Saikosaponin B3 potentiates doxorubicin-induced apoptosis in modulating Akt and GSK3-β in breast cancer cell lines

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EXAMIANTION COMMITTEE

We hereby recommend that the dissertation prepared under our supervision by Quratulain Malik (NUST201463163MSMME62414F). Titled: Saikosaponin B3 potentiates doxorubicin-induced apoptosis by modulating Akt and GSK3-β in breast cancer cell lines be accepted in partial fulfillment of the requirements for the award of Biomedical Science degree with (A grade).

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ABSTRACT

Resistance is intrinsic to cancer but as therapy becomes effective, acquired resistance has become common. Drug resistance is often associated to decreased levels of caspase-3. PTEN acts as a tumor suppressor and inhibits PTEN from phosphorylating Akt which further promotes cell proliferation. Resistance sustains due to insensitivity of cancerous cells to chemotherapeutic agents. Studies on mechanisms of cancer drug resistance have yielded important information about how to circumvent this resistance to improve cancer chemotherapy and have implications for pharmacokinetics of many commonly used drugs. Our results suggest that combination of doxorubicin and saikosaponin can be used as combination to treat cancerous cells effectively and increased activity of caspase-3 can promote cell apoptosis result and result in recovering cells from tumorigenesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

SB3 Saikosaponin B3

Doxo Doxorubicin

WHO World Health Organization

% Percentage

CHAPTER 1

INTRODUCTION

Doxorubicin, an anthracycline drug which is reported effective in various types of cancer mainly in breast cancer. The favorable therapeutic response of doxorubicin is often associated with severe toxicity. Doxorubicin most desired therapeutic response is seldom associated with severe toxicity. A major challenge facing chemotherapy of solid tumors is the limited efficacy and selectivity of cytotoxic drugs. Doxorubicin is an anthracycline drug, along with other drugs of same nature are used on a wide scale in the treatment of breast cancer [1]. Acquired resistance against chemotherapeutic drugs is a major problem in cancer cases. Mechanism of the resistance acquired varies along with the type of cancer under study and is mostly undefined at the moment. Studies have suggested that failure of the cells to carry out apoptosis plays a supporting role in this [2]. Apoptosis may be triggered by an external or internal stimuli or any chemical change from inside the body [3]. Process is followed by the activation of variety of special group of proteins called Caspases [4, 5]. Caspases when activated induce certain biochemical and physical changes [6]. Caspase-3 plays a key role in this process [7-11]. Caspase-3 mutation is reported in case of MCF-7 breast cancer cells, this happens due to a mutation in exon 3 of cells [12]. Aggravated levels of caspase-3 in MCF-7 cells shows that it plays a facilitating role in pathways such as death-receptor pathway and mitochondria led apoptotic-pathways [12-15] .

Saikosaponin B3 is a bioactive phytochemical of Bupleurum marginatum and B. falcatum. Saikosaponin is known for its pharmacological properties such as anti inflammatory activity and anti- oxidant activity [16]. These features of saikosaponin gained attention and it was considered for cancer treatment. Chinese and Japanese also use it as a common medicinal plant, and are also registered in WHO [17, 18].

Studies are conducted to study the role of saikosaponin in regulating the anti oxidant activity. The mechanism of action varies with the nature of problem. This research was conducted to study the effect of saikosaponin B3 in breast cancer cells. For this purpose, anti cancer and anti oxidant properties were regulated to treat breast cancer and to understand the mechanism of action.

Breast cancer is dealt as a global health concern for a long time now, especially in the western countries. MCF-7 cell lines were bought from KRL hospital Islamabad. MCF-7 is basically a breast cancer cell lines. In the present study, we investigated the role of doxorubicin-induced apoptosis by modulating Akt and GSK3-β. It depends on the growth of malignant cells, which have the tendency to spread to other parts of body. MCF-7 model is well established for research of anti-estrogen therapy, but it was found equally suitable in this case of cell model [19]. In this study, cell lines were cultured in RPMI medium and were given low dose of doxorubicin to activate drug resistance mechanism. Cell lines used were non- adherent, therefore the process involves suspension cells. Akt pathway plays a vital role in cell survival and proliferation, and is found deregulated in a various types of cancers. This pathway is under numerous researches due to its role in the

activation of kinase inhibitors which are further involved in controlling tumor growth [20]. GSK3β is a substrate of Akt. When GSK3β is phosphorylated, the activity of GSK3β is blocked. This happens due to GSK3β pro-apoptotic nature, blocking the activity of GSK3β is expected to dampen apoptosis [1].

