Wine by-products as source for bioactive compounds with anti-atherothrombotic activities

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Scope

Taking into consideration the whole winemaking process, global wine production is around 27 billion litres a year, whereas the wine sector is responsible for approximately 0.3% of annual greenhouse gases emission. The winemaking process results in the concomitant production of grape pomace (GP), by-products which account for 20-25% of the weight of the grapes used. Only a small amount of the latter by-products is reutilized and its disposal raises environmental concerns, while representing a high cost to the industry that could be used in [1]. There is evidence-based data that support the possible protective effects of low-to-moderate wine consumption on cardiovascular health. However, wine cannot be consumed to an extensive extent due to the presence of ethanol. Alternatively, GP is a source of wine-like micro-constituents which can be useful for food fortification. Our previous studies revealed that wine contains micro-constituents of proper quality and quantity that exert cardioprotective actions, partly through inhibiting Platelet Activating Factor (PAF) [2], a potent mediator of inflammation and thrombosis. The aim of the present study was to examine the anti-platelet and anti-inflammatory activity of a specific grape pomace extract [3].

Methodology

Grape pomace from red grape varieties (Cabernet Sauvignon, Cabernet Franc, Syrah, Sour Black) were extracted with 80% ethanol.

Anti-platelet properties*:* Platelet aggregation was measured in healthy volunteers' platelet rich plasma by the light transmittance method, against three agonists: PAF, ADP and TRAP. The results were expressed as IC_{50} values (μg of extract that cause 50% inhibition of aggregation) and EC_{50} values (agonist concentration that causes 50% of the maximum aggregation) in the extract's presence (150 μg) and absence.

Anti-inflammatory properties*:* Peripheral blood mononuclear cells (PBMC's) from healthy volunteers were pre-incubated with different extract concentrations, which were tested for their effect on cell viability, for 1h and then stimulated with LPS (100ng/mL assay) for 4h. Secretion of IL-1β and TNF-a was measured with ELISA and normalized with the cell protein. The MTT assay was used in order to test cells viability.

Results

The presence of 500 and 1000 μg/mL of extract reduced the % LPS-induced TNF-a secretion at 38.2% (22.2-90.1) and 6% (-2.1-10), respectively and the %LPS-induced IL-1β secretion at 82.1% (69.6-94.3) and 62.9 (40.7-96.4) respectively (**Fig. 1**).

The extract's IC_{50} was calculated at 162.1 ± 66.9, 181.2 ± 82.3 and 156.3 ± 97.5μg against PAF, ADP and TRAP, respectively (**Fig.2**). From the linear part of the aggregation-agonist concentration curve, EC_{50} values were calculated for each agonist in the absence and presence of 150μg extract at 0.00031mM, 0.0072mM, 0.0047mM and 0.00062mM, 0.010mM, 0.0053mM for PAF, ADP and TRAP respectively, revealing lower platelet aggregation sensitivity. Hence, the EC_{50} values in the presence of 150μg extract were increased approximately at 100%, 45% and 13% against PAF, ADP and TRAP respectively, compared to EC_{50} values in the absence of extract (**Fig.3**).

