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An in Vitro Model Study on the Effectiveness of Electrical Pulse Mediated Herbal Therapy on Breast Cancer

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ABSTRACT

Background: Breast cancer is the most life-threatening cancer in women all over the world. Considering the detrimental side effects of standard chemo drugs, plant-based drugs can be used effectively. This work aims to investigate the effect of herbal extracts mediated by electrical pulses on breast cancer cell lines.

Methods: Three different samples were chosen (Turmeric, Pepper, Neem), shade dried, and extracted using the soxhlet method, with ethanol as a solvent. These extracts were used as an anticancer drug against three breast cancer cell lines (MCF-10A, MCF-7, MDA-MB-231). Further, BTX ECM830 electroporator was used to generate electrical pulses of different parameters to enhance the uptake of extracts into the cancer cells. Realtime MT Assay was used to obtain the viability of breast cancer cell lines. A comparative study was conducted to assess the effect of each treatment on different breast cancer cell lines.

Results: Treatments were more susceptive to cancerous cell lines than normal cells. Low intensity, high duration electrical pulses with herbal extracts showed a higher cytotoxic effect than other treatments. A notable increase in cell death was observed in combination treatments compared to single treatments. The lowest viability of 4% was obtained for synergetic treatment of electrical pulses and herbal extract.

Conclusion: In the current study, we found for the first time that herbal extracts in combination with electrical pulses exhibited strong anticancer activity against breast cancer cell lines. This promising treatment can be used efficiently to treat breast cancer without any side effects

INTRODUCTION

Breast cancer is the most common cancer in women and is the second most leading cancer after lung cancer worldwide. As per statistics, every 12 minutes, 6 women develop breast cancer and one dies in the US.[1] The incidence and mortality have been increasing over the years. This increasing trend is the same in India too, accounting for 14% of cancers in Indian women. Every four minutes, an Indian woman is diagnosed with breast cancer.[1,2] Many potential drugs developed to treat cancer have had limited success, owing to a lack of safe and efficient delivery methods. These drugs are unable to access hydrophilic and hydrophobic lipid bilayers of cell membranes which are normally impermeable.[2] Many anticancer drugs lack specificity and are equally toxic to normal healthy cells causing several adverse effects.[3,4] Further, the body becomes resistant to either single or a combination of chemo drugs.[4] Hence, using electrical pulses for the active delivery of drug molecules to the interior of cells offers exciting prospects.

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Electroporation is a viable physical technique that utilizes precisely controlled electric fields of high intensity and short duration pulses to open up transient aqueous pathways through semi or non-permeable membranes and tissues, allowing targeted delivery of drugs, antibodies, and genes.[5,6] Since the application of pulses is only for a very short duration, the cell membranes eventually reseal, forcing the drug molecules to act specifically within the cell in various ways, such as diffusion, electro-osmotic and colloid-osmotic flow.[7] This process offers up to 1000-fold improved therapeutic benefit compared to using the drug alone and is gaining acceptance as a viable procedure to enhance the efficacy of drug delivery for cancer treatment.[8] When cells are exposed to an external electric field, they experience an increase in the transmembrane potential. If this potential exceeds a critical value (typically 1V), transient pores are formed in the membrane. The magnitude of membrane potential can be defined,[9] by the following equation:

$$V=1.5\,ER \quad (1)$$

where V is a voltage induced on the cell membrane, E is electric field intensity applied and R is the radius of the cancer cell. This equation can be modified as

$$E=4V/(3D) \quad (2)$$

where D is the diameter of the cancer cell.

When electroporation is combined with the injection of cytotoxic drugs, this combination treatment is called Electrochemotherapy, which increases the diffusion of chemotherapeutic drugs into the interior of tumor cells. This is a local, site-specific and physical technique with fewer side effects and less costs, and can be performed as an outpatient treatment, requiring no costly outfits such as a radiation facility.[10,11]

Current treatments used to treat breast cancer include chemotherapy, radiotherapy, and chemically derived drugs. Treatments such as chemotherapy have caused much stress, and have damaged patients' health.[12] To reduce the side effects and to cure the disease without any side effects, naturally available medicinal plants are used to treat various diseases. Medicinal plants have played a pivotal role in the prophylaxis and treatment of several serious diseases. From long, herbal medicines have been used and are still used in many countries as the primary source in prevention, drug development, and treatment. Most herbal plants exhibit potential activities such as anticancer, analgesic, antibacterial, antiviral, anti-inflammatory, and anti-diabetic activity.[12, 13] According to World Health Organisation (WHO), about 80% of the world's population solely depends upon integrative traditional herbal medicine for treating and preventing diseases. Much research has been conducted to investigate the potential properties and uses of plant extracts to develop drugs for diseases including cancer.[11-14]

