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RESEARCH PAPER

Anti-cancer effects of nanoemulsion prepared using *Zingiber Officinale* L. tincture against PC3 prostate cancer cells

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ABSTRACT

Objective(s): The purpose of this work was to estimate the anti-cancer properties of the nanoemulsions synthesized by *Zingiber officinale* L. tincture against PC3 prostate cancer cells.

Materials and Methods: Fresh ginger was initially procured from a local market, and extraction was performed after complete washing. In the next step, a nanoemulsion containing ginger extract was prepared using Tween 80, and its size and zeta potential were determined by a Zetasizer. The prepared nanoemulsion was assessed by transmission electron microscopy (TEM) to confirm the size and morphology of the particles. The toxicity of the nanoemulsion containing ginger extract against PC3 prostate cancer cells and normal HFF skin cells was evaluated using the MTT assay. To determine apoptosis, flow cytometry was used to assess cell cycle changes. In addition, the antioxidant activity of the nanoemulsion was estimated by DPPH and ABTS free radical scavenging tests.

Results: The results showed that the prepared nanoparticles had a size of 67 nm (confirmed by TEM electron microscopy) and a zeta potential of -25.05 mV. The results of the MTT assay showed inverse dose-dependent toxicity for different concentrations of ginger nanoemulsion against PC3 cells. In addition to anti-cancer activity, the nanoemulsion showed a potent ability to scavenge DPPH and ABTS free radicals.

Conclusion: Our results showed that the nanoemulsions containing ginger extract had toxicity against PC3 cancer cells but not normal cells, indicating their applicability as a suitable option for treating PC.

Keywords: *Cell cycle, Free radical, Nanoparticle, Prostate cancer, Zingiber Officinale*

How to cite this article

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INTRODUCTION

Cancer, a disease with different etiologies, is the second most common reason for death worldwide [1, 2]. Research has shown that reactive oxygen species (ROS) play a major role in cancer development [3, 4]. Excessive production of ROS or the lack of competent or adequate anti-oxidants in cells can lead to DNA damage [5, 6]. Oxidative stress-related damage has been shown to affect all chemical and molecular steps of tumor development [7, 8]. There are also reports that cancer cells reduce the activity of some anti-oxidant enzymes, leading to the accumulation of ROS in these cells [9, 10]. In traditional drug delivery methods, the drug is aimlessly distributed all over the body, and target cells may absorb only a part of the drug, depending on their locations. Therefore, significant parts of the drug are removed from the body without being used. The most important disadvantages of this type of drug delivery are drug waste, overdose reactions, high costs of raw drug materials, physicochemical incompatibilities, and unwanted drug interactions [11]. Nanocarriers are mainly used to address these disadvantages and increase drug delivery to target areas [12-14]. Along with the advances of the pharmaceutical industry in the treatment of diseases, the science of nanotechnology, as one of the branches of pharmaceutical science, has been associated with promising outcomes, especially in cancer treatment [15-19].

Emulsions are colloidal suspensions with at least two immiscible liquids, forming an unbalanced system that does not appear spontaneously [20].

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The structure of emulsions consists of droplets from one liquid (i.e., the suspended or internal phase) scattered into another liquid (i.e., the continuous or external phase). For example, oil-in-water nanoemulsions comprise small oil droplets (less than 100 nm in diameter) dispersed in an aqueous phase, in which each oil droplet is attached to thin layers of emulsifier particles. Due to their smaller particle sizes, nanoemulsions have higher surface areas than conventional emulsions and are more efficient transporters.

Emulsions with droplet sizes between 20 and 200 nm are referred to as mini-emulsions, nanoemulsions, ultrafine emulsions, sub-micron emulsions, etc. Although interest in nanoemulsions has increased in recent years, the most direct applications of nanoemulsions have been in the pharmaceutical and cosmetic industries [21]. Recent studies have highlighted the necessity of optimizing nanoemulsion production methods. As oil-water dispersions with 100-nm sizes, nanoemulsions are known as thermodynamically stable structures that can increase the bioavailability of drugs. Besides, it cannot be more emphasized that we need to develop proficient drug carriers to boost their efficacy. In this regard, nanoemulsions have been proposed as valuable vehicles and are subjected to extensive studies.

