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Expression of Matrix Metalloproteinase-9, P53 and B-cl2 Genes in Human Osteosarcoma Cell Line Treated with Ginger Extract

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Aim: The current study was designed to explore the potential effect of ginger extract on the osteosarcoma cell line (MG-63).

Material and Methods: Human osteosarcoma cell line (MG-63) was acquired from Nawah-Scientific. The tested drug was ginger extract prepared by maceration in semi-liquid form. The potential impact of ginger extract upon MG-63 cells was evaluated using cytotoxicity assay, microscopic evaluation, Enzyme-Linked Immunosorbent Assay for evaluation of Matrix Metalloproteinase-9 (MMP-9), and Real-Time Polymerase Chain Reaction for detection of the expression levels of the pro-apoptotic gene (P53) and antiapoptotic gene B-cell lymphoma 2 (B-cl2) in ginger extract-treated cells. Then the data collected was statistically assessed.

Results: The outcomes demonstrated that ginger extract's cytotoxic impact upon MG-63 cells was dose-dependent, as viability increased as long as the concentration decreased. Under the microscope, MG-63 cells treated with ginger extract displayed apoptotic signs such as chromatin condensation, cell shrinkage, and plasma membrane blebbing. Data about MMP-9 showed that, ginger extract dramatically and dose-dependently reduced MMP-9 levels. Regarding P53 and B-cl2, data revealed that ginger extract significantly increased P53 levels and decreased B-cl2 in a dose-dependent manner.

Conclusion: Ginger extract substantially diminishes the viability of osteosarcoma cells because of its strong cytotoxic activity against MG-63 cells.

Key words: Ginger extract, osteosarcoma, apoptosis, P53, B-cl2, and Polymerase Chain Reaction.

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Introduction

Osteosarcomas (OSs) are the most prevalent type of bone cancer, representing approximately 40-60% of all primary bone tumors. This aggressive cancer typically strikes the growing bones of children and adolescents, often targeting the metaphysis of the long bones. A rare and less common form of OS is jaw osteosarcoma (JO), which accounts for only 6% of all OS cases. [1]

The underlying causes of OS are still not well understood. Unlike many other types of cancer, there is no single genetic alteration that is commonly found in OS cases. Instead, cancer is often characterized by a complex genetic makeup. [2]

Epidemiologic, genetic, and environmental variables, such as chromosomal abnormalities, ionizing radiation, and alkylating chemicals, all contribute to the etiology of OS. [3]

Furthermore, there is an elevated mutation rate in the tumor suppressor genes RB1 and P53, which could contribute to the early stages of the disease. Studies have repeatedly demonstrated that the environment of the bone is important for the development and resistance to treatment of OS. [4]

OS is treated using a multidisciplinary approach. Chemotherapy and surgery are the main treatments for OS. [5]

High-dose methotrexate and combinations of cisplatin, etoposide, ifosfamide, and doxorubicin are the most often utilized chemotherapeutic medications.[6]

When treating metastatic lesions in particular, chemotherapy is a very successful treatment strategy. Unfortunately, chemotherapy may not always completely destroy all cancerous cells, and some cells may reappear after a short period of time. [7]

Cancer chemotherapy side effects can be temporary or long-term, such as hypersensitivity reactions, chemotherapy-induced nausea and vomiting, constipation and diarrhea, mucositis, tiredness, and neurotoxicity. [8]

Scientists are turning their attention to naturally-derived chemicals since they are thought to have less side effects than chemotherapy. [9]

We have access to a wide variety of therapeutic plants in nature that can improve our health. Research has shown that a broad range of plant species, along with their bioactive constituents, possess anti-cancer properties that inhibit the growth and spread of cancerous cells. [10]

Naturally generated plant chemicals are often more tolerated and non-toxic to normal human cells. [9]

Ginger (Zingiber officinale Roscoe), an evergreen with a lengthy history of use, has been prized for its culinary and medicinal properties. [11]

