Original Article

Apoptotic Effects of Ginger Extract (*Zingiber officinale*) on Esophageal Cancer Cells ESO26: An *In Vitro* Study

Abbas Abbasi[1], Ali Azizi[2], Seidmostafa Nachvak[1], Elaheh Alizadeh[1], Rezvan Abbsavaran[1], Elham Mirtaheri[1], Mehdi Moradinazar[3], Mehrali Rahimi[4]

[1]Faculty of Nutrition and Food Industry, Pharmaceutical Science Research Center, [2]Department of Community Medicine, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran, [3]Assistant Professor in Epidemiology, Behavioral Disease Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran, [4]Diabetes Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Aim: Ginger is a natural dietary rhizome with antioxidant, anti-inflammatory, and anticarcinogenic properties. It has many medical beneficial properties such as anti-proliferation and antiapoptotic effects on cancerous esophageal cells. **Materials and Methods:** Esophageal cancer cells ESO26 were cultured in the presence and absence of ginger extract at various concentrations for 12, 18, and 24 h. Then, the viability was determined by 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay. Western blot analysis of caspase-3 was performed to detect apoptosis. p21, *Bax*, and *Bcl-2* gene expression was measured using quantitative polymerase chain reaction (PCR). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. **Results:** The ginger extract increased the cleavage of caspase-3 in cells ($P < 0.05$). Results of real-time PCR have shown that ginger decreased the expression of *Bcl-2* and increased *Bax* and *p21* gene expression ($P < 0.05$). **Conclusions:** Results showed that the process of cell proliferation has been stopped. Also, this study indicated that ginger might exert a chemopreventive effect on esophageal cancer through the suppression of proliferation and the growth of tumor cells as well as the induction of apoptosis.

Keywords: *Apoptosis, caspase-3, esophageal cancer, ginger*

Introduction

In esophageal cancer, esophagus tissue cells are involved.[1] Inhibiting cell proliferation and increasing apoptosis in tumor cells are effective strategies to prevent tumor growth.[2] Apoptosis is an essential process during development and is also an active form of cell suicide controlled by a network of genes, which plays a key role in the pathogenesis of diseases, including cancer.[3] Several studies strongly suggested that defect in the process of apoptosis is the main factor in the onset and progression of cancer.[4]

Ginger has been prescribed for many diseases[5] due to its antioxidant, anti-inflammatory, and anticarcinogenic properties.[6] Some of the ginger components are gingerols, shogaols, paradols, zerumbone, and gingerdiols.[7] A study showed that the major components of ginger (6-shogaol and 6-gingerol) have anti-invasive activity against hepatoma cells.[7] Ethanol extract of ginger decreased the size, incidence, number, and multiplicity of cells in mouse skin[8] and human colorectal cancer cells.[9] Ginger supplementation suppresses colon carcinogenesis in the presence of procarcinogen.[10] Recent studies showed that 6-gingerol as main component of ginger inhibited nuclear factor kappa B (NF-κB) activation by impairing the nuclear translocation of NF-κB, suppression of cIAP expression and increased trial-induced caspase-3/7 activation in gastric cancer cells.[11] The inhibitory effect of 6-gingerol on proliferation of hepatoma cells has been shown in cell culture.[12] One study suggested that ginger arrested cell cycle and induced apoptosis.[11] Animal studies showed that 6-shogaol prohibited growth of pancreatic cancer and increased the effects of gemcitabine in the suppression of tumor growth.[13,14] Also 6-gingerol induces apoptosis in the prostate cancer cell.[15,16] Also, zerumbone could inhibit Vascular endothelial growth factor (VEGF) expression, cell proliferation, and NF-κB activation in gastric cancer cell lines.[17,18]

There is a lot of information about the anticancer effects of the ginger. However, its molecular mechanisms in esophageal cancer cells are not well known. The herbal extract has more anticancer effects than the purified components of ginger.[19] This study was undertaken to investigate impacts of ginger ethanol extract on the growth of esophageal

How to cite this article: Abbasi A, Azizi A, Nachvak S, Alizadeh E, Abbsavaran R, Mirtaheri E, *et al*. Apoptotic effects of ginger extract (*Zingiber officinale*) on esophageal cancer cells ESO26: An *in vitro* study. J Rep Pharma Sci 2020;9:183-8.

