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Estimate the in Vitro Effect of Panax Ginseng Root Extract on the Expression of p53 gene of MCF-7 Cancer Cell Lines

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A B S T R A C T

Breast cancer is a worldwide health issue that affects women. The majority of the drugs now used to treat breast cancer have harmful side effects. Oriental medicine ginseng root provides numerous health advantages and may have direct anti-cancer effects. The purpose of this study was to evaluate how ginseng affected the MCF-7 Cancer Cell Lines' p53 gene expression. Ginseng root's cytotoxic effects on MCF-7 cell lines were evaluated using the MTT cell viability test in 96-well plates. The number of cell lines seeded each well was 1x104. Cells were treated with 1 and 2 mg/well of the evaluated ginseng root extract after 24 hours, or when a confluent monolayer was achieved. The RNeasy® Mini Kit was used to extract all of the genomic RNA from MCF-7 cell lines in order to measure the p53 gene's expression both before and after therapy. The cytotoxicity rate for a concentration of 2 mg/well reached 78.4%, while for a concentration of 1 mg/well, the cytotoxicity rate reached 39.7%, which exhibited significant ($P \leqslant 0.05$) changes then the untreated control group. Folding changes ranged from (0.747-1.214) in 2mg/well group, with an average of 1.009565, while in 1mg/well group, folding changes ranged from (1.765-11.471) with an average of 4.328188. The results exhibited a significant (P=0.001) elevated in gene expression in treated groups in contrast to the control group. Where in control group, folding changes average was 3.394422, This means that ginseng root extract increased gene expression at both concentrations, but was better at the 2mg/well concentration. It was found that ginseng root extract has cytotoxic effects on the MCF-7 cancer cell line, as the cytotoxicity rates were very high. The root extract also led to an increase in the gene expression of p53, which encodes a tumor suppressor protein that plays a vital role in regulating the cell cycle and preventing tumor formation

Keywords: Panax genus, MCF-7, Breast cancer, p53 gene

1. Introduction

Ginseng is a perennial herb that is a member of the Panax genus and the Araliaceae family. Because it contains active ingredients, the root of the plant is favored [1]. According to the method of processing, ginseng is classified as follows: sun ginseng, which is made by steaming white ginseng under high pressure and temperature, white ginseng, which is between four and six years old, prepared by peeling and oven- or air-drying, and red ginseng, which is six years old and steamed without peeling [2]. Ginseng is considered to have a number of pharmacological qualities and is one of the most widely used herbal treatments in Asia [3]. Ginsenosides, commonly known as steroid-like saponins, are found only in ginseng species [4]. Numerous studies have demonstrated the efficacy of ginsenosides in the treatment of neurological and cardiovascular disorders [5,6], and in the prevention of breast, lung, and colon cancer [7,8]. Specifically, ginsenoside enhanced quality of life following chemotherapy for epithelial uterine cancer, according to a clinical investigation [9]. According to certain research, several ginsenosides can cause cancer cell lines to undergo apoptosis and suppress the NF-κB signaling pathway and cell growth [10,11,12]. Worldwide, the most prevalent malignant tumor in women is breast cancer. Patients with breast cancer make for much to 36% of cancer patients. It is expected that in 2018, 2.089 million women received a breast cancer diagnosis [13]. Many carcinogenic elements continuously induce ductal hyper-proliferation, which is the primary cause of breast tumors. The microenvironment of the tumor affects how breast cancer starts and develops [14,15]. Over the past few decades, medicinal plants have come to be recognized as efficient and affordable sources of synthesized new chemotherapeutic chemicals. As a result, many researchers have directed their attention into this exciting field [16]. It has been observed that patients typically self-medicate with plant items. They employ goods made from whole or parts of plants, which is a widespread practice in all global cultures [17]. This study was performed to assess the effects of ginseng on the expression of p53 gene of MCF-7 Cancer Cell Lines.

2. Materials and Methods

2.1 Maintenance of Cell Line Cultures

The human breast cancer cell line MCF-7 was cultured and maintained in RPMI-1640 media supplemented with 100 µg/mL streptomycin, 100 U/mL penicillin, and 10% fetal bovine serum (Capricorn, Germany). Following two weekly reseedings at 80% confluence with Trypsin-EDTA, the cell passage was cultivated at 37° C in 5% CO2 [16].

