Journal Pre-proof

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PII: S0899-9007(20)30066-6
DOI: https://doi.org/10.1016/j.nut.2020.110783
Reference: NUT 110783

To appear in: *Nutrition*

Received date: 18 September 2019
Revised date: 13 January 2020
Accepted date: 15 February 2020

Please cite this article as: Carla Petrella , Valentina Carito , Claudio Carere , Giampiero Ferraguti , Stefania Ciafrè , Fausta Natella , Cristiano Bello , Antonio Greco , Massimo Ralli , Rosanna Mancinelli , Marisa Patrizia Messina , Marco Fiore , Mauro Ceccanti , Oxidative Stress Inhibition by Resveratrol in Alcohol Dependent Mice, *Nutrition* (2020), doi: https://doi.org/10.1016/j.nut.2020.110783

Highlights:

- Chronic alcohol consumption determines metabolic and pathological alterations mainly due to the oxidative stress in the mouse.
- Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a non-flavonoid phenol with antioxidant properties.
- Resveratrol consumption prevents free oxygen radicals' formation measured in the serum of alcohol dependent mice.
- Resveratrol metabolites counteracts alcohol-induced alteration of BDNF in the liver, a target tissue of alcohol intoxication.

Oxidative Stress Inhibition by Resveratrol in Alcohol Dependent Mice

Carla Petrella[1]*, Valentina Carito[1], Claudio Carere[2], Giampiero Ferraguti[3], Stefania Ciafrè[1], Fausta Natella[4], Cristiano Bello[4], Antonio Greco[5], Massimo Ralli[5], Rosanna Mancinelli[6], Marisa Patrizia Messina[7], Marco Fiore[1]*, Mauro Ceccanti[8]

[1] Institute of Biochemistry and Cell Biology, IBBC−CNR , Rome, Italy
[2] Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy
[3] Department of Experimental Medicine, Sapienza University of Rome, Italy
[4] CREA-AN, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Food and Nutrition Research Centre, Rome, Italy
[5] Department of Sense Organs, Sapienza University of Rome, Italy
[6] Centro Nazionale Sostanze Chimiche, ISS, Rome, Italy
[7] Department of Gynecological and Obstetrics Sciences, Sapienza University of Rome, Italy
[8] Centro Alcologico della Regione Lazio (CRARL), ASL Roma 1, Rome, Italy

Corresponding Authors*:

Dr. Marco Fiore PhD

Institute of Biochemistry and Cell Biology, IBBC−CNR

Via del Policlinico 155, 00161 Roma, Italy.

E-mail address: marco.fiore@cnr.it

Dr. Carla Petrella PhD

Institute of Biochemistry and Cell Biology, IBBC−CNR

Via del Policlinico 155, 00161 Roma, Italy.

E-mail address: carla.petrella@cnr.it

Abstract

Objective**:** uncontrolled ingestion of alcohol has dramatic consequences on the entire organism also associated with the oxidation process induced by alcohol by elevating radical oxygen species (ROS). Resveratrol, a non-flavonoid phenol, shows well-documented antioxidant properties. We investigated the potential antioxidant ability of this natural compound in a mouse model of alcohol addiction.

Methods: we administered (per os) for two months 10 mg/kg/day of resveratrol in alcoholic adult male mice. Oxidative stress was evaluated by measuring serum free oxygen radicals defense (FORD) and free oxygen radicals (FORT) levels. Resveratrol metabolites were measured in the serum of mice administered with resveratrol. Finally, the effect of resveratrol on alcohol-induced alteration of BDNF in the liver was investigated.

Results: prolonged consumption of resveratrol strongly counteracts serum ROS formation caused by chronic alcohol intake, without effects on natural, free oxygen radical defense. The presence of resveratrol metabolites only in the serum of animals supplemented with resveratrol potentiates the evidence that the antioxidant effect observed is due to the ingestion of the natural compound. Moreover, resveratrol supplementation can counteract alcohol-induced BDNF elevation in the liver, the main target of organ alcohol-induced damage.

