WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 3, 670-680 Research Article ISSN 2278 – 4357

NANO CURCUMIN A NOVEL HERBAL DRUG ENHANCED APOPTOSIS AND INHIBIT EHRLICH ASCITES TUMOR CELLS IN VIVO

Suman Nagpal[1]*, Meeta Gera[1], Ramesh Kumar[2] and V. K. Jain[1]

[1]Amity Institute of Advance Research and Studies (M&D), Amity University, Noida-201303, India.

[2]Virology Section, Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi, India.

Article Received on
27 December 2017,

Revised on 17 Jan. 2018,
Accepted on 07 Feb. 2018,

DOI: 10.20959/wjpps20183-11051

***Corresponding Author**
Suman Nagpal
Amity Institute of Advance Research and Studies (M&D), Amity University, Noida-201303, India.

ABSTRACT

Cancer is a hyperproliferative disorder that is usually treated by chemotherapeutic agents which are toxic, not only to tumor cells, but also to normal cells leading to severe side effects. In addition, these agents are very expensive and beyond the reach of many patients. Curcumin, a yellow coloured poly-phenol, is the main curcuminoid of the Indian spice turmeric (Curcuma longa). It is known for its non-toxicity, affordability and effectiveness in treating cancer cells. The only disadvantage of free curcumin is its low aqueous solubility and poor bioavailability which set major limitations for its therapeutic use. To overcome this, we in our lab for the first time prepared curcumin nanoparticles by wet-milling technique and characterized using a suite of characterization techniques. Nanocurcumin antitumor and cytotoxic properties were also evaluated in Ehrlich ascites tumor cell (EAT Cell)-bearing mice. The EAT cell-bearing animals were observed for their survival, body weight variations and also the tumors grown at the site of injection were evaluated for histopathological changes. The present study revealed that only 0.75mg (nanocurcumin drug)/30gm mice weight is sufficient to reduce the tumor volume upto 33% which shows the therapeutic efficiency of natural curcumin significantly enhanced by converting it into nano sized particle.

KEYWORDS: Nanocurcumin, wet milling, bioavailability, Ehrlich ascites tumor cell (EAT Cell), anti cancerous.

1. INTRODUCTION

Cancer is an aggressive disorder and a prompt action is very necessary to check its growth. Cancer is very different from most body disorders and so is its treatment. It is usually treated by chemotherapeutic agents, which are toxic, not only to tumor cells, but also to normal cells. These agents are also known for causing severe side effects. In addition, these agents are very expensive and beyond the reach of many cancer patients.

Today, alternative treatments of cancer are been explored in great detail and a very promising one is curcumin – active ingredient of spice turmeric. Numerous studies on animals and humans have shown positive effects of curcumin on various types of cancer and scientist believe it to be a great adjunct to traditional methods to treat cancer.[1,5] Turmeric and its active ingredient "curcumin" are being studied upon as chemotherapeutic agent in various diseases. Moreover, curcumin-based treatments have not shown any significant toxic side effects. The only disadvantage of curcumin is of low aqueous solubility and poor availability which set major limitations for its therapeutic use.

The advent of nanotechnology has brought with it an astounding number of possible applications in the field of medicine, which involved in the design, synthesis, characterization, and application of nanomaterials and their devices.[6,7] It provides an effective method to improve the water solubility of this hydrophobic drug. Over a period of time, a lot of emphasis has been given to improve the bio-distribution of natural curcumin, but it is only recently that the application of nano therapeutics which has significantly improved its therapeutic efficacy. This is through the development of nanorange formulations of curcumin.

Although number of reports are available for encapsulation of curcumin in various nanomaterials like self-assembling peptide hydrogel,[8] casein nanocapsules,[9] alginate – chitosan pluronic[10] and polymeric micelles,[11] for enhancing their efficacy and bioavailability, all of these studies reported the use of curcumin in their natural form encapsulated in some matrix, But none of the earlier reported techniques have shown the actual conversion of natural curcumin into nano range.

