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on Fundamental and Applied Aspects of
Physical Chemistry

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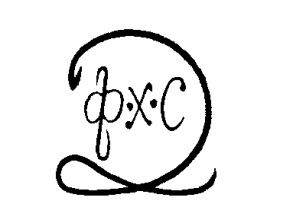

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EXAMINATION OF PROOXIDATIVE ACTIVITY OF RED WINE IN MELANOMA CELLS

N. Đorđević [1], L. Korićanac [1], J. Žakula [1], N. Todorović Vukotić [1], S. Pejić [1], V. Tešević [2] and S. B. Pajović [1]

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ABSTRACT
Melanoma is responsible for 75% of all deaths from skin cancer. Its lethality arises from its rapid progression, easy metastasis and drug-resistance as well. Red wine is a natural product rich in polyphenolic compounds with potent anticancer activities. It seems that in cancer cells these compounds behave as prooxidants initiating reactive oxygen species mediated cellular DNA breakage and consequent cell death. The aim of this study was to investigate prooxidative activity of red wine samples (Merlot variety, commercial as well as VCR1 and VCR101 clonal wines) in melanoma A375 cells, through measuring the relationship of reduced and oxidized form of glutathione (GSH/GSSG) and comparison with the GSH/GSSG ratio in control (untreated melanoma cells). The data obtained showed that tested red wine samples decrease GSH/GSSG ratio in A375 cells compared to control (4.6 ± 0), with the largest decrease noticed in treatment with VCR101 wine (0.66 ± 0.05).

INTRODUCTION
Wine is a natural product with well-known beneficial effects on human health. Especially the red one is rich with the polyphenols, compounds with wide range of biological activities, like antioxidant, anti-inflammatory and chemopreventive activities. Since novel studies pointed out a connection between inflammation and cancer development, consummation of phenolic rich natural products, like red wine, could be very useful for cancer prevention [1].

Antioxidant activity of the polyphenolic compounds is one of the most important features related to biological activity of these compounds in healthy cells. On the other hand, polyphenols exert a wide variety of anticancer effects. They modulate activities of ROS-scavenging enzymes, take part in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness [2]. An increasing number of data indicate that polyphenols can act as prooxidants due to the action of reactive oxygen species, and thus initiate the breakdown of cellular DNA, and therefore cell death [3].

Melanoma is less common, but the most malignant skin tumor. It belongs to one of the most malignant tumors in humans. The incidence of melanoma is constantly increasing in recent decades, so for the last 20 years an increase of up to 200% over has been reported [4]. This report investigated prooxidative activity of commercial and red wine obtained from specific vine clones (VCR1 and VCR101) of Merlot variety in melanoma A375 cells aiming to complete biological characterization of specific vine clones.

METHODS
In order to examine the prooxidative effect of red wine in cancer cells, melanoma A375 cells were treated with 5% concentration of analysed wine in exponential growth phase. The wines used in this study were of Merlot variety, both, commercial wine and wines obtained from two vine clones (VCR1 and VCR101). The melanoma cells were prepared according to method of Rahman et al. [5]. After

24 h incubation, A375 cells were washed with 3 mL of cold 0.1 M phosphate buffer pH 7.4. Thereafter, cells were tripsinized with 0.5 mL Try-EDTA for 5 min at room temperature and collected in 2 mL of cold medium. After centrifugation for 3 min at 2000 g on room temperature, the supernatant was pour off and the residue was resuspended in 2 mL of 0.1 M phosphate buffer pH 7.4. The cells were again centrifuged at 4000 g, 10 min and resuspended in 500 μL of extraction buffer (0,1 M potassium phosphate buffer pH 7,5 with 5 mM EDTA, 0,1% Triton X100 and 0.6% sulfosalicylic acid). Finally, melanoma cells were lysed for 30 min on ice with vortexing. After centrifugation at 4000 g, 20 min at 4 °C, the resulting supernatant was collected in chilled tubes.

The content of reduced and oxidized glutathione was determined according to modified method of Salbitani et al. [6]. The reaction mixture (sample) contained 600 μL of extraction buffer, 40 μL 0.4% DTNB and 50 μL of cell extract/GSH standard. Blank contained 650 μL of extraction buffer and 40 μL 0.4% DTNB. After 5 min of incubation at room temperature, the absorbance was measured on 412 nm. The content of GSH was calculated from standard curve. Thereafter, 50 μL 0.4% NADPH and 1 μL 0.5 U of glutathione reductase were added in reaction mixture. After 30 min of incubation at room temperature, the absorbance at 412 nm was measured, and the concentration of total glutathione was calculated from the standard curve for GSH. The concentration of GSSG was determined by subtraction of the content of GSH from the determined concentration of total glutathione. The obtained GSH/GSSG ratio in analysed samples was compared with the GSH/GSSG ratio in control (untreated melanoma cells). All data were displayed as mean value ± SD and analyzed by two-way ANOVA followed by Tukey's HSD test.

