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Synthesis Of Andrographolide Containing Silver Nanoparticle For Enhancement Of Anticancer Drug Delivery

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ABSTRACT:

By encasing andrographolide, which is isolated from Andrographis paniculata Nees, in silver nanoparticles, the suggested study enhances its anticancer potential. Using the Soxhlet process, andrographolide was isolated from Andrographis paniculata Nees. Its limited absorption and high hydrophobicity limit its clinical use as a chemopreventative drug. In order to improve solubility, absorption, and affectivity against tumours, its silver nanoparticles were created. The formation of nanoparticles was monitored using a variety of analytical techniques and assays, including drug release, haemolytic toxicity study, in vitro cytotoxicity study, zeta potential, particle size, Atomic Force Microscopy (AFM), Differential Scanning Colorimetric analysis (DSC), X-ray diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). Atomic force microscopy and particle size analysis showed the presence of silver nanoparticles with an average size of 17.98 nm, and a zeta potential of -4.68 mV was determined. An-AgNPs were able to release 95.11% of the andrographolide in 12 hours, while the andrographolide was released 94.21% in 4 hours. The results of haemolytic toxicity showed that An-AgNPs exhibits less hemotoxicity than plain andrographolide. X-ray diffraction was used to determine the synthesised nanoparticles' crystalline nature. According to the findings of the invitro cytotoxicity investigation, andrographolide-induced An-AgNPs cytotoxicity to the tumour cells was dosage dependant and more cytotoxic than that of plain andrographolide. This study demonstrates the potential anti-cancer effects of

silver nanoparticles encapsulated in andrographolide.

Keywords: *Nanotechnology, Silver Particles, Andrographolide, Andrographis paniculata Anticancer activity,*

INTRODUCTION:

Numerous treatments, such as chemotherapy, hormone therapy, surgery, radiation, immunotherapy, and targeted therapy, are used to treat cancer, a complex, multifactorial disease marked by the unchecked growth and spread of abnormal cells brought on by a combination of genetic, external, internal, and environmental factors [1]. The fight against cancer is still one of the biggest issues facing science, even with significant advances in research. Drug resistance, poor selectivity, and unwanted side effects are only a few of the serious problems with current cancer treatments. Finding effective, affordable, and sensitive lead compounds with cell-targeted selectivity and improving their sensitivity is therefore a challenge.

The development of nanoinstruments for oncological applications is the aim of a new research area called nanomedicine for cancer. Enabling early tumour detection, precise diagnosis, and customised treatment is the ultimate goal of cancer nanomedicine. The main advantage of cancer nanomedicine on the road to more individualised treatment is the use of nanoparticles with molecular-level functionality. According to the American Society for Testing and Materials' (ASTM) E2456-06 standard, nanoparticles are particles that range in size from 1 to 100 nm. But not all authors have followed these guidelines exactly. Therefore, particles larger than 100 nm are considered nanoparticles (NPs) in several investigations [2, 3, 4]. Nanoparticles can be classified as polymeric, metallic, magnetic, carbon nanotubes, liposomes, dendrimers, or quantum dots depending on their composition. [5, 6].

Numerous nanoparticles have been investigated for cancer diagnosis and treatment in recent years, including carbon nanotubes [7], paramagnetic nanoparticles [8], liposomes [9], gold nanoparticles [10], and numerous others [11]. To improve the pharmacokinetic and pharmacodynamic properties of existing anticancer medications and cancer treatment combinations, for example, nanoparticles may be employed [12, 13]. Silver nanoparticles (AgNPs) have unique properties that allow them to be utilised as catalysts, [14] in spectrally selective coatings for solar energy absorption as optical sensors, [15] in fabric tailoring and electronics devices, [16] and in a variety of bactericidal agents for medicinal purposes. [17, 18] Because of their strong reactivity, AgNPs play a significant role in preventing microbial (bacterial) growth in both solid and liquid culture mediums. [18] AgNPs prepared by green synthesis phenomena is effective in biological environmental systems. [19, 20]

