Pharmacogn. Mag.
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ORIGINAL ARTICLE

Bio-Based Synthesised and Characterized Monodispersed *Curcuma longa* Silver Nanoparticles Induces Targeted Anticancer Activity in Breast Cancer Cells

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Submitted: 20-02-2018 Revised: 11-04-2018 Published: 10-09-2018

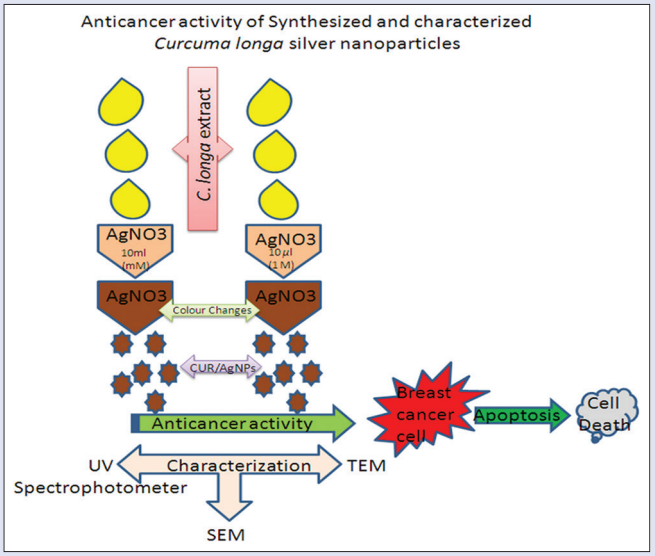
ABSTRACT

Background: Among the various metal nanoparticles, plant extract-based silver nanoparticles are gradually getting preference because the bioreduction potential of plant extracts is much faster for silver ions. The silver nanoparticles have the bactericidal activity and have shown to possess anticancer potential for developing effective and safe novel therapeutic agents. **Objectives:** The objective of the study was to synthesize *Curcuma longa* silver nanoparticles for the induction of anticancer activity in breast cancer cell. **Materials and Methods:** The 20 ml and 10 ml of *C. longa* stock extract were mixed with 10 ml and 10 µl of $AgNO_3$ solution (A) 1 mM and (B) 1M, respectively; reduction process initiates the formation of nanoparticles as detected by color change of $AgNO_3$ from brown to dark brown; thereafter, characterization done by ultraviolet–visible spectrophotometer, Scanning electron microscope, and transmission electron microscopy. **Results:** The synthesized and characterized *C. longa* silver nanoparticles induce targeted anticancer activity in breast cancer cells.

Key words: Anticancer, apoptosis, green synthesis, nanomedicine, nanoparticles

SUMMARY

- Nanotechnology deals with synthesis of nanoparticles of variable sizes, shapes, and chemical composition and can be prepared by physical, chemical, and biological methods. The Green chemistry is the most accepted method for the formation of nanoparticles by the use of plant materials. As far as the safety of biological synthesis procedures is concerned, the plant extract-mediated approach may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. In the present study, *Curcuma longa* rhizomes were selected for the synthesis of silver nanoparticles because *C. longa* known for its pharmacological potential, but it is also known for the less bioavailability. The pharmacologically active phytochemicals of *C. longa* are flavonoids and its diverse class responsible for its traditional remedial properties including aromatic, nonaromatic, volatile, and nonvolatile constituents. Among them, curcuminoids (diphenylheptanoids) include curcumin and its derivatives, demethoxycurcumin and bisdemethoxycurcumin being the major bioactive compounds in *C. longa*. The curcuminoids are yellow natural phenols that have antioxidant, anticancer, anti-inflammatory, and chemotherapeutic activities, but it is also known for the less bioavailability. The silver nanoparticles have the bactericidal activity and have shown to possess better antioxidant and anticancer potential for developing effective and safe novel therapeutic agents. Therefore, *C. longa* silver nanoparticles (CUR/AgNPs) have been synthesized and characterized for the prominent delivery and inducing targeted anticancer activity in MDA-MB-231 breast cancer cells. When the 20 ml and 10 ml of *C. longa* stock extract was mixed with 10 ml and 10 µl of $AgNO_3$ solution (a) 1 mM and (b) 1M, respectively, the biosynthesis reaction started within few minutes and it was detected by color change and was monitored by ultraviolet–visible (UV-Vis) spectrophotometer. UV-Vis absorption spectra showed that bands contain peaks (410 for sample A to 425 nm for sample B) of CUR/AgNPs. In addition, transmission electron microscopy (TEM) analysis detailed that nanoparticles appeared to be spherical and single crystalline. It was also observed by SEM analysis that sample (A) consists particle size distribution which were <35.6 nm and a smaller size (~1.10 nm), whereas in sample (B) particle size distribution with being <58.6 nm and a smaller size (~2.34 nm). Finally, anticancer properties of synthesized CUR/AgNPs (A) and (B) were screened against MDA-MB-231 breast cancer cells. It was found that CUR/AgNPs (A) and (B) induce the cancer cell death by decreasing cell proliferation, inducing genotoxicity and apoptosis. Synthesized CUR/AgNPs (A) and (B) showed dose- and time-dependent anticancer activity. Additional research efforts are needed to enlighten the signaling mechanism for the lead to develop more effective actions for breast cancer.