Raw fibers, lipids, phenols, terpenes, polysaccharides, and organic acids are all found in ginger. The phenolic components of ginger, which include pinene, zingiberol, shogaols, gingerols, capsaicin, and gingerdione, are primarily responsible for the health benefits of ginger; the most common active ingredient is gingerol. [12]

Ginger has been shown in numerous research to possess anti-inflammatory, antibacterial, cardiovascular, neuroprotective, anticancer, respiratory, anti-obesity, antidiabetic, anti-nausea, and anti-emetic qualities. [13]

According to studies, different types of cancer cells grow more slowly and die more quickly in lab conditions when ginger and its constituents are present. This indicates that ginger has anti-cancer capabilities. This shows that ginger could be helpful as a natural cancer treatment or prevention agent.[14]

Ginger extracts possessed a strong cytotoxic effect on ovarian cancer cell line through different mechanisms. [15]

Ginger extract exhibited significant cytotoxic activity and highly powerful inhibitory properties against the cell lines of breast cancer (MCF7) and hepatocellular carcinoma (HePG2). [16]

Ginger and its constituents have demonstrated anticancer impact by downregulating the proliferation and inducing cell death in a variety of cancer cell types in vitro. [17]

Usually, discussions of cell death are binary, focusing on either necrosis or apoptosis. Apoptosis, often referred to as coordinated cell death, is a process of sequential pattern of cell death. [18]

Apoptosis causes morphological shrinkage and pyknosis in cells; this is characterized by chromatin condensation, DNA breakage, cytoplasmic compacting, and plasma membrane blebbing. [19]

A sudden breakdown of plasma membrane integrity during necrosis causes a proinflammatory, unintentional type of cell death. [20]

Organelles enlarge, the cell's plasma membrane ruptures and lyses, and the internal contents seep into the surrounding tissue, resulting in tissue damage when a cell undergoes necrosis. [21]

Thus, the role of this research was to explore the effect of ginger extract on osteosarcoma cell line (MG-63).

Material and Methods

Ethical statement: The current study was executed according to the ethical guidelines set forth by the Research Ethics Committee of the Faculty of Dentistry, Minia University, Egypt, under approval number 95 on March 28, 2023.

I.Material

A.Cell line, Culture and Treatment

Osteosarcoma cell line (MG-63) was obtained from Nawah-Scientific, Al Mokattam Egypt. MG-63 cells were brought from the "American Type Culture Collection (ATCC)" with the reference number "CRL-1427™".

The growth media consisted of 500 mL of RPMI-1640 plain medium supplemented with 50 mL of heat-inactivated fetal bovine serum (FBS) and 5 mL of penicillin and streptomycin.

Trypsin EDTA 0.25% and FBS were purchased from GIBO COBRAL Limited in Scotland and stored at -20° C until needed. Phosphate buffer saline (1.15g Na2 HPO, 0.20 gm KHPO4, 9 gm KCI, 8 gm Nacl, and 1000 ml double distilled water) was autoclaved for twenty minutes at 121° C with HCI or NaOH to alter the pH.

B.The Tested Agent

Ginger extract was prepared in Nawah-Scientific in a semi-liquid form.

Extraction of Ginger Root by Maceration

The provided sample (750 g) was divided into little pieces, dried by hot air and ground using a grinder (model: RRH-1000AK, KARIZMA, Italy) then mixed with 1.5 L of ethanol 70% at room temperature. The ethanolic extracts were collected and allowed to evaporate under vacuum at 40°C, yielding 17.6 g.

Study Design

In the present study, we had four groups based on the viability test:

Group I: Control group MG-63 cells treated with dimethyl sulfoxide (DMSO).

Group II: MG-63 cells were treated with the pre IC_{50} concentration of ginger extract for 24 hours.

Group III: MG-63 cells were treated with the IC_{50} concentration of ginger extract for 24 hours.

