# Turmeric Herbal Chemo-Therapy for Metastatic Triple negative Breast cancer

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***Abstract*—Big pharma is only around for about 100 years, while the natural/herbal remedies are in vogue for thousands of years. It is especially useful for cancer treatment, which is not a single disease, but is a bunch of a diseases. Turmeric is one of these herbs, which has been in use for over 5000 years, both in medicine and in daily cooking. Its use has been investigated in this research for its efficacy in killing the aggressive and metastatic triple negative breast cancer (TNBC) cell line, MDA-MB-231, along with electrical pulses (EP) for enhanced uptake. For this, eight 1200V/cm, 100µs pulses were used. Four groups, untreated control, turmeric only, EP only and EP+turmeric were studied. The viability results indicate there is noticeable cell death, when measured immediately and after 24h with the EP+turmeric treatment. The highest cell death was 40% immediately after and it was 95.3% after 24h for the EP+turmeric sample. This shows the promise of use of this molecule for the unmet need of treating TNBC.**

## I. Introduction

Turmeric is a natural herbal spice that has been in use for over 5000 years, both in Ayurveda (Science of Life) medicine and in cooking. It is a healing powerhouse, richly loaded with antioxidants, anti-cancer agents and other phytochemicals. It is found useful to treat over 150 diseases, including many cancers [1, 2]. However, it has reduced bioavailability and solubility issues. To enhance both its cellular uptake and its potency, electrical pulses have been used in this research [3, 4] to study their effect on the cell death of MDA-MB-231, an invasive and highly metastatic cell line of triple negative breast cancer (TNBC). It is of practical interest to study TNBC, as, as the name implies, it lacks of all the three receptors, and hence, it is not responsive to the commonly administered chemo drugs, that attack one or more these triple receptors. Given the poor prognosis of TNBC, new therapies are required to target them. In this study, we explore the use of Electrochemotherapy with turmeric (Curcuma longa) against these aggressive TNBC cells.

## II. Materials and Methods

### *A. Cell Preparation*

Basal type human adenocarcinoma epithelial triple negative breast cancer cells MDA-MB-231 (ATTC®) were used. Dulbecco's modified Eagle's medium (DMEM; Gibco™, USA) with 10% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA, USA) and 1% penicillin-streptomycin (PS; Life Technologies, USA) was used to culture the cells in monolayer. Cells were incubated at 70-80% humidity, 95% air, 5% CO2, and 37°C. The cultured cells were collected with trypsinization and were centrifuged for 5 min at 1000rpm at 4°C. The supernatant was discarded, and the cell were resuspended in fresh DMEM media to make a cell suspension at $1\times10^6$ cells/mL for the turmeric treatment.

### *B. Turmeric Treatment*

Commercially available, powder form turmeric that is used cooking is used in this research. A 100mg/mL stock solution of turmeric was prepared in dimethyl sulfoxide (DMSO). The required volume from 100mg/mL stock solution was added into media containing $1\times10^6$ cells/mL to make 2mg/mL final concentration of turmeric in cells for the electroporation treatment. Cells for control samples did not receive any turmeric.

### *C. Electroporation Treatment*

A 600µL volume of cells with turmeric treatment or control were transferred into BTX electroporation cuvettes with electrode gap distance of 4 mm for the electrical pulse application. A BTX ECM 830 electroporator (Genetronics Inc., CA, USA) was used to generate and apply eight square wave unipolar pulses of 1Hz frequency at 1200V/cm with 100µs pulse duration to cuvettes with cells. The experiments were performed

in triplicates. We had total four different treatment groups: control (no treatment), 1200V/cm, 100µs, 8pulses only (EP only), 2mg/mL turmeric only and 2mg/mL turmeric + 1200V/cm, 100µs, 8pulses (EP+turmeric).

### *D. Trypan Blue Assay*

Following the electroporation, 20µL of treated cells were mixed into 20µL of trypan blue. 20µL of this mixture was used to determine the cell viability using Nexcelom Bioscience Cellometer®. Viability values were normalized with control viability (100%) and were reported.