Research Objectives:

- Breast cancer is considered a global issue due to its high rate of occurrence in developing countries. Causes of the disease vary from person to person which includes race, hormonal levels and physical factors. Variety of studies explains the relationship between breast cancer and hormonal imbalance.
- The purpose of the study conducted was to study the anti-oxidant and anti cancer activity of saikosaponin B3. The drug resistance mechanism was first activated by low dose levels of doxorubicin, and then saikosaponoin B3 was administered to recover the cell from drug resistance.
- Anti-oxidant and anti-cancer properties were studied with the help of changes in Akt pathway.
- The activity of proteins which contributed to cell apoptosis and facilitated anti-cancer and anti-oxidant activities were highlighted.

Chapter 2

2. LITERATURE REVIEW

2.1. Breast cancer:

Breast cancer is among the most recurrent type of cancers in women worldwide. Despite of other factors, that may trigger initiation of breast cancer, family history is one of the prominent factors seen in majority of cases [21]. Progression of cancer goes through several stages, starting with hyperpoliferation which later transforms into in situ carcinomas and eventually into metastatic form of disease [22, 23]. The process of development of breast cancer follows a succession of events in a cell, which also involves genetic and epigenetic events [21].

Previously, to understand how disease progresses in human body molecular analysis of invasive breast cancer was done. Various types of analyzation techniques have been brought to use to characterize the lesions formed as a result of breast cancer. As a result of this analysis, a definite relationship among genetic and physiological factors has been reported. The analysis also catered various pathways that play a supporting role in the progression of this disease [24]. MCF-7 is a breast cancer cell line, reported to be deficient in caspase-3 and shows selective sensitivity to several components possessing

chemotherapeutic properties. The decreased level of caspase-3 was found to be associated with resistance against drugs [25].

Breast cancer is linked to multiple causes such as germline mutations which is seen in almost 5-10 percent of cases. Research revealed that over 130 mutations in germline are noticed in case of breast cancer. A study was conducted to investigate based on 208 cases diagnosed with breast cancer lying within age group of 21-44 years. Analysis of the frequency achieved through this study showed a decreasing trend with age of diagnosis. 23.1 percent of cases were reported till the age of 30 whereas 3.4 percent of cases were reported between 40-44 years. 192 out of 208 cases under investigation showed 12 BRCA1 mutations. Just like the relation between age of diagnosis and disease occurrence, younger aged cases showed relatively high frequency of mutations as compared to the ones who were relatively more aged. The results showed considerable difference in the figure: 11.4 percent of mutations found in the cases with younger age group such as below 30 years whereas 4.7 percent were reported beyond this age group. The variation in the frequency was found to have a strong relationship with history of the family.

On investigation of ovarian cancer family history BRCA1 mutation again aroused as a triggering factor in promoting disease. This also yields an important relationship between chance of occurrence of disease and patient with a close family history such as mother, sister or an aunt or grandmother who had a BRCA1 mutation. For the age group with ages within age group thirty, two out of eight women with a close family history of ovarian cancer such as an aunt or grandmother suffering from breast cancer had been detected with brca1 mutation. As the number of acquaintances grows in a group of case, the frequencies with which mutations appear tend to increase manifold. Similarly, the number of mutations tends to descend if the subject has no close relation affected with breast cancer. This phenomenon was seen both in case of ovarian and breast cancer. Research shortlisted the most common mutation at molecular level, which were seen in majority of cases [26].

Heritable variation in case of breast cancer which involves BRCA1 and 2 are mainly responsible for exposing women to risk of disease. The proportion or tendency of suffering from disease was also seen in early phase of women's life. Mutations also result due to the deletion of any specific sequence or a pair of sequence which makes up DNA sequence. In this case guanine and adenine were reported as mutated but this only

accounts for one percent of the population. On average, mutations in BRCA1 were more commonly reported and diagnosed than that of BRCA2. Therefore, mutations in both of these cases were analyzed critically to underline the major causative factor that triggers the development of disease in all those subjects. Results supported the idea that hereditary variations in BRCA2 constitute a very minute population of patients, so this can be concluded that this gene does not play a major role in causing the disease. Whereas, presence of such variants which contribute or in short promote these genes can eventually add to the risk of causing disease early in life [27].

Mutations of these genes are on average seen in half of the cases reported with family history of disease [28-33]. Deletions caused by BRCA2 mutations reported in the previous research point the fingers at the proteins responsible for it. Apart from proteins another determining factor is the location of the deletion [27].