Considering all the above, we have focussed on an alternative treatment that integrates electroporation techniques and extracts of medicinal plants as anti-cancer drugs. Extracts from Turmeric (*Curcuma longa L*), Black Pepper (*Piper nigrum*), and Neem (*Azadirachta indica*) are used as anticancer drugs against three breast cancer cell lines, MCF-10A, MCF-7, and MDA-MB-231. These medicinal plants are loaded with numerous bioactive compounds and are extensively used in Ayurveda, Siddha, and Unani medicine to treat various diseases.[14,15,16] Turmeric and its compounds have been found to possess effective anticancer activities by affecting diverse biological pathways involved in cell cycle regulation, tumorigenesis, metastasis, apoptosis, oncogene expression, and mutagenesis. Curcumin, a major compound of turmeric induces apoptotic cell death by damaging the DNA in human cancer cell lines, such as MCF-7, TK-10, and UACC-62 through topoisomerase II poison.[16,17] Methanolic extracts of neem leaves have exhibited significant anticancer activity against various cell lines including lung cancer with an IC value of 70.66. Neem seed oil has been tested against breast cancer cell lines resulting in growth inhibition of cancer cells by apoptosis induction and G1 phase arrest.[17,18] Similarly, in the case of pepper, a hot pungent spice is well known for its wide spectrum of exceptional medicinal properties. Pepper extracts potentially inhibited breast, lung, CNS, stomach and colon cancer proliferation by 23%, 34.7%, 28.6%, 55.2% and 42.4% respectively 19.[18] Further, they showed strong cytotoxicity against various other cancer cell lines too.[19,20] Previous studies have emphasized the anticancer potentiality of turmeric, neem, and pepper which motivated us to opt for these medicinal plants for our work. This work aims to investigate the efficiency of electrical pulse mediated delivery of herbal extracts against breast cancer cell lines.

METHODS

The methodology involved in this study is illustrated in Figure 1 by the stepwise process. Herbal extracts were extracted and used to treat breast cell lines (Noncancerous MCF-10A, Estrogen receptor-positive MCF-7, and Triple-negative MDA-MB-231). The efficiency of extracts with and without the electrical pulses was investigated.

Collection of plant samples

Commercially available dried rhizomes of turmeric, and dried fruits of black pepper were purchased from an organic store in Chennai, India. Fresh neem leaves were collected from Anna University Campus,

Chennai, India, and washed thoroughly with distilled water to remove dirt and impurities and shade dried for 10 days. All the samples were powdered using an electric mixer and stored for further use.

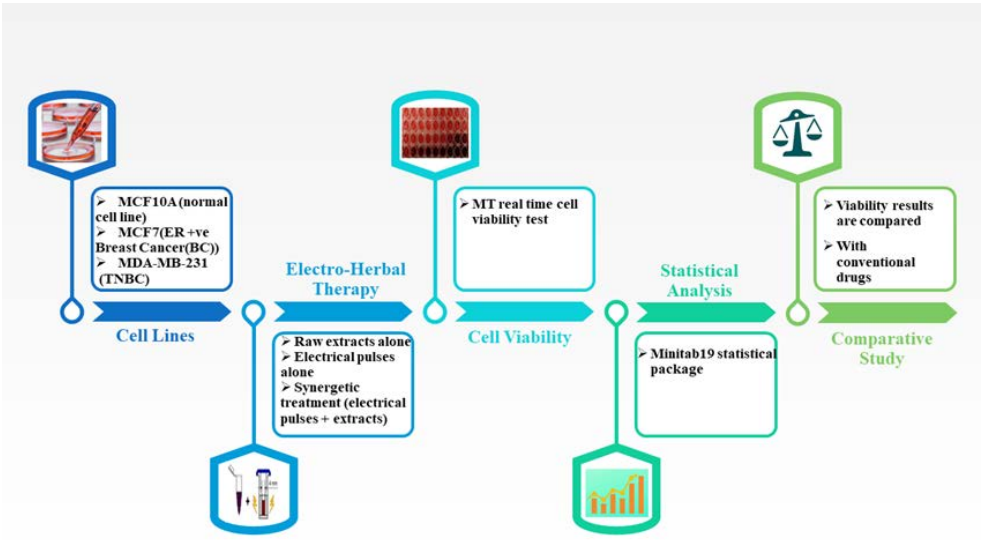

Figure 1. Experimental methodology

Preparation of extracts

For the study, 25g of powdered turmeric, neem leaf, and pepper were taken into a thimble and placed in a Soxhlets apparatus, with 100ml of solvent (ethanol). This extraction process continued for about 7 to 10 hours. Crude extract obtained was filtered using Whatman No.41 filter paper to remove particles and the solvent was removed by using a rotary evaporator. The obtained extracts were stored in an airtight container at 4°C.[20,21]

Preparation of cell lines

Hormone responsive breast cancer MCF-7 cells, non-cancerous epithelial MCF-10A cells, and human adenocarcinoma epithelial triple-negative cancer cells (ATTC®) were used in this study. MCF-7 cells were cultured as a monolayer in DMEM with 10% Fetal Bovine Serum (FBS), and 1% Penicillin/Streptomycin. MCF-10A was immortal but not transformed (non-tumorigenic) and it was adherent. MCF-10A cells were cultured in a 1:1 ratio of DMEM: Ham's F12 supplemented with 5% horse serum, 20ng/ml human epidermal growth factor, 0.5mg/ml hydrocortisone, 100ng/ml cholera toxin, 10µg/ml bovine insulin, 100 IU/ml penicillin and 100µg/ml streptomycin. The cells were grown at 37°C with 5% CO_2 in an incubator. TNBC cells were cultured as a monolayer in DMEM with 10% FBS and 1% penicillin-streptomycin and incubated at 70-80% humidity, 5% of and 37°C. The cells were detached using trypsin and centrifuged at 1000rpm, 4°C for 5min, and re-suspended in fresh media for treatment.[21,22]

Cell viability

Real-time MT Assay was used to obtain the viability of breast cancer cell lines. The treated cells were added to the 96 well plates containing 55µL of media. Then, 25µL of RealTime-GloTM reagent (Promega, USA) was added to assess the viability. As per the manufacturer's protocol, the final concentration of MT reagent was maintained at 1X. Luminescence was measured for 1000ms integration time using a SpectraMaxM5 multi-plate reader (Molecular Devices, USA). To determine the number of viable cells, the obtained experimental luminescence was normalized to control luminescence.[9,10,22,23]

$$cell\ viability\ \% = \frac{\text{Experimental luminescence value}}{\text{Control luminescence value}} \times 100 \quad (3)$$

