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## GROWTH INHIBITORY ACTIVITY OF ARTIFICIAL LIGNIN ON HUMAN CELL LINES

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### ABSTRACT

Lignin, together with cellulose and hemicellulose is a main structural polymer in the cell wall of any higher plant. It plays an essential constitutive and functional role in plant cell walls. It has been suggested that lignin-related plant extracts might be beneficial in cancer chemotherapy. The aim of the study was to evaluate the growth activity of *in vitro* enzymatically polymerized dehydrogenate polymer (DHP) of coniferyl alcohol on tumor cell lines. Cytotoxicity and growth inhibitory activity were measured by colorimetric tetrazolium assay (MTT) on MCF7 - human breast adenocarcinoma cell line and MRC- human lung fibroblast cell line. Cells were exposed to DHP for various exposure periods. Two sets of experiments were performed. In one, cells were exposed to complexes 72 h "in continuo" and in another, cells were left for recovering in fresh medium 72h following 4h-exposure. Both cell lines were found to be sensitive to DHP only for the 72h exposure. Cytotoxic effect was concentration and time-dependent. The most profound growth inhibitory effect was achieved at concentration of 1mg/mL. The preliminary results of this study suggest the necessity to evaluate the growth activity effect on other tumor cell lines.

Key words: cancer chemotherapy, tumor cell lines, lignin

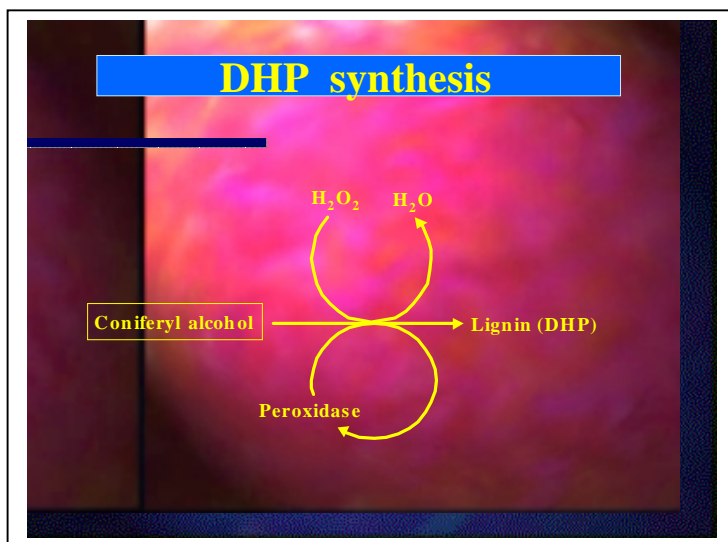
### BACKGROUND

Lignin, one of the main structural polymers in the cell wall of higher plants, is the second most abundant organic compound on the earth's surface. It is a complex phenolic heteropolymer covalently associated with both polysaccharides and proteins. Lignin is known to be responsible for the mechanical stress protection of plant cell and for providing a support of the plants against gravity.

The ability of plants to synthesize lignin, as well as antioxidative capacity of this macromolecule, are known to be involved in plant disease resistance. The copolymerization

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of toxic xenobiotics with lignins may be an effective form for inactivating (and/or immobilizing) these dangerous metabolites. Thus, in all senses, the acquisition of lignified cell walls by land plants must be considered a major step in their evolution and adaptation to the earth.



Although lignin exhibits a high degree of structural variability, its final structure is generally attained by aggregation of subunits. These subunits are polymers of phenolic monomers. Lignin structure consists of the low-molecular (3000-4000) and high-molecular (80000-100000) fraction.

Natural (isolated) and synthetic lignins have been shown to exhibit antiviral, antitumor and immunopotentiating activities.

### AIM

The aim of the study was to evaluate the growth activity of *in vitro* enzymatically polymerised dehydrogenative polymer (DHP) of coniferyl alcohol on tumor cell line (MCF7)

### MATERIALS AND METHODS

**Lignin** -The DHP was synthesized from  $5 \cdot 10^{-3}$  M coniferyl alcohol and  $5 \cdot 10^{-3}$  peroxidase, in  $5 \cdot 10^{-2}$  M phosphate buffer, pH 7.6. The polymerization was finished after M  $H_2O_2$  in presence of  $2.5 \cdot 10^{-8}$  mol/l horseradish two days and resulting suspension of DHP was centrifuged at 5000 rpm. The precipitate was resuspended in twice distilled water and centrifuged. This procedure was repeated twice. Finally, the precipitate was air-dried.

