A Comparative Study On Viability Of MCF-7 Human Breast Cancer Cell Lines Using Piperine and Tamoxifen – An In Vitro Study with a Novel Mishmash

Thulasi Gokul, D. Anusha * and Darling Chellathai David

Department Of Pharmacology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai - 600116, India.
*Corresponding author E-mail : drdanusha@gmail.com

http://dx.doi.org/10.13005/bpj/1568

This breast cancer is a disease which is common in India with mortality incidence ratio of 66 in rural and 8 in urban. Breast cancer can be cured once it is diagnosed appropriately and when treatment is started at right time with right drug in proper dosage. But chemotherapy itself have many adverse events, to come over these problems, natural products or extracts are being used to aid in reducing the complications. To evaluate Anti-Cancer activity of piperine and tamoxifen and in both as Combination on the MCF 7 human breast cancer cell lines. Breast cancer cells (MCF 7) have been treated with piperine and incubated at 37 c, the drug samples were added, incubated for 3 hours then followed by tetrazolium dye treatment and incubated. One ml of dimethyl sulfoxide is added and incubated. Absorbance at 537nm was measured with UV spectrophotometer using dimethyl sulfoxide as the blank. Then IC50 was determined in graphical representation according to percentage of cell viability and concentration of sample. The anti-cancer effect of piperine and tamoxifen and in Combination, when treated with MCF 7 human breast cancer cell lines, starting from minimum to maximum dose concentration (μg/ml), the percentage of cell viability is 51.49 at the dose of 62.5 μg/ml, 51.09 at 125 μg/ml, 52 at the dose of 32.5 for piperine, tamoxifen and combination respectively. This study concludes tamoxifen in combination with piperine have significant anti-cancer activity, which would probably play a role as cytotoxic agent in tumour cells.

Keywords: Breast cancer, Oestrogen receptor antagonist, cytotoxic activity.
in combination with GnRH Agonist for estrogen receptor positive breast cancer is as effective as cyclophosphamide, methotrexate and 5 fluorouracil (CMF Regimen), and hence this may be used as alternate therapy. ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial suggested that tamoxifen can also prevent the contra lateral breast cancer5. This showed importance of tamoxifen in treatment of breast cancer5. It is well known that combination of the drug is very compliant in treating the disease, and simultaneous use of natural products in treating the disease for a better outcome. Centuries old plants have always been an integral part of treating various types of cancers though allopathy medicines have been the mainstay of treatment. However numerous studies have quoted the effects of piperine on various types of carcinomas, by increasing bioavailability and reducing adverse effects of the prescribed therapeutic drug6. Piperine treatment alone has shown beneficial effects in breast carcinoma targeting cancer stem cell renewal properties7. According to Callaghan et al 1995, tamoxifen was inhibited by p-glycoprotein8. Another study demonstrated the inhibitory property of piperine with p-glycoprotein9. With this information, we hypothesised that the combination of tamoxifen with piperine extract would show beneficial effect therapeutically in Michigan Cancer Foundation-7 (MCF-7) cells. MCF-7 cell line was developed by Herbert D. Soule from an excision of chest wall nodules and pleural effusion on his seventh attempt at Michigan Cancer Foundation, hence the name MCF-710. This excision was collected from a patient Helen Marion who was suffering from metastatic disease, and developed MCF-7 cell line11. This study explores the cytotoxic effects of piperine and tamoxifen combination against MCF-7 human breast carcinoma cell lines.

MATERIALS AND METHODS

MCF-7 cell line (Human Breast Cancer Cell lines) was purchased from National centre for cell sciences Pune (NCCS). The cells were cultured in MEM (Minimal Essential Medium). The MEM culture medium consisted 10% Fetal Bovine serum (FBS), Streptomycin (100 μg/ml), Penicillin (100 U/ml) with a well maintained humidified atmosphere and temperature (50 μg/ml CO2, 37 °C). Media and chemicals were obtained from Hi Media Laboratories (Reagents, MEM), Cistron laboratories (Fetal Bovine Serum), Sisco research laboratory chemicals Mumbai (Trypsin, methylthiazolyl diphenyl- tetrazolium bromide and Dimethyl sulfoxide), Sigma Aldrich Mumbai (Piperine and other chemicals, reagents).

3(4, 5- dimethyl – 2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) Assay

As described previously12, assay have been performed. Initially in the 24 well plate cells were plated and at the temperature of 37°c with5% CO2 these cells are incubated in an aseptic conditions. When these cells reach to the stage of confluence, in different concentrations samples were added and incubated for next 1 day or 24 hours of duration. Once the process of incubation is done, the sample were removed from the well and cleaned or washed with saline that is Phosphate buffered with PH 7.4. In each well, 100 μl of 0.5% MTT was supplemented and then for the next 4 hours samples are incubated.

