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## **Effect of x- ray on the treatment of breast cancer combined with amygdalin and doxorubicin separately**

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**Abstract---Background:** Radiation therapy has the ability to destroy healthy cells in addition to cancer cells in the area being treated. However, when radiation combines with doxorubicin, it becomes more effective on breast cancer treatment. **Objective:** This study aims to clarify the effect of X-ray from LINAC combined with amygdalin and doxorubicin on breast cancer treatment, and the possibility of using amygdalin with X-ray instead of doxorubicin for the breast cancer treatment. **Method:** Two cell lines were used in this study, the first one was MCF-7 cell line and second one was WRL- 68 normal cell line. These cells were preserved in liquid nitrogen, prepared, developed and tested in the (place). The effect of three x-ray doses combined with amygdalin and with doxorubicin was studied on these strains. **Results:** Combination of radiation with amygdalin and with doxorubicin, separately, exam revealed no statistically significant difference between x-rays doses (1Gy, 3Gy and 5 Gy) combined with amygdalin and x-rays doses (1Gy, 3Gy and 5 Gy) combined with doxorubicin for MCF-7 and WRL-68. **In conclusion:** there is possible to be considered amygdalin as a promise breast cancer treatment instead of doxorubicin combined with x-ray.

**Keywords---**Radiotherapy, x-ray, MCF-7, Amygdalin, Doxorubicin.

## Introduction

Cancer is a common disease in the world where some cells of the human body grow out of control, they are divided faster than normal cells and not organized, and can spread to all parts of the body. Cancer cells can strike anywhere in the body, which consists of a very large number of cells, amounting to trillions of cells (1). Human cells grow and multiply by a process called the cell division to produce new cells depending on the needs of the human body. When cells aged or damaged, they die and are replaced by new cells.

Breast cancer is a disease in which the cells of the breast tissue changes and divides in out of control, usually resulting in a lump or mass. Most breast cancers start in the milk glands or in the ducts between the milk glands and the nipple (2). Breast cancer treatment includes various medical methods, the most common of which are radiotherapy, surgical as well as chemotherapy, in addition to many methods that are often used simultaneously with radiation to achieve a synergistic effect such as chemicals (doxorubicin) also such as the use of natural products including amygdalin (3).

Electromagnetic ionizing radiation "X- ray from a linear accelerator or gamma rays" causes several changes within cells that can lead to the initiation or promotion of cancer in the affected area, and it is also believed that the effects of ionizing radiation on non-target cells may lead increase in the risk of cancer (4). Although radiotherapy is one of the most common procedures for treating a wide range of tumors including breast cancer and the main target is killing cancer cells by damaging the DNA of the cells, the radioresistance of cancer cells remains a major limitation in the application of radiotherapy procedures (5). Double strand break of DNA in the cell by X-ray and gamma ray are the deadliest that damage cellular DNA completely, whereas if the damaged part of the cells is successfully repaired, the cancer cells will survive (6). Radiation therapy has the ability to destroy healthy cells in addition to cancer cells in the area being treated. However, Radiation is more effective at killing cancer cells, but it has less of an effect on healthy cells, which are able to survive the treatment and repair themselves and therefore it is difficult for cancer cells repairing the damage caused by radiation.

Doxorubicin is widely regarded as one of the most effective treatments for breast cancer, and it is considered one of the most active cytotoxic agents for breast cancer, though the concern about congestive cardiomyopathy requires a cumulative dose must be limited to minimize the risk of its toxicity (7). It is common knowledge that doses of chemotherapeutic agents lower than standard dosing can activate adaptive cellular response mechanisms, which can lead to the development of acquired drug resistance (8). According to a number of studies, the neoplastic carcinoma cells remaining in the patient after chemotherapy possess distinct properties that promote their survival and proliferation for new tumor formation (9).

Amygdalin is a naturally occurring disaccharide as a source of HCN; it's particularly presented in the seeds of many fruits such as bitter almonds, apricot and peach (10). Amygdalin consists of two glucose molecules, benzaldehyde and

hydrogen cyanide and can be as two R and S epimers, where R-amygdalin is a natural amygdalin and S-amygdalin is called neoamygdalin (11). Amygdalin induces apoptosis through regulation of Bax and Bcl-2 expressions in human DU145 and LNCaP prostate cancer cells (12).