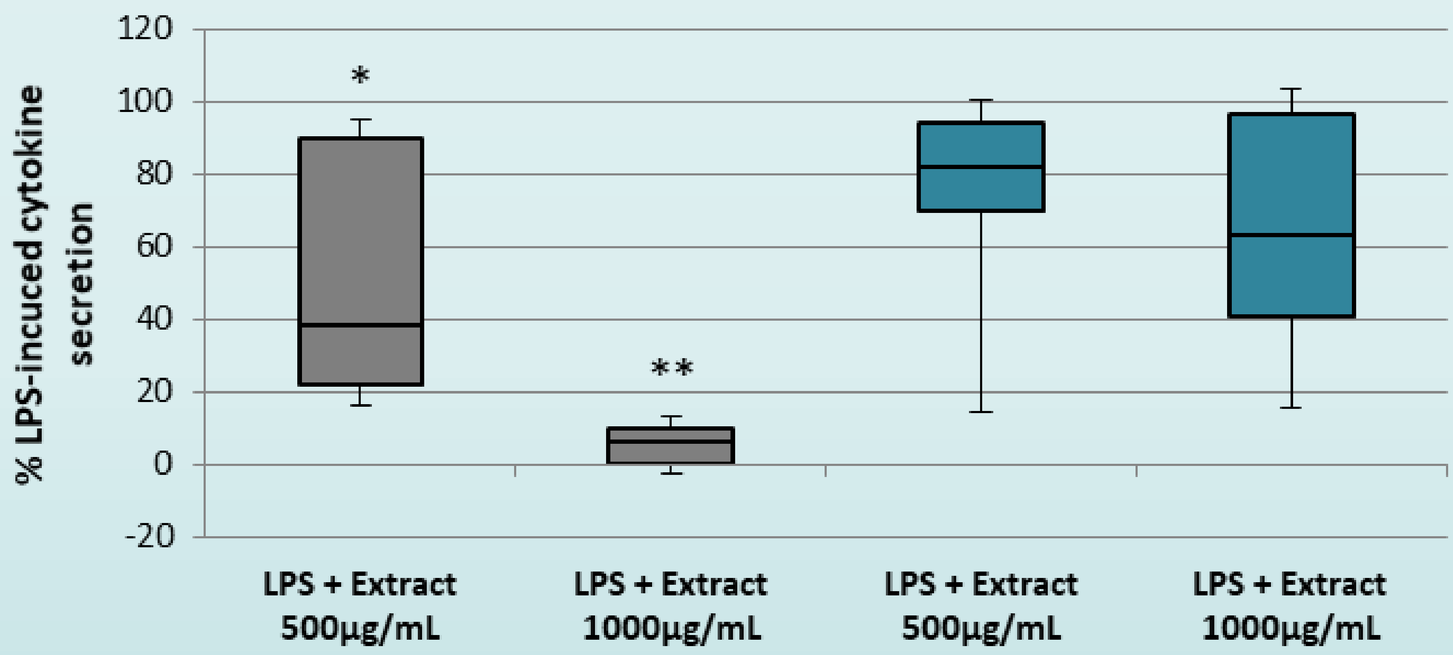

Fig. 1. % of LPS-induced secretion of TNF-a (grey) and IL-1β (blue) by PBMC's after pre-incubation with extract. Data represent the median (25-75) of three independent experiments. Independent Samples Kruskal Wallis Test. (*p=0.04), (**p<0.000).

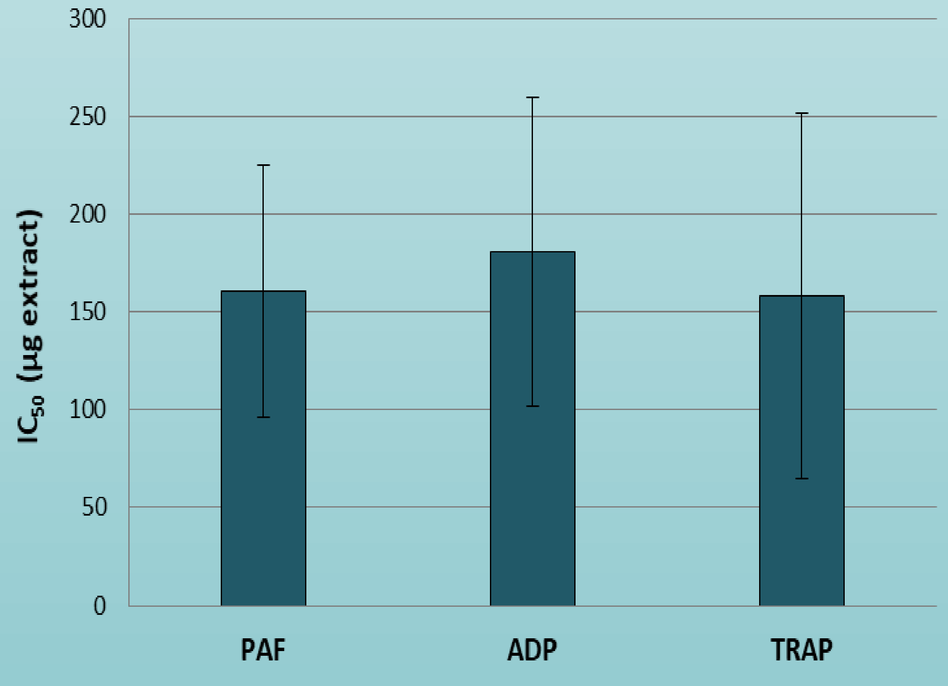

Fig. 2. The extract's IC_{50} values against PAF, ADP and TRAP. Data represent the mean ± sd of three independent experiments.

