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# Nano-Encapsulation and Apoptotic Impact of Green Tea Polyphenol and Epigallocatechin-3-Gallate on Breast Cancer Cells *In vitro*

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## Abstract

Breast cancer (BC) is an important cause of mortality globally. The current study aims to determine the anti-cancer effects of green tea polyphenols (PP) and epigallocatechin-3-gallate (EG), either individually or encapsulated in nanoparticles (NPs), on two different human breast cancer cell lines *in vitro*. The proliferation of MCF-7 and MDA-MB-231 cell lines was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and apoptosis was examined using flow cytometry. We also measured entrapment efficiency (EE) using dialysis technology. The particle size (PZ) and zeta potential (ZP) of the proposed nanoparticles were investigated. The cytotoxicity test was done to examine the cytotoxic effects of polyphenols (PP) and EG on human breast cancer. Cell viability in MTT assays of cancer cells incubated with PP and EG encapsulated in nanoparticles showed significant cytotoxicity; besides, we determined the half-maximal inhibitory concentration ($IC_{50}$) and fold change (FC) of the drug. The flow cytometry analysis showed a quantitative analysis of different degrees of apoptosis. Our results proved that PP NPs had the highest cytotoxic effects at 100 µM with 9.39±0.47 and 10.76±0.53 % of MCF-7 and MDA-MB-231 cell viabilities, respectively. The MCF-7 is more sensitive than the MDA-MB-231 to PP and EG NPs. PP NPs are highly effective against late apoptosis in MCF-7 (13.6%) compared to late apoptosis in MDA-MB-231 (11.8%). In conclusion, introducing PP NPs and EG NPs could have great potential against resistant human breast cancer cells *via* the mediation of cell death-mediated apoptosis.

**Keywords:** Green Tea, Polyphenols, MCF-7 cells, MDA-MB-231 cells.

## 1. Introduction

Breast cancer (BC) is the most frequent invasive ailment and the second leading cause of women's deaths, accounting for approximately 29% [1]. BC is a heterogeneous group of cancer cells that can be classified based on their morphological and biological characteristics, behaviour, and treatment response. There is no permanent treatment for cancer. Surgery, chemotherapy, radiation therapy, and palliative care are options for treatment. Chemoprevention is a viable solution among the widely used cancer disease prevention strategies [2]. Due to the appearance of resistance, most patients responded poorly to conventional chemotherapy. As a result, it is vital to create new treatment strategies to increase cancer cells' sensitivity to chemotherapy-induced cell death [3]. There are many medicinal herbs that can treat dangerous and fatal diseases, such as breast cancer. To cure such cancer, taking a biological agent with no side effects is highly important [4]. Natural products obtained from various sources may have the ability to activate many biochemical processes that tackle cancer. Natural substances obtained from dietary sources can target different breast cancer-related ways, acting as a powerful defense against

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cancer and playing a key role in breast cancer protection [5].

In cell cultures and animal models, it has been discovered that GT, a putative cancer chemotherapeutic agent generated from dietary components, plays a substantial role in overcoming cancer resistance, including that of breast, lung, liver, and skin cancers [2]. GT is made from the *Camellia sinensis* plant's leaves and is one of the most popular drinks on the planet [6]. GT PP is the most common type of GT ingredient and is increasingly used in biomedical research to treat diseases such as cancer, obesity, cardiovascular disease, and diabetes mellitus. GT PPs (catechins and flavanols), caffeine, theanine, vitamins, and minerals are among the 200 bioactive compounds contained in GT [1]. The catechin profile of a typical green tea leaf extract contains 10%–15% epigallocatechin-3-gallate (EG), 6%–10% epigallocatechin (EGC), 2%–3% epicatechin-3-gallate (ECG), and 2% Epicatechin (EC). EG is the most prevalent polyphenol in green tea [7]. EG is essential in preventing cervical cancer proliferation, which is linked to the induction of apoptosis. It inhibits cell propagation in addition to inducing cancer apoptosis in breast, lung, and prostate cancers [8,9]. According to **Dai *et al.* (2017),** treatment with EG reduced the proliferation of breast cancer cells by deactivating the β-catenin signalling pathway and inhibiting the viability, expression, and phosphorylation of protein kinase B in MDA-MB-231 cells [10]. **Wu *et al.* (2019)** showed that EG dose-dependently increased the apoptotic index, suggesting that the mitochondria-mediated apoptotic pathway was activated. In human thyroid cancer cells, EG induces apoptosis that is mediated through the mitochondria [9]. Chemotherapeutic chemicals now in use are unable to distinguish cancer cells from normal cells and hence destroy both types of cells. The use of nanoparticle versions of these drugs can overcome this chemotherapeutic-associated problem [11]. Nanoparticles (NPs) have many advantages compared to chemotherapies, such as protection against cancer resistance and induction of apoptosis [12,13]. The use of NP versions of these drugs can overcome this chemotherapeutic-associated problem.

Other distinctive advantages of the nanoparticles as therapeutic carriers include increased therapeutic efficacy, decreased toxicity, and the capacity to encapsulate and distribute poorly soluble medicines [11]. Specific characteristics of nano-delivery systems, such as their small size and advantageous features, make it possible for them to modify the pharmacokinetics [14]. Nanotechnology has the ability to improve the biological transport of natural chemicals with medicinal potential [15]. Generally, but not always, many different forms of NPs used as drug carriers are made of lipids or polymers [16]. Many macromolecules have been employed to enhance the stability and cancer cell-treating properties of tea catechins, including polyethylene glycol (PEG), keratin, bull serum albumin, and chitosan (CS) [17].

In general, the current work intends to investigate the anti-cancer and pro-apoptotic effects *in vitro* of GT polyphenols (PP) and epigallocatechin-3-gallate (EG) against human breast cancer MCF-7 and MDA-MB-231 cell lines, either individually or when encapsulated in nanoparticles.

## Materials and Methods

### Chemicals

Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, antibiotic-antimycotic, l-glutamine (LG), phosphate buffered saline (PBS), annexin V apoptosis kit (Ann V), and propidium iodide (PI) kit were purchased from Thermo Fisher Scientific. Chitosan (CS), polyethylene glycol (PEG), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and polyvinyl alcohol (PVA). High-performance liquid chromatography (HPLC) grades (dichloromethane, ethanol, ascorbic acid, acetonitrile, and acetic acid) were purchased from Sigma-Aldrich Co., USA. Doxorubicin (DOX) was purchased from Sandoz, a Novartis company. The green tea (GT), *Camellia sinensis*, Code No. Y20140020, was cordially gifted from Hebei Baicao Kangshen Pharmaceutical Co., Xiyuan Hospital, and the China Academy of Chinese Medical Sciences. All chemicals were of the highest commercially available analytical grade and purity.

### Cell line

Human breast cancer cell lines MCF7 and MDA-MB-231 were purchased from the Holding Company for Vaccines, Sera, and Drugs (VACSERA), Giza, Egypt, tissue culture laboratory as an 80% confluent sheet, which supplied them from the American Type Culture Collection (ATCC, USA).

### Methods

#### 1. Polyphenols extraction from dried green tea leaves

Hot water (60ºC) was added to 30 g of crushed dried GT leaves in a 1:20 ratio (while moving periodically to deactivate enzymes). The filtrate from the boiling mixture was collected three times. The GT solution was concentrated using a rotavapour (a water bath set at 60ºC). The concentration had a 245 ml volume. Dichloromethane was added in an equal volume (245 ml) and mixed into the concentration. The upper portion was the undissolved residual concentrate (specific gravity of dichloromethane > specific gravity of water), and the lower portion was caffeine

(chlorophyll, lipid, carbohydrate, etc.) dissolved in dichloromethane.

