Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

The Molecular Activity of Gingerol on Inhibits Proliferation of Breast Cancer Cell Line (MCF7) Through Caspase Activity

Mohammed Subhi Mohammed
Kufa Technical Institute, Al-Furat Al-Awsat Technical University Al-Najaf, Iraq.
*Corresponding Author *E-mail*: mohammed.mohammed@atu.edu.iq

Abstract:

This study was conducted to determine the role and molecular function of phenolic gingerol in regulating the cell proliferation of MCF-7 breast cancer cells, a compound isolated from Zingiberofficinale. Different gingerol concentrations from (1-1000) µg/ml were prepared to assess gingerol's cytotoxic effect on MCF-7 cell lines, cell line exposure periods were measured in (24) hours.Under complete sterile conditions on a microtitration plate. Results showed that, after exposure to cell line for 24 hours, Cells treated with gingerol showed higher cell-death percentagethan untreated cells as quantified bythe MTT assay, with a 100 µg/mL inhibition rate of MCF-7 of 83.2 percent following 24 hour of exposure.The capacity of gingerol to induce apoptosis to an infected cell under a fluorescence microscope has been studied by crystal violet stain to measure the percentage of induction of apoptosis. A significant increase of the expression of caspase-3, caspase-8 and caspase-9 in comparison with untreated cells has revealed the gingerol effect on the MCF-7 line. The role of apoptosis, its high potential as an antitumor agent, was demonstrated in this study.

Keywords: Gingerol, molecular activity, caspase.

Introduction:

Breast cancer is the leading cause of cancer death in women and is the most common type of cancer(1). About 70% of patients with breast cancer are positive for estrogen receptor (ER) and these are suitable for treatment with antiestrogen. ER-negative breast cancer is often more aggressive or malignant than ER-positive(2). In addition to recurring and metastatic breast cancer, over-expression of an epidermal growth factor receptor (EGFR) or human EGFR-2 (HER2) is correlated with(3). These receptors are widely recognized as a therapeutic goal for breast cancer and its downstream signaling pathways.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

Although early detection methods and multimodal approaches have been developed for breast cancer treatment, only small steps have been taken to improve the clinical effects of women with metastases. There is therefore the possibility to provide insights into therapeutic goals and strategies for treating breast cancer in detail on the biology and its molecular mechanics behind the progression of the disease.Ginger is a natural dietary rhizome commonly employed as a herb and aromatic agent. Ginger contains several bioactive components, such as gingerols, shogaols, paradols and zingerone, which show the pharmacological role of anti-inflammatory and antitumor mediators (4).6-gingerol and six-shogaol were widely reported in ginger bioactive ingredients to perform anti-tumor activities in a range of cancers through cell proliferation, migration, and invasion or induction of apoptosis(5). One of the major ginger isolated phenolic compounds, gingerol has been reported to have antitumor activity in inhibiting or inducing apoptosis against oval-, colon-, lung- and prostate cancer cells (6), However, gingerol's effects and molecular mechanisms on the growth and progression of breast cancer cells are poorly understandable. In this study we studied the regulatory effects and signalized pathways of 10-gingerol in MCF-7 breast cancer cell proliferation and invasion.

Material and methods:

The dried officinaleZingiber was bought from the local market (Z. officinale). Three times at room temperature for 24 h, Z. officinale was extracted of ethanol. The extract was concentrated, hung in water and subsequently partitioned by n-hexane three times. Silk gel column chromatography was performed with n-hexane (24 g) extract. The sixth fraction (0,4 g) was further separated by semi-preparative HPLC among eight fractions eluted from column column chromatography.