RESULTS AND DISCUSSION

The obtained data revealed that GSH/GSSG ratio in melanoma A375 cells treated with all three samples of analysed wines was significantly lower ($p < 0.001$) than GSH/GSSG ratio in control cells (4.6 ± 0). This ratio was lower in melanoma cells treated with clonal (VCR1 and VCR101) wines compared to commercial wine. Furthermore, the treatment of melanoma cells with VCR101 clonal wine mostly reduces the GSH/GSSG ratio (0.66 ± 0.05). Prooxidative effect of wine samples could be attributed to their polyphenolic compounds. Previous literature data have just pointed out prooxidative activity of some polyphenols, mostly flavonoids, through their various ways of cytotoxic effects on cancer cells [7,8]. On the other hand, previous research has also shown that red wine and its polyphenols increase GSH intracellular level in human erythrocytes of healthy donors mainly due to the elevated activity of glutathione reductase [9,10]. Namely, flavonoids are potent antioxidants under normal and pro-oxidants under pathological conditions, when they can target apoptotic signalling cascade activating apoptosis and also can suppress proliferation and inflammation.

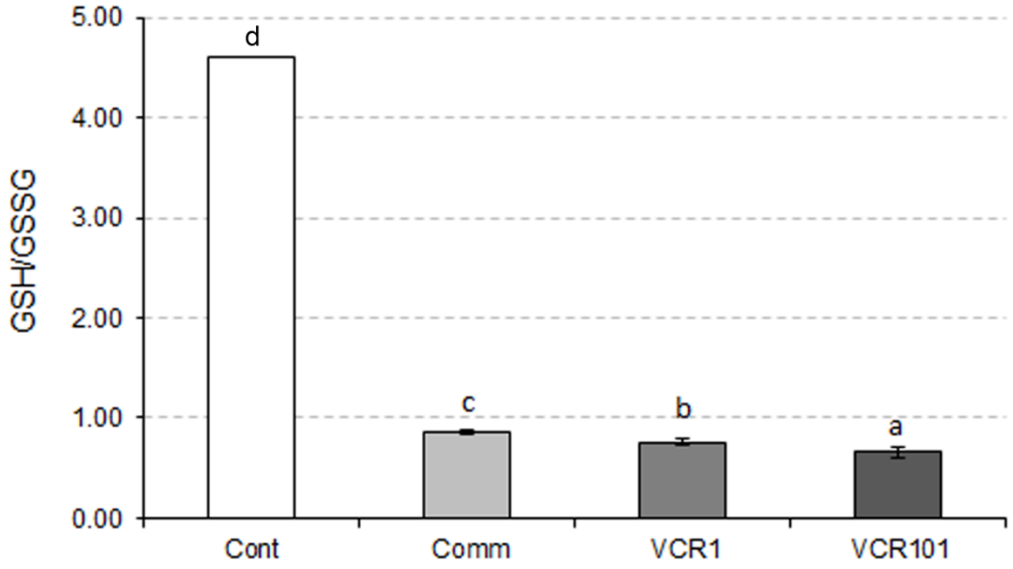

Figure 1. GSH/GSSG ratio in control A375 cells (Cont) and A375 cells treated with Merlot red wine samples, commercial wine (Comm) and wine obtained from two vine clones VCR1 and VCR101. All measurements were done in triplicate and the obtained results are expressed as mean value ± SD. Different letters show significant differences between obtained values ($p < 0.05$), according to Tukey's HSD test.

The obtained results further complement previously published results about chemical composition and biological activity of specific red wine clones of Merlot variety, VCR1 and VCR101 [11]. These results also further recommended mentioned clones for use in standard vinification procedures.

CONCLUSION

From the obtained results, it can be concluded that the cause of the shifted GSH/GSSG balance towards GSSG is probably due to the prooxidative effect of red wines in A375 cells. This result is very important having in mind the chemo- and radio-resistance of melanoma cells. The given conclusion is in accordance with the previous scientific assumptions and findings that polyphenolic compounds of plant origin exhibit their anticancer activity by prooxidative action. Finally, this study highlights red wine itself as polyphenolic-rich food that, in moderate consumption, could reduce the risk of cancer.

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