Soybean Seed Extract induced Inhibition of Jab1 in Breast Cancer Cell Line

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Abstract

Scientific research has suggested that the modification of signaling networks has a strong relationship with the progression of breast cancer and alterations by phytocompounds could offer a promising approach for treating and managing the disease. Therefore, we evaluated the inhibitory potency of soybean seed extract against Jab1 in breast cancer using several in vitro assays. Our findings revealed that treatment with soybean seed extract in breast cancer cells resulted in a dose- and time-dependent reduction in cell growth, with an IC_{50} value of 67.68 µg/ml. Furthermore, the antioxidant activity of the soybean seed extract was confirmed by the DPPH assay. Additionally, soybean seed extract-treated triple-negative breast cancer cells exhibited increased nuclear condensation, indicating apoptosis.

Real-time PCR results demonstrated the cell growth inhibitory potential of the soybean seed extract by downregulating Jab1 expression. At the same time, there was a significant increase in Bax and caspase 3 expression, which suggests cell cycle arrest at the G0/G1 phase. Moreover, increased caspase 3 activation elucidates one of the possible mechanisms behind the inhibitory effect of soybean seed extract against Jab1 in breast cancer cells (MDA-MB-231). In conclusion, our study suggests that the bioactive compounds in soybean may serve as potent therapeutic agents for targeting the oncogene Jab1 in breast cancer.

Keywords: Soybean seed extract, Breast cancer, MDA-MB-231, Apoptosis, Jab1, Bax, Caspase 3.

Introduction

For decades, plants have been extensively exploited for bioprospecting novel and potent bioactive compounds with broad pharmaceutical applications. Globally, these plant-derived compounds are widely used in traditional medicine, as they are relatively cheaper, less toxic, have fewer side effects and are more accessible to rural populations compared to modern medicine[28].

According to the World Health Organization, approximately 80% of people living in developing countries still rely on herbal medicines for their primary healthcare needs[23]. To date, only about 1,000 plants have been identified for their anti-cancer properties[6].

The WHO has also reported that breast cancer is a growing global concern and the second leading cause of death among women. One in ten women is diagnosed with breast cancer at some point in their lives[9]. The rising incidence of breast cancer is alarming, with cases projected to reach 6.99 million by 2040[14]. Breast cancer remains one of the most common cancers worldwide and continues to be the leading cause of death among women in both developed and developing countries. Therefore, therapeutic research is essential to combat the increasing incidence of breast cancer and the challenges faced during treatment[4]. Although cancer rates are increasing, research efforts to combat this deadly disease are also gaining momentum.

Chemotherapy is currently the most widely used treatment for cancer and has proven effective; however, it comes with numerous side effects for patients undergoing treatment[29]. One major drawback of chemotherapy is the lack of specificity in cancer drugs[42]. Cancer is a progressive disease often resulting from genomic instability caused by chromosomal translocations, leading to the aberrant expression of oncogenes such as *Jab1*/COPS5 or MYC. *Jab1* regulates numerous signaling factors by influencing their subcellular localization, degradation and deneddylation[25] and it inactivates several tumor-related genes including p53, p27 and thioredoxin[21,30]. Elevated *Jab1* expression has been observed in various cancers and is associated with poor survival rates[31-33].

Additionally, Jab1 overexpression correlates with poor prognosis in breast cancer patients, suggesting that *Jab1* could be a potential therapeutic target for breast cancer treatment[26]. Numerous scientific reports have explored the relationship between *Jab1* and other signaling pathways, reinforcing the notion that *Jab1* is a viable therapeutic target for treating various cancers including breast cancer.

Plant-based drugs are considered a safer alternative for cancer treatment, with around 80% of the population relying on them for minor health issues[13]. Developed countries such as Australia, North America and those in Europe, have begun recognizing the potential of natural drugs as alternative therapies[3,8]. This recognition is largely due to their higher safety, lower toxicity, cost-effectiveness and the enhancement of quality through advanced technologies[5].

These factors have driven the demand for novel and effective plant-based drugs. Soybean, an economically important crop from the Fabaceae family, stands out from other legumes due to its high content of fiber, proteins, phytosterols and isoflavones[15,39].

Soybean extract and its pure compounds have been shown to possess potent antioxidant and anti-cancer properties[10,17]. The alarming increase in breast cancer-related mortality globally has intensified the search for effective plant-based compounds that can inhibit genes responsible for cancer progression, paving the way for the development of targeted therapeutic strategies[4]. This prompted us to investigate the potential of soybean seed extract in downregulating genes involved in cancer progression. While soybean's anti-cancer properties are well-documented, the inhibitory role of soybean seed extract against *Jab1* has not yet been explored. Soybean has been shown to be effective against prostate[12,16] and breast cancer[19] and is used as an alternative therapy to radiation in treating these cancers, often in combination with other anti-cancer treatments[37].

