

Cytotoxicity and Antioxidant Effect of Ginger Gold Nanoparticles on Thyroid Carcinoma Cells

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Abstract:

The purpose of this study was to evaluate the antioxidant and cytotoxic effects of the ginger extract-AuNP on the FTC-133 cell line. The biosynthesis of gold nanoparticles was achieved by reduction of gold (III) chloride hydrate (HAuCl₄) using Zingiber officinale extract. Characterization of biosynthetic gold (AuNPs) nanoparticles by ultraviolet-visible spectroscopy, atomic force microscopy (AFM), EDX and SEM. The cytotoxicity of FTC-133 cell viability showed that the nanoethanol extract caused a decrease in cell viability in a dose-dependent manner for 48 hours, which resulted in a significant ($p \leq 0.05$)-dependent decrease in the activity of FTC-133 cells at 400 $\mu\text{g mL}^{-1}$. A cell death of 75% was achieved with an IC₅₀ of 85.2, which was significantly different from the ethanol extract of 242.1 $\mu\text{g mL}^{-1}$. The antioxidant properties of the synthesized nanoparticles were effective, and the IC₅₀ for scavenging DPPH radicals was determined to be 40.55 $\mu\text{g/mL}$. Mechanism of Affected Living Cells to Apoptosis High-content screening (HCS) assay showed a significant increase in cell membrane permeability, cytochrome c and nuclear concentration (200 $\mu\text{g/mL}$) concentration (dose-dependent) ($p < 0.0001$)) Nano ethanol extract when compared with doxorubicin as standard. The effect of nano-ethanol extract on the mechanism of apoptosis showed that when the concentration was increased (dose-dependent) cell viability and mitochondrial membrane permeability (200 $\mu\text{g/mL}$) compared with doxorubicin as a positive control ($p < 0.0001$) reduce.

Keywords: Zingiber officinale, Gold nanoparticles, MTT, DPPH, HCS

INTRODUCTION

Nanotechnology is a scientific field involving the production, manipulation and use of nanomaterials. [1] Since the beginning of the 20th century, the synthesis of gold nanoparticles (AuNPs) has been fascinated by the scientific community due to its unique physical and chemical properties. Because of these special properties, many research groups have tried to use AuNP to solve socially important problems in various fields, including medicine, cosmetology, biology, clinical chemistry and pharmacology [2, 3]. There are more applications, due to the high environmental pollution caused by chemical synthesis, so green synthesis is needed, including clean, non-toxic and environmentally friendly nanoparticle synthesis methods with sustainable commercial viability [4]. Green synthesis utilizes environmentally friendly, non-toxic and safe materials [5], such as plant leaf extracts, bacteria, fungi and enzymes, for the synthesis of metal nanoparticles, providing many environmental benefits and compatibility with pharmaceutical and other biomedical applications. Ginger Zingiber officinale is widely used as a spice or folk medicine. It is a perennial herb that grows about one meter high in stems each year, with narrow green leaves and yellow flowers [6]. It has a long history as a herbal medicine for the treatment of various diseases, including nausea and vomiting, constipation, indigestion (dyspepsia), pain and cold syndrome. Recently, it has been reported that ginger and its bioactive compounds including alkaloids, flavonoids, zingiberene, gingerol, shogaol, paradols and zingerone have anti-cancer, anti-clotting, anti-diabetic, anti-inflammatory, arthritic, heart disease and Antioxidant properties because it removes superoxide anion and hydroxyl radicals [7]. It also reported its antifungal and antibacterial properties [8]. Safety assessment studies have shown that ginger is well tolerated, even at very high doses, without any toxic effects. Therefore, ginger and its

biologically active ingredients have the potential to develop modern medicine for the treatment of anemia and inflammation-related diseases [9]. Ginger extract showed selective anticancer activity. It is considered a promising chemopreventive agent against colon and skin cancer. The plant also has antioxidant activity and significantly reduces lipid peroxidation by maintaining superoxide dismutase, catalase and glutathione peroxidase activity [10]. This study was designed to evaluate the potential anti-cancer and anti-oxidant properties of ginger extract in combination with nanoparticle AuNPs in vitro.

MATERIALS AND METHODS

Plant Collection

Z. officinale rhizome was collected in March 2018 from the local market in Cairo - Egypt. The plant was submitted to the University of Baghdad/Scientific Herbarium, certified by Dr. Sukeyna A. Aliwy, Ph.D. in Plant Taxonomy, and the voucher code is assigned: Z. Officinale 50781. Wash the roots to remove dust and allow it to dry completely. The dried roots were ground to a fine powder using a Wiley Mill grinder (Standard Model 3) and stored in a refrigerator at 4 °C. An ethanol extract (99.9%) was prepared using the Soxhlet apparatus according to the method described in [11]. (10 g) fine powder was placed in a porous cellulose sleeve, and 100 mL of ethanol was added to the round bottom flask. The extraction procedure was carried out at 70 °C for 8 hours and the resulting solvent extract was concentrated at 40 °C using a rotary evaporator to complete evaporation. The collected dry extracts were weighed and stored for storage and stored in a refrigerator at 4 °C.

