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# 1, 4 Benzoquinone Induced Apoptosis on MCF-7 Breast Cancer Cell Line

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**Abstract:- Drugs belonging Quinones group have been successfully explored for their anti-cancer activity (such as Adriamycin, mitoxantron, etc.) 1,4 Benzoquinone (BZQ) is an oxidative stress agent that belonging to this family. BZQ has been tested for its potential of anti-cancer in U2OS Osteosarcoma cell line but not MCF-7 Breast Cancer cell line. In the present study, the anti- cancer activity of BZQ on MCF-7 cancer cell lines were studied by MTT assay, Trypan blue assay and morphological analysis. The results showed that BZQ induced MCF-7 cell death. Further analysis showed that the type of the death is apoptosis which is time-dependent and dose-dependent. Further studies using antioxidants suggest the mechanism to induce apoptosis is by disrupting the antioxidant system. This results support the capacity of BZQ as a potent anti-cancer drug in therapeutic regimen.**

***Keywords:-*** *Cancer, MCF-7, 1,4-Benzoquinone, Oxidative Stress.*

## I. INTRODUCTION

Cancer is an abnormal growth of cell due to mutation at the DNA, according to World health organization (WHO) cancer is included in the top 6 of worldwide death causes.[1] By data, breast cancer has one of the biggest mortality rate with nearly 627.000 number of death per year.[2] Breast cancer is classified as adenocarcinoma, cancer that is originated from epithelial cells which produce mucus, and will start to enlarge and be a tumor. The effect of this tumor can lead to organ, nerve, endocrine and circulation problem.[1]

Some of the difference between cancer cell and healthy cell is the free radical and antioxidant regulation. Compared to healthy cell, cancer is more vulnerable to damage from disturbance in free radical and antioxidant balance. This instability would result oxidative stress that would induce apoptosis through several mechanism.[3, 4] The management of cancer can use several method, the main ones are through surgery, radiotherapy and chemotherapy.[1] But there is still challenges in the management of cancer such as high side effect, improve able efficacy and the growing resistance to cancer treatment. This situation encourages researcher to find and develop new drugs for cancer. Some of the drug has already used oxidative stress inducing mechanism as a way to induce cell death the example is Pertuzumab, Atezolizumab and Doxorubicin.[5] Therefore finding new drug which mechanism is inducing oxidative stress have a great potential to be explored.

One of stress inducing agent that has been reviewed as anticancer agent comes from quinone group. Quinone can be natural or synthesis molecule that contributes in the transfer of electron at photosynthesis.[6] The ability of quinone in inducing oxidative stress is caused by its capacity to make hydroquinone and semiquinone which act radically and helped by enzyme which can activate redox cycle in producing ROS.[7, 8] Quinone also have the ability to induce inflammation, anticancer and toxicity.[9]

Through the reasons listed above, the potential of quinone group to become a new candidate in cancer management is high. This research will focus on analyzing the death of breast cancer cell MCF-7 by using 1,4-benzoquinon. MCF-7 is used because of its ability to retain most characteristic of breast epithelium, easy to spread and have low growth rate.[10]

## II. MATERIAL AND METHODS

This research was conducted through lab examination by creating culture cell with 70% confluence for control examination and for variable variation, where every variable examination was given Benzoquinone with distinct amount of concentration, 25μM, 50μM, and 100μM respectively. Then, all cells was observed during specified amount of time interval, 3 hours, 6 hours, 8 hours, and 24 hours.

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Methods conducted on this research are:

- Trypan Blue assay
- MTT Assay
- Morphological Analysis

- *Cell lines:*

- MCF-7-Human Breast Carcinoma Cell Line (From Sigma aldrich)

- *Materials*

- 1,4 Benzoquinone (From Sigma aldrich)
- GSH (From Sigma aldrich)
- NAC (From Sigma aldrich)

## III. RESULTS

The lab results are given below on these following table. The value of the entries on these tables are the percentage of the dead cell on every culture cell tested by using Trypan blue assay.

Based on the experiment, the results infers that the cell death on medium cells (or control cells) percentage are increased as the time passes by.

Based on lab experiment on treatment cells, the increment of Benzoquinone concentration will lead to increment of cell death percentage. The cell death percentage also increases for the time being.

The data will be visualized on the following charts and the lab examination evidence will be presented.

