VOLUME 8 ISSUE 2 2022

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, No. 2, 765-773 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Synthesis of Gold Nanoparticles Using *Centella Asiatica* Extract and its *In Vitro* Cytotoxic Activity Against Human Breast Adenocarcinoma MCF-7 and Normal Vero Cell Line

Sampath Uvarajan[1]* and Durai Prabhu[2]

[1]Department of Biochemistry, Government Arts and Science College, Thennangur, Vandavasi 604408, Tamil Nadu, India
[2]Department of Zoology, Government Arts and Science College, Thennangur, Vandavasi 604408, Tamil Nadu, India

**Corresponding Author*

Received: 10th October, 2022; **Accepted:** 16th November, 2022; **Published online:** 4th December, 2022

https://doi.org/10.33745/ijzi.2022.v08i02.092

Abstract: In the past few decades, the gold nanoparticles (AuNPs) have attained much attention among all metal nanoparticles and it is greatly contributed for cancer photo-diagnostics, photo-thermal therapy, bio-labelling, drug delivery etc. In the present investigation, AuNPs were green synthesized using *Centella asiatica* leaf aqueous extract. The physicochemical properties of synthesized AuNPs were characterized using UV–visible and Transmission Electron Microscopy (TEM) analysis. Further, *in vitro* anticancer efficacy of biosynthesized AuNPs were assessed against human breast adenocarcinoma (MCF-7) and normal African green monkey kidney Vero cell line. Bio approach synthesized gold nanoparticles were highly stable, monodisperse particles in the size ranging <100 nm. Cytotoxic activity has been evaluated using MTT, morphological observation, Annexin-V/PI Double-Staining Assay, Comet assay for apoptosis. AuNPs were inhibited MCF7 cancer cells and were not toxic to the normal Vero cells at 48 h exposure, therefore, the present finding confirms that the bio-approach synthesized metal AuNPs possess *in vitro* cytotoxic effects and it may be used as an anticancer substance.

Keywords: *Centella asiatica,* Gold nanoparticles, MCF-7, Vero, Cytotoxicity, Apoptosis

Citation: Sampath Uvarajan and Durai Prabhu: Synthesis of gold nanoparticles using *Centella Asiatica* extract and its *in vitro* cytotoxic activity against human breast adenocarcinoma mcf-7 and normal Vero cell line. Intern. J. Zool. Invest. 8(2): 765-773, 2022.

https://doi.org/10.33745/ijzi.2022.v08i02.092

Introduction

Nanotechnology is an interdisciplinary approach to innovation of the most active areas of research in the field of modern materials science (Prashanth *et al.*, 2011). Nanoparticles have gained significant attention due to their application in cancer therapy and imaging, angiogenesis, genetic disease and genetic disorder diagnosis, photothermal therapy and photoimaging (El-Sayed *et al.*, 2005; Sadeghi *et al.,* 2015). Cancer is a complicated disease which

occurs in cells of specific tissue with no longer receptive to signals that regulate cellular differentiation, survival proliferation, and death (Martin *et al.,* 2013). Cancer is the second leading cause of death worldwide next to diabetes mellitus and responsible for 9.6 million death in 2018 (Ackermann and Mrowka, 2019). Globally, statistics for the occurrence of common cancers are lung cancer: 2.09 million, breast cancer: 2.09 million, colorectal: 1.80 million, prostate cancer: 1.28 million, skin cancer (non-melanoma): 1.04 million, and stomach cancer: 1.03 million. In particular, breast cancer is most common cancer diagnosed in women world wide (El Hilali *et al.,* 2019). At the present time, systematic chemotherapy combined with medical procedure is acted in emergency clinics. Though, poor biodistribution and non-specific delivery of chemotherapeutic drugs are considered to be a fundamental problem (Ud Din *et al.*, 2017). Therefore, researchers are keen to develop such a delivery system that can efficiently penetrate both cellular and outer mitochondrial membranes in triggering apoptosis (Jeyarani *et al*., 2020).

Gold nanoparticles (AuNPs) can be synthesized in the lab chemically, physically and biologically methods. Among these methods the bio-approach of nanoparticles synthesis has various advantages compared to other methods such as biocompatibility and extensive application in biology and medicine, simplicity of the method, use of natural resources (microorganisms, algae, fungi, plants), non-toxic method making the synthesized NPs suitable for pharmaceutical and biomedical applications, external capping or stabilizing agents are not needed for synthesis of NPs, cost effective method because of minimum or non-requirement of energy, simplicity in scaling up for large-scale synthesis (Sperling *et al.*, 2008; Ghosh *et al.*, 2008; Boisselier and Astruc, 2009; Swaha *et al.*, 2019). Plant-derived natural compounds can be used as efficient reducing and stabilizations agents which can confer stable size and agglomeration of synthesized AuNPs (Khan *et al.*, 2019). The stabilizing ligands of nanoparticles are usually formed from alkanoids, flavonoids, alkaloids, proteins or starch (Jayaprakash *et al.*, 2017). The medicinal values of *C. asiatica* are due to the presence of various phyto-constituents such as terpenes, phenols, vitamins, minerals, polyacetylene, and fatty acids (Archana *et al.*, 2019). The herb is also known for possessing strong anti-inflammatory, anti-asthmatic, anti-oxidative, anti-cancer, hepatoprotective, and neuroprotective activities (Barnes *et al.*, 2007; Huang *et al.*, 2011; Bian *et al*., 2012; Orhan, 2012). In this present investigation, we focused on the biosynthesis of gold nanoparticles using medicinal herb *C. asiatica* and to investigate the biocompatibility and *in vitro* cytotoxicity effect against normal green monkey kidney Vero cell line and breast cancer (MCF7) cell lines.