Received: 14 Sep 2019
Accepted: 10 May 2020
Published: 07 Oct 2020

Address for correspondence:
Dr. Mehrali Rahimi, Department of Internal Medicine, Kermanshah University of Medical Science, Kermanshah, Iran.
E-mail: majavarda@yahoo.com

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_98_19

Quick Response Code:

cancer cell lines. Although chemopreventive role of ginger has been studied,[18-20] very little information is available about anticancer role of ginger in esophageal cancer.

Materials and Methods

Ginger extract

Ginger crude extract was obtained using ethanol extraction provided by Noor Azian Murad protocol of Lipids Engineering Applied Research (CLEAR) Center, University Technology Malaysia.[21] The preparation steps include cutting, washing, drying, and grinding. At the end, a little alcoholic extract of ginger was obtained. To dry the ginger pieces, they were placed in an incubator at 45°C. The ginger was dried before the extraction using rotary evaporator in ethanol (1 L) for 6 h. The solvent was removed using vacuum at 500 mbar within 2 h, followed by 400 and 300 mbar in the next 3 h to yield extract, a brown viscous liquid (9.80 g, 4.9%).

Cell culture

ESO26 cells were cultured primarily in Dulbecco's Modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 110 mg/L sodium pyruvate, and 3.7 g/L sodium bicarbonate at 37°C and 5% CO_2. Penicillin (100 U/mL), streptomycin (100 mg/mL), and amphotericin B (2.5 mg/L) were added to the culture media for the inhibition of bacterial and fungal contamination. Every 2 days and at the confluent stage, cells were trypsinized and subcultured in three new flasks at a density of 1×10^6 cells per 25 cm^2 flask.

MTT assay

The 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine the cytotoxicity. The extraction of ginger was dissolved in ethanol, and the final organic solvent concentration in the cell culture was 1% (vol/vol). Control cultures were exposed to the solvent only. Cells were seeded in MTT (Sigma, USA) supplemented with phosphate-buffered saline (PBS) (0.1 mg) in the presence and absence (as controls) of the extraction of ginger, which was added into each well at different concentrations and then incubated at 37°C for 4 h. The MTT formazan crystals, 1-(4, 5-dimethylthiazol-2-yl)-3, 5-diphenylformazan, were dissolved by the addition of acid–isopropanol (0.04 N HCl in isopropanol) to stop the cleavage of the tetrazolium ring by dehydrogenase enzymes, which convert MTT to an insoluble purple formazan in living cells and mixed at room temperature. After 20 min, the level of colored formazan derivative was determined by measuring the optical density (OD) with a microplate reader (model 3550; Bio-Rad, USA) at 570 nm (OD 570–620). The mean OD value of the content of four wells was used for assessing the cell viability expressed as percentage of control; Viable cells (%) = [(total cells – dead cells)/total cells] × 100%.

SDS-PAGE and Western blot analysis

Cell lysate was performed using lysis buffer (20 mM Tris–HCl pH 7.4, containing 50 mM NaCl, 1% NP-40, 10% glycerol, 1 mM PMSF, and 2 mM ethylenediaminetetraacetic acid [EDTA]). Freshly added protease inhibitor cocktail (Sigma). After 10 min keeping in 0°C, the suspension was centrifuged in 14,000×*g* for 15 min at 4°C, and the supernatant was collected. Then, the protein extracts were quantified using Bradford assay, and equal amounts of total protein (20 μg) were boiled in sodium docecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for 10 min and loaded per lane and segregated by 12.5% SDS-polyacrylamide gel at 150 V. In the next step, protein bands were transferred to polyvinylidenedifluoride (PVDF). The membranes were washed three times with PBS containing 0.05% (vol/vol) Tween 20, and then blocked in PBS–bovine serum albumin (BSA) 2% (wt/vol) for 2 h. Then it was incubated overnight at 4°C in the presence of primary antibodies against caspase-3, for 2 h in secondary (rabbit anti-mouse IgG, horseradish peroxidase [HRP] conjugated) antibodies after washing five times in each step. Finally, the blots were exposed to HRP substrate solution (3,3¢,5,5¢-Tetramethylbenzidine (TMB) and H_2O_2) to detect target proteins. All Western blot analyses were repeated at least three times on different cell culture samples. Blots were quantified by scanning densitometry using Image J software (developed by Wayne Rasband, National Institutes of Health, and Bethesda, Maryland; available at http://rsb.info.nih.gov/ij/index.html).

