

Comparison of Anticancer Activity Between Thymoquinone and Tamoxifen , Thymoquinone + Tamoxifen On MCF-7 Cell Line of Human Breast Cancer –An Invitro Study.

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This study aims at evaluating the anticancer effect on the MCF-7 (Michigan Cancer Foundation-7) cell line of human breast cancer using Thymoquinone and Tamoxifen alone as well as in combination therapy by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) Test. NCCS in Pune provided the MCF-7 cell line. The cells were kept at 37°C in a humidified medium of 50µg/ml CO₂ in Minimal Essential Medium added with 10percent FBS (Foetal Bovine Serum), streptomycin (100µg/ml), as well as penicillin (100U/ml). MTT-(3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl tetrazolium bromide) test was conducted on MCF-7 cell line for Thymoquinone and Tamoxifen as sole and combination therapy. Measurements were performed using UV (Ultra-Violet)-spectrophotometer at 570-nanometre absorbance and the content needed for a 50 percent inhibitory concentration (IC₅₀) was calculated and evaluated graphically. IC₅₀ of Thymoquinone on MCF 7 was found to be at 31.2 µg/ml and Tamoxifen was at 62.5 µg/ml were as in combination therapy the IC₅₀ was found to be at 7.8 µg/ml. There is a remarkable reduction in concentration to achieve IC₅₀ percentage in combination therapy with a comparison with individual therapy. Therefore, the combination therapy of Thymoquinone and Tamoxifen on the MCF-7 cell line is more efficacious when compared to individual treatment on cell viability inhibition.

Keywords: Inhibitory Concentration; Michigan Cancer Foundation-7, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Tamoxifen; Thymoquinone.

Breast cancer among women varies hugely in incidence around the world.¹ It is the 2nd most frequent cause of death among carcinoma deaths in females which occurs due to metastasis.^{2,3} Though there is major progress in the treatment strategies, Breast carcinomas have a high mortality rate.^{4,5}

Cell lines play a major role in studying specific alterations in cell structure.⁶

Mechanisms behind drug resistance, which is the main cause for chemotherapy failure in patients with breast cancer, can be studied using the MCF-7 cell line.^{7,8}

Herbert D. Soule developed the MCF-7 cell line from a chest wall nodule excision of the patient who was diagnosed to have metastatic disease at Michigan Cancer Foundation.⁹

Thymoquinone has various properties like anti-diabetic, anti-oxidant, anti-allergic, as well as anti-tumor substance properties.¹⁰ It was established that Thymoquinone promotes apoptosis in carcinoma cells by XIAP (X-Linked Inhibitor of Apoptosis Protein) mediated PKB (Protein Kinase B).¹¹

Tamoxifen is an estrogen receptor modulator that is non-steroidal. It inhibits the actions of estrogen on breast cancer cells by competitively inhibiting estrogen binding to estrogen receptors, which is frequently utilized in hormone treatment of receptor-positive breast tumors in pre-menopausal as well as post-menopausal females.¹²

Recent studies have revealed that Tamoxifen promotes both cell death as well as cell cycle arrest in carcinoma cells of the breast by activation of ERK1/2 through signaling induction that causes MAPK and caspase pathway modification in an ER ("Estrogen Receptor")-positive breast carcinoma cell line (MCF-7) promptly.^{13,14} Tamoxifen is prescribed at the dosage of 20mg/kg body weight per day for the treatment of ER-positive breast cancer.

Combination therapy plays a major role in improving the efficacy in the treatment of patients having breast carcinoma.¹⁵

Hence, in this research, we have assessed the anti-cancerous impact of Tamoxifen to inhibit cell proliferation in breast carcinomas, in combination with Thymoquinone.

MATERIALS AND METHODS

NCCS (National Center for Cell Science) in Pune provided the MCF-7 cell line. The cells were kept at 37°C in a humidified medium of 50µg/ml CO₂ in "Minimal Essential Medium" added with 10 percent FBS, streptomycin (100µg/ml), and penicillin (100U/ml).

