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ENCAPSULATION OF CURCUMIN IN SILVER NANOPARTICLE FOR ENHANCEMENT OF ANTICANCER DRUG DELIVERY

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ABSTRACT: In the proposed work, enhancement of anticancer potential of curcumin extracted from *Curcuma longa* Linn. by encapsulated in the silver nanoparticle. Curcumin was extracted from *Curcuma longa* by Soxhlet method. Curcumin is a kind of poly phenols which was widely used because of its medicinal application. Curcumin shows poor solubility and low absorption rate therefore its silver nanoparticles was formed to increase solubility, absorption as well as affectivity against tumor. Various analytical methods and assay such as Particle size, Zeta potential, Atomic Force Microscopy (AFM), Differential Scanning Colorimetric analysis (DSC), X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR), Drug Release, Hemolytic toxicity study and *In-vitro* cytotoxicity study were employed for monitoring the formation of nanoparticles. Presence of silver nanoparticles with an average size of 17.98 nm was revealed by Atomic Force Microscopy and particle size analyzer and zeta potential may obtained -4.68 mV. The result of hemolytic toxicity was demonstrated that Cur-AgNPs shows lesser hemotoxicity in comparison with plain curcumin and Cur-AgNPs were able to release 93.15% curcumin in 12 hrs whereas the plain curcumin was released 93.31% in 4 hrs. The crystalline nature of synthesized nanoparticles was done by X-ray diffraction. The results obtained by *in-vitro* cytotoxicity study, demonstrated that Cur-AgNPs cytotoxicity induced by curcumin to the tumor cells were dose dependent and had a greater cytotoxic effect on the tumor cells than plain curcumin. This work shows that curcumin encapsulated silver nanoparticles shows potential activity against cancer cell.

INTRODUCTION: Nanotechnology can be defined as the formation, development, enhancement and exploration of nano sized materials having size range of (1-100 nm). It works with the substance which has specific properties such as physical, chemical and biological.

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Now a day's nanotechnology works on the design and development of many novel formulations for the prevention, treatment and diagnosis of many critical diseases, [1 - 3] like cancer, T.B. and cardio-vascular diseases *etc*. Because of their specific characteristics, silver nanoparticles (AgNPs) may used as catalysts, [4] in spectrally selective coatings for absorption of solar energy as optical sensors, [5] in fabric tailoring as well as in electronics devices [6] and in various therapeutic activity of bactericidal agents.[7, 8]

AgNPs have a important role in prohibiting microbial (bacterial) growth in solid and liquid

culture media because of its high reactivity.[8] AgNPs prepared by green synthesis phenomena is effective in biological environmental systems. [9, 10]

Curcumin differentiates between the normal cells and the cancer cells. It will not kill the normal cells when it destroys the cancer cells. Such a medicine is hard to come by. But the problem is that the curcumin has very less bio availability. It is not soluble in water. So it is not present in the viable form in blood for long time after consumption to be effective for the treatment. The bio availability of nano curcumin is more than that of the conventional type.

Attempts to improve the bioavailability of this novel compound curcumin are underway at various places as we speak. Adjuvants which absorb, retain curcumin and then release it in the blood stream after some time are being discovered. Nano particles of curcumin are being studied. Liposomes and phospholipid complexes are used to improve the delivery of curcumin.

Turmeric is safe and non-toxic for most patients, it has been shown to have diverse biological effects in humans and animals. Turmeric/curcumin is a potent anti-inflammatory and antioxidant. The evidence suggests that it can suppress tumor genesis, tumor promotion, and metastasis and, therefore, has enormous potential as an anticancer agent. Further study is needed to determine whether it, like other antioxidants, should be avoided during chemotherapy. Pure curcumin and the crude ethanol extract have great potential in the prevention and cure of cancer.[11]

Extraction of curcuminoids from turmeric powder with the help of methanol as solvent was carried out and curcuminoids were isolated in the form of crystals with 95% purity and 75% yield. The isolated crystals show very good anti-inflammatory activity against inflammation induced edema. [12] For curcumin extraction dichloromethane is the best solvent for extraction. Cost-effective, simple and rapid identification method for authentication of *C. longa* rhizome have been developed.[13] The ethanol extract of curcuma rhizome exhibited significant antibacterial activity. Ethanol extract is effective than aqueous extract against bacteria and fungi. [14] Isolated turmeric extracts and pure curcumin showed very weak activity against the studied mycobacteria but showed very good antioxidant activity. [15] It is one of the best medicines to treat various life threatening diseases like Alzheimer, Huntington's disease, various types of cancer and AIDS.