Materials and Methods

Chemical:

Human breast adenocarcinoma cancer cell line (MCF7) and normal green monkey kidney cell line (Vero) were obtained from National Centre for Cell Science (NCCS), Pune, India. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), antibiotics and chloroauric acid ($HAuCl_4$) were purchased from HiMedia (Mumbai, India). Annexin V-FITC/ PI apoptosis detection kit was purchased from Sigma Aldrich (Saint Louis, Missouri, USA).

Biosynthesis of gold nanoparticles:

Biosynthesis of gold nanoparticles were performed by following method of Mubarakali *et al.* (2011). *C. asiatica* aqueous leaf extract (1 ml) was taken and added 9.0 ml of 1.0 mM of $HAuCl_4$ separately in conical flask. The reaction was performed in dark at room temperature for 12 h in a static condition and color changes were recorded.

Characterization of gold nanoparticles:

After 12 h the ruby red color formation was confirmed as gold nanoparticles. In addition to the biosynthesis nanoparticles, *C. asiatica* aqueous leaf

extract, chloroauric acid solutions were recorded for their absorbance from 300 to 800 nm using UV–visible spectrum (Shimadzu-1800 spectrophotometer). Size and shape of AuNPs were characterized by higher resolution transmission electron microscope (HRTEM) (FEI, TECNAI T30). Briefly, the sample 2 µl was placed on the carbon-coated copper grid then immediately transfer to the microscope.

Cell viability assay:

The effect of AuNPs synthesized from *C. asiatica* aqueous leaf extract on cancer cell line MCF7 and Vero cell line was determined by the MTT assay (Mossmann, 1983). Briefly, DMEM added with L-glutamine (2 mM), penicillin (100U/ml), streptomycin (100 µg/ml), FBS (10%) was used for cell culture. Cells were cultured in 25 cm^2 flasks at 37ºC in a 5% CO_2 atmosphere. For further dosage studies, cells were seeded into 96 well plates (1×10^6 cells in each well) and incubated at 37ºC for 24 and 48 h. MCF 7 breast cancer cells and normal Vero cells were treated with AuNPs and $HAuCl_4$ alone at different concentration (5, 10, 20, 40, 80 and 160 µg/ml). Plates were incubated for 24 and 48 h with treatment followed by cell viability test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT solution (10 µl) was added to each well at concentration of 5 mg/ml, plates were incubated at 37ºC for 4 h. Formazan crystals of purple color appeared were dissolved in 100 µl of dimethyl sulfoxide (DMSO). Using a multi-well ELISA plate reader, the optical density (OD) was measured at 570 nm. Percentage of cell viability was evaluated by using the following formula:

Percentage cell viability (%) = OD/OD of control × 100

Cytomorphological observation:

The MCF7 cells were seeded at $1x10^6$ cells/well into a six well chamber plate and incubated overnight. Later, the medium was replaced with maintenance DMEM with 10% FBS containing inhibitory concentration (IC_{50}) of AuNPs synthesized from *C. asiatica* aqueous leaf extract and incubated for 48 h. The cells morphology was examined under inverted light microscope.

Apoptosis assay:

MCF7 cells were plated at 1×10^6cells/well into a six well chamber plate. At >90% confluence, the cells were treated with an inhibitory concentration of AuNPs synthesized from *C. asiatica* aqueous leaf extract and control alone for 48 h. Then cells were washed with PBS, fixed in methanol: acetic acid (3:1, v/v) for 10 min and stained with 5 µl of annexin V-FITC conjugated and 10 µl of propidium iodide (40 µg/ml) for 20 min. Nuclear morphology of cells were examined under Carl Zeiss Axio vision fluorescent microscope (Software: Axiovision 4.8).