Ginger is an important medicinal plant possessing diverse biological properties, including anti-nausea, cardiotonic, anticoagulative, antibacterial, anti-oxidant, anti-cough, anti-hepatotoxic, anti-inflammatory, diuretic, antispasmodic, immunostimulatory, and anti-flatulence effects [22-24]. Ginger has been reported to increase gastrointestinal secretions, reduce blood cholesterol, and direct blood flow toward the brain [25]. Ginger has also been effective in preventing nausea, vomiting, anorexia, intestinal spasms, bronchitis, and rheumatic problems [26, 27]. Ginger is also used as a condiment in the food industry [28]. Experimental studies have shown that ginger has anti-cancer properties, suppressing the growth of cancer cells, especially colon cancer, in humans [29, 30]. The phytochemical compounds of ginger, which have been mostly characterized, include essential oils, phenolic compounds, carbohydrates, proteins, alkaloids, glycosides, steroids, terpenoids, saponins, and tannins. These ingredients seem to be indispensable for the anti-cancer and other biological properties of this plant. Fresh ginger contains 2.3% protein, 0.9% fat (such as glycerides, phosphatidic acid, and lecithin), 1.2% minerals, 12.3% carbohydrates, and 2.4% fiber [31]. Ginger also contains vitamins such as thiamine and riboflavin. Of course, the types of the vitamins vary depending on the class, species, growing conditions, and drying and storage methods. The chemical constituents of ginger include gingerols (such as 6-gingerols), 6-shuagols (6-gingerol hydroxylized analogs), 6- and 10-dihydrogenergion, 6- and 10-valinoids, B- and A-gingerd, 6-paradols, and galangal zingerone. Among these, 6-, 8-, and 10-gingerols, along with shuagols are the main components. Gingerols are responsible for the effects of fresh ginger and include a series of phenolic compounds, with 6-gingerols being the most important part. Dried ginger's biological effects are due to shovels, which are the dehydrated forms of gingerol [32, 33].

The high rate of mortality renders prostate cancer (PCa) a major health concern worldwide. This cancer is generally therapy-resistant, and many research efforts have been dedicated to increasing the efficacy of treatments in patients with this type of cancer. Similar to many cancers, targeted therapy is a viable option for PCa as well [34]. A variety of targeted-therapy systems have been developed, employing a wide range of materials such as nanoparticles (magnetic, solid-lipid, etc.), peptide-based or antibody-based compounds, aptamers, liposomes, as well as nanoemulsions. The use of nanocarriers has yielded promising outcomes in terms of drug delivery in cancer treatment, including in PCa [35]. *In vitro* studies on PCa cell lines can help divulge the potential benefits of nano-based carries, such as nanoemulsions. Among available human PCa cell lines (i.e., TSU-PnI, LNCaP, PC3, and DU145), PC3 cells have shown growth kinetics similar to that of *in vivo* tumors and, therefore, are suitable for cancer research [36].

Considering the properties of ginger and nanoemulsions, we aimed to assess the anti-oxidant and growth inhibitory properties of the nanoemulsions synthesized by ginger tincture against prostate cancer (PC) cell line.

MATERIALS AND METHODS

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma Chemicals

Co. (St. Louis, USA). Dulbecco's Modified Eagle Medium (DMEM), FBS, trypsin, antibiotic,3,4,5-Dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT), and acridine orange dye were purchased from Sigma-Aldrich Company, Ltd. (Poole, United Kingdom). The PC cell line (PC3) and skin fibroblasts (HFF) were obtained from Iran's Pasteur Institute.

Preparation of ginger root tincture

First, ginger root was prepared and washed well with distilled water. It was then dried at 25 ˚C and pounded in a mortar until powdered. To prepare the tincture, 10 grams of ginger root powder was initially mixed with 75 mL of 96% ethanol, and after adding 25 mL of distilled water and 5 mL of glycerin, the mixture was transferred into a glass container and placed on a stirrer (three times a day for 1 hr). After 15 to 21 days, the tincture was ready [6].