In 24-well plates, cells (1×10⁵/well) were plated and incubated at 37°C with 5 percent CO₂ conditions. Samples were introduced at various concentrations and incubated for 24hrs after reaching cell confluence. It was taken from the well after incubation and rinsed in DMEM without serum or phosphate-buffered saline (pH 7.4). 0.5percent "3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl—tetrazolium bromide (MTT)" was applied to 100µl/well (5mg/ml) and incubated for 4hrs. DMSO was applied to all of the wells which were used as a blank and the absorbance was recorded at 570nm in UV-Spectrophotometer after the incubation period. Measurements were conducted and the content needed for a 50percent inhibition (IC50) was calculated and evaluated graphically.

The following formula was used to calculate the % cell viability:

"%Cell viability=A570 of treated cells/A570 of control cells×100"

Cell viability assessments were compared between the control and test samples in each assay and were plotted on the graphs.

Table 1. Anticancer effect of Thymoquinone, Tamoxifen and Thymoquinone + Tamoxifen on MCF 7 cell line

S. No.	Concentration (µg/ml)	Dilutions	Thymoquinone		Tamoxifen		Thymoquinone + Tamoxifen	
			Absorbance (O.D*)	Cell Viability (%)	Absorbance (O.D*)	Cell Viability (%)	Absorbance (O.D*)	Cell Viability (%)
1	1000	Undiluted	0.235	17.66	0.380	28.57	0.224	16.84
2	500	1:1	0.332	24.96	0.435	34.21	0.290	21.80
3	250	1:2	0.413	31.05	0.528	39.69	0.357	26.84
4	125	1:4	0.496	37.29	0.602	45.26	0.424	31.87
5	62.5	1:8	0.579	43.53	0.673	50.60	0.490	36.84
6	31.2	1:16	0.665	50.00	0.741	55.71	0.557	41.87
7	15.6	1:32	0.748	56.24	0.814	61.20	0.624	46.91
8	7.8	1:64	0.848	63.75	0.885	66.54	0.691	51.95
9	Cell control	-	1.330	100	1.330	100	1.330	100