Comet assay:

Comet assay was performed to analyze the level of DNA damage caused due to the effect of AuNPs following method of Dhawan *et al.* (2003) with slight modifications. Briefly, cells were subjected to centrifugation, supernatants were discarded and washed with PBS to obtain the yield of $1x10^6$ cells/ml. Cells treated with AuNPs synthesized from *C. asiatica* aqueous leaf extract were mixed with LMPA (Low melting point agarose) and were spread on fully frozen microscopic slides containing NMA (Normal melting agarose). The slides were then immersed in the lysis solution for nearly 2 to 6 h at 4°C, arranged side by side on a gel electrophoresis apparatus and were allowed to sit in an alkaline buffer for around 20 min for the unwinding of DNA and the expression of alkaline labile DNA damage. Further, the electric field was applied by adjusting the current of 300Ma and electrophoresis was performed for 30 min. Slides were washed using distilled water followed by chilled 70% ethanol and were air dried. Finally, the slides were stained with 75 µl of propidium iodide (40 µg/ml) and kept for 5 min. Further, the slides were observed under a fluorescent microscope.

Results and Discussion

In this study, after the addition of the solution containing Au(III) ions to the fresh *C. asiatica* aqueous leaf extracts, a color change from yellow

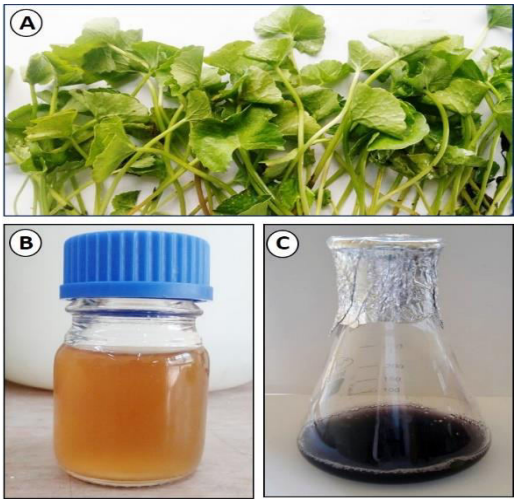

Fig.1: (A) Photographs of *Centella asiatica* leaves, (B) aqueous leaf extract of *C. asiatica* and (C) synthesized gold nanoparticles using *C. asiatica* leaf extract which indicates ruby red color solution.

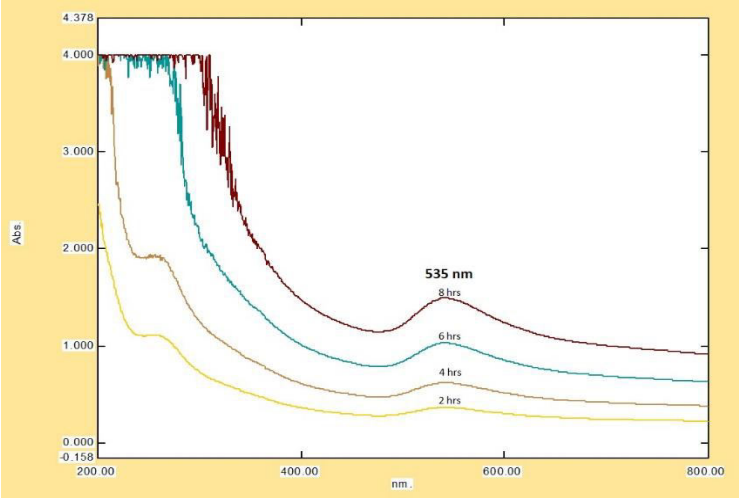

Fig. 2: UV–Vis spectrum analysis of gold nanoparticles were observed at different time intervals (2, 4, 6 and 8 h) and peaks obtained at 535 nm.

to ruby red was observed (Fig. 1). The colloidal dispersion of AuNPs lead to a change in the color of nanofluids from yellow to ruby red or bluish due to absorption or scattering of the light that passes through synthesized solution as previously described by Cademartiri and Ozin (2009). Figure 2 shows the UV-Visible absorption spectra of AuNPs solutions observed at different time intervals (2, 4, 6 and 8 h) and revealed a well-defined surface plasmon band centred at around 535 nm. Based on the above-stated phenomenon of absorption and scattering of the light by AuNPs, the color change of the reaction mixtures was consistent with occurrence of the maximum (λ_{max}) of the localized surface plasmon resonance (LSPR) absorption band, which for gold nanofluids typically occurs in the range of 520–570 nm (Pal and Kryschi, 2015). In order to determine the size and shape of the produced AuNPs, TEM analyses were performed. The synthesized gold nanoparticles were spherical in shapes and ranged from <100 nm as shown in Figure 3A. Selected area of electron diffraction pattern image showing of AuNPs with ring arisen due to the reflections from 111, 200, 220 and 300 and confirmed the polycrystalline nature of synthesized AuNPs (Fig. 3B). The results are in accordance with the earlier reports of synthesized gold nanoparticles using