Preparation Ginger Nanoparticle

50 mg of ginger extract was extracted with deionized water, and 5 ml of a 1 mM HAuCl₄·3H₂O solution was slowly added under magnetic stirring to uniformly coat the gold.

This extract was used as a reducing and stabilizing agent for 1 mM HAuCl₄·3H₂O. [12]

Characterization of synthesized GNPs

In order to determine the time point of maximum yield of HAuCl₄·3H₂O (sigma.co) nanoparticles, the absorption spectrum of the sample was taken to be 200-1100 nm using a UV-vis spectrophotometer (shimadzu). Use deionized water as a blank. Samples from the maximum production time point of gold nanoparticles were air dried and characterized by atomic force microscopy (NT-MDT, Ntegra, Russian Federation) for their detail size, morphology and gold agglomeration. The AFM image is a cantilever with a force constant of 0.02-0.77 N / m and a tip height of 10-15 nm in contact mode. A thin film of the sample was prepared on a carbon coated copper grid by dropping a sample on the grid for one minute. Movie on SEM grid Dry for 5 minutes under mercury lamp .The measurement uses (TESCAN, Vega III, Czech Republic) SEM machine. The presence of elemental gold was confirmed by EDAX. The EDAX spectrum records one of the dense gold nanoparticle regions on the surface of the film in a dot pattern. Nanocrystals were analyzed using an SEM machine.

Cytotoxicity assay:

Tests were performed in triplicate and the IC50 values of the samples were calculated using a log dose inhibition curve. Cell line culture: Human follicular thyroid carcinoma, lymph node metastasis (FTC-133) was cultured in 100 µl of RPMI 1640 (Roswell Park Memorial). Institute medium) Medium containing 10% fetal bovine serum (FBS). FTC-133 cells were incubated overnight at 37 ° C, 5% CO₂ for cell attachment.

MTT cytotoxicity assay: Follow the manufacturer's instructions for agreement [13]. The cells (1 x 10⁴ to 1 x 10⁶ cells mL⁻¹) were cultured in 96-well plates to a final volume of 200 mL well⁻¹. The plates were covered with a sterile parafilm, gently stirred and incubated for 24 hours at 37 ° C, 5% CO₂ . After the incubation, the medium was removed, and 200 mL of a 2-fold serial dilution of the crude extract of Z. officinale (25, 50, 100, 200, 400 mg mL⁻¹) was added to the wells. Triplicate was performed at each concentration and control. The plates were incubated for 48 hours at 37 ° C, 5% CO₂ . After exposure to the extract, 10 mL of MTT solution was added to each well. The plates were further incubated for 4 hours at 37 ° C, 5% CO₂ . The medium was then carefully removed, 100 mL of the dissolution solution was added to each well and incubated for 5 minutes. Absorbance was measured using an ELISA reader (Bio-rad, Germany) at a wavelength of 575 nm. Statistical analysis was performed on the optical density readings to calculate the IC50. According to the following equation

Viability(%) = (optical density of sample/optical density of control)×100

Free radical antioxidant activity

The antioxidant activity of the ethanol extract conjugate and AuNP was examined using a free radical scavenging test (DPPH) [14]. The scavenging activity against DPPH free radicals was determined using spectrophotometry (shimadzu). Colorimetric changes were measured at 517 nm. In this experiment, a set of concentrations (12.5, 25, 50 and 100 µg mL⁻¹) was used for each identified compound. Ascorbic acid was used as a control. Calculated based on the percentage of free radicals

$$\% \text{ inhibition} = \frac{\text{Absorbance of - ve control} - \text{Absorbance of sample}}{\text{Absorbance of - ve control}} \times 100$$

Multi-parameter cytotoxic assay: A multiparametric cytotoxicity assay [15] was performed to measure five orthogonal FTC-133 cell health parameters after exposure to nano ethanolic ginger extract in vitro. Parameters were: viable cell count, total nuclear intensity, cell membrane permeability, mitochondrial membrane permeability, and cytochrome c release. Briefly, treated FTC-133 cells were stained with a cell staining solution (MMP dye + osmotic dye) at 37 ° C for 30 minutes after exposure to different concentrations of the nano ethanolic ginger extract for 24 hours. Cells were fixed, permeabilized and blocked, and then probed with primary cytochrome C antibody and a second DyLight 649-conjugated goat anti-mouse IgG for 60 minutes each. The plates were analyzed using an ArrayScan HCS Analyzer (Thermo Scientific, USA).