Abbreviations used: µl: Microlitre; µg: Microgram; mM: Milli Molar; M: Molar; rpm: Revolution per minute; IC_{50}: Inhibitory concentration at which 50% cells get died; PBS: Phosphate-buffered saline solution; DMSO: Dimethylsulfoxide; MTT: 3-4,5-dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide; DMEM: Modified eagle's media; DPPH-2,2-diphenyl-1 picrylhydrazyl; Abs: Absorbance.

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DOI: 10.4103/pm.pm_71_18

Access this article online
Website: www.phcog.com
Quick Response Code:

Cite this article as: Sheikh E, B. Bhatt ML, Tripathi M. Bio-based synthesised and characterized monodispersed *Curcuma longa* silver nanoparticles induces targeted anticancer activity in breast cancer cells. Phcog Mag 2018;14:S340-5.

INTRODUCTION

The biological amalgamation strategies for developing nanomedicine(s) are first-line therapeutic drugs to improve human health, especially if they are proposed for invasive applications. Among the various metal nanoparticles, plant extract-based silver nanoparticles are gradually getting preference since 2009 because the bioreduction potential of plant extracts is much faster for silver ions than the gold ions.[1] The silver nanoparticles have the bactericidal activity and have shown to possess better antioxidant and anticancer potential for developing effective and safe novel therapeutic agents.[2] As far as the safety of biological synthesis procedures is concerned, the plant extract-mediated approach may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.[3] Thus, the biological synthesis of silver nanoparticles using plant extract-based protocol seems to be natural, simplistic, cost-effective, and safe for human therapeutic use and suitable for large-scale production.[4] In the present study, *Curcuma longa* (family Zingiberaceae) rhizomes were selected for the synthesis of nanoparticles because it is used as traditional medicine for various diseases and has also been reported for possessing multiple pharmacological activities including antioxidant, anticarcinogenic, and immunological ones.[5,6] The pharmacologically active phytochemicals of *C. longa* are flavonoids and its diverse class responsible for its traditional remedial properties including aromatic, nonaromatic, volatile, and nonvolatile constituents.[7] Among them, curcuminoids (diphenylheptanoids) include curcumin and its derivatives, demethoxycurcumin, and bisdemethoxycurcumin being the major bioactive compounds in *C. longa*. The curcuminoids are yellow natural phenols that have antioxidant, anticancer, anti-inflammatory, and chemotherapeutic activities,[8] but it is also known for the less bioavailability. Therefore, *C. longa* silver nanoparticles (CUR/AgNPs) have been synthesized and characterized for the prominent delivery and inducing targeted anticancer activity in MDA-MB-231 breast cancer cells.

MATERIALS AND METHODS

Materials

Fresh rhizomes of *C. longa* were collected from local market of Lucknow, and the taxonomic identification was made by National Botanical Research Institute (NBRI), Lucknow, India. The voucher specimen was numbered and kept in our research laboratory for further reference. The Modified Eagle's media (MEM), fetal bovine serum (FBS), antibiotics for animal cell culture used in the study were obtained from HiMedia. Silver nitrate, ethanol, MTT salt, acridine orange (AO), propidium iodide, 4',6-diamidino-2-phenylindole (DAPI) dye, and other chemicals were obtained by Sigma-Aldrich, USA. The breast cancer cells (MDA-MB-231) were obtained from National Centre for Cell Sciences (NCCS) Pune, India.

