ORIGINAL ARTICLE

Upregulation Mechanism of *CCND1* in Apoptosis on MCF-7 Cell Line upon Treatment with Quranic Verses

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ABSTRACT

INTRODUCTION: Ruqyah Shar'iyyah, one of the complementary and alternative medicine (CAM) has shown therapeutic benefits in breast cancer by utilizing Quranic verses in reducing symptoms and enhancing their quality of life as a result of invasive standard therapies. However, scientific evidence is required to demonstrate Ruqyah Shar'iyyah's effectiveness on gene expression and molecular pathways in carcinogenesis. Therefore, this study was conducted to evaluate the effectiveness of Ruqyah Shar'iyyah on breast cancer cell line on apoptosis and *CCND1* expression in related signaling pathways. **MATERIALS AND METHODS**: MCF-7 cell lines were treated with direct recitation of several Quranic verses for 12 hours and 24 hours. Cell viability was observed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The expression of *CCND1* was determined using RT-qPCR technique. The molecular mechanism and signalling pathways were evaluated using Reactome database for *in silico* analysis. **RESULTS:** Cell viability data showed 95.69% and 93.54% exhibiting slight reduction for 12-hour treatment in untreated and treated MCF-7, respectively. Meanwhile more reduction was observed in 24-hour treatment with 95.11% in untreated cell line as compared to 92.34% in treated cell line. The cell morphology also exhibited apoptotic activity in the treated group for both two time points. Gene expression analysis of *CCNDI* also demonstrated upregulation with 1.81-fold change. The data was supported by the *in-silico* analysis in which 25 relevant significant signalling pathways related to *CCND1* highlighting the role of the gene in breast cancer development. **CONCLUSION:** *CCND1* may have a function in signalling pathways that control the proliferation of mammary epithelial cells.

Keywords
apoptosis, breast cancer, Quranic verses, CCND1, mechanism

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INTRODUCTION

In 2020, Malaysia recorded 8,418 new cases of breast cancer, highlighting it as a significant health concern requiring attention and effective treatment. The precise cause of breast cancer remains unknown and is attributed to various potential risk factors. Some key factors such as gender, are uncontrollable, with women being 100 times more susceptible than men.[1] Moreover, advancing age is also linked to an increased incidence of breast cancer, with most diagnoses occurring in women aged 55 and above.[1] Notably, a study in 2016 revealed that American women over 40 and 60 contribute to 99.3% and 71.2% of breast cancer-related deaths, respectively.[2] To address this, it is recommended that women over 40 undergo regular mammography screenings for early detection of breast cancer.

Moreover, the likelihood of developing breast cancer is higher in women with a family history of the disease. A United Kingdom cohort study indicates that women with first-degree relatives (mother, sister, or daughter) diagnosed with breast cancer face a 1.75-fold increased risk compared to those with unaffected relatives. The risk further escalates to 2.5-fold higher for women with two or more first-degree relatives affected by breast cancer.[3] This inherited susceptibility is associated with genetic mutations in the BRCA1 and/or BRCA2 genes.[4]

Presently, the primary treatment modes available for breast cancer include surgical procedures combined with adjuvant therapies like radiation therapy, chemotherapy, targeted therapy, hormonal therapy, and immunotherapy.[4,5] However, these treatments may result in undesired side effects, prompting some patients to consider complementary and alternative medicine (CAM) in conjunction with conventional approaches. The National Cancer Institute defines CAM as medical products and practices outside the realm of standard medical care. It encompasses five main categories: alternative medical systems, exemplified by traditional medicine and homeopathy; mind-body interventions, including practices like yoga and cupping; biologically based therapies, such as dietary supplements; manipulative and body-based methods, like reflexology and massage; and energy therapies, encompassing practices like Qigong and Tai Chi.[6,7]

