Drug and Chemical Toxicology, 1:69–83, 2006

ISSN: 0148-0545 print
DOI: 10.1080/01480540500408655

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Evaluation of Cytotoxic and Mutagenic Effects of *Coriolus versicolor* and *Funalia trogii* Extracts on Mammalian Cells

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This study examined the *in vitro* cytotoxic activities of standardized aqueous bioactive extracts prepared from *Coriolus versicolor* and *Funalia trogii*ATCC 200800 on HeLa and fibroblast cell lines using a MTT (3-[4,5-dimetiltiazol-2-]-2–5-difeniltetrazolium bromide) cytotoxicity assay. *F. trogii* and *C. versicolor* extracts were cytotoxic to both cell lines. At 10 μL treatment level, *F. trogii* and *C. versicolor* extracts inhibited proliferation of HeLa cancer cells by 71.5% and 45%, respectively, compared with controls. Toxicity was lower toward normal fibroblasts. In the latter case, treatment at 10 μL level with *F. trogii* and *C. versicolor* extracts reduced cell proliferation by 51.3% and 38.7%, respectively. In separate experiments, the mitotic index (MI) obtained with 3 μL treatment level of unheated extracts of the two fungi was comparable to the MI value obtained by treatment with 4 μg/mL MMC (anticancer agent mitomycin-C). A significant induction of sister chromatid exchange (SCE) was observed in normal cultured lymphocytes treated with MMC (4 μg/mL). MMC treatment reduced replication index compared with treatment with unheated *F. trogii* extract and negative controls ($p < 0.001$). In contrast to MMC, *F. trogii* extracts did not affect the proliferation of human lymphocytes compared with controls ($p > 0.05$). Laccase and peroxidase enzyme activities in *F. trogii* extract were implicated in their inhibitory effect on cancer cells. *F. trogii* extract was concluded to have antitumor activity.

Keywords Antitumor effect, *Coriolus versicolor*, Cytotoxicity, Fibroblast, *Funalia trogii*, HeLa

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INTRODUCTION

Medicinal mushrooms have an established history of use in traditional oriental therapies of cancer. Modern clinical practice in Japan, China, Korea, and other Asian countries continues to rely on mushroom-derived preparations for therapy (Muzino, 1995). Medicinal effects have been demonstrated for many traditionally used mushrooms (Ooi and Liu, 1999), including extracts of *Favolus alveolarius* (Chang et al., 1988), *Phellinus linteus* (Chung et al., 1993; Kim et al., 2001), *Agaricus campestris* (Gray and Flatt, 1998), *Pestalotiopsis* sp. (Kiho et al., 1997), *Lentinus edodes* (Everett et al., 1985; Kim and Park, 1979; Song et al., 1998), *Pleurotus ostreatus* (Kim and Park, 1979), *Tricholoma* sp. (Liu et al., 1996, Wang et al., 1995, 1996a), and *Coriolus versicolor* (Chu et al., 2002; Cui and Chisti 2003; Fujita et al., 1988; Han et al., 1996; Li et al., 1990; Mao and Gridley, 1998; Mayer and Drews, 1980; Ng, 1998; Ooi and Liu, 1999; Yang et al., 1992a).

Among various bioactive components derived from *C. versicolor*, polysaccharopeptide (PSP) appears to be the most effective as an antitumor and immunomodulatory agent. PSP is a protein-bound polysaccharide isolated from the deep-layer cultivated mycelia of *C. versicolor* COV-1 strain (Yang et al., 1992b). The polysaccharide moiety of PSP is a (1,3)-glucan branching at 4V and 6V positions and consists of five different sugars including arabinose, glucose, galactose, mannose, and xylose. Its polysaccharide moiety is rich in aspartic and glutamic acids. The substance has a molecular weight of about 100,000 Da and is highly water-soluble (Ng, 1998; Wang et al., 1996b). *In vitro* studies have reported that PSP and PSK (protein-bound polysaccharide Krestin) (Sakagami et al., 1991) can directly inhibit the proliferation of leukemia, lymphoma, hepatoma, breast, and lung tumor cell lines in a dose-dependent manner (Chow et al., 2003; Dong et al., 1996, 1997; Hsieh and Wu, 2001; Hsieh et al., 2002; Kidd, 2000; Wang et al., 1995). Although recent studies have demonstrated its antitumor activity on cancer cells *in vitro* and *in vivo*, the exact mechanisms of action are not fully understood (Lau et al., 2004). This work reports on cytotoxic and other effects of *Funalia trogii* ATCC 200800 and *Coriolus versicolor* extracts on HeLa cervical cancer and normal human fibroblast cells. These effects are compared with the effects produced by the anticancer agent mitoycin-C (MMC).