Group IV: MG-63 cells were treated with the post IC_{50} concentration of ginger extract for 24 hours.

II.Methods

1. **Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening**

Adherent MG-63 cells were grown to ~80% confluency. Cell density was determined using a hemocytometer. Plates were incubated for 24 hours at 37°C in a humidified incubator with 5% CO_2.

Detached cells were washed out using PBS, the % of remaining live cells was determined using SRB Stain by adding 50 µl of SRB solution for each well. Plates were incubated at room temperature in dark conditions for 15 minutes. The following formula was used to determine the proportion of cytotoxicity:

$$\text{Cytotoxicity}\% = \frac{\text{OD}_{\text{DMSO}} - \text{OD}_{\text{sample}} \times 100}{\text{OD}_{\text{DMSO}}}$$

This assay was used to ascertain the pre-IC_{50}, half-maximal inhibitory concentration (IC_{50}), and post-IC_{50} concentrations of ginger extract.

2. Microscopic Examination using Haematoxylin and Eosin Stains

For cytological analysis, pelleted cells were re-suspended in PBS, and a portion (50 µL) was placed on the glass slide that had been cleaned with ethanol, allowed to air dry, and fixed with methanol.

After three minutes of immersion in filtered haematoxylin stain, the slides were twice cleaned with distilled water. After five seconds of immersion in filtered eosin stain, the slides were cleaned with distilled water. After being mounted with Canada balsam and submerged in xylene, the dried slides were covered with coverslips and allowed to dry.

To capture microscopic fields, oil immersion photomicroscopy was used at a power of x1000. A digital video camera (C5060, Olympus, Japan) mounted on a BX60 light microscope (Olympus, Japan) was used to do this at Minia University. After that, pictures were added to the computer system so they could be examined.

3. Enzyme-Linked Immunosorbent Assay

The concentration of Matrix Metalloproteinase-9 (MMP-9) in ginger extract-treated cells was evaluated using Abcam's MMP-9 Human ELISA kit. The assay was performed following the manufacturer's instructions.

Each standard concentration-treated cells were dispensed into different wells of ELISA plate as 100 µL in the suitable wells. After covering the plate, it allowed to incubate either overnight or for 2.5 hours at room temperature. at 4°C while being gently shaken.

After discarding the test and standard solutions, the plate was washed four times using a wash buffer. Each well received 100 µL of 1X Biotinylated MMP-9 Detection Antibody, which was applied and gently shaken for an hour at room temperature. The plate was washed as previously. Each well received 100 µL of 1X HRP-Streptavidin solution, which was then gently shaken and left for forty-five minutes at room temperature.

Each one well received 100 µL of the Tetramethylbenzidine (TMB) One-Step Substrate Reagent. After giving the mixture a quick shake, it was left at room temperature for 30 minutes in the dark. Following the addition of 50 µL of Stop Solution to each well, a 450 nm scan was performed immediately. The mean absorbance of every duplicate standard set was ascertained.

4. Real-Time Polymerase Chain Reaction

Using Real-Time Polymerase Chain Reaction (RT-PCR), the P53 and B-cell lymphoma 2 (B-cl2) gene expression levels in cells treated with ginger extract were investigated.

The one-step SYBR-Green I-based real-time RT-PCR was carried out using the iScriptTM One-Step RT-PCR Kit with SYBR® Green premix (Biorad, Hercules, California, USA) and the iCycler Real-Time

PCR apparatus (Biorad, Hercules, California, USA).

The specimens were analyzed in a 25 µl solution with 0.2 µM of each primer, 5 µl of isolated RNA, 0.25 µl of RNA transcriptase, and 12.5 µl of iScriptTM SYBR® Green premix 2X.

5. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 26.0 window program was used to conduct One-Way Analysis of Variance (ANOVA) on the experimental data to determine the significance of differences between groups according to MMP-9 activity assay. The results of the ANOVA test were shown as mean ± standard deviation. A *P*-value of less than 0.05 was regarded statistically significant.