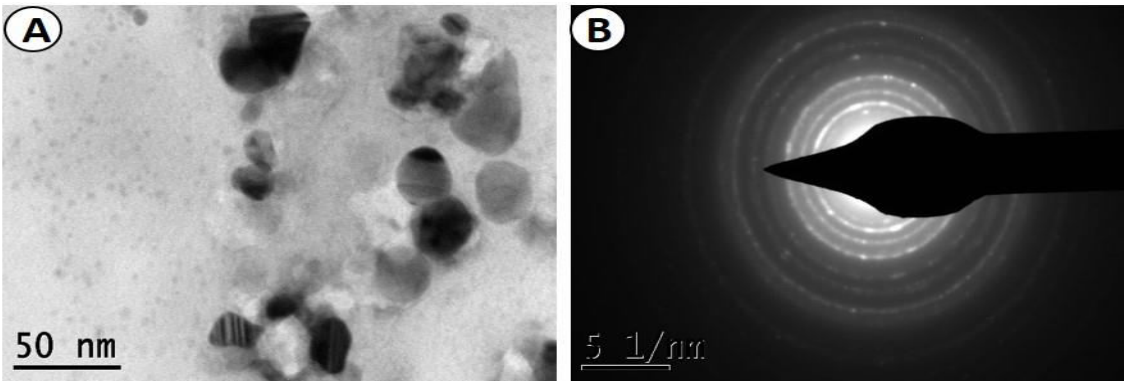

Fig. 3: (A) TEM micrographs of gold nanoparticles. Bar represents 50 nm. (B) Polycrystalline nature of synthesized AuNPs confirmed by Selected Area Electron Diffraction (SAED) pattern.

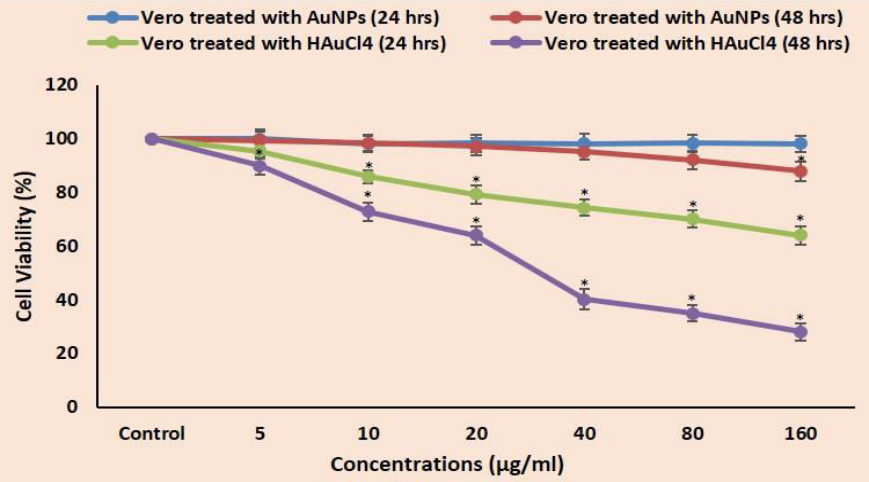

Fig. 4A: Cytotoxicity effect of $HAuCl_4$ and AuNPs on normal kidney (Vero) cells at 24 and 48 h. Values are given as mean ± S.D. for each concentration. (*) represents the treated concentrations statistically significant ($p < 0.05$) when compared to control.

the aqueous leaf extract of *Acalypha indica* (Krishnaraj *et al.,* 2014) and Biogenic gold nanoparticles using *Commiphora wightii* and their cytotoxic effects on MCF7 breast cancer cell line (Uzma *et al.,* 2020).

The present study evaluated the cytotoxicity and biocompatibility of synthesized gold nanoparticles against metabolically active proliferating cells. To ensure this, different concentrations (5, 10, 20, 40, 80, and 160 µg/ml) of gold nanoparticles and $HAuCl_4$ alone were tested against breast cancer cell line (MCF7) and normal green monkey kidney cell line (Vero). MTT is a tetrazolium salt which readily reacts with mitochondrial dehydrogenase of metabolically active cells to yield coloured formazan product. This can be measured spectrophotometrically for the determination of viable or active cells present (Mossmann, 1983). The inhibitory concentration (IC_{50}) of AuNPs to inhibit MCF7 breast cancer cells was found to be 20 µg/ml at 48 h. In contrast, inhibitory concentration of $HAuCl_4$ to inhibit cancer cells was found to be 160 µg/ml at 24 h. In normal Vero cells treated with AuNPs, 87.77 % of cell viability was found to be 160 µg/ml at 48 h. while the same concentration of $HAuCl_4$ were used to treat normal Vero cells showed only 28% of cell viability (Figs. 4A, B). Based on the above-stated phenomenon, the activity of AuNPs on Vero cell line was constantly less at experimented dilutions