Antitumor, cytotoxic, and possible beneficial properties of bioactive extracts on mammalian cells can be assessed *in vitro* by a number of tests including the ability of the extracts to induce production of certain enzymes in the cells. Genotoxic potential of extracts can be assessed with the sister chromatid exchange (SCE) test that is highly sensitive (Aydemir et al., 2005; Çelik and Akbaş, 2005; Krause et al., 2003; Lopez-Nigro et al., 2003; Tucker and Preston, 1996). SCE test uses genetic damage induced in cells by a compound as an indicator of its potential for mutagenicity. SCE test involves exchange of DNA segments between two sister chromatids in a chromosome during cell replication. An increased frequency of SCE in presence of the compound indicates its mutagenicity (Hoerauf

et al., 1999; Holz et al., 1995). SCE has been widely used to determine cytogenetic response to chemical exposure, and dose-response relationships have been established in many *in vivo* and *in vitro* studies (Aydemir et al., 2005; Çelik and Akbaş, 2005; Lopez-Nigro et al., 2003). The response of the SCE test is typically assessed in comparison with a standard. The prototype bioreductive agent mitomycin-C (MMC), which is used in treatment of cancer (Spanswick et al., 1998), is a suitable positive standard. MMC has genotoxic, mutagenic, and cytotoxic effects.

One possible mechanism by which certain extracts can protect mammalian cells is by possessing enzymes that deactivate compounds that can cause damage to cells. Ability of *F. trogii* extracts to protect cells against reactive oxygen species (ROS) was assessed by measuring the contents of protective enzymes in the extract preparations. ROS are capable of unrestricted oxidation of various cellular components and can lead to oxidative destruction of the cell (Bor et al., 2003; Mittler, 2002). Cells are normally protected against ROS by intricate antioxidative systems comprising enzymatic and non-enzymatic components (Kumar et al., 2002; Menezes Benavente et al., 2004; Shigeoka et al., 2002). Enzymes implicated in protecting cells against ROS include superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), glutathione reductase (GR; EC 1.6.4.2), and catalase (CAT; EC 1.11.1.6). Phenols, chlorophenols, dimetoxiphenols, and nitrophenols can be oxidized by a number of fungal and plant oxidative enzymes such as peroxidase (HrP; E.C. 1.11.1.7) (Cooper and Nicel, 1996) and laccase (Ullah et al. 2000) toxicity. Laccases (EC 1.10.3.2) are multicopper proteins belonging to the family of blue-oxidases enzymes, which can oxidize a variety of aromatic compounds with concomitant reduction of oxygen to water (Torres et al. 2003). A bioactive extract that possess these enzymes, or induces the cells to produce them, is potentially capable of mitigating oxidative damage to cells.

MATERIALS AND METHODS

Organisms

Macrofungi *Funalia trogii* and *Coriolus versicolor* were obtained from the Environmental Biotechnology Laboratory, Environmental Engineering Department, University of Mersin, Turkey. The fungi were maintained on Potato Dextrose Agar (PDA; Merck, Germany) slants and incubated at 30°C for 5 days and stored at 4°C.