To overcome this, we, for the first time used a novel technique for the conversion of natural curcumin absolutely in the nanorange. i.e., <100nm based on wet-milling process (Application 670 /DEL/2009, filed 2009-03-31),[12] which significantly enhanced its poor

aqueous solubility and bioavailability[13] and was much more effective than natural curcumin in term of their anti-microbial effects.[14] This unique technique of curcumin nano formulation overcomes all the problems associated with the earlier reported studies. i.e. using the synthetic chemical or encapsulated nanoparticles to enhance its bioavailability.

The present research study focuses on the preparation of curcumin nanoparticles as a potential, herbal, non-toxic, bio-active drug with enhanced bioavailability which is further investigated by its therapeutic and preventive effects on Ehrlich ascites tumor cells in Swiss albino mice.

2. MATERIAL AND METHODS

2.1 Preparation of Curcumin Nanoparticles

The preparation based on the wet-milling technique involved spraying the curcumin solution in a volatile organic solvent into hot water under ultrasonication, followed by concentrating the aqueous solution under reduced pressure, and then freeze-drying it to obtain a powder.

2.2 Characterization of Curcumin Nanoparticles

The synthesized nanoparticles were characterized by UV-visible spectroscopy, Fourier-transform infrared (FTIR), Dynamic light scattering (DLS) and Scanning electron microscopy (SEM) analysis. The UV-vis spectroscopic studies were carried out using Spectro UV-vis Dual beam and Auto Cell UVS-2700, Labomed, INC [Germany]. For this study nano particles of curcumin [1mg/ml] were used to measure the absorption spectra. The FTIR spectra of curcumin and nanocurcumin bandages were recorded on a FTIR analyzer [BRUKER]. The mean particle diameter of curcumin nanoparticles was measured by dynamic light scattering (DLS) performed on Malvern Zetasizer S90 series. A scanning electron micrograph (SEM) of the aqueous dispersion of curcumin nanoparticles was recorded on an EVO-18, special edition, Carl Zeiss [Germany]. The particle size analysis and distribution of nanoparticles was performed by SEM, and DLS analysis.

2.3 Animals

Inbred Swiss albino mice, 5-6 weeks old, weighing 25+ 5 g, of either sex were used for the experiments. The animals were housed in standard size polycarbonate cages under standard laboratory conditions (26+1 oC, 12-h light; 12-h dark cycle), humidity (50-60%) with standard pellet diet and tap water.

2.4 Tumor Implantation

Recipient mice was inoculated intraperitoneally with fixed number of Ehrlich ascites tumor cells (15 x 10^6) suspended in a total volume of 200µl collected from donor mice. The day of inoculation (tumor implantation) was assigned as day 'zero'.

Ehrich ascites tumor bearing mice were randomized into two cohorts of six animals each and administered separately. Group-I animals were kept as a control (untreated) while Group-II animals were injected with the nanocurcumin drug at a dose of 50mg/kg intraperitoneally daily (For 30gm mice weight =1.5mg, 225µl of NPC). All the above groups of animals were administered for 4 weeks. All experiments have been approved by the University animal ethics committee.

2.5 Physical Observations

Physical parameters such as body weight, food & water intake, ILS, MST were monitored every day.

2.6 Ascites Volume and Cell Number

In animal studies tumor size is used to assess responses to anticancer therapy. Ehrlich ascites tumor cells grow as ascites tumor, and increase in volume and cell number is an indicator of tumor progression. Hence in this study we analyzed both these parameters to monitor the effect of the nanocurcumin drug on tumor cell proliferation and compared with that of control.

2.7 Calculation of Tumor Volume

Four weeks after implantation of tumor cells (tumor size 20 – 250 mm3) volumes of 12 (6 control and 6 treated) tumors were determined in vivo by external caliper.

In order to determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volume based on caliper measurements were calculated by the modified ellipsoidal formula[15,16]

$$Tumor\ volume = 1/2(length \times width\ 2)$$

2.8 Histopathological Studies

Tumors removed from sacrificed mice were immediately fixed in 10% formalin fixative for 24h. The tissues were then dehydrated in ascending series of alcohol, kept in 1:1 mixture of absolute alcohol and benzene and then in benzene for 1h each. Finally, tissue pieces were embedded in paraffin wax and 7 micron thick sections were cut and spread on glass slides,

stained with hematoxylin and eosin, slides mounted in DPX and viewed under light microscope and photographed.