2.2 Ginseng root extract

50g of ginseng root powder was taken. A round-bottom flask was used to measure and store this powder. The root powder was placed in a round-bottom flask and filled with 200 milliliters of 70% ethanol. After being put in a reflux extractor, the mixture remained for 12 hrs. After the extraction phase, the mixture was filtered through Whatman filter paper (No. 31) to separate the extracted solution from the residual. After filtering, the extract was placed in a previously cleaned petri dish and let to dry naturally in the shade. The dried ginseng extract is collected and stored for later use at 4°C in a firmly sealed container [18,19].

2.3 Cytotoxicity assays

Ginseng root's cytotoxic effects on MCF-7 cell lines were evaluated using the MTT cell viability test in 96-well plates. The number of cell lines seeded each well was $1x10^4$. Cells were treated with 1 and 2 mg/well of the tested ginseng root extract after 24hr., or when a confluent monolayer was achieved [20]. To assess the viability of the cells, the medium was taken out after 72 hours of treatment, 28 µL of 2 mg/mL solution of MTT was added, and the cells were allowed to culture for 2.5 hr. at 37°C. A microplate reader was used to measure the absorbance at 492 nm during the triplicate assay. The following formula was used to calculate the percentage of cytotoxicity, or the inhibition rate of cell growth [21]:

$$Cytotoxicity = A - B / A * 100$$

where A and B are the optical density values of control and tested samples, respectively

2.4 RNA extraction

A total of genomic RNA was extracted from MCF-7 cell lines by using the RNeasy® Mini Kit (QIAGEN).

2.5 Quantitative PCR (qPCR)

RNA was extracted from total RNA samples and reverse-transcribed using a high-capacity cDNA kit (ProtoScript® II First Strand cDNA Synthesis Kit). Following the manufacturer's instructions, Trizol was used to extract RNAs from MCF-7 cell lines. With 2 µg of total RNA, cDNA synthesis was accomplished using the Revert AidT MH Minus First Strand cDNA Synthesis Kit (Canada). MiRNA gene amplification was performed with specific primers, as listed in Table (1).

Table 1 - PCR Primers.

Primer name		**Sequence ('5---- 3')**	**Ref.**
p53 gene	Forward	5-TTGAGGTGCGTGTTGTG-3	[22]
	Reverse	5-CTTCAGTGGCTGGGAGTG-3	
GAPDH	Forward	5-ACCACAGTCCATGCCATCAC-3	
	Reverse	5-TCCACCACCCTGTTGCTGTG-3	

mRNA levels were measured by RT-qPCR using SYBR-Green Reagents. Following 40 cycles of denaturation at 94°C for 20 seconds, annealing step at 60°C for 1 minute, and extension at 72°C for 30 seconds, the amplification conditions were determined. As indicated in table (2), the Real-Time PCR program was set up in accordance with the prescribed thermocycling methodology

Table 2 - Real time-PCR program for amplification.

Cycle Step	**Temperature °C**	**Time**	**Cycles**
First Denaturation	95°C	60sec	1
Denaturation	95°C	15sec	40
Extension	60°C	30sec	
Melt Curve	60-95°C	40 min.	1

2.7 Statistical Analysis

For statistical analysis, Microsoft Excel 2019 was utilized. Descriptive analysis of the parameters under investigation is displayed by error bars.

3. Results & Discussion

Figure (1) shows the cytotoxicity rate of ginseng root extract, where the cytotoxicity rate for a concentration of 2 mg/well reached 78.4%, while for a concentration of 1 mg/well, the cytotoxicity rate reached 39.7%, which showed exhibited (P≤0.05) changes then to the untreated control group.

