Evaluation of the biological activity of *Pleurotus ostreatus* and *Ganoderma lucidum*, edible Basidiomycetes of Greek habitats with enhanced β-glucan content

Vlachou-Efstathiou, P.M.[1,4], Vlassopoulou, M.[1,3], Boulaka, A.[1], Bekiaris, G.[2], Koutrotsios, G.[2], Zervakis, G.I.[2] , Kyriacou, A.[3], Tsitsiloni, O.[4], Georgiadis, P.[1] and Pletsa, V.[1]

[1] *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece*
[2] *Laboratory of General and Agricultural Microbiology, Department of Crop Science, Agricultural University of Athens, Greece*
[3] *Department of Nutrition and Dietetics, Harokopio University, Athens, Greece*
[4] *Section of Animal & Human Physiology, Faculty of Biology, School of Science, National & Kapodistrian University of Athens, Greece*

INTRODUCTION

Basidiomycetes are known worldwide for their health-promoting properties. β-Glucans, a group of β-D-glucose polysaccharides naturally occurring in the fungal cell walls, are considered responsible for their potential prebiotic, immuno-modulating and anti-tumor effects. It is, thus, of great importance to use indigenous fungal genetic resources in order to isolate fungal bioactive compounds for the development of nutraceuticals.

MATERIALS & METHODS

We examined the biological activity of the Greek *Pleurotus ostreatus* and *Ganoderma lucidum*. Lyophilized whole mushroom and their β-glucan-enriched extracts where examined, using the MTT method, for their ability to affect cell proliferation of human epithelial colorectal adenocarcinoma cells (Caco-2 and HT-29), peripheral blood monocytes (U937) and human peripheral blood lymphocytes (PBLs) of healthy donors, after 48 h of incubation. Their cytotoxic effect and the cell death mechanism were investigated via Trypan blue exclusion assay, flow cytometry and Western blot analysis. Furthermore, pre- and anti-inflammatory cytokine production was estimated in PBLs to investigate their potential immune-modulatory effect.

RESULTS I. Inhibition of cell proliferation (MTT assay)

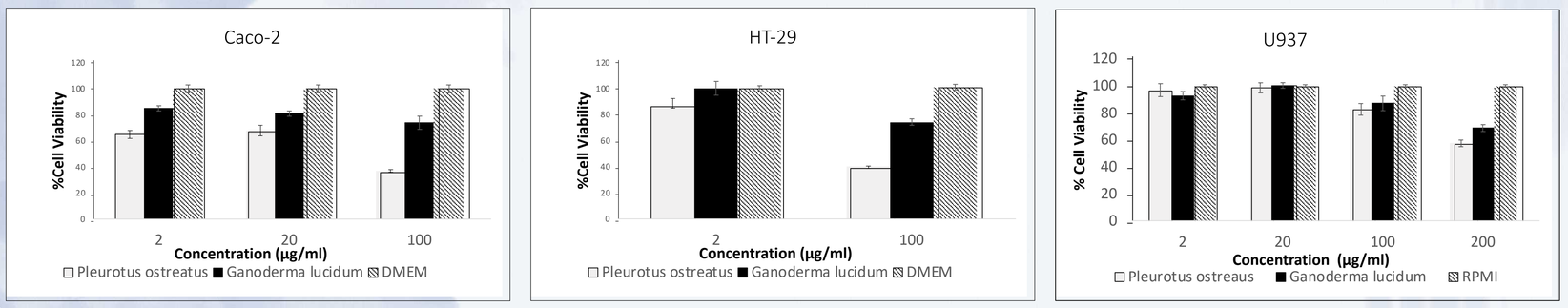

Figure 1: *Pleurotus ostreatus inhibits cell proliferation more effectively than Ganoderma lucidum in Caco-2 and HT-29 cells in a dose-dependent manner, whereas only the highest concentration (200μg/ml) of the tested lyophilized samples exhibits cytotoxic activity in U937 cells.*