Once the incubation is done, dimethyl sulfoxide added in all the wells. Absorbance at 570 nm was taken using ultraviolet visible spectrophotometer, keepingdimethyl sulfoxide as blank. IC50 was plotted taking dosage values against percentage cell viability. The concentration required to show the 50% of inhibition is known as Inhibitory Concentration. The percentage cell viability was calculated as- dividing treated cells with control cells multiplied by 100.

RESULTS

The Anticancer effect of piperine, tamoxifen and their Combination, when treated on MCF 7 cell lines starting from minimum to maximum dose concentration (µg/ml) was determined, the Percentage of cell viability is shown in Table 1.

The percentage of maximum cell viability of piperine treated cells was 78.92% (minimum cell inhibition- 21.08%) observed at 7.8μg/ml whereas minimum cell viability of piperine was 10.13%(maximum cell inhibition -89.87%) observed at 1000µg/ml. In tamoxifen treated cells, maximum cell viability was 84.86%(minimum cell inhibition –15.31%) and minimum cell viability was 18.68%(maximum cell inhibition -81.32). In
Table 1. Anti cancer effects of Piperine, Tamoxifen and Piperine+Tamoxifen combination

<table>
<thead>
<tr>
<th>S. No</th>
<th>Dose (µg/ml)</th>
<th>Piperine</th>
<th>Tamoxifen</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
<td>78.92</td>
<td>84.69</td>
<td>66.06</td>
</tr>
<tr>
<td>3</td>
<td>15.6</td>
<td>70.97</td>
<td>78.92</td>
<td>59.43</td>
</tr>
<tr>
<td>4</td>
<td>31.2</td>
<td>60.43</td>
<td>69.18</td>
<td>52.00</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>51.49</td>
<td>58.84</td>
<td>42.97</td>
</tr>
<tr>
<td>6</td>
<td>125</td>
<td>40.55</td>
<td>51.09</td>
<td>32.73</td>
</tr>
<tr>
<td>7</td>
<td>250</td>
<td>27.03</td>
<td>38.56</td>
<td>24.29</td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>19.48</td>
<td>24.85</td>
<td>17.26</td>
</tr>
<tr>
<td>9</td>
<td>1000</td>
<td>10.13</td>
<td>18.68</td>
<td>7.63</td>
</tr>
</tbody>
</table>

combination treatment with tamoxifen and piperine, maximum cell viability was 66.06% (minimum cell inhibition – 33.94) and minimum cell viability was 7.63 (maximum cell inhibition – 93.47%) at minimum concentration of 7.8 µg/ml and maximum concentration of 1000 µg/ml respectively. The IC50 value is plotted accordingly in graph and depicted in Figure 1-3.

DISCUSSION

Our data indicate that piperine might increase tamoxifen efficacy by interacting with
P-glycoprotein. Recent studies suggest tamoxifen as a directly acting drug in treatment of breast cancer and found beneficial with good efficacy, tolerability and oral bioavailability. P-glycoprotein was reported to limit the concentration of active metabolites of tamoxifen in breast cancer cells causing a compromise in the treatment. This was thought to be a clinical implication for acquired or innate resistance to tamoxifen treatment by breast cancers. In untreated breast cancer cells, approximately 40% of P-glycoprotein expression was reported and this was increased with initiation of chemotherapy which decreased response to treatment. Many drugs decreasing drug resistance showed specific and competitive (with cytotoxic drugs) binding to P-glycoprotein for active transportation. Many P-glycoprotein inhibitors are in clinical trials and many failed due to unfavourable drug properties and toxicity. Piperine showed satisfactory drug properties and inhibition of P-glycoprotein efflux activity. Piperine reversed drug resistance of KB and SW480 cancer cell lines to chemotherapy. In our study piperine combination showed maximum therapeutic effect in comparison to individual drug treatments.

**CONCLUSION**

This study concludes that combination of piperine and tamoxifen shows enhanced cell Inhibitory activity on growth of MCF 7 human breast cancer cell line, in comparison to individual Inhibitory activity of piperine and tamoxifen. This combination can be taken as novel drug combination aiding to treat breast carcinoma. Further studies are required to characterise and prove other effects of this combination.

**ACKNOWLEDGEMENTS**

I acknowledge Dr. P. Elango, Professor, Department of Pharmacology, SRMC & RI-DU; Dr. Sharanya and Dr. Jyothsna Pinni for providing their expertise support during the study.

**REFERENCES**

7. Minh Truong Do, Hyung Gyun Kim, Jae Ho Choi,


