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Cytotoxic Activities of *Funalia trogii* (Berk.) Bond. Et. Singer ATCC 200800 Bioactive Extract on HeLa Cells and Fibroblast Cells

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This study demonstrated the cytotoxic and cell growth inhibitory effect of a bioactive extract of *Funalia trogii* (Berk.) Bond. et Singer, which was grown in solid-state fermentation at room temperature, on He-La and fibroblast and the mitotic index of lymphocytes. The effect demonstrated by the bioactive extract of *F. trogii* on laccase, peroxidase, SOD, ascorbate peroxidase, catalase, and glutation peroxidase is shown in Table 1.

A study on the possible cytotoxic and cell growth inhibitory effects of the bioactive extracts of two white-rot fungi showed that these extracts had significant cytotoxic and antiproliferative properties on the HeLa cancer cell line. Hence, the aim of this study was to examine the *in vitro* cytotoxic activities of standardized aqueous bioactive extracts prepared from *Funalia trogii* on HeLa and fibroblast cell lines using a MTT (3-[4,5-Dimetiltiazol-2-]-2-5-difeniltetrazolium bromide) cytotoxicity assay. *F. trogii* solutions were found to exhibit cytotoxic effects on HeLa cell lines. Based on the data, it was found that toxicity ratios of 0.05 µL of *F. trogii* extract solutions were 71.5%. Furthermore, *F. trogii* extract solutions were also cytotoxic on fibroblast cell lines.

Analysis of the data indicated that the toxicity ratio of 0.05 µL of *F. trogii* solutions was 51.3%. These results showed that the extracts had a substantial cytotoxic action on HeLa cell lines but less on fibroblast cells. In separate experiments the mitotic index reached nearly the same value at 4 µg/mL MMC, 75 µL concentrations of non-heated fungal extract. Therefore, in order to compare the mutagenic potential of fungal extract and MMC, we used concentration values in SCE analysis. Table 2 represents the SCE frequency of cultures treated with non-heated fungal extract, positive control, and negative controls. A significant induction of SCE was observed in cultured lymphocytes treated with MMC (4 µg/mL) compared with non-heated fungal extract and negative control ($p < 0.001$). There was no significant difference between negative control and non-heated fungal extract ($p > 0.05$, $p = 0.73$). In conclusion, we did not observe any genotoxic effect.

In bioactive extracts of fungi, we revealed the determination of enzyme or enzymes responsible for cytotoxic effect on HeLa cell line. As a result, antitumor activitiy was shown by two enzymes—laccase and peroxidase—produced by fungi. Bioactive extracts have natural quinone substances from lignin by production of peroxidase and laccase. These enzymes acted more selectively on HeLa cells, arresting the cell in the G-phase of the cell cycle and inducing apoptosis. The basis of our work

TABLE 1. Effect of Bioactive Extract of *Funalia trogii* on Mitotic Index Values in Cultured Human Blood Lymphocytes and Bioactive Extract Enzyme Activities

Bioactive extracts	Normal mitotic index	*F. trogii* extract		
		Untreated	Treated	
	Negative control	Room temperature	65 °C	85 °C
Blood sample 1	7.8	3.70	3.20	4.80
Blood sample 2	8.2	3.33	3.40	5.20
Average	8.0	3.50	3.30	5.00
Laccase Activity (IU)	—	704.24 (8.5 CU)	674.23 (7.8 CU)	84.62 (0.43 CU)
Peroxidase Activity (IU)	—	780.32 (8.8 CU)	695.67 (6.0 CU)	50.76 (0.19 CU)
SOD (IU)	—	59.0	57.0	56.0
Ascorbate peroxidase (IU)	—	19.0	8.0	4.0
Catalase (nmol/min)	—	2.72	1.57	0.22
Glutathion reductase (IU)	—	3.3	3.1	2.2

was that bioreductive activation was also a highly specific delivery mechanism for targeting a variety of processes and was important for tumor growth. Thus, bioreduction of a quinone in the hypoxic region of a tumor would result in the formation of an intermediate semiquinone or hydroquinone (depending on 1-e or 2-e reduction). Hence, we proved that the covalent bond in quinone is metabolically stable, the effector quinone substance is only released within the hypoxic regions of tumors, and the desired large differential between the effects of the quinone is attained. We are able to produce the quinones naturally, which can act as excellent subtrates for NAD(P) H-quinone oxidoreductase (DT-diaphorase) and thus target tumors rich in this enzyme, thereby providing cytotoxic activation by a hypoxia independent mechanism.

This study provides evidence for *in vitro* antitumor activity of a bioactive extract from *F. trogii*. Therefore, upon *in vivo* data, which will follow this study, it may become a promising cytotoxic product for treatment of various types of cancer.

TABLE 2. SCE Frequency in Cultured Human Blood Lymphocytes Treated with Bioactive Extract and Negative and Positive Controls

Groups	Examined second metaphases	SCE/metaphase mean ± SE
Bioactive Extract (0.015 µL)	50	3.98 ± 0.10*
PC (MMC 4 µg/mL)	50	10.41 ± 0.01 **
NC	50	3.71 ± 0.065

** $p < 0.001$, * $p > 0.05$ compared with negative control, NC: negative control, PC: positive control, MMC: mitomycin C