Results

1. Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening

The 24-hour assessment focused on the cytotoxic impact of ginger extract on MG-63 cells. The cytotoxic pattern was dose-dependent, according to the collected data.

As the concentration of ginger extract increased, the mean viability percentage of MG-63 treated cells decreased relative to control cells, as exhibited in figure (1) and table (1).

Table 1: The estimated mean viability percentage (%) of MG-63 cells treated with ginger extract for 24 hours at three different concentrations: low concentration (296.4µg/ml), medium concentration (420.11µg/ml), and high concentration (982µg/ml).

Sample	**MG-63 treated cells with ginger extract for 24 hours' incubation**		
Concentration µg/ml	**Pre IC_{50}** (296.4)	**IC_{50}** (420.11)	**Post IC_{50}** (982)
Viability%	75%	50%	25%

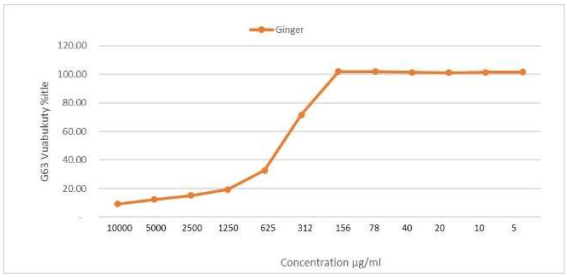

Figure 1: The cytotoxic effect of 12 different concentrations of ginger extract on MG-63 cells for 24 h with IC50 concentration recording 420.11µg/ml.

2. Microscopic Examination using Haematoxylin and Eosin Stains

Control Group

MG-63 cells exhibit dysplasia-related characteristics like cellular and nuclear pleomorphism, increased nuclear cytoplasmic ratio, nuclear hyperchromatism, and few cells showed signs of apoptosis such membrane blebbing, as showing in figure (2A).

Pre IC_{50} Treated Group

As presented in figure (2B), this group showed early apoptotic signs including: apoptotic cells with peripheral chromatin condensation, and membrane blebbing.

IC_{50} Treated Group

This group exhibited apoptosis in certain forms: membrane blebbing, apoptotic cells with peripheral chromatin condensation, apoptotic bodies (black arrow), and swollen necrotic cells with swollen nucleus, as revealed in figure (2C).

Post IC_{50} Treated Group

This group exhibited both signs of apoptosis and marked necrosis such as: apoptotic cells with peripheral chromatin condensation, apoptotic bodies, shrunken apoptotic cells, swollen necrotic cells with swollen nuclei, and necrotic debris (orange arrow), as illustrated in figure (2D).