Electrical pulse application

BTX ECM 830 Electroporator (Harvard apparatus, USA) was used to generate electrical pulses of different parameters, EP1=1200V/cm, 100µs, 8 pulses, EP2=500 V/cm, 100µs, 8 pulses, EP3= 500V/cm, 20ms, 8 pulses. [17,30-31]

Test protocol

The cultured breast cancer cell lines (MCF-10A, MCF-7, and MDA MB-231) were subjected to various treatments as follows:

Statistical analysis

Minitab 19 statistical package was used to perform the statistical analysis. Data were checked for normality and homoscedasticity assumptions. Statistical significance for cell viability study was assessed using Analysis of variance (ANOVA), followed by Tukey's posthoc test for multiple comparisons. The following statistical model was used

$$A_{ijk} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + (e_{ijk}) \quad (4)$$

where, A_{ijk} is predicted response, μ is the overall effect, A_i is the effect of i^{th} level of treatment, B_j is the effect of j^{th} level of time, C_k is the effect of k^{th} level of the cell line, AB_{ij}, AC_{ik}, BC_{jk}, and ABC_{ijk}, are the interaction effects between treatment, time, and patient, e_{ijk} is a random error with l=3 (no. of replicates).[24] A probability value (P<0.5) was considered statistically significant for the whole study.

RESULTS

A comparative study was done to assess the effect of electrical pulse mediated herbal therapy on breast cancer (MCF-7 (Estrogen receptor-positive) & MDA-MB-231(more aggressive, Triple-negative)) and non-cancerous MCF-10A cell lines.

Dosage curve

To start with, a dose-dependent viability study was performed with three breast cell lines (Noncancerous MCF10A, Estrogen receptor-positive cancer MCF7, and Triple-negative cancer MDA-MB-231). The cell lines were cultured and exposed to different doses of neem extracts (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8μg/ml). Cell viabilities were checked after 4h, 12h, 24h, 36h, 48h, 60h, and 72h to investigate the potential of the treatments. Further, the dose required for a 50% inhibition (IC_{50} value) was determined graphically as illustrated in Figure 2.

When different doses of neem extracts were given to cell lines, the lowest cell viability of 19.8% was observed in MDA-MB-231 cell lines at 72hours, whereas the highest cell viability of 128.72% was observed in MCF-10A cell lines at 24hours. When MCF-7 cells were exposed to neem extracts, the lowest half-maximal inhibitory concentration (IC_{50}) of 62.5μg/ml was observed. A constant dosage of 62.5μg/ml was used for further studies with other extracts and treatments. Figure 2 clearly indicates that the neem extract treatments did not cause any detrimental effect on healthy breast cells (MCF-10A), causing more cell death in breast cancer cell lines (MCF-7, MDA-MD-231).

Cytotoxicity on normal breast cell lines (MCF-10A cells)

The effect of electro-herbal therapy on noncancerous breast cell lines (MCF-10A) was investigated to find out the virulence of extracts in healthy cells over 4 to 72 hours. The impact of electrical pulses and the extracts (turmeric, pepper & neem) on normal cell lines is depicted in Figure 3.
When the cell lines were treated with neem extracts, we could observe an increase of 2 to 3% in cell viability. Further, when treated with electrical pulses of (EP1) 1200V/cm, 100μs, 8 pulses, cell viabilities decreased up to 4% after 72 hours of treatment.

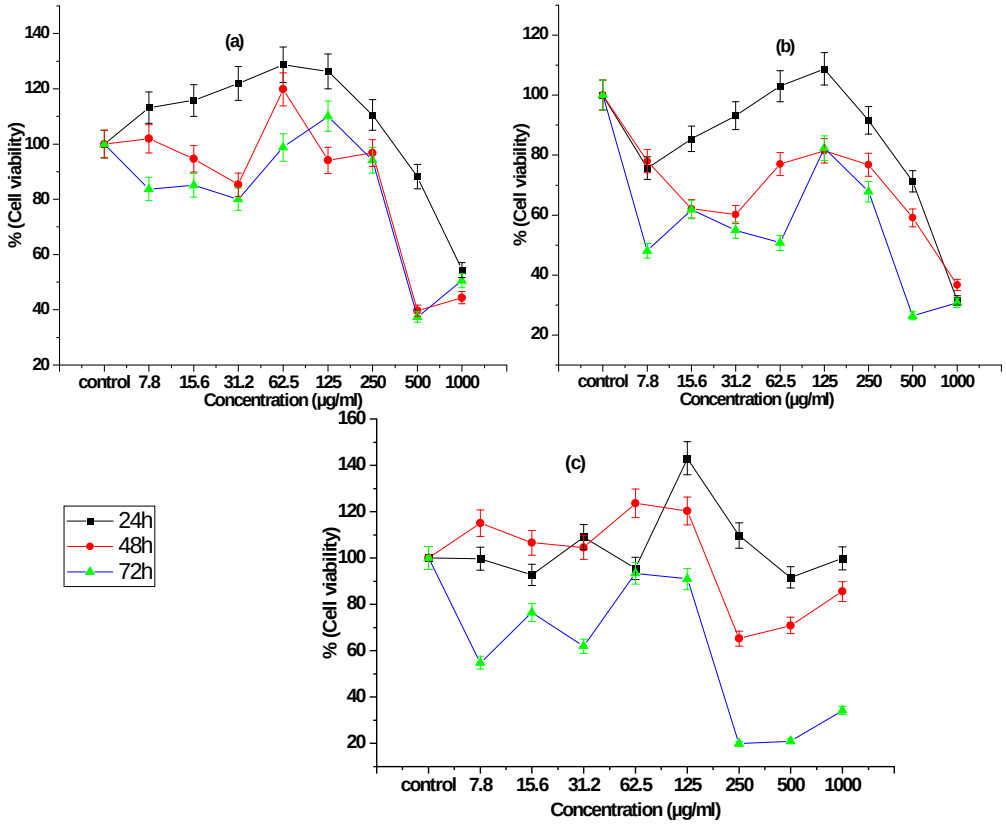

Figure 2. Dose dependent curves of neem extracts on (a) MCF-10A, (b) MCF-7, (c) MDA-MB-231 cell lines