With a molecular formula of $C_{20}H_{30}O_5$ and a molecular weight of 350.455 g/mol[3], andrographolide (An), a pure isolate of chemical synthesis derived from sambiloto plants (Andrographis paniculata Nees.), is an extremely bitter substance that takes the form of colourless, needle-shaped crystals. An has a maximum wavelength of 223 nm in the UV spectrum and a melting point of 228–230 °C in ethanol. [21] Its pharmacological effects include anti-inflammatory, antihyperlipidemic, hepatoprotective, antidiabetic, antimalarial, anti-arthritic, anticancer, antibacterial, immunomodulator, and antiparasitic. [22] It also inhibits the replication of the HIV virus. An has been shown in pre-clinical tests to have anti-cancer properties against a variety of cancer cell lines, according to mounting data. [23] However, An's poor bioavailability and high hydrophobicity significantly limit its clinical research and use. Therefore, to improve bioavailability and anti-cancer activity, a high-efficiency drug delivery system is needed. It's interesting to note that both in vitro and in vivo experiments revealed that loaded silver nanoparticles greatly improved the oral absorption and bioavailability of An. [24] Our hypothesis for the current investigation was that An-AgNPs have anti-cancer properties.

MATERIAL AND METHODS:

Material: Andrographis paniculata were collected from Botanical garden, Bareilly, U.P., 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: After gathering Andrographis paniculata, the leaves and stem were cleansed with deionised water. It was allowed to dry at ambient temperature in a pollution-free environment. It was ground into a powder using a mixer grinder after a few days. Using ethanol as a solvent, 20 g of the powder was transferred into a thimble and put in a Soxhlet apparatus. The final result was a dark yellow extraction that was filtered. For roughly 20 minutes, the filtrate was placed in an ultrasonicator set to a frequency of 20 KHz in order to decrease the andrographolide's particle size. [25]

Synthesis of silver nanoparticle: After precisely weighing and dissolving 0.017 grammes of silver nitrate in 100 millilitres of double-distilled water, the mixture was placed in an amber-colored bottle for later use. One millilitre of 1 mM silver nitrate (AgNO3) was brought to a boil. Ten millilitres of andrographolide extract were added to this suitable solution in order to reduce the silver ions (Ag+). The solution was then allowed to sit at room temperature for an hour, and the colour change was promptly seen. The creation of silver nanoparticles was shown by the colour changing from yellow to yellowish brown. It was shown that the ethanolic extract of andrographolide could decrease the aqueous silver ions to produce extremely stable silver nanoparticles. For later application, the produced silver nanoparticle was lyopillized. [26]

Characterization techniques:

FTIR analysis: An FT-IR spectrophotometer (Cary-630 FTIR, Agilent Technologies) was used to determine the FTIR spectra of AgNPs produced with andrographolide (An-AgNPs). [27]

Particle Size and Zeta potential Determination: Using a particle size analysis device (Malvern Instrument, United Kingdom), the zeta potential and particle size of silver nanoparticles of ethanolic extract of andrographolide (An-AgNPs) were ascertained. [28]

Atomic Force Microscopic analysis: Using AFM (AIST-NT Smart SPM 1000, CA), the structure and surface properties of andrographolide (An-AgNPs) were ascertained. The nanoparticles' AFM was achieved in AC mode on a glass substrate. [29]

X-Ray Diffraction (XRD) analysis: Using an X-ray diffractometer (Bruker, Munich, Germany), the crystalline nature of the silver nanoparticles of the ethanolic extract of andrographolide (An-AgNPs) was verified. The scanning rate was 2θ/min with an interval of 0.02º and a 2θ range of 0–40º. [30]

DSC analysis: A DSC instrument (Mettler Toledo DSC 822e) was used to conduct a DSC research of silver nanoparticles of ethanolic extract of andrographolide (An-AgNPs). After weighing and enclosing An-AgNPs in an aluminium pan with a pinholed cover, thermograms were taken in nitrogenous air and heated from room temperature to 350ºC at a rate of 700ºC in 7 minutes. [31]