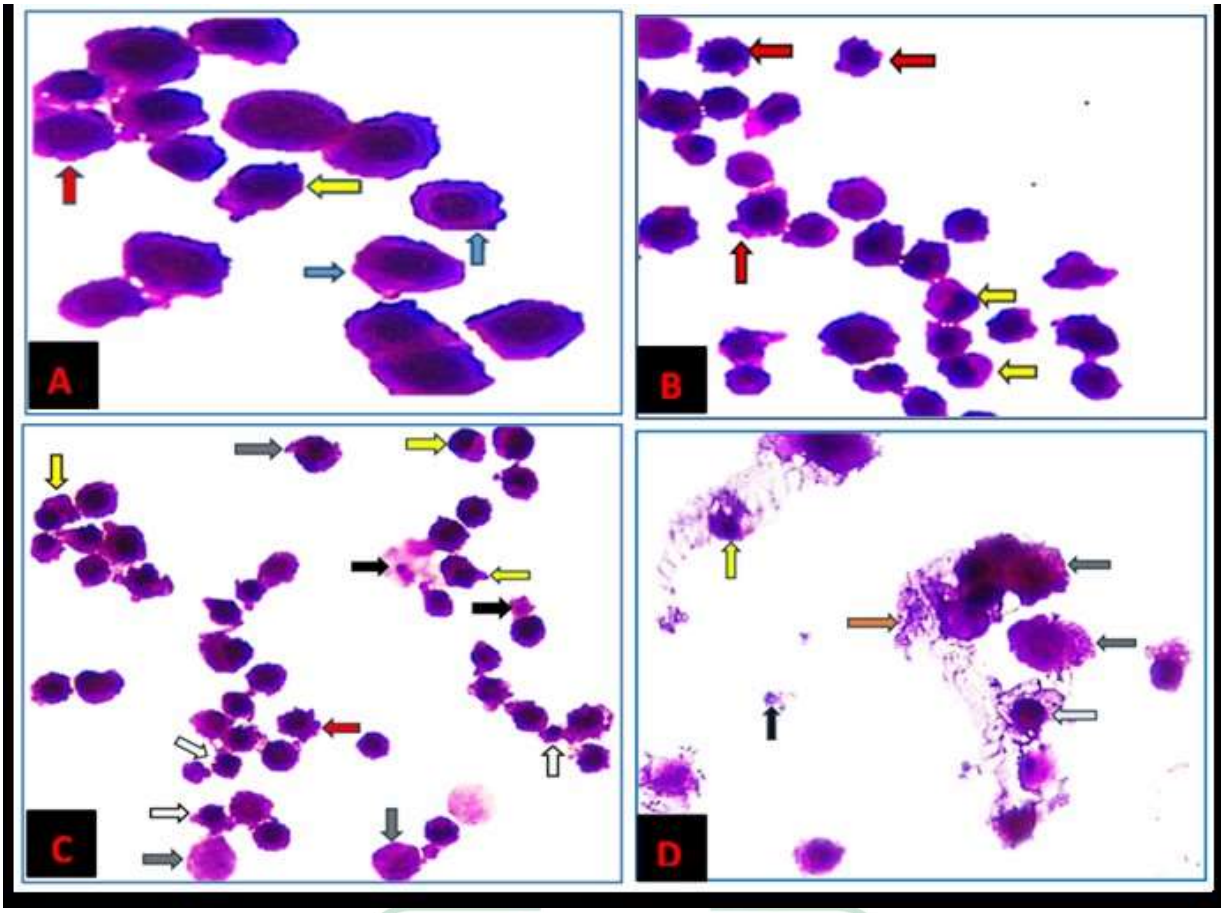

Figure 2: A photomicrograph displaying: (A) control cells showing nuclear hyperchromatism (red arrow), increased nuclear cytoplasmic ratio (blue arrow), and membrane blebbing (yellow arrow), (B) pre-IC50 treated cells demonstrating peripheral chromatin condensation (yellow arrow), and membrane blebbing (red arrow), (C) IC50 treated cells revealed membrane blebbing (red arrow), apoptotic cells with peripheral chromatin condensation (yellow arrow), shrunken apoptotic cells (white arrow), apoptotic bodies (black arrow), and swollen necrotic cells with swollen nucleus (grey arrow), and (D) Post-IC50 treated cells illustrated peripheral chromatin condensation (yellow arrow), apoptotic bodies (black arrow), shrunken apoptotic cells (white arrow), swollen necrotic cells with swollen nuclei (grey arrow), and necrotic debris (orange arrow) (H and E, oil, 1000X original magnification).

3. **Enzyme-Linked Immunosorbent Assay**

There was a significant decrease in matrix metalloproteinase-9 (MMP-9) expression as contrasted to the control group. Definitely, the expression of *MMP-9* remarkably decreased in dose dependent manner from 1144.5 pg/ml in control group to 477.8 pg/ml, 230.2 pg/ml, and 183.5 pg/ml in pre-IC_{50}, IC_{50}, and post-IC_{50} groups respectively, as illustrated in table (2).

