Multifunctional Green Synthetic Hematite ($\alpha$-$\text{Fe}_2\text{O}_3$) Nanoparticles Mediated by Camellia Sinensis (Indian Tea Plant) Extracts towards Antioxidant and Antiproliferative Activities against MCF-7 Cell Lines

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Abstract

Among nanoparticles used for medical applications, Camellia Sinensis Nanoparticles (CSNPs) are among the least investigated. This study was undertaken to develop CSNPs by green synthesis using Camellia sinensis tea (Theaceae) plant extract to produce the NPs. The Camellia sinensis, Indian tea plant used from ancient time to increase appetite. Other medicinal uses have also been employed for the synthesis of super paramagnetic $\alpha$-$\text{Fe}_2\text{O}_3$ nanoparticles (NPs). The plant extracts revealed the phenolic groups bifunctional nature and capping nature through the -OH bonding over the nanoparticles (NPs) surface. The prepared nanoparticles (NPs) shows $\alpha$-$\text{Fe}_2\text{O}_3$ phase among iron oxides and spherical morphology with an average size around 5 nm. The magnetic measurements proved the superparamagnetic behaviour of NPs with non-saturating MS value of 8.5 emu/g at room temperature (300 K). The CSNPs were characterized by UV-V is spectroscopy and X-ray Diffractometry, and evaluated with Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Fourier-Transform Infrared (FTIR). The CSNPs were spherical (size 7-20 nm) and contained phenols and flavonoids acquired from the Camellia sinensis extract. CSNPs has good 1-Diphenyl-2-Picrylhydrazyl (DPPH), OH, and NO-scavenging properties. MTT assay showed that CSNPs (IC50 = 0.006 μM) were more antiproliferative toward the human MCF-7 cells than the Camellia sinensis tea extract (IC50 = 0.894 μM), Gemcitabin (IC50 = 2.133 μM). The anticancer cell effects of CSNPs on MCF 7 are mediated through the induction of apoptosis and G2/M cell-cycle arrest.

Keywords: Cytotoxicity, green synthesis, MCF-7 cells, hematite, nanoparticles, Camellia sinensis

Abbreviations: CS: Camellia Sinensis; NPs: Nanoparticles; CSNPs: Camellia Sinensis Nanoparticles; TEM: Transmission Electron Microscopy; SEM: Scanning Electron Microscopy; XRD: X-ray Diffractometry; FTIR: Fourier-transform infrared; MTT assay: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay; MCF-7: Michigan Cancer Foundation-7; Ms Value: Mass Spectroscopy Value
Introduction

Iron oxide-based nanoparticles (NPs) have been drawing attention in differing fields, due to their exceptional physico-chemical properties at the nanoscale [1,2]. Among various stages of iron oxides, the most contemplated form of iron oxides are hematite (α-Fe$_2$O$_3$), maghemite (γ-Fe$_2$O$_3$) and magnetite (Fe$_3$O$_4$). Though some of them show moderately poor magnetite properties, affordable costs and high resistance, maghemite can be considered as an elective alternative for magnetite. The hematite is one of the most active and stable phase in our environment and hence forth it broadly utilized in other non-bio applications, for example, catalysis, sensing and so on [3,4]. In view of these applications diverse physical and chemical process, for example, sono-compound, co-precipitation, sol-gel and others have been developed and utilized to synthesize the NPs [5].

In recent days green synthesis techniques have received overwhelmed attention due to its eco-friendly and therapeutic approaches [6,7]. Green synthetic approach has some additional advantages such as economic viability, less waste generation and simple in process. Hence various naturally available resources such as plants products, magnetitatic bacteria, algae, yeast and viruses are utilized for the green synthesis of NPs [8]. The plant-based extracts give the impression of being the best reducing or capping agents for their availability, suitability for bulk production and making non-toxic waste compared to other microbial extracts [9]. The additional advantages of this green medicinal plant extract mediated NPs synthesis is that, the NPs surface can be stabilized by the non toxic plant components. Thus these NPs show the nontoxic behaviour towards the normal human cells.

The platinum (II)–cisplatin complex, metal-based compounds are touted as potential drug delivery systems for cancers [3]. For coating purposes we have used chitosan with the plant extracts. The green synthesized possess remarkable antioxidant activities [10,7]. Plant-derived materials seem to have potential for application in management of cancer because of having toxicity to the cancer cells [9,11].

In this study, we demonstrated a simple one-step green process to synthesize CSNPs using Camellia sinensis extract from unfermented young tea leaves or unopened buds [12]. The Camellia sinensis extract has been used as an efficient reducing and capping agent. The extract contains several polyphenolic compounds belonging to the Quercetin that possess a wide range of biological activities, including antioxidant, anticancer, antiviral, antibacterial, and antifungal effects [13-15]. Nanoparticles development in medical treatment is an approach to achieve unique tasks in a novel way by the design of the drug delivery system in the mode of the treatment sequence, the formulation of nanoparticles from the plants in this study for breast cancer treatment is highly innovative.

Tea plant extract-derived on MCF-7 cells has not been established practically so far. From the treatment options for breast cancer are chemotherapy, radiation therapy, stem cell transplantation, biological or immune therapy, and targeted therapy. Chemotherapy and radiation are plagued by side effects that include lumps in breast, hair loss, fatigue, gastrointestinal disorders, and susceptibility to bleeding and infections, whereas other treatments are effective in some receptive patients only. For these reasons, more effective alternative cancer therapeutic compounds with minimal side effects are desperately being sought.

Materials and Methods

Preparation of materials

The fresh Camellia sinensis tea plant leaves were purchased from a local herbal store in Guwahati, Assam and washed several times using distilled water to remove impurities. The leaves were sun-dried and then crushed into powder. Chitosan Cl$_2$ (99.98%) was used as a precursor and it was supplied from Merck (Darmstadt, Germany). All solutions were prepared with deionized water. The plant was authenticated by Department of Botany, Utkal University, and the voucher has been deposited. The powder (10 gm) was soaked in methanol at Room Temperature (RT) for 2 days and centrifuged at 10000 rpm for 10 min. Finally, the extract was filtered through 0.45 μM filter membrane and stored at 4 °C until further use.

Preparation of chitosan based delivery nanoparticles

The NPs were synthesized by addition of FeCl$_3$·6H$_2$O (0.4 mM) in a 3:1 volume ratio with addition of sodium acetate (2 M) at RT and heated at 80°C for 2 h. The prepared α-Fe$_2$O$_3$ NPs were washed magnetically with water and air-dried.
Characterization of synthesized CSNPs

Camellia sinensis nanoparticles by various techniques

The structural and morphological studies of green synthesized CSNPs was quantitated by UV-Vis spectrophotometry (Lambda 25-Perkin Elmer, Waltham, MA, USA) over wavelength range of 200–800 nm, and the chemical composition was characterized by Fourier-transform infrared (FTIR, Shimadzu) spectrometry in the range of 400–4,000 cm⁻¹. The phase purity and particle size of CSNPs were determined by using the X-ray diffractometer (XRD-Bruker AXS) at 40 kV with nickel-filtered Cu (λ=1.542 Å) in the range of 10° to 80°. Morphological analysis of CSNPs was conducted by using transmission electron microscopy (TEM; HITACHI H-7650, Tokyo, Japan) at voltage 120 kV. The sample suspension was drop-casted on a carbon-coated copper grid and allowed to air-dry at room temperature overnight. The powdered sample was put on the carbon stub using carbon tape and then gold-coated using a sputter coater for ultrastructural examination via scanning electron microscopy (Philips XL-30) [4].

Phenolic and flavonoid content quantification

The phenolic and flavonoid contents of CSNPs and crude Camellia sinensis tea extract were quantified.

Total phenolic content

Phenolic content was determined by the Folin–Ciocalteu assay as described by Singleton and Rossi, [16] with minor modifications. Briefly, 10 μL sample solution and 500 μL Folin-Ciocalteu reagents were placed in each well of 96-well plates. Then, 350 μL of 10% of Na₂CO₃ was added to the wells, and the plate was incubated in the dark at room temperature for 2h. The absorbance was then recorded spectrophotometrically (Agilent 8453 Spectrophotometer, USA) at 765 nm against 10% DMSO as the negative control. Phenolic content was estimated using the gallic acid calibration curve (R²=0.97), and was expressed as gallic acid equivalent (μg GAE).

Total flavonoid content

Total flavonoid content was determined by an aluminium chloride colorimetric method as described previously [17]. In this test, the reaction mixture was prepared by mixing 100 μL CSNPs or Camellia sinensis tea extract, 10μL 10% aluminium chloride, 10μL 5% (CH₃CO₂) K, and 30 μL distilled water. The mixture was incubated at room temperature for 30 min, and absorbance was read at 415 nm. The calibration curve (R²=0.99) was obtained using the quercetin solutions at concentrations of 0.0–10 μg/mL and the flavonoid content.