Preparation of Bioactive Extracts

The solid-substrate fermentation (SSF) medium used for producing the fungal biomass consisted of wheat bran 36 g and soybean flour 4 g. The substrate was humidified with a 0.1 M, pH 6.0 sodium phosphate buffer added at 60% v/w. The humidified medium was placed in 1-L Erlenmeyer flasks and autoclaved

(120°C, 60 min). The autoclaved medium was inoculated with the fungal stock cultures that had been grown on PDA. The flasks were incubated for 10 days at 30°C. After this, the contents of the flasks were dried (Sanyo MIR 152 incubator, Japan) for 24h at 40°C. The dried material was ground in a coffee grinder for 2 min.

The ground biomass powder (1 g) was suspended in potassium phosphate buffer (10 mL, 0.1 M, pH 6.0) for 15 min. Solids were removed by centrifugation at 12, 000 × g (Hettich Micro 22R, Germany) for 15 min (Ünyayar et al., 2005). The supernatant (i.e. the bioactive extract) was sterilized using a 0.22-μm filter and diluted with aforementioned phosphate buffer to desired concentrations. In this study, 1 to 10 μL bioactive extracts including 0.057 to 0.574 μg protein/mL for *F. trogii* and 0.038 to 0.376 μg protein/mL for *C. versicolor* were used per well. Hot water or alcohols were not used for extraction in any step of this work.

Enzyme Assays

Activities of the following enzymes in the fungal extracts were quantified: superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), glutathione reductase (GR; EC 1.6.4.2), ascorbate peroxidase (APX; EC 1.11.1.11), laccase (E.C. 1.10.3.2), and horseradish peroxidase (HrP; E.C. 1.11.1.7).

Total SOD activity was assayed by monitoring the inhibition of photochemical reaction of nitroblue tetrazolium (NBT) according to the method of Beyer and Fridowich (1987). One unit of SOD activity was defined as the amount of the enzyme that was required to cause 50% inhibition of the consumption of NBT as monitored at 560 nm. CAT activity was assayed by measuring the rate of decomposition of H_2O_2 at 240 nm, as described by Aebi (1983). The initial rate was measured by noting the decrease in absorbance after 30 s of incubation. Glutathione reductase (GR) activity was measured by following the change in absorbance at 340 nm, according to the method of Carlberg and Mannervik (1985). APX was determined according to the method of Bonnet et al. (2000). A decrease in absorbance at 290 nm was measured. APX activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Laccase and horseradish peroxidase (HrP) activities were determined spectrophotometrically by monitoring the oxidation of guaicol as quinone according to Hiroi and Ericksson (1976).

Cell Cultures

HeLa cells, an epithelial-like adherent cell line from human cervix adenocarcinoma, and fibroblast cells were purchased from Sap Institute Culture Collection (Ankara, Turkey). The cell lines were grown and maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2. DMEM F-12 medium, supplemented with 10% fetal calf serum and 1% antibiotics (100 units of penicillin and 100 mg streptomycin), was used. The cells were subcultured twice a week, using a dissociation medium of Trypsin-EDTA (pH 7.4). After being harvested from culture flasks, the cells were counted using a hemocytometer,

and cell viability was determined by trypan blue exclusion. Fibroblast and HeLa cells were plated at 5×10^5 cells per well on 6-well plates or 3×10^5 cells/mL on 96-well plates. Twelve hours after seeding, *F. trogii* and *C. versicolor* extract solutions (1–10 μL per 200 μL well) were added and the cell cultures were incubated for 24 h at 37°C under 5% CO_2. After the incubation, the fungal extract–induced cells and controls were analyzed and compared.

Cytotoxic Effects of Fungal Extracts by Measurements of Cell Viability

Antitumor activity and cytotoxic effects of *F. trogii* and *C. versicolor* extracts on HeLa and fibroblast cells lines were determined at extract application levels of between 1 and 10 μL, using the 3-[4,5-dimetiltiazol-2-]-2–5-difeniltetrazolium bromide (MTT) assay. The MTT assay for cell injury is based on the ability of mitochondrial dehydrogenases of viable cells to reduce MTT to a purple formazan product that can be quantified spectrophotometrically (570 nm) (Coulter Micro Plate Reader, Fullerton, CA, USA) after solubilization in isopropyl alcohol containing 0.08 M HCl.

