CYTOTOXIC ACTIVITY OF PLEUROTUS CITRINOPILEATUS EXTRACTS AGAINST HEPG2 CELL LINE

Saroja Preethy¹, Dr. Anbuselvi²
¹²Industrial Biotechnology Department, Bharath Institute of Higher Education and Research, Selaiyur, Chennai 600073
rathna07@hotmail.com

ABSTRACT

Background and Objective: Oyster mushrooms being one of the largely cultivated and harvested species only next to button and milky white mushrooms in India, have numerous medicinal values and one such is the cytotoxic activity exhibited by the glycoprotein extracts against various cell line like U937 leukemia cells. The aim of the present study is to find the degree of cytotoxicity caused by the extracts of Pleurotus citrinopileatus against HepG2 cell lines.

Methods: Herein, we are going to compare the degree of cytotoxicity caused by three different extracts [acetone, ethanol and aqueous] of Pleurotus citrinopileatus using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay.

Result: P. citrinopileatus acetone extracts showed higher cytotoxic activity indicated by MTT assay with the half maximal inhibitory concentration of IC50 value 37.03 µg/ml against the HepG2 cell lines compared to ethanol and aqueous extracts.

Conclusion: P. citrinopileatus acetone extract appears to contain the biologically active compounds that might help reducing the cancer cell viability.

Novelty: This study gives us further insight into the promising drug candidates from the P. citrinopileatus extracts for combating different cancers like leukemia, liver, breast and many more.

Keywords: Acetone, aqueous extract, Pleurotus citrinopileatus, cell viability, cytotoxic activity, ethanolic extracts.

I. INTRODUCTION

Liver cancer is one of the fastest emerging cancer in India with over 50,000 cases reported annually. With years passing by many cancer management options have come into existence with the first line of treatment including surgical removal, chemotherapy, radiations, ablations, freezing of cancer cells, drug targeted therapy, embolization, chemo-embolization, immunotherapy and palliative care.

Use of non-toxic mushrooms as immunotherapeutic drugs or adjuvant drugs has been into practice late 20th and early 21st century. In this study, we are to investigate the cytotoxic activity in the extract of Pleurotus citrinopileatus against the hepatocarcinoma cell line (HepG2 cell line).

The in vitro determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple
formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

II. MATERIALS & METHODS

MTT reagent (the solution is filtered through a 0.2 μm filter and stored at 2–8 °C for frequent use or frozen for extended periods)

1 DMSO
2 CO₂ incubator
3 Micro Plate reader
4 Inverted microscope
5 Refrigerated centrifuge
6 Mushroom extracts – aqueous, ethanol and acetone extracts.
7 Cisplatin

MUSHROOM EXTRACTS

Freshly collected fruiting bodies of *P. citrinopileatus* was sun dried for 96 hours and finely pulverized. Five grams of powdered samples were extracted with 100 ml of aqueous solution, 80% of ethanol and 80% of acetone with stirring at 150 rpm for 24 hours at 25°C to obtain aqueous, ethanol and acetone extracts, respectively. The mixture was then filtered through two layers of Whatman No. 1 filter paper. The residue was then extracted with two 100 ml aliquots of aqueous solution, ethanol and acetone as described above. Then the extracts were evaporated and remaining solvent was removed with freeze drier. These are considered the test solutions.

Preparation of test solutions

For Anticancer studies, serial two fold dilutions (3.125-100μg/ml) were prepared from this for carrying out anticancer studies.

Cell lines and culture medium

Human liver carcinoma cell lines (HepG2) were procured from NCCS, stock cells was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) in an humidified atmosphere of 5% CO₂ at 37°C until confluent. The cell was dissociated with TPVG solution.

www.turkjphysiotherrehabil.org 14470
(0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5% CO₂ incubator.

**Source of reagents:** DMEM, FBS, Pen strip, Trypsin procured from Himedia.

**Procedure**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁴ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100μl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100μl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO₂ atmosphere. After incubation the test solutions in the wells were discarded and 100μl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100μl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line.

**IC₅₀ Value:**

The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half.

The IC₅₀ of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC₅₀ values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC₅₀ values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve.

The direct microscopic observations of drug treated images of cell lines by inverted biological microscope was performed and percentage of HepG2 cell viability against of test samples of serial two fold dilutions (3.125-100μg/ml) were assessed and compared with cisplatin, a commonly used anticancer agent in chemotherapy for hepatocarcinoma patients.

**III. RESULTS:**

The direct microscopic observations of drug treated images of cell lines by inverted biological microscope shows the number of live cells and change in cell morphology is studied. HepG2 cell lines treated with acetone extract and cisplatin exhibited vast morphological changes, apoptosis related characteristics such as shrinkage, nuclear collapse, blebbing cell membrane and apoptotic bodies compared to ethanol or aqueous extracts [Fig. 1]. Figure 2 shows the does-response curve formed from three different extracts concentration against the HepG2 cell line which shows significant degree of cytotoxicity in cell line treated with acetone extract.
Fig. 1. Effect of a) acetone extract, b) ethanol extract, c) aqueous extract and d) cisplatin in HepG2 cell line.

The IC\textsubscript{50} value of the given samples (Acetone, Ethanol, Aqueous) is 37.03, 39.57, >100 \mu g/ml, which proves that the 100 \mu g/ml of acetone extract has greater degree of cytotoxicity compared to ethanol and aqueous extracts.

IV. DISCUSSION

In our study the finding that the acetone extracts of \textit{P. citrinopileatus} fruiting bodies exhibited strong inhibitory activity against the HepG2 cells compared to ethanol or aqueous extracts which completely contradicts the study by Younis and his colleagues in which the water extract of \textit{Pleurotus} species shows highest cytotoxic effect against HepG2 and 2 other cancer cell lines.\textsuperscript{5}

More of water extracts of \textit{Pleurotus} species have exhibited higher cytotoxicity so far, followed by methanolic extracts rather than acetone or ethanolic extracts of the oyster mushroom.

In our study, the use of the acetone extract as an adjuvant enhanced the drug sensitivity to the chemotherapeutic drug, cisplatin, bringing the IC50 value to 4.31 \mu g/ml. The results obtained were very similar to that conducted by Xu and his colleagues where exposure of liver cancer cells to polysaccharide-protein complex isolated from
oyster mushrooms reduced the in vitro cancer cell proliferation and invasion but also enhanced the drug-sensitivity to Cisplatin.

V. CONCLUSION

Through the study, the fact to use oyster mushroom extract as an adjuvant drug is reiterated.

Acknowledgment

The authors are grateful to Greensmed labs for technical assistance.

REFERENCES: