Cytotoxic and gene expression study of KRAS gene in lung cancer cell line of a549 treated with Tinospora cordifolia extract

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INTRODUCTION

Normally, normal cells multiply to form new cells and worn out cells are removed in an orderly manner. This is regulated by process controlled by many enzymes and checkpoints. Cancer is initiated when this controlled process is deregulated and cells in any particular part of the body start multiplying in an uncontrolled fashion. These cancerous cells are different from normal cells in many ways which include their growth pattern and cell death mechanism.[1] Cancerous cells do not die as they deregulate the normal cell death mechanisms and continuously multiply. These altered cells also gain the ability to invade other tissues and parts of the body to form secondary tumors.

The main reason for alteration of normal cells into cancerous cells is DNA damage. This is because DNA is the genetic material which is responsible for every process taking place in cell and every protein catalyzing that process. When a normal cell is considered, if the DNA gets damaged by either physical or chemical agents, various cellular mechanisms repair the damage; if these mechanisms are unable to repair the damage, the cell undergoes controlled death pathway called as apoptosis. These cells are removed because if the cell divides, these DNA alterations will be continued which can be harmful for the body.[2]

Medicinal plants are those plants that are used in treating and preventing specific human diseases. It can be treated by using medications and should not be used in most harmful cases. Chemical drugs cannot be used to treat such type of harmful diseases. Still, the use of plants as a source of medicine is very much important for human beings. Identify medicinal and how to use them is so important.

KEY WORDS: Degeneration, Drugs, Macromolecules, Toxicity

ABSTRACT

Introduction: There has been global resurgence of interest in herbal drugs in the recent past. Though herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used. Materials and Methods: Therefore, these herbal drugs deserve detailed studies in the light of modern medicine. In spite of synthetic drugs, herbal drugs have their place in therapy. Results and Discussion: Their effectiveness, low-cost and comparative freedom from serious toxic effects makes these medicines not only popular but also an acceptable mode of treating diseases even in modern times. Medicinal plants are those plants that are used in treating and preventing specific human diseases. Conclusion: It can be treated by using medications and should not be used in most harmful cases. Chemical drugs cannot be used to treat such type of harmful diseases. Still, the use of plants as a source of medicine is very much important for human beings. Identify medicinal and how to use them is so important.

KEY WORDS: Degeneration, Drugs, Macromolecules, Toxicity
Lung cancer can be diagnosed with many techniques such as radiographs and CT scans. These initial diagnostic techniques are followed by confirmatory tests such as biopsy. Treatment options are considered based on the stage of the disease and health of the patient.

MATERIALS AND METHODS

Collection of the Material

Antibacterial activity
- Inoculum Preparation
- Luria-Bertani broth

Cell culture
1. Neutralization
2. Splitting or culturing the cells

Cell Viability Test

Composition of preservation medium

Preservation of cells
- The cells were split with minimal essential medium, i.e., after trypsinization and addition of medium into centrifuge tube
- To provide slow cooling, we have to arrange cotton in a container and wipe it with isopropanol and again cotton, cryovials were kept and again cover with cotton (it should contain isopropanol so that it will not dry up).

Anticancer activity

Maintenance of cell line
The A549, liver cancer cell line was purchased from NCCS, Pune, and the cells were maintained in DMEM medium supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL⁻¹), in atmosphere of 5% CO₂/95% air at 37°C [Figure 1 and 2].

The zone of inhibition for the given antibiotics with sensitivity and resistant zone is also described [Table 1 and 2].

RESULTS

Antibacterial Activity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Zone of inhibition (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10 µg 25 µg 50 µg 75 µg 100 µg</td>
</tr>
<tr>
<td>Gram positive</td>
<td>9 10 11 14 16</td>
</tr>
<tr>
<td>Gram negative</td>
<td>10 11 13 15 16</td>
</tr>
</tbody>
</table>

Anticancer activity

Maintenance of cell line

Table 1: Zone of inhibition representing in mm

A: Gram-positive standard norfloxacin

B: Gram-negative standard ciprofloxacin

Table 2: Zone of inhibition in mm shown in Staphylococcus aureus and Bacillus antibacterial activity in chloroform extract

DISCUSSION

Since ancient times, medicinal plants have been used as an important source of drugs for curing various diseases. Many plant extracts were used in clinical practice. Pharmacologically active compounds found in plant kingdom, higher plants are arguably the most important groups. Many plants are used with medicinal properties and they cover a broad spectrum of pharmacological effects.

It is especially meaningful in tropical countries due to the great variety of plants belonging to their ecosystem. In India, many medicinal plants have long been used to treat different kinds of diseases. Today, there is an increasing desire to unravel the role of ethnobotanical studies in trapping the centuries-old traditional folk knowledge as well as in searching new plant resources of food, drug, etc. (Jain, 1987, 1991). People living in the developing countries rely quite effectively on...
The antimicrobial activity was performed for all chloroform and methanolic extract and ethanolic fraction of *Tinospora cordifolia*. The antimicrobial screening was done for antibacterial (both Gram-positive and Gram-negative microorganism). The antibacterial activity was undertaken out for the disc plate and cup plate agar diffusion assay using Gram-positive and Gram-negative bacteria. Extracts of *Tinospora cordifolia* showed a potent activity. The antibacterial activity of *Tinospora cordifolia* plant extract was found to be higher in hexane extract than methanol extract and chloroform extract. The plant extract exhibited highest antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, *Pseudomonas aeruginosa* for 100 μg/ml.

A549 and HEK293 cell lines treated extracts have been subjected to RNA extraction for gene expression analysis. The differential expression study was carried out using real-time polymerase chain reaction method. Diabetes-specific genes of interest (genetic variants in the gene encoding for KRAS gene have been associated with type 2 diabetes and impaired β-cell function) are chosen and the expression level of these genes will be examined quantitatively. The expected findings from *in vitro* may reveal the antidiabetic properties and suggest that the plant extract may be useful for the management of the disease.

**REFERENCES**


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