This study aimed to investigate the inhibitory role of the methanolic extract of soybean seeds against *Jab1* in MDA-MB-231 breast cancer cells. Additionally, this study seeks to unravel the potential of soybean seed extract in regulating other genes, such as *Bax*, *Bcl2* and *caspase 3*, which are biomarkers for cancer progression. Soybean extract has already been proven to induce cell death in colon[45] and prostate cancer cell lines[18]. As studies continue to focus on identifying plant-derived bioactive compounds that target oncogenes or tumor-inducing genes, it becomes increasingly important to assess the ability of these compounds to downregulate tumor-promoting gene expression. The soybean seed extract-mediated downregulation of *Jab1* presents a promising and rational approach in developing targeted therapies for breast cancer management.

Material and Methods

Soybean seeds (*Glycine max* L.) cv. JS335 were obtained from the Indian Council for Agricultural Research (ICAR) - Indian Institute of Soybean Research (IISR), Indore, Madhya Pradesh, India. The seeds were maintained and propagated in the field at the Department of Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India. The seeds were shade-dried for one week and finely powdered using a commercial mixer. Soxhlet extraction was performed by packing 10 grams of seed powder in a thimble and extracting it with 250 ml of methanol for 20 cycles. The extracted sample was evaporated using a rotary evaporator and the yield percentage of the methanolic soybean seed extract was determined to be 34.43%. Subsequently, 1 mg of the condensed sample was dissolved in 1 ml of methanol for further studies.

Biochemical characterization

GC-MS analysis: The GC–MS analysis of the methanolic extract of soybean cv. JS335 was performed using a TSQ™ 8000 Evo Triple Quadrupole GC–MS/MS system (Thermo Fisher Scientific) equipped with an Elite-5 capillary column (30 nm × 0.25 mm ID × 0.25 µm df). The mass detector was operated in electron impact (EI) mode with a full scan range of 50–550 amu. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector was set at 290°C and the oven temperature was programmed as follows: 50°C, increase at 8°C/min to 200°C (held for 5 min) and then at 7°C/min to 290°C (held for 10 min). The peaks in the chromatogram were identified based on their mass spectra. Interpretation of the mass spectra was done using the National Institute of Standards and Technology (NIST) database, with the spectra of phytochemicals compared to known compounds stored in the NIST library.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: The DPPH assay was carried out according to Brand-Williams et al[7]. Briefly, methanolic extract from soybean seeds was taken in different concentrations and to these samples, 1.5 ml of DPPH methanolic solution (20 mg/l) was added. Later, the absorbance was measured at 517 nm after 20 minutes of reaction. The percentage of radical scavenging activity of the sample was estimated according to the equation:

Radical scavenging activity % = (Absorbance of control-Absorbance of sample/Absorbance of control) × 100.

In-vitro anticancer studies

Maintenance of cell culture: The human breast adenocarcinoma cell line (MDA-MB-231), procured from the National Center for Cell Sciences, Pune, was cultured in RPMI (Roswell Park Memorial Institute) and DMEM (Dulbecco's Modified Eagle's) media. Both RPMI and DMEM media contained 10% heat-inactivated fetal calf serum, antibiotics, glutamine and 1% antimycotic solution. The cell cultures were incubated at 37°C in a CO_2 incubator.

Determination of cell viability using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay: The monolayer cell culture was trypsinized and the cell count was adjusted to 1×10^4 cells/ml using medium containing 10% FBS. To each well of a 96-well microtiter plate, 0.2 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer had formed, the supernatant was discarded and the monolayer was washed once. Various concentrations of soybean seed extract (25, 50, 75 and 100 mg/ml) diluted in media were added and the cells were incubated for 2 hours to assess the effect of the extract on cell viability in a dose-dependent manner. After 2 hours, 20 µl of MTT in 200 µl of media was added to each well and incubated for 4 hours at 37°C.

Following this, the MTT solution was discarded and 200 µl of DMSO was added to dissolve the dark blue formazan crystals. After a few minutes, the absorbance was measured at 570 nm. The percentage of cell viability was calculated using the following formula:

% Viability = OD of test/ OD of control × 100

Wound healing assay: The inhibition of tumor cell migration by soybean seed extract was assessed using a wound-healing assay. Briefly, cells were allowed to grow to full confluence in 6-well plates, after which a vertical wound was created using a 200 µl sterile pipette tip. The cell debris was removed and fresh complete medium containing the desired concentration of soybean seed extract was added. The cells were incubated at 37°C and 5% CO_2 for 24 hours and photographed using a microscope at two time points (0 and 24 hours). The area of migration was measured and analyzed using Image J software[20].