Preparation of nanoemulsions using ginger root tincture

Ultrasound was used to fabricate nanoparticles. First, 5 mg of ginger tincture, 100 µL tween 20, 100 µL tween 80, and 500 µL ethylene glycol were transferred into a 50-ml falcon tube, and the volume was reached to 50 mL by adding distilled water. A magnet was placed inside an Erlenmeyer flask, and the flask was placed on a hot plate (styrofoam) to homogenize the constituents. Then the mixture was transferred into an ultrasonic device for half an hour to produce a stable solution. In the next step, the solution was placed into an ultrasonic bath (UP400S Hielscher, Germany, temperature 25 °C) for 15 min and then into a sonicator device (three repetitions each for five min) [37].

Determining the average size of nanoemulsions

After preparation, 20 µL of the nanoemulsion was diluted by 980 µL of a phosphate buffer by pipetting several times. The diluted sample was then transferred into a special sized measuring compartment and analyzed by a Particle Size Analyzer (Nano-ZS, Malvern, UK).

Determination of zeta potential of nanoemulsions

The nanoemulsion was diluted by a phosphate buffer as noted in the previous section. After it became completely uniform, the sample was transferred to a Zeta device for measuring the Zeta potential and analyzed by a Particle Size Analyzer (Nano-ZS, Malvern, UK).

Transmission electron microscopy

Transmission electron microscopy (TEM) (JEOL, Japan) is one of the most suitable tools available for analyzing the morphology of nanostructures [38, 39]. In fact, TEM's ability to examine the size of materials is unparalleled and has many advantages over light microscopy. In this method, images are produced by transmitting an electron beam over a sample's surface. The wavelengths of electrons are shorter than those of light photons, and therefore, shorter wavelengths provide a clearer and better resolution, as well as more information. Three-dimensional images of the sample's structure were obtained. In order to determine the size and morphology of the nanoemulsions synthesized, 20 µL of the sample was poured on a slide, and the slide was allowed to dry in the laboratory environment. It was then mounted on a TEM to determine the sizes of the nanoemulsion and nanoparticles [10, 40].

Evaluation of the stability of the nanoemulsions

The stability of the nanoemulsions was analyzed by placing them at room temperature. Over three months, the sizes of the droplets were assessed by using a DLS assay. Change in the size, ζ-potential, and PDI value of the nanoemulsions was analyzed during this period.

Cell culture

The PC3 cell line and skin fibroblasts (HFF) were purchased from Iran's Pasteur Institute. For cell culture, the RPMI medium (GIBCO, USA) was used, supplemented with 10% bovine fetal serum (GIBCO, USA) and 1% penicillin/streptomycin (GIBCO, USA). Both cell lines were incubated at 37 °C, 5% CO_2, and 95% humidity.

MTT Test

Cancer cells' survival was assessed using the MTT assay, as described in previous studies. The basis of this test is the conversion of the MTT reagent (a yellow liquid) to purple formazan crystals by the act of mitochondrial succinate dehydrogenase, which is present only in living cells [41, 42]. Approximately 2,000 cells were implanted in each well of a 96-well plate, and the cells were incubated for 24 hr to adhere to the bottom of the plate. Then they were treated

with the nanoemulsion at doses of 12.5, 25, 50, and 100 µg/mL. The light absorption of each well was determined 48 hr after treatment with the nanoemulsion, assuming a linear relationship between the number of living cells and light absorption. In each plate, 10 µL of the MTT solution (5 mg/mL in PBS) was initially added to each well. After a 4-hr incubation period, the liquid inside the wells was drained, then 100 µL DMSO was added to each well, and the mixture was shaken for 20 min. Finally, the light absorption of the wells was measured at 570 nm by an ELISA Reader (Epoch, Biotek, United Kingdom) [43]. The cytotoxicity of the nanoemulsion against the PC3 cancer cell line and HFF cutaneous fibroblasts was measured using the following formula:

Cell viability (%) = (Testabs / controlabs)*100

Flow cytometry

Flow cytometry was used to assess apoptosis based on cell cycle changes. This technique is a valid and broadly-employed tool to identify and characterize cells of various origins. The basis of cell detection in this technique is light scattering. In this method, fluorescent dyes, such as PI, are used to record fluorescence emission. After culturing cells in 6-well plates, the cells were initially washed with PBS and then treated with various concentrations of nanoparticles. Then the cells were exposed to PI for 30 min. Finally, the ratios of cells in various cell cycle phases were determined via a flow cytometer (BD FACSCalibur] according to the manufacturer's guidelines [44].

