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Research Article

Anticancer effects of falsa (Grewia asiatica) extracts against breast cancer cells

Khadija Rashid, Mahwish Aftab
Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan

Abstract

Background: Breast cancer is the most common cancers among females around the globe. There are treatment options for breast cancer, but with substantial side effects. Low survival rate and advanced stages highlight the need to discover new therapeutic compounds. Purpose of this study was to investigate the effects of *Grewia asiatica* (falsa) extracts exposure on cell viability and expressional modulation of cell cycle genes (CCND1, CCND2 and CDKN1A) in breast cancer cells.

Methods: As a first step, toxic effect of falsa extracts were identified against breast cancer cell line MCF-7. Cell viability was identified by MTT dye reduction assay. Afterwards, expressions of the genes (CCND1, CCND2 and CDKN1A) were identified by real time PCR methodology. Untreated samples were used as controls while the data was analyzed by 2-ΔΔCT method to identify fold changes. The difference in fold changes was compared with the untreated controls.

Results: The results indicated that the extracts showed moderate anti-proliferative effects against MCF-7 cell line. Real time PCR results showed potential of the fruit extracts to alter expression of genes (CCND1, CCND2 and CDKN1A) in MCF-7. CDKN1A (up to 2.3fold) and CCND1 (up to 1.4fold) genes were upregulated in MCF-7 cells treated with the extracts while CCND2 was downregulated (up to -1.7fold).

Conclusion: Falsa extracts induce cell death and alter the expression of cell cycle related genes to halt the growth process in breast cancer cells.

Key Words: Cancer, Grewia asiatica, Anticancer, Cyclins

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer worldwide and a leading cause of cancer-related mortality among women. According to GLOBOCAN 2020, breast cancer accounts for approximately 2.3 million new cases annually, representing 11.7% of all cancer diagnoses globally [1]. Despite major advances in detection and treatment, breast cancer remains a significant public health challenge, particularly in low- and middle-income countries where late-stage diagnosis is common due to limited awareness and screening facilities [2].

Early detection plays a critical role in reducing breast cancer mortality. Screening techniques such as mammography, ultrasound, and magnetic resonance imaging have substantially improved early diagnosis and survival rates. However, disparities in access to these technologies contribute to variations in incidence and mortality across regions [3]. Lifestyle and environmental factors, including obesity, physical inactivity, alcohol consumption, smoking and adoption of Western dietary patterns are well-established contributors to breast cancer risk, emphasizing the importance of preventive strategies [4]. Breast cancer is a biologically heterogeneous disease, characterized by diverse molecular and genetic alterations. Germline mutations in genes significantly elevate lifetime breast cancer risk by impairing DNA repair pathways, leading to genomic instability [5]. Advances in molecular profiling have enabled classification of breast cancer into distinct subtypes. Luminal A, Luminal B, HER2-

Corresponding Author: Khadija Rashid
Email: kdrashid123@gmail.com
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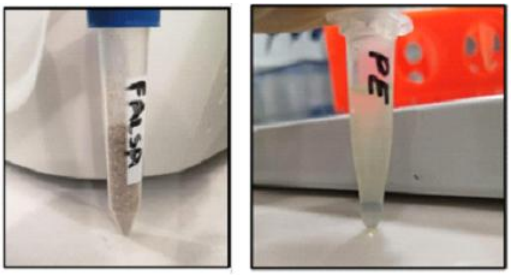
positive and triple-negative, each subtype is associated with different prognoses and therapeutic responses [6]. This molecular understanding has paved the way for personalized and targeted treatment approaches. Recent therapeutic advances, such as CDK4/6 inhibitors, PARP inhibitors, HER2-targeted agents, and immune checkpoint inhibitors, have significantly improved outcomes in both early-stage and metastatic breast cancer [7, 8]. Long-term survivors experience treatment-related complications, including fatigue, neuropathy, lymphedema, musculoskeletal dysfunction and reduced quality of life, highlighting the need for comprehensive survivorship care [9]. In parallel with conventional therapies, increasing attention has been given to the role of dietary and plant-derived bioactive compounds in cancer prevention and adjunct treatment [10, 11]. Polyphenols, flavonoids and proanthocyanidins, commonly found in fruits such as grapes and falsa exhibit antioxidant, anti-inflammatory, anti-proliferative and pro-apoptotic properties in preclinical cancer models [12-14].