Akt pathway plays a vital role in cell survival and proliferation, and is found deregulated in various types of cancers. This pathway is under numerous researches due to its role in the activation of kinase inhibitors which are further involved in controlling tumor growth [20]. GSK3β is a substrate of Akt. When GSK3β is phosphorylated, the activity of GSK3β is blocked. This happens due to GSK3β pro-apoptotic nature, blocking the

activity of GSK3β is expected to dampen apoptosis [1]. Akt pathway is reported deregulated in numerous types of cancers and it plays a major role in cell proliferation which tends to promote cancer development.

2.2. Therapeutic potential of Doxorubicin:

The favorable therapeutic response of doxorubicin is often associated with severe toxicity. Doxorubicin most desired therapeutic response is seldom associated with severe toxicity. A major challenge facing chemotherapy of solid tumors is the limited efficacy and selectivity of cytotoxic drugs. Doxorubicin is an anthracycline drug, along with other drugs of same nature are used on a wide scale in the treatment of breast cancer [1].

Acquired resistance against chemotherapeutic drugs is a major problem in cancer cases. Mechanism of the resistance acquired varies along with the type of cancer under study and is mostly undefined at the start. Studies have suggested that failure of the cells to carry out apoptosis plays a supporting role in this [2]. Process is followed by the activation of variety of special group of proteins called Caspases [4, 5]. Caspase-3 mutation is reported in case of MCF-7 breast cancer cells, this happens due to a mutation in exon 3 of cells [12]. Aggravated levels of caspase-3 in MCF-7 cells shows that it plays

a facilitating role in pathways such as death-receptor pathway and mitochondria led apoptotic-pathways [12-15].

2.3. Anti-inflammatory activity of saikosaponin B3:

Saikosaponin B3 is a bioactive phytochemical of Bupleurum marginatum and B. falcatum. Saikosaponin is known for its pharmacological properties such as anti inflammatory activity and anti- oxidant activity [16]. These features of saikosaponin gained attention and it was considered for cancer treatment. Chinese and Japanese use this as a medicinal plant and is registered in WHO. This researched focused on the anti-inflammatory and anti-cancer properties of saikosaponin B3.

2.4. Anti-oxidant activity of saikosaponin:

Saikosaponin B3 is responsible for the activation of enzymes involved in carrying out antioxidant activities. Researchers revealed a negative side of oxygen species; where aggravated level of heat stress triggers oxidative damage in kidneys. Saikosaponin D is extracted from the same source i.e Bupleurum falcatum plant from where saikosaponoin B3 is derived helps in protecting organs from any sort of damage. When mechanism of action was studied, research revealed the role of Saikosaponin D in down regulating the oxidative damage [34].

CHAPTER 3

MATERIALS AND METHOD

3.1. Chemicals and reagents

10mg of Doxorubicin hydrochloride was bought from Shifa International Hospital, chemotherapy department (product catalogue# L01D B01).

3.2.Cell culturing

Media containing 10% fetal bovine serum (FBS) and 1% penicillin was kept at 37^{o} C in water bath for half an hour. Cells were then transferred with the help of pipette to 5ml of media contained in a falcon tube. Centrifugation of these cells was done at 10,000 rpm for 5 minutes. After removing the supernatant media cells were incubated at room temperature for full growth. Later, these cells were washed with 1X PBS for couple of times. 150 ml of RIPA buffer was added and cells were centrifuged at 10,000 rpm for 10 minutes. After removing the supernatant the cells were vortexed for 10 minutes and then placed in ice. This process is known as thaw, was repeated six times for 10 minutes each after vortexing each sample for two minutes.

3.3.Study Design:

Besides culturing the breast cancer cells, these cells were administered with low dose (5µM) of doxorubicin at regular intervals. Due to the consistent dose plan of doxorubicin, cells developed resistance against the given drug. Cells were later treated with 20µM saikosaponin B3 which is a phytochemical. Ultimately, combination of drugs was given

to overcome the acquired drug resistance. The combined effect of drugs was studied through pathways of Akt and GSK3β. The study formulated can be understood through following diagram:

