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ORIGINAL ARTICLE

Changes in Lung Cancer Cell Line Affected by Cytotoxicity of Lagenaria Siceraria Plant Extract

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ABSTRACT

Chemotherapy is a type of cancer treatment in which the lack of selective cytotoxicity often leads to intolerable side effects. Today, the use of medicinal plants is essential in treating cancer due to their fewer side effects. Lagenaria siceraria Standl is critical for cytotoxicity studies due to its polyphenolic, cucurbitacins, pectin, flavonoids, and saponin compounds. In this study, the cytotoxic effects of plant fruit extract were investigated on lung cancer cell lines. To this end, the hydroalcoholic extract of the plant fruit was initially prepared by the percolation method. Then, the effects of solutions containing samples with different concentrations (5000, 500, 1000, 100, 100, 250, 10, 1, 0.1µg.ml^{-1}) were investigated by MTT assay on lung cancer cell line (A549). Cisplatin was considered as a positive control. Statistical calculations were carried out using Prism V.3 software to compare IC_{50}, and the data were analyzed by analysis of variance (ANOVA) and t-test. The results indicated that the IC50 level of cisplatin anti-cancer drug, as a common drug in the market, is significantly lower than Lagenaria siceraria extract. However, the extract of this plant revealed a significant growth inhibitory effect on lung cancer cells. The results also showed that Lagenaria siceraria extract is an effective cytotoxic compound on lung cancer cells. More extensive studies are needed to find effective plant extracts compounds to find and design new and effective cancer treatment drugs.

Keywords: Lagenaria siceraria, Cell line, Lung cancer, IC50, MTTassay.

INTRODUCTION

Nowadays, various cancers cause a large proportion of deaths across the world. There are various treatments for cancer, such as surgery, chemotherapy, radiotherapy, hormone therapy, immunotherapy, and etc. Various methods are also used in the case of lung cancer. Systemic chemotherapy is one of which, but the lack of selective toxicity often leads to intolerable side effects. Today, medicinal plants (mainly native plants) are essential in cancer treatment apart from the commonly mentioned methods. Medicinal plants are still recognized as a cheap and safe natural resource as less toxic drugs worldwide. Many developing countries have relied more on traditional herbs than chemical drugs to treat their diseases (Mariod et al., 2020; Kubde et al., 2010). On the other hand, cell culture has been dramatically progressed to measure the cytotoxicity of chemicals, drugs, pesticides, and in vitro analysis of all-natural and synthetic compounds, etc. (Dorsley, 1996).

The Medicinal plant, Calabash, with the scientific name of Lagenaria siceraria, belongs to the Cucurbitaceae family, which has approximately 118 genera and 825 species. This genus has a creeping annual plant species, usually scattered in hot areas and mainly planted because it is ornamental. This plant is known as Calabash in Persian, but it is known as Bottle Gourd in English and Lauki in Hindi and Urdu. Lagenaria siceraria is originally native to India and Africa (Mozaffarian, 2007). Lagenaria Siceraria fruit is a source of vitamin C, beta-carotene, B vitamins, pectin, high choline levels as a lipotropic factor, saponin, essential oils, and cucurbitacin B, D, G, H (Tyagi et al., 2017). Cucurbitacins are a group of quaternary triterpenoids that have been considered due to having cytotoxic and antitumor properties. Cucurbitacins are conventionally divided into 12 groups, including A-T, especially cucurbitacins having cytokine effects. The E extract of Lagenaria siceraria is due to flavonoids, tannins, steroids such as fucosterol and campesterol, phenol, and glycoside in addition to the above cases (Shah and Seth, 2010).

A new ribosomal activity inhibitor (RIP) protein called Lagenin has also been isolated from the lyophilized aqueous extract of the seeds of Lagenaria siceraria, which has immunosuppressive, antiviral, and anti-HIV properties (Dhiman et al., 2012).

This plant has antioxidant, cardioprotective, hepatoprotective, analgesic, anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, diuretic, and immune system regulating effects. Ghosh et al. (2009) showed that the water-soluble polysaccharide from Lagenaria siceraria was identified by the NMR method, and its toxicity effect on the human breast adenocarcinoma cell line (MCF-7) was determined by MTTassay (Tyagi et al., 2012; Sankari et al., 2010; Ghosh et al., 2009).

Saha et al. (2011) examined the antitumor effect of methanolic extract of Lagenaria siceraria shoots on Ehrlich and Ascites carcinoma cells by induction in rats and compared the results with FU-5. Finally, its antitumor effect was proven by measuring the rats' lifespan and measuring hematological and biochemical parameters of rat liver tissue (Saha et al., 2011).

Studies show that no study has been conducted on the fruit of the studied species of this plant. Moreover, the effects of this plant's hydroalcoholic extract on lung cancer cell lines have not been studied. Since no similar study of Lagenaria siceraria has been performed on lung cancer cell line in Iran (Tyagi et al., 2012; Deshpande et al., 2008), the purpose of this research is to study the effect of hydroalcoholic extract of this plant to inhibit the growth of target cells (lung cancer).