One of the practices within CAM involves Islamic spiritual healing. This is manifested through the recitation of Quranic verses known as Ruqyah Shar'iyyah. This approach is commonly embraced by Muslim patients, underscoring the profound influence of religion, particularly when confronted with life-threatening illnesses. Islamic spiritual healing employs recitation of Quranic verses or the traditions of Prophet Muhammad (peace be upon Him), known as sunnah, for healing purposes. This is practiced globally and even among non-Muslim regions. There are two main approaches to Islamic spiritual healing: direct recitation of Quran to patients and the use of herbs or water (healing water) infused with pre-recited Quranic verses.[8] Muslims attribute healing powers to Quranic recitation, citing verses such as Surah Al-Isra' verse 82, which states, "We send down (stage by stage) in the Quran that which is a healing and a mercy to those who believe..." Additionally, Surah Fussilat verse 44 emphasizes the Quran as a guide and healing for believers, and Surah Ash-Shu'ara verse 80 mentions, "And when I am ill, it is He (Allah) who cures me." Previous studies have indicated that Islamic spiritual healing significantly contributes to enhancing the mood, coping with symptoms and side effects, and alleviating anxiety in breast cancer patients, facilitating spiritual and psychological healing. It is important to note that complementary medicine is intended to mitigate the side effects of breast cancer or enhance quality of life and should not replace conventional medical treatments.

Numerous articles assert that Islamic spiritual healing positively influences cancer patients in terms of mood, spirits, and psyche. However, only a limited number of articles delve into the impact of Ruqyah Shar'iyyah on cell cultures and the associated genetic mechanisms.[9] As mentioned earlier, genetic factors significantly contribute to breast cancer development. Multiple genes play a role in breast cancer pathogenesis across various signalling pathways, necessitating an exploration of their functions. Among these genes is Cyclin D1 (*CCND1*), a candidate gene involved in cell cycle regulation. *CCND1* encodes a cyclin protein crucial for CDK kinase regulation, forming a complex with CDK4 or CDK6 to facilitate G1/S transition during the cell cycle. Alterations to *CCND1*, such as missense mutations, in-frame deletions, insertions, amplifications, and overexpression, have been observed in various carcinomas, including breast carcinoma, impacting cell cycle progression. A previous study reported *CCND1* mutations seen in 16.28% of breast carcinomas. *CCND1*'s role in breast cancer can influence tumour growth, cell cycle, and cell migration, as evidenced by cyclin protein expression in breast cancer tissue samples. Consequently, *CCND1* has been associated with the prognosis of breast cancer, with higher expression levels correlating with poor metastasis-free or recurrence-free survival.[9]

Given that the prevalent practice of Islamic spiritual healing is favoured in Malaysia, particularly among Muslim cancer patients, this study aimed to evaluate the efficacy of Ruqyah Shar'iyyah in regulating *CCND1* by inhibiting cell proliferation of breast cancer cells. Our investigation focused on assessing the expression level of the *CCND1* gene and its impact on the cell proliferation and apoptosis of the MCF-7 breast cancer cell line after treatment with Ruqyah Sha'iyyah.

MATERIALS AND METHODS

Selection of Quranic verses

General and specific Quranic verses were selected in this study. Table 1 shows a list of Quranic verses in Rukyah Shar'iyyah that commonly used in breast cancer treatment.[11,12] The selection of Qur'anic verses was based on the several well-established endorsed by Islamic healers who were used in breast cancer treatment. Six Islamic healers in Malaysia were chosen for the Quranic verses selection in which they use similar verses in their breast cancer treatment

Table 1: Series of Qur'anic verses used in Ruqyah Shar'iyyah

Category	Surah and Verses
General	al-Fatihah:1-7, al-Baqarah: 255, al-Hasyr:21-24, al-Ikhlas: 1-4, al-Falaq:1-5, al-Ikhlas:1-6
Specific	al-Baqarah:1-5, al-Baqarah:163-164, al-Baqarah:284-286, Ali `Imran:18, al-A`raf:54-56, al-Mu'minun:116-118, al-Jin:3, al-Saffat:1-10, Ali `Imran: 4, al-'An`am:133-135, Yunus:69-70, Yunus:73,Yunus:88, al-A`raf:90-93, Ibrahim:13-17, Ibrahim:22, Ghafir:70, Ghafir:75-77, al-Kahfi:29, al-Kahfi:59, al-Kahfi:52-52, al-Kahfi:102, al-Kahfi:105-106, al-Nahl:45-46, al-Nahl:85, al-Nahl:88, al-Anbiya':39-41, al-Anbiya':76, al-Anbiya':98, al-Hajj:44, al-Hajj:48, al-Hajj:57, al-Hajj:72, al-Nur:57, al-Zumar:47, al-zumar:51, Fussilat:41, Fussilat:50, al-Saffat:66, al-Saffat:111, al-Furqan:12, al-Furqan:19, al-ahzab:64, al-Isra':97, Taha:97, Taha:100, Hud:39, Hud:58, Hud:66, al-Qasas:81, l-Zukhruf:51, Maryam:37, al-Fil:1-5.

