Growth Inhibition of Human Tumor Cell Lines of Different Origins with Medicinal Mushroom Preparations

Ksenija Durgo[a], Draženka Komes[a], Ana Belščak-Cvitanović[a], Dunja Horžić[a], Jasna Franekić[a] & Ivan Jakopović[b]

[a] Faculty of Food Technology and Biotechnology, Zagreb, Croatia

[b] Dr. Myko san- Health from Mushrooms Co., Zagreb, Croatia

Mushrooms

- biologically active compounds
  - polysaccharides
  - polyphenols
  - flavonids
  - vitamin sources

-antioxidant activity

-enhancement of immunity, antibiotic activity and tumor retarding effects

- Biological effects - multidimensional
- Mechanism of action - a cascade of events s
- Inhibition or induction of expression of certain proteins
- Modification of basic metabolic processes in the cell
- Differentiation of the redox status of the cell

Aim of the investigation

- previous investigations-inhibition of tumor growth
- mechanisms of inhibition?
- new insight in basic mechanisms of inhibition of growth

Aim of the investigation

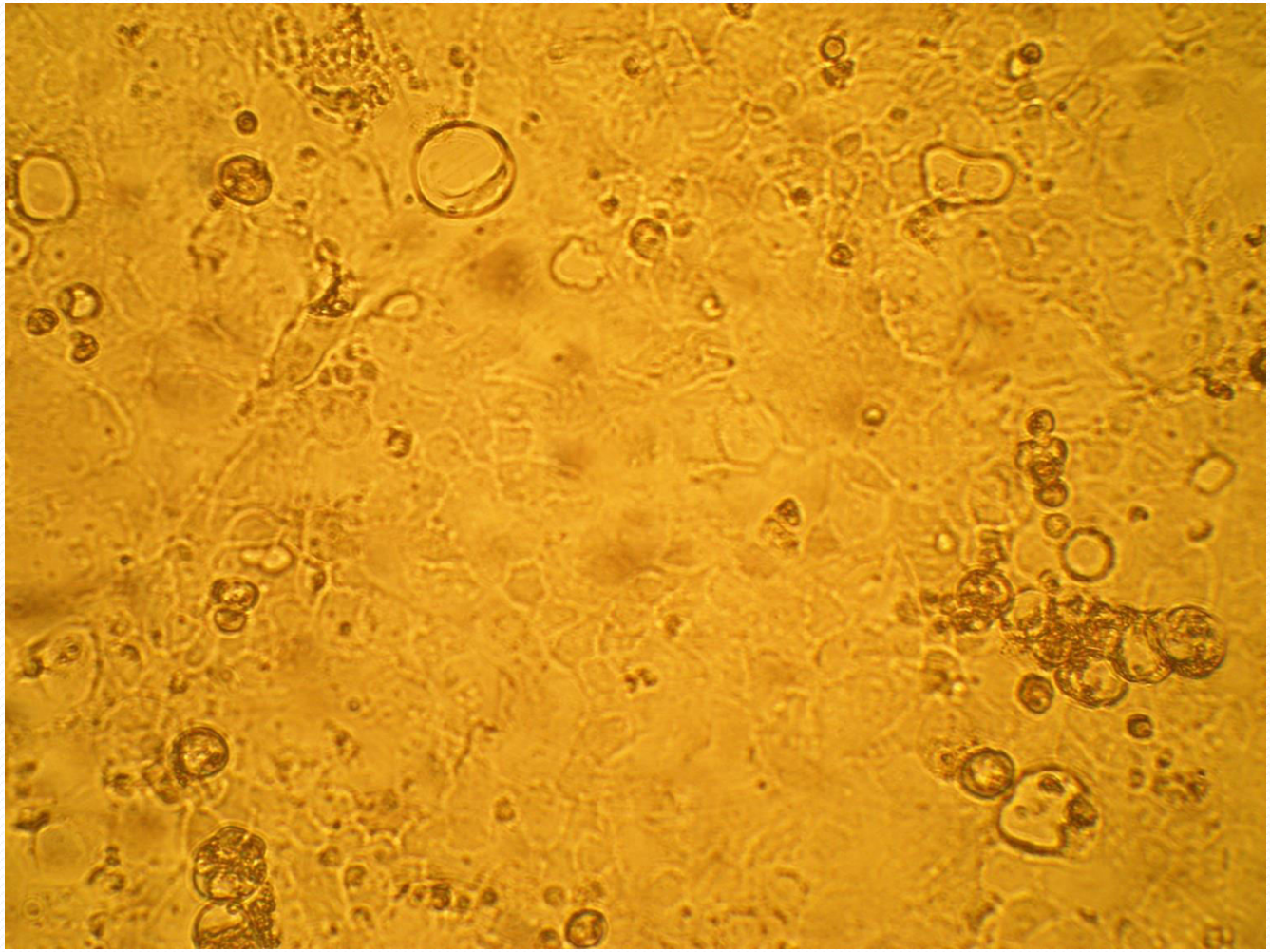
- Determination of the cytotoxic, anti-oxidative and pro-oxidative nature of blended mushroom extracts
  - Lentifom
  - Superpolyporin
  - Agarikon
  - Agarikon+
  - Agarikon.1 (tablets)
  - Mycoprotect.1 (tablets)

...produced by Dr. Myko san- Health from Mushrooms Co.