3. RESULTS AND DISCUSSION

3.1 Preparation of Curcumin Nanoparticles

Nano curcumin powder as prepared above when re-suspended in water, the lyophilized powder formed a very fine dispersion and appeared to be soluble, unlike curcumin, which is not soluble in water, without using any stabilizers and surfactant the finished product entirely consisted of curcumin in the form of nanoparticles.

3.2 Characterization of curcumin nanoparticles

3.2.1 DLS and SEM

DLS of an aqueous dispersion of nano curcumin revealed the formation of nanoparticles with an average hydrodynamic diameter of 40nm. SEM of the powdered sample and liquid sample before lyophilization showed the particles to be approximately 40-70nm (Fig 1).

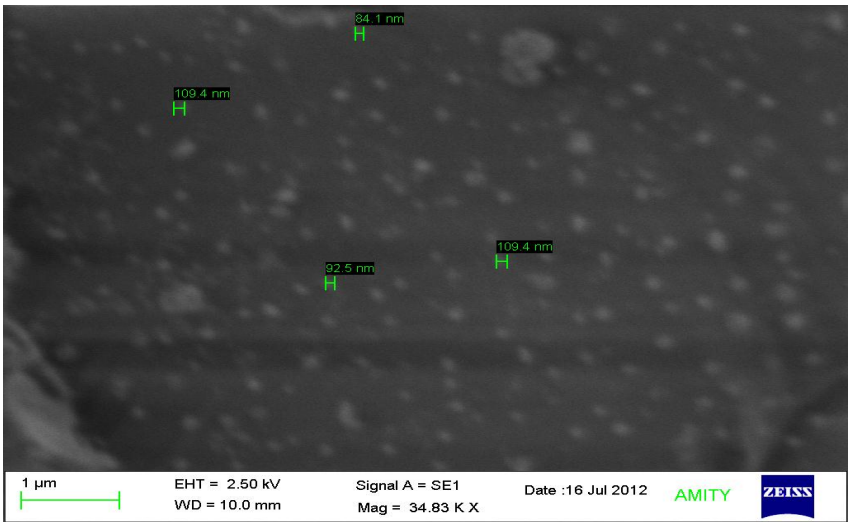


Figure 1: Photograph showing SEM of Nano curcumin.

3.2.2 UV-Visible Spectroscopy

The ultraviolet [UV] spectra of the nanoparticles of curcumin solution showed a characteristic peak at 427-437 nm.

3.2.3 FTIR Spectra

The FTIR spectra of curcumin and nanocurcumin particles were studied. The curcumin nanoparticles has shown absorption peaks at 1664 cm-1 and 1325 cm-1 relating to amide I and III of C=O stretching, N-H/C-N stretching and CH_2 wagging coupled with OH groups of

curcumin respectively. The absorption peak observed at 3414 cm-1 indicates the hydrogen bonding nature of OH/NH2 stretching due to glutaraldehyde activation. All the above observations found in the IR spectra of films confirm the presence of curcumin in curcumin nanoparticles (Fig 2).

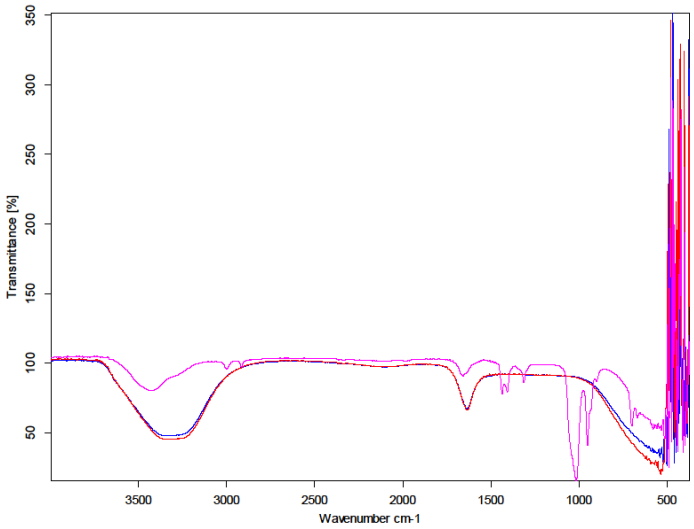


Figure 2: FTIR spectra of Nano curcumin.