DAPI staining: The occurrence of apoptosis was evaluated using DAPI (4',6-diamidino-2-phenylindole) staining. Cells were seeded and incubated with soybean seed extract for 24 hours. Prior to staining, the treated cells were fixed with 3.7% paraformaldehyde for 5 minutes at room temperature and then washed twice with phosphate-buffered saline (PBS). DAPI was added to the fixed cells for 5 minutes, after which they were examined using fluorescence microscopy. Apoptotic cells were identified by the condensation and fragmentation of the nuclei. The percentage of apoptotic cells was calculated as the ratio of apoptotic cells to the total number of cells counted, multiplied by 100. A minimum of 300 cells was counted for each treatment group[41].

Detection of apoptosis by acridine orange and ethidium bromide double staining: Cells were seeded and incubated with soybean seed extract for 24 hours. The control and treated cells were then trypsinized and mixed thoroughly to obtain a single-cell suspension. Trypsin was neutralized by adding complete medium and 20 ml of the cell suspension was incubated with 10 ml of a 100 mg/ml ethidium bromide and acridine orange mixture for 5 minutes. The cells were subsequently imaged using a fluorescence microscope[43].

RT-PCR analysis: Cells were seeded and incubated with soybean seed extract for 24 hours. RNA was isolated from the cells treated with soybean seed extract using the RNase mini kit method and the quantity and purity of the isolated RNA were determined using a Nanodrop spectrophotometer. The isolated RNA was converted into cDNA and RT-PCR was performed using the PrimeScript RT-PCR kit to measure the expression of four genes (*Bax*, *Bcl2*, *Caspase 3* and *Jab1*). After amplification, the PCR products were separated by agarose gel electrophoresis. The sequences of the primers used in RT-PCR are provided in table 1.

Results and discussion

Biochemical characterization: The GC-MS analysis of soybean seed extract revealed the presence of several phytochemicals. A total of 14 compounds were identified based on peak area, retention time and molecular formula (Figure 1). Various bioactive compounds have been previously reported in soybean seed extract including phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids[25]. The first compound identified had a retention time of 9.32 min and was guanosine, while 3,9-Epoxypregn-16-ene-14-18-diol-20-one was the last compound identified with the longest retention time of 39.58 min. The list of compounds detected through GC-MS analysis is provided in table 2. Additionally, soybean seed extract showed an increasing free radical scavenging activity with increasing concentrations of the extract (Table 3).

