

## 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

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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 dyetreatment 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 ( $\mu\text{g}/\text{ml}$ ), the percentage of cell viability is 51.49 at the dose of 62.5  $\mu\text{g}/\text{ml}$ , 51.09 at 125  $\mu\text{g}/\text{ml}$ , 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.

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Cancer is the primary cause of death worldwide and in India as well<sup>1</sup>. Though there are many types of cancers in world, out of all these breast cancer was the major cancer type in India<sup>2</sup>. This breast cancer is the one which is affecting people across the country with mortality incidence ratio of 66 in rural and 8 in urban according to recent statistics In India<sup>3</sup>. Breast cancer can be cured once it is diagnosed appropriately and when treatment is started at right time with right drug in proper dosage. Since there are many adverse effects

in cancer chemotherapy this should be addressed in an intense way with the help of combination of drugs. This can prevent resistance, increase the bioavailability of the drug and decrease the adverse effects of the present drug in chemotherapy. Approximately 70 % of breast cancers are estrogen receptor positive or endocrine sensitive type<sup>4</sup>. Tamoxifen is an estrogen receptor antagonist prescribed at 20 mg/kg/day dosage for the treatment of endocrine sensitive breast cancer. There is a footnote in the same study saying that tamoxifen

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 cancer<sup>5</sup>. This showed importance of tamoxifen in treatment of breast cancer<sup>5</sup>. 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 drug<sup>6</sup>. Piperine treatment alone has shown beneficial effects in breast carcinoma targeting cancer stem cell renewal properties<sup>7</sup>. According to Callaghan *et al* 1995, tamoxifen was inhibited by p-glycoprotein<sup>8</sup>. Another study demonstrated the inhibitory property of piperine with p-glycoprotein<sup>9</sup>. 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-7<sup>10</sup>. This excision was collected from a patient Helen Marion who was suffering from metastatic disease, and developed MCF-7 cell line<sup>11</sup>. 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

CO<sub>2</sub>, 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 previously<sup>12</sup>, assay have been performed. Initially in the 24 well plate cells were plated and at the temperature of 37°C with 5% CO<sub>2</sub> 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, keeping dimethyl sulfoxide as blank. IC<sub>50</sub> 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 7.8 µg/ml whereas minimum cell viability of piperine was 10.13% (maximum cell inhibition -89.87%) observed at 1000 µg/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

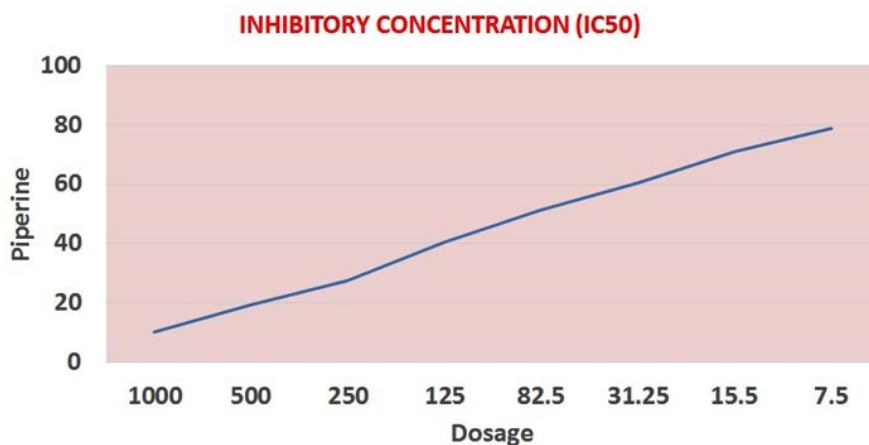
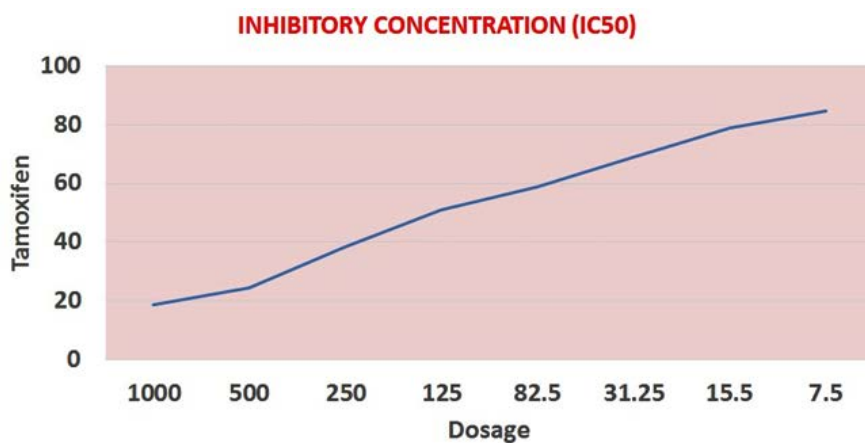
**Table 1.** Anti cancer effects of Piperine, Tamoxifen and Piperine+Tamoxifen combination

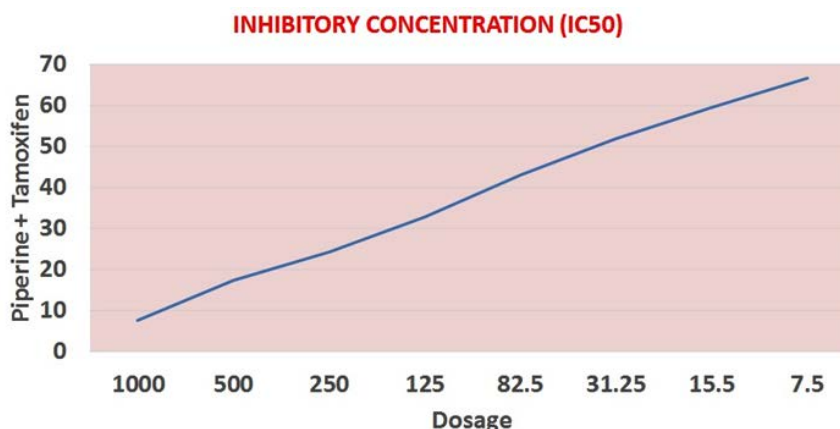
S. No	Dose ( $\mu\text{g/ml}$ )	Piperine	Tamoxifen	Combination
1	Control	100	100	100
2	7.8	78.92	84.69	66.06
3	15.6	70.97	78.92	59.43
4	31.2	60.43	69.18	52.00
5	62.5	51.49	58.84	42.97
6	125	40.55	51.09	32.73
7	250	27.03	38.56	24.29
8	500	19.48	24.85	17.26
9	1000	10.13	18.68	7.63

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  $\mu\text{g/ml}$  and maximum concentration of 1000  $\mu\text{g/ml}$  respectively. The  $\text{IC}_{50}$  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

**Fig. 1.** Cell viability in MCF 7 culture of Piperine treated group**Fig. 2.** Cell viability in MCF 7 culture of Tamoxifen treated group



**Fig. 3.** Cell viability in MCF 7 culture of Piperine and Tamoxifen combination treated group

P-glycoprotein. Recent studies suggest tamoxifen as directly acting drug in treatment of breast cancer and found beneficial with good efficacy, tolerability and oral bioavailability<sup>13-15</sup>. 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<sup>16,17</sup>. Many drugs decreasing drug resistance showed specific and competitive (with cytotoxic drugs) binding to P-glycoprotein for active transportation<sup>18-20</sup>. Many P-glycoprotein inhibitors are in clinical trials and many failed due to unfavourable drug properties and toxicity<sup>21-27</sup>. 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<sup>28</sup>. 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.

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