RESULTS II. Cell death characterization

(a) Caco-2

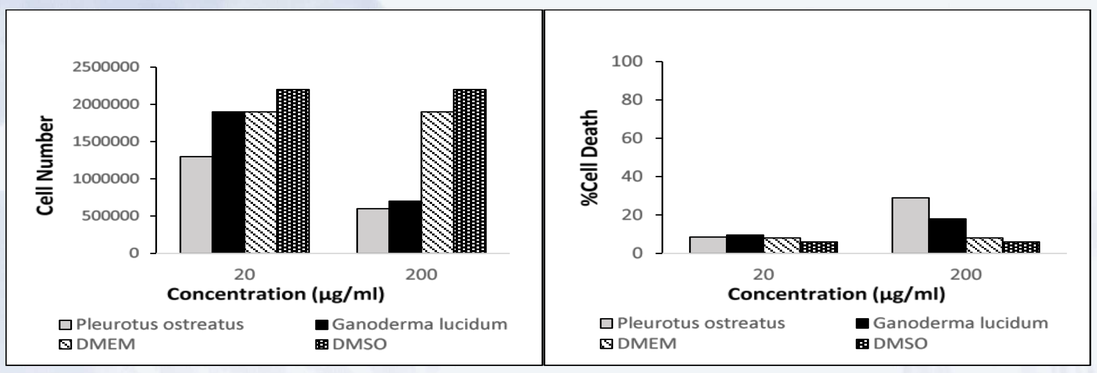

Figure 2: *The percentage of cell death in Caco-2 was assessed by Trypan blue exclusion assay*

(b) U937

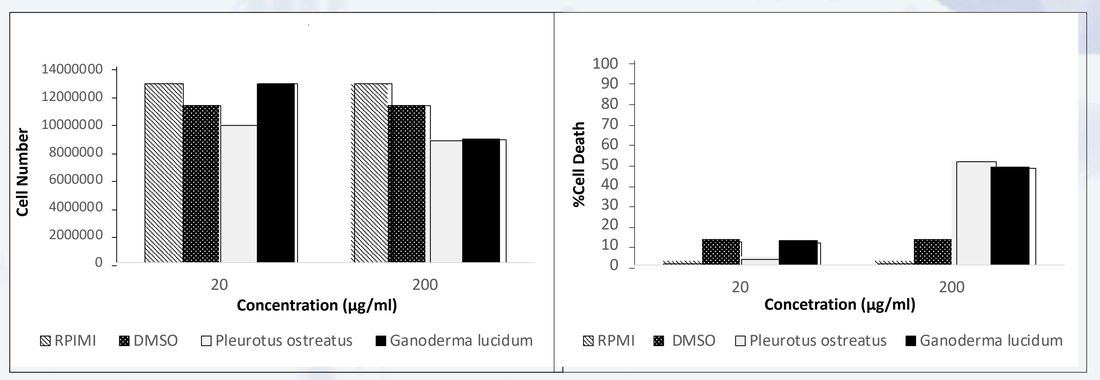

Figure 4: *The percentage of cell death in U937 was assessed by Trypan blue exclusion assay*

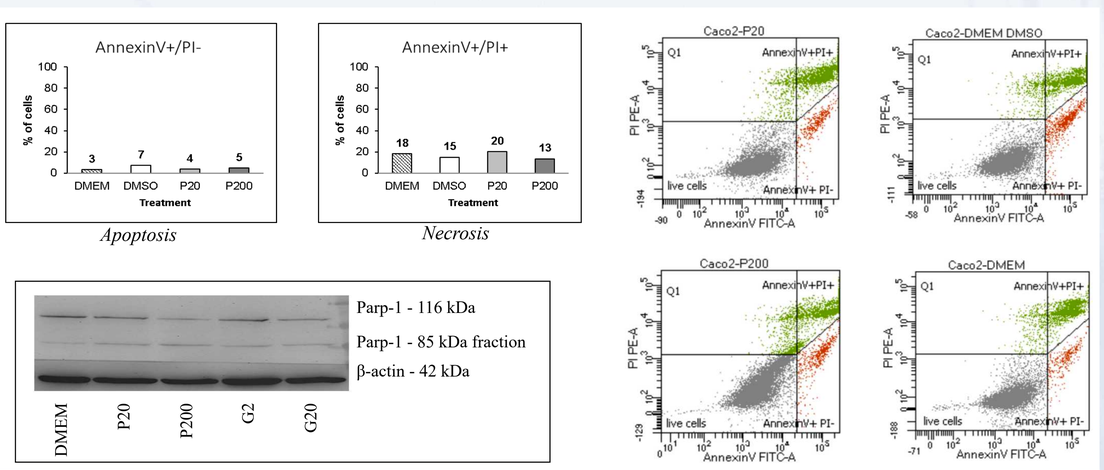

Figure 3: *FACS and Western Blot analysis revealed that the cytotoxic effect of both Basidiomycetes in Caco-2 cells is due to necrosis.*