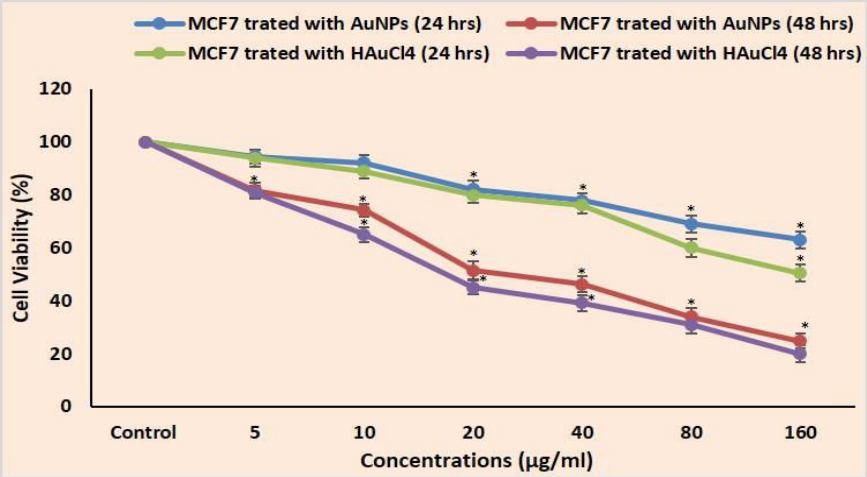

Fig. 4B: Cytotoxicity effect of $HAuCl_4$ and AuNPs on MCF7 breast cancer cells at 24 and 48 h. Values are given as mean ± S.D. for each concentration. (*) represents the treated concentrations statistically significant ($p < 0.05$) when compared to control.

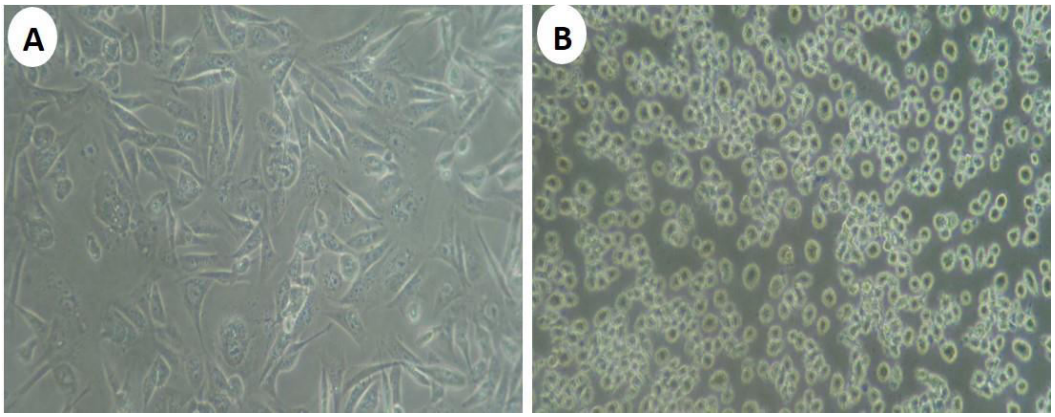

Fig. 5: Cytomorphological effects of MCF7 cancer cells. (A) Control (untreated), (B) MCF7 treated with 20 µg/ml of AuNPs at 48 h.

as compared with cancer cell line. Also, in the different concentrations of AuNPs, MCF7 cells showed highly significant ($p<0.05$) reduced cell viability when compared to Vero cells. Overall, AuNPs from *C. asiatica* aqueous leaf extracts were toxic to breast cancer cells and non-toxic to normal Vero cells, respectively. Jeyarani *et al.* (2020) reported the cytotoxic effects of novel biomimetic gold nanoparticles from *Geranium pusillum* against normal (HEK-293) and cancer (MDA-MB-231) cell lines. Swaha *et al.* (2019) reported the cytotoxic effects of novel eco-friendly approach for biosynthesis of gold nanoparticles (AuNPs) using *Hygrophila spinosa* extract against MCF-7 and MDA-B-231 (breast), SKOV-3 (ovarian) and U-87 (brain) cancer cell lines.

C. asiatica aqueous extract mediated AuNPs and $HAuCl_4$ alone used to treated and untreated MCF7 cells at 48 h and observed under inverted microscope. Untreated (control) MCF7 showed irregular confluent aggregates with round polygonal cells appeared normal (Fig. 5A). AuNPs treated MCF7 cells showed persuade cell reduction which lead to the changes in membrane integrity and cell shrinkage due to appearances of apoptotic cell demise (Fig. 5B). HepG2 cell line treated with the phytosynthesized AuNPs at 30 µg/ml showed cell death up to 51%. In addition the cells showed apoptotic lead to cell death as evidenced by showing changes in loss of membrane integrity, inhibition of cell growth and cytoplasmic condensation (Ashokkumar *et al.*,