The undissolved residual GT (upper part) was mixed with 250 ml of ethanol aqueous solution with different concentrations (0, 40, 60, 80, and 100%, v/v) and 0.1 g of ascorbic acid (to stop oxidation) in a separating funnel. The lower portion was the remaining tea solution (oil, fats, lipids, etc.), and the upper yellow portion was PP dissolved in an aqueous ethanol solution. The mixture was immiscible. Five more times, this liquid-liquid extraction was carried out without the addition of ascorbic acid. The PP was then dried and kept in a desiccator to prevent sticking once the solution was concentrated. The PPs broke down when exposed to air. The PP concentration weighs 5.8 g in total. The amount of PP was extracted from GT at various extraction times (20, 40, 80, 100, 140, and 180 min) [18].

**2. Extraction of the constituents of polyphenol**

The HPLC technique indicates the percentage of PP constituents. The HPLC systems used for the analysis consisted of a 486 detector (M 7200 Absorbance Detector, Young-In Scientific Co.), a 426 HPLC pump (Alltech Co.), and a Reodyne injection valve (20 µL sample loop). The data was collected using an Autochro-WIN data collection device (Ver. 1.42, Young-In Scientific Co.). After each injection, sufficient time was given to settle the column and detector signals. We had experimented with the mobile phases of water, methanol, acetonitrile, and acetic acid. A commercial analytical chromatographic column, RS-tech (0.46×25 cm, 5 µm, C18, Daejeon, Korea), was used in this experiment. The mobile phase flow rate was 1.0 mL/min, and the injection volume was 20 µL. The UV detector's wavelength was set at 280 nm. A 50 ml volumetric flask containing 0.5 g of the dry powdered extract of PP and 5 ml of HPLC-grade acetonitrile is used. Then it was diluted with various amounts of water, ascorbic acid, and acetonitrile. The percentage of polyphenolic constituents that the HPLC system has detected [19].

**3. EG extraction from dried green tea leaves**

In order to prepare the samples, 4 mL of ethanol aqueous solution was mixed with 2 mg of the standard chemicals (catechins (EG)), and the isoflavone content was adjusted to 500 ppm. The UV detector's wavelength was set to 280 nm, and the HPLC equipment was utilised for analysis. We made dry powder samples by grinding and sieving (< 30 µm) GT that contained catechins (EG). Three grammes of GT powder were added to 100 mL each of pure ethanol, pure water, and different concentrations of aqueous ethanol (0, 40, 60, 80, and 100%, v/v). By using the dipping method, catechins (EG) were extracted from each sample at different extraction times (20, 40, 80, 100, 140, and 180 min). Prior to HPLC analysis, the extraction solution was filtered through a 0.2-µm membrane filter [19].

In this work, the effects of extraction time (20, 40, 80, 100, 140, and 180 min) and solvent composition (ethanol) (0, 40, 60, 80, and 100 vol%) on the amounts of catechins (EG) extracted from GT were investigated.

**4. Preparation of nanoparticles**

Using polymeric nanoparticles CS and PEG, researchers were able to overcome cancer cells' resistance to chemotherapy by promoting mitochondrial malfunction and activating apoptosis.

**4.1. Preparation of PEG NPs and CS NPs (nano-void)**

PEG and CS NPs were prepared by a modified single-emulsion solvent evaporation method [20]. Briefly, after being produced in 1% glacial acetic acid and filtered, the PEG or CS solution (12% w/w) was added to the aqueous PVA solution (12 mL, 2% w/v).

**4.2. Preparation of PP and EG nanoparticles**

To entrap the PP and EG inside the polymer nanocapsule, we added a certain amount of them while we were preparing the nanocapsule using 3.75 mM N-hydroxy succinimide (NHS) and 1.5 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), which were stirred for 1 hr to activate the interaction between 5 mg of the CS NPs and PEG NPs with either PP or EG. This new mixture was stirred overnight at 4 °C, after which new NPs of CS were decorated with PEG as surface ligands outside the NPs.

**4.3. Characterization of nanoparticles**

**4.3.1. Particle size (PZ) distribution and zeta potential (ZP)**

All nanoparticles' PZ, ZP, and polydispersity index (PDI) were investigated using the Malvern Zeta Sizer (Nano ZS, UK) by photon correlation spectroscopy (PCS) at 25ºC. Every reading was performed in triplicate, and the mean ± standard error (SE) values were used.

**4.3.2. Measurement of the drug encapsulation efficiency (EE%)**

The free cells (non-conjugated cells with NPs) were removed using the dialysis tubing technique using a membrane bag (Membrane, Spectrum Lab., USA; molecular weight cut-off, MWCO: 25 KDa) [20]. Using this method, the concentrations of the nanoconjugated cells were measured. Using a UV-based enzyme-linked immunosorbent assay (ELISA) method, the free and conjugated forms of cells were detected using a variable-wavelength detector. After using the dialysis technique, encapsulating (entrapment) efficiency (EE%) was measured.

**4.3.3. Cell culture and maintenance**

*In vitro*, the cell proliferation and maintenance of human breast MCF-7 and MDA-MB-231 cells were studied in tissue culture flasks containing DMEM with 1% LG. 1% antibiotic-antimycotic mixture and

5% FBS were added to both cell lines as supplements. To obtain sufficient cell numbers, the cells were incubated at 5% $CO_2$ humidity and a temperature of 37°C. The finished mixture was then thrown away and cleaned three times cleaned with PBS. To harvest the cells, 5% FBS was added after the cells had been detached using 0.05% trypsin and 0.02% EDTA to separate adherent cells [21].

**4.4. Measurement of the cytotoxicity**

Using breast cells, the MTT test was used to evaluate all drug groups. All cell lines' growth was evaluated using an MTT assay in the presence of PP, EG, and nanoparticles. This assay kit was widely used for the cytotoxicity evaluation of the cell proliferation of these nanoparticles. DOX as a standard drug (positive control) was utilised as well. MTT is a water-soluble tetrazolium salt that allows succinate dehydrogenase in the mitochondria to cleave, producing an insoluble purple formazan. Since the formazan product cannot pass through living cells' membranes, it accumulates inside them. For negative control, media were added without drugs [22].

Briefly, the cells were cultured at a density of $1 \times 10^4$ cells/well in a 96-well tissue culture microtiter plastic plate and incubated for 24 hours at 37°C in 5% $CO_2$ in a water-jacketed carbon dioxide incubator. This allowed the cells to adhere to the plate. After 24 hours, all drugs (PP, PP NPs, EG, and EG NPs) and DOX with their described concentrations (0, 5, 10, 20, 40, 60, 80, and 100 µM) were added into the media over these cells. Culture media treated with nano-void (*i.e.,* nano-capsules without loaded drugs) were used as a control group to compare nano-drugs with them. In addition, culture media treated with PBS was used as a negative control to compare free drugs with it. A positive control was doxorubicin. MTT dissolved in 5 mg/mL PBS was added to each well after 24 hours of incubation, and the samples were then incubated at 37°C for 4 hours. Cells' ability to reduce MTT was used to measure cell proliferation. The water-insoluble dark blue formazan crystals that were created during MTT cleavage in active metabolising cells were dissolved by adding 150 µl of DMSO (solubilizing solution) (10%) into each well to stop the reaction. The plate should be left in the incubator for all of the night in an atmosphere with humidity (37°C, 5% $CO_2$). A microplate reader (BMG Labtech, Germany) was used to read the plates' absorbance at 540 nm. All assays were carried out three times in triplicate for each concentration, and the average of all the experiments has been shown as cell proliferation as a percentage of cell viability ± standard error (SE) in comparison with the negative control.