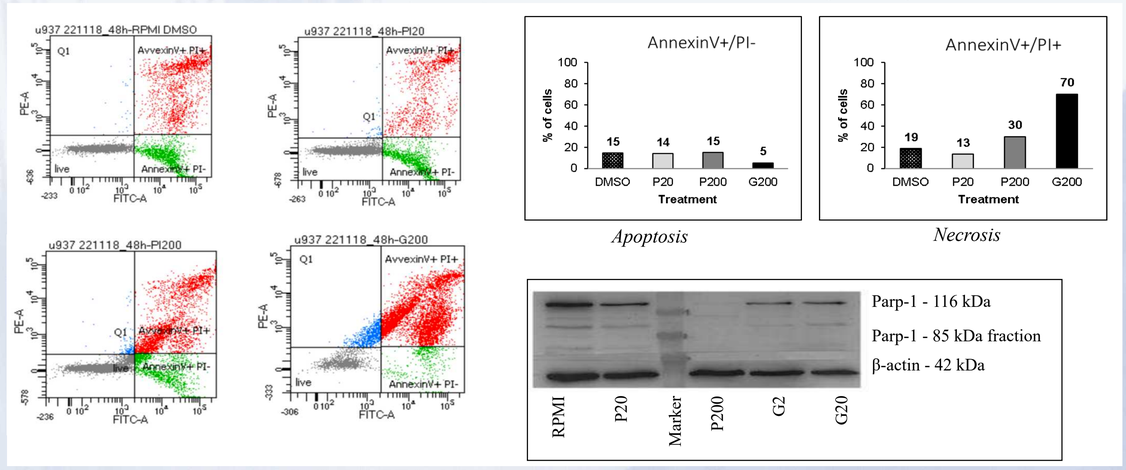

Figure 5: *FACS and Western Blot analysis revealed that the cytotoxic effect of both Basidiomycetes in U937 cells is due to necrosis.*

RESULTS III. Cytokine induction

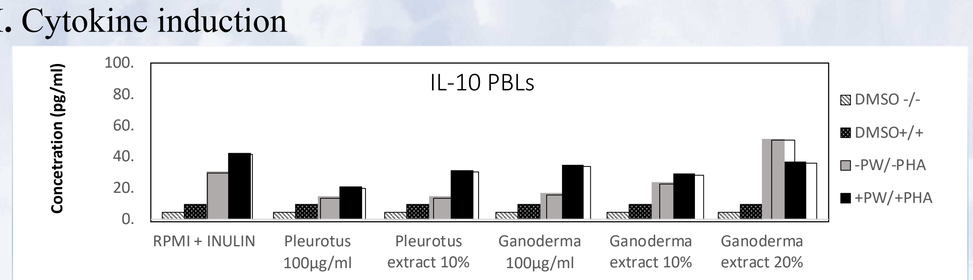

Figure 6: *Both lyophilized samples and the matched β-glucan enriched extracts induce anti-inflammatory IL-10 levels in PBLs, in a dose-dependent manner, in the presence and absence of the mitogens Pokeweed (PW) & Phytohematogglutinin M (PHA M).*

CONCLUSIONS

Our initial findings support the anticancer and immuno-modulating potential of the Greek *P.ostreatus* and *G.lucidum*. Further investigation is currently ongoing to establish their prebiotic activity and unravel the mechanisms underlying their biological activities as a whole.

PERSPECTIVES

These preliminary data will be followed by:

- further screening of several β-glucan rich Basidiomycetes,
- *in vitro* fermentation of the most active fungal substrates by fecal slurry of healthy volunteers and metabolomic analysis of the fermentation products,
- NGS analysis to assess their impact on gut microbiome, and
- further *in vitro, ex-vivo* and *in vivo* evaluation of their anti-cancer and immunomodulating activities.

ACKNOWLEDGEMENTS

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ AGRICULTURAL UNIVERSITY OF ATHENS
ΧΑΡΟΚΟΠΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ HAROKOPIO UNIVERSITY
ΠΑΠΑΔΟΠΟΥΛΟΣ
Ευρωπαϊκή Ένωση Ευρωπαϊκό Ταμείο Περιφερειακής Ανάπτυξης
ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ ΥΠΟΥΡΓΕΙΟ ΟΙΚΟΝΟΜΙΑΣ & ΑΝΑΠΤΥΞΗΣ ΕΙΔΙΚΗ ΓΡΑΜΜΑΤΕΙΑ ΕΠΑ & ΤΣ
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