Cell growth inhibition values were calculated as follows:

$$\text{Percent inhibition of cell growth } (\%) = \frac{(A_b - A_a)}{A_b} \times 100$$

where A_b is the viability of control group of cells, and A_a was the viability of cells that had been treated with the fungal extract.

HeLa and fibroblast cells were plated at 3×10^5 cells/mL on 96-well plates. Twenty-four hours after seeding, *F. trogii* and *C. versicolor* extracts were added, and the plates were incubated at 37°C. After 24 h, the culture medium was aspirated and 13 μL MTT solution (5 mg/mL in RPMI-1640 without phenol-red) and 100 μL fresh medium were added to both the control cells and cells that had been treated with the fungal extracts. The plates were incubated for 4 h at 37°C. After this period, the medium was aspirated. The formazan crystals that had formed were dissolved by adding 100 μL of 0.08 M HCl in isopropyl alcohol; the solutions were read at 570 nm. The lines were cultured in medium alone or with fungal extract at five different concentrations (ranged from 0.057 to 0.574 μg protein/mL for *F. trogii* and 0.038 to 0.376 μg protein/mL for *C. versicolor*) in a 96-well microculture plate.

Microscopic Evaluations

Lymphocyte Cultures and Sister Chromatid Exchange Analysis in Peripheral Blood Lymphocytes

Three tests were performed: mitotic index (MI) for cytotoxicity, sister chromatid exchange (SCE) analysis for mutagenicity, and replication index

(RI) for evaluation of cytostatic effects. Normal cultured human blood lymphocytes were used. Fresh blood samples were collected from four healthy donors (two males and two females, aged 22 ± 2 years), as required, throughout this study. For each donor, lymphocyte cultures were prepared according to the method of Moorhead et al. (1960).

Mitotic Index (MI)

MI was used as one measure of cytotoxicity of *F. trogii* extracts (heated to 65°C and 85°C and nonheated) in comparison with the cytotoxicity of the well-known anticancer agent mitomycin-C (MMC). MI was calculated as the proportion of metaphase cells by observing a total of 3000 cells. All scoring and counting procedures were performed by the same investigator using a nonautomated microscope.

Cell Proliferation Kinetics (CPK)

Cells were analyzed for the relative frequency of first-division metaphase (M1, identifiable by uniform staining of both chromatids) and second-division metaphase (M2, identifiable by differential staining of both sister chromatids) and third and subsequent division metaphase (M3, identifiable by nonuniform patterns of staining of both chromatids). Replication or proliferation index (RI or PRI) is the average number of replications completed by metaphase cells and is calculated using the following equation (Lamberti et al., 1983):

$$\text{RI or PRI} = [1 \times (\text{M1}) + 2 \times (\text{M2}) + 3 \times (\text{M3})]/100$$

where M1, M2, and M3 are percentages of cells in phases M1, M2, and M3, respectively.

Sister Chromatid Exchange Analysis

SCE analysis was carried out for evaluation of mutagenic potential of *F. trogii* extracts relative to MMC. Heparinized whole blood (0.8 mL) was added to 4.5 mL of F10 cell culture medium (Gibco, USA) that was supplemented with 18.5% v/v fetal calf serum (Gibco, USA), 0.2 mL phytohemagglutinin (Gibco, USA), and antibiotics (10,000 IU/mL penicillin and 10,000 IU/mL streptomycin). 5-Bromo-2-deoxyuridine (9 μg/mL; Sigma, USA) was added to cultures at the beginning of the 72-h incubation period. Incubation was carried out at 37°C. Lymphocytes were cultured in the dark for 72 h, and metaphases were blocked during the last 1.5 h with colcemid added at a final concentration of 0.2 mg/mL.