Similarly, for EP2 (500V/cm, 100µs, 8 pulses) and EP3 (500V/cm, 20ms, 8 pulses) treat-ments, 1-8% decrease was observed in cell viabilities. When EP2 was used, less cell death was observed compared to other electrical parameters (EP1 & EP3). This may be due to the effect of lower intensity for a lower duration.

In the case of electro-neem treatment, electrical pulses (EP1, EP2& EP3) were used to enhance the uptake of extracts into the cells. When EP2 with neem extracts was used, cell viabilities were two times higher than in EP3+neem treatment and 1.8 times higher than in EP1+neem treatment. This could be due to the lower intensity of electrical pulses applied for the lower duration. Cell viabilities after 4 hours of treatment reduced slightly in certain cases. But after 12 hours, cell viabilities increased showing the efficiency of neem and electrical pulses as a safer method (less cell death is observed) as depicted in Figure 3.

Similarly, pepper extracts were used to treat noncancerous breast cell lines, in which ~3% increase in cell viability was observed, whereas when media-ted by electrical pulses, about 3 to 6% decrease in cell viability was observed. With the application of electrical pulses, we could observe a minimal reduction in viable cells. Over the duration of 4 to 72 hours, cell viabilities followed an increasing pattern, which indicates the normal growth of cells. When the EP2 parameter was used, less cell death was noted. The results observed indicate that the electro-pepper treatment does not affect the normal healthy cells.

With turmeric extracts, cell viabilities increased up to 10% whereas they reduced up to 5% when electrical pulsed extracts were used. Further, when turmeric extracts with electrical pulses were used, the lowest cell death of ~4% was observed. This indicates that the combined effect of electrical pulses and turmeric extracts yields a lower cell death than turmeric alone treatment.

Cell viability after 4 hours of treatment reduce slightly compared to the control (100%) in certain cases. But it was closer to the control values after 72 hours of treatment (an increasing pattern was observed from 4 h to 72 h). Cell viabilities of MCF-10A were checked for significance and were not statistically ($P>0.05$) different from each other. Hence, cell viabilities do not vary much when compared with control values (100%). With the obtained results, the above treatments discussed indicate that they do not affect the normal healthy cells. Hence, these treatments are safer on normal breast cells. Electrical parameter (EP2) can be chosen to achieve less cell death as it involves a low intensity of 500V/cm and less duration of 100µs.

Cytotoxicity on Estrogen receptor breast cancer cell lines (MCF7 cells)

Similarly, the efficiency of electrical pulses in delivering extracts to MCF-7 human breast cancer cell lines was explored over 4 to 72 hours. When cell lines were treated with neem extracts, we could observe a decrease of nearly 2% in cell viability compared to the control (100%) after 72hours of treatment as presented in Figure 4. Treatment with electrical pulses (EP1=1200V/cm, 100µs, 8 pulses) alone yielded a cell viability of 18.15% after 24 hours, which dropped by 2.85% to reach 15.30% after 12 hours, and by 2.05% to reach 13.25% after 60 hours of treatment and a slight increase (14.22%) was observed after 72 hours of treatment. Similarly, when experimented with low intensity and low duration electrical pulses (EP2), cell viabilities of 104.22% after 4 hours, 100.19% after 36 hours, and 100.29% after 72 hours of treatment were observed. When low intensity, high duration electrical pulses were used, cell viabilities of 18.23% after 4 hours were observed, followed by 14.25% after 24 hours and 10.06% after 36 hours which dropped to 9.37% and 9.80% after 48 and 60hours of treatment.

For different electrical pulse treatments, the results obtained can be given as EP3 > EP1> EP2. Hence, EP3, low intensity, high duration electrical pulses contributed to higher cell death, followed by EP1 (high intensity, low duration) and EP2 (low-intensity low duration electrical pulses. It is clear that the electrical pulses alone have produced more cell death than the extracts. For synergetic treatments, viabilities observed were ~22 times lower compared to neem alone treatment. Neem extracts with EP3 show higher cell death (95.54%) than other treatments, which is nearly ~15 times more than control values.

When pepper alone was given to MCF-7 breast cancer cell lines, cell viabilities were nearly equal to control values, without showing much reduction in cells as illustrated in Figure 4. But with electrical pulses alone, a decrease of 89% in cell viability was observed. When pepper extracts were mediated by electrical pulses, we could observe a decrease of 94% in cell viability when compared to pepper treatment alone. Hence, pepper extracts when mediated by electrical pulses (EP3) can be used for effective treatment.