MCF-7 cell culture

The human breast cancer cell line (MCF-7) ATCC HTB-22tm) (Human breast adenocarcinoma) was cultured in 25 cm2 cell culture flask in Roswell Park Memorial Institute (RPMI) 1640 medium and supplemented with 10% (v/v) foetal bovine serum (FBS; TICO Europe, Netherlands) and 1% (v/v) penicillin-streptomycin (Gibco, Thermo Fisher Scientific, USA). The cells were cultivated in the incubator containing humidified atmosphere of 5% CO_2 at 37°C.

Treatment Procedure

The treatment sessions were carried out in a carbon dioxide (CO_2) incubator to ensure controlled in-vitro environment for optimum cell culture growth throughout the procedure. MCF-7 cells were divided into two groups: untreated and treated. Cells in the untreated group served as a control and were not exposed to Qur'anic recitation. The treated group was exposed to Qur'anic recitation for 12 hours and 24 hours using a sound level meter at 50-60 decibel played in the closed chamber. The plates containing the cells were placed inside a sterile chamber with four sterile speakers at each corner to avoid any other interfering factors. During the treatment sessions, a compilation of several Qur'anic verses from the Qur'an was played by the speakers. The Qur'anic verses used are as shown as in Table I according to the category of either general Qur'anic verses or specific Qur'anic verses. General verses were first introduced in the treatment, followed by the specific verses for the breast cancer treatment.

Cell Proliferation Assay

The effect of Islamic spiritual healing on the proliferation of MCF-7 cell line was assessed using Trypan blue exclusion assay. Cells at a density of 3x104 cells /ml (100 ul/well) were seeded in 6-well plate and incubated overnight under 5% CO_2 at 37 °C, followed by exposure to Quranic verses recitation using speaker for 12 hours and 24 hours incubation. The cells without the treatment of Islamic spiritual healing served as the control group. After treatment, MCF-7 cells were trypsinised and incubated in Trypan blue dye (0.2%) for 5 minutes at room temperature. A 20uL aliquot was removed and placed on a Neubauer haemocytometer. The number of viable and non-viable cells were counted under a microscope. The percentage of cell viability was calculated using the following equation in which the non-treated cells were set as 100%.

$$\text{Cell viability (\%)} = \frac{\text{Treated cell OD} - \text{black OD} \times 100}{\text{Non treated cell OD} - \text{blank OD}}$$

Morphological Evaluation using Phase-Contrast Microscopy

Morphological evaluation following the exposure to Islamic spiritual healing on MCF-7 cells was performed using a phase-contrast microscopy. Changes in cell morphology of treated cells were imaged at 100X magnification using an inverted phase contrast microscope (Nikon Instruments, Tokyo, Japan) and were compared to the control group.

Gene expression level

CCNDI gene was determined for the expression level upon Rukyah Shar'iyyah treatment to the MCF-7 in triplicates. Based on the percentage of cell viability, the reduction of cell growth was prominent after 24-hour treatment. Thus, this time point was selected for gene expression analysis. After 24-hour treatment session, MCF-7 cells were harvested, and total RNA was extracted using the innuPREP RNA Mini Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's instructions. RNA quality was measured, quantified, and analyzed using NanoDrop (Thermo Scientific).

50 µL of RNA was eluted from each sample by using Rnase-free water provided with the kit. Only 1 µL of RNA was dropped into the well of QuickDrop™ Micro-Volume Absorbance Spectrophotometer (SpectraMax, USA) to obtain a ratio of 260/280 and 260/230 reading to determine the purity of RNA sample. RNA samples were diluted and standardized to 100 ng/µL prior to cDNA conversion process.

cDNA synthesis

Reverse transcription was performed using the Viva cDNA Synthesis Kit (Vivantis Technologies Sdn Bhd, Malaysia) following the manufacturer's recommended protocol. The following mixture was prepared to give a 10 ul reverse transcriptase (RT) solution: 1 ug/ul of total RNA was added to 1 ul of random hexamers, 11 ul of 10 mM dNTPs mix and top up to 10 ul with nuclease-free water. After proper mixing, the mixture was incubated at 65℃ for 5 min and chill on ice for 2 minutes. Then, a mixture of 2 ul of 10x buffer M-MuLV, 100 unit of M-MuLV reverse Transcriptase with a final volume of 10 ul with nuclease-free water was added to the previous mixture and was mixed gently prior to incubation at 42℃ for 60 min. Later, reverse transcription was inactivated at 85℃ for 5 min and later the mixture was stored at -20℃.

