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PRELIMINARY STUDY FOR THE EFFECT OF DIONYSIA AUCHERI METHANOLIC EXTRACT ON THE VIABILITY OF SOME CELL LINES

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ABSTRACT

The purpose of present study is a cytotoxic investigation for Dionysia aucheri methanolic extract on different cell lines. Methods used for that: HPLC fractionation; the fractions proceeded according to protocol of the pharmacology department, university of Malaya, Kuala Lumpur, Malaysia, and the largest purified fraction was used for MTT test. Four types of cell lines (Normal human hepatic cell line (WRL), Human lung adenocarcinoma cell line (A549), Human hepatic carcinoma cell line (HEPG2) and Human prostate carcinoma cell line (PC-3)) were treated with six two fold concentrations (100,50,25,12.5, 6.25 and 3.1285) µg/ml, at 24 hour interval. Results showed that the extract affected all types of cell line in a dose dependent manner. The most important conclusion in this study was the significant effect of the all D.aucheri extract concentrations on the viability of human hepatic carcinoma (HepG-2) cell line, while little cytotoxic effect of the methanolic extract was on the human prostate carcinoma (PC-3) cells which might give the plant an importance to be a promise phyto protective agent. This made an imported demand to search and investigates the plant active components that affect even normal cells.

KEYWORDS: (cytotoxic investigation, Dionysia aucheri, methanolic extract, HPLC, WRL cell line, HEPG-2 cell line, A549 cell line, PC-3 cell line, Phytoprotective agent).

INTRODUCTION

As there is a pressing need for the development of new drugs due to disease resistance toward conventional treatments as well as the drugs side effects; the natural products are available source as a novel active drug for many diseases.[1] Dionysia aucheri family Primulaceae is one of the thousands natural plants that rich with active components need for investigation, among them essential oils and flavonoids.[5] Screening potential new substance toxicity is an essential aspect for drug discovery process and any changes in cell viability are directly correlated to the toxic effect of that tested compounds.[2]

The MTT based cytotoxicity assay is widely choses as a cell viability-measurement for optimal end point.MTT is a yellow water-soluble tetrazolium dye {3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide} reduced by live, but not the dead cells to a purple formazan product that is insoluble in aqueous solution. The amount of MTT-formazan produced can be determined spectrophotometrically once solubilized in suitable solvent after cells have been espoused to different concentration at different time intervals to that material of concerned.[3]

MATERIALS AND METHODS

Plant extract

About 20 g. of the dried powdered aerial part of the plant Dianysia aucheri was macerated in 150 ml of 80%methanolic solution for 24 hours in a dry dark place then filtered. The filtrate was concentrated by rotary evaporator to get a brown viscous residue with about 5.6 g. weight kept in a cool place till use. A 200mg was sent for chromatography and compound isolation library.[4]

Chromatography and compound isolation

The dried (200mg) methanolic extract of Dionysia aucheri was fractionated using reversed phase C18 flash column chromatography into many fractions according to protocol of the pharmacology department, university of Malaya, Kuala Lumpur, Malaysia. After dissolving the residue with methanol and applied gradient and fractionation HPLC to yield the following fractions (1):Fraction A: MeOH:H2O (20:80); B : MeOH:H2O (30:70); C :MeOH:H2O (40:60) D : MeOH:H2O (50:50); E as D ;F:MeOH:H2O(60:40); G:MeOH:H2O(100:0). All fractions were analyzed using liquid chromatography mass spectrometry (LC-MS) (Shimadzu ITTOF) .The largest fraction was separated and subjected to preparative liquid chromatography HPLC using a Waters Novapak C18

column(25X100mm,6µm).The extract was eluted at rate of 12 ml/min over 60 minutes and the gradient started at 30% solvent B(acetonitrile with 0.1%formic acid) and 70% solvent A(water with 0.1%formic acid)for 5 minutes, then 30-37%(B)for 5 minutes. The gradient then changed from 37% to 50%(B) over 45 minutes, followed by 50-100% of (B) for 5 minutes. Finally, there was an isocratic elution of 100%(B)for 50-60 minutes. The largest purified fraction was used for this study.

Preparation of the extract solutions

Six serial two folds dilution of purified fraction in a growth media DMEM were prepared :(100,50,25,12.5, 6.25 and 3.1285) µg/ml, then sterilized through 0.22µm Millipore filter. All were kept in dark cool place till use.[1]

Type of cell lines used in the study

All types are supplied by Department of pharmacology, University of Malaya, Kuala Lumpur (1): Normal human hepatic cell line (WRL), Human lung adenocarcinoma cell line (A549), Human hepatic carcinoma cell line (HEPG2) and Human prostate carcinoma cell line (PC-3).

Cell viability and the MTT-based cytotoxicity assay[1,3]

Cell cultures were maintained in high glucose DMEM with 10% FBS using aseptic techniques. Cells from 70-90% confluent flasks with >90% viability were seeded into 96-well Microtiter culture plates. The viability of the cells was assessed using the MTT colorimetric assay. Four types of cell lines (WRL,PC-3,A459 and HepG2)were either left untreated(as control) or were treated with different consternation(100,50,25,12.5,6.25 and 3.1285µg/ml)from the isolated fraction in the previous step then following 4 hours incubation, the medium was replaced with 100 µl fresh DMEM and 20 µl of 5 mg/ml MTT dye solution and further incubated for one hour. Subsequently, the cell medium was aspirated and 100 µl of 100% DMSO was added to all wells to dissolve the insoluble purple formazan product into a coloured solution; the absorbance of which was measured at a wavelength of 570 nm using a microplate reader. The optical density (OD) of the samples was compared to that of the control to obtain the percentage viability as follow:

Cell viability (%) = [(OD at570) of sample/ (ODat570) of control] X100.