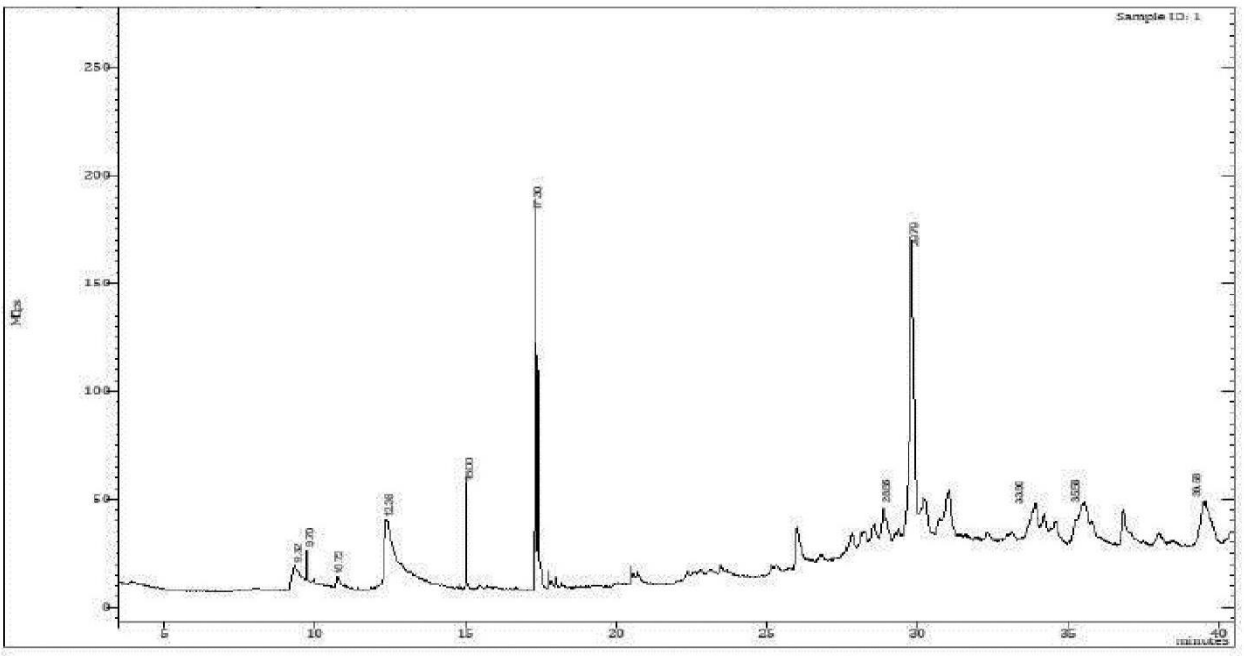

Figure 1: Gas Chromatography-Mass Spectrometry (GC-MS) chromatogram of methanolic seed extract of soybean. The peak numbers present in the figure specify the compounds in table 2.

Table 1
Primers used for gene expression studies

Gene	Forward/Reverse	Sequence 5' to 3'
Bax	Forward	AGGGTGGCTGGGAAGGC
Bax	Reverse	TGAGCGAGGCGGTGAG
Bcl2	Forward	ATCGCTCTGTGGATGACTGAGTAC
Bcl2	Reverse	AGAGACAGCCAGGAGAAATCAAAC
Jab 1	Forward	GGCGCCTTTAGGACATACC
Jab 1	Reverse	CATGAAACTCCCTCGTCCC
Caspase 3	Forward	ACCAAAGATCATACATGGAAGCGA
Caspase 3	Reverse	CGAGATGTCATTCCAGTGCT
β actin	Forward	ACCCAGAAGACTGTGGATGC
β actin	Reverse	CAGTGAGCTTCCCGTTCAG

The sequence of primers used in RT-PCR analysis for determining the mRNA expression of apoptotic genes (*Bax, caspase-3*), anti-apoptotic genes (*Bcl2, Jab1*) in methanolic seed extract of soybean treated MDA-MB-231 cells compared with the control (untreated MDA-MB-231cells).

Table 2
Phytocompounds identified from methanolic seed extract of soybean through Gas Chromatography-Mass Spectrometry (GC-MS)

S.N.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1	9.32	Guanosine	$C_{10}H_{13}N_5O_5$	283	1.18
2	9.70	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180	1.13
3	10.72	Neocurdione	$C_{15}H_{24}O_2$	236	0.73
4	12.36	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	8.81
5	15.00	Dodecanoic acid, 10-methyl-, methyl ester	$C_{14}H_{28}O_2$	228	3.58
6	17.30	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	13.83
7	17.39	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	9.47
8	28.85	Benz[e]azulene-3,8-dione, 3a,4,6a 7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-5-(hydroxymethyl)-7-(1-hydroxy-1-methylethyl)-2,10-dimethyl-, [3aR-(3aα,6aα,7α,10β,10aβ,10bβ)]-	$C_{20}H_{28}O_6$	364	3.48
9	29.79	i-Propyl 9-hexadecenoate	$C_{19}H_{36}O_2$	296	44.02
10	30.21	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	3.09
11	33.90	i-Propyl 9-tetradecenoate	$C_{17}H_{32}O_2$	268	0.35
12	35.56	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	1.13
13	36.85	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	4.74
14	39.58	3,9-Epoxypregn-16-ene-14-18-diol-20-one, 7,11-diacetoxy-3-methoxy-	$C_{26}H_{36}O_9$	492	4.46

Table 3
Assessment of antioxidant activity in methanolic seed extracts of soybean using DPPH assay

Extract concentration (µg/ml)	Radical scavenging activity(%)
20	21.1 ± 0.27^e
40	32.8 ± 0.49^d
60	44.5 ± 0.39^c
80	63.6 ± 0.34^b
100	72.76 ± 0.45^a

Data represents mean values ± standard error (n = 5 × 3).
Values with different letters are significantly different according to DMRT at 5% level.

***In vitro* anticancer activity of soybean extract:** The *in vitro* anticancer efficacy of soybean extract in MDA-MB-231 cells was analyzed using various methods including cell viability assays, wound healing assays, DAPI staining, double staining and RT-PCR analysis.

MTT assay: The MTT assay measures the metabolic activity of cells by quantifying NADPH-mediated cellular oxidoreductase enzymes which reduce the MTT dye into an insoluble formazan product. The cytotoxic activity of soybean extract on MDA-MB-231 cells was assessed using this assay. The results indicated that soybean extract exhibits good inhibitory activity by decreasing the viability of cancer cells in a time- and dose-dependent manner, with an IC_{50} value of 67.68 µg/ml (Figure 2). These findings demonstrate the potential of soybean as a stable entity with cytotoxic properties. The results from the MTT assay imply that soybean possesses a potent cytotoxic effect on tumor cells. The viability of the MDA-MB-231 cell population gradually decreased with both time and dose in response to soybean extract treatment.

