DIMITRIOS STAGOS

Title of thesis: «Study of biological activities of extracts and plant polyphenols from Greek grape varieties (*Vitis vinifera*)»

The three member consulting committee:
Kouretas Dimitrios, Professor (Supervisor)
Mamuris Zissis, Professor
Stathopoulos Konstantinos, Assistant Professor

ABSTRACT

In the present study, extracts (methanolic and water) and fractions enriched in polyphenols from two Greek grape varieties, Mandilaria Santorini (red grapes) and Assyrtiko Santorini (white grapes), as well as monomeric plant polyphenols present in them were tested for their biological activity. The tested plant polyphenols were: three hydroxycinnamic acids (cafeic acid, ferulic acid and coumaric acid), two hydroxybenzoic acids (gallic acid and protocatechuic acid), four flavonoids (quercetin, rutin, (+)-catechin and (-)-epicatechin) and *trans*-resveratrol. The following biological properties were investigated: 1) The antioxidant activity by using the DPPH method. 2) The effect of the tested compounds on mutagenicity induced by oxidative factors. In this case, the following methods were used: i) DNA strand breakages induced by mitomycin C in plasmid DNA, ii) mutations induced by bleomycin and hydrogen peroxide in bacterial cells (*Salmonella typhimurium* TA102) and iii) SCEs induced by mitomycin C in cultures of human lymphocytes. 3) The effect of tested compounds on ozone-induced oxidation of SP-A protein (which is one of the major proteins of lung surfactant). 4) The effect of tested compounds on the activity of topoisomerase I enzyme.

The results from the DPPH method showed the following: i) The extracts and the fractions exhibited strong antioxidant activity in low concentrations (IC_{50} values between 19-92 μg/ml). ii) Some of the polyphenol enriched fractions had less antioxidant activity than their corresponding extracts, and so the results suggested that the antioxidant activity of extracts is not attributed only to the polyphenols but also to other compounds or to a synergistic effect between polyphenols or between polyphenols and other compounds present in extracts. iii) The potential order of the polyphenols was: gallic acid > caffeic acid = quercetin = (-)-epicatechin > (+)-catechin > rutin > protocatechuic acid > ferulic acid > *trans*-resveratrol > coumaric

acid. Moreover, the IC_{50} values some of them were in concentrations which can be achieved in the human organism through the diet.

The conclusions coming from the effects of the tested compounds on the mutagenic activity induced by oxidative factors were the following: i) The extracts from both grape varieties inhibited at low concentrations the bleomycin-induced mutagenicity in bacterial cells as well as the mitomycin C-induced DNA strand breakages in plasmid DNA. Furthermore, the methanolic extracts inhibited the hydrogen peroxide-induced mutagenicity in bacterial cells. Thus, the results indicated that the inhibitory activity of grape extracts against DNA mutations induced by oxidative factors may be one of the mechanisms accounting for their chemopreventive action. ii) The antimutagenic activity of extracts could not be attributed to any of the tested polyphenols, since the concentrations of those polyphenols exerted antimutagenic activity were much lower than the concentrations of the polyphenols in the extracts. Consequently, the inhibitory activity of extracts may not be attributed to the polyphenols or there may be a synergistic activity between polyphenols or between the polyphenols and some other compounds of the extracts. iii) From the tested polyphenols, caffeic acid inhibited the bleomycin-induced mutagenicity in bacterial cells and quercetin inhibited the hydrogen peroxide-induced mutagenicity in bacterial cells as well as the mitomycin C-induced SCEs in human lymphocytes. The inhibitory activity of these polyphenols could probably be attributed not only to their strong antioxidant properties but also to their metal chelating properties.

The results from the effects of the tested compounds on topoisomerase I activity showed the following: i) The extracts from both grape varieties inhibited the catalytic activity of both topoisomerase I from wheat germ and human topoisomerase I. Therefore, the inhibition of this enzyme may be one of the mechanisms accounting for the anticarcinogenic activity of grape extracts observed in other studies. ii) Some of the polyphenol enriched fractions were less potent than their corresponding extracts. Thus, the inhibitory activity of extracts against topoisomerase I may be due not only to the polyphenols but also to other compounds present in extracts. iii) From the tested polyphenols, caffeic acid, quercetin and protocatechuic acid exerted inhibition against the activity of topoisomerase I.

The conclusions resulting from the effects of the tested compounds on ozone-induced oxidation of SP-A were the following: i) The flavonoids, (+)-catechin, (-)-epicatechin and rutin, inhibited in a dose-dependent manner the SP-A oxidation. ii) The

hydroxybenzoic acids, gallic acid and protocatechuic acid, inhibited SP-A oxidation but they were less potent than the flavonoids and their inhibitory activity reached a plateau at 50μM. iii) The hydroxycinnamic acids, caffeic acid, ferulic acid and coumaric acid, exhibited inhibition at medium concentrations, while there was not statistically significant inhibition at high concentrations. In general, these results suggested that plant polyphenols could inhibit the ozone-induced oxidation of SP-A, which plays an important role in normal lung function and innate host defense. Therefore, plant polyphenols found in dietary foods could possibly protect from the detrimental effects of air pollutants on lungs.