Hemolytic toxicity: As outlined in our earlier study, whole human blood was drawn and kept in HiAnticlot blood collection jars. After centrifuging the human blood, the red blood cells were extracted and reconstituted in a 10% haematocrit normal saline solution. Five millilitres of distilled water (used as a 100% haemolytic

standard) and five millilitres of normal saline (used as a blank for spectrophotometric analysis) were individually incubated with one millilitre of the red blood cell suspension. Additionally, 5.0 mL of regular saline was mixed with 1 mL of suitably diluted andrographolide (An-AgNPs), which then reacted with the 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. [32]

Drug Release Study: A dialysis tube (MWCO 2000 Da) was filled with 10 milligrammes of andrographolide (An-AgNPs) that had been dissolved or dispersed in 5 millilitres of PBS (pH 7.4). The dialysis tube was submerged in 100 millilitres of PBS (pH 7.4) aqueous recipient medium. After the medium was stirred at 100 rpm and 37 2°C, the sample was continuously released through the dialysis tube, taken out at predetermined intervals, and subjected to HPLC analysis. To keep the sink condition stable, the same volume of fresh PBS (pH 7.4) was added. After that, HPLC was used to analyse the samples. [32]

In-vitro cytotoxicity assay: Tetrazolium dye-based MTT test was used to measure cellular cytotoxicity in accordance with a previously documented protocol.In a humidified incubator with 5% CO2, 17 MDA-MB-435 and A-549 cells were kept at 37°C in RPMI-1640 media supplemented with 10% heat-inactivated foetal bovine serum and antibiotics. For 24 hours, the cells were exposed to different doses of andrographolide (An-AgNPs) (10, 20, 40, and 80 lg/mL). Based on the formulation's drug content, the amount of formulation required to prepare molar equivalents of andrographolide was determined. Control was administered without the use of drugs. After adding MTT and incubating the plates for three more hours, the medium was removed and 250 lL of DMSO was added. An ELISA plate reader was used to measure each well's absorbance at 570 nm at 25°C. The formula was used to determine the survival fraction of cells by subtracting the average values of the triplicate from the average value of the control. [33, 34]

RESULTS AND DISCUSSION:

FTIR analysis of Andrographolide (An-AgNPs): FTIR analysis was done to measure the characterization and conjugation of potent biomolecules of andrographolide with silver nanoparticles. According to the FTIR spectra of Andrographolide (An-AgNPs) different peaks was obtained which indicates different functional groups and intermolecular bonding of Andrographolide (An-AgNPs).

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 NO3-group, C=C bonding, and =C-H stretch are indicated by the spectra obtained at 621, 971, 1033, 1080, 1402, 1544, and 1649, respectively. Thus, it makes sense that a variety of water-soluble heterocyclic chemicals, such as flavonoids and alkaloids, function as capping ligands to create andrographolide (An-AgNPs).