Determination of free radical scavenging activity dpph assay

The DPPH molecule is a chemical compound used to quantify free radicals in a system [45]. After being dissolved in ethanol, the molecule converts to its radical form, which has the highest absorption at 517 nm. The radical produced is removed after reaction with a reducing substance, leading to a decline in 517 nm absorption. First, 1 mL of the nanoemulsion in different final concentrations (125, 250, 500, and 750 µg/mL) was prepared. Then 4 mL of DPPH with a concentration of 0.15 mM (dissolved in 95% ethanol) was added to nanoemulsion samples. The solution was vigorously mixed by a vortex for three minutes and then kept in the dark for 30 min before reading its absorbance at 517 nm (1800 spectrophotometer, Shimadzu, Kyoto, Japan). In this experiment, BHA was employed as a control anti-oxidant.

ABTS assay

The ABTS molecule is commonly used to measure the anti-oxidant activity of various materials. To prepare the ABTS solution, its reagent was combined with potassium persulfate and water and then kept dark for 16 hours until turning into a blue-green liquid (i.e., a free radical with a peak absorption at 734 nm). In this method, ABTS (cationic state) radicals were initially generated and then mixed with the nanoemulsion to record the decrease in absorption. A diluted ABTS radical solution was mixed with the nanoemulsion (the ratio of 1: 1) at different concentrations, and after incubation for 1 hr at 37 °C, the absorption of the solution was recorded at 734 nm (1800 spectrophotometer, Shimadzu, Kyoto, Japan). This experiment was performed three times; the mean values were used for calculating anti-oxidant activity, and BHA was employed as a control anti-oxidant [46].

Statistical analysis

The anti-oxidant activities of the nanoemulsion and control reagent were initially calculated by inserting their respective ODs using specific formulas. The resulting values were then placed into SPSS software and compared using one-way ANOVA and the least significant differences (LSD) method. Error bars on graphs, means, standard deviations, and 5% confidence intervals were employed for calculations.

RESULTS

Characterization of nanoemulsions

The results showed that the average size of the nanoemulsions was 67.49 nm. Most of the nanoemulsions had hydrodynamic sizes between 9.34 and 32.37 nm, and the mean diameter was obtained at 15.45 nm. Based on intensity distribution, the nanoemulsions had a mean diameter of 89.49 nm. Alternatively, the droplet volumes of the nanoemulsions varied from 9.34 to 81.30 nm with a mean particle volume of 34.35 nm (Fig. 1a, b, c). The PDI value of the nanoemulsions was 0.411, which was greater than 0.3, indicating nonhomogeneous dispersion and presence of large nanoparticles in the solution. As shown in Fig. 1, although particles larger than 400 nm were present in the solution, they comprised a very low percentage (i.e., less than 0.01% of all particles).

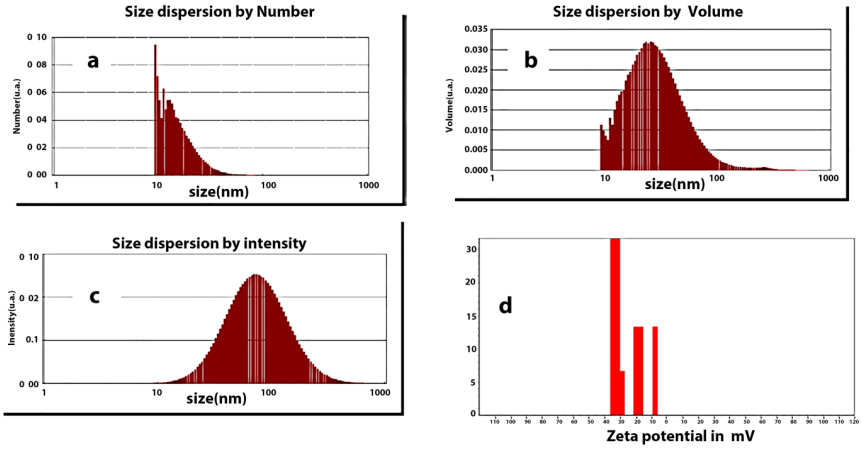


Fig 1. Particle size of the nanoemulsion produced by *Zingiber Officinale* L. tincture. (a) Most of the particles had hydrodynamic sizes between 9.34 and 32.37 nm, and the mean diameter was 15.45 nm. (b) Considering the intensity distribution, the nanoemulsions had a mean diameter of 89.49 nm. (c) Nanoemulsion droplet volume varied from 9.34 to 81.30 nm with a mean particle volume of 34.35 nm. (d) Zeta potential of the nanoemulsions synthesized by *Zingiber Officinale* L. tincture was measured, showing a net charge of -25.05 ± 9.17 mV and mean mobility of -195 +0.71 µm/s/V/cm.