Conclusion**:** the consumption of resveratrol through metabolite formation may play a protective role, by decreasing free radical formation, and by modulating BDNF involved in hepatic disruption induced by chronic alcohol consumption. Further investigation about the mechanism underlying the protective effect could reinforce the potential use of resveratrol as a dietary supplement to prevent damage associated with chronic alcohol abuse.

Keywords: addiction; alcohol use disorders; antioxidant; polyphenols; resveratrol; BDNF.

Introduction

Chronic alcohol consumption represents, in many countries, a social and public health problem. Scientific research has constantly focused on finding solutions not only for the management of psychological dependence but also for the treatment of the numerous pathologies resulting from the abuse of alcohol [1–3].

Uncontrolled and continuous ingestion of alcohol has dramatic consequences on the entire organism. Indeed, long-term alcohol abuse can cause several clinical conditions, including cirrhosis of the liver, chronic pancreatitis, epilepsy, polyneuropathy, heart disease, nutritional deficiencies and neuro-behavioral problems [4–13].

In human adults, ethanol is oxidized to acetaldehyde using NAD+, mainly via the hepatic enzyme alcohol dehydrogenase (ADH). In fetuses and adults, ethanol is metabolized by different enzymes (cytochrome P-450 superfamily, in particular by CYP2E1). In the presence of ethanol and oxygen, CYP2E1 releases superoxide radicals and induce the oxidation of polyunsaturated fatty acids to toxic aldehyde products. Acetaldehyde is a compound highly unstable and quickly forms free radicals that are highly toxic if not extinguished by antioxidants [13,14]. In the fetus, these free radicals can damage embryonic neural crest cells and can lead to severe birth defects, mental retardation and physical abnormalities in the newborns (Fetal Alcohol Spectrum Disorder, FASD) [15,16].

In chronic alcoholics, prolonged exposure of the kidney and liver to these compounds can lead to severe damage. Acetaldehyde is transformed into acetic acid by aldehyde dehydrogenase (ALDH2). Finally, the acetic acid is transformed in acetyl-CoA by acyl-CoA synthetase and by acetyl-CoA-synthase-2 localized in the mitochondria. Once acetyl-CoA is formed, it enters the normal citric acid cycle [13,14].

In human adults, the excess of both acetate and NADH cofactor inhibits the normal aerobic metabolism of the Krebs cycle, shifting towards lipid metabolism, with the synthesis of

triglycerides, leading to the steatosis of the liver [14]. These alterations are generally associated with the oxidation process of alcohol and more particularly with the oxidative stress resulting in the production of free radicals and lipid peroxidation [17]. As consequences, these processes result in an excessive production of acetaldehyde, formation of adducts at the level of cellular proteins, inactivation of enzymes, interference with the mechanisms of DNA repair and depletion of antioxidant systems [13]. The oxidative stress in cells, derived from alcohol consumption and associated with the altered lipidic metabolism, also induces morphological and functional alterations both in different brain regions [18,19] and in peripheral tissues [7–11].

Neurotrophic factors, like Brain-Derived Neurotrophic Factor (BDNF), have been largely studied for their involvement in neuronal development and plasticity. It's well known that they also regulate responses to drug abuse, including alcohol [20,21]. Numerous experimental pieces of evidences show that ethanol alters the expression of different neurotrophins in various brain areas [21,22], however little is known about the alcohol effects in non-neuronal tissues.

Epidemiological evidence supports the hypothesis that modifiable lifestyle-related factors are associated with neurological impairments, opening new avenues for the prevention of central disorders [23]. In particular, the contribution of diet has become the object of intense research and, as recently suggested, some of the neurodegenerative processes associated with central nervous system disorders may be influenced by a targeted diet [24,25]. Indeed, great attention focused on the role of different dietary constituents in the prevention or in counteraction of brain disorders [26].

Between dietary components, antioxidants are considered of interest in the context of alcohol-related disorders. We have recently demonstrated in mice that polyphenols, organic molecules of natural origin, widely present in the plant reign, produce beneficial effects to counteract the damage induced by chronic ethanol consumption, because of the antioxidant and anti-inflammatory properties [27–31].

Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a non-flavonoid phenol, is one of the phytoalexins naturally produced by several plants (Table 1), as a defensive mechanism against

pathogenic agents. Resveratrol shows a plethora of well-documented favorable actions in humans, as well as in experimental models. Anti-inflammatory [32–34] and protective vessel/endothelium [35,36] properties are of great interest for managing different pathological conditions.

The present study aimed to investigate the effect of oral administration of resveratrol in a mouse model of chronic alcohol addiction. We have evaluated the potential antioxidant ability of this natural compound, by measuring serum Free Oxygen Radicals Defense (FORD) and Free Oxygen Radicals Test (FORT) levels, in mice chronically addicted to alcohol. Moreover, we assessed the serum levels of resveratrol metabolites to support the hypothesis that the effects observed could be related to the compound. Finally, the effects of resveratrol on alcohol-induced alteration of BDNF in the liver, the main target tissue of alcohol intoxication, were investigated.

Methods

Animals

CD-1 outbred male mice were used in this experiment. All animals were three months old and housed in groups of 5 mice at the beginning of the experiments in Plexiglas cages (33 x 13 x 14 cm) under standardized conditions with pellet food (enriched standard diet purchased from Mucedola, Settimo Milanese, Italy). Food (Purina Lab Chow # 5015) and water were available ad libitum. A 12 L:12 D lighting regime was used. 40 male CD-1 mice were randomly divided into four groups: *(i)* a group of mice (CTR, n =10) received sucrose dissolved in water at equivalent caloric intake of the ethanol group and was used as control group; *(ii)* another group of mice (EtOH, n = 10) received ad libitum, as only source of liquid, after an habituation period, ethanol 11% dissolved in water for 60 days; *(iii)* the third group of mice (Resv, n =10) received resveratrol (10mg/Kg/day) dissolved in sucrose; and *(iv)* a further group of mice (EtOH+Resv, n = 10) received resveratrol (10mg/Kg/day) dissolved in ethanol 11% (again following an habituation period). The control and the ethanol groups were established, according to indication previously released [27].

Ethanol used for the preparation of the drinking solution was obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and was of analytical grade. Fluid intake was measured regularly and the amounts consumed were calculated according to methods previously established [37]. All groups received pellet food ad libitum as above. Food intake was measured regularly and the daily amounts consumed were calculated. Two months after treatment, mice were sacrificed for the experiments. All efforts were made to minimize and reduce animal suffering and for limiting the number of animals used. All animal experiments were carried out following the procedure described by the guidelines of the European Community Council Directive of 24 November 1986 (86/609/EEC). Permission number 08-2014 of February 3, 2014.

Drugs

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) was purchased from Sigma-Aldrich (Italy) and freshly prepared by suspending in water just before administration.

Blood and tissues preparation

Animals were sacrificed by a guillotine. The blood was collected in vials and quickly centrifuged at 10000 rpm for 15 min for serum preparation (n = 5 per group) or in heparin vials for the measurement of blood ethanol levels (n = 5 per group). Serum aliquots were then stored at -80°C, while heparin vials were stored at 4°C. Tissue samples were homogenized by ultrasonication in RIPA buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 5 mM EDTA; 1% Triton X-100; 0.1% SDS; 0.5% sodium deoxycholate; 1 mM PMSF; 1 mg/mL leupeptin), kept in a cold room on a rotary shaker for 2 h to allow the complete tissue disaggregation and cell lysis, and then centrifuged at 10,000 g for 30 min at 4°C. The supernatant was stored at -80°C till the day of analyses.