Cytotoxicity Assay

The cells were seeded on 96-well plate and washed in various doses (1, 6, 12.5, 25, 50, 100, 200, 400, 600, 800, 1000 μg \ml) with phosphate buffered saline (PBS). The medium was aspired to the 24, 48, 72 hours of incubation and 100 μl MTT solution were added to each well (5 mg/mL in PBS, pH 7.2). Plate incubation at 37 °C for 2 hours. After incubation, the formosanic dye was added 50 microns of dimethyl sulfoxide and sweetened for 45 minutes. At 584 nm wavelength, the absorption was established by a microplate reader. The trial for each condition was

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

performed three times (7). The inhibiting rate of cell growth (the percentage of cytotoxicity was calculated as (IG%)=(A−B)/A×100, where A is the mean optical density of untreated wells, and B is the optical density of treated wells (8)

Samples for RT-PCR:

Incubation for a period of 24 hours at 37 C was carried out for the flakes (25cm2) which are linked to the tissue culture containing 16 cells/flask. During inoculation, the medium was removed from the flasks. Free serum media have also been used to treat the negative control only(9)For 24 hours, each of the bottles was re-incubated at 37 Celsius.

RNA extraction:

The total RNA was obtained from negative and infected control cells harvested at 24 using the full RNA (AccuZol TM) extraction kit. In addition, the detection of RNA concentrates in the ng/μL and purity was achieved using a nano drop estimate by estimating the optical density ratio of (260/280nm). The purity of the RNA sample is accepted (1.7 and 1.9) (10)

Quantitative real-time polymerase chain reaction (qRT-PCR):

The primers were chosen from the RT-PCR in Table 1. The RT-PCR reactions are achieved by manufacturers' procedures (Agilent Tech. Stratagene, U.S.). Furthermore the isolated RNA in double-stranded cDNAs is reversed with the reverse transcriptase-polymerase enzyme by means of Quanti-Fast Green PCR Master MixKit (abm Kit). Also, reaction's amplification condition is in the following way: 15mins at 42 Celsius, denaturation for 19mins at 95 Celsius, and 40 cycles of amplification, each of them for 3seconds and at denaturation temperature 95Celsius, the annealing temperature at 58^0C while the elongation temperature at for 20 seconds at 72 Celsius. Each one of the tests has been achieved in RT-PCR tests; a single sample with no cDNA is utilized for each pair of primers that contain just RNase free water. Furthermore, the ratio of the expression with the calibrator sample has been estimated: the Ct in next formula: CT (testing) = CT gene of interest (targeted, testing) – CT internal control. Gene s average CT was estimated in every one of the samples (a triplicate for every one of them) and applied for normalizing the expression level with the use of ΔΔ CT approach: threshold cycle (Ct) has been estimated,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

whereas the relative gene expression has been indicated in the following way: fold change = 2^ (−ΔΔCt) (11).