MATERIAL AND METHODS:

Material: *Curcuma longa* were collected from Botanical garden, Guru Ramdas Khalsa Institute of Science and Technology (Pharmacy), Jabalpur, MP, India. $AgNO_3$ and ethanol and other chemicals were purchased from Chemical drug house, New Delhi, India and other chemical used analytical reagent grade.

Extraction of Curcumin from *Curcuma longa* (Turmeric): Extraction of curcumin *Curcuma longa* (turmeric) were collected and rhizomes were cleaned using deionized water, then rhizomes were scrapped. It was dried in pollution free atmosphere at room temperature. After few days it was powdered using mixer grinder. 20 g of the turmeric powder was taken into a thimble and placed in a Soxhlet apparatus, with ethanol as a solvent. Finally dark yellow extraction was obtained and filtered. The filtrate was kept in Ultrasonicator at 20 KHz frequency for about 20 minutes to reduce the particle size of the curcumin.

Synthesis of Silver Nanoparticle: A 0.017gm of silver nitrate was accurately weighed and dissolved in 100 ml of double distilled water and stored in amber coloured bottle until further use**.** A 100 ml of 1mM silver nitrate ($AgNO_3$) was heated to boiling. To this appropriate solution, 10 ml of curcumin extract was poured for reduction of silver ions (Ag^+), then kept at room temperature for 1 hour, the change in colour of solution was examined timely. The color change from yellow to yellowish brown indicated the formation of silver nanoparticle. It observed that the aqueous silver ions could be reduced by the ethanolic extract of curcumin to generate very stable silver nanoparticles. The prepared silver nanoparticle was lyopillized for further use.

Characterization Techniques:

Fourier Transform Infra-Red Spectroscopy (FTIR analysis): FTIR Spectra of AgNPs formed with curcumin (Cur-AgNPs) was determined with a

FT-IR spectrophotometer (Cary- 630 FTIR, Agilent Technologies).

Particle Size and Zeta Potential Determination: The particle size and zeta potential of silver nanoparticle of ethanolic extract of curcumin (Cur-AgNPs) was determined using particle size analysis instrument (Malvern Instrument, United Kingdom).

Morphology:

Atomic Force Microscopic Analysis: The structure and surface characterstics of Cur-AgNPs was determined by AFM (AIST-NT Smart SPM 1000, CA). AFM of the nanoparticles was accomplished at glass substrate in AC mode.

X-Ray Differaction (XRD) Analysis: Crystalline nature of silver nanoparticle of ethanolic extract of curcumin (Cur-AgNPs) was confirmed by X-ray diffractometer (Bruker, Munich, Germany). The scanning rate was 2θ/min over a 2θ range of 0 - 40º and with an interval of 0.02º.

Differential Scanning Colorimetry (DSC) Analysis: DSC study of silver nanoparticle of ethanolic extract of curcumin (Cur-AgNPs) was performed by using DSC instrument (Mettler Toledo DSC 822e). Cur-AgNPs were weighted into aluminum pan and confined with a pin-holed lid, then thermograms was observed under nitrogenenous atmospheric condition from ambient to 350 ºC at a heating rate of 700 ºC in 7 min.

Hemolytic toxicity: Whole human blood was collected and stored in HiAnticlot blood collection vials as described in our previous paper. [16, 17] The human blood was centrifuged then red blood cells were separated and resuspended in normal saline solution (10% hematocrit). One ml of the red blood cell suspension was individual incubated with 5 mL of distilled water (taken as 100% hemolytic standard) and 5 mL of normal saline (taken as blank for spectrophotometric evaluation).

Further, 1 mL of adequately diluted Cur-AgNPs was added to 5.0 mL of normal saline and interacted with RBC suspension. The suspension was centrifuged for 10 min at 2000 rpm and the absorbance of supernatants was measured at 540 nm, which was used to evaluate the percentage hemolysis using distilled water as 100% hemolytic standard.

Drug Release Study: Ten milligrams of Cur-AgNPs was dissolved/dispersed in 5 mL of PBS (pH 7.4) and then placed into a dialysis tube (MWCO 2000 Da). The dialysis tube was placed into 100 mL of aqueous recipient medium of PBS (pH 7.4). The medium was placed in the stirring condition at 100 rpm at 37 ± 2 °C, then the sample are released through the dialysis tube continuously and then the sample were withdrawn at specific time interval and analyzed on HPLC and the same volume of fresh PBS (pH 7.4) was added to maintain the sink condition. Then the samples were examined by using HPLC.