**4.5. Determination of the 50% inhibitory concentration ($IC_{50}$)**

For the antitumour activity assays on MCF-7 and MDA-MB-231, the $IC_{50}$ value was determined as the effective concentration that reduces the growth of cells to 50% of the control value, resulting in 50% cytotoxicity. The results were used to calculate the $IC_{50}$ values of the drug.

**4.6. Quantitative determination of apoptosis and necrosis**

The effects of the extract on MCF-7 and MDA-MB-231 resulted in the two main types of cell death, necrosis and apoptosis, which were detected using flow cytometry and the FITC Annexin V and Propidium Iodide (Ann V-PI) kit.

$2\times10^5$ cells/ml of MCF-7 and MDA-MB-231 were grown in 10 ml Petri dishes of DMEM with 10% FBS. The obtained $IC_{50}$ doses of the proposed free and nano EG and PP were added. As mentioned above, the control was 0.0% of these extracts (without drug). The cells were extracted by adding trypsin and centrifuging for 5 minutes at 1000x after being incubated for 24 hours. The cells were then given two cold PBS washes. The cells were gently mixed with 5 µl of FITC Annexin V and 5 µl of propidium iodide solution before being incubated for 15 min at room temperature in the dark. The cells stained according to the kit's protocols were then given the binding buffer and then analysed by a flow cytometer (Beckman Coulter, USA) using 488 nm as the excitation wavelength [20]. The determinations were performed in duplicate.

**5. Statistical analysis**

The data obtained in the present work were represented in figures and tables as the mean ± standard error (SE). All assays were repeated three times (n = 3). A one-way ANOVA analysis (SPSS, version 20) was used. *P* values < 0.05 were considered statistically significant when comparing the treated groups with the control.

**Results**

**1. Extraction of the constituents of polyphenol**

The components present in the polyphenol are identified by comparing them with the HPLC system, as shown in **table 1**.

**Table 1.**Percentage concentrations of the components in polyphenols

| Component | Concentration (%) |
|---|---|
| Gallic acid | 2.81 |
| Epigallocatechin (EGC) | 1.87 |
| Epigallocatechin-3- gallate (EG) | 7.99 |
| Epicatechin-3- gallate (ECG) | 1.66 |

The most component abundant pp in green tea is EG. Atypical PPs in GT leaves extract contains 7.99% EG, 2.81% Gallic acid, 1.87% EGC, and 1.66% ECG.

## 2. EG extraction from dried green tea leaves

In the case of the extraction time (20 min) remained constant, **Table 2** displays the amount of PP and EG present in the GT extracts produced using various ethanol solvent compositions by HPLC system.

**Table 2.** Amount of PP and EG extracted from GT leaves upon solvent composition, fixing time 20 min.

| Solvent composition (Ethanol, %) | Amount mg/g | |
|---|---|---|
| | PP | EG |
| 0 | 7.76 | 1.1 |
| 40 | 8.2 | 0.9 |
| 60 | 7.2 | 0.87 |
| 80 | 6.08 | 0.7 |
| 100 | 2.83 | 0.57 |

GT extracts contain between 8.2 ~ 2.83 and 1.1 ~ 0.57 mg/g, respectively, of PP and EG. It is clear that PP was extracted from GT at a higher rate than EG was at the same concentration of 40% (8.2) ethanol solution (*i.e.*, 60% water). This might be because EG and PP are dissolved differently in water, with EG having a higher solubility than PP. The amount of EG extracted from green tea increased as ethanol concentration decreased, recording the highest extracted amount at 0% (1.1) ethanol (*i.e.*, 100% water) and the lowest at 100% (0.57).

The levels of PP and EG in GT extract that were produced using various extraction times are shown in **Table 3**. As can be indicated, GT contains the largest amount of PP extracted after 100 minutes (10.65 mg/g) and the lowest amount after 20 minutes (8.2 mg/g). The GT yields the highest amount of EG (1.86 mg/g) at 80 minutes and the lowest amount (0.82 mg/g) at 140 minutes.

**Table 3.** Amount of EG and PP extracted from green tea upon Extraction time

| Extraction time (min) | Amount mg/g | |
|---|---|---|
| | PP | EG |
| 20 | 8.2 | 1.1 |
| 40 | 8.56 | 1.56 |
| 80 | 9.14 | 1.86 |
| 100 | 10.65 | 0.96 |
| 140 | 8.57 | 0.82 |
| 180 | 8.68 | 1.00 |

## 3. Characterization of nanoparticles

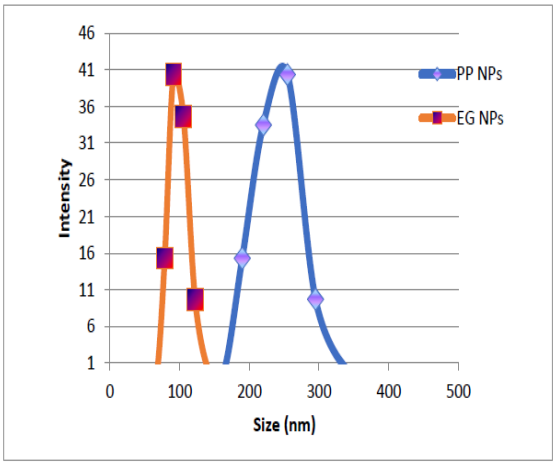
The data illustrated in **Table 4** show the nano-void, PP NPs, and EG NPs characterizations. We noticed that the nano-formulation had the smallest average nano-size for the nano-void (88.24 nm ± 1.68 nm), and the highest average nano-size (255.2 nm ± 7.42 nm) was for the PP nano-formulation. The presence of PP and EG increased the size of the nanoparticles. Nano-voids were the most stable, with the lowest positively charged zeta potential (+15.6±2.6 mV) on their surfaces and a very low polydispersity index recorded at (0.01±0.00). The zeta potential of EG NPs was the highest (+37.64±6.32), which is less stable on their surfaces with very high entrapment efficiency (93.5%).

**Table (4):** Average size, polydispersity index (PDI), Zeta potential (ZP), and entrapment efficiency (EE) of the synthesized nanoparticles.

| NPs type | Mean ± SE | | | EE, % |
|---|---|---|---|---|
| | Size, nm | PDI | ZP, mV | |
| nano-void | 88.24 ±1.68 | 0.01 ± 0.00 | +15.6 ±2.6 | - |
| PP NPs | 255.2±7.42 | 0.5 ± 0.00 | +27.23 ±4.5 | 87 |
| EG NPs | 91.13 ±2.5 | 0.3 ± 0.01 | +37.64 ±6.32 | 93.5 |

Data were represented in terms of mean ± standard error (SE), n=3. PDI: polydispersity index; ZP: Zeta potential; EE%: entrapment efficiency. On the other hand, the size distribution graph describes the intensity based on the size distribution, and the Malvern Zeta-Sizer analysis displays the polydispersity index. The analysis indicated that the size distribution by intensity has two peaks, recorded as around 100 nm (91.13±2.5) for peak 1 of EG nanoparticles and about 250 nm (255.2±7.42) for peak 2 of PP nanoparticles (**Fig. 1**).

