

ANTICANCER ACTIVITY OF *CAMELLIA SINENSIS* MEDIATED COPPER NANOPARTICLES AGAINST HT-29, MCF-7, AND MOLT-4 HUMAN CANCER CELL LINES

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ABSTRACT

Objective: With the advancement in nanotechnology, it is imperative to unearth its applications in medicine. The present investigation deals with the copper nanoparticles (CuNPs) biosynthesizing capability of the leaves of medicinally important plant, *Camellia sinensis*.

Methods: The phytosynthesized CuNPs were characterized by energy-dispersive X-ray spectroscopy (EDX), nanoparticle tracking analysis, X-ray diffraction, scanning electron microscopy, transmission electron microscopy, and Fourier transform infrared spectroscopy analysis. In the current study, we have made attempts to exploit the anticancer ability of the CuNPs against human colon cancer cell line (HT-29), human breast cancer cell line (MCF-7), and human leukemia cancer cell line (MOLT-4) through sulforhodamine B assay. We also carried out the synergistic activity with the standard drug adriamycin (ADR).

Results: The synthesis of CuNPs was confirmed using EDX analysis where the presence of a strong optical absorption peak was observed at 1 keV, which is typical for the absorption of metallic CuNPs. According to the results obtained, CuNPs showed good antiproliferative results in a dose-dependent manner on HT-29 and MCF-7 cell lines, with 80 µg/ml concentration giving the best result. The synergistic effect of CuNPs+ADR was even better than that of CuNPs alone on all the cell lines. The synergism drug combinations showed high responsive results on the leukemia cell line compared to individual drugs.

Conclusion: Among all the treatments and cell lines studied, the most favorable and responsive antiproliferative impact was recorded for CuNPs+ADR combination treatment at 40 µg/ml concentration on MCF-7 breast cancer cell line.

Keywords: CuNPs, *Camellia sinensis*, Anticancer, Sulforhodamine B assay, HT-29, MCF-7, MOLT-4.

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INTRODUCTION

Cancer is one of the frequent causes of death in the world. Methods such as surgery, chemotherapy, and radiotherapy are used extensively in cancer treatment, but these methods have numerous side effects apart from being expensive. Therefore, there is a strong demand for effective, inexpensive, and non-toxic treatments with minimal side effects. Continuous demand for new anticancer drugs has stimulated chemotherapeutic research based on the use of metals since potential drugs developed in this way may be less toxic and more prone to exhibit antiproliferative activity against tumors [1,2]. Transition metal complexes have been extensively studied for their nuclease-like activity using the redox properties of the metal and dioxygen to produce reactive oxygen species (ROS) to promote deoxyribonucleic acid (DNA) cleavage by direct strand scission or base modification [3]. The most recent trend in this area has been testing of metal nanoparticles for DNA degradation studies. Use of metal nanoparticles can be particularly advantageous in generating singlet oxygen. A recent report by Zhang *et al.* demonstrated that the presence of metal nanoparticles can enhance singlet oxygen generation [4].

Copper is a trace element essential for human life. It is a building element of several important enzymes (e.g., superoxide dismutase, cytochrome oxidase, and tyrosinase) and it regulates the intracellular redox potential, while its complexes possess antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties. Anticancer activity of copper compounds may be a result of different mechanisms. Anticancer activity of copper complex compounds is related to their ability to produce ROS and they are also thought to have nuclease activity.

Therefore, the present work focused on the green synthesis of copper nanoparticles (CuNPs) using *Camellia sinensis* leaf extract in response

to the quest for search of a novel anticancer agent against HT-29, MCF-7, and MOLT-4 human cancer cell lines. The importance of the present work is that the synthesized nanoparticles may contain the surface coating of green tea bioactive flavonoids and polyphenols and hence it may become a theme aspect of future research, and the novel aspect of this research work is that there are no reported studies of anticancer effects of CuNPs on colon and leukemia cancer cell lines.

METHODS

Preparation of plant extract

C. sinensis plant was chosen as the experimental model for synthesizing CuNPs and it was procured from Ooty, Tamil Nadu, in the month of April 2014 and grown in the Botanical garden of Institute of Science, Mumbai. Leaves of tea plant were collected, surface sterilized, air dried, and grounded to produce fine powder. For the synthesis of CuNPs, aqueous extract was prepared by mixing 1 g of leaf powder in 100 ml distilled water and boiled for 20 minutes followed by filtering through the Whatman filter paper no. 1 to obtain a clear solution.

Experimental synthesis of CuNPs

An air-tight flask containing 50 ml of 10 mM of CuCl₂·2H₂O solution and 8 ml tea extract was heated at 90°C in a water bath shaker. About 1.0 ml of ascorbic acid was added drop wise to the flask. The heating and mixing were continued till the color changes to yellow, orange, brown, and finally dark brown.