Blood ethanol levels by gas chromatography/head space procedure

Gas chromatography/Head Space procedure was applied in this research to determine blood alcohol concentration in whole blood samples (n 1⁄4 5 for each group). In this research, a Clarus 600 Gas Chromatography Perkin Elmer and a TurboMatrix 40 Trap HeadSpace Sampler Perkin Elmer with FID detector were used. Analytical conditions were set up and the method was validated by a previous study [12]. From each blood sample was collected 100 mL of whole blood with a micropipette and transferred this volume into a gas chromatography vial. The gases which are formed inside gas chromatography vial were collected to be analyzed. If the sample was not analyzed in the same day as its collection, it was important to firmly close the vial to prevent the evaporation of ethanol during the time and to conserve the vial inside of a refrigerator. Standard solutions were set up for calibration curve at 100 mg, 50 mg, 25 mg, 12.5 mg, 6.2 mg, and 3.1 mg of ethanol and were obtained by consequent dilutions of pure ethanol in distilled water.

Free Oxygen Radicals Defense (FORD) and Free Oxygen Radicals Test (FORT)

FORD and FORT tests were carried out using two specific kits (both purchased by Callegari, Parma, Italy) following the instruction provided by the manufacturer with minor modification [38,39].

Briefly, this test uses a preformed stable and colored radical and determines the decrease in absorbance that is proportional to the antioxidant concentration of the sample according to Lambert Beer's law [40]. In the presence of an acidic buffer (pH= 5.2) and a suitable oxidant (FeCl3) the chromogen, which contains 4-amino-N,N-diethylaniline sulfate forms a stable and colored radical cation photometrically detectable at 505 nm. Antioxidant compounds in the sample reduce the radical cation of the chromogen quenching the color and producing a discoloration of the solution, which is proportional to their concentration. The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E commonly used as a reference. By contrast, FORT test allows the determination of free oxygen radicals (ROSs) through a colorimetric assay based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxides (ROOH) into derivative radicals, according to Fenton's reaction (Pavlatou MG, 2009). Briefly, when 20 mL of the blood serum sample was dissolved in an acidic buffer, the hydroperoxides reacted with the transition metal ions liberated from the proteins in the acidic medium and were converted to alkoxy- (RO-) and peroxy- (ROO-) radicals. The radical species produced by the reaction interact with an additive (phenylendiamine derivative (2 CrNH2)) that forms a colored, fairly long-lived radical cation evaluable by spectrophotometer at 505 nm (linear kinetic-based reaction, 37°C). The intensity of the color correlates directly to the quantity of radical compounds and to the hydroperoxide concentration and, consequently, to the oxidative status of the sample according to the Lambert-Beer law (Form CR 2000; Callegari, Parma, Italy).

Serum Resveratrol Metabolites Measurement

The serum for resveratrol metabolites measurement was immediately prepared by centrifugation at 1500g for 30 min at 20°C. Serum aliquots were acidified at pH 3 adding formic acid and stored at -80°C. 50 µl of samples were added with 150 µl ice-cold acetonitrile (ACN) and with 5 µl of Internal Standard (ethyl gallate 0.3 mg/ml). After vortexing, the samples were centrifuged at 14000 rpm for 10 min at 4°C. The supernatants were filtered through 0.20-µm syringe PVDF filters before MS analysis.

Quantitative on-line HPLC-ESI-MS/MS analyses were performed using HPLC system interfaced to an Applied Biosystems (Foster City, CA, USA) API3200 Q-Trap instrument working with triple quadrupole analyzer in Multiple Reaction Monitoring (MRM) mode. LC analyses were conducted using a system equipped with a 200-binary pump (Perkin-Elmer, USA). Samples were injected (15 µl) into an Ultra II Aqueous C18 (Restek) (100 x 2.1 mm i.d., 3µm) and eluted at a flow rate of 0.25 ml min^{-1}. Mobile phase A was H_2O containing 0.1% formic acid while mobile phase B was acetonitrile containing 0.1% formic acid. Elution was carried out using a gradient commencing at 90% A for 7 min, then changing to 20% A in 6 min, remaining at 20% A for 9 min, and finally returning to 90% A in 10 min. The column was kept at 30 °C, using a Peltier Column Oven Series 200 (Perkin Elmer). The flow from the chromatograph was injected directly into the ESI source. The API 3200 ES source was tuned by infusing solutions of Resveratrol and ethyl gallate (1 µg ml^{-1} in methanol 50%) into the source at a flow rate of 10 ml min^{-1}. The MS operated with an electrospray voltage at -4500V and with source temperature of 500°C. Nitrogen was used as ion spray (GS1), drying (GS2) and curtain gas at 40, 20 arbitrary units, respectively. The declustering potential (DP), collision energy (CE) and entrance potential (EP) for resveratrol and ethyl gallate were; -45, -35, -7; while for resveratrol metabolites they were: 45, -25, -7. Resveratrol sulfate, dihydro-resveratrol glucuronide dihydro-resveratrol sulfate and ethyl gallate were detected with MRM transition of 307/227, 405/228, 309/229 and 197/124 [M-H], respectively. Data