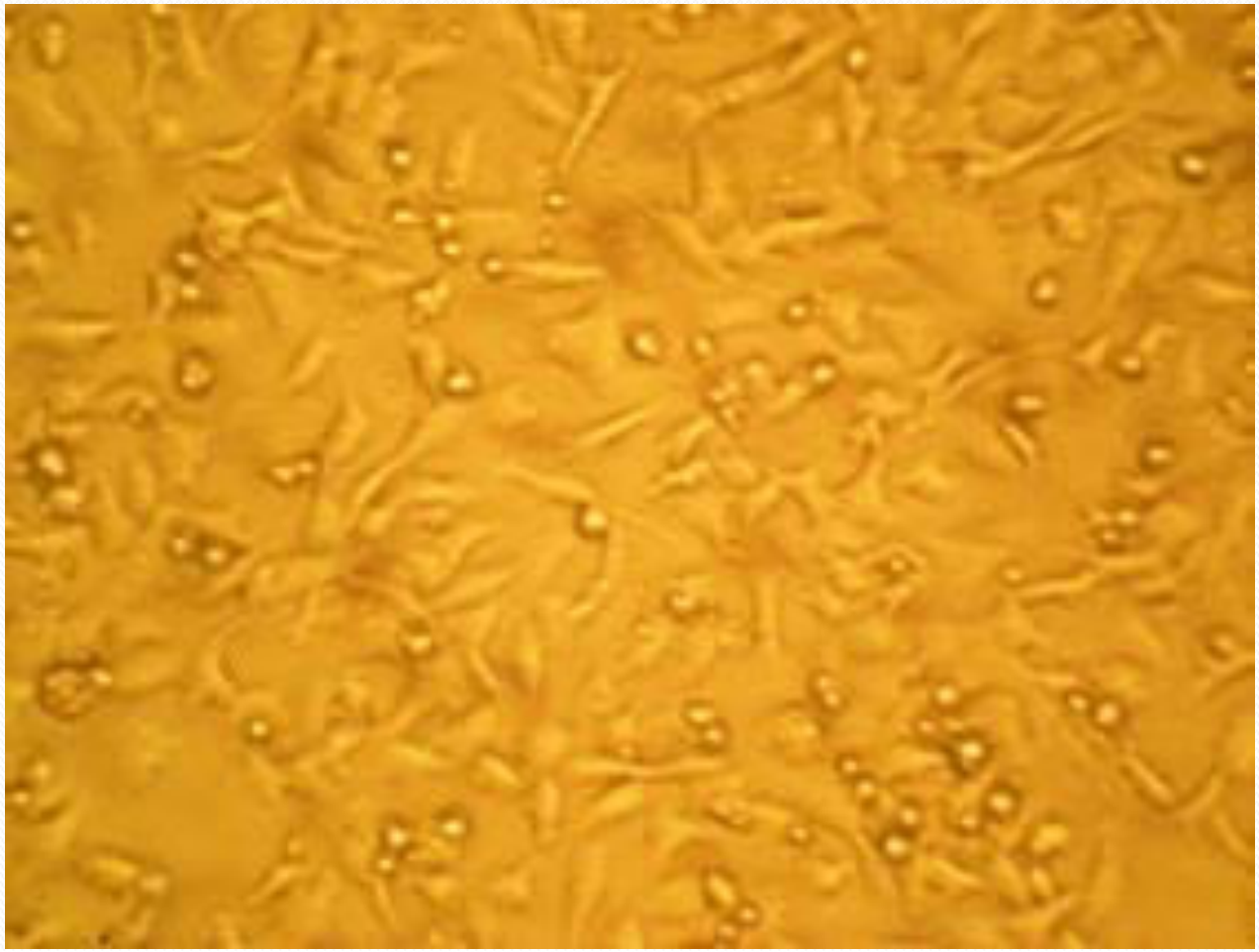
Biological test system

- human cell lines of different origin;