Characterization of synthesized CuNPs

Energy-dispersive X-ray (EDX) spectroscopy was obtained using ZEISS Ultra55 field emission scanning electron microscopy (SEM) for the elemental analysis of CuNPs. Nanoparticle tracking analysis (NTA) was

done using NTA model, LM 20 (NanoSight, UK), for the visualization and analysis of nanoparticles for their particle size [5]. Crystalline nature of the nanoparticles was analyzed by X-ray diffraction (XRD) at 2θ diffraction angle that ranges from 20° to 80° using Rigaku MiniFlex benchtop X-ray spectrophotometer. The surface morphology of CuNPs was analyzed using ZEISS Ultra55 Field Emission SEM. The particle size and surface morphology were determined using FEI Tecnai T20 transmission electron microscopy (TEM) instrument which was operated at an accelerating voltage of 200 kV with the resolution of 0.22 nm. The chemical composition of the synthesized CuNPs was studied using Fourier transform infrared spectroscopy (FTIR) spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer), and the dried powders were characterized in the range of $4000\text{-}400\text{ cm}^{-1}$ using KBr pellet method.

Anticancer studies using sulforhodamine B (SRB) Assay

HT-29, MCF-7, and MOLT-4 cancer cell lines were selected as the experimental models to carry out anticancer studies in the present work. These cell lines were obtained from Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Mumbai.

The cell lines were grown in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates in 100 μL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C , 5% CO_2 , 95% air, and 100% relative humidity for 24 hrs prior to the addition of experimental drugs. The final concentrations of the experimental drugs used were 10 $\mu\text{g}/\text{ml}$, 20 $\mu\text{g}/\text{ml}$, 40 $\mu\text{g}/\text{ml}$, and 80 $\mu\text{g}/\text{ml}$.

After compound addition, the plates were incubated at standard conditions for 48 hrs, and the assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by the gentle addition of 50 μl of cold 30% (w/v) TCA and incubated for 60 minutes at 4°C . The supernatant was discarded; the plates were washed five times with tap water and air dried. SRB solution (50 μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and the plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength [6].

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells $\times 100$.

$$\% \text{ Growth} = \frac{\text{Average absorbance of test}}{\text{Average absorbance of control}} \times 100$$

The results obtained in the cytotoxicity testing were expressed in three formats. They are median lethal concentration (LC50), 50% growth inhibition (GI50), and total growth inhibition (TGI). The LC50 value is the concentration of drug causing 50% cell lethality. The GI50 value is the concentration of drug which causes 50% inhibition in the growth of cells. The TGI value is the concentration of a drug that leads to total inhibition of cell growth. Apart from the experimental samples, a positive control drug named adriamycin (ADR) was used in this study. This drug is an anticancer chemotherapy drug. The final readings of the cytotoxicity assays were presented as mean of at least three independent measurements.

RESULTS AND DISCUSSION

The results obtained related to the green synthesis of CuNPs using *C. sinensis*, their characterization, and anticancer activities on selected cancer cell lines were discussed below.

Characterization of CuNPs

The formation of CuNPs was initially confirmed visually by change in color. The color change for CuNPs was from straw yellow (Fig. 1a) to dark brown (Fig. 1b) after heating at 90°C in a water bath; the reaction mixture comprising plant extract, 10 mM CuCl_2 , and 1 M ascorbic acid. EDX analysis of copper nanoparticle sample showed the presence of the elemental copper, carbon, oxygen, and chlorine (Fig. 2). A strong optical absorption peak was observed at 1 keV, which is typical for the absorption of metallic CuNPs [7]. According to the NTA analysis of CuNPs, the mean size was recorded as 28 nm (Fig. 3) and the concentration was found to be 2.4×10^8 particles/ml. The XRD spectra of CuNPs exhibited three peaks at 2θ values of 43.4° , 50.7° , and 74.4° corresponding to (111), (200), and (220) planes of copper and were well matched with the standard powder diffraction card of the Joint Committee on Powder Diffraction Standards, copper file No. 04-0836 (Fig. 4). SEM analysis revealed spherical, uniform, and aggregated CuNPs with diameters that range from 10 to 50 nm (Fig. 5). TEM analysis of CuNPs exhibited spherical morphologies with their size ranging from 10 to 40 nm (Fig. 6). Most of the CuNPs were roughly circular with smooth edges.

The FTIR spectrum of green tea extract (Fig. 7) shows a band at 3437.3 cm^{-1} which is due to stretching vibrations of O-H groups and N-H stretching in amines. The C-H stretch in alkanes and O-H stretch in carboxylic acid appear at 2926 and 2864 cm^{-1} , respectively. An important band at 1631.8 cm^{-1} attributes to the C=C stretch in aromatic ring and C=O stretch in polyphenols. The C-N stretch of amide-I in protein gives the band at 1407.1 cm^{-1} . The C-O stretching in amino acid causes a band at 1106.6 cm^{-1} . Hence, we can conclude that green tea leaf extract is rich in polyphenols, carboxylic acid, polysaccharide, amino acid, and proteins [8]. The FTIR spectrum of CuNPs (Fig. 8) reveals a peak at 3456 cm^{-1} that can be assigned to -OH group of polyols such as catechins. A peak is observed at 2901 cm^{-1} , which can be attributed to the stretching vibrations of -CH (alkane) or secondary amines. The band at 1404.1 cm^{-1} corresponds to the C-N stretching vibration of aliphatic amines or to alcohols/phenols. The C-O-C stretching in polysaccharides gives a band at 1785 cm^{-1} and C-O stretching in amino acid causes a band at 1121 cm^{-1} . The strong band at 1633.2 cm^{-1} is attributed to the C=C stretch in aromatic ring and C=O stretch in polyphenols. Therefore, by taking account of the above-mentioned observations, we can estimate that the polyphenol groups present in the tea extract are responsible for reduction and capping of CuNPs.