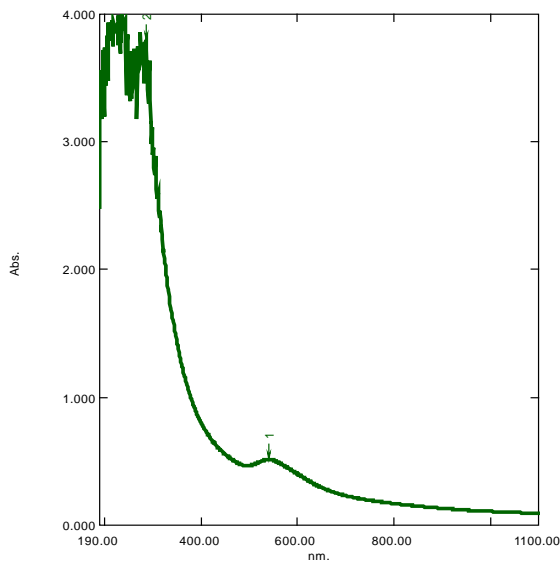
RESULT AND DISCUSSION :

Characterization of AuNPs : UV visible spectra

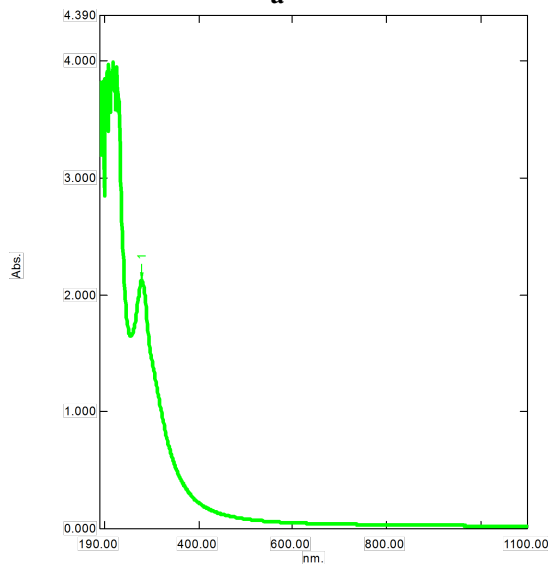
The aim of this study was to screen the synthesis of ginger roots AuNPs and their cytotoxic activity against FTC-133 cells. Ultraviolet-visible spectroscopy is an important technique for obtaining this spectrum formation and stabilization of metal nanoparticles. The addition of 1 mM aqueous solution of HAuCl₄·3H₂O to Z. officinale extract resulted in a change in color from yellowish brown to purple or black due to absorption or scattering of light by them and formation of gold nanoparticles [16]. Therefore, the first evidence generated by AuNPs is the visible color change of the reaction mixture. The color change of the reaction mixture is consistent with the occurrence of the local maximum surface plasmon resonance (LSPR) (λ_{max}) [12] In order to find the position of (λ_{max}) of the LSPR absorption band, UV / Vis absorption spectrophotometry was applied. The λ_{max} position of the LSPR absorption band of the Au nanofluid synthesized using the ethanolic plant extract is summarized in (Table 1). The area and localization of λ_{max} of LSPR depends on the size and shape of AuNP, the concentration of AuNPs precursor, the type of solvent and the reaction temperature [19].

Table(1):UV-visible spectra of ethanol extract for ginger and nano.

Ginger extract	Wavelength (λ)	Abs of ginger extract	Wavelength (λ)Of nano ginger extract	Abs of nano ginger extract
Ethanol	278.00	2.124	541.00	0.514



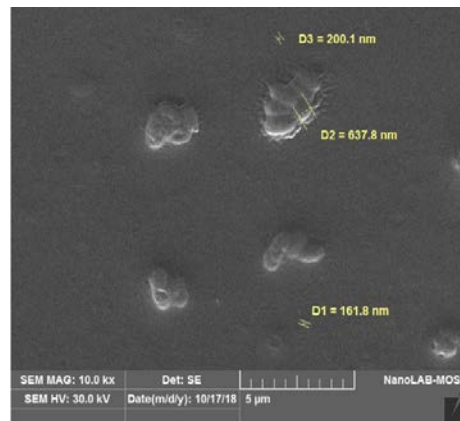
a



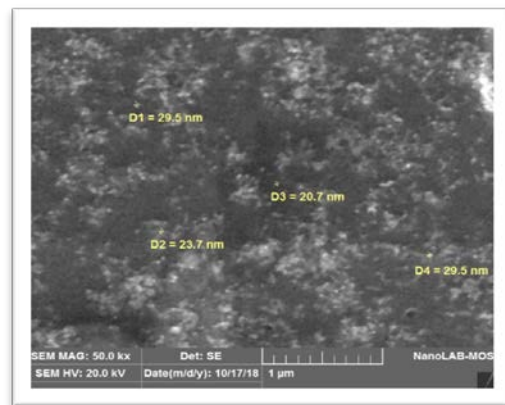
b

Figure (1) :UV-visible spectra of **a-** nano ethanol and **b-** ethanol extract right