For each lymphocyte test culture, 0.1 mL of *F. trogii* extract that had been sterilized with a 0.22-μm pore membrane was added at the beginning of the culture period. The cells were harvested by replacing the culture medium with KCl (0.075 M) in which cells were held for 20 min at 37°C. The cells were fixed in Carnoy's fixative (five treatments with methanol:acetic acid, 3:1, v/v) and

the glass slides were held at room temperature overnight. Air-dried slides were stained according to fluorescence-plus Giemsa (FPG) method (Pery and Wolf, 1974). SCEs were determined by examining 50 complete second metaphases: one SCE being counted each time two adjacent segments of one of the chromatids in a chromosome were stained differently.

Because the mitotic index values were nearly identical for 4 μg/mL MMC and 75 μL nonheated extract of *F. trogii*, the mutagenic potential of MMC and the extract were compared at these concentrations in the SCE analysis.

Statistical Analysis

Data were compared by one-way analysis of variance (ANOVA). Statistical analysis was performed using the SPSS for Windows 9.05 software package (SPSS Inc., Chicago, IL, USA). Multiple comparisons were performed by least significant difference (LSD) test for SCE, MI, and RI values. $P < 0.05$ was considered as the level of significance. ANOVA was performed using the Statisca for Windows software (Release 5.0; StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Percent inhibition of growth of cells measured with the MTT assay is shown in Table 1 relative to controls. Extracts of both fungi inhibited growth of HeLa cancer cells and normal fibroblasts in a dose-dependent manner in the concentration range tested. Both extracts had a stronger inhibitory effect on cancer cells compared with their effect on normal cells. For extract application levels of ≥3 μL, *F. trogii* extract had a significantly greater cytotoxic potency toward HeLa compared with the *C. versicolor* extract (Table 1). For the entire range of extract concentrations, the cytotoxicity of *F. trogii* extracts toward normal cells was comparable to that of the *C. versicolor* extracts (Table 1). ANOVA data (Table 2) confirmed that the effects of fungal species and the extract concentration on mortality of HeLa and fibroblast cells were statistically significant ($p = 0.05$). The interaction between the two main effects was also significant ($p = 0.05$) (Table 2).

The average mortality rates of HeLa and fibroblast cells are shown in Table 3 for various extract concentrations of the two fungi. For both cell types and fungi, increasing the extract concentration increased cell mortality. The extracts generally had greater inhibitory influence on HeLa cells, compared with their influence on fibroblasts. Microscopic observations confirmed that both the fungal extracts produced visually observable cytotoxic effects in fibroblasts and HeLa cells. The significant antiproliferative effect of *F. trogii* extract on HeLa cells reflects its potential therapeutic value in cancer treatment.

Our results were consistent with previous work on *C. versicolor* extracts. For example, PSP and PSK extracted from cultured *C. versicolor* were previously

Table 1: Growth inhibition by fungal extracts.

Extract	Extract application level (μL)	Growth inhibition (%)	
		HeLa	Fibroblast
Controls	0.0	0.0	0.0
F. trogii	1	27.2	17.2
	3	39.7	29.0
	5	56.1	36.0
	7	59.5	37.6
	10	71.5	51.3
C. versicolor	1	27.0	13.3
	3	27.5	18.5
	5	31.8	26.6
	7	40.8	29.8
	10	45.0	38.7

Table 2: Mean squares of ANOVA for effects of fungal species and extract concentration on mortality of cells.

Source of variation	Degrees of freedom	Mean square	
		HeLa	Fibroblast
Fungus (F)	1	0.085[a]	0.022[a]
Concentration (C)	4	0.052[a]	0.046[a]
F × C	4	0.008[a]	0.003[a]
Error	36	0.0001	0.0001

[a]$p < 0.05$.

shown to posses cytotoxic antitumor activity. PSP was found to dose-dependently inhibit the proliferation of HL-60 and Hep-G2 cells without significantly affecting the normal human peripheral blood lymphocytes and fetal hepatocytes (Dong et al., 1996, 1997). *C. versicolor* extracts at 50 to 800 μg/mL exhibited significant dose-dependent inhibitory effects on the proliferation of Raji, NB-4, and HL-60 cells, with more than 90% suppression of growth at the high application level (Lau et al., 2004). Unlike PSP and PSK that are hot extracted from fungal biomass, our results are for biomass extracted with cold buffer.

