Molecular Docking Assisted Isolation of Azadirachtin-A, from Seeds of Azadirachta indica Extract against Cervical Cancer

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Abstract

Objectives: To isolate Azadirachtin-A, from Seeds of Azadirachta indica and to perform molecular docking studies for antiproliferative activity against cervical cancer. Methods: The Current studies on Azadirachtin-A, a potent cervical anticancer agent, was designed for the isolation of Azadirachtin-A, from neem fruits collected from local area (Ibrahimpatnam), the seeds were first separated, dried and made in to coarse powder. The seed kernels was defatted and extracted with methanol using soxhlet apparatus. Methanolic extract of seeds were subjected to direct reverse phase High Performance Liquid Chromatography (HPLC) for separation of Azadirachtin-A, by molecular docking of Azadirachtin-A, using Molecular Operating Environment (MOE) 2008 software Findings: This revealed that Azadirachtin-A, has an affinity of 59.2% and 72% towards amino acids like Glycine 131 and Lysine 89 with polar intractions. Azadirachtin-A, found to interact with Cyclin E, was retrieved from the Protein Data Bank (PDB code: 1W98). The Azadirachtin-A, showed excellent, Increase Life Span (63.44 and 82.56), reduced the viable cells (30.4±1.5 and 24.5±1.6), and tumor volume (1.2±0.05 and 1.0±0.05) when compared to standard drug 5-Fluorouracil. Application: Azadirachtin-A, showed significant docking interaction with Cyclin E, active site using windows 2002, MOE 2008 software. Result showed that Azadirachtin-A, may lead to potent cervical anticancer agent.

Keywords: Azadirachtin-A, Azadirachta indica, Cervical Cancer, 5-Fluorouracil, (HeLa) Henrietta Lacks Cell Line, Molecular Docking

1. Introduction

Cervical Cancer is defined as recurrent growth of tumor at cervical area, microinvasion of more than 3 millimeter depth of stomach to spread by metastasis not more than 7 millimetre, that extends through pelvic sidewall to the uterus and further into lower third of the vagina. Chemotherapy is a major mode of cervical cancer treatment, in which drugs like 5-fluorouracil, cisplatin, scaboplatin, paclitaxel, cyclophosphamide, topotecan, gemcitabine, docetaxel, irinotecan, mitomycin and doxorubicin are used. All the above stated drugs have certain serious side effects. Hence there is a need for development of safe and natural anticancer agent against cervical cancer. In this regard, some phytochemicals were identified, such as taxol, and vincristine¹. There is growing number of publications on Neem and its extract to combat against cervical cancer. Neem is a tropical, evergreen, profusely branched tree with oblique shaped ever green leaves and trunk of timber values with insect repellent properties². Broadly speaking, Neem shows anticancer activity by inducing the antioxidant enzyme,
and by modifying intracellular components necessary for cancer growth development; such as Cyclin D, Cyclin B, CyclinB1, Cyclin1, Cyclin E, P53, PCNA, P21, GST-P, NFkB, IkB, EAS, BCL2, BAX, APF1, Cytochrome C, Survivin, Caspase 3, 6, 8, 9 and PARP. Azadirachtin-A, reported to interfere with cell cycle kinetics in cancer cells by inducing cell cycle arrest at G1/S or G2M phase through repression of Cyclin, CDKs and PCNA. Azadirachtin-A, can be extracted from neem kernel powder using ethyl acetate as a solvent microwave-assisted extraction, refluxed for 12 hrs. Hundred grams of neem kernel powder gives about one gram of Azadirachtin-A. The Studies of Molecular docking was done and gave an insight into the binding mode of Azadirachtin-A, using Molecular Operating Environment (MOE) 2008 software. Current studies revealed that Azadirachtin-A, has an affinity of 59.2% and 72% towards amino acids like Glycine 131 and Lysine 89 with polar interactions. Azadirachtin-A known to interact with Cyclin E, causes phosphorylation of the same, prevents the G1/S phase protein expression.