Scanning Electron Microscope (SEM) Scanning electron microscopy (SEM) micrographs of the obtained AuNPs show that they are spherical as shown in fig (2a,b) SEM analysis of AuNPs showed the formation of well dispersed AuNP. Morphological examination also shows nanoparticle crystal size (D1=29.5,D2=23.7,D3=20.7,D4=29.5nm) with speherical in shape while ethanol extract(D1=200.1,D2=637.8,D3=161.8nm) . This result is similar to the particle size mentioned in [20, 21]. X-ray energy dispersion analysis (EDAX) gives qualitative and quantitative states of the elements that may be involved in GNP formation. The characteristic peaks obtained in the (EDX) image (3a, b) according to the structural view of the SEM confirmed the efficient synthesis of the gold nanoparticles.



a



b

Figure (2): SEM images of ethanol ginger extract(**a**) and nano(**b**)

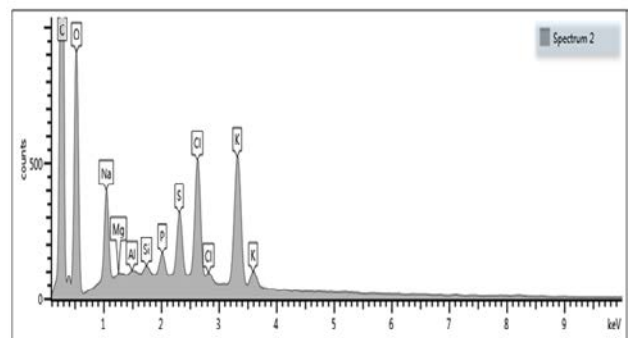


Figure (3a): EDAX profiles for ethanol extract

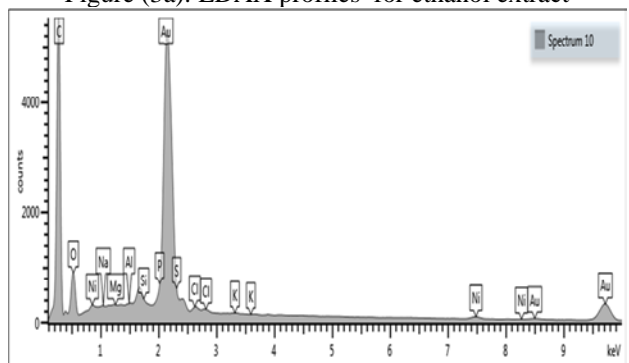


Figure (3b): EDAX profiles of GNPs for ethanol extract along with the elemental composition of Au

Atomic-force microscopy (AFM)

Nanoparticle analysis is an important challenge in nanoscale metrology today. Therefore, their correct characterization is very important. By measuring the height of the nanoparticle image, the size of the nanoparticles can be easily determined from the AFM image [22]. Nanoparticle analysis can be performed using scanning probe microscopy (AFM), which has several advantages over SEM/TEM for characterizing nanoparticles. The image from the AFM represents three-dimensional data, so the height of the nanoparticles can be quantitatively measured. Using SEM, the measured image is only two-dimensional, and the AFM scan speed is slower than SEM [23]. From this analysis, the size of the nanoparticles was easily detected before testing GNP on the cell line. As shown in (4), the 3D image of the synthesized GNP and the darker color mean that very small particles and a lighter color mean larger particles.