The antioxidant effects of plant bioactive compounds show significant cytotoxic and pro-apoptotic effects on breast cancer cells. Exposure to soybean extract may induce distinctive cytotoxic stress in breast cancer cells, potentially due to the activation of apoptotic signaling cascades leading to mitochondrial membrane destruction. These results are supported by previous studies conducted by Amaani and Dwira[2] which found that the IC_{50} of *Glycine soja* ethanolic extract on A549 lung cancer cells was 114.5 µg/ml.

Wound healing assay: Cancer cell migration and invasion play a pivotal role in growth, proliferation and metastasis. The scratch assay was used to identify the inhibitory and anti-tumorigenic effects of soybean extract on the migration of triple-negative breast cancer (TNBC) cells. The cells were seeded in six-well plates and incubated at 37 °C until they reached confluence. Subsequently, a scratch was created in the cell monolayer using a sterile pipette tip for both soybean extract-treated and untreated cells. The wound gap was photographed using an inverted microscope at 0, 24 and 48 hours. After 48 hours of observation, the untreated cancer cells had closed around 10% of the 400 µm gap due to the invasion and migration of adjacent cells into the scratched area.

In contrast, cancer cells treated with the IC_{50} value of soybean extract did not migrate, leaving a visible gap at the scratched site (Figure 3). This confirms that soybean extract strongly inhibits the invasion and migration of TNBC cells. The results of the wound healing assay demonstrated that soybean extract aids in restricting cell migration and invasion by inhibiting the Jab1-associated pathway. Since cancer cell invasion and migration are directly related to cell death, the results indicate that soybean extract effectively inhibits metastasis. The results obtained are supported by *in vitro* assessments of the wound healing properties of the hydroalcoholic extract of Jaboticaba (*Plinia peruviana*)[35].

DAPI staining: DAPI nuclear staining was performed to observe the characteristic morphological changes of the MDA-MB-231 cell nucleus with induced apoptosis under a fluorescent microscope. The fluorescent dye DAPI (4,6-diamidino-2-phenylindole) is a DNA-specific dye that forms a fluorescent complex by binding within the AT-rich sequences, emitting blue fluorescence. Breast cancer cells treated with soybean extract at an IC_{50} value of 92 µg/ml showed a significant increase in the number of apoptotic cells compared to untreated cells (Figure 4).

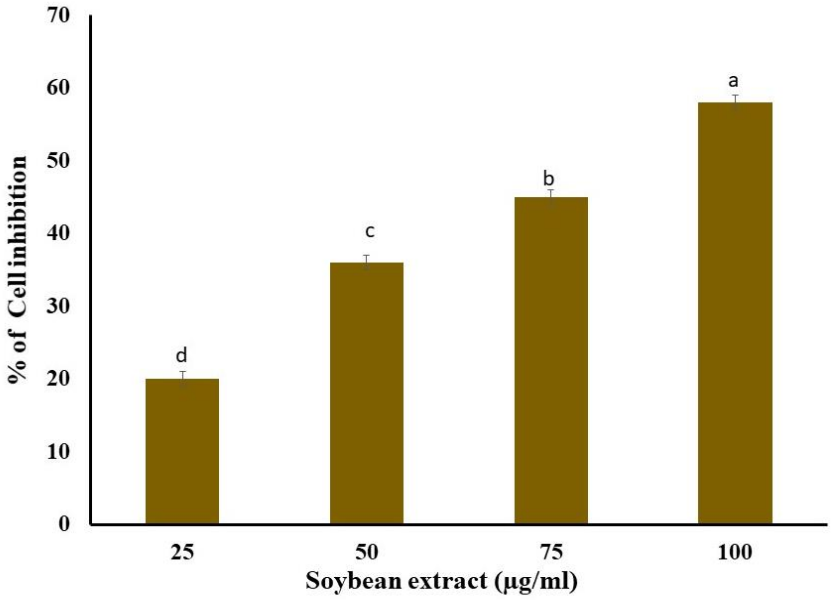

Figure 2: Cytotoxic activity of methanolic seed extract of soybean in MDA-MB 231 breast cancer cell lines. The percentage of cell inhibition was measured using MTT assay. Each bars signifies the mean ± SE of three independent experiments. Bars with different letters are significantly different according to DMRT at 5% level.