Anticancer studies

Joshi *et al.* used ADR as a reference standard drug for the testing of anticancer activity using the SRB assay [9]. In the present work, we evaluated our compounds - CuNPs, *C. sinensis* leaf extract and their synergistic activity with ADR for their anticancer properties *in vitro* against HT-29, MCF-7, and MOLT-4 cell lines. Results obtained are

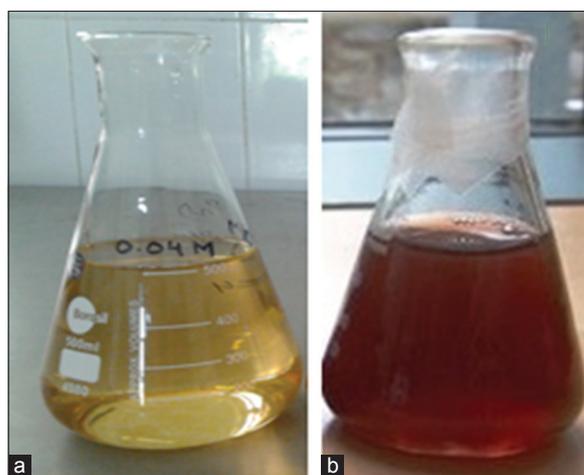


Fig. 1: (a) *Camellia sinensis* leaf broth, (b) formation of copper nanoparticle

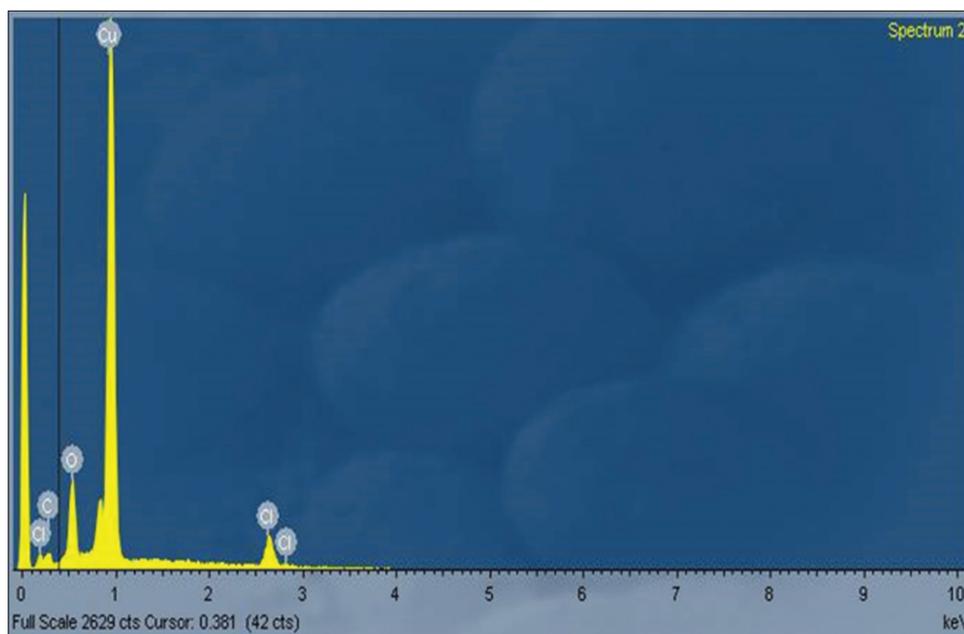


Fig. 2: Energy-dispersive X-ray spectroscopy spectra of copper nanoparticles synthesized using *Camellia sinensis* leaf extract