4. **Real-Time Polymerase Chain Reaction**

RT-PCR analysis of the P53 and B-cell lymphoma 2 (B-cl2) genes in MG-63 cells revealed that, relative to the control group (1-fold), the P53 gene values increased to 3.0553-fold in pre-IC50 to 4.4605-fold in

IC50, then 6.6729-fold in post-IC50 of ginger extract. This is shown in table (3).

The B-cl2 gene values were 1fold, 0.6934-fold, 0.472-fold, and 0.3543-fold for control, pre-IC_{50}, IC_{50} and post IC_{50} groups correspondingly, as shown in table (3).

Table 2: Matrix metalloproteinase-9 activity assay among the studied groups.

Ser	Code	MMP-9 (pg/ml)
1	Control MG-63 cells	1144.5
2	Pre IC_{50}-treated cells	477.8
3	IC_{50}-treated cells	230.2
4	Post IC_{50}-treated cells	183.5

Table 3: The expression levels of P53 and B-cell lymphoma 2 genes in MG-63 cells treated with various doses of ginger extract using Real-Time PCR analysis.

Ser	Code	P53 (µg/ml)	B-cl2 (µg/ml)
1	Control MG-63 cells	1	1
2	Pre IC_{50}-treated cells	3.0553	0.6934
3	IC_{50}-treated cells	4.4605	0.472
4	Post IC_{50}-treated cells	6.6729	0.3543

5. Statistical Analysis

A statistically significant difference was found between MG-63 cells treated with different doses of ginger extract for a 24-hour period and control MG-63 cells, according to a one-way ANOVA test. *P*-value was less than 0.0001, indicating statistical significance between the studied groups according to MMP-9 activity assay, as shown in table (4).

Table 4: Descriptive statistics of one-way ANOVA test for the mean values of MMP-9 activity assay in different groups of MG-63 cells.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1446177.250	3	482059.083	63.603	< 0.0001
Within Groups	30316.950	4	7579.238		
Total	1476494.200	7			

Discussion

Osteosarcoma (OS) is a primary bone malignancy that is extremely aggressive and affects 3.4 million people worldwide each year.[22] Three to four percent of all cancers in young people are caused by OS. [23]

OS occurs sporadically, with only a few cases linked to known inherited cell cycle regulatory disorders, but over 70% of tumor tissues exhibit a chromosomal aberration. Alterations in tumor suppressor genes or DNA helicases are usually involved.[22]

The current course of treatment for recently diagnosed OS comprises neoadjuvant chemotherapy, adjuvant chemotherapy following surgery, and surgical excision of the primary tumor and any clinically discernible metastatic disease.[24]

However, due to the nature of chemotherapy, it kills both cancer and healthy cells resulting in adverse effects. Chemotherapy side effects have an impact on an individual's emotional, physical, and overall well-being. Reducing the dosage of chemotherapy can manage these side effects.[25]

The use of herbal remedies as anticancer drugs dates back ten years. They have been displayed to have anti-

inflammatory qualities and to contain a variety of components with anticancer properties, including direct cytotoxic impacts and indirect regulation in the tumor microenvironment, cancer immunity, and chemotherapy progression. [26]

Several studies have revealed that the mix of natural pharmaceuticals and chemotherapy can be utilized to motivate the effectiveness and reduce the adverse outcomes and difficulties of medical operations. [27]

Ginger contains bioactive substances such as phenolic compounds, flavonoid compounds, and essential oils, all of which contribute to pharmacological activity. Gingerol is the most common phenolic molecule found in ginger rhizomes, which also contain shogaol, paradol, zingerol, gingerones, and gingerdiones. [28]

Pharmacological studies have illustrated the possible anticancer effects of ginger and the related bioactive components. [29] Ginger has anticancer effects against many cancer types such as melanoma, breast cancer, ovarian cancer, and head and neck squamous carcinoma. [30]

This experiment's objective was to explore the potential effect of ginger extract on MG-63 cells. This was done by employing the cytotoxicity assay, histological examination, Enzyme-Linked Immunosorbent Assay (ELIZA) and Real-Time Polymerase Chain Reaction (RT-PCR).