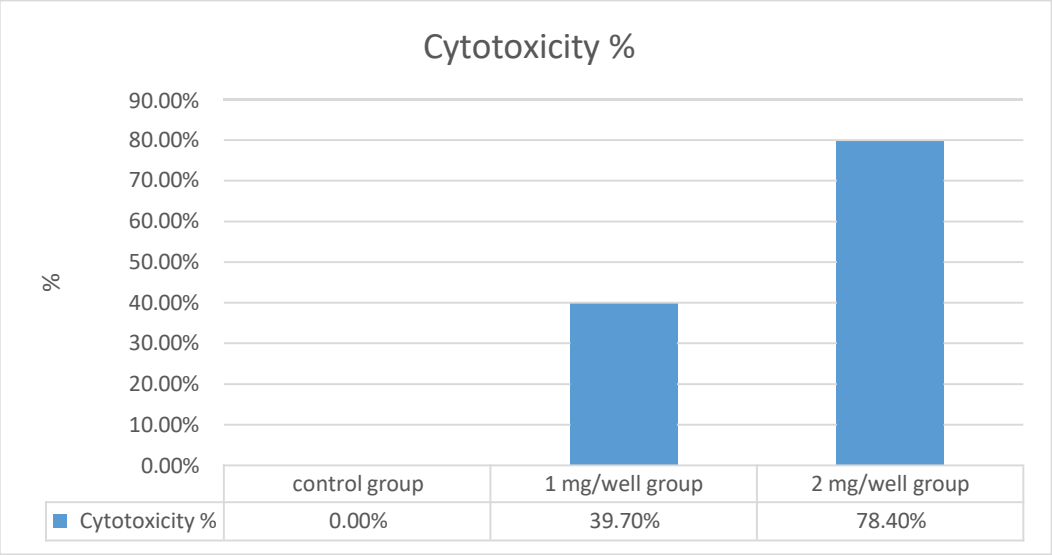

Fig. 1 - The cytotoxicity rate of ginseng root extract.

Because breast cancer is aggressive and can spread to distant locations, it is the most common cause of death for women [23]. Breast cancer is a worldwide health issue [24]. Natural products are currently receiving increased attention in the fight against cancer. Natural compounds have demonstrated a number of anticancer benefits [25, 26]. Numerous research have demonstrated ginseng's anticancer properties against a variety of cancer types [27,28,29,30]. The current study examines the effect of ginseng extract on breast cancer cell lines. Ginseng suppresses the proliferation of breast cancer cells in a dose-dependent manner, according to the current study. The treated groups' reduced cell counts could be the result of growth inhibition and/or apoptosis. In a different study on breast cancer, ginsenoside Rd therapy decreased lung tumor lesions in an experimental and spontaneous metastatic paradigm. Ginsenoside 2 was linked to this process because it increased Smad2 expression while decreasing miR-18a expression [31]. Furthermore, ginsenoside 20(S)-protopanaxadiol was shown to strongly suppress the growth and lung metastasis of triple-negative breast cancer in a prior in vitro and in vivo investigation. The process involved ginsenoside 20(S)-protopanaxadiol reversing EMT and inhibiting the EGFR-mediated MAPK signaling pathway [32]. Ginsenosides have been shown in numerous studies to inhibit different kinds of breast cancer cells. Cell cycle arrest in triple-negative breast cancer cells was facilitated by ginsenosides Rk1 and Rp1 [33, 34]

3.1 P53 gene expression

Table (3) shows that gene expression in positive samples, based on ΔCT, ranged from (6.1-8.7) in control group, while in 1mg/well group, ΔCT ranged from (6.3-8.3) and in 2 mg/well ΔCT ranged from (5-6.3). Folding changes ranged from (0.747-1.214) in 2mg/well group, with an average of 1.009565, while in 1mg/well group, folding changes ranged from (1.765-11.471) with an average of 4.328188. The results showed a significant (P=0.001) increase in gene expression in treated groups compared to the control group. Where in control group, folding changes average was 3.394422, This means that ginseng root extract increased gene expression at both concentrations, but was better at the 2mg/well concentration.

Table 3 - The expression level of the p53 gene in treated and control groups.