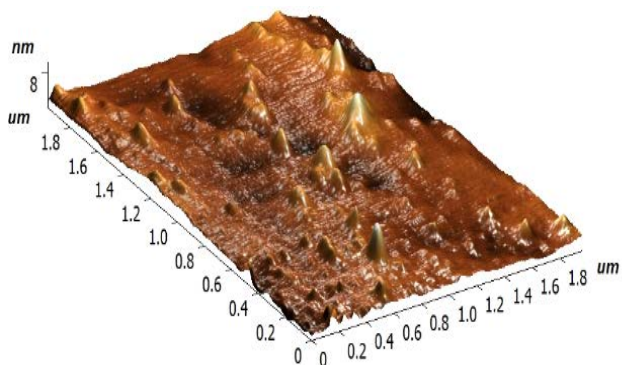
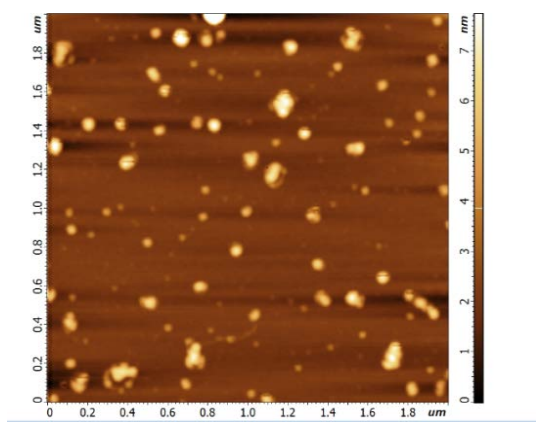
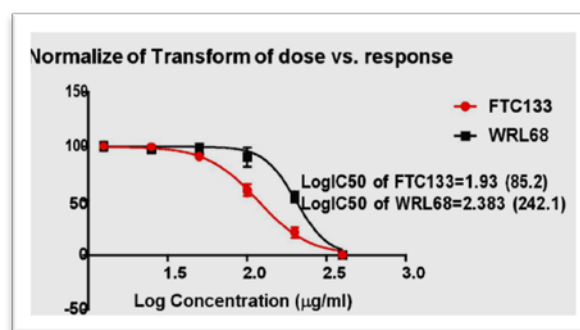
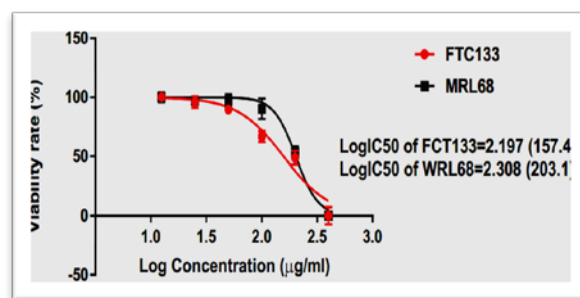


Figure (4): AFM images of synthesized of AuNPs of ethanol ginger extract

Viability assay(MTT):

The cytotoxicity results of FTC-133 cell viability after 48 hours of treatment with different concentrations of ethanol extract (12.5 to 400 µg mL⁻¹). Ethanol extracts resulted in a decrease in cell viability in a dose-dependent manner, and nano ethanol extracts resulted in a significant (p < 0.05) decrease in FTC-133 cell survival in a dose-dependent manner, reaching 75% cell death at 400 µg/mL. The IC₅₀ was 85.2 and the ethanol extract was significantly different from 242.1 µg mL⁻¹ (Fig. 5a, b).



a

Figure (5):Cytotoxicity of Ethanol ginger extract(a) and nano(b) with FTC133 and normal cell

Evidence from in vitro, animal and epidemiological studies indicates that ginger and its active ingredients inhibit growth and induce apoptosis in a variety of cancer types, including skin, ovary, colon, breast, cervix, mouth, kidney, prostate, stomach, Pancreas, liver. And brain cancer. These characteristics of ginger and its constituents may be related to antioxidant, anti-inflammatory, anti-mutagenic properties and other biological activities [24, 25]. Ginger and its general compounds such as gingerol, shogaol, paradols and zingerone exert immunomodulation, anti-apoptosis, anti-tumor, anti-inflammatory, anti-hyperglycemia, anti-hyperlipidemia, anti-oxidation and anti-emetic activity [26] Ginger extract exhibits chemical sensitization of certain tumor cells in vitro and in vivo. To support this, another study showed that ginger can reverse the delay in gastric emptying caused by cisplatin, suggesting that ginger can be used as an antiemetic agent for cancer chemotherapy [27]. Based on the results obtained from the MTT assay, the prepared extract showed a dose-dependent cytotoxic activity against cancer cells. Some compounds present in ginger can exert a cancer preventive effect by inducing apoptosis in cancer cells or transformed cells. The oleoresin from ginger root contains 6-gingerol, the main pharmacologically active ingredient and a relatively small amount of structurally related vanilloid, 6-paradol [28]. These results indicate the potent cytotoxicity and antitumor activity of GE and its potential as an anticancer agent. Based on the results obtained from the cytotoxicity assay, ethanol-AuNP is the most potent extract in FTC-133, which is used only in all of the following assays.