Quantitative real-time polymerase chain reaction

Total ribonucleic acid (RNA) of cells was extracted using high pure isolation kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. Total RNA was assessed qualitatively and quantitatively using a spectrophotometer (NanoDrop 2000, USA), and samples were stored at -80°C for further investigations. A one-step quantitative test was performed on RNA expression using CYBR Green kit. Cells were treated with ginger crude extract for 18 h. The primer sequences were completely similar to those performed in our previous study. The synthesis of complementary deoxyribonucleic acid (cDNA) was carried out at thermal cycler conditions corresponding to 15 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C to denature the DNA and 45 s at 60°C to anneal and extend the template. The melting curve was analyzed at temperatures in the range of 65°C–95°C with a temperature transient rate of 0.1°C/s. All reactions were performed in triplicate in a Stratagene MX 3000P system (USA). The values obtained for the target gene expression were normalized to β-actin, and analyzed by the relative gene expression -ΔΔCT method, where -ΔΔCT = (CT target – CT β-actin) unknown – (CT target – CT β-actin) calibrator.

Statistical analysis

In this study, all the experiments were conducted in triplicates, and reported values were represented as the mean value ± standard error of mean (SEM). The one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test was performed to compare the results. The statistical significance of variations was confirmed at $P < 0.05$.

Results

Effect of ginger extract on cell survival

We determined the effects of ginger extract in different concentrations (0.0, 0.2, 0.4, and 0.8 mg/mL) on survival of human ESO26 cells after incubation with the extract for 12, 18, 24, and 48 h before being harvested. As shown in Figure 1, our results indicated that the ethanol extract of ginger has a dose-and time-dependent anti-proliferative effects on viability of ESO26 cells. Moreover, according to Figure 1B, the IC_{50} concentration of ginger was 0.84 mg/mL. This study showed that the ginger extract had a stronger anti-proliferative potentiality on ESO26 cells, and even at low concentrations, ginger significantly inhibited the cell proliferation. In addition, the maximum effect of the ethanol extract was approximately 82% reduction in cell viability, which has been observed after 18 h of treatment with the highest dose (0.8 mg/mL). As depicted in Figure 1B, ginger extract at concentrations of 0.2–0.8 mg/mL reduced the survival of ESO26 cells. At concentration of 0.8 mg/mL, reductions in ESO26 cells survival were 4%, 24%, 65%, and 82% after 12, 18, 24, and 48 h of treatment, respectively. Then cells treated with concentration (0/8 mg/mL) of ginger.

Effects of ginger on morphological changes

To examine whether ginger is capable of increasing cell death by inducing apoptosis, the morphology of cells before and after treatments was observed. It could be observed that ginger significantly increased the percentage of apoptotic cells in a dose-dependent manner [Figure 2]. The morphological abnormalities (shrinkage and vacuolization), growth inhibition, and detachment of cells were evident in the ginger-treated cells, especially at the highest dose (0.8 mg/mL), whereas no significant abnormality and cellular death were observed after exposure of ginger at 0.2 mg/mL. It was found that ginger had apoptotic effect on the ESO26 cells.

Determination of caspase-3 activity in ESO26 cells

Cells were treated with ginger extract for 18 h, and Western blot analysis was performed to assess apoptosis. Our results showed that ginger had an anti-proliferative effect on ESO26 cells; these results indicated that the ethanol extract of ginger has a potential to induce apoptosis and inhibit proliferation cells. Results of blotting presented in Figure 3 reveals that ginger extract could induce apoptosis in ESO26 cells. Currently, there are two known pathways that activate the apoptotic caspase cascade: intrinsic and extrinsic pathways.[22] Both of them (internal and external) trigger the activation of caspase-3, which means that caspase-3 is a 32-kDa inactive precursor. Activation of Caspase 3 (17 kDa fragment) has caused othersCaspases are activated in the cytoplasm or cell nucleus.[23] The ginger extract increased the cleavage of caspase-3 in ESO26 cells. Therefore, our results indicated that ginger extract induces apoptosis in ESO26 cells.