3.3 Morphological of Tumor and Body Weight

Tumor appearances in mice are shown in Fig. 3a and b. In both the group of animals, tumor inoculated by EAT cells at the injection site were prominently monitored. In case of control animals, tumor have exhibited fast growth and size progression, and bulging of skin as well as complete loss of hairs at the injection site. In contrast, group-II, i.e., treated animals, the skin overlying the tumor had normal morphology and was not invaded by tumor cells without accompanied any hair loss.

(a) **(b)**

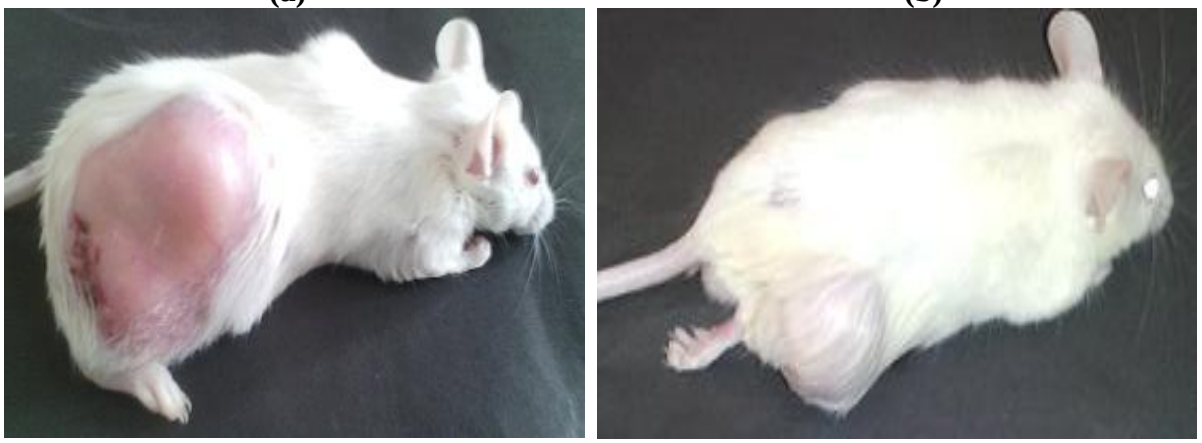


Figure 3: Morphological view of a. control Swiss albino mice (Untreated) b. Nano Curcumin treated Mice.

The average body weight of mice from all treatment groups are shown in Table 1. Group-I animals were taken as an initial body weight of 27.8gm which later on gets constant after day 20 (40.4gm) while the group-II animals has an initial weight of 28.0gm and gradually increases up to the day 21 and after that it gets constant to 36.8gm till the date of surgery. The amount of food uptake in the control group became constant during the whole observation period but, the treated group of animals showed very small escalation after the day 19 and there was no significant difference found in the amount of water uptake in both the group of animals under observation.

No evidence of loss in body weight as well as any signs of systemic toxicity or behavioural abnormalities was found during the course of treatment.

Table 1: Average Body weight of control and nano-curcumin treated Mice during the period of study.

Days	Control (Mean ± SD)	Treated (Mean ± SD)
0	27.8± 1.12	28.0±0.95
21	40.4±1.78	36.8±1.42

3.4 Ascites Volume and Cell Number

One week after the injection of tumor cells, subcutaneous tumor volumes (V) were measured with digital Vernier calipers. EAT cells begin their exponential growth from the seventh day after tumor cell injection and the animal succumbed to the ascites tumor burden on day 20-25 after injection.

The mean value of tumor volume in control and treated group of animals was found to be 11473.05mm3 and 1747.36mm3 respectively (Fig 4a and 4b). The results showed, when compared to the 100% growth of Ehrlich ascites tumor (EAT) in the peritoneum of mice there was about 33% reduction in the growth of tumor in the mice treated with curcumin nanoparticles (For 30gm mice weight = 0.75mg, 200µl of NPC daily) for tumor growth period, indicating that proliferation rate of tumor cells in treated mice was inhibited by curcumin nanoparticles due to decrease in tumor burden and the result showed compressed tumor weight of 1.18gm compared to the control group of animals (group-I) was 3.62gm.