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) :

The scavenging activities of the nanoethanol extract of *Z. officinale* roots were estimated using the following concentrations: 100, 50, 25 and 12.5 µg mL⁻¹. The results (Figure 6) show the effective free radical scavenging activity compared to vitamin C, with calculated IC₅₀ values of 40.55 µg/mL compared with vitamin C 36.48 µg/mL, the nano ethanol extract reduced the pattern of DPPH significantly (p ≤ 0.05).

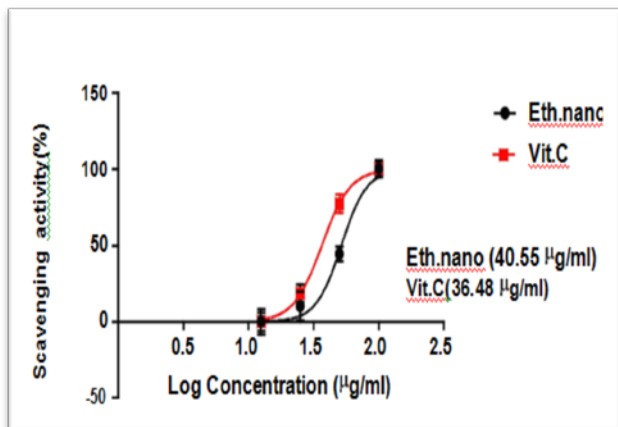


Figure 6: DPPH scavenging activity of nano ethanolic extracts of ginger

The DPPH test is based on the ability of DPPH radicals to react with H-donors including phenolic compounds [29]. DPPH• Free radicals are one of the few stable organic nitrogen radicals with a deep purple color. The antioxidant is capable of reducing the stable free radical DPPH to yellow diphenyl-picrylhydrazide (DPPH-H). Oxidation of biomolecules induces a variety of pathological conditions, including atherosclerosis or cancer. These damages are caused by the presence of free radicals [30]. The result may be due to the presence of 6-gingerol, which shows strong antioxidant activity *in vitro* [31]. Ginger can be considered a repository of antioxidants. It has the special properties of scavenging reactive oxygen species (ROS), free radicals, peroxides and various other harmful oxidants. The active ingredients such as gingerol, shogaol and zingerone present in ginger have antioxidant activity. It inhibits the enzyme, xanthine oxidase, which is primarily involved in the production of reactive oxygen species. The first evidence for the antioxidant properties exhibited by zingerone is that zingerone has the ability to degrade free radicals produced by various food radiations. It has been reported that Zingerone protects DNA *in vitro* from stannous chloride-induced oxidative damage of ROS. Another exciting fact about zingerone is its high antioxidant activity compared to ascorbic acid [32]. Zingerone provides a direct adaptive effect by preventing oxidative stress on intestinal smooth muscle [33].

Multi-parameter cytotoxic activity of *Z.officinalis* extract: Multiparametric cytotoxic activity with *Z. officinalis* nanoethanol extract was performed in HCS using FTC-133 cells. Five different measurements (cell

count viability, nuclear intensity, cell membrane permeability, mitochondrial membrane potential and cytochrome C release) were detected in this assay, and also processed (extract and doxorubicin) with captured Images of untreated FTC-133 cells.

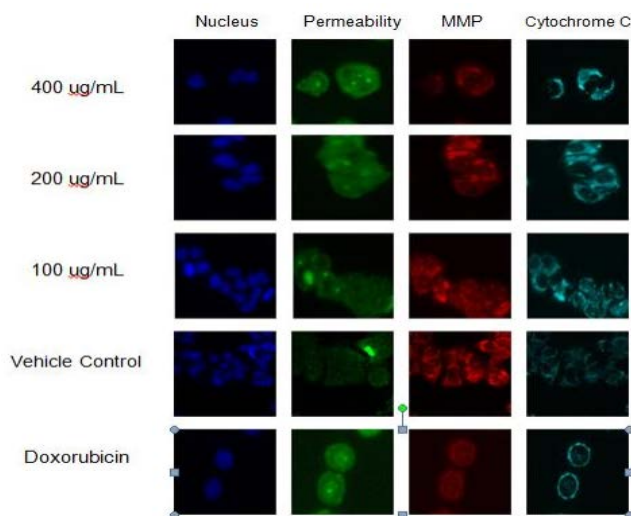


Figure 7 shows the effect of representative images obtained from multi-parameter (nuclear intensity, cell membrane permeability, mitochondrial membrane potential and cytochrome C release) cytotoxicity on the nuclear and mitochondria of FTC-133 cells.

