American Journal of Cancer Prevention, 2018, Vol. 6, No. 2, 35-40
Available online at http://pubs.sciepub.com/ajcp/6/2/5

DOI:10.12691/ajcp-6-2-5

Laser Ablation Synthesized Copper Nanoparticles for Cancer Treatment: An Animal Cell Line Studies

Mahadevaiah[1,#], Nawneet K. Kurrey[2,#], Gowtham G. K[3], Thejas Urs G[1], Somashekar R[1,*]

[1]Center for Materials Science, Vijnana Bhavan, University of Mysore, Manasagangotri, Mysore – 570006
[2]Department of Biochemistry and Nutrition, CSIR - Central Food Technological Research Institute, Mysuru – 570020
[2]Department of Physics, Yuvarajas College, University of Mysore, Mysore – 570005
[#]These authors contributed equally to this work.
*Corresponding author: rs@physics.uni-mysore.ac.in

Received August 28, 2018; Revised October 12, 2018; Accepted November 15, 2018

Abstract Drive of nanoparticles in all arenas of science, have essentially attracted the field of medicine and medical sciences. Role of nano particles in drug delivery and targeting is been on its peak in alluring researchers. In this concern, to evaluate and check the adaptability we have made an attempt in using pure metal nanoparticles for cancer inhibition. Pure Copper nanoparticles (CuNPs) were synthesized employing laser ablation in distilled water media. Formations of CuNPs were confirmed by EDAX and this is supported by UV-Visible spectra. The particle size these metal clusters were analyzed by DLS. To understand the biological inhibiting potential of these NPs we have studied on normal and cancers cells. MTT assay result indicates no evidence of in vitro cytotoxity in the normal cells treated with CuNPs. Cell morphology, cell proliferation and viability were examined for exposed cell lines and the effects were quantified in terms cells cytotoxicity using standard procedures. Study reveals the effect of nanoparticles on cancerous cells of Breast, Melanoma and Colon origin and normal fibroblast cells. Furthermore, result demonstrated the distinct role of nanoparticles in normal and cancer cells of different origin with an inference that specific nanoparticles were effective in controlling particular cancer. This study provides fundamental evidence for the easy, simple and safe mode of nanoparticles synthesis and their application in inhibiting/killing cancer cells.

Keywords: *copper nanoparticles, laser ablation, cancer treatment, cell line studies, nanoparticles*

Cite This Article: Mahadevaiah, Nawneet K. Kurrey, Gowtham G. K, Thejas Urs G, and Somashekar R, "Laser Ablation Synthesized Copper Nanoparticles for Cancer Treatment: An Animal Cell Line Studies." *American Journal of Cancer Prevention*, vol. 6, no. 2 (2018): 35-40. doi: 10.12691/ajcp-6-2-5.

1. Introduction

Nanoparticles have become the most interesting and promising field of research, which have almost seen in each and every facts of human life. The diverse properties of this tiny cluster of a material have attracted the human crud to check for its potential applicability in each and every fields. In this perspective to check the inhibition properties of metal nanoparticles, we have carried out this study to understand the potential of these properties. For this purpose nanoparticles prepared by physical laser ablation technique is used. One can refer article Htain Lin Aye et al (2010) for detailed report on ablation. Various metal and semiconducting nano materials have been prepared employing Laser ablation using different suspension media such as water and other solvents.

In our study we have noticed, that not only the preparation of these nano clusters, but also the size of the ablated particles cab be controlled. Results of size controlled particle preparation and the effect of these nanoparticles on inhibition of cancer cells are detailed in this report.