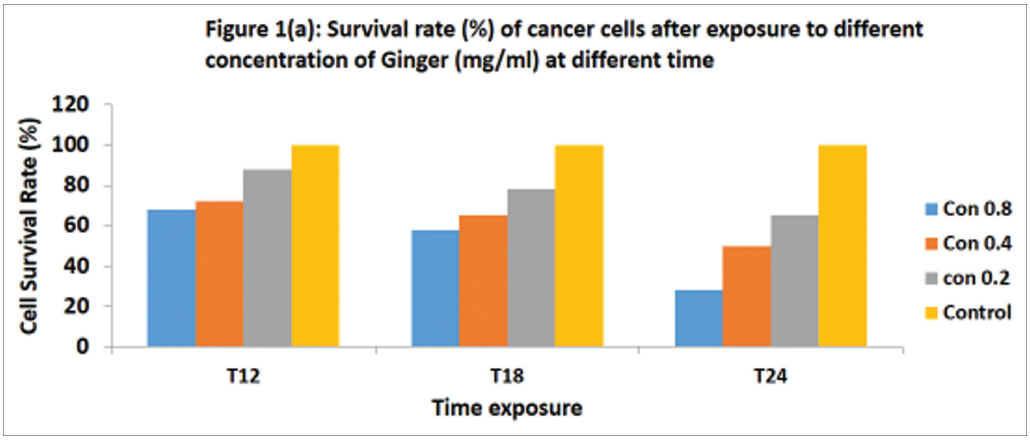

Figure 1: (A, B) Influence of ginger on viability of cell lines ESO26. Cell viability was monitored by 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay. Data are expressed as the mean ± standard error of mean (SEM) of the three separate experiments

Ginger treatment modulates expression of apoptosis-related genes

Apoptotic cascade is regulated by a variety of factors, among them Bcl-2 protein family plays central role in apoptotic events. This family includes antiapoptotic members such as Bcl-2, which is specifically considered as an important antiapoptotic protein that inhibits cell death. On the contrary, proapoptotic members such as Bax and p21 play important role in apoptosis induction. In Figure 4a, the ginger extract decreased the expression of Bcl-2 and increased the expression of the apoptosis regulatory gene Bax and p21, which was revealed in Figure 4b and c. Our results showed that ginger extract modulates the expression of a number of genes, which may induce apoptosis in esophageal cancer cells.

Discussion

Gastrointestinal (GI) cancer is one of the prevalent cancers (20%) in newly diagnosed cancer cases.[24] *In vitro* studies have shown that ginger has an anticancer role in colon cancer, and crude ethanol extract of ginger has anticancer activity and antioxidant role in GI cancer cells.[24] Ginger exerts its anticancer effects through inhibition of the NF-κB activation and downregulation of cytosolic inhibitors of apoptosis (cIAP)-1. Park *et al.*[25] showed that 6-gingerol via cell cycle arrest at G1 phase and independent of p53 molecule inhibits the growth of the BxPC-3 and HPAC cells. Similarly, ginger has been shown to suppress the growth of cancerous cells (BxPC-3 and HPAC) through cell cycle arrest at G1 phase and independent of p53 status.[26] It has been shown that the major components of ginger, 6-gingerol and 6-shogaol, have an anticancer role in liver cancer.[18] Numerous *in vitro* studies showed that ginger and its active components inhibit growth and proliferation of colorectal cancer cells.[2-27] A significant number of *in vitro* and *in vivo* studies reported that ginger and its active compounds have anti-cancer effect against GI cancer.[9-27]

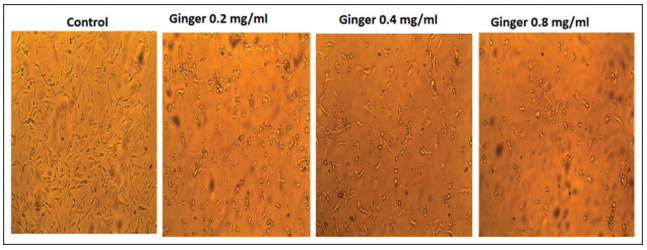

Figure 2: ESO26 cells morphological changes treated with different concentrations of ginger extract

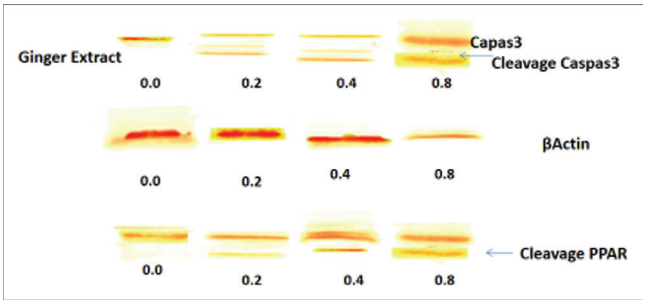

Figure 3: Cleavage of PPAR and caspase-3 in ESO26 cells after treatment with ginger extract. Data are expressed as the mean ± standard error of mean (SEM) of the three separate experiments