In the present study we had four groups; Group I: was the control group MG-63 treated with dimethyl sulfoxide (DMSO) to exclude its role, Group II: MG-63 cells were treated with the pre-IC_{50} concentration of ginger extract, Group III: MG-63 cells were treated with the IC_{50} concentration of ginger extract, and Group IV: MG-63 cells were treated with the post-IC_{50} concentration of ginger extract.

First, Sulforhodamine B Colorimetric (SRB) cytotoxicity assay was done to explore the impact of the ginger extract on MG-63 after 24 hours' incubation and to calculate the pre-IC_{50}, IC_{50}, and post-IC_{50} values of ginger extract.

The viability of MG-63 cells was found to be concentration-dependent 24 hours after treatment. The values of the pre-IC_{50}, IC_{50}, and post-IC_{50} were recorded in the following order: 296.4 µg/ml, 420.11 µg/ml, and 982µg/ml, respectively. The corresponding viability percentages were 75%, 50%, and 25% for the pre-IC_{50}, IC_{50}, and post-IC_{50}. SRB indicated that ginger extract has strong anti-growth and cell death properties against MG-63 cells.

El-Sayeh et al.[16] corroborated the present findings and demonstrated that an 80% methanolic ginger extract had a noticeable cytotoxic activity and was discovered to have extremely strong inhibitory properties against the cell lines for breast cancer (MCF7) and hepatocellular carcinoma (HepG2). Reactive oxygen species were reduced by the extract's IC_{50} when it was applied to the HepG2 and MCF7 cell lines.

According to experimental studies, ginger derivatives' chemo-preventive properties include the ability to alter the evolution of the cell cycle. Both monolayer and cancer-stem cell-like spheroids showed a stopping of the cell cycle in the G2/M phase following the treatment of breast cancer cells (MCF-7 and MDA-MB-231). Additionally, 6-shogaol inhibited stem cell self-renewal. [30]

In terms of the histological analysis, when MG-63 cells were exposed to ginger extract at the pre-IC_{50} dose, they displayed early signs of apoptosis, including peripheral chromatin condensation and membrane blebbing.

The IC_{50}-treated group showed apoptotic indicators such as peripheral chromatin condensation, apoptotic bodies, cellular and nuclear shrinkage, and membrane blebbing. The cell viability

dropped and there were more evidence of necrosis and apoptosis in the group that had received post-IC_{50} treatment.

Most anticancer drugs currently utilized in clinical oncology rely on intact apoptotic signaling pathways to cause cancer cell death without triggering inflammatory response. [31]

Since matrix metalloproteinase-9 (MMP-9) can cleave a large number of extracellular matrix (ECM) proteins to control ECM remodeling, it has a major impact on the advancement of OS by encouraging tumor growth, invasion, and metastasis. In order to liberate numerous plasma surface proteins from the cell surface, it can also break them. [32]

Using ELIZA, this study confirmed that ginger had anti-invasion and anti-metastasis properties on MG-63 cells by decreasing MMP-9 expression levels from 1144.5 pg/ml in the control group to 477.8 pg/ml, 230.2 pg/ml, and 183.5 pg/ml at 24 hours for pre-IC_{50}, IC_{50}, and post-IC_{50} dosages of ginger extract, respectively.

Corrosponding to the current data, Pashaei-Asl et al.[15] revealed that investigations using the MTT assay showed that the ginger extracts had significant cytotoxic consequences on ovarian cancer, SKOV-3 accompanied by a dramatically decreased MMP-9.