Cancer is a major chronic disease involving unusual cell growth with the likely to invade or extend to other parts of the body. Though enormous advancements have come in the scientific research, cancer is still one of the major leading causes of death for people. In the United States, 1 out of every four people dies from cancer [7]. India has nearly three million cancer patients and will add about one million every year. Based on the studies, it is clear that cancer incidences are increasing due to changes in the lifestyle, environment, including genetic variations. Cancer percentage is growing, and novel anti-cancer drugs with new mechanisms of action are essential for future chemotherapy in order to combat disease, which is of prime priority to public health [8]. Therefore, it is worth evaluating the anti-cancer properties of Copper Nanoparticles synthesized by LASER ablation. CuNPs can be an alternative solution to allopathic medicine which comes with severe side effects. Thus, cancer patients who already got crippled with this disease, which is further burdened by drug-induced toxic side effects, have now turned to seek help from the complementary and alternative medicine hoping for a better cure. With previously established information regarding the significant role of nanoparticles synthesized by various

methods is appropriate to address the anti-cancer potency [9]. The objective of this study is to examine the cytotoxicity of the CuNPs in the different cancer cell and compare with normal cells. The CuNps prepared by ablation are of various sizes, which are obtained for different time intervals. The study is carried out for all these CuNps of various sizes to understand the size dependent inhibition property.

2. Materials and Methods

2.1. Preparation and Characterization of Copper Nanoparticles

The copper sheet was purchased in local metal shop and used as target for laser ablation. The laser is focused on this copper sheet which placed in petri dish containing 50 ml of double distilled water. The Nd-YAG (type RS BPW 21) laser with pulse duration of 9 ns and 10 Hz repetition rate of wavelength 1064nm and the focal length of the lens is 10 cm. The energy of laser source 2watts, this beam was made to vertically focus the metal target using appropriate mirrors and lens to get sharp intense spot [3]. The pictorial representation of ablation setup and solutions containing nanoparticles are shown in Figure 1.

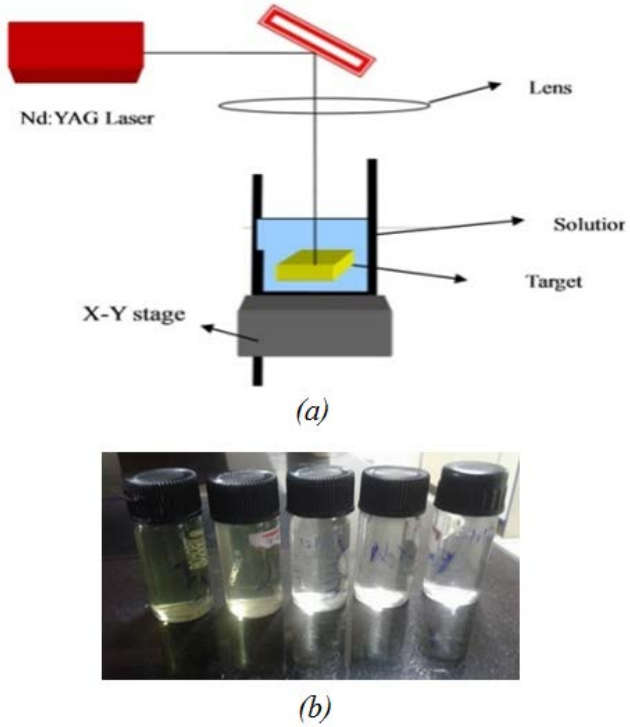

Figure 1. Ablation setup (a) and the solutions containing copper nano particles (b)

Table 1. Copper sheet weight and copper nano particles weight % in Distilled Water for different Ablation time intervals as shown below ((1) 30 and (2) 60 (3)90 (4) 120 (5) 150 mins of time Ablation durations

Ablation time intervals	Copper target initial weight (mg)	Final weight (mg)	Weight of nanoparticles in solution (mg)	Minimum size (nm)	Maximum size (nm)	Mean average size (nm)	CS (M^2/CC) Specific Surface Area
30min	1000	999.99	0.01	1.010	1.060	1.04	5.75
60min	999.99	999.97	0.012	35.60	246.0	70.30	85.35
90min	999.97	999.96	0.015	46.80	3,510	671.0	8.94
120min	999.96	999.94	0.019	707	4,570	3,190	4.31
150min	999.94	999.91	0.025	445	3,310	2,891	2.07