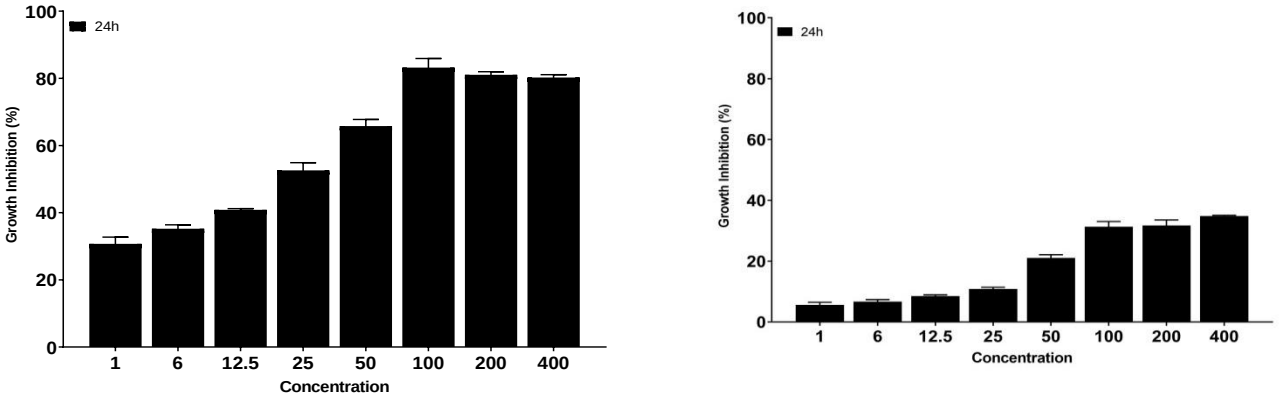
Table 1: Primers

NO	Target genes	F\R	Sequence '5 - … - '3
1	CASP-8 (Caspase-8)	F	GACCACGACCTTTG AAGAGCTTC
		R	CAGCCTCATCCGTA TATC
2	CASP-9 (Caspase-9)	F	CTCTTGAGCAGTGG CTTGTC
		R	GCTGATCTATGAGC GATACT
3	CASP-3 (Caspase-3)	F	GTGGAACTGACGATGATATGGC
		R	CGCAAAGTGACTGGATGAACC
5	18S rRNA Housekeeping gene	F	GGAGTATGGTTGCAAAGCTGA
		R	ATCTGTCAATCCTGTCCGTGT

Results:

Cytotoxicity Assay:

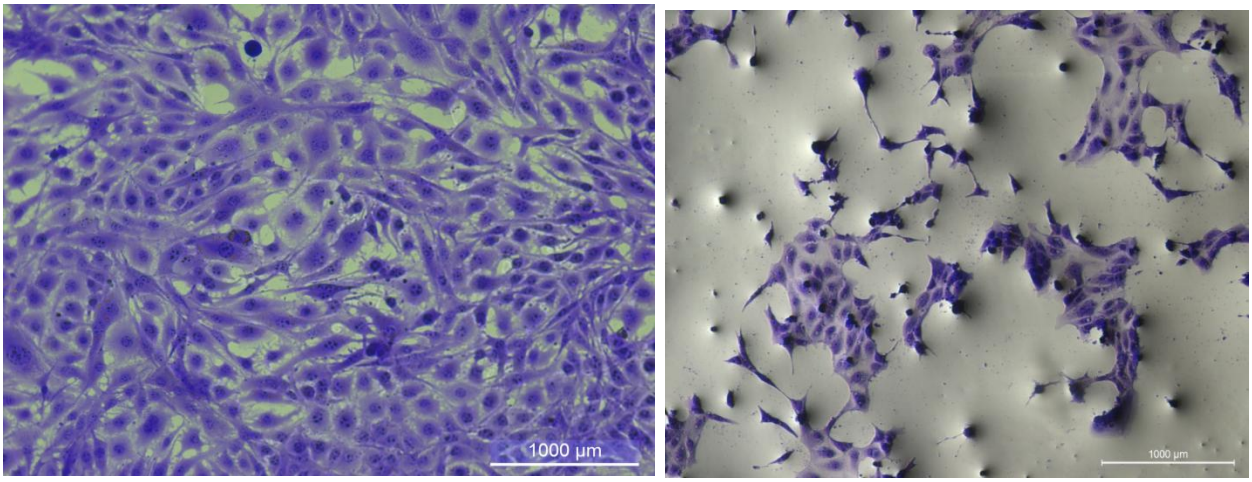
The in vitro cytotoxicity tests for the calculation of gingerol effects on cancer cell cell lines have been evaluated by MCF-7 cell lines using various series of gingerol dilutes. In case of 100 µg\ml of gingerol exposure, maximum MCF 7-cell line cytotoxicities were obtained and gave 83.2%). In the case of the exposure 100 µg\ml Gingerol was 34.6 percent cytotoxicity of normal cell lines of REF can be seen in Figure 1.



(A) **(B)**

Figure 1: Cytotoxicity assay of gingerolexposure on (A) MCF-7 (B) REF cell line.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.