CaCo2 – human colon cancer cells

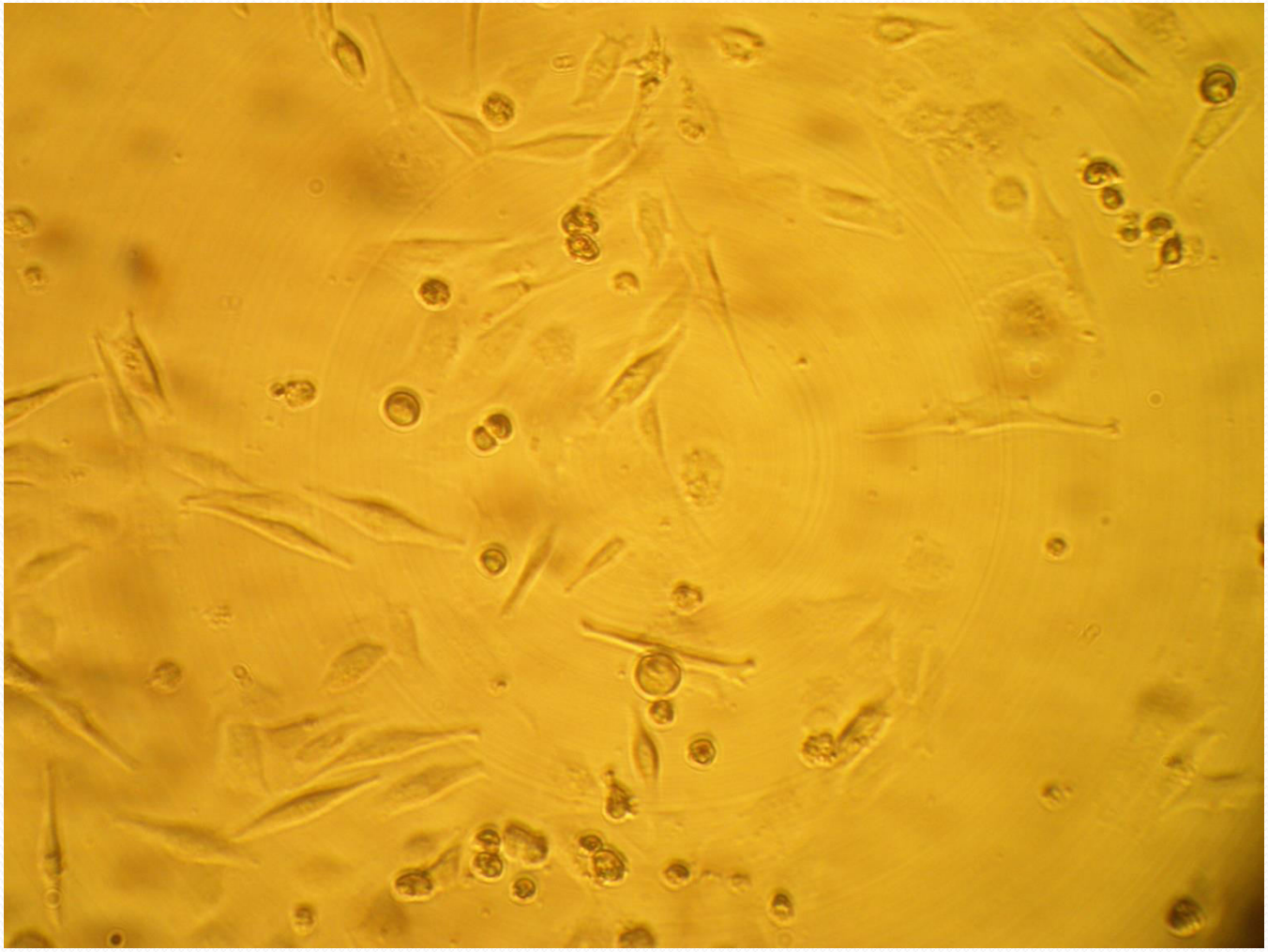


SKLU-1 human lung adenocarcinoma cells



H69V-small cell lung carcinoma cells

CCTG-1 – human astrocytoma cells

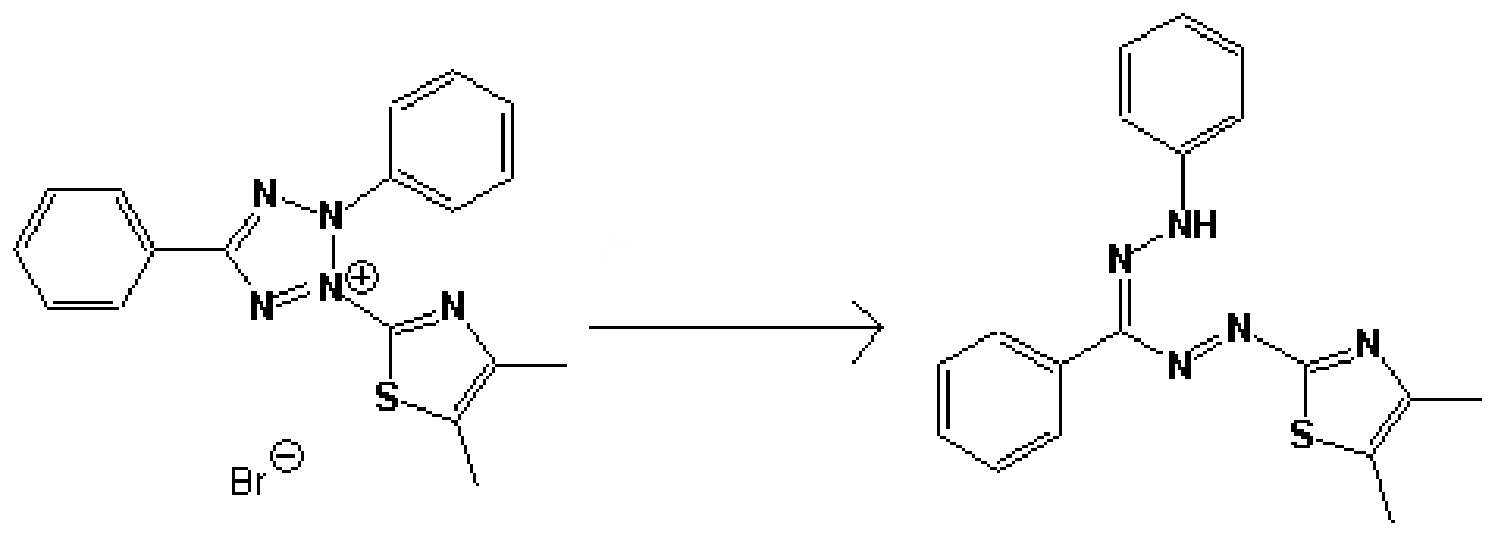


Experimental design

- for all cell lines growth curve was determined
- cells were treated with mushroom extracts for three days (three cell deivisions) durong exponential phase of growth