Quantitative real time PCR (RT-qPCR)

Quantitative Real time PCR reactions were performed by TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays using a StepOne™Plus Real-Time PCR Systems (Applied Biosystems, USA). The assay identification number for targeted gene used in this study was *CCND1*: Hs00765553_m1. In a 20 ul reaction solution. 2 ul of cDNA template, 1 ul of TaqMan Gene Expression Assay, 10 ul of TaqMan Universal PCR Master Mix was added to 7 ul of RNAse-free water. The reaction was completed with the following steps: 10 min at 95°C, then followed by 40 cycles of 15 sec at 95°C, 1 min at 60° C. The relative expression of target gene was normalized with b-actin as the endogenous control and calculated using the 2ΔΔCt method. All samples were in measured independently in triplicates.

Statistical Analysis

PRISM 5 Software (GraphPad, La Jolla, CA, USA) was used to perform statistical analysis. The statistical significance of data was obtained by performing one-way ANOVA for multiple-group comparison. p values less than 0.05 were considered to indicate statistical significance.

In silico analysis

Reactome database from Cytoscape software version 3.7.1 was used to perform the in-silico analysis for functional interaction and gene enrichment. The most relevant signaling was based on false discovery rate (FDR) value after rejecting the type 1 error.

RESULTS

Cell viability

The inhibitory effects of Quranic verses used in this study on the cell proliferation is shown in Table 2 and Figure 1. The study demonstrated that the inhibitory effect on MCF-7 cell line gradually increased with the duration of exposure time. After 12-hour treatment with Quranic verses, the non-treated cell lines yielded 95.69% of mean cell viability as compared to the treated group with 93.54%. In a longer duration of treatment with 24 hours, the reduction on cell proliferation was higher with 95.11% for non-treated as compared to 92.34% for treated MCF-7 cell lines.

Table 2: Summary for mean cell viability (%) for 12-hour and 24-hour treatment

Time	Group	Experiment	Cell viability (%)			Mean cell viability (%)	Std. deviation
			Replicate 1	Replicate 2	Replicate 3		
12h	Control	1	96.25	94.74	97.40	95.69	1.535
		2	97.73	95.65	93.83		
		3	96.34	93.15	96.15		
	Treated	1	94.59	94.44	91.55	93.54	1.857
		2	90.70	94.05	92.13		
		3	96.51	95.00	92.86		
24h	Control	1	97.12	95.50	95.15	95.11	1.466
		2	93.58	97.48	94.69		
		3	93.39	93.75	95.33		
	Treated	1	93.20	92.00	92.52	92.34	0.916
		2	91.07	93.16	92.17		
		3	92.31	90.99	93.64		

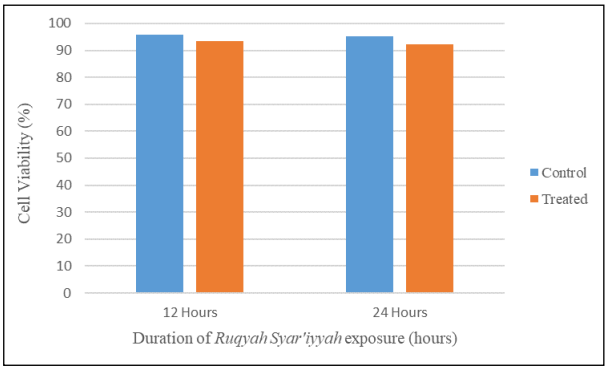

Figure 1: Cell viability (%) of MCF-7 cells after being treated with Ruqyah Shar'iyyah recitation for 12 and 24 hours. The data represents the mean of three independent experiments done in triplicates (n=12) using one-way ANOVA.