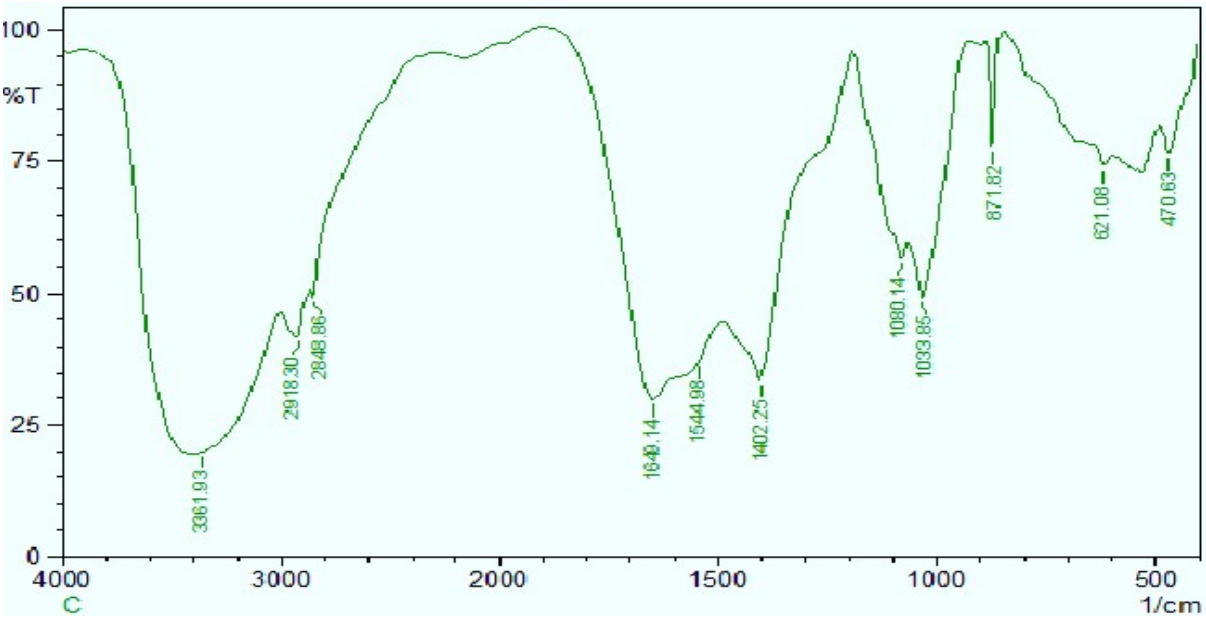

Fig. 1: FTIR of An-AgNPs

Particle Size and Zeta potential Determination: The zeta potential of andrographolide (An-AgNPs) was found to be -4.68 mV (Figure 3), while the average particle size was 17.98±0.16 nm (Figure 2), with the distribution of particle sizes measured at 71.3% of 20.31 nm, 17.5% of 16.67 nm, and 11.2% of 14.5 nm.