Biosynthesis of *Curcuma longa*-silver nanoparticles

The fresh rhizomes of *C. longa* were washed with distilled water and shade dried for more than 1 week and then sliced for making a fine powder of dried rhizomes by mortar and pestle. This powder was stored in a closed container for future use. *C. longa* fresh rhizome powder (1 g) was added to 1000 ml distilled water (solvent) and left for 24 h at room temperature. After 24 h, temperature was slightly increased for dissolving the rhizome powder which was followed by vigorous stirring for 2 h. At last, extract was filtered by Whatman Filter Paper No. 1 and filtrate was stored at 4°C for further uses. Two concentrations of $AgNo_3$, 10 ml (1×10^{-3} mM) and 10 µl (1 M) were added to 20 ml and 10 ml, respectively, filtrate of *C. longa* followed by stirring 2 h at room temperature and left for 24 h in dark to avoid any photochemical reactions at room temperature. The reduction proceeded which was detected by the color changing of the formulation (A) and (B). Formulations can be stored at 4°C for >2 weeks.[9]

Characterization of *Curcuma longa*-silver nanoparticles

Ultraviolet–visible spectrophotometer

Characterization of CUR/AgNPs is essential to evaluate the functional aspects of the synthesized particles. After formation, CUR/AgNPs was subjected to ultraviolet–visible (UV-Vis) spectrophotometer (1800 Shimadzu) over the wavelength range of 300–800 nm after diluting the sample with Millipore water. Spectrum bands were influenced by the size, shape, morphology, composition, and dielectric environment of the prepared nanoparticles.[9]

Scanning electron microscope

Scanning electron microscope is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons; the electrons interact with the atoms in the sample, producing various signals that contain information about the sample's surface and composition. Both samples (A) and (B) were characterized by SEM Sample fixation and dehydration on a glass grid and analyzed using SEM.[10]

Transmission electron microscopy

The applications of AgNPs are highly dependent on the chemical composition, shape, size, and monodispersity of the particles. Two samples (A) and (B) were characterized by TEM to check the morphology and size of prepared CUR/AgNPs. Ten microliters sample was adsorbed on the carbon-coated copper grid and analyzed under a Jeol JEM 1400 TEM at 100 KV. Images were acquired using a Gatan Orius CCD camera and Digital Micrograph software.[11]

Cancer cell culture

Human breast cancer cells (MDA-MB-231) were obtained from NCCS, India. These cells were grown as a monolayer in MEM, which was supplemented with 10% FBS and 1% antibiotic and incubated at 37°C in 5% CO_2 with 95% humidity. The cells were maintained in 75 cm^2 tissue culture flask.

Viability of *Curcuma longa*-silver nanoparticles treated MDA-MB-231 cells – MTT assay

The MTT assay is a colorimetric, nonradioactive assay for measuring cell viability through increased metabolization of tetrazolium salt. MDA-MB-231 cells in the concentration of 1×10^6 cells/ml were taken into 96 well plates. Then, the cells were treated with different concentration of CUR/AgNPs (0–50 µg/ml) and incubated in the presence of 5% CO_2 and 95% humidity at 37°C for 24and 48 h. MTT (5 mg/ml) was added to the incubated cells and then further incubated for another 4 h. The crystals were dissolved in 200 µl of dimethyl sulfoxide (DMSO), and the absorbance was measured by colorimetric at 570 nm with reference filter as 655 nm.[12]

Acridine Orange and ethidium bromide dual staining to detect induction of apoptosis in cancer cells

AO and ethidium bromide (EB) dual staining was carried out to detect induction of apoptosis on CUR/AgNPs treated (24 and 48 h) MDA-MB-231 cells. AO/EB dual staining is used to study the apoptotic and necrotic cells to visualize the cellular and nuclear changes with respect

to live cells morphology. For this assay, 0.5×10^6 cells were treated with CUR/AgNPs for 24 and 48 h at 37°C. The cells were initially washed with phosphate-buffered saline (PBS) and then stained with EB (100 µg/ml) and AO (100 µg/ml). The cells were fixed in 3:1 ratio of methanol and glacial acetic acid for 1 h at room temperature and were examined under a UV illumination fluorescence microscope using the 20× objectives (Nikon 80i Eclipse, Japan) and the digitized images were captured.[13]

Evaluation of nuclear changes on *Curcuma longa*-silver nanoparticles-treated MDA-MB-231 by DAPI