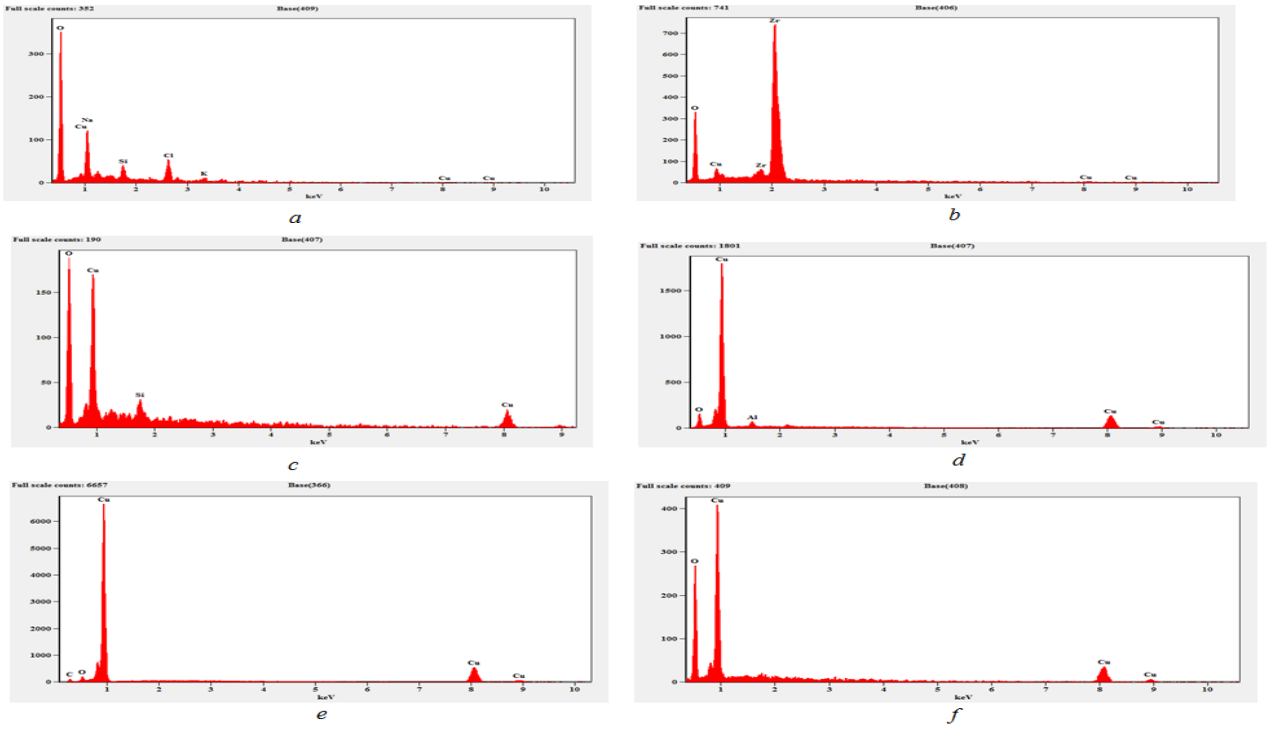

Figure 2. Elemental analysis which shows the percentage of Cu. (a) 30min b) 60 mins c) 90mins (d) 120 and (e) 150 mins of time durations (f) Pure Copper Target

Here the CS is the specific surface area of particles (M^2/CC). Computation assumes smooth, solid and specific particles. This does not reflect porosity or unique topographic characteristic particles.

The weight of nanoparticles are determined by physical measurement of the copper target material, using a weighing balance. This is measured each and every time before and after ablation. The weight parameters are tabulated in Table 1 for detailing. Elemental analysis using EDAX were carried out to confirm the presence of copper and also to quantify the percentage presence. This was done using EDAX equipped SEM instrument HITACHI S3400N [4]. Results of EDAX, confirms the presence of the copper in all the ablated solution of various time intervals and confirms an increase in concentration of copper nanoparticles with time. The Figure 2 shows the EDAX results.

The size distributions of the synthesized Cu nanoparticles were analyzed using Microtrac DLS Instrument [5]. Results of DLS shows an interesting variation i.e dependence of size with the ablation time duration. This time controlled growth of nanoparticles is an important aspect in this study, by which one achieve the required size of nanoparticle based on the intent of application. The size distribution of particles obtained from the results of DLS are shown in Table 1.