**Fig. (1):** Size distribution of PP NPs and EG NPs



## 4. Cytotoxicity

### 4.1. MTT assay

The cytotoxic effects of PP, PP NPs, EG, EG NPs, and DOX on MCF-7 and MDA-MB-231 cell lines cultured *in vitro* were measured by MTT assay. Cells were assayed for cell viability with 24 hours of exposure to different doses of drugs. **Figs. 2 and 3** show the anticancer effects of PP and EG as free anticancer drugs and their nano-formulations (PP NPs and EG NPs) against MCF-7 and MDA-MB-231. Zero concentration in **Figs. 2 and 3** illustrates the cancer cells without any treatments compared to the free drug and the cancer cells with nanoparticle drugs.

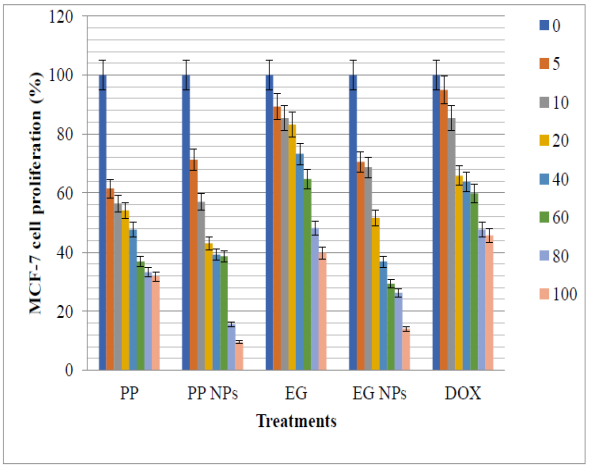



**Fig. (2):** The cytotoxic-based mitochondrial activity of the MCF7 cell line upon PP, PP NPs, EG, and EG NPs treatments. Three independent runs (n = 3) were done and illustrated in the figure.

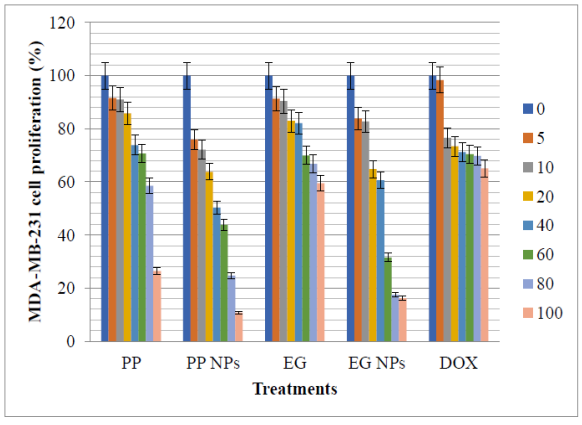



**Fig. (3):** The cytotoxic-based mitochondrial activity MDA-MB-231 cell line upon PP, PP NPs, EG, and EG NPs treatments. Three independent runs (n = 3) were done and illustrated in the figure.

There was a significant decrease in all free and nano-formulations over MCF-7 and MDA-MB-23. We used DOX as a well-known positive standard anticancer control. The results showed that the MCF-7 and MDA-MB-231 had a dose-dependent decrease in cell viability; in other words, the cell viability declined with increasing the concentration of drugs. Furthermore, at 100 M, PP NPs displayed the most cytotoxic effects, reducing the viability of MCF-7 and MDA-MB-231 by 9.39±0.47 and 10.76±0.53 %, respectively.

The results showed that after 24 hours, PP NPs and EG NPs significantly reduced the viability of the MCF-7 and MDA-MB-231 compared to the control group.

### 4.2. The 50% inhibitory concentration ($IC_{50}$) and fold change (FC)

The MCF-7 and MDA-MB-231 were used to create a dose-response curve that yielded the 50% inhibitory concentration ($IC_{50}$) for the free and nanoparticle drugs in this study.

To determine the cytotoxic FC between the drug-encapsulated nanoparticles and their free counterparts, the $IC_{50}$ of the free PP and EG is divided by the $IC_{50}$ of each nanoplatform. The $IC_{50}$s and FC of all free- and nano-formulations over MCF-7 and MDA-MB-231 are demonstrated in **Table 5**. The $IC_{50}$s of PP NPs and EG NPs over the MCF-7 were 26.85 µM and 27.76 µM, with 1.09 and 2.91 FC, respectively, compared to the MDA-MB-231, with the $IC_{50}$s of PP NPs and EG NPs being 41.86 µM and 41.59 µM, and FC of 1.93 and 0.97, respectively, the decrease in the $IC_{50}$ value, meaning that this nano-platform is more cytotoxic against cell lines.

**Table 5:** $IC_{50}$ and fold change1 (FC1= free drug/NPs)

| Cell lines (µg/mL) | PP | PP NPs | FC | EG | EG NPs | FC |
|---|---|---|---|---|---|---|
| MCF-7 cells | 29.49 | 26.849 | 1.0986 | 80.831 | 27.759 | 2.912 |
| MDA-MB-231 cells | 81.07 | 41.868 | 1.9364 | 40.375 | 41.596 | 0.971 |

PP: polyphenols; NPs: nanoparticles; FC: fold change

The $IC_{50}$ and cytotoxic FC2 of all free- and nano-formulations over MCF-7 and MDA-MB-231 were demonstrated in **Table 6.** We used DOX as a well-known positive standard anticancer control.

**Table 6:** IC50 and fold change 2 (FC2 = DOX/Drug)

| Cell lines/ FC2 | DOX | PP | PP NPs | EG | EG NPs |
|---|---|---|---|---|---|
| MCF-7 | 97.940 | 29.497 | 26.849 | 80.831 | 27.759 |
| FC2 | ref | 3.320 | 3.648 | 1.212 | 3.528 |
| MDA-MB-231 | 97.115 | 81.071 | 41.868 | 40.375 | 41.596 |
| FC2 | ref | 1.198 | 2.319 | 2.405 | 2.335 |

PP: polyphenols; NPs: nanoparticles; FC: fold change; DOX: doxorubicin.

According to the MTT assay, all $IC_{50}$ percentages at 24 hours ($IC_{50}$@24h) of the PP, PP NPs, EG, and EG NPs were less than those of DOX with a different percentage of the drug. The cytotoxicity of PP NPs induced in the MCF-7 was greater than that of the MDA-MB-231 due to the lowest $IC_{50}$ value. The lowest exposure level required to inhibit the growth of 50% of MCF-7 in vitro after 24 h was found in PP NPs, followed by EG NPs. Generally, the MCF-7 was more sensitive than MDA-MB-231 to PP NPs and EG NPs, where the $IC_{50}$@24h over MCF-7 and MDA-MB-231 was 26.85, 27.75, 41.86, and 41.59, respectively.

**5. Apoptotic induction**

The majority of the cells were healthy in the double negative quadrant (negative annexin/negative PI), early apoptotic cells (positive annexin/negative PI), late apoptosis in the double positive quadrant (positive annexin/positive PI), and necrotic cells (negative annexin/positive PI).

The number of early and late stages of apoptotic and necrotic MCF-7 in the control drug was significantly different. The trusted late apoptotic and necrotic MCF-7 were markedly less than the early apoptotic cells **(Fig. 4 C)**.

In the treatments with EG and EG NPs, the number of necrotic MCF-7 cells showed increased significantly in comparison with late apoptotic cells. In addition, the counted number of the late apoptotic cells at the corresponding treatments with EG was significantly more than that of the early apoptotic ones. In the cells exposed to PP or PP NPs, the number of necrotic MCF-7 cells was markedly less than that of the late stage of apoptosis and higher than that of the early stages.