***In-vitro* Cytotoxicity Assay:** Cellular cytotoxicity was assessed by tetrazolium dye-based MTT assay following a previously reported procedure.[17] MDA-MB-435 and A-549 cells were maintained in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum and antibiotics at 37°C in a humidified incubator containing 5% CO_2. The cells were treated with Cur-AgNPs in various concentrations (10, 20, 40, and 80 lg/mL) for 24 h.

The amount of formulation needed to prepare molar equivalents of curcumin was calculated, based on the drug content in the formulation. Control was taken without any drug treatment. Subsequently, MTT was added and plates were then incubated for another 3 h, the medium was pippetted off and 250 lL DMSO was added. The absorbance of individual wells was noted at 570 nm *via* an ELISA plate reader at 25 °C. Average values from triplicate were subtracted from average value of control and the survival fraction of cells was calculated by the formula.

RESULT AND DISCUSSION:

FTIR Analysis of Cur-AgNPs: FTIR analysis was done to measure the characterization and conjugation of potent biomolecules of curcumin with silver nanoparticles. According to the FTIR spectra of Cur-AgNPs (**Fig. 1**) different peaks was obtained which indicates different functional groups and intermolecular bonding of Cur-AgNPs.

The spectra are obtained at 682, 972, 1081, 1178, 1309, 1443, 1555 and 1686 cm-1 indicates the presence of aromatic C-H bending, N-H stretch and O-H carboxylic acid, C-O-C stretch, C-N stretching of aromatic amine group and C-N stretching ester

and ethers in the residual solution and NO_3- group, C=C bonding, =C-H stretch respectively. The variety of functional groups indicated in FTIR spectra are mainly obtained from heterocyclic substances that are generally water soluble compounds of curcumin. So it can be understood that various water soluble heterocyclic compounds like alkaloids and flavonoids works as the capping ligands for the formation of Cur-AgNPs .[18]

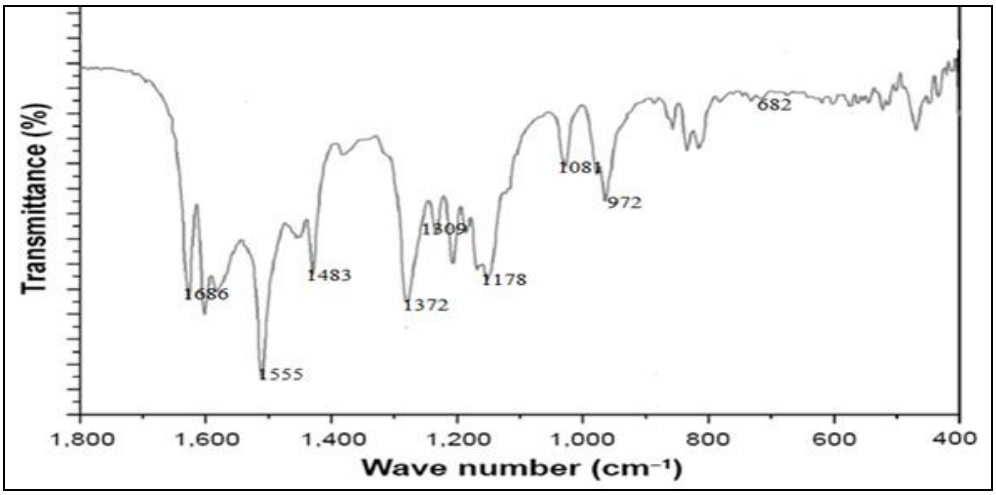

FIG. 1: FTIR OF CUR-AgNPs

Particle Size and Zeta Potential Determination: The average particle size of Cur-AgNPs were found to be 17.98±0.16 nm (**Fig. 2**) and distribution of particles size of Cur-AgNPs were found to be 71.3% of 20.31nm, 17.5% of 16.67nm and 11.2% of 14.5nm and zeta potential of Cur-AgNPs was found to be -4.68 mV (**Fig. 3**).