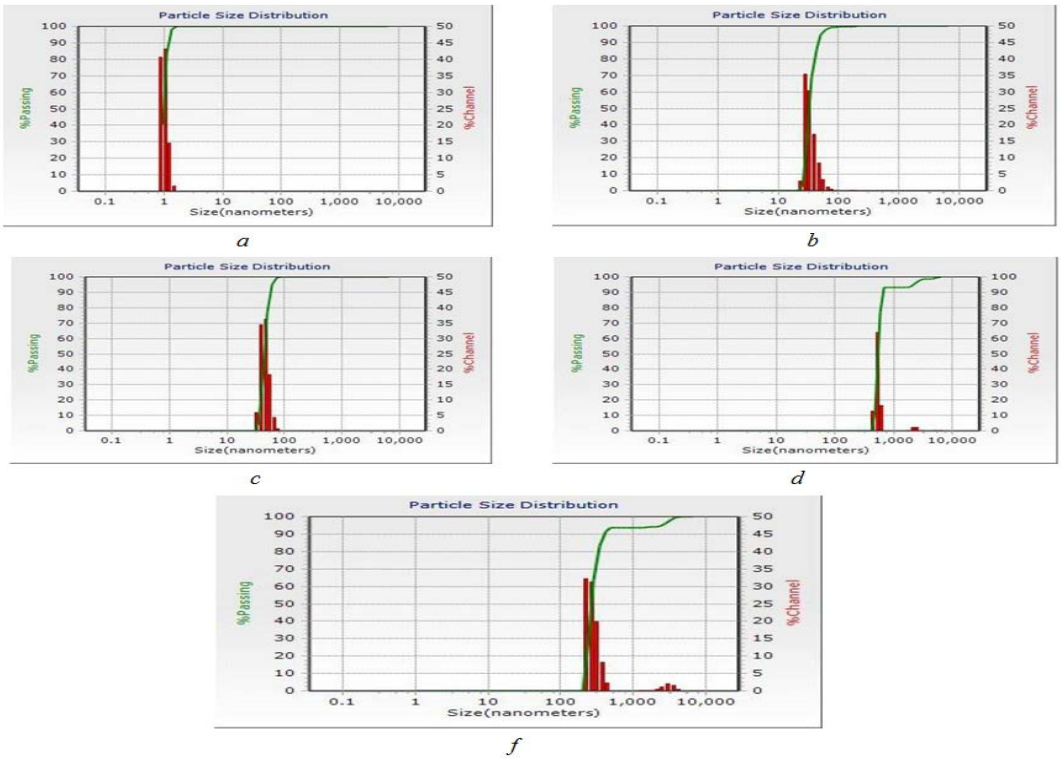

Figure 3. Particle size distribution obtained for the solutions (a) 30 (b) 60 (c) 90 (d) 120 (e) 150 mins of time durations

