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ORIGINAL ARTICLE

The assessment of anti-tumoral activity of polysaccharide extracted from terrestrial filamentous fungus

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Abstract Fungal polysaccharides are well-known for the medicinal properties such as antitumor and immunomodulating effects. Hence, this study evaluated antitumor effects of polysaccharide extracted from *Fusarium* sp. isolated from soil samples of Karaj district, Alborz, Iran along with its taxonomic study. The filamentous fungus strain FK1 was isolated from the soil sample of Karaj, Iran. The strain was identified based on cultural, morphological and 18 S rRNA gene parameters as *Fusarium*. Further, the strain *Fusarium* was cultured in fermented broth of modified (PDB) for 10 days at 25 °C. The polysaccharide of strain FK1 was extracted from the mycelium free supernatant by boiling water method and evaluated for antitoxicity effect on two human cancer cell lines: HeLa cell line and Lymphoblastoid cell line (LCL) by MTT method. Findings revealed that water-extracted from mycelia polysaccharide of strain FK1 had the highest cytotoxicity effect against LCL which is the cause of B lymphocyte cancer, at 50 μg/ml concentration dose (114 ± 1.63) followed by 100 μg/ml (105 ± 0.57) and 10 μg/ml (104 ± 0.57), while it did not have a considerable effect on HeLa cell line. *Fusarium* could be alternative sources as an antitumor component.

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1. Introduction

Among microorganisms, fungi have been recognized as the most fruitful sources to produce a varied range of secondary bioactive metabolites such as antimicrobial, antioxidant, antitoxic and anticancer compounds with the various types of pharmaceutical and therapeutic applications (Lavi et al.,

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2006; Li et al., 2011; Mizuno et al., 1990; Yu et al., 2010). These bioactive compounds could be mainly classified as terpenoids, alkaloids, quinines, steroid, isocoumarins, ligase, phenols, phenyl propanoids and polysaccharides (Yu et al., 2010; Zhang et al., 2007). Among them, fungal polysaccharides are well known for their nutritional and extracted from medicinal plants. They are long carbohydrate molecules and edible fungi who live extensively in algae, plants, animals, and microorganisms such as fungi and bacteria (Tang et al., 2014; Wang et al., 2014; Zhao et al., 2014; Zhu et al., 2016a). During the past years according to unique biological activities of polysaccharides extracted have attracted increasing attention. The several studies have been reported that the biological actives such as anti-oxidation, anti-inflammatory, anti-tumor, anti-aging, anti-viral, anti-ulcer, neuroprotective and immunological activities (Tang et al., 2014; Wang et al., 2014; Zhao et al., 2014). In recent years, several studies on antitumor activity of polysaccharide extracted from basidiomycete mushrooms such as *Morchella esculenta*, *Agaricus brasiliensis*, *Ganoderma Lucidum* and *Lentinus edodes* have been reported (Chan et al., 2008; Duncan et al., 2002; Hsu et al., 2011; Mizuno et al., 1990; Yap and Ng, 2001; Zhang et al., 2007). Multiple new methods have been used for the extraction of polysaccharides including enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) (Zhu et al., 2016b). The genus *Fusarium* belongs to the *Nectriaceae* family, order *Hypocreales*, *Sordariomycetes* class and Phylum Ascomycota. *Fusarium* species is widely distributed in various habitats. Soil is the most common habitat for this organism. Up to now, not much work on the antitoxicity activities of the polysaccharide extracted from soil *Fusarium* species has been done. The main purpose of this study was to evaluate an antitumor effect of polysaccharide extracted from *Fusarium* spp. isolated from soil samples of Karaj district, Alborz, Iran along with its taxonomic study.

2. Materials and methods

2.1. Isolation of filamentous fungus

Soil samples were collected randomly from the agricultural lands of Karaj district, Alborze province, Iran during the year 2012–2013. Isolation of *Fusarium* species from soil samples were performed by serial dilution method (Leslie and Summerell, 2006) and plated on Sabouraud Dextrose Agar (SDA) medium procured from Merk, Germany. After 7 days, the incubated plates at 25 °C were visualized under the stereo binocular microscope (Mangus MS24) for the presence of *Fusarium* sp. The isolate was subculture on SDA slants supplemented with 50 μg/ml chloramphenicol as antibacterial antibiotic using a single spore method (Leslie and Summerell, 2006). The pure culture of *Fusarium* strain was maintained at 4 °C for further study.

2.2. Taxonomic characterization of the strain FK1

Strain of FK1 was identified based on cultural, morphological and sequence analysis of 18S rRNA gene parameters.

2.3. Cultural and morphological characteristics

Cultural characteristic of the *Fusarium* was studied on SDA medium by visual and stereo binocular microscopic (Mangus MS24) examination. Morphological characters such as macroconidia, microconidia, chlamydospores and conidiogenous cells were also studied by light microscope (Leslie and Summerell, 2006; Gerlach and Nirenberg, 1982).

2.4. DNA extraction

The isolated *Fusarium* was grown in 500 ml flasks containing 100 ml of PDB medium for 5 days at 25 °C by agitation to form pellets of vegetative cells. Total DNA (100 mg was extracted from the mycelium of the isolate using a Fermentase kit (Fermentas Inc., Hanover, MD) according to the manufactures instructions.

2.5. Amplification and sequencing of 18S rRNA Gene

The 18S rRNA gene was amplified using PCR with Taq DNA polymerase and universal fungal primer pairs 0817F (5- TTAGCATGGAATAATRRAATAGGA-3) and 1196 R (5- TCTGGACCTGGTGAGTTTCC-3). The procedure was performed using Thermal Cycler PCR (Eppendorf, Germany), in a total volume of 25 μl containing 50 ng/μl DNA, 10 μmol each primer, 10 mM dNTPs, 2.5 μl 10X PCR buffer and 0.25 Unit *Taq* DNA polymerase Fermentase kit (Fermentas Inc., Hanover, MD). PCR conditions include; initial denaturation at 95 °C for 30 min, followed by 40 cycles of denaturation at 95 °C for 30 s, primer annealing at 52 °C for 30 min and primer extension at 72 °C for 30 s and the final extension at 72 °C for 10 min. The PCR products were detected by 1% (w/v) agarose gel containing DNA safe stain (15 μl) and were visualized by Ultraviolet (UV) fluorescence gel documentation system (UTP-Bio Doc, USA). Sequencing of PCR product was carried out after PCR product clean up. The eluted pure PCR products were, subsequently sequenced by an automated gene sequencer (3730 xl DNA analyzer, Applied Biosystems, U.S.A).

2.6. Fermentation and extraction of polysaccharide compounds from strain Fusarium fungus

The fungus was cultured in SDA medium and incubated at 25 °C for 5 days. Fermentation was carried out in a 1 L Erlenmeyer flask containing 250 ml of modified SDB medium supplemented with 20% of glucose (Shuler and Karagi, 1992). One 6 mm agar disk was used as a seed culture to inoculate the fermented broth and incubated in an orbital shaker (150 rpm) for 10 days at 28 °C. After the incubation period, the mycelium biomass was separated from the culture supernatant through two layer Watchman No. 1 filter paper. Then, the mycelium biomass was used for further analysis. The polysaccharide was extracted from the mycelium free supernatant which was washed by phosphate buffer for three times. Extraction of the polysaccharide was done by boiling water method (Mizuno et al., 1984; Mizuno, 1999).

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