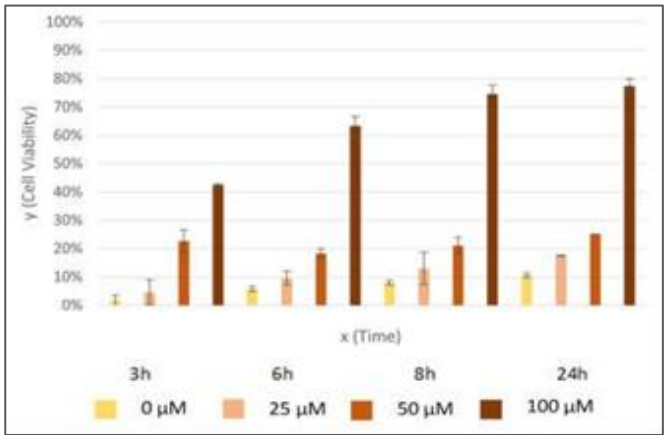


Fig 1:- BZQ-Induced Apoptosis on MCF-7 Cell Line. (Time Dependent)

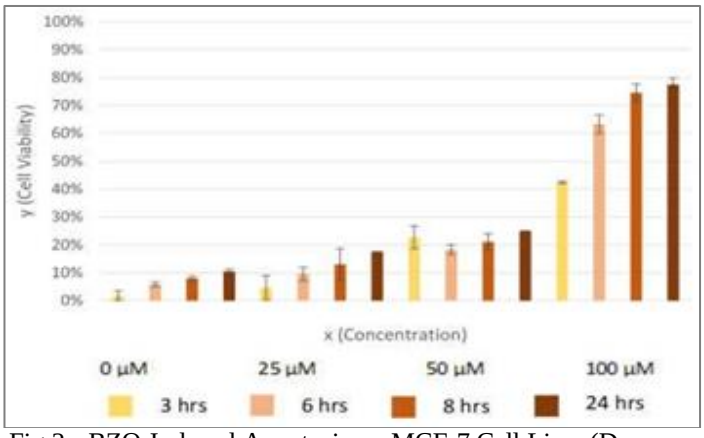


Fig 2:- BZQ-Induced Apoptosis on MCF-7 Cell Line. (Dose-Dependent)


Fig 3:- 3 Hours Lab Examination Results


Fig 4:- 6 Hours Lab Examination Results


Fig 5:- 8 Hours Lab Examination Results


Fig 6:- 24 Hours Lab Examination Results

Result of Trypan blue assay is tested statistically using t-test, P value lower than 0,05 means it have significant result. So the value start to become significant at 3 horus on 50 µM dose, means it is the early dose that benzoquinone starts to be effective at the least time.

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| | Control vs 25 μM | Control vs 50 μM | Control vs 100 μM |
|---|---|---|---|
| 3 h | 0.3141 | 0.03921 | 0.01105 |
| 6 h | 0.1745 | 0.02227 | 0.01377 |
| 8 h | 0.2662 | 0.05865 | 0.01065 |
| 24h | 0.03358 | 0.0177 | 0.006782 |

Table 1:- T-test result

Examination using microscope founds apoptotic body and shrinkage of cell, that suggest the mechanism of cell death is apoptosis.

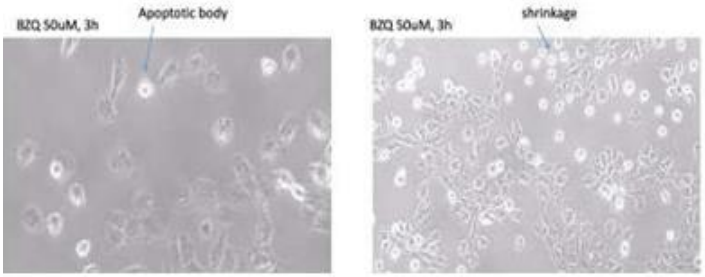


Fig 7:- Apoptotic vs Shrinkage 3 hours comparison

After doing some experiments on Benzoquinone death rate, there are additional lab examination to identify the mechanism of Benzoquinone to eradicate cancer cells. There are 6 control cells, and grouped into three groups, each consists of 2 cells. Each group are given different treatments, one acts as normal control, one acts as NAC-injected control, and one acts as GSH- injected control. And three other group was given 50μM concentration of Benzoquinone during 3 hours (Chosen because the dose have started been significant in the earliest time), grouped by the same method of its control cells. Tested using trypan blue assay.

The result shows that both NAC and GSH acts as resistance layer for Benzoquinone, as indicated on the table that the percentage is lower than the medium 50μM normal Benzoquinone. Averagely, GSH injected MCF-7 show the least value of cell death percentage, compared to the NAC-induced and Benzoquinone treated cell, where Anti-Oxidants prevent cell death.