Also, particles with sizes of about 9.34 nm constituted approximately 0.09% of the particles. Overall, most of the particles were less than 50 nm in size, indicating an acceptable threshold for biological studies.

The zeta potential of the nanoemulsions produced using ginger tincture was -25.05± 9.17 mV, and the mean mobility was obtained at -195 +0.71µm/s/V/cm (Fig. 1d). According to Salopek *et al*., a zeta potential in the range of -30 to -16 mV reflects the stability of a nanoemulsion. Therefore, it can be said that the zeta potential of the nanoemulsions prepared from ginger tincture was close to the stability range. The sizes of the nanoemulsions prepared were also assessed by electron microscopy, showing an overall particle size of less than 100 nm. Besides, TEM analysis showed that the nanoemulsions were spherical in shape (Fig. 2).

Twenty microliter of the sample was placed on the instrument's carbon-coated copper grid, and the gride was allowed to dry in the laboratory environment. The morphology of the particles was observed by TEM microscopy applying an accelerating voltage of 120 kV, revealing spherical particles. The nanoemulsions seemed to be highly stable at room temperature (Table 1). A slight increase in the size of the droplets was noticed after 90 when the nanoemulsions were stored at room temperature.

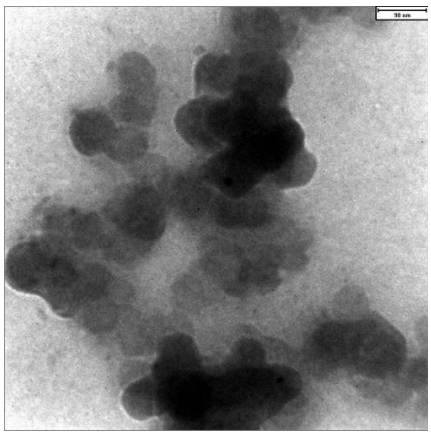
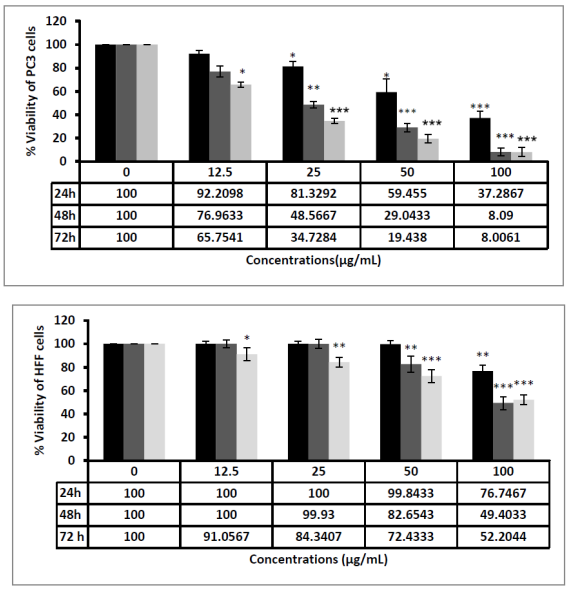


Fig. 2. TEM images of the nanoemulsion fabricated using *Zingiber Officinale* L tincture.

Table 1. Size, ζ-potential, PDI values of synthesized nanoemulsions after 3 months

Time interval (day)	Size (nm) ± SD	ζ- pot (mv) ± SD	PDI ± SD	Viscosity(AU)±SD
1	67.49±0.27	-25.05± 9.17	0.411±0.05	0.844±0.01
30	68.21±0.33	-19.14± 5.22	0.389±0.02	0.858±0.12
60	68.19±0.16	-23.25± 6.47	0.378±0.09	0.861±0.09
90	71.08±0.11	-28.49± 8.11	0.399±0.03	0.878±0.04