MATERIALS AND METHODS

The applied method in this research can be divided into three stages, including extraction, storage, cell culture, and

finally, investigating the toxicity of the studied plant, which will be explained in detail below:

Extraction in 2020: This research has employed an experimental laboratory study. The first stage was to collect Lagenaria siceraria from Dangsarak village, Neka city, Mazandaran province, in the early fall of 2020, which was matched with a herbarium sample in Tehran School of Pharmacy, and the scientific name of the plant was approved by a relevant expert. The next step was to dry the fruit, which was done under the hood at room temperature until the plant was dehydrated. The dried plant was crushed with a mortar, and the seeds were separated from the fleshy shell. Then, the required fruit peel was crushed using a hand mill. In the next stage, 500g of the dried shell was extracted by percolation method with 80% methanol solvent. The extraction process was performed 3 times with an interval of 48 hours.

The extract was concentrated using a rotary evaporate distillation apparatus and completely dried for 3 days using a freeze dryer at -45°C to form a powder. Finally, the dry powder was stored in a sealed glass container in the refrigerator, away from heat and light until it was time for cell culture experiments.

Cell culture and storage: In this research, the A549 cell line of human lung cancer was purchased from Pasteur Institute Cell Bank of Tehran. Cell passages between 26 and 31 in DMEM medium with 10% fetal bovine serum (FBS), 100mM sodium pyruvate, 1.5g.l^{-1} sodium bicarbonate and 1% penicillin-streptomycin in an incubator (BINDER, USA) were used at 37°C, with sufficient humidity and 5% carbon dioxide. When the cells reached at least 70% cell growth, they were separated from the bottom of the flask by trypsin-ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500rpm for 5 minutes. The cell sediment was prepared in suspension in 1cc of culture medium, and the viability percentage of cells in cell suspension was determined by mixing an equal proportion of trypan blue using a hemocytometer slide and examination by light microscopy. After ensuring that the cells were not infected, cells with viability above 90% were used for testing (Tyagi et al., 2012; Ghosh et al., 2009).

Cytotoxicity of Lagenaria siceraria through MTT assay: MTTI colorimetric method was used to investigate the effect of Lagenaria siceraria extract on the growth and proliferation of cancer cells. This method is a competitive mitochondrial metabolic test based on the decomposition of tetrazolium salt by the enzyme mitochondrial succinate dehydrogenase of living cells. In this method, 100µl of culture medium containing 104 cells was placed in each 96-well plate well, and 0.1, 1, 10, 100, 250, 500, 1000, and 5000µg per ml of Lagenaria siceraria extract were added to the cells after 24 hours of incubation and incubated for 72 hours. Then, 20µl of MTT (5mg.ml^{-1} from Sigma Company) was added to each well and incubated in the dark for another 4 hours. The culture medium containing MTTI was carefully removed after the necessary time, and 50µl of diluted DMSO solution was added to each plate house to dissolve the purple formazan. The light absorption of each well was read using ELISA at 490 and 630nm after 15 minutes of incubation at room temperature. The results are reported as the percentage of cell survival against the concentration of the extract. The percentage of cell survival is calculated as follows:

Cell survival rate = (test light absorption / control light absorption) × 100

The statistical analysis was carried out by ANOVA and T-test.

Research findings: The IC_{50} value of Lagenaria siceraria extract on lung cancer cell (A549) is 93.094±6.5µg.ml^{-1}, which is as much as 5.37±0.35µg.ml^{-1} for cisplatin anticancer drug. The comparison of IC_{50} data of plant extract and cisplatin on lung cancer cell line (A549) showed that there was a significant difference between all data ($p<0.05$).

The results indicated that IC_{50} of cisplatin drug was significantly lower than IC50 of plant extract ($p<0.01$) (Figure 1).

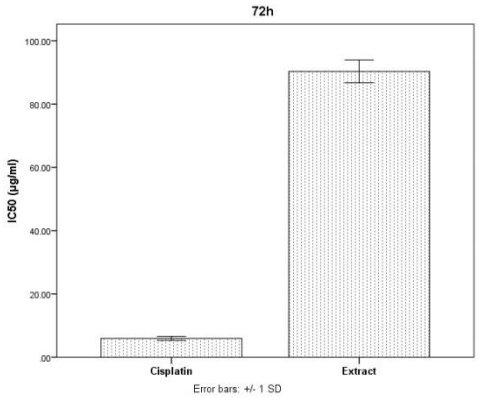

Figure 1. Comparison of IC_{50} of Lagenaria siceraria extract and cisplatin on A549 cell line during 72 hours of incubation ($p<0.01$)

The cell viability (%) was determined after exposure to Lagenaria siceraria extract and completion of MTTassay using the read adsorption values in the A549 cell line (Figure 2 and 3).