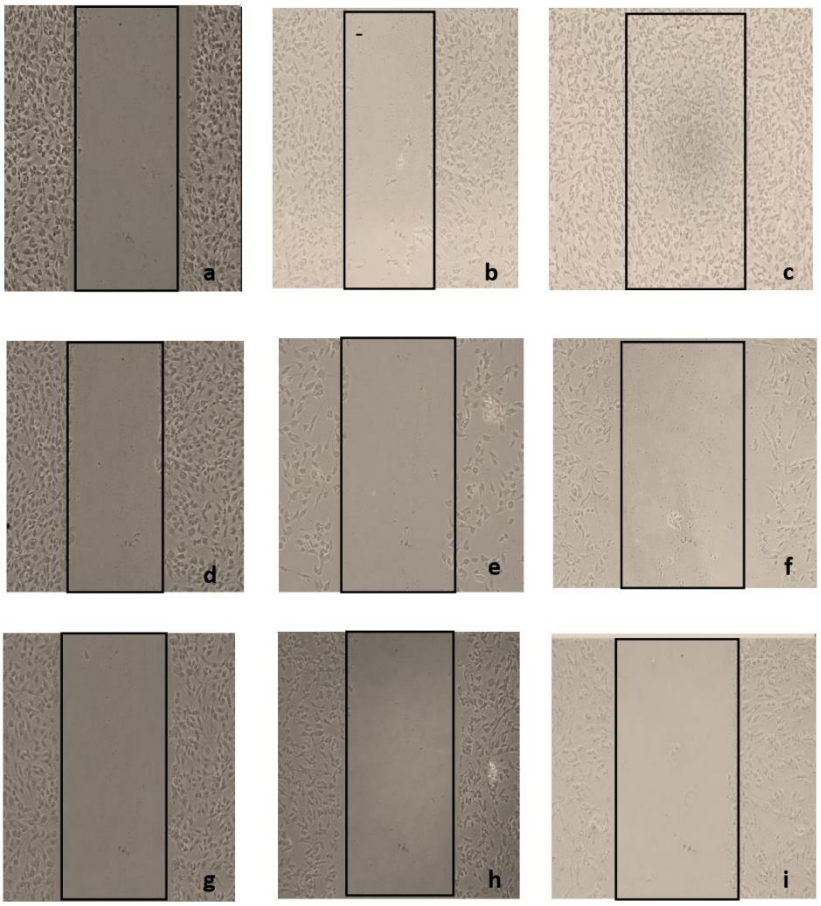

Figure 3: Wound healing assay: Effect of methanolic seed extract of soybean on the migration capability of MDA-MB-231 breast cancer cell lines. a-c: untreated MDA-MB-231 cells (a: 0th hour; b: 24 hours; c: 48 hours); d-f: cisplatin treated MDA-MB-231 cells (d: 0th hour; e: 24 hours; f: 48 hours); g-i: soybean seed extract MDA-MB-231 cells (g: 0th hour; h: 24 hours; i: 48 hours)

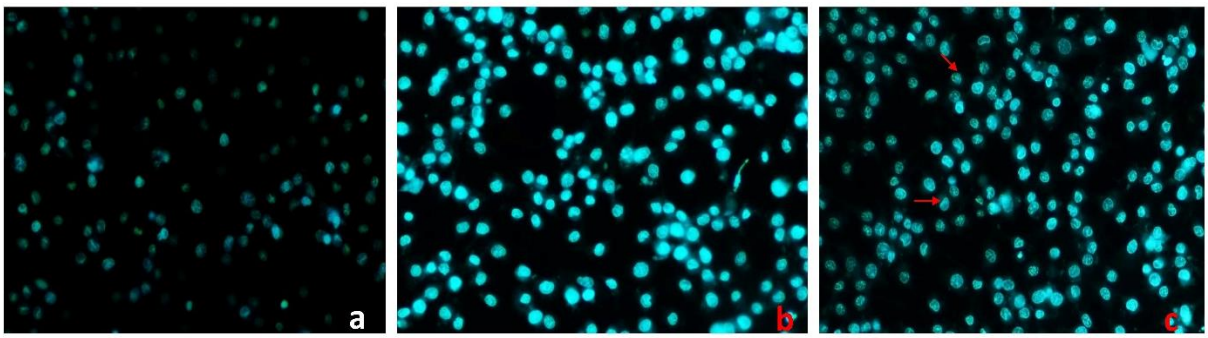

Figure 4: DAPI (4'-6-diamidino-2-phenylindole) nuclear staining of MDA-MB-231 breast cancer cell lines treated with methanolic seed extract of soybean. a: untreated MDA-MB-231 cells; b: cisplatin treated MDA-MB-231 cells; c: soybean seed extract treated MDA-MB-231 cell lines showing cells undergoing apoptosis. Red arrows indicate chromatin condensation, cell shrinkage and nuclear fragmentation in soybean treated MDA-MB-231 cells

The results obtained from DAPI staining indicate that soybean could induce apoptosis in MDA-MB-231 cell lines. The morphological changes such as chromatin condensation, cell shrinkage and nuclear fragmentation observed in soybean-treated cells, may be due to the inhibition of the *Jab1* signaling pathway, which cleaves specific cell surface proteins and alters the anatomy of chromatin, resulting in DNA degradation. Choudhary et al[11] reported that polyphenols extracted from faba beans were investigated on the 3T3-L1 cell line for their hypoglycemic effects, oxidative stress potential and xanthine oxidase activity.