Apoptosis

The cell proliferation was consistent with cellular changes for these two time points upon treatment with Quranic verses. The observation using phase-contract microscopy demonstrated the evidence of apoptosis activity with membrane blebbing, cell shrinkage, karyorrhexis and pyknotic bodies. The cellular changes due to apoptosis was more observed in 24-hour treatment as compared to 12-hour treatment. Meanwhile the cellular features such as halo and shade-off contrast patters were also present as shown in Figure 2.

Upregulation of CCND1

The expression level of CCND1 was upregulated upon treatment with Rukyah Sya'iyyah for 24 hours as exhibited in Table 3 with 1.81-fold change based on threshold cycle.

Table 3: CCND1 expression level

Sample	Ct (mean)	Ct of ACTB (mean)	ΔCt	ΔΔCT (ΔCt Treated - ΔCt Non Treated (mean))	RQ
Non-Treated	29.292	36.728	-7.436	-0.858	1.813
Treated	27.454	35.748	-8.294		

Abbreviation: Ct=Threshold cycle, ΔCt=delta threshold cycle, ΔΔCT=delta delta threshold cycle, RQ=relative quantification

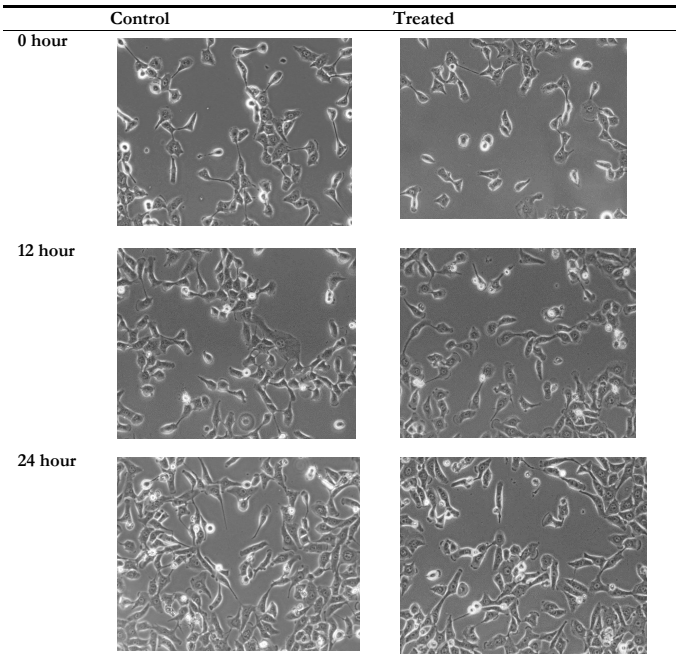

Figure 2: Morphology analysis by phase-contrast microscopy of MCF-7 cells treated with Ruqyah Shar'iyyah recitation for 12- and 24 hours. All images were obtained at a magnification of x100.

Mechanism of CCND1

According to the pathway analysis generated by Reactome database, there were 25 most relevant signalling pathways sorted by p-value for *CCND1*. The top five pathways were *RUNX3*-regulates *WNT* signalling (FDR: 2.05e-05), Oestrogen-dependent nuclear events downstream of *ESR*-membrane signalling (FDR: 8.44e-05) and Transcriptional regulation by *VENTX* (FDR: 1.51e-04), Extra-nuclear oestrogen signalling (FDR: 5.47e-04), Transcriptional regulation by *RUNX3* (FDR: 6.07e-04). Figure 3 and Table 4 represents the top 25 most significant pathways related to *CCND1*.

Figure 3: Genome-wide overview of the results of pathway analysis for *CCND1* generated using Reactome

DISCUSSION

Breast cancer has emerged as a silent threat and killer affecting women across the global population. Complementary and alternative medicine (CAM) has gained significant traction among breast cancer patients

who are Muslims worldwide, serving to alleviate signs and symptoms associated with both pre- and post-operative conventional invasive therapies. It is advisable to incorporate CAM as a supportive measure alongside conventional medicine to address treatment-related side effects.[13] In the present study, the impact of *CCND1* in upregulating multiple signalling pathways in breast cancer was demonstrated, showcasing a decrease in the numbers of cancer cell proliferation and apoptotic activity following treatment with Quranic verses. Notably, there was a slight marked decline in cell viability percentages observed after 12-hour and 24-hour treatments.

Table 4: The 25 most relevant pathways related to CCND1 sorted by p-value.