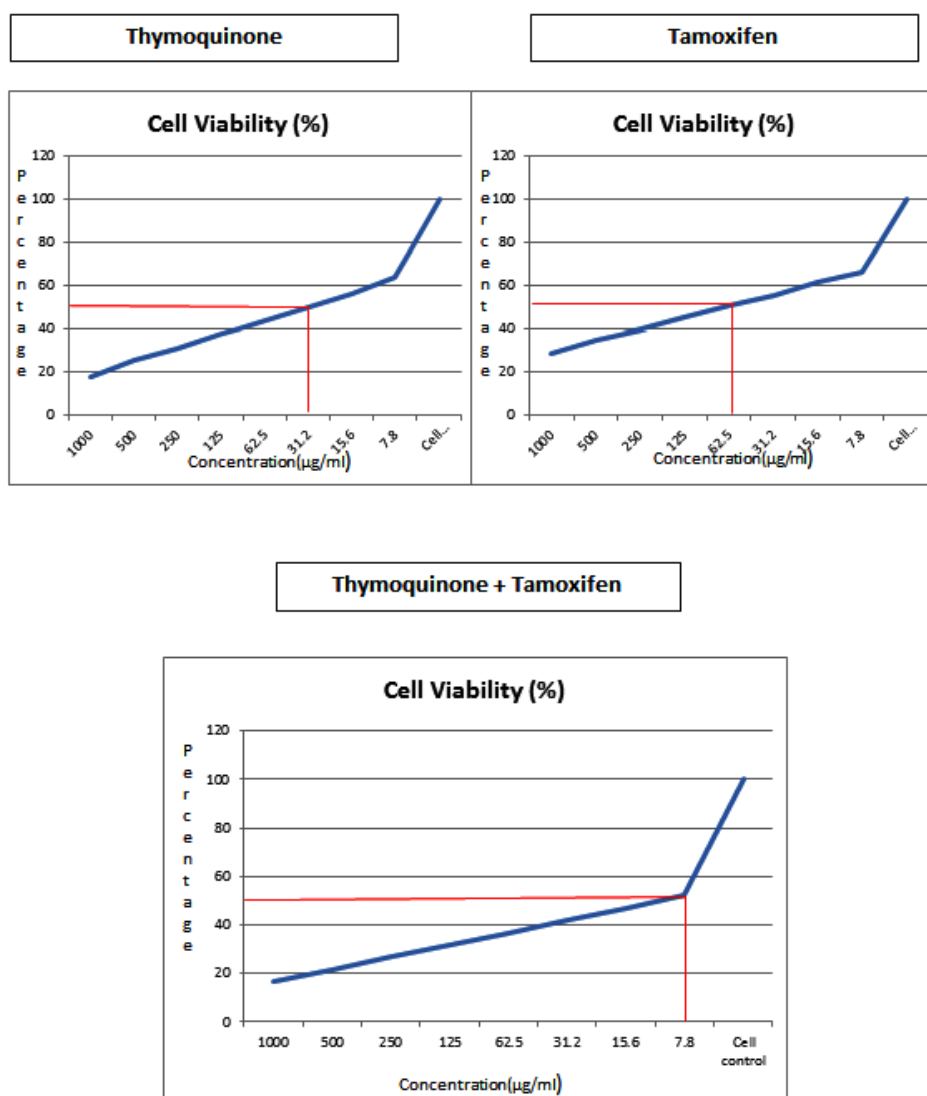
*O.D- Optical Density

RESULTS AND DISCUSSION

The anti-cancerous activity of Thymoquinone, Tamoxifen, and in combination was studied on the MCF-7 cell line at various concentrations that range from 7.8-1000 μ g/ml and the cell viability percentage was measured correspondingly and was tabulated in the table-1.

The findings revealed the cytotoxic effects of Thymoquinone and Tamoxifen were seen even at a minimal concentration on MCF-7 cells using the MTT test.

The maximum cell viability percentage of Thymoquinone treated cells was found to be 63.75 % at 7.8 μ g/ml with minimal cell inhibition of 36.25% and the minimum cell viability percentage of Thymoquinone was 17.66 % at 1000 μ g/ml with maximum cell inhibition of 82.34% respectively. Similarly, in cell lines treated with Tamoxifen, the maximum cell viability percentage was 66.54% at 7.8 μ g/ml with minimal cell inhibition of 33.46 % and the minimum cell viability percentage of Tamoxifen was 28.57 % at 1000 μ g/ml with



Graph 1. Graphical representation of cell viability percentage of individual treatment of Thymoquinone, Tamoxifen, and the combination (Thymoquinone + Tamoxifen) treatment at various concentrations