In the early apoptotic stage, the highest-counted cells were detected in the culture exposed to EG NPs. For the rest of the treatment, there was a descending order: PP NPs, PP, EG, and finally, the control. In the late apoptotic stage, the lowest count of MCF-7 cells was recorded for the cells unexposed to any treatment, significantly less than the count found in cancer cells exposed to the PP, EG, EG NPs, and PP NPs. The counted necrotic MCF-7 cells showed that the highest percentage was observed in cells exposed to EG, whereas the lowest percentage was recorded in those exposed to the control.

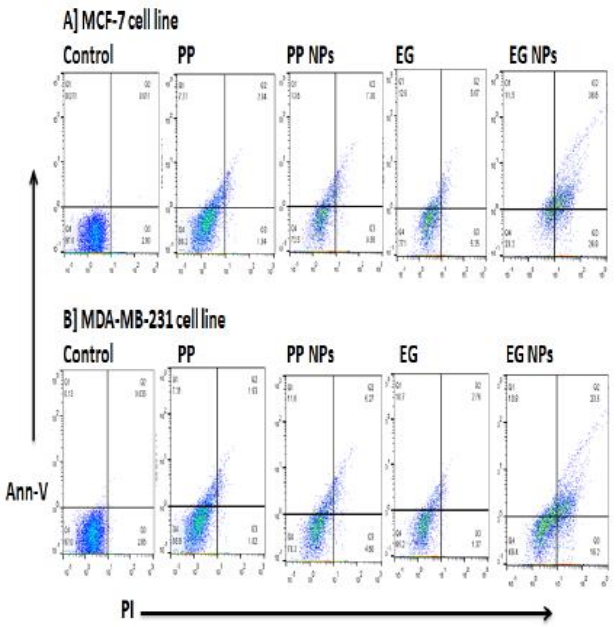
The number of early and late stages of apoptotic and necrotic MDA-MB-231 cells was significantly different in the control drug, and the counted late apoptotic and necrotic MDA-MB-231 cells were markedly less than the early apoptotic cells **(Fig. 4 D)**.

In the treatments with PP and PP NPs, the number of necrotic MDA-MB-231 cells showed a significant decrease in comparison with the late apoptotic cancer cells. In addition, the counted number of the late apoptotic cells treated with EG and PP NPs was significantly higher than that of the early apoptotic ones at the corresponding treatments with EG NPs. In the cells exposed to EG NPs, the number of necrotic MDA-MB-231 cells was higher than in the late and early stages of apoptosis.

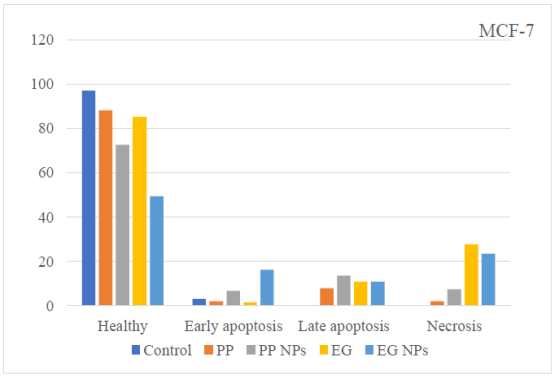
The quantity of dead MCF-7 cells was much higher than that of MDA-MB-231 cells, according to flow cytometry. Both types of cells treated with PP, PP NPs, EG, and EG NPs showed signs of apoptosis and necrosis. Our results proved that PPNPs are highly effective against late apoptosis in MCF-7 (13.6%) compared to late apoptosis in the MDA-MB-231 (11.8%). EG NPs are highly effective against necrotic MDA-MB-231 (38.6%) compared to the necrotic MCF-7 cell line (23.5%).

**Discussion**

Despite the expansion of new therapies against cancer, cancer resistance to drugs still represents a major impediment to successful treatment [23]. The development of new drugs from natural resources in the oncology field represents a promising hope because conventional chemotherapeutic treatments may have serious side effects and cannot be tolerated by patients with deteriorating health conditions. Moreover, chemoresistance has become another rising obstacle when conventional chemotherapeutic drugs are considered. Studies have recently concentrated on the anticarcinogenic properties of green tea extracts, hoping that this line of treatment, when used in combination with chemotherapeutic drugs, would increase their efficiency and reduce their doses [3, 5, 7].




**C] MCF-7 cell line**




**D] MDA-MB-231**

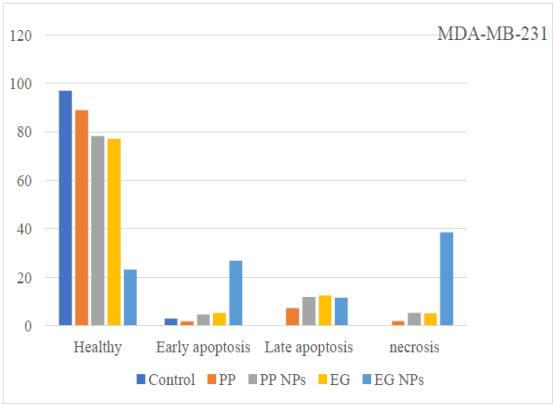



**Fig. (4): A]** MCF-7 cancer cell line apoptosis using a flow cytometer, **B]** MDA-MB-231 cancer cell line apoptosis using a flow cytometer, Annexin V/PI positively (and/or) negatively for cancer cells treated with PP, PP NPs, EG, and EG NPs compared to control. **C]** The early and late stages of apoptosis as well as necrotic MCF-7 cancer cells and those exposed for 24 hours to the drugs (PP, PP NPs, EG, and EG NPs). **D]** The early and late stages of apoptosis as well as necrotic MDA-MB-231 cancer cells, and those exposed for 24 hours to the drugs (PP, PP NPs, EG, and EG NPs).

In the present study, the green tea was extracted into PP and EG. On the other hand, its effect was either individually or encapsulated in nanoparticles. Encapsulation of the medications in polymeric NPs is a great technique for accurate delivery [24]. The MCF-7 and MDA-MB-231 were chosen as cancer cell models due to the widespread incidence of breast cancer, which represents the most common cancer-causing morbidity and mortality among women worldwide [25].The results of the cytotoxicity studies showed that PP NPs, EG NPs, and PP and EG were cytotoxic to MCF-7 and MDA-MB-231 *in vitro* in a descending manner, whereas nano-void was devoid of a detectable antitumour effect. These results may be due to the fact that GT and its extracts are composed of caffeine (chlorophyll, lipid, carbohydrate, oil, fats, *etc.*). Having various molecular weights, catechins and flavanols also comprise low molecular weight compounds (PP and EG), which are stable in a wide range of pH levels, from acidic to alkaline. In neutral and alkaline mediums, EG is less stable because the basic medium traces and attacks the hydroxyl groups of the phenyl ring of EG, resulting in EG oxidation [1]. The current study output is in parallel with our recently published papers regarding CS and hyaluronic acid nanoparticles for treating colon and breast cancer. The synthesized NPs had a specific binding affinity with the CD44 receptor on cancer cells, which allowed the NPs to tackle the colorectal cells in a targeted manner [20, 26, 27].

Previous research has demonstrated that the PPs found in green tea leaves are a strong antioxidants. Its curative properties for a variety of pathological conditions, diseases, and disorders have been researched. As it displayed no significant toxicity at regular levels, it is a substance that can be consumed without risk. Strongly effective against human breast cancer cells are EG and PP [2, 8, 28-30].