- range of concentrations set to daily dose
  - 0.1x-100x
- 1x – recomended daily dose, recalculated from dose/bw to mL(mg) of mushroom extract/mL of growth medium

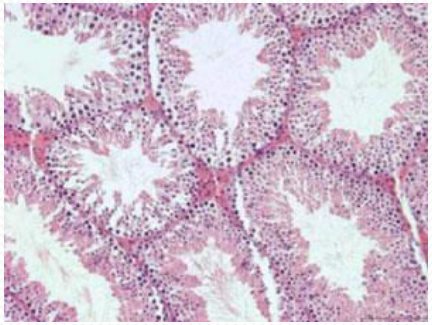
Experimental design-cytotoxicity

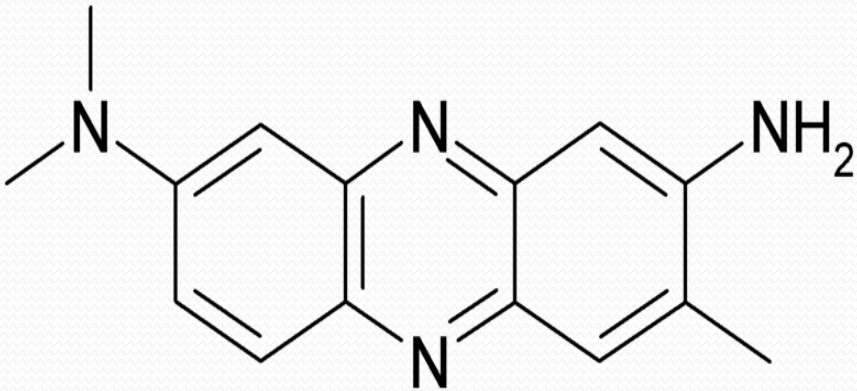
MTT assay – activity of mitochondrial dehydrogenases as a measure of cell survival