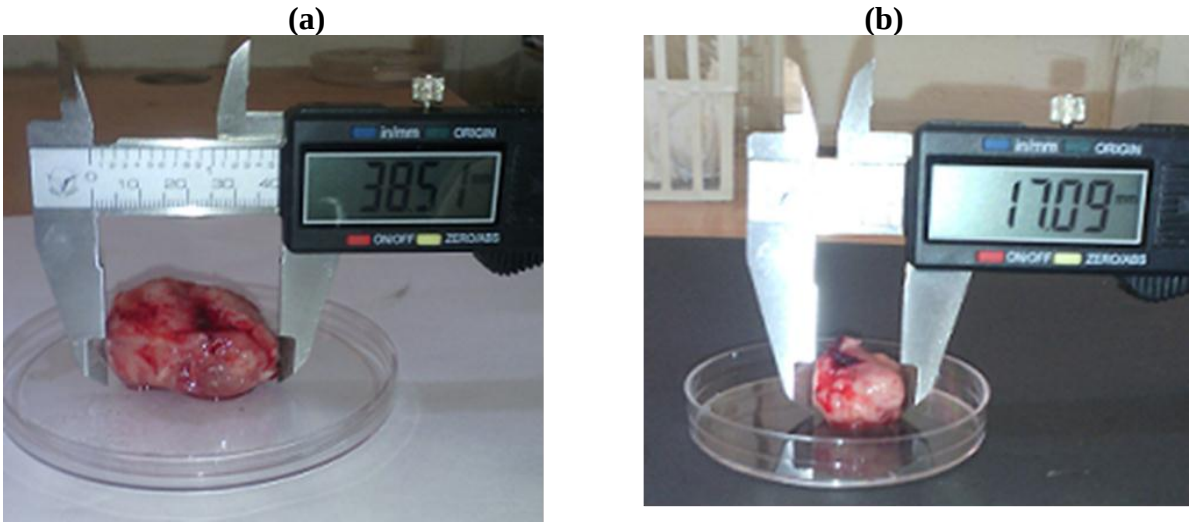
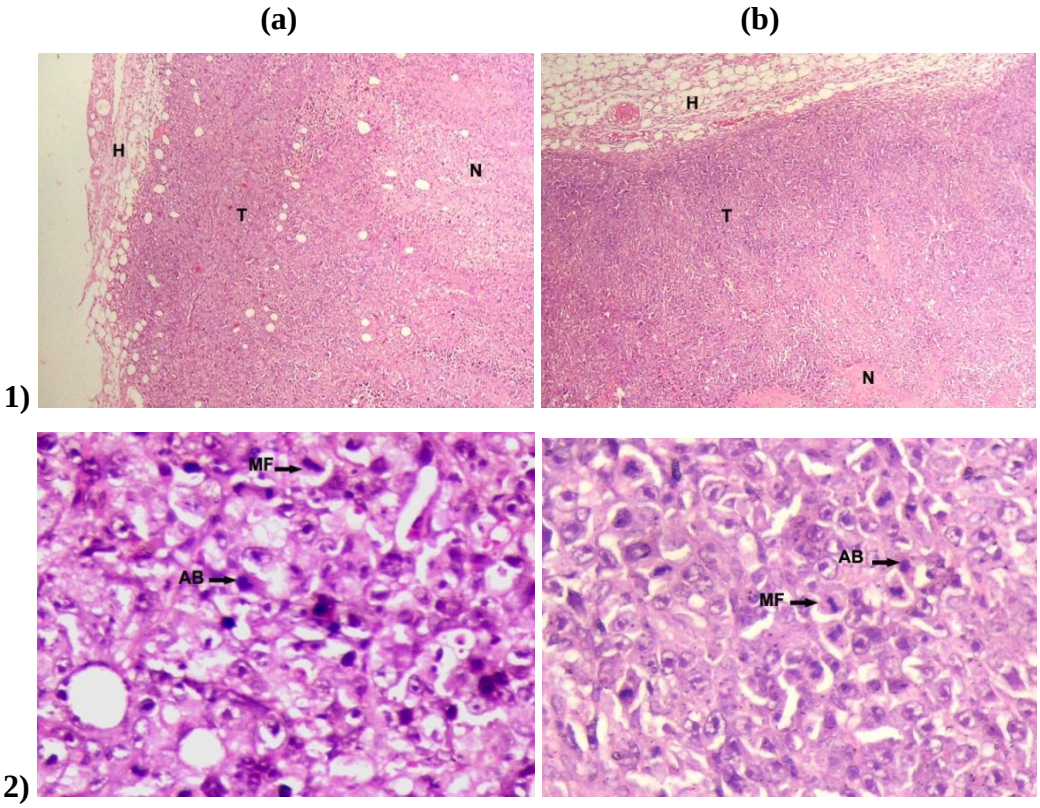