When used for their antitumour properties, PPs have anti-cancer effects by regulating apoptosis, development, invasion, and angiogenesis. Moreover, inhibiting cancer cell development, metastasis, invasion, and activating apoptosis in cancer cells are only a few of the ways that EG can interfere with cellular signalling and metabolic pathways. However, it has no effect on normal cells [6, 9, 31, 32].

Researchers studying cancer have mostly accepted a novel therapeutic approach that combines GT

catechins with anticancer drugs. Tea PPs can enhance anticancer activities and functional differences [7]. **Bhattacharjee and Bharadwaz** reported that solvent extraction is a successful method for removing PPs and caffeine from dried tea leaves. It has been discovered that this approach depends on factors such as solvent type, temperature, solution pH, *etc*. The effectiveness of the equipment used determines how many extraction phases are necessary. It is the technique now used in industry for massive production processes. In the present study, we used the solvent extraction method (HPLC system) to extract PPs from green tea leaves, agreeing with this method because of its low-cost equipment, rapidity, and easy of handling [18].

The present results showed that the component with the highest percentage concentration of PPs is EG (7.99%). **Bhattacharjee and Bharadwaz,** in their study on the extraction of PPs from dried tea leaves, found that the highest percentage concentration was EG (5.099%). **Lee and Lee** studied catechin (EG) extraction behaviour using GT to gather first-hand information on extracting material. The extracted solution's catechins (EG) were identified using the analytical HPLC system. The amount of EG in green tea extracted was 0.90~0.30 mg/g [19]. It can be seen that the present results showed that the same amount of EG in green tea was extracted by the HPLC system using ethanol solvent compositions when the extraction time (20 min) remained constant in the range of 1.1~0.57 mg/g. The vast majority of herbal phytoconstituents now use nanoparticles as a drug delivery method to increase their therapeutic efficacy [33].

**Luo** extracted four catechins from green tea polyphenols by HPLC analysis, including two minor non-ester catechins (EGC and EC) and two major ester catechins (EG and ECG). Of these four catechins obtained by three conventional methods (UAE-ethanol, ethanol extraction, and hot water extraction), the content of EG was the highest, followed by ECG, EGC, and EC. The catechins were reported to mainly contribute to the antioxidant properties of the green tea infusions [34]. The results obtained herein approved it, showing that EG had the highest content.

In this investigation, we created PEG-Cs nanoparticles loaded with PP and EG and assessed their anticancer potential against breast cancer cell lines. Regarding this, a solution of polyethylene glycol or chitosan was prepared and then added to a solution of aqueous polyvinyl alcohol (PVA). A technique used to create nanoparticles is the modified single emulsion solvent evaporation method. This technique creates polymer nanoparticles with great stability [21].

Another study performed a more rigorous analysis of self-assembled nanoparticles' size-dependent tumour penetrating capability. It showed that smaller particles could penetrate cancerous cells more than their larger free counterparts [20]. These observations of the nano-sizes of the PP and EG NPs were in agreement with the current NPs' physicochemical properties, where our results recorded that the particle size of the synthesized nanoparticles was around 250 (255.2$\pm$7.42) nm for the PP nano-formulation and around 91.13$\pm$2.5 nm for the EG nano-formulation. The polydispersity index was 0.5$\pm$0.00 and 0.3$\pm$0.01, respectively. The zeta potential was (+27.2 $\pm$4.5 and +37.64$\pm$6.32) mV, and the EE% of PPNPs and EGNPs were 87% and 93.5%, respectively. This means that EGNPs are more stable with a very high EE (93.5%) because of their low average nanosize.

The results of the cytotoxicity studies recorded in the present work revealed that the PP NPs were more toxic to the cell lines than any of its extractions with a lower $IC_{50}$ value. It seems that the more toxic components available, the higher the chance of developing cytotoxic outcomes in cancerous cells. This interpretation is supported by the present results, which show that the $IC_{50}$ values of the PP and EG were higher than those of the nano-PP and EG. The observation was that the PP and EG NPs were more toxic than PP and EG. In addition, increasing cytotoxicity led to decreasing cell viability, which was concentration-dependent. According to [35], in their study on the mechanism of EG promoting apoptosis of the MCF-7 cells, and **Yiannakopoulou,** in his study on the mechanisms of action of green tea catechins in breast cancer, focusing on the interaction between survival and apoptosis, this effect appears to be common with cell lines [36]. Additionally, in their research on gold nanoparticles, **Balakrishnan** found that MCF-7 and MDA-MB-231 were subjected to apoptosis [37].

The decrease in cell viability in the cancerous cells was in agreement with previous studies, which suggested that liver and lung cell death may be caused by knocking down HOTTIP epigenetic expression [38] and that long noncoding RNAs may be enrolled as epigenetic modulation [39].

In MCF-7, **Yiannakopoulou** demonstrated the anti-tumour activity of EG. For 24 hours, MCF-7 cells were grown with or without EG. Increasing EG concentrations and a cell proliferation assay were used to assess the impact of EG on MCF-7 [35]. **Balakrishnan** discovered that free Qu and AuNPs-Qu-5 (gold nanoparticles) reduced the viability. In MCF-7, AuNPs-Qu-5 showed an $IC_{50}$ value of 50 μM, while after 24 h, AuNPs-Qu-5 treated MDA-MB-231 showed an $IC_{50}$ value of 100μM. The $IC_{50}$ values for free Qu for MCF-7 and MDA-MB-231 were 100 μM and 125 μM, respectively. No significant cytotoxicity was displayed by free AuNPs towards cancer cell lines [37].

In the present work, the cytotoxicity studies showed that the $IC_{50}$ values of PP NPs and EG NPs over the MCF-7 were 26.85 μM and 27.76 μM, respectively, compared to the MDA-MB-231, where the $IC_{50}$ values of PP NPs and EG NPs were 41.86 μM and 41.59 μM, respectively. The decrease in the $IC_{50}$ value of PP NPs against MCF-

7 is more cytotoxic. Furthermore, PP NPs had the highest cytotoxic effects at 100 µM with 9.39±0.47 and 10.76±0.53 % of MCF-7 and MDA-MB-231, respectively. On the other hand, the cytotoxic effects of EG NPs at 100 µM were observed in 14.01±0.70 and 16.17±0.81 % of MCF-7 and MDA-MB-231, respectively.

The current finding is consistent with that of **Chao You Huang,** who found that EG treatment significantly increased the apoptotic rate in MCF-7 cells compared to the control. MCF-7 treated with EG (0 ~ 120 µM) had a dose-dependent inhibition of their ability to grow, with an $IC_{50}$ (the half maximal inhibitory concentration) of 37.684 µmol/l. Cells treated with EG at a high concentration (120 µM) showed severe inhibition of their ability to grow [40].

A distinct and significant form of programmed cell death called apoptosis includes the elimination of abnormal cells that are detrimental to tissue homeostasis and growth. The induction of apoptosis is frequently an anticancer drug's most important mode of action [37].

Our morphological and ultrastructural results in the present study using flow cytometry assays demonstrated various degrees of cell damage that reached apoptosis and necrosis. In the treatments with EG and EG NPs, the number of necrotic MCF-7 cells showed a significant increase (27.6% and 23.5%, respectively), compared with PP and PP NPs, where the number of late apoptotic cells increased by 7.77% and 13.6%, respectively. On the other hand, the counted number of the late apoptotic cells treated with EG and PP NPs was significantly higher than that of the early apoptotic ones at the corresponding treatments with EG NPs. In the cells exposed to EG NPs, the number of necrotic cells in MDA-MB-231 was greater than that of those in the late and early stages of apoptosis.