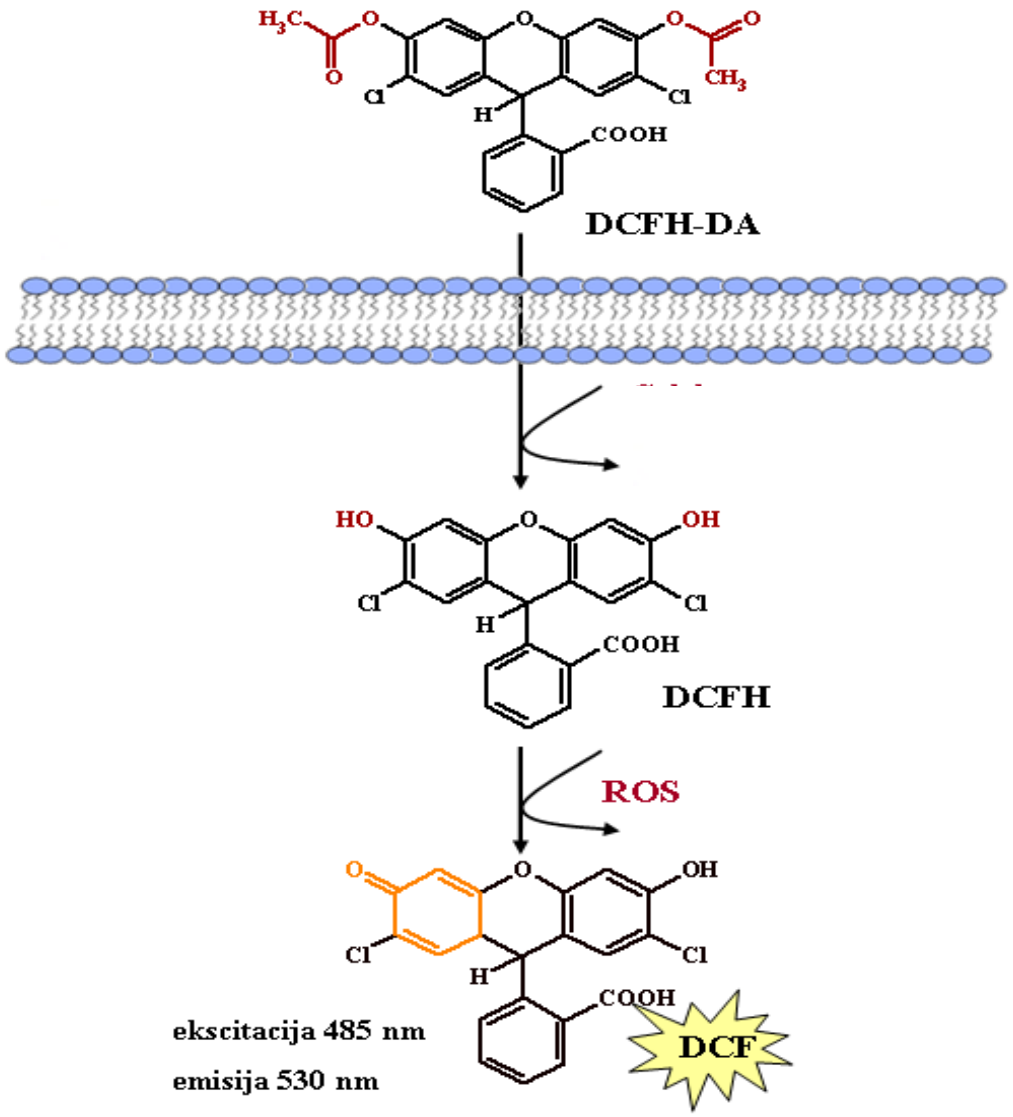
Experimental design-cytotoxicity

- Neutral red assay (NR) – accumulation of NR in lysosomes of viable cells





Experimental design – determination of reactive oxygen species



Determination of the prooxidative and antioxidative nature of mushroom extracts

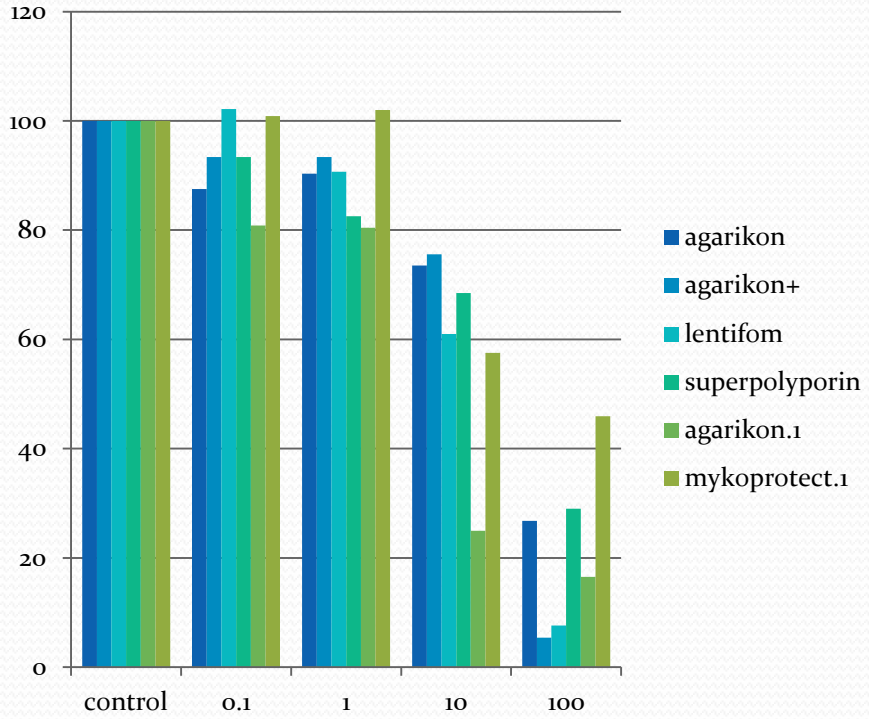
- prooxidative nature: increase/decrease of ROS formation in comparison to control