### *E. MT Viability Assay*

After electroporation, 10µL of treated cells from each sample was added into a 96 well plate (10,000 cells/well) containing 150µL of fresh media with 1× RealTime-GloTM reagent and incubated for 24h. The RealTime-GloTM was added to assess the viability at 24h and prepared in DMEM media using MT cell viability Substrate and NanoLuc® as per the manufacturer's protocol. Following 24h incubation, luminescence (Lum) values were recorded for 1s integration time using SpectraMax M5 multi-detection microplate reader system (Molecular Devices, Sunnyvale, CA, USA). Experimental Lum values were normalized with raw control Lum to determine the cell viability at 24h using Equation 1 and were reported.

$$Cell\ Viability\ (\%) = \frac{Experimental\ Lum\ value}{Control\ Lum\ value\ at\ 24\ h} \times 100 \qquad (1)$$

### *F. Statistical Analysis*

One-way analysis of variance (ANOVA) [4], and Tukey's test were performed to find the significant difference among treatment [4]. The model statistical model used in this study is shown in Equation 2:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \qquad (2)$$

where, $\sum_{i=1}^{4} T_i = 0$ and $\varepsilon_{ij} \approx NID(0, \sigma^2)$

In this model, $Y_{ij}$ is the overall response, µ is a constant overall mean, common to all treatments. $T_i$ is the effect of $i^{th}$ level of 'Treatment', and $\varepsilon_{ij}$ is a random error component, which is assumed to be independently and normally distributed random variable with mean 0 and variance $\sigma^2$. The trypan blue assay data satisfied the normality and equal variance assumption required for ANOVA test. However, Box-Cox transformation ($\lambda = 0.2$) was used to transform the MT viability data to satisfy assumptions.

ANOVA test was run to check for statistically significant difference ($p < 0.05$) among the means of the four treatment groups. The hypothesis of the interest in the ANOVA test is shown in Equation 3 [4].

$$\begin{aligned} &H_0: \ \mu_1 = \mu_2 = \mu_3 \ldots = \mu_n \\ &H_1: \ \text{Not all means are equal} \end{aligned} \qquad (3)$$

where, n = number of independent treatment groups (4), and $H_0$ and $H_1$ are the null and alternate hypothesis.

When p-value from ANOVA test was less than 0.05, we rejected the null hypothesis ($H_0$) and concluded that at least one of the effects was significant. Post ANOVA significance ($p < 0.05$), Tukey's test was performed to compare all six possible pairs among four treatment means to identify treatment means significantly different ($p < 0.05$) from each other. The letter report obtained from Tukey's test was reported with results, where each treatment is represented by a letter or a group of letters. Treatments not connected by the same letter were significantly different from each other ($p < 0.05$).

## III. Results and Discussion

### *A. Trypan Blue Assay*

Fig. 1 shows the cell viability results obtained immediately after pulsing the cells for the various sample conditions, viz. untreated control, ep only, turmeric only and EP+turmeric. Normalizing the viability of the untreated control sample, as 100%, the other samples have the viabilities of 75.9%, 87.9% and 59.7% respectively. This indicates the potency of the combination of turmeric and EP, which caused max cell death. The statistical analysis, as shown below indicates that all the four viabilities are statistically different.

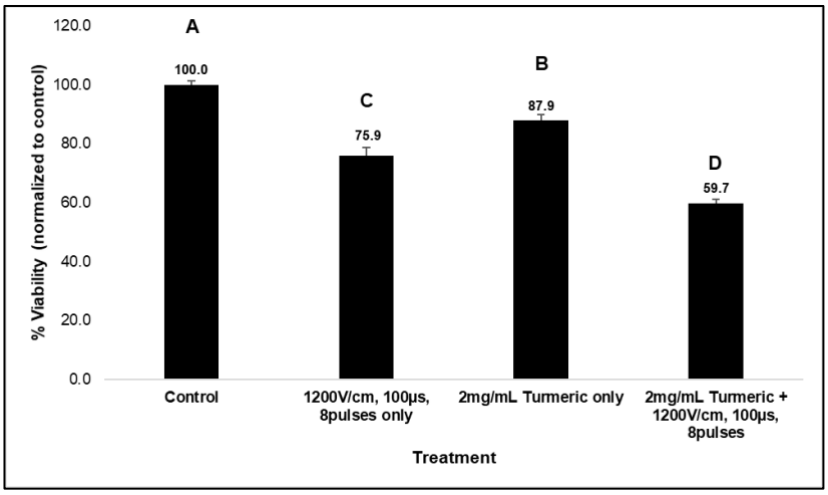


Fig. 1. The viability of MDA-MB-231 cells immediately after different treatments. All values are normalized with the viability of control (100%).

Table 1 shows the details of ANOVA test performed on the model, such as Degree of Freedom (DF), F ratio of F-test and P-value. The DF is three because there are total four treatment groups in our model. The P-value obtained for the Model is <0.0001, which is less than 0.05 indicating that at least one of the treatments in our model is significant.