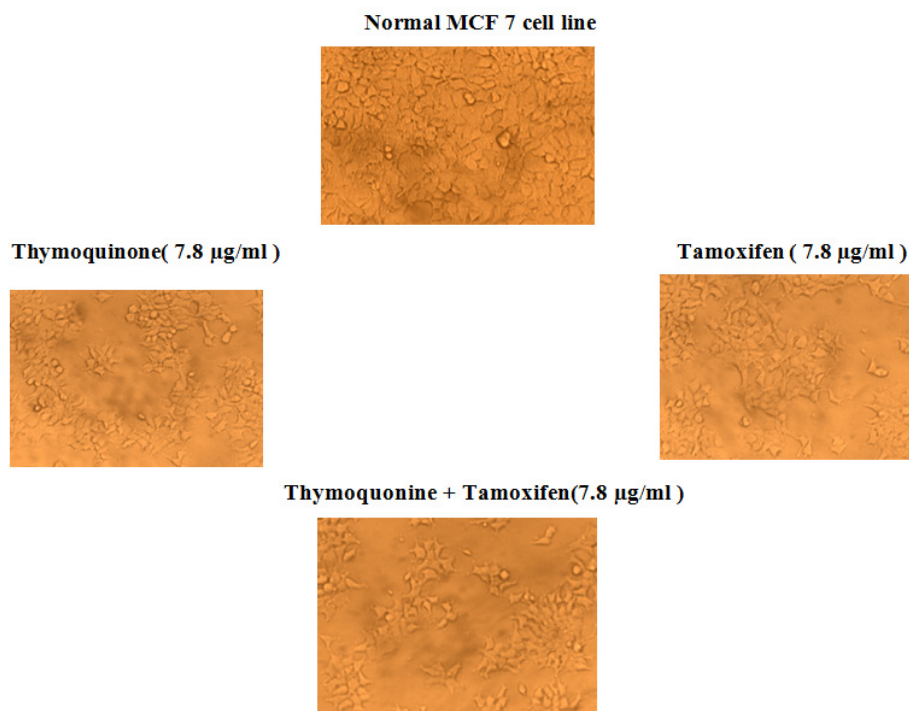


Fig. 1. Anticancer effect of Thymoquinone on MCF 7 cell line

maximum cell inhibition of 71.43 % respectively. When comparing the MCF-7 cells which were treated with the combination of Thymoquinone and Tamoxifen it was found that the maximum cell viability percentage was 51.95% at 7.8 µg/ml with minimal cell inhibition of 48.05 % and the minimum cell viability percentage of Thymoquinone and Tamoxifen was 16.84% at 1000 µg/ml with maximum cell inhibition of 83.16 % respectively.

The cell viability percentage of individual treatment of Thymoquinone, Tamoxifen, and the combination (Thymoquinone + Tamoxifen) treatment at various concentrations were plotted and were represented in graph-1. The cell viability picture showing the anti-cancerous activity of both individual and combination therapy at various concentrations has been depicted in figure-1.

Tamoxifen causes a change in plasma membrane permeability due to second messenger formation through the phospholipase pathway and the sustained activation of protein kinase C. This change in cell membrane permeability is responsible for its apoptotic response.^{16,17} The increase in

Tamoxifen concentration increases the intensity of MCF7 mitochondrial membrane permeability, which leads to the loss of mitochondrial membrane potential and the release of cytochrome C from the mitochondria causing cytotoxic effects in MCF-7 breast cancer cell lines.¹⁸ Studies have suggested that tamoxifen is efficacious with good tolerance and bioavailability.¹⁹⁻²¹

Thymoquinone causes apoptosis in cancer cells by various multiple target modulation such as p53-dependent, and p53-independent pathway, activation of caspases, increases in p53 expression, anti-apoptotic Bcl-2 up-regulation and decrease in cyclins B1 and D1.²² Recent studies have revealed that NF-kB is a potential target for inducing apoptosis in cancer cells.²³

Metastasis is the major limitation of Tamoxifen treatment in breast carcinoma patients which occurs due to overexpression of Transforming Growth Factor- α (TGF- α) on prolonged therapy.²⁴⁻²⁶

Moreover, resistance to Tamoxifen treatment is another consequence of reducing its efficacy. Inquisitively, shreds of evidence

revealed that in chronic treatments -Tamoxifen acts as an estrogen-like structure, promoting tumor progression.²⁷

Tamoxifen concentration is reduced in breast carcinoma cells owing to the presence of P-glycoprotein, causing a treatment compromise. This was believed to be a cause for the expansion of innate resistance in breast carcinoma patients treated with Tamoxifen.¹¹

Considering the above-mentioned concepts, this study specifies the need for combination therapy in place of monotherapy. It has recently been proved that Thymoquinone produces a synergistic impact on Tamoxifen through XIAP-mediated Akt regulation in estrogen receptor-positive and negative breast carcinomas when compared to monotherapy with Tamoxifen. In addition to this, decrease in the levels of anti-apoptotic Bcl-2 and Bcl-xL expression, and an increase in the levels of pro-apoptotic Bax, p27, cytosolic AIF and cytochrome C proteins is responsible for increased apoptotic effect in MCF-7 treated with Thymoquinone and Tamoxifen. Besides they induce apoptosis by activation of Caspase-9 and by decreased level of p-Bad (*BCL2 Antagonist of cell Death*), p-MAPK and p-GSK-3 β in Akt pathway.¹¹

Literature states that though Thymoquinone has anti-cancerous activity, evidence revealed that the addition of this drug with Tamoxifen improved the efficacy.¹¹

In this study, it was observed that combination therapy of Thymoquinone and Tamoxifen had better efficacy and additive anti-cancerous activity in the MCF-7 cell line than sole therapy.

CONCLUSION

In this study, it is determined that the combined effect of Thymoquinone and Tamoxifen has the better activity of cell inhibitory on MCF 7 cell line growth when compared with the individual effect of Thymoquinone and Tamoxifen. Hence the above combination can be considered for better efficacy in the treatment of breast carcinoma patients. Further In-Vivo evaluations are needed in the future to aid its anti-cancerous effect.

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Conflict of Interest

There is no any conflict of interest between authors.

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