	0	12.5	25	50	100
24h	100	92.2098	81.3292	59.455	37.2867
48h	100	76.9633	48.5667	29.0433	8.09
72h	100	65.7541	34.7284	19.438	8.0061

	0	12.5	25	50	100
24h	100	100	100	99.8433	76.7467
48h	100	100	99.93	82.6543	49.4033
72 h	100	91.0567	84.3407	72.4333	52.2044

Fig 3. Toxicity of the fabricated nanoemulsion against the PC3 prostate cancer cell line (a) and the normal HFF cell line (b) was measured at different concentrations and times in triplicate (N= 3). The IC_{50} values for the PC3 cell line were 42, 26, and 17 µg/mL at 24, 48, and 72 hr after treatment, respectively. The differences between the test and control groups were compared using analysis of variance (ANOVA) and the LSD test. P< 0.05 was considered statistically significant. The results have been presented as mean ± SD (n = 3), *; P<0.05, ***; P<0.01, and ***; P < 0.001

Nanoemulsion toxicity against PC3 cancer cells

The toxicity of different concentrations of the synthesized nanoemulsions against the prostate cancer cell line (PC3) and normal HFF cell line was assessed using the MTT assay after 24, 48, and 72 hr of treatment (Fig. 3a and b). As shown in Fig. 3, the viability and proliferation of the PC3 cells exposed to nanoemulsions decreased with the increasing nanoemulsion concentration. In addition, the results showed that the lowest concentration used in this test had the most significant anti-cancer effects (*P*-value <0.001). Normal HFF cells were less affected by the presence of the nanoemulsion as compared with cancer cells. Viability assessment for PC3 cells demonstrated the IC_{50} values of 42, 26, and 17 µg/mL after 24, 48, and 72 hr of treatment, respectively.

Analysis of cell cycle changes and apoptosis by flow cytometry

The growth inhibitory effects (via arresting the cell cycle) of the synthesized nanoemulsions against PC3 cancer cells were assessed by flow cytometry. The PC3 cells were exposed to different concentrations of the nanoemulsions for 48 hr and then analyzed by flow cytometry. Compared with the control, our findings showed that the number of the cancerous cells halted in the subG1 phase of the cell cycle significantly increased after treatment with the nanoemulsions (Fig. 4), reflecting a rise in

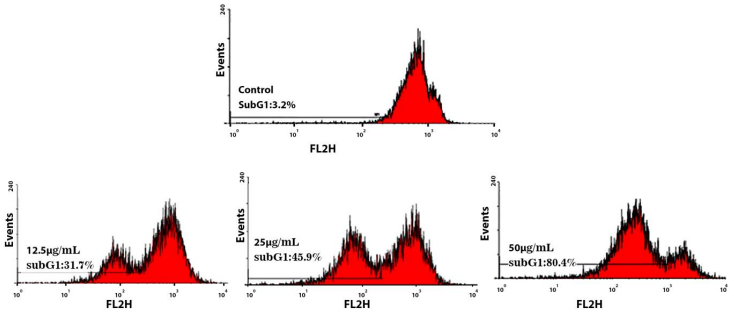


Fig 4. Apoptosis and cell cycle analyses via flow cytometry. PC3 prostate cancer cells were treated with different concentrations of nanoemulsions (12.5, 25, and 50 µg/mL). Then the cells were exposed to PI for 30 min. The ratios of cells in various cell cycle phases was determined using a flow cytometer (BD FACSCalibur) according to the manufacturer's guidelines. The ratio of cells in the SubG1 phase of the cell cycle significantly and dose-dependently increased, indicating a dose-dependent rise in apoptotic cells