acquisition and processing were performed using Analyst software 1.5.1. The quantification of resveratrol metabolites was performed by using a calibration curve of Resveratrol.

BDNF determination

BDNF evaluation was carried out with ELISA kit ‘‘BDNF EmaxTM ImmunoAssay System” number G7611 by Promega (Madison, WI, USA) following the instructions provided by the manufacturer [41]. The colorimetric reaction product was measured at 450 nm using a microplate reader (Dynatech MR 5000, PBI International, USA). BDNF concentrations was determined, from the regression line for the BDNF standard (ranging from 7.8 to 500 pg/ml purified BDNF) incubated under similar conditions in each assay. The BDNF sensitivity of the assay was about 15 pg/ml of wet tissue and cross-reactivity with other related neurotrophic factors (NGF, neurotrophin-3 and neurotrophin-4) was less than 3%. Data are represented as pg/mg total proteins and all assays were performed in duplicate which were averaged for statistical comparison.

Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) considering as main factors ethanol and resveratrol administration. Post-hoc comparisons were performed using the Tukey’s HSD test. Data are illustrated as mean ± SEM. Statistical significance was accepted at a P value of less than .05.

Results

Blood ethanol levels and Resveratrol effects on food, liquid intake and body weight:

Under the present experimental conditions, ANOVA did not show significant changes in body weight during two months of treatment and at the end of the treatment between groups. Moreover, food and liquid intake during two months of treatment were comparable between groups. The ethanol blood levels in the ethanol groups ranged from 3.2 to 21.2 mg/100 mL [27].

Oxidative stress evaluation

In order to evaluate whether prolonged consumption of resveratrol might exert an antioxidant effect in alcohol addicted mice, we analyzed FORT and FORD levels in the serum. We found that FORT levels in blood serum were significantly higher in EtOH mice as compared to controls, $p<0.01$ in the ANOVA. Quite interestingly, the consumption of resveratrol partially counteracted this effect, Fig. 1A for post-hoc comparisons. However, the levels of FORD in blood serum were significantly lower in ethanol-treated mice (EtOH and Resv + EtOH) as compared to CTR group, $p<0.01$ in the ANOVA, see Fig. 1B for post-hoc comparisons. However, resveratrol consumption did revert the effect of ethanol on the endogenous defense system as disclosed by FORD ANOVA data.

Serum Resveratrol metabolites

Table 2 shows the mean values of resveratrol metabolites expressed as µg/ml: resveratrol sulfate (RVS), dihydro-resveratrol glucuronide (DHRVG), dihydro-resveratrol sulfate (DHRVS) in each experimental group. In the serum of CTR mice and EtOH mice, no resveratrol metabolites were observed. As predicted, the presence of RVS, DHRVG and DHRVS was observed in the serum of Resv and Resv + EtOH groups.

BDNF determination

Figure 2 shows the BDNF data in the liver. Chronic alcohol consumption increases BDNF levels $p<0.05$ in the ANOVA. Resveratrol per se did not modify endogenous BDNF liver levels, however, Resveratrol partially counteracted this increment as revealed by post-hoc comparisons.