Several studies related to chemoprevention uses DAPI staining to observe the apoptotic changes at DNA level as a reliable apoptotic assay. Cells were cultured in a 24-well tissue culture grade plate for 24 and 48 h. After incubation with IC_{50} concentrations (µg/ml) of CUR/AgNPs, MDA-MB-231 cells were washed in PBS, fixed with 2% paraformaldehyde for 15 min, and were treated with 0.2% Triton X-100 in PBS for 15 min at room temperature. Cells after washing with PBS were stained with DAPI (2 µg/ml) and incubated in dark for 30 min. The cells were then examined and photographed using a fluorescence microscope (Nikon ECLIPSE Ti-S, Japan).[12]

Cytotoxicity of *Curcuma longa*-silver nanoparticles-treated MDA-MB-231 cells detected by propidium iodide staining

The CUR/AgNPs treated (24 and 48 h) and un-treated MDA-MB-231 cells (20×10^3 cells per well on 24 well plate) were washed in ice-cold PBS and fixed in 75% methanol and 25% glacial acetic acid for 30 min in ice. Fixed cells were washed twice by PBS and stained with propidium iodide (PI; 30 mg/ml; Sigma) containing DNase-free RNase A (1 mg/ml) for 30 min at 37°C. Stained cells were washed in PBS and observed under a fluorescence microscope (Nikon).[12]

RESULTS

Detection and characterization of *Curcuma longa*-silver nanoparticles

Fresh rhizomes of *C. longa* were used for the study. They were grown in controlled conditions in our laboratory taken from local market and identified by NBRI [Figure 1]. When the 20 ml and 10 ml of *C. longa* stock extract was mixed with 10 ml and 10 µl of $AgNO_3$ solution (A) 1 mM and (B) 1M, respectively, the biosynthesis reaction started within few minutes and it was detected by color change, clear $AgNO_3$ solution was changed into brown and dark brown color due to the excitation of surface plasmon resonance (SPR) which indicates the formation of CUR/AgNPs [Figure 2] The reduction process initiates the formation of nanoparticles that was monitored by UV-Vis spectrophotometer by diluting the sample with Millipore water and it was also used as blank. UV-Vis absorption spectra showed that bands contain peaks (410 for sample A to 425 nm for sample B) of CUR/AgNPs. We have obtained the well-synthesized AgNPs using two concentrations of silver nitrate (1 mM and 1M) with the help of exploring the effects of *C. longa* extract. The reactions were observed under the UV-vis spectroscopy to examine the bioreduction of AgNPs from aqueous $AgNo_3$ solution. The shift changes of absorption peak appeared indicating difference in the size of the formed silver nanoparticles particles. There was a wavelength with absorption peak of 410–425 nm when different concentrations of *C. longa* extract reacted with different concentrations of 1 mM and 1M of $AgNo_3$. The SPR wavelength shifts toward long wavelength region which indicated larger particle size [Figure 3]. The UV-vis absorption

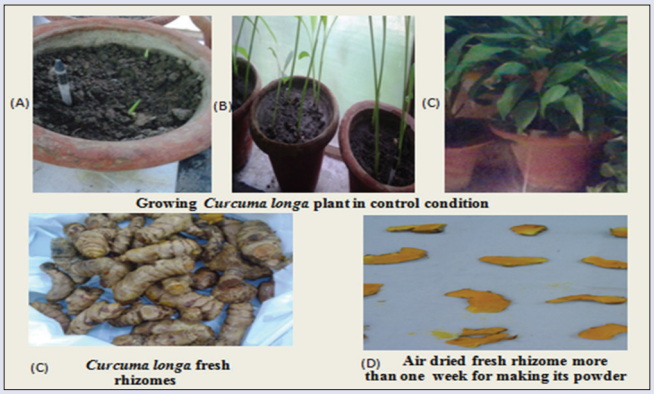

Figure 1: *Curcuma longa* plant in control condition in our laboratory. The *Curcuma longa* were grown in controlled conditions in our laboratory taken from local market and identified by National Botanical Research Institute