Grewia asiatica, commonly known as falsa, is a medicinally important fruit-bearing plant belonging to the family Malvaceae. It is widely cultivated in South Asia and has been traditionally used for its nutritional and therapeutic properties. The fruit and other plant parts are rich in bioactive compounds, including anthocyanins (cyanidin-3-glucoside), flavonoids, phenolic acids, vitamin C and carotenoids, which are known for their antioxidant and anticancer potential. Several *in vitro* and *in vivo* studies have demonstrated the anticancer activity of *G. asiatica* extracts against multiple cancer types. Marya et al. (2011) evaluated aqueous extracts of different parts of *G. asiatica* using the MTT assay and reported significant cytotoxic activity against breast and liver cancer cell lines, indicating selective anticancer effects. Importantly, these extracts showed minimal toxicity toward normal cells, suggesting a favorable therapeutic index [15]. Further investigations using methanolic extracts of *G. asiatica* confirmed cytotoxic effects against a range of cancer cell lines, including breast (MCF-7), lung (NCI-H522), kidney (HEK-293), cervical (HeLa) and laryngeal (Hep-2). The extracts exhibited stronger inhibitory effects on breast and lung cancer cells, while limited activity was observed against normal cell lines, highlighting cancer-specific cytotoxicity. *In vivo* studies have also supported the anticancer potential of *G. asiatica*. Administration of methanolic fruit extracts at doses of 250 and 500 mg/kg significantly inhibited tumor growth in Ehrlich's ascites carcinoma (EAC)-bearing mice and increased survival rates by 42% and 61%, respectively. These effects were attributed to the induction of apoptosis, inhibition of tumor cell proliferation and enhancement of antioxidant defense mechanisms [16]. Overall, existing preclinical evidence suggests that *G. asiatica* possesses promising anticancer properties, particularly against breast cancer. As the integration of dietary interventions with standard treatments may provide a supportive approach to reducing treatment-related toxicity and improving patient outcomes. Continued research focusing on prevention, personalized medicine and integration of natural bioactive compounds is essential to further improve breast cancer outcomes worldwide.

METHODS

Preparation of the extracts

Seeds of falsa were collected from the available fruits from market. Briefly, 2 grams of falsa seeds were weighed and placed in falcon tube. 5 ml of 50% methanol was added and mixed vigorously followed by placement in stirring incubator for 15 minutes at 37 ºC. After stirring, liquid fractions were eluted by centrifugation at 3000 rpm for 10 minutes at room temperature and supernatant were removed. The supernatant was poured in eppendorf and placed in speed vac at 45 ºC under vacuum till the evaporation of methanol present in tube. After the evaporation of methanol, the weight of the extract was measured and dissolved in culture medium.



Falsa seeds Supernatant of falsa seeds

Breast cancer cell culture

Breast cancer cell line MCF-7 obtained from ATCC was cultured using RPMI-1640 media. Media was made with 10% fetal bovine serum, penicillin and streptomycin. Breast cancer cell line cell stock, stored at -80 ºC, was taken out and to nullify the effect of DMSO present in stock solution, five times volume of media was added and centrifuged. Supernatant media was discarded and fresh complete new media was added to resuspend the pallet. The cell cultures were maintained at standard incubation conditions and were used for subsequent experiments.

MTT assay for cytotoxicity

Cytotoxic effect of falsa seeds extracts was investigated by MTT dye reduction assay. For this purpose, the cells were cultured in 96-well plates (4000 cells/well/100µl media) and treated next day with the fruit seed extracts for 24, 48 and 72 hours (3.7-250µg/ml) to see the concentration and time dependent effects. Afterwards, plates were incubated in incubator at 37 ºC. Surviving cell fractions from the treated and untreated cells (control group) were examined by adding 10µl/well MTT solutions (5 mg/ml in PBS) and after 3 hours of incubation, formed crystals were dissolved by adding 50µl of DMSO. Optical density was measured by ELISA plate reader at wavelength 540 nm with 690 nm reference filter. Surviving cells were shown as percentages as compared to untreated control set to 100% as reference. Experiment was executed with at least three replicates of each group.

Expression profiling of genes

MCF-7 cells were cultured in 6 well plates at a density of 200,000 cells/well /2ml media and exposed to different concentrations (100, 200, 300µg/ml). The cells were exposed for 48 hours, followed by collection of cell palettes and storage at -80 ºC immediately. Untreated MCF-7 cells were used as controls in these experiments. Total RNA content from collected cell pellets was extracted by using a commercially available kit (Thermo Fisher, Cat#K0731) following the manufacture's protocol. RNA was quantified by using Nanodrop and a total of 500 ng extracted RNA/sample was used to synthesize cDNA by using Revert Aid First Stand cDNA Synthesis Kit (Thermo Fisher Cat#K1622). For the verification of cDNA synthesis process, a reference gene HPRT1 was amplified via conventional PCR methodology. Primers for the selected genes were designed using Primer 3 software and were optimized by using gradient PCR method. Real time PCR was performed by using SybrGreen fluorescence dye for the selected genes by using prepared cDNA samples from MCF-7 cells treated with different concentrations of extracts of falsa for 48 hours. Following the amplification procedures, 2-ΔΔCT method was used to calculate expressional changes in the selected genes (CCND1, CCND2, CDKN1A) by comparing Ct values of experimental and untreated control samples.