Amygdalin has been demonstrated to inhibit MCF-7 growth by increasing sensitivity to the effects of oxidative stress *in vitro*. In addition, amygdalin inhibited the proliferation of the MCF-7 cell line. This inhibition of tumor cell expansion may be due to the variation in susceptibility to oxidative stress. Therefore, the mechanism of the effect of amygdalin on breast cancer cells is mainly by the motivation of oxidative stress (13).

One researcher reported that amygdalin stimulates apoptosis and prevents the adhesion of breast cancer cells, and the results indicate the possibility of applying amygdalin as a promise chemotherapy helping to prevent or mitigate the development of breast cancer (14). In addition, the mechanism of DXR is to promote apoptosis, as DXR leads to DNA damage that also leads to cell cycle arrest (15). At the same time, low doses of doxorubicin with less cytotoxicity could be an efficient chemotherapy for breast cancer when used in combination with X-ray or gamma rays (16). Therefore, the main objective of this study is to clarify the effect of X-ray from LINAC combined with amygdalin and doxorubicin on breast cancer, and the possibility of using amygdalin with X-ray instead of doxorubicin for the breast cancer treatment.

## **Material and Method**

### **Cell lines in the study**

Two cell lines were used in this study, the first one was MCF7 cell line and second one was WRL 68 normal cell line. These cells were preserved in liquid nitrogen, prepared, developed and tested in the Malaya university/Facuality of medicine/pharmacology Department, Malaysia - kualalampur. The effect of x-ray, amygdalin and doxorubicin was studied on these strains. Exposing the MCF-7 cell line and the WRL-68 normal cell line to three X-ray doses with the plant extract (amygdalin) and with the chemical doxorubicin in a mixed form, separately, then reading the effects after 24 hours.

After the creation of breast cancer MCF-7 cell line and WRL-68 normal cell line. These cell lines will be transferred to the bowl implant after shaking gently to Beaker sterile. Using a micropipette, 200ul of cells stuck were placed in holes drilled plate 96-multi well (so containing each hole to approximately  $3 \times 10^4$  cells / hole). The plates were covered tightly with a transparent adhesive, and then the plates were incubated for 24 hours in an incubator equipped with 5% CO<sub>2</sub> at a temperature of 37 C until a single layer of cells was obtained in each hole.

The next step (After the incubation period of the plates culture), the culture medium was withdrawn from each hole using a micropipette. The cells will be exposed to the plant extract (Amygdalin) at a concentration of 100 µg/ml, as well as the chemical doxorubicin at a concentration of 20 µM/mL, which was prepared according to paragraph (2-2-8) (Fruehauf et al., 1993) and with three repetitions

for each dilution, whereby (3 plates) containing the cell lines are prepared. At the same these cells will be exposed to three x-ray doses from LINAC instrument, separately.

### **Irradiation of samples method**

After adding the plant extract (Amygdalin) and chemical material (Doxorubicin), the samples of irradiation were performed directly exposed to X-rays using medical linear accelerator instrument (LINAC) Elekta Synergy 3630 (Elekta Instrument AB, Stockholm, Sweden) with power of 6 MV, the distance of irradiation was 50 cm from the window and the depth of the exposed was 10 mm. Then the irradiation of the samples begins by exposing the first sample to 1Gy, the second sample to 3Gy and the third sample to 5Gy

### **Reading method**

These plates are incubated directly in the incubator and under appropriate conditions for 24 hours. After 24 hours, the plates were taken out of the incubator. Six wells containing MCF 7 cells and six wells containing WRL-68 cells to be controlled by adding only the serum-free medium (2-4-1-9) without exposed. Other wells will expose by an amygdalin with x-ray, and by a doxorubicin with x-ray, separately.

The contents of each well were withdrawn and 10 µl of MTT dye was added to each hole. Then the plate was incubated in the incubator for 4 hours. The dye is removed by washing the plates with tap water several times to get rid of the excess dye.