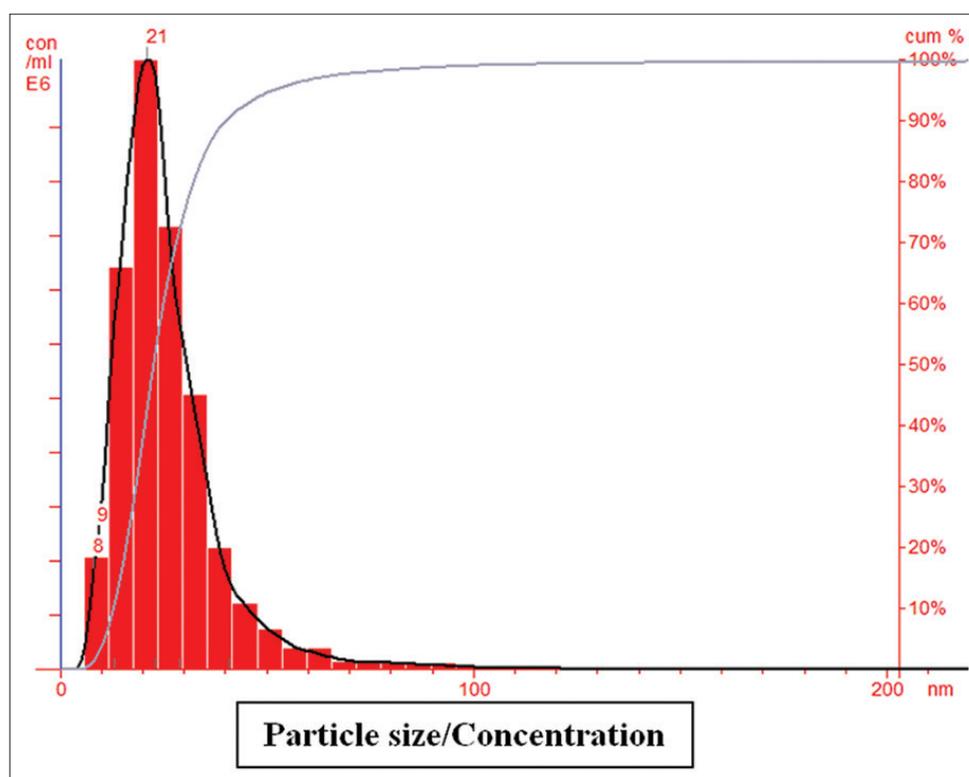


Fig. 3: Frequency of occurrence of varying sizes of synthesized copper nanoparticles calculated using nanoparticle tracking analyzer

presented in the form of LC50, TGI, and GI50 values and these are summarized in Table 1.

Application on human colon cancer cell line (HT-29)

CuNPs show 56.8% of control growth at the highest concentration of 80 $\mu\text{g}/\text{ml}$ but do not show much effect in the preceding concentrations. But, in combination with ADR, the treatment shows synergistic activity with better result at all the four concentrations when compared to CuNPs alone. (Fig. 9) This result was prominently noted at 10 $\mu\text{g}/\text{ml}$ giving the best result (Table 2). Copper nanoparticle shows the best

action against HT-29 cell line compared to other treatments at 80 $\mu\text{g}/\text{ml}$ concentration. CuNPs treatment showed GI50 value of 33 $\mu\text{g}/\text{ml}$ but its synergistic effect with ADR shows GI50 to be <10 $\mu\text{g}/\text{ml}$ (Table 1). In the context of percent cell growth inhibition, ADR showed a linear cytotoxic response with respect to drug concentration.

No reports were found showing the cytotoxic effects of CuNPs on colon cancer cell lines, but there are reports for anticancer activity of CuNPs on adenocarcinomic human alveolar basal epithelial cells (A549) [10] and cervical cancer (HeLa) cells [11]. Badawi *et al.* investigated the

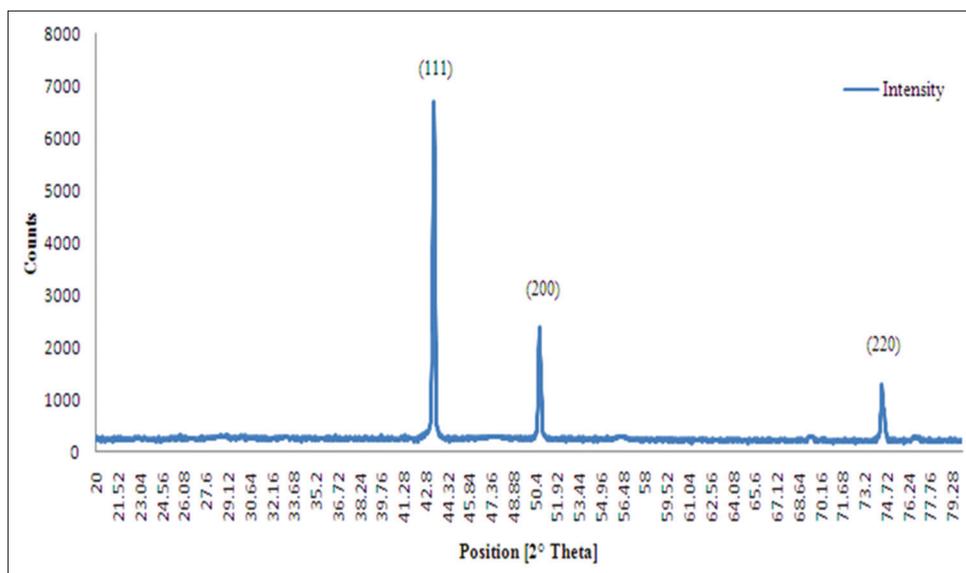


Fig. 4: X-ray diffraction spectra of copper nanoparticles synthesized using *Camellia sinensis* leaf extract

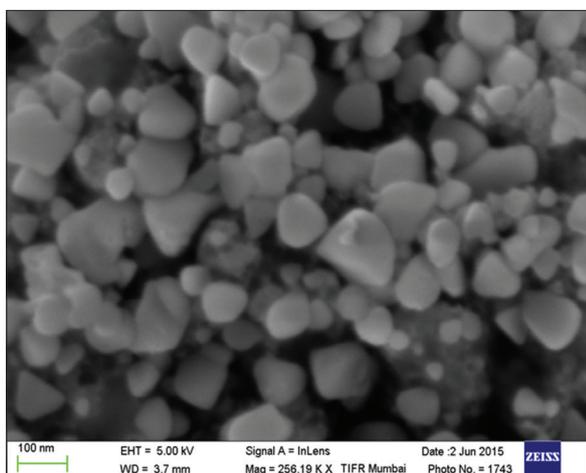


Fig. 5: Scanning electron microscopy image of *Camellia sinensis* extract-mediated synthesized copper nanoparticles at 100 nm scale