**Wei** investigated the theory that EG in nano-formulation induces apoptosis, which results in a reduction in cell proliferation [30]. According to **Huang,** EG-treated MCF-7 cells had a considerably higher rate of apoptosis than the control group. In this instance, the EG and the control groups' respective apoptotic rates were 1.37 and 5.83%, respectively [3]. The results demonstrate that EG inhibits MCF-7 cells from proliferating while promoting apoptosis in these cells.

**Conclusion:** The synthesized PP NPs and EG NPs provided a great effect against resistant human breast cancer cells *via* the modulation of cell death-mediated apoptosis.

**Conflicts of interest:** There are no conflicts to declare.

**References**

**[1] Zeng, L.; Yan, J.; Luo, L.; Ma, M. and Zhu, H. (2017):** Preparation and characterization of epigallocatechin-3-gallate (EGCG)-loaded nanoparticles, and their inhibitory effects on human breast cancer MCF-7 cells. ***Scientific Reports,*** 7(November 2016), pp. 1 – 15. doi: 10.1038/srep45521.

**[2] Liu, S. min, Ou, S. yi and Huang, H. hua (2017):** Green tea polyphenols induce cell death in breast cancer MCF-7 cells through induction of cell cycle arrest and mitochondrial-mediated apoptosis. ***Journal of Zhejiang University: Science B,*** 18(2), pp. 89 – 98. doi: 10.1631/jzus.B1600022.

**[3] Huang, C. Y.; Ju, D. T.; Chang, C. F.; Muralidhar Reddy, P. and Velmurugan, B. K. (2017):** A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. ***BioMedicine (France),*** 7(4), 12–23. https://doi.org/10.1051/bmdcn/2017070423.

**[4] Batrawi, M.; El Sayed, H.; Hussein, H.; Abdel Mawella, S.; Madbouly, N. and Fawzi, S. (2017):** Qualitative versus quantitative study of distress in a sample of young age breast cancer survivors. ***Egyptian Journal of Psychiatry,*** 38(3), 172. https://doi.org/10.4103/ejpsy.ejpsy_20_17.

**[5] Mitra, S., and Dash, R. (2018):** Natural products for the management and prevention of breast cancer. ***Evidence-Based Complementary and Alternative Medicine,*** 2018. https://doi.org/10.1155/2018/8324696.

**[6] Miyata, Y.; ScienMatsuoces, T.; Araki, K.; Nakamura, Y.; Sagara, Y.; Ohba, K.; Sakai, H.; Hayakawa, S.; Ohishi, T.; Miyoshi, N. and Oishi, Y. (2018):** Anticancer effects of green tea and the underlying molecular mechanisms in bladder cancer. ***Medicines,*** 5(3), 87. https://doi.org/10.3390/medicines5030087.

**[7] Cao, J.; Han, J.; Xiao, H.; Qiao, J. and Han, M. (2016):** Effect of tea polyphenol compounds on anticancer drugs in terms of anti-tumor activity, toxicology, and pharmacokinetics. ***Nutrients,*** 8(12). https://doi.org/10.3390/nu8120762.

**[8] Anand, A.; Chawla, J.; Mahajan, A.; Sharma, N. and Khurana, N. (2017a):** Therapeutic potential of epigallocatechin gallate. ***International Journal of Green Pharmacy,*** 11(3), S364–S370. https://doi.org/10.22377/ijgp.v11i03.1143.

**[9] Wu, D.; Liu, Z.; Li, J.; Zhang, Q.; Zhong, P.; Teng, T.; Chen, M.; Xie, Z.; Ji, A. and Li, Y. (2019):** Epigallocatechin-3-gallate inhibits the growth and increases the apoptosis of human thyroid

carcinoma cells through suppression of EGFR/RAS/RAF/MEK/ERK signaling pathway. ***Cancer Cell International,*** 19(1), 1–17. https://doi.org/10.1186/s12935-019-0762-9.

**[10] Dai, Xiaofeng, Hongye Cheng, Zhonghu Bai, and Jia Li. (2017):** Breast cancer cell line classification and its relevance with breast tumor subtyping. ***Journal of Cancer***, 8(16):3131–41. doi: 10.7150/jca.18457.

**[11] Mostafa Alizade- Harakiyan, Jangjoo, A. G.; Tohid Jafari-Koshki, Ali Fatemi and Mesbahi, A. (2018):** Radiobiological modeling of acute esophagitis after radiation therapy of head, neck, and thorax tumors: The influence of chemo-radiation. ***Journal of Cancer Research and Therapeutics,*** 14(7), 1525–1534. https://doi.org/10.4103/jcrt.JCRT.

**[12] Abd-Rabou, A. A.; Ahmed, H. H. and Kishta, M. S. (2022a):** Implication of extrinsic and intrinsic apoptotic pathways in the targeted therapy of hepatocellular carcinoma using aptamer-labeled viramidine nanoparticles. ***BMC Cancer,*** 22(1): 1–14.

**[13] Hashim, A. F.; Abd-Rabou, A. A. and El-Sayed, H. S. (2022):** Functional nanoemulsion and nanocomposite microparticles as an anticolorectal cancer and antimicrobial agent: applied in yogurt. ***Biomass Conv Bioref***, https://doi.org/10.1007/s13399-022-03313-3.

**[14] Sun, Y. S.; Zhao, Z.; Yang, Z. N.; Xu, F.; Lu, H. J.; Zhu, Z. Y.; Shi, W.; Jiang, J.; Yao, P. P. and Zhu, H. P. (2017):** Risk factors and preventions of breast cancer. ***International Journal of Biological Sciences,*** 13(11): 1387–1397. https://doi.org/10.7150/ijbs.21635.

**[15] Saw, P. E.; Lee, S. and Jon, S. (2019):** Naturally occurring bioactive compound-derived nanoparticles for biomedical applications. ***Advanced Therapeutics,*** 2(5): 1800146. https://doi.org/10.1002/adtp.201800146.

**[16] Haider, M.; Abdin, S. M.; Kamal, L. and Orive, G. (2020):** Nanostructured lipid carriers for delivery of chemotherapeutics: A review. ***In Pharmaceutics* (Vol. 12, Issue 3). MDPI AG,** https://doi.org/10.3390/pharmaceutics12030288.

**[17] Siddiqui, I. A. and Sanna, V. (2016):** Impact of nanotechnology on the delivery of natural products for cancer prevention and therapy. ***Molecular Nutrition & Food Research,*** 60(6): 1330–1341. https://doi.org/10.1002/mnfr.201600035.

**[18] Bhattacharjee, C. and Bharadwaz, A. (2012):** Extraction of polyphenols from dried tea leaves. ***International Journal of Scientific and Engineering Research,*** 3(5).

**[19] Lee, K. J. and Lee, S. H. (2008):** Extraction behavior of caffeine and EGCG from green and black tea. ***Biotechnology and Bioprocess Engineering,*** 13(5): 646–649. https://doi.org/10.1007/s12257-008-0034-3.

**[20] Almutairi, F. M.; Abd-Rabou, A. A. and Mohamed, M. S. (2019):** Raloxifene-encapsulated hyaluronic acid-decorated chitosan nanoparticles selectively induce apoptosis in lung cancer cells. ***Bioorganic and Medicinal Chemistry,*** 27(8): 1629–1638. https://doi.org/10.1016/j.bmc.2019.03.004.