Apoptosis is a very important process for selective cell removal, which plays a critical role in many cellular processes. Caspases are important factors of the apoptotic system. Caspase-3 is an enzyme that is activated during apoptosis in a wide variety of tissues.[28] In this study, the ginger extract increased the cleavage of caspase-3 in ESO26 cells. These cleavages occur at apoptosis, and are markers of apoptosis.[29] As shown in Figure 3, our results showed that the cytotoxic effect reported in response to the treatment with ginger is related with the induction of apoptosis in the ESO26 cells. Several studies have revealed that ginger extract could induce apoptosis.[27-29]

Numerous studies have shown that ginger has the ability to inhibit NF-κB. Inhibition of NF-κB is due to the anti-cancer role of ginger.[30,31] Many studies have shown that ginger reduces Bcl-2 and increases Bax.[32,33] Results of real-time PCR in Figure 4B showed that ginger extract decreased the expression of Bcl-2, which is a very important protein involved in the regulation of antiapoptotic processes. Also, Figure 4A and C shows that the ginger extract increased the expression of apoptotic regulatory genes (*Bax* and *p21*). These proteins destroy the potential of the mitochondrial membrane, resulting in the release of cytochrome C from the mitochondria. The expression of these genes is regulated by the p53 tumor suppressor.[34] The p21 protein is involved in the induction of apoptosis. Several studies have reported that ginger increased *p21* expression.[32-35] Our results showed that ginger extract modulated the expression of a number of genes that play a role in the survival and growth of esophageal cancer cells. Results showed that ginger decreased the expression of *Bcl-2* and increased the expression of apoptosis regulatory genes *Bax* and *p21*. Decreasing of *Bcl-2* by ginger plays an important role in the treatment of cancer cells. Simultaneous with the changes in the expression of these genes, the level of caspase-3 also increased. Coactivation of caspase-3 and increased levels of p21 and Bax led to the death of cancer cells.

Overall, according to the results of this study and previous study, it can be concluded that ginger has the potential for inducing apoptosis in esophageal cancer cells. Ginger (as a popular spice and effective factor in reducing the incidence of cancer) is a relatively cheap spice that is found throughout the world. Thus, due to its different biological roles as well as its low toxicity reported in studies, it should be used reasonably in a daily diet.

One of the most important limitations of our study was that we could not simultaneously examine several cancer cells. Also, we could not examine the role of ginger components separately in cancer cells. But one of the most important strengths of this research was that we used several techniques simultaneously to determine a metabolic pathway.

Conclusion

In general, this study indicates that ginger causes a series of fundamental changes in the mitochondrial membrane potential, activation of caspases on the upregulation of *Bax*, *p21*, and downregulation of *Bcl-2* gene in esophageal cancer cells. The findings of this study indicated that ginger might exert a chemopreventive effect on esophageal cancer through the suppression of proliferation and induction of apoptosis. Collectively, these data suggest that ginger can be introduced as an effective factor in reducing and preventing cancer.

Acknowledgement

The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences, Kermanshah, I.R. Iran.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

