Cytotoxic and Gene Expression Research on Kras Gene in Lung Cancer Cell Line of A549 Treated with Tinospora Cordifolia Extract

T. Jayalakshmi, R. Priya, M. Harish

ABSTRACT--- 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 unscientically exploited or improperly used. 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. 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 and human has been using herbs for generations around the world, due to charm needed to cure the disease, many people have come to the conclusion that even chemical drugs their answers may already be sick of these medications may be harmful for health them in the future. 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.

Index Terms:— Degeneration, Drugs, macromolecules, toxicity.

1. INTRODUCTION

Normally, normal cells multiply to form new cells and worn out cells are removed in an orderly manner. This is a 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 starts multiplying in an uncontrolled fashion. These cancerous cells are different from normal cells in many ways which includes 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 repairs 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]

Collection of the Material:

2.1 Anti-Bacterial 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 in to 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).

Anti-Cancer Activity:

Maintenance of Cell Line: The A549, Liver Cancer cell line were 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.

3. RESULTS

Anti Bacterial Activity:

### Gram Positive Strain

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gram Positive Strain</th>
<th>Gram Negative Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Drug</td>
<td>Staphylococcus aureus</td>
<td>E. coli</td>
</tr>
<tr>
<td>for Gram Positive</td>
<td>Norfloxacin</td>
<td></td>
</tr>
<tr>
<td>for Gram Negative</td>
<td>Ciprofloxacin</td>
<td></td>
</tr>
</tbody>
</table>

3.1 RESULTS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.No.</td>
<td>Compound</td>
</tr>
<tr>
<td>1</td>
<td>Chloroform Extract</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform Extract</td>
</tr>
</tbody>
</table>

### Table 2: Zone of Inhibition in mm shown in staphylococcus aureus and Bacillus Anti Bacterial Activity in Chloroform Extract

Figure 1: Zone of Inhibition shown Anti Bacterial Activity; A: Gram Positive Standard Norfloxacin; B: Gram Negative Standard Ciprofloxacin

3.2 RESULTS

A

B

3.3 RESULTS

Table 1: Zone of Inhibition in mm shown in staphylococcus aureus and Bacillus

Antibacterial Activity

4. 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 eco-system. 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 ethno-botanical 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 traditional medicine for primary needs (Sullivan and Shealy 1997; Singh, 2002). In this context it is very important to document the ethno-botanical information on medicinal plants before it completely lost.

5. CONCLUSION:

The antimicrobial activity was performed for all chloroform and Methanolic extract and ethanolic fraction of Tinosporacordifolia. 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 Tinosporacordifolia, showed...
a potent activity. The antibacterial activity of Tinosporacordifolia 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 E.Coli, Pseudomonas aeruginosa for 100μg/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 by using real time RT-PCR method. Diabetes-specific genes of interest (Genetic variants in the gene encoding for KRAS gene have been associated with type 2 diabetes (T2D) and impaired β cell function,) are chosen and the expression level of these genes will be examined quantitatively. The expected findings from invitro may reveal the anti-diabetic properties and suggest that the plant extract may be useful for the management of the disease.

6. REFERENCES:

9. "Tobacco Smoke and Involuntary Smoking" (PDF).IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (WHO International Agency for Research on Cancer) 83. 2004. There is sufficient evidence that involuntary smoking (exposure to secondhand or 'environmental' tobacco smoke) causes lung cancer in humans. ... Involuntary smoking (exposure to secondhand or 'environmental' tobacco smoke) is carcinogenic to humans (Group 1).