Fucoidan Extract Induces Apoptosis In MCF-7 Cell Line Via Bcl2 And Caspase3 Assay In An In-Vitro Condition

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Abstract: Fucoidan extract (FE) is a type of sulphated polysaccharide extracted from Turbinaria conoides, it plays a vital role in treating many severe ailments. In our study, FE extracted from Turbinaria conoides was used for analyzing the induction of apoptotic effect of MCF7 breast cancer cell line using the Bcl2 and Caspase3 assay and it was compared with the standard camptothecin by flow cytometry instrument. From our results it was found that FE induced apoptosis expressions against the MCF7 breast cancer cell line compared to the standard camptothecin. Thus our apoptotic results indicate that FE obtained from the Turbinaia conoides was found to have possible therapeutic activity in invitro condition against breast cancer cell line. It can be further analyzed to confirm its anticancer activity using the invivo studies.

keywords: Apoptosis, Bcl2, Caspase3 assay, MCF7, Programmed cell death.

1.INTRODUCTION
Breast cancer is one of the main cause of death according to the recent survey of American Cancer Society (2019) it is estimated that around 268,600 new cases of invasive breast cancer diagnosed in women; 2,670 cases diagnosed in men; and an additional 62,930 cases of in situ breast lesions (ductal carcinoma in situ [DCIS] or lobular carcinoma in situ [LCIS]) diagnosed in women. MCF7 is a type of breast cancer cell line isolated from metastatic cells of a 69 years old women as it was determined by Dr. Soule and his co-workers in the year 1937[1]. In recent research, it is found that breast cancer can be overcome by the natural sources that too, brown seaweeds are found to be a good source of anticancer. Fucoidan is a kind of sulphated polysaccharide which is present in most of the marine macroalgae and it is reported to have significant biological activities like antioxidant, Immunomodulatory, antivirus, antithrombotic and anticoagulant effects in them[2]. In this study, the apoptotic activity of the fucoidan extracted from turbinaria conoides against the breast cancer cell line MCF7 was analysed in invitro condition using Bcl2 and caspase assay. Apoptosis is a kind of natural cell death process which is otherwise called as programmed cell death. It is led by the flattening of the cytoplasm, shortening of the nucleus, cleavage of nucleosomal and DNA, all these changes lead to the complete destruction of the plasma membrane in the initial stage of apoptosis, and a shift in the internal and external phospholipid phosphatidylserine (PS).

Bcl2 is a type of gene that is present in the B-cells of lymphoma, that acts as regulatory protein of anti-apoptosis and caspase (Cysteine-aspartic proteases, cysteine aspartates or cysteine-dependent aspartate-directed proteases) is an enzyme belongs to the protease family which plays an important role in causing the apoptosis as well as inflammation. There are different types of cysteine proteases that are responsible for mammalian cancer. Caspase 2,8,9,10 are termed as initiator caspase, 3,6, and 7 respectively. Thus, in this study, MCF7 breast cancer cell line was exposed to FE induced to Bcl2 and caspase 3 assays to find its invitro apoptotic activity.

2.MATERIALS AND METHODOLOGY
Fucoidan Extracted from Turbinaria Conoides, MCF7-Human Breast Adenocarcinoma Cell line (NCCS, Pune), Cell culture medium, DMEM, Fetal Bovine Serum, D-PBS Himedia, PE Mouse Anti-Human Bcl-2, Camptothecin Sigma, 6 well cell culture plate.

2.1.SAMPLE COLLECTION & EXTRACTION
Turbinaria conoides a type of brown seaweed was collected from Mandapam coastal area and it was cleaned and air dried and it was further dried using hot air oven at 50° C and it was made into powder form and it was subjected to extraction procedure of soaked water method[3] was followed with slight modification[4].

2.2.Bcl2 Assay:
MCF7 breast cancer cells were cultured in a microtitre plate in a CO2 incubator at 37°C for 24 hours. The spent medium was aspirated and the cultured cells were treated with the test sample fucoidan extracted from the Turbinaria conoides and controls were filled with 2ml of the culture medium and incubated at 24 hours. The culture medium from the wells was removed and washed with PBS solution after the removal of PBS solution 200 µl of trypsin – EDTA solution was added and incubated at 37°C for 3-4 minutes, Again the culture medium was harvested into 12x75 mm polystyrene tubes. The harvested polystyrene tubes were centrifuged at 300 x g at 25° c. supernat was removed carefully and the obtained pellets were washed twice with PBS.PBS treated cells was treated with 1ml of pre-chilled 70% ethanol and vortexed to avoid the clumping of cells. Ethanol treated cells
were centrifuged at a higher centrifugal rate compared to the ethanol untreated cells. Then the ethanol-treated cells were washed with PBS solution twice. 10 μL of PE-Bcl2 antibody was added and mixed thoroughly and incubated for 30 minutes in dark at room temperature. The above mixture was washed with 0.5ml of 1X PBS, the mixture was mixed thoroughly and analysed with Flow cytometry.