Discussion

In this study, we demonstrated that prolonged consumption of Resveratrol strongly counteracts serum-free oxygen radical formation, caused by chronic alcohol intake, without influencing the natural free oxygen radical defense in a mouse model of alcohol addiction. We did also demonstrate for the first time that the presence of resveratrol metabolites only in the serum of animals supplemented with resveratrol, strongly supports the evidence that the antioxidant effects observed are due to the ingestion of this natural compound.

Although the medical use of natural compounds is of very ancient origin, only recently the availability of studies conducted with the scientific method has allowed to rigorously and reproducibly document the efficacy of some substances supplemented in the diet.

Concerning ethanol addiction, it is well known that the chronic consumption of alcoholic drinks causes not only psychological dependence [42], but it severely compromises physical health [18]. Among factors that influence alcoholic liver disease (the main side effect of alcohol abuse), the role of oxygen is certainly relevant [43]. The normal production of molecular fragments containing oxygen represents the side effects of many metabolic reactions and is neutralized by antioxidant compounds present inside the cells (such as glutathione and vitamin A and E). When the production of free radicals is in excess or the anti-oxidant defenses are inadequate, the aggression of the radicals leads to lipids peroxidation in the cellular membranes. Based on this consideration, the use of antioxidants in the diet could be a quite useful tool to counteract the formation of free radicals. In a previous study, we have demonstrated that olive polyphenols supplementation may partially counteract the alcohol pro-oxidant effects in a mouse model of alcohol addiction [27]. A powerful antioxidant action [44–46] is common to the chemical class of polyphenols also containing resveratrol; this makes resveratrol an extremely interesting compound for its potential use in pathological conditions where the redox system is altered [44].

The present studies confirm and extend other investigations [47,48] describing the antioxidant effect of resveratrol in rodent models of ethanol addiction. In particular, oxidative stress modulation

together with anti-inflammatory properties of resveratrol significantly prevented cognitive deficits induced by chronic ethanol exposure in different brain regions of ethanol-treated rats. Also in alcohol-addicted mice, resveratrol ameliorated ethanol-induced oxidative challenges and angiogenesis processes [47].

However, the route of administration for resveratrol chosen in our experimental protocol (drinking water) allowed us to mimic a widely accepted method of intake in humans. In this sense, we do strongly believe that the results obtained in our study may provide translational confirmations for a potential for use in humans.

In the present experimental conditions of a chronic alcohol addiction in the mouse, for the first time, serum metabolites of resveratrol have been measured, demonstrating that *i)* resveratrol may be completely absorbed by the gastrointestinal tract; *ii)* the beneficial antioxidant properties observed might be, also, due to the effects of the Resveratrol metabolites. The absence of resveratrol or its metabolites in the serum of control mice indicates that resveratrol is rapidly metabolized and transformed in its main products: resveratrol sulfate, dihydro–resveratrol glucuronide, dihydro-resveratrol sulfate. Indeed, recent crucial findings support the hypothesis that the resveratrol metabolites may play a key role in the pharmacological effect of resveratrol. In particular, glucuronide and sulfate conjugates show antioxidant and anti-inflammatory activities, but also cytotoxicity against various cancer cell lines [49]. Furthermore, another study clearly demonstrated that resveratrol metabolites may elicit comparable functions to those induced by resveratrol further demonstrating their critical roles in contributing to the *in vivo* biological activities of the parent molecule. Finally, some anti-inflammatory properties of the metabolites of resveratrol have been disclosed in the regulation of chemokine levels in macrophages activated by LPS *in vitro* [50].

Another novel interesting finding of the present work regards the modulation of BDNF in peripheral tissues. BDNF, as other neurotrophic factors, is primarily involved in the development and maintenance of neuronal integrity and functionality [51–53], but its role also in non-neuronal