One hundred µl of the DMSO solute solution was added to each well and incubated for 5 min. The absorbance will read using an ELISA device (Scanning multiwell spectrophotometer reader) at a wavelength of 940 nm for adherent cells in the well, and then the percentage of cell inhibition was estimated by measurement with the control (Freshney et al., 2000). The Inhibitory rate will calculate according to state in ((Batncur- Glavis et al., 1999) by converting the reading of the absorbance of the optical density in the ELISA device into percentages according to the following equation: -

$$\text{Inhibition rate} = \frac{\text{O.D (control)} - \text{O.D (sample)}}{\text{O.D(control)}} \times 100$$

O.D represents Optical density

### **Statistical analysis**

Statistical analyses were done using SPSS for windows (IBM, inc) version 22. Statistical analysis was carried out on absorbance readings to calculating IC50. The differences among treated of MCF-7 and among treated of WRL-68, separately, and differences between MCF-7 and WRL-68 were analyzed using paired t-test. Mean and Standard Deviation were reported and p value of significant was equal or greater than 0.05.

## Result

The results of the MTT test showed that X-rays showed activity against MCF-7 cells, which caused a decrease in their viability after exposure that amounted to 24.27, 33.65 and 47.32% at doses (1, 3 and 5 Gy), respectively. In addition, the decrease in the percentage of vitality of cells exposed to the three doses of x-rays with amygdalin was 48.11, 54.06 and 59.9%, respectively, while the decrease in the percentage of vitality of cells exposed to the three doses of X-rays with doxorubicin was 50.39, 60.69 and 65.86 %, respectively.

The results MCF-7 showed that there was a decrease in the survival ratios of cells exposed to 1 Gy of X-rays alone, 1 Gy of X-rays with amygdalin and 1 Gy of X-rays with doxorubicin, which were 24.27, 48.11 and 50.39%, respectively. When exposed to 3 Gy of X-ray alone, 3 Gy with amygdalin, and 3G with doxorubicin, the percentages of decreases in cell viability were 33.65, 54.06, and 60.69%, respectively. By increasing the X-ray dose to 5 Gy, the percentages decreased to 47.32, 59.9 and 65.86%, respectively, as shown in Table (4-1) and Figure (4-1).

Table (4-1): Effect of different doses of X-ray, amygdalin and doxorubicin on the viability of WRL-68 and MCF-7 cells using MTT assay for a period of 24 hours exposure and at a temperature of 37 °C

Treatments	WRL-68			MCF-7		
	1 Gy	3 Gy	5 Gy	1 Gy	3 Gy	5 Gy
Radiation	75.54 ± 3.7 a, A	67.11 ± 6.93 a, B	52.35 ± 3.38 a, C	75.73 ± 2.93 a, A	66.35 ± 5.36 a, B	52.68 ± 4.88 a, C
Rad. + Amygdalin 100 µg/mL	69.59 ± 6.77 a, A	52.52 ± 4.33 b, B	45.15 ± 5.29 b, C	51.89 ± 5.65 b, A	45.94 ± 5.43 b, A	40.1 ± 1.8 b, B
Rad. + DOXO 20 µM/mL	68.24 ± 7.97 a, A	59.99 ± 7.19 ab, A	43.61 ± 7.82 b, B	49.61 ± 3.09 b, A	39.31 ± 3.81 b, B	34.14 ± 5.49 b, B

Different small letters (a, b) in the column are considered significant at  $p \leq 0.05$ , SD: standard deviation. Different small letters (A, B) are considered in the row significant at  $p \leq 0.05$ .