Figure 4: Tumor weight of a. control Swiss albino mice (Untreated) b. Nano curcumin treated Mice.

3.5 Histopathological Studies

The histological changes in the mice tumors and skin were studied with hematoxylin and eosin staining of the paraffin sections in treatment groups. The histopathological examination of the tumor of scarified mice was performed after 4 week of treatment of mice with nanocurcumin as shown in Figures 5b1,5b2 and 5b3 in comparison with control Figure 5a 1, 5a 2 and 5a 3



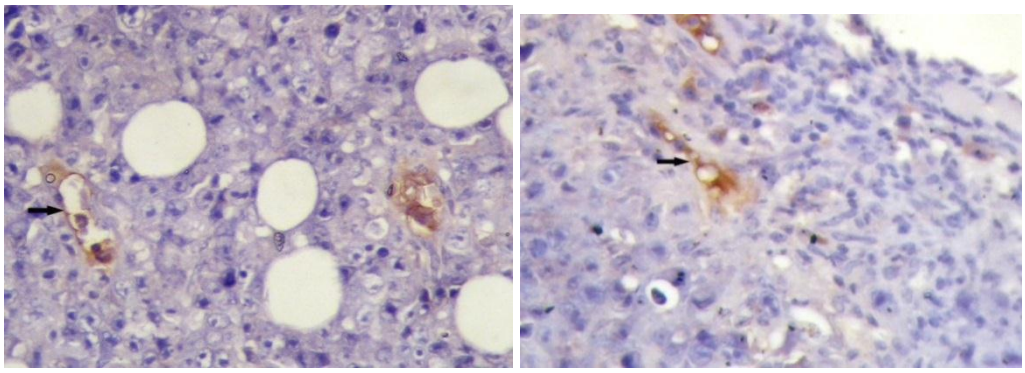
3) 

Figure 5: Histopathological Cross sectional images of the a. Control and b. Nano curcumin treated.

Cross sectional images of the control group showed more viable tumor cell and non-homogeneous growth of cells (5a) while nanocurcumin treated group showed necrosis in the central area of viable tumor cells and homogeneous growth of cells in the periphery (5b). Photographs also demonstrate that nanocurcumin reduced the number of mitotic figures (MF) and increased necrosis (5b) as compared to control with enhance mitosis and mitotic figure and apoptosis (5a).

3.6 Nano Curcumin Readily Overcomes the Bioavailability Pitfall of Free Curcumin in Vivo

To improve the systemic bioavailability of free curcumin, in our lab, we for the first time prepared curcumin nano particles using wet-milling method to reduce the average particle size of curcumin to 40-80nm. We found that nano curcumin prepared by this method had good chemical and physical stability, stored in the powder form at room temperature, and was freely dispersible in water. The present study revealed that only 0.75mg (nanocurcumin drug)/30gm mice weight is sufficient to reduce the tumor volume upto 33% which shows the therapeutic efficiency of natural curcumin significantly enhanced by converting it into nano sized particle.

4. CONCLUSION

The present research work demonstrates a novel method of preparation of nanocurcumin with absolutely in nano size range, i.e., 40-80nm. The curcumin nanoparticles prepared have shown higher aqueous solubility which proved to be significant in its anti-angiogenic, anti-cancerous and anti-metastatic effects as the results indicates nearly 33% reduction of tumor in the treated group of animals. The results also indicates significant reduction in tumor cell number and not exhibit any kind of adverse morphological effects like hair loss as observed

in case of synthetic or chemotherapeutic treatment of cancer. In addition to this, the developed nanoparticles of curcumin can readily be stored at room temperature as a lyophilized powder, and can also be transported as such, requiring only reconstitution in an aqueous phase at the point of destination, all of which should facilitate eventual application in a clinical setting.