RESULTS

The highest composition fraction from theDionysia aucheri methanolic extract after purification then subjected to a cytotoxic assay showed different effects toward different cell lines. The normal hepatic cell line(WRL) was affected by the highest concentration (100)µg/ml, table-1- show a significant cytotoxicity effect and cause about 29% inhibition to cell viability. The toxic effect of the plant extract was decreased as the concentration decreased.

Table-1-Effect of different concentration of D.aucheri on WRL Cell viability percentage

Concentration µg/ml	Cells Viability (%)	Standard deviation
100	70.83969	2.21
50	98.43511	3.54
25	104.3893	4.01
12.5	108.2061	1.25
6.25	111.6412	0.25
3.1285	116.1069	2.54

The extract showed cytotoxic effect on the human adenocarcinoma (A549) cell line. As shownin table-2- the potent cytotoxic effect was at the concentration 100µg/ml then decreased significantly with the decreased of extract concentration.

Tablet-2-Effect of different concentrations of D.auchei methanolic extract on A549 cell viability

Concentration µg/ml	Cells Viability (%)	Standard deviation
100	77.63636	3.59
50	88.42424	4.25
25	91.27273	3.95
12.5	91.69697	3.58
6.25	91.93939	4.01
3.1285	95.81818	2.54

The most important result in this study was the significant effect of the all D.aucheri extract concentration on the viability of human hepatic carcinoma (HepG-2) cell line table-3-. About half cells viability was affected by 100µg/ml plant extract, then declined but till had toxic effect even at the lowest concentration 6.25µg/ml.

Table-3-Effect of different concentration of D.aucheri onHepG-2 cell viability

Concentration µg/ml	Cells Viability (%)	Standard deviation
100	59.93884	3.2
50	70.69317	2.40
25	77.11519	1.20
12.5	85.57594	0.90
6.25	89.60245	2.22
3.1285	98.36901	4.11

The fourth type of cell line subjected to different concentration of the methanolic extract was the human prostate carcinoma (PC-3) cells. The extract show different behaveour, there was little cytotoxic effect and the potent action was at concentration 100µg/ml which caused less than 5% cell inhibition,table-4.

Table-4-Effect of different concentration o f D.aucheri onPC-3 cell viability

Concentration µg/ml	Cells Viability (%)	Standard deviation
100	95.18072	3.01
50	98.79518	1.08
25	101.6064	0.98
12.5	104.0161	1.59
6.25	106.0241	2.57
3.1285	108.4337	3.54

DISCUSSION

The conclusion of this study reviled that even many plants and their extract had been used in public and folklore medicine ,an extensive study should be done to stand on their actual benefits avoiding the expected cytotoxic effect on normal cells. The study proofed that this plant extract exhibit different effects , as there was toxic effect toward cancerous cell lines in does dependent manner, at the same time high concentration showed potent toxicity toward normal cell line and this emphasized the importance of the plant researches to investigate their effect on various cells and tissues. Carcinogenesis is a multistep process, and oxidative damage is linked to the formation of tumors through several mechanisms.[9,10] Oxidative stress induced by free radicals causes DNA damage, which, when left unrepaired, can lead to base mutation, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement.[10] This potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Studies to date have demonstrated that phytochemicals in common fruits and vegetables

can have complementary and overlapping mechanisms of action including antioxidant activity and scavenging free radicals; regulation of gene expression in cell proliferation, cell differentiation, oncogenes, and tumor suppressor genes; induction of cell-cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation, and reduction; stimulation of the immune system; regulation of hormone metabolism; and antibacterial and antiviral effects.[7,8,15,16]

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents, some of which are necessary for life. These agents may be present in air, food, and water, or they may be produced by metabolic activity within cells. The key factor is to maintain a balance between oxidants and antioxidants to sustain optimal physiological conditions. Overproduction of oxidants can cause an imbalance, leading to oxidative stress, especially in chronic bacterial, viral, and parasitic infections.[9] Oxidative stress can cause oxidative damage to large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk for cancer and CVD (cardio-vascular disease).[6,9,10] To prevent or slow the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Fruits, vegetables, and whole grains contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics and carotenoids, and may help protect cellular systems from oxidative damage and also may lower the risk of chronic diseases.[7,8,11,12] Strong epidemiological evidence suggests that regular consumption of fruits and vegetables can reduce cancer risk. Block et al.[13] reviewed about 200 epidemiological studies that examined the relationship between intake of fruits and vegetables and cancer of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary. As fruits and vegetable were significantly protective in cancer of the esophagus, oral cavity, larynx , the pancreas and stomach in 26 studies and for colorectal and bladder cancer in 23 of studies ,Dionysia aucheri is a promissing phytochemical rich with flavonoids and essential oils need for further investigation and studies. A prospective study involving 9959 men and women in Finland showed an inverse association between the intake of flavonoids and incidence of cancer at all sites combined[14] the risk of lung cancer was reduced by 50% in the highest quartile of flavonol intake. Consumption of quercetin from onions and apples was found to be inversely associated with lung cancer risk.[15] The effect of onions was particularly strong against squamous-cell carcinoma. Boyle et al.[16] showed that increased plasma levels of

quercetin was accompanied by increased resistance to strand breakage by lymphocyte DNA and decreased levels of some oxidative metabolites in the urine.

The resutls showed that there were some cytotoxic effects on normal cell line(WRL)cell line which may explaine the toxic dose (toxic level) of plant extract with all active constituents applied in this research ,however most essential oils have been found to be cytotoxic without being mutagenic, it is likely that most of them are also devoid of carcinogenicity and may be considered as secondary carcinogens after metabolic activation.[17] For this reasons there are important demands to make further investigations and researches about Dionysia aucheri active components and their effects.

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