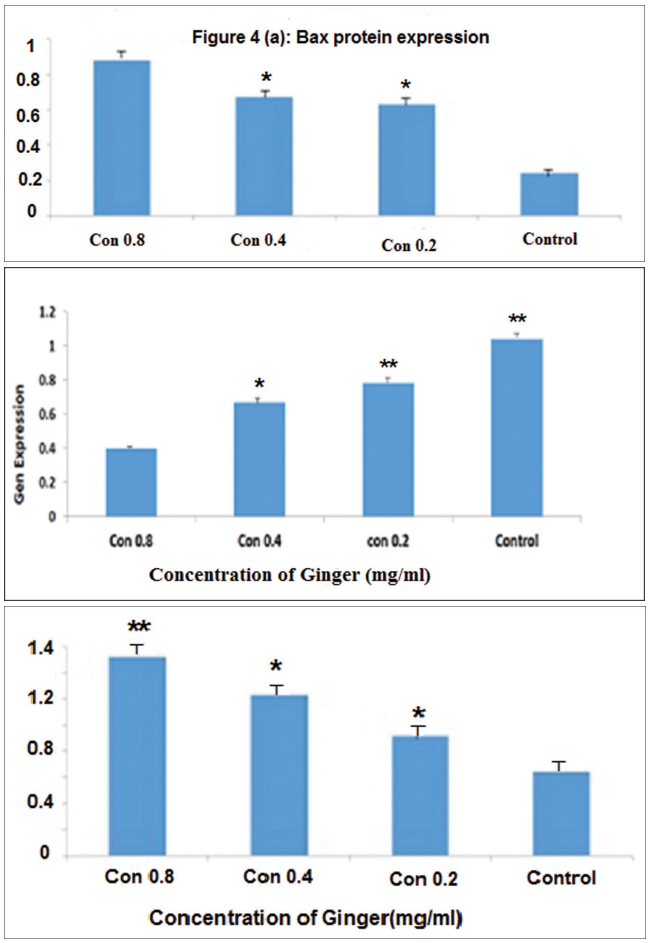

Figure 4: (A, B, and C) Effects of ginger on expressions of *Bax* and *Bcl-2* genes of U87MG cells. Ribonucleic acid (RNA) was isolated, reverse transcribed to complementary deoxyribonucleic acid (cDNA), and then amplified by a real-time polymerase chain reaction (PCR) detection system to measure messenger RNA (mRNA) levels of *Bax*, *p21*, and *Bcl-2*. **P* < 0.05, ***P* < 0.001. Data are presented as the mean ± standard error of mean (SEM) from three independent experiments

References

1. Tanaka H, Shimada Y, Imamura M, Shibagaki I, Ishizaki K. Multiple types of aberrations in the p16 (INK4A) and the p15 (INK4B) genes in 30 esophageal squamous-cell-carcinoma cell lines. Int J Cancer 1997;70:437-42.
2. Kim EJ, Lee YJ, Shin HK, Park JH. Induction of apoptosis by the aqueous of Rubuscoreanum in HT-29 human colon cancer cells. Nutr 2005;21:1141-8.
3. Dueñas CV, Camarero IR, Cobaleda C, García IS. Function of oncogenes in cancer development. A changing paradigm. EMBO J 2013;32:1502-13.
4. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 2006;71:1397-421.
5. Shukla Y, Singh M. Cancer preventive properties of ginger: A brief review. Food Chem Toxicol 2007;45:683-90.
6. Baliga MS, Haniadka R, Pereira MM, D'Souza JJ, Pallaty PL, Bhat HP, *et al*. Update on the chemopreventive effects of ginger and its phytochemicals. Crit Rev Food Sci Nutr 2011;51:499-523.
7. Wohlmuth H, Smith MK, Brooks LO, Myers SP, Leach DN. Essential oil composition of diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe) grown in Australia. J Agric Food Chem 2006;54:1414-9.
8. Katiyar SK, Agarwal R, Mukhtar H. Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of *Zingiber officinale* rhizome. Cancer Res 1996;56:1023-30.
9. Lee SH, Cekanova M, Baek SJ. Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. Mol Carcinog 2008;47:197-208.
10. Manju V, Nalini N. Chemopreventive efficacy of ginger as naturally occurring anticarcinogen during the initiation, post-initiation stages of 1, 2 dimethylhydrazine-induced colon cancer. Clinica Chimica Acta 2005;358:60-6.
11. Kazuhiro I, Ando T, Maeda O, Ohmiya N, Niwa Y, Kadomatsu K, *et al*. Ginger ingredients reduce viability of gastric cancer cells via mechanisms. Biochem Biophy Res Commun 2007;362:218-23.
12. Chen CY, Liu TZ, Liu YW, Tseng WC, Liu RH, Lu FJ, *et al*. 6-shogaol (alkanone from ginger) induces apoptotic cell death of human hepatoma p53 mutant Mahlavu subline via an oxidative stress-mediated caspase-dependent mechanism. J Agric Food Chem 2007;55:948-54.
13. Zhou L, Qi L, Jiang L, Zhou P, Ma J, Xu X, *et al*. Antitumor activity of gemcitabine can be potentiated in pancreatic cancer through modulation of TLR4/NF-κB signaling by 6-shogaol. AAPS J 2014;16:246-57.
14. Gasparian AV, Yao YJ, Kowalczyk D, Lyakh LA, Karseladze A, Slaga TJ, *et al*. The role of IKK in constitutive activation of NF-κB transcription factor in prostate carcinoma cells. J Cell Sci 2002;115:141-51.
15. Shukla Y, Prasad S, Tripathi C, Singh M, George J, Kalra N. *In vitro* and *in vivo* modulation of testosterone mediated alterations in apoptosis related proteins by [6]-gingerol. Mol Nutr Food Res 2007;51:1492-502.
16. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. Food Chem Toxicol 2002;40:1091-7.
17. Tsuboi K, Matsuo Y, Shamoto T, Shibata T, Koide S, Morimoto M, *et al*. Zerumbone inhibits tumor angiogenesis via NF-κb in gastric cancer. Oncol Rep 2014;31:57-64.
18. Weng CJ, Wu CF, Huang HW, Ho CT, Yen GC. Anti-invasion effects of 6-shogaol and 6-gingerol, two active components in ginger, on human hepatocarcinoma cells. Mol Nutr Food Res 2010;54:1618-27.