UV –Visible Spectra graphs

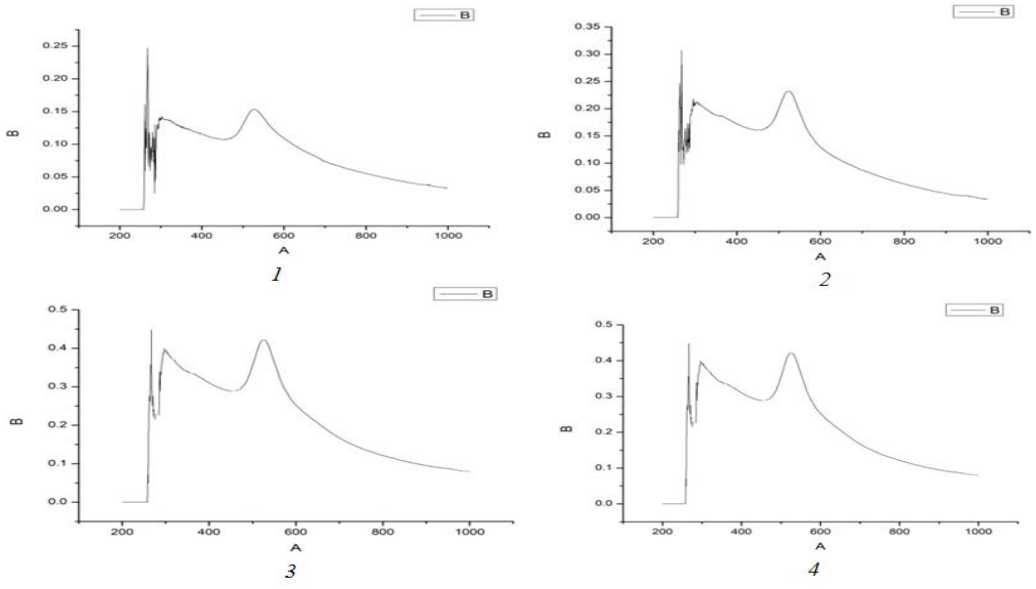

Figure 4. UV –Visible Results show different Ablation Duration, 1)30 mins 2) 60 mins 3) 90 mins 4)120 mins

UV/Visible spectroscopy is carried out to understand the characteristic absorption of the sample, using Bexkman counter DU 730 life science UV/Vis spectro photometer**.** The results show an absorption in spectral range of 600nm, which is attributed copper; this confirms the presence of Cu nanoparticles in the ablated solutions [6]. The UV/Vis absorption spectrum obtained for the samples are shown in Figure 4.

2.2. Cell Culture and Maintenance

Human breast cancer cell lines MDA-MB-231; murine Melanoma- B16F10; human colon Cancer- HCT-15 and Murine fibroblast cells- NIH3T3 were procured from Cell Repository, National Centre for Cell Science, Pune, India. All cell lines were grown and maintained in DMEM (Himedia, Mumbai India) medium supplemented with heat inactivated Fetal Bovine Serum (FBS) (Himedia, Mumbai India) at 37°C in an atmosphere of 5% CO_2 in 95% humidified air.

2.3. Cytotoxicity Assay (MTT Assay)

Cytotoxicity assay was performed using MTT reagent (Himedia, Mumbai India) as described earlier (Yashawini PS 2017). Cells were seeded in 96 well plate at 10,000 cells per well and incubated for 24 hours at 37°C to allow the adhere to the bottom of the plate. After 24 hours media was removed and replaced with copper nanoparticles at different concentration (5-20% volume/volume) in total of 150µl volume per well in triplicates and incubated for 48 hours. After the incubation 15µl of MTT reagent (5mg/ml in 1X Phosphate buffer saline) was added in each well and incubate in dark at 37°C for 3-4 hours. Subsequently the MTT reagent containing culture media was aspirated and the formazan precipitate was formed was dissolved in 100µl Dimethyl Sulphoxide. The dissolved formazan precipitate was measured using 96 well plate reader at 570nm. Cell viability expressed as a percentage of the untreated control (100% cell viability). The effect of the CuNPs on the viability of the cells was measured in triplicate, and the experiments were repeated at least twice.

3. Results and Discussion

3.1. Cytotoxicity Property of Copper Nanoparticles by MTT Assay

The effect of copper nanoparticles synthesized by LASER ablation at different time points (60, 90,120 and 150 minutes) on cytotoxicity on normal cells (NIH3T3) and cancer cells (MDA-MB-231, B16F10 and HCT-15) were evaluated by MTT assay. Cells were treated with increasing concentration of CuNPs ranging from 5-20%. Copper nanoparticles at varying concentration did not exhibit any cytotoxic effect in noncancerous NIH3T3 cells (Figure 5). However, as shown in Figures 6, 7 & 8 CuNPs had a concentration dependent cytotoxic effect on cell proliferation of murine melanoma, human colon and breast cancer cells. The effects of these NPs vary from cells to cells, our result showed that the Human breast cancer cells MDA-MB-231 cells were maximum effected compared to other cells at even lower dose (5%).

