Protective effect of stilbenes in rats with severe acute liver failure. A new role for grapevine.

Fernandes JC[1;2]; Schemitt EG[2], Hartmann RM[2], Lima A[1,3], Marroni NP[2], Ferreira RB[1]

[1]Universidade de Lisboa, Instituto Superior de Agronomia, LEAF, Tapada da Ajuda, 1349-017 Lisbon, Portugal.
[2] Laboratory of Experimental Hepatology and Gastroenterology, Hospital de Clínicas de Porto Alegre, Porto Alegre 90040060, Brazil
[3]Faculty of Veterinary Medicine, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 376, 1749-024 Lisboa, Portugal

Introdution

Grapevine (*Vitis vinifera* L.) is one of the most economically important fruit crops in the world[1]. Grapevine health-promoting secondary metabolites such as stilbenes can be induced by various environmental stress factors. *V. vinifera* has been used in medicine due to the biological activities of several of its secondary metabolites in which stilbenes (*trans*-resveratrol and viniferins) are included[2]. Preliminary experiments performed by our group have shown that some viniferins isolated from stressed grapevines inhibited MMP-9[3], a group of proteases involved in cancer development and inflammation, and well-known mediators in liver disorders[4]. Due to the prominence of liver diseases, it becomes important to further study these secondary metabolites at physiological and molecular levels.
The goal of this report was to investigate the combined effect of viniferin and *trans*-resveratrol on severe acute liver failure induced by thioacetamide (TAA).

Experimental Procedures

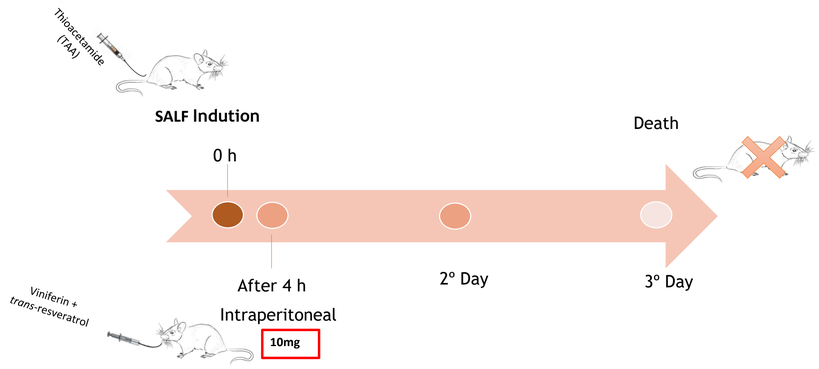

Fig. 1 Experimental timeline for severe acute liver failure (SALF) model. Male Wistar rats (n = 28) were divided into four groups: control, control+VR, TAA, and TAA + VR. Two TAA doses (400 mg/kg) were administered intraperitoneally, 8 h apart. The ε-viniferin + resveratrol (VR) (10 mg/kg +10 mg/kg) was administered at 30 min, 24 h, and 36 h.

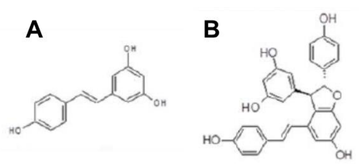

Fig. 2 Molecular structures of *trans*-resveratrol (A) and ε-viniferin (B).

Results

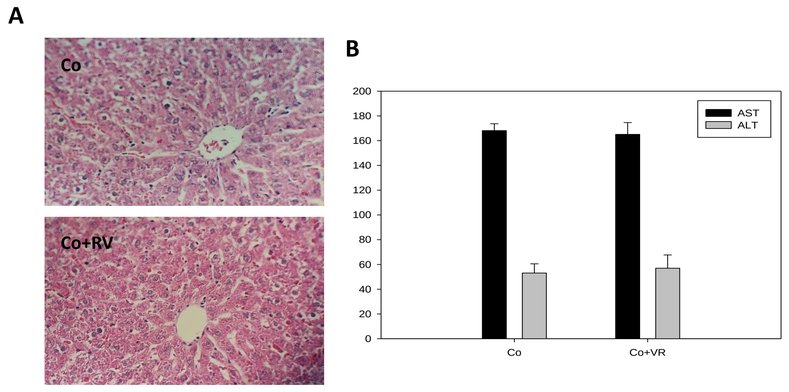

Fig. 3 Effect of Viniferin+Resveratrol (VR) on liver injury in animals exposed to an experimental model of severe acute liver failure. A) Representative photomicrographs; original magnification, 200 ×. Hematoxylin and eosin (HE) stain. B) Effect of Viniferin+Resveratrol on hepatic integrity levels in rats with severe acute liver failure. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