Because at concentrations of 5, 7, and 10 μL the *F. trogii* extracts were more effective against HeLa than were the *C. versicolor* extracts (Table 3), further work focused only on *F. trogii* extracts. The cytotoxic activity of *F. trogii* extracts was inactivated by heating to 85°C, implying the involvement of heat-inactivated enzymes in the observed bioactivity (Table 4). Enzymatic analysis showed that four important scavenger enzymes were found in *F. trogii* extract:

Table 3: Effect of fungal extracts on mortality rates of HeLa and fibroblast cells.

Fungus	Extract volume (μL) in 200 μL well	Absorbance	
		HeLa	Fibroblast
F. trogii	1	0.1326[e]	0.0952[h]
	3	0.1992[c, d]	0.1626[e, f]
	5	0.2882[b]	0.2138[c, d]
	7	0.3220[b]	0.2440[b, c]
	10	0.3804[a]	0.3046[a]
C. versicolor	1	0.1330[e]	0.1094[g, h]
	3	0.1372[e]	0.1286[f, g, h]
	5	0.1852[d]	0.1410[e, f, g]
	7	0.2208[c, d]	0.1740[d, e]
	10	0.2336[c]	0.2588[b]

Mean absorbance values followed by the same letter in the same column for a given fungus are not significantly different.

Table 4: Effect of heat treatment of *F. trogii* extract on cell growth inhibition and enzyme activity of extract.

	Heat treatment		
	None	65°C	85°C
HeLa growth inhibition (%)	72	71	0.0
Fibroblast growth inhibition (%)	51	49	0.0
Laccase activity (IU)	8.5	7.8	0.43
Peroxidase activity (IU)	8.8	6.0	0.19
SOD activity (IU)	59.0	57.0	56.0
Ascorbate peroxidase activity (IU)	19.0	8.0	4.0
Catalase activity (nmol/min)	2.72	1.57	0.22
Glutathione reductase activity (IU)	3.3	3.1	2.2

SOD, superoxide dismutase.

superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR). The extract also contained laccase and peroxidase. Heat treatment of the extract at 65°C and 85°C greatly reduced the activity of some of these enzymes (Table 4). Heat treatment did not affect the activity of SOD proving that *F. trogii* SOD is more heat stable than SOD activity of some other fungi and plants (Ali et al., 2005; Bai at al., 2003). Compared with unheated extract, heat treatment at 65°C had no effect on growth inhibitory activity of the extract toward HeLa and fibroblast cells. Treatment at 65 °C did reduce the activities of APX and CAT enzymes in the extract by 57% and 42%, respectively. However, the activities of laccase, peroxidase, and GR were only marginally (<11%) reduced. These results suggested that laccase, peroxidase, and GR enzymes were involved in cytotoxicity of extract, but APX

and CAT did not contribute to it. To better identify the enzymes responsible for cytotoxicity, extract that had been heated to 85°C and cooled was used in cytotoxicity assays. This treatment reduced activities of laccase, peroxidase, and GR enzymes by approximately 88%, 93%, and 33%, respectively (Table 4). The treated extract displayed no inhibition of HeLa and fibroblasts. These results indicated that cell growth inhibition was particularly influenced by the enzymes laccase and peroxidase. Although APX and CAT were not associated with the cytotoxic effect of the extracts, presence of these enzymes in the extracts suggests that *F. trogii* has a strong antioxidative defense system.

Antitumor activity of laccase and peroxidase is explained by the likely presence in the extract of natural quinone substances produced by the action of these enzymes on lignin substrate. Tumor cells are rich in NAD(P) H-quinone oxidoreductase (DT-diaphorase), which converts quinones to substances that are toxic to the cell (Fryatt et al., 2004; Moody and Wardman, 2002). Therefore, the fungal extracts are preferentially cytotoxic to tumor cells. Indeed, antitumor drugs such as MMC and geldanamycin rely on selective activation of quinones and quinone-like molecules to cytotoxic agents within the tumor cells.