2. Materials and Methods

2.1 From Azadirachta indica seeds

Extraction of Azadirachtin-A

Azadirachtin-A is an enriched concentrate containing 60% active ingredient. The neem fruits were collected from local area then seeds were separated from fruits. The seeds were dried by shade drying. Then, seeds were made in to coarse powder. The Azadirachta indica seed kernels were defatted using ethyl acetic acid as a dissolvable microwave-assisted extraction, refluxed for 12 hrs. Hundred grams of neem kernel powder gives about one gram of Azadirachtin-A. The Studies of Molecular docking was done and gave an insight into the binding mode of Azadirachtin-A, using Molecular Operating Environment (MOE) 2008 software. Current studies revealed that Azadirachtin-A, has an affinity of 59.2% and 72% towards amino acids like Glycine 131 and Lysine 89 with polar interactions. Azadirachtin-A known to interact with Cyclin E, causes phosphorylation of the same, prevents the G1/S phase protein expression.

2.1.1. Studies Which Relates to Molecular Docking

By Molecular Operating Environment 2008 docking study was done. From the bank of protein data Cyclin E was received and by using sequence option receptor was visualized and the further deletion of cofactors were done. By force field method the partial protein charge was adjusted and by energy refinement the Building of Assisted Model (AMBER 99) was adjusted. Later 3D protonation was subjected by the protein at cut off 12.0 and according to standard geometry hydrogen was added and by using force field energy minimized to receptor, at 0.01 KJ mole gradients Merck Molecular Force Field (MMFF94x). With the help of builder module the ligand structures were formed and with the method of Hamilton MMFF94 force field partial charges were adjusted and subsequently as according to standard geometry 3D protonation and hydrogen were added. At 0.01KJ mole gradient of force field MMFF94x energy was minimized to ligands at cutoff 12. By using the option simulation docking was performed and it was followed by dock on the active sites of selected amino acids with the help of sequence option and eventually the settings options such as receptor and solvent, selected residues, force field refinement, alpha triangle and best 30 pose the docking was done. From the 30 best posed of each structure of chemical after getting the results the attainment of best will be obtain. In the series the best pose of resultant score values were used for docking analysis and interaction.

2.1.2. Protein Data Bank, Azadirachtin-A of Molecular Docking (PDB Code: 1W98) is shown in Figure 1 and 2.
2.1.3. Activity of Anticancer

In an institutional Animal ethics committee an experimental protocol on mice for the pharmacological screening were done in Guru Nanak Institute of Pharmacy, Hyderabad, India (Reg no: 1374/ac/10/CPCSEA). For the screening of anticancer activity a Male Albino Swiss which weighs up to 20-25 g were selected. Under the conditions of standard laboratory, animals were kept and maintained for free access to water and food ad libitum. Prior to in vivo experiment for the period of seven days the selected animals in the environment of laboratory were allowed to acclimatize. The animals which were selected, divided into 5 batches containing 12 animals each. From the donor mice HeLa cell lines got and were suspended in a 0.9% NaCl normal saline. The count of cell was adjusted to 2×10⁶ cells/mL. Through the intraperitoneal route HeLa cell line were treated in all groups except the normal group which is in control on day zero. Normal saline (5 mL/kg body weight) is treated in group 1, HeLa cell line in group 2 and 5-flourouracil (20 mg/kg body weight) in group 3. At a dose of 50 and 100 mg/kg body weight the compound test which belongs to group 4 and 5 was administered through intraperitoneal route. After tumor transplantation 5-flourouracil and the test compound were treated for 9 days. Each group of 6 animals was sacrificed after 9 days. Against Cyclin-E the evaluation of tumor was done. For the remaining each group of 6 mice was recorded for Mean survival time¹⁰,¹¹ are shown in Table 1.

2.1.4. Cell Count of Tumor

The 6 mice were done for the process of dissection and from the peritoneal cavity the harvestation of total ascetic fluid were done. With the help of graduated centrifuge tube the volume was measured and by centrifuge PCV was noted at 1000 rpm for 5 min and by trypan blue dye exclusion test was done to check viable cells and in Neubauers counting chamber the cells were counted¹²,¹³.

2.1.5. Increase Life Span % and Mean Survival Time

On tumor growth the Azadirachtin-A, activity was evaluated and mortality recording is done within the observation period.