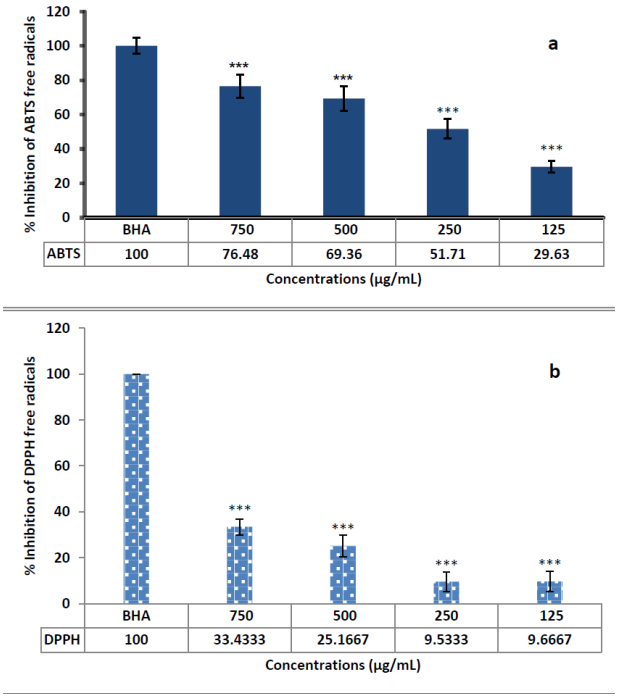


Fig 5. ABTS (a) and DPPH (b) scavenging activities of the nanoemulsion produced from *Zingiber Officinale* L. tincture. The nanoemulsion efficiently eliminated these free radicals in a concentration-dependent manner. All the tests were performed in triplicate, and BHA was applied as the positive control. The differences between the test and control groups were compared using the analysis of variance (ANOVA) and the LSD test. P<0.05 was considered statistically significant. (***; P<0.001, indicating a significant difference as compared with BHA).

the ratio of apoptotic cells in a dose-dependent manner. According to the results, the ratio of the cells in the SubG1 phase of the cell cycle increased significantly and dose-dependently (31.7%, 45.9%, and 80.4% in the groups exposed to 12.5, 25, and 50 µg/mL of the nanoemulsion, respectively, in comparison with the control group) (Fig. 4), indicating a dose-dependent rise in the ratio of apoptotic cells. Therefore, the nanoemulsions could promote their anti-cancer effects by inducing apoptosis and arresting the cell cycle of cancerous cells, hindering tumor growth.

Radical scavenging activity

Anti-oxidants are used to neutralize reactive oxygen species (ROS), which are known to predispose to cancer [47, 48] via destabilizing DNA, proteins, and lipids and causing mutations in key oncogenes or tumor suppressors [49, 50]. In this experiment, the anti-oxidant activity of the nanoemulsions was measured based on their ability to neutralize DPPH and ABTS free radicals. The results showed that the nanoemulsion had a dose-dependent radical scavenging activity (Fig. 5 a and b).

DISCUSSION

The history of the use of medicinal plants is as old as human beings, that is, with the emergence of the first diseases, man instinctively began to treat them using surrounding materials, and of course, many different plants were around and at his disposal [51-54]. Over time, humans gradually became acquainted with the miraculous power of plants for treating diseases and illnesses and used them for this purpose [55]. Although interest in these useful plants has been low for years, fortunately, they have received more attention recently [56]. Until about half a century ago, plants were among the main sources of drug production, but after the development of organic chemistry sciences, efforts were diverted towards producing complex and synthetic chemical drugs [57]. One of the diseases that has attracted the attention of researchers is cancer. Therefore, this study aimed to investigate the anti-cancer and anti-oxidant effects of the nanoemulsions produced from ginger tincture. Prostate cancer (PC) is one of the most prevalent cancers in men, in which prostate gland cells mutate and transform into cancer cells. One way to treat cancer is to use the active ingredients

of plants. On the other hand, nanotechnology has promised breakthroughs in cancer treatment in recent years [58], and nano-formulations of cytotoxic natural products have been suggested as potent anti-cancer agents [59].

The results of this work displayed that the nanoemulsions containing ginger tincture could appropriately suppress the growth of the PC3 prostate cancer cell line without any major cytotoxicity against normal HFF cells. Ginger nanoemulsion was reported to suppress PC3 cells' proliferation dose- and time-dependently. Ginger, as a medicinal plant, contains many biologically active compounds, some of which can have anti-cancer activity. Generally, the permeability of tumor vessels is a key factor for the successful delivery of chemotherapeutics by nanoemulsion carriers [60]. It is estimated that the average size of tumor vascular cavities is 800-100 nm, which is significantly larger than the cavities in the normal vascular endothelium (> 6 nm). Therefore, nanoemulsions with a diameter of about 50-200 nm are small enough to inactively penetrate tumor vascular endothelium after intravenous injection. In addition, the formation of a discontinuous endothelial lining in tumor vessels during angiogenesis facilitates the release of nanoemulsions into the interstitial space of the tumor. Besides, these nanoemulsions are too large to enter the endothelium of normal tissues [61, 62]. Therefore, nanoemulsions with a diameter less than 200 nm can effectively accumulate in tumor tissues.