Pathway Name	Entities	
	p-value	FDR*
RUNX3 regulates *WNT* signaling	4.36e-07	2.05e-05
Estrogen-dependent nuclear events downstream of *ESR*-membrane signaling	3.67e-06	8.44e-05
Transcriptional Regulation by *VENTX*	1.00e-05	1.51e-04
Extra-nuclear estrogen signaling	5.37e-05	5.47e-04
Transcriptional regulation by *RUNX3*	6.07e-05	5.47e-04
Estrogen-dependent gene expression	1.03e-04	7.24e-04
Interleukin-4 and Interleukin-13 signaling	1.94e-04	0.001
ESR-mediated signaling	2.88e-04	0.001
Signaling by Nuclear Receptors	6.53e-04	0.003
Drug-mediated inhibition of CDK4/CDK6 activity	7.92e-04	0.003
PTK6 Regulates Cell Cycle	0.001	0.004
Signaling by interleukins	0.002	0.006
RUNX3 regulates p14-ARF	0.002	0.006
Defective binding of *RB1* mutants to E2F1, E2F2, E2F3	0.002	0.007
Aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects	0.002	0.007
Regulation of RUNX1 expression and activity	0.004	0.008
Aberrant regulation of mitotic cell cycle due to RB1 defects	0.005	0.008
Cytokine signaling in immune system	0.005	0.008
Cyclin D associated events in G1	0.007	0.008
G1 phase	0.007	0.008
Ubiquitin-dependent degradation of cyclin D	0.007	0.008
RMTs methylated histone arginine	0.007	0.008

Since this study only covered up to exposure of 24 hours to Qur'anic recitation, the results were not significant enough to draw any definitive conclusion. Therefore, it is hypothesized that longer treatment durations would may produce more meaningful and significant outcomes, as suggested by Syed Bidin et al. (2020).[14] Therefore, future research should extend the exposure period to 72 or 96 hours to better understand the effects of Ruqyah Shar'iyyah on cancer cells, as indicated by several studies.[14] Additionally, it is worthwhile to further investigate using water as a medium, which might offer better effects. The vibrations from the sound wavelengths can penetrate and alter the water molecules, potentially enhancing their effectiveness in killing cancer cells.[15, 16]

Significantly, alterations in cellular membranes were evident, characterized by cell shrinkage, blebbing of the cellular membrane, and the formation of pyknotic bodies, indicative of the apoptosis mechanism. Despite the minimal difference observed between the 12-hour and 24-hour treatments, a more pronounced effect may potentially be achieved with prolonged exposure and repetitive treatments, as recommended by Quranic healers for their patients. Previous reports have highlighted the therapeutic properties of Quranic verses, demonstrating their ability to impede cell proliferation in normal chondrocytes during the process of wound healing.[17]

The direct recitation of Quranic verses through a speaker emitting 50-60 decibels within an enclosed chamber aims to facilitate wave transmission into cells, potentially inducing alterations in the cell membrane and nucleic acid within the nucleus.[15] Furthermore, the utilization of an anechoic chamber in this research ensures the exclusion of environmental interference, including noise contamination and sound inconsistencies.[15]

Regarding cellular changes, this study delved into the molecular level by examining gene expression. Numerous genes have been implicated in the development of breast cancer, serving as either negative or positive regulators in signalling pathways. Genetic aberrations can alter the normal functioning of these expressed genes. To substantiate the involvement of genes, the study focused on evaluating CCND1 gene expression in the MCF-7 cell line treated with Quranic verses compared to the untreated MCF-7 cell line, revealing a 1.81-fold change in its threshold cycle. *CCND1* was overexpressed in 50% of breast cancers, and its gene amplification is linked to breast cancer recurrence and decreased chemosensitivity.[18,19,20] Cyclin D1 (*CCND1*) encodes crucial oncoproteins involved in cancer cell proliferation across various cancers, including breast carcinoma. Located on chromosome 11q13, its upregulation has been observed in tamoxifen-resistant breast cancer cells. Suppression of *CCND1* leads to a reduction in Cyclin D1

protein, thereby inhibiting the proliferation of cancer cells in tamoxifen-resistant breast cancer cell lines.[21]