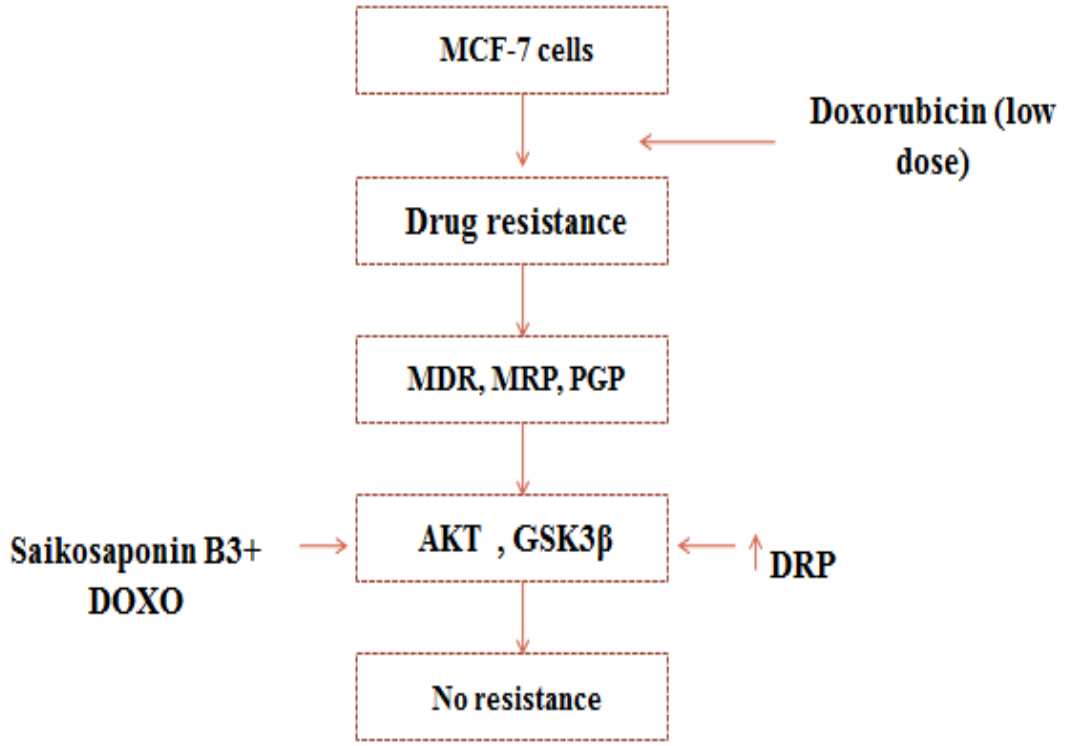

Figure 1: Schematic representation of mechanism of action of Saikosaponin B3 and doxorubicin.

3.4.Sample groups for study:

The experiment was split into four sample groups named as control which was left untreated, second named as doxorubicin in which cell lines were administered with

doxorubicin. Subsequently the third one was named as saikosaponin B3 where the cell lines were treated with saikosaponin and the last sample group was given a combination of drugs.

Table 1: Experimental design: Control group consisted of untreated cancerous cells. Doxorubicin group contained cell lines treated with doxorubicin, whereas saikosaponin B3 group was treated with saikosaponin B3 and these drugs were given as a combination for treatment of combination group.

Serial No	Sample groups	Details	Time
1.	Control	Control (MCF-7 cells), Untreated	24 hours
2.	Doxorubicin	Cell lines treated with Doxorubicin	24 hours
3.	Saikosaponin B3(SB3)	Cell lines treated with Saikosaponin B3	24 hours
4.	Doxorubicin+ Saikosaponin B3	Cell lines treated with Doxorubicin and Saikosaponin B3	24 hours

3.4.1. Doxorubicin– treated cell line:

Cancerous cell lines were treated with 5µM of doxorubicin at regular intervals of one hour for twenty four hours. The low dose of doxorubicin was planned due to its highly

toxic nature. The solution was prepared by diluting 5.8mg of drug per ml of water. Doxorubicin is a commercially available chemotherapeutic drug, commercially known as Adriamycin. It was given at regular intervals in low dose to activate drug resistance mechanism. The impact of drug was noticed by the change in the levels of cellular proteins involved in Akt pathway. Akt pathway plays major role in cell proliferation and survival and is found deregulated in variety of cancers. Studies suggest that Akt pathway is activated at early phase in case of breast cancer.

3.4.2. Saikosaponin B3- treated cell lines:

One of the sample group containing cancerous cell lines was treated with 20µM of saikosaponin B3 per ml. The solution was made by diluting 10mM of drug per ml of water. Saikosaponin B3 is a phytochemical. The administration of this drug was stretched over a period of twenty four hours. Drug possesses strong anti-oxidant and anti-cancer properties. Research was mainly designed to evaluate the effect of saikosaponin B3 in breast cancer cell lines.

3.4.3. Combination of drugs:

This group was treated with a combination of drugs including doxorubicin and saikosaponin B3 in their desired concentrations mentioned in the study design. The purpose of combination was to recover the cells from acquired drug resistance and to successfully treat those cells. Resistance against doxorubicin was induced to check whether saikosaponin B3 alone can produce a significant effect in triggering apoptosis of cancerous cells which could possibly be masked in case of combined effect of the drugs.