Mutagenic and cytotoxic affects of *F. trogii* extract and the anticancer drug MMC were compared using the sister chromatid exchange assay and measurements of mitotic index and replication index. Table 5 shows the MI values observed in cultured human blood lymphocytes treated with various concentrations of MMC and *F. trogii* extracts. Data are shown both for unheated and heat-treated extracts. Compared with negative control, treatment of cells with unheated extract significantly reduced the MI value demonstrating a toxic effect of the extract on human peripheral blood lymphocytes *in vitro*. The cytotoxic effect of 3 μL unheated extract was comparable to that of 3 μL MMC (Table 5). Heat treatment at 65°C only marginally reduced the cytotoxicity, but treatment at 85°C greatly diminished it. There was no significant difference between 65°C-heated extract (3 μL) and treatment with 4 μg/mL concentration of MMC.

Table 6 shows data on the SCE frequency and replication index (RI) of lymphocytes treated with unheated *F. trogii* extract, positive control (i.e.,

Table 5: Mitotic index (MI) values of cultured human blood lymphocytes.

Group	Concentration	MI (%)[a] mean ± SE
Negative control	–	7.04 ± 0.060
F. trogii extract (unheated)	1 μL	5.09 ± 0.035
F. trogii extract (unheated)	3 μL	3.50 ± 0.185
MMC	4 μg/mL	3.53 ± 0.10
MMC	8 μg/mL	1.35 ± 0.071
F. trogii extract (65°C treatment)	3 μL	3.30 ± 0.10
F. trogii extract (85°C treatment)	3 μL	5.00 ± 0.20

Mmc, mitonycin-co.
[a]$p < 0.001$ compared with negative control; SE: standard error.

Table 6: SCE frequency and replication index (RI) values on cultured human blood lymphocytes.

Group	SCE/metaphase mean ± SE	RI mean ± SE
F. trogii extract (3 µL)	3.98 ± 0.10*a*	1.97 ± 0.025*a*
MMC (4 µg/mL)	10.41 ± 0.01*b*	1.86 ± 0.020*c*
Negative controls	3.71 ± 0.065	2.08 ± 0.035

MMC, mitomycin C.
[a]p > 0.05, [b]p < 0.001, [c]p < 0.05 compared with negative control; SE: standard error.

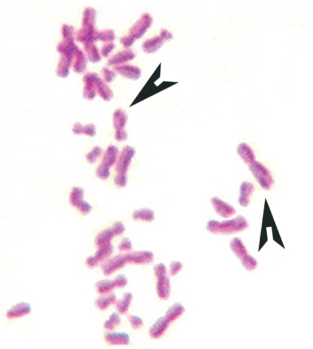

Figure 1: The SCE (arrowheads) in cultured human blood lymphocytes treated with MMC (4 µg/mL).

MMC), and negative controls. A significant induction of SCE was observed in lymphocytes treated with MMC (4 µg/mL) (Figure 1) compared with cells that had been treated with fungal extract and negative control ($p < 0.001$). There was no significant difference between SCE values of negative control and cells that had been treated with the unheated fungal extract ($p > 0.05$, $p = 0.73$).

Replication index (RI) is a measure of the kinetics of the cell cycle. MMC at 4 µg/mL significantly lowered the RI value compared with controls (Table 6). In contrast, *F. trogii* extract only marginally affected proliferation of human lymphocytes.

CONCLUSION

Cytotoxicity and cell growth inhibition studies performed with extracts of *Funalia trogii* ATCC 200800 showed the extracts to have significant cytotoxic and antiproliferative properties on the HeLa cancer cell line. The extract inhibited proliferation of HeLa cells in a dose-dependent manner. The source of the extract's cytotoxic activity was linked with the presence of laccase and

peroxidase enzymes in the extract. The extract did not have any mutagenic effects on human blood lymphocytes.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ersan Karababa for statistical analysis and Prof. Dr. Yusuf Chisti for valuable discussions and suggestions.

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