Groups	P53	GAPDH	ΔCT	ΔΔCT	2-ΔΔCt	Folding	Mean	P value
	30.3	25.3	5	-0.88	1.840375301	1.01395948		
	30.1	25.1	5	-0.88	1.840375301	1.086734863		
2 mg/well	31.3	25	6.3	0.42	0.747424624	1.164733586	1.009565	
	31	25.2	5.8	-0.08	1.057018041	0.768437591		
	30.7	25.1	5.6	-0.28	1.214194884	1.01395948		
	31.8	25.5	6.3	0.42	0.747424624	2.329467173		
	31.4	25	6.4	0.52	0.697371833	1.765405993		
	33.6	25.3	8.3	2.42	0.186856156	3.530811985		
1 mg/well	32.7	25.1	7.6	1.72	0.303548721	2.67585511	4.328188	
	33.6	25.8	7.8	1.92	0.26425451	11.47164198		0.001
	33.5	25.7	7.8	1.92	0.26425451	4.993322196		
	33.9	24.5	9.4	3.52	0.087171479	3.530811985		
Control	32.3	24.6	7.7	1.82	0.283220971	1.337927555		
	33.9	25.2	8.7	2.82	0.141610486	1.892115293		
	33.4	24.8	8.6	2.72	0.151774361	1.647182035		
	32	25.9	6.1	0.22	0.858565436	6.147500725	3.394422	
	33.4	25.9	7.5	1.62	0.325335464	3.073750363		
	32.7	25.3	7.4	1.52	0.348685917	6.588728138		
	32.8	25.1	7.7	1.82	0.283220971	3.073750363		

In Figure (2) Curves of amplification were the X-axes represent the number of cycles while Y-axes represent in testing Florence. Positive samples showed curve shape of amplification at different cycle number on roy channel, while negative specimens display know curve amplification at any point of the cycle number and will remain flit blow the threshold level of amplification.

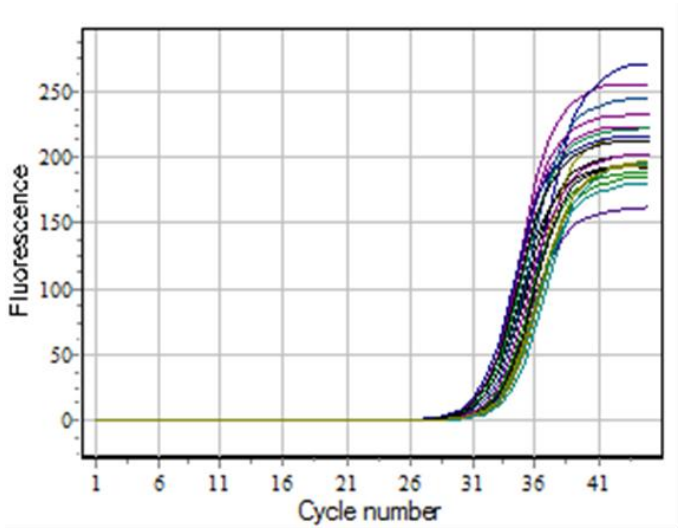

Fig. 2 - Cy5 channel p53 gene amplification shows a positive sample with a curve shape at a distinct cycle number.

Upstream regulatory proteins like p53 cause downstream genes to become active, which in turn causes either cell death through apoptosis or cell repair (cyclearrest). Different techniques, such as downregulating special genes, transactivating unique genes, and separate transcription mechanisms, are used to accomplish the various pathways [35]. The elevation of p53 in MCF-7 cancer cell lines after ginseng root extract treatment is described for the first time in this paper. Other plant extracts have shown how the plant extract affects the expression of the p53 gene in different cancer cell lines. In A549

lung cancer cells, Euryale feroxSalisb extract caused Akt to be downregulated and p53 to be upregulated, resulting in apoptotic behavior and cell cycle arrest [36]. Although the importance of extracts and the other essential oils as anticancer medications for promising cancer treatment has been examined in a number of studies, additional research, such as safety and toxicity studies, is required before they enter clinical trials. Previously, the formation of ROS [37] was associated with the position of p53 in the mitochondria and the release of cytochrome-c in the cytoplasm [38] in cell death brought on by p53 over-expression using traditional markers of mammalian apoptosis.

4. Conclusions

It was found that ginseng root extract has cytotoxic effects on the MCF-7 cancer cell line, as the cytotoxicity rates were very high. Additionally, p53 gene expression increased as a result of the root extract. P53 encodes a tumor suppressor protein that is essential for controlling the cell cycle and halting their growth.

Acknowledgements

Acknowledgements and Reference heading should be left justified, bold, with the first letter capitalized but have no numbers. Text below continues as normal.

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