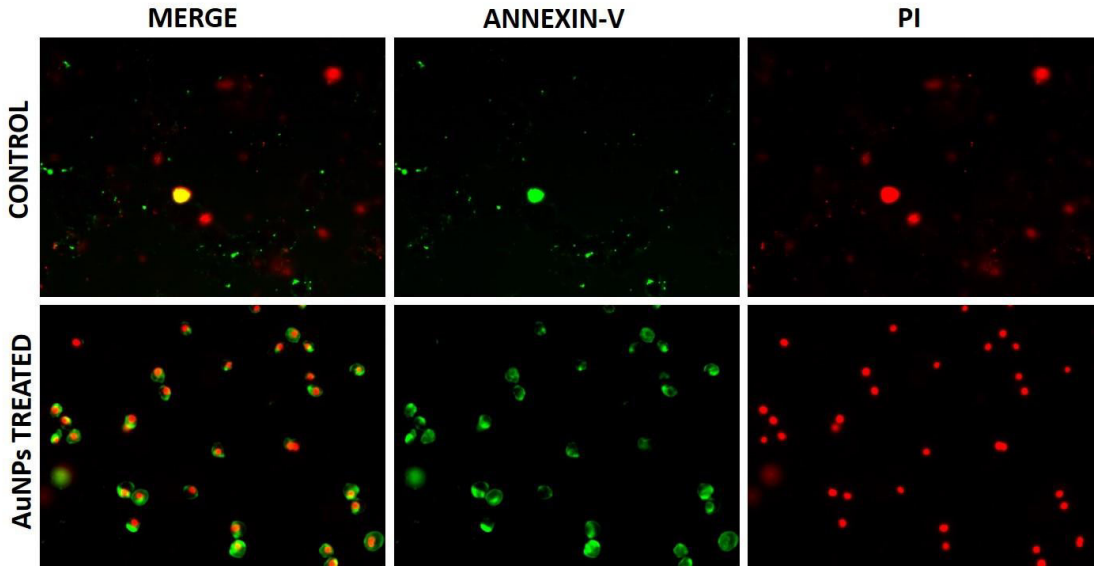

Fig. 6: AuNPs induced apoptosis of MCF7 cells as established through phosphatidylserine (PS) translocation using a fluorescent microscope. Control cells displayed no Annexin V-FITC/PI staining. Cells treated with AuNPs (IC_{50} 20 µg/ml at 48 h) showed double staining of Annexin V-FITC/PI, indicating apoptosis.

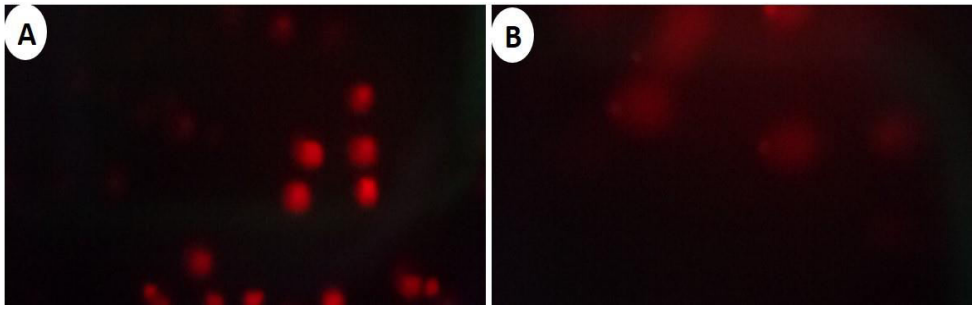

Fig. 7: Apoptotic inducing effect of AuNPs on MCF7 cells assessed by comet assay. Control (A), MCF7 cells treated with AuNPs showing olive tail movements which indicates DNA damage (B).

2014). Moreover, the phytosynthesized gold nanoparticles (AuNPs) showed cytotoxicity effect on three different cancer cell lines such as HepG2, HeLa and MCF7 and cytomorphological changes were observed (Balashanmugam *et al.*, 2016).

A fluorescence-based assay was employed to test the effect of synthesized gold nanoparticles on breast cancer cells (MCF7) using annexin V-FITC/PI dual staining (Fig. 6). MCF7 untreated cells stained negative for annexin V-FITC and PI. MCF7 cells treated with IC_{50} of *C. asiatica* aqueous extract mediated AuNPs showed intense positive staining with annexin V and PI induces apoptosis. Recently, Mahalakshmi *et al.* (2019) have synthesized gold nano conjugates which showed apoptotic effects on HeLa cells upon treatment with IC_{50} concentration used with dual stain. Moreover, Prabhu *et al*. (2014) documented apoptotic effects on human colon cancer cell lines HCT15 and HT-29 treated with synthesized AgNPs mediated isolated pure compound from *Vitex negundo* leaves using annexin V-FITC/PI dual stain.