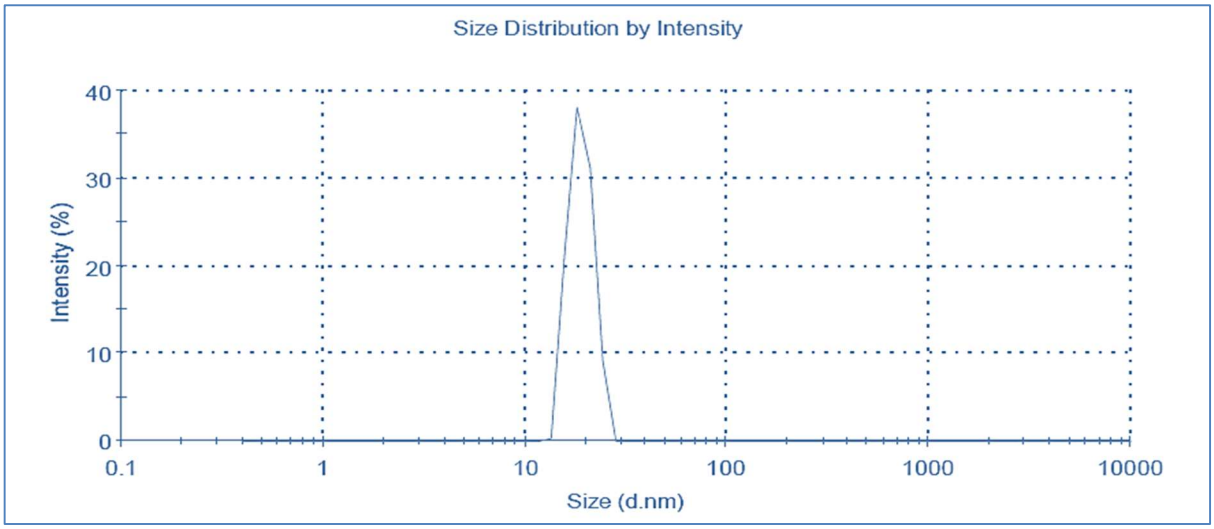

Fig. 2: Particle Size Study of An-AgNPs

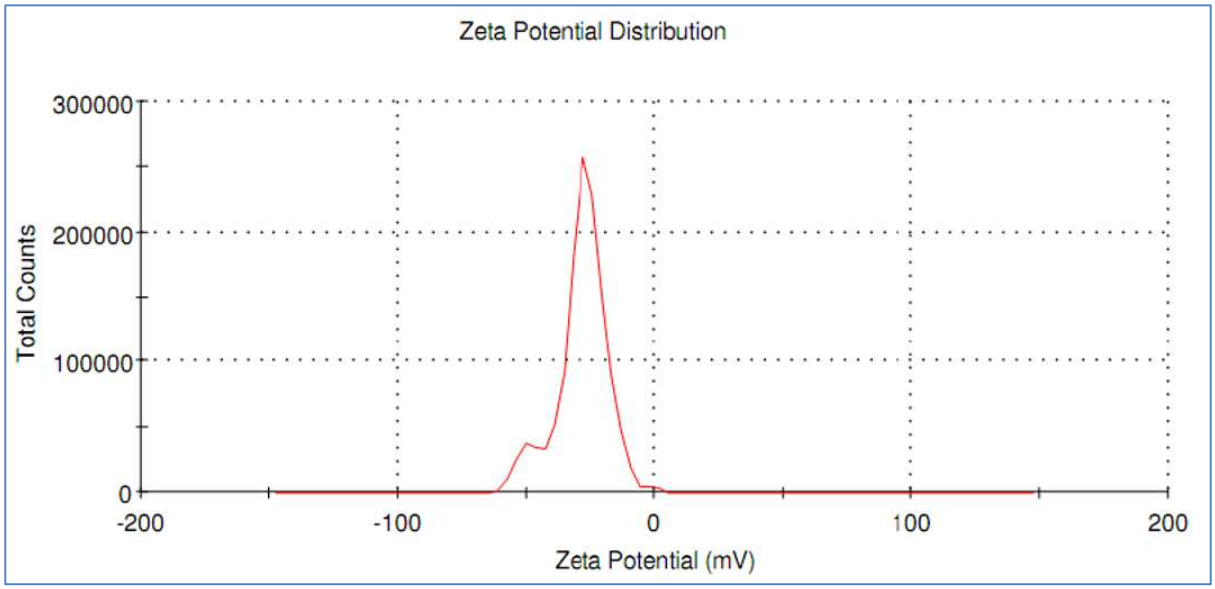

Fig. 3: Zeta Potential Study of An-AgNPs

Atomic Force Microscopic analysis: Atomic force microscopy was used to characterise the structure and surface properties of andrographolide (An-AgNPs), and a picture was produced. According to the AFM image (Figure 4), the andrographolide (An-AgNPs) was visullized in a spherical shape and within the nanometric size range of 18 nm.