Antioxidant activity

The antioxidant potential of the CSNPs was determined through 1-diphenyl-2-picrylhydrazyl (DPPH) and radical (−OH and −NO) scavenging activities.

Scavenging activity of DPPH

The DPPH scavenging activity of CSNPs was determined using the method described by Blois [18]. In brief, approximately 20 μL each of CSNPs and Camellia sinensis tea extract at concentrations ranging from 0.156 to 10 μM were added to 100 μL 0.1 mM methanolic DPPH solution. The mixture was incubated for 30 min at room temperature with constant shaking, and the absorbance was recorded at 517 nm. Butyl hydroxyl toluene was used as the reference. The DPPH radical scavenging activity (RSA) was calculated as percent inhibition using the following equation 1:

\[ \text{RSA} (%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \]

Where \( A_{\text{control}} \) and \( A_{\text{sample}} \) are the absorbance of the control and sample, respectively.

Scavenging activity of OH

Hydroxyl radical scavenging activity was determined by the degradation of 2-deoxyribose after condensation with thiobarbituric acid to produce OH radicals [19]. The final reaction mixture containing 100 μM FeCl₃, 100 μM ascorbic acid, 1 mM H₂O₂, 20 mM KOH buffer (pH 7.4), 100 μM EDTA, and 2.8 mM 2-deoxyribose was mixed with different concentrations (0.156–10 μM) of the CSNPs or control and incubated at 37°C for 1 h. Then, 0.5 mL reaction mixtures were added to 1 mL of 0.5% thiobarbituric acid diluted with NaOH (0.025 M) and 1 mL of 2.8% trichloroacetic acid. The mixtures were again incubated at 100°C for 30 min to obtain the chromogenic adduct. After cooling to room temperature, the concentration of chromogen was quantified at 532 nm. Gallic acid was used as the reference, and the percent inhibition of radical scavenging activity calculated using Eqn 1.

Scavenging activity of NO

The NO scavenging activity was determined spectrophotometrically (Agilent 8453 Spectrophotometer, Golden Valley, MN, USA) [19]. Approximately 50 μL
of 5 mM sodium nitroprusside was added to various concentrations (0.156–10 μM) of CSNPs or control, and the mixture was incubated at 25°C for 1 h. Then, 1.5 mL of this mixture was obtained and treated with 1.5 mL Griess’ reagent (Sigma-Aldrich Co., St Louis, MO, USA) to diazotize and form chromophore. Gallic acid was used as the reference. The concentration of the chromophore was determined at 546 nm, and percent inhibition of scavenging activity was calculated using Eqn 1.

Antiproliferative activity

The antiproliferative effects of CSNPs and *Camellia sinensis* tea extract was determined on the acute human malignant mammary cell (MCF-7) cell line (American Type Culture Collection [ATCC], Manassas, VA, USA), with Cisplatin and Gemcitabin as reference drugs [20]. The analyses used the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay kit (Sigma-Aldrich Co.). Briefly, cells were allowed to grow in a 75 m² cell culture flask until 95% confluent before seeding at 1×10⁵ cells/mL into each well of a 96-well plate. These cells were then treated with various concentrations (0.001–3.5 μM) of CSNPs, *Camellia sinensis* tea plant extract, Cisplatin, and Gemcitabin. Normal adult human mammary (MCF-7) cell line was used as the control. After incubation for 48 h at 37°C at pH 7.5 and under 5% CO₂, 25 μL 5.5 mg/mL MTT solution was added to each well and the plate was covered with aluminium foil and incubated for a further 3 h in the dark. The medium was immediately aspirated and the purple formazanlysed with MTT solution. The assay was conducted in triplicates. The absorbance was determined at 570 nm using the Spectra Max plus 384 UV–Vis plate reader. The half maximum inhibitory concentration (IC₅₀) was determined by non-regression analysis using Graph Pad Prism V. 5.03 (Graph Pad Software Inc., CA, USA).