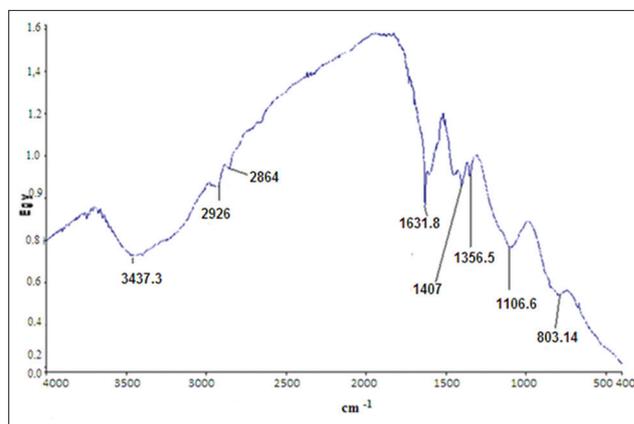


Fig. 7: Fourier transform infrared spectroscopy spectra of *Camellia sinensis* leaf extract

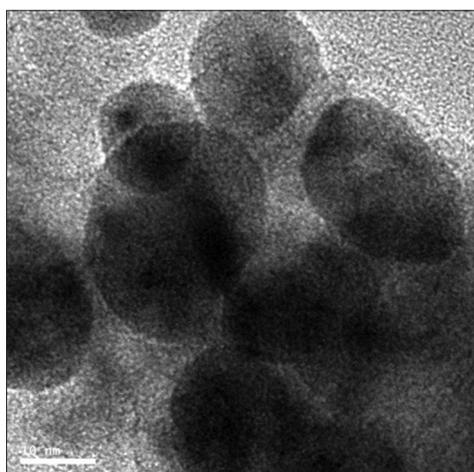


Fig. 6: Transmission electron microscopic image of *Camellia sinensis* extract-mediated synthesized copper nanoparticles at 10 nm scale

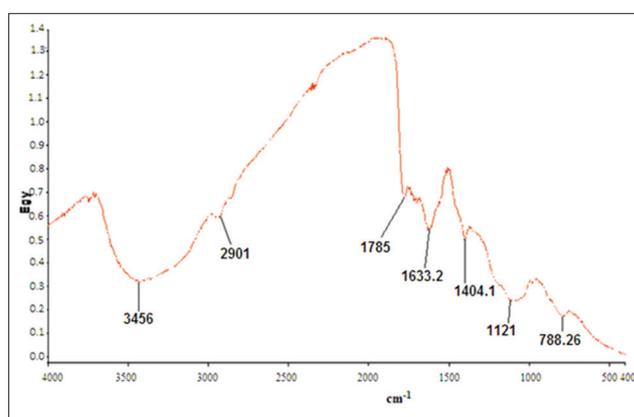


Fig. 8: Fourier transform infrared spectroscopy spectra of copper nanoparticles synthesized using *Camellia sinensis* leaf extract

in vitro anticancer activity of copper cetyltrimethylammonium bromide surfactant-loaded cyclodextrin nanoparticles on Ehrlich ascites carcinoma, colon cancer cells (HCT116), liver cancer cells (HepG-2), breast cancer cells (MCF-7), and cervical cancer cells (HeLa) using the

Table 1: LC50, TGI, and GI50 values of HT-29, MCF-7, and MOLT-4 cell lines after treatment with test drugs

Cell lines	Drug concentrations ($\mu\text{g/ml}$) calculated from graph								
	HT-29			MCF-7			MOLT-4		
	LC50	TGI	GI50*	LC50	TGI	GI50*	LC50	TGI	GI50*
CuNP-tea	78.5	55.8	33.0	78.5	84.9	50.3	>80	>80	>80
CuNP-tea+10 μg ADR	NE	NE	<10	<10	<10	<10	<10	<10	<10
<i>C. sinensis</i> extract	>80	>80	>80	>80	>80	>80	>80	>80	>80
<i>C. sinensis</i> extract+10 μg ADR	NE	NE	<10	<10	<10	<10	<10	<10	<10
ADR	NE	<10	<10	<10	<10	<10	<10	<10	<10

*Yellow highlighted test values under GI50 column indicate activity. *C. sinensis*: *Camellia sinensis*, ADR: Adriamycin, CuNPs: Copper nanoparticles, SD: Standard deviation, TGI: Total growth inhibition, LC50: Median lethal concentration, GI50: 50% growth inhibition

Table 2: Percent control growth of HT-29 cell line after treatment with test drugs

Human colon cancer cell line HT-29				
Percent control growth				
Drug concentrations ($\mu\text{g/ml}$)				
Mean \pm SD				
Concentrations	10	20	40	80
CuNP-tea	81.1 \pm 25.3	78.6 \pm 17.1	43.0 \pm 8.3	-56.8 \pm 2.8
CuNP-tea+10 μg ADR	-13.7 \pm 1.2	-5.8 \pm 4.2	-1.8 \pm 17.5	-0.1 \pm 12.2
<i>C. sinensis</i> extract	102.1 \pm 1.9	99.0 \pm 14.6	104.2 \pm 2.3	112.3 \pm 6.9
<i>C. sinensis</i> extract+10 μg ADR	1.2 \pm 7.9	27.9 \pm 2.3	48.3 \pm 4.7	27.9 \pm 4.8
ADR	-9.4 \pm 7.7	-18.4 \pm 5.2	-19.9 \pm 2.7	-25.1 \pm 8.9

n=3 (Minus values indicate positive response). *C. sinensis*: *Camellia sinensis*, ADR: Adriamycin, CuNPs: Copper nanoparticles, SD: Standard deviation

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [12].