The results shown in Figure 8 indicate that the viable count of FTC-133 cells decreased as the concentration of *Z. officinalis* extract at 200 µg mL⁻¹ compared to untreated cells. For cells treated with 25, 50, 100 and 200 µg/mL of *Z. officinalis* nanoethanol extract, a reduction in cell count was observed at 30% is significantly different from control and Doxo while other concentrations are not significantly different from control. This result highly indicates that the nanoethanol extract is cytotoxic to FTC-133 cells, as observed by MTT assay, where the decrease in cell count is dose dependent and the most significant decrease occurs after application of high concentrations (p < 0.0001). Dose (200 µg mL⁻¹) extract.

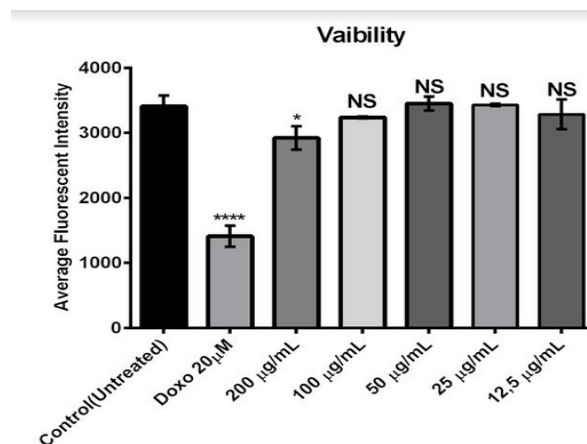


Figure 8: Effect of nano ethanolic extract treatment on cell viability in FTC-133

Cell viability is an important toxicity measurement parameter and is directly related to the toxic effects of drugs [34]. The decrease was found to depend on cell density and nanoethanol extract concentration. This effect may be due to cytostatic and cytotoxic effects that may down-regulate telomerase activity [35]. Exposure of higher concentrations of FTC-133 cells to *Z. officinale* extract resulted in an increase in nuclear size due to increased nuclear swelling and cell membrane permeability. The morphology of the nucleus (Hoechst Blue) showed nuclear concentration, which typically occurred in high concentrations of *Z. officinale* extract (100 and 200 µg mL⁻¹). Both effects were significantly different from untreated cells (Figure 9) ($p < 0.1$ and $p < 0.0001$, respectively), and no such events were induced at lower concentrations of *Z. officinale*.

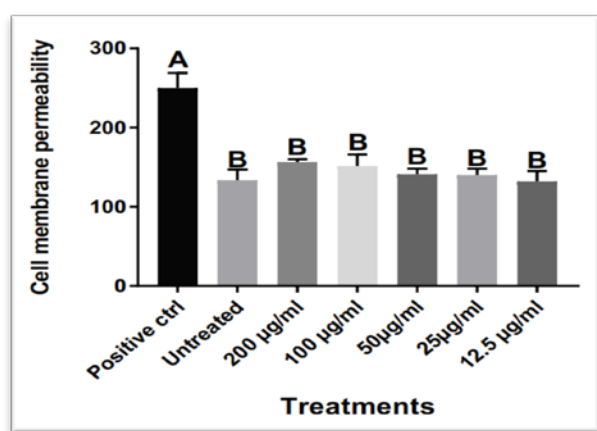


Figure 9: Effect of nano ethanolic extract treatment on cell membrane permeability in FTC-133

Changes in cell membrane permeability are often associated with toxic or apoptotic responses. In addition, cytotoxicity can lead to loss of cell membrane integrity [36]. Nano ethanol extracts can alter the composition and physical order of lipids in cells and in the plasma membrane. This effect leads to a change in plasma membrane permeability due to the continued activation of the second messenger and protein kinase C by the phospholipase pathway. Increased plasma membrane permeability and sustained protein kinase C activity can affect binding outside the rod. Paragraph [37]. On the other hand, Figure 10 shows that the intensity of FTC-133 cell membrane permeability (green) gradually increased in a dose-dependent manner, and a significant increase was observed after exposure to *Z. officinale* of 100 and 200 µg mL⁻¹. The fluorescence intensity of the extract and doxorubicin-treated cells showed a significant increase in nuclear intensity of approximately 1.3 after exposure to 200 µg mL⁻¹ extract compared to 20 µM doxorubicin (a strong antineoplastic agent) compared to untreated cells. Times. Chemotherapy embeds a DNA molecule that causes a large amount of DNA damage, which is twice as strong.