Because then it would be nearly impossible to distinguish whether doxorubicin or saikosaponin B3 played essential role.

3.5. MTT Assay

MTT is a cell viability assay, which is done to determine the count of living or dead cells. This is a standard practice done in research to study the impact of any chemical (drugs in this case). Cells were cultivated in 6-well plates with each partition containing a separate sample. This research includes four sample groups: DC (control), DD (doxorubicin), DT (saikosaponin B3) and DDT (combination of drugs). The process involves use of a chemical which shows different colors in case of dead and living cells. The change in color can be detected at the desired wavelength by spectrophotometer. Degree of absorption varies with the solvent.

3.6. Protein Extraction:

Cell lines were preserved in WiseCryo at -80^{o} C and proteins were extracted by using RIPA buffer. The solution was homogenized by vigorous pipetting. Samples were centrifuged for ten minutes, which resulted in cell pellet (cell debris) and media. Media was separated by using micropipette. The cell line used during research was a suspension cell line therefore majority of protein were supposed to be present in supernatant. The proteins were collected in eppendorf and stored in refrigerator.

3.7. Protein Quantification:

Process was carried on by making samples and pouring them into cuvettes. Prior to experimentation, these cuvettes were sterilized using 70% ethanol to ensure de-colorization. The process known as Bradford protein assay is done to quantify the concentrations of protein. Bovine serum albumin (BSA) was used as a standard to prepare samples which were analyzed by using UV spectrophotometer. The experiment measures the varying change in absorbance with the changes in concentration of samples. Cuvettes were loaded with 1ml of sample each time. Due to light sensitive nature of BSA, the experiment was done in a controlled environment with minimal to no light exposure. Once the samples are ready, they were vortexed to experience the color change due to Bradford reagent. After vortexing the samples were kept in incubator for 5-10 minutes at room temperature. Data was plotted across an optimal wavelength of 595nm, among percentage cell viability and absorbance. Initially, a blank was run which consisted of plain water and Bradford reagent with no trace of sample. Same procedure was repeated for sample groups such as control, doxorubicin, saikosaponin B3 and combination (Doxo+SB3).

Table 2: Varying concentrations of BSA samples and Sample groups along with their activity recorded at 595nm.

BSA+sample concentrations	Water	Bradford reagent	Total volume	Absorbance
0µl	800 µl	200 µl	1000 µl	0
20 µl	780 µl	200 µl	1000 µl	0.0421
40 µl	760 µl	200 µl	1000 µl	0.0860
60 µl	740 µl	200 µl	1000 µl	0.1986
80 µl	720 µl	200 µl	1000 µl	0.2000
100 µl	700 µl	200 µl	1000 µl	0.2354
Control 2 µl	798 µl	200 µl	1000 µl	0.96
Doxorubicin 2 µl	798 µl	200 µl	1000 µl	1.24
Saikosaponin B3 2 µl	798 µl	200 µl	1000 µl	0.92
Combination(doxorubicin+ saikosaponin B3) 2 µl	798 µl	200 µl	1000 µl	0.95

The experimentation renders a curve along with corresponding absorbance values. Further, the loading values are derived out of them. For the given case, the values of samples (control, doxorubicin, saikosaponin B3 and combination) turned out to be 2.59, 2.01, 2.71 and 2.62 respectively. These values were derived through a slope equation for given curve obtained and is written as $y=0.025x - 0.0044$. The standard curve can be seen below.