C. sinensis leaf extract showed 112.3% control growth at the highest dose concentration on HT-29 cell line, but when combined with ADR, the percent control growth decreased drastically and it may be due to the effect of ADR alone. GI50 value for tea extract is >80 $\mu\text{g/ml}$, but for tea extract+ADR, it is <10 $\mu\text{g/ml}$, hence we can say that this effect is due to the presence of ADR because tea extract alone does not show any significant action. According to epidemiological studies, the antiproliferative effects of tea extract against colon cancer [13] maybe due to tea polyphenols and their protective activity is related to the strong free radical scavenging and antioxidative capacity of tea [14].

Application on human breast cancer cell line (MCF-7)

The treatment of CuNPs, CuNPs+ADR, tea leaf extract, and tea leaf extract+ADR was administered on human breast cancer cell line (MCF-7) by performing SRB assay.

Among the CuNPs, only 80 $\mu\text{g/ml}$ concentration exhibits a reasonable percent control growth of 5.6%, but the combined effect of CuNPs+ADR shows the maximum activity at the 40 $\mu\text{g/ml}$ concentration followed by 80 $\mu\text{g/ml}$. CuNPs showed dose-dependent cytotoxic activity where the highest dose concentration showed the maximum effect. Lower activities were found in *C. sinensis* leaf extract, showing control growth values of 73.7%, the lowest at 40 $\mu\text{g/ml}$ concentration. After combining tea extract with ADR for its synergistic effect, 10 $\mu\text{g/ml}$ concentration showed the lowest percent growth value of 23.8%, but we can attribute this effect to the activity of ADR alone since tea extract alone show minimal activity at the studied dose levels (Table 3). The microscopic photographs of MCF-7 cells treated with our experimental drugs showed morphological changes such as shrinkage and irregular shape (Fig. 10).

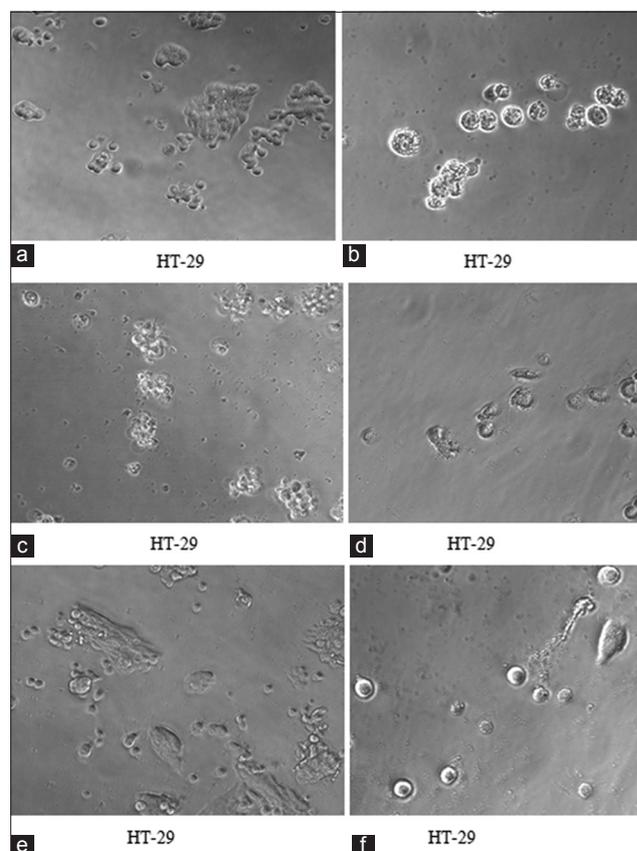


Fig. 9: Effect of test drugs on human colon cancer cell line (HT-29) as observed under the inverted microscope. (a) Before treatment, (b) adriamycin (ADR), (c) copper nanoparticle (CuNP)-tea, (d) CuNP-tea+ADR, (e) *Camellia sinensis* extract, (f) *C. sinensis* extract+ADR

CuNPs exhibited GI50 value of 50.3 $\mu\text{g/ml}$ whereas its synergistic effect with ADR showed <10 $\mu\text{g/ml}$ value (Table 1). Hence, we can conclude that the synergistic effect of CuNPs+ADR indicates high responsive effect on MCF-7 cell line. According to a study by Baskar *et al.*, cytotoxicity was studied on the MCF-7 cell line using CuNPs synthesized in *Catharanthus roseus* flower extract [15]. The cytotoxicity of 21.17, 41.52, and 62.96% was reported for 50, 100, and 150 $\mu\text{g/ml}$ concentration, respectively. The positive control used was cyclophosphamide which displayed 73.82% cytotoxicity. It was observed from these results that the increase in the concentration of copper nanobiocomposite increased the cell toxicity, which matched the trend of our results. In yet another study, *Acalypha indica*-mediated synthesized copper oxide nanoparticles exerted cytotoxic effects on the MCF-7 cancer cell line [16].