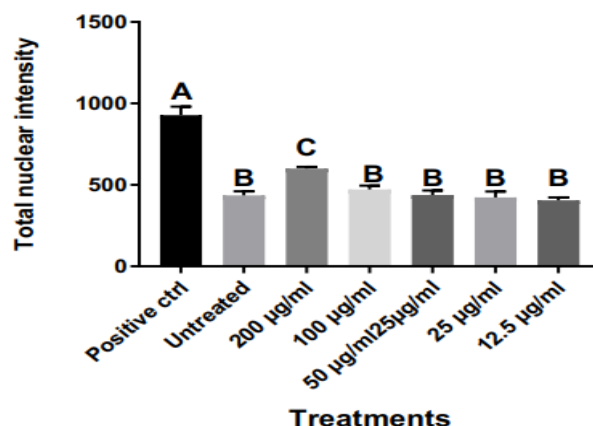


Figure 10: Effect of nano ethanolic extract treatment on nuclear intensity in FTC-133

Two additional parameters, mitochondrial membrane potential and cytochrome C release, were also measured. In contrast, as shown in Figure 11, only a higher concentration of *Z. officinale* extract (200 µg mL⁻¹) induced a significant decrease in mitochondrial membrane potential ($p < 0.0001$) (40.3), whereas positive doxorubicin control resulted in a decrease to 71.9%.

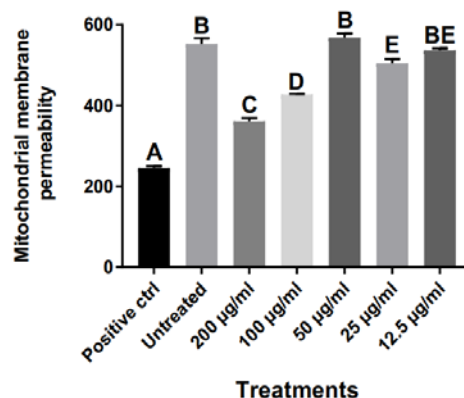


Figure 11: Effect of Nano-ethanol Extract Treatment on Mitochondrial Membrane Potential of FTC-133

The measurement of the mitochondrial membrane potential depends on the average intensity of the MMP dye, which penetrates the mitochondria in each nuclear cytoplasmic region and the lower the fluorescence intensity, the higher the effect on the mitochondria. On the other hand, the release of cytochrome C was only significantly ($p = 0.0005$) induced after exposure of FTC-133 cells to 200 µg mL⁻¹ extract (Fig. 12). The release of cytochrome C was measured by a 0.7-fold increase in the mean fluorescence intensity of treated FTC-133 cells compared to untreated cells, while the intensity of doxorubicin was increased 1.5-fold.

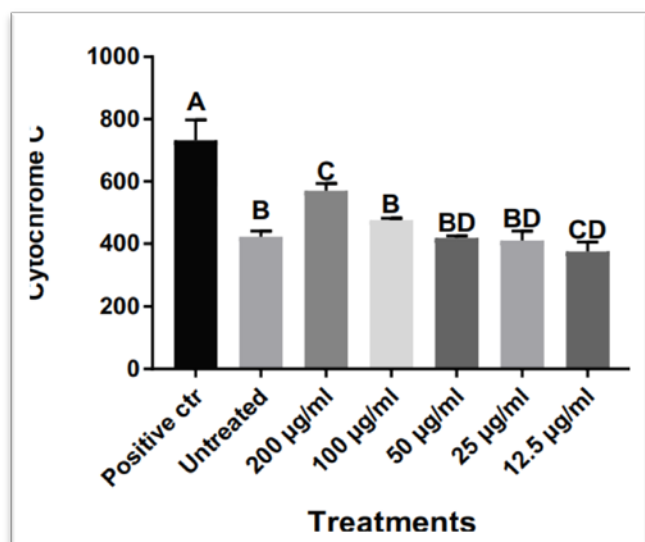


Figure 12: Effect of nano ethanolic extract treatment on cytochrome c in FTC-133

Cytochrome c plays an important role in apoptosis and can be released from the mitochondria into the cytoplasm [38]. Compared to doxorubicin, FTC-133 cells treated with nano ethanol extract showed strong nuclear staining of cytochrome c [Fig. 12]. Clearly, treatment of thyroid cancer cells with nano ethanol extracts mimics cytochrome c from mitochondria to cytoplasm.

CONCLUSIONS

The results showed that the nano ethanol ginger extract had a good scavenging effect on DPPH free radicals. These results indicate that ginger extract has strong cytotoxicity and antitumor activity and may be developed as an anticancer agent. These results indicate the potential of ginger extract as an additive in the food and pharmaceutical industries,. Nano ethanol extracts exhibit cytotoxic effects in FTC-133 thyroid cancer cells. The HCS assay showed that the nano ethanol extract had a toxic effect in 200 µg/mL on FTC-133 cells in a dose-dependent manner, and nuclear intensity, membrane permeability and cytochrome c were observed to increase. In addition, cell viability decreased while mitochondrial membrane potential changed. and further work is needed to understand the molecular mechanism of action of the ginger extract.

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