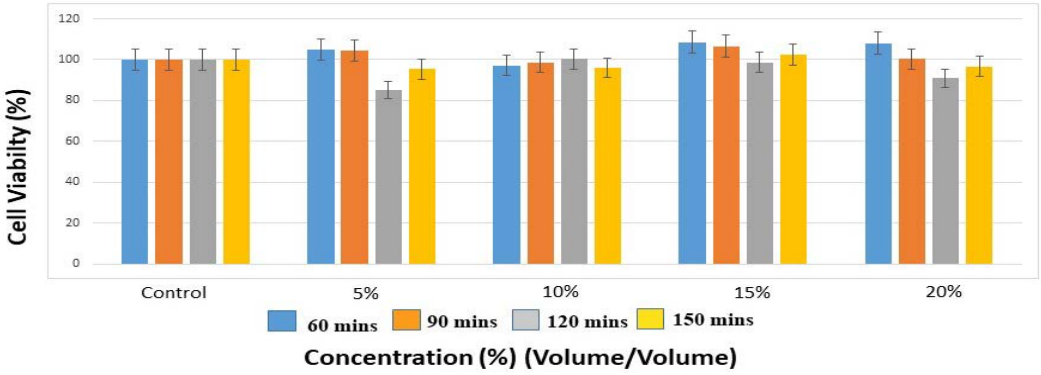

Figure 5. MTT assay for cytotoxicity (%) of CuNPs in noncancerous NIH3T3 cells

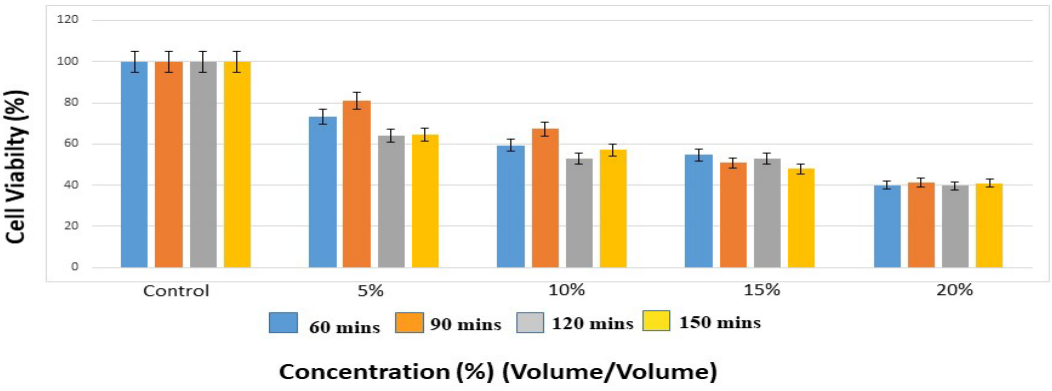

Figure 6. MTT assay for cytotoxicity (%) of CuNPs in human breast cancer MDA-MB-231 cells: Bar graph showing the cell viability (%) of cells treated with different CuNPs of varying concentration