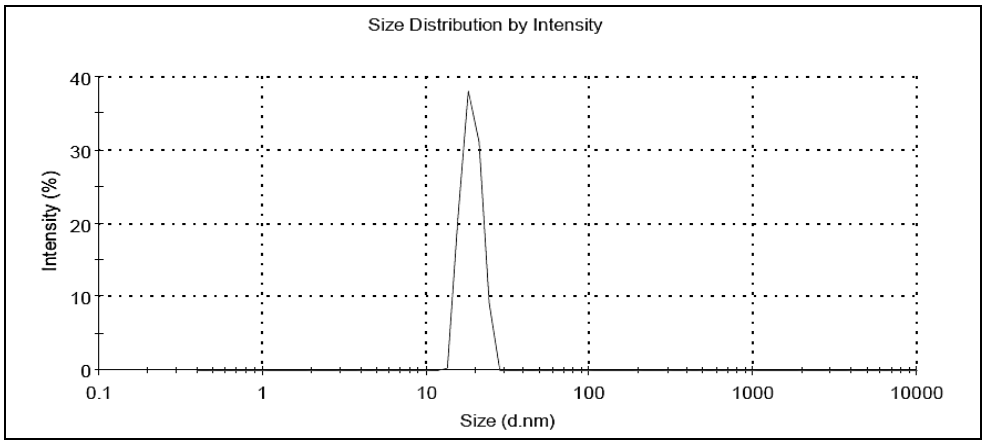

FIG. 2: PARTICLE SIZE STUDY OF Cur-AgNPs

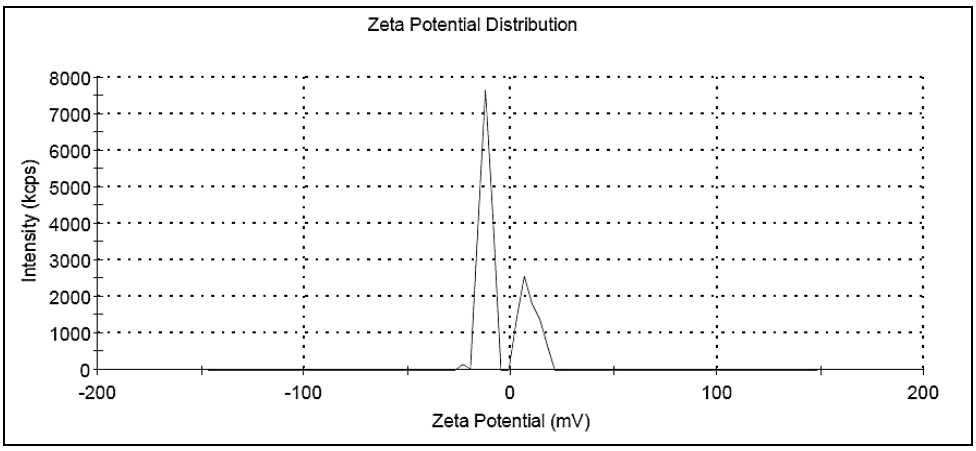

FIG. 3: ZETA POTENTIAL STUDY OF CUR-AgNPs

Morphology:

Atomic Force Microscopic Analysis: The structure and surface characterstics of Cur-AgNPs was characterized by atomic force microscope and image was obtained. The Cur-AgNPs was visullized in spherical shaped and in nanometric size range (18nm) as estimated by AFM image (**Fig. 4**).

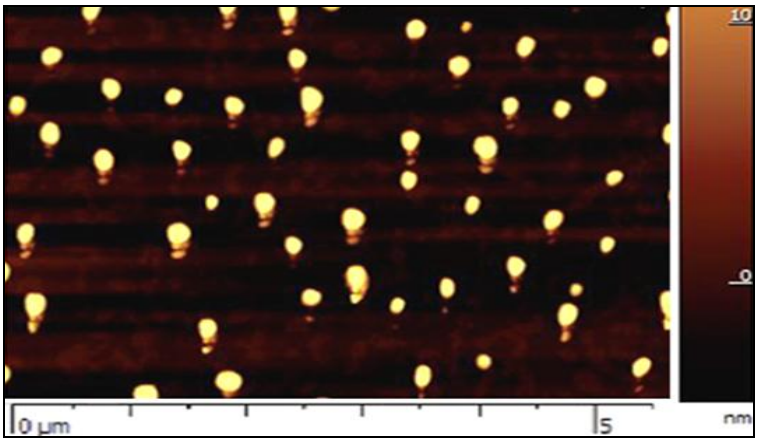

FIG. 4: AFM IMAGE OF CUR-AgNPs

XRD Analysis of Cur-AgNPs: XRD is a very potent technique that is normally used to analyze the characteristics and details of structure nanoparticles. The XRD patterns are observed by measuring the angles at which an X-ray beam is diffracted by the crystalline phases in the object.