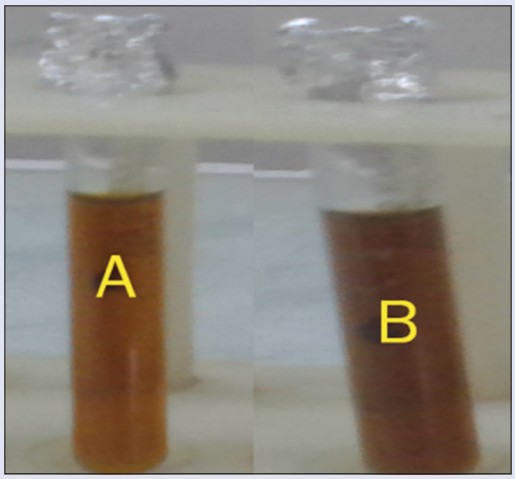

Figure 2: Biosynthesis reaction detected by colour change. After treating the *Curcuma longa* filtrate with Milimolar Concentration of $AgNo_3$ for 24 h, color changes to brown named as *C. longa* silver nanoparticles (A), while treating the *Curcuma longa* filtrate with Molar Concentration of $AgNO_3$ for 24 h, color changes to dark brown named as *Curcuma longa* silver nanoparticles (B)

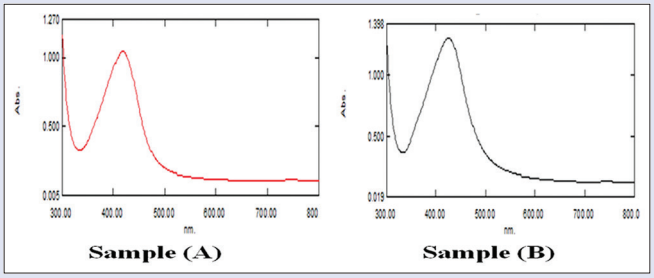

Figure 3: Characterization of nanoparticles by ultraviolet–visible spectrophotometer. The reduction process initiates the formation of nanoparticles that was monitored by ultraviolet–visible spectrophotometer. The ultraviolet–visible spectrum bands were influenced by the size, shape, morphology, composition, and dielectric environment of the prepared nanoparticles. Ultraviolet–visible absorption spectra showed that bands contain peaks at (410-425 nm) of Sample (A) and (B) of *Curcuma longa* silver nanoparticles

spectrum of the obtained CUR/AgNPs was compared under room temperature and by slightly increasing the temperature at different time intervals. The process under slightly increasing the temperature is more advantageous as it results in the formation of nanoparticles at a higher yield and slightly smaller size when compared with room temperature with a narrow size range < 58.6 nm are detectable [Figure 4]. In addition, TEM analysis detailed that nanoparticles appeared to be spherical and monodispersed. It was also observed by SEM analysis that sample (A) consists of particle size distribution which were <35.6 nm and a smaller size (~1.10 nm), whereas in sample (B), particle size distribution with being <58.6 nm and a smaller size (~2.34 nm) [Figure 5].

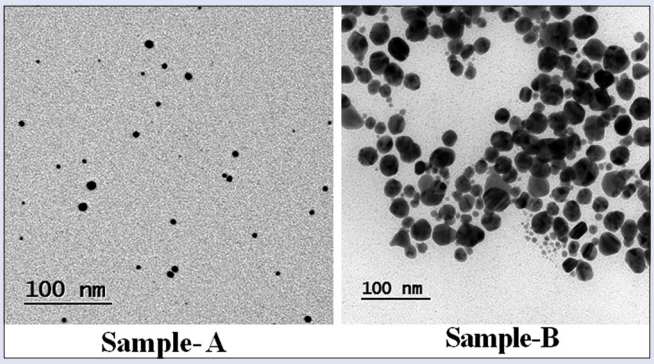

Figure 4: Characterization of nanoparticles by transmission electron microscopy analysis. *Curcuma longa* silver nanoparticles (A) and (B) were characterized by transmission electron microscopy detailed that nanoparticles were of size <100 nm and are spherical as shown in figure

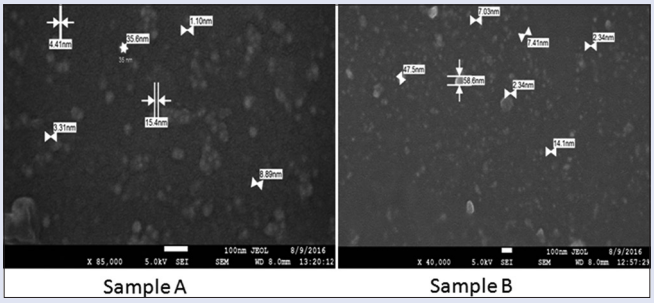

Figure 5: Characterization of nanoparticles by scanning electron microscopy analysis. The Sample (A) and (B) were characterized by scanning electron microscopy to check the size of prepared *Curcuma longa* silver nanoparticles. In sample A the particle size distribution ranges from 1.10 nm to 35.6 nm, whereas in Sample B particle size distribution ranges from 2.34 nm to 58.6 nm