Cell death detection using comet assay allows the recognition of DNA comet tails for the detection of extant of DNA damage using the PI. An important aspect of DNA damage is the establishment of free radicals that mainly influence the formation of single-strand breaks. The comets originating from damaged cells have a distinct head with a tail (Babu *et al.*, 2019). In our study, alkaline comet assay were used to detect

the DNA strand breaks in the MCF7 cells following exposure to the *C. asiatica* aqueous extract mediated AuNPs. The levels of DNA damage in the cells treated at IC_{50} concentrations of AuNPs when compared to untreated control cells. The results revealed that the MCF7 cells treated at IC_{50} concentrations (20 µg/ml at 48 h) showed significant DNA damage with olive moments while DNA damage was not observed in untreated control cells, as the halo surrounding cell nuclei was clearly visible (Fig. 7). Similarly, DNA damage in the MCF7 cells was noticed after treatment with different concentrations of AuNPs synthesized from *Commiphora wightii* aqueous extract (Uzma *et al.*, 2020). Prabhu *et al.* (2013) reported apoptotic inducing effect of biologically synthesized AgNPs on HCT15 cells assessed by comet assay. Moreover, the DNA damage was induced by p-GNPs on cell lines (MCF-7 and MDA-MB-231) and drastically increased the length of comet tail at IC_{50} concentration of p-GNPs (Uma Suganya *et al.,* 2016).

Conclusion

In the present study, we established a rapid bioapproach synthesis of AuNPs using herb, *C. asiatica* aqueous extract. The characterization methods revealed that terpenes and phenolic compounds were involved in the *C. asiatica* extract-mediated synthesis of AuNPs by using UV-visible and Transmission Electron Microscope. Cytotoxicity of AuNPs synthesized using *C. asiatica* extract against MCF7 breast cancer adenocarcinoma cell line is evident from the reduction of cell viability in treated cells. AuNPs induced apoptosis confirmed by dual staining method and significant DNA damage was evident from the AuNPs treated MCF7 cells showed comet tails analyzed by Comet assay. Based on the *in vitro* studies, the significant cytotoxic and apoptotic effects could be proven against MCF7 cancer cell line treated with biosynthesis AuNPs.

References

Ackermann S and Mrowka R. (2019) Cancer-An ongoing fight searching for reasons and therapies. Acta Physiologica 226(1): 13275.

Archana P, Mathur AK and Archana M. (2019) Advances and emerging research trends for modulation of centelloside biosynthesis in *Centella asiatica* (L.) Urban- A review. Indust Crops Prod. 141: 111768.

Ashokkumar T, Prabhu D, Geetha R, Govindaraju K, Manikandan R, Arulvasu C and Singaravelu G. (2014) Apoptosis in liver cancer (HepG2) cells induced by functionalized gold nanoparticles. Colloids Surf B: Biointerfaces 123: 549-556.

Babu G, Prabhu D, Krishnapriya MV and Arulvasu C. (2020) A novel phytosterol isolated from *Datura inoxia*, RinoxiaB is a potential cure colon cancer agent by targeting BAX/Bcl2 pathway. Bioorganic Med Chem. 28:115242.

Balashanmugam P, Prabhu D, Balakumaran MD and Kalaichelvan PT. (2016) Phytosynthesized gold nanoparticles from *C. roxburghii* DC. leaf and their toxic effects on normal and cancer cell lines. J Photochem Photobiol B Biol. 165: 163-173.

Barnes J, Anderson LA and Phillipson JD. (2007) Herbal Medicines. Pharmaceutical Press, RPS Publishing, London UK.

Bian D, Liu M, Li Y, Xia Y, Gong Z and Dai Y. (2012) Madecassoside, a triterpenoid saponin isolated from *Centella asiatica* herbs, protects endothelial cells against oxidative stress. J Biochem Mol Toxic. 26: 399-406.

Boisselier E and Astruc D. (2009) Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev. 38: 1759-1782.

Cademartiri L and Ozin GA. (2009) Concepts of Nanochemistry. Wiley-VCH, Weinheim, pp. 85-110.

Dhawan A, Bajpayee MM, Pandey AK and Parmar D. (2003) Protocol for the single cell gel electrophoresis/comet assay for rapid genotoxicity assessment. https://www.academia.edu/27064201/ITRC_THE_SCGE_COMET_ASSAY_PROTOCOL_PROTOCOL_FOR_THE_SINGLE_CELL_GEL_ELECTROPHORESIS_COMET_ASSAY_FOR_RAPID_GENOTOXICITY_ASSESSMENT.

El Hilali H, El Hilali F, Traore B and Laziri F. (2019) Anatomical pathology laboratory activity in Meknes, Morocco. Open J Pathol. 9(3): 50.

El-Sayed IH, Huang X and El-Sayed MA. (2005) Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. Nano Lett. 5: 829-834.

Ghosh P, Han G, De M, Kim CK and Rotello VM. (2008) Gold nanoparticles in delivery applications. Adv Drug Deliv Rev. 60: 1307-1315.