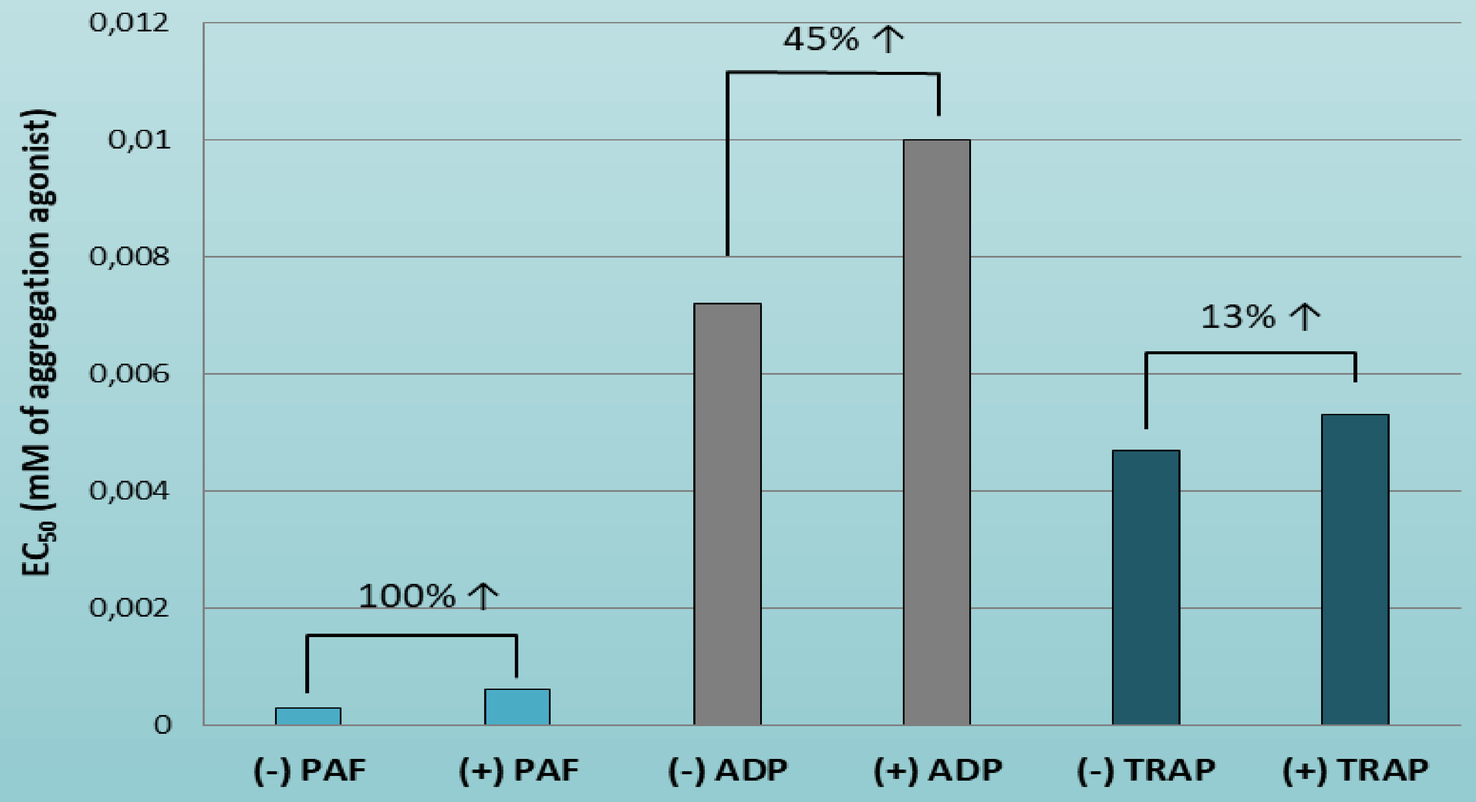

Fig. 3. The extract's EC_{50} values against PAF, ADP and TRAP in the absence (-) and presence (+) of 150μg of extract. Data represent the mean ± sd of three independent experiments.

Conclusions

Wine by-products could be used for the isolation of an aqueous ethanol extract with potent anti-platelet and anti-inflammatory actions that could lead to the production of functional foods with cardioprotective properties. Additionally, the sustainable exploitation of GP may be a useful strategy for reducing environmental contamination and as an alternative to reduce the carbon footprint in the winemaking process.

Acknowledgements

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