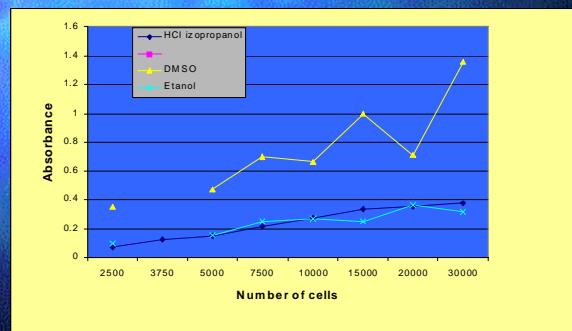
**Cell line** - The human breast adenocarcinoma cell line (MCF7) was used. MCF7 cells were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 5 to 10% of fetal calf serum and antibiotic: 100 IU/ml of penicillin, 100 $\mu$ g/ml of strptomycin. Cells

were cultivated in flasks at 37 °C in the atmosphere of 100% humidity and 5% of CO<sub>2</sub> (Heraeus)

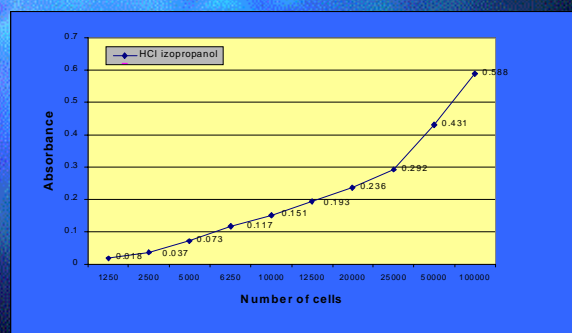
Colorimetric MTT assay- The MTT method involving reduction by living cells of tetrazolium salt to blue formazan product, the concentration of which can be measured spectrophotometrically. This method can be used to measure cytotoxicity, proliferation or activation.

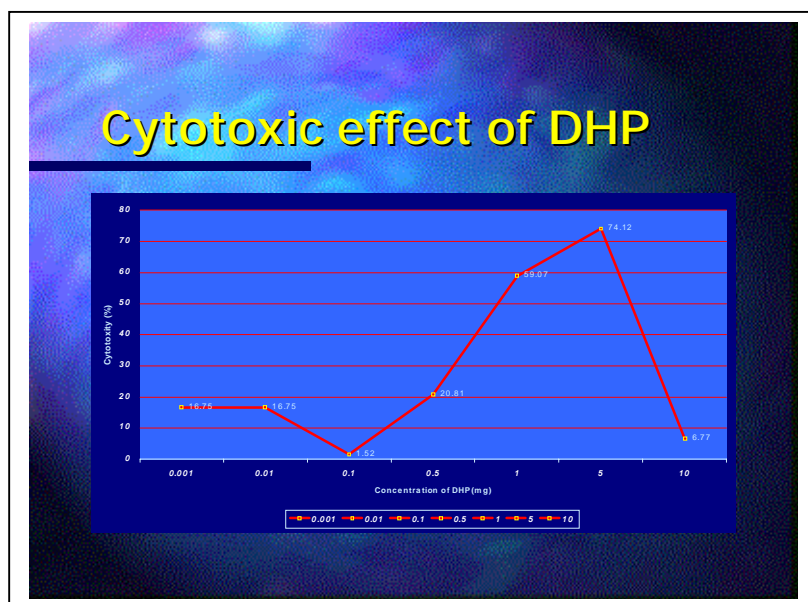
The hydrogen acceptor MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is commonly utilized to estimate cellular viability in drug screening protocols.

### The organic solvent to dissolve the blue formazan product



### Linearity of the MTT assay





#### FUTURE EXPERIMENTS

Search for other solvent (methanol, chloroform-methanol, acetone, N,N-dimethylformamide, pyridine).

Antiproliferative effect of DHP synthesized from different monomers, as well as of lignin isolated from certain tree species.

It is possible to separate the two lignin fractions and study their antiproliferative effect on various tumor cell lines