Acridine orange and ethidium bromide double staining technique: The apoptotic events, necrosis and associated morphological changes of the cells were analyzed under fluorescent microscopy using the acridine orange/ethidium bromide (AO/EtBr) dual staining technique. As depicted in figure 5c, cells treated with soybean extract at the IC_{50} value

emitted yellow and reddish-orange fluorescence, indicating the presence of early and late apoptosis. In contrast, untreated cells appeared as green bodies, signifying viable cells that had not undergone apoptosis. The reddish-orange fluorescence indicates the occurrence of induced apoptotic events and the inhibition of *Jab1* in cells due to the loss of membrane integrity (Figure 5).

The double staining technique distinguishes between different stages of apoptosis acridine orange stains both live cells and cells undergoing early apoptosis, while ethidium bromide distinguishes only stains cells undergoing late apoptosis. Consequently, live cells were stained green and cells undergoing early and late apoptosis were stained for yellow and reddish-orange respectively. The results obtained from the double staining were supported by Singh et al[40], who highlighted the cellular and anticancer activity of *Amoora rohituka* leaf extracts in human breast cancer cells.

Reverse transcriptase-PCR (RT-PCR) analysis: This study demonstrates the apoptotic effect of soybean methanolic extract on MDA-MB-231 breast cancer cell lines. The results indicate that the extract inhibits the *Jab1* oncogene, which is significantly expressed in cancerous cells (Figure 6). Quantitative PCR results reveal a downregulation of the Jab1 gene by soybean seed extract, showing a significant reduction in expression compared to the negative control, with no substantial difference observed when compared to the positive control.

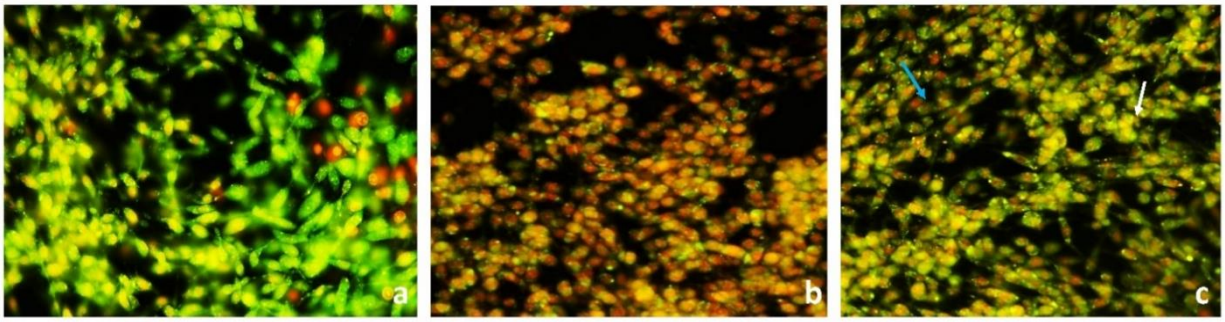

Figure 5: AO/EB (acridine orange/ethidium bromide) double-staining of MDA-MB-231 breast cancer cells treated with methanolic seed extract of soybean. a: untreated MDA-MB-231 cells; b: cisplatin treated MDA-MB-231 cells; c. soybean seed extract treated MDA-MB-231 cells. Blue arrow indicates a reddish-orange fluorescence which confirms the augmentation of early and late apoptosis and white arrow indicates late apoptosis of the treated cells which is seen as yellow color. Live cells are seen in green color