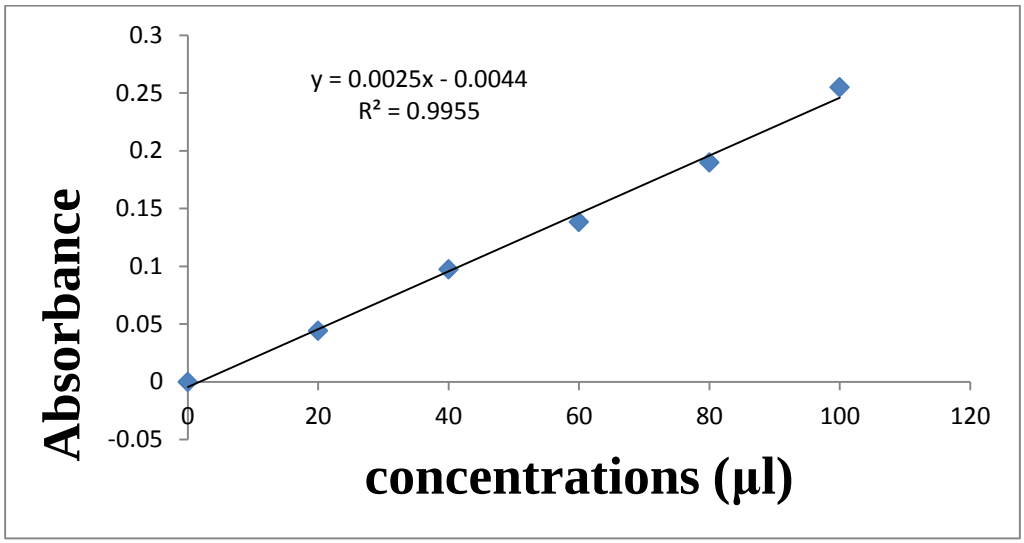

Figure 2: Curve obtained by using BSA as a standard.

3.8. Western blot:

3.8.1. Gel preparation

Western blot also known as immune-blotting was done to identify specific proteins. 12% separation gel was prepared based on the size of proteins to be detected. Prior to loading it between the plates, it was given a run on the vortex to facilitate mixing. Isopropanol was used to avoid any air bubbles within gel. Gel took almost 20- 25 minutes to solidify. Once the gel is set, isopropanol was removed from the top. In the meanwhile, stacking gel of 5% was prepared and was loaded at the top of separating gel after vortexing. Gel comb is placed on top of it, this helps to form wells. Gel is solidified in around 10 minutes.

Samples were prepared according to the values obtained through Bradford assay. Samples contained 4X buffer and distilled water (which is optional in case of cell lines because this will further dilute the samples).

Table 3: calculations for samples loading in gel

Samples	absorbance	2ug	1ug	50ug loading	Water	4X buffer	total
control	0.96	38.56	19.28	2.59	3.41	1	7
doxorubicin	1.24	49.76	24.88	2.01	3.99	1	7
Saikosaponin B3	0.92	36.96	18.48	2.71	3.29	1	7
combination	0.95	38.16	19.08	2.62	3.38	1	7

Absorbance values in the table above came from the Bradford assay and are further used to calculate the values of samples which will be loaded on gel. The values of absorbance are replaced in place of y for each sample and subsequent values of loading for each sample were obtained.

50µg= Desired amount loaded divided by the value of the given sample in 1 µg concentration.

3.8.2. Sample preparation

Samples were prepared by adding 4X buffer and protein sample. These samples when ready were kept in hot water bath for 10 minutes to facilitate protein denaturation. Samples were again centrifuged to avoid formation of any membranes. Samples are loaded onto gel after removing the comb. 1X running buffer was added into the tank after

setting the plates in assembly. In case of two gels running side by side, the tank is filled upto the mark of two. Wells are loaded in the manner of following order:

4X buffer	Protein marker	Sample 1	Sample 2	Sample 3	Sample 4

3.8.3. Running gel

Gel is set to run for 120 minutes at 90 volts. If the gel runs successfully, you can see the air bubbles once the electric supply is provided. After running, the gel is transferred on nitrocellulose membranes known as NC membranes. The assembly was prepared in the following manner: initially, the sponges were dipped in the transfer buffer properly. Between the sponges filter papers were placed on both sides. On top of filter paper, gel is placed carefully. At this point make sure the gel is hydrated, do not let it dry. NC membrane should cover the gel. Cassettes were closed and were transferred to the transferring assembly and the tank was filled with transfer buffer this time. The transfer is set to run for 25 minutes at 90 volts, to get excellent results the position was swapped after 25 minutes. After completion of transfer, NC was shifted to a box containing ponceau solution to visualize the bands.

Figure 3: NC membranes when exposed to ponceau solution shows visible band patterns.

3.8.4. Transferring bands on NC membrane

Further the NC membrane was washed in distilled water and cut according to the molecular weights of the proteins to be detected. Protein marker shows the gradual levels of proteins. After this pieces of NC membrane were paced in 5% non-fat milk for two hours. This process is known as blocking, this blocks the undesired binding of proteins with antibodies. Once the time is over, membranes were shifted into petri plates containing primary antibodies such as β-actin, caspase-3 and Akt. These antibodies were obtained from Santa Cruz Biotechnology (SCBT), Inc. After an exposure of over 16-18 hours to primary antibody, membranes were washed with TBST for almost an hour. Then these membranes were treated with secondary antibody for good 2-4 hours. After this

membranes were again washed with TBST. Later to visualize the bands, substrate named as “NBT detection substrate” was poured on the membranes which made the bands visible.