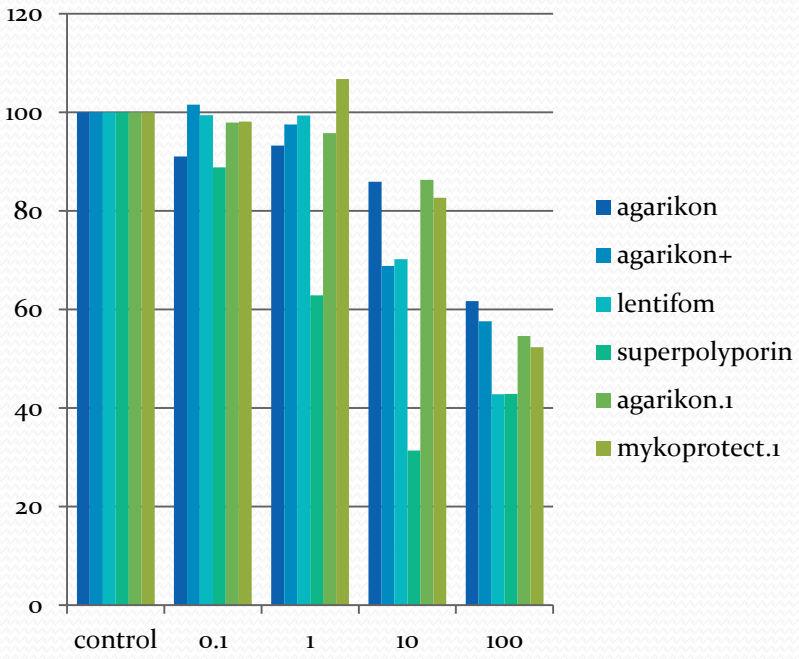
- antioxidative nature: increase/decrease of ROS formation in comparison to positive control (hydrogen peroxide)

Results-polysaccharides, polyphenols and flavonoids content

	dry matter	polysaccharides	polyphenols	flavonoids
	%	mg/g s.tv.	mg GAE/L	mg GAE/L
Agarikon	4.02 ± 0.02	579.35 ± 25.62	839.17 ± 7.07	455.83 ± 9.43
Agarikon+	4.60 ± 0.03	490.42 ± 40.79	908.33 ± 9.43	542.08 ± 0.59
Lentifom	1.71 ± 0.01	402.61 ± 37.50	339.58 ± 8.84	174.17 ± 1.18
Superpolyporin	3.61 ± 0.01	467.09 ± 29.00	788.75 ± 0.59	305.83 ± 25.93
Agarikon 1	93.00 ± 0.32	1053.88 ± 57.89	448.75 ± 17.09	240.83 ± 7.07
Mycoprotect 1	92.94 ± 0.15	935.35 ± 35.70	635.42 ± 24.19	362.92 ± 7.66