(A) **(B)**

Figure 2: Cancer cell line which have been stained by the crystal violet stains and examined under the inverted microscope (A) MCF-7 control (B)Gingerol treated cell of the MCF-7

Gene expression analysis:

In the cell-line MCF-7, gingerol effects were assessed in three distinct genes cspase-8, caspase-9 and caspase-3. The over-expression of gingerol was treated in the MCF-7 cell line case-8, case-9and case-3. Fig 2 shows the impact of the gene epression on optimal gingerol.

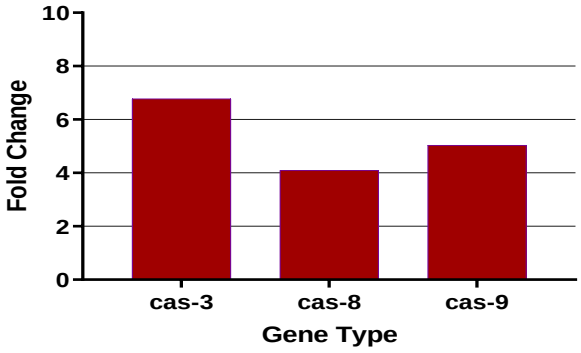


Figure (3): Gene expression analysis of Cas-3, Cas-8 and Cas-9 for 6- gingerolbt effected on MCF-7 cell line.

***T-test = (t=6.761, df=3, *p.v*<0.0021).**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

Discussion:

There are concerns about cancer-related morbidity and death, despite significant progress with chemical and radiation therapy development. Additionally, there are few and limited methods of medicinal prevention. Our current study aimed at examining the anti-cancer potential of routine food ingredients. Such food components may be potentially acceptable and easily implemented in preventive medicine.

The purpose of this study was to study caspase activity involvement in the treatment of apoptosis with gingerol. MCF-7 cancer cells were shown to have strong cytotoxicity by gingerol.
The increasing concentrations of gingerol have increased the cytotoxicity. Gingerol IC50 values at 100 μg/ml showed significant cytotoxicity for 24 hour exposures in MCF-7 cells.
Eternal sources of active compounds are natural products. The therapeutic use of natural products has increased from folk use of the entire plant to the use of standardized extracts by natural entities unprocessed (12), following the isolation of specified molecular structure compounds(13). Grouping of compounds with similar molecular structure with an intimate pharmacological activity might highlight the relationship between structure and activity and encourage chemists to initiate lead optimisation.

There have been reports behind these properties of different molecular mechanisms. The inhibitorous effects of [6]-gingerol were previously reported on cancers of different origins. [6]-gingerol has been shown to be especially effective in preventing skin carcinoma and an angiogenesis in endothelial cells (14, 15). Ginger is a dietary supplement and a major ingredient in many traditional medicines. The effects of [6]ingerol on gastrointestinal cancer, like colon cancer, can be studied logically. In addition, pharmacocinetic studies in people on the active ginger components of oral ginger extracts found significant levels of [6]-gingerol in plasma and colon tissue free or conjugated (16). Another bioavailability study in rats for [6]-gingerol has suggested that 10 minutes after oral administration it can be detected in plasma at a concentration of 4.3μg/ml. The same study also determined its high distribution in tissues of the gastrointestinal tract with the highest concentration. The plasma [6]-gingerol tissue ration was reported to be >1 and its lipophilicity was attributed (17). All these data support a study of the effects of colon cancer on anti-cancer[6]-gingerol. There was an increasing number of studies in the first decade of the 21st century characterizing the mechanisms behind phytochemicals' anti-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

cancer effects. Mechanistic evaluation studies[6]-gingerol's anti-cancer properties against various kinds of cancer offer valuable insight into their various mechanisms of action to achieve cytotoxic or pro-apoptotic effects in cancer cells. A few recent reports on [5]-gingerol's anti-cancer activity against colon cancer show various mechanisms to deal with various lines of colon cancer. (18).The MTT test suggests that dose-related action is based on the various immortal cell lines (MCF-7), and that induced by gingerol in MCF-7 cell lines, relatively higher apoptotic cell levels. The estrogen receptor (ER) can be present in MCF-7 (19).

