Cytotoxicity assay of *Pleurotus eryngii* fruiting body extract as an anti-cancer in vitro

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Abstract

*Pleurotus eryngii* one of the main edible mushrooms which is rich with important bioactive components. This work, designed to evaluate the bioactivity of purified crud fruiting body from *Pleurotuseryngii* as an anticancer effect on human colon cancer cell (HCT116) and Human epithelial type 2(Laryngeal ) (HEp-2) cells (Laryngeal Carcinoma Cells Lines with study its effect on normal Kidney (MDCK). The crystal violates method was used to determine the inhibition rate, live-cell number (optical density), and dose-response curve, and inhibition concentration of fifty number of cells (IC50). The effect of *Pleurotuseryngii* fruiting body extract on the above cells line at 2000, 1000, 500, 250, 125, and 62.5μg/ml concentrations for 24h. The results of this study show that the inhibition of cancer cells is directly proportional to the concentration of fruiting body extract.

Keywords: P eryngii, mushroom, bioactive componente


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Introduction

Mushrooms are considered functional foods, which can provide health benefits beyond the traditional nutrients they contain. Medical mushrooms are believed to possess many medical functions, including antitumor, immunomodulation, antioxidants, radical scavenging, cardiovascular, anti-hypercholesterolemia, anti-fungal, anti-parasitic, anti-fungal, swelling, detoxification, liver and many others, high basidiomycetes contain bioactive compounds in the fruit body, cultured mycelium (Cheung, 2008). Oyster mushroom and other species of mushrooms are widely relevant in the world and industry and are regarded to be one of the commercially valuable species in many countries on such a large and small scale (Adebayo et al. 2012a) as well as are the greatest wood decomposers, growing on a wide range of forest and agricultural waste than any other fungal community species (Adebayo et al. 2014a).
As a way to overcome this problem, the escalating need for food due to an increase in the world's population may have led to an interest in eating fungi, including Mushrooms (Abirami and Ananthi, 2015). *Pleurotus* species are considered to be one of the most desirable species in the field because of high nutritional value (Tesfaw et al. 2015). *Pleurotus* species as a good source of fatty acids especially oleic acids and linolenic which are essentially needed for building blocks of the human dietary (Solomko et al. 1984)

*Pleurotus eryngii*, commonly called the king oyster mushroom due to its remarkable flavor and nutritional value, has rapidly become a highly valued species among consumers in North Africa, Europe, and Asia (Jeong, et al. 2010).

The present search aimed

To determine the effect of fruiting body extract from *P. eryngii* as an anticancer to the colon and laryngeal cancer in vitro.

**Materials and Methods**

1. Culture Media: Liquid medium was prepared from powdered medium according to the US biological product manual according to Spector et al. (1998).

2. Maintenance media: Its preparation by way similar to those of tissue culture media listed above except it was free of fetal bovine serum (FBS) (Freshney, 1994).

3. Freezing media: Ten ml of stock solution was prepared from the following constituents: 6 ml serum-free media, 3 ml fetal bovine serum, 1 ml dimethyl sulfoxide (DMSO) was added drop by drop with mixing. The stock was stored at -20°C in between uses (Smith and Tata, 1991).

4. Prepared for fungal extracts: The fruiting bodies were grinding using a blender and then suspended in cold distal water after that incubated at 37°C. The suspension was filtered by using filter paper Whatman No. 2 to remove the biomass, this procedure repeated twice. The supernatant was concentrated by a rotary evaporator at 40°C under reduced pressure. The dried biomass dissolved in distilled water to make stocks 300 mg/ml and stored at 4°C. (Jedinak and Sliva, 2008).

5. Anticancer activity assays:

   1. Preparation of cell line for cytotoxicity assays: HCT-116, Hep2, and MDCK cells line in the frozen vial were obtained from the consultation office in the medicine college Babylon University and grown in 25 ml culture flask in growth medium containing 10%( FBS and antibiotics, incubated at 37°C).

   2. Freezing of cells line: The cell lines source was kept frozen at -180°C in a Nitrogen tank according to protocol mention by (Specter et al. 1998).
3. Thawing of cells line: The cell thawing according to Freshney, 1994.

4. Harvesting of cells: Harvesting is a technique that uses the proteolytic enzyme trypsin, to detach and disaggregate the adherent monolayer cells from the bottom of the culture vessel. This procedure was performed whenever the cells need to be harvested for passage and cell count (Freshney, 1994).

When the growth of cells enters a single layer and before the exponential process, the medium will be removed after that he cells washed with 3ml of warm PBS solution. 1ml of warm trypsin EDTA solution will be added to cover the monolayer with the rocking of the flask dish gentle for 4-5 times to flood the monolayer and incubated at 37°C until the monolayer detached. The separation of the cells completed by rocking the flask from side to side. The cells were gently piped up and down to destroy single-cell globs. In a growth medium with 5-10% FBS, which was used to inactivate the trypsin, cells were counted and re-suspended to the target amount on the culture plate, the cells were subcultured into two flasks or cultivated.