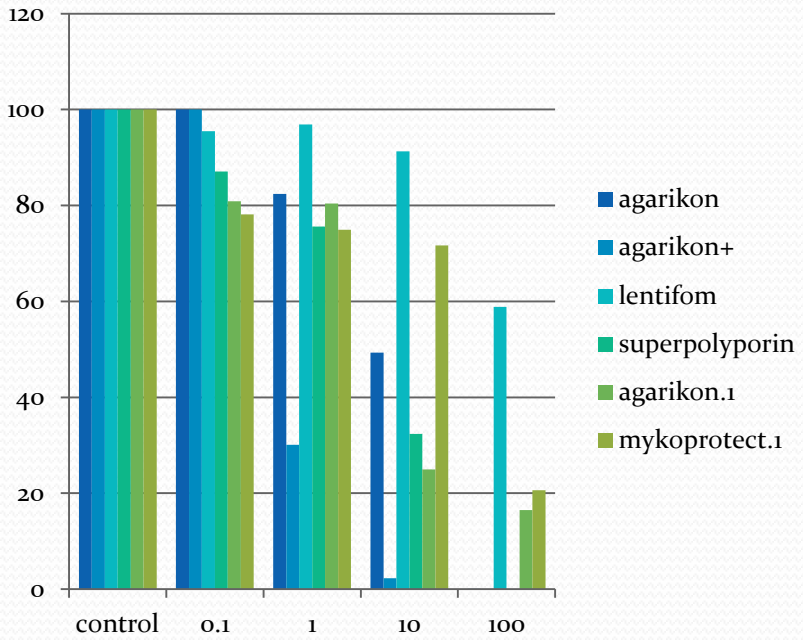
Results – MTT/NR assay CaCo2 cells

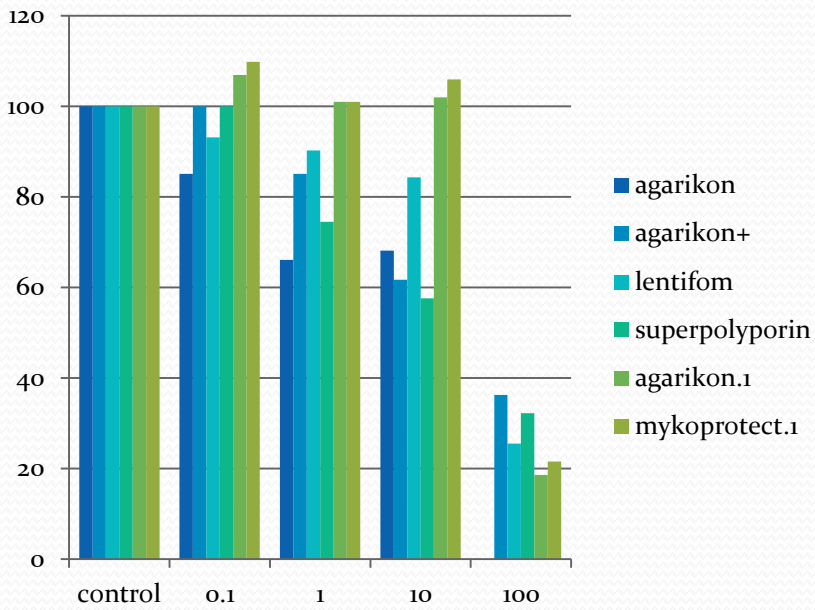




Colon cancer cells are resistant to all investigated extracts at the concentrations recomended as a daily intake. Higher concentrations decrease cell survival significantly, specially lentifom and superpolyporin characterized with low levels of polysaccharides, polyphenols and flavonoids.

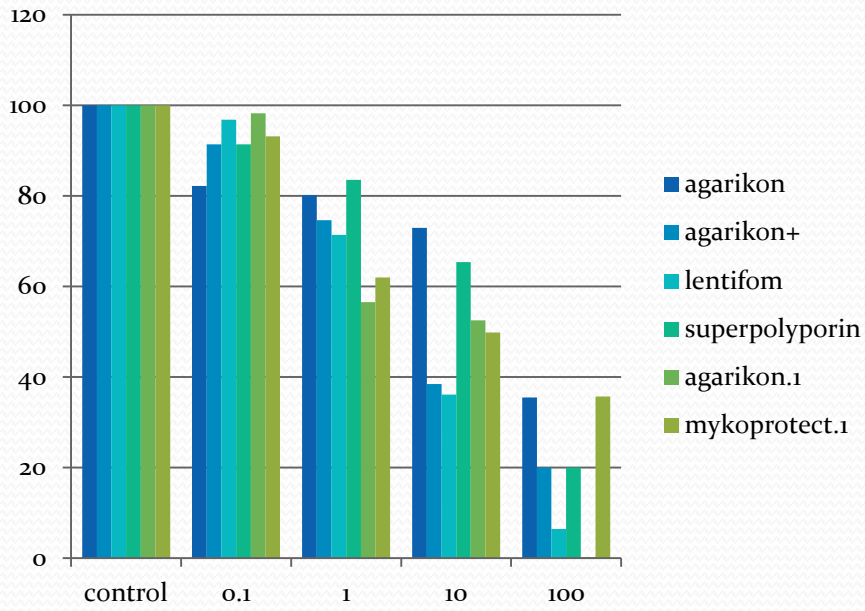
Results – MTT/NR assay SKLU-1 cells

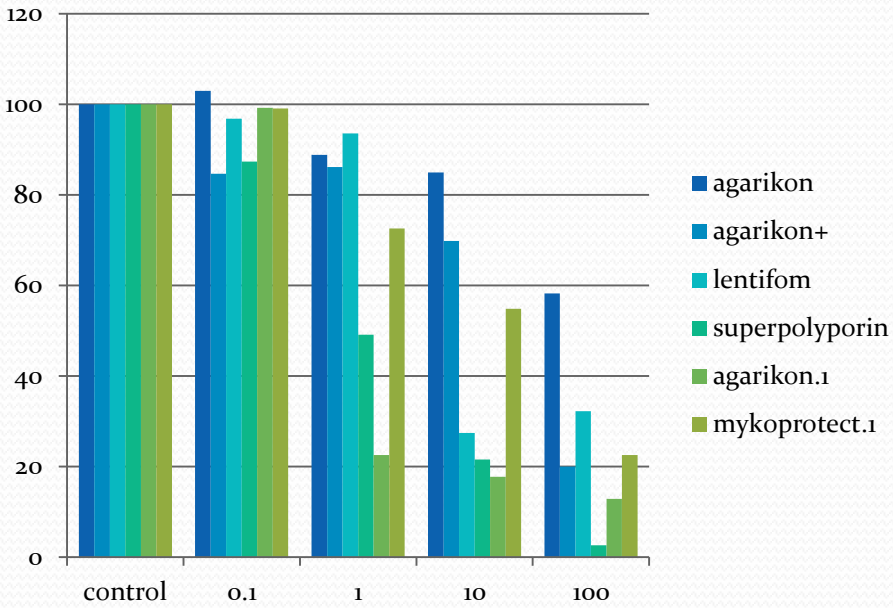




Agarikon+ and agarikon, extracts with high polyphenol and flavonoids content showed strong cytotoxic effect on this type of cells. Superpolyporin also decreased cell survival at daily recomended doses.