Anticancer potential of *Curcuma longa*-silver nanoparticles

In the present study, CUR/AgNPs (A) and (B) synthesized and characterized and its anticancer activity on MDA-MB-231 cells was evaluated. It was observed that, after the treatment CUR/AgNPs (A) and (B), the viability of breast cancer cells was inhibited assessed by MTT assay. For the evaluation of inhibition of cell viability of cells, different doses of CUR/AgNPs (A) and (B) (0–50 µg/ml) were administered for 24 and 48 h to MDA-MB-231 cells. It was found from MTT assay that, viability of cells decreased as the dose and time increases [Figure 6] and at approximately 10 and 14 µg/ml treatment dose of CUR/AgNPs (A) and (B) respectively for 48 h of treatment, 50% cells viability was lost, i.e., IC_{50}, as compared to untreated cells (control). The CUR/AgNPs (A) and (B) inducing early apoptosis detected by AO staining at 24 h of treatment, whereas EB, known to identify only dead cells, detected the MDA-MB-231 dead cells at 48 h of treatment. The dual staining of AO/EB detects the induction of apoptosis in MDA-MB-231 cells by CUR/AgNPs and changes associated with apoptotic cell death. The cells treated with concentrations of CUR/AgNPs (A) and (B) for 24 h [Figure 7] showed marked changes in morphology such as irregular shape, membrane blebbing, and the early apoptotic cells displayed greenish yellow nucleus with condensed chromatin at both the concentration of CUR/AgNPs (A) and (B) as compared to untreated (control), whereas at 48 h, the late apoptotic cells showed orange red nucleus with chromatin condensation and nuclear fragmentation. Apoptosis or programmed cell death is characterized by apoptotic morphology which includes membrane blabbing, chromatin condensation, degradation of DNA, and apoptotic body formation. The pictorial data of PI staining showed a marked difference between the control and treated CUR/AgNPs (a) and (b) MDA-MB-231 cells as morphological changes similar to apoptotic/dead cells were observed in the treated ones, whereas the untreated cells appeared round without any changes. The PI positive (apoptotic/dead cells) detected as bright yellowish red fluorescent cells at 48 h of CUR/AgNPs (a) and (b) cytotoxic treatment indicated by arrows in Figure 8. The genotoxic effect was detected with DAPI staining in MDA-MB-231 cells at 48 h treatment of CUR/AgNPs (a) and (b). The apoptotic cells were determined by condensed chromatin and fragmented nuclei which were shown by arrows in blue fluorescent-treated cells as shown in Figure 9 as compared to control. It was also demonstrated from DAPI staining that CUR/AgNPs-induced apoptosis, which was consistent with the results of

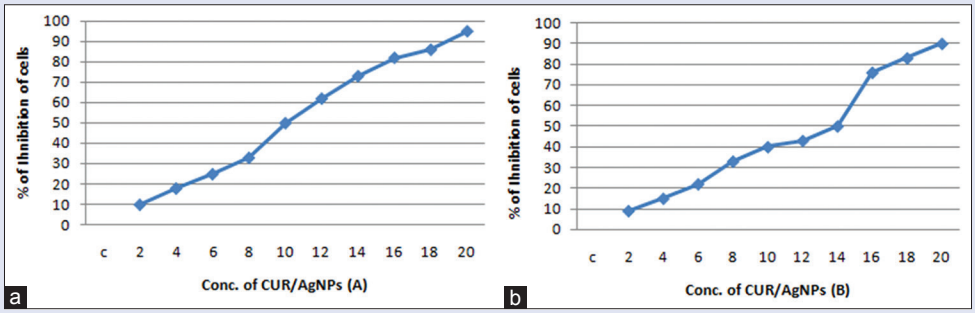

Figure 6: Graph of % inhibition of cells. (a) The MTT assay shows the 50% inhibition of MDA-MB-231 cells after treatment of *Curcuma longa* silver nanoparticles (a) at dose of 10 µg/ml (IC_{50}) at 48 h. At 24 h no such significant cytotoxic effect of *Curcuma longa* silver nanoparticles (a) were seen. (b) The MTT assay shows the 50% inhibition of MDA-MB-231 cells after treatment of *Curcuma longa* silver nanoparticles (b) at dose of 14 µg/ml (IC_{50}) at 48 h. At 24 h no such significant cytotoxic effect of *Curcuma longa* silver nanoparticles (b) were seen