Annexin-V/PI double-staining assay

Phosphatidylserine translocation in MCF-7 cells treated with CSNPs was determined by the Annexin-V-FITC/propidium iodide (PI) apoptosis detection kit (Sigma-Aldrich Co.). In brief, cells treated with 0.006 μM CSNPs for 12, 24, or 48 h were harvested, washed with PBS twice, resuspended in binding buffer, and incubated with Annexin V-FITC and propidium iodide solutions at room temperature in the dark for 30 min. Apoptosis was determined by flow cytometry using FACS Calibur (BD Biosciences, San Jose, CA, USA) with 15,000 ungated cells.

Cell-cycle analysis

MCF-7 cell-cycle distribution after treatment with CSNPs was determined by flow cytometry according to the method described previously [21]. Briefly, MCF-7 cells after incubation for 12, 24, or 48 h with 0.006 μM CSNPs were collected and centrifuged at 200× g for 5 min before washing with a mixture of PBS and sodium azide. The harvested cells were fixed with chilled 70% ethanol at −20°C for 5 days and then centrifuged at 200× g for 5 min. The supernatant was discarded and the cells were again washed twice with PBS and sodium azide; stained with PBS-staining buffer containing 0.1% triton X-100, 10 mM Methyleneediamine tetra-acetic acid, 50 μg/mL RNAase A, and 3 μg/mL PI; and incubated on ice in the dark for 30 min. Flow cytometric analysis was conducted using the BD FACS Calibur flow cytometer (BD), and data analysis was conducted using the Cell Quest Pro software with a DNA histogram to express the proportion of cells in cell-cycle phases. By quantifying the sub-G1 peak, apoptotic cells with hypodiploid DNA content were detected.

Protease activity of caspases 3 and 9

The caspase-3 and -9 activities were determined by a colorimetric assay kit (Gene script kit, code: L00289, GenScript, Piscataway, NJ, USA) in MCF-7 cells. Cells treated with 0.006 μM CSNPs for 12, 24, or 48 h were washed twice with ice-cold PBS, centrifuged at 200× g for 5 min, and the medium was discarded before harvesting cells. The cell pellet was suspended in cells lysis buffer and incubated on ice for 1 h. Untreated MCF-7 cells incubated for 12, 24, or 48 h served as controls. The protein concentrations were determined using the Bradford method. Approximately 1×10⁸ MCF-7 treated cells were transferred to the 96-well plate and 5 μL caspase substrate was added to the cells; the plate was wrapped with aluminum foil and incubated at 37°C for 4 h. After incubation, the plate was read at 405 nm in a microplate reader (Universal Microplate reader, Biotech, Inc., Winooski, VT, USA) to determine caspase activity, and the results are presented as optical density (OD).

Results and Discussion

Characterization of CSNPs

*Camellia sinensis* tea contains several polyphenols and flavonoids. The CSNPs suspension prepared from *Camellia sinensis* tea was dark brown (Figure 1), which is due the CSNPs [22] formed through quercetin. Because hydroxyl groups are abundant in flavonoids, the reduction of quercetin involved oxidation of the hydroxyl groups.
groups, with concomitant decrease in pH of the reaction suspension [23].

UV–Vis spectroscopy is a useful method to validate the presence of metal NPs. Using this technique, aqueous Camellia sinensis tea solution showed an intense absorption peak at 250 nm, which is associated with the benzoyl $\pi\rightarrow\pi^*$ transitions – an indication of the presence of flavonoids [8] (Figure 2). The *Camellia sinensis* tea extract with quercetin solution exhibited another discrete absorption peak at 410 nm, which is attributed to the quercetin ion content (Table 1). CSNPs produced a wide, continuous, absorption spectrum without the typical peaks of *Camellia sinensis* tea extract or *Camellia sinensis* tea with quercetin solution, indicating formation of CSNPs.

Figure 1: Formation of nanoparticles and its characterization.

![Figure 1](image)

Figure 2: UV–visible spectra of CSNPs, CS plant extract, CS with Chitosan.

![Figure 2](image)

Table 1: Total phenolic and flavonoid contents of camellia sinensis leaf extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolic contents (πg,-GAE/mg)</th>
<th>Total Flavonoids (πg, QE/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Tea</td>
<td>229.05±1.21</td>
<td>27.45±0.97</td>
</tr>
<tr>
<td>CSNPs</td>
<td>28.5±.58</td>
<td>8.85±.33</td>
</tr>
</tbody>
</table>