Most importantly, our study reinforces the emerging notion that nanotechnology provides a highly appropriate avenue for harnessing the full potential of promising, highly bioavailable and natural anticancer drug nanocurcumin. This study also suggests that the systemic administration of curcumin nanoparticles is safe and deserves to be investigated for further clinical applications. Thus, to the best of our knowledge, our study is the first to rigorously show that the nanocurcumin prepared by this novel wet-milling approach has tangible therapeutic and chemo preventive effects in vivo. In addition to this, our work, albeit not "regulatory grade" by any means, provides some degree of reassurance that the formulation and dosing regimen used does not entail obvious systemic adverse effects.

ACKNOWLEDGEMENT

We wish to express our gratitude to the Founder President of Amity University, Dr Ashok K. Chauhan, for his encouragement and guidance

CONFLICT OF INTEREST

There is no conflict of interest in this work.

REFERENCES

1. Chen, H.W., et. al., (2008), "Curcumin inhibits lung cancer cell invasion and metastasis through the tumor suppressor HLJ1" *Cancer Res*, 68: 7428–7438.
2. Yodkeeree S, et. al., (2009) "Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the down-regulation of MMPs and uPA", *J Nutr Biochem.,* 20: 87–95.
3. Saha A, et.al., (2010) " Apoptosis of human lung cancer cells by curcumin mediated through up-regulation of 'growth arrest and DNA damage inducible genes 45 and 153", *Biol Pharm Bull*,33: 1291–1299.
4. Kanai M, et. al., (2011) "A phase I/II study of gemcitabine based chemotherapy plus curcumin for patients with gemcitabine resistant pancreatic cancer", *Cancer Chemother. Pharmacol.*, 68: 157–164.

5. Itokawa H, et.al., (2008) "Recent advances in the investigation of curcuminoids", *Chinese Medecine.*, 3: 11.
6. Altunbas A, et.al., (2011) "Encapsulation of curcumin in self-assembling peptide hydrogels as injectable drug delivery vehicles" *Biomaterials.*, 32(25): 5906-14.
7. Kang, P., et. al., (2013) "Enhanced Dispersibility and Bioactivity of Curcumin by Encapsulation in Casein Nanocapsules", *J. Agric. Food Chem.*, 61(25): 6036–6043.
8. Das, R.K., et. al., (2010) "Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells" *Nanomedicine: Nanotechnology, Biology and Medicine*, 6: 153–160.
9. Gou, M. I., et. al., (2011) "Curcumin-loaded biodegradable polymeric micelles for colon cancer therapy in vitro and in vivo", *Nanoscale*, 4: 1558-67.
10. Yallapu, M.M., et. al., (2010) "Curcumin induces chemo/radio-sensitization in ovarian cancer cells and curcumin nanoparticles inhibit ovarian cancer cell growth", *Journal of Ovarian Research*, 3: 11 10.1186/1757-2215.
11. Mulik, R.S., et. al., (2010) "Transferrin mediated solid lipid nanoparticles containing curcumin: Enhanced in vitro anticancer activity by induction of apoptosis" *International Journal of Pharmaceutics*, 398: 190-203.
12. Jain, N., et al., (2009) "Preparation of curcumin nanoparticles and evaluation of their bio-efficacy", Indian patent *Application 670 /DEL/2009, filed 2009-03-31.*
13. Bhawana, et al., (2011) "Curcumin nanoparticles preparation characterization and their antimicrobial study", *J Agric Food Chem*, 59(5): 2056-61.
14. Gera, M., et. al., (2015) "Preparation of a Novel Nanocurcumin Loaded Drug Releasing Medicated Patch with Enhanced Bioactivity Against Microbes" *Adv. Sci. Eng. Technol*, 7(6): 485-491.
15. Euhus, D.M., et. al., (1986) "Tumor measurement in the nude mouse" *J Surg Oncol*., 31: 229-234. 10.1002/jso.2930310402.
16. Tomayko, M.M., and Reynolds C.P., (1989) "Determination of subcutaneous tumor size in athymic (nude) mice" Cancer Chemother. Pharmacol., 24: 148-154. Google Scholar.