ANTI-CANCER ACTIVITY OF Withania somnifera AGAINST HUMAN LIVER CANCER CELLS - IN VITRO

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Abstract

Introduction: Cancer, one of the most researched diseases worldwide, researches is being done to develop new technology and more effective treatments. Still, there are many gaps in the cancer treatments that are now on the market that have a negative impact on patients' health in the form of side effects. A better approach to treat this awful disease is to use herbal drugs made from medicinal plants. Since ancient times, different illnesses have been treated with the well-known Ayurvedic medicinal plant Withania somnifera L. Dunal (Solanaceae). The aim of this study is to analyze the anti-cancer activity of Withaniasomnifera against human liver cancer cells.

Materials and methods: Withania somnifera root powder was extracted as viscous mass by undergoing soxhlet extraction and then rotary evaporation. After culturing of HepG2 cells and treatment with the extract, it was subjected to MTT assay and Real time PCR to identify the anti-cancer activity.

Results: MTT assay showed that as the concentration of treated W.somnifera ethanolic extract increases, the cancer cell viability decreases. As the concentration of the extract increased, the cell viability decreased. The W.sominifera performed by altering the expression of the apoptotic signaling molecules Bcl 2 and BclXl to suppress the growth of cancer cells.

Conclusion: The ethanolic extract of Withaniasomnifera down regulating the expression of Bcl 2 and Bcl XL mRNA on human liver cancer cells and induced apoptosis of the cells. The most effective anticancer substances are found in W.somnifera, which also induces apoptosis without causing any negative side effects.

Keywords: Anti-cancer, apoptosis, cancer, HepG2 cells, Withaniasomnifera.

INTRODUCTION

Cancer is defined as the over-proliferation of abnormal cells with high capacity for invasion and replication. In terms of non-communicable diseases, cancer stands as the second leading cause of death. Of all cancers, lung cancer constitutes about 29% of cases. Breast cancer is the second-leading cause of death in women after lung cancer, whereas prostate cancer is the second-leading cause of morbidity in men [1]. In terms of cancer-related mortality, liver cancer is in third place. Hepatocellular carcinoma (HCC) is the sixth most common type of cancer in the world and the second largest contributor to cancer mortality. The likelihood of dying from cancer is constantly increasing.

Different therapies are used alone or in combination for treating a wide variety of tumors [2]. There are various treatments available which have many side-effects, toxic and harmful for patients. There is now a recent trend towards the use of traditional plant based medicines for treating cancer [3]. The majority of anticancer medications on the market today are made from natural ingredients [4]. A whopping 74 percent of the FDA-approved medications now on the market are derived from plant products [5]. Many of the medicines that are now being used have used natural compounds as core molecules. Therefore, it is possible that the phytochemicals found in diverse plants and herbal remedies will be sufficient to thwart or treat a variety of human cancers. The assumption that herbal medications offer benefits over those of allopathic medicines while being less harmful may be the reason for the rise in popularity of herbal treatments for cancer therapy [6].
Ashwagandha (Withaniasomnifera) belonging to the family Solanaceae, is an ayurvedic herb also known as Indian Winter Cherry and Indian Ginseng [7]. The word “somnifera,” which means “sleep inducer” underlines the significance of this plant for stress relief. It is one of the most important herbs in ayurveda which have been used for more than 3000 years in lowering inflammation, blood sugar levels, cortisol, anxiety and depression. This medicinal plant has been found to have many therapeutic properties like anti-inflammatory, anti-arhritic, anti-coagulant, anti-oxidant, anti-diabetic, analgesic, regenerating and anti-epileptic properties [8]. Besides all the above mentioned properties of Withaniasomnifera, it has also been identified to possess anti-tumor/ anti-cancer properties [9]. There are still many research gaps, such as the anticancer potential of some isolated compounds, their molecular mechanisms, and the safety and toxicity aspects of this plant, despite the fact that its antitumor and anticancer potential has already been reviewed by some scientists [10]. Our team has extensive knowledge and research experience that has translated into high quality of publications [11][12–25]

The purpose of the current essay is to fill these knowledge gaps and provide new guidance for the development of anti-cancer medications derived from this plant. The main aim of this study is to analyze the anti-cancer activity of Withaniasomnifera against human liver cancer cells.

MATERIALS AND METHODS:

Chemicals

Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco’s modified Eagle’s medium (DMEM) and phosphate buffered saline (PBS) were purchased from Gibco, Canada. JC-1 (5,5,6,6 - tetrachloro-1,1,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and real time PCR kit (MESA Green) were purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

Extract preparation

The Withaniasomnifera powder was extracted with 70% ethanol. The extract was then filtered with Whatman no. 1 filter paper and the solvent evaporated at reduced pressure by using a Rotary evaporator apparatus to get a viscous mass, which was then stored at 4°C until used.