19. Pashaei-Asl R, Pashaei-Asl F, Mostafa Gharabaghi P, Khodadadi K, Ebrahimi M, Ebrahimie E, *et al*. The inhibitory effect of ginger extract on ovarian cancer cell line; application of systems biology. Adv Pharm Bull 2017;7:241-9.
20. Hahm ER, Singh SV. Sulforaphane inhibits constitutive and interleukin-6-induced activation of signal transducer and activator of transcription 3 in prostate cancer cells. Cancer Prev Res (Phila) 2010;3:484-94.
21. Anisa NI, Azian N, Sharizan M, Iwai Y. Temperature effects on diffusion coefficient for 6-gingerol and 6-shogaol in subcritical water extraction. J Physics Conference Series 2014;495:012009.
22. Nguyen C, Pandey S. Exploiting mitochondrial vulnerabilities to trigger apoptosis selectively in cancer cells. Cancers 2019;11: 1-20.
23. Mortezaee K, Salehi E, Mirtavoos-Mahyari H, Motevaseli E, Najafi M, Farhood B, *et al*. Mechanisms of apoptosis modulation by curcumin: Implications for cancer therapy. J Cell Physiol 2019;234: 12537-50.
24. Plengsuriyakarn T, Viyanant V, Eursitthichai V, Tesana S, Chaijaroenkul W, Itharat A, *et al*. Cytotoxicity, toxicity and anticancer activity of *Zingiber officinale* Roscoe against cholangiocarcinoma. Asian Pac J Cancer Prev 2012;13:4597-606.
25. Park YJ, Wen J, Bang S, Park SW, Song SY. [6]-gingerol induces cell cycle arrest and cell death of mutant p53-expressing pancreatic cancer cells. Yonsei Med J 2006;47:688-97.
26. Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, *et al*. Mitochondrial dysfunction in drosophila PINK1 mutants is complemented by parkin. Nature 2006;441:1157-61.
27. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
28. Lavrik IN, Golks A, Krammer PH. Caspases: Pharmacological manipulation of cell death. J Clin Invest 2005;115: 2665-72.
29. Lossi L, Castagna C, Merighi A. Caspase-3 mediated cell death in the normal development of the mammalian cerebellum. Int J Mol Sci 2018;19:3999.
30. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
31. Pacifico F, Leonardi A. NF-κB in solid tumors. Biochem Pharmacol 2006;72:1142-52.
32. Ramachandran C, Quirin KW, Escalon EA, Lollett IV, Melnick SJ. Therapeutic effect of supercritical CO_2 extracts of Curcuma species with cancer drugs in rhabdomyosarcoma cell lines. Phytother Res 2015;29:1152-60.
33. Choudhury D, Das A, Bhattacharya A, Chakrabarti G. Aqueous extract of ginger shows antiproliferative activity through disruption of microtubule network of cancer cells. Food Chem Toxicol 2010;48:2872-80.
34. Yue Y, Stone S, Lin W. Role of nuclear factor κb in multiple sclerosis and experimental autoimmune encephalomyelitis. Neural Regen Res 2018;13:1507-15.
35. Nikkhah Bodagh M, Maleki I, Hekmatdoost A. Ginger in gastrointestinal disorders: A systematic review of clinical trials. Food Sci Nutr 2019;7:96-108.