sites is growing. A recent research shows an altered level of BDNF in the brain and in the liver of patients affected by different psychiatric disorders, suggesting that these abnormalities could participate in the pathophysiology of such central diseases [54]. Also, diet could modulate BDNF levels in the hepatic gland. As previously shown, the brain and liver of mice fed with different fat content in the diet, present altered BDNF expression with circadian oscillation, supporting its role in the pathogenesis of feeding disorders, as obesity [55]. It's well known that chronic alcohol consumption has a dramatic impact on the CNS, target for direct and indirect consequences of alcohol abuse [56]. While it is well established that drug and alcohol consumption may cause also severe disruptions in neurotrophic factors levels, but only a few studies investigated their impact on peripheral tissues. Concerning alcohol addiction, we previously demonstrated that, in a mouse model of FASD, offspring of addicted mice were characterized by alterations in BDNF and other growth factors expression in the liver [37,57]. In the present work, we evidence a BDNF modulation induced by resveratrol in the liver, the main target organ of alcohol-induced injury. BDNF binding to hepatocytes leads to the activation of catabolic pathways, such as fatty acid oxidation and gluconeogenesis inhibition [58]. Additionally, a recent work of Berna et al. shows how BDNF could prevent the apoptosis of cells in the liver and kidney, and increasing cell survival and contributing to tissue integrity [59]. Hepatic stellate cells were found to express BDNF and TrkA but not TrkB [60] demonstrating that the liver is a powerful source of several neurotrophins and neurotrophin receptors. These findings correspond with the known involvement of hepatic stellate cells in liver remodeling, in the production of extracellular matrix components and in cell proliferation in acute necrotizing liver pathology. In analogy with findings in other organs and systems, neurotrophins are hypothesized to play a role in the pathophysiology of liver diseases. Based on these considerations, the increased BDNF levels found in the liver of alcohol-addicted mice are in line with the postulation of a compensatory system that attempts to react to the hepatic damage induced by alcohol consumption [60]. The consumption of resveratrol may play a hepato-

protective role also through preventing the increase in neurotrophic factors in peripheral sites potentiating the maintenance of a physiological homeostatic balance.

Most of the current research lines are focused on the use of natural products as adjuvants in the treatment of different conditions that alter the body's homeostasis. Among these, certainly anti-oxidant compounds receive the attention of the scientific community for their potential wide use in multiple pathological circumstances. The results of the present work confirm and extend many other experimental pieces of evidence on the use of resveratrol, as well as of other antioxidant polyphenols, capable to prevent oxidative imbalance induced by chronic alcohol consumption [28,29]. Resveratrol is present in red wine whose consumption in moderate doses was speculated, although strongly debated [61], to elicit beneficial cardiovascular effects [62]. Indeed, the French paradox summarizes the apparently paradoxical epidemiological observation that French people have a relatively low incidence of coronary heart disease while having a diet relatively rich in saturated fats, in apparent contradiction to the widely held belief that the high consumption of such fats is a risk factor for coronary heart disease. As a first difference compared to the other European populations, it was speculated that the greater consumption of wine, mainly the red one, could bring greater antioxidant content, in particular by resveratrol, capable of playing a protective antioxidant role and therefore fundamental for the prevention of cardiovascular diseases. However, the amount of resveratrol needed to manifest these effects cannot be achieved through moderate wine consumption or throughout the consumption of other natural sources of resveratrol (see Table 1). Indeed, total resveratrol contained in red wine (1.90-12.60 mg/L) is not at all sufficient to carry out a cardiovascular protection effect, however, it could contribute significantly. Quite interestingly, previous studies on a FASD mouse model showed differences in ethanol-induced toxicity (but not enough to remove the alcohol damage) when ethanol is administered alone or in the red wine demonstrating a sort of minimal neurotrophic/neurobehavioral protection by red wine probably because containing compounds with antioxidant properties as polyphenols [37,57,63,64]. Anyway, those studies always stressed the point that women must avoid drinking alcohol during gestation.

In conclusion, the present findings strongly provide evidence that resveratrol consumption may elicit powerful antioxidant properties. Accordingly, resveratrol could be considered an interesting and valuable adjuvant compound in those conditions where the alteration of the redox state plays a decisive role in determining a pathological circumstance. Furthermore, these basic research data may be also of interest to those researchers working in the fields of human dependence.