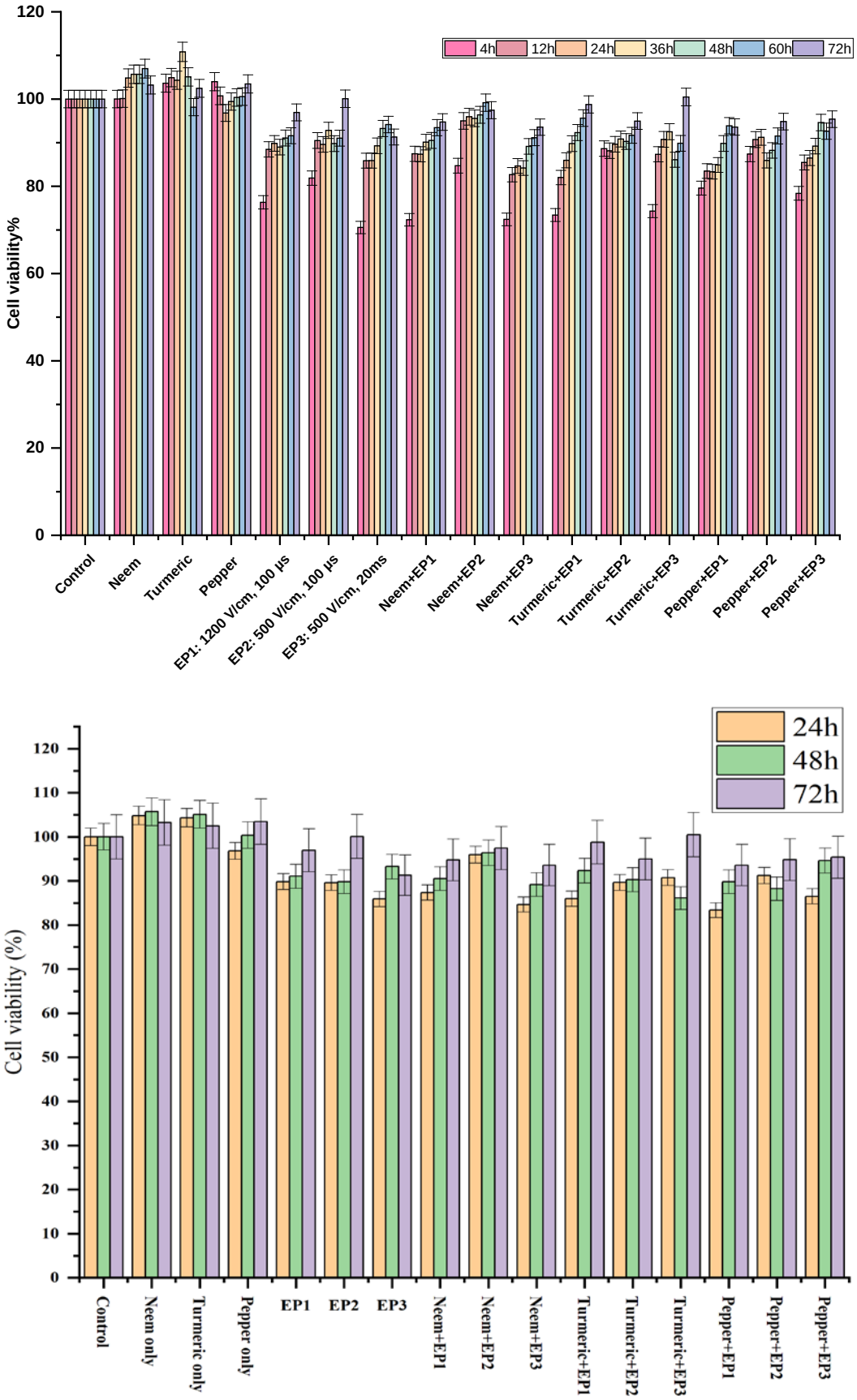

Figure 3. Viabilities of MCF10A cell lines after treatments for different time intervals

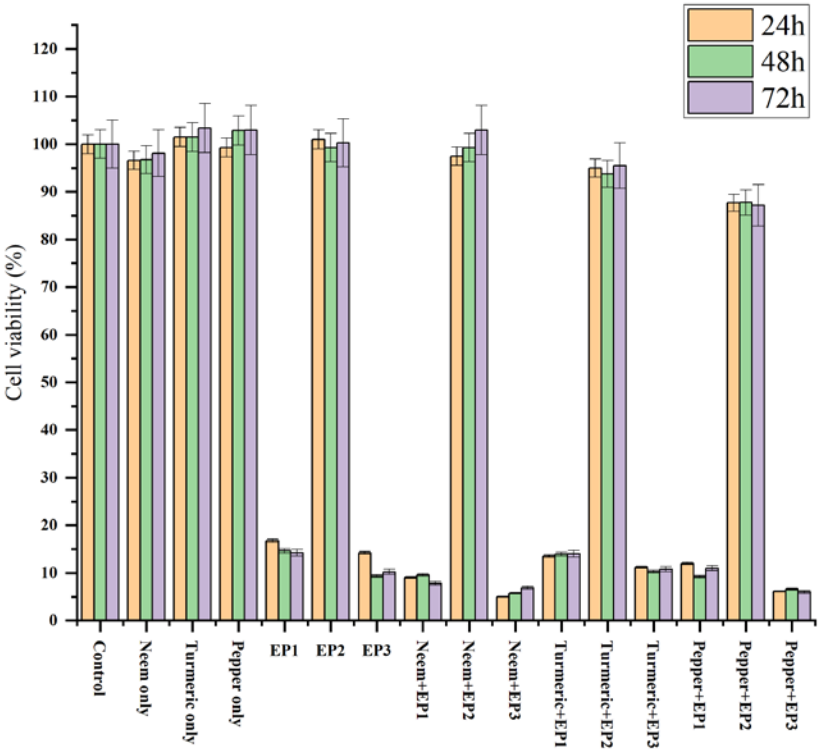

Figure 4. Viabilities of MCF-7 cell lines after treatments for different time intervals. *P<0.05 significantly different from control

The lowest cell viability of 98.80% was achieved with turmeric alone, and the lowest viability of 9.37% was achieved with electrical pulses alone, but when turmeric extracts were given with electrical pulses, the cell viability drastically reduced to 10.78% which was an 89.09% decrease in cell viability than the extract alone treatment and 90.52% decrease in cell viability than electrical pulse treatment alone. A significant decrease in the values of cell viability was observed in the case of EP1 treatment, EP3 treatment, and synergetic treatments (EP1 and EP3 with turmeric extracts) (P<0.05) as illustrated in Figure 4. This clearly shows that the synergetic treatments with EP3 and EP1 cause a strong cytotoxic effect.