There was minimal activity shown by tea extract because GI50 value of this treatment was >80 $\mu\text{g/ml}$ but its synergistic effect with ADR

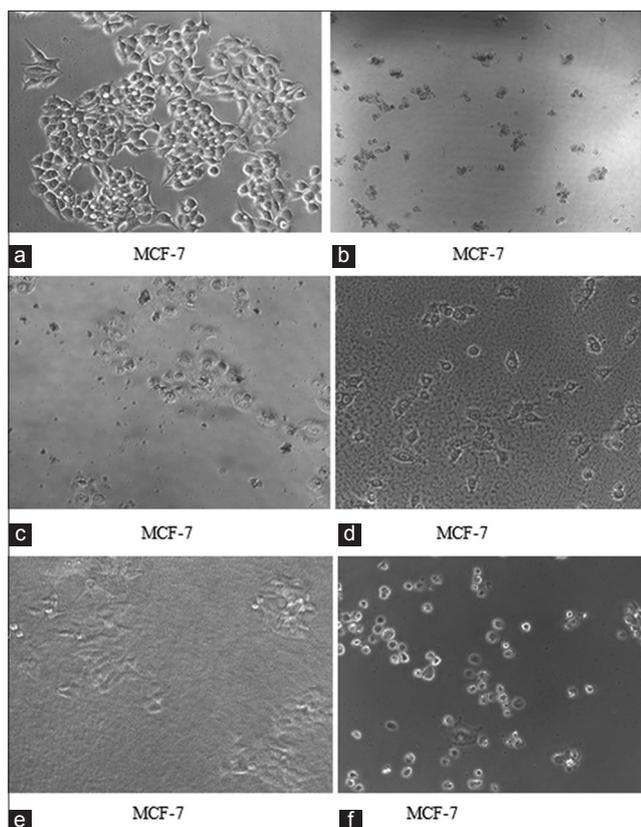


Fig. 10: Effect of test drugs on human breast cancer cell line (MCF-7) as observed under the inverted microscope. (a) Before treatment, (b) adriamycin (ADR), (c) copper nanoparticle (CuNP)-tea, (d) CuNP-tea+ADR, (e) *Camellia sinensis* extract, (f) *C. sinensis* extract+ADR

indicated $<10 \mu\text{g/ml}$ GI50 values, which clearly indicates its cytotoxic activity and this is due to the presence of ADR alone. In a study, black tea extract showed high cytotoxic effect against MCF-7 cells after 24 h of influence where IC50 was lower than the lowest concentration used ($0.00078 \mu\text{g/ml}$) [17].

Application on human leukemia cancer cell line (MOLT-4)

The treatments of the two test drugs (CuNPs and *C. sinensis* leaf extract) and their synergistic effects with standard drug (ADR) through the SRB assay were evaluated on human leukemia cancer cell line (MOLT-4). Both the test compounds exhibited low activity for the first three concentrations, but the $80 \mu\text{g/ml}$ concentration exhibited the best activity for CuNPs and tea extract. CuNPs+ADR showed the strongest activity followed by tea extract+ADR, CuNPs, and *C. sinensis* extract (Table 4). The synergistic antiproliferative activities were clearly evident in MOLT-4 cell line. CuNPs and tea extract when combined with ADR showed better results compared to the test drugs alone treatments. All the synergism combination drugs yielded $<10 \mu\text{g/ml}$ values for GI50, TGI, and LC50. These results were visualized under an inverted microscope before and after SRB staining, showing morphological changes such as shrinkage, low cell count, and irregular shape, which is the characteristic of cytotoxicity (Fig. 11).

There are no reports of cytotoxic studies on MOLT-4 cell line using CuNPs and *C. sinensis* extract. However, there was a report in which the antiproliferative effect of green tea polyphenols on promyelocytic leukemia cell line (HL-60) was evaluated through the MTT assay and it reported moderate response on HL-60 cell line with IC50 value $>10 \mu\text{g/ml}$ [18].

Table 3: Percent control growth of MCF-7 cell line after treatment with test drugs

Human breast cancer cell line MCF-7				
Percent control growth				
Drug concentrations ($\mu\text{g/ml}$)				
Mean \pm SD				
Concentrations	10	20	40	80
CuNP-tea	103.3 \pm 12.6	98.5 \pm 19.9	66.8 \pm 13.4	5.6 \pm 9.1
CuNP-tea+10 μg ADR	-39.5 \pm 4.5	-41.7 \pm 4.6	-54.1 \pm 0.6	-53.0 \pm 1.9
<i>C. sinensis</i> extract	92.7 \pm 11.9	76.4 \pm 9.1	73.7 \pm 16.2	87.4 \pm 23.6
<i>C. sinensis</i> extract+10 μg ADR	-23.8 \pm 6	-12.3 \pm 0.9	4.0 \pm 2.5	5.1 \pm 2.1
ADR	-35.4 \pm 6.9	-42.2 \pm 5.4	-61.9 \pm 1.1	-71.1 \pm 5.5

n=3 (Minus values indicate positive response). *C. sinensis*: *Camellia sinensis*, ADR: Adriamycin, CuNP: Copper nanoparticles, SD: Standard deviation