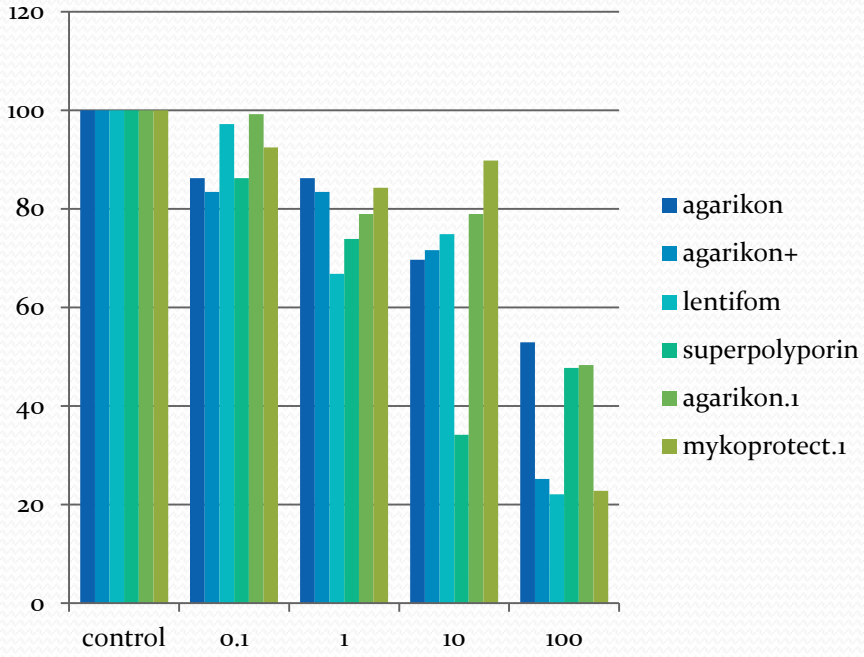
Results – MTT/NR assay H69 V cells

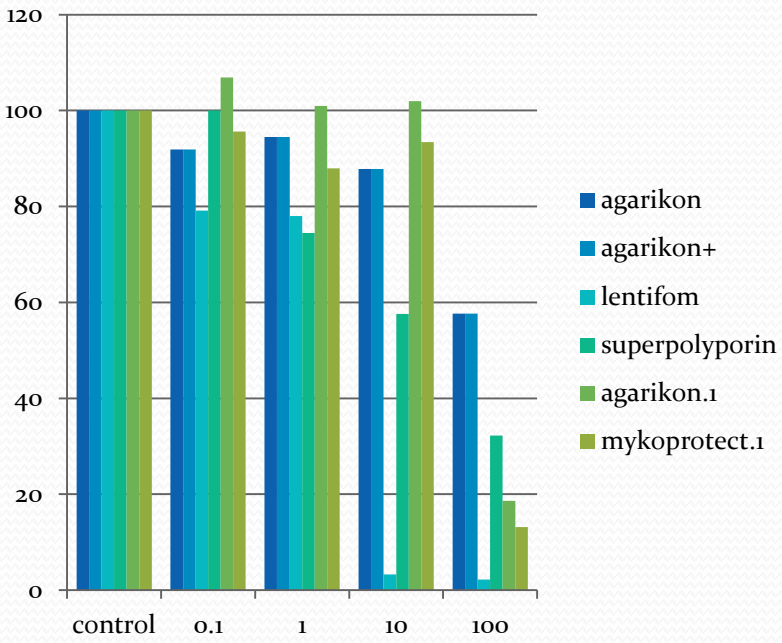




Agarikon.1 showed the highest cytotoxic effect at 1x concentration. It is a crude compound with the highest polysaccharide content.

Results – MTT/NR assay astrocytoma cells





Lentifom and superpolyporin showed strong cytotoxic effect, although these extracts contain low levels of polysaccharides, flavonoids and polyphenols.

Results-antioxidative/prooxidative nature of mushroom extracts

- Agarikon .1 increased prooxidative effect of hydrogen peroxide on CaCo2 cells. In other cases-strong antioxidative behaviour was detected.
- Mycoprotect.1 - prooxidant on astrocytoma cells; increased prooxidative nature of hydrogen peroxide on CaCo2 cells
- lentifom decreased free radical formation induced by hydrogen peroxide on all cell lines except on CaCo2 cells
- Agarikon – prooxidant at lower concentrations (0.1 and 1x) on SKLU-1, H69V and CaCo2 cells

Results-antioxidative/prooxidative nature of mushroom extracts

- Agarikon+ - prooxidant at lower concentrations (0.1 and 1x) on SKLU-1, H69V and CaCo2 cells
- Superpolyporin - prooxidant at lower concentrations on H69V and CaCo2 cells

Conclusions

- Overall effect of six examined mushroom extracts strongly depends upon cell type
- The highest concentration of polysaccharides, polyphenols or flavonoides in certain extract will not determine it effect on cell survival
- Influence of ROS formation in CaCo2 cell line and its high percentage of survival will be determined
- in further work, possible apoptotic events as a cause of cell death will be determined

Thank you for attention!