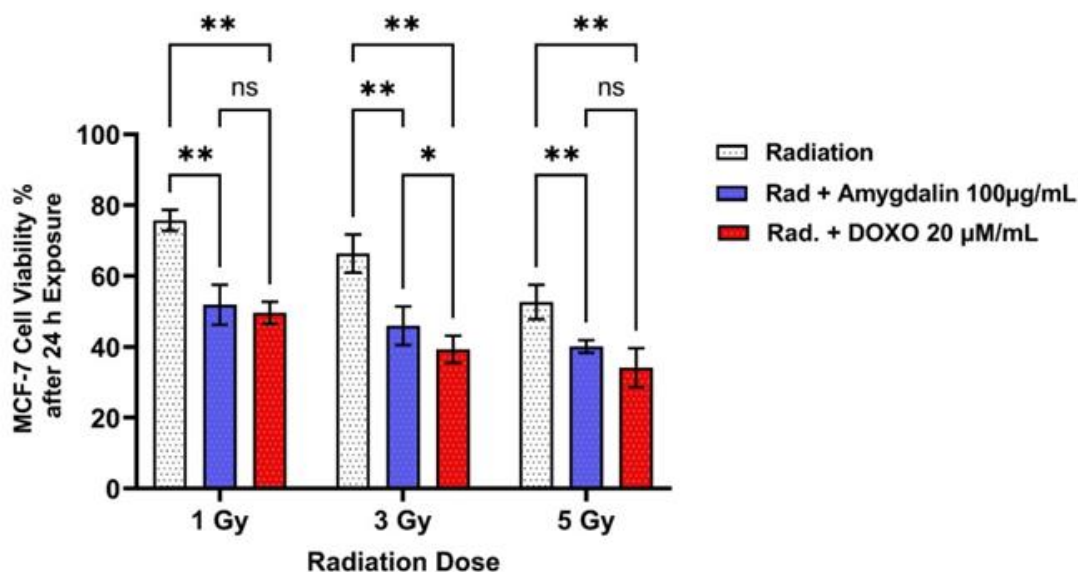


Figure (4-1): The effect of statistically different doses of X-ray, amygdalin and doxorubicin on the viability of MCF-7 cells using MTT assay for a period of exposure of 24 hours at a temperature of 37 °C.

At the same time, it can be seen that there was a decrease in the percentage of viability of WRL-68 cells, as the cells were inhibited by 24.46, 32.89, and 47.65 with doses (1, 3 and 5 Gy) Gy, respectively, while the decrease in the percentage of vitality of cells exposed to the three doses of X-ray was with amygdalin 30.41, 47.48 and 54.85 in doses, respectively. The decrease in the percentages of viability of cells exposed to the three doses of x-rays with doxorubicin was 31.76, 40.01 and 56.39, respectively.

In addition, the results show a decrease in the percentage of viability of WRL-68 cells exposed to 1 Gy from X-ray alone, also with amygdalin, and doxorubicin at percentages of 24.46, 30.41 and 31.76, respectively. The decrease in the percentages of viability of cells exposed to 3 Gy from X-rays alone, also with amygdalin and with doxorubicin was 32.89, 47.48 and 40.01, respectively, and the decrease in the percentages of viability of cells exposed to a 5 Gy dose of X-rays alone, also with amygdalin and with doxorubicin was 47.65, 54.85 and 56.39, respectively, as shown in Table (4-1) and Figure (4-2).

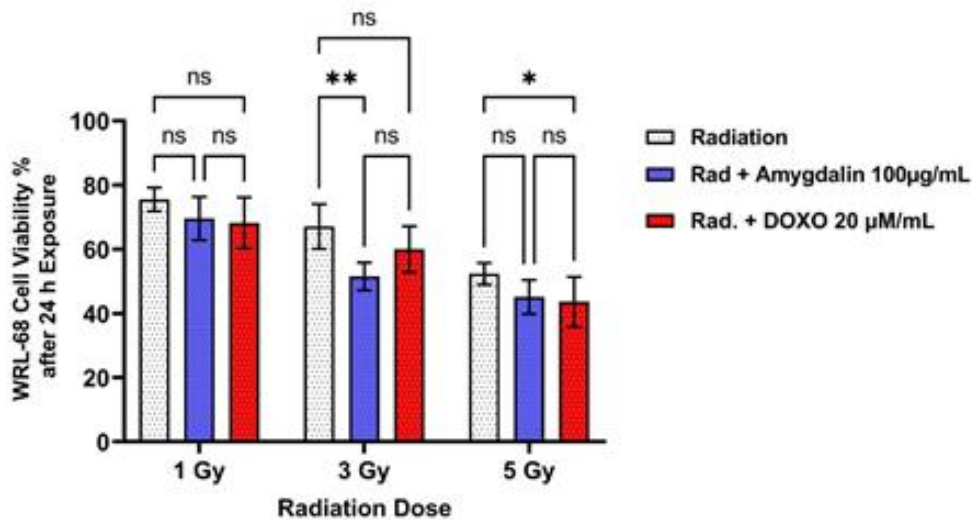


Figure (4-1): The effect of statistically different doses of X-ray, amygdalin and doxorubicin on the viability of WRL-68 cells using MTT assay for a period of exposure of 24 hours at a temperature of 37 °C

#### Summary of a statistical comparison of the percentages of decrease in the vitality of MCF-7 cells after exposure to the three doses of X-ray, amygdalin and doxorubicin

For MCF-7, there were no significant differences between 1Gy of X-rays with amygdalin and 1Gy of X-rays with doxorubicin, while the difference was 1Gy of X-rays with amygdalin and 3 Gy and 5Gy of X-rays with doxorubicin there was It is a significant difference  $P < 0.0004$  and  $P < 0.0001$ , respectively. There was no significant difference between 3Gy X-rays with amygdalin and 1Gy and 3Gy X-rays with doxorubicin, while the difference was significant between 3gy X-rays with amygdalin and 5Gy X-rays with doxorubicin. In addition, the difference between 5Gy X-rays with amygdalin and 1Gy X-rays with doxorubicin was significant ( $P < 0.0009$ ), while there was no significant difference between 5Gy X-rays with amygdalin and 3 Gy and 5 Gy X-rays with doxorubicin as shown in Table (4-2).