Acknowledgements

Authors thank Sapienza University of Rome and IBBC-CNR for supporting their study.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure Captions

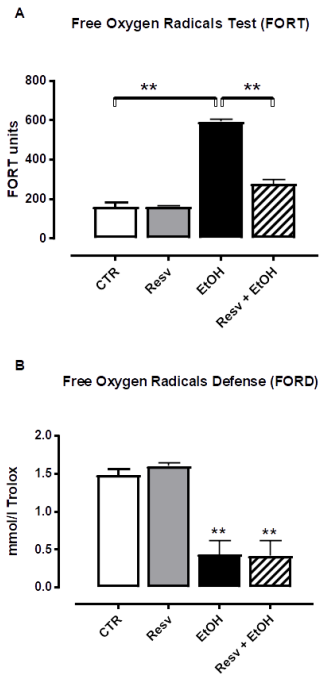

Figure 1

FORT (A, expressed as FORT unit; see Methods) and FORD levels (B, expressed as mmol/Trolox eq; see Methods) in blood serum of control or alcoholic mice treated or not with 10mg/kg/day resveratrol for 60 days. The vertical lines in the figures indicate pooled standard error means (SEM) derived from appropriate error mean square in the ANOVA. Asterisks indicate significant differences between groups (**P < 0.01); in panel B asterisks indicate significant differences between EtOH treated groups and the other experimental groups.

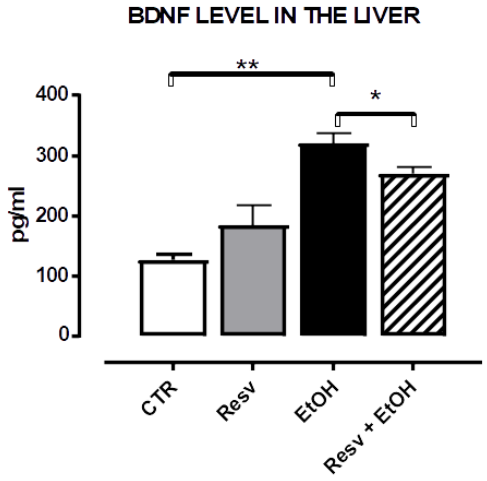

Figure 2

Liver BDNF expressed as pg/mg total proteins of control or alcoholic mice treated or not with 10 mg/kg/day resveratrol for 60 days. The vertical lines in the figures indicate pooled standard error means (SEM) derived from appropriate error mean square in the ANOVA. Asterisks indicate significant differences between groups (*P < 0.05; **P<0.01).

Table 1: Resveratrol concentration found in some natural sources.

Source	**Resveratrol concentration**	**References**
Bilberries	≈ 16 ng/g	[65]
Blueberries	≈ 32 ng/g	[65]
Cocoa powder	0.28 – 0.46 mg/cup	[66–68]
Cranberry raw juice	≈ 0.2 mg/L	[65]
Green peanuts	0.087 µg/g	[69]
Peanut butter	0.04-0.13 mg/cup	[66–68]
Peanuts (boiled)	0.32-1.28 mg/cup	[66–68]
Peanuts (raw)	0.01-0.26 mg/cup	[66–68]
Red grapes	0.24-1.25 mg/cup	[66–68]
Red wine (global)	1.9 ± 1.7 mg/L	[70]

Table 2: Resveratrol and its metabolites expressed in µg/ml (mean ± SEM) in the serum of mice administered daily for 2 months with 10mg/kg of resveratrol and their respective controls. RV: resveratrol, RVS: resveratrol sulfate, DHRVG: dihydro-resveratrol glucuronide, DHRVS: dihydro-resveratrol sulfate.

Groups	Resveratrol Metabolites			
	RV	**RVS**	**DHRVG**	**DHRVS**
CTR	n.d.	n.d.	n.d.	n.d.
EtOH	n.d.	n.d.	n.d.	n.d.
Resv	n.d.	0.045 ± 0.015	0.025 ± 0.001	0.051 ± 0.001
Resv + EtOH	n.d.	0.0955 ± 0.013	0.0615 ± 0.01	0.0798 ± 0.03

Graphical abstract