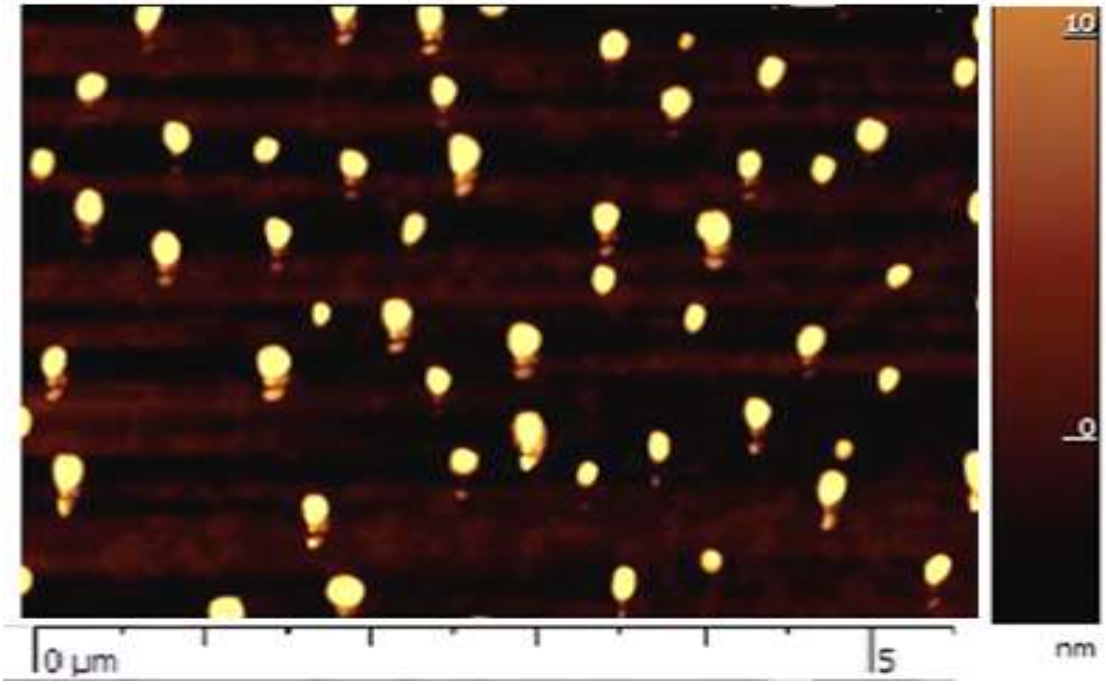

Fig. 4: AFM Image Of An-AgNPs

XRD analysis of Andrographolide (An-AgNPs): X-ray diffraction (XRD) is a powerful technique that is typically used to examine the intricacies and properties of nanoparticle structures. The angles at which an X-ray beam is diffracted by the object's crystalline phases are measured in order to view the XRD patterns. The crystalline nature of silver nanoparticles was demonstrated by the conspicuous peaks at 111, 200°, 220°, 38°, 311°, and 222° (2θ) in the XRD pattern of synthesised Ag-NPs, which is displayed in figure 5. It is known from the XRD pattern that andrographolide (An-AgNPs) was created utilising crystalline in nature.

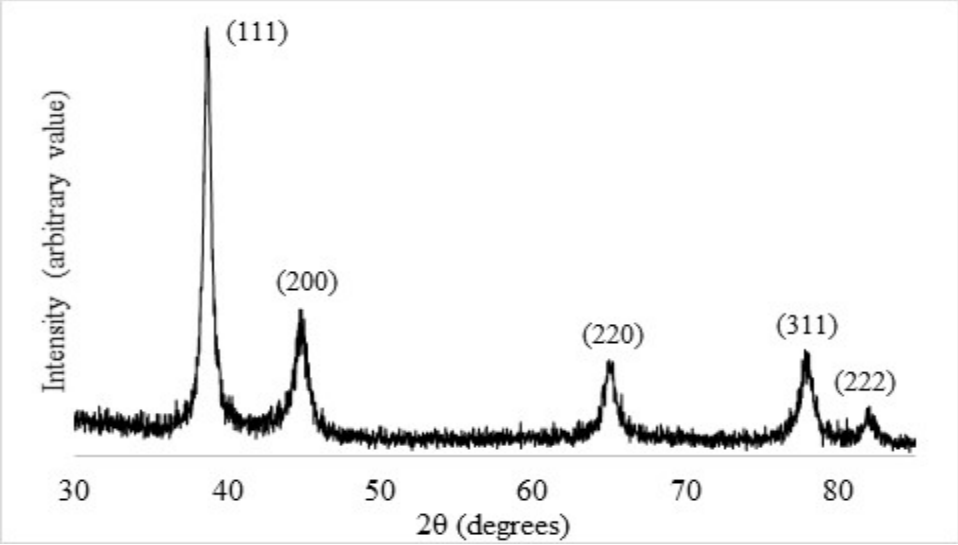

Fig. 5: XRD Image Of An-AgNPs

DSC analysis: The DSC thermogram of andrographolide and andrographolide (An-AgNPs) is displayed in Figure 6. For andrographolide, an endothermic peak was obtained at 264ºC, whereas an exothermic peak was seen at 36, 77, 166, and 191ºC. For Andrographolide (An-AgNPs), an endothermic peak was found at 264ºC, while an exothermic peak was found at 38, 165, 181, and 192ºC. But in the DSC thermogram of drug-loaded andrographolide (An-AgNPs), the exotherm of andrographolide is found at 166ºC, suggesting that the drug is present in crystalline form within the nanoparticles.