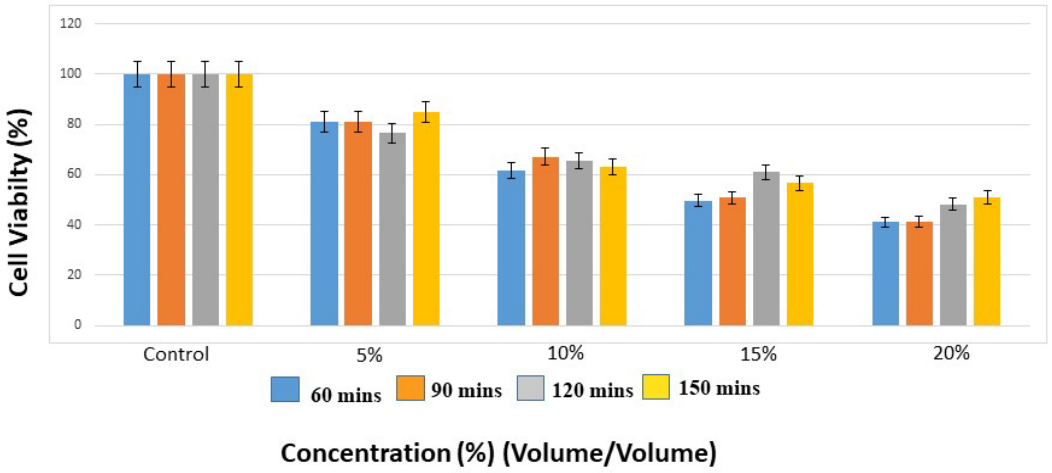

Figure 7. Comparison of cytotoxicity (%) of the murine melanoma B16F10 cells exposed to CuNPs done by MTT assay. Bar graph showing the cell viability (%) of cells treated with different CuNPs of varying concentration

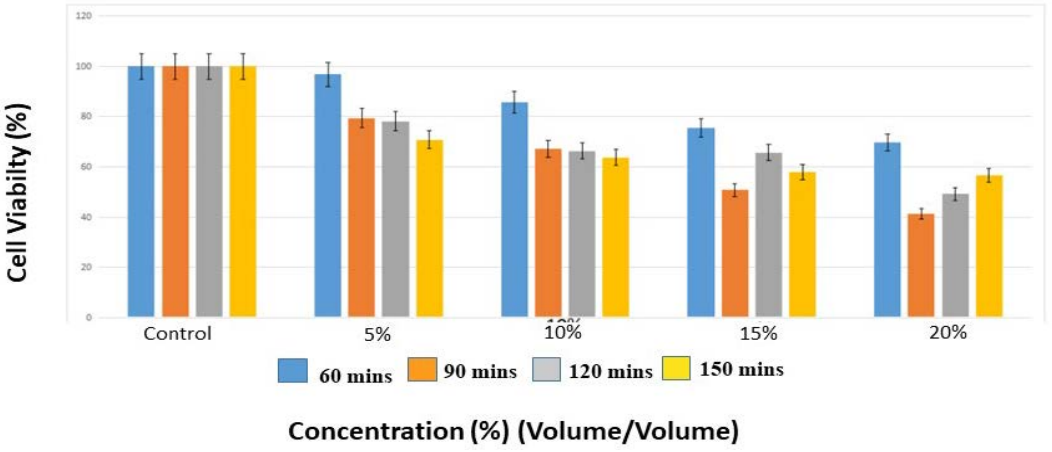

Figure 8. MTT assay for cytotoxicity (%) of CuNPs in human colon cancer HCT-15 cells: Bar graph showing the cell viability (%) of cells treated with different CuNPs of varying concentration

Another important observation was that these CuNPs were not effective at lower concentration in B16F10 and HCT-15 cells, while at higher concentration they demonstrated profound cell killing including in MDA-MB-231 cells. Result showed that all cells except NIH3T3 were rounded up and detached after treatment with CuNPs, indicating these cells were not in healthy state and prone to cell death.

The results, clearly showed that the CuNPs synthesized by Laser ablation remarkably anticancer property in cancer cells and non-toxic to normal cells. Overall, our cell lines studies signifies the distinct role of CuNPs synthesized at different points tested in noncancerous and cancerous cells of different origin, indicating that its preferential ability to kill cancerous ass compared with noncancerous cells. CuNPs may be effective in controlling particular cancer and harmless to normal cells.

Here we would like to add the historical importance associated with miracle dip (shown in Figure 9) in river Ganges which according to our result in this paper, may be due to the presence of nanoparticles in the Ganges river. Of course over the years the river has been polluted. Nanoparticles have a very large surface charge density and can very easily get into the body through the pores regions and these have affinity to get attached to the cancerous or diseased cells because of surface charge density and lead to death of cancerous /diseased cells as per our study reported here. This needs a little more research investigations and at this stage we can only prophesy this aspect.

