



## Extended Abstract In Vitro Effect of Fungal Chitosan on Tumoral Cells and Microbial Cultures <sup>+</sup>

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The ability of marine chitosan to be processed as films or hydrogels makes it a suitable candidate for applications in key areas, such as medicine, environmental protection, agriculture, and cosmetics [1]. Recently, some polysaccharides from *Ganoderma lucidum* have been reported as a potential alternatives to commercial shrimp chitosan for biomedical applications [2,3]. The aim of this study was to evaluate the in vitro interaction of fungal chitosan-rich preparations, extracted by chemical and enzymatic methods from *G. lucidum* with normal, tumoral, and microbial cultures.

Chitosan was chemically extracted (C1) by serial treatment of *G. lucidum* powder with 4M NaOH and 1% HCl. Enzymatically extracted chitosan (C2) was obtained by alkaline treatment in 11M NaOH, at 100 °C and incubation with  $\alpha$ -amylase (Protemyl) at 85 °C, for 3 h. The chitosan extracted from shrimps (CS) was purchased from Sigma-Aldrich and used as a control. The anti-proliferative activity of chitosan was determined in Hep-2 carcinoma cells and cytotoxicity in normal fibroblasts from NCTC clone L929 cell line using methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay [4]. Several concentrations of extracts were added in the culture medium and cultivated for 48 h. Cell morphology observations were performed in treated cell cultures after Giemsa staining. The antimicrobial activity of chitosan extracts was investigated on two pathogenic bacterial species, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) using disc diffusion method and incubation at 37 °C, for 24 h.

The in vitro test results showed that C1 and C2 fungal chitosan extracts, as well as the CS control sample, did not affect the cell viability of normal NCTC cells in the range of concentrations 50–1000  $\mu$ g/mL. Incubation of samples with Hep-2 carcinoma cells indicated a significant anti-proliferative activity in the range of concentrations 500–1000  $\mu$ g/mL, similar to CS control sample. Cell morphology observations showed a normal phenotype of fibroblast cells cultured in the presence of both chitosan extracts, while concentrations of over 500  $\mu$ g/mL led to morphological changes of Hep-2 tumoral cells. All tested samples interfered with bacterial growth. Thus, the images of bacterial cultures showed the appearance of inhibition zones around chitosan extracts. The measured diameter was higher for C1 extract than that for C2 extract.

Fungal chitosan extracts exhibited anti-proliferative effects on human carcinoma cells and efficiently inhibited microbial growth, suggesting significant biomedical potential.

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