Depending on the origin and type of cells, the ability to bind with the AMN-3 plasma membrane receptor differ. The cell surface comprises a variety of highly specified receptor molecules. These sites are recognition sites that are often transferred to the cell by chemical signal. These signals may trigger a variety of chemicals and eventually disable certain genes (20). 6-gingerol pungent ingredient of ginger was suggested to be antibacterial, anti-inflammatory, antimicrobial and inhibit angiogenesis and this plant may be helpful to treat tumors and other diseases dependent on angiogenesy(15). In the inhibition of the carcinogenic process, both nutrient and non-nutrient components of the Diet play important role. Different mechanisms allow for the use of the non-nutritional components: 1. Due to its anti-oxidant properties. 2. The carcinogenes are disabled. 3. Or to enhance the protective enzyme tissue levels in the body. The body defense system detoxifies toxic metabolites of harmful medicines and chemicals. Phytochemicals in turmeric, mustard and alliums may be more than one way to give benefits. It was hypertonic and may cause dosage-dependent osmotic shock in cancer cell lines Different constituents in ginger extract(21). In every treatment, the metabolic pathways differed between the lines. These facts have been mentioned in several studies that examined the effects on various cellular lines of different plant extracts(22). Antioxidants may delay or inhibit lipid or other molecular oxidation by inhibiting the initiation or propagation of oxidative chain reactions. Ginger has powerous antioxidant and chemopreventative properties in bioactive phenolic substances (23). The antioxidant activity of PH compounds has mainly their effect on absorption and neutralization, single and treble oxygen, or peroxide decomposition. They are important in absorbing and neutralizing free radicals. Phenolic compounds, including ginger, can directly contribute to antioxidant effects in many plants and vegetables. Polyphenolic compounds are reported to inhibit mutagenesis and carcinogenesis in humans, if a diet rich in fruits and vegetables consumes up to 1.0 g daily (24)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

Apoptosis is an essential form of cell death for normal growth and homeostasis maintenance. Moreover, the cells of apoptosis trend are likely to undergo present therapy, chemotherapy and radiation therapy, and this process has a clear therapeutic implication (25). The ability to inhibit or enhance apoptosis by plant extraits depends on various factors including; multiple micronutrients extract concentration and concerted action(26).

References:

1. Waks AG, Winer EP. Breast cancer treatment: a review. Jama. 2019;321(3):288-300.
2. Guo M, Wang M, Deng H, Zhang X, Wang Z-Y. A novel anticancer agent Broussoflavonol B downregulates estrogen receptor (ER)-α36 expression and inhibits growth of ER-negative breast cancer MDA-MB-231 cells. European journal of pharmacology. 2013;714(1-3):56-64.
3. Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. Breast cancer research and treatment. 2012;136(2):331-45.
4. Mao Q-Q, Xu X-Y, Cao S-Y, Gan R-Y, Corke H, Li H-B. Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods. 2019;8(6):185.
5. Liu R, Heiss EH, Sider N, Schinkovitz A, Gröblacher B, Guo D, et al. Identification and characterization of [6]-shogaol from ginger as inhibitor of vascular smooth muscle cell proliferation. Molecular nutrition & food research. 2015;59(5):843-52.
6. Lechner JF, Stoner GD. Gingers and their purified components as cancer chemopreventative agents. Molecules. 2019;24(16):2859.
7. Mohammed MS, Al-Taee MF, Al-Shammari AM. Caspase dependent and independent anti-hematological malignancy activity of AMHA1 attenuated newcastle disease virus. International Journal of Molecular and Cellular Medicine. 2019;8(3):211.
8. Shahneh FZ, Valiyari S, Azadmehr A, Hajiaghaee R, Yaripour S, Bandehagh A, et al. Inhibition of growth and induction of apoptosis in fibrosarcoma cell lines by Echinophora platyloba DC: in vitro analysis. Advances in pharmacological sciences. 2013;2013.
9. Helms HC, Abbott NJ, Burek M, Cecchelli R, Couraud P-O, Deli MA, et al. In vitro models of the blood–brain barrier: an overview of commonly used brain endothelial cell culture models and guidelines for their use. Journal of Cerebral Blood Flow & Metabolism. 2016;36(5):862-90.
10. Scientific T. Interpretation of nucleic acid 260/280 ratios. T123 Technical Bulletin Wilmington, DE, USA: Thermo Scientific. 2012.
11. Mohammed MS, Altaee MF, Al-Shammari AM. Retinoic Acid Treatment of Human Hematological Malignancies Induces Caspase Dependent and Independent Apoptotic Cell Death. Indian Journal of Forensic Medicine & Toxicology. 2021;15(1):1323.
12. Aboul-Enein AM, Al-Abd AM, Shalaby E, Abul-Ela F, Nasr-Allah AA, Mahmoud AM, et al. Eichhornia crassipes (Mart) solms: from water parasite to potential medicinal remedy. Plant Signaling & Behavior. 2011;6(6):834-6.
13. Ibrahim SR, Abdallah HM, El-Halawany AM, Mohamed GA. Naturally occurring thiophenes: isolation, purification, structural elucidation, and evaluation of bioactivities. Phytochemistry reviews. 2016;15(2):197-220.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11095 - 11103
Received 05 March 2021; Accepted 01 April 2021.