Figure 9. Hindu devotees taking holy bath at river Ganges

4. Conclusion

These results suggest that copper nanoparticles do kill the healthy state of a cancerous cell and invariably tend to die. They also have varying levels of cytotoxicity due to presence of copper nanoparticles which was observed in

different cancer cell types. It is quite evident from the results that CuNPs do exhibit a pronounced cancer cell killing mechanism without causing damage to normal cells. This study provides a new perspective for cell biology research in nanomedicine. CuNPs treatment can be effectively used for direct target placement in an appropriate solution which is region specific and for killing cancer cells in a short period of time. This study may pave the way for the simple and safe procedure of nanoparticle synthesis and their application in controlling cancer. Further experiments can be performed to decipher the molecular mechanism of cytotoxicity and cell death for the better understanding of effects and successful treatment of cancers. Image of control and NPs treated cells are shown in Figure 10 below.

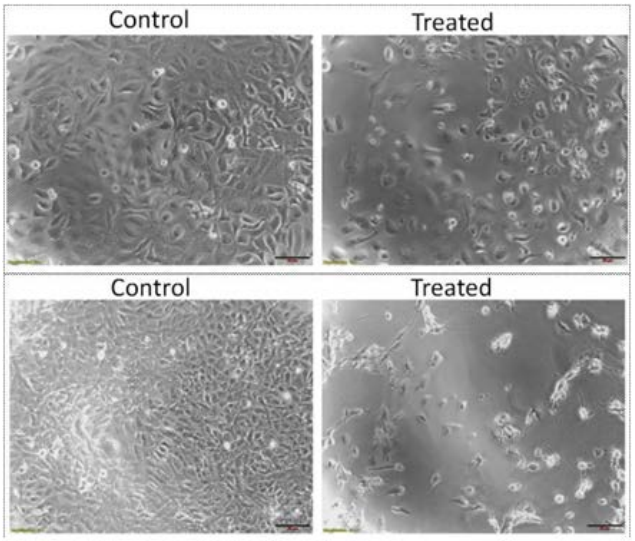

Figure 10. Representative phase contrast images of control and NPs treated cells. (Top) Comparison of cytotoxicity (%) of the murine melanoma B16F10 cells exposed to NPs done by MTT assay (Bottom) MTT assay for cytotoxicity (%) of NPs in human colon cancer HT-29 cells

Funding

None.

Conflicts of Interest

None. All the authors of this article declare that, there is no conflicts of interest among us in publication of this article.

Acknowledgements

Authors thank UGC, India for their support to the University of Mysore through UPE and CPEPA major projects.

References

[1] Aye HL, Choopun S, Chairuangsri T. Preparation of nanoparticles by laser ablation on copper target in distilled water. InAdvanced Materials Research 2010: 93: 83-86.

[2] Tilaki RM, Mahdavi SM. Size, composition and optical properties of copper nanoparticles prepared by laser ablation in liquids. Applied Physics A. 2007:88(2): 415-419.

[3] Muslim DS, Dham ZA, Mohammed DN. Synthesis and characterization of nanoparticles conjugated tannase and using it for enhancement of antibacterial activity of tannase produced by Serratia marcescens. Microbial pathogenesis. 2017; 110: 484-93.

[4] SEM Instruments Manual, Make Japan, Model No,S-3400N, EDAX make USA, model Noren System Company Thermo Fisher Scientific.

[5] Microtrac DLS instrument Manual, Made in USA.

[6] Kazakevich PV, Voronov VV, Simakin AV, Shafeev GA. Production of copper and brass nanoparticles upon laser ablation in liquids. Quantum Electronics. 2004; 34(10): 951-6.

[7] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: a cancer journal for clinicians. 2014; 64(1): 9-29.

[8] Hubbell JA, Chilkoti A. Nanomaterials for drug delivery. Science. 2012; 337(6092): 303-305.

[9] Tang W, Yuan Y, Liu C, Wu Y, Lu X, Qian J. Differential cytotoxicity and particle action of hydroxyapatite nanoparticles in human cancer cells. Nanomedicine. 2014; 9(3): 397-412.

[10] Yashaswini PS, Kurrey NK, Singh SA. Encapsulation of sesamol in phosphatidyl choline micelles: Enhanced bioavailability and anti-inflammatory activity. Food chemistry. 2017; 228: 330-337.