In one study, the anti-oxidant activity and cytotoxicity of *Zingiber officinale* rhizome oil were investigated *in vitro*, and GC-MS analysis revealed that the essential oil contained at least seven anti-cancer compounds, about 90% of which were present in ginger oil. Of these seven compounds, α-zingiberene (20%), β-pinene (14.20%), and β-sesquiphellandrene (12.11%) had the highest cytotoxicity (63). A study by Wang *et al*. supported the anti-oxidant properties of ginger oil, as evidenced by the DPPH free radical scavenging test. The IC_{50} value of ginger essential oil was reported at 0.55% (v/v), showing strong cell toxicity toward two human cancer cell lines (HO-8910 and Bel-7402 with the values of 0.00643 and 0.00256% (v/v), respectively). In addition, red ginger roots oleoresin (Zingiber officinale Roxb. Var. Rubrum Theilade) has been reported to contain antioxidant compounds [64].

Some of the bioactive compounds of ginger have been found to be easily degraded by oxidation. Microencapsulation can be used to stabilize these bioactive compounds. In a study by Anggasta *et al.*, oleoresin was used as a coating material by okra mucilage, and in comparison with another industrial coating substance (maltodextrin), the results showed that microencapsulation of ginger by the natural okra coating could confer stronger anti-oxidant effects [65]. In the study of Mulia *et al.*, the oleoresin nanoemulsion from ginger extract (*Zingiber officinale* var. Roscoe) was produced using vegetable oil and prepared for encapsulating bioactive substances [66]. Oleoresin is known as a natural antitumor, antimicrobial, and anti-oxidant agent, but it has low bioavailability due to its unique physicochemical properties such as low solubility of oleoresin-derived phenolic complexes in the gastrointestinal tract. Therefore, preparation of its nanoemulsion form can overcome this limitation and increase its applications in the pharmaceutical industry. In one study, the anti-cancer and anti-proliferative effects of ginger against Hela cells were shown [67].

In this study, flow cytometry analysis showed that the synthesized nanoemulsions interfered with the cell cycle of cancerous cells, either at G2/M or G1/S transition checkpoint and in this way, suppressed their proliferation. The PC3 cells treated with the nanoemulsion for 48 hr also revealed a halt in cell cycle progression at G2/M and S phases, triggering apoptosis in the cancerous cells.

Cell cycle interruption (at the S phase) seemed to be a major mechanism through which the synthesized nanoemulsions suppressed the growth and proliferation of cancer cells. Deregulation of the cell cycle, especially at the main transition points such as the S-phase transition, can markedly suppress cellular division. A halt in the S phase accompanied by persistent E2F-1 activity can trigger cellular apoptosis and programmed death [68]. As we observed, the cancerous cells treated with the *Zingiber Officinale* L. Tincture nanoemulsion displayed a disrupted cell cycle at the G2/M transition point, terminating in their apoptosis. Therefore, the fabricated nanoemulsions can be regarded as potential anti-tumor agents suppressing cell cycle progression in tumor cells.

CONCLUSION

In this study, a nanoemulsion was synthesized

using *Zingiber Officinale* L. Tincture. The characteristics of the nanoemulsion were investigated using DLS, zeta potential, and TEM techniques. The average size of the nanoemulsions containing ginger was about 67 nm. The nanoemulsion conferred high stability and efficiency and possessed anti-oxidant and cytotoxic properties, which were more potent against prostate cancer cells than normal cells. The PC3 cells treated with the nanoemulsion for 48 hr revealed a halt in cell cycle progression at G2/M and S phases, triggering apoptosis in the cancerous cells. Based on these findings, cell cycle arrest at G2/M transition can be one of the anti-cancer mechanisms exploited by the nanoemulsion produced. In addition, this nanoemulsion showed an acceptable capacity to eliminate DPPH and ABTS free radicals, indicating its anti-oxidant activity. So, these bioproperties render the nanoemulsion produced via *Zingiber Officinale* L. Tincture as a potential anti-tumor agent that can be applied in nanomedicine to treat cancers.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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