TABLE 1: ANOVA TEST RESULTS OF FOR IMMEDIATE VIABILITY

| Source | DF | F Ratio | Prob > F |
|---|---|---|---|
| Model | 3 | 74.3938 | <.0001* |
| Error | 8 | | |
| Total | 11 | | |

Post ANOVA significance, Tukey's test was conducted, and the obtained letter report for different treatment is indicated in Fig. 1. Letter report indicates that the four treatments control, EP only, turmeric only, and EP+turmeric are significantly different from each other since they are represented by different letters 'A', 'C', 'B', and 'D', respectively.

### *B. MT Viability Assay*

Fig. 2 shows the cell viability results obtained 24h after pulsing the cells using MT assay for the various sample conditions, viz. untreated control, EP only, turmeric only and EP+turmeric. Normalizing the viability of the untreated control sample, as 100%, the other samples have the viabilities of 11.5%, 3.3% and 4.7% respectively. This indicates the potency of the EP+turmeric combination, which caused increased cell death. There is drastic reduction in cell viability after 24h, indicating that the turmeric could still be effective after 24h. The statistical analysis indicates that there is no statistical difference between the viabilities of the turmeric only and EP+turmeric samples, after 24h pulsing. They were statistically different immediately after pulsing. This indicates the change in mechanism of cell death with time. The 48h and 72h viability studies are on-going.

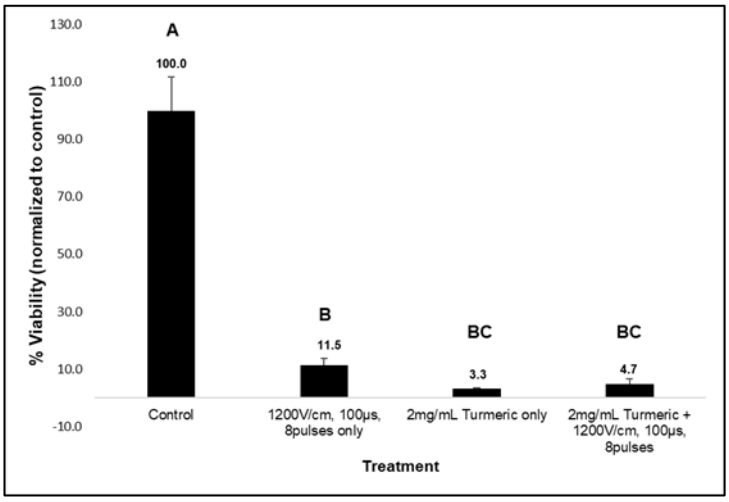


Fig. 2. The viability of MDA-MB-231 cells at 24h for different treatments. All values are normalized with the viability of control (100%).

Table 2 shows the details of ANOVA test performed on the model, such as DF, F ratio of F-test and P-value. The DF is three because there are total four treatment groups in our model. The P-value obtained for the Model is <0.0001, which indicates that the at least one of the treatments in our model is significant.

TABLE 2: ANOVA TEST RESULTS OF FOR THE VIABILITY AT 24H

| Source | DF | F Ratio | Prob > F |
|---|---|---|---|
| Model | 3 | 68.9053 | <.0001* |
| Error | 8 | | |
| Total | 11 | | |

Post ANOVA significance, Tukey's test was conducted, and the obtained letter report for different treatment is indicated in Fig. 2. Letter report indicates that the control, represented by letter 'A' is significantly different from three treatment groups EP only, turmeric only, and EP+turmeric, as letter 'A' is different from letters 'B' and 'BC'. This is expected since no treatment was done to control samples. The treatment with EP only is significantly different from control, but it is not significantly different from turmeric only and EP+turmeric, as EP only shares the letter 'B' in 'BC' with turmeric only and EP+turmeric. Turmeric and EP+turmeric are not significantly different from each other since they are represented by the same letters 'BC'.

## IV. CONCLUSION

- Electro-turmeric therapy is designed as an effective method to increase therapeutic efficacy and reduce side effects, if any.
- The turmeric+eight 1200V/cm, 100µs pulses are effective in reducing the viability to 4.75%, which is statistically not different from the 3.3%, obtained for turmeric only.
- The use of electrical pulses is preferable due to the multimodality treatment that could be obtained due to synergy of electrical pulses and turmeric.
- This therapy is cheap and effective, and has the potential to treat the unmet need for aggressive TNBC.

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