2.3. Caspase Assay
MCF7 cells were cultured in the culture medium in microtitre plate and kept for overnight incubated at 37°C for 24 hours. Spent medium was aspirated and it was treated with test compound FE from Turbinaria conoides and control in a separate well and it was incubated for 24 hours. After 24 hours, the medium was removed and the wells were washed with PBS solution and incubated at 37°C for 2-4 minutes. To that PBS treated 2ml of culture, the medium was added and the cells were directly harvested in 12x75 mm polystyrene tubes and the tubes were centrifuged at 300 mg at 25°C and the supernatant was discarded carefully. The pellet containing the cells was washed with PBS solution and then PBS was decanted completely. The above mixture Pre-cooled lm of the 70% ethanol was added and vortexed to avoid the clumping of cells. It was centrifuged at a very high speed compared to the pre-cooled cells and care should be taken while aspirating the supernatant not to lose the pellet. Collected pellets were washed twice with PBS solution. 5ml of FITC caspase 3 antibodies were added to the pellets and mixed thoroughly and incubated at 30°C in the dark at 20-25°C, pellets were again washed with 1X PBS solution with 0.1% sodium azide, and then 0.5ml of PBS solution was added and mixed thoroughly and subjected to flow cytometry.

3. RESULTS

3.1. Bcl-2 Expression Study of the FE against MCF7 Cell line
The Statistical data of Bcl2 expression Study using flow cytometry indicates that, in MCF7 cells, the Expression of Bcl2 is very high in Untreated MCF7 Cells (81.49MFU) compared to Camptothecin (25uM) is 19.71MFU (Mean Fluorescence Intensity). The Test Compound FE showed 22.09MFU of Bcl2 expression with IC 50 Concentration (115.21uG/mL) (FIG. 1).

3.2. CASPASE 3 Expression Study of the FE against MCF7 Cell line:
The Statistical data of CASPASE 3 expression Study using flow cytometry indicates that the Expression of CASPASE 3 is very low in Untreated MCF7 Cells (12.75 MFI) Mean Fluorescence Intensity compared to Std Drug Camptothecin (25uM) showing 54MFI. The Test Compound FE showed Caspase3 expression at 31.48 in terms of Mean Fluorescence Intensity with IC 50 Concentration (115.21uG/mL) (FIG 2).

4. DISCUSSION
In our study, it is found that FE induced apoptosis by down-regulating the Bcl2 genes and up-regulating Caspase3 assay with a moderately significant result compared to the standard camptothecin. The mechanism behind the apoptosis activity is due to the pro-apoptotic activity protein BAX and BAK gene was activated by the induction of FE in MCF7 cell line. These BAX and BAK acts on the outer membrane of mitochondria and makes the mitochondria permeable for the entry of BAX and BAK genes and release the signals called cytochrome C and ROS from the mitochondria to cytosol, this will lead to the activation of caspase assay for apoptotic activity the same mechanism was found in the 4T1 cell line by the induction of fucoidan and it is also found effective in the invivo animal model by the Intraperitoneal injection of fucoidan in breast cancer.
injection of fucoidan [5] it was also proved that the induction of fucoidan resulted in the release of a large amount of cytochrome C in the cytoplasm which leads to activation of caspase-3 which will release the cascade of apoptotic effect and decreased the growth of the cancer cells. In the previous work it was found that FE upregulates the pro-apoptotic protein Bax and Bad expression and down-regulates the anti-apoptotic protein activity of BCL-2 and Bcl-XI [6] It is also concluded that fucoidan extract inhibits the growth of MCF7 cell line through inducing the caspase-independent mitochondrial-mediated apoptotic activity [7]

5. CONCLUSION
From our results, it is clear that the FE induced apoptosis by Bcl2 and caspase3. FE Found to inhibit the growth of MCF7 breast cancer cell line through downregulating the Bcl2 & upregulating the Caspase assay. Thus this indicates that the FE extracted from Turbinaria conoides as the potential to induce apoptosis in MCF7 cell line in in-vitro condition. It can be further analyzed using the animal models in in-vivo condition and the anticancer property against breast cancer can be proved. so that, it can be used as a potential regimen in future.

5. REFERENCES
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