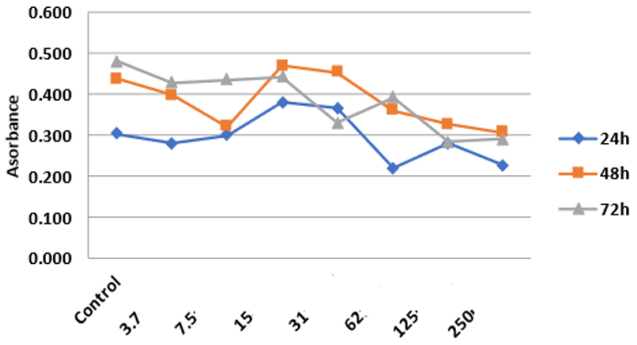
RESULTS

Cytotoxicity of falsa seeds extract

Falsa extract exposure inhibited the proliferation of MCF-7 cells. Lighter color of formazan crystals in response to treatment, produced in purple in 96 well plate, showed the lesser number of viable cells present (data not shown here). The effects were calculated numerically as absorbance in MS Excel and shown Figure 2. The decline in curved showed decreased optical density as measured by ELISA reader, which in turn reflected the light color produced by a smaller number of viable cells. The effects were concentration dependent as minimum inhibition of proliferation were observed with lower concentration of the extracts while maximum inhibition of proliferation were observed with increased concentration of extracts.

Expression changes induced by falsa extracts

The cDNA synthesized from cell line MCF-7 after exposure with the fruit extract at different concentrations (100µg/ml, 200µg/ml and 300µg/ml) were used to investigate expressional modulation in different genes. qRT-PCR methodology was used to investigate the expressional changes while cDNA samples of untreated cells were used for comparison as controls. HPRT1 was used as reference gene to normalize the data sets. All the cDNA samples were processed in triplicates. The impact of falsa extracts exposure on genes is shown as bar charts (fold changes). At low concentration of the extract, it showed moderate induction (1.42fold) in CCND1 gene but showed high induction at further higher concentration of the compound. Falsa extract showed inhibition of CCND2 gene (-1.72fold) at 100µg/ml concentrations but interestingly, at 200 and 300µg/ml concentration, falsa extract showed almost no change in induction of gene. Falsa extract showed continuous induction of CDKN1A gene (1.13, 1.89 and 2.07fold changes respectively) at all selected concentrations (100, 200 and 300µg/ml). In case of CDKN1A gene, the induction of this gene was directly proportional to concentrations that meant at high concentrations, higher induction of gene was observed.



Time interval	Control	3.7µg/ml	7.5µg/ml	15µg/ml	31µg/ml	62µg/ml	125µg/ml	250µg/ml
24h	0.304	0.280	0.301	0.381	0.366	0.219	0.280	0.227
48h	0.438	0.398	0.321	0.470	0.454	0.360	0.327	0.307
72h	0.480	0.429	0.436	0.442	0.330	0.393	0.285	0.292

Figure 2. The breast cancer cells were exposed to falsa extracts and cell viability was measured by using MTT assay. The absorbance values were used to plot the line graphs while the readings obtained are also shown in the tabular form. The extracts inhibited proliferation of the tested cancer cell line.