viability and cytotoxicity assay. The difference in size of the nanoparticles of sample A and B may be responsible for different doses concentration of IC_{50}. As long as the anticancer activity of CUR/AgNPs (a) and (b) are taken into account, there is no such marked difference, but the IC_{50} doses of both samples are different.

DISCUSSION

The plant crude extracts contain novel secondary metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids, which are mainly responsible for the reduction of ionic metal into bulk metallic nanoparticles.[14] Primary and secondary metabolites are constantly involved in redox reactions required to synthesize eco-friendly nanoparticles. Biosynthesis reactions can be modulated to transform the shape and size of nanoparticles using different metal concentrations and amounts of plant extract in the reaction medium.[15,16] Gericke and Pinches[17] reported that the size of particles that form intracellular could be controlled by altering key factors such as pH, temperature, substrate concentration, and time of exposure to the substrate. However, the biochemical and molecular mechanisms of AgNPs biosynthesis remain feebly characterized and should be investigated to further optimize the process. For instance, characterization of biochemical mechanisms underscored the importance of phytochemicals, which may mediate biosynthesis. Improvements in chemical composition, size, shape, and dispersity of nanoparticles would permit the use of nanobiotechnology in a variety of other applications. AgNPs, especially their sizes, are of critical importance while depicting various activities.[18] It has been observed that the AgNPs with smaller particle size have a stronger penetration ability and greater toxicity.[19] A size dependent-controlled targeting of AgNPs can improve effectiveness in cancer treatment, as the cancer cells are different in pore size compared to other cells.[20] It has been observed that synthesized CUR/AgNPs was spherical with such a size which is wildly recognized among a good range for anticancer drug delivery applications.[21] Therefore, it is essential to execute biologically safe as well as effective natural compounds as anticancer and chemopreventive drugs that have already been extensively used in humans. We have synthesized the *C. longa* silver nanoparticles with size ranges from (~1.10–35.6 nm) of sample (A) and (~2.34–58.6 nm) of sample (B). Various phytochemicals such as flavonoids, phytoalexins, and phenolic compounds have been used as adjuvants in the chemotherapeutic treatment of cancer. They can also act as biological response modifiers supporting immune system function and protect living cells against damage from free radicals.[22] One such promising natural polyphenol compound found in *C. longa* is curcumin, known to inhibit the proliferation of various tumor cells in culture including breast cancer cells[23] and prevents carcinogen-induced cancers in rodents. Chiu and Su[24] showed that curcumin inhibited the proliferation of MDA-MB-231 cells either through upregulating p21 expression or upregulating the Bax to Bcl-2 ratio. Banerjee *et al.*[25] reported that curcumin-induced G2/M arrest and apoptosis, inhibited cell proliferation by inhibiting the assembly dynamics of microtubules, and further activated the mitotic checkpoint in MCF-7 cells. Nevertheless, the preclinical and clinical studies related with oral administration of curcumin have revealed its very poor bioavailability.[26] It has been found that CUR/AgNPs induces apoptosis which leads to breast cancer cell death. Consequently, *C. longa* silver nanoparticles synthesized and characterized accordingly in such a way so that it can overcome this problem and exemplify the effectiveness of curcumin and other phytochemicals. Further research is required to focus on the characterization of the mechanism behind the anticancer of CUR/AgNPs (A) and (B) may be the act same but follows different mechanisms of action.