CHAPTER 4

RESULTS

3.9. Cell viability Assay results:

MTT is a cell viability assay, which is done to determine the count of living or dead cells. The effect of doxorubicin and saikosaponin on cell viability was studied. Initially, low dose of doxorubicin was given to activate drug resistance against it. Doxorubicin is declared a very effective drug in cancer treatment. The purpose of induced resistance was to check the effectiveness of saikosaponin B3. Due to the activation of drug resistance, doxorubicin did not produce considerable impact on cell viability. But saikosaponin was found to produce significant results that can be seen in the figure below. Whereas, combination of drugs showed drastically significant results as compared to when drugs were individually administered. The main purpose of combination was to recover the cells from resistance and to treat the cells at the same time. When the drugs produced by the combination of drugs were compared with the results produced by doxorubicin alone, they showed significance.

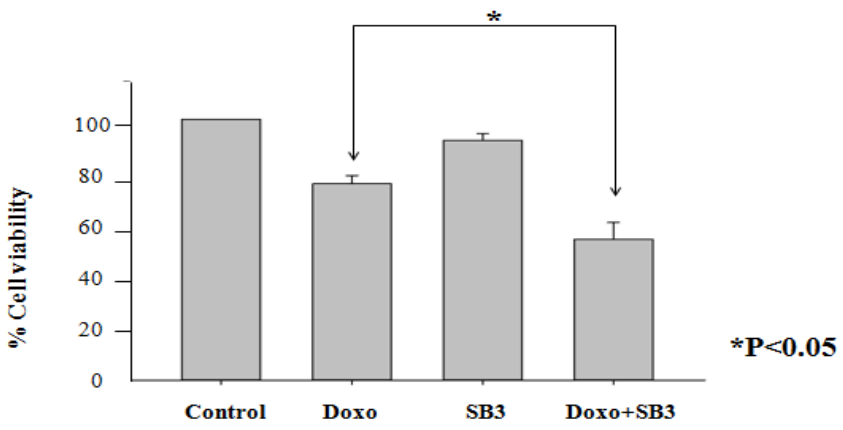

Figure 4: Effect of drugs on cell viability.

3.10. Effect of Doxorubicin and Saikosaponin-B3 on MCF-7 cell lines:

Akt pathway is reported deregulated in numerous types of cancers and it plays a major role in cell proliferation which tends to promote cancer development.

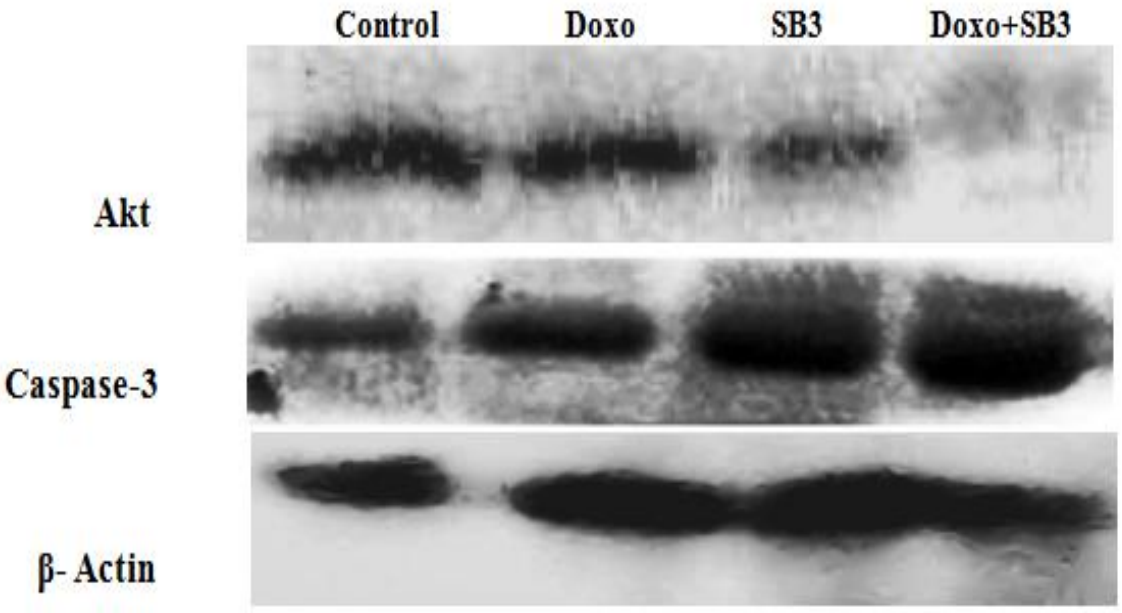

Figure 5: Western blot analysis of proteins

Results show β-actin activity in all groups. Whereas, caspase-3 showed increase activity in drug combination group. While Akt is active promoting cell proliferation in control group but in case of treatment groups its activity decreases considerably.