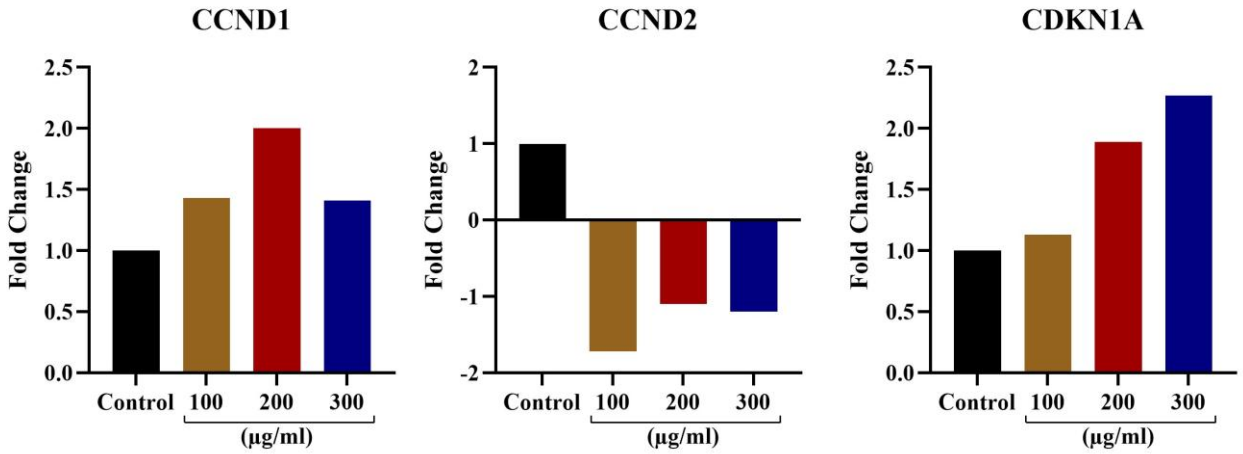


Figure 3. Breast cancer cells were exposed to falsa extracts and changes in expression data of the genes were evaluated by real time PCR method. Fold changes were calculated while normalizing the data sets and compared with untreated control sets as one.

DISCUSSION

Because of low treatment effectiveness and significant adverse effects, researchers are trying to detect natural compounds that have therapeutic potential and minimum negative impacts against cancers [17, 18]. The present findings demonstrate that falsa extract significantly inhibits the proliferation of MCF-7 breast cancer cells in a concentration-dependent manner. The reduction in cell viability, as evidenced by decreased formazan crystal formation in the MTT assay and corresponding absorbance values, indicated a marked cytotoxic effect of the extract on the cancer cells. The lighter purple coloration observed in treated wells compared to controls reflected reduced mitochondrial metabolic activity, which correlates directly with lower number of viable cells. Quantitative analysis of optical density further confirmed these observations, as declining absorbance values corresponded with increasing extract concentration. The progressive decrease in optical density from 24 to 72 hours suggests that the inhibitory effect of the extract indicating sustained bioactivity and possible

cumulative cellular damage. These findings align with growing evidence that plant-derived phytochemicals possess significant anticancer properties. Enhanced inhibitory effect at higher concentrations further strengthens the argument for the presence of potent bioactive compounds within the extract. Similar results have been reported by other researchers [19-21]. Overall, the results suggest that falsa extract exhibits promising antiproliferative activity against MCF-7 cells and may serve as a potential candidate for further investigation in breast cancer therapeutics.

To figure out molecular reasons, expression analysis of cell cycle related genes was conducted. The gene expression analysis demonstrated that falsa extract modulates key regulators of cell cycle progression in MCF-7 cells in a concentration-dependent manner. The observed changes in CCND1, CCND2 and CDKN1A expression suggest that the extract influences molecular pathways governing cell proliferation and growth control. The selected cyclins and inhibitor play a very important role in cell cycle progression. The upregulation of CCND1 at increasing concentrations indicates that the extract may initially stimulate CCND1 associated signaling. However, the concurrent and progressively stronger induction of CDKN1A suggests activation of cell cycle inhibitory mechanisms at the same time. Since CDKN1A is a well-known cyclin-dependent kinase inhibitor, its dose-dependent induction implies potential enforcement of cell cycle arrest despite CCND1 modulation. The direct proportionality between extract concentration and CDKN1A expression strengthens the hypothesis that higher doses promote stronger growth-inhibitory signaling. In contrast, CCND2 expression was suppressed at the lowest concentration but returned to near-basal levels at higher doses, indicating a differential regulatory response. This biphasic pattern may reflect selective pathway targeting or compensatory regulatory mechanisms within the cyclin network.

Overall, the gene expression profile supports the antiproliferative effects observed in viability assays, suggesting that falsa extract may exert its anticancer activity through modulation of cell cycle regulators, particularly via induction of CDKN1A-mediated growth arrest. Further mechanistic studies are required to clarify the signaling pathways involved and to determine whether these transcriptional changes translate into functional cell cycle arrest or apoptosis.

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Declaration of generative AI

During the preparation of this work, the authors used ChatGPT (GPT-5.1; free version) developed by OpenAI to improve the English language and clarity of the manuscript. Afterwards, the authors carefully reviewed and edited the content and take full responsibility for the content of the article.

Author Contributions: Khadija Rashid executed experiments and wrote the manuscript. Mahwish Aftab helped with data analysis and interpretations.

Competing Interests: Authors declare no competing interests.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.