The XRD pattern of synthesized Ag-NPs is shown in **Fig. 5**, the following prominent peaks at 5°, 7°, 18°, 28°, 33°, 38°, 44°, 50°, 58°, 64° and 77° (2θ) indicated the crystalline property of silver nanoparticles, from the XRD pattern it is known that Cur-AgNPs formed using crystalline in nature.

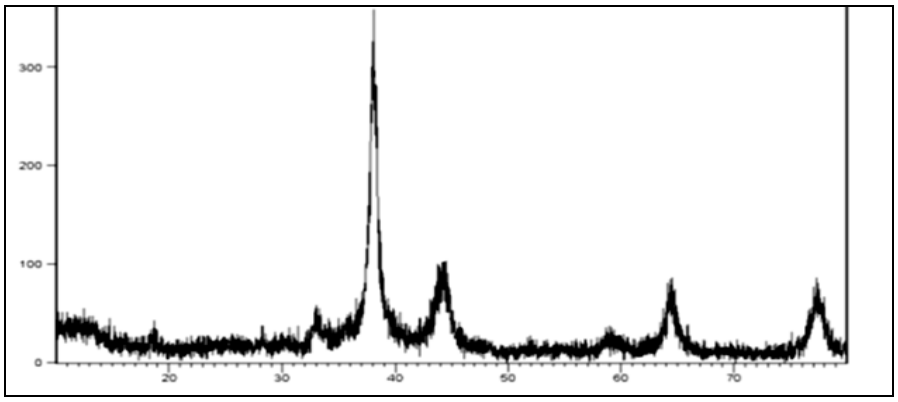

FIG. 5: XRD IMAGE OF CUR-AgNPs

DSC Analysis: DSC thermogram of Curcumin and Cur-AgNPs shown in **Fig. 6**.

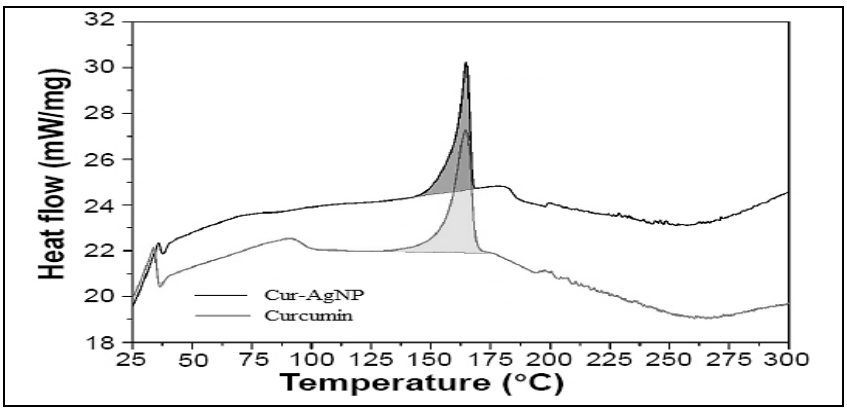

FIG. 6: DSC IMAGE OF CUR-AgNPs

In the case of curcumin, an exothermic peak was observed 36, 77, 166 and 191 ºC and endothermic peak was obtained at 264 ºC. In case of Cur-AgNPs, an exothermic peak was obtained at 38, 165, 181and in 192 ºC and endothermic peak was obtained at 264 ºC However, the exotherm of curcumin is detected at 166 ºC in the DSC thermogram of drug loaded NPs (Cur-AgNPs), indicating that the drug exists in crystalline form inside the nanoparticles.

Hemolytic Toxicity: Hemolytic toxicity study was executed to evaluate the hemotoxic effect of the Cur-AgNPs. The plain curcumin and Cur-AgNPs have exhibited hemolytic toxicity upto 15.16 ± 1.10% and 2.54 ± 0.5% respectively. The curcumin concentration for silver nano particulate formulations was determined on the basis of the drug content. Feasibly, the encapsulation of drug molecules in the AgNPs and consequential delayed release results in a significant reduction in the hemolytic toxicity. Moreover, Cur-AgNPs demonstrated lesser hemotoxicity in comparison with plain curcumin.

***In-vitro* Drug Release:** The release of curcumin from the Cur-AgNPs demonstrated that the AgNPs have sustained release property (**Fig. 7**). The conclusions established the fact that there was a prominent time prolongation of curcumin release from Cur-AgNPs system. Cur-AgNPs were able to release 93.15% curcumin in 12 hrs whereas the plain curcumin was released 93.31% in 4 hrs.