Procurement and culture of human liver cancer cell line

The liver cancer cell line was obtained from The National Centre for Cell Science (NCCS), Pune, India and cultured according to the cell culture instructions provided. Briefly, liver cancer cells were grown in DMEM containing 10% FBS at 37˚C in an atmosphere containing 5% CO2.

Cell viability assay:

Human liver cancer cells were seeded at a density of 5x105 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentrations of Withaniasomnifera in triplicate and incubated at 37˚C in a 5% humidified CO2 incubator for 24 h. Subsequently, MTT was added to each well, and incubation was continued for a further 4 h at 37˚C. To dissolve the formazan formed from MTT, the cells were resuspended in 200 µl dimethyl sulfoxide (DMSO), and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated 3 times, independently. The mean optical density (OD) ± SD for each group of replicates was calculated. The entire procedure was repeated 3 times. The inhibitory rate of cell growth was calculated using the equation: % Growth inhibition = (1 - OD extract treated)/ OD negative control x 100.

Gene expression analysis by Real Time PCR

mRNA expression levels were examined using real-time PCR. The total RNA was isolated by using Tri Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial Superscript III first strand cDNA synthesis kit (Invitrogen, USA) according to the manufacturer’s protocol. Real time-PCR was carried out in a MX3000p PCR system (Stratagene, Europe). Reaction was performed using MESA Green PCR master mix (It contains all the PCR components along
with SYBR green dye.) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pairs. The data were analyzed by comparative CT method and the fold change is calculated by 2−CT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

Statistical analysis

Data were expressed as the means ± SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and p<0.05 was considered to indicate a statistically significant result.

RESULT AND DISCUSSION:

Ayurveda is a highly recognized and traditional system which utilizes many natural products in various forms for medicinal and therapeutic purposes. Thousands of plants mentioned in the Ayurvedic system are useful in treatment and prevention of diseases. One potential plant of the Ayurvedic system is “Ashwagandha” [Withaniasomnifera (L.) Dunal], commonly regarded as Indian Ginseng which possesses various therapeutic activities, such as neuroprotective, hypoglycemic, hepatoprotective, antiarthritic, and anticancer effects [26]. Liver cancer is one of the most frequent and lethal malignancies worldwide. While hepatocellular carcinoma (HCC) is one amongst the four of the leading malignant tumors in adults. Epidemiological studies have established a close association between the occurrence of Hepatocellular carcinoma and chronic hepatitis B infection.

In Ayurveda, Ashwagandha is claimed to have potent rejuvenative and life prolonging properties. It is also used for the treatment of nervous exhaustion, memory related conditions and insomnia. In previous researches, it was found to exhibit stimulatory effects, both in vitro and in vivo, on the generation of cytotoxic T lymphocytes, and demonstrated the potential to reduce tumor growth. Apoptosis or programmed cell death plays an important role in embryonic development, tissue remodeling, immune regulation and tumor regression. Two groups of molecules (Bcl-2 family and “Death factor” family) are involved in regulating apoptosis [27].

The ethanolic extract of Withaniasomnifera possesses potent anti-cancer and apoptotic inductive potential against HepG2 cells. From the results of the MTT assay we could see that when 50 µg concentration of Withaniasomnifera extract is used, the cell viability is 80%, for 100 µg concentration, viability is 60% and for 200 µg concentration, viability is 50% (Figure 1). This shows that as the concentration of the extract increases, there is a significant decrease in the cell viability. Bcl2 mRNA gene is a protein that helps control whether a cell is alive or dead by blocking apoptosis. BclxL mRNA gene is found mainly in cytoplasm of liver cancer cells which also inhibits apoptosis. The Bcl2 mRNA gives no significant change among the group compared to the control (Figure 2). The BclxL mRNA gene gives a significant change among the group for the concentration 100 mg/ml and 200 mg/ml when compared to control and 50 mg/ml (Figure 3). This research illustrated that ethanolic extract of Withaniasomnifera were cytotoxic against Bcl2 and BclxL mRNA on human liver cancer cells and induced apoptosis.

Previous research suggested that the only extract from the roots of Withaniasomnifera could be a source of novel chemicals that can reduce the spread of cancer [28]. Furthermore, it has been demonstrated that the leaves of Withaniasomnifera can suppress the proliferation of human cancer cell lines in a manner similar to adriamycin. The leaf extract of W.somnifera exhibited antiproliferative action against lung, intestinal, and breast tumor cell lines [29].

Extensive research will need to confirm whether Withaniasomnifera has the potential to enhance the anticancer effects of anti-hepatocarcinomic medications in in vivo models. This may give a better idea for the pharmacological uses in the industries.
CONCLUSION:

Ashwagandha (Withaniasomnifera) ethanolic extract is a powerful anti-oxidant and has potential anti-cancer properties in HepG2 cells by inducing apoptosis in those cells.

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REFERENCES


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