Table (4-2): a summary of the comparison of MCF-7 cell viability after exposure to radiation, amygdalin and doxorubicin for a period of 24 hours at a temperature of 37 °C

Treatments	Rad. 1 Gy + Amygdalin 100 µg/mL	Rad. 3 Gy + Amygdalin 100 µg/mL	Rad. 5 Gy + Amygdalin 100 µg/mL
Rad. 1 Gy + DOXO 20 µM/mL	0.7336 NS	0.3906 NS	0.0009 **
Rad. 3 Gy + DOXO 20 µM/mL	0.0004 **	0.0533 NS	0.9686 NS

Rad. 5 Gy + DOXO 20 $\mu$ M/mL	<0.0001 **	0.0007 **	0.326 NS
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It was also found in WRL-68 cells that there were no significant differences between 1Gy and 3Gy x-rays with amygdalin and 1Gy and 3Gy x-rays with doxorubicin, while there was a significant difference between 1Gy x-rays with amygdalin and 5Gy x-rays with doxorubicin ( $P < 0.0001$ ). There was a significant difference ( $P < 0.0014$ ) between 3 Gy of X-rays with amygdalin and 1Gy of X-rays with doxorubicin, while there was no significant difference between 3Gy of X-rays with amygdalin and 3 Gy and 5 Gy of X-rays with doxorubicin. In addition, the difference between 5Gy x-rays with amygdalin and 1-Gy and 3Gy x-rays with doxorubicin had significant difference ( $P < 0.0001$ ) and ( $P < 0.005$ ), respectively), While there is no significant difference between 5Gy x-rays with amygdalin and 5Gy x-rays with doxorubicin. As shown in Table (4-3).

Table (4-3): a summary of the comparison of WRL-68 cell viability after exposure after exposure to radiation, amygdalin and doxorubicin for a period of 24 hours at a temperature of 37 C

Treatments	Rad. 1 Gy + Amygdalin 100 $\mu$ g/mL	Rad. 3 Gy + Amygdalin 100 $\mu$ g/mL	Rad. 5 Gy + Amygdalin 100 $\mu$ g/mL
Rad. 1 Gy + DOXO 20 $\mu$ M/mL	0.9789 NS	0.0014 **	<0.0001 **
Rad. 3 Gy + DOXO 20 $\mu$ M/mL	0.0927 NS	0.1192 NS	0.0050 **
Rad. 5 Gy + DOXO 20 $\mu$ M/mL	<0.0001 **	0.1536 NS	0.9653 NS

## Discussion

Although X-rays and doxorubicin are effective treatments, as well as amygdalin promising and effective, the clinical advantages of radiotherapy and chemotherapy - as with treatments for cancer cells - have a number of side effects such as toxicity (through free radicals and oxidative stress). As well as resistance of cancer cells to radiotherapy and chemotherapy. Free radicals are continually generated from oxygen by a variety of cellular metabolic systems. Therefore, mitochondria use up to eighty percent of the molecular oxygen present, and five percent of this oxygen is converted to superoxide and hydroxyl radical (17). As a result, free radicals play an important role in the normal metabolic processes of cells. However, its presence poses significant risks, especially for large molecules such as nucleic acids and proteins, which are more susceptible to damage when oxygenated free radicals are present (18).

Oxidative stress is defined as a physiological condition in which the metabolism of antioxidants generates reactive oxygen species (ROS) and free radicals (19). In addition to the production of ROS and free radicals, regular cellular metabolism plays an important role in cell signaling pathways (20). Several studies suggest that oxidative stress may have an essential link with human pathophysiological disorders (21). Specifically, from It is well established that oxidative stress

damages the DNA molecule, modulates signaling pathways, and regulates the growth of many malignancies, including breast, lung and liver cancers.<sup>22</sup> In addition, it has been claimed that the entire DNA molecule can bind to hydroxyl radicals, resulting in to damage the deoxygenated pentaglyceride, including purine and pyrimidine bases. During these destructive processes, 8-OH deoxyguanosine (8-OHdG) may be generated, which may significantly increase the risk of mutations (23).