A comparison of the obtained results shows that the highest cell death has been achieved in the following order:

(Neem + EP3) > (Pepper + EP3) > (Neem + EP1) > (Pepper + EP1) > (Turmeric + EP3) > (Turmeric + EP1) > EP3 > EP1 > (Pepper + EP2) > (Turmeric + EP2) > (Neem + EP2) > Neem > Pepper > Turmeric > EP2

Hence, it is clear that synergistic treatments have exhibited higher cell death than other treatments.

Cytotoxicity on Triple-negative breast cancer cell lines TNBC (MDA-MB-231)

Due to lack of receptors, Triple-negative breast cancers are very difficult to treat and often termed aggressive breast cancer. The effect of electrical pulse mediated herbal extracts on aggressive TNBC cell lines was studied. When MDA-MB-231 cells were treated with neem extracts alone, the lowest cell viability of 85.10% was observed after 4 hours of treatment, whereas when electrical pulses alone were used, viabilities dropped up to 6.44% for EP3 treatment after 4 hours. When neem extracts were mediated by electrical pulses, a decrease of ~60% to ~93% in cell viability was observed. With the application of electrical pulses, cell viability decreased drastically as illustrated in Figure 5. Hence, the electrical pulses have increased the uptake of extracts into TNBC cell lines.

Similarly, when pepper extracts were used to treat MDA-MB-231 cells, cell viabilities varied from 81% to 106.48%. In the case of electrical pulse alone treatment, a decrease of 55.46% was noted for EP1 treatment and an increase of 11% for EP2 treatment was noted. In the case of EP3 treatment, a decrease of 93.79% was observed after 24 hours of treatment, when compared to pepper alone treatment. Low-intensity high duration (EP3) electrical pulses exhibited a higher cell death rate of 94.62%, followed by high-intensity low duration EP1 and then by EP2 as depicted in Figure 5. When pepper alone was given, 18.76% cell death was observed, whereas when this was mediated by electrical pulses, we observed a higher cell death rate of 95.96%, which was ~5 times more than in the pepper alone treatment.

Turmeric treatment yielded cell viability of 89.50% after 24 hours of treatment, but when turmeric extracts

with electrical pulses were used, there was a decrease of 61.51% (EP1), an increase of 1.7% (EP2), and a decrease of 92.87% (EP3) compared to turmeric alone treatment. This shows the efficacy of synergetic effects in reducing the cell viabilities of MDA-MB-231 cells.

Based on the results, a combination of pepper and electrical pulses (EP3) was more effective in treating MDA-MB-231 breast cancers. The lowest viability of 5.08% with the highest cell death rate of 94.92% was observed by electro-pepper-treatment on triple-negative cells. Treatments that caused the highest to lowest cell death are arranged in the following order:

(Pepper + EP3) > (Neem + EP3) > (Turmeric + EP3) > EP3 > (Turmeric + EP1) > (Neem + EP1) > (Pepper + EP1) > EP1 > Pepper > Neem > Turmeric > (Neem + EP2) > (Turmeric + EP2) > (Pepper + EP2) > EP2.

DISCUSSION

Any drug or therapy that kills cancerous cells has to be tested on healthy cells too. This helps in assessing the safety of drug/therapy on normal cells. Hence, different human breast cancer cell lines were chosen and checked for their effectiveness. Based on the results, it can be said that the MCF-7 cells and MDA MB 231 cells were more sensitive for the treatments than MCF-10A cells as depicted in Figure 6.

Higher cell death was achieved for synergetic treatments than single treatments for all extracts. Further, low-intensity high duration EP3, with herbal extracts yielded higher cell death on MCF-7 and MDA-MB-231 cells, without sparing normal cells. Treatments with neem extract alone exhibited the lowest cell viability of 96.52% for MCF-7 cell line, whereas for MDA-MB-231, cell viability of 85.10% was observed and for MCF-10A, the figure was 100.01%. Further, with turmeric extracts, cell viability of 98.80% (MCF-7), 89.50% (MDA-MB-231) and 103.64% (MCF-10A) was observed. For pepper extract treatments, 99.27% (MCF-7), 81.24% (MDA-MB-231) and 96.80% (MCF-10A) cells survived. These results clearly show that the herbal extracts target more MDA-MB-231 cells than MCF-7 and MCF-10A cells. Herbal treatments show a potent cytotoxic effect on cancerous cells, without damaging normal cells and, hence, these treatments are much safer than using conventional synthetic drugs.