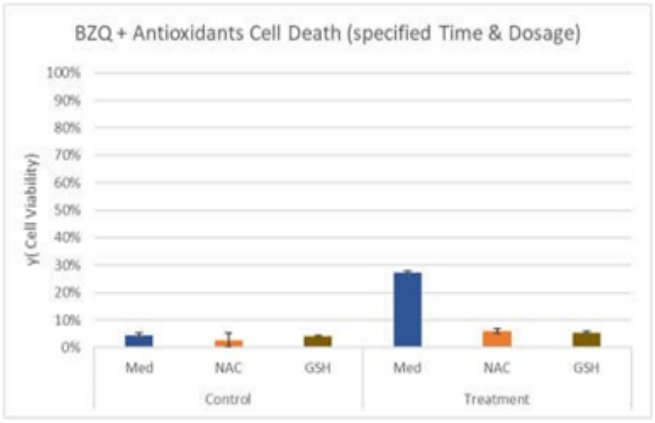


Fig 8:- Comparison of BZQ vs Non-BZQ Cells

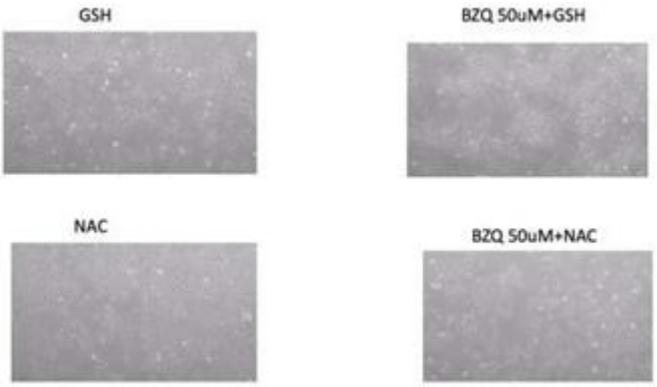


Fig 9:- BZQ + Anti-Oxidants 3 hours results

The following table shows the experiment result of MTT Assay Test, MTT Assay is based on the reduction of MTT (3-(4,5- dimethyl thiazolyl)-2,5- diphenyl-tetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product.

From the experiment, the average absorbance result for Benzoquinone and Control are 0.0913 and 518.1402, respectively. This means that the most of MTT indicators are being absorbed, leaving a small leftovers, thus making the value is really small compared to its control.

The following chart compares % of Cell viability on all of these 3 cells.

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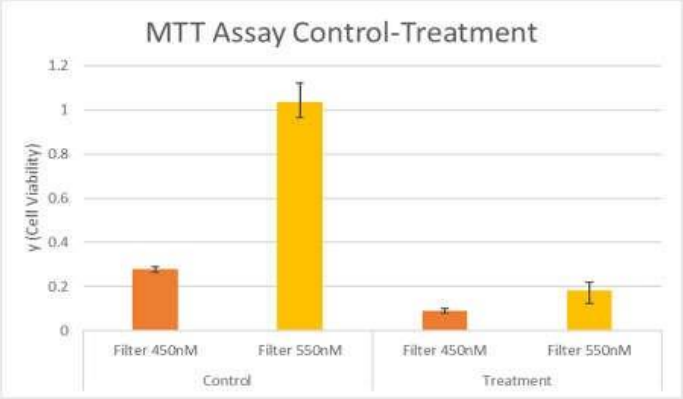

Fig 10:- Comparison of Cell Viability Percentage

From Fig. 7, the percentage of cell viability on Benzoquinone-injected cells is small compared by its control. This evidence also supports the previous arguments that most of MTT indicators are being absorbed. This implies that if the cell viability has significantly small value indicate the reduced metabolism of MCF-7 cancer cell

## IV. DISCUSSION

The wide range of therapeutic and pharmacological potential of quinones was revealed in several previous studies. 1,4-Benzoquinone one of the main types of quinone have never been tested on its anti-cancer potential, and this matter will be assessed. The anti- proliferative effect of 1,4-benzoquinone were evident from the results obtained from MTT assay. Number of viable cells was found to be decreasing. Morphological changes in extract treated cells were examined and compared with control cells using microscope. The number of cell death was assessed from the results of trypan blue assay. All these data reflects the anti-cancer potential of 1,4-benzoquinone. Testing using antioxidants as a combination therapy also suggest that the mechanism of death is induced by interfering with antioxidant system in cancer cells.

## V. CONCLUSIONS

The results of this study support the efficiency 1,4 Benzoquinone as an anti-cancer agent for MCF-7 cancer cell line. The MTT assay, trypan blue and morphological analysis of the effect of 1,4 Benzoquinone justifies scientifically, this experiment is paving the way for future studies on 1,4-Benzoquinone and can put forth the possibility of formulating effective anti-cancer drug in therapeutic regimen.

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