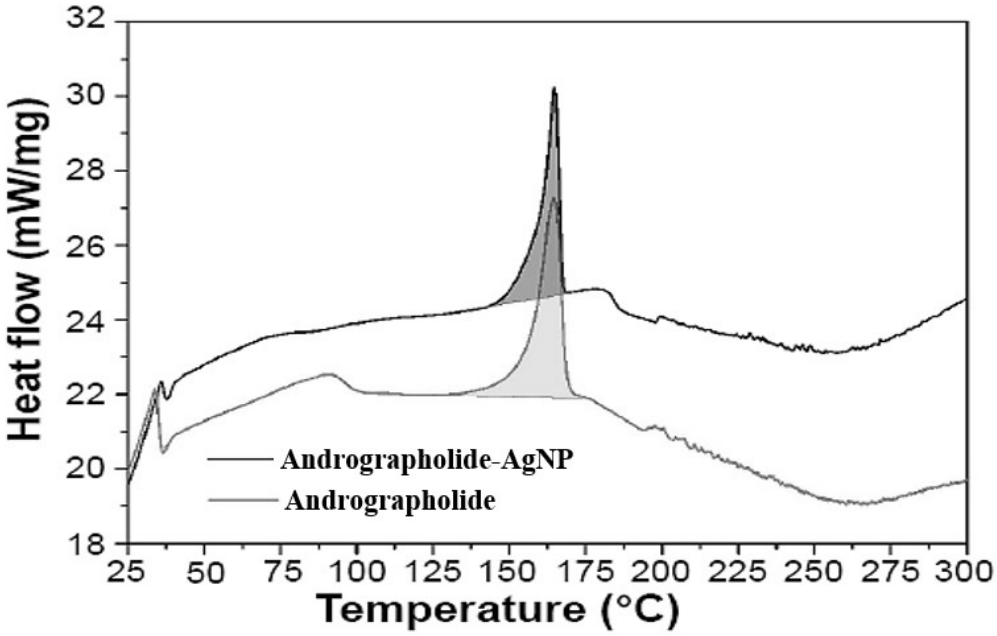

Fig. 6: DSC image of An-AgNPs.

Hemolytic toxicity: In order to assess the hemotoxic effect of andrographolide (An-AgNPs), a haemolytic toxicity study was conducted. Both andrographolide (An-AgNPs) and plain andrographolide have demonstrated haemolytic toxicity up to 3.11±0.5% and 16.22±1.10%, respectively. The drug content was used to calculate the andrographolide concentration for silver nanoparticulate formulations. It is conceivable that the haemolytic toxicity is significantly decreased by the encapsulating of drug molecules in AgNPs and the ensuing delayed release. Furthermore, compared to simple andrographolide (An-AgNPs), andrographolide (An-AgNPs) showed less hemotoxicity.

In-vitro drug release: The andrographolide's (An-AgNPs) and andrographolide release showed that the AgNPs had a sustained release characteristic (Figure 7). The findings shown that the andrographolide and Andrographolide (An-AgNPs) release system exhibited a noticeable temporal prolongation. Whereas simple andrographolide released 94.21% in 4 hours, andrographolide (An-AgNPs) was able to release 95.11% in 12 hours.