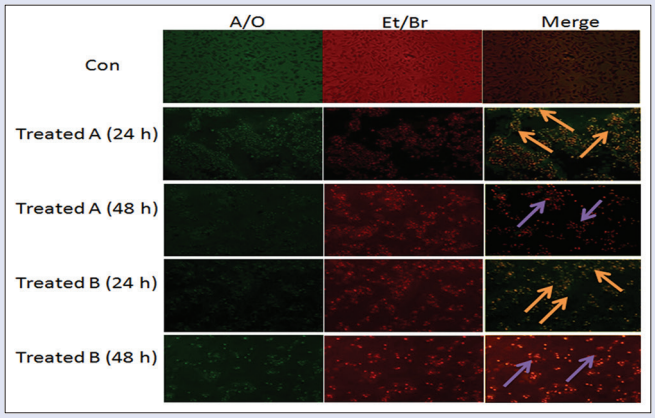

Figure 7: Dual staining by Acridine orange and Ethidium bromide shows the apoptotic activity of *Curcuma longa* silver nanoparticles (A) and (B). The *Curcuma longa* silver nanoparticles (A) and (B) induces the early apoptosis detected by Acridine orange after 24 h of CUR/AgNPs treatment indicated by yellow arrows in figure, whereas dead cells detected by Ethidium bromide staining after 48 h of treatment. The dual staining shows that yellowish cells are early apoptotic cells found at 24 h of treatment, while red fluorescence shown by blue arrows are dead cells found at 48 h of treatment in merge section

CONCLUSION

In outline, CUR/AgNPs have been successfully synthesized biologically using the *C. longa* extract as reducing agent and were characterized by various parameters. Finally, anticancer properties of synthesized CUR/AgNPs (A) and (B) were screened against MDA-MB-231 breast cancer cells. It was found that CUR/AgNPs (A) and (B) induces the cancer cell death by decreasing cell proliferation, inducing genotoxicity and apoptosis. Synthesized CUR/AgNPs (A) and (B) showed dose- and time-dependent anticancer activity. Additional research efforts are needed to enlighten the signaling mechanism for the lead to develop more effective actions for breast cancer.

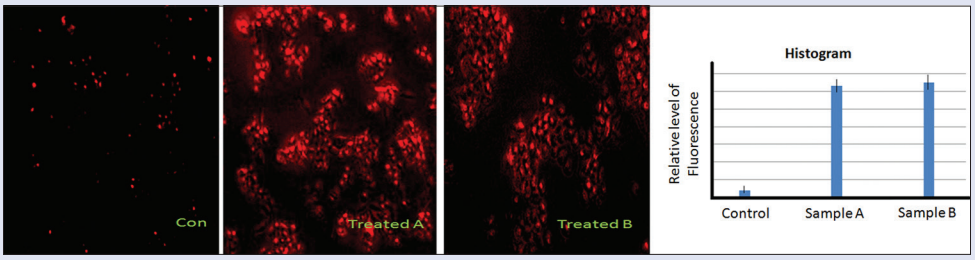

Figure 8: Cytotoxic effect of *Curcuma longa* silver nanoparticles (A) and (B) detected by propidium iodide staining. Propidium iodide staining reveals that the number of propidium iodide-positive cells indicating dead cells as after the treatment of *Curcuma longa* silver nanoparticles (A) and (B) as compared to control also shown in histogram

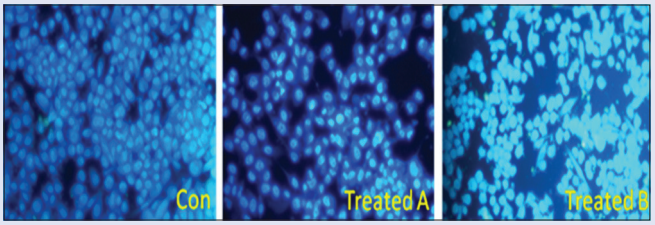

Figure 9: Genotoxic effect was detected with DAPI staining in MDA-MB-231 cells. The DAPI staining shows condensed chromatin and fragmented nuclei in the apoptotic blue fluorescent cells in of *Curcuma longa* silver nanoparticles (a and b) treated ones shown in figure whereas low fluorescence in control section. However the no. of cells also decreased after the *Curcuma longa* silver nanoparticles (A) and (B) treatment after 48 h

Acknowledgements

Authors are very thankful to Dr. Omkar (HOD), Department of Zoology, University of Lucknow for departmental research support and Dr. M. Arshad, Assistant Professor, for his valuable suggestions. The infrastructural support accorded by the Department of Zoology, University of Lucknow, Lucknow, is acknowledged.

Financial support and sponsorship

The author, Eram Sheikh thanks University Grants Commission, Government of India, for providing the Maulana Azad National Fellowship (senior research fellowship) for research.

Conflicts of interest

There are no conflicts of interest.

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