Huang SS, Chiu CS, Chen HJ, Hou WC, Sheu MJ, Lin YC, Shie PH and Huang GJ. (2011) Antinociceptive activities and the mechanisms of anti-inflammation of asiatic acid in mice. Evid Based Complement Alternat Med. 2011: 895857.

Jayaprakash N, Vijaya JJ, Kaviyarasu K, Kombaiah K, Kennedy LJ, Ramalingam RJ, Munusamy MA and Al-Lohedan HA. (2017) Green synthesis of Ag nanoparticles using Tamarind fruit extract for the antibacterial studies. J Photochem Photobiol B Biol. 169: 178-185.

Jeyarani S, Vinita NM, Puja P, Senthamilselvi S, Devan U, Velangani AJ, Biruntha M, Pugazhendhi A and Kumar P. (2020) Biomimetic gold nanoparticles for its cytotoxicity and biocompatibility evidenced by fluorescence-based assays in cancer (MDA-MB-231) and noncancerous (HEK-293) cells. J Photochem Photobiol B Biol. 202: 111715.

Khan MA, Khan T and Ali H. (2019) Plant cell culture strategies for the production of terpenes as green solvents. Ind Appl Green Solvents 50: 1-20.

Krishnaraj C, Muthukumaran P, Ramachandran R, Balakumaran M and Kalaichelvan P. (2014) *Acalypha indica* Linn: biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. Biotechnol Rep. 4: 42-49.

Mahalakshmi M and Kumar P. (2019) Phloroglucinol-conjugated gold nanoparticles targeting mitochondrial membrane potential of human cervical (HeLa) cancer cell lines. Spectrochimica Acta Part A Molec Biomolecr Spectrosc. 219: 450-456.

Martin TA, Ye L, Sanders AJ, Lane J and Jiang WG. (2013) Cancer invasion and metastasis: molecular and cellular perspective. In: Metastatic Cancer Clinical and Biological Perspectives, (ed.) Jandial Rahul, Austin, TX: Landes Bioscience, pp. 135-168.

Mossmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

Mubarakali D, Thajuddin N, Jeganathan K and Gunashekaran M. (2011) Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerf. 85(2): 360-365.

Orhan IE. (2012) *Centella asiatica* (L.) Urban: from traditional medicine to modern medicine with neuroprotective potential. Evid Based Complement Alternat Med. 2012:946259.

Pal NB and Kryschi C. (2015) A facile one-pot synthesis of blue and red luminescent thiol stabilized gold nanoclusters: a thorough optical and microscopy study. Phys Chem Chem Phys. 17: 21423-21431.

Prabhu D, Arulvasu C, Babu G, Manikandan R and Srinivasan P. (2013) Biologically synthesized green silver nanoparticles from leaf extract of *Vitex negundo* L. induce growth-inhibitory effect on human colon cancer cell line HCT15. Process Biochem. 48: 317-324.

Prabhu D, Arulvasu C, Babu G, Manikandan R, Srinivasan P, Govindaraju K and Ashokkumar T. (2014) Synthesis and characterization of silver nanoparticles using crystal compound of sodium para-hydroxybenzoate tetrahydrate isolated from *Vitex negundo* L leaves and its apoptotic effect on human colon cancer cell lines. European J Med Chem. 84: 90-99.

Prashanth S, Menaka I, Muthezhilan R and Sharma NK. (2011) Synthesis of plant-mediated silver nano particles using medicinal plant extract and evaluation of its antimicrobial activities. Int J Eng Sci Tech. 3(8): 6235-6250.

Sadeghi B, Mohammadzadeh M and Babakhani B. (2015) Green synthesis of gold nanoparticles using *Stevia rebaudiana* leaf extracts: Characterization and their stability. J Photochem Photobiol B Biol. 148:101-106.

Sperling RA, Gil PR, Zhang F, Zanella M and Parak WJ. (2008) Biological applications of gold nanoparticles. Chem Soc Rev. 37: 1896-1908.

Swaha S, Arjun P, Bharti A and Muhammad Delwar H. (2019) Process optimization for green synthesis of gold nanoparticles mediated by extract of *Hygrophila spinosa* T. Anders and their biological applications. Physica E Low-dimensional Systems Nanostruct. 22: 113830.

Ud Din F, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S and Zeb A. (2017) Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomed. 12: 7291-7309.

Uma Suganya KS, Govindaraju K, Ganesh Kumar V, Karthick K and Parthasarathy V. (2016) Pectin mediated gold nanoparticles induces apoptosis in mammary adenocarcinoma cell lines. Int J Biol Macromol. 93: 1030-1040.

Uzma M, Sunayana N, Raghavendra VB, Madhu CS, Rajasree S and Brindhadevi K. (2020) Biogenic synthesis of gold nanoparticles using *Commiphora wightii* and their cytotoxic effects on breast cancer cell line (MCF-7). Process Biochem. 92: 269-276.