CYTOTOXIC ACTIVITY OF GYMNEMA SYLVESTRE

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ABSTRACT

The cytotoxic activity of 95% alcoholic extract of Gymnema sylvestre, Gymnemagenin and deacyl Gymnemicacid was carried in vitro on MCF7 cell lines. The extract and the pure compounds have exhibited considerable and significant cytotoxic activity as compared to the standard compound Etoposide. As deacyl Gymnemicacid has shown higher activity, this compound is further subjected to Annexin V assay using the flowcytometry technique to know the apoptic induction potential. Deacyl Gymnemicacid has shown 53.3 % and 64.8 % apoptosis induced cells at 5µg and 10µg dose levels. From this study, it can be concluded that deacyl Gymnemicacid possess potent cytotoxic activity.

Keywords: Gymnema sylvestre, MCF7 cells, Cytotoxic activity. Anticancer activity, Annexin V assay, Apoptosis.

INTRODUCTION

Gymnema sylvestre (Asclepidaceae)[1] is a woody climbing plant native to the tropical forests of southern and central India. The plant is commonly known as Gurmar which means destroyer of sugar. It has been used in India for the treatment of diabetes for over 2,000 years. The plant is acrid, anti-inflammatory, liver tonic, emetic, diuretic, stomachic, and refrigerant, astringent. It is also used in hepatosplenomegaly, dyspepsia, constipation, jaundice, helminthiasis and amenorrhoeia[2].

The major bioactive constituents of Gymnema sylvestre are oleanane type of triterpenoid saponins known as Gymnemic acids[3]. It also contains flavones, anthraquinones, pentatriacontane, hexatriacontane, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, ß- amyrin, related glycosides & stigmasterol.

The plant has been thoroughly investigated for anti-diabetic activity by various models. Both alloxan and streptozotocin induced diabetic models were studied. In both the models the plant has shown positive effect on almost all parameters[4-7]. The plant
also has been studied for hypolipidimic & antiatherosclerotic activity\cite{8} on experimentally induced hyperlipidemic rats. It has shown positive effect by reducing various lipid parameters. The plant was also reported to possess good anti-inflammatory activity\cite{9}.

Gymnema sylvestre has been extensively studied for the antidiabetic activity. However the cytotoxic activity of the plant has not been explored to any greater extent. In recent years the cancer has become widespread disease and there is a need to find a safe and potential cytotoxic medicaments from plant sources. Therefore in our present research work we have selected Gymnema sylvestre to investigate for the cytotoxic activity.

EXPERIMENTAL

Plant material

The leaves of Gymnema sylvestre were obtained from Yucca enterprises, Pune. The leaves were powdered and stored in air tight containers.

Extraction

The dried leaf powder (1 kg) was extracted with 95% alcohol by maceration method for 7 days. The contents were filtered and the filtrate was concentrated under reduced pressure by rotary flash evaporator, dried in a dessicator & stored in air tight container. Gymnemagenin and deacyl Gymnemic acid were obtained from Natural Remedies, Bangalore.

Cytotoxic Activity (MTT Assay)\cite{10}

MCF7 cell lines were maintained at 37°C with 5% CO₂ in CO₂ incubator. 1x 10⁵ cells were taken in each well of 96 well plate. To the wells different concentrations of extract, standard & pure compounds were added. Then incubated at 37°C with 5% CO₂ for 45 hrs. At 45th hour, 20 μl of 0.5mg/ml solution of MTT \{3-(4, 5-dimethyl thiazyl-2-yl)-2, 5-diphenyl tetrazolium bromide\} was added and incubated for 3 hrs. Then, absorbance was measured at 570 nm in a multiwell plate reader. The experiment was performed in triplicate. Survival ratio (%) was calculated using the following equation.

\[
\text{Survival ratio} \% = \frac{A_{\text{TREATMENT}}}{A_{\text{CONTROL}}} \times 100
\]

Where \( A = \text{Absorbance} \)
Annexin V Assay\textsuperscript{[11]}

K562 cells were grown in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin and 2mM L-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO$_2$ at 37$^\circ$C. The cultured cells were subcultured twice each week, seeding at a density of about 2×10$^3$ cells/ml. Cell viability was determined by the trypan blue dye exclusion method. Annexin V assay was performed on Guava Easy Cyte Flowcytometer. K562 cells were seeded at a density of 1×10$^5$ cells/ml in 6-well culture plates, cultured in 10% FBS with sample/compound for 24 h. After treatment, cells were harvested, washed with PBS, stained and analyzed by flowcytometer.

\textbf{RESULTS & DISCUSSION}

The alcohol extract, Gymnemagenin and deacyl Gymnemic acid were tested for cytotoxic activity on MCF7 cell lines by MTT assay. Alcohol extract is tested at 25, 50 and 100 µg/ml and the compounds at 5, 10, 25 µg/ml concentrations. The compound deacyl Gymnemic acid has shown significant and good cytotoxic activity compared to standard drug Etoposide. Gymnemagenin has also shown the activity, but however not considerable as compared to deacyl Gymnemicacid and Etoposide. The cytotoxic activity of deacyl Gymnemicacid is increasing with the dose.

\textbf{TABLE 1: CYTOTOXIC ACTIVITY OF GYMNEMA SYLVESTRE COMPOUNDS AND EXTRACT.}

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Conc. µg/ml</th>
<th>ETOPOSIDE</th>
<th>DEACYL GYMNEMEIC ACID</th>
<th>GYMNEMA-GENIN</th>
<th>ALCOHOL EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% LIVE CELLS</td>
<td>% LIVE CELLS</td>
<td>% LIVE CELLS</td>
<td>µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>55.65±2.3</td>
<td>60.35±2.6</td>
<td>78.85±2.8</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>45.85±1.8</td>
<td>40.35±1.9</td>
<td>65.00±2.1</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>20.35±1.2</td>
<td>32.65±1.3</td>
<td>60.22±2.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Results are Mean±SEM to the mean.
Annexin V Assay

One of the biochemical features of apoptotic cells is the expression of cell surface markers achieved by flip-flop movement of the phosphatidylserine from inner membrane to the outer membrane of the plasma membrane. Annexin V, a recombinant phosphatidylserine-binding protein, interacts strongly and specifically with phosphatidylserine residues and can be used for the detection of apoptosis. As the deacetylagnememicacid has showed considerable and significant activity in MTT assay, this compound is subjected to Annexin V assay using flowcytometer. The deacetyl Gymnemic acid is tested at 5 µg/ml and 10 µg/ml concentrations. It has induced apoptosis at the both the concentrations. This is indicated by the density of the colored area in the apoptic zone of the control, 5ug and 10ug plots. In the control plot the % of Annexin V positive (apoptic) cells in the apoptic zone (quadrant) is 9.4 %. The % of Annexin V positive cells treated with deacetyl Gymnemic acid is 53.3 % and 64.8 % at 5 µg and 10 µg respectively. These results indicate that deacetyl Gymnemicacid possess potent cytotoxic activity.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Annexin V +ve (%)</th>
<th>7-AAD +ve (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.4%</td>
<td>12.5%</td>
</tr>
<tr>
<td>5ug</td>
<td>53.3%</td>
<td>11.6%</td>
</tr>
<tr>
<td>10ug</td>
<td>64.8%</td>
<td>10.7%</td>
</tr>
</tbody>
</table>
CONCLUSION

This Annexin V assay study gives a further positive evidence for the cytotoxic activity of Gymnema Sylvestre.

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