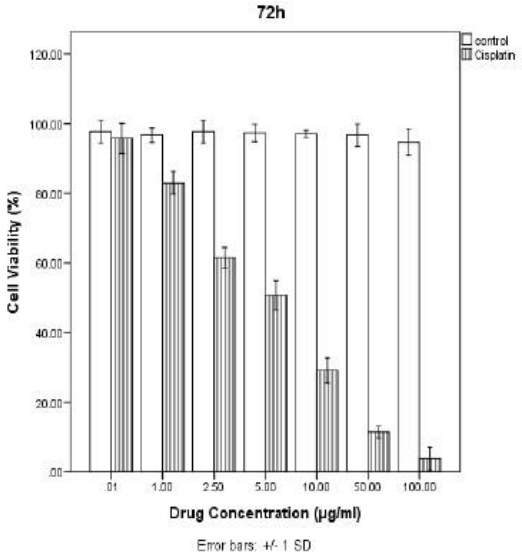

Figure 2. Cell viability (%) of A549 cell line exposed to cisplatin during 72 hours of incubation

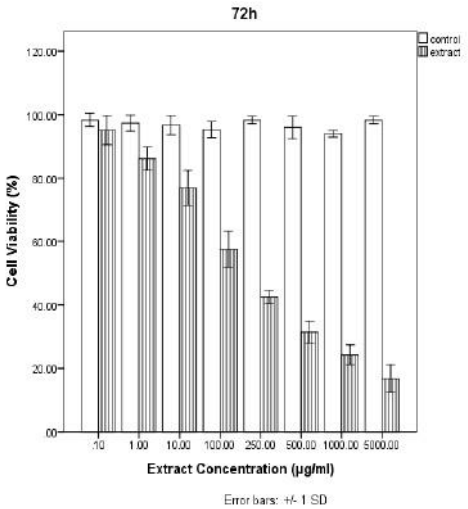

Figure 3. Cell viability (%) of A549 cell line exposed to Lagenaria siceraria extract during 72 hours of incubation

DISCUSSION AND CONCLUSION

The cell culture methods give a much deeper understanding of the effects of different drugs on cancer and normal cells. This method makes it possible to assess the effects and changes that different compounds such as cisplatin and Lagenaria siceraria extract (which were studied in this study) on cells in a controlled and observable cell culture space, identify their mechanisms and biological effects more accurate, and examine their effect on different intracellular factors.

These facilities lead to better identification of intracellular processes and interactions during cancer drug therapy, leading to improved therapies (Deshpande et al., 2008). Compounds with toxic effects, especially cytotoxicity, are among the most critical candidates in the synthesis of anti-cancer drugs for use in cancer chemotherapy, whose cytotoxicity can be measured on cell and tissue culture using toxicity assay methods (Monglli, 2000). Today, the compounds of natural origin (plant, animal, and mineral) are the focus of pharmacists and physicians to synthesize new drugs and treat diseases - especially diseases for which there is no definitive or effective treatment and medication regimen- due to their abundance, side effects, and fewer drug interactions, cheapness, etc.

The results showed that this plant's extract had a significant growth inhibitory effect on the lung cancer cell line (A549), although there is a significant difference between the IC_{50} value of Lagenaria siceraria extract and the cisplatin anticancer drug. Cisplatin is an official drug widely used in treating lung, ovarian, testicular, bladder, lymphoma cancers, etc. The reason for the significant difference between IC_{50} of cisplatin and Lagenaria siceraria extract in the evaluated cell line is that cisplatin is a pure chemical compound, Lagenaria siceraria extract is a mixture of many compounds, including cucurbitacin, polysaccharide inhibitor lagenin, flavonoids, etc. As a result of further studies, purified compounds may be a viable alternative to cisplatin with greater efficacy and fewer side effects.

Other studies on the components of this plant (seeds, flowers, etc.) showed that Lagenaria siceraria is a valuable medicinal plant due to its content of vitamin C, beta-carotene, B vitamins, pectin, high levels of choline as a lipotropic factor, saponin, cucurbitacin B, flavonoids, tannins, colloids, steroids such as fucosterol and compsterol, phenol, glycoside, a ribosomal activity inhibitor protein called Lagenin, and water-soluble polysaccharide on MCF-7, HepG2, and EAC cancer cell lines. The effect of hydraulic extract of this plant fruit on lung cancer cell line (A549) was investigated in this study. It was found that the fruit extract of this plant was able to inhibit this cancer cell line significantly. Pure compounds can be isolated by further studies on the fruit of this plant, and a compound can be obtained that can be used therapeutically in the treatment of lung cancer. Hence, it is suggested to investigate the effects of other total extracts (aqueous acetone-ethyl acetate, etc.) on cancer cell lines in addition to this type of extract (hydroalcoholic). Also, it is recommended to isolate the active ingredients of different organs of this plant (leaves, flowers, fruits, seeds, etc.) and then evaluate their effects on inhibiting the growth of different cancer cell lines and different growth inhibition mechanisms.

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