By using formula the life span in % was calculated¹⁴,¹⁵,¹⁶

\[
\text{Percentage ILS} = \frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of control group}} \times 100
\]

\[
\text{Mean Survival Time} = \frac{\text{Survival time of each mice in a group in days}}{\text{Total number of mice}}
\]

3. Results and Discussions

The study of docking test was done on windows 2007 using MOE 2008. From the protein data bank(PDB code: IW98),

### Table 1. List of concentrations of Standard and Test drug tested for cervical cancer activity along with number of cells and cancer cell lines

<table>
<thead>
<tr>
<th>No of groups</th>
<th>Saline Name of cancer cell - line</th>
<th>No. of cells</th>
<th>Name of test</th>
<th>mg/kg/ body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>-</td>
<td>HeLa</td>
<td>2×10⁶ cells</td>
<td>Control</td>
</tr>
<tr>
<td>3.</td>
<td>-</td>
<td>HeLa</td>
<td>2×10⁶ cells</td>
<td>5-Flourouracil (S) 20 mg/kg body weight</td>
</tr>
<tr>
<td>4.</td>
<td>-</td>
<td>HeLa</td>
<td>2×10⁶ cells</td>
<td>Azadirachtin-A (T) 50 mg/kg body weight</td>
</tr>
<tr>
<td>5.</td>
<td>-</td>
<td>HeLa</td>
<td>2×10⁶ cells</td>
<td>Azadirachtin-A (T) 100 mg/kg body weight</td>
</tr>
</tbody>
</table>
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the Cyclin E was retrieved, current studies revealed that Azadirachtin-A, has an affinity of 59.2% and 72% towards amino acids like Glycine 131 and Lysine 89 with polar interactions. Azadirachtin-A, known to interact with Cyclin E, causes phosphorylation of the same, prevents the G1/S phase protein expression. The test solutions were considered as standard and Azadirachtin-A, (50 and 100 mg/kg body weight from the values of decrease in tumor cell count, %ILS 5-fluorouracil (20 mg/kg body weight) and MST. The results were shown in Table 2. The activity of antitumor of tested Azadirachtin-A, on HeLa cell line in vivo studies depends on no. of viable cells and tumor volume. In tumor volume and cell number the Azadirachtin-A, significantly exhibited in decrease as compared to control. The Azadirachtin-A, showed good %ILS (63.44 and 82.56), reduced the viable cells (30.4±1.5 and 24.5±1.6), and tumor volume (1.2±0.05 and 1.0±0.05) respectively as compared with 5-Fluorouracil. The Azadirachtin-A, showed an excellent interaction in docking of molecule and the cervical anticancer guidance were provided. The amino acids such as Asp-A86, Ile-A10, Gly-A131, Asn-A132, Asp-A145 interact with Cyclin E at the active site. Gly-A131 with the binding of 59.2% involves polar interaction, Lys89 with the binding of 72% involving polar interaction.

Table 2. In Vivo Antitumor Activity of Azadirachtin-A, against (HeLa) Cell lines bearing Mice

<table>
<thead>
<tr>
<th>No of groups</th>
<th>MST ± SE</th>
<th>ILS (%)</th>
<th>Tumor volume (ml)</th>
<th>Viable cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>18.5±0.23</td>
<td>-</td>
<td>3.4±0.2</td>
<td>94.5±3.6</td>
</tr>
<tr>
<td>3.</td>
<td>36.6±0.48</td>
<td>97.83</td>
<td>0.9±0.02</td>
<td>18.1±1.8</td>
</tr>
<tr>
<td>4.</td>
<td>28.2±0.52</td>
<td>63.44</td>
<td>1.2±0.05</td>
<td>30.4±1.5</td>
</tr>
<tr>
<td>5.</td>
<td>32.4±0.34</td>
<td>82.56</td>
<td>1.0±0.05</td>
<td>24.5±1.6</td>
</tr>
</tbody>
</table>

4. Conclusion

In the present study in vivo anticancer activity of Azadirachtin-A, was performed. The Azadirachtin-A, was found to have activity of anticancer potent when compared to standard drug 5-Fluorouracil. Moreover, the Azadirachtin-A, showed significant docking interaction with Cyclin E, active site using windows 2002, MOE 2008 software. Result showed that Azadirachtin-A, may lead to potent cervical anticancer agent.

5. Acknowledgements

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6. References

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