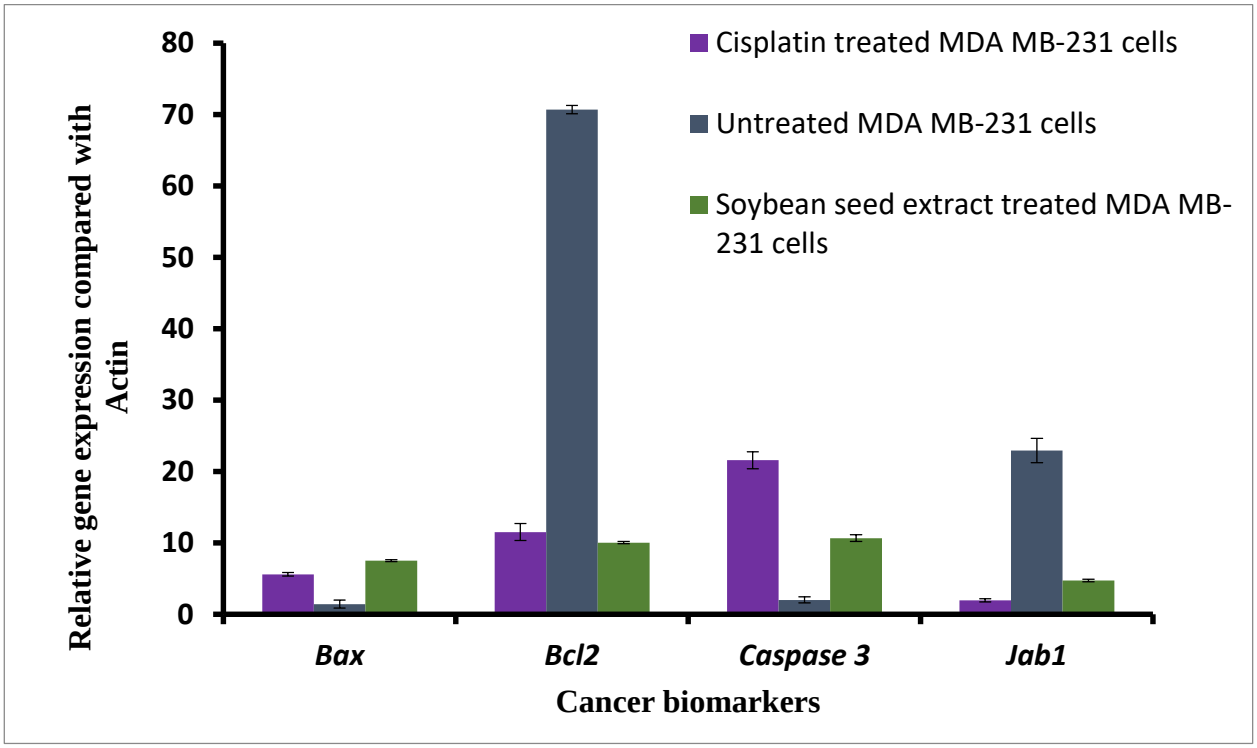

Figure 6: Expression levels of (a) Bax gene; (b) Bcl2; (c) Caspase 3; (d) Jab1 genes in cisplatin treated MDA-MB-231 cells, untreated MDA-MB-231 cells and in soybean seed extract treated MDA-MB-231 cells. Data represents mean value ± standard error of three independent experiments (P<0.05)

Additionally, the extract downregulated the expression of the *Bcl2* gene while upregulating the expression of *Bax* and *caspase-3* genes. This modulation consequently leads to the inhibition of cancer cells by inducing apoptosis[44]. It is well-established that Bax, a pro-apoptotic protein, plays a crucial role in regulating the apoptotic pathway by forming pores in the outer mitochondrial membrane, resulting in cytochrome c leakage, which is repressed by the anti-apoptotic protein *Bcl2*[34]. Numerous reports highlight the role of the mitochondrial apoptotic pathway in the apoptotic potential of plant-derived bioactive compounds across various cancers[27,36,38]. Soybean treatment enhances the release of cytochrome c into the cytosol, which is essential for the subsequent activation of caspase-3. These findings suggest that the regulation of apoptosis-related genes is a significant molecular mechanism underlying the induction of apoptosis in MDA-MB-231 cells treated with soybean seed extract.

Statistical analysis: Each experiment was conducted independently three times for each biological sample. The data for the DPPH assay were expressed as mean values ± SE (n = 5 × 3). Statistical differences were analyzed using SPSS software version 20, employing Duncan's Multiple Range Test (DMRT) at a significance level of $P < 0.05$.

Conclusion

Our study demonstrated the significant cell growth inhibitory effect of soybean seed extract on breast cancer cells. We tried to establish a direct link between the downregulation of *Jab1* and the cell growth inhibitory activity of soybean seed extract in MDA-MB-231 breast cancer cells. Additionally, various *in vitro* assays were conducted to assess the apoptotic activity of the extract. The soybean seed extract induced apoptosis in breast cancer cells through *Jab1* downregulation, as well as caspase- and ROS-dependent mitochondrial pathways.

Therefore, our findings suggest that soybean seed extract could serve as a promising therapeutic agent for the enhanced management of breast cancer, with minimal side effects and toxicity to normal cells. However, further research is necessary to explore the anticancer potential of soybean seed extract *in vivo*.

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