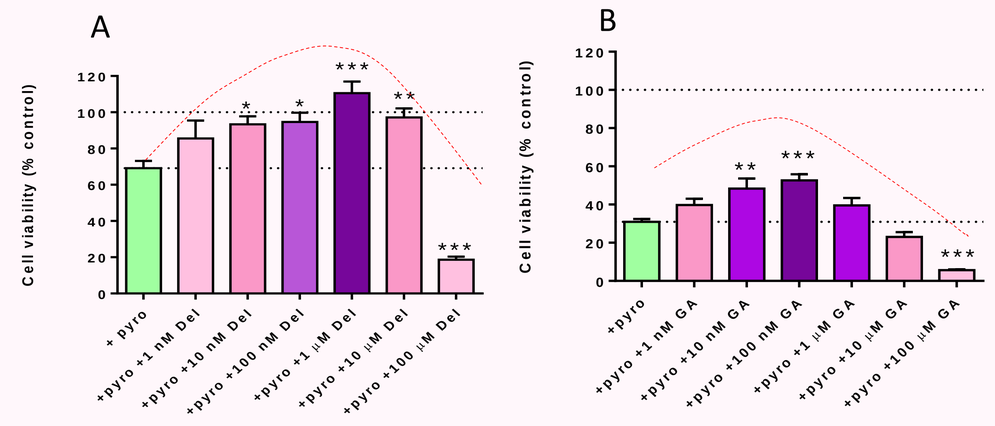


The EPR data (A) generated showed that both delphinidin and its major metabolite gallic acid exhibit pro-oxidant activities at ≥10 µM, with some modest free radical scavenging effects (< 1 µM). FRAP analysis (B) only detected reducing activity for delphinidin and gallic acid at concentrations of ≥10 µM.

Protection

MTT assay

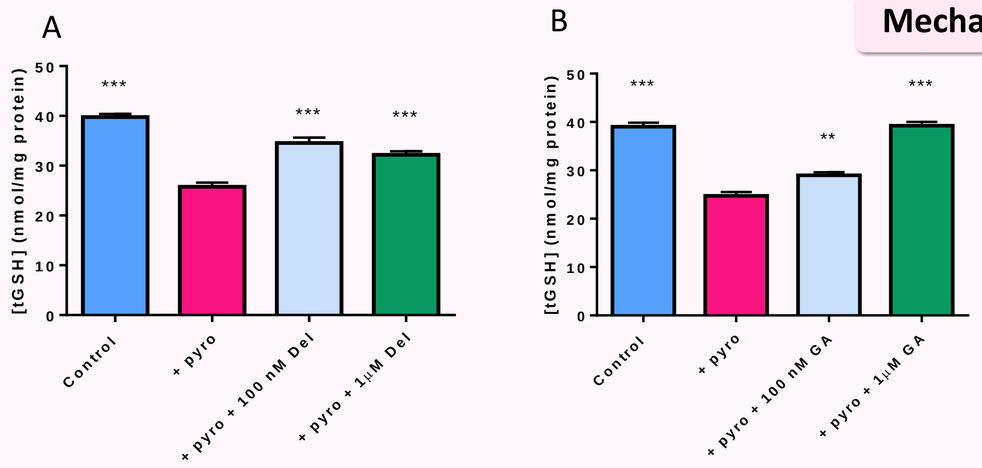
Both phenolics protected endothelial cells against chemically induced oxidative stress.



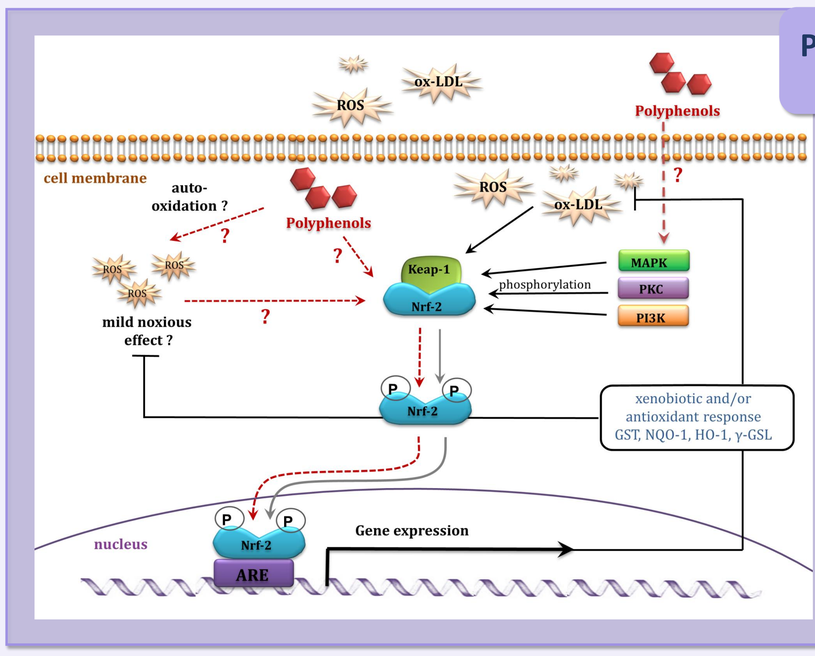
The indirect antioxidant protective effects of both phenolics displayed a **hormesic** profile: delphinidin (A) and gallic acid (B) caused similar protective effects against oxidative stress-induced cell death when present at concentrations at or below 1 µM (physiologically relevant). Both had significant cytotoxic effects towards HUVECs at high concentrations.

Mechanism

Total GSH assay



Both phenolics (Del (A), GA (B)) significantly protected against pyrogallol-induced depression of total glutathione in HUVECs.



Proposed mechanism of action:

It is hypothesized that polyphenols impose a mild noxious effect, driving intracellular defences and xenobiotic-metabolizing enzymes to invoke cytoprotection.

Conclusions:

The results confirm that physiologically relevant concentrations of delphinidin and its major metabolite, gallic acid, are sufficient to induce antioxidant benefits, but via an indirect, xenobiotic mechanism that induces upregulation of endogenous antioxidant capacity.

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