The present observation was corroborated by Wang et al.[33] 6-shogaol and 6-gingerol efficiently stop the invasion and metastasis of hepatocellular carcinoma by suppressing the MAPK and PI3k/Akt pathways, downregulating NF-κB and STAT3 activities, and inhibiting MMP-2/-9 and uPA.

Then, to study the molecular mechanism of ginger extract-induced apoptosis in MG-63 cells, RT-PCR utilized to measure the expression level of P53 and B-cell lymphoma 2 (B-cl2) genes in control and studied groups.

The P53 is considered the "guardian of the genome" due to its crucial role in keeping the genome intact. When cells are in a healthy state, they can engage many transcriptional programs such as cell cycle arrest, DNA repair, senescence, and death, which limit tumor formation, by activating the P53 signaling pathway. [34]

It was noticed that the pro-apoptotic gene (P53) showed a significant upregulation that indicated that ginger extract treatment triggered cell death in MG-63 cells. The values rose from 1-fold in control group to 3.0553-fold, 4.4605-fold, and 6.6729-fold in the ginger extract for pre-IC_{50}, IC_{50}, and post-IC_{50} concentrations, respectively.

Moreover, Pant et al.[35] agreed with the current findings and stated that P53-target genes are significant in arrest of cell cycle by encoding P21protein and apoptotic pathway by activation of apoptotic regulators Bax and Bak.

The current reported expression profile following the outcomes of Pashaei-Asl et al.[15] demonstrated that, in contrast to the control group, the ovarian cancer cell line treated with ginger extract had P53 expression levels that were almost seven times higher.

The anti-apoptotic gene (Bcl-2) exhibited a significant downregulation which improved the apoptotic properties of ginger extract on the MG-63 cells as the values decreased from 1-fold in control group to 0.6934-fold, 0.472-fold, and 0.3543-fold in pre-IC_{50}, IC_{50}, and post IC_{50} concentration of ginger extract correspondingly.

Bcl-2 proteins are key regulators with anti-apoptotic activities. Actually, they have the ability to permanently prevent cells from going through apoptosis and turn them into malignant clones. [36]

Over-expression of anti-apoptotic Bcl-2 genes or reduction of pro-apoptotic P53 family proteins, both resulting in

decrease in apoptosis, are frequently detected in many cancer. [37]

The outcomes of Pashaei-Asl et al.[15] were matched with the present expression profile reporting that Bcl-2 expression was reduced by more than 0.4 times following a 48-hour ginger intervention as contrasted with the control group.

Fan et al.[38] concurred with the present findings and approved that 6-gingerol treatment raised the levels of cleaved caspase-3, caspase-8, and caspase-9 as well as the mRNA ratio of Bax to Bcl-2, indicating that 6-gingerol may change Bcl-2 proteins and trigger the osteosarcoma cells' apoptotic mechanisms.

Similarly, Wang et al.[33] indicated the effect of 6-gingerol on promyelocytic leukemia-induced apoptosis and suppression of Bcl-2 expression. HL-60 cell.

Lastly, statistical analysis displayed a statistically significant difference among the different studied groups. The P-value was less than 0.0001. This corroborated the study findings, demonstrating a positive association between ginger extract concentrations and lower MG-63 cells viability.

Conclusions

Ginger extract dramatically reduced the viability of human osteosarcoma cells. Ginger extract had a cytotoxic activity against human osteosarcoma by enhancing the expression of the P53 gene and downregulating the expression of the B-cell lymphoma 2 gene (B-cl2) and matrix metalloproteinase-9.

Conflict of Interest

The authors have no financial conflicts of interest.

Funding

No fund.

Data availability

All data generated or analyzed during this study are included in this published article.

Ethical approval and consent to participate

The current study was executed according to the ethical guidelines set forth by the Research Ethics Committee of the Faculty of Dentistry, Minia University, Egypt, under approval number 95 on March 28, 2023.

Competing interests

The authors declare no competing interests

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