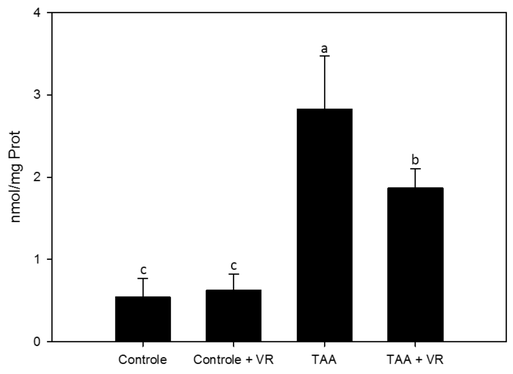

Fig. 4 Lipid peroxidation by the TBARS method revealed a significant increase in the TAA group in relation to the CO and CO + VR groups ($P < 0.001$). A significant reductions were observed in the TAA + VR group as compared with the TAA group ($P < 0.001$).

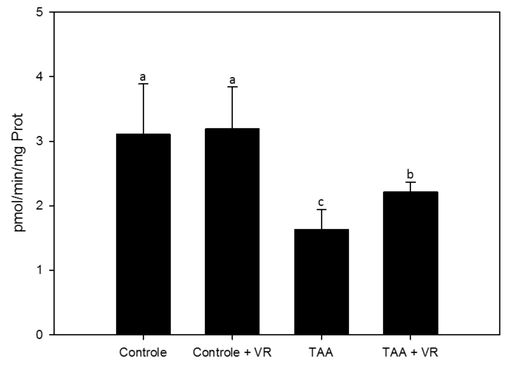

Fig. 5 Effect of VR on enzyme activity of glutathione *S*-transferases (GST) in the liver of rats with severe acute liver failure. The ε-viniferin+resveratrol treatment increased the values of GST enzyme activity closer to that of the control group ($P < 0.01$).

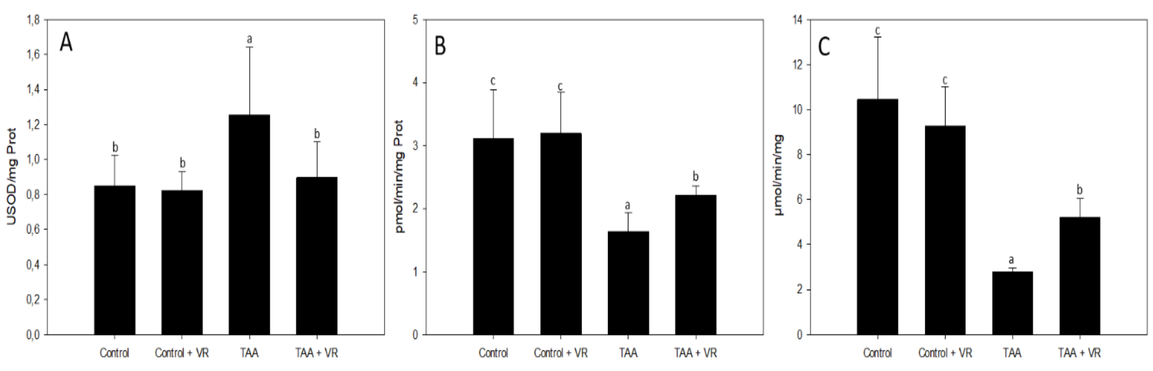

Fig. 6 Analysis of activity of the antioxidant enzymes SOD (A), CAT (B) and C) GST. CAT enzyme activity decreased in the TAA group as compared with the CO and CO + VR groups ($P < 0.001$), the administration of VR increased its activity compared with the TAA group ($P < 0.01$). SOD activity exhibited the inverse behavior; was higher in the TAA group than control ($P < 0.01$) and after the administration of VR a reduction in its activity ($P < 0.01$). GST had the same behavior as CAT.