Table 4: Percent control growth of MOLT-4 cell line after treatment with test drugs

Human leukemia cancer cell line MOLT-4				
Percent control growth				
Drug concentrations ($\mu\text{g/ml}$)				
Mean \pm SD				
Concentrations	10	20	40	80
CuNP-tea	83.2 \pm 6.2	78.7 \pm 2.2	74.5 \pm 7.6	50.8 \pm 1.6
CuNP-tea+10 μg ADR	-52.6 \pm 2.1	-51.2 \pm 5.7	-43.2 \pm 8.8	-36.6 \pm 14.3
<i>C. sinensis</i> extract	85.2 \pm 1.3	91.9 \pm 2.7	88.7 \pm 0.9	82.9 \pm 1.6
<i>C. sinensis</i> extract+10 μg ADR	-53.7 \pm 1.1	-48.1 \pm 5.9	-33.1 \pm 7.1	-23.0 \pm 8.3
ADR	-54.8 \pm 5.4	-56.2 \pm 9.2	-48.4 \pm 14.7	-43.8 \pm 17.8

n=3 (Minus values indicate positive response). *C. sinensis*: *Camellia sinensis*, ADR: Adriamycin, CuNP: Copper nanoparticles, SD: Standard deviation

CONCLUSION

We carried out the phytosynthesis of CuNPs using *C. sinensis* leaf extract, its characterization, and anticancer applications. Visual observation of color change from yellow to dark brown confirmed the synthesis of CuNPs. Further, EDX analysis was employed to confirm CuNP formation that recorded a strong absorption peak at 1keV which confirmed the presence of metallic copper. XRD spectra exhibited three 2θ peaks which correspond to the planes of copper. NTA analysis revealed the mean size of CuNPs as 28 nm with the frequency of 2.4×10^8 particles/ml. The spherical morphology and aggregation of nanoparticles were confirmed by SEM and TEM findings, respectively. FTIR analysis revealed the presence of polyphenolic biomolecules as the major factor for the reduction and capping of CuNPs. The anticancer abilities of CuNPs were studied with HT-29 colon cancer, MCF-7 breast cancer, and MOLT-4 cancer cell lines with reference of standard anticancer drug ADR using SRB assay. According to the cytotoxic applications, CuNPs are good antiproliferative agents on HT-29 and MCF-7 cell lines, giving dose-dependent results. As the treatment drug dose increases, the cytotoxic effect of CuNPs increases, with $80 \mu\text{g/ml}$ concentration giving the best response. The synergistic drug combinations gave better results on the entire three cell lines, with $40 \mu\text{g/ml}$ concentration of CuNPs+ADR indicating most favorable antiproliferative impact on MCF-7 cell line followed by $10 \mu\text{g/ml}$ concentration on MOLT-4 cell line. The findings of this study exhibited efficient antiproliferative results of *C. sinensis*-mediated CuNPs on studied human cancer cell lines.

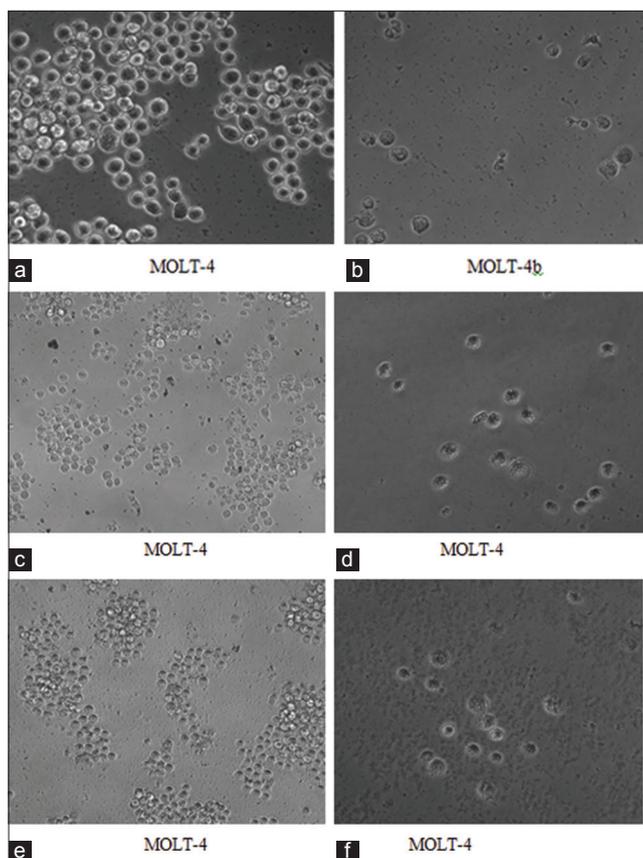


Fig. 11: Effect of test drugs on human leukemia cancer cell line (MOLT-4) as observed under the inverted microscope. (a) Before treatment, (b) adriamycin (ADR), (c) copper nanoparticle (CuNP)-tea, (d) CuNP-tea+ADR, (e) *Camellia sinensis* extract, (f) *C. sinensis* extract+ADR

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