Various studies have shown the effects of radiation, doxorubicin, and amygdalin separately on breast cancer cells (24-25), but the controversy in this case is whether amygdalin can be used as an alternative to doxorubicin with X-rays in the treatment of breast cancer. The results of our study revealed a decrease in the percentage of viability of MCF-7 and WRL-68 cells due to exposing them to X-rays, indicating an increase in the percentage of cell death (Table 1 and Figure 1 and 2). This may be because ionizing radiation directly hitting the DNA generates charged particles that carry the energy provided by the photons, causing the phosphodiester bonds to break. This accounts for up to 30 percent of DNA damage (26). The rest of the damage is caused by the activity of free radicals. • OH, which is of great biological importance, results from the interaction of ionizing radiation (such as X-rays or gamma rays) with a water molecule, a process known as "radiolysis" of water. The deposition of radioactive energy results in hydrogen atoms, fluidized electrons, and other chemical by-products. These include molecular hydrogen, hydrogen peroxide, and peroxytrite, which form DNA-damaging chemicals, causing double-stranded DNA breaks and preventing repair of damaged DNA (27). As mentioned earlier, free radicals are among the main carcinogens.

Studies have indicated that radiation generates free radicals • OH which is closely related to cell death under hypoxic conditions and • NO appears to act as a radiosensitizer by mimicking the effects of oxygen on radiation-induced DNA damage (28). This is consistent with our findings, where cell death occurs either through direct effect, causing filament breakage or indirect effect through generation of free radicals. Several studies have shown that doxorubicin affects cancer cells in two ways: directly, by breaking down DNA strands and preventing their repair by topoisomerase-II, and indirectly, where doxorubicin creates free radicals that destroy cell membranes, DNA, and proteins (29).

In addition, doxorubicin is oxidized to the unstable semiquinone metabolite, which is further converted to doxorubicin during the production of reactive oxygen species and this can cause lipid oxidation, membrane damage, DNA damage, oxidative stress, and apoptosis (30). Recent research has shown that several natural compounds, including amygdalin, inhibit cancer cell proliferation and proliferation, and induce apoptosis of cancer cells, which may warrant future clinical research in breast cancer (31). Our study showed that exposure to doxorubicin combined with X-ray and amygdalin combined with X-ray reduced viability of MCF-7 and WRL-68 cells, indicating an increase in cell death (Table 1 and Figure 1 and 2). Therefore, when x-rays and doxorubicin or x-rays and amygdalin are combined, the effect on breast cancer cells increases and the rate of cell death accelerates.

In this study, Tables 2 and 3 show that there are no significant differences between the effects of X-ray exposure (1Gy) combined with amygdalin and X-ray exposure (1Gy) combined with doxorubicin on the viability of MCF-7 or WRL-68 cells. Also, there were no significant differences between exposure to x-rays (3Gy) with amygdalin and doxorubicin, as well as to x-rays (5Gy) with amygdalin and doxorubicin. Accordingly, the mechanism of effect of amygdalin and doxorubicin is to activate apoptosis, which leads to DNA damage and this was confirmed by (32). Aghaee, F., et al. (2013) also reported that combination therapy offers the advantage of reducing treatment side effects than high therapeutic doses of medication or radiation alone (33).

Therefore, it is possible to treat breast cancer with amygdalin instead of doxorubicin with x-rays. It is worth noting that there are no previous studies dealing with the use of radiation with amygdalin (at the same time) as an effective treatment for breast cancer. It is therefore necessary to carry out extensive studies, including different types of cancer. In addition, based on our findings, we recommend combining different concentrations of amygdalin with different doses of X-rays in order to obtain more accurate data.

## **Conclusion**

The results show x-rays from LINAC combined with doxorubicin or with amygdalin have a high significance statistically on MCF-7 and WRL-68 cell viability. There is no significant difference between x-ray combined with doxorubicin when comparison with x-rays combined with amygdalin on MCF-7 and WRL-68 cell viability. Therefore, it is possible to be considered amygdalin as a promise breast cancer treatment instead of doxorubicin combined with x-ray.

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