Abbreviations: NPs- Nanoparticles; CSNPs-Camellia sinensis nanoparticles;
An FTIR analysis was used to identify molecules responsible for the reduction of quercetin and coating of the CSNPs. The FTIR spectrum of *Camellia sinensis* tea extract showed peaks at 3,345, 2,860, 1,712, 1,667, 1,520, 1,389, 1,124, and 720 cm\(^{-1}\), representing free OH, and stretching of CH group of aldehydes and alkanes, carbonyl group (C=O), (NH)–C=O group, C=C aromatic ring, C–OH, and phenyl (C–H), respectively (Figure 3). These peaks showed that flavonols and other phenolics molecules are present in the *Camellia sinensis* tea extract. With presence of quercetin, the position and intensity of peaks changed, especially by the decrease in the hydroxyl peak and appearance of the carbonyl peak, indicating the involvement of phytochemical sites in synthesis and binding of CSNPs. Thus, the results confirmed that reduction of quercetin occurs through oxidation of hydroxyl to carbonyl groups.

The CSNPs were examined under X-ray diffraction to determine crystalline structure (Figure 4). There are five well-defined and characteristic diffraction peaks at 40.1°, 46.3°, 58.5°, 70.1°, and 82.2° representing reflections from (111), (200), (220), (311), and (222) planes, respectively, of face-centered cubic crystal structure of quercetin. The presence of a broad and intense diffraction peak at 20 of 13.5° may be assigned to the chemical contents of the *Camellia sinensis* tea extract. Using the Scherrer equation, the average size of CSNPs was calculated at 15 nm.

**Figure 3:** FTIR spectra of CSNPs and CS extract with instrument.

**Figure 4:** X-ray diffraction pattern of the CSNPs.
Under scanning electron microscopy (SEM) and transmission electron microscopy (TEM), CSNPs were shown to be spherical with narrow size distribution (Figure 5). The particles did not appear to aggregate, although some were present in clumps that suggested passive contact. The ultra-structure studies showed that CSNPs are stable and had little tendency to aggregate, which is attributed to the presence of phenols and flavonoids in NPs. It is proposed that, while inhibiting particle aggregation, phenols and flavonoids play a major role in the chelation of NPs to ligands. The size distribution of CSNPs is in the range of 6–18 nm, averaging at 11 nm, which is marginally smaller than the value obtained by X-ray diffraction determination.

Figure 5: The (A) SEM and (B) TEM images.

Radical scavenging activity

To assess the antioxidant activity of Camellia sinensis tea extract and CSNPs, DPPH radical scavenging activity was determined in MCF-7 cells (Figure 6). It is assumed that the content of phenolic and flavonoid compounds of the extract and CSNPs would afford them promising radical scavenging activity because these compounds are known to be scavengers of DPPH, OH, and NO [19,23]. The radical scavenging activities of Camellia sinensis tea extract for DPPH was high even at low doses and was not dose-dependent (Figure 7). On the other hand, the DPPH scavenging activity of CSNPs was low at low concentrations and only began to approach antioxidation efficacy of Camellia sinensis tea extract only after reaching high doses.

Figure 6: (A) DPPH, (B) Oh, and (C) NO scavenging activity of white tea extract and CSNPs.
Both *Camellia sinensis* plant extract and CSNPs showed dose-dependent scavenging activities of OH and NO. However, overall, *Camellia sinensis* tea extract is still more efficient than the CSNPs at OH and NO scavenging. This phenomenon can be attributed to the concentration of phenolic and flavonoid compounds, which were much higher in the crude extract than CSNPs (Table 2).

### In vitro cytotoxicity

The antiproliferative activity of the *Camellia sinensis* tea extract and CSNPs was determined on the MCF-7 cell line with Cisplatin and Gemcitabin for comparison (Figure 8 and Figure 9). CSNPs were highly toxic to MCF-7 cells, with effect increasing with increase in concentration. In fact, based on IC_{50}, CSNPs are >2, >100, and >3,000 times more toxic to MCF-7 cells than Ciplastin, crude *Camellia sinensis* tea extract, and Gemcitabin, respectively (Table 3). These findings show that CSNPs have potential as an anticancer compound. Both CSNPs and crude *Camellia sinensis* extract showed low toxicity to the normal MCF-7 cell line. It is proposed that the high cytotoxicity of CSNPs to the cancer cell line is due to the presence of Quercetin, which interacts physicochemically with the functional groups of cellular proteins, nitrogen bases, and phosphate groups.
**Figure 8:** Viability of MCF-7.