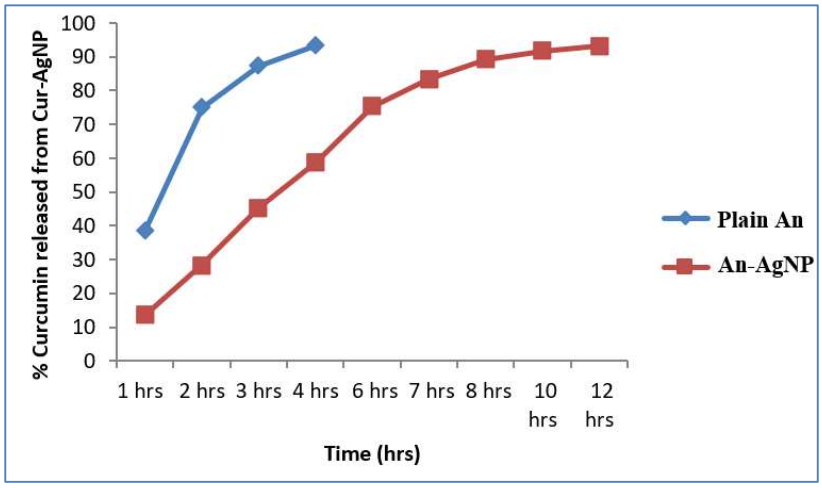

Fig. 7: Drug Release of Plain andrographolide (An) and Andrographolide (An-AgNPs)

In-vitro cytotoxicity assay: Using the MTT assay, MDA-MB-435 and A-549 cells were examined. The findings unequivocally pointed to dose-dependent cytotoxicity, or decreased cellular viability as andrographolide concentration rose. Figure 8 displays the percentage of cells that survive after being incubated with both plain andrographolide and andrographolide (An-AgNPs) formulation at different concentrations. Following incubation, the formulation of andrographolide (An-AgNPs) has an inhibitory impact on cell development; as the quantity of andrographolide increased, the percentage of control growth decreased. Furthermore, increasing the amount of andrographolide in the AgNPs or in free form resulted in a decrease in cell viability. The cytotoxicity of andrographolide (An-AgNPs) was higher than that of ordinary andrographolide in the concentration range of 10–80 lg/mL. This is consistent with AgNPs' ability to attach to carbohydrate receptors. These findings showed that the cytotoxicity of andrographolide (An-AgNPs) to the tumour cells was dosage dependant and that the cytotoxicity was more pronounced than that of plain andrographolide (An-AgNPs).

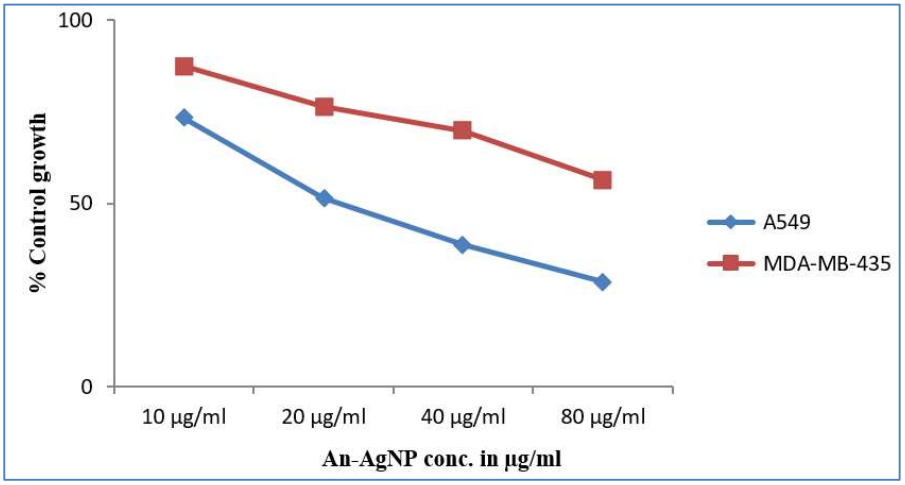

Fig. 8: In-Vitro Percentage Control Growth of An-AgNPs of Cancer Cell lines.

CONCLUSION:

We found in this work that andrographolide, which is taken from Andrographis paniculata, could be a useful source for AgNP production. Among the many benefits of this method for producing AgNPs from Andrographis paniculata ethanolic extract are its economic feasibility and ease of scaling up. This approach has the potential to be interesting for the large-scale synthesis of various inorganic materials (nanomaterials) due to the utilisation of such environmentally benign nanoparticles as an anticancer agent in medicinal applications. By using nanotechnology to create silver nanoparticles, the anticancer properties of andrographolide—which is produced from an ethanolic extract of Andrographis paniculata—can be strengthened. An-AgNPs had potent anticancer action against cancer cell lines, according to the results. Therefore, it can be said that andrographolide nanoparticles were more effective than larger ones at inhibiting cancer.

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