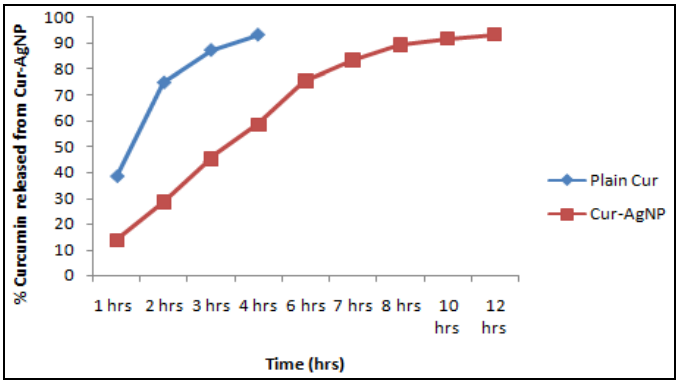

FIG. 7: DRUG RELEASE PATTERN OF PLAIN CURCUMIN (CUR) AND CUR-AgNPs

***In-vitro* cytotoxicity assay:** MDA-MB-435 and A-549 cells was investigated by MTT assay. The results clearly suggested a dose-dependent cytotoxicity, *i.e*. reduced cellular viability upon increasing the concentration of curcumin. The survival fraction of cells upon incubation of plain curcumin and Cur-AgNPs formulation in varying concentration is shown in **Fig. 8**.

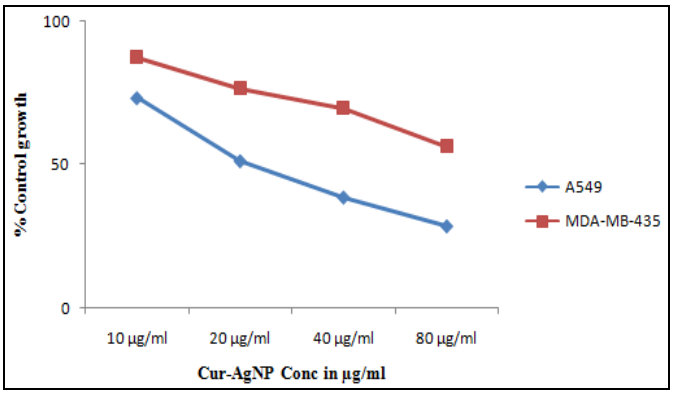

FIG. 8: *IN-VITRO* PERCENTAGE CONTROL GROWTH OF CUR-AgNPs IN A549 AND MDA-MB-435 CANCER CELLLINES

After incubation, Cur-AgNPs formulation shows inhibitory effect on cell growth, the % control growth was reduced with the increasing the concentration of curcumin. Further, the cell viability decreased when the concentration of curcumin either in free form or inside the AgNPs was increased. In the concentration range of 10–80 lg/mL, Cur-AgNPs were cytotoxic to a greater degree in comparison to plain curcumin. This is concordant with carbohydrate receptor-binding capacity exhibited by AgNPs. These results demonstrated that Cur-AgNPs cytotoxicity induced by curcumin to the tumor cells were dose dependent and had a greater cytotoxic effect on the tumor cells than did plain curcumin.

CONCLUSION: The parts of plant like root, leaf, rhizomes and their extracts can be widely and effectively used in the formulation of silver nanoparticles and drug delivery. The shape and size of nanoparticles can be very easily controlled with the use of plants. In the present study we observed that curcumin extracted from *Curcuma longa* rhizome may be be good source for the synthesis of AgNPs. This approach for synthesis of AgNPs of ethanolic extract of *Curcuma longa* has many advantages including the ease with which the process can be scaled up and its economic viability. The use of such eco-friendly nanoparticles as an anticancer agent in medical applications, renders this method potentially exciting for a large-scale synthesis of other inorganic materials

(nanomaterials). The effect of anticancer activity of curcumin obtained after ethanolic extract of *Curcuma longa* rhizome can be enhanced via formation of silver nanparticle by nanotechnology process. Results revealed that Cur-AgNPs showed effective anticancer activity towards cancer cell lines.

Hence it can be concluded that nanosize particle of curcumin was more effective in cancer inhibitory better than the larger size particles.

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DECLARATION OF INTEREST: The authors report no conflicts of interest.

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