Given the evidence of cellular changes, cell proliferation, and *CCND1* upregulation, the Reactome database furnished information on 25 associated signalling pathways. Among these, the top five most significant pathways predominantly participate in regulating cellular proliferation and cell survival by targeting the oestrogen receptor. As shown in Table 4, the pathway most closely linked to *CCND1* is the *WNT* signalling pathway, regulated by *RUNX3*. The binding interaction between *RUNX3*, beta-catenin (*CTNNB1)*, and *TCF/LEF* prevents the *CCND1* and MYC gene promoters, thereby interfering with the *WNT* signalling-mediated activation of *CCND1* and *MYC1* transcription. Consequently, this regulation inhibits *WNT*-induced cellular proliferation.[22] Additional pathways associated with *CCND1* include *MAPK* and *PI3K/AKT*, whose activation can influence oestrogen levels and contribute to cell proliferation through gene dysregulation.[23,24] These pathways, specifically *MAPK* and *PI3K/AKT*, play a role in stimulating the secretion of MMP9, a proteolytic enzyme that degrades the extracellular matrix (ECM), thereby promoting cell invasion and metastasis.[25] It is noteworthy that oestrogen, recognized as a mitogenic agent, disrupts the cell cycle by shortening the G1 phase and advancing it to the S phase, with aberrant expression of *CCND1*.[26] This mechanism has been implicated in over 50% of mammary tumour development.

Regarding apoptosis activity, *VENTX* plays a role in inducing cell cycle arrest by promoting the transcription of cell cycle inhibitors *TP53* and *p16INK4A*. This activation subsequently triggers tumour suppressor pathways regulated by *TP53* and *p16INK4A*, leading to cell differentiation.[27] Additionally, *VENTX* inhibits the transcription of *CCND1*. This mechanism has been observed in the development of various cancers, including colon and breast cancer.[28]

CCND1 has been shown to participate in oestrogen-dependent transcription, where beta oestradiol (E2) binds to receptors on the plasma membrane, serving a non-genomic role in extra-nuclear signaling.[29,30] This extra-nuclear signalling induced by E2 operates independently of the transcriptional activity of oestrogen receptors. The ongoing mechanism activates various signalling pathways, including the *RAF/MAK* kinase cascade and *PI3K/AKT* signaling cascade, governing processes such as apoptosis, cellular proliferation, and metastasis.[24] Furthermore, extra -nuclear signalling engages in cross-talk with nuclear oestrogen receptor signalling and is essential for controlling ER protein stability, thereby contributing to the development of breast cancer.[31]

RUNX3 functions as a transcription factor, playing a crucial role in DNA binding through heterodimerization with *CBFB* (CBF-beta), thereby regulating gene expression in various essential developmental pathways.[32] Its participation in neurogenesis and its identification as a tumour suppressor gene in carcinogenesis have been demonstrated. Previous studies have revealed the inactivation of *RUNX3* in human breast cancer cell lines, evident through hypermethylation of the RUNX3 promoter, hemizygous deletion of the RUNX3 gene, and cytoplasmic sequestration of the RUNX3 protein.[28] Consequently, the silencing of *RUNX3* via promoter hypermethylation in breast cancer predisposes Runx3+/- individuals to the development of breast cancer by targeting the oestrogen receptor alpha (ER+SR1) protein.[33] RUNX3 inactivation typically occurs as an early event in breast cancer progression, characterized by downregulation.[34]

CONCLUSION

Our results indicate that Ruqyah Shar'iyyah as a complementary medicine is a promising non-invasive treatment for breast cancer patients to use alongside conventional treatments such as surgery, radiotherapy, and chemotherapy. The treated breast cancer cells exhibited cell death due to apoptotic activity. Although the differences between the control and treatment groups were minimal and insignificant, the treatment groups did show positive effects, particularly over extended exposure times. It can be concluded that longer treatment durations may yield better results. Therefore, it is recommended to practice Qur'anic recitation consistently as a supplementary therapy along

with conventional medicine to improve the condition of breast cancer patients.

This suggestive evidence reveals the reduction on cell viability of MCF-7 breast cancer cell line was due to the upregulation of *CCND1*. The identification of associated signalling pathways with *CCND1* elucidates potential biomarker implicated in the pathogenesis of breast cancer upon treatment with selected Quranic verses in Ruqyah Shar'iyyah.

CONFLICT OF INTEREST

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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