5. Cells line Subculture: After the cells converged as monolayers, the subculture was carried out by the protocol specified by Ng and Schantz, (2010).

6. Crystal violet assay: Using plate reader, crystal violet (C.V) reading was used to assess the optical density of cell growth in each microtiter plate well. The maintenance medium with the research material was discarded after the cytotoxicity check endpoint and the wells were washed by an automated pipette with 100 μl of cold PBS. Next, at room temperature, the cell cultures were fixed with 10 percent formalin buffered for 20 min. The fixing solution was discarded and an aqueous CV solution of 0.1 percent was applied to each well. By gentle shaking, the samples were incubated at room temperature for 20 minutes and washed in running tap water for 15 min. The plates allowed to dry in the air and the absorbance was read at 570 nm by a microplate reader (Castro-Garza et al. 2007). The percentage of inhibition was calculated according to the following equation: (Chiang et al. 2004).

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\text{Inhibition Rate (I.R) \%} = \frac{\text{optical density of control wells} - \text{optical density of test wells}}{\text{optical density of control wells}} \times 100.
\]

The percentage of viability was calculated according to the following equation: Viable cell number = (optical density of treatment wells X 105).

The concentration required for a 50% inhibition of viability (IC50) was determined using an excel sheet and fitted by blotting graphically of relative cell inhibition percentage in the Y-axis versus the concentration of each compound used in the X-axis.

1- Study design and cytotoxicity assays

The cytotoxicity assays were applied to determine the effect of various concentrations of fungal extracts on cell line culture, according to Freshney (1994). When the growth in the flask was monolayer before it
entered the exponential phase the cell monolayer was extracted and re-suspended in a concentration with a growth medium. In a concentration of $5 \times 10^5$ cell/ml and seeded in a 96 well microtiter plate. Since the cell growth reaches 80%, the wells were exposed to serial dilutions of the test chemicals as in the following experiments:

-Experiment No.1: The effect of the fungal extract on HCT116, HEP2, and MDCK cell line at 2000, 1000,500,250,125, and 62.5μg/ml concentrations for 24h.

Microtiter plates were seeded with HCT116, HEP2, and MDCK cells. Each cell type alone, Four replicates wells were considered as a control group and each one of the wells was exposed to 200 ul of each of the serial dilutions. Then the plate was covered with a self-plastic lid and incubated for 24hrs. After the end of the exposure, the wells washed with 200 μl of a sterile PBS. The effect of the extract on the cell line growth was assessed by C.V assay.

**Result**

**Assay of cytotoxic activity of the P. eryngii fruiting body extracts against carcinoma cells lines**

Two carcinoma cell lines were used to test the cytotoxic activity of the *P. eryngii* extracts, including human colonic epithelial carcinoma ((HCT-116)) (ATCC® CCL-247™) caused colorectal carcinoma and Human epithelial type 2 (HEp-2) cells, considered to originate from a human laryngeal carcinoma.

1: Cytotoxicity activities of *P. eryngii* fruiting body extract (HCT-116) cells

The figure (1) showed that all extract concentrations of *P. eryngii* used are inhibit of colon cancer cells(HCT-116)and exhibited significantly increasing inhibition of cancer in comparison with control treatments of viable cells number. And is markedly decreased cell proliferation of colon cancer with the increased of concentration (625Ug/ml to2000Ug/ml)

![Cytotoxic Activity of P. eryngii Extracts on HCT-116 Cells](chart)

Figure (1): cytotoxic activity of the *P. eryngii* extracts on (HCT-116) cells
2: Cytotoxicity activities of *P. eryngii* fruiting body extracts against (HEp-2) cells.

The results in figure (2) showed that all extract concentrations of *P. eryngii* used are inhibit the laryngeal carcinoma and exhibited a significantly increasing of inhibition of cancer with a dose-dependent manner (625Ug/ml to 2000Ug/ml) in compared with control treatments.

Figure (2) cytotoxic activity of the *P. eryngii* extracts on (HEP-2) cells

3: Assay of Cytotoxic effect of *P. eryngii* fruiting body extract on MDCK cell (Normal cell)

MDCK cells line in the frozen vial were obtained from the consultation office in the medicine college Babylon University. It was grown in a 25 ml culture flask with a growth medium containing 10% FBS and antibiotics, incubated at 37°C.

The fruiting extracted from *P. eryngii* exhibited a strong increase in cell size in concentration depended on a manor. (625Ug/ml to 2000Ug/ml) in comparison with the control cell (none treated cell) as shown in figure (3).