Table 1 Evaluation of the genotoxic activity of ε-viniferin+resveratrol treatment using the alkaline version of the comet assay (n>3). Study indicate that ε-viniferin+resveratrol treatment can reverse, to some extent, the damage caused by TAA.

Group	Damage index	Damage frequency
CO	69.57± 9.79^{c}	38.57± 4.03^{c}
CO+VR	81.57± 22.58^{c}	42.14± 9.90^{c}
TAA	297± 15.16^{a}	91.75± 3.40^{a}
TAA+VR	176± 32.04^{b}	71.66± 13.57^{b}

DI: damage index: varies from 0 (no damage, 100 cells×0) to 400 (maximum damage, 100×4); DF (%): damage frequency: percentage of cells presenting damage. Different letters indicate significant differences at p<0.001.

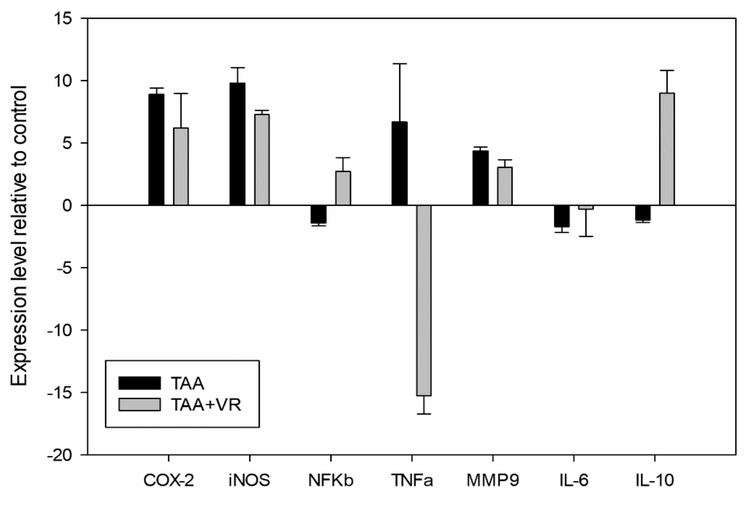

Fig. 6 Transcriptional responses to the application of viniferin and resveratrol in the liver of rats with experimental severe acute liver failure of several genes related to inflammation and oxidative stress. In this study iNOS and COX-2 show an up-regulation on TAA and TAA+VR relative to the control, but viniferin and resveratrol reduce the expression of these genes compered to TAA treated animals. The expression of the proinflammatory cytokines TNFα genes increased, and IL-6 was not altered in the animals that received TAA as a hepatotoxicant when compared to the control. The stilbenoid application reversed this state reduced the expression of TNFα compered to TAA group with a severer downregulation of this gene compared to the control, conversely the administration of viniferin and resveratrol did not affect the expression of IL-6 . The administration of viniferin and resveratrol also reduce the expression of MMP9 compared to TAA

Conclusions

Overall, our work shows that stilbenes might have an important role in the prevention of oxidative damage in the liver. The *in vivo* studies demonstrated that ε-viniferin+resveratrol administration in rats suffering from severe acute liver failure induced by TAA had a protective role in the antioxidant pathway. In this way, ε-viniferin+resveratrol may activate the antioxidant system, minimizing damage from the inflammation process. The results show a significant correlation between stereoisomers of *trans*-resveratrol oligomers and the prevention of liver diseases. In light of the present results and in the current worldwide situation, where we observe an increase in the frequency of liver diseases, the finding of novel molecules which can improve, or prevent, these pathologies can be of significant importance and should be considered as a top priority.

References

[1] Torregrosa L et al. (2015) Methods Mol Biol 1224:177-94; [2] Schmidlin L et al. (2008) Plant Physiol 148:1630-9; [3] Medina C (2006) J Pharmacol Exp Ther 318:933-8; [4] Faghihzadeh F (2016) J Res Med Sci 20:797–810

CEP/CEUA CEUA: 2018/494, ULBRA.

Acknowledgements

The research was funded by Fundação para a Ciência e a Tecnologia (FCT) grant SFRH/BPD/123393/2016 to JCF. LEAF grant Uid/agr/04129/2013.