**[21] Abd-Rabou, A. A.; Shalby, A. B. and Ahmed, H. H. (2019):** Selenium nanoparticles induce the chemo-sensitivity of fluorouracil nanoparticles in breast and colon cancer cells. ***Biological Trace Element Research,*** 187(1): 80–91. https://doi.org/10.1007/s12011-018-1360-8.

**[22] Al-harthy, S. E.; Al-Motairi, M. S.; Al-Kreathy, H. M.; Kamel, F. O.; Sayed-Ahmed, M. M.; Huwait, E. A.; Al-Malkey, H. S. and Osman, A.-M. M. (2019):** Enhancement of doxorubicin cytotoxicity by verapamil in human breast cancer cells. ***Journal of Pharmaceutical Research International,*** 27(6): 1–10. https://doi.org/10.9734/jpri/2019/v27i630188.

**[23] Mukherjee, A. K.; Saviola, A. J.; Burns, P. D. and Mackessy, S. P. (2015):** Apoptosis induction in human breast cancer (MCF-7) cells by a novel venom l-amino acid oxidase (Rusvinoxidase) is independent of its enzymatic activity and is accompanied by caspase-7 activation and reactive oxygen species production. ***Apoptosis,*** 20(10): 1358–1372. https://doi.org/10.1007/s10495-015-1157-6.

**[24] El-Hamed, A.; Abd-Rabou, A. A. and Faramawy, A. (2022):** Therapeutic efficacy of curcuma and pomelo-loaded chitosan nanoparticles in intestinal murine trichinellosis. ***Egypt J Chem***, 65(2):551–564.

**[25] Perez-Ruiz, A. G.; Ganem, A.; Olivares-Corichi, I. M. and García-Sánchez, J. R. (2018):** Lecithin-chitosan-TPGS nanoparticles as nanocarriers of (-)-epicatechin enhanced its anticancer activity in breast cancer cells. ***RSC Advances,*** 8(61): 34773–34782. https://doi.org/10.1039/c8ra06327c.

**[26] Abd-Rabou, A. A.; Ahmed, H. H.; Kishta, M. S. and Zoheir, K. (2022b):** Selective viramidine-loaded aptamer-nanoparticles trigger cell cycle arrest in nucleolin-expressed hepatoma cells through modulation of CDC25A/p53/PI3k pathway. ***J Clust Sci,*** 34: 1–14.

**[27] Abd-Rabou, A. A.; Abdelaziz, A. M.; Shaker, O. G. and Ayeldeen, G. (2023):** Hyaluronated nanoparticles deliver raloxifene to CD44-expressed colon cancer cells and regulate lncRNAs/miRNAs epigenetic cascade. ***Cancer Nanotechnology,*** volume 14, 32.

**[28] Weisburg, J. H.; Weissman, D. B.; Sedaghat, T. and Babich, H. (2004):** *In vitro* cytotoxicity of epigallocatechin gallate and tea extracts to cancerous and normal cells from the human oral cavity. ***Basic and Clinical Pharmacology and Toxicology***, 95(4), 191–200. https://doi.org/10.1111/j.1742-7843.2004.pto_950407.

**[29] Thangapazham; Rajesh, L.; Neena Passi, and Radha K. Maheshwari. (2007):** Green tea polyphenol and epigallocatechin gallate induce apoptosis and inhibit invasion in human breast cancer cells. ***Cancer Biology and Therapy***, 6(12):1938–43.

doi: 10.4161/cbt.6.12.4974.
**[30] Wei, R.; Mao, L.; Xu, P.; Zheng, X.; Hackman, R. M.; MacKenzie, G. G. and Wang, Y. (2018):** Suppressing glucose metabolism with epigallocatechin-3-gallate (EGCG) reduces breast cancer cell growth in preclinical models. ***Food and Function,*** 9(11): 5682–5696. https://doi.org/10.1039/c8fo01397g.
**[31] Gianfredi, V.; Vannini, S.; Moretti, M.; Villarini, M.; Bragazzi, N. L.; Izzotti, A. and Nucci, D. (2017):** Sulforaphane and epigallocatechin gallate restore estrogen receptor expression by modulating epigenetic events in the breast cancer cell line MDA-MB-231: A Systematic Review and Meta-Analysis. ***Journal of Nutrigenetics and Nutrigenomics,*** 10(3–4): 126–135. https://doi.org/10.1159/000480636.
**[32] Cheng, Z.; Zhang, Z.; Han, Y.; Wang, J.; Wang, Y.; Chen, X.; Shao, Y.; Cheng, Y.; Zhou, W.; Lu, X. and Wu, Z. (2020):** A review on anti-cancer effect of green tea catechins. ***Journal of Functional Foods,*** 74(September), 104172. https://doi.org/10.1016/j.jff.2020.104172
**[33] Satheesh, B. N.; Suyakala, P. C.; Anjaneyulu, V. and Senthil, R. D. (2019):** Green tea catechin-loaded nanodelivery systems for the treatment of pandemic diseases. ***Asian Journal of Pharmaceutical and Clinical Research,*** 12(5): 1–7. https://doi.org/10.22159/ajpcr.2019.v12i5.27308.
**[34] Luo, Q., Zhang, J. R., Li, H. Bin, Wu, D. T., Geng, F., Corke, H., Wei, X. L., & Gan, R. Y. (2020):** Green extraction of antioxidant polyphenols from green tea (*Camellia sinensis*). ***Antioxidants,*** 9(9), 1–15. https://doi.org/10.3390/antiox9090785.
**[35] Huang, C. Y.; Han, Z.; Li, X.; Xie, H. H. and Zhu, S. S. (2017):** Mechanism of EGCG promoting apoptosis of MCF–7 cell line in human breast cancer. ***Oncology Letters,*** 14(3): 3623–3627. https://doi.org/10.3892/ol.2017.6641.
**[36] Yiannakopoulou, E. (2014):** Green tea catechins: proposed mechanisms of action in breast cancer focusing on the interplay between survival and apoptosis. ***Anti-Cancer Agents in Medicinal Chemistry,*** 14(2), 290–295. https://doi.org/10.2174/18715206113136660339.
**[37] Balakrishnan, S.; Mukherjee, S.; Das, S.; Bhat, F. A.; Raja Singh, P.; Patra, C. R. and Arunakaran, J. (2017):** Gold nanoparticles-conjugated quercetin induces apoptosis *via* inhibition of EGFR/PI3K/Akt–mediated pathway in breast cancer cell lines (MCF-7 and MDA-MB-231). ***Cell Biochemistry and Function,*** 35(4): 217–231. https://doi.org/10.1002/cbf.3266.
**[38] Abd-Rabou, A. A.; Abd El-Salam, N. M.; Sharada, H. M.; *et al.* (2021a):** Thymoquinone crosstalks with DR5 to sensitize TRAIL resistance and stimulate ROS-mediated cancer apoptosis. ***Asian Pac J Cancer Prev APJCP,*** 22(9): 2855.
**[39] Abd-Rabou, A. A.; Abdelaziz, A. M.; Shaker, O. G. and Ayeldeen, G. (2021b):** Metformin-loaded lecithin nanoparticles induce colorectal cancer cytotoxicity *via* epigenetic modulation of noncoding RNAs. ***Mol Biol Rep,*** 48(10): 6805–6820.
**[40] Huang, Chao You, Zheng Han, Xi Li, Hui Hua Xie, and Shan Shan Zhu. (2017):** Mechanism of EGCG promoting apoptosis of MCF–7 cell Line in human breast cancer. ***Oncology Letters,*** 14(3):3623–27. doi: 10.3892/ol.2017.6641.