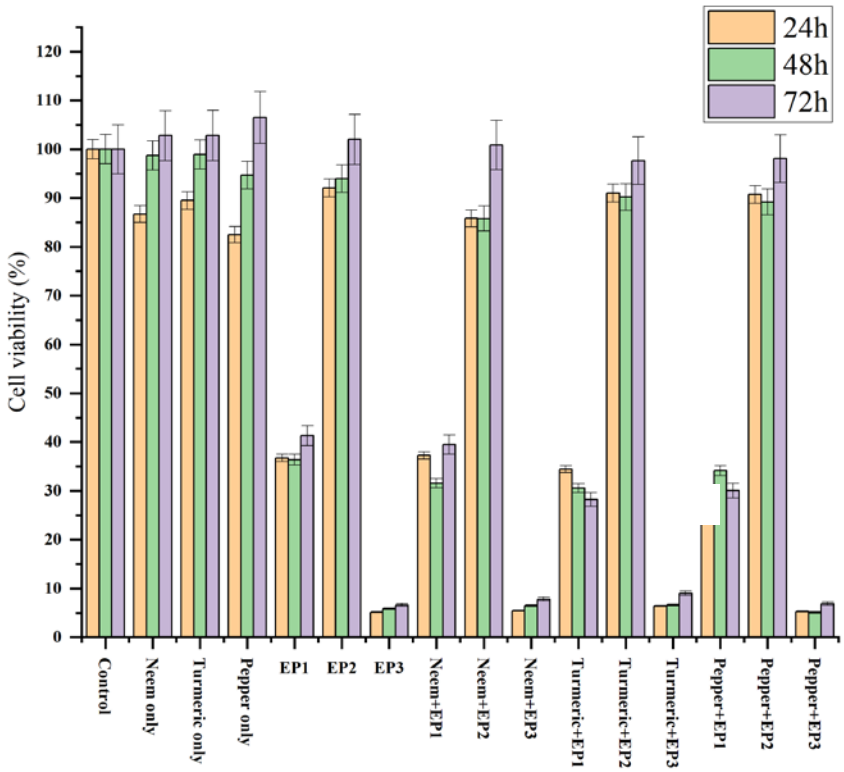

Figure 5. Viabilities of *MDA-MB-231* cell lines after treatments for different time intervals. **P*<0.05 significantly different from control.

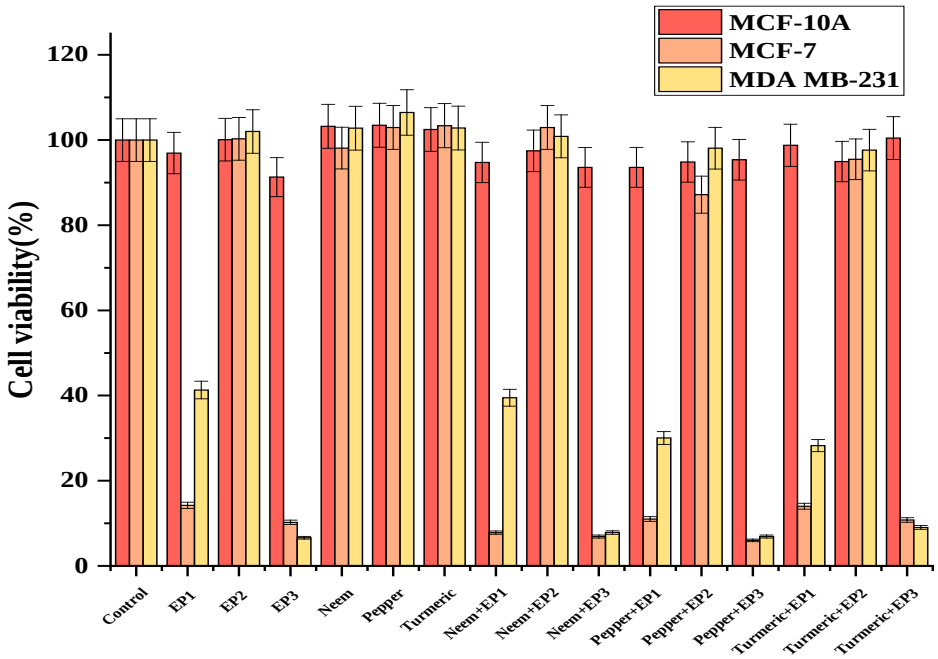

Figure 6. Effect of treatments on cell viabilities of breast cell lines (MCF-10A, MCF-7, MDA-MB-231) after 72 hours of treatment

A higher percentage of cell death was observed in MCF7 and MDA MB-231 than in MCF10A cells for all the treatments, indicating the treatments to be safer as they did not cause much damage to healthy cells. These results are in line with previous studies regarding the effect of with turmeric, neem, and pepper.[16,17,24,25]

Breast cancer cell lines under different pulse conditions exhibited different cell viabilities over different intervals of time. Under the pulse conditions of 500 V/cm, 100 µs, 99.27% of MCF-7 cells survived, while under 1200 V/cm, 100 µs, viability reduced to 13.25%, which further reduced to 9.37% for 500 V/cm, 20 ms. For MDA-MB-231cells, viability was about 92.04% for 500 V/cm, 100 µs, which reduced to 35.83% for 1200V/cm, 100 µs, and a further drop to 5.12% for 500 V/cm, 20ms was observed. These results indicate that more energy is required to permeabilize the aggressive TNBC cells than MCF-7 cells. This could be due to the phenotype difference among the cells.[10] Further, low intensity, higher duration electrical pulses (500 V/cm, 20ms) exhibited the lowest cell viabilities than other treatments. This could be due to the differences in sensitivities of the cells to the electrical pulses, based on their phenotype, morphology, and other physiological and biological characteristics. The obtained results corroborate the results of previous research.[10]