**Figure 9:** Induction of MCF-7 cell apoptosis by CSNPs after staining with FITC-conjugated annexin V-FITC. (A1–C1) untreated (control) MCF-7 cells at 12, 24, and 48 h, respectively. (A2–C2): MCF cells after treatment with CSNPs at 12, 24, and 48 h, respectively.
of the DNA, [24] thus causing cell death. Moreover, previous studies showed that quercetin causes production of free radical, [25] lactate dehydrogenase leakage, [9] and cell-cycle disturbances that could be among mechanisms of the anticancer effects of CSNPs.

Flavonoid compounds can control metabolic activities of cancer cells[26]. The anticancer effects of flavonoids occur through oxidative destruction, inhibition of proliferation, inactivation of carcinogen, promotion of differentiation, induction of cell-cycle arrest and apoptosis, impairment of tumor angiogenesis, and suppression of metastasis [27-29]. Flavonoids can interact with xenobiotic metabolizing enzymes and inhibit involvement of kinases signal transduction, interact with estrogen type II binding sites, and alter gene expression patterns, [30,31] with resultant promotion of antiproliferative activity of CSNPs.

The size of NPs is an important factor in their cytotoxic effects. Small-sized NPs can exert greater cytotoxicity than large ones [32,33]. At an average of 11 or 15 nm, CSNPs can evade the mononuclear phagocytic system whereas easily crossing cell membranes [34] to exert anticancer effects. The antiproliferative effect of CSNPs on the MCF-7 is selective because these NPs are relatively innocuous to normal mammary cells. The IC₅₀ values of Camellia sinensis tea extract and CSNPs on the MCF-7 cells were considerably higher than on the MDAMB 231 cells. It is suggested that surface modifications of the CSNPs by the flavonoids acquired from Camellia sinensis tea extract are responsible for the toxic effect of CSNPs on cancers without adversely affecting normal cells [31,35].

Table 2: The IC50 of CSNPs, tea extract, cisplatin, gemcitabine

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC50 μM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CSNPs</td>
</tr>
<tr>
<td>MCF-7</td>
<td>0.004± 0.004</td>
</tr>
<tr>
<td>MDAMB-231</td>
<td>3.212± 0.78</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as Mean ± (SD= 0.001) of three replicants; Abbreviations: CSNPs-Camellia sinensis nanoparticles, MCF-7-Cell lines, NPs- nanoparticles;

Table 3: Flow cytometry analysis of FITC conjugated annexin V/PI staining.

<table>
<thead>
<tr>
<th>Cell stage</th>
<th>Cell (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 Hr.</td>
</tr>
<tr>
<td>Viable</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>94.4± 0.35</td>
</tr>
<tr>
<td>Early Apoptosis</td>
<td>2.72± 0.17</td>
</tr>
<tr>
<td>Late Apoptosis/Necrosis</td>
<td>1.67± 0.37</td>
</tr>
</tbody>
</table>

Notes: Values are expressed as mean ± SD of triplicate experiments. Data has been analyzed using one-way ANOVA. * indicates significant value (P - 0.05) different from control. Abbreviation: FITC: Fluorescein isothiocyanate;
Caspases

Caspase-3 and -9 are the primary executioners of apoptosis. In MCF-7 cells, treatment with CSNPs significantly increased the activities of caspase-3 and caspase-9. Caspase pathway-mediated apoptosis seems to be the common mode of cell death instituted by NPs; for example, with zerumbone-loaded nanostructured lipid carriers and magnetic iron oxide NPs on Jurkat cells, [39] amine-modified polystyrene NPs on astrocytoma cells, [40] and nickel-zinc ferrite NPs on the HepG2, HT29, and MCF-7 cell lines [41].

Conclusion

We tried to develop an eco-friendly and efficient process for the synthesis of CSNPs with Camellia sinensis tea extract as the medium. The mild reaction condition and easy workup procedure as well as the scope of pharmacological and medicinal applications are making our methodology a valuable contribution to the processes for green synthesis of NPs. The CSNPs with size range between 6 and 18 nm are ideal as a drug carrier system, because it avoids clearance by the monocyte–phagocytic system while easily penetrating cell membranes. The CSNPs has potent radical scavenging capability and are antiproliferative to the MCF-7 cells without adversely affecting the normal mammary cells. The antiproliferative of CSNPs is via apoptosis and G2/M cell-cycle arrest. Thus, CSNPs has the potential to be developed into anticancer agent.

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CYP1-mediated antiproliferative activity of dietary
flavonoids in MDA-MB-468 breast cancer cells.


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