14. Kim SO, Kundu JK, Shin YK, Park J-H, Cho M-H, Kim T-Y, et al. [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-κ B in phorbol ester-stimulated mouse skin. Oncogene. 2005;24(15):2558-67.
15. Kim E-C, Min J-K, Kim T-Y, Lee S-J, Yang H-O, Han S, et al. [6]-Gingerol, a pungent ingredient of ginger, inhibits angiogenesis in vitro and in vivo. Biochemical and biophysical research communications. 2005;335(2):300-8.
16. Yu Y, Zick S, Li X, Zou P, Wright B, Sun D. Examination of the pharmacokinetics of active ingredients of ginger in humans. The AAPS journal. 2011;13(3):417-26.
17. Jiang Sz, Wang Ns, Mi Sq. Plasma pharmacokinetics and tissue distribution of [6]-gingerol in rats. Biopharmaceutics & drug disposition. 2008;29(9):529-37.
18. Radhakrishnan E, Bava SV, Narayanan SS, Nath LR, Thulasidasan AKT, Soniya EV, et al. [6]-Gingerol induces caspase-dependent apoptosis and prevents PMA-induced proliferation in colon cancer cells by inhibiting MAPK/AP-1 signaling. PloS one. 2014;9(8):e104401.
19. Vladusic E, Hornby A, Guerra-Vladusic F, Lakins J, Lupu R. Expression and regulation of estrogen receptor beta in human breast tumors and cell lines. Oncology reports. 2000;7(1):157-224.
20. Al-Zobaidi MSM, Shawkat MS, Galoob AA-AN. Effect of bioactive compounds in ginger extract on cancer cell lines In vitro. Jornal of Biotechnology Research Center. 2012;6(2).
21. Mohammed MS. Effect of CurcumineExtract (Curcuma Longa) On Cancer Cell Lines inVitro. Journal of Kufa for Nursing Science Vol. 2013;3(2).
22. Chierrito D, Villas-Boas CB, Tonin FS, Fernandez-Llimos F, Sanches AC, de Mello JC. Using Cell Cultures for the Investigation of Treatments for Attention Deficit Hyperactivity Disorder: A Systematic Review. Current neuropharmacology. 2019;17(10):916-25.
23. Tan BL, Norhaizan ME, Liew W-P-P, Sulaiman Rahman H. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Frontiers in pharmacology. 2018;9:1162.
24. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009;2(5):270-8.
25. Obeng E. Apoptosis (programmed cell death) and its signals-A review. Brazilian Journal of Biology. 2021;81(4):1133-43.
26. Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. Nutrients. 2020;12(1):236.