The use of electrical pulses did not cause more cell death in normal cells, unlike cancerous cells. This may be due to the fact that cancer cells exhibit different electrochemical properties and different electrical charge distribution compared to normal cells. Further, membrane potential plays a major role as healthy cells have a membrane potential of about −60 to −100mV, whereas the membrane potential of cancer cells falls around -15mV only, which is less than the value for healthy cells.[26] An increase in cell viability from 12h to 72h was observed in certain cases which may be due to the continuous proliferation of a few leftover live cancer cells. Hence, the impacts observed on viabilities of MDA-MB-231 and MCF7 cells are much larger and time sustained when compared to non-cancerous MCF10A cells as discussed in the work of Lakshya *et al.*[27]

Also, results (Figure 6) indicate that more energy was required to permeabilize the aggressive TNBC cells than MCF7 cells. This could be due to the phenotype difference among the cells. Further, low intensity, higher duration electrical pulses (500V/cm, 20ms) exhibited the lowest cell viabilities than other treatments. This could be due to the differences in cell sensitivities to the electrical pulses, based on their phenotype, morphology, and other physiological and biological characteristics. The obtained results corroborate the results of previous research.[10]

Viability as low as 4% was obtained with the synergetic treatment of electrical pulses and herbal extracts. A notable increase in cell death, compared to single treatments, indicates enhanced cytotoxicity caused by increased uptake of herbal extracts due to applied electrical pulses. These results corroborate previous work on turmeric, pepper, tulsi, mint and neem extracts.[27-29,31] It can be seen that synergetic treatments of extracts with different electrical parameters cause more cell death in cancerous cells than noncancerous cells. Hence, these treatments may overcome drug-resistant tumors. Also, desirable cell

death can be achieved by appropriately selecting EP parameters for a fixed dosage.

Further, considering the cost and side-effects caused by using conventional drugs, natural herbal extracts can be opted instead. The increasing cost of conventional treatments and the lack of effective drugs to cure breast cancers have drawn attention to the use of medicinal plants. This is because herbal medicines are natural and no significant side effects are observed compared to synthetic drugs. Previously, when cisplatin (a commonly used drug to treat breast cancer) was used to treat MCF7, viabilities varied from 34.5% to 74.5% for different concentrations. For a constant dose of 62.5 µg/mL, viabilities of 60.4% were observed.[32] But viabilities as low as 4% can be observed by using electro herbal treatment and, hence, there was about a 92% decrease in cells survival compared to cisplatin treatment as indicated in Figure 7. Hence, treatment with natural extracts can be chosen to avoid the side effects associated with the usage of synthetic drugs.

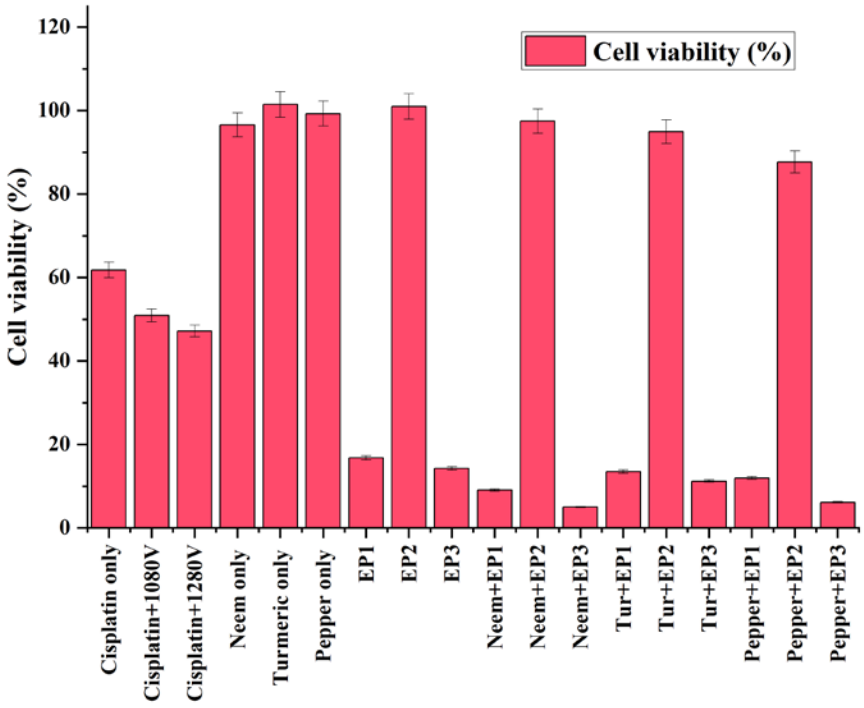

Figure 7. Comparison of cell viabilities with conventional drug (cisplatin) after 24 hours of treatment

CONCLUSION

Breast cancer is a major problem among females. ER-positive receptor breast cancer is the most commonly occurring breast cancer. Further, triple-negative breast cancers are hard to treat due to the lack of three receptors and hence a majority of treatments fail. In this study, the feasibility of herbal extracts and electroporation as alternate therapy was explored. To do this, herbal extracts (Turmeric, Pepper, and Neem) at a concentration of 62.5µg/mL were used in combination with electrical pulses against MCF-10A, MCF-7, and MDA-MB-231 cell lines. Various electrical pulses of different intensity and pulsed duration were used to enhance the uptake of extracts against the cell membrane.

Results indicate that a combination of herbal extracts and electrical pulses is more effective for treating breast cancer. This treatment did not affect the non-cancerous cells and targeted only the breast cancer cells which is the main advantage of our technique. Low intensity, high duration electrical pulses in combination with herbal extracts showed a higher cytotoxic effect than other treatments. Instead of using expensive synthetic drugs and treatments, our technique can be implemented for an efficient outcome without any associated side effects. Optimal efficiency and desired cell death could be achieved by optimizing the parameters of applied electrical pulses. This treatment could be transferred to clinical practice as an alternate therapy against breast cancers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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