Figure (3) the effect of *P. eryngii* fruiting body extract on MDCK cell

Discussion

1: Cytotoxicity effect of *P. eryngii* fruiting body extracts against human (HCT-116) cells

*P. eryngii*, commonly called the king oyster mushroom due to its remarkable flavor and nutritional value, has rapidly become a highly valued species among consumers in North Africa, Europe, and Asia (Jeong, *et al.* 2010). (Zhang *et al.* 2014) evaluate the antitumor activity of PEP extracted from *P. eryngii* in vivo, murine renal cancer allograft model was established by s.c. injection of Rencacell (2×105 cells, 0.1 mL of PBS)

The polysaccharides extracted from *P. eryngii* have been demonstrated to have multiple functions, such as antitumor (Hwang, *et al.* 2003; Kim *et al.* 2004), antioxidant Kim *et al.*, (2004), (hepatoprotective (Chen, *et al.* 2012, Chen, *et al.* 2012), enhancing immunity (Kang *et al.* 2004)
Barrios-González (2018) investigated a newly identified protein from Pleurotus eryngii and showed that PEP had cytostatic effects on colon cancer cells through the induction of cell cycle arrest and cellular apoptosis. PEP significantly inhibited the colony formation of colon cancer cells, so the tumor volume was significantly suppressed in a dose-dependent manner, whereas the tumor growth did not exhibit any delay in the control group. The PPEP showed significant inhibition of the colon cancer cell growth without any cytotoxicity on normal colon cells at the same concentrations.

2: Cytotoxicity effect of *P. eryngii* fruiting body extracts against (HEp-2) cells

Compound from *P. eryngii* exhibited strong anti-cancer effects against a human laryngeal carcinoma in a concentration dependent on a manner. The result showed that cell cancer inhabited with the increasing concentration (625 μg/ml to 2000 μg/ml) in comparison with the control cell (none treated cell) as shown in figure (2).

The study also reported that *P. eryngii* has the anti-cancer effect dependent on the GC-Mass analysis method. It has been reported that the antioxidant activity of *P. eryngii* fruiting body extracts was well correlated with the content of their phenolic compounds. Phenolic compounds made a significant contribution to the antioxidant activity (Cheung *et al*. 2003; Gan *et al*. 2013).

(Hu *et al*. 2018) reported that the polyphenol-rich *P. eryngii* extract (PPEP) cause cytotoxic effects at the concentrations from (0 to 200 μg mL−1). In RAW264.7 cells (p < 0.05).

It was found that the fruiting bodies of *Ganoderma lucidum*, *Lentinus edodes*, and *Grifola frondosa*, have antitumor activity against the A549 human lung adenocarcinoma cell line and HEp-2 human laryngeal epidermoid carcinoma cells (Zhu *et al*. 2015).

3: Cytotoxic effect of *P. eryngii* fruiting body extract on MDCK cell (Normal cell)

Nutritional Value, The nutritional value of edible mushrooms is due to their high protein, fiber, vitamin and mineral contents, and low-fat level (Barro *et al*. 2008), edible mushrooms are a good source of protein, 200–250 g/kg of dry matter; leucine, valine, glutamine, glutamic and aspartic acids are the most abundant. Mushrooms are low-calorie foods since they provide low amounts of fat, 20–30 g/kg of dry matter, being linoleic (C18:2), oleic (C18:1), and palmitic (C16:0) the main fatty acids. Edible mushrooms contain high amounts of ash, 80–120 g/kg of dry matter (mainly potassium, phosphorus, magnesium, calcium, copper, iron, and zinc). Carbohydrates are found in high proportions in edible mushrooms, including chitin, glycogen, trehalose, and mannitol; besides, they contain fiberglucans, hemicelluloses, and pectic substances, also a good source of vitamins with high levels of riboflavin (vitamin B2), niacin, folates, and traces of vitamin C, B1, B12, D a *Pleurotus ostreatus* has highest nutritional value depend upon the presence of high-level of essential amino acids (arginine, alanine, glutamine, and glutamic acid).
carbohydrates (no starch, but found the glucans, mannitol, and trehalose), water content (from 80 to 90%), protein (40%), vitamin B, C, D, K, thiamine, riboflavin, folic acid and niacin (Çalarırmak, 2007; Wiater et al., 2015), minerals (Ca, P, Fe, K, Mn, Cu, Zn, Mg and Se (Roupas et al. 2012). Besides, it includes also essentials unsaturated fatty acids including oleic, linoleic, -linolenic and palmitic acids (Ça larırmak, 2007)

Conclusions

1. The fruit extract of the fungus P. eryngii was potent in inhibiting or killing cancer cells, including cancer of the colon and larynx cancer

2. The fungus P. eryngii does not have a toxic effect on normal cells but works as a nutrient-rich substance to increase cell growth.

References


Ça larırmak, N. (2007). The nutrients of exotic mushrooms (Lentinula edodes and Pleurotus species) and an estimated approach to the volatile compounds. Food chemistry, 105(3), 1188-1194.

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