3.11. Effect on anti-oxidant activity:

Anti oxidant activity of the drugs were checked with the help of dpph. The solution had violet color which neutralizes as a result of reaction. The experiment was run against sample groups of doxorubicin, saikosaponin B3 and combination of drugs. Combination of drugs showed highest value among all the groups. According the protocol, results of these groups were compared to the result when the same protocol was done with ascorbic acid. Ascorbic acid showed significance in results apart from combination group.

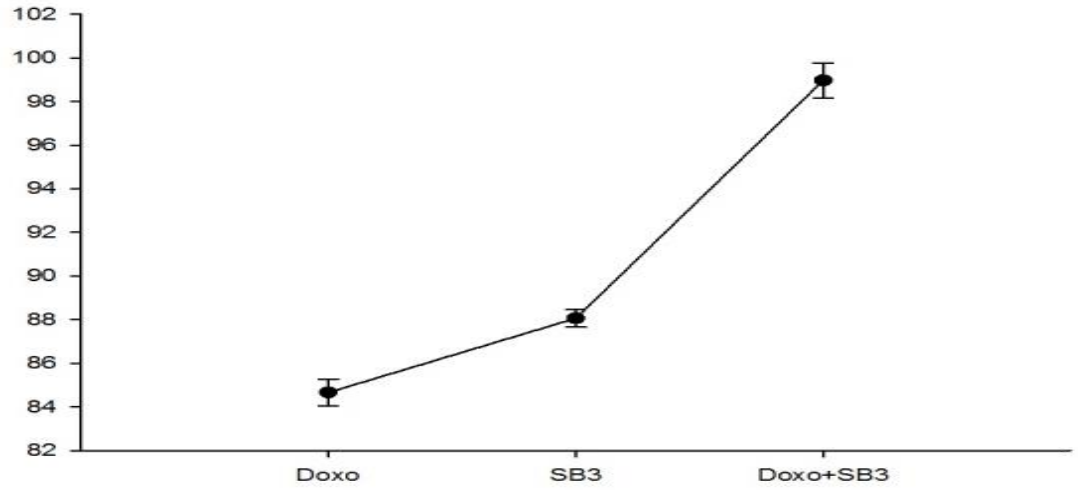

Figure 6: Anti-oxidant activity and its variance with drugs

CHAPTER 4

DISCUSSION

3.12. Increased Akt activity in Breast cancer:

Akt pathway plays major role in cell proliferation and cell survival and is often deregulated in majority of cases. This pathway is under numerous researches due to its role in the activation of kinase inhibitors which are further involved in controlling tumor growth [20]. Akt plays key role in cancer progress and has potential utility in prevention of cancer [21].This study was conducted to study the impact of doxorubicin and saikosaponin B3 in breast cancer cells. Due to its role in cell proliferation, Akt was found highly active in cancerous cells. PTEN opposes PI3K from phosphorylation of Akt, therefore acts as a tumor suppressor [22, 23]. Loss or mutation of PTEN results in the uncontrolled behavior of Akt, because PTEN inhibits PI3K which phosphorylates Akt. PTEN is reportedly mutated in majority of cancers up to a significantly high ratio of 83% in endometrial cancer with an average mutation rate of 16% [24]. Use of inhibitors for PI3K pathway in breast cancer can play an essential role in controlling the growth of disease. Loss of PTEN also play key role in progression of tumor cells [25]. Studies also correlate the loss of PTEN to activation of Rho family proteins, which results in increased invasion and migration of cells [20].

3.13. Increased Caspase-3 activity:

Caspase-3 is a key promoter of apoptosis, but is found deficient of in breast cancer cells. Studies suggest that decrease in levels of caspase-3 can contribute to resistance against chemotherapeutic drugs such as doxorubicin [26]. Caspase-3 mutation is reported in case of MCF-7 breast cancer cells, this happens due to a mutation in exon-3 of cells [12]. Aggravated levels of caspase-3 in MCF-7 cells shows that it plays a facilitating role in pathways such as death-receptor pathway and mitochondria led apoptotic-pathways [12-15]. Due to its pro-apoptotic nature results of study showed considerably high levels of caspase-3 in treated cell lines which refers to cells entering apoptotic stage successfully.

CONCLUSION

- Concomitant effect of combination of drugs successfully enhanced the anti-cancer activity.
- Combination of